POPULATION ECOLOGY OF THE DIATOM GENUS *PSEUDO-NITZSCHIA* WITHIN THE SOUTH SLOUGH, CHARLESTON, OREGON

by

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A THESIS

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"Population Ecology of the Diatom Genus *Pseudo-nitzschia* within the South Slough, Charleston, Oregon," a thesis prepared by Andrew Ohana-Richardson in partial fulfillment of the requirements for the Master of Science degree in the Department of Biology. This thesis has been approved and accepted by:

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Andrew Ohana-Richardson for the degree of Master of Science

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Title: POPULATION ECOLOGY OF THE DIATOM GENUS *PSEUDO-NITZSCHIA* WITHIN THE SOUTH SLOUGH, CHARLESTON, OREGON

Approved: _____
Dr. Lynda Shapiro

Environmental factors may affect the population ecology of potentially toxigenic *Pseudo-nitzschia* spp. within the South Slough, Charleston, Oregon. Stationary and channel sites were sampled in 2005 and 2006 for: physical parameters including temperature, salinity, and nitrogen (as nitrate + nitrite) as well as biological variables chlorophyll *a*, *Pseudo-nitzschia* cell concentrations, species identifications, and domoic acid levels (particulate and dissolved DA). Linkages between physical and biological parameters were sought statistically.

The results indicate temperature and salinity are important predictors of *Pseudo-nitzschia* cell concentrations throughout the sampling periods. This indicates high abundances in autumn based on historical predictors. pDA levels, likely from *P. australis* and *P. pungens*, increased with temperature. dDA decreased with increasing pDA. This does not follow the historical trend of phytoplankton bloom abundance driven

by nitrogen values within the South Slough. Further, these results document the presence of *Pseudo-nitzschia* and DA within an estuary and may enhance management practices.

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TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

LIST OF TABLES

CHAPTER I

INTRODUCTION

Harmful algal blooms, HABs, are the sudden and exponential increase in a species or group of phytoplankton such that they become a nuisance to humans. HABs can occur in both freshwater and marine ecosystems and are global problems affecting many different parts of the world. Many different protists are known to form HABs, with the most well studied forms being the dinoflagellates. The only known HAB phenomenon associated with diatoms, Bacillariophyta, is termed Amnesic Shellfish Poisoning (ASP) or more recently Domoic Acid Poisoning (DAP). DAP refers to a suite of mammalian symptoms that include vomiting, diarrhea, confusion, memory loss, disorientation, coma, and even death (Perl et al. 1990). DAP was known as ASP due to the linkage of amnesia with consumption of shellfish following the initial identification of domoic acid as a toxin (Wright et al. 1989).

The amino acid domoic acid (DA), a homolog of the neurotransmitter glutamate, was originally isolated from the red alga *Chondria armata* and used for medicinal purposes in the treatment of intestinal pinworm in Japan (Takemoto and Daigo 1958). DA is able to bind to the glutamate receptors on mammalian neurons, causing neuronal depolarization in the hippocampus that is followed by swelling which triggers memory loss (Teitelbaum et al. 1990). The first documented DAP event occurred in 1987 on

Prince Edward Island (PEI), Canada (Wright et al. 1989) when more than 100 illnesses and at least three deaths were directly linked to consumption of the blue mussel, *Mytilus edulis* (Perl et al. 1990). The origination of the toxin was quickly linked to a large bloom of *Pseudo-nitzschia multiseries* (previously *Nitzschia pungens* Grunow f. *multiseries*, Hasle et al. 1996) found off Cardigan Bay in the autumn of that year (Bates et al. 1989).

During 1987, more than 15 million *P. multiseries* and *P. pungens* cells L^{-1} were found around the time that domoic acid concentrations reached 790 $\mu g\ g^{-1}$ of mussel tissue. Current closure levels for shellfish toxicity are set at 20 $\mu g\ g^{-1}$ where toxigenic *Pseudo-nitzschia* spp. are known. The climatology (dry summer followed by unusually wet autumn) is a likely explanation for inititaion of the large bloom event off PEI. It is theorized that stratification followed by heavy freshwater runoff could have provided nutrients important in bloom development (Smith 1993). The following year was also high in mussel domoic acid levels as well as population abundance, but in subsequent years, with drier autumns, concentrations declined until levels were negligible. A time delay of peak domoic acid level nine days after peak *Pseudo-nitzschia* concentrations was also obvious (Smith et al. 1990).

In 1991, another large DAP event occurred, this time on the west coast of North America and involving seabirds instead of humans. In Monterey Bay, California, more than 100 brown pelicans and Brandt's cormorants died due to consumption of DA (Work et al. 1993). The DA came from a different species, *P. australis*, that was initially fed upon by anchovies, which were in turn consumed by the birds (Buck et al. 1992; Fritz et al. 1992). During this bloom, population abundances were greater than 10 million cells

L^{-1}. DA concentrations found within the anchovies were as high as 2000 μg g^{-1} of tissue. Peak particulate DA (pg $cell^{-1}$) corresponded to peaks in cell abundance during the spring and autumn months throughout 1991 to 1994. The spring bloom is thought to be triggered by upwelling conditions while the larger autumn bloom is related to high stratification of surface waters and lower nutrient concentrations, the relaxation following upwelling (Walz et al. 1994).

There are currently ten diatom species within the genus *Pseudo-nitzschia* that are described to be toxigenic in culture (Bates 2000). Most of these are considered cosmopolitan, with the well studied *P. multiseries*, *P. pungens*, and *P. australis* thought to occur globally (Hasle 2002). As previously discussed, these diatoms can bloom suddenly creating large, dense populations which may move onshore and inundate the benthic or pelagic food web with toxigenic cells. Many different studies have investigated the development of blooms and initiation of domoic acid production and most of these studies have been performed with cultured isolates.

Nutrients are likely to play an important role in both bloom development and domoic acid production due to the requirement of phytoplankton for molecules required for photosynthesis (Redfield et al. 1963). During the PEI DAP event, low levels of silicate were found in the coastal waters (Subba Rao et al. 1988). Silicate is important for frustule development of diatoms and in low concentrations is inhibitory to the growth of *Pseudo-nitzschia* cells (Bates et al. 1991; Pan et al. 1991). Silicate has also been negatively correlated with cellular DA concentrations (Pan et al. 1996). Nitrogen, utilized by all phototsynthetic organisms, is another important nutrient linked to *Pseudo-*

nitzschia ecology. Nitrogen (N), either in the form of nitrate, nitrite, or ammonium, can be used directly by phytoplankton in the production of amino acids, including DA. It has been demonstrated that cultures of *P. multiseries* will not produce domoic acid in low N batch cultures during stationary phase when DA is normally abundant (Bates et al. 1991). Of great ecological importance is the speed at which nutrients may be added to or depleted from a coastal ecosystem. This is why coastal upwelling plays such an important role in phytoplankton bloom maintenance.

Upwelling is the process by which cold, nutrient rich water is brought to the surface from depth. It occurs along eastern boundary coastlines where strong winds carry surface water offshore through Ekman transport. Downwelling/relaxation events occur following the cessation or reversal of winds causing the upwelling. Essentially, downwelling brings warmer, oligotrophic surface water back towards shore, which then moves over recently upwelled water. This provides for a nutrient rich front at depth above the critical depth, or depth at which photosynthetic rate equals or exceeds respiration rate, allowing phytoplankton cell growth. Along the West Coast of North America, upwelling dominates during the spring and summer when Ekman transport is driven by strong, northwesterly winds. For this reason, spring and late summer blooms are common off the Western United States inshore of the California Current system.

Upwelling and downwelling events also play an important role not only for open coastal waters but also for coastal embayments where nutrient rich water may entrain from offshore upwelling sources during sustained wind relaxation events as seen off Oregon (Smith et al. 1966). Relaxation events have been shown to allow movement of

Pseudo-nitzschia spp., as well as other phytoplankton, into coastal embayments (i.e. Sunset Bay, OR) (Shanks and McCulloch 2003). This process should be considered important for estuaries as well as open marine dominated inlets.

Estuaries are an important land and sea interface where physical and chemical factors change with the ebb and flood of tydes. Estuaries are some of the most productive marine ecosystems (Odum 1971). They are important habitats for many organisms including birds, plants, fishes, invertebrates, and mammals. The South Slough, in Charleston, Oregon, is the southern arm of the productive Coos Estuary and has been protected, along with 24 other estuaries nationwide, by the National Estuarine Research Reserve System. The Coos Estuary is the fifth largest estuary in the Pacific Northwest and is the home of a large aquaculture industry (Munson and Fishman 1984). Coos Bay is the largest producer of Pacific Oysters within the state (Rumrill 2007).

Odum (1969) notes that estuaries are kept in an early and fertile stage by the rapid nutrient cycling caused by tidal forces. The influence of the tide plays and important role in supplying many primary producers, coastal marine phytoplankton, to the South Slough. Trainer et al. (2000) determined that the flow of cells and nutrients from headlands into stratified embayments can provide for phytoplankton bloom development. Roegner and Shanks (2001) showed that chlorophyll *a*, an important measure of phytoplankton abundance, was coastally derived and advected into the South Slough with the flooding tide. Hughes (1997) determined that a difference in phytoplankton abundance existed between coastal and riverine ends of the South Slough. She also found seasonal variation in phytoplankton abundance occurred due to the tide domination

in the non-winter months.

Cziesla (1999) determined that *Pseudo-nitzschia* populations in the coastal waters of the Coos Bay region increased during a downwelling/relaxation event during the late summer upwelling season. He found that nutrient concentrations and wind direction were optimized to keep a population blooming just outside the mouth of Coos Bay. The stratification created by this downwelling/relaxation period kept *Pseudo-nitzschia* within 10 km of the shore. Cziesla (1999) concluded that the estuarine population Hughes (1997) described entered the bay and subsequently South Slough with every tidal flood.

Even with the knowledge of bloom movement onshore and up-estuary as well as the fact that *Pseudo-nitzschia* blooms have occurred on the West Coast at least since the 1920s (Fryxell et al. 1997), little is known about the ecology behind bloom populations once they move within estuaries. Given that estuaries such as the South Slough in link marine and terrestrial food webs, it is paramount that more be determined related to the physical limitations of toxigenic species. The large Pacific Oyster fishery within the Coos Estuary (Rumrill 2007) is especially vulnerable to environmental problems of the coastal waters. Because of their potential as a food source for the oysters and other estuarine residents, it is necessary to determine factors influencing the source and advection of potentially toxigenic *Pseudo-nitzschia* spp. within estuarine waters. The purpose of this study is to characterize the important physical parameters governing *Pseudo-nitzschia* spp. bloom persistence as well as toxin production within the South Slough in hopes that this may be utilized in future studies on DAP events in estuarine ecosystems.

This thesis addresses the following questions:

I. What are the background physical parameters important for phytoplankton growth within the South Slough? This will be answered with data collected in the summer of 2005. That data includes temperature, salinity, nitrogen, and chlorophyll *a* concentrations collected at three fixed station sites corresponding to lower, middle, and upper estuary.

II. When are *Pseudo-nitzschia* abundant within estuarine waters? This will be answered with data collected in summer and autumn 2005 and spring 2006. The data includes temperature, salinity, nitrogen, and *Pseudo-nitzschia* concentrations collected at the three fixed station sites as well as within the estuarine channel at various sites following flood tide at one hour before high tide. This data will also be utilized to create statistical models for forecasting purposes.

III. What species of *Pseudo-nitzschia* are present? This will be answered with data collected in 2005 and 2006 (see II). The data includes taxonomic identity of cells in whole water samples collected at the same sites as II.

IV. Are these species toxigenic? Again, this will be answered with the data collected in 2005 and 2006. The data includes analysis of particulate (pDA) and dissolved (dDA) domoic acid concentrations from high cellular abundance sites in II.

I propose that physical parameters within estuarine waters directly influence all marine

phytoplankton. More specifically, it is hypothesized that *Pseudo-nitzschia* spp. abundance limitations determined by physical parameters can be utilized to forecast bloom persistence. It is also thought that DA concentrations should be present in estuarine waters given presence of toxigenic species and that DA production is also directly linked with governing physical parameters.

CHAPTER II

MATERIALS AND METHODS

2.1 Description of Study Site

The South Slough is the southern arm of the Coos Estuary (43.35ºN and 124.34ºW) that exists about halfway between Puget Sound and San Francisco Bay. The Coos Estuary is the largest estuary within Oregon at more than 54 km^2 (Proctor et al. 1980). The South Slough is characterized as a drowned river mouth estuary which is typically well mixed in the dry season when rainfall drops below 10 cm per month. The South Slough National Estuarine Research Reserve, roughly 24% of the South Slough, is managed by state and federal agencies that are part of the NERR system. The area is very important commercially for shellfish harvesting (i.e. *Crassostrea gigas*, Rumrill 2006).

Samples were collected within the South Slough at three fixed station sites from June through August 2005 and in channel at varying intervals (Fig. 1) in October 2005 and May through June 2006. The three fixed station sites encompass the entire tidally influenced portion of the Winchester arm of the South Slough and were used in a previous study of phytoplankton variability (Hughes 1997). The most oceanic of these is the Boat House (site x, Fig. 1) at the Oregon Institute of Marine Biology (OIMB), University of Oregon, which is characterized by well mixed neritic water throughout the

year. The second site is at the South Slough Pilings (site y, Fig. 1), with a usual salinity gradient of 20-30 psu throughout the year (Hughes 1997). The last site is located at Hinch Lane Bridge (site 12, Fig. 1). This most southern site is characterized by extreme salinity gradients of fresh water (near 0 psu) in the winter months to near 32 psu in the summer months (Hughes 1997). Seasonal variation of water parameters within the South Slough is summarized by Rumrill (2007).

Samples collected in channel (sites a-m and 1-12, Fig. 1) were collected at approximate 10 minute intervals near the center of the channel following any tidal flow that could be seen, such as characterized by floating debris. Collection began during each sampling event at the entrance of the Charleston Boat Basin (oceanic) and continued up estuary to riverine sites in Winchester and Sengstaken arms. Thus large ranges of salinity were sampled during the event.

2.2 Sample Collection

During each sampling event, the flooding tide was followed up-estuary starting at one hour before high tide. Whole water samples were collected at all sites using a bucket sampler to collect the top meter of water column. These were immediately placed on ice until further analysis. From these, samples were collected for nitrogen and chlorophyll *a* analysis. An additional whole water sample was collected at each site and immediately fixed with acid Lugol's solution to be analyzed later for *Pseudo-nitzschia* abundance and composition. The same protocol was followed for both fixed station and channel sampling. Timing of the channel sampling was based on plankton tows which were

performed from the OIMB Boat House at least weekly from October 2005 through June 2006 to determine *Pseudo-nitzschia* abundance in the water column. If it was at least the third most dominant organism visualized with light microscopy, channel sampling was performed the following day and continued until abundance dropped below these criteria.

During sample collection, temperature and salinity measurements were recorded with an YSI 30 meter (Yellow Springs, OH, USA). Coordinates were taken with a Magellan GPS Tracker (San Dimas, CA, USA).

2.3 Historical and Experimental Nitrogen Analysis

Monthly nitrogen (nitrate + nitrite, mg L^{-1}) and chlorophyll *a* (μg L^{-1}) data (N = 86) collected by S. Rumrill in 2003 and 2004 (NOAA's Central Data Management Office 2004) at three sites (Boat House, Valino Island, and Winchester Arm corresponding to sites x, 10, and 12 in Fig. 1) were log transformed and regressed using the method of least squares. Nitrogen was treated as the predictor and chlorophyll *a* as the dependent variable (Fig. 3). Significant relationship was tested with Analysis of Variance (ANOVA, $\alpha = 0.05$; see Appendix for statistical tables; all analyses pefromed with SPSS 14.0). The model equation took the form of Eq. (1):

$$Y = \beta_{00} + \beta_{01}N \qquad (1)$$

where N is log transformed nitrogen (mg L^{-1}) and Y is the predicted log transformed chlorophyll (μg L^{-1}).

Whole water samples collected in 2005 and 2006 on dates with >5000 *Pseudo-nitzschia* cells L^{-1} at site x (Table 1) were processed for nitrogen (nitrate + nitrite) concentration spectrophotometrically (South Slough 2005). To 25 mL of sample was added 75 mL ammonium chloride-EDTA solution which was then reduced using a cadmium-copper column. Reduced sample was collected at a rate of ~10 mL min^{-1}. The first 50 mL collected was discarded. To 25 mL of reduced sample was added 1 mL sulfanilamide color reagent. This solution was swirled and allowed to sit for 10 min. Absorbance was then measured at 543 nm against a nanopure water blank on a spectrophotometer (Aquamate). A standard curve was measured using standards created from commercial nitrate-nitrogen (Fischer) at the following concentrations: 0, 0.4, 0.8, 1.2, 1.6, 2.0 (ppm). Final concentrations of sample nitrogen were calculated from standard curve. Concentrations were recorded in mg L^{-1} and log transformed for further analysis.

Log transformed nitrogen concentrations from 2005 and 2006 were treated as three populations defined by season of year (Table 2) independent of sampling year or site. The three seasons were spring (N = 24), summer (N = 30), and autumn (N =13). Winter sampling was not performed due to low abundance of *Pseudo-nitzschia* spp. within estuarine waters (Hughes 1997). Nitrogen concentrations were compared across seasons using a non-parametric Krustal Wallis mean rank difference test ($\alpha = 0.05$). Means and ranges of nitrogen were plotted across seasons (Fig. 8). Differences across fixed station sites and regions (Kruskal Wallis mean rank difference) were also tested for significance. Historical nitrogen concentrations (collected by S. Rumrill) were compared

with collected collected samples using the Mann-Whitney-Wilcoxan rank difference test.

Table 1. Sampling dates (when *Pseudo-nitzschia* spp. >5000 cells L^{-1}) used in analyses listed by day and month, sites correspond to notation on area map (Fig. 1). Fixed station sites located at: x = 43º20.988'N, 124º19.798'W; y = 43º17.981'N, 124º19.421'W, 12 = 43º26.581'N, 124º19.183'W. * Used in nitrogen regression analysis.

Year	Site
2005	Fixed station sites: 18-May (y not sampled); 03-Jun; 07-Jun; 10-Jun; *04-Jul; *05-Jul; *06-Jul; *08-Jul; *17-Aug; *18-Aug; *19-Aug; *22-Aug; *23-Aug; *29-Aug Channel sites: *11-Oct 1-6,11; *17-Oct 7-12
2006	Channel sites: *14-Jun a-g; *19-Jun h-m

Table 2. Sampling dates for nitrogen analysis divided into season of collection. Seasons: 1 = spring, 2 = summer, 3 = autumn.

Season	Site
1	18-May-05; 03-Jun-05; 07-Jun-05; 10-Jun-05; 14-Jun-06; 19-Jun-06
2	04-Jul-05; 05-Jul-05; 06-Jul-05; 08-Jul-05; 17-Aug-05; 18-Aug-05; 19-Aug-05; 22-Aug-05; 23-Aug-05; 29-Aug-05
3	11-Oct-05; 17-Oct-05

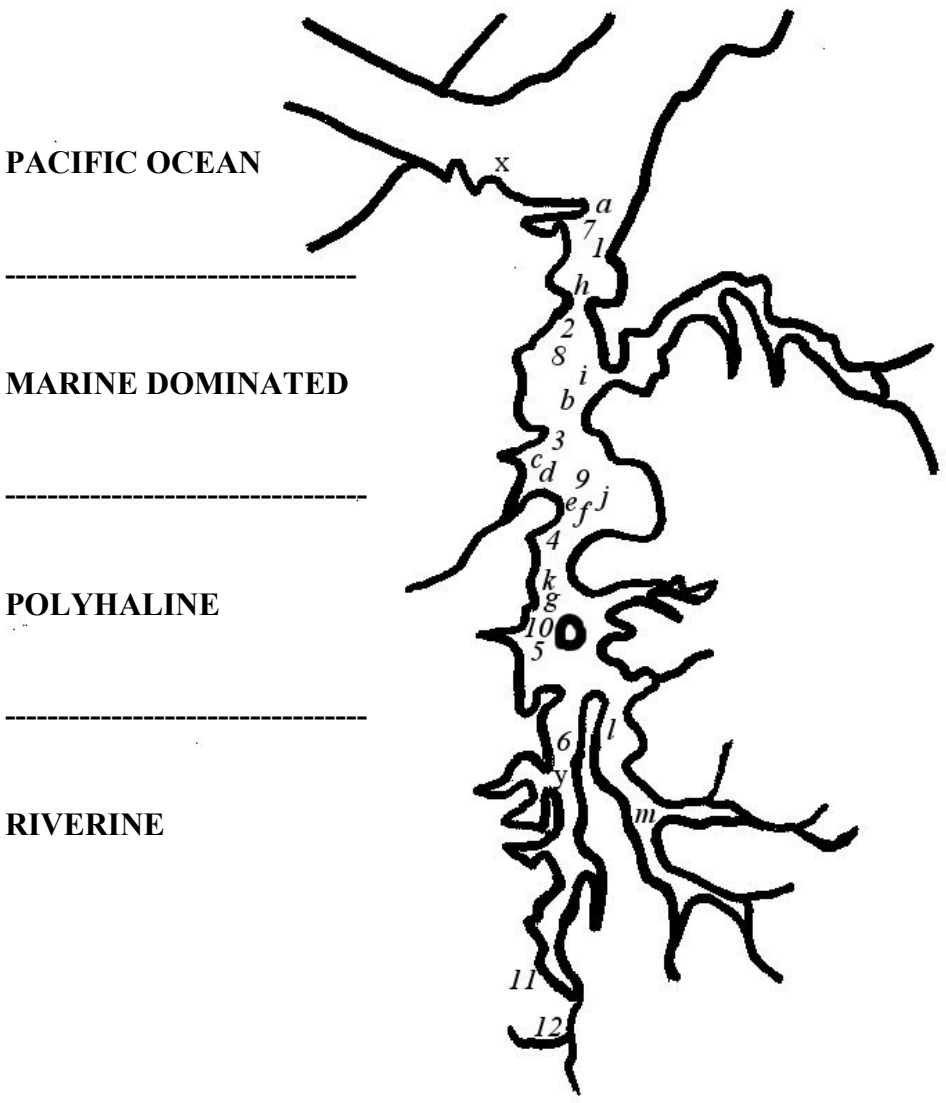

Figure 1. South Slough sampling sites, Charleston, OR. Fixed station sites at OIMB Boat House (x), S. S. Pilings (y), and Hinch Lane Bridge (12). Boat sites in 2005 (1-12) and 2006 (a-m) were collected in channel.

2.4 Chlorophyll *a* Analysis

Chlorophyll *a* extracts were made from whole water by filtering 100mL of gentle inversion-mixed sample through 47mm Whatman GF/C filters (Sigma-Aldrich) using gentle vacuum filtration (<6 in. Hg) as in US EPA Method 445.0 (1997). Each filter was rolled, placed in a glass, foil-wrapped culture tube and stored at -20°C. Tubes were stored for a minimum of 24hours and not longer than 1 week until analysis. Extraction was performed by placing 5mL of 90% acetone into each tube and refrigerating at 4°C overnight. Following refrigeration, tubes were warmed to ambient room temperature while kept in the dark. Once at room temperature, tubes were centrifuged for 10 min. and supernatant was pipetted into fluorometry tubes (Parsons, Maita, and Lalli 1984). Samples were then analyzed using a TD-700 Laboratory Fluorometer (Turner Designs) for direct concentration of chlorophyll *a* following standardized calibration and blanking with 90% acetone solution. All measurements were recorded in concentrations of μg of Chlorophyll *a* per liter of seawater.

Chlorophyll *a* data from fixed station sites with greater than 5 μg chl *a* L^{-1} (N = 55, Table 3) were log transformed for a plot of means with 95% confidence intervals (Fig. 12). Means across sites (Boat House N = 30, S. S. Pilings N = 6, Hinch Lane Bridge N = 19) were compared using a One-way ANOVA with α set at 0.05. Differences were compared, following significant ANOVA results, with Tukey's HSD. Similar tests were performed for determination of seasonal (Kruskal Wallis mean rank difference) and regional (ANOVA) differences.

Log transformed chlorophyll *a* data from fixed station sites with greater than 5 μg

chl *a* L^{-1} (N = 55, Table 3) were regressed with method of least squares (Fig. 16) using backwards-selection stepwise analysis with temperature, salinity, and tidal height data (NOAA) as explanatory variables. Log transformed temperature was found to be the significant explanatory variable ($\alpha = 0.05$). The model equation took the form of Eq. (2):

$$Y = \beta_{00} + \beta_{01}T \quad (2)$$

where $\underline{T}$ is log transformed temperature (°C) and $\underline{Y}$ is the predicted log transformed chlorophyll *a* ($\mu g\ L^{-1}$) concentration.

Table 3. Sites (>5 µg chl *a* L^{-1}) listed by day and month, sites correspond to notation on area map (Fig. 1). Fixed station sites located at: x = 43º20.988'N, 124º19.798'W; y = 43º17.981'N, 124º19.421'W; 12 = 43º26.581'N, 124º19.183'W.

Year	Site
2005	01-Jul 12; 04-Jul x,12; 05-Jul x,12; 06-Jul x,12; 07-Jul x,12; 08-Jul x,12; 20-Jul x,12; 21-Jul x; 25-Jul x; 26-Jul x; 27-Jul x; 28-Jul x; 01-Aug x,12; 02-Aug x,12; 03-Aug x,y,12; 04-Aug x,12; 05-Aug x,y,12; 09-Aug x,12; 10-Aug x,12; 11-Aug x; 12-Aug x; 15-Aug x; 16-Aug x,y,12; 17-Aug x,12; 18-Aug x,y,12; 19-Aug x,y,12; 22-Aug x; 23-Aug x; 25-Aug x; 29-Aug x; 30-Aug x,y,12

2.5 Cell Abundance Quantification

For determination of cell abundance, cell counts were performed from whole water samples preserved with acidic Lugol's solution using a modified Utermöhl (1931) method. Following gentle inversion mixing for 1minute, 5mL of each sample was micropipetted into a settling chamber. Samples were allowed to settle for a minimum of 2h (see abundance quantification optimality experiment, Chapter 2.6). Cell counts were then performed using an inverted compound microscope (Leica Microsystems, Wetzlar, Germany) at 100x magnification scanning the entire settling chamber. Care was taken to ensure that cells were only counted that contained visible cellular content (e.g. intact chloroplasts) as representative of being alive upon fixation. Counts were calculated to cells per liter of seawater. Data was then log transformed for normality in further analyses.

2.6 Abundance Quantification Optimality Experiment

To determine the optimal settling time for *Pseudo-nitzschia* cells fixed with acidic Lugol's solution, three replicates of 5mL of a known high abundance sample (05-July, 2005 Boat House site) were settled for 2h, 5h, 24h, and 48h. Cell counts were performed after each time interval. A comparison of treatment means using ANOVA ($\alpha = 0.05$) was performed to determine any significant differences between settlement times (Fig. 2, SPSS 14.0).

The optimality experiment did not result in any significant differences between count means of different treatment times ($F = 0.806$, $p = 0.525$). Two hours (4.25 +/-

0.02) of settlement yielded similar results to 5 (4.26 +/- 0.03), 24 (4.24 +/- 0.02), and 48 (4.22 +/- 0.02) hours for *Pseudo-nitzschia* cells that were fixed with Lugol's solution (Fig. 2).

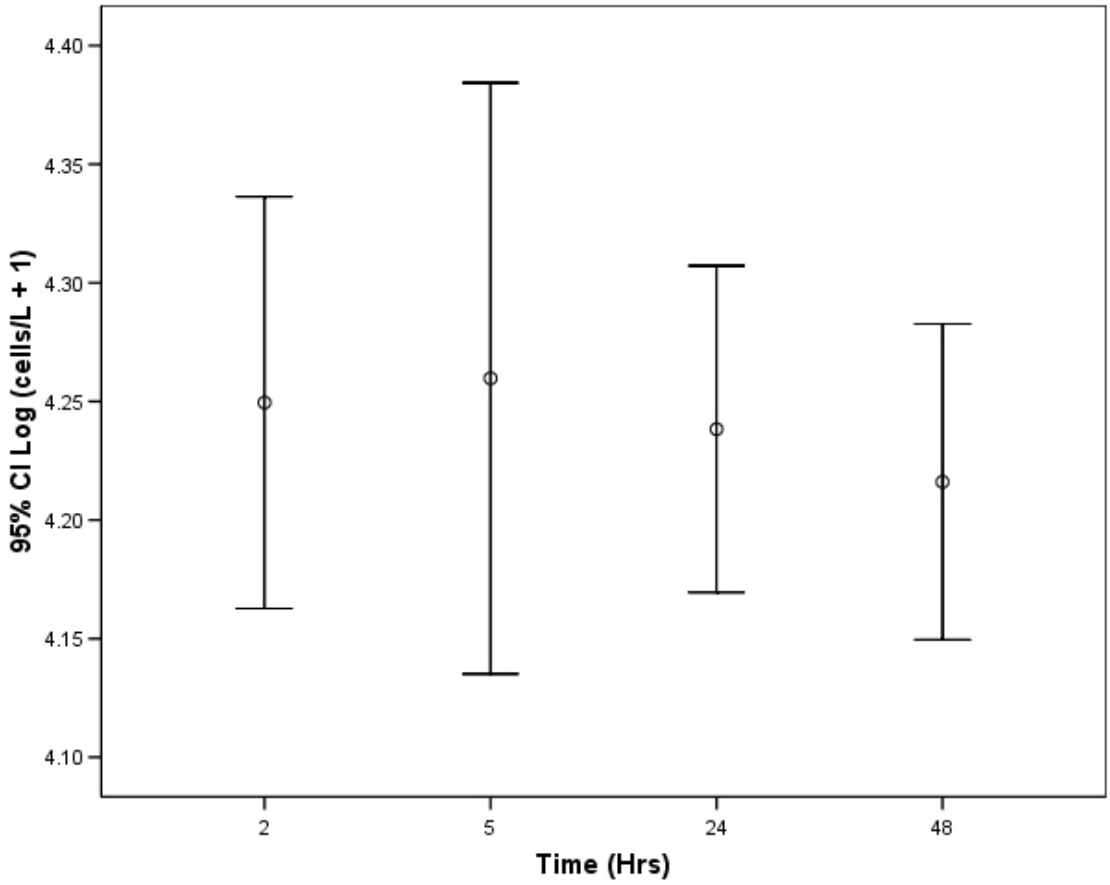

Figure 2. Comparison of means across treatments. Sample from 05-July, 2005. F = 0.806, p = 0.525, (SPSS 14.0).

2.7 Salinity and Temperature Analysis

Near daily salinity (psu) and temperature (°C) data collected by S. Rumrill at Valino Island (site 10, Fig. 1) from 2000 (N = 341), 2001 (N = 358), 2002 (N= 365), 2003 (N = 365), and 2004 (N=363; NOAA's Central Data Management Office 2004) was log transformed and averaged through seasons (winter = January through March, spring = April through June, summer = July through September, and autumn = October through December). These were plotted with 95% confidence intervals (Figs. 4 and 5). Means across seasons were compared using a One-way ANOVA with α set at 0.05. Differences were compared, following significant ANOVA results, with Tukey's HSD.

Salinity and temperature data (Fig. 23) were analyzed as predictors in fitting single (Figs. 24 and 25) and multiple (Fig. 26) linear regression models with the method of least squares while log transformed cell count data was treated as the dependent variable. Only data (N=13) from 2006 was used in analysis. Zero values were omitted from analysis entirely. The multiple regression model equation took the form of Eq. (3):

$$Y = \beta_{00} + \beta_{01}S + \beta_{02}T \qquad (3)$$

where S is the log transformed salinity (psu), T is the log transformed temperature (°C), and Y is the predicted log transformed cell concentration (cells L^{-1}). Correlations with cell concentrations were found for both explanatory variables independently using the Pearson Correlation Coefficient.

Data from 2005 was used to test the model's predictive capabilities. Predicted

and observed cell concentrations were regressed with method of least squares, observed cell concentration was treated as the independent variable (Figs. 27 through 32). Data from 2000 through 2004 collected by S. Rumrill was processed through the models to determine times when salinity and temperature conditions were optimal for *Pseudo-nitzschia* population growth (Figs. 33 and 34).

2.8 Cell Identification

Pseudo-nitzschia cells were identified to species level using a scanning electron microscope (Zeiss Ultra 55 SEM, Micro Analytical Facility, University of Oregon). A modification of the EPA standard method (LG401) was utilized for digestion of cells to be analyzed with SEM, whereby 8-15mL of samples (from dates with greater than 5000 cells L^{-1}, Table 1, N = 18) were centrifuged for 4min. at 3000xg in centrifuge tubes and decanted. Pellets were resuspended using deionized water. Centrifugation and resuspension was repeated until the supernatant ran clear. Cells were resuspended in 2mL of 30% hydrogen peroxide and mixed. Samples were loosely capped and then placed in a sand bath at 80°C for 2-4h. Samples were cooled and supernatant was decanted. Tubes were then filled with 10mL of deionized water, mixed to resuspension, centrifuged and decanted. Centrifugation was repeated twice. Pellet was then resuspended in 1mL deionized water. A drop of resuspended material was dried overnight on 15mm aluminum specimen mount stubs with 12mm carbon tabs (Electron Microscopy Sciences). Stubs were gold sputter coated and analyzed using digital imaging.

Cells from each site were identified to species level using two or more of the following morphological characteristics: valve length; valve width; number of striae within 10 μm; number of poroid rows within each stria; presence or absence of a central large interspace; and number of fibulae within 10 μm. Identification was aided by the use of *Pseudo-nitzshchia*-lator (5.0) software published by J. Ehrman (Mt. Allison University, 2003).

2.9 Domoic Acid Analysis

Samples (Table 1) from October 2005 and June 2006 were analyzed for both particulate (>5000 cells L^{-1}) and dissolved (>0 cells L^{-1}) domoic acid concentrations. Particulate domoic acid (pg $cell^{-1}$) and dissolved domoic acid (pg mL^{-1}) were both analyzed using a direct competition ELISA (Enzyme Linked Immunosorbent Assay) with the Biosense Laboratories ASP ELISA KIT (A31300401 pre-release). The following protocol for preparation of samples and analysis is published by Biosense Laboratories. 100 mL of whole water was vacuum filtered through GF/C (Sigma Aldrich) filters saving both filter (particulate) and filtrate (dissolved), samples were frozen at -80 ºC until analyzed. 10 mL of 20% methanol were added to filters and vortexed. Samples were centrifuged at 3000 xg for 10 minutes. Supernatant containing particulate samples were diluted 1:10 with sample buffer. Filtrate was diluted with sample buffer to 1:25 for dissolved analysis.

Domoic acid samples were incubated in prepared 96 well plates with anti-domoic acid HRP conjugated primary antibodies for 1 h and developed with TMB peroxidase.

Following addition of H_2SO_4, plates were read at 450nm on a microplate spectrophotometer (Perkin Elmer). Fitting a standard curve allowed for calculation of domoic acid concentrations. Particulate domoic acid concentrations were regressed to temperature collected simultaneously (Fig. 43). Dissolved domoic acid concentrations were regressed to particulate domoic acid concentrations (Fig. 44).

CHAPTER III

RESULTS

3.1 Historical Analysis

Nitrogen data collected by S. Rumrill (NOAA) for 2003 and 2004 showed a strong negative linear relationship to chlorophyll *a* values collected simultaneously. A strong decline in chlorophyll *a* could be explained by increasing nitrogen concentrations (Fig. 3). This relationship ($R^2 = 0.48$) was highly significant ($p << 0.05$) indicating that around half of the time phytoplankton bloom dynamics were tightly linked with estuarine nitrogen availability. The equation for line of best fit is Eq. (4):

$$Y = 0.663 - 2.438N \qquad (4).$$

Compilation of temperature and salinity data from Valino Island (NOAA CDMO) indicates that across years sampled (2000 to 2004), both physical parameter means are highest in the summer season and lowest in the winter (Figs. 4 and 5). Spring and autumn exist as transitions between summer and winter. Autumn has lower mean temperatures than spring across years while salinity ranges are similar.

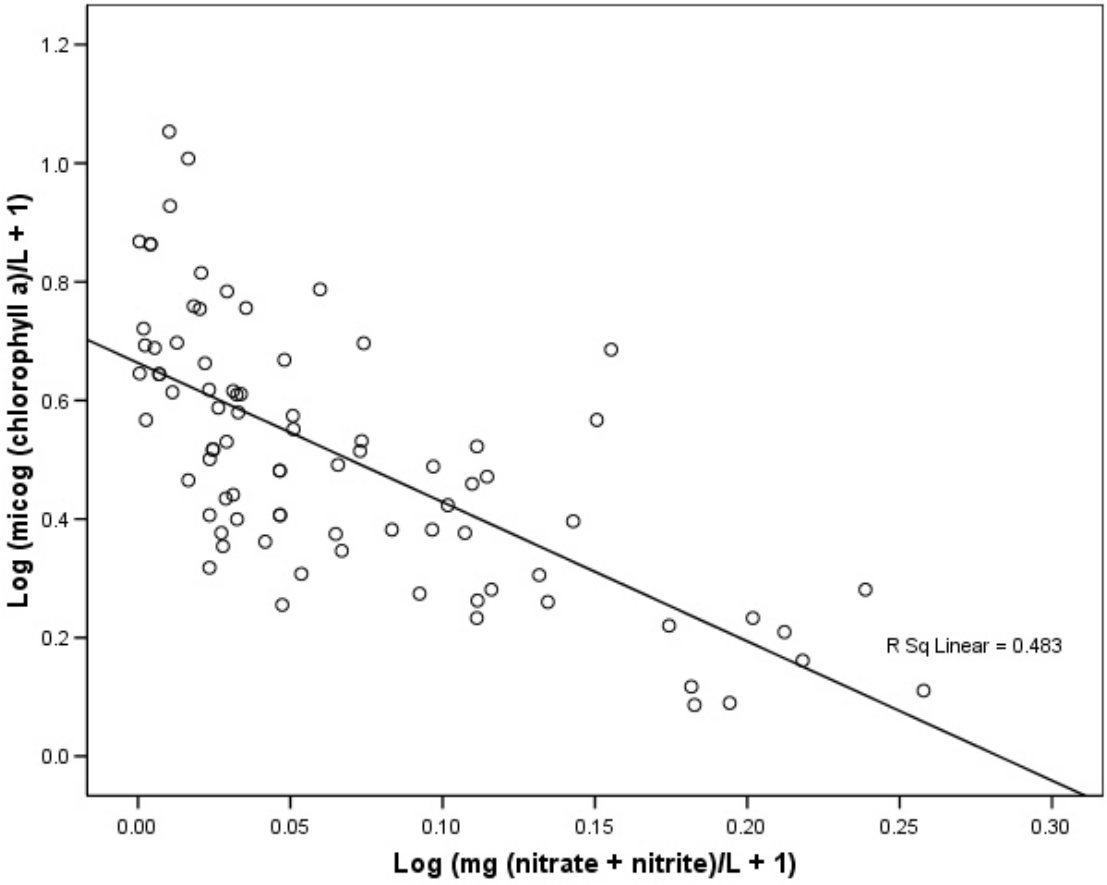

Figure 3. Relationship between nitrogen (as nitrate + nitrite) and chlorophyll *a* concentrations within the South Slough estuary. Data sets collected by S. Rumrill at various sites in 2003 and 2004 as part of the NERR Systemwide Monitoring Program (SWMP/NOAA-CDMO). Linear regression, $R^2 = 0.48$, $p << 0.05$, (SPSS 14.0).

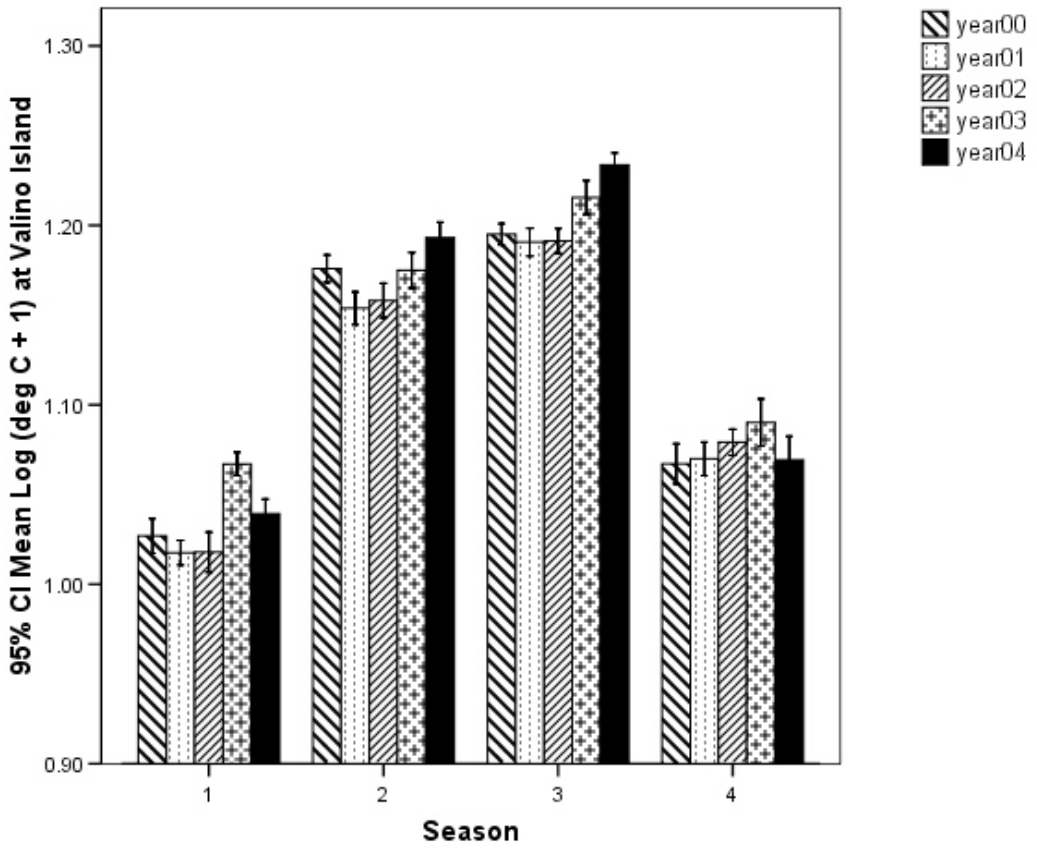

Figure 4. Comparison of seasonal temperature (°C) means across years at Valino Island, South Slough. Seasons correspond to: 1 = winter, 2 = spring, 3 = summer, 4 = autumn. Note that the summer consistently has higher temperatures than other three seasons. Winter generally has the lowest temperatures. Data set collected by S. Rumrill as part of the NERR Systemwide Monitoring Program (SWMP/NOAA-CDMO). (SPSS 14.0).

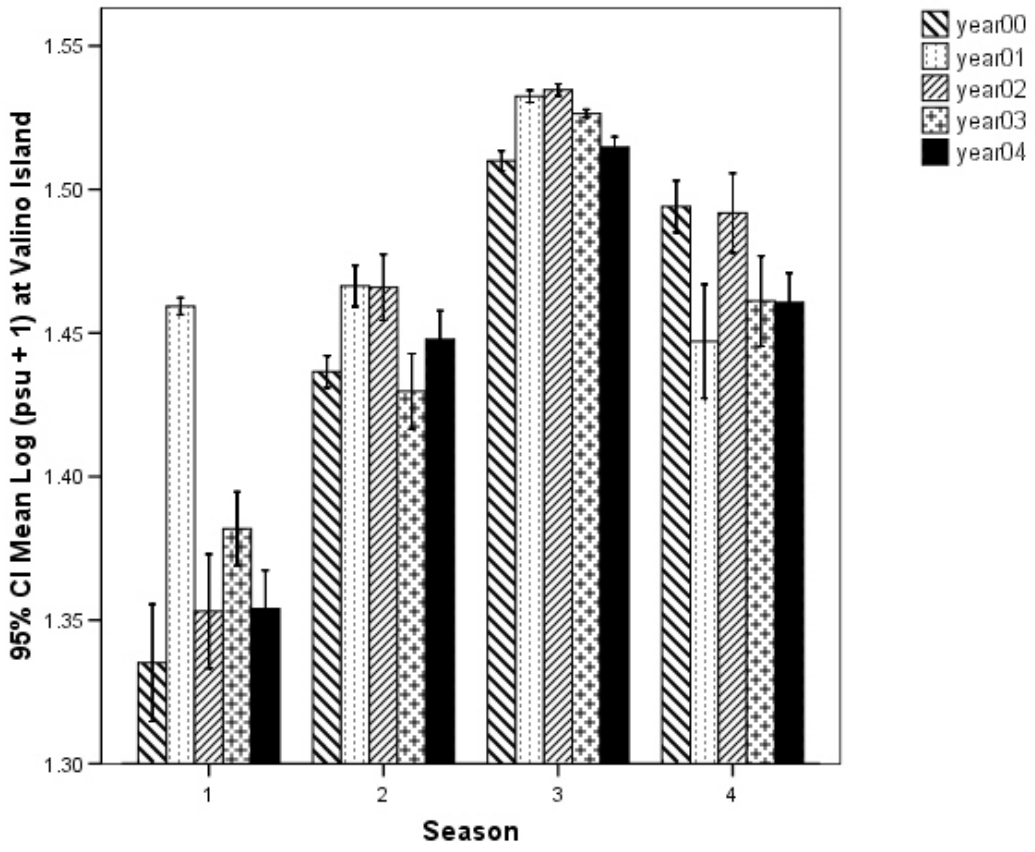

Figure 5. Comparison of seasonal salinity (psu) means across years at Valino Island, South Slough. Seasons correspond to: 1 = winter, 2 = spring, 3 = summer, 4 = autumn. Note that the summer consistently has higher salinities than other three seasons. Winter generally has the lowest salinities. Data set collected by S. Rumrill as part of the NERR Systemwide Monitoring Program (SWMP/NOAA-CDMO). (SPSS 14.0).

3.2 Nitrogen Analysis

As seen in Fig. 6 the nitrogen concentrations were most variable (with minimal variability between sites on most dates) in the late spring and early summer of 2005 with a decrease in overall concentrations across sites by summers end. The Kruskal-Wallis mean rank difference test indicates no significant differences in nitrogen concentrations between the 2005 fixed station sites of Boat House (n = 14), South Slough Pilings (n = 13), and Hinch Lane Bridge (n = 14, Fig. 7, $p = 0.246$). A test of seasonal differences in 2005 fixed station and channel sites indicates a highly significant difference between ranked nitrogen concentrations (Fig. 8, $p << 0.05$) with concentrations decreasing from spring (n = 11), through summer (n = 30), to autumn (n = 14). A test of regional concentrations from 2005 and 2006 channel data indicate no significant differences in nitrogen concentrations between lower (n = 8), middle (n = 12), and upper (n = 7) estuarine regions (Fig. 9, $p = 0.763$). Recent analysis by Rumrill et al. (2007) indicates that the source of nitrogen switches from watershed inputs in the winter and spring to oceanic inputs in the summer.

No significant difference ($p = 0.163$) between historical data (n = 86) and data collected in 2005 and 2006 at >5000 *Pseudo-nitzschia* spp. cells L^{-1} (n = 68, Fig. 9) was found using the non-parametric Mann-Whitney-Wilcoxon test of ranked difference for nitrogen concentrations.

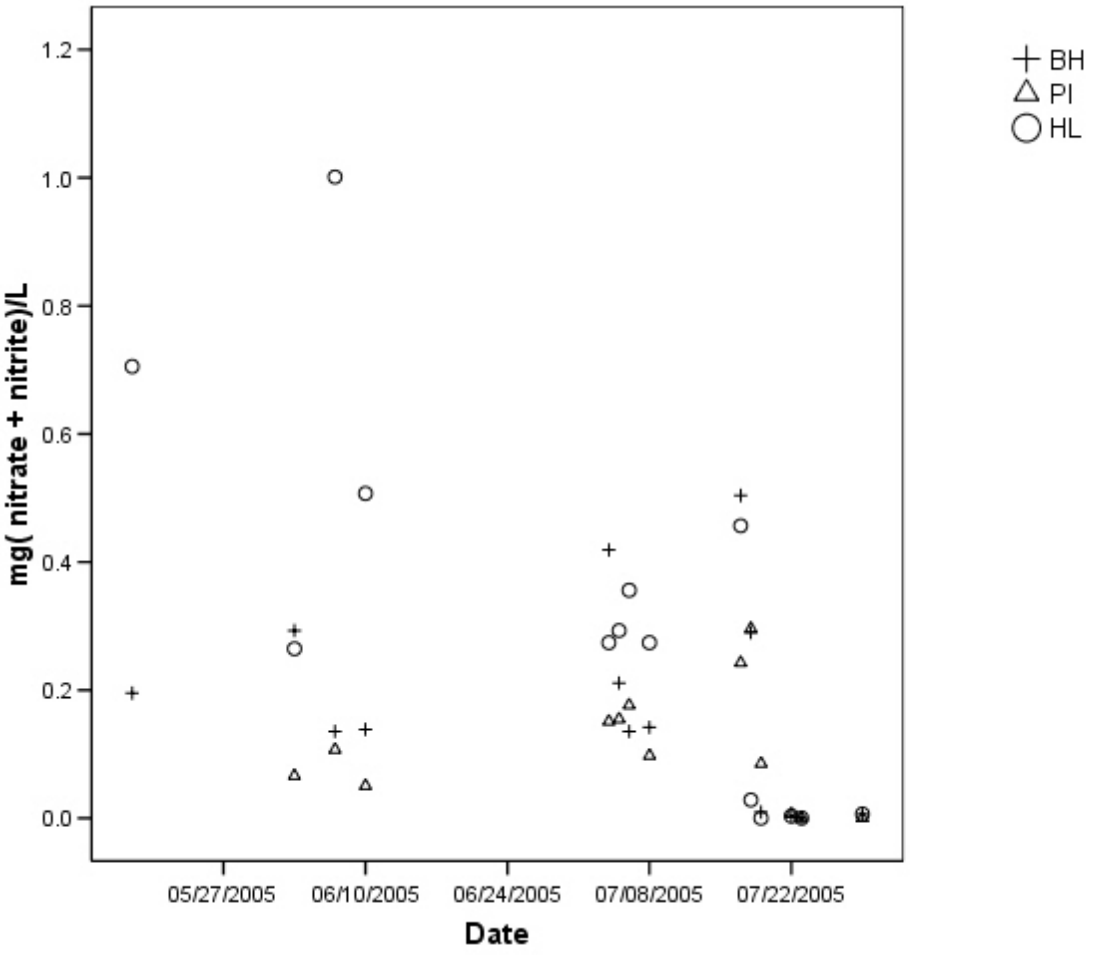

Figure 6. Nitrogen (as nitrate + nitrite) concentrations on 2005 fixed station sampling dates. Sample sites denoted as: BH = Boat House; PI = South Slough Pilings; and HL = Hinch Lane Bridge (SPSS 14.0).

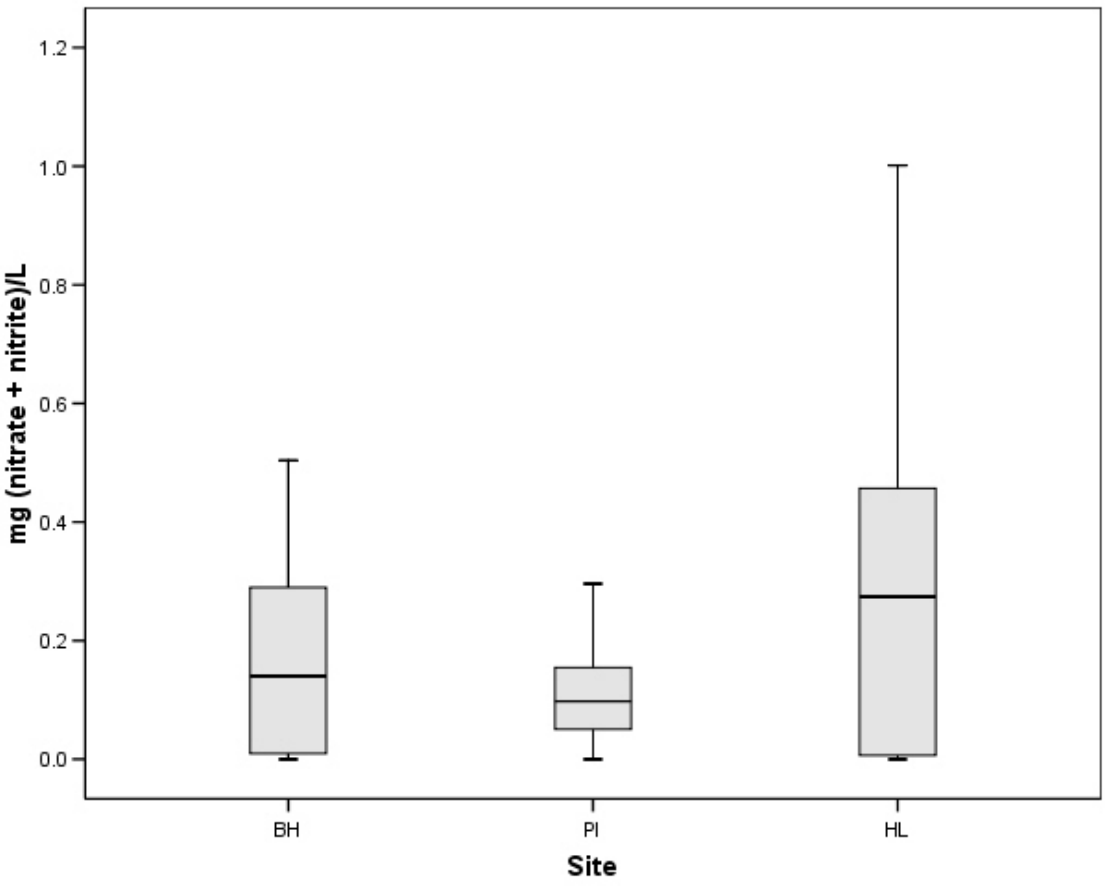

Figure 7. Box-and-whisker plot of 2005 nitrogen (as nitrate + nitrite) across site without regard to date collected. Sample sites denoted as: BH = Boat House; PI = South Slough Pilings; and HL = Hinch Lane Bridge. All samples collected *at* fixed station sites (SPSS 14.0).

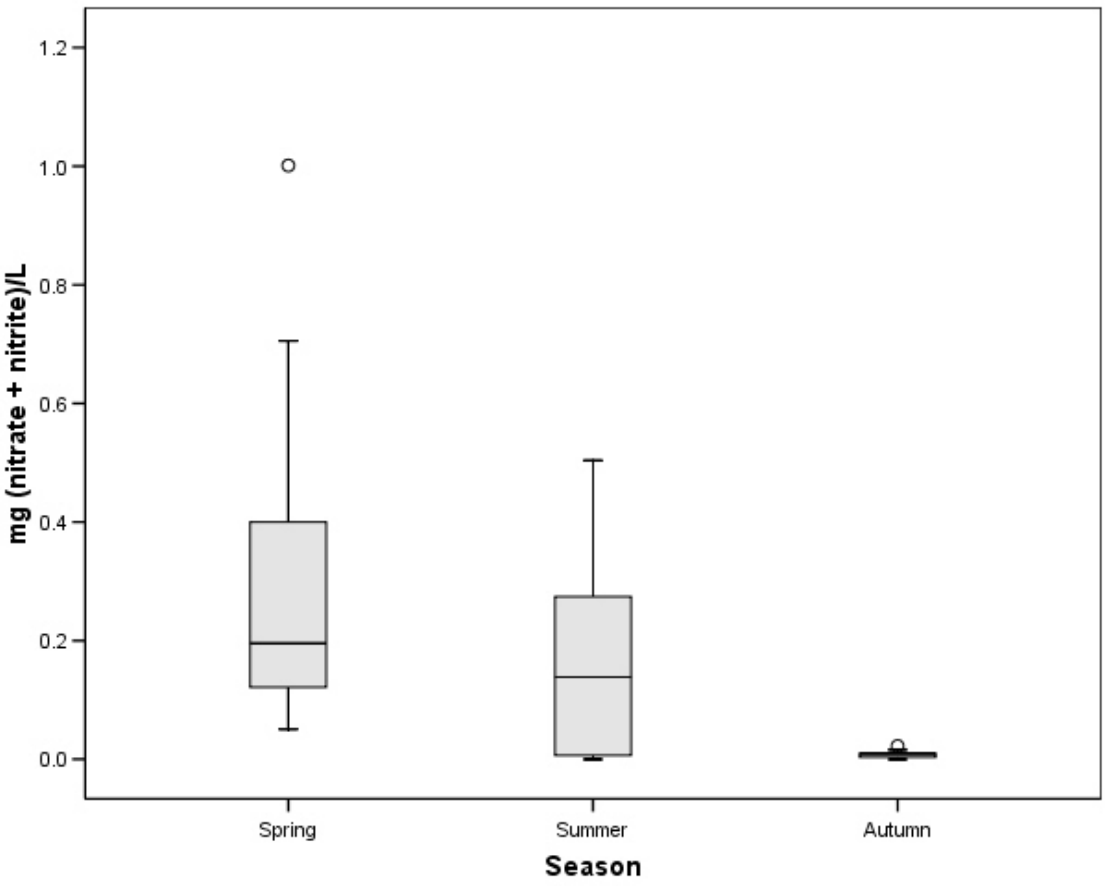

Figure 8. Box-and-whisker plot of 2005 nitrogen (as nitrate + nitrite) across seasons without regard to site or sampling method. Spring and summer samples collected at fixed station sites, autumn samples collected in channel (SPSS 14.0).

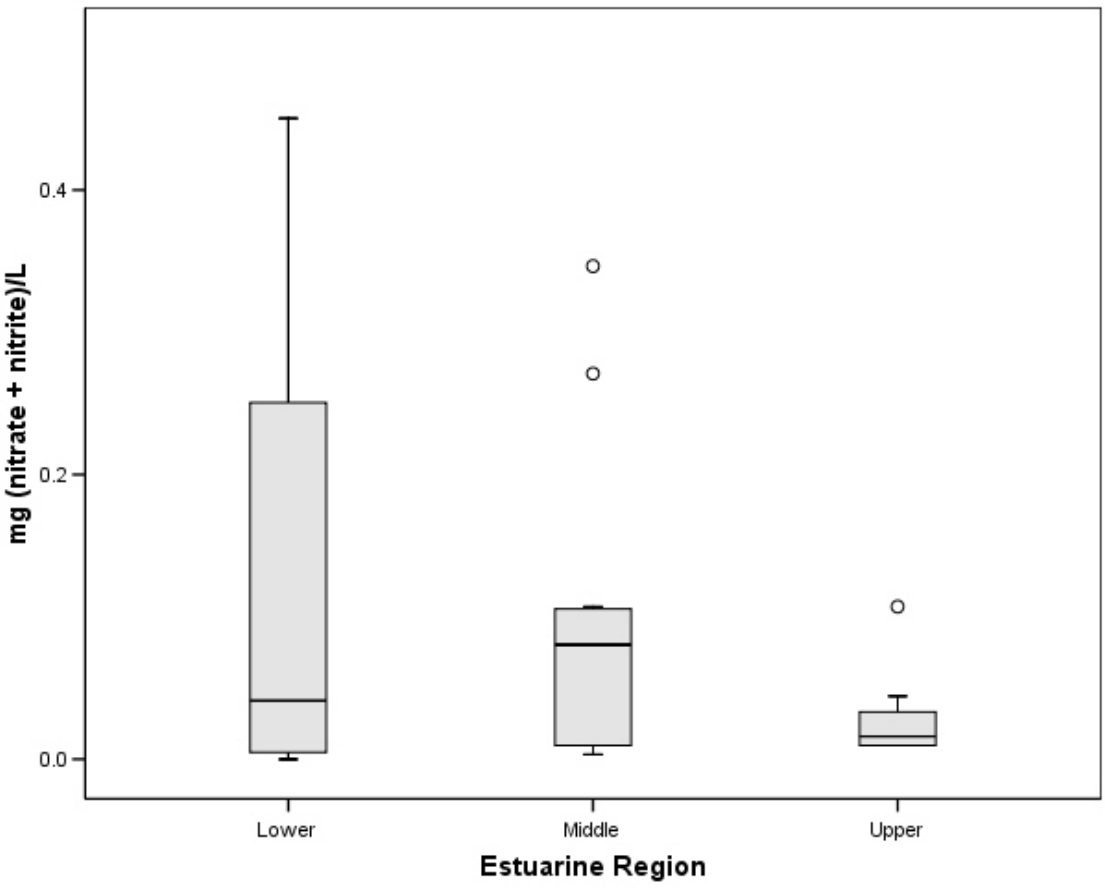

Figure 9. Box-and-whisker plot of 2005 and 2006 nitrogen (as nitrate + nitrite) across regions without regard to date collected. Samples collected in channel (SPSS 14.0).

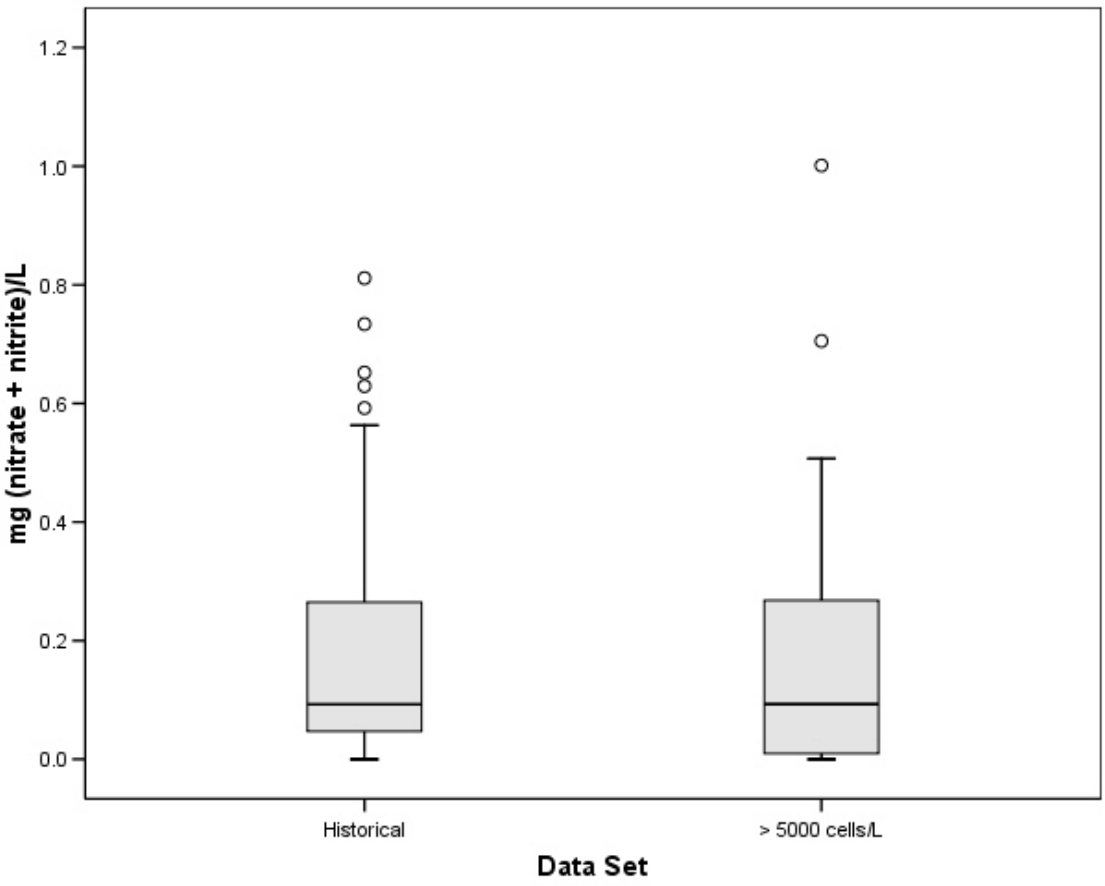

Figure 10. Box-and-whisker plot of nitrogen (as nitrate + nitrite) at historical sites (2003 and 2004, NOAA NERR CDMO) and sampling dates with >5000 *Pseudo-nitzschia* spp. cells/L (2005 and 2006) without regard to sampling method or date collected. *Pseudo-nitzschia* spp. cell concentrations were not recorded for historical data (SPSS 14.0).

3.3 Chlorophyll *a* Analysis

Chlorophyll *a* concentrations increased from spring through summer at 2005 fixed station sites with highest concentrations and most variability occurring at the marine dominated site (Boat House) and lowest concentrations with least variability occurring at the middle estuarine site (South Slough Pilings, Fig. 11). During this period concentrations peaked at near 40 μg chlorophyll *a* L^{-1} in July at Boat House. A comparison of log transformed chlorophyll *a* concentration means across fixed station sites in 2005 (Fig. 12) indicates that concentrations are statistically different across sites ($F = 47.652$, $p << 0.05$). Using Tukey's HSD, differences are found between all sites with highest concentrations existing at Boat House (n = 36), followed by Hinch Lane Bridge (n = 35), and finally South Slough Pilings (n = 33; $p << 0.05$).

Chlorophyll *a* samples collected in 2005 at both fixed station and channel sites are significantly ($X^2 = 8.282$, $p < 0.05$) different across seasons (Fig. 13) as determined by the Kruskal-Wallis mean rank difference test. Summer (n = 99), ranks higher (65.34) than either spring (n = 9, 31.67) or autumn (n = 14, 53.54). 2005 and 2006 chlorophyll *a* concentrations (Fig. 14) are not significantly different between lower, middle, and upper estuarine regions. A significant difference exists between historically collected chlorophyll *a* concentrations (n = 84) and those collected on dates with greater than 5000 *Pseudo-nitzschia* spp. cells L^{-1} in channel and at fixed stations in 2005 and 2006 (n = 66; Fig. 15). The historical mean is lower than the mean of chlorophyll *a* on dates with a bloom ($t = 7.544$, $p << 0.05$).

Regression analysis of chlorophyll *a* in relation to temperature for both 2005 and

2006 sampling data (Fig. 16) indicates a negative relationship with moderate explanatory power ($n = 126$, $R^2 = 0.311$, $p << 0.05$). This suggests increasing temperatures are linked to decreasing chlorophyll *a* concentrations.

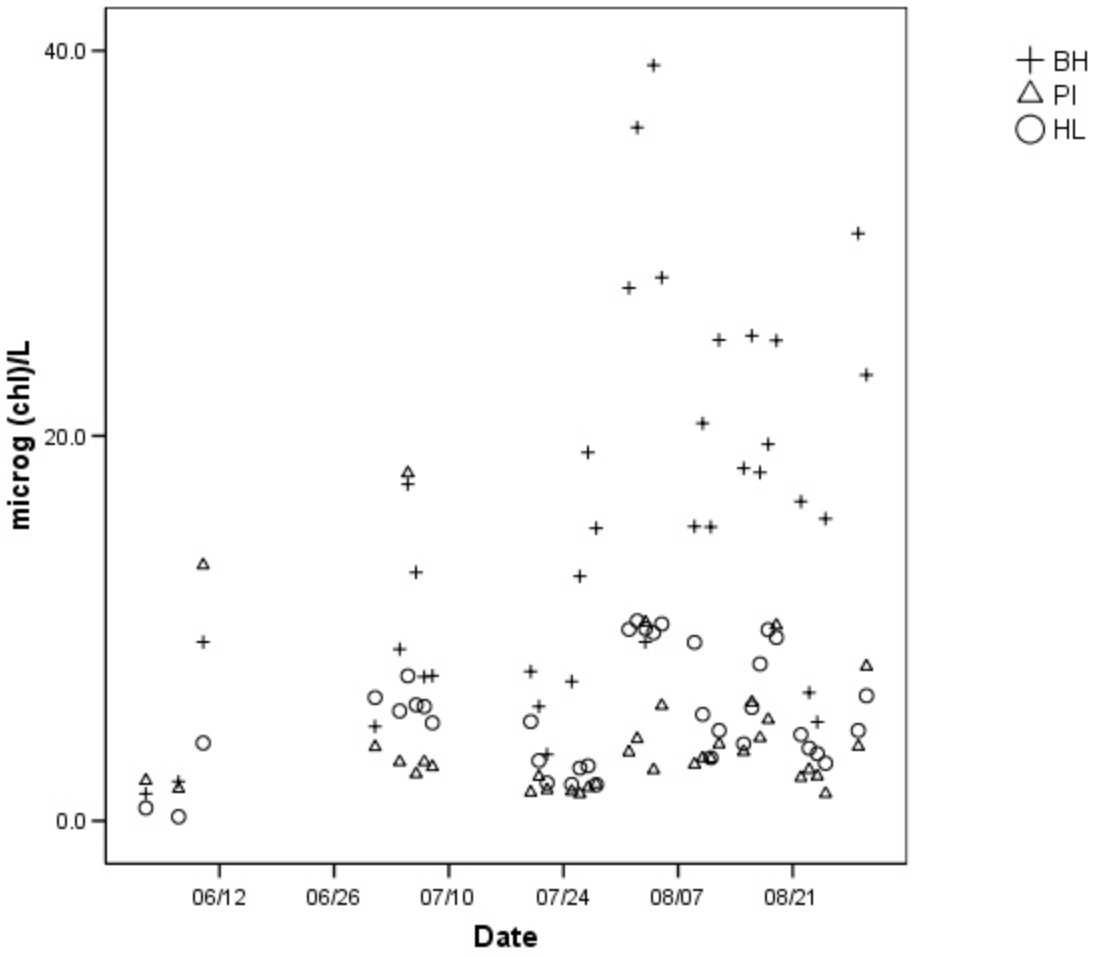

Figure 11. Chlorophyll *a* concentrations on 2005 fixed station sampling dates. Sample sites denoted as: BH = Boat House; PI = South Slough Pilings; and HL = Hinch Lane Bridge (SPSS 14.0).

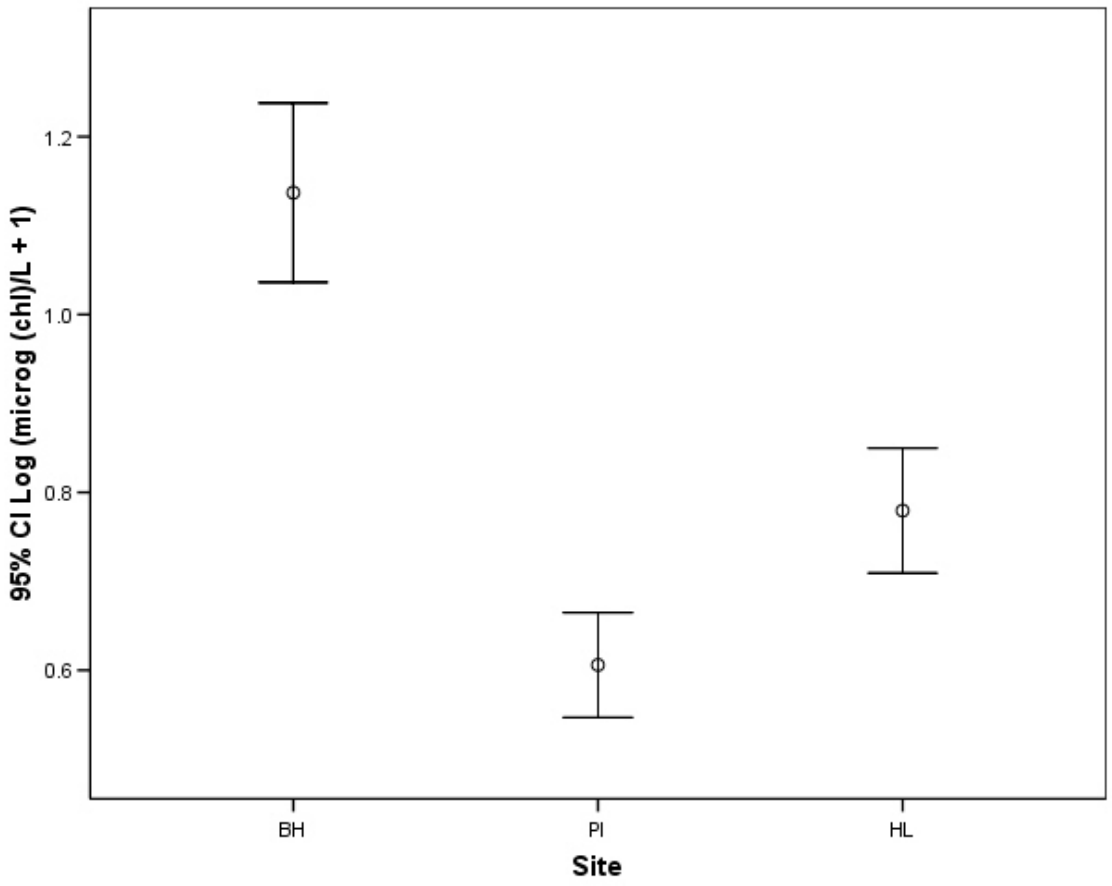

Figure 12. Comparison of log transformed chlorophyll means across sites without regard to season in 2005. Sample sites denoted as: BH = Boat House; PI = South Slough Pilings; HL = Hinch Lane Bridge. All samples collected at fixed station sites (SPSS 14.0).

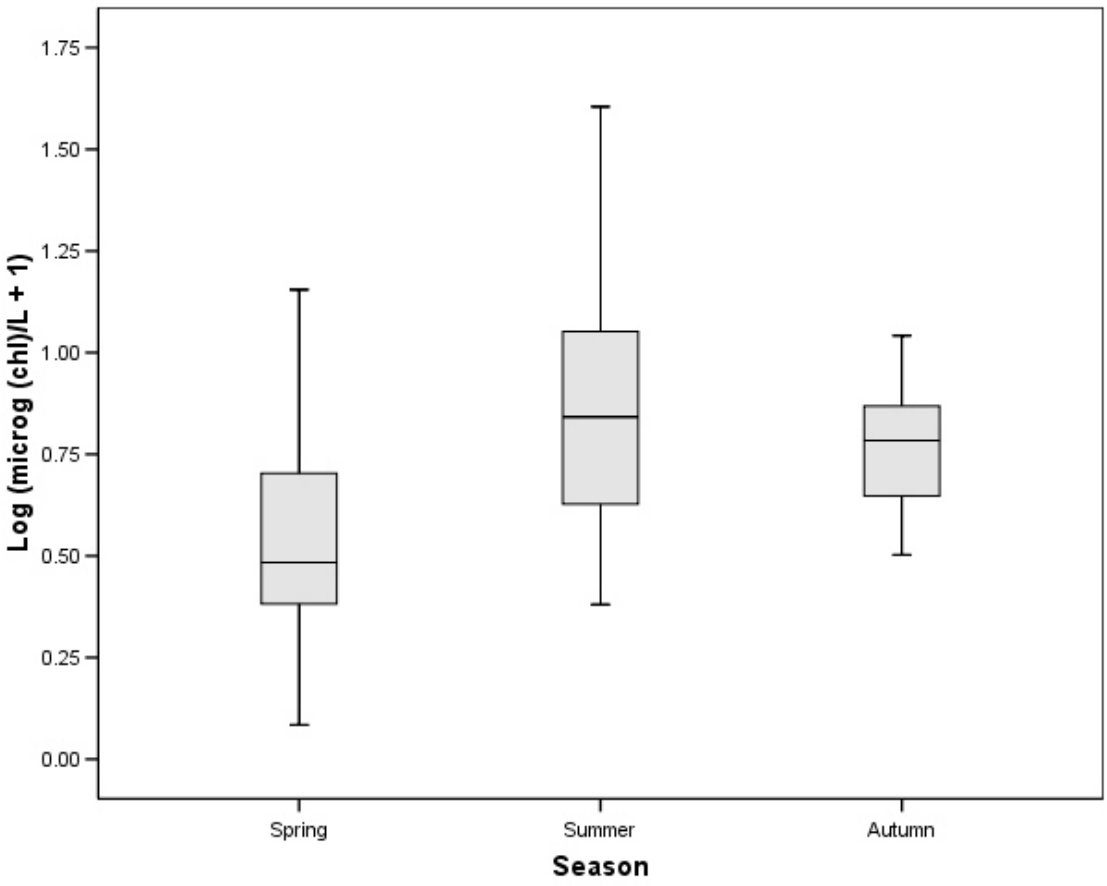

Figure 13. Box-and-whisker plot of 2005 chlorophyll *a* across seasons without regard to site or sampling method. Spring and summer samples collected at fixed station sites, autumn samples collected in channel (SPSS 14.0).

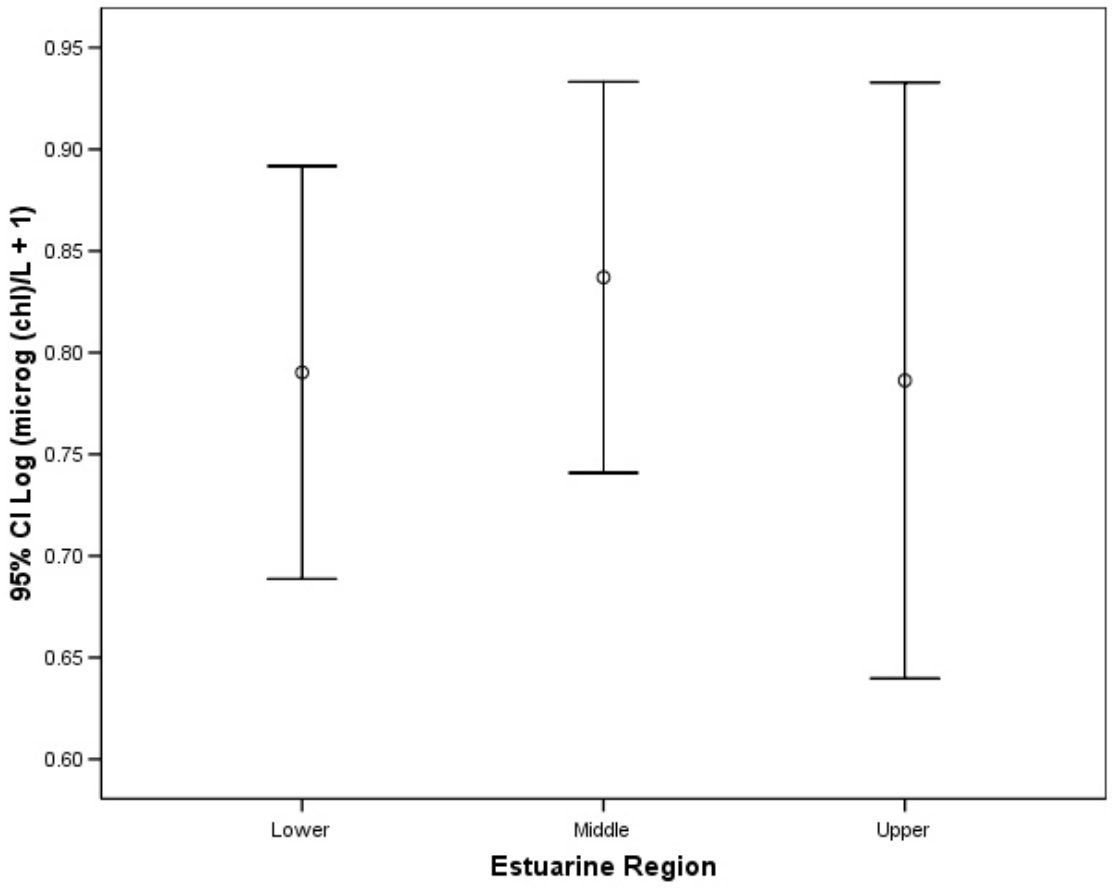

Figure 14. Comparison of log transformed chlorophyll means across estuarine regions without regard to seasons in 2005 and 2006. Samples collected in channel (SPSS 14.0).

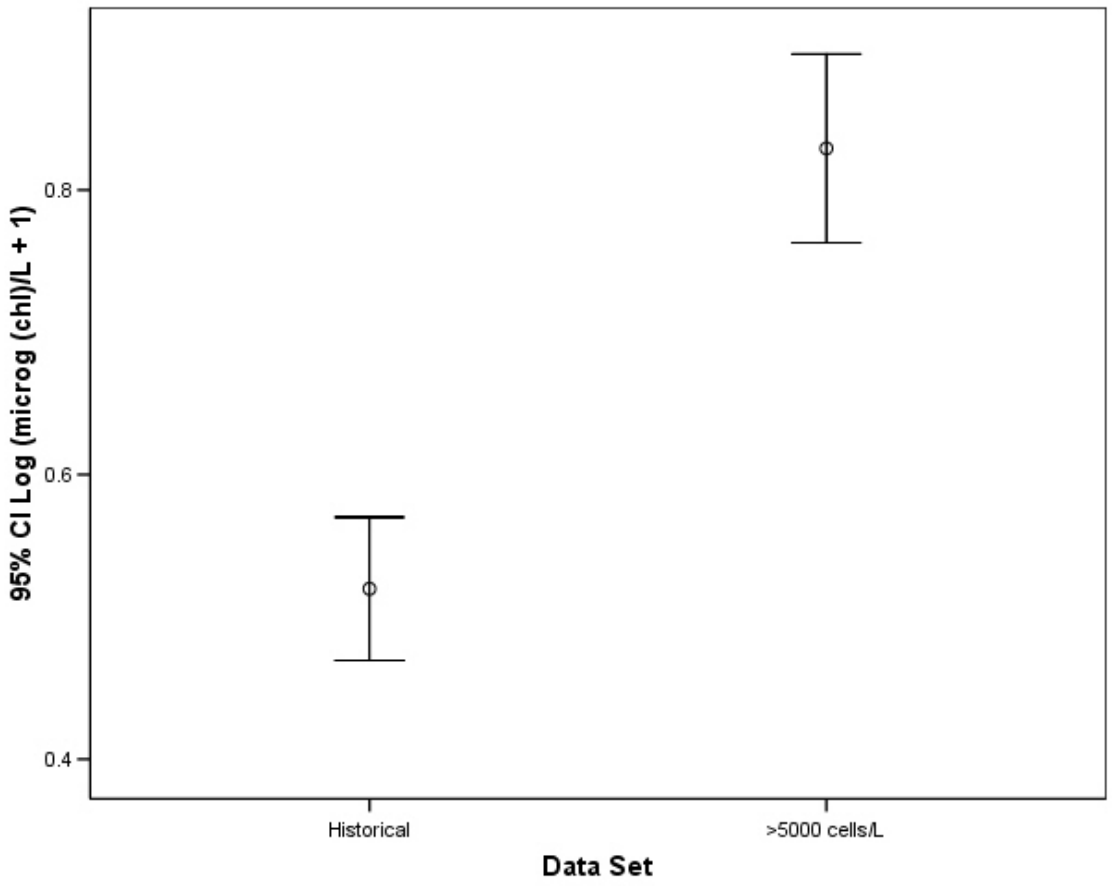

Figure 15. Comparison of log transformed chlorophyll means at historical sites (2003 and 2004, NOAA NERR CDMO) and sampling dates with >5000 *Pseudo-nitzschia* spp. cells/L (2005 and 2006) without regard to sampling method or date collected. *Pseudo-nitzschia* spp. cell concentrations were not recorded for historical data (SPSS 14.0).

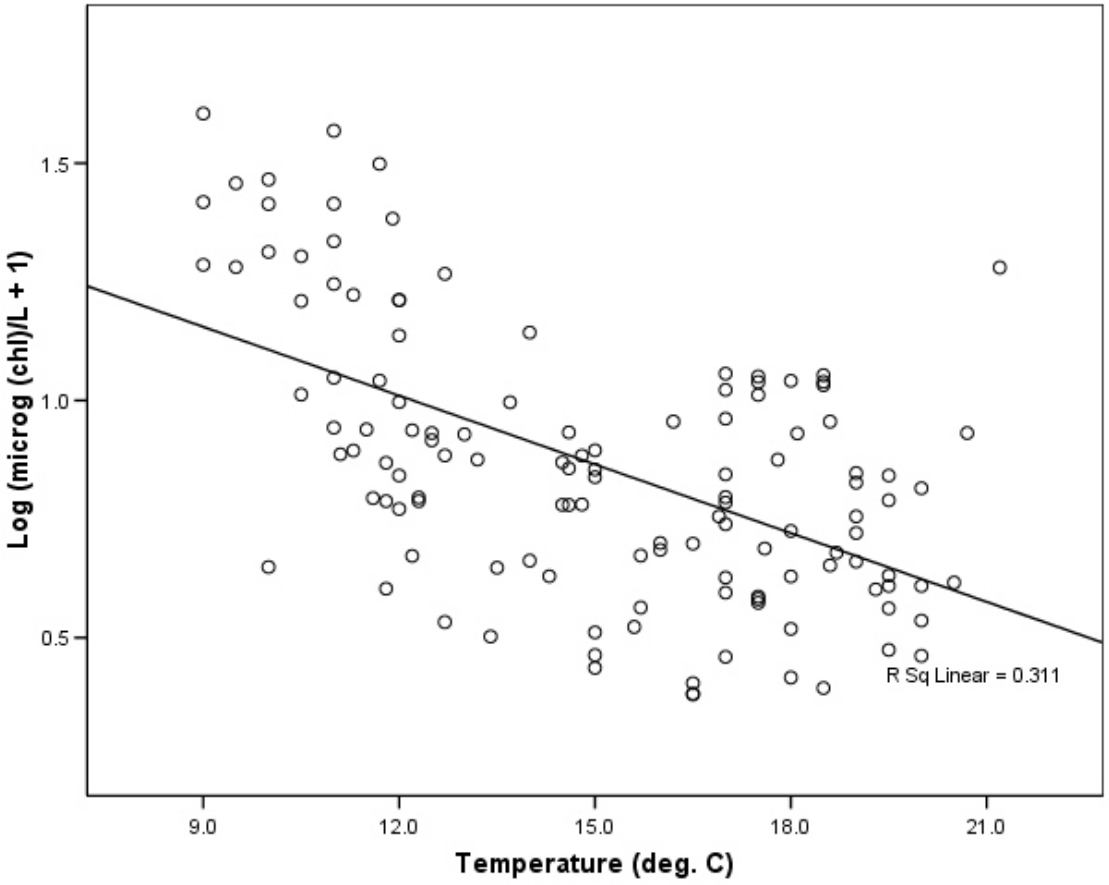

Figure 16. Relationship between temperature (ºC) and chlorophyll *a* concentrations within the South Slough estuary. Linear regression of log-transformed chlorophyll *a* data for pooled samples (sites in 2005 and 2006). $R^2 = 0.311$, $p << 0.05$ (SPSS 14.0).

3.4 Cell Abundance Quantification

Pseudo-nitzschia spp. cell concentrations (Fig. 17) were determined to be above 100,000 cells L^{-1} in spring fixed station sampling months and decreased to below 10,000 cells L^{-1} through summer at Boat House. South Slough Pilings samples remained between 100 and 1000 cells L^{-1} through sampling period. Hinch Lane Bridge samples peaked in spring months and declined to undetectable levels throughout summer. Comparing ranked means ($X^2 = 58.811$, $p << 0.05$) indicates that Boat House has highest mean rank (n = 36, 83.08) followed by South Slough Pilings (n = 34, 45.91) and Hinch Lane Bridge (n = 36, 31.08; Fig. 18).

Analysis of 2005 channel and fixed station data for seasonal cell concentrations (Fig. 19) indicates the highest mean rank (Kruskal-Wallis) is in the autumn (n = 14, 95.39) followed by spring (n = 16, 82.06) and summer (n = 90, 51.24). Differences among seasons are significant (X^2, $p << 0.05$). Mean difference comparisons of 2005 and 2006 estuarine region data (Fig. 20) indicate that the only significance exists between lower and upper regions ($F = 6.032$, $p << 0.05$). In this comparison, lower estuary has highest mean concentrations although lower is not significantly different from middle as determined using Tukey's HSD.

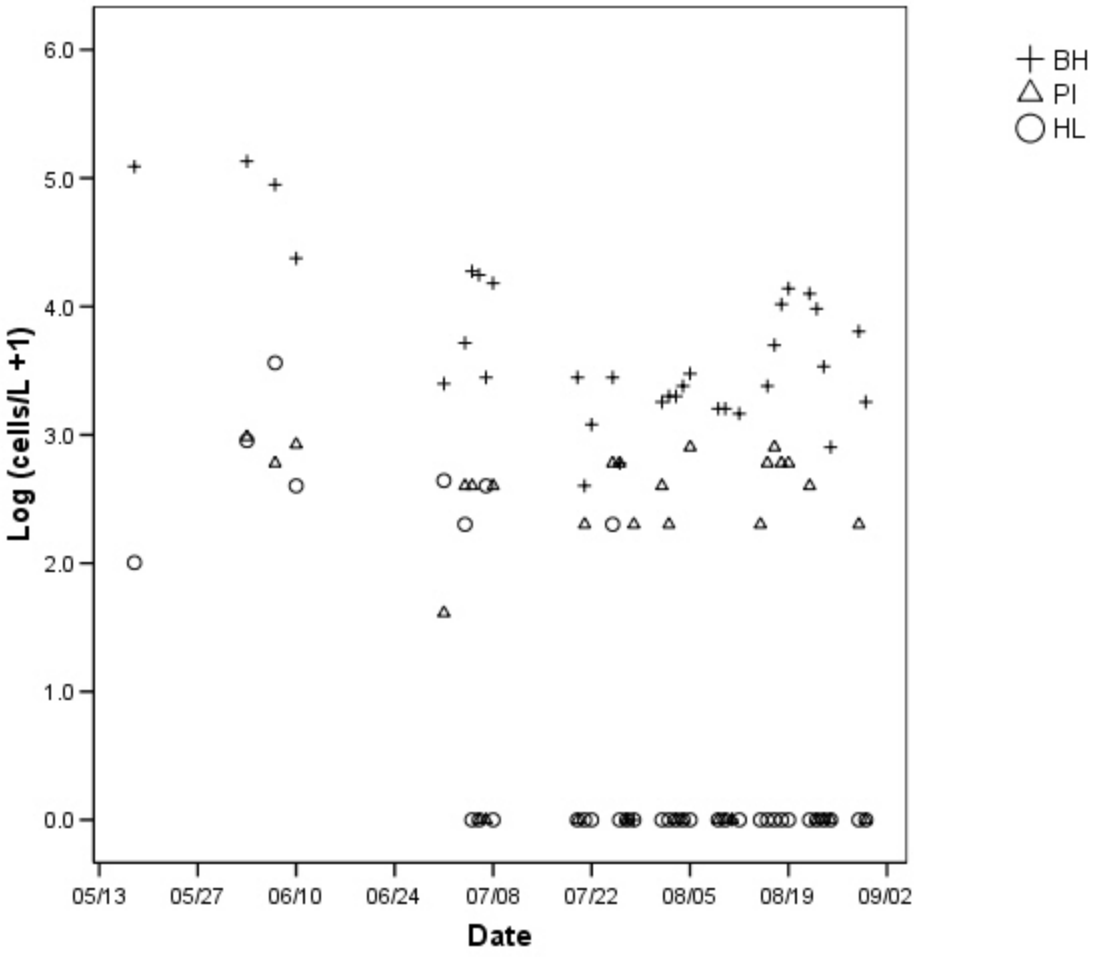

Figure 17. *Pseudo-nitzschia* spp. concentrations on 2005 fixed station sampling dates. Sample sites denoted as: BH = Boat House; PI = South Slough Pilings; and HL = Hinch Lane Bridge (SPSS 14.0).

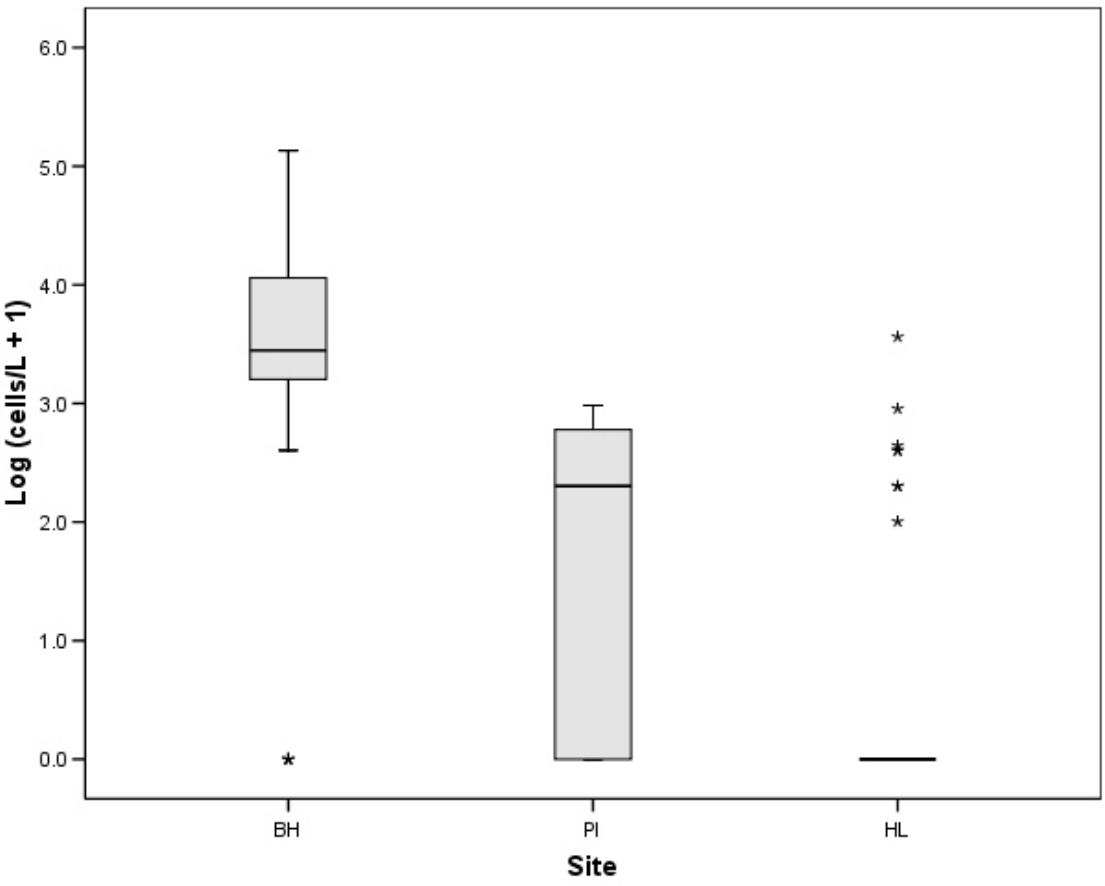

Figure 18. Box-and-whisker plot of *Pseudo-nitzschia* spp. concentrations across sites without regard to season in 2005. Sample sites denoted as: BH = Boat House; PI = South Slough Pilings; HL = Hinch Lane Bridge. All samples collected *at* fixed station *sites* (SPSS 14.0).

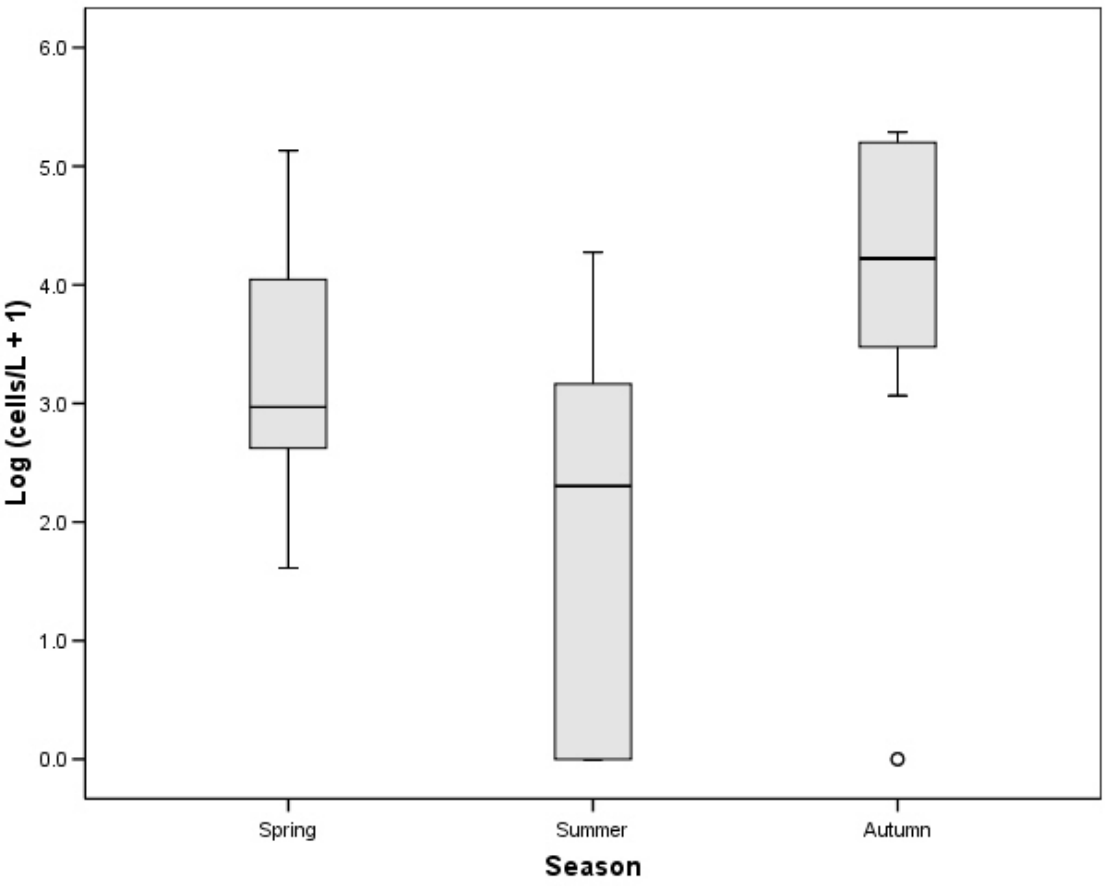

Figure 19. Box-and-whisker plot of log transformed *Pseudo-nitzschia* spp. concentrations across seasons without regard to site or sampling method in 2005 (SPSS 14.0).

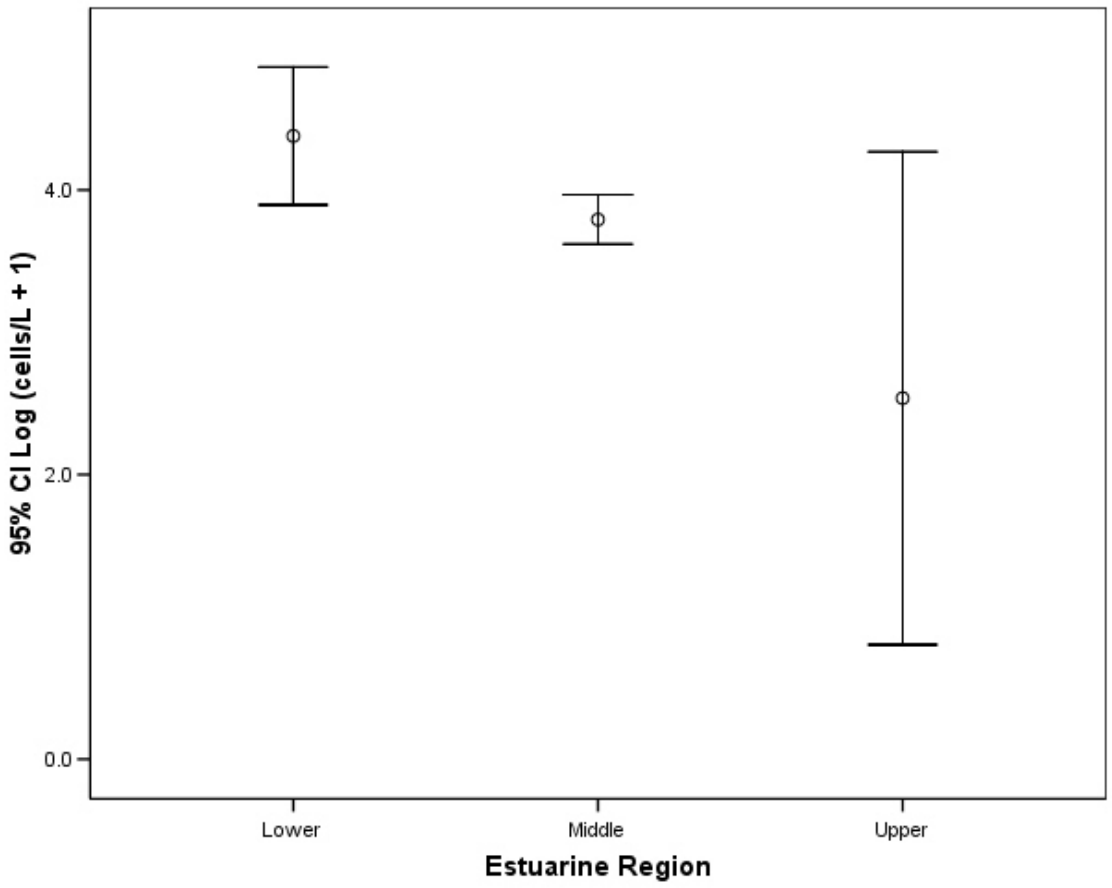

Figure 20. Comparison of log transformed *Pseudo-nitzshia* spp. concentration means across estuarine regions without regard to seasons in 2005 and 2006. Samples collected in channel (SPSS 14.0).

3.5 Salinity and Temperature in Relation to Population Abundance

Temperature data recorded for fixed station sampling period in 2005 (Fig. 21) indicates highest temperatures occurred at Hinch Lane Bridge (riverine) and South Slough Pilings (middle estuarine) while Boat House (marine dominated) had the lowest temperatures. The salinity recorded for the same samples (Fig. 22) demonstrate highest values at Boat House followed closely by South Slough Pilings with lowest values found at Hinch Lane Bridge. A temperature-salinity (T-S) plot indicates that the lowest temperatures are tightly linked to the highest salinities, with higher temperatures occurring throughout most of the salinity range (Fig. 23).

Log transformed cell concentration data of 2006 is strongly negatively correlated with temperature data (Fig. 24) indicating that as temperature increased, Pseudo-nitzschia spp. cell abundance decreased ($R^2 = 0.648$, $p << 0.05$). The equation for the line of best fit in the regression analysis is Eq. (5):

$$Y = 5.99 - 0.16T \quad (5)$$

Log transformed cell concentration data of 2006 was positively correlated with salinity data (Fig. 25) indicating that as salinity increased, Pseudo-nitzschia spp. cell abundance did as well ($R^2 = 0.446$, $p << 0.05$). The equation for the line of best fit in the regression analysis is Eq. (6):

$$Y = -4.75 + 0.27S \quad (6)$$

Taken together, these relationships indicate that *Pseudo-nitzschia* becomes significantly more abundant in the marine dominated waters that the mouth the Coos Estuary.

Multiple regression analysis using both temperature and salinity as predictors for log transformed Pseudo-nitzschia spp. concentration data (Fig. 26) provides the equation for line of best fit, Eq. (7):

$$Y = -3.603 - 0.137T + 0.064S \quad (7)$$

This multiple regression model is powerful (adjusted $R^2 = 0.592$) and highly significant ($p << 0.05$).

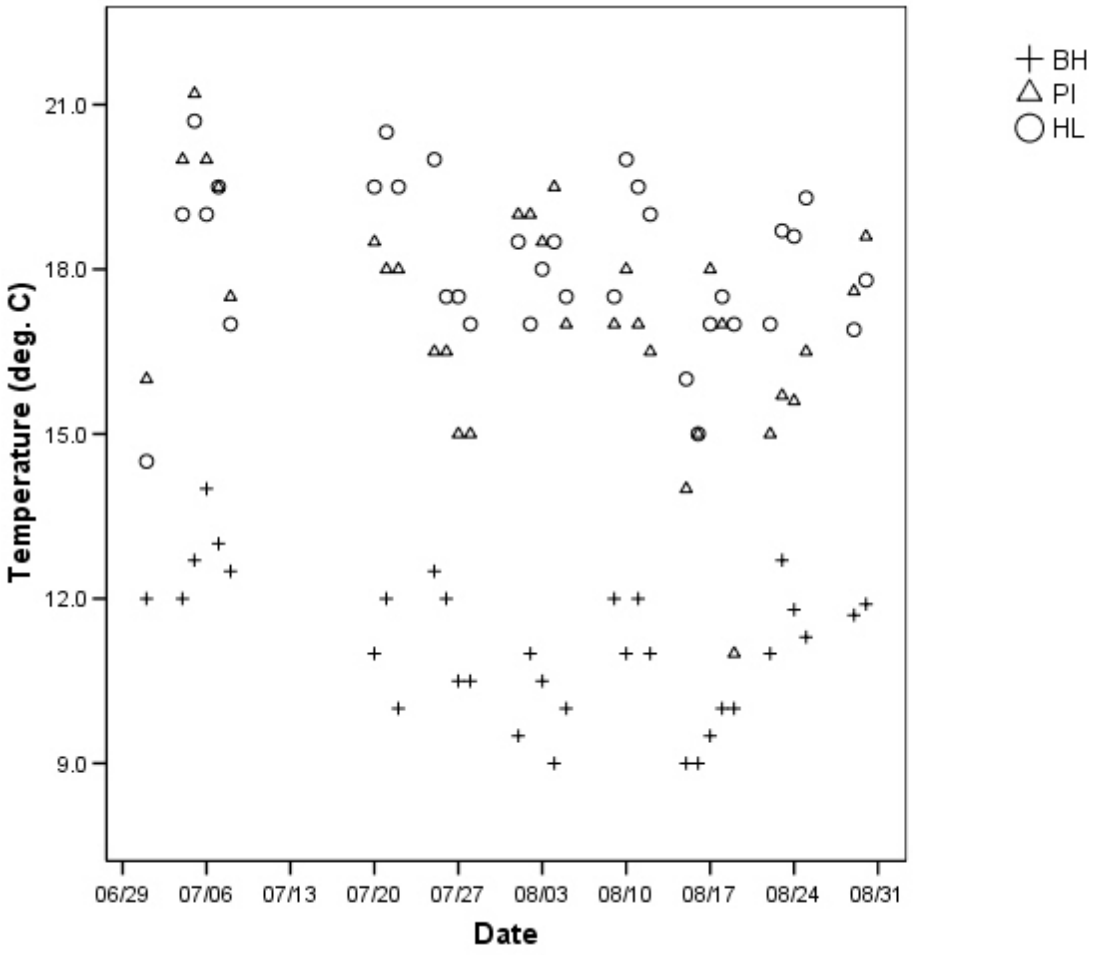

Figure 21. Temperature (°C) on 2005 fixed station sampling dates. Sample sites denoted as: BH = Boat House; PI = South Slough Pilings; and HL = Hinch Lane Bridge (SPSS 14.0).

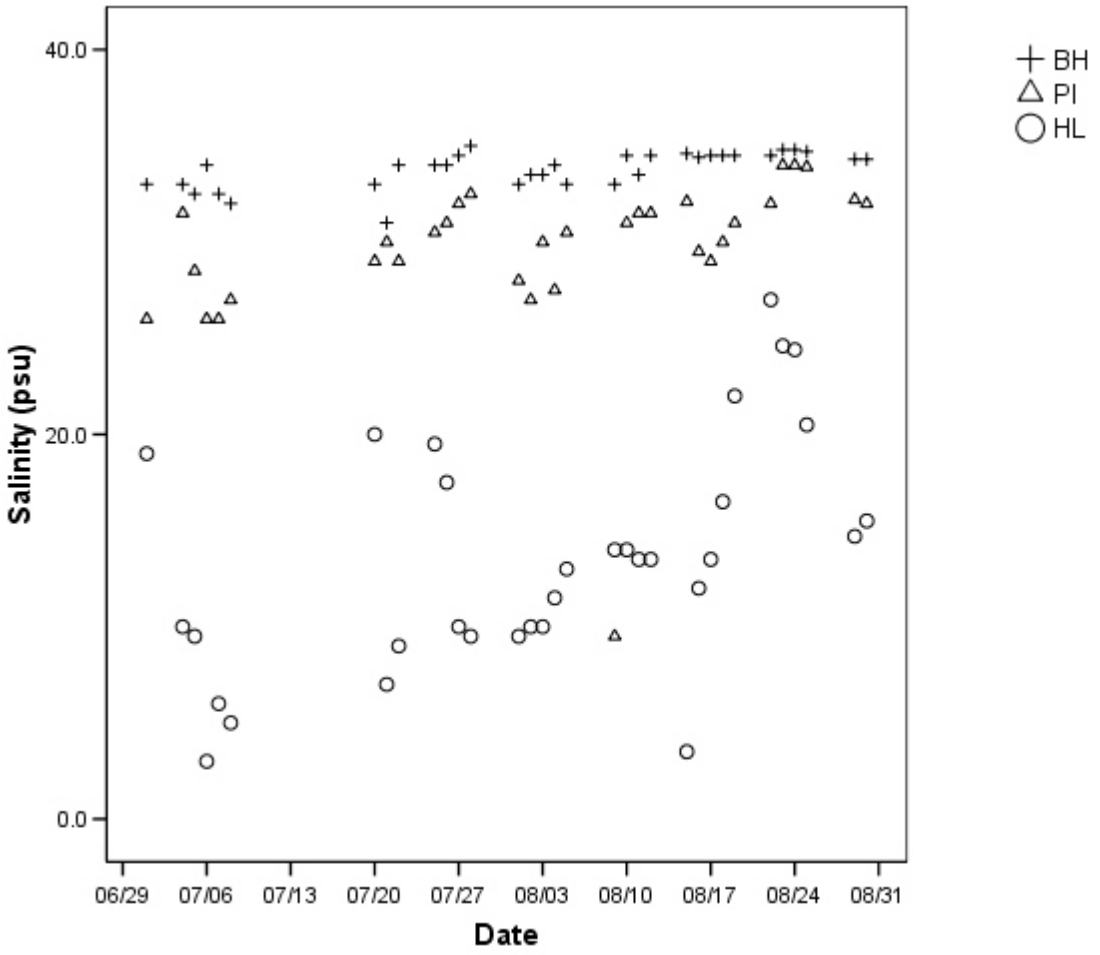

Figure 22. Salinity (psu) on 2005 fixed station sampling dates. Sample sites denoted as: BH = Boat House; PI = South Slough Pilings; and HL = Hinch Lane Bridge (SPSS 14.0).

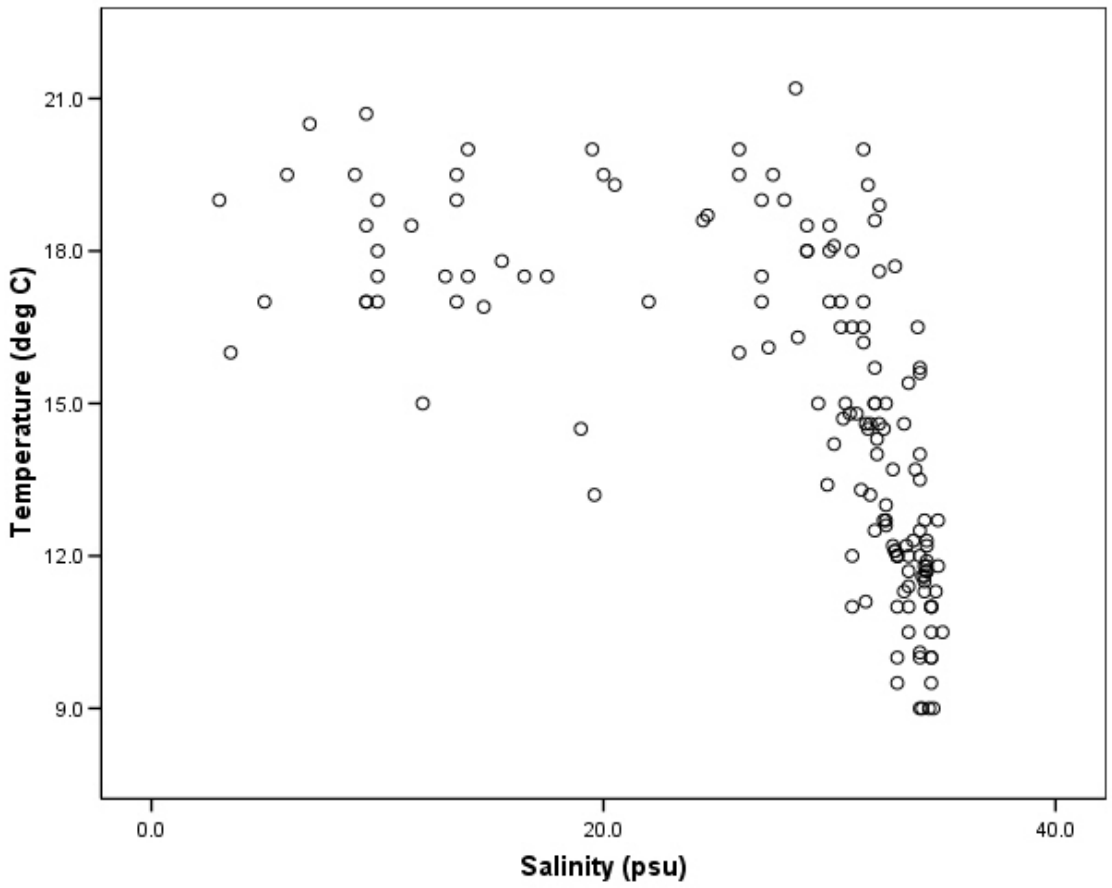

Figure 23. Temperature-Salinity plot for all sites in 2005 and 2006 (SPSS 14.0).

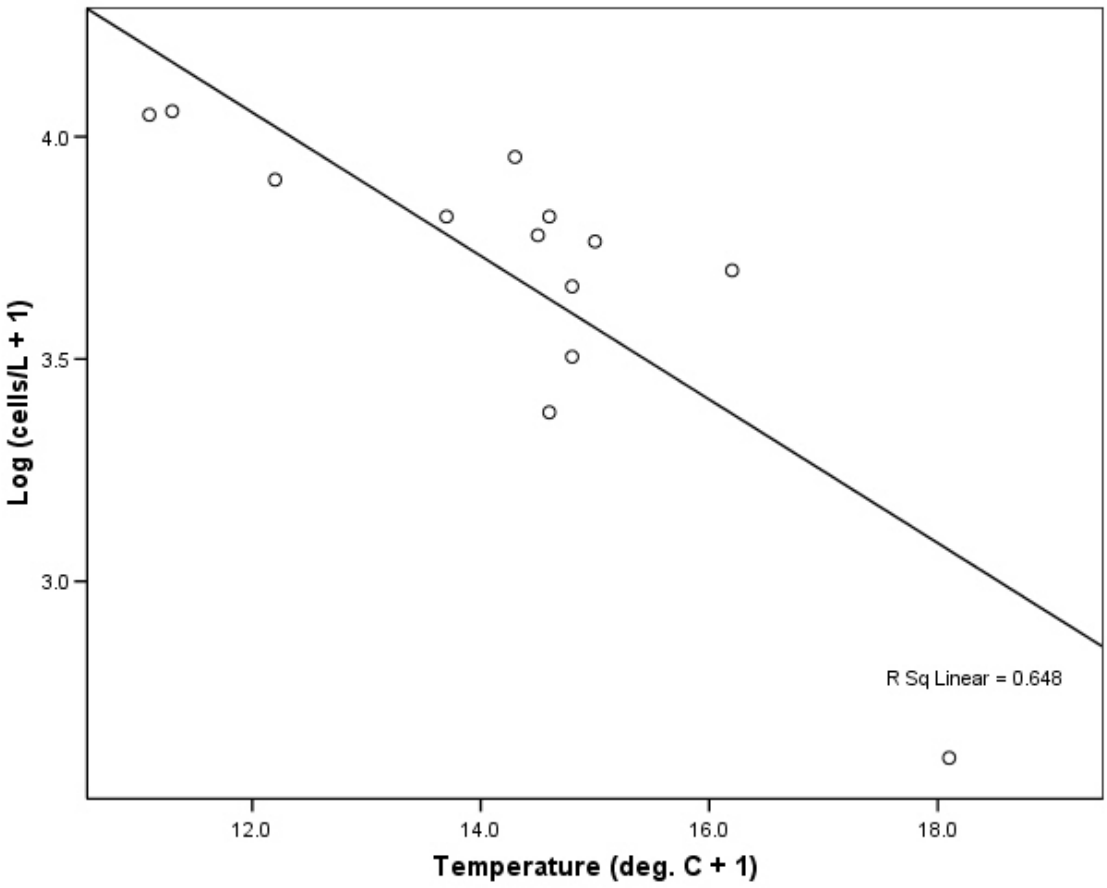

Figure 24. Regression analysis of 2006 log transformed *Pseudo-nitzschia* spp. cell concentrations related to temperature (°C) without regard to site or date collected ($R^2 = 0.648$, $p << 0.05$, SPSS 14.0).

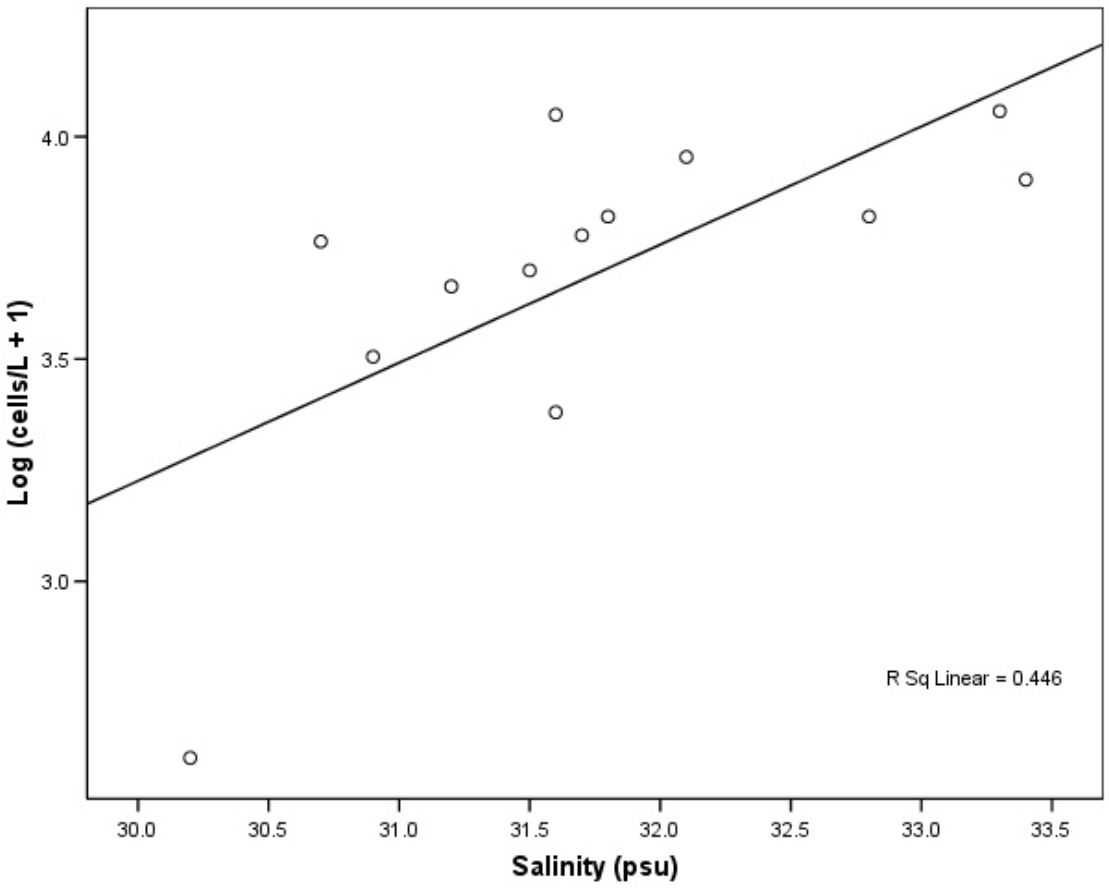

Figure 25. Regression analysis of 2006 log transformed *Pseudo-nitzschia* spp. cell concentrations related to salinity (psu) without regard to site or date collected (R^2 = 0.446, p << 0.05, SPSS 14.0).

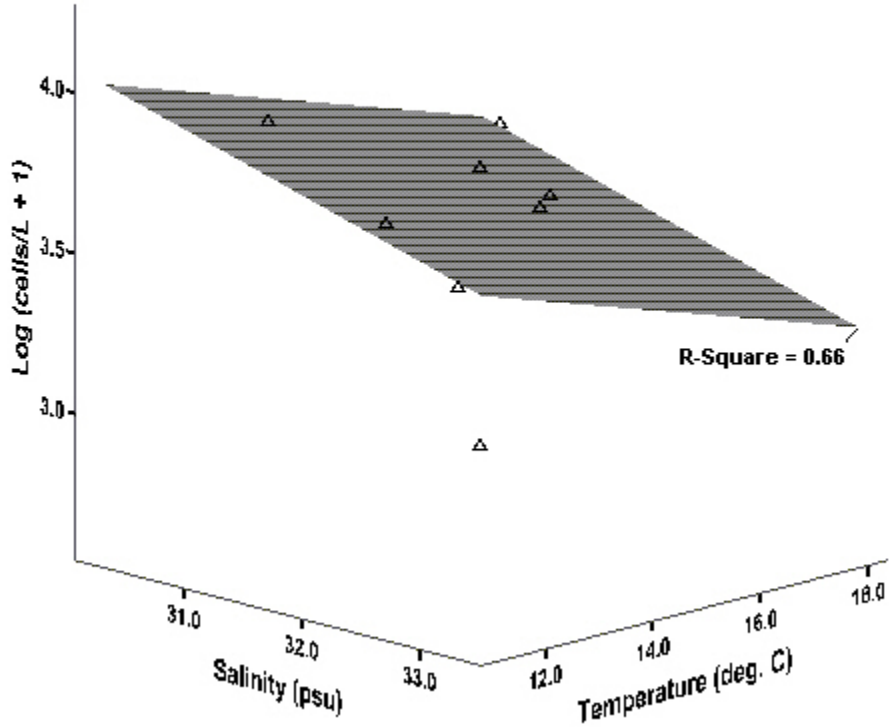

Figure 26. Multiple regression analysis of 2006 log transformed cell concentrations from channel sites as explained by both temperature (°C) and salinity (psu) without regard to site or date collected. $R^2 = 0.66$, $p << 0.05$, (SPSS 14.0).

3.6 Model Prediction

Regression analysis demonstrates a moderate capacity for prediction of the *Pseudo-nitzschia* spp. concentrations based on temperature measurements from 2005 fixed station sites (Fig. 27, $R^2 = 0.424$, $p << 0.05$). Similar results are found with the salinity model predictions and 2005 fixed station site observed concentrations (Fig. 28, $R^2 = 0.465$, $p << 0.05$).

Using 2005 channel data to determine predictive capabilities demonstrates strong predictive capabilities based on the temperature (Fig. 29, $R^2 = 0.819$, $p << 0.05$) regression model alone. The predictive capacity is much weaker with the salinity model in predicting 2005 channel data (Fig. 30, $R^2 = 0.296$, $p > 0.05$). Thus the temperature model has better overall predictive capabilities that are consistent with either fixed station or channel sites.

Strong correlations are found in testing the multiple regression model that incorporates both temperature and salinity (Figs. 31 and 32). This model has good predictive capabilities ($R^2 = 0.426$) that are highly significant for fixed station site data ($p << 0.05$). It also strongly predicts observed data from 2005 channel sites ($R^2 = 0.757$, $p << 0.05$) and can be used consistently across sampling methods.

Model predictions using compiled historical temperature and salinity data indicate peaks of *Pseudo-nitzschia* spp. blooms are possible in late summer and early autumn (Fig. 33) when both temperature and salinity models converge on increasing concentrations. The multiple regression model using both temperature and salinity produces similar results, but with more variable peaks, also indicating late summer and

early fall high cell concentrations (Fig. 34).

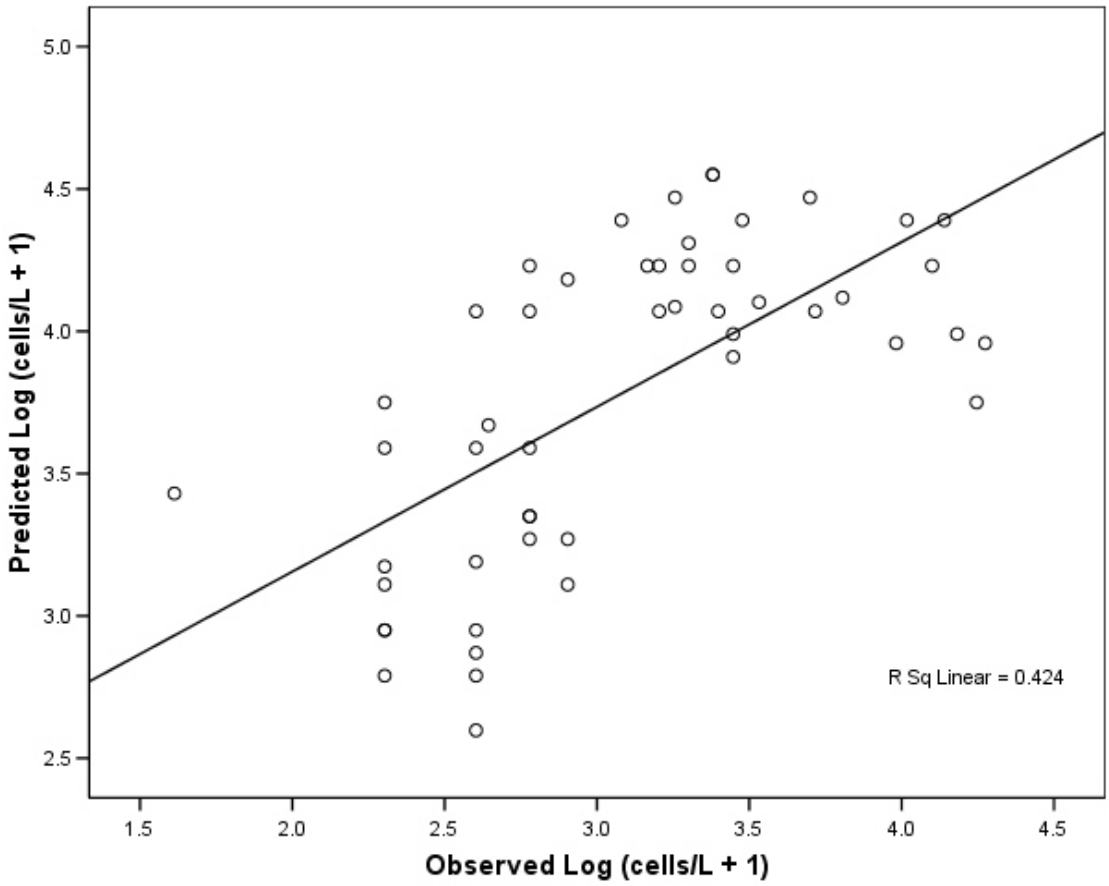

Figure 27. Regression analysis of temperature model predicted log transformed cell concentrations related to observed log transformed cell concentrations from fixed station sites without regard to site or date collected in 2005. $R^2 = 0.424$, $p << 0.05$, (SPSS 14.0).

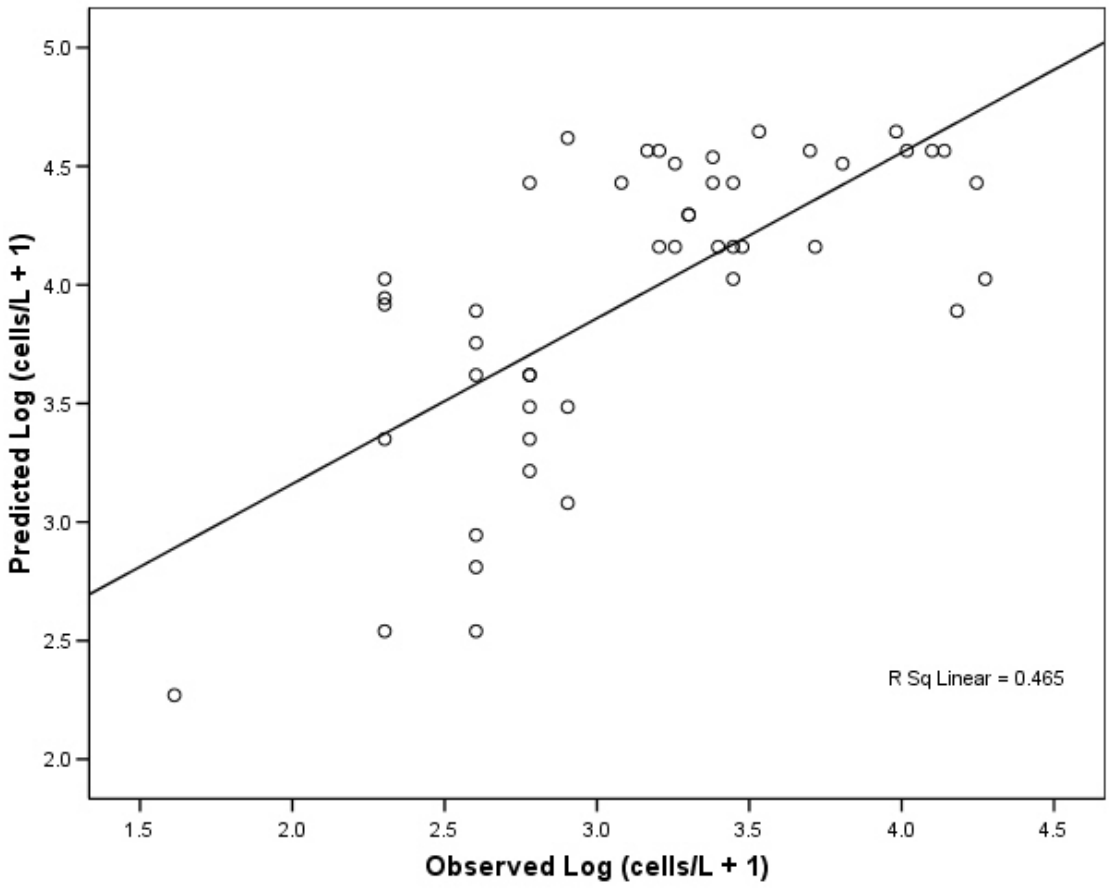

Figure 28. Regression analysis of salinity model predicted log transformed cell concentrations related to observed log transformed cell concentrations from fixed station sites without regard to site or date collected in 2005. $R^2 = 0.465$, $p << 0.05$, (SPSS 14.0).

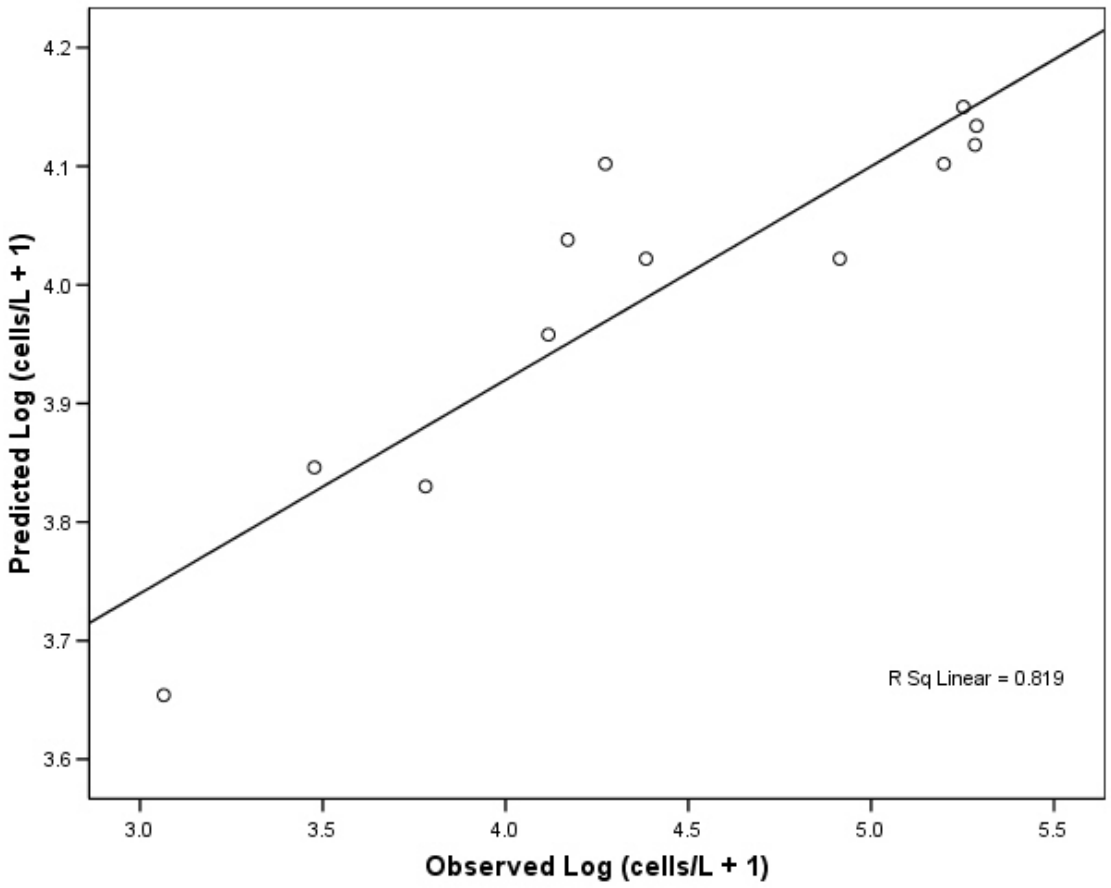

Figure 29. Regression analysis of temperature model predicted log transformed cell concentrations related to observed log transformed cell concentrations from channel sites without regard to site or date collected in 2005. $R^2 = 0.819$, $p << 0.05$, (SPSS 14.0).

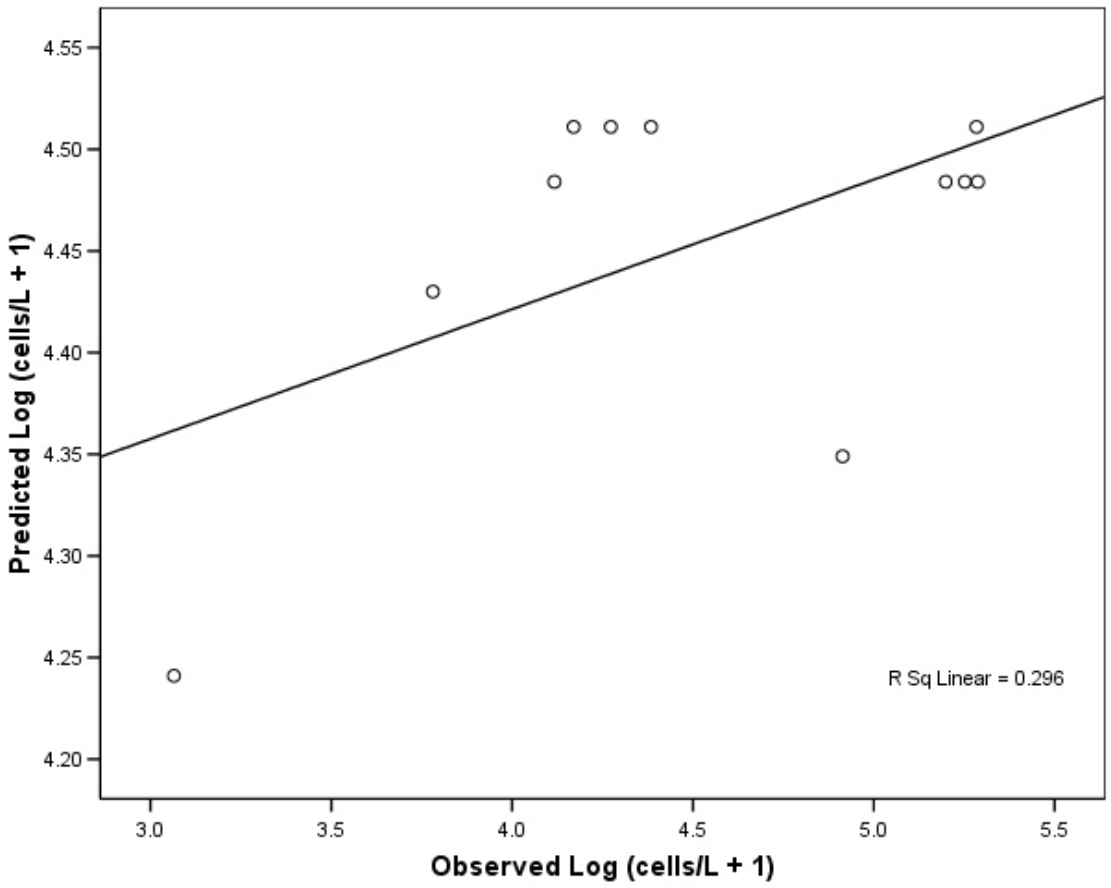

Figure 30. Regression analysis of salinity model predicted log transformed cell concentrations related to observed log transformed cell concentrations from channel sites without regard to site or date collected in 2005. $R^2 = 0.296$, $p << 0.05$, (SPSS 14.0).

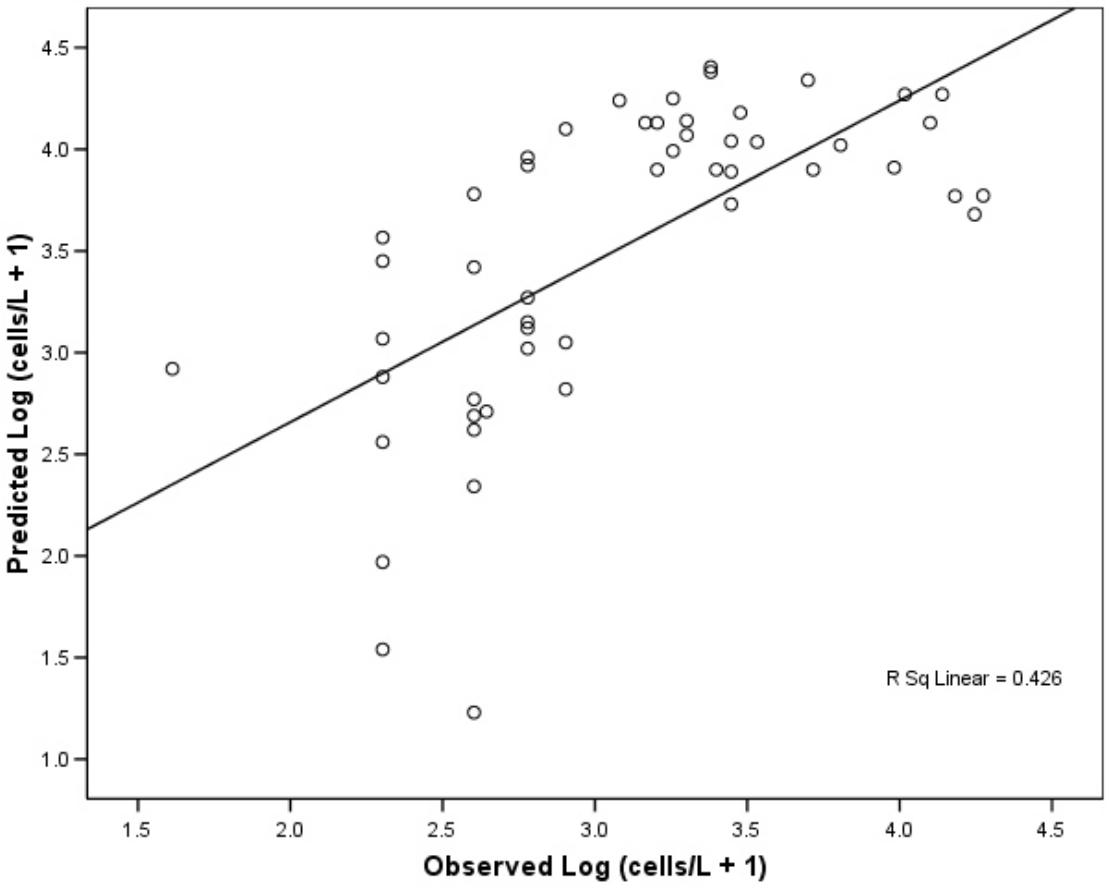

Figure 31. Regression analysis of temperature and salinity model predicted log transformed cell concentrations related to observed log transformed cell concentrations from fixed station sites without regard to site or date collected in 2005. $R^2 = 0.426$, $p << 0.05$, (SPSS 14.0).

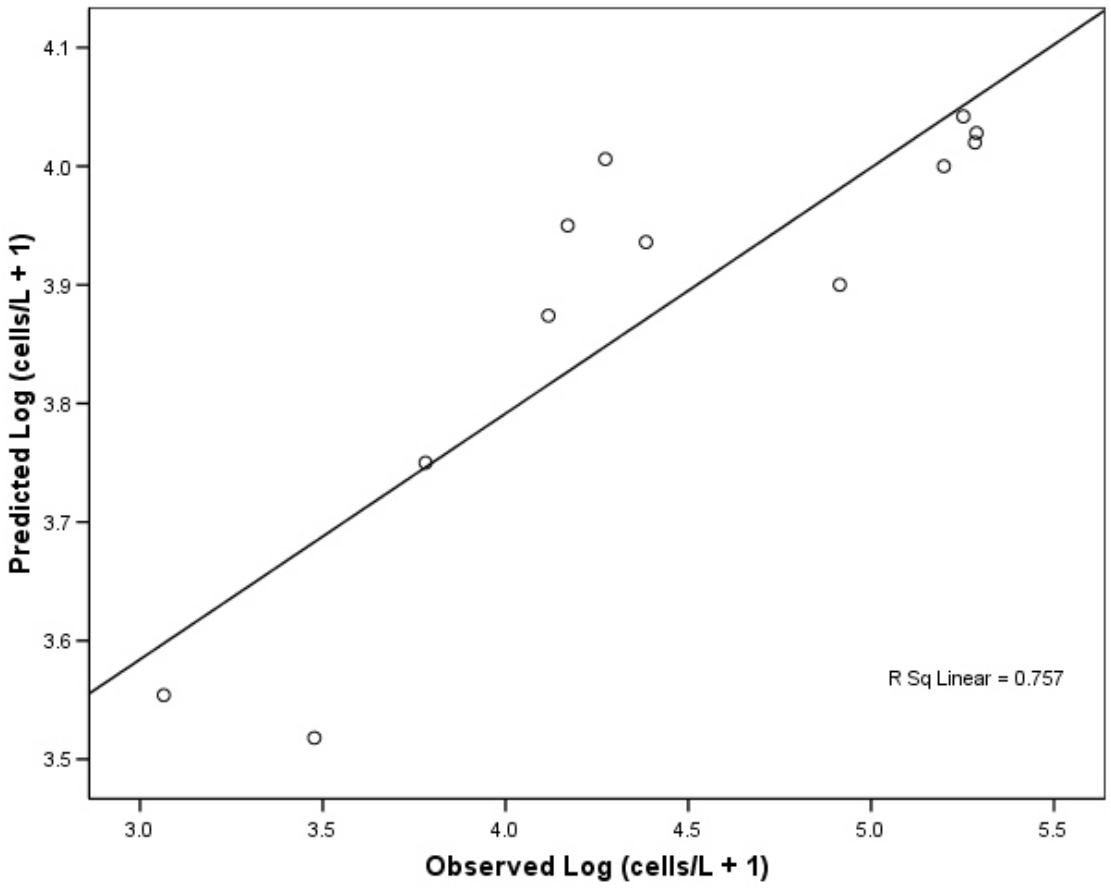

Figure 32. Regression analysis of temperature and salinity model predicted log transformed cell concentrations related to observed log transformed cell concentrations from channel sites without regard to site or date collected in 2005. $R^2 = 0.757$, $p << 0.05$, (SPSS 14.0).

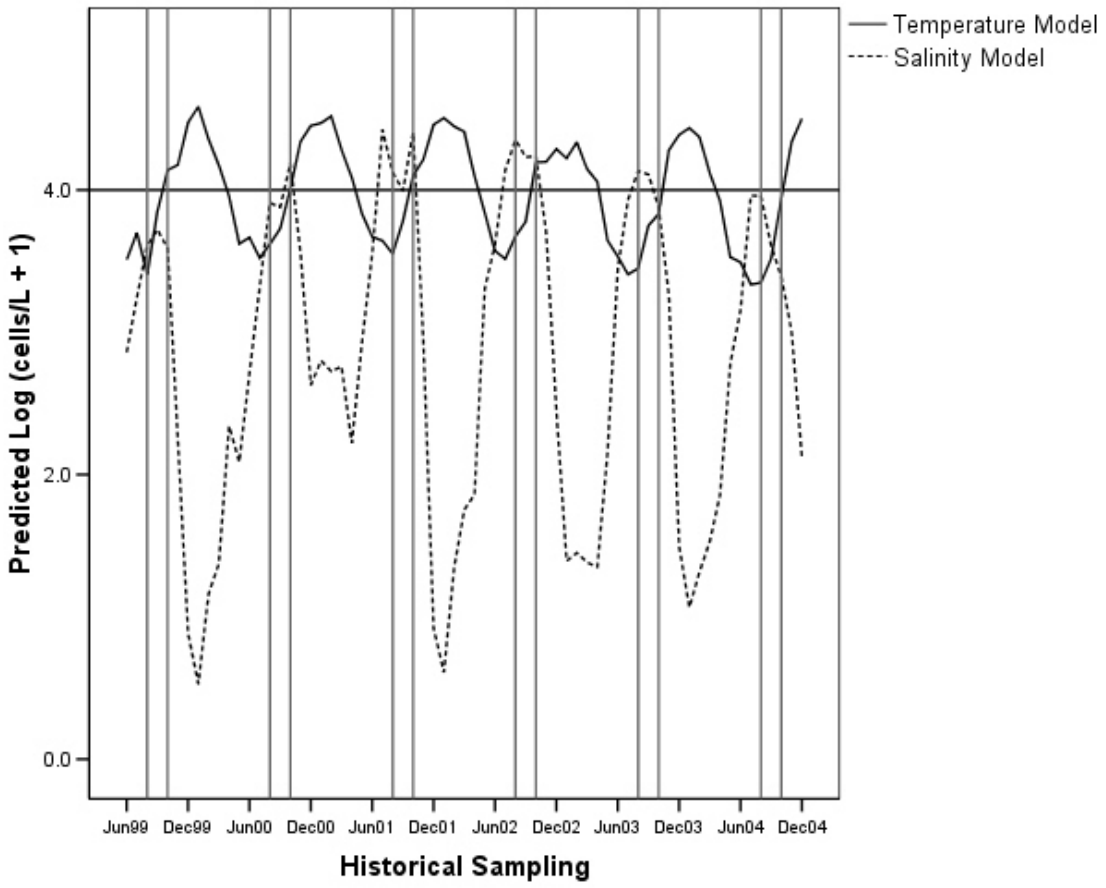

Figure 33. Single predictor models *Pseudo-nitzschia* cell concentrations for 1999 through 2004 from temperature (°C) and salinity (psu) data collected by S. Rumrill at Valino Island. Vertical bars indicate two month period between September 1st and November 1st. Note peaks of potential bloom events in late summer, early autumn (SPSS 14.0).

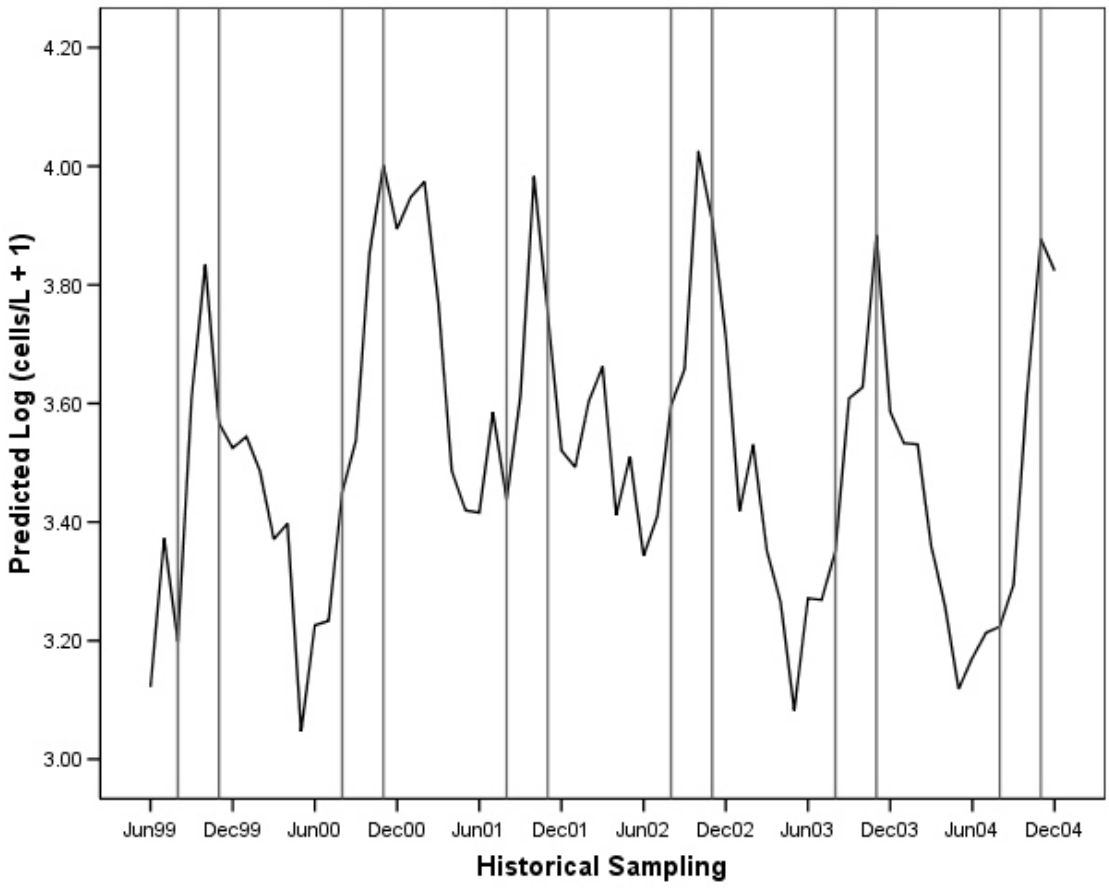

Figure 34. Multiple regression model predicted *Pseudo-nitzschia* cell concentrations for 1999 through 2004 from temperature (°C) and salinity (psu) data collected by S. Rumrill. Vertical bars indicate two month period between September 1st and November 1st. Note peaks of potential bloom events from August to November (SPSS 14.0).

3.7 Species Identification

Identification of species for morphologically intact specimens following digestions are listed in Table A.14. The morphological determination of specimens to the species level only provides concrete identifications for ten of thirteen sampling dates tested. The positive identifications only exist for two species: *Pseudo-nitzschia australis* and *P. pungens*. *P. australis* was dominant in samples from spring through early summer 2005. *P. pungens* was codominant with *P. australis* for the rest of the summer and autumn samples of 2005. *P. pungens* was the only species positively identified from June 2006.

Figures 35 through 40 demonstrate positively identified specimens of *P. australis* from 2005. Note the wide cell theca (> 6 μm) with two poroid rows within striae, larger number of striae 10 μm^{-1} (> 14), and absence of central large interspace. Figures 41 and 42 demonstrate positively identified specimens of *P. pungens* from 2005 and 2006. Note the narrow cell theca (< 6 μm) with two poroid rows within striae, smaller number of striae 10 μm^{-1} (< 14), and absence of central large interspace. Determination to species level is difficult due to similarity between morphological characters, especially for *P. australis* and *P. pungens*.

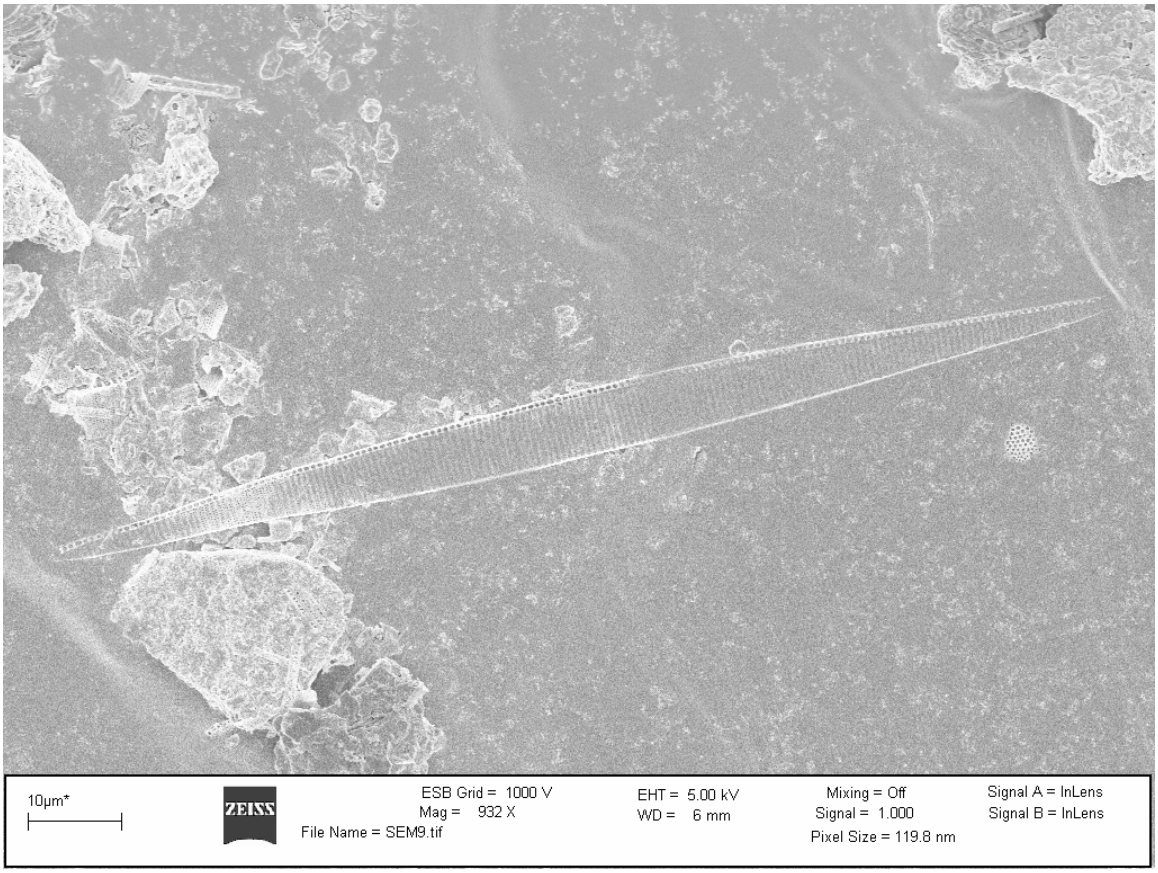

Figure 35. Scanning electron micrograph of *Pseudo-nitzschia australis* collected from Boat House on 18-May, 2005 (CAMCOR Facility, University of Oregon).

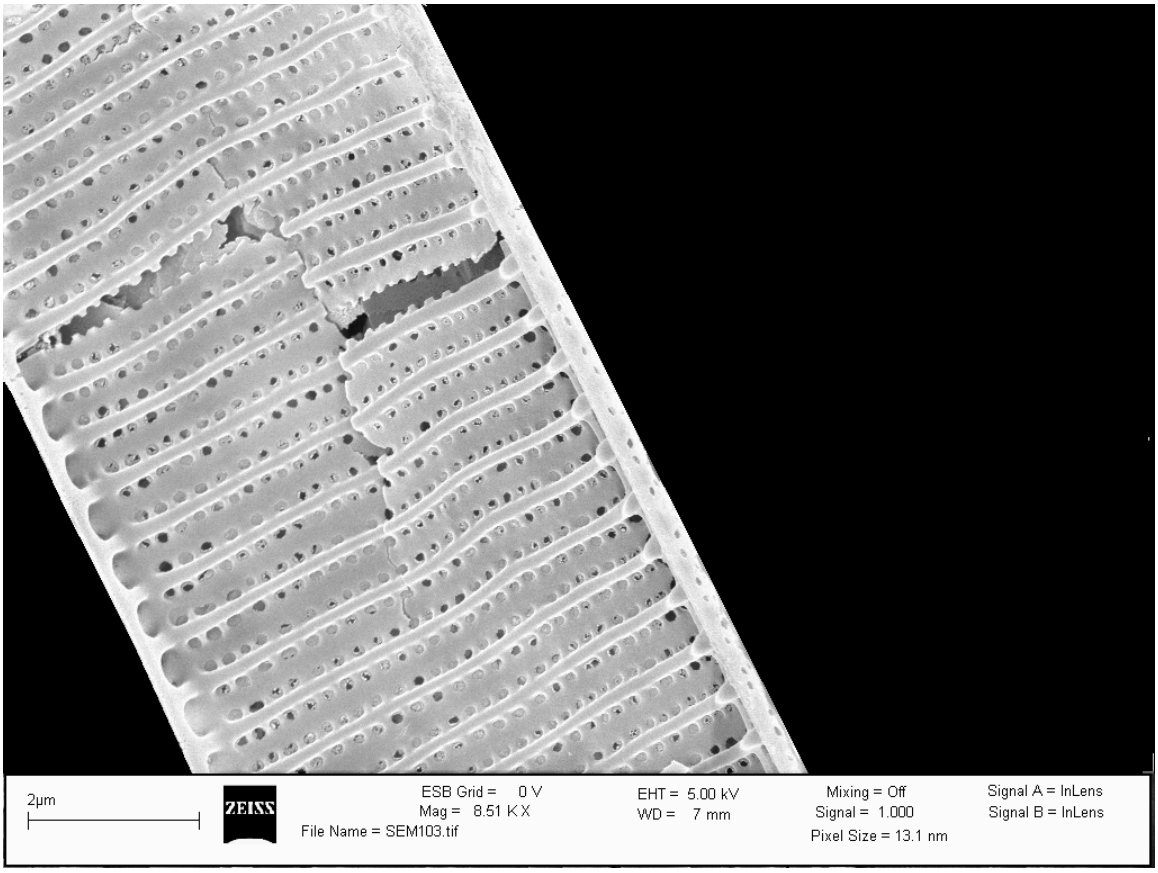

Figure 36. Scanning electron micrograph of *Pseudo-nitzschia australis* collected from Boat House on 18-May, 2005 (CAMCOR Facility, University of Oregon).

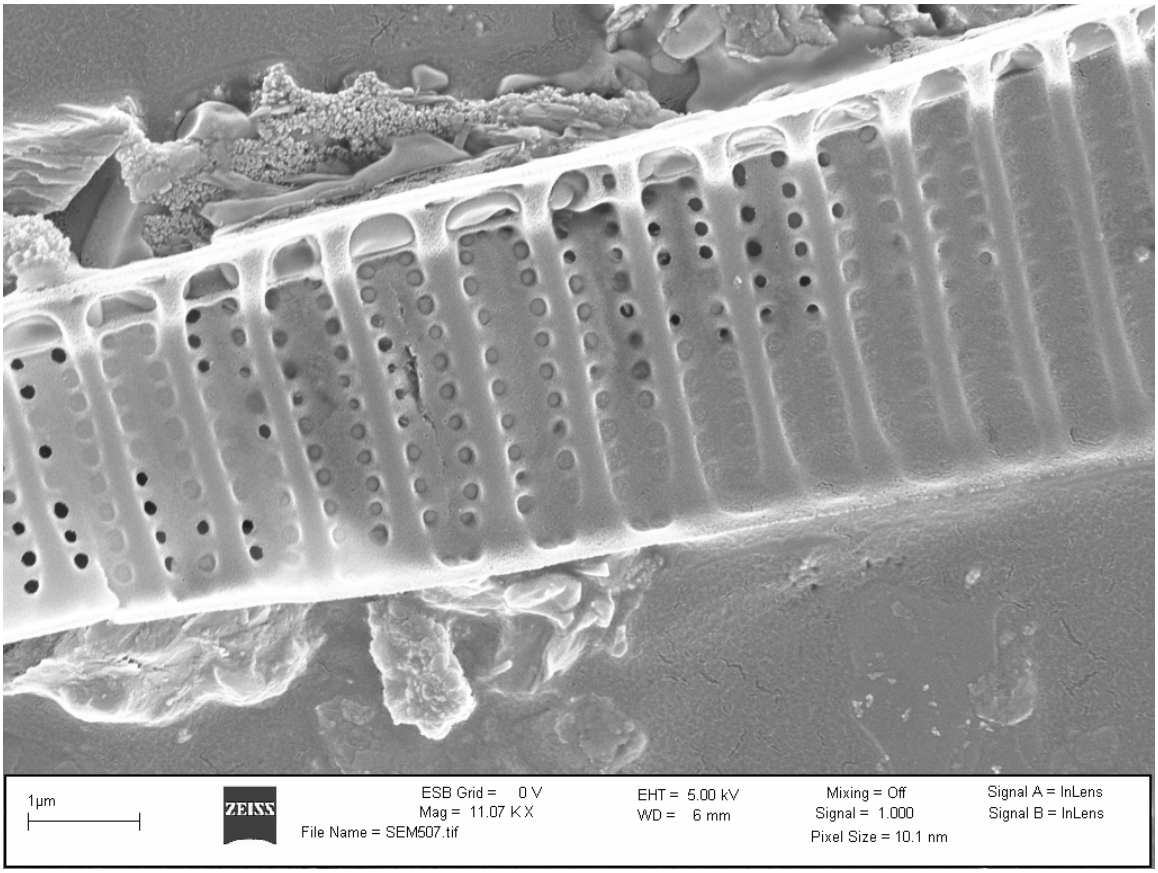

Figure 37. Scanning electron micrograph of *Pseudo-nitzschia australis* collected from Boat House on 7-June, 2005 (CAMCOR Facility, University of Oregon).

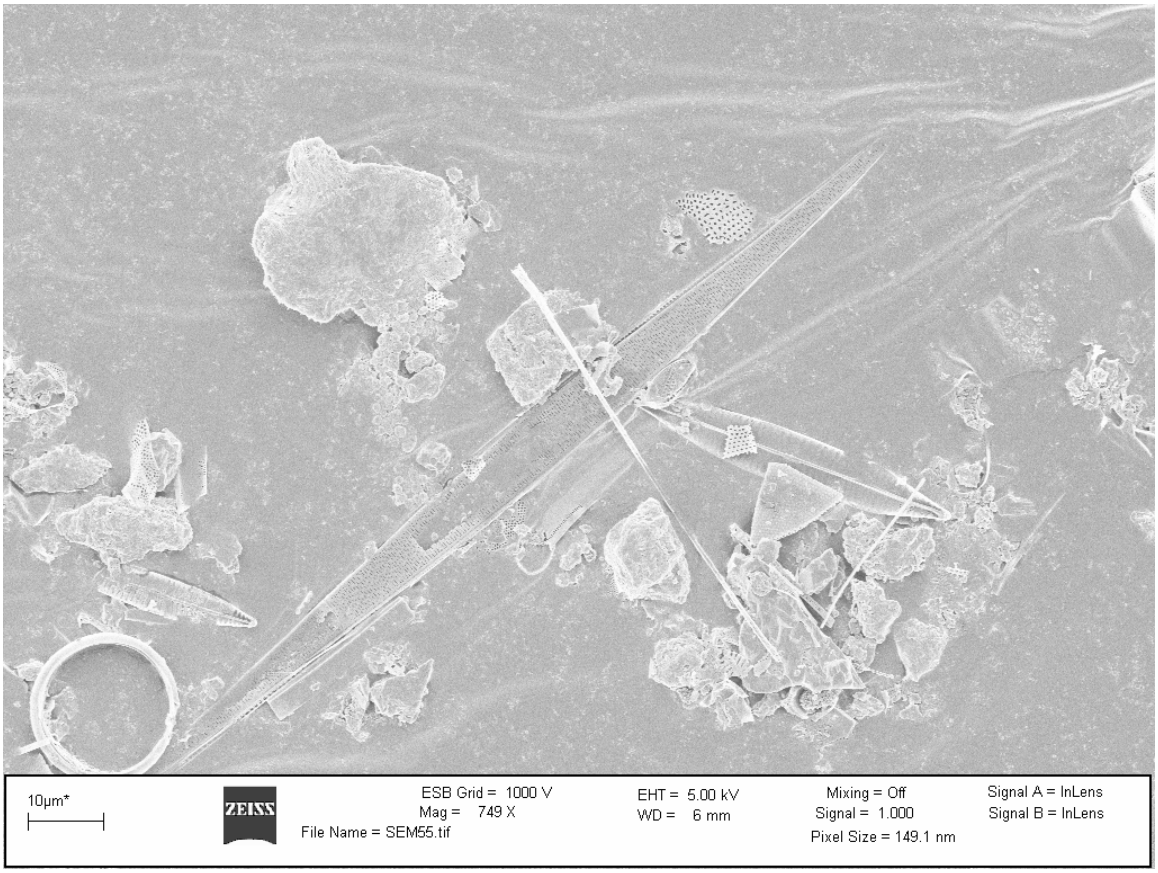

Figure 38. Scanning electron micrograph of *Pseudo-nitzschia australis* collected from Boat House on 5-July, 2005 (CAMCOR Facility, University of Oregon).

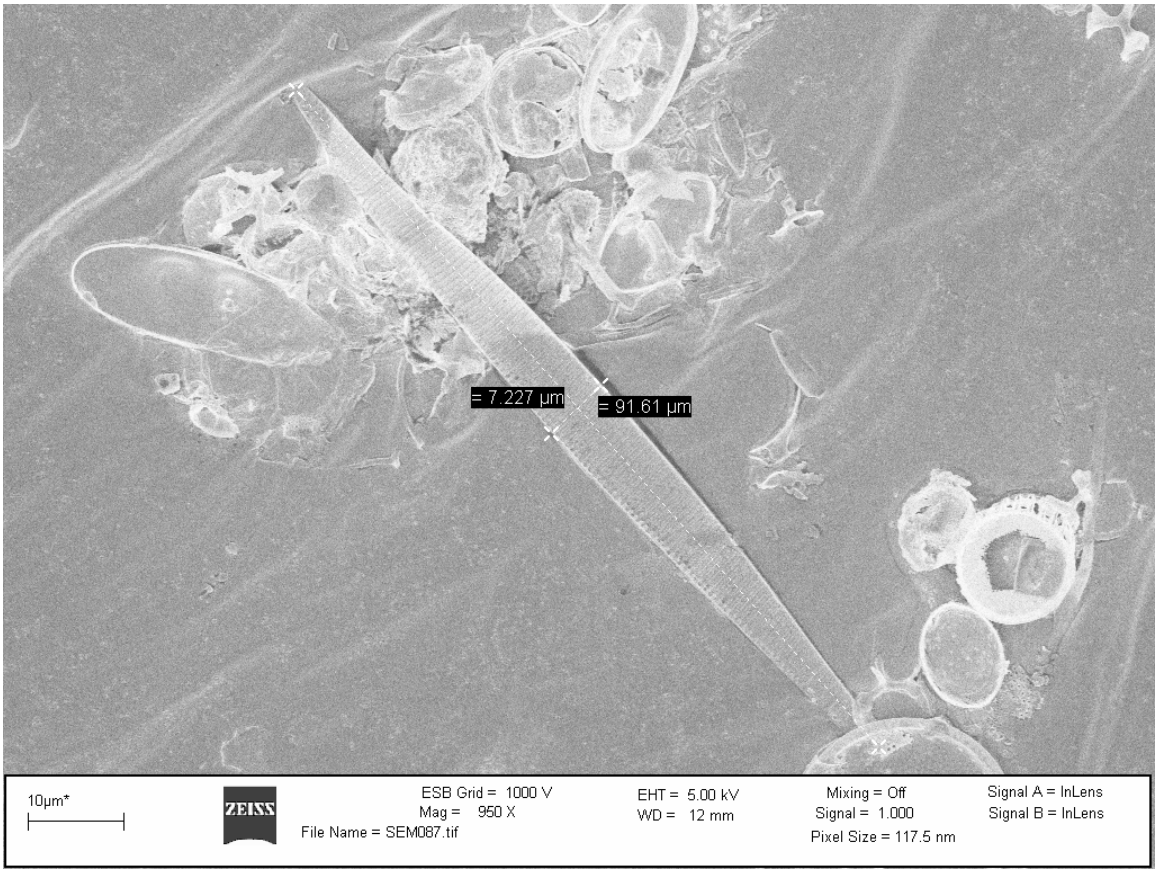

Figure 39. Scanning electron micrograph of *Pseudo-nitzschia australis* collected from Boat House on 23-August, 2005 (CAMCOR Facility, University of Oregon).

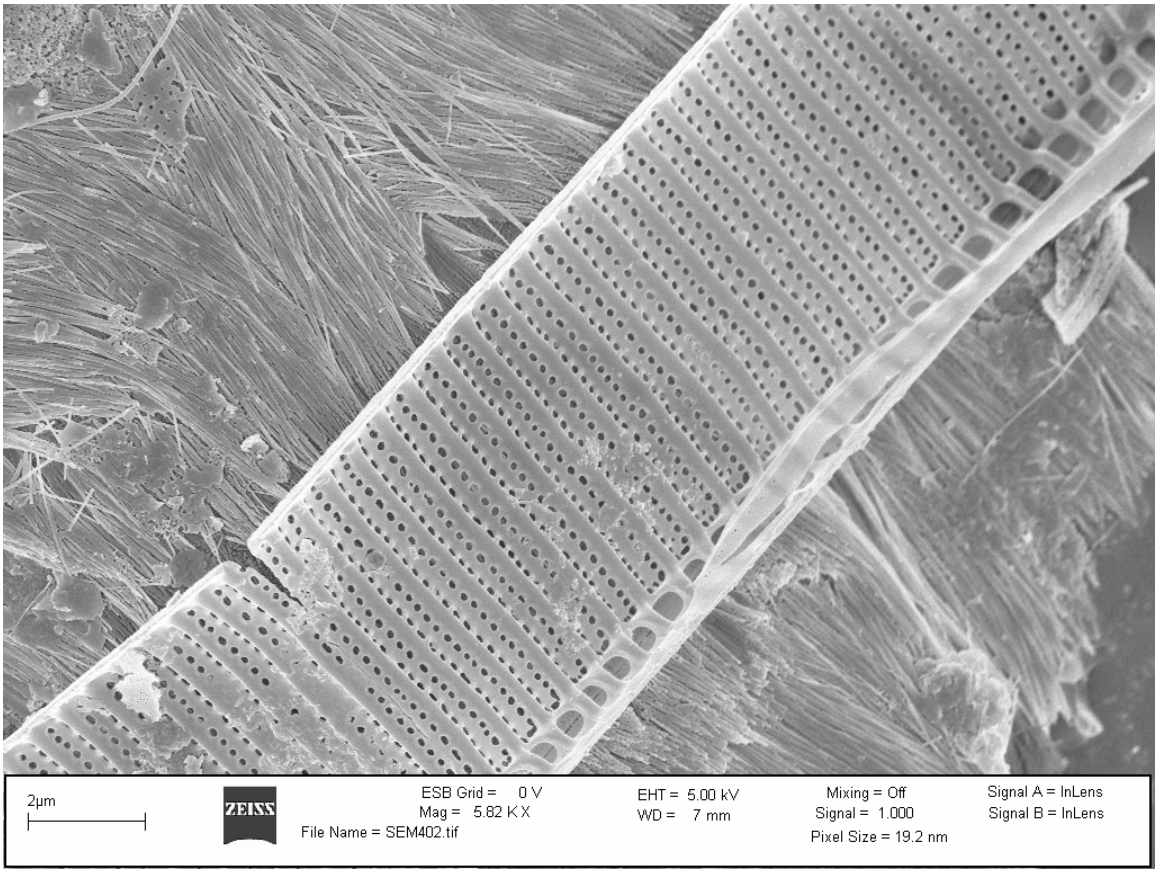

Figure 40. Scanning electron micrograph of *Pseudo-nitzschia australis* collected in channel (site 1, Fig. 1) on 17-October, 2005 (CAMCOR Facility, University of Oregon).

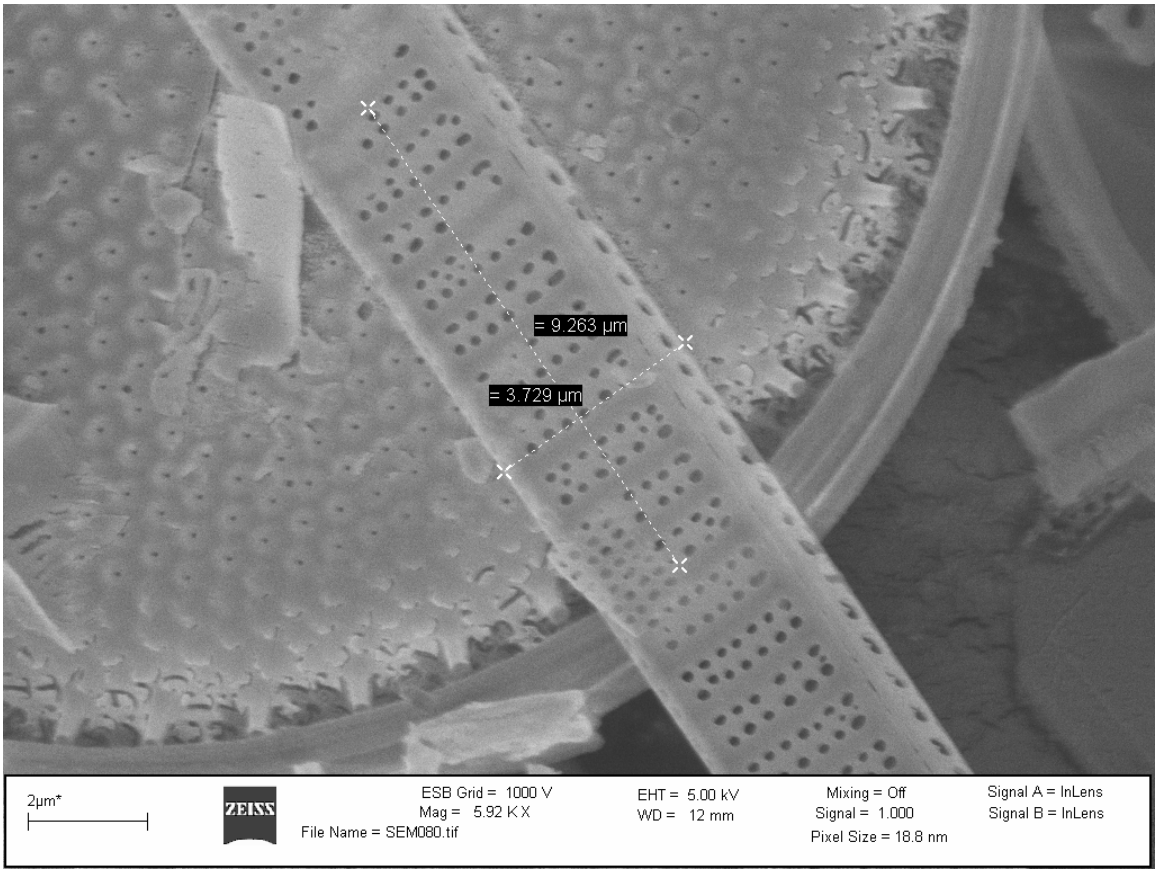

Figure 41. Scanning electron micrograph of *Pseudo-nitzschia pungens* collected at Boat House on 19-August, 2005 (CAMCOR Facility, University of Oregon).

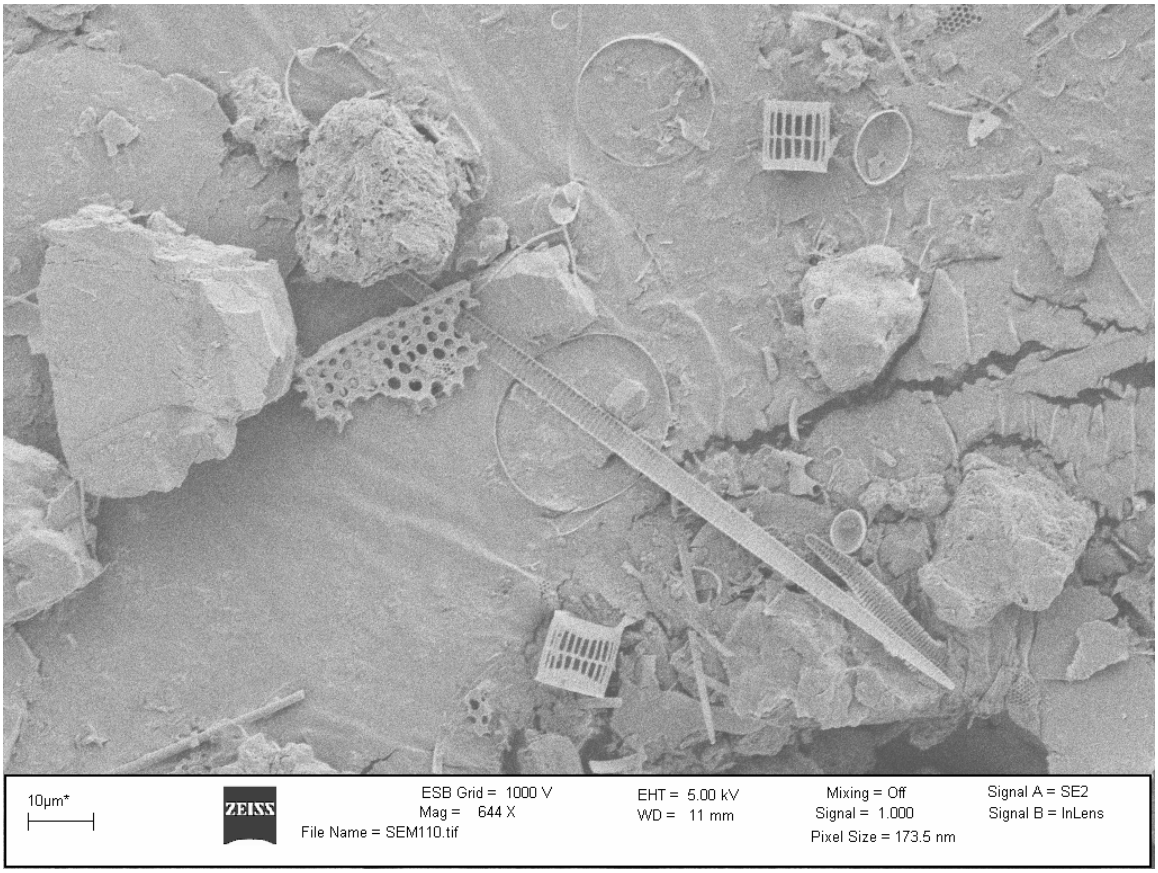

Figure 42. Scanning electron micrograph of *Pseudo-nitzschia pungens* collected in channel (site h, Fig. 1) on 19-June, 2006 (CAMCOR Facility, University of Oregon).

3.8 Domoic Acid Analysis

Cellular or particulate domoic acid concentrations increased with increasing temperature in October 2005 and June 2006 (Fig. 43). Temperature is highly explanatory of particulate domoic acid concentrations ($R^2 = 0.634$, $p << 0.05$). Non-cellular dissolved domoic acid concentrations were found to be negatively related to particulate domoic acid concentrations (Fig. 44). This significantly high relationship ($R^2 = 0.592$, $p << 0.05$) presents decreasing dissolved domoic acid values with increasing particulate concentrations.

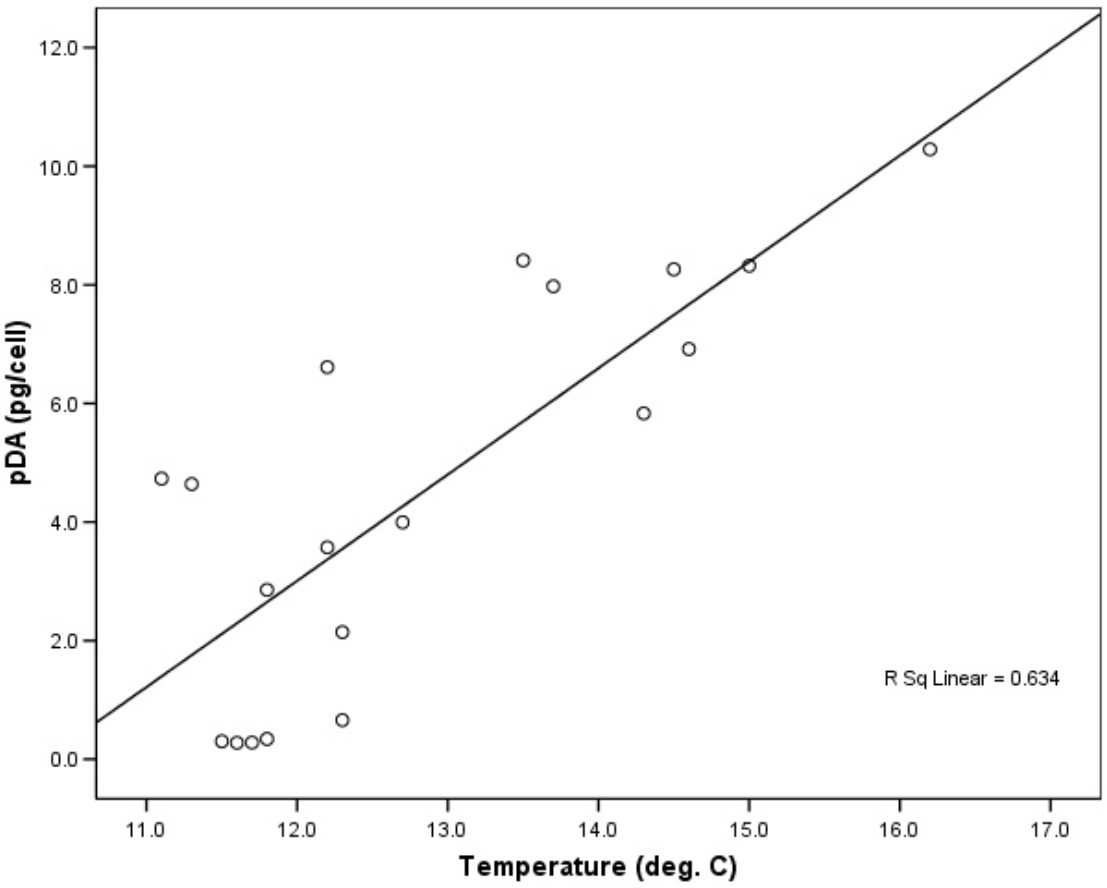

Figure 43. Regression analysis of 2005 and 2006 particulate domoic acid (pg cell^{-1}) related to temperature (ºC) without regard to site or date collected. Samples collected in channel on dates with >5000 cells L^{-1} (SPSS 14.0).

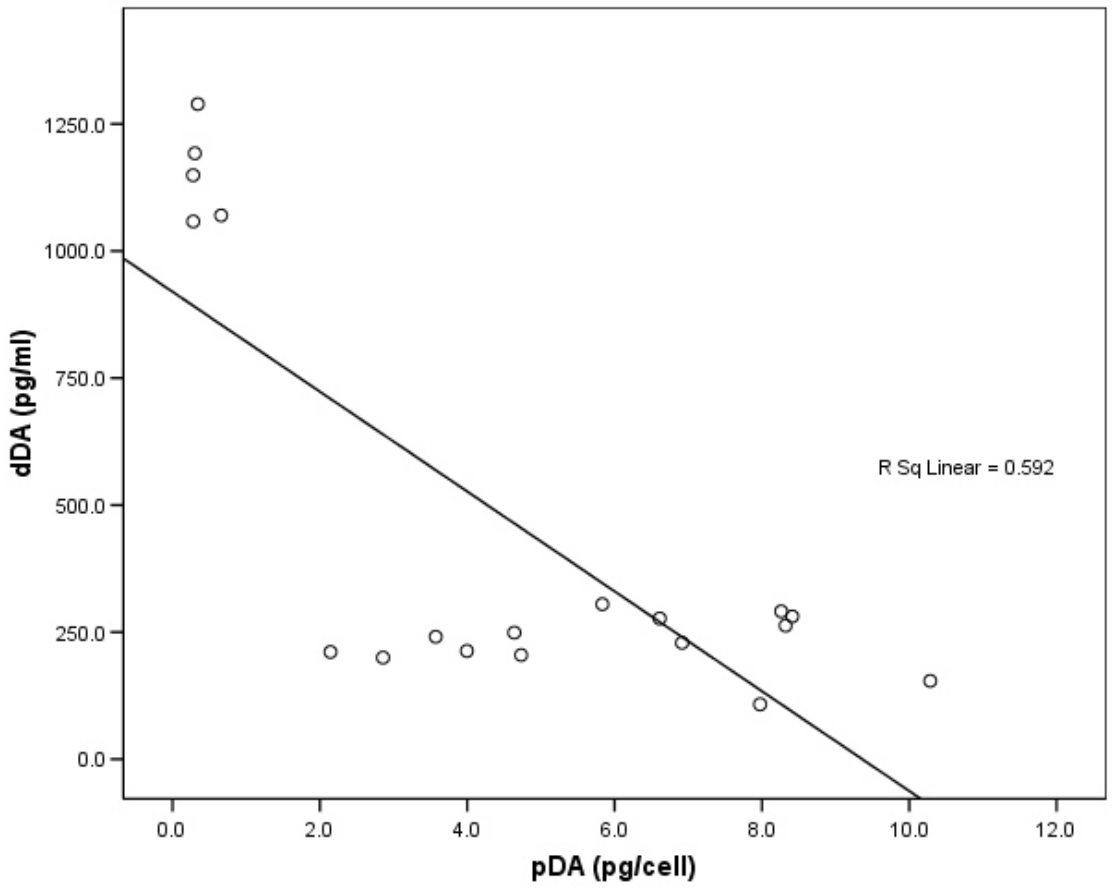

Figure 44. Regression analysis of 2005 and 2006 dissolved domoic acid (pg mL^{-1}) related to particulate domoic acid (pg $cell^{-1}$) without regard to site or date collected. Samples collected in channel on dates with >5000 cells L^{-1} (SPSS 14.0).

CHAPTER IV

DISCUSSION

Harmful algal blooms (HABs) are global phenomena with varying distributional and ecological patterns that should be studied in relation to the environments in which individual blooms exist. There are many different types of HABs, ranging from the oxygen depleting species which alter the environment, affecting many organisms, to toxigenic and ambush-predatory species that affect organisms more specifically. This type can cause difficulty for managers and scientists who attempt to understand, generalize, and predict HAB occurrences. The studies related to the ecological, physiological, biochemical, oceanographic, and limnological pathways associated with HABs will most certainly assist in the development of warning systems and possible protection of impacted ecosystems.

The Pacific Coast of North America does not have the diversity in HABs that is seen on the Atlantic Coast of North America. This should enable researchers here to focus more specifically on the two main forms that do exist: the dinoflagellates that cause Paralytic Shellfish Poisoning (PSP) and the diatoms that cause Domoic Acid Poisoning (DAP or ASP for Amnesic Shellfish Poisoning). While PSP is a well characterized event, it is principally caused by the dinoflagellates in the *Alexandrium tamarensis* complex (Scholin et al. 1994) and has been known possibly since the Vancouver expedition of 1793 (Lalli and Parsons 1997), DAP is a relatively recent (1991) discovery

off of this coast (Work et al. 1993). As such, less is known about DAP and the ecology of the diatoms that cause it.

With the possible exception of *Amphora coffaeformis* (Shimizu et al. 1989), diatoms in the genus *Pseudo-nitzschia* are the sole known bacillariophyte producers of the potent neurotoxin domoic acid (DA). *Pseudo-nitzschia* spp. have been known off the West Coast of North America at least since 1920 (previously known as *Nitzschia seriata*). Their distribution extends from Baja to Alaska (see Fryxell et al. 1997, Bates et al. 1998 for review). The species that have been present during the time of DAP events along this coast include *P. australis*, *P. multiseries*, *P. delicatissima*, *P. psuedodelicatissima*, and *P. pungens* (van Apeldoorn et al. 1999). While DAP associated *Pseudo-nitzschia* blooms off the East Coast of North America are typically monospecific, those on the West Coast often contain more than one toxigenic species (Bates et al. 1998). However, there is substantial evidence that the principally toxigenic *P. australis* populations drive the largest DAP events (Garrison et al. 1992; Horner et al. 1996; Trainer et al. 1998; Scholin et al. 2000). In the Pacific Northwest, *P. australis* and *P. pseudodelicatissima* are the dominant toxigenic species (Horner 2001).

Extensive research in the state of Washington related *Pseudo-nitzschia* spp. blooms with coastal oceanographic conditions. A 1992 study found a mixture of *Pseudo-nitzschia* spp., including the toxigenic *P. australis* and *P. multiseries*, were common in coastal waters during a warm weather period attributed to the el Niño Southern Oscillation (ENSO). Domoic acid was correspondingly discovered in razor clams, blue mussels, oysters, and Dungeness crabs within the Puget Sound region (Horner and Postel

1993). In 1997 and 1998, peak concentrations in a bloom of *P. pseudodelicatissima* corresponded with the highest dissolved domoic acid (dDA) levels within the large Juan de Fuca eddy. It was then hypothesized that the eddy, a cold counterclockwise oceanographic feature, serves as a potential source of toxigenic blooms to the coast. The mechanism by which entrained cells move out of the eddy and along the coast appears to linked to prolonged upwelling. Early autumn storms then transport the blooms onshore (Trainer et al. 2002), where *Pseudo-nitzschia* can become the primary food source for suspension feeding littoral organisms, thus leading to large toxification events as evident in razor clams (*Siliqua patula*) collected along the Washington coast in September of 1998 (Adams et al. 2000). Ongoing research into the oceanography of the Juan de Fuca eddy and the ecology of *Pseudo-nitzschia* blooms that the eddy supports are the current goals of the ECOHAB-PNW project, a multi-disciplinary research group funded by NOAA and NSF. Research of this complexity is essential for developing predictive parameters that enable managers to minimize costly shellfish harvesting closures (worth ~$20 million in 1991, http://www.ecohabpnw.org/) and determine the limits of toxigenic events.

Extensive studies on *Pseudo-nitzschia* ecology have been sparse in Oregon. Even though the shellfish industry in Oregon is much smaller than in Washington state, Oregon still harvests >6 million lbs. of shellfish annually (2005 projections, $4.71 million from the harvest of *Crassostrea gigas*, Pacific Shellfish Growers Association, http://www.pcsga.org/). For this reason, the state of Oregon, maintains a sampling program for the detection of DA in *Siliqua patula* and *Mytilus edulis* as well as

monitoring the abundance of *Pseudo-nitzschia* spp. (http://www.oregon.gov/ODA/). This is limited however, in the extent of physical and ecological parameters measured, a result of funding and human resources. DA measurements in shellfish can be problematic due to varying depuration rates: within 72 hours in *M. edulis* (Novaczek, et al. 1992) and longer than 16 months in *S. patula* (Wekell et al. 1994).

In the study of HABs, it is essential to quantify the extent of a bloom. This usually entails a series of point samples that provide cell concentrations which are extrapolated over the measured or probable distance of bloom movement. Spot sampling at single site locations can provide misleading results as blooms exist in a fluid environment and as such, are constantly moving. Knowledge of the underlying mechanisms driving this movement can assist in sample design as well as determining a bloom's extent based on sampling events.

The California Current, a slow (<0.25 m s^{-1}), wide (>1000 km), southerly flowing eastern boundary current, runs along the West Coast of North America. The California Current is a part of the North Pacific Gyre. Its flow is relatively close to shore in the summer and offshore in the winter when it is replaced nearshore by the northward flowing Davidson Current (Hickey 1989). The subarctic waters flowing south with the California Current, together with the process of upwelling, provide cold, nutrient rich waters to much of the Pacific Coast (Washington state to Pt. Conception, California) during the spring and summer months. Upwelling is a common seasonal phenomenon that occurs through the movement of strong northwesterly winds that drive surface waters seaward (Ekman transport). This movement away from shore allows cold deep waters to

rise towards the surface. The deep water brings with it a replenishing supply of nutrients into the euphotic zone where it becomes readily available to phytoplankton.

The seasonal upwelling is imperative for net primary productivity, the amount of photosynthesis minus the total algal respiration rate, as it allows for nutrients to move into the mixing depth where phytoplankton exist. In the spring and summer, this mixing depth (10-40 m) is shallower than the critical depth, or the depth at which photosynthetic production equals respiration. In the winter, when light levels are low and strong winds drive down the depth of mixing (70-80 m), the critical depth exists higher than the mixing depth and there is no net primary productivity even though the necessary nutrients are readily abundant. Characteristic of stratified, temperate coastal waters there is a period of transition that occurs when strong mixing relaxes in the early spring and when it sets up again in the late summer (Anderson 1964).

During a series of cruises off the Oregon and Washington coasts in 1961 and 1962, Anderson (1964) determined that the spring phytoplankton bloom, driven by periodic intervals of thermal stratification and strong mixing. Correspondingly, the autumn bloom is likely due to upwelling during optimal irradiance periods. Spring blooms are generally larger than those in the autumn along the Oregon coast. Upwelling is known to occur throughout summer, although intensity varies with wind, and is enough to maintain standing stocks (measured as chlorophyll *a* concentrations) of phytoplankton coastally. However, the optimal temperatures, irradiance levels, and nutrient concentrations in spring and autumn drive two peaks in seasonal phytoplankton productivity (measured as carbon production). Any winter productivity is usually driven

by freshwater effluent, principally from the Columbia River outflow. Anderson (1964) concluded that off of the Oregon coast, silicate and phosphate were abundant enough to maintain standing stocks, although levels did decrease with increasing productivity. He also concluded that it was likely that nitrate-nitrogen was likely to be the nutrient limiting coastal productivity (levels dropped to 0 $\mu g\ L^{-1}$ in summer months). These nutrients, while replenished with winter mixing, had to be upwardly advected with upwelling in the spring through autumn. Anderson (1964) demonstrated that it was important to determine where primary productivity occurs as well as what drives it.

As Odum noted in 1971, some the most productive waters on earth exist within estuaries. Estuaries, which are partially closed coastal regions with freshwater inflow (Lalli and Parsons 1997), act as biologically important land and sea interfaces where marine phytoplankton and nutrients can accumulate with flood tide. Within an estuary, nutrients are derived from both fresh water and marine sources. In the South Slough, fresh water inflow dominates in the winter months (>142 cm of rainfall), flooding the estuarine waters with high nutrient loads and organic runoff. This source of nutrient influx drops during the dry summer season (<10 cm of rainfall) when salinities increase. During this season, upwelling is the principal source of nutrients to the South Slough (Rumrill 2006).

Chlorophyll and nitrate-nitrogen in the South Slough, vary tidally on a seasonal basis with concentrations completely out-of-phase with tidal cycling during the wet season and in-phase during the dry season (Rumrill et al. 2007). This has been explained as a shift from fresh-water derived to marine derived chlorophyll *a*. Without

regard to where the chlorophyll *a* originates, regression analysis of historical data indicates a strong negative relationship between phytoplankton populations, as approximated with chlorophyll *a* concentrations, and nitrogen abundance (Fig. 3). This negative relationship indicates that, throughout the year, nitrogen is depressed during periods of increased photosynthetic capacity. In this context it is noted that nitrogen plays a key role for estuarine phytoplankton populations as a nutrient important in bloom dynamics.

On a temporal scale, chlorophyll *a* concentrations are highest in the spring, summer, and autumn months while nitrogen is lowest at the same time period (Rumrill 2006). Data from the current study support those findings, indicating that nitrogen is highest in the spring and lowest in the autumn (Fig. 8), while chlorophyll *a* is found to be highest in the summer months (Fig. 13). Hughes (1997) found two phytoplankton blooms occur in estuarine waters on a seasonal basis, the spring and fall blooms. She determined that the fall bloom tended to be larger on basis of cell abundance alone. She suggested this pattern, along with low winter abundances, is indicates time periods in the year when irradiance and nutrient levels are optimal for phytoplankton growth.

The spring and fall blooms also correspond with temperatures and salinities that exist between the high extremes found in summer and the low extremes found in winter. Historical data show that salinities of spring and autumn are similar (Fig. 5) while mean temperatures are lower in the autumn than the spring (Fig. 4). As determined by 2005 and 2006 data, chlorophyll *a* is correlative with temperature values (Fig. 16), suggesting that increased temperature imparts a stress on phytoplankton populations. In

addition to nutrient and irradiance conditions, the data presented here suggests that salinity and temperature are also very important physical stressors on South Slough bloom dynamics, at least for members in the genus *Pseudo-nitzschia*.

Stressors are here defined as environmental conditions that can decrease phytoplankton productivity. These stresses usually exhibit influence within a range of values that can be elucidated from manipulations of cultures in a laboratory. It is not always evident, however, that the physiological limits of lab cultures can be directly applied to field populations. For this reason, other methods have been sought to determine stress parameters in an ecological context. A popular method in determination of the extent a given stressor has on a population of cells is the measure of productivity (cellular C:N ratios). This method produces misleading results when species are able to tolerate long periods of stress induction before changes in productivity rates are quantifiable (Berges and Falkowski 1998). A seemingly more robust method, due to quicker response times, is the direct measurement of molecular markers which are directly linked to onset of a physiological stressor (Falkowski et al. 1992). This method has been validated in studies involving light and nutrient limitations as potential stressors (e.g. La Roche et al. 1995; Berges and Falkowski 1998; Timmermans et al. 1998). In the context of the current study, temperature and salinity are the measured environmental variables that can reach stressful levels. If DA acts as a secondary metabolite produced in response to stress, correlations between the occurrence of DA and particular temperature or salinity values may be indicative of the conditions when these variables act as stressors. Previous studies, demonstrating the linkage of increased cellular

concentration with decreased temperature (Dortch et al. 1997) and increased salinity (Thessen et al. 2005) support the use of these parameters for predictive analyses. The use of measurements of DA production as a potential indicator for physiologically stressed cells will be discussed later.

Beyond physiological limits, spatial scales are also important in determining bloom dynamics, with upper estuarine regions being dominated by freshwater or halo-tolerant species. Lower estuarine regions within the South Slough are dominated by marine derived autotrophs and heterotrophs (Hughes 1997). In the current study it was found that during the sampled months, marine dominated sites (BH in Fig. 12) have the highest chlorophyll concentrations while middle estuarine sites have the highest nitrogen concentrations (Fig. 9).

While historical data indicate linkage between nitrogen and chlorophyll *a* concentrations, data collected during periods of increased *Pseudo-nitzschia* spp. concentrations indicate otherwise. Figure 10 presents a comparison of historical and high cell concentration samples that are not significantly different for nitrogen concentrations, yet in a similar analysis chlorophyll *a* is much greater in samples with *Pseudo-nitzschia* spp. than in historical data (Fig. 15). This disparity suggests that other physical parameters may be primarily influencing *Pseudo-nitzschia* spp. bloom abundance within estuarine waters.

Hughes (1997) found multiple blooms of *Pseudo-nitzschia* spp. occurring between spring and early autumn. The abundance quantification was low at the upper estuarine

sites and she concluded that blooms prefer higher salinity, coastally derived waters. These conclusions are supported by data collected by Cziesla (1999) that indicate *Pseudo-nitzschia* spp. blooms develop coastally around relaxation of upwelling events and are tidally transported into the Coos Estuary. This movement is most likely propagated by internal waves (Shanks and McCulloch 2003). Data from 2005 support the data collected by Hughes (1997), as seen in Figure 18 where Boat House has the highest cell abundances (pattern similar to Fig. 20). These high abundances were mostly found in the spring and autumn (Fig. 19), indicating the temporal scale of optimal bloom conditions.

The temperature and salinity values found in spring and autumn fall within known tolerance ranges for *Pseudo-nitzschia* spp. (van Apeldoorn et al. 1999). Lowest temperatures coincide with highest salinities and highest estuarine temperatures correspond with the broadest salinity range (Fig. 23). This indicates that low salinity waters (i.e. upper estuary) have the most stress potential for marine derived phytoplankton as both temperatures and salinities reach extreme tolerance limits. The direct measurement of temperature and salinity along with the calculation of cellular abundance levels provides insight into the unique relationship of *Pseudo-nitzschia* spp. populations with the physical environment.

It was found that cellular concentrations decrease with increasing temperatures in 2006 (Fig. 24). The strong linear relationship provides predictive capabilities that were tested with 2005 data (Figs. 27 and 29). Moderate to high predictive capacity as found potentially indicates the impact temperature may have on estuarine populations, given

that temperature is the stressor acting directly upon the population and not an unmeasured covariant. A statistical salinity model (Fig. 25) demonstrates similar, although less predictive, results (Figs. 28 and 30) where populations positively correlate with measured salinities. Utilizing both temperature and salinity together as explanatory variables for cellular concentrations provides a strong multiple regression model (Fig. 26) with high predictive capacity (Figs. 31 and 32). It is obvious that using either physical parameter alone is strongly linked to a significant proportion of the variation found within the population abundances. However, there are important reasons why both explanatory variables should be utilized.

Historical temperature and salinity data in the models provided a means of highlighting optimal time periods for bloom abundance within a given year. As seen when this data was plotted for the single models, abundances were nearly out of phase for either explanatory variable (Fig. 33). However, the small region of convergence in late summer early autumn in single model analyses was greatly enhanced with the multiple regression model predictions (Fig. 34). This demonstrated that the period from late August to early November was consistently optimal in terms of temperature and salinity, or something that covaries, in every year analyzed. These predictions are consistent with previously published results on Coos Bay from 1991 (Villac et al. 1993) and 1994 through 1995 (Hughes 1997). As Hughes (1997) suggested, the nutrient and irradiance levels may not be conducive to bloom sustenance until the spring and fall, however, these results suggest a new link to bloom propagation within the South Slough. Temperature, driven up by increased irradiance and low flushing rates of shallow estuarine waters, may

be too high throughout summer to sustain cold water phytoplankton. Salinity is decreased in the winter below the tolerance level of *Pseudo-nitzschia* spp. and is only optimal from spring to autumn during the dry season when rainfall is minimal. Then it becomes primarily the autumn months when the temperatures begin to drop and freshwater inflow has not yet started that conditions are optimal for estuarine bloom persistence. This hypothesis is supported by the reversal from upwelling favorable to downwelling favorable conditions as measured by the upwelling index off coastal Oregon (see Appendix Fig. A.1). It is noted that this does not suggest a mechanism of coastal bloom development, which likely is nutrient dependent (Cziesla 1999).

The ability to predict bloom persistence becomes increasingly relevant with the possibility of toxigenic species residing within estuarine waters. Of the ten known species within the genus that are toxigenic (Bates 2000), only *Pseudo-nitzschia australis* (Buck et al. 1992; Fritz et al. 1992; Scholin et al. 2000; Trainer et al. 2000), *P. multiseries* (Scholin et al. 2000; Trainer et al. 2000), and *P. pseudodelicatissima* (Adams et al. 2000; Horner et al. 2000) have been linked to West Coast toxification events. Along the Oregon coast, only *P. australis* has been linked to domoic acid accumulation within the food web (*Siliqua patula*, Trainer et al. 2000). Even with these historical insights into the most common species linked to toxigenic events, identification of blooms to the species level is important in the current study. Scanning electron microscopy (Figs. 35 - 42) allowed for identification of the toxigenic *P. australis* (Garrison et al. 1992) and *P. pungens* (Rhodes et al. 1996). The presence of these species within the South Slough should alert the reader to potential for toxin production within

estuarine waters.

The next logical step to elucidate the ecological extent of these potentially toxigenic blooms is to test for presence of toxin. Particulate domoic acid (Fig. 43) concentrations can be explained by temperature values (note no other explanatory variable measured was significantly correlated). The increase in pDA with increasing temperature strongly supports the previously discussed hypothesis that temperature is a physical stressor. It is not only at the population level that this stressor acts as in analysis of bloom abundance, but also at the cellular level. The role of domoic acid in cellular processes is not yet determined; however, it is known that cells produce the neurotoxin under stressful conditions (Bates et al. 1998) which may include temperature as shown in highly toxigenic *P. multiseries* grown above 20ºC (Lewis et al. 1993). It is thought that primary metabolism shuts down allowing secondary metabolic processes, such as domoic acid production, to occur during stressful periods (Pan et al. 1998). These results suggest that high temperatures are indeed stressful to cells within the estuarine environment.

The tight inverse linkage between dissolved domoic acid and pDA (Fig. 44), further suggests that domoic acid is biochemically synthesized during stressful conditions. Experiments with *P. multiseries*, showed that DA was not produced until stationary phase in the bloom growth cycle (Bates et al. 1991). Stationary phase indicates environmental limitations inhibitory to population growth. Individual cells begin to lyse due to these limitations, causing a decrease in bloom abundance (death phase, Eppley 1977), most likely releasing biochemical metabolites such as domoic acid into the water column. Due to the shut down of metabolic processes and cell death, dDA likely

increases with decreasing pDA, as presented in this study. The production of DA as a secondary metabolite should be seriously considered in the determination of the physiological state of individual cells. If the biochemical pathway leading to DA production can be elucidated, rates of DA production can provide important information as to the specific parameters that initially stress a cell. At present at least, DA may be utilized as a molecular marker for determining overall levels of physiologic stress that cells can tolerate.

While the link between ecology and production of domoic acid by toxigenic species within the genus *Pseudo-nitzschia* is a complex process about which much is unknown (van Apeldoorn et al. 1999), there are some basic principals that may govern the occurrence of blooms in estuarine regions. As found in this study, measurable physical parameters play a large role in defining the conditions that can have the potential to support toxigenic blooms of *Pseudo-nitzschia*. Temperature, and to a lesser extent salinity, may be important stressors and they can be used as markers for bloom extent and may for the basis of a statistical model that may permit the calculation of where (based on the measured parameters) and more importantly when (based on historical data) a bloom is likely to occur and persist. It was demonstrated that the time frame optimal for persistence, based on temperature and salinity, occurs during the late summer and early autumn within the South Slough. This time corresponds to the relaxation of summer upwelling conditions as measured by the upwelling index.

Beyond a statistical model for bloom abundance, toxin production by toxigenic species found within the estuary also correlated significantly with temperature stressor.

The extreme temperatures found within shallow slough waters can limit both bloom persistence and cellular metabolism. The potential importance of temperature as a stressor at the population and the individual level highlighted here, and the clear covariance, should provide a tool for advising managers of estuarine waters that are concerned with toxigenic *Pseudo-nitzschia* spp. Given the use of cell abundance values for both *P. pungens* and *P. australis*, found primarily in different seasons, in calculation and verification of the models presented, conclusions can be made to supporting the validity of multi-species models in a given estuary.

It should be noted that with given flushing rates of approximately 3 days (Rumrill 2006) and *Pseudo-nitzschia* doubling times of around 2 days (Garrison 1992), populations have potential for periods of residence within estuarine waters. Thus, even if physical parameters support growth upon cells entering the estuary, conditions may become stressful while cells are in residence. In this instance, populations, without regard to where blooms initiate (coastally or upestuary), can reside here long enough for cells to become toxigenic and increase the potential for estuarine DAP events. This demonstrates the coastal inlets, like the South Slough, should be considered for future HAB monitoring of coastal waters.

It is the hopes that this study will be a basis for future research in the area of ecosystem level stressors on the population ecology of *Pseudo-nitzschia* spp. Given the need for spatial and temporal predictive capabilities regarding DA producing events (Wood et al. 1992), this research should be utilized as a basis for addition of measured effectors to bloom persistence so that predictions can eventually be made conclusive.

Furthermore, this research should demonstrate that covariance models of phytoplankton population ecology can be elucidated with determination of simple and quantifiable physical parameters.

APPENDIX

DATA AND ANALYSES

Table A.1. Regression analysis of historical chlorophyll and nitrogen (as nitrate + nitrite) data (NOAA NERR CDMO). Nitrogen used as the predictor for chlorophyll. Note high explanatory capability ($R^2 = 0.483$) with very high significance in ANOVA of $p << 0.05$ (SPSS 14.0).

Model Summary(b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
0.695(a)	0.483	0.477	0.155476

a. Predictors: (Constant), logN

b. Dependent Variable: logchl

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	1.810	1	1.810	74.873	0.000(a)
Residual	1.934	80	0.024		
Total	3.744	81			

a. Predictors: (Constant), logN

b. Dependent Variable: logchl

Coefficients(a)

	Unstandardized Coefficients			
variable	B	Std. Error	t	p
(Constant)	0.663	0.025	26.457	0.000
logN	-2.348	0.271	-8.653	0.000

a. Dependent Variable: logchl

Table A.2. Non-parametric Kruskal-Wallis mean rank difference test of nitrogen (as nitrate + nitrite) for 2005 fixed station sites. Note non-significance between sample sites ($p > 0.05$, SPSS 14.0).

Ranks

site	N	Mean Rank
BH	14	21.07
PI	13	16.96
HL	14	24.68
Total	41	

Test Statistics(a,b)

	N
Chi-Square	2.801
Degrees of freedom	2
p	0.246

a. Kruskal Wallis Test

b. Grouping Variable: site

Table A.3. Non-parametric Kruskal-Wallis mean rank difference test of nitrogen (as nitrate + nitrite) for seasons in 2005. Note high significance between seasons ($p << 0.05$) with spring ranking highest and autumn ranking lowest (SPSS 14.0).

Ranks

season	N	Mean Rank
Spring	11	40.18
Summer	30	29.05
Autumn	14	16.18
Total	55	

Test Statistics(a,b)

	N
Chi-Square	14.174
Degrees of freedom	2
p	0.001

a. Kruskal Wallis Test

b. Grouping Variable: season

Table A.4. Non-parametric Kruskal-Wallis mean rank difference test of nitrogen (as nitrate + nitrite) for estuarine regions in 2005 and 2006. Note non-significance between regions ($p > 0.05$, SPSS 14.0).

Ranks

region	N	Mean Rank
Lower	8	13.38
Middle	12	15.21
Upper	7	12.64
Total	27	

Test Statistics(a,b)

	N
Chi-Square	0.542
Degrees of freedom	2
p	0.763

a. Kruskal Wallis Test

b. Grouping Variable: region

Table A.5. Non-parametric Mann-Whitney-Wilcoxon rank difference test of nitrogen (as nitrate + nitrite) at historical sites (2003 and 2004, NOAA NERR CDMO) and sampling dates with >5000 *Pseudo-nitzschia* spp. cells/L (2005 and 2006). Note non-significance between data sets ($p > 0.05$, SPSS 14.0).

Ranks

data	N	Mean Rank	Sum of Ranks
Historical	86	81.96	7,048.50
>5000c/L	68	71.86	4,886.50
Total	154		

Test Statistics(a)

	N
Mann-Whitney U	2,540.500
Wilcoxon W	4,886.500
Z	-1.396
p (2-tailed)	0.163

a. Grouping Variable: nitrogen

Table A.6. Comparison of means test using ANOVA and Tukey's Honestly Significant Difference test of chlorophyll *a* for 2005 fixed station sites. Sample sites denoted as: BH = Boat House; PI = South Slough Pilings; HL = Hinch Lane Bridge. Note high significance between data sets as determined in ANOVA ($p << 0.05$). BH has highest mean chlorophyll values, followed by HL and PI (SPSS 14.0).

Descriptives

	N	Mean	Std. Deviation	95% CI Lower Bound	95% CI Upper Bound	Minimum	Maximum
BH	36	1.1370	0.29755	1.0363	1.2377	0.38	1.60
PI	33	0.6059	0.16617	0.5470	0.6649	0.38	1.05
HL	35	0.7795	0.20486	0.7091	0.8499	0.22	1.06
Total	104	0.8482	0.31949	0.7860	0.9103	0.22	1.60

ANOVA

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Between Groups	5.104	2	2.552	47.652	0.000
Within Groups	5.409	101	0.054		
Total	10.513	103			

Multiple Comparisons

Tukey HSD

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% CI Lower Bound	95% CI Upper Bound
BH	PI	.53103(*)	0.05577	0.000	0.3984	0.6637
	HL	.35750(*)	0.05494	0.000	0.2268	0.4882
PI	BH	-.53103(*)	0.05577	0.000	-0.6637	-0.3984
	HL	-.17354(*)	0.05615	0.007	-0.3071	-0.0400
HL	BH	-.35750(*)	0.05494	0.000	-0.4882	-0.2268
	PI	.17354(*)	0.05615	0.007	0.0400	0.3071

*. The mean difference is significant at the .05 level.

Table A.7. Non-parametric Kruskal-Wallis mean rank difference test of chlorophyll *a* for seasons in 2005. Note significance between data sets ($p < 0.05$) with summer ranking highest and spring ranking lowest (SPSS 14.0).

Ranks

Season	N	Mean Rank
Spring	9	31.67
Summer	99	65.34
Autumn	14	53.54
Total	122	

Test Statistics(a,b)

	logchl
Chi-Square	8.282
Degrees of freedom	2
p	0.016

a. Kruskal Wallis Test

b. Grouping Variable: season

Table A.8. Comparison of means test of chlorophyll *a* using ANOVA for 2005 and 2006 channel sites by estuarine region. Note non-significance between data sets ($p > 0.05$, SPSS 14.0).

Descriptives

	N	Mean	Std. Deviation	95% CI		Minimum	Maximum
				Lower Bound	Upper Bound		
Lower	8	0.790220	0.1214068	0.688721	0.891719	0.6031	0.9388
Middle	12	0.837019	0.1513966	0.740826	0.933211	0.5328	1.0416
Upper	7	0.786300	0.1585246	0.639689	0.932910	0.5024	0.9554
Total	27	0.810003	0.1416808	0.753956	0.866050	0.5024	1.0416

ANOVA

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Between Groups	0.016	2	0.008	0.375	0.691
Within Groups	0.506	24	0.021		
Total	0.522	26			

Table A.9. Comparison of means t-test for log transformed chlorophyll at historical sites (2003 and 2004, NOAA NERR CDMO) and sampling dates with >5000 *Pseudo-nitzschia* spp. cells L^{-1} (2005 and 2006). Note high significance between data sets ($p << 0.05$) for equal variances (SPSS 14.0).

data	N	Mean	Std. Deviation	Std. Error Mean
Historical	84	0.51972	0.231982	0.025311
>5000c/L	66	0.82919	0.269970	0.033231

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% CI	
	F	Sig.	t	Degrees of freedom	p (2-tailed)	Mean Diff.	Std. Err Dif.	Lower	Upper
Equal variances assumed	0.751	0.388	-7.544	148	0.000	-0.309473	0.041020	-0.390533	-0.228413
Equal variances not assumed			-7.408	128.442	0.000	-0.309473	0.041773	-0.392125	-0.226821

Table A.10. Regression analysis of log transformed chlorophyll of 2005 and 2006. Temperature used as the predictor for chlorophyll. Note explanatory capability (R^2 = 0.311) with very high significance in ANOVA of p << 0.05 (SPSS 14.0).

Model Summary(b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.558(a)	0.311	0.306	0.23778

a. Predictors: (Constant), VAR00002

b. Dependent Variable: VAR00001

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	3.168	1	3.168	56.035	.000(a)
Residual	7.011	124	0.057		
Total	10.179	125			

a. Predictors: (Constant), VAR00002

b. Dependent Variable: VAR00001

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	1.590	0.099		15.988	0.000
VAR00002	-0.048	0.006	-0.558	-7.486	0.000

a. Dependent Variable: VAR00001

Table A.11. Non-parametric Kruskal-Wallis mean rank difference test of *Pseudo-nitzschia* spp. concentrations for sites in 2005. Note significance between data sets ($p << 0.05$) with Boat House ranking highest and Hinch Lane Bridge ranking lowest (SPSS 14.0).

Ranks

site	N	Mean Rank
BH	36	83.08
PI	34	45.91
HL	36	31.08
Total	106	

Test Statistics(a,b)

	logcells
Chi-Square	58.811
Degrees of freedom	2
p	0.000

a. Kruskal Wallis Test

b. Grouping Variable: site

Table A.12. Non-parametric Kruskal-Wallis mean rank difference test of *Pseudo-nitzschia* spp. concentrations for seasons in 2005. Note significance between data sets ($p << 0.05$) with Autumn ranking highest and Summer ranking lowest (SPSS 14.0).

Ranks

season	N	Mean Rank
Spring	16	82.06
Summer	90	51.24
Autumn	14	95.39
Total	120	

Test Statistics(a,b)

	logcells
Chi-Square	28.224
Degrees of freedom	2
p	0.000

a. Kruskal Wallis Test

b. Grouping Variable: season

Table A.13. Comparison of means test using ANOVA and Tukey's Honestly Significant Difference test of *Pseudo-nitzschia* spp. concentrations for 2005 and 2006 estuarine regions. Note high significance between data sets as determined in ANOVA ($p << 0.05$). Differences exist between Lower and Upper ($p << 0.05$) in Tukey's HSD (SPSS 14.0).

Descriptives

	N	Mean	Std. Deviation	95% CI Lower Bound	95% CI Upper Bound	Minimum	Maximum
Lower	8	4.3794	0.57991	3.8946	4.8642	3.78	5.29
Middle	10	3.7923	0.24314	3.6184	3.9662	3.38	4.17
Upper	7	2.5369	1.87260	0.8050	4.2687	0.00	4.91
Total	25	3.6286	1.24242	3.1158	4.1415	0.00	5.29

ANOVA

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Between Groups	13.121	2	6.560	6.032	0.008
Within Groups	23.926	22	1.088		
Total	37.047	24			

Multiple Comparisons

Tukey HSD

(I) region	(J) region	Mean Difference (I-J)	Std. Error	p	95% CI Lower Bound	95% CI Upper Bound
Lower	Middle	0.58708	0.49467	0.473	-0.6556	1.8297
	Upper	1.84252(*)	0.53973	0.007	0.4867	3.1983
Middle	Lower	-0.58708	0.49467	0.473	-1.8297	0.6556
	Upper	1.25544	0.51392	0.058	-0.0356	2.5465
Upper	Lower	-1.84252(*)	0.53973	0.007	-3.1983	-0.4867
	Middle	-1.25544	0.51392	0.058	-2.5465	0.0356

*. The mean difference is significant at the .05 level.

Table A.14. Identifications of *Pseudo-nitzschia* spp. found in 2005 and 2006 using scanning electron microscopy. Positive identification noted with single species listed. Species potentially present using morphological characters are: *P. australis* (*a*); *P. pungens* (*p*); *P. multiseries* (*m*); *P. fraudulenta* (*f*); and *P. seriata* (*s*). Note positive identifications for only *P. australis* and *P. pungens*.

Date	Species	Interspace	Length	Width	Striae/10microns	Rows of poroids
05/18/05	*a*	n	.	7.2	15	2
05/18/05	*a,p*	.	.	.	14	2
05/18/05	*a,p,s,m*	n	.	.	15	
05/18/05	*a*	n	116	6.7	15	2
05/18/05	*a*	n	96	7.7	15	2
05/18/05	*a,p*	.	.	.	15	2
05/18/05	*a,p*	.	.	.	14	2
05/18/05	*a,p*	n	.	.	16	2
06/03/05	*a,f*	.	.	6.6	.	2
06/03/05	*a,p*	.	.	.	14	2
06/03/05	*a,p*	.	.	.	16	2
06/03/05	*a,p*	.	.	.	14	2
06/03/05	*a,p*	.	.	.	13	2
06/07/05	*a*	n	119	6.7	14	2
06/07/05	*a,p,m*	n	.	.	12	.
06/07/05	*a,p*	n	.	.	15	2
06/07/05	*a,p*	.	.	.	15	2
06/07/05	*a,p*	.	.	.	14	2
06/10/05	*a,p*	.	.	.	14	2
06/10/05	*a*	.	.	.	17	2
06/10/05	*a,p*	.	.	.	15	2
07/04/05	*a,p*	.	.	.	16	2
07/05/05	*a*	n	.	7.1	15	2
07/05/05	*a*	.	129.2	7.2	13	2
07/06/05	*a*	n	111	7.1	.	2
07/06/05	*a,p*	.	.	.	14	2
07/08/05	*a,p*	.	.	.	15	2
07/08/05	*a*	n	.	7.4	.	2
07/08/05	*a*	n	.	.	17	2
07/08/05	*a*		.	.	17	2
08/19/05	*p*	n	.	3.7	10	2
08/19/05	*p*	n	113	4	.	2
08/22/05	*a,p*	n	.	.	14	2
08/23/05	*a*	n	91	7.2	15	2
08/23/05	*p*	n	111	4.4	13	2
08/23/05	*a*	n	.	7.5	14	2
08/23/05	*p*	n	116	4.2	13	2
08/23/05	*a*	n	98	7.4	14	2
10/17/05	*a*	n	116	6.8	13	2
06/19/06	*p*	n	118	4.2	11	2

Table A.15. Regression analysis of 2006 *Pseudo-nitzschia* spp. concentrations as a function of temperature. Note level of high explanatory power ($R^2 = 0.648$) and high significance ($p << 0.05$, SPSS 14.0).

Model Summary(b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.805(a)	0.648	0.616	0.23589

a. Predictors: (Constant), temp

b. Dependent Variable: cells

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	1.126	1	1.126	20.241	.001(a)
Residual	0.612	11	0.056		
Total	1.738	12			

a. Predictors: (Constant), temp

b. Dependent Variable: cells

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	5.991	0.515		11.628	0.000
temp	-0.161	0.036	-0.805	-4.499	0.001

a. Dependent Variable: cells

Table A.16. Regression analysis of 2006 *Pseudo-nitzschia* spp. concentrations as a function salinity (SPSS 14.0). Note level of moderate explanatory power ($R^2 = 0.446$) and high significance ($p << 0.05$, SPSS 14.0).

Model Summary(b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.668(a)	0.446	0.396	0.29576

a. Predictors: (Constant), sal

b. Dependent Variable: cells

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	0.776	1	0.776	8.873	.013(a)
Residual	0.962	11	0.087		
Total	1.738	12			

a. Predictors: (Constant), sal

b. Dependent Variable: cells

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	-4.746	2.834		-1.675	0.122
sal	0.266	0.089	0.668	2.979	0.013

a. Dependent Variable: cells

Table A.17. Multiple regression analysis of 2006 log transformed *Pseudo-nitzschia* cell concentrations as explained by both temperature (°C) and salinity (psu) without regard to site or date collected. Note level of predictive capabilities ($R^2 = 0.592$) and high significance ($p << 0.05$, SPSS 14.0).

Model Summary(b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.812(a)	0.660	0.592	0.24317

a. Predictors: (Constant), VAR00003, VAR00002

b. Dependent Variable: VAR00001

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Regression	1.147	2	0.573	9.699	.005(a)
Residual	0.591	10	0.059		
Total	1.738	12			

a. Predictors: (Constant), VAR00003, VAR00002

b. Dependent Variable: VAR00001

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	3.603	4.067		0.886	0.396
VAR00002	-0.137	0.055	-0.685	-2.504	0.031
VAR00003	0.064	0.109	0.162	0.592	0.567

a. Dependent Variable: VAR00001

Table A.18. Regression analysis of 2005 fixed station site temperature model predicted *Pseudo-nitzschia* spp. concentrations as explained by observed concentrations (SPSS 14.0). Note level of moderate predictive capabilities ($R^2 = 0.424$) and high significance ($p << 0.05$, SPSS 14.0).

Model Summary(b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.651(a)	0.424	0.412	0.42243

a. Predictors: (Constant), obs

b. Dependent Variable: pred

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	6.439	1	6.439	36.087	.000(a)
Residual	8.744	49	0.178		
Total	15.183	50			

a. Predictors: (Constant), obs

b. Dependent Variable: pred

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	1.998	0.303		6.586	0.000
obs	0.579	0.096	0.651	6.007	0.000

a. Dependent Variable: pred

Table A.19. Regression analysis of 2005 fixed station site salinity model predicted *Pseudo-nitzschia* spp. concentrations as explained by observed concentrations (SPSS 14.0). Note level of moderate predictive capabilities ($R^2 = 0.465$) and high significance ($p << 0.05$, SPSS 14.0).

Model Summary(b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.682(a)	0.465	0.453	0.46587

a. Predictors: (Constant), obs

b. Dependent Variable: pred

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	8.495	1	8.495	39.139	.000(a)
Residual	9.767	45	0.217		
Total	18.261	46			

a. Predictors: (Constant), obs

b. Dependent Variable: pred

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	1.764	0.357		4.941	0.000
obs	0.698	0.112	0.682	6.256	0.000

a. Dependent Variable: pred

Table A.20. Regression analysis of 2005 channel site temperature model predicted *Pseudo-nitzschia* spp. concentrations as explained by observed concentrations (SPSS 14.0). Note level of high predictive capabilities ($R^2 = 0.819$) and high significance ($p << 0.05$, SPSS 14.0).

Model Summary(b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.905(a)	0.819	0.800	0.06757

a. Predictors: (Constant), obs

b. Dependent Variable: pred

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	0.206	1	0.206	45.117	.000(a)
Residual	0.046	10	0.005		
Total	0.252	11			

a. Predictors: (Constant), obs

b. Dependent Variable: pred

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	3.200	0.120		26.561	0.000
obs	0.180	0.027	0.905	6.717	0.000

a. Dependent Variable: pred

Table A.21. Regression analysis of 2005 channel site salinity model predicted *Pseudo-nitzschia* spp. concentrations as explained by observed concentrations (SPSS 14.0). Note level of predictive capabilities ($R^2 = 0.296$) and non-significance ($p > 0.05$, SPSS 14.0).

Model Summary(b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.544(a)	0.296	0.218	0.07587

a. Predictors: (Constant), obs

b. Dependent Variable: pred

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	0.022	1	0.022	3.781	.084(a)
Residual	0.052	9	0.006		
Total	0.074	10			

a. Predictors: (Constant), obs

b. Dependent Variable: pred

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	4.166	0.150		27.778	0.000
obs	0.064	0.033	0.544	1.945	0.084

a. Dependent Variable: pred

Table A.22. Regression analysis of 2005 fixed station site multiple regression model predicted *Pseudo-nitzschia* spp. concentrations as explained by observed concentrations (SPSS 14.0). Note level of predictive capabilities ($R^2 = 0.426$) and high significance ($p << 0.05$, SPSS 14.0).

Model Summary

R	R Square	Adjusted R Square	Std. Error of the Estimate
.653(a)	0.426	0.414	0.57464

a. Predictors: (Constant), VAR00003

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	12.018	1	12.018	36.394	.000(a)
Residual	16.180	49	0.330		
Total	28.198	50			

a. Predictors: (Constant), VAR00003

b. Dependent Variable: VAR00002

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	1.076	0.413		2.608	0.012
VAR00003	0.791	0.131	0.653	6.033	0.000

a. Dependent Variable: VAR00002

Table A.23. Regression analysis of 2005 channel site multiple regression model predicted *Pseudo-nitzschia* spp. concentrations as explained by observed concentrations (SPSS 14.0). Note level of predictive capabilities ($R^2 = 0.757$) and high significance ($p << 0.05$, SPSS 14.0).

Model Summary

R	R Square	Adjusted R Square	Std. Error of the Estimate
.870(a)	0.757	0.733	0.09354

a. Predictors: (Constant), VAR00004

ANOVA(b)

	Sum of Squares	Degrees of freedom	Mean Square	F	p
Regression	0.273	1	0.273	31.222	.000(a)
Residual	0.087	10	0.009		
Total	0.361	11			

a. Predictors: (Constant), VAR00004

b. Dependent Variable: VAR00005

Coefficients(a)

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	p
(Constant)	2.962	0.167		17.762	0.000
VAR00004	0.207	0.037	0.870	5.588	0.000

a. Dependent Variable: VAR00005

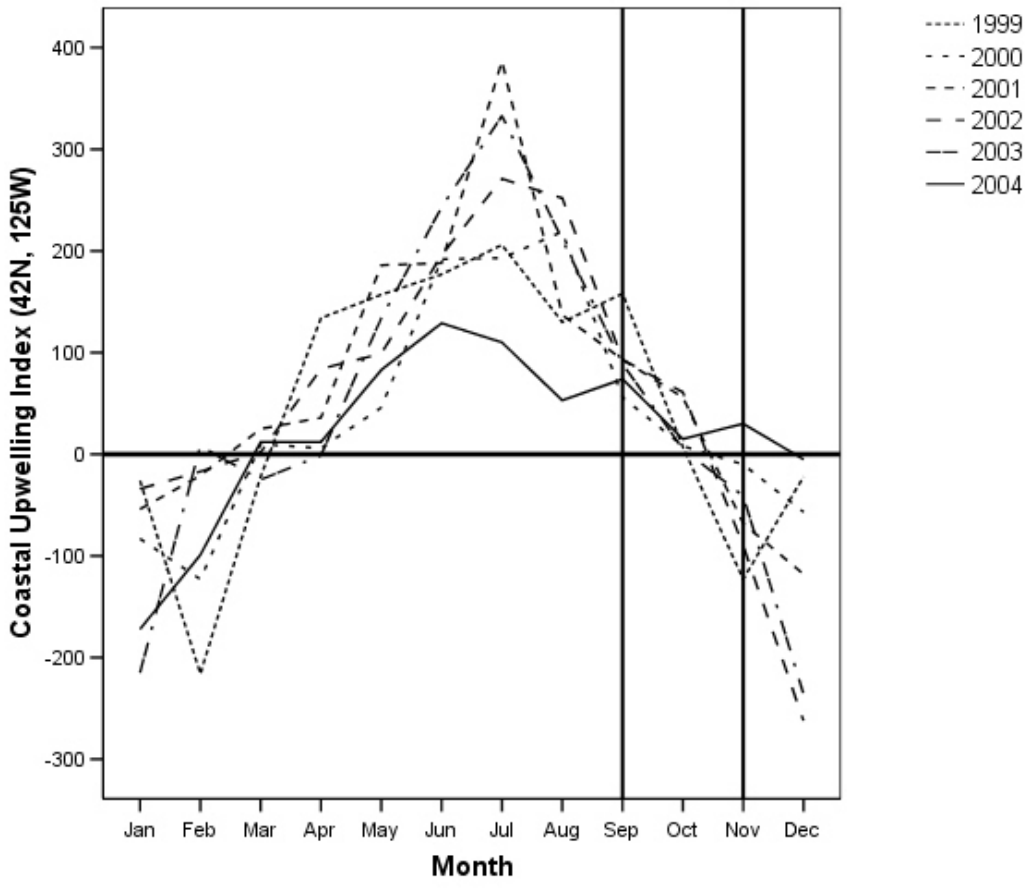

Figure A.1. Oregon Coastal Upwelling Index for years corresponding to historical South Slough temperature and salinity data (SPSS 14.0). Note the period from September through October when hydrographic conditions indicate relaxation following summer upwelling that peaks in July.

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