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Publication Date

2021

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UNIVERSITY OF CALIFORNIA

SANTA CRUZ

**THE ENVIRONMENTAL PARAMETERS AND TRENDS DRIVING
TOXIGENIC *ALEXANDRIUM CATENELLA* BLOOMS IN MONTEREY BAY,
CALIFORNIA FROM 2001-2019**

A thesis submitted in partial satisfaction
of the requirements for the degree of

MASTER OF SCIENCE

In

OCEAN SCIENCES

by

Regina Leigh Radan

March 2021

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TABLE OF CONTENTS

List of Tables and Figures.....	v
Abstract.....	xii
Acknowledgments.....	xiv
CHAPTER 1: Background and Introduction	1
References.....	5
Chapter 2: The Environmental Parameters and Trends Driving Toxigenic <i>Alexandrium catenella</i> Blooms in Monterey Bay, California from 2001-2019	9
1. Introduction.....	9
2. Materials and Methods.....	12
2.1. <i>Data Collection</i>	12
2.2. <i>Water sample collection and processing</i>	13
2.3. <i>Statistical model</i>	15
3. Results.....	18
3.1. <i>RAI (2002 -2019)</i>	18
3.2. <i>Time series (2001-2019)</i>	19
3.2.1. <i>A. catenella</i>	19
3.2.2. <i>Paralytic shellfish toxin</i>	19
3.2.3. <i>Blooms</i>	20
3.2.4. <i>Sea surface temperature</i>	21
3.2.5. <i>Nitrate [NO₃⁻] concentrations</i>	21

3.2.6. <i>Chlorophyll-a</i>	22
3.2.7. <i>San Lorenzo River Discharge</i>	22
3.3. <i>Statistical analysis</i>	23
3.4. <i>Model validation</i>	26
4. Discussion.....	31
5. Summary.....	38
Tables and Figures.....	40
References.....	62
CHAPTER 3: Conclusion and Future Direction.....	67
References.....	72

List of Tables and Figures

Chapter 2: The Environmental Parameters and Trends Driving Toxigenic

Alexandrium catenella Blooms in Monterey Bay, California from 2001-2019

Table 1. Biological and physical parameters used to determine the environmental factors affecting the presence of *Alexandrium catenella* and PSTs..40

Table 2. Generalized Linear Model results of the categorical representation of the phytoplankton found to cluster with *Alexandrium catenella* in the hierarchical cluster analysis (Figure 3). These are the most commonly found phytoplankton genera at the SCW from 2002 to 2019.....41

Table 3. The averages, and ranges of *Alexandrium catenella*), paralytic shellfish toxins, sea surface temperature, nitrate, chlorophyll-a, and the rate of San Lorenzo River discharge during the given time intervals.....42

Table 4. Generalized Linear Model results using the PC terms, calculated from the Principal Component Analysis, employed to determine the statistical correlation with the categorical representation of *Alexandrium catenella* (cells L⁻¹), and the environmental parameters for the annual, spring and fall/winter models. Numbers in parentheses are the factor estimates from the PC analysis43

Table 5. Generalized Linear Model results using the PC terms, calculated from the Principal Component Analysis, used to determine the statistical correlation with the categorical representation of *Alexandrium catenella* blooms, and the

environmental parameters for the annual, spring and fall/winter models.

Numbers in parentheses are the factor estimates from the PC analysis.44

Table 6. Generalized Linear Model results using the PC terms, calculated from the Principal Component Analysis, used to determine the statistical correlation with the categorical representation of paralytic shellfish Toxins (PSTs) ($\mu\text{g STX equiv. /100 g shellfish}$), and the environmental parameters for the annual, spring and fall/winter models. Numbers in parentheses, are the factor estimates from the PC analysis45

Table 7. Generalized Linear Model results using the PC terms, calculated from the Principal Component Analysis, used to determine the statistical correlation with the 3-month moving average of Pacific decadal oscillation (PDO), North Pacific Gyre Oscillation (NPGO), and Oceanic Niño Index (ONI), and the categorical representation of the response variables *Alexandrium catenella* (cells L^{-1}), paralytic shellfish toxins (PSTs) or blooms of *Alexandrium catenella* for the annual, spring and fall/winter models. Numbers in parentheses are the factor estimates from the PC analysis.....46

Table 8. The prediction and failure rates (%) of the categorical representation, 1-present, 0- not present, of the response variables, *Alexandrium catenella*, *Alexandrium catenella* blooms, and paralytic shellfish toxins (PSTs) compared to the annual, spring, and fall/winter principal components of the environmental data. “False negative” predicts an absence, when there is

actually a presence. “False positive” predicts a presence, when there is actually an absence.....47

Table 9. The prediction and failure rates (%) of the categorical representation, 1- present, 0- not present, of the response variables, of *Alexandrium catenella*, *Alexandrium catenella* blooms, and paralytic shellfish toxins (PSTs) compared to the principal components of the 3- month moving averages of Pacific decadal oscillation (PDO), North Pacific Gyre Oscillation (NPGO), and Oceanic Niño Index (ONI). “False negative” predicts an absence, when there is actually a presence. “False positive” predicts a presence, when there is actually an absence.....48

Figure 1. The Relative Abundance Index (RAI) data from 2002 to 2019 showing the distribution of the most common diatoms and dinoflagellates at the Santa Cruz Wharf, Santa Cruz, CA. The colors represent absent, rare, present, common and abundant in the direction of the arrow at the top, the white representing absent and the darker red representing abundant. The top row of the graph displays the changes in chlorophyll-a ($\mu\text{g L}^{-1}$) (white represents the lowest chlorophyll-a ($0.5 \mu\text{g L}^{-1}$), and the dark blue represents the highest concentration of chlorophyll-a ($289.07 \mu\text{g L}^{-1}$) from 2002 to 2019..49

Figure 2. A hierarchical cluster analysis using the Relative Abundance Index (RAI) data from 2002 to 2019 of the most commonly seen A) dinoflagellates and B) diatoms at the Santa Cruz Wharf Santa Cruz, CA. The red cluster in A and B

represents a lower number of cells whereas the green cluster represents higher numbers of phytoplankton for each group.....50

Figure 3. A hierarchical cluster analysis using the Relative Abundance Index (RAI) data provides a graphical representation of the relationship among the most common phytoplankton found at the Santa Cruz Wharf, Santa Cruz, CA from 2002 to 2019.....51

Figure 4. (A). *Alexandrium catenella*, and (B). Paralytic shellfish toxins (PSTs) weekly data from the Santa Cruz Wharf, Santa Cruz, CA from 2001 to 2019. The solid gray line in (B) represents the regulatory limit of 80 µg Saxitoxin (STX) equiv. /100 g shellfish.....52

Figure 5. (A). *Alexandrium catenella* and (B). Paralytic shellfish toxins (PSTs) annual data from the Santa Cruz Wharf, Santa Cruz, CA from 2001 to 2019. Error bars represent the standard deviation for the mean of twelve months for each year.....53

Figure 6. Constellation plot from the hierarchical cluster analysis using *Alexandrium catenella* (cells L⁻¹) for the Santa Cruz Wharf, Santa Cruz, CA from 2001 to 2019.....54

Figure 7. Annual averages of the independent and dependent variables as a function of time from 2001 to 2019, (A-F), and anomalies of these variables at the Santa Cruz Warf, (G-L). (A) *Alexandrium catenella*, (B) Paralytic shellfish toxins (PSTs), (C) Sea surface temperature, (D) Nitrate, (E) Chlorophyll-a, (F) San Lorenzo River discharge, (G) *Alexandrium catenella* anomalies, (H) PST

anomalies, (I) Sea surface temperature anomalies, (J) Nitrate anomalies, (K) Chlorophyll-a anomalies, and (L) San Lorenzo River discharge anomalies.

Error bars represent the standard deviation of twelve data points..55

Figure 8. Monthly averages of the independent and dependent variables as a function of time from 2001 to 2019, (A-F), and anomalies of these variables at the Santa Cruz Warf, (G-L). (A) *Alexandrium catenella*, (B) Paralytic shellfish toxins (PSTs), (C) Sea surface temperature, (D) Nitrate, (E) Chlorophyll-a, (F) San Lorenzo River discharge, (G) *Alexandrium catenella* anomalies, (H) PST anomalies, (I) Sea surface temperature anomalies, (J) Nitrate anomalies, (K) Chlorophyll-a anomalies), and (L) San Lorenzo River discharge anomalies.

Error bars represent the standard deviation of the monthly data points..56

Figure 9. The relationship between the predicted values of *Alexandrium catenella* from the annual model, and A) Factors 1 [(+) Sea surface temperature (°C), and (-) Nitrate (µM)], Factor 2 [(+) Chlorophyll-a (µg L⁻¹)], and Factor 3 (-) [San Lorenzo River discharge (m⁻³s⁻¹)], and B) temperature °C, log₁₀ nitrate (µM), and log₁₀ San Lorenzo River discharge (m⁻³ s⁻¹).....57

Figure 10. The relationship between the predicted values of *Alexandrium catenella* from the spring model and A) Factors 1 [(+) Sea surface temperature (°C), and (-) Nitrate (µM)], Factor 2 [(+) Chlorophyll-a (µg L⁻¹)], and Factor 3 [(-) San Lorenzo River discharge (m⁻³s⁻¹)], and B) Factor 2 [(+) Chlorophyll-a (µg L⁻¹)], and Factor 3 [(-) San Lorenzo River discharge (m⁻³s⁻¹)] for the fall model.....58

Figure 11. The relationship between the predicted values of *Alexandrium catenella* blooms, and A) Factor 2 [(+) Chlorophyll-a ($\mu\text{g L}^{-1}$)], and Factor 3 [(-) San Lorenzo River (m^3s^{-1})] for the annual model, B) Factors 1 [(+) Sea surface temperature ($^{\circ}\text{C}$), and (-) Nitrate (μM)] for the spring model, and C) Factor 2 [(+) Chlorophyll-a ($\mu\text{g L}^{-1}$)], and Factor 3 [(-) San Lorenzo River discharge (m^3s^{-1})] for the fall model.....59

Figure 12. The relationship between the predicted values of PSTs, and A) Factors 1 [Sea surface temperature ($^{\circ}\text{C}$), and (-) Nitrate (μM)], and sea surface temperature ($^{\circ}\text{C}$), \log_{10} nitrate (μM) for the annual model, B) factor 1 [(+) Sea surface temperature ($^{\circ}\text{C}$), and (-) Nitrate (μM)], and Factor 3 [(-) San Lorenzo River discharge (m^3s^{-1})] for the spring model, and C) Factors 1 [(+) Sea surface temperature ($^{\circ}\text{C}$), and (-) Nitrate (μM)], and Sea surface temperature $^{\circ}\text{C}$, \log_{10} nitrate (μM) for the fall model.....60

Figure 13. The relationship between the predicted values of the categorical representation of the response variables *Alexandrium* (cells L^{-1}) (A-C), Blooms (D-F), and Paralytic shellfish toxins (PSTs) (G-I) and Factor 2, North Pacific Gyre Oscillation (NPGO), for the annual, spring, and fall/winter model. Predicted values from the annual models for, A) *Alexandrium* (cells L^{-1}), D) Blooms, and G) Paralytic shellfish toxins (PSTs) and NPGO. Predicted values for the Spring models, B) *Alexandrium* (cells L^{-1}) E) Blooms (1,349 cells L^{-1}) and H) Paralytic shellfish toxins (PSTs), and NPGO. Fall/winter

model predicted values, C) *Alexandrium* (cells L⁻¹) F) Blooms (1,349 cells L⁻¹), and I) Paralytic shellfish toxins (PSTs), and NPGO.....61

CHAPTER 3: Conclusion and Future Direction

Figure 1: Annual averages of the independent and dependent variables as a function of time from 2001 to 2019, (A-F), and anomalies of these variables at the Santa Cruz Warf, (G-L). (A) *Alexandrium catenella*, (B) Paralytic shellfish toxins (PSTs), (C) Sea surface temperature, (D) Nitrate, (E) Chlorophyll-a, (F) San Lorenzo River discharge, (G) *Alexandrium catenella* anomalies, (H) PST anomalies, (I) Sea surface temperature anomalies, (J) Nitrate anomalies, (K) Chlorophyll-a anomalies, and (L) San Lorenzo River discharge anomalies. Error bars represent the standard deviation of twelve data points...71

Abstract

THE ENVIRONMENTAL PARAMETERS AND TRENDS DRIVING TOXIGENIC *ALEXANDRIUM CATENELLA* BLOOMS IN MONTEREY BAY, CALIFORNIA FROM 2001-2019

by
Regina L. Radan

An 18-year data set of samples collected weekly from the Santa Cruz Municipal Wharf (SCW) in Santa Cruz, California, was used to investigate the environmental parameters driving *Alexandrium catenella* abundance and toxicity in northern Monterey Bay, California. *Alexandrium catenella* is the causative organism for Paralytic Shellfish Poisoning (PSP) in California coastal waters and is routinely monitored. A semi-quantitative index of relative abundance and quantitative cell counts of *A. catenella* (cells L⁻¹) reveals an increase in relative and absolute abundance of *A. catenella* from 2003-2007, and again from 2016-2019. Using a Generalized Linear Model (GLM), both annual and seasonal models were developed using 12 biological and physical parameters to explain the dependent variables, *A. catenella* abundance, and Paralytic Shellfish Toxins (PST) concentration. Together, (-) NO₃⁻ (μM) and sea surface temperature (°C) ($p = 0.0031$), (-) San Lorenzo River discharge (m³s⁻¹) ($p = 0.0002$), and (+) chlorophyll-a (μg L⁻¹) ($p < 0.0001$), were all strongly correlated with the presence of *A. catenella* at the SCW in the annual model. Spring and fall/winter models showed similar patterns. Blooms of *A. catenella* are defined as abundances greater than one standard deviation from the climatological mean (1349 cells L⁻¹). Blooms were strongly correlated with (-) San Lorenzo River discharge ($p = 0.0192$) and (+) chlorophyll-a ($p < 0.0001$) in the annual model but

showed different patterns for the seasonal models. When lagging PSTs by three weeks, the annual model identified a strong negative correlation with NO_3^- and a positive correlation with sea surface temperature ($^{\circ}\text{C}$) ($p=0.0024$). This study demonstrates the importance of examining more than one environmental factor when determining the drivers for toxic blooms. These models could potentially be used to forecast future blooms and the presence of PSTs in Monterey Bay, California, and elsewhere where dinoflagellates are common members of the phytoplankton assemblage.

Acknowledgments

My sincere gratitude to my advisor Dr. Raphael Kudela, for his incredible patience, and encouragement while mentoring me through my thesis. I thank my committee members, Drs. Jonathan Zehr, Marilou Sison-Mangus, and William Cochlan for your edits, and suggestions for this research. I would especially like to thank Dr. Cochlan for his mentoring, and friendship over these many years. I would like to thank Kendra Negrey for imparting your knowledge, and advice to me for my thesis work, and for being a friend through it all. I would like to thank the past and present members of the Kudela Lab, especially Cori Gibble, Meridith McPherson, Niky Taylor, and Cristian Garrido for your friendship and support. I would also like to thank my parents for always believing in me. Finally, I would like to thank my loving husband, Mitchell Kimbrough, and my daughters, Liliani and Angeli, for your love, patience, and encouragement throughout this journey.

Chapter 1: Background and Introduction

Harmful algal blooms (HABs) are the accumulation of phytoplankton that may cause illness or death in humans, marine mammals, and birds, and are a global threat (Anderson et al., 2012). The occurrence of HABs appears to be increasing in both their frequency and intensity in recent years (e.g., see reviews by Hallegraeff, 1993; Anderson et al., 2002; Glibert et al., 2006, Kudela et al, 2008). Phytoplankton blooms whether harmful or not, occur when phytoplankton grow under optimal environmental conditions such as nutrients, sea surface temperature, salinity, and irradiance. Alternatively, harm can be caused by the production of toxins that accumulate in filter-feeding marine organisms such as molluscan shellfish and planktivorous nekton, ultimately causing illness and/or death of organisms in the higher trophic levels (e.g., marine mammals and humans) that in turn consume these filter-feeding animals (e.g., Anderson et al., 2002). In addition to health concerns for humans and wildlife, HABs often adversely impact local economies dependent on tourism and fisheries. In 2015, after a large bloom of the diatom *Pseudo-nitzschia australis* on the west coast, the Dungeness crab industry was shut down for five months in California, and one month in both Washington, and Oregon, leading to a \$97.5 million loss of revenue (Trainer, et al, 2020). Detection of saxitoxin and other toxins, such as domoic acid in shellfish leads to yearly closures of recreational shellfish harvesting up and down the west coast of the United States from May through October (Price et al., 1991, Lewitus et al., 2012). There are many species of HABs around the globe, but the ones found most frequently on the west coast are

dinoflagellates; *Alexandrium catenella*, and *Dinophysis*, diatoms; *Pseudo-nitzschia*, and a freshwater cyanobacteria; *Microcystis* (Lewitus, et al., 2012). These HABs, produce toxins that pose both health risks and associated economic losses due to harvesting closures and beach closures, and have led to the focus in extensive monitoring and research of HABs.

A. catenella is a dinoflagellate with a worldwide distribution (Hallegraeff, 1993), and is found along the west coast of North America, from Alaska to Mexico (Lewitus et al., 2012). *A. catenella* is 20 to 48 μm wide, and 18 to 32 μm in length. It is a chain forming dinoflagellate, with two flagella that enable it to swim through the water column and obtain nutrients at depth. Diel vertical migration (DVM) is a mode of swimming vertically through the water column adapted by some dinoflagellates, using their two flagella (Cullen, 1985). This may provide *A. catenella* with an advantage over other non-motile phytoplankton (Smayda, 2010), particularly when DVM is used to access nutrient rich deeper depths (Dortch and Maske, 1982).

A. catenella, part of the *Alexandrium tamarense* species complex, consisting of *A. mediterraneum*, *A. tamarense*, *A. pacificum*, and *A. australiense* (Wang, et al., 2014, John, et al., 2014, Litaker, et al., 2018), produces saxitoxin and related compounds (collectively referred to as paralytic shellfish toxins, or PSTs) that cause paralytic shellfish poisoning (PSP). Saxitoxin is considered a biological weapon because it is one of the most potent neurotoxins, 1,000 times more toxic than the nerve gas sarin. Saxitoxin binds and blocks the sodium channels of nerve endings, preventing the signal from the nerve impulse from going through. PSTs are

transferred to higher trophic levels by bioaccumulating in shellfish such as mussels, clams, and oysters. When ingested by humans or marine mammals, PSTs, lead to symptoms including numbness and tingling of the lips, mouth and face, nausea and vomiting, and in severe cases paralysis and death (Kao and Nishiyama, 1965). A worldwide regulatory limit of 80 µg saxitoxin (STX) equivalent /100 grams of shellfish was established in the 1930s for PSTs (Price et al. 1991). PSTs are routinely identified in weekly mussel samples from the Santa Cruz Municipal Wharf as part of the California biotoxin monitoring program (California Department of Public Health; CDPH; Price et al., 1991, Langlois, 2014).

Monterey Bay, California is a region where fisheries and tourism are important, and where *A. catenella* is prevalent (Jester et al., 2009a). Monterey Bay is located within the California Current Eastern Boundary Upwelling System, and seasonal upwelling is a strong driver and regulator of plankton biomass and diversity (Chavez and Messié, 2009, Kudela et al., 2010, Bakun et al., 2015). In the fall, when there is a period of relaxation after the upwelling period (Ryan et al., 2009), warm sea surface temperatures, low nutrient conditions, along with stratification, have been identified as important drivers for the presence of *A. catenella*, and toxicity (Nishitani and Chew, 1984, Ryan et al., 2009, Moore et al., 2009, 2011, 2015, Bill et al., 2016) on the west coast. Vertically migrating dinoflagellates, such as *A. catenella*, can access the nutrients below the surface layer; these available nutrients often facilitate a fall bloom (Langlois, 2001, Kudela et al., 2005, Figueiras et al. 2006, Ryan et al., 2009, Smayda and Trainer, 2010). Despite *A. catenella* being a small part of the

community of phytoplankton in Monterey Bay, California, there was evidence of an increase in abundance and toxicity on the west coast (Moore et al., 2008).

Increases in sea surface temperatures, due to climate change, has been hypothesized to lead to an increase in frequency and presence of *A. catenella* and associated toxins (Moore et al., 2011, Wells, et al., 2019). Studies done in Puget Sound (Moore et al., 2015), and the Gulf of Maine (Bucci et al., 2020), identified that with increasing sea surface temperatures, and other factors, one can expect blooms of *A. catenella* to begin earlier in the year, and last later in the season. Besides, sea surface temperature, other environmental and climate parameters, such as low nutrients, calm winds, water stratification, and NPGO, PDO and ONI (Moore et al., 2010, 2011, 2015, Bucci et al., 2020) are drivers of the presence of *A. catenella* and toxins. It is crucial to evaluate the combination of environmental variables that may be contributing to the rise of *A. catenella* and the level of PSTs occurring along the California coast to understand the interannual variability that leads to periods of more or less abundance and toxicity.

The main objectives of this study were to determine the relationship between environmental parameters, and the presence of *Alexandrium catenella*, blooms and toxicity over the 18 years. Once the environmental drivers were determined, a statistical model was developed to predict the probability of future *Alexandrium catenella*, blooms and toxicity. The following chapter provides a statistical analysis of the environmental, and climate variables driving the presence of *Alexandrium catenella*, blooms and toxicity in Monterey Bay, California from 2001-2019.

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Chapter 2: The environmental parameters and trends driving toxigenic

***Alexandrium catenella* blooms in Monterey Bay, California from 2001-2019**

1. Introduction

Along the west coast of California, *Alexandrium catenella*, a chain-forming, toxigenic dinoflagellate, has the potential to develop into harmful algal blooms (HABs). *Alexandrium catenella*, part of the *Alexandrium tamarense* species complex, consisting of *A. mediterraneum*, *A. tamarense*, *A. pacificum*, and *A. australiense* (Wang, et al., 2014, John, et al., 2014, Litaker, et al., 2018) produces saxitoxin and related compounds (collectively referred to as paralytic shellfish toxins, or PSTs) that cause paralytic shellfish poisoning (PSP). Paralytic shellfish toxins may cause illness and death in humans, marine mammals, fish, and seabirds from the ingestion of contaminated fish or shellfish (Anderson et al., 2012). Several incidents of PSP events occurred along the west coast prior to identification of PSTs as the causative agent, including Sonoma County in 1903 and Santa Cruz County in 1915 (Sommer and Meyer, 1937; Horner et al., 1997, Lewitus et al., 2012). Subsequently, in 1927 a monitoring program was established in California in response to these events (Price et al., 1991, Horner et al., 1997). A worldwide regulatory limit of 80 µg saxitoxin (STX) equivalent /100 grams of shellfish was established in the 1930s for PSTs (Price et al. 1991). In addition to health concerns for humans and wildlife, HABs often adversely impact local economies dependent on tourism and fisheries. Detection of saxitoxin and other toxins, such as domoic acid in shellfish, has now led to the annual banning of recreational shellfish harvesting up and down the west coast

of the United States from (typically) May through October (Price et al., 1991, Lewitus et al., 2012).

Monterey Bay, California is one region where fisheries and tourism are important, and where *A. catenella* is prevalent (Jester et al., 2009a). Monterey Bay is located within the California Current Eastern Boundary Upwelling System, and seasonal upwelling is a strong driver and regulator of plankton biomass and diversity (Chavez and Messié, 2009, Kudela et al., 2010, Bakun et al., 2015). This upwelling supplies the nutrients to support phytoplankton blooms in the spring, summer, and fall (Skogsberg, 1936, Pennington and Chavez, 2000, Kudela et al., 2005), but upwelling diminishes in the fall and is typically followed by a prolonged period of relaxation (Ryan et al., 2009). This relaxation period results in seasonally warm, and stratified waters (before the onset of winter storms). During this period vertically migrating dinoflagellates, such as *A. catenella*, can access the nutrients below the surface layer; these available nutrients often facilitate a fall bloom (Langlois, 2001, Kudela et al., 2005, Figueiras et al. 2006, Ryan et al., 2009, Smayda and Trainer, 2010).

Paralytic shellfish toxins are routinely identified in weekly mussel samples from the SCW as part of the California biotoxin monitoring program (California Department of Public Health; CDPH; Price et al., 1991, Langlois, 2014). An increase in PSTs was observed after a shift from a predominantly diatom-dominated community to a dinoflagellate-dominated community during the summer of 2004 to the winter of 2006 in Monterey Bay, CA (Jester et al., 2009b). The environmental variables driving this shift are not fully understood. This unique shift was suggested

to be related to the delayed onset and weaker upwelling in the California Current System (CCS) in the spring/summer. This delayed onset and weaker upwelling were followed by a period of stronger upwelling than is usually observed along with warmer sea surface temperatures in the CCS in the fall/winter season (Jester et al., 2009b and references therein). Increases in PSTs are also seen in other areas such as Puget Sound, WA during this time period, which is causing concern as elevated sea surface temperatures due to anthropogenic climate change has been hypothesized to lead to an increase in frequency and abundance of *A. catenella* and associated toxins (Moore et al., 2011, Wells, et al., 2019).

It is crucial to evaluate the combination of environmental variables that may be contributing to the rise of *A. catenella* and the level of PSTs occurring along the California coast to understand the interannual variability that leads to periods of more or less abundance and toxicity. The significance of multiple environmental variables were evaluated using a generalized linear model (GLM). A GLM is a linear regression-based model that allows the response variables to have other than a normal distribution (Quinn and Keough, 2002). This allows continuous environmental data to be related to categorical representations of the presence of *A. catenella* or PST concentrations using logistic regression. Similar methods have been used for other HAB organisms and events (Lane et al., 2009 and Anderson et al., 2009 for *Pseudo-nitzschia* blooms).

The main objectives of this study were twofold. First, to determine the relationship between environmental parameters and the presence of *A. catenella* and

PST concentration over these 18-years, encompassing the previously described an increase in cell/toxin concentrations (Jester et al., 2009b). Second, after identifying environmental drivers, to develop a statistical model predicting the probability of future *A. catenella* abundance, toxicity, and blooms in the Monterey Bay area. This is the first-time the presence of *A. catenella*, blooms, and toxicity have been explicitly modeled for Monterey Bay, California, allowing us to identify the causative mechanisms for the periodic resurgence of *A. catenella* and PSTs in the California Current System.

2. Materials and Methods

2.1. Data collection

The 18- year (2001-2019), monitoring data set used for this study, was compiled from previously published observations (Jester et al., 2008, 2009), and weekly sampling as part of the California Department of Public Health (CDPH) time series; these latter observations were initially collected as part of the NOAA-funded California Program for Regional Enhanced Monitoring for PhycoToxins (Cal-PreEMPT), and later with funding from the Central and Northern California Ocean Observing System (CeNCOOS). To evaluate the potential relationships between the presence of *A. catenella*, PSTs, and environmental parameters, seven physical and biological variables were collected weekly (Table 1) from the Santa Cruz Municipal Wharf (SCW), in Santa Cruz, California (36° 95' N, 122° 02' W) from July 2001 to October 2019.

In addition to the above environmental variables, river discharge rates for the San Lorenzo River were obtained from the United States Geological Survey National Water Information System (<http://waterdata.usgs.gov/nwis/>). Bakun upwelling indices for the Monterey Bay area (36°N 122°W) were obtained from the National Oceanographic and Atmospheric Administration Pacific Environmental Research Division (<https://www.pfeg.noaa.gov/products/PFELData/upwell/daily/>). Basin-scale indices for the Pacific decadal oscillation (PDO) (<http://jisao.washington.edu/pdo>), North Pacific Gyre Oscillation (NPGO) (www.03d.org/npgo) and Oceanic Niño Index (ONI) (http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml) were also used for this analysis from July 2001 to October 2019 (Table 1).

2.2. Water sample collection and processing

Whole water samples were collected weekly from 2001 to 2006, samples were only collected from the surface (< 5m) using a PVC bucket but starting in mid- 2006, water samples were collected from three depths (0, 5, and 10 m) then mixed to create an “integrated” water sample. A concurrent net tow was taken by dropping a net (mesh size – 20µm) 5 times down to 10 m (=50 m vertical effort). Whole water was filtered through a Whatman GF/F filter (nominal pore size 0.7 µm) and analyzed for inorganic nutrients using a Lachat Instruments QuickChem 8000 Series Flow Injection Automated Ion Analyzer using standard colorimetric techniques for the analysis of nitrate + nitrite (hereafter referred to as nitrate (NO₃⁻); Smith and Bogren 2001), phosphate (PO₄⁻) (Knepel and Bogren 2002), and silicic acid (Si) (Wolters,

2002). Samples for ammonium (NH_4^+) were analyzed using the fluorometric method of Holmes *et al.* (1999); whereas urea samples were analyzed with the spectrophotometric method (Price and Harrison, 1987) as modified by Cochlan *et al.*, 2008. Chlorophyll-a (chl-*a*) samples were collected and processed using the non-acidified fluorometric method (Welschmeyer 1994) using a Turner Designs 10AU fluorometer.

A live net tow sample was viewed under a dissecting microscope, and the relative abundance index (RAI) of the most commonly observed genera of dinoflagellates and diatoms were recorded. The RAI data was categorized as: 0 – absent (0%), 1- rare (< 1%), 2- present (1 to 10%), 3- common (11 to 50%), or 4- abundant (>50%). RAI data collected by CDPH from January 31, 2002, to August 29, 2007, for *Akashiwo*, were lumped together under *Gymnodinium* spp. Therefore, RAI data collected after August 29, 2007, for *Akashiwo* and *Gymnodinium* were combined using the average of the minimum and maximum to keep the data consistent. RAI data were not collected until January 2002; therefore, RAI data were available and analyzed from January 2002 until October 2019.

A. catenella cells were identified and counted using a Zeiss Standard 18 compound microscope equipped with a fluorescence Illuminator 100 (Zeiss) using species-specific large subunit ribosomal RNA-targeted fluorescent DNA probes following protocols described in Miller and Scholin (1998). To evaluate “blooms” for this study, a “bloom” was identified as cell counts of *A. catenella* greater than one standard deviation above the climatological mean density of, 1,349 cells L^{-1} .

Mussels used for monitoring toxins at the SCW were collected from Davenport State Beach, Davenport, CA, and transported and bagged at the University of California Santa Cruz Long Marine Lab (LML), Santa Cruz, CA. Bags of approximately 30 mussels were stored in seawater tables at LML until they were taken to the SCW and hung in the water to be used to monitor for harmful algal toxins. Mussels remained at the SCW for at least one week before harvesting. The mussels were then processed in the lab and sent to CDPH to be analyzed for PSTs using the mouse bioassay (MBA,) as part of the Biotxin Monitoring Program.

2.3. Statistical Model

Statistical analyses were conducted using the JMP ® Pro Version 14.2.0 software program. To assess the presence/absence of *Alexandrium* cells, PSTs, or blooms of *A. catenella* ($1349 \text{ cells L}^{-1}$), a categorical representation of the response variables was set up as 0/1 where 0 = not present, 1 = present. This categorical representation was the best way to depict the data where *A. catenella* cells, PSTs, and blooms were not always present. For model development, the nutrient parameters along with chl-*a* and San Lorenzo river discharge rate ($\text{m}^{-3}\text{s}^{-1}$) were log-transformed to account for non-linear distribution. The data were analyzed to determine the lead-lag relationships between all independent variables for one to four weeks in response to the dependent variables.

A Generalized Linear Model (GLM) was used to further explore the relationship between the continuous predictor variables, \log_{10} of nitrate [NO_3^-], \log_{10} of ammonium [NH_4^+], \log_{10} of urea, \log_{10} of phosphate, \log_{10} of chlorophyll-*a*, sea

surface temperature (°C), and \log_{10} of San Lorenzo river discharge along with the Pacific decadal oscillation (PDO), North Pacific Gyre Oscillation (NPGO) and Oceanic Niño Index (ONI), and the categorical representations of the presence of *A. catenella* and PST concentrations. Initial statistical analyses were completed using NH_4^+ , urea, silicic acid, phosphate, and upwelling data (data not shown). In both a forward and backward stepwise regression, approach; these terms were subsequently eliminated from the model because they were not significantly correlated with the response variables, and nitrate was the only macronutrient retained in further analysis. Results were considered significantly positive or negative at the $p \leq 0.05$ level for all statistical analyses.

Before performing a GLM, a Principal Component Analysis (PCA) (Quinn and Keough, 2002) was completed to deal with the multicollinearity of the data. A varimax rotation was utilized to clarify the relationship between the different factors. Ultimately, the Principal Component (PC) factors were used in the GLM to study the response of *A. catenella* abundance, toxicity, and bloom development to these factors. Annual and seasonal models, spring (March –June) and fall/winter (July- February), were developed using a GLM. The seasonal models were set up based on the seasonal periods described by Pennington and Chavez (2000), and Lane et al. (2009) in Monterey Bay, CA.

In a GLM, the relationship of the independent variables to the response variable is fitted by using maximum likelihood estimation. We can determine the

probability that *A. catenella* is “blooming” or not or the probability that PSTs are present or not. The data are fitted into the following GLM formula:

$$\ln [p/(1-p)] = \beta_0 + \beta_1 x_{i1} + \dots + \beta_k x_{ik} \quad (1.1)$$

Where: term $[p/ (1-p)]$ is the probability of *A. catenella* being present or “bloom” events, β_0 is the intercept, and β_1 is the regression coefficient of the k variables x (Quinn and Keough, 2002). This represents the rate of change of one variable as the function of the change in the other.

A cross-validation technique was used to determine the effectiveness of the predictability of the models. Using the validation function in JMP ® Pro Version 14.2.0 software, the columns of data applied to the models were divided into three sets, the training set (60% of the data, N=577), the validation set (20% of the data, N=192), and the test set (20% of the data, N=193). The training set estimates the parameters of the model; the validation set evaluates the predictability of the model, and finally, the test set assesses the final model fit. A nominal logistic regression model was run with the validation column, and the previously described PC terms, to make predictions of the presence of *Alexandrium*, PSTs, and blooms, for the annual and seasonal models. A confusion matrix was analyzed to determine the accuracy of the models.

The biological and physical parameters used for the statistical analysis of this study are listed in Table 1.

3. Results

3.1. RAI (2002- 2019)

A heat map (Figure 1), and a hierarchical cluster analysis (Figure 2A), generated from the RAI data for the distribution of the 13 most common phytoplankton genera at the SCW from 2002 – 2019 (Figure 1, 2A), illustrates an increase in the abundance of dinoflagellates from 2003 to 2007 ($p= 0.0068$, Fisher's Exact Test), a decrease in the relative abundance of dinoflagellates was seen from 2008 to 2015 ($p = 0.0131$) (Figure 1, 2A), and a subsequent increase from 2016 to 2019 ($p <.0001$).

From 2005 to 2006, during the relative increase in the dinoflagellate abundance, diatoms decreased in abundance ($p= 0.0126$) but increased from 2008 to 2019 ($p = 0.0237$, Fisher's Exact Test) (Figure 1, 2B). A hierarchical cluster analysis was set up using the RAI data for a graphical representation of the relationship among the most common phytoplankton found at the SCW from 2002 to 2019. *A. catenella* was found to cluster with *Margalefidinium* (previously *Cochlodinium*), *Rhizosolenia*, *Gymnodinium*, *Coscinodiscus*, and *Eucampia* (Figure 3). Using a GLM, the annual model, *Margalefidinium*, (<0.0001), *Gymnodinium* ($p= 0.0013$), and *Eucampia* ($p= 0.0070$) had significant positive correlations with the presence of *A. catenella* (Table 2). For the spring model, *Margalefidinium*, ($p= 0.0114$) and *Gymnodinium* ($p= 0.0016$) had positive correlations with the presence of *A. catenella* (Table 2). The fall/winter model exhibited positive correlations with *A. catenella*, and the presence of *Margalefidinium*, (<0.0001), and *Eucampia* ($p= 0.0193$) (Table 2).

3.2. Time series (2001-2019)

Observations

3.2.1. *A. catenella*

The average *A. catenella* over this 18-year data set, was 301 (cells L⁻¹) with a range of 0 to 21,950 (cells L⁻¹) (Figure 4A, 5A, Table 3). A constellation plot based on the hierarchical cluster analysis, (Figure 6) shows clusters of the most coupled years of *A. catenella* data. From 2003 to 2007, during the increase in *A. catenella* ($p = 0.0041$), the average *A. catenella* cell counts were 461 cells L⁻¹, with a range of 0 to 17,387 cells L⁻¹ (Table 3), 160 more cells than the average number of cells of this 18-year study. When *A. catenella* cell counts were the highest, in 2005, the average *A. catenella* cell counts were 1051 (cells L⁻¹), with a range of 0 to 17,387 cells L⁻¹, 3.5-fold greater than the average number of cells of this 18-year study. After 2007, *A. catenella* activity was diminished until fall 2011 (Figure 4A, 5A, 7A). Increases in *A. catenella* were seen during the summer and fall of 2013, and 2014 (Figure 5A, 7A, B). From 2016 to 2019, the average cell counts (475 cells L⁻¹, range 0 – 21,950 cells L⁻¹) (Table 3) significantly increased since this earlier period 2008-2015 (139 cells L⁻¹, range 0 – 7067 cells L⁻¹) ($p = 0.0022$) and is shown graphically. *A. catenella* were most present from May through November, particularly in May-June and again in October-November (Figure 8A, G).

3.2.2. Paralytic Shellfish Toxin

The average PST concentration over this 18-year data set was, 24.09 µg STX equiv. /100 g shellfish with a range of 0 to 770 µg STX equiv. /100 g shellfish

(Figure 4B, Table 3). From 2003 to 2007, the average PSTs were 38 μg STX equiv. /100 g shellfish (0 to 770 μg STX equiv. /100 g shellfish) and during 2005, 33 μg STX equiv. /100 g shellfish (0 to 232 μg STX equiv. /100 g shellfish) (Figure 5B, 7B, Table 3). PSTs, as expected, follow similar trends as the presence of *A. catenella* with lower cell counts in 2008-2015 (17 μg STX equiv. /100 g shellfish (0 to 656 μg STX equiv. /100 g shellfish)) compared to 2003-2007 ($p = 0.0062$) and again in 2016-2019 ($p = 0.0001$) (Figure 4B, 5B, 7B, H). Though PSTs remained below the regulatory limits after 2007, except in November 2010 (147 μg STX equiv. /100 g shellfish), during the winter of 2013 (544, 585, 685, and 111 μg STX equiv. /100 g shellfish), fall of 2016 (127 μg STX equiv. /100 g shellfish), and the spring of 2019 (235 and 231 μg STX equiv. /100 g shellfish), the toxins are usually present from 2008 to 2019 (Figure 4B, 5B, 7B, H). When observing average monthly trends, the highest concentrations of PSTs were during July, August, November, and December (Figure 8B, H).

3.2.3. Blooms

Blooms occurred 11.5 % of the time that *A. catenella* cell were present over this 18-year study using a threshold of 1349 cells L^{-1} . During the 18-year study, *A. catenella* was present 43.4 % of the time, and PSTs were present 61.7 % of the time. From 2003 to 2007, when the presence of *A. catenella* increased, *A. catenella* was present 60 % of the time, blooms occurred 13.5% of the time, and PSTs were present 87.1 % of the time. In 2005, *A. catenella* was present 66% of the time, blooms occurred 15.1% of the time, and PSTs were present 80.5 % of the time. From 2008-

2015, *A. catenella* was present 29.5 % of the time, blooms occurred 3% of the time, and PSTs were present 40.2 % of the time. From 2016 to 2019, *A. catenella* was present 68.4 % of the time, and PSTs were present 62 % of the time, but blooms of *A. catenella* only occurred 6 % of the time.

3.2.4. Sea surface temperature

The average sea surface temperature over these 18-years, was 13.7 °C, with a range of 8.8 to 19.1 °C (Figure 7C, Table 3). From 2003 to 2007 and 2016 to 2019, when there was an increase seen in *A. catenella* cell counts, and PSTs, the average sea surface temperature was 13.6 °C (9.8 to 19.1 °C) and 14.5 °C (10.3 to 18.8 °C), respectively (Table 3). During 2005, the average sea surface temperature was 13.8 °C (10 to 19.1°C), and from 2008 to 2015, the average sea surface temperatures were 13.4 °C (8.8 to 18.6 °C) (Figure 7C, I, Table 3). Sea surface temperature monthly anomalies based on the 13.6 °C average from the monthly data show the highest temperatures in Monterey Bay, CA, were seen from July through October (Figure 8C, I).

3.2.5. Nitrate [NO_3^-] concentrations

The average nitrate [NO_3^-] (μM) concentration over these 18-years, was 4.3 μM , with a range of 0 to 47.2 μM (Figure 7D, Table 3). During 2003 to 2007, the average NO_3^- was 5.2 μM (0 to 47.2 μM) and 2016 to 2019, 3.3 μM (0 to 14.5 μM). The average NO_3^- in 2005 was 5.1 μM (0 to 24.1 μM) (Figure 7D, J, Table 3). When *A. catenella* had the lowest cell counts, 2008-2015, average NO_3^- was 4.3 μM (0 to 24 μM) (Table 3). The average yearly anomalies of NO_3^- (Figure 7J) display above-

average NO_3^- concentrations from 2004 to 2009. The monthly averages of NO_3^- (Figure 8D) especially, the average monthly anomalies of NO_3^- (Figure 8J), display above-average NO_3^- concentrations during December through April.

3.2.6. Chlorophyll-a

The average chlorophyll-a concentration from 2001 to 2019, was $8.2 \mu\text{g L}^{-1}$, the range was 0.3 to $289.1 \mu\text{g L}^{-1}$ (Figure 7E, Table 3). During the years when *A. catenella* cell counts were the highest, the yearly averages of chlorophyll-a were, $10.4 \mu\text{g L}^{-1}$ (0.5 to $289.1 \mu\text{g L}^{-1}$) (2003-2007), and $8.57 \mu\text{g L}^{-1}$ (0.6 to $137.6 \mu\text{g L}^{-1}$) (2016-2019) (Table 3). From 2008 to 2015, yearly averages of chlorophyll-a were $6.9 \mu\text{g L}^{-1}$ (0.9 to $53.3 \mu\text{g L}^{-1}$) (Figure 7I, J, Table 3). The yearly averages and average yearly anomalies of chlorophyll-a (Figure 7E, K) show an increase and above-average chlorophyll-a during 2004, 2006, 2007, 2011, 2016, and 2017. The average monthly (Figure 8I) and average monthly anomalies (Figure 8E, K) of chlorophyll-a concentrations were positive during May, June, July, October, and November.

3.2.7. San Lorenzo River Discharge

The average San Lorenzo River Discharge rates from 2001 through 2019, was $3.6 \text{ m}^3\text{s}^{-1}$, the lowest discharge rate was $0.01 \text{ m}^3\text{s}^{-1}$, and the highest discharge rate was $123.46 \text{ m}^3\text{s}^{-1}$ (Figure 7F, Table 3). During the years 2003 to 2007 and 2016 to 2019, the average discharge rates were, $3.85 \text{ m}^3\text{s}^{-1}$ (0.02 to $91.46 \text{ m}^3\text{s}^{-1}$) and $6.16 \text{ m}^3\text{s}^{-1}$ (0.17 to $120.91 \text{ m}^3\text{s}^{-1}$) (Figure 7F, Table 3), respectively. The average discharge rates from 2008 to 2015 were, $2.24 \text{ m}^3\text{s}^{-1}$ (0.01 to $123.46 \text{ m}^3\text{s}^{-1}$) (Figure 7F, Table 3). The average yearly anomalies of San Lorenzo River Discharge rates display the

highest discharge rates were seen in 2005, 2006, 2010, 2017, and 2019 (Figure 7L). The highest San Lorenzo River Discharge rates were seen during December through April (Figure 8F, L).

3.3. Statistical analysis

For the annual model, PC1 produced from the PCA consisted of NO_3^- and sea surface temperature (37.82 variance explained), PC2 was comprised of chlorophyll-a (26.22 % variance explained) and PC3 was San Lorenzo River Discharge rates (25.39% variance explained). These PC factors were first used to generate a model for the categorical representation of the presence of *A. catenella*. The GLM results showed a strong correlation with all variables in the annual model (Table 4). The presence of *A. catenella* had a strong negative correlation with NO_3^- , and a positive correlation with sea surface temperature ($p < 0.0001$), a strong positive correlation with chlorophyll-a ($p < 0.0001$), and a negative correlation with San Lorenzo River Discharge rates ($p = 0.0497$).

For the spring model, similar factors were generated from the PCA analysis. PC1 consisted of NO_3^- (μM) and sea surface temperature (36.01 % variance explained), PC2 was comprised of chlorophyll-a (26.62 % variance explained), and PC3 was San Lorenzo River discharge rates (25.63 % variance explained). The presence of *A. catenella* was significant with all factors in the spring model (Table 4). There was a strong negative correlation with NO_3^- , and a positive correlation with sea surface temperature ($p = 0.0003$), a positive correlation with chlorophyll-a ($\mu\text{g L}^{-1}$) ($p =$

0.0147), and a strong negative correlation with San Lorenzo River discharge rates ($p=0.0142$).

For the fall/winter model, similar to the spring and annual model, PC1 consisted of NO_3^- and sea surface temperature (38.02 % variance explained), PC2 was comprised of chlorophyll-a (26.45 % variance explained) and PC3 was San Lorenzo River discharge rates (25.31% variance explained). *A. catenella* had a negative correlation with NO_3^- and a positive correlation with sea surface temperature ($p=0.0031$). A positive correlation was seen with chlorophyll-a ($p= <0.0001$), and there was no correlation with San Lorenzo River Discharge rates in the fall/winter model (Table 4).

The presence of *A. catenella* blooms (1349 cells L^{-1}) in the annual model (Table 5), did not show a correlation with sea surface temperature, and NO_3^- . However, a positive correlation was seen with chlorophyll-a ($p= < 0.0001$), and a negative correlation was seen in San Lorenzo River discharge rates ($p=0.0263$) with *A. catenella* blooms.

In the spring model, *A. catenella* blooms demonstrated a strong negative correlation with NO_3^- , and a positive correlation with sea surface temperature ($p=0.0286$). No significant relationship was seen between *A. catenella* blooms and chlorophyll-a or San Lorenzo river discharge (Table 5). *A. catenella* blooms had a positive response for the presence of chlorophyll-a in the fall/winter model ($p <.0001$) along with a negative response to the San Lorenzo River Discharge rates ($p=0.0193$) (Table 5).

When PSTs lagged by 3 weeks, the annual model showed a strong negative correlation with NO_3^- and a positive correlation with sea surface temperature ($p=0.0024$) (Table 6). With a 2-week lag, PSTs had a strong negative correlation with NO_3^- (μM) and a positive correlation with sea surface temperature ($p=0.0451$), and a negative correlation with San Lorenzo River Discharge rates ($p=0.0061$) in the spring model (Table 6). In the fall/winter model, PSTs showed a strong negative correlation with NO_3^- and a positive correlation with sea surface temperature ($p=0.0009$) (Table 6). PSTs were also strongly correlated with the presence of *A. catenella* (<0.0001).

Using the provided 3-month moving average of NPGO, PDO, and ONI, a PCA was performed, and revealed results of PC1 for the annual model, PDO and ONI (51.282 % variance explained) and PC2, NPGO (37.229 % variance explained). A GLM revealed the presence of *A. catenella*, *A. catenella* blooms (1349 cells L^{-1}), and PSTs were strongly correlated with the presence of NPGO ($p= <.0001$, 0.0180 and 0.0031, respectively) during the annual model (Table 7). The PCA for the spring model, PC1 PDO and ONI (48.475 % variance explained) and PC2, NPGO (43.115 % variance explained). The GLM showed the presence of *A. catenella*, *A. catenella* blooms (1349 cells L^{-1}), and PSTs had a strong correlation with NPGO ($p <.0001$, 0.0311, and 0.0011, respectively) (Table 7). The fall/winter model had a PC1 of PDO and ONI (52.230 % variance explained), and PC2, NPGO (35.190 % variance explained). The presence of *A. catenella* had a positive correlation with PDO and ONI ($p = 0.0256$) and with NPGO ($p = 0.0001$) (Table 7). When lagging *A. catenella* blooms (1349 cells L^{-1}) by 4 weeks, there was a positive correlation with NPGO ($p =$

0.0163). PSTs had a positive correlation with NPGO in the fall/winter model when lagged by 1 week ($p = 0.0033$) (Table 7).

3.4. Model validation

The summary of the results from the prediction models is seen in Table 8. Using the PC results from the annual and seasonal models along with the validation column, a logistic regression model was run to predict the effectiveness of the model.

Using the confusion matrix produced by the nominal logistic regression, for the annual model for the presence, or absence of *A.catenella*, the accuracy of our model, or how often the classify is correct, was 63%. The model misclassified the presence or absence of *A.catenella*, 37% of the time. The true positive rate, which tells us when *A.catenella* was actually present was 58%, and the false -positive rate was 33%. The precision of the annual model, or how often the model is correct when it predicts *A.catenella* is present, was 62%. For the spring model, the accuracy of the model was 59%. The presence of *A.catenella* was successfully predicted 32% of the time, the model predicted incorrectly 28% of the time. The precision for the spring model was only 35%. For the fall/winter model, the true positive rate was 33%, and the false-positive rate was 12%. The accuracy for the fall/winter model was 59% and the precision of the model 75%. The true negative rate was 88%.

The presence of *A. catenella* blooms ($1349 \text{ cells L}^{-1}$) in the annual, spring, and fall models, were not successfully predicted. The models had a true positive rate of 0, and a false positive rate of 0. Though the model predicted the absence of *A. catenella* blooms 100% of the time.

The annual model for the presence of PSTs, had an accuracy of 56%. The model misclassified the presence or absence of PSTs, 44% of the time. The true positive rate was 99% and the false-positive rate was 1%. The precision of the annual model was 56%. For the spring model, the true positive rate, which tells us when PSTs were present was 71% and the false-positive rate was 75%. The model had a false negative rate of 1% and 5% for the annual and spring model, respectively. The precision of the spring model was 60%. For the fall/winter model, the presence or absence of PSTs was successfully identified 67% of the time, the model predicted incorrectly, or misclassified, 33% of the time. The true positive rate was 95% and the false-positive rate was 83%. The precision of the fall model was 67%.

The summary of the results from the prediction models for the presence of *A. catenella*, blooms, and toxicity in the presence of NPGO, and PDO and ONI, are in Table 9. For the annual model, for the presence of *A. catenella* when NPGO is occurring, the accuracy of the model was 76%. The true positive rate, which tells us when *A. catenella* was present was 85%, and the false-positive rate was 44%. The precision of the model was 80%. For the spring model, the accuracy of the model was 79%, whereas the misclassification was 21%. The true positive rate, and the false positive rate, were 80% and 20%, respectively. The presence of *A. catenella* in the fall model, when NPGO and PDO and ONI were occurring, had a true positive rate of 95%, and a false positive rate of 57%. The accuracy of the model was 81%, and a precision of 82%.

Similarly, to the previous models, the presence of *A. catenella* blooms in the annual model, spring, and fall models, were not successfully predicted in the presence of NPGO. The models had a true positive rate of 0, and a false positive rate of 0. The predictability of PSTs in the presence of NPGO, was also unsuccessful with true positive rates of 100%, and false positive rates of 100%.

Using the residuals of the predicted values of *A. catenella*, blooms, and toxicity, from the annual and seasonal GLM models, graphs were produced to validate the relationship between the environmental variables, and the response variables. A graphical representation of *A. catenella* (cells L⁻¹), and factor 1 ((-) NO₃⁻ (μM) and (+) sea surface temperature (°C)) showed an R² value of 0.182, factor 2 ((+) chlorophyll-a (μg L⁻¹)) had an R² value of 0.511 and factor 3 ((-) San Lorenzo River Discharge rates (m³s⁻¹)) produced an R² value of 0.271 (Figure 9A). The environmental variables were also evaluated against the predicted values, and produced R² values of 0.244, for sea surface temperature (°C), R² = 0.370 for NO₃⁻ (μM), R² = 0.630 for chlorophyll-a (μg L⁻¹), and R² = 0.472 for San Lorenzo River Discharge rates (m³s⁻¹) (Figure 9B). For the presence of *A. catenella* for the spring model, factor 1 (sea surface temperature (°C) and (-) NO₃⁻ (μM)) had the highest R² of 0.512 (Figure 10A). The environmental variables were again evaluated against the predicted values but produced low R² values (Figure 9A). The fall/winter model, factor 2 ((+) chlorophyll-a (μg L⁻¹)) had an R² of 0.196, and factor 3, ((-) San Lorenzo River Discharge rates (m³s⁻¹)), a R² of 0.782 (Figure 10B).

Overall, the predicted values for *A. catenella* blooms were not strongly depicted which may be indicative of the low bloom observations (Figure 11A, B, C). However, factor 2, ((+) chlorophyll-a ($\mu\text{g L}^{-1}$)) produced an R^2 of 0.617 and 0.546 for the annual and fall/winter models, respectively (Figure 11A, C). For the spring model, factor 1, ((+) sea surface temperature ($^{\circ}\text{C}$), and (-) NO_3^- (μM)) had an R^2 of 0.528 (Figure 11B). The predicted values for PSTs had the best overall predictions (Figure 12A, B, C). The annual, for factor 1 (sea surface temperature ($^{\circ}\text{C}$) and (-) NO_3^- (μM)) had an R^2 of 0.890 (Figure 12A). When the environmental variables for factor 1 (sea surface temperature ($^{\circ}\text{C}$) and (-) NO_3^- (μM)) were evaluated against the predicted values, sea surface temperature ($^{\circ}\text{C}$) had an R^2 value of 0.442, and (-) NO_3^- (μM) an R^2 value of 0.569 (Figure 12A). The spring model had an R^2 value of 0.662 for Factor 1 (sea surface temperature ($^{\circ}\text{C}$) and (-) NO_3^- (μM)) (Figure 12B). The fall model for the presence of PSTs and Factor 1 (sea surface temperature ($^{\circ}\text{C}$) and (-) NO_3^- (μM)) had a with an R^2 value of 0.842 (Figure 12C).

Similar analysis of using the predicted values of *A. catenella*, blooms, and toxicity to evaluate the predictability of their presence when NPGO and PDO and ONI were present were completed (Figure 13A-H). A graphical representation of *A. catenella* in the annual, spring and fall models in the presence of NPGO (PDO and ONI in the fall model), had R^2 values of 0.921, 0.886, and 0.693, respectively (Figure 13A-C). Overall, the predicted values for *A. catenella* blooms and NPGO, were not strongly depicted which may be indicative of the low bloom observations (Figure 13C- E). However, in the annual model, the R^2 value was 0.590 (Figure 13C). For

the annual, spring and fall models, the predicted values PSTs, were graphed against Factor 1 of the PCA, (NPGO) and resulted in R^2 values of 0.920, 0.903, and 0.942, respectively (Figure 13F-H).

When evaluating our models using the data from 2003 to 2007, when *Alexandrium* amplified from the 18-year time series, similar patterns were observed. The PCA terms were similar for the annual, spring, and fall/winter model with PC1 set up with sea surface temperature ($^{\circ}\text{C}$) and NO_3^- (μM), PC2 chlorophyll-a ($\mu\text{g L}^{-1}$), and PC3 San Lorenzo River Discharge rates (m^3s^{-1}). For the annual model, the presence of *A. catenella* had a strong negative correlation with NO_3^- (μM), and positive correlation with sea surface temperature ($^{\circ}\text{C}$) ($p=0.0025$), a strong negative correlation with San Lorenzo River Discharge rates (m^3s^{-1}) ($p=0.0005$), and a positive correlation with chlorophyll-a ($\mu\text{g L}^{-1}$) ($p < 0.0030$). For the spring model, *A. catenella* cells L^{-1} had a strong negative correlation with NO_3^- (μM) and a positive correlation with sea surface temperature ($^{\circ}\text{C}$) ($p=0.0029$), a strong negative correlation with San Lorenzo River Discharge rates (m^3s^{-1}) ($p=0.0013$), and a positive correlation with chlorophyll-a ($\mu\text{g L}^{-1}$) ($p=0.0024$). Finally, for the winter/fall model, the presence of *Alexandrium* correlated similarly than the full 18-year model, for this model, there was a strong negative correlation with NO_3^- (μM) and positive correlation with sea surface temperature ($^{\circ}\text{C}$) ($p=0.0407$), and positive correlation with chlorophyll-a ($\mu\text{g L}^{-1}$) ($p=0.00291$) but again did not correlate with San Lorenzo River discharge rates (m^3s^{-1}).

4. Discussion

Environmental triggers of *A. catenella* and PSTs have been examined in various studies (Jester, et al, 2009b, Moore et al., 2009, 2015, Bill et al., 2016). However, the potential combination of factors proven to be important for growth and toxicity in those studies, in both the annual and seasonal models, have been limited. The analysis done on this 18-year data set, shows the importance of not only warm sea surface temperatures (°C), but low NO₃⁻ (μM), and San Lorenzo River discharge rates, along with the presence of NPGO, and PDO and ONI in the fall/winter model.

In the present study, a semi-quantitative index of relative abundance, and quantitative cell counts of *A. catenella* (cells L⁻¹) increased from 2003 to 2007 in agreement with previous studies in Monterey Bay, CA (Jester et al., 2009b), which documented an increase in dinoflagellates, and a decrease in diatoms during the time period of 2004-2006. As a result of the extensive 18- year data set used here, the environmental factors contributing to this increase could be examined more thoroughly. This increase in dinoflagellates did not continue past 2007 but did increase in 2016-2019 (Figure 1 and 2A).

The semi-quantitative index of relative abundance data was observed for patterns in the species composition. A hierarchical cluster analysis of the data of the most common species of phytoplankton seen at the SCW (Figure 3) demonstrates similar groupings as Smayda et al.,2001, who described nine habitat-specific dinoflagellate types. Specifically of interest to this study, HAB forming dinoflagellates tend to form 3 groups, Type IV, frontal zone, Type V, upwelling

relaxation, and Type VI coastally entrained. The dinoflagellates *A. tamerense* and *fundyense* along with *Gymnodinium* tend to group into Type VI but also can mix with IV and V types. Similarly, *A. catenella* is found in a combination of these habitats, stratified, coastal, warm waters, and can adapt to high turbulence by forming chains and being strong swimmers (Smayda, T.J., 2010). Though we don't have data for all of the phytoplankton species by Smayda, and our data is only grouped into 2 clusters, we do see similar patterns of *Alexandrium* and *Gymnodinium* grouping together, while *Ceratium*, *Dinophysis*, and *Procentrum* group separately (Figure 3, Table 2). The significance of *Alexandrium* and *Gymnodinium* grouping together along with *Margalefidinium* in the annual and seasonal models (Table 2) provides us with the ability to predict the presence of *Alexandrium* when these other phytoplankton are present in our SCW weekly samples.

The reasoning behind this increase from 2003-2007, was thought to be due to an increase in upwelling events in the CCS, followed by warmer temperatures in the fall (Jester, et al., 2009b). Upwelling data were not used for this analysis, however, NO_3^- , and sea surface temperature, can be used as a proxy for upwelling, whereas upwelled waters would indicate colder sea surface temperatures, and greater nutrient concentrations. Our model correlates with differing trends which indicates a probable negative association with upwelling. As mentioned in Lane et al., 2009, and Jester, et al, 2009b the lower upwelling trends could have contributed to the increase in *A. catenella* and PTS during this time.

Similar to this study, Schullien et al. (2017) reported fall blooms, mainly consisting of dinoflagellates, occurred in 2006, 2011, and 2013. Moore et al. (2009, 2015) found the combination of warm air temperatures and sea surface temperature ($^{\circ}\text{C}$), and low winds contributed to PSTs found in mussels in Puget Sound. They also suggest that warmer temperatures may even cause two blooms of *A. catenella* a year instead of the one normally witnessed in the Pacific Northwest today. Bill et al. (2016) also found temperature to be an important environmental factor driving blooms of *Alexandrium* in the Salish Sea. They were able to successfully predict higher risk time frames of blooms in the Salish Sea when applying a polynomial fit to growth rates and comparing this to in situ temperature and salinity data. In the present study, the years of low sea surface temperatures ($^{\circ}\text{C}$) correspond to the years of low *A. catenella* densities (Figure 7G and I), another indication of the importance of elevated sea surface temperatures ($^{\circ}\text{C}$) for the presence of *A. catenella*.

As sea surface temperatures increased, resulting in increased biomass (as measured by chlorophyll-a), NO_3^- reserves were depleted; this macronutrient depletion has been demonstrated to be another important factor coincident with the increase in *A. catenella*, and number of PSTs during this study. However, during the 2003 to 2007 period, NO_3^- anomalies (Figure 7K) showed NO_3^- concentrations that were above the climatological mean but, during 2016-2019, where increased abundances were seen, NO_3^- was found to be below average (Figure 7G, K). The monthly averages of NO_3^- (Figure 8D), especially the average monthly anomalies of NO_3^- (Figure 8J), during the period of June and October, when *A. catenella* would

normally be seen, were below average. We typically see blooms of phytoplankton once the NO_3^- concentrations have been depleted at the surface. Though only NO_3^- as a nitrogen source was used in our model, NH_4^+ and urea from river runoff may also support dinoflagellate blooms and should be considered during upwelling relaxation times (Kudela and Cochlan, 2000, Kudela et al., 2010). NH_4^+ and urea may even be the preferred sources of nitrogen if available and may contribute to higher growth rates (Anderson et. al., 2012).

During the years when the presence of *A. catenella* were the highest, chlorophyll-a (Figure 7G, J), as expected, were also found to be in higher concentrations. During the months of June-October, when *A. catenella* would be present, the monthly average concentrations of chlorophyll-a were above average. This is in agreement with Schullien et al., (2017), who found increases in the monthly average chlorophyll-a concentrations during the fall/winter months.

Higher-than-normal river discharge, during 2005-2006 (Figure 7F, L) have been suggested as a contributing factor to the increase in dinoflagellates and a decrease in diatoms during this period (Lane et al., 2009). As mentioned previously, our model shows a consistent negative relationship with San Lorenzo River discharge rates, and the presence of *A. catenella* and PSTs (Table 4) in our annual, and spring models. However, the average monthly discharge rates and average monthly anomalies (Figure 8F, L) demonstrate that *A. catenella* tends to be present during the months of low discharge rates (Figure 8A, G). Though we found, San Lorenzo River

discharge rates were not significant in our fall/winter model for *A. catenella* (cells L⁻¹), it was significant for the presence of *A. catenella* blooms.

In Puget Sound, it was found that the warm phase and El Nino, during the PDO, had the potential to influence blooms and toxicity of *A. catenella* (Moore, et al., 2010). This warm phase may extend the months of the annual warm sea surface temperatures, and therefore facilitate more blooms and potentially longer blooms. Similarly, a positive correlation was seen between ONI and PDO, and the presence of *A. catenella* in the fall/winter model although warm waters generated by ONI and PDO do not usually last through the summer/fall when we normally witness *A. catenella* at the SCW. In the present study, NPGO was strongly correlated with all response variables during the annual and seasonal models (Table 7). NPGO is associated with increases in NO₃⁻, salinity, and chlorophyll-a and exhibits large-scale changes in sea surface height and temperature. Three of these variables, NO₃⁻ (μM), chlorophyll-a (μg L⁻¹), and sea surface temperature (°C) were found to be important environmental triggers for the presence of *A. catenella*, blooms, and toxicity during this study. It is important to observe climate patterns as well as environmental variables when observing correlations with the presence of *A. catenella* and toxicity.

4.1 Model Validation

The accuracy of our environmental models for the presence of *A. catenella* in Monterey Bay, were not optimal to be used for a forecasting model. Though this analysis was done on a large data set, *A. catenella* were inconsistently present throughout the 18-year study, which may account for the low true positive rates, and

accuracy. The model predicts *A. catenella* blooms will never be present, indicated by the 100% true negative rate. *A. catenella* blooms were only present 11.5% of the time during this study which would account for this error. Throughout this study, PSTs are present, even at low concentrations, especially during the years when the presence of *A. catenella* was the greatest, 2003-2007 (87%), and 2016-2019 (62%). Though the accuracy of the models for PSTs were low, the true positive rates were high, and the false negative rates were low. The model predicts PSTs will always be present, but for managers trying to determine when to expect PSTs, a low false negative rate is important in order not to underestimate the presence of PSTs. Similar statistical models were done on studies of *Pseudo-nitzschia* blooms in Monterey Bay, CA (Lane et al., 2009), and the Santa Barbara Channel, (Anderson, et al., 2009), as well as phytoplankton, and microbial abundance, and bloom dynamics in Monterey Bay, CA (Schulien, et. al, 2017). Both Lane et al. (2009), and Anderson et al. (2009), produced successful models for predicting *Pseudo-nitzschia* blooms. *Pseudo-nitzschia* (cells L⁻¹), are present every year in Monterey Bay which may contribute to the success of their models.

Using the residuals from the predicted values of the GLM models to determine the effectiveness of our models, we had R² values ranging from 0.095 to 0.888 for the environmental variables, and for the climate models, the R² values for the presence of *A. catenella* and PSTs were around 0.9. Though the R² values varied, the graphs successfully gave us insight to how the variables will work together and represent the relationships between the independent and dependent variables as our

models showed. Schullien et al. (2017), similar to our study, found significance among the variables in their partial least regression models, but also had low R-squared values. The low R^2 values indicate the interannual variability that is unaccounted for in our models. Though we did use an extensive suite of environmental variables from the SCW for our models, other biological and physical parameters must be considered when evaluating the presence of *A. catenella* and PSTs such as mixotrophy, vertical migration, and cysts. Their omission in our models, may contribute to the low R-squared values.

Monterey Bay is part of the California Current System (CCS). The CCS is seasonally driven by the wind patterns, and in the spring, and summer contribute to the nutrient, cold upwelled waters on the west coast, which ultimately supports phytoplankton growth and toxicity. The increase in warming expected from climate change, will affect the CCS by bringing reduced upwelling and stratified waters to Monterey Bay. When considering these relationships, particularly with temperature, climate change has the potential to increase the capabilities of *A. catenella* to thrive as sea surface temperatures rise and the waters become more stratified (Moore et al., 2008, 2009, 2011). This will be advantageous to dinoflagellates like *A. catenella* that have the ability to vertically migrate to obtain the nutrients below the nitricline. Diel vertical migration (DVM) is a mode of swimming vertically through the water column adapted by some dinoflagellates and used to obtain nutrients at depth or in the stratified layers of the ocean (Cullen, 1985).

Despite the role of inorganic nutrients in maintaining toxic blooms of the dinoflagellate *Alexandrium catenella*, there is strong evidence for the potential use of mixotrophy (Jeong et al., 2005a, Stoecker et al., 2006, Burkholder et al., 2008). *Alexandrium spp.* have been observed to ingest *Synechococcus* along with taking up inorganic nutrients (Jeong et al., 2005a, b). *Synechococcus* is a unicellular cyanobacterium found year-round in Monterey Bay (Paerl et al., 2012), and higher abundances are found during the late summer and fall (Paerl et al., 2012, Schullien et al., 2017) associated with warmer temperatures, low river discharge, and low nitrate in the fall as seen here with *A. catenella*. This may be another competitive advantage *A. catenella* has over other phytoplankton. Finally, *A. catenella* cysts survive for a long time in the seabed and may contribute to the sustainability of *A. catenella* and PSTs (Anderson et al., 2012), and thus should be taken into account when discussing what contributes to the presence of and future studies at the SCW.

5. Summary

In conclusion, despite *A. catenella* normally being a minor member of the phytoplankton community Monterey Bay, there has been evidence of an increase in abundance and toxicity on the west coast (Moore et al., 2008). This extremely toxic phytoplankton was a dominant part of the community during the shift from diatoms to dinoflagellates in the summer of 2003 to 2007 (Jester et al., 2009b), and were seen in higher concentrations in 2016-2019, when sea surface temperatures were the greatest in this 18-year study, and NO_3^- concentrations were the lowest. Though PSTs have remained below the regulatory limits except in November 2010 and during the winter

of 2013, the toxins are still present most of the year and need to be taken into account given the lethality of *Alexandrium spp.* on the US west coast and the possible geographic and temporal expansion of its harmful blooms (Moore et al. 2008). Studies done in Puget Sound (Moore et al., 2015), and the Gulf of Maine (Bucci et al., 2020), identified that with increasing sea surface temperatures, and other factors, one can expect blooms of *A. catenella* to begin earlier in the year, and last later in the season.

This study includes the first statistical model done to evaluate the presence of *A. catenella* and toxicity in Monterey Bay, CA. When attempting to forecast the presence of *A. catenella*, blooms, and toxicity, these models can be used to identify large scale patterns, but they are difficult to use for specific events. The interannual variability must be taken into account when developing models. Weekly samples are only a snapshot of what is happening in Monterey Bay, and other factors must be considered. The overarching results of this study, in all models, both environmental and climate, the presence of *A. catenella* and toxicity, is associated with increases in sea surface temperature, and chlorophyll a, and a decrease in nutrients, and this is a reasonable description of our future. These results have clear and broad benefits to agencies charged with monitoring coastal systems for the protection of economic and public health. In particular, organizations responsible for taking care of local fisheries, allowing delayed or earlier harvesting when the optimal environmental conditions are expected to occur.

Table 1. Biological and physical parameters used to determine the environmental factors affecting the presence of *Alexandrium catenella* and PSTs.

Parameters	Units
Environmental Variables	
Chlorophyll- a	$\mu\text{g/L}$
Sea Surface Temperature	$^{\circ}\text{C}$
Nitrate	μM
Silicic acid	μM
Phosphate	μM
Ammonium	μM
Urea	μM
San Lorenzo River discharge	$\text{m}^3 \text{s}^{-1}$
Bakun Upwelling Index	$\text{m}^3 \text{s}^{-1}$
Pacific Decadal Oscillation Index	1 st EOF of Sea Surface Temperature
North Pacific Gyre Oscillation Index	2 nd EOF of Sea Surface Height
Oceanic Niño Index	Mean SST anomalies in the Nino-3.4 region
Response Variables	
<i>A. catenella</i>	cells L^{-1}
PSTs concentration	$\mu\text{g}/100\text{g}$

Table 2. Generalized Linear Model results of the categorical representation of the phytoplankton found to cluster with *Alexandrium catenella* in the hierarchical cluster analysis (Figure 3). These are the most commonly found phytoplankton genera at the SCW from 2002 to 2019.

	Species	<i>p</i> -value
Annual	<i>Margalefidinium</i>	<.0001
	<i>Gymnodinium</i>	0.0013
	<i>Coscincodiscus</i>	0.6740
	<i>Eucampia</i>	0.0070
	<i>Rhizosolenia</i>	0.4757
Spring/Summer	<i>Margalefidinium</i>	0.0114
	<i>Gymnodinium</i>	0.0016
	<i>Coscincodiscus</i>	0.8628
	<i>Eucampia</i>	0.2034
	<i>Rhizosolenia</i>	0.5476
Fall/Winter	<i>Margalefidinium</i>	<.0001
	<i>Gymnodinium</i>	0.0889
	<i>Coscincodiscus</i>	0.5755
	<i>Eucampia</i>	0.0193
	<i>Rhizosolenia</i>	0.5998

Table 3. The averages, and ranges of *Alexandrium catenella*), paralytic shellfish toxins, sea surface temperature, nitrate, chlorophyll-a, and the rate of San Lorenzo River discharge during the given time intervals.

	18- year average (range)	2003- 2007 average (range)	2005 average (range)	2008- 2015 average (range)	2016-2019 average (range)
<i>Alexandrium catenella</i> (cells L ⁻¹)	301 (0-21,950)	461 (0-17,387)	1051 (0-17,387)	139 (0-7067)	475 (0-21,950)
Paralytic shellfish toxins (µg STX equiv. /100 g shellfish)	24 (0-770)	38 (0-770)	33 (0-232)	17 (0-656)	26 (0-235)
Sea surface temperature (°C)	13.7 (8.8-19.1)	13.6 (9.8-19.1)	13.8 (10 -19.1)	13.4 (8.8 –18.6)	14.5 (10.3-18.8)
Nitrate (µM)	4.3 (0-47.2)	5.2 (0-47.2)	5.1 (0-24.1)	4.3 (0-24)	3.3 (0-14.5)
Chlorophyll-a (µg/L)	8.2 (0.34-289.1)	10.4 (0.5-289.1)	8.2 (0.5-49.5)	6.9 (0.85-53.3)	8.6 (0.6-137.6)
San Lorenzo River discharge (m ⁻³ s ⁻¹)	3.6 (0.01-123.5)	3.9 (0.02-91.5)	5.58 (0.34-63.2)	2.24 (0.01-123.5)	6.16 (0.2-120.9)

Table 4. Generalized Linear Model results using the PC terms, calculated from the Principal Component Analysis, employed to determine the statistical correlation with the categorical representation of *Alexandrium catenella* (cells L⁻¹), and the environmental parameters for the annual, spring and fall/winter models. Numbers in parentheses are the factor estimates from the PC analysis.

<i>A. catenella</i>		Variables	Estimates	<i>p</i> -values
Annual	PC1	Nitrate (-0.838) Temperature (0.853)	0.355	<.0001
	PC2	Chlorophyll a (0.970)	0.428	<.0001
	PC3	San Lorenzo River (0.952)	-0.149	0.0497
Spring	PC1	Nitrate (0.881) Temperature (-0.795)	-0.506	0.0003
	PC2	Chlorophyll a (0.947)	0.343	0.0147
	PC3	San Lorenzo River (0.948)	-0.337	0.0142
Fall/Winter	PC1	Nitrate (-0.793) Temperature (0.894)	0.256	0.0031
	PC2	Chlorophyll a (0.966)	0.541	<0.0001
	PC3	San Lorenzo River (0.957)	0.060	0.5042

Table 5. Generalized Linear Model results using the PC terms, calculated from the Principal Component Analysis, used to determine the statistical correlation with the categorical representation of *Alexandrium catenella* blooms, and the environmental parameters for the annual, spring and fall/winter models. Numbers in parentheses are the factor estimates from the PC analysis.

Blooms (1 std deviation above the mean, 1349 cells L ⁻¹)		Variables	Estimates	<i>p</i> -values
Annual	PC1	Nitrate (-0.838) Temperature (0.853)	0.017	0.9224
	PC2	Chlorophyll a (0.970)	0.715	<0.0001
	PC3	San Lorenzo River (0.952)	-0.446	0.0263
Spring	PC1	Nitrate (0.881) Temperature (-0.795)	-0.645	0.0286
	PC2	Chlorophyll a (0.947)	0.065	0.8335
	PC3	San Lorenzo River (0.948)	-0.300	0.3027
Fall/Winter	PC1	Nitrate (-0.793) Temperature (0.894)	0.303	0.2127
	PC2	Chlorophyll a (0.966)	1.02	<.0001
	PC3	San Lorenzo River (0.957)	-0.607	0.0193

Table 6. Generalized Linear Model results using the PC terms, calculated from the Principal Component Analysis, used to determine the statistical correlation with the categorical representation of paralytic shellfish Toxins (PSTs) ($\mu\text{g STX equiv. /100 g shellfish}$), and the environmental parameters for the annual, spring and fall/winter models. Numbers in parentheses, are the factor estimates from the PC analysis.

<i>PSTs</i>		Variables	Estimates	<i>p</i> -values
Annual 3-week Lag	PC1	Nitrate (-0.838) Temperature (0.853)	0.178	0.0024
	PC2	Chlorophyll a (0.970)	0.021	0.7893
	PC3	San Lorenzo River (0.952)	-0.060	0.4364
Spring 2-week Lag	PC1	Nitrate (0.881) Temperature (-0.795)	-0.211	0.0451
	PC2	Chlorophyll a (0.947)	0.158	0.2343
	PC3	San Lorenzo River (0.948)	-0.347	0.0061
Fall/Winter	PC1	Nitrate (-0.793) Temperature (0.894)	0.312	0.0009
	PC2	Chlorophyll a (0.966)	0.135	0.2137
	PC3	San Lorenzo River (0.957)	-0.043	0.6779

Table 7. Generalized Linear Model results using the PC terms, calculated from the Principal Component Analysis, used to determine the statistical correlation with the 3-month moving average of Pacific decadal oscillation (PDO), North Pacific Gyre Oscillation (NPGO), and Oceanic Niño Index (ONI), and the categorical representation of the response variables *Alexandrium catenella* (cells L⁻¹), paralytic shellfish toxins (PSTs) or blooms of *Alexandrium catenella* for the annual, spring and fall/winter models. Numbers in parentheses are the factor estimates from the PC analysis.

		Variables	Response variable	Estimates	<i>p</i> -values
Annual	PC1	PDO (0.783) ONI (0.949)	<i>A. catenella</i>	0.159	0.3200
			<i>A. catenella</i> blooms	0.561	0.1712
			PSTs	0.103	0.5412
	PC2	NPGO (0.972)	<i>A. catenella</i>	1.128	<.0001
			<i>A. catenella</i> blooms	0.769	0.0180
			PSTs	0.582	0.0031
Spring	PC1	PDO (0.835) ONI (0.919)	<i>A. catenella</i>	0.346	0.2310
			<i>A. catenella</i> blooms	0.186	0.8974
			PSTs	0.060	0.8321
	PC2	NPGO (0.979)	<i>A. catenella</i>	1.426	<.0001
			<i>A. catenella</i> blooms	2.955	0.0311
			PSTs	1.102	0.0011
Fall/Winter	PC1	PDO (0.709) ONI (0.958)	<i>A. catenella</i>	0.462	0.0256
			<i>A. catenella</i> blooms	1.605	0.1338
			PSTs	0.089	0.6843
	PC2	NPGO (0.962)	<i>A. catenella</i>	0.952	0.0001
			<i>A. catenella</i> blooms (4-week lag)	1.549	0.0163
			PSTs (1-week lag)	0.743	0.0033

Table 8. The prediction and failure rates (%) of the categorical representation, 1- present, 0- not present, of the response variables, *Alexandrium catenella*, *Alexandrium catenella* blooms, and paralytic shellfish toxins (PSTs) compared to the annual, spring, and fall/winter principal components of the environmental data. “False negative” predicts an absence, when there is actually a presence. “False positive” predicts a presence, when there is actually an absence.

		Annual	Spring	Fall/ Winter
Presence (1) Successfully Predicted	<i>A. catenella</i>	58	32	33
	<i>A. catenella</i> blooms	0	0	0
	PSTs	99	71	95
Absence (0) Successfully Predicted	<i>A. catenella</i>	67	72	88
	<i>A. catenella</i> blooms	100	100	100
	PSTs	0	25	17
False Negative	<i>A. catenella</i>	42	68	67
	<i>A. catenella</i> blooms	100	100	100
	PSTs	1	29	5
False Positive	<i>A. catenella</i>	33	28	12
	<i>A. catenella</i> blooms	0	0	0
	PSTs	1	75	83

Table 9. The prediction and failure rates (%) of the categorical representation, 1- present, 0- not present, of the response variables, of *Alexandrium catenella*, *Alexandrium catenella* blooms, and paralytic shellfish toxins (PSTs) compared to the principal components of the 3-month moving averages of Pacific decadal oscillation (PDO), North Pacific Gyre Oscillation (NPGO), and Oceanic Niño Index (ONI). “False negative” predicts an absence, when there is actually a presence. “False positive” predicts a presence, when there is actually an absence.

		Annual	Spring	Fall/ Winter
Presence (1) Successfully Predicted	<i>A.catenella</i>	85	80	95
	<i>A.catenella</i> blooms	0	0	0
	PSTs	100	100	100
Absence (0) Successfully Predicted	<i>A.catenella</i>	56	78	43
	<i>A.catenella</i> blooms	100	100	100
	PSTs	0	0	0
False Negative	<i>A.catenella</i>	15	20	5
	<i>A.catenella</i> blooms	100	100	100
	PSTs	0	0	0
False Positive	<i>A.catenella</i>	44	22	57
	<i>A.catenella</i> blooms	0	0	0
	PSTs	100	100	100

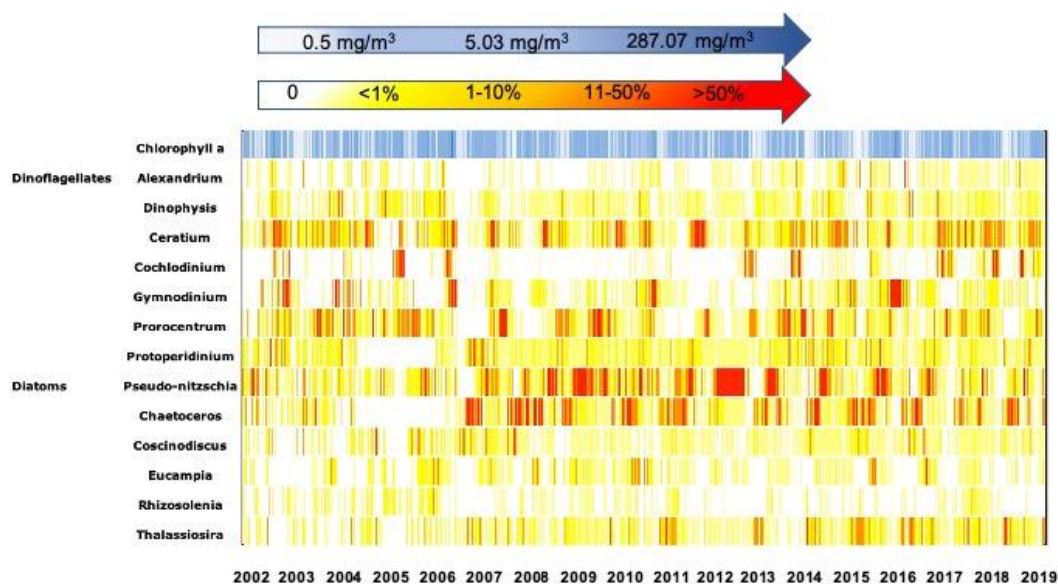


Figure 1. The Relative Abundance Index (RAI) data from 2002 to 2019 showing the distribution of the most common diatoms and dinoflagellates at the Santa Cruz Wharf, Santa Cruz, CA. The colors represent absent, rare, present, common and abundant in the direction of the arrow at the top, the white representing absent and the darker red representing abundant. The top row of the graph displays the changes in chlorophyll-a ($\mu\text{g L}^{-1}$) (white represents the lowest chlorophyll-a ($0.5 \mu\text{g L}^{-1}$), and the dark blue represents the highest concentration of chlorophyll-a ($289.07 \mu\text{g L}^{-1}$) from 2002 to 2019.

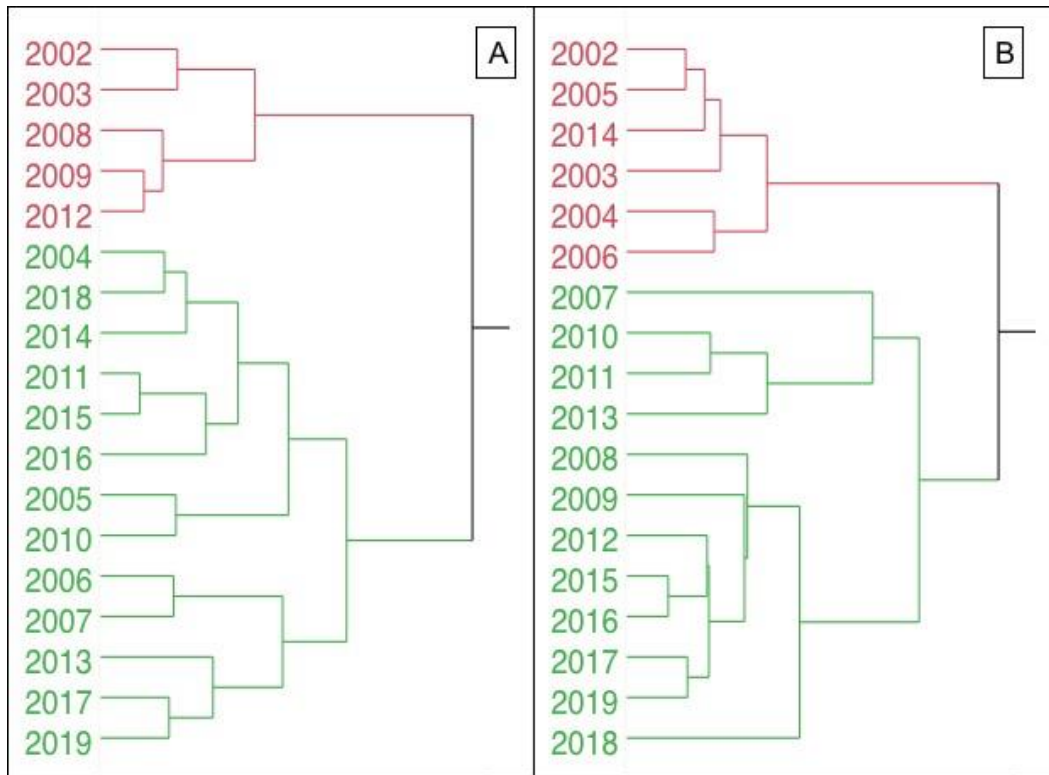


Figure 2. A hierarchical cluster analysis using the Relative Abundance Index (RAI) data from 2002 to 2019 of the most commonly seen A) dinoflagellates and B) diatoms at the Santa Cruz Wharf Santa Cruz, CA. The red cluster in A and B represents a lower number of cells whereas the green cluster represents higher numbers of phytoplankton for each group.



Figure 3. A hierarchical cluster analysis using the Relative Abundance Index (RAI) data provides a graphical representation of the relationship among the most common phytoplankton found at the Santa Cruz Wharf, Santa Cruz, CA from 2002 to 2019.

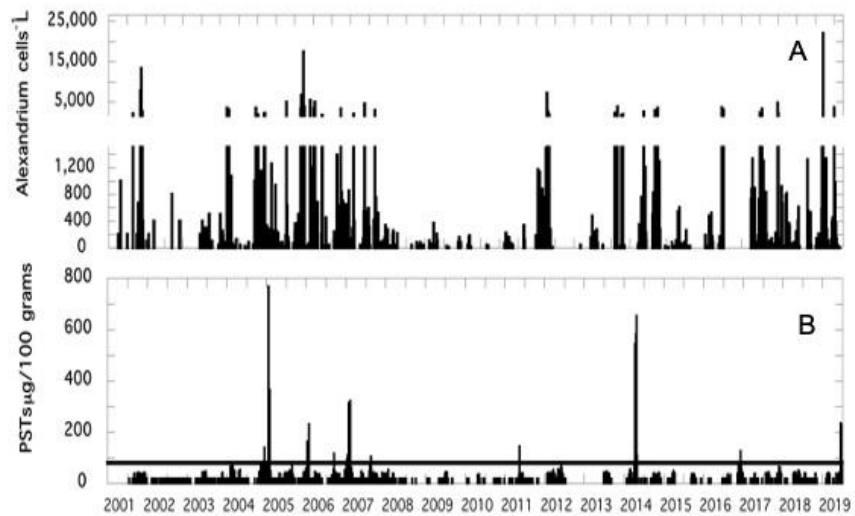


Figure 4. (A). *Alexandrium catenella*, and (B). Paralytic shellfish toxins (PSTs) weekly data from the Santa Cruz Wharf, Santa Cruz, CA from 2001 to 2019. The solid gray line in (B) represents the regulatory limit of 80 µg Saxitoxin (STX) equiv. /100 g shellfish.

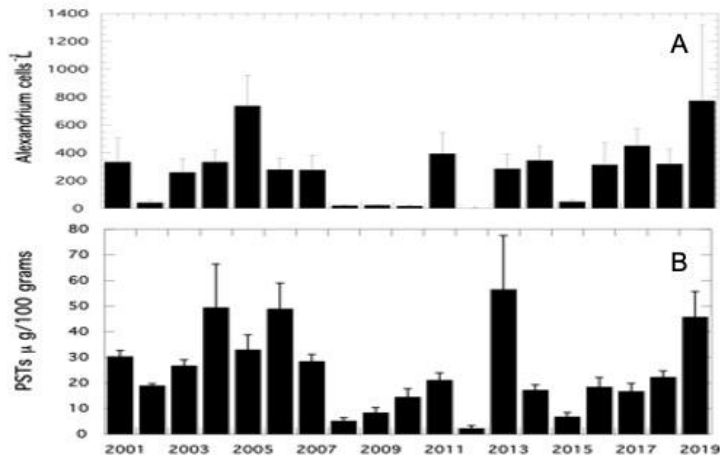


Figure 5. (A). *Alexandrium catenella* and (B). Paralytic shellfish toxins (PSTs) annual data from the Santa Cruz Wharf, Santa Cruz, CA from 2001 to 2019. Error bars represent the standard deviation for the mean of twelve months for each year.

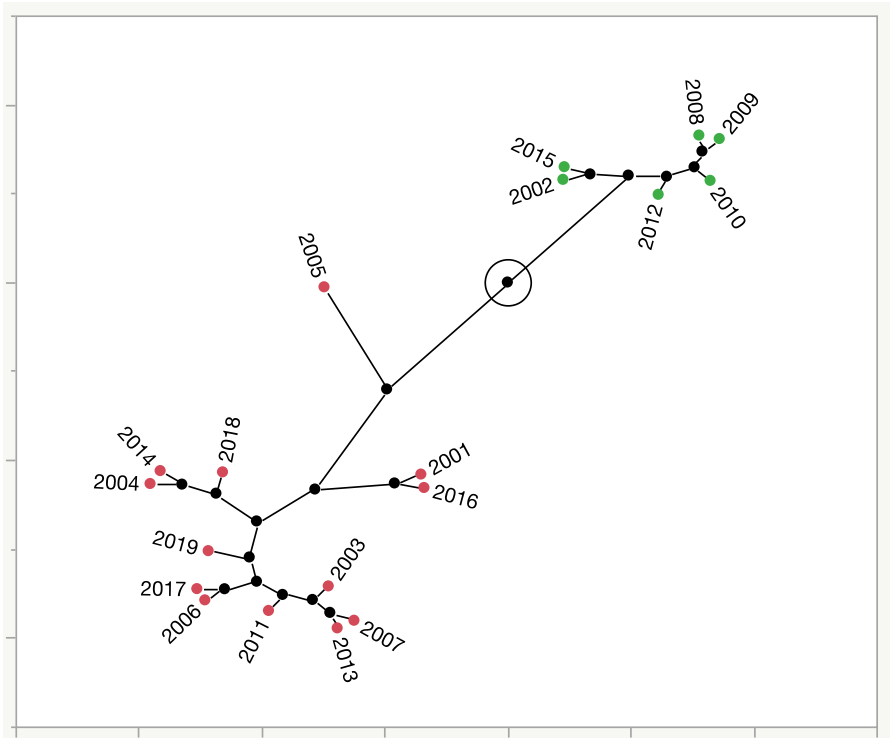


Figure 6. Constellation plot from the hierarchical cluster analysis using *Alexandrium catenella* (cells L⁻¹) for the Santa Cruz Wharf, Santa Cruz, CA from 2001 to 2019.

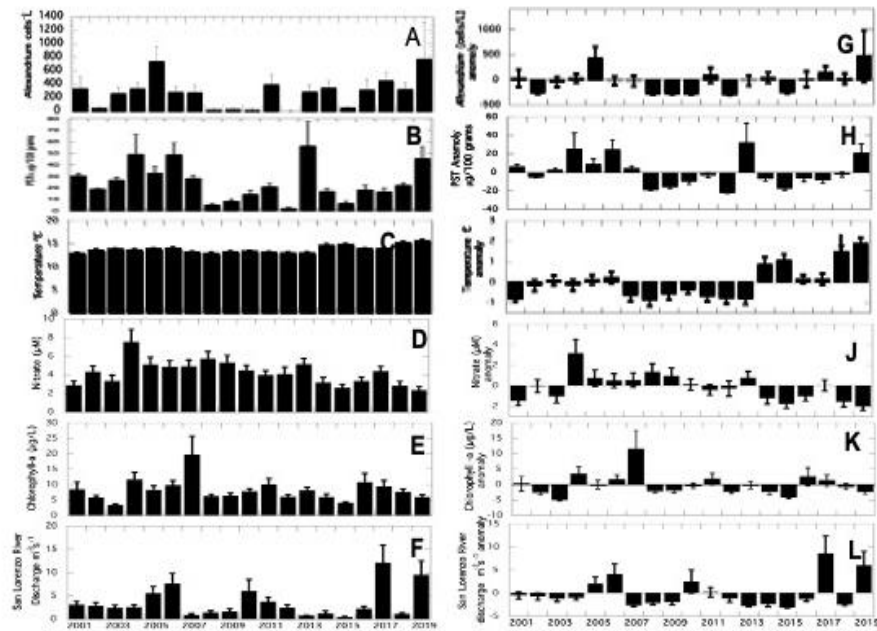


Figure 7. Annual averages of the independent and dependent variables as a function of time from 2001 to 2019, (A-F), and anomalies of these variables at the Santa Cruz Warf, (G-L). (A) *Alexandrium catenella*, (B) Paralytic shellfish toxins (PSTs), (C) Sea surface temperature, (D) Nitrate, (E) Chlorophyll-a, (F) San Lorenzo River discharge, (G) *Alexandrium catenella* anomalies, (H) PST anomalies, (I) Sea surface temperature anomalies, (J) Nitrate anomalies, (K) Chlorophyll-a anomalies, and (L) San Lorenzo River discharge anomalies. Error bars represent the standard deviation of twelve data points.

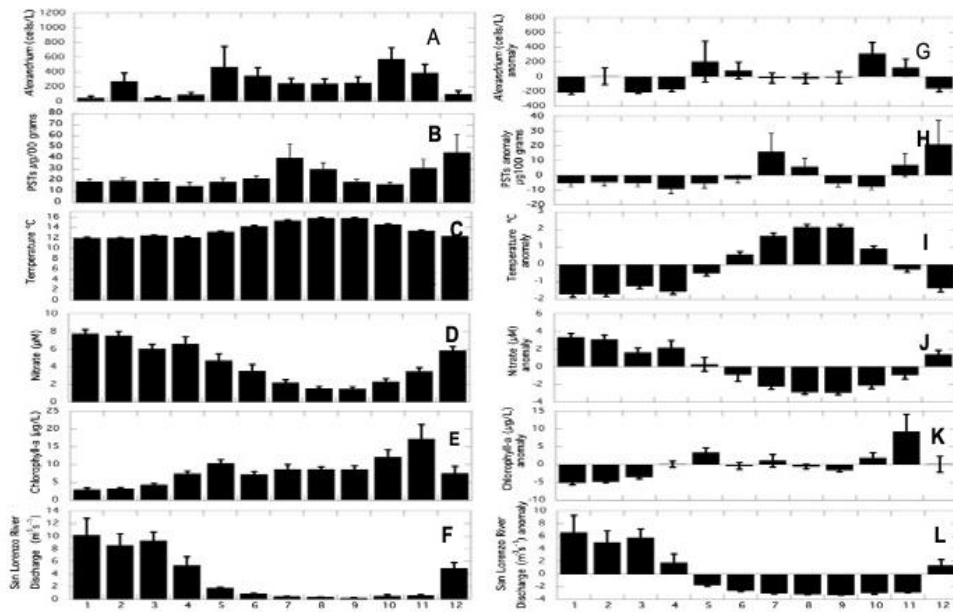


Figure 8. Monthly averages of the independent and dependent variables as a function of time from 2001 to 2019, (A-F), and anomalies of these variables at the Santa Cruz Warf, (G-L). (A) *Alexandrium catenella*, (B) Paralytic shellfish toxins (PSTs), (C) Sea surface temperature, (D) Nitrate, (E) Chlorophyll-a, (F) San Lorenzo River discharge, (G) *Alexandrium catenella* anomalies, (H) PST anomalies, (I) Sea surface temperature anomalies, (J) Nitrate anomalies, (K) Chlorophyll-a anomalies ($\mu\text{g L}^{-1}$), and (L) San Lorenzo River discharge anomalies. Error bars represent the standard deviation of the monthly data points.

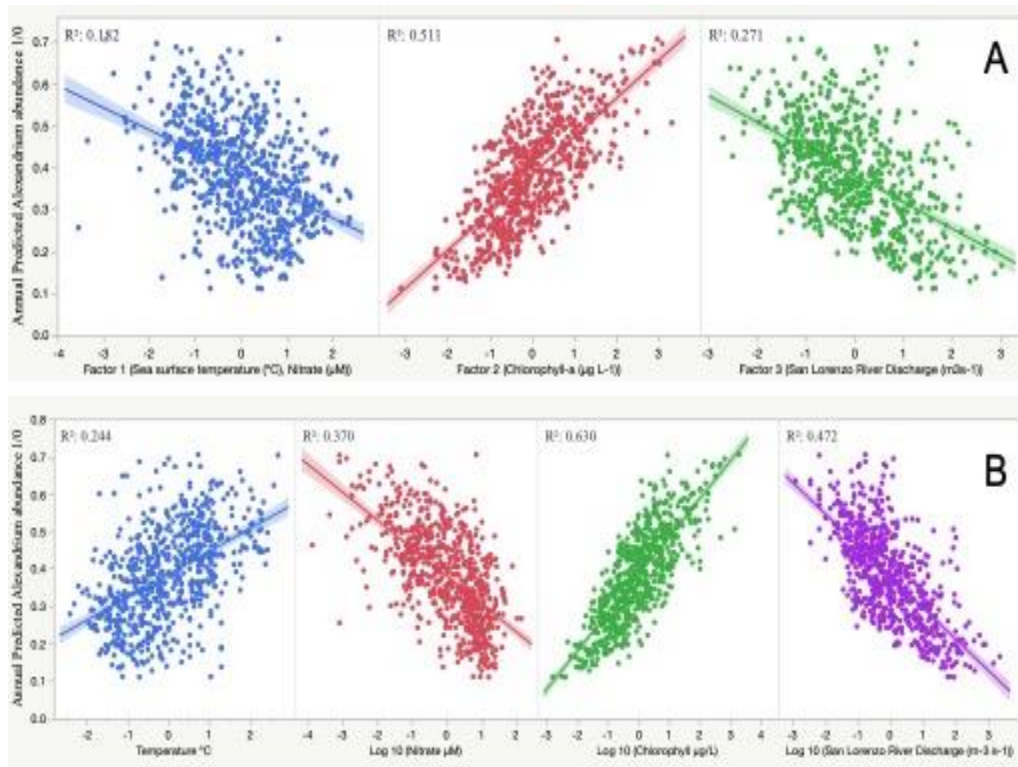


Figure 9. The relationship between the predicted values of *Alexandrium catenella* from the annual model, and A) Factors 1 [(+) Sea surface temperature (°C), and (-) Nitrate (μM)], Factor 2 [(+) Chlorophyll-a (μg L⁻¹)], and Factor 3 (-) [San Lorenzo River discharge (m³s⁻¹)], and B) temperature °C, log₁₀ nitrate (μM), and log₁₀ San Lorenzo River discharge (m³ s⁻¹).

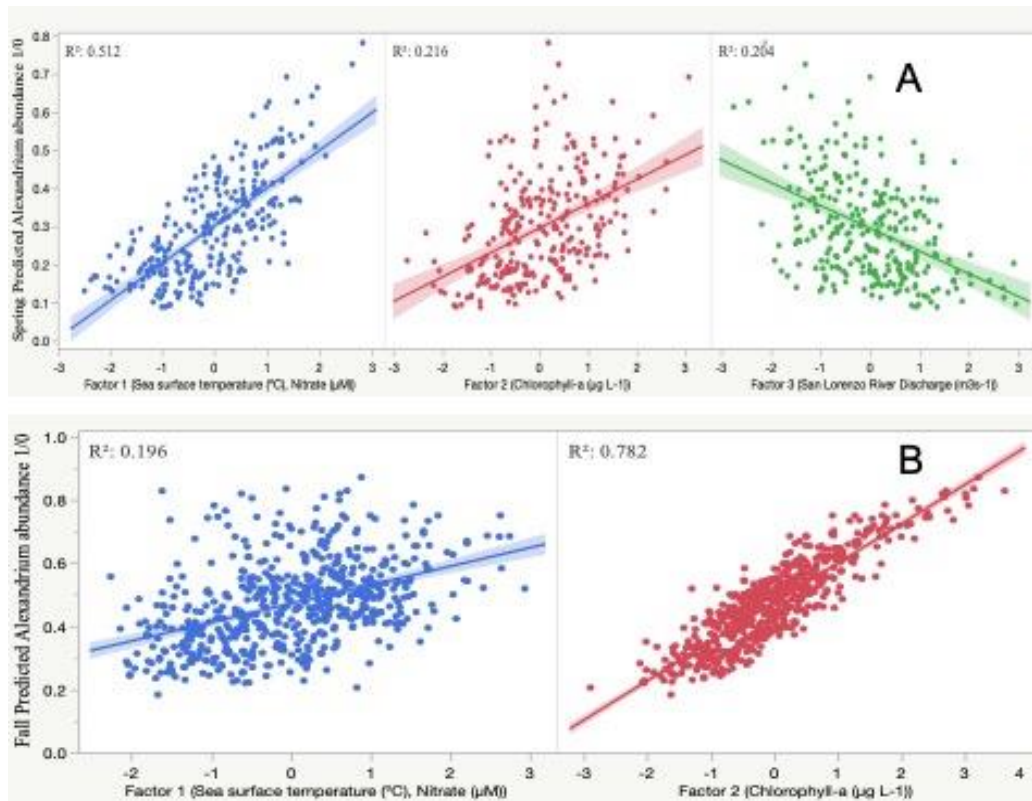


Figure 10. The relationship between the predicted values of *Alexandrium catenella* from the spring model and A) Factors 1 [(+) Sea surface temperature (°C), and (-) Nitrate (μM)], Factor 2 [(+) Chlorophyll-a (μg L⁻¹), and Factor 3 [(-) San Lorenzo River discharge (m⁻³s⁻¹)], and B) Factor 2 [(+) Chlorophyll-a (μg L⁻¹), and Factor 3 [(-) San Lorenzo River discharge (m⁻³s⁻¹)] for the fall model.

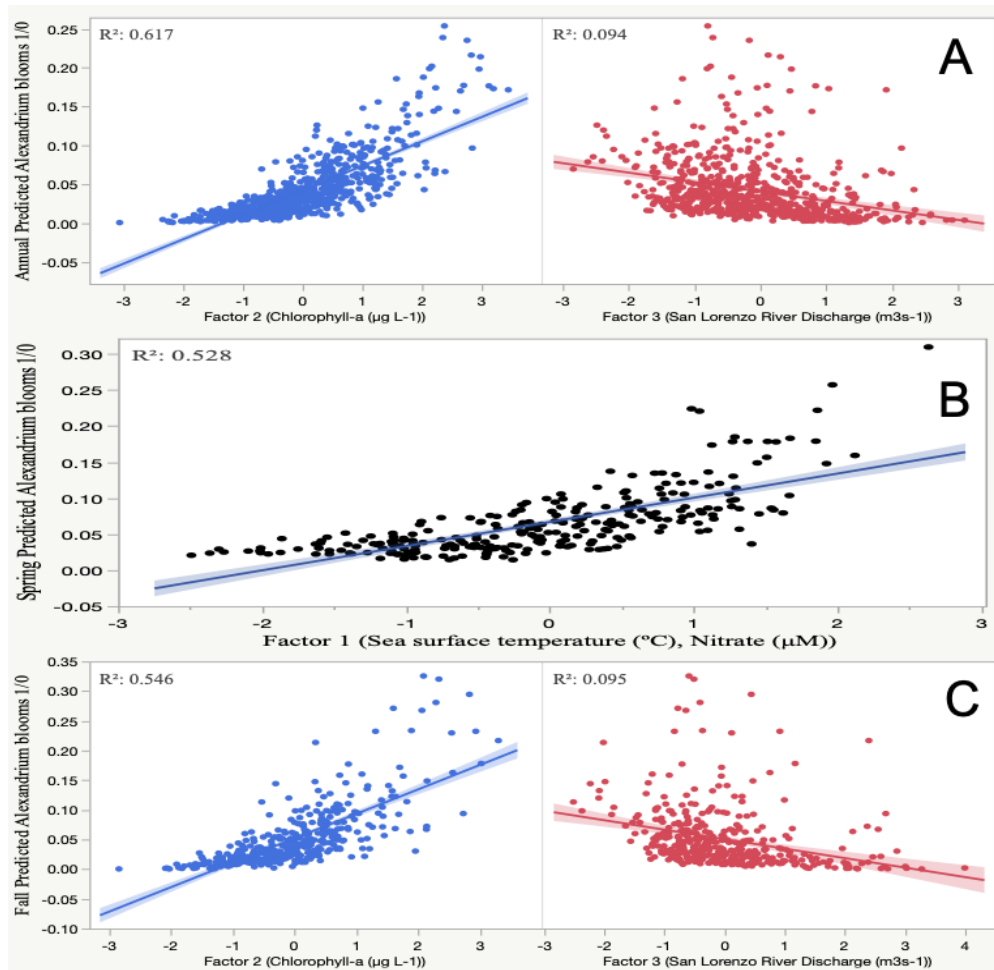


Figure 11. The relationship between the predicted values of *Alexandrium catenella* blooms, and A) Factor 2 [(+) Chlorophyll-a ($\mu\text{g L}^{-1}$)], and Factor 3 [(-) San Lorenzo River discharge (m^3s^{-1})] for the annual model, B) Factors 1 [(+) Sea surface temperature ($^{\circ}\text{C}$), and (-) Nitrate (μM)] for the spring model, and C) Factor 2 [(+) Chlorophyll-a ($\mu\text{g L}^{-1}$)], and Factor 3 [(-) San Lorenzo River discharge (m^3s^{-1})] for the fall model.

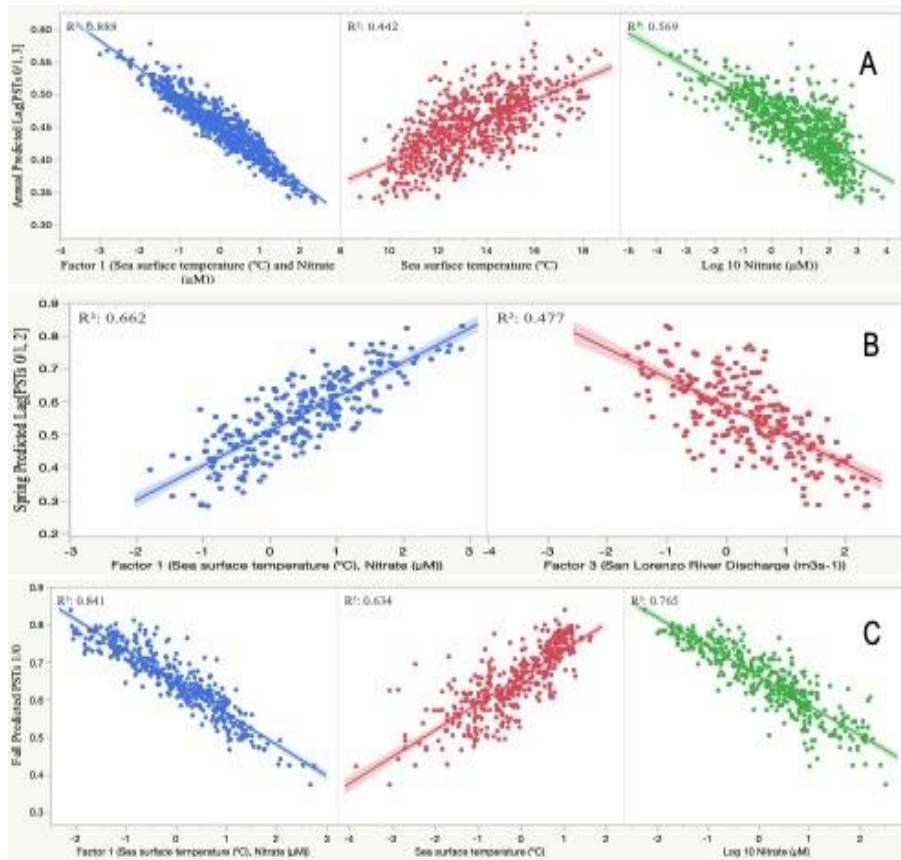


Figure 12. The relationship between the predicted values of PSTs, and A) Factors 1 [Sea surface temperature ($^{\circ}\text{C}$), and (-) Nitrate (μM)], and sea surface temperature ($^{\circ}\text{C}$), \log_{10} nitrate (μM) for the annual model, B) factor 1 [(+) Sea surface temperature ($^{\circ}\text{C}$), and (-) Nitrate (μM)], and Factor 3 [(-) San Lorenzo River discharge (m^3s^{-1})] for the spring model, and C) Factors 1 [(+) Sea surface temperature ($^{\circ}\text{C}$), and (-) Nitrate (μM)], and Sea surface temperature $^{\circ}\text{C}$, \log_{10} nitrate (μM) for the fall model.

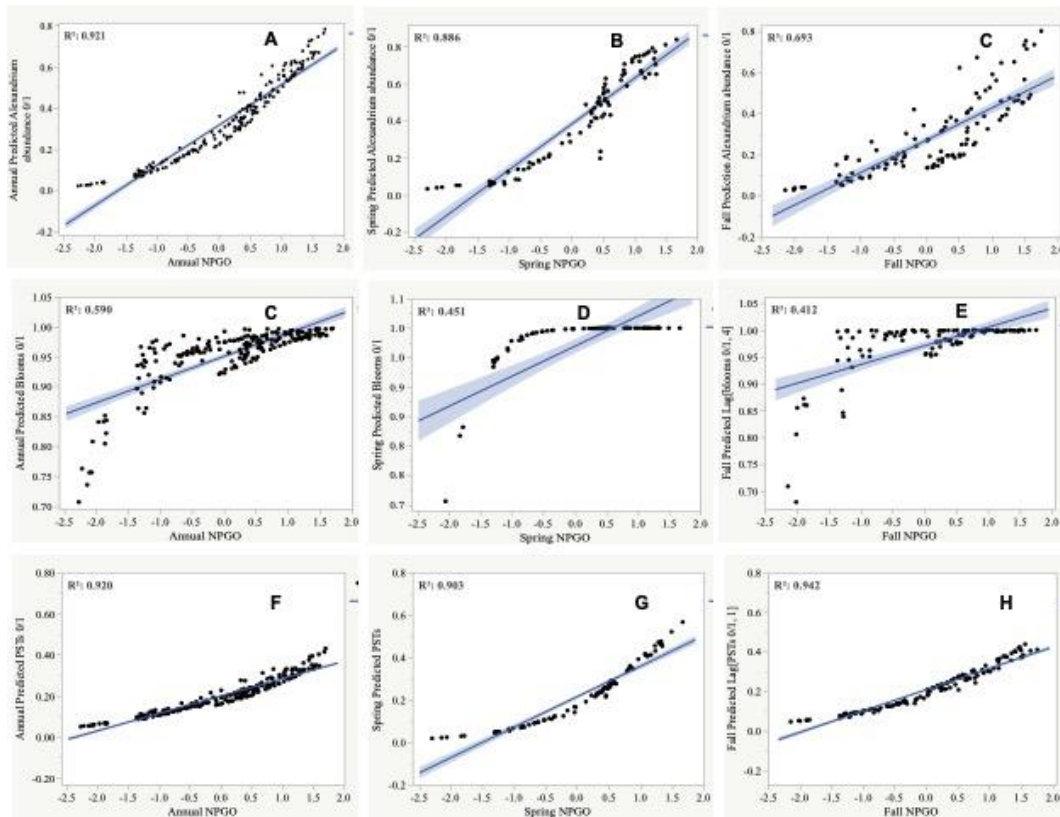


Figure 13. The relationship between the predicted values of the categorical representation of the response variables *Alexandrium* (cells L⁻¹) (A-C), Blooms (D-F), and Paralytic shellfish toxins (PSTs) (G-I) and Factor 2, North Pacific Gyre Oscillation (NPGO), for the annual, spring, and fall/winter model. Predicted values from the annual models for, A) *Alexandrium* (cells L⁻¹), D) Blooms, and G) Paralytic shellfish toxins (PSTs) and NPGO. Predicted values for the Spring models, B) *Alexandrium* (cells L⁻¹) E) Blooms (1,349 cells L⁻¹) and H) Paralytic shellfish toxins (PSTs), and NPGO. Fall/winter model predicted values, C) *Alexandrium* (cells L⁻¹) F) Blooms (1,349 cells L⁻¹), and I) Paralytic shellfish toxins (PSTs), and NPGO.

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Chapter 3: Conclusions and Future Directions

This study includes the first statistical model done to evaluate the presence of *A. catenella* and toxicity in Monterey Bay, CA. This 18-year data set, reveals that the combination of increase sea surface temperatures ($^{\circ}\text{C}$), the depletion in NO_3^- (μM), (-) San Lorenzo River Discharge rates (m^3s^{-1}), and the increase in chlorophyll-a ($\mu\text{g L}^{-1}$), along with the presence of NPGO may be the optimal environmental conditions for an increase in *A. catenella* and PSTs in Monterey Bay, CA. Though our models, were not appropriate for forecasting future abundance and toxicity, the results were similar to other models published for the presence of *A. catenella* and toxicity (McGillicuddy et al., 2011, Moore et al., 2009, Bill et al., 2006, Bucci et al., 2020).

Forecasting blooms of *A. catenella* is difficult in Monterey Bay, because blooms are rarely seen. In areas where *A. catenella* blooms are seen on a yearly basis, such as Puget Sound (Moore et al., 2009, 2011), forecasting using statistical models is more attainable. Though our models for the presence of *A. catenella* and toxicity were not optimal, the significance of these environmental and climate variables, were clearly seen in the patterns of the graphical representations of the residuals from the predicted values of the GLM models and the PC factors. This successfully gave us insight into how the variables would work together. It is difficult to use these models for a specific event but, they can be utilized to look at large scale patterns over time. When attempting to forecast the presence of *A. catenella* and toxicity, one must be aware of interannual variability.

The weekly data obtained from the Santa Cruz Municipal Wharf in Santa Cruz, California (SCW), is just a snapshot of what is happening in Monterey Bay, CA on a weekly basis. Jester et al., 2009, found a shift from a diatom dominated community to a dinoflagellate dominated community in 2004. It was suggested that this may have been due to the lower upwelling index during the spring and summer of these years. Though the upwelling index was not statistically significant in our models, sea surface temperature and nutrients are often used as a proxy for upwelling. *A. catenella* cells L^{-1} are typically seen when sea surface temperatures are warm, and nutrients are low (Nishitani and Chew, 1984, Ryan et al., 2009, Moore et al., 2009, 2011, 2015, Bill et al., 2016) which would be seen when the upwelling index is negative. Though these parameters were significant in our models, when observing the yearly averages and anomalies of these variables (Figure 1), the variables do not always line up with the years we see *A. catenella* and PSTs. This is an indication, that we must be aware of what is happening in the months and years before *A. catenella* is present in Monterey Bay, CA, in order to fully understand why these dinoflagellates are present.

As sea surface temperatures increases due to climate change, it is important to consider the significance of increases in sea surface temperatures, along with the depletion in NO_3^- and reduction in San Lorenzo River discharge rates together with the increase in chlorophyll-a, in our models. Increases in sea surface temperatures, due to climate change, has been hypothesized to lead to an increase in frequency and abundance of *A. catenella* and associated toxins (Moore et al., 2011, Wells, et al.,

2019). However, as parameters change, due to climate change in Monterey Bay, CA, these models may become less informative as new environmental, and climate variables become more significant to the presence of *A. catenella* and toxicity.

Along with interannual variability, and climate change, other factors should be considered for the presence of *A. catenella* and toxicity in Monterey Bay, CA in future models. In Puget Sound and the Gulf of Maine, cysts of *A. catenella* in the sediments on the seafloor (Horner et al., 2011, Bucci et al., 2020, Moore et al., 2015) are indicative of bloom dynamics. *A. catenella* has a resting cyst stage when environmental conditions are not suitable for growth (Moore et al., 2015). The environmental conditions contributing to the bloom development from cysts are similar to what we have seen with the presence of *A. catenella* and toxicity (Moore et al., 2015, Bucci et al., 2020) warm sea surface temperatures, sunlight, and nutrients are all associated with cysts. As climate changes, leading to warmer sea surface temperatures, cysts may contribute to the development of blooms. The role of cysts in bloom development and toxicity have not been studied in Monterey Bay, CA, but should be considered when studying the presence of *A. catenella* and toxicity in Monterey Bay.

Another factor that should be studied is mixotrophy. *A. catenella* has the potential to be mixotrophic, combining both autotrophy (photosynthesis) and heterotrophy (consumption of prey items; Jeong et al., 2005a, Stoecker et al., 2006, Burkholder et al., 2008) but the feeding strategies of *A. catenella* are still unclear. *Alexandrium spp.* have been observed to ingest both *Isochysis galbana* and

Synechococcus (Jeong et al., 2005a, b). *Synechococcus* is a unicellular cyanobacteria found year-round in Monterey Bay (Paerl et al., 2012), and higher abundance are found during the late summer and fall (Paerl et al., 2012) when *A. catenella* is present. It has been hypothesized that mixotrophic dinoflagellates will have lower growth rates due to increased energy costs of using photosynthetic organelles, as well as a feeding apparatus (Raven, 1997); however, the use of mixotrophy may still provide a competitive advantage during conditions of low nutrients (Raven, 1997). The ability to utilize mixotrophy allows dinoflagellates more opportunities for nutrition than those species that can only access one resource (Barton et al., 2013). Mixotrophy may be a major mode of nutrition for species in eutrophic waters and likely important in the development and maintenance of blooms (Burkholder, 2008). However, information on the role of mixotrophy in nutrition, growth and bloom development, as well as the importance of the use of toxicity is largely unknown (Burkholder, 2008).

As the CCS is affected in the near future by climate change, upwelling indices and nutrients will diminish, and waters will become stratified due to the increase in sea surface temperatures. These environmental variables, along with the climate variable, NPGO, will provide the necessary environment for the presence of *A. catenella* and toxicity to thrive. Both cysts and mixotrophy potentially contribute to the variability in our models, and a fully physiological (rather than statistical) model may capture this variability. However, for now, these models do not exist, and our statistical model is the best indicator of future conditions on decadal timescales.

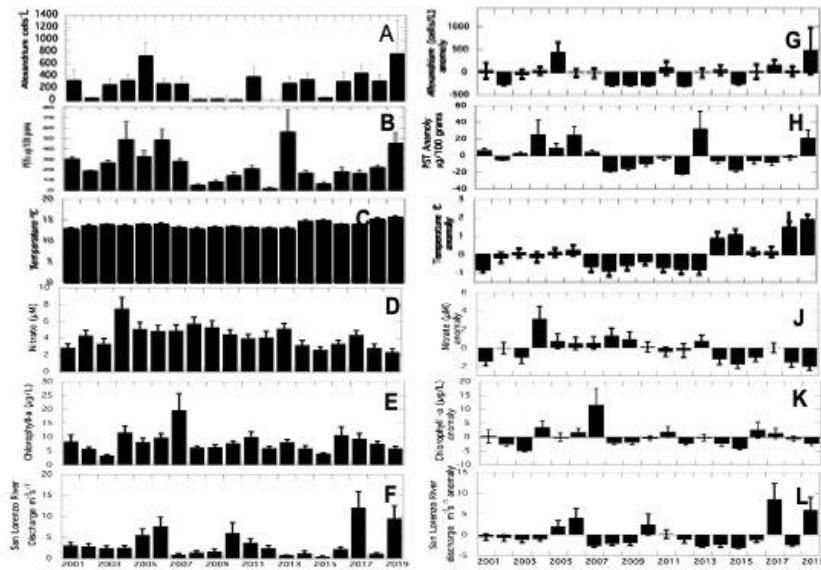


Figure 1. Annual averages of the independent and dependent variables as a function of time from 2001 to 2019, (A-F), and anomalies of these variables at the Santa Cruz Warf, (G-L). (A) *Alexandrium catenella*, (B) Paralytic shellfish toxins (PSTs); $\mu\text{g}/100$ grams), (C) Sea surface temperature ($^{\circ}\text{C}$), (D) Nitrate (μM), (E) Chlorophyll-a ($\mu\text{g L}^{-1}$) (F) San Lorenzo River Discharge (m^3s^{-1}), (G) *Alexandrium catenella* anomalies, (H) PST anomalies, (I) Sea surface temperature anomalies, (J) Nitrate anomalies (μM), (K) Chlorophyll-a anomalies ($\mu\text{g L}^{-1}$), and (L) San Lorenzo River Discharge anomalies (m^3s^{-1}). Error bars represent the standard deviation of twelve data points.

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