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***Alexandrium* spp. and *Pseudo-nitzschia* spp. Cohabitation in the Gulf of Maine**

By

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THESIS

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Biological Sciences: Marine Biology

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This thesis was examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences: Marine Biology by:

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Abstract

Globally, harmful algal blooms are a severe issue for marine ecosystems, animals, and humans both ecologically and economically. The Gulf of Maine is no stranger to harmful algal blooms, specifically the dinoflagellate, *Alexandrium catenella*, that has routinely been forming toxic blooms since the early 1970s. In 2016, a new species, the diatom *Pseudo-nitzschia* spp. formed a massive bloom and has had annual blooms since. Anecdotally, these two species do not bloom concurrently, suggesting there may be specific environmental conditions that promote bloom formation for each species. The intent of this research is to uncover potential parameters that are affecting bloom formation in each species. Analysis of a dataset of harmful algal bloom observations from coastal NH during 2017 to 2022 suggests that neither species has significantly increased in abundance over this time period. While *Pseudo-nitzschia* spp. is present in greater abundance than *Alexandrium* spp. its presence has not prevented *Alexandrium* spp. from blooming. Additionally, *Pseudo-nitzschia* spp. was present in the water column for a longer period relative to *Alexandrium* spp., suggesting environmental factors may play a role in mediating the population abundances of these two HAB alga. In an analysis that included nutrient concentration, temperature, and salinity, phosphorus was found to be an important factor in driving the cell abundance of each species. A correlation analysis indicated a negative and significant correlation between *Alexandrium* spp. and phosphorus, whereas a positive and significant trend between large sized *Pseudo-nitzschia* spp. and phosphorus was observed. These findings will provide an enhanced understanding into how the environment influences the abundance of these two species allowing for better predictions of the future trajectory of these species in a warming Gulf of Maine.

Introduction

Marine ecosystems are vital in our global society providing immeasurable services to humans, including food production, climate regulation, tourism, and transportation. Microscopic algae, called phytoplankton, are at the center of all marine ecosystems. Phytoplankton form the base of the food web, contribute to primary productivity, produce significant amounts of oxygen, and directly contribute to the cycling of nutrients (Henson et al., 2021). Phytoplankton community structure and abundance is driven by interactions with other organisms, nutrient availability, physical oceanographic processes, and geography. The various factors that influence phytoplankton growth and mortality often align to produce excessive community growth, known as a 'bloom'. Some blooms, particularly of toxin containing phytoplankton, can be harmful to humans and animals (Gobler, 2020; Sterling et al., 2021) and the ecosystems in which they occur (Hallegraeff et al., 2021).

Harmful algal blooms (HABs) are a significant problem worldwide (Anderson et al., 2021) and can last for weeks or months, affecting hundreds of kilometers of coastline (Erdner et al., 2011). Harmful algal blooms cause morbidity/mortality of marine organisms, and ecosystem scale impacts on ecology and biogeochemistry of the ocean (Erdner et al., 2011), as well as influencing our economy (Anderson et al., 2021). All bloom events have one, or more, of the following characteristics: (1) produce water discoloration, mucilage, scum, or foam; (2) cause accumulation of biotoxins in seafood; (3) cause bans of shellfish or other invertebrate harvesting and closures of beaches to protect human health; (4) are classified as any event where humans or animals are negatively affected by microalgae (Zingone and Oksfeldt Enevoldsen, 2000; Hallegraeff et al., 2021). In summation, a bloom is any event where a specific phytoplankton

population has reached sufficient density to cause negative impacts on other organisms directly or indirectly and make the environment unsuitable for living (Zingone and Oksfeldt Enevoldsen, 2000; Matrai et al., 2005).

There are a multitude of factors that can influence bloom development (Zingone and Oksfeldt Enevoldsen, 2000; Anderson et al., 2021); in most cases, a combination of factors co-occur simultaneously to stimulate bloom formation (Zingone and Oksfeldt Enevoldsen, 2000). The required environmental criteria for blooms also vary between algal life stages and within a species making it possible for HAB formation of a single species to occur under several environmental conditions. Past research has shown that eutrophication, introduction of non-native species, natural climactic variability, aquaculture (Hallegraeff et al., 2021), climate change (Gobler, 2020), and geography (Trainer et al., 2012) have all been found to influence bloom development (Fernandes et al., 2014; Anderson et al., 2021). However, no one factor can account for all phytoplankton blooms, and it is the complicated combination of many factors that make HAB dynamics hard to predict (Zingone and Oksfeldt Enevoldsen, 2000). In addition to these complex dynamics, toxins are not produced by a single species but instead span several genera and within a single genus not all species are toxic. Research has also shown that there is not always a correlation between cell abundance and toxin concentration (Busch et al., 2016), suggesting that the factors that mediate bloom formation and those that influence toxin dynamics could be different. All these factors combined makes the issue of understanding HABs highly concerning and difficult to predict.

Like the rest of the global ocean the Gulf of Maine (GOM) has been impacted by HABs. In the GOM, the most frequently occurring HAB has historically been caused by the dinoflagellate *Alexandrium catenella*, responsible for shellfish closures due to paralytic shellfish

poisoning (PSP) risk (Anderson, 1997; Matrai et al., 2005; McGillicuddy et al., 2005; Fernandes et al., 2014; McGillicuddy et al., 2014; Li et al., 2020; Anderson et al., 2021). Paralytic shellfish poisoning is caused by a potent neurotoxin known as saxitoxin (Anderson, 1997; McGillicuddy et al., 2005; Erdner et al., 2011). Saxitoxin itself can be produced by multiple genera but in the GOM *A. catenella* is the primary contributor (Fernandes et al., 2014; Anderson et al., 2021). This toxin can also be produced across multiple *A. catenella* cell concentrations (McGillicuddy et al., 2014). Annually, *A. catenella* has been responsible for shellfish harvesting closures along the Maine, New Hampshire, and Massachusetts coastline (Anderson, 1997; Matrai et al., 2005; Kleindinst et al., 2014) as saxitoxin bioaccumulates in the tissue of shellfish (Anderson, 1997; McGillicuddy et al., 2005; Anderson et al., 2021). Bioaccumulation in shellfish is not only harmful to humans but can be transferred to higher trophic levels (reviewed by Anderson et al., 2021). Observed concentrations of *A. catenella* have reached approximately hundreds of thousands to one million cells per liter (Li et al., 2020) in the GOM, well beyond the New Hampshire shellfish testing threshold limit of 7.5 cells L⁻¹.

For the past several decades understanding the seasonal and interannual variability of this species has been a major focus of the scientific community (Li et al., 2020). *Alexandrium catenella* appears to bloom seasonally, restricted to the summer months (Anderson, 1997; Matrai et al., 2005; Kleindinst et al., 2014). Research suggests blooms are driven by a combination of factors including temperature, nutrients, water column stratification (reviewed by Matrai et al., 2005; Anderson et al., 2021), vertical migration (Anderson et al., 2021), swimming, and ocean currents (McGillicuddy et al., 2005). These factors impact both the growth rate and life cycles of the of the alga. During a bloom vegetative cells reproduce asexually (Matrai et al., 2005; Erdner et al., 2011) allowing the alga to rapidly divide and reach high population concentrations. As the

bloom declines, vegetative cells will form cysts, a dormant type of cell, that will sink into the sediment ensuring the survival of *A. catenella* throughout the winter (Anderson, 1997; Erdner et al., 2011; McGillicuddy et al., 2014). As the water warms in the spring *A. catenella* cysts are triggered to excyst (Anderson et al., 2021), and cells then begin to grow faster as they become dependent on nutrients in June through August (McGillicuddy et al., 2005). Cyst formation is thought to be driving the annual observations of *A. catenella* in the GOM since 1972 when the first massive red tide appeared on the east coast.

Recently, another HAB species has been seen in the GOM at bloom densities. In this case, *Pseudo-nitzschia* spp., a diatom, is frequently found at concentrations from tens of thousands to nearly 200,000 cells per liter (Fernandes et al., 2014; Clark et al., 2019). The current state of NH threshold limits to initiate toxin testing for *Pseudo-nitzschia* spp. are broken into two size classifications, with the threshold for large *Pseudo-nitzschia* spp. ($\geq 3\mu\text{m}$) at 2,000 cells L^{-1} and small ($< 3\mu\text{m}$) diameter cells at 15,000 cells L^{-1} . There are more than 50 known species of *Pseudo-nitzschia* spp., however this species is quite cryptic in its morphology, making it visually difficult to distinguish between species. Of the available methods, light microscopy does not provide enough resolution for us to distinguish among species, making electron microscopy and molecular sequencing the only reliable methods for species differentiation (Fernandes et al., 2014; Bates et al., 2018; reviewed in Sterling et al., 2021). Of the entire genus twenty-six species are known to produce domoic acid (DA; Bates et al., 2018; Anderson et al., 2021), spread among both the large and small size classifications.

Exposure to domoic acid can result in an illness known as amnesic shellfish poisoning (ASP; Bates et al., 2018; Sterling et al., 2021). This poisoning causes symptoms in humans including seizures, permanent short term memory loss, and even death (Sterling et al., 2021).

These symptoms have also been observed in marine mammals and seabirds (Sterling et al., 2021), and domoic acid poisoning has been observed to be more common in marine organisms than saxitoxin poisonings (Anderson et al., 2021). This HAB species gained recognition on the east coast of North America in Canada in 1987 (Clark et al., 2019), and upon its acknowledgement, local agencies began setting up monitoring for *Pseud-nitzschia* spp. (Fernandes et al., 2014). Prior to 2016, both *Pseudo-nitzschia* spp. and DA had been observed in the GOM, but never in concentrations that were a risk to human health. In 2016, this streak ended, with a large bloom of *Pseudo-nitzschia* spp. and high DA concentrations observed in the GOM. This has led to an annual bloom of *Pseudo-nitzschia* spp. every year since (Sterling et al., 2021) causing economic loss to shellfish and planktivorous fisheries (Cochlan et al., 2008).

Unlike *A. catenella*, *Pseudo-nitzschia* spp. does not appear to have seasonal restrictions as to when it can grow. This diatom is a highly versatile species in its ability to grow, and bloom, under a variety of conditions (Clark et al., 2019; Anderson et al., 2021). It is able to prosper even when nutrients are limiting and temperature conditions are elevated (Trainer et al., 2009). It has a competitive affinity for nitrogen and can utilize organic or inorganic supplies of nitrogen when the nutrient is low (Cochlan et al., 2008). This has made it difficult to determine what the environmental and/or biological requirements are for bloom formation and DA production (Trainer et al., 2009; Anderson et al., 2021). Lab research suggests several factors that may impact HAB formation and DA production such as phytoplankton biomass (Trainer et al., 2009), presence of bacteria (Sterling et al., 2021), nutrient levels (Cochlan et al., 2008; Trainer et al., 2009) including phosphate (PO_4^{3-}), orthosilicic acid ($\text{Si}(\text{OH})_4$), nitrate (NO_3^-) (Sterling et al., 2021), iron and copper limitation, elevated copper (Cochlan et al., 2008), increased salinity, increased pH, predators, and light availability (reviewed by Clark et al., 2019).

However, as with many biological and ecological studies, lab research does not always transfer directly to the natural environment (Clark et al., 2019). Trainer et al. (2009) specifically found that phosphate (PO_4^{3-}), orthosilicic acid (Si(OH)_4), and nitrate (NO_3^-) have no effect on the concentration of *Pseudo-nitzschia* spp. cells or DA in the natural environment. Despite the information presented here, we still know very little about how *Pseudo-nitzschia* spp. interacts with the GOM ecosystem (Clark et al., 2019), compared to other places such as the west coast where it has been a longer standing problem (Anderson et al., 2021; review in Sterling et al., 2021). Further, it is unclear if *Pseudo-nitzschia* spp. is slowly outcompeting *A. catenella* to become a new dominant HAB species in the GOM system. Understanding the mechanisms that control HAB formation has long been a goal in the field of biological oceanography to better predict bloom occurrence. Here, cell abundance of each HAB species were analyzed along with a suite of environmental variables to answer two distinct questions: (1) How is the abundance of these two species changing over time? (2) What are the important environmental factors that drive observed population abundances of each species?

Methods

Cell Abundance Data Collection

Data for this research was collected from two sources. First, cell abundances for *Alexandrium* spp. and *Pseudo-nitzschia* spp. was taken from publicly available data from the Watershed Management Bureau of New Hampshire Department of Environmental Services (NHDES). They monitor several sites but only two locations were used in this analysis, the Judd Gregg Coastal Marine Lab in New Castle, NH, labeled UNH Pier (43.072147, -70.710907; Figure 1) and Hampton Harbor in Hampton, NH, labeled HHHR2 (42.899308, -70.817986; Figure 1). UNH Pier was used for both time series analysis of population dynamics and environmental drivers of observed cell abundances, whereas HHHR2 was only used for time series analysis in relation to population dynamics. After receiving the first time series dataset, emails were sent weekly with single week measurements from Brooke Dejadon, a shellfish program specialist. The following information was taken from these datasets: Date (MM/DD/YYYY format), Site (UNH Pier or HHHR2), cell abundance of *Alexandrium* spp. (Alex; cells L⁻¹), *Pseudo-nitzschia* spp. both small (PN-small; cell width < 3µm; cells L⁻¹) and large (PN-large; cell width ≥ 3µm; cells L⁻¹) size classes. Microsoft Excel (Version 2205 Build 16.0.15225.20172) was used to copy this information into two .csv files, one for each location.

Minor data manipulation was performed on both data sets using Microsoft Excel equations to prepare the documents for later analysis using R. Three equations were used to separate the MM/DD/YYYY format into individual cells for month, day, and year. The equations used for this were ‘=MONTH(*insert cell number*)’, ‘=DAY(*insert cell number*)’, and ‘=Year(*insert cell number*)’. This created three individual columns that were added next to the

original date pulled from the times series data set. The data sets were updated every few months with new weekly data. New addition of data ceased September 2022 allowing for summer blooms to be taken into consideration during analysis.

Environmental Parameters Data Collection

To better identify potential environmental drivers influencing the observed population distributions of these two HAB species, data from the Piscataqua River Estuaries Partnership (PREP) was utilized. Several environmental parameters that affect phytoplankton growth and bloom formation were collected from various sampling locations (Table 1). The locations used vary in distance from the UNH pier site, progressing well into the Great Bay Estuary (Figure 1). This was done to retrieve as much environmental data that matched the cell abundance sampling days as possible. The environmental sampling sites are listed in the order in which the data was added into the UNH Pier data set. The order was determined by distance to the sampling site. For instance, GRBCML was used first and all possible matching dates were pulled. The next closest site used was BCHNWCNWCLF and any missing dates were filled in using this location. This was continued for all six sampling locations. After downloading each file as .csv files the same date equations used on the cell abundance data were used on these data files.

Table 1. Piscataqua River Estuaries Partnership Site Code (Latitude, Longitude), Environmental Parameters, and Date Range(s) Used

Site Code	Environmental Parameters	Date Range(s) Used
GRBCML (43.072361, -70.710303)	Chlorophyll a, Corrected for Pheophytin Water Quality	4/19/2017 - 12/4/2017
	Nitrogen, Nitrite (No2) + Nitrate (No3) As N Diss Water Quality	
	Phosphorus, Orthophosphate as P Dissolved Water Quality	
	Salinity Water Quality	1/1/2017 - 2/23/2018
	Temperature Water Water Quality	
	Solids, Suspended Total Water Quality	

BCHNWCNWCLF (43.067764, -70.713275)	Salinity Water Quality	5/29/2018 - 9/1/2021
	Temperature Water Water Quality	
04-SAG (43.050833, -70.770833)	Chlorophyll a, Corrected for Pheophytin Water Quality	7/2/2018 - 12/3/2018
	Nitrogen, Nitrite (No2) + Nitrate (No3) As N Diss Water Quality	
GRBULB (43.10738, -70.86337)	Chlorophyll a, Corrected for Pheophytin Water Quality	5/4/2018 - 6/30/2018
	Nitrogen, Nitrite (No2) + Nitrate (No3) As N Diss Water Quality	4/29/2020 - 12/8/2020
	Phosphorus, Orthophosphate as P Dissolved Water Quality	5/22/2018 - 12/8/2020
	Solids, Suspended Total Water Quality	4/11/2018 - 12/6/2019
	Salinity Water Quality	4/11/2018 - 4/11/2018 9/1/2018 - 12/1/2018 4/17/2019 - 4/30/2019 9/1/2019 - 12/10/2019 4/23/2020 - 5/31/2020 9/1/2020 - 12/9/2020
	Temperature Water Water Quality	4/11/2018 - 4/11/2018 9/1/2018 - 12/1/2018 4/25/2019 9/18/2019 - 12/6/2019 4/29/2020 - 5/27/2020 9/10/2020 - 11/20/2020
GRBOR (43.134, -70.911)	Chlorophyll a, Corrected for Pheophytin Water Quality	4/10/2018 - 4/10/2018 4/23/2019 - 12/4/2019 5/11/2020 - 12/7/2020
GRBGB (43.0722, -70.8694)	Nitrogen, Nitrite (No2) + Nitrate (No3) As N Diss Water Quality	4/10/2018 - 6/19/2018 4/23/2019 - 12/4/2019

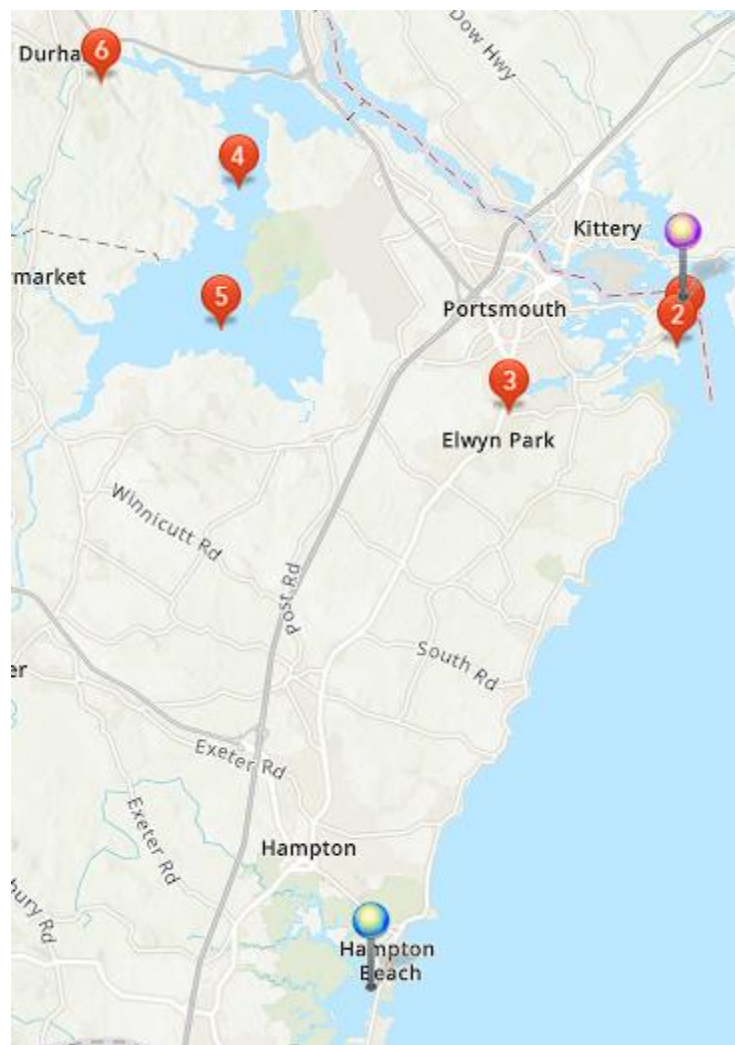


Figure 1. Map of the Piscataqua River Estuary Partnership sampling locations used for analysis created using ArcGIS. Points are numbered 1-6 in the order they were added to the CML cell abundance data set. Order is based off distance from the UNH Pier location. Nutrient locations are labeled with red location markers 1 = GRBCML, 2 = BCHNWCNWCLF, 3 = 04-SAG, 4 = GRBULB, 5 = GRBGB, and 6 = GRBOR. The UNH Pier location is labeled with a purple pin and the HHR2 location is labeled with a blue pin at the bottom of the map.

Data Analysis

The UNH Pier and all environmental data sets were uploaded to RStudio (2022.07.2 Build 576) to combine environmental data with the UNH Pier cell abundance data. Packages used for data manipulation were *dplyr*, *tidyr*, *formattable*, *forcats*, and *tidyverse*. Monthly averages of cell abundance and environmental variables were calculated and combined by date in one master data set. The data set was kept in wide format and only changed to long if analysis required it. A series of graphs examining the relationship between the *Alexandrium* spp. cell abundance and small and large *Pseudo-nitzschia* spp. at both the UNH Pier and HHR2

locations were created. Packages involved in the graphing procedures were *ggplot2*, *dplyr*, *tidyr*, *forcats*, *tidyverse*, *ggtext*, *ggpubr*, *formattable*, *scales*, *lubridate*, *lemon*, and *viridis*. Graphs went through several iterations before the finals were created and included in the results. A Kendall Rank Correlation was performed comparing the *Alexandrium* spp. cell concentrations to the *Pseudo-nitzschia* spp. cell concentrations, using each size class individually. The packages used for the Kendall Rank Correlation were *nlme*, *DescTools*, and *mblm* in addition to the above-mentioned graphing packages that were used for organizational purposes.

For environmental analysis only the UNH Pier location was used due to lack of environmental data surrounding the HHHR2 location. RStudio was used to create several graphs with cell abundances for each species and size class graphed against various environmental data. The environmental parameters used were temperature ($^{\circ}\text{C}$), salinity (ppt), nitrogen (mg L^{-1}), phosphorus (mg L^{-1}), nitrogen:phosphorus ratio, and chlorophyll *a* ($\mu\text{g L}^{-1}$). The packages used for this round of graphing were the same as the packages used for the cell abundance data. Graphs went through several iterations before being finalized and included in the results section. After graphing was complete statistical analysis was performed on the data. A partial least squares regression (PLS) was run followed by a variable importance in projection (VIP) as performed by Anderson and Harvey (2019). This was done to investigate which variables were important, if any, to the PLS model. Once those parameters were determined another Kendall Rank Correlation analysis was performed to ascertain the relationship and significance between cell abundance and relevant environmental parameters. The packages used to run the PLS, and VIP were *pls* and *plsvarsel*. The packages used to run the Kendall Rank Correlation were the same as before. All data analysis can be located here: <https://github.com/Tawinter/phyto-thesis>.

Results

Cell Abundance Trends

Both species and size classes display high variability in abundance over monthly and annual time scales, as well as between sampling locations. Results from analysis of temporal trends indicate that *Alexandrium* spp. has a seasonal pattern while *Pseudo-nitzschia* spp. does not (Figure 2). *Alexandrium* spp., is only observed from April to August at the HHHR2 location and from March to August at the UNH Pier location. In contrast, *Pseudo-nitzschia* spp., is observed throughout the entire year, from January through December. To understand how often the cells were observed at any concentration, the frequency of observation was calculated per year for each HAB species (Figure 3). Overall, *Alexandrium* spp. was observed 2-51% of the time with variation between the sites consisting of 10-43% at UNH Pier and 2-51% at HHHR2. Large *Pseudo-nitzschia* spp. was observed 32-65% of the time while being present 32-58% at UNH Pier and 37-65% at HHHR2. Lastly, small *Pseudo-nitzschia* spp. was seen in 22-90% of samples with a variation of 30-75% at UNH Pier and 22-90% at HHHR2 (Table 2). These percentages varied widely between years examined, and this high variability led to no clear trends in cell abundance temporally.

High variability was also observed in the total sum of HAB cells observed on an annual basis (Figure 5). In general, it was observed that *Pseudo-nitzschia* spp. has a higher cell count than *Alexandrium* spp. but neither species appears to be increasing or decreasing in their annual sum abundance. A correlation analysis between *Pseudo-nitzschia* spp. and *Alexandrium* spp. was conducted to determine if high cell abundance in one species was correlated with low cell abundance in the other species, potentially indicating competition (Figure 6). The results from a

Kendall rank correlation analysis, found that the correlation was weak and negative for all comparisons, and there was only a significant correlation between large *Pseudo-nitzschia* spp. and *Alexandrium* spp. at the UNH Pier location (large PN HHR2: $\tau = -0.053$, p-value = 0.703; small PN HHR2: $\tau = -0.024$, p-value = 0.873; large PN UNH Pier: $\tau = -0.432$, p-value = 0.022; small PN UNH Pier: $\tau = -0.034$, p-value = 0.842). At this location there was a higher cell abundance of *Pseudo-nitzschia* spp. and lower cell abundance of *Alexandrium* spp.

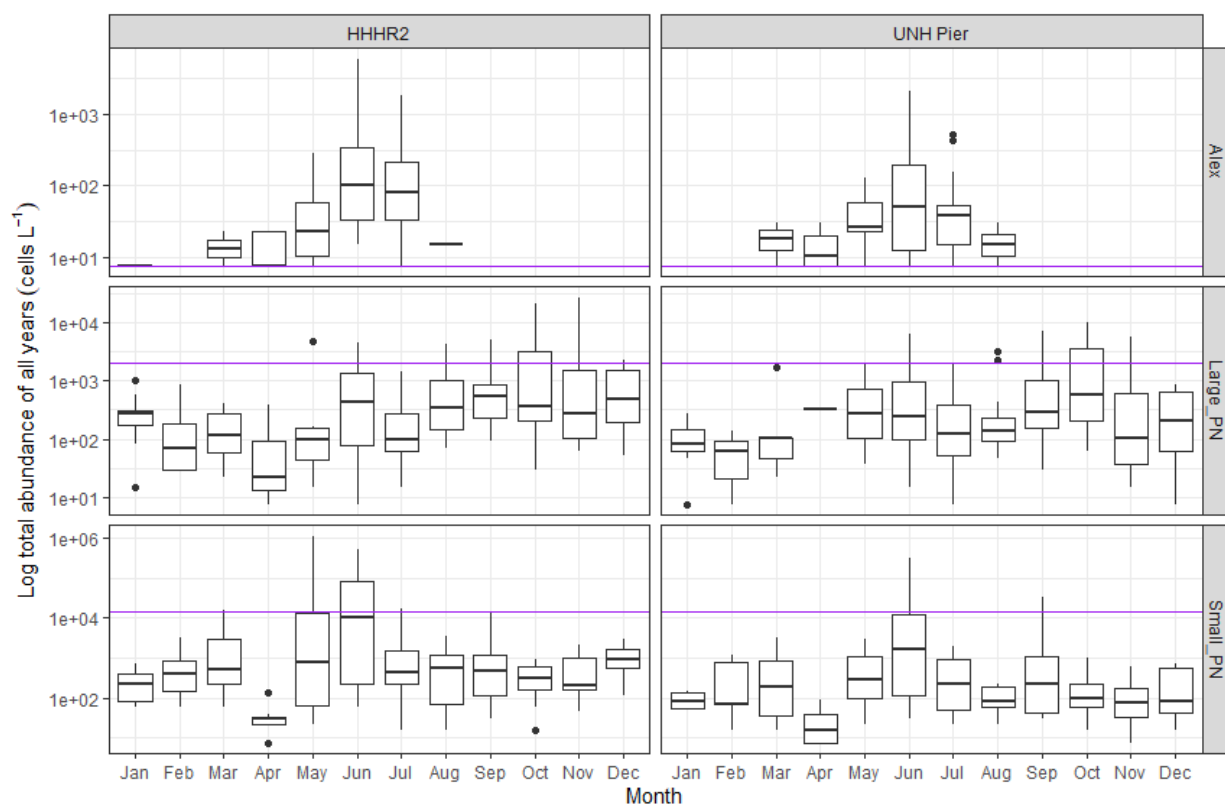


Figure 2. Monthly seasonal patterns for *Alexandrium* spp. (cells L⁻¹) and *Pseudo-nitzschia* spp. (cells L⁻¹) at both the UNH Pier and HHR2 sampling locations. Note, the data is graphed on a log scale due to high abundances of *Pseudo-nitzschia* spp. *Alexandrium* spp. is limited in growth from March to August at the UNH Pier location and April to August at the HHR2 location. *Pseudo-nitzschia* spp. displays the ability to grow from January to December. The horizontal bar within the box represents the median of the data while the vertical lines represent the max and min cell abundances. The purple line represents the log of the limit for each species in New Hampshire (Alex = 0.875 cells L⁻¹, Large_PN = 3.301 cells L⁻¹, Small_PN = 4.176 cells L⁻¹).

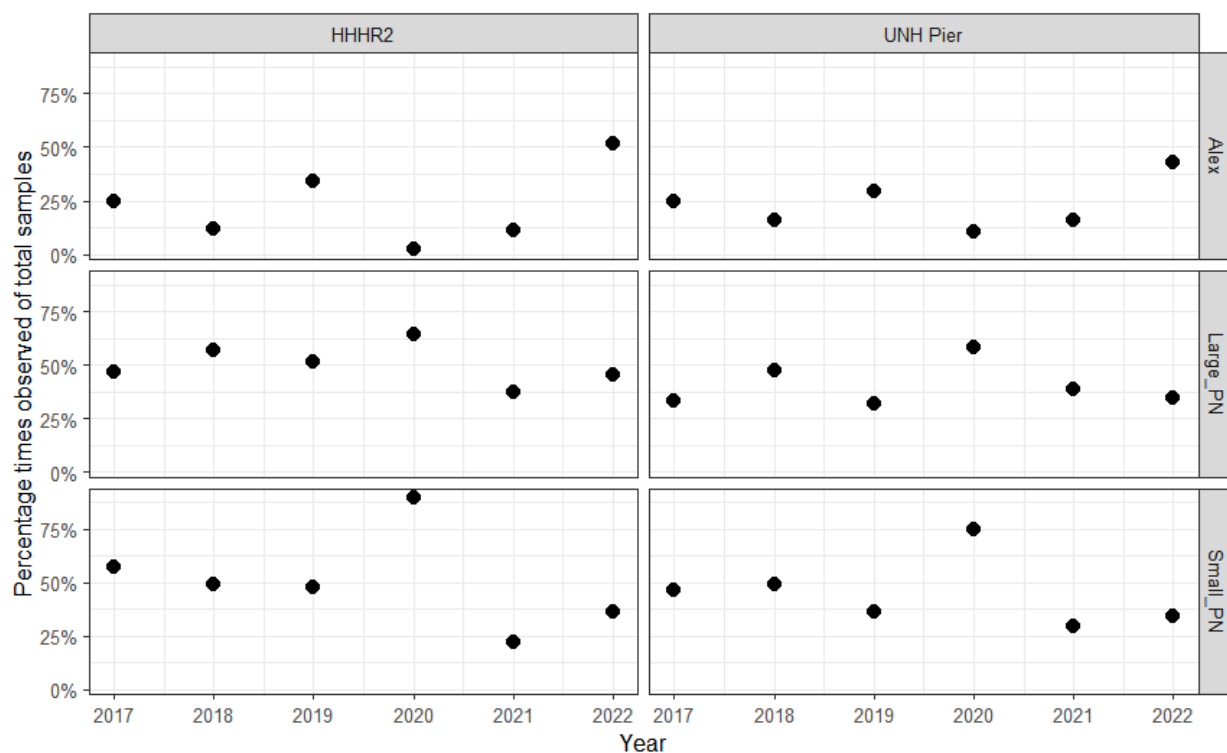


Figure 3. Frequency of observation (%) of *Alexandrium* spp. and *Pseudo-nitzschia* spp. at two sampling locations UNH Pier and HHHR2. Overall, *Alexandrium* spp. was observed 2-51% of the time, large *Pseudo-nitzschia* spp. was observed 32-65% of the time, and small *Pseudo-nitzschia* spp. was seen in 22-90% of the time.

Table 2. Detailed summary of observances, samples, and percentages that are displayed in Figure 3.

Year	Station	Species	Times Obs	Total Samples	Percent
2017	UNH Pier	Alex	11	45	24%
		lg PN	15	45	33%
		sm PN	21	45	47%
	HHHR2	Alex	11	45	24%
		lg PN	20	42	47%
		sm PN	24	43	57%
2018	UNH Pier	Alex	8	51	16%
		lg PN	24	51	47%
		sm PN	25	51	49%
	HHHR2	Alex	6	51	12%
		lg PN	29	51	57%
		sm PN	25	51	49%

2019	UNH Pier	Alex	13	44	30%
		lg PN	14	44	32%
		sm PN	16	44	36%
	HHHR2	Alex	15	44	34%
		lg PN	22	43	51%
		sm PN	21	44	48%
2020	UNH Pier	Alex	5	48	10%
		lg PN	28	48	58%
		sm PN	36	48	75%
	HHHR2	Alex	1	48	2%
		lg PN	31	48	65%
		sm PN	43	48	90%
2021	UNH Pier	Alex	7	44	16%
		lg PN	17	44	39%
		sm PN	13	44	30%
	HHHR2	Alex	3	27	11%
		lg PN	10	27	37%
		sm PN	6	27	22%
2022	UNH Pier	Alex	15	35	43%
		lg PN	12	35	34%
		sm PN	12	35	34%
	HHHR2	Alex	17	33	51%
		lg PN	15	33	45%
		sm PN	12	33	36%

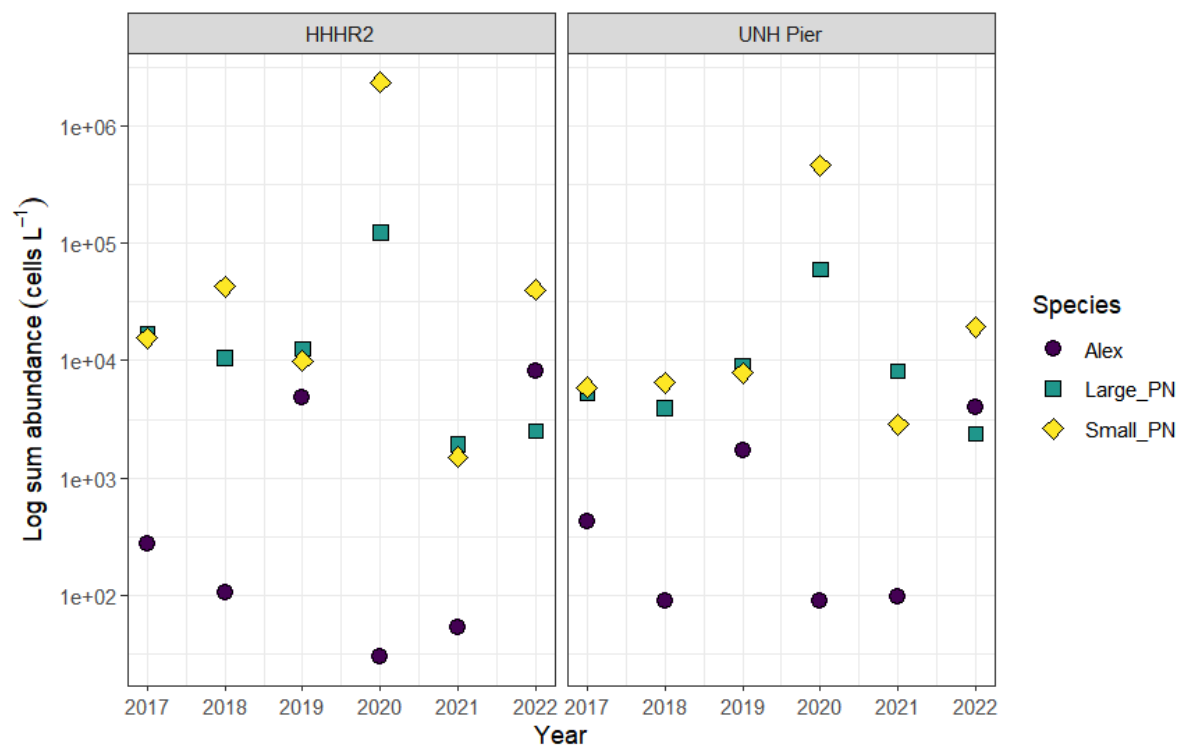


Figure 4. Sum cell abundances for *Alexandrium* spp. (cells L⁻¹) and *Pseudo-nitzschia* spp. (cells L⁻¹) at the UNH Pier and HHR2 sampling locations. The data is graphed on a log scale due to the high quantities of *Pseudo-nitzschia* spp.. In general, *Pseudo-nitzschia* spp. has higher cell abundances than *Alexandrium* spp. but there does not appear to be a trend of either species decreasing or increasing.

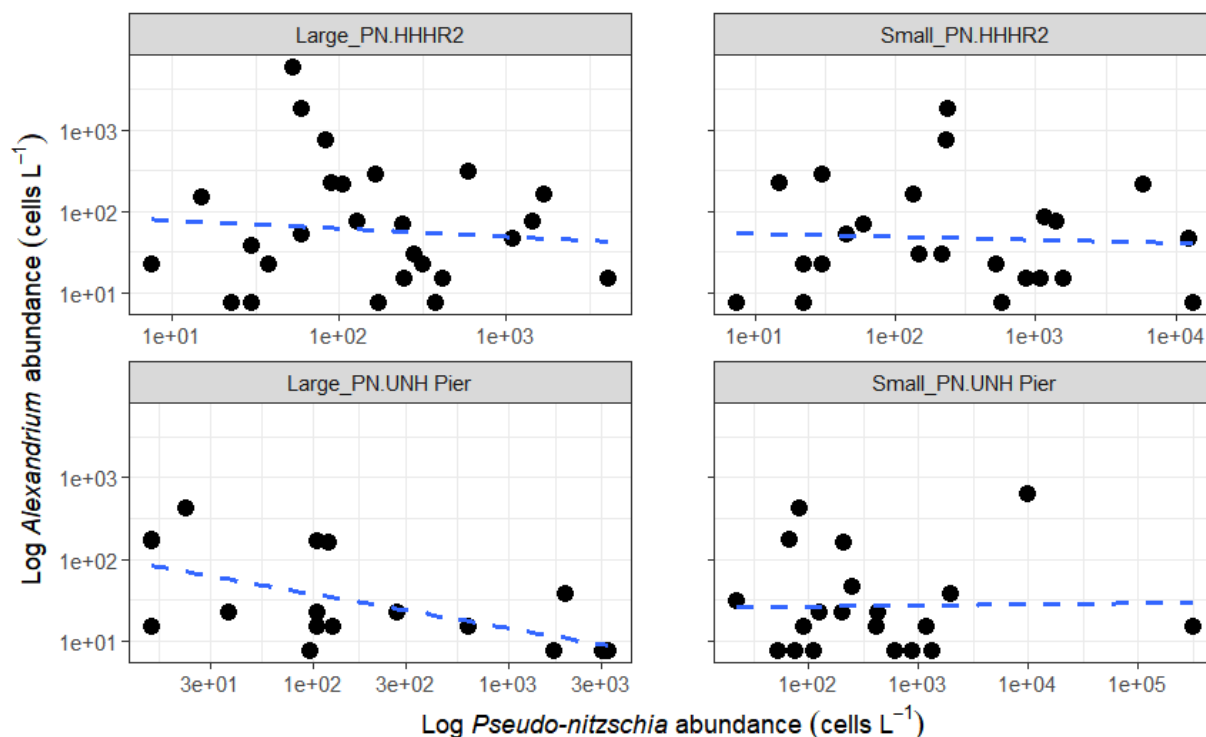


Figure 5. Correlations between the abundance of *Alexandrium* spp. (cells L⁻¹) and *Pseudo-nitzschia* spp. (cells L⁻¹). The four graphs display size class of *Pseudo-nitzschia* spp. and sampling location. There is very little correlation between the two species as also shown by the Kendall (τ_b) rank correlation analysis. Large PN HHHR2: $\tau = -0.053$, p-value = 0.703; small PN HHHR2: $\tau = -0.024$, p-value = 0.873; large PN UNH Pier: $\tau = -0.432$, p-value = 0.022; small PN UNH Pier: $\tau = -0.034$, p-value = 0.842.

Table 3. Number of *Alexandrium* spp. observations concurrent with *Pseudo-nitzschia* spp. presence. The observations are broken down into the year, station, and size class of *Pseudo-nitzschia* spp..

Year	Station	<i>Pseudo-nitzschia</i> spp. Size Class	# <i>Alexandrium</i> spp. observations
2017	UNH Pier	Small	1
		Large	1
	HHHR2	Small	5
		Large	5
2018	UNH Pier	Small	3
		Large	3
	HHHR2	Small	3
		Large	4
2019	UNH Pier	Small	5
		Large	5
	HHHR2	Small	8

		Large	10
2020	UNH Pier	Small	4
		Large	2
	HHHR2	Small	1
		Large	0
2021	UNH Pier	Small	3
		Large	2
	HHHR2	Small	0
		Large	0
2022	UNH Pier	Small	4
		Large	4
	HHHR2	Small	6
		Large	8

Environmental Trends

Only data from the UNH Pier sampling location was compared to environmental data as there was little to no data to compare to the HHHR2 sampling location. Additionally, only the years 2017 to 2021 were considered as the 2022 samples have not been added yet. Under all nutrient conditions *Pseudo-nitzschia* spp. had higher cell abundances than *Alexandrium* spp. There were variable trends for both species within each of the six environmental parameters (temperature, salinity, chlorophyll *a*, nitrogen, phosphorus, and nitrogen:phosphorus). Both species appeared to inhabit the same temperature range with a cut off at 5°C, with only *Pseudo-nitzschia* spp. showing a few exceptions lower than this in 2018. In terms of salinity, *Pseudo-nitzschia* spp. does appear to have a higher tolerance for lower salinity relative to *Alexandrium* spp. *Pseudo-nitzschia* spp. was observed in salinities as low as 18 ppt and more frequently between 18-28 ppt than *Alexandrium* spp.

For abundance in relation to chlorophyll *a* concentration, no clear differences between cell abundance and chlorophyll *a* were observed. One point was removed from both small and large size classes of *Pseudo-nitzschia* spp., at approximately 50 µg L⁻¹, to allow for less

compression of the data. For nitrogen, no *Alexandrium* spp. was observed above 0.13 mg L^{-1} , whereas there were several observations of PN at higher nitrogen concentrations. Additionally, a single outlier of 0.25 mg L^{-1} for the small and large size classes of *Pseudo-nitzschia* spp. was removed, to better observe the overall trend. For phosphorus, *Alexandrium* spp. is not observed above 0.020 mg L^{-1} while *Pseudo-nitzschia* spp. is observed far above this phosphorus value (Figure 10). The ratio of nitrogen:phosphorus was compared, but no distinct trends or observation cutoffs were noted.

The results from a partial least square regression analysis showed that phosphorus was the most important factor driving species abundance for all species examined (*Alexandrium* spp.: 1.897; small *Pseudo-nitzschia* spp.: 1.195; and large *Pseudo-nitzschia* spp.: 1.597). A Kendall Rank Correlation found that *Alexandrium* spp. was negatively but significantly correlated with phosphorus levels (τ : -0.513 and p-value: $2.206e^{-4}$) while large *Pseudo-nitzschia* spp. was significant and positively correlated (τ : 0.387 and p-value: $2.272e^{-3}$). Small *Pseudo-nitzschia* spp. was the least correlated with phosphorus and its correlation was not significant (τ : 0.122 and p-value: $3.295e^{-1}$). Beyond phosphorus, important environmental variables that were found to drive abundance were species specific including (in order of importance to that species): *Alexandrium* spp.: nitrogen (1.114); small *Pseudo-nitzschia* spp.: temperature (1.189) and N:P (1.075); and large *Pseudo-nitzschia* spp.: N:P (1.010).

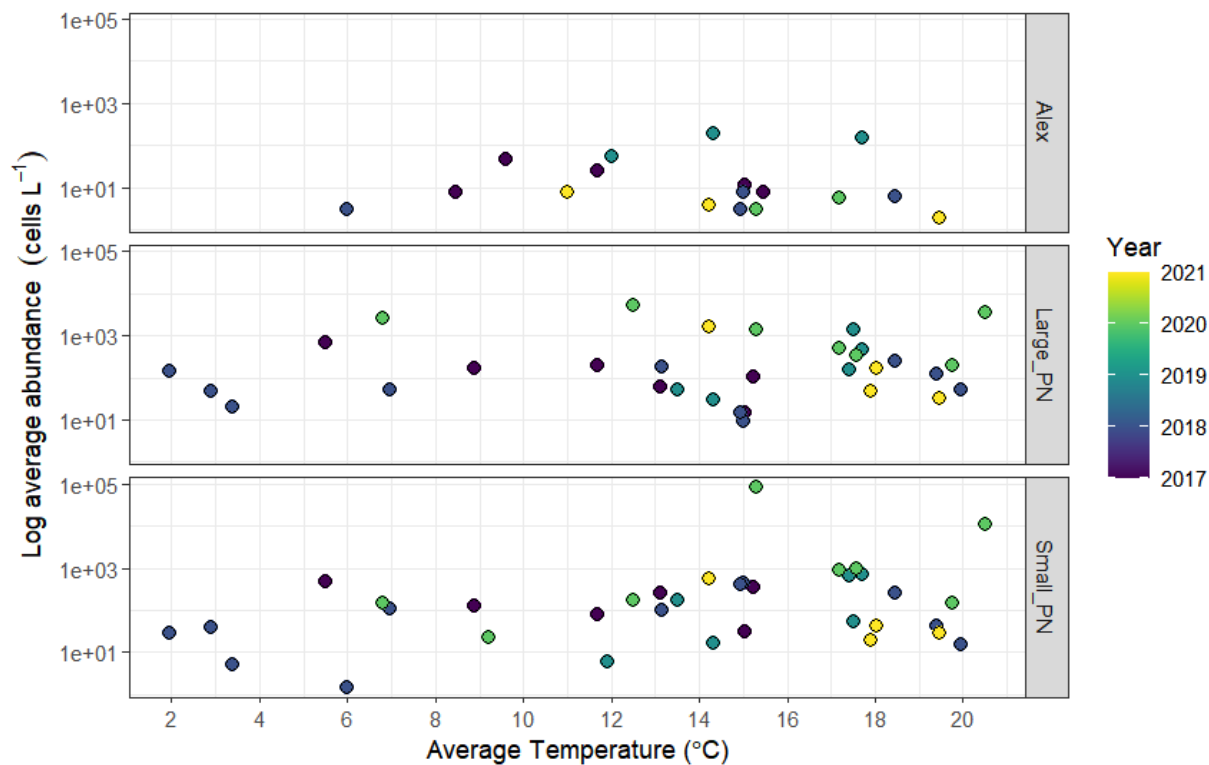


Figure 6. Abundance of *Alexandrium* spp. (cells L⁻¹) and *Pseudo-nitzschia* spp. (cells L⁻¹) in relation to temperature (°C) using monthly averages from 2017 to 2021. The species are shown on a log scale due to the high abundances of *Pseudo-nitzschia* spp.. Overall, each species appears to inhabit the same temperature range with only *Pseudo-nitzschia* spp. appearing below 5°C.

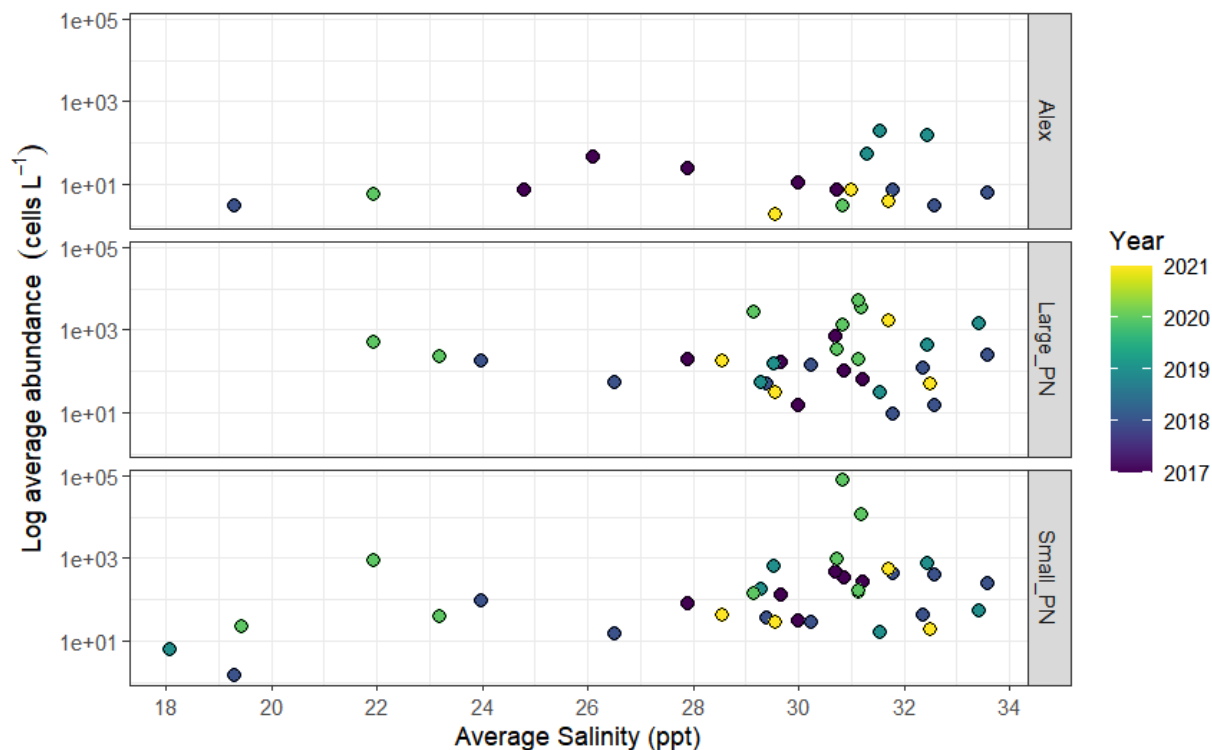


Figure 7. Abundance of *Alexandrium* spp. (cells L⁻¹) and *Pseudo-nitzschia* spp. (cells L⁻¹) in relation to salinity (ppt) from 2017 to 2021. Abundance is on a log scale due to high quantities of *Pseudo-nitzschia* spp.. *Pseudo-nitzschia* spp. does appear to tolerate lower salinities better than *Alexandrium* spp. *Pseudo-nitzschia* spp. appears at salinities as low as 18 ppt and more frequently between 18 and 28 ppt.

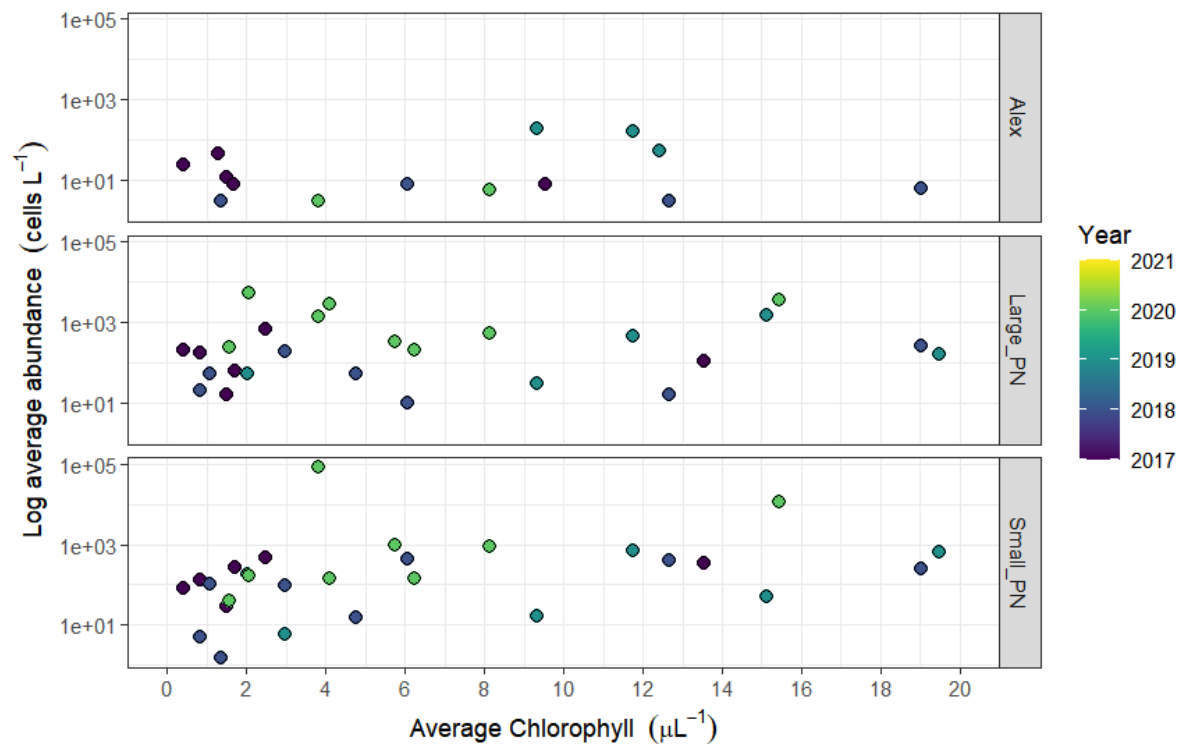


Figure 8. Abundance of *Alexandrium* spp. (cells L⁻¹) and *Pseudo-nitzschia* spp. (cells L⁻¹) in relation to chlorophyll *a* (μg L⁻¹) concentration from 2017 to 2021. One point was removed from large and small size classes that was close to 50 μg L⁻¹ for ease of viewing remaining data. No clear pattern was observed in the data.

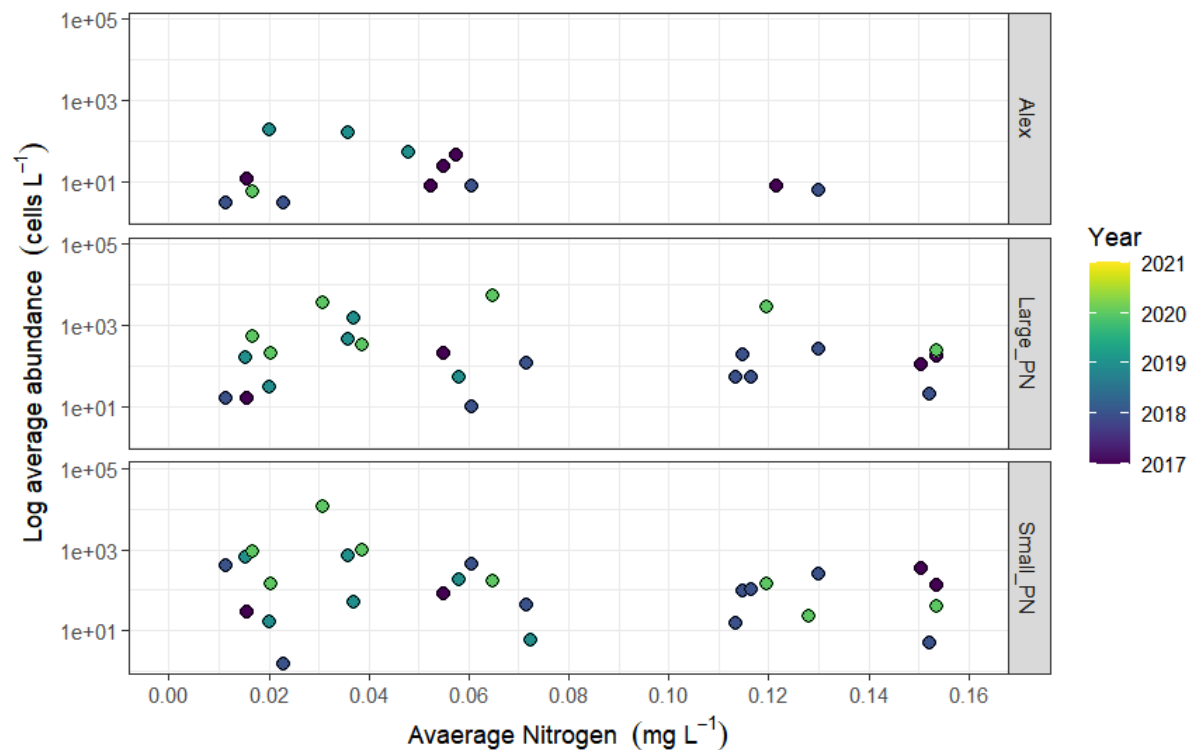


Figure 9. Abundance of *Alexandrium* spp. (cells L⁻¹) and *Pseudo-nitzschia* spp. (cells L⁻¹) in relation to nitrogen (mg L⁻¹) concentrations from 2017 to 2021. One outlier for both small and large *Pseudo-nitzschia* spp. was removed at 25 mg L⁻¹ to allow for a better view of the data. At higher levels (12+ mg L⁻¹) of nitrogen *Pseudo-nitzschia* spp. appears more frequently than *Alexandrium* spp. With *Alexandrium* spp. disappearing completely after 13 mg L⁻¹.

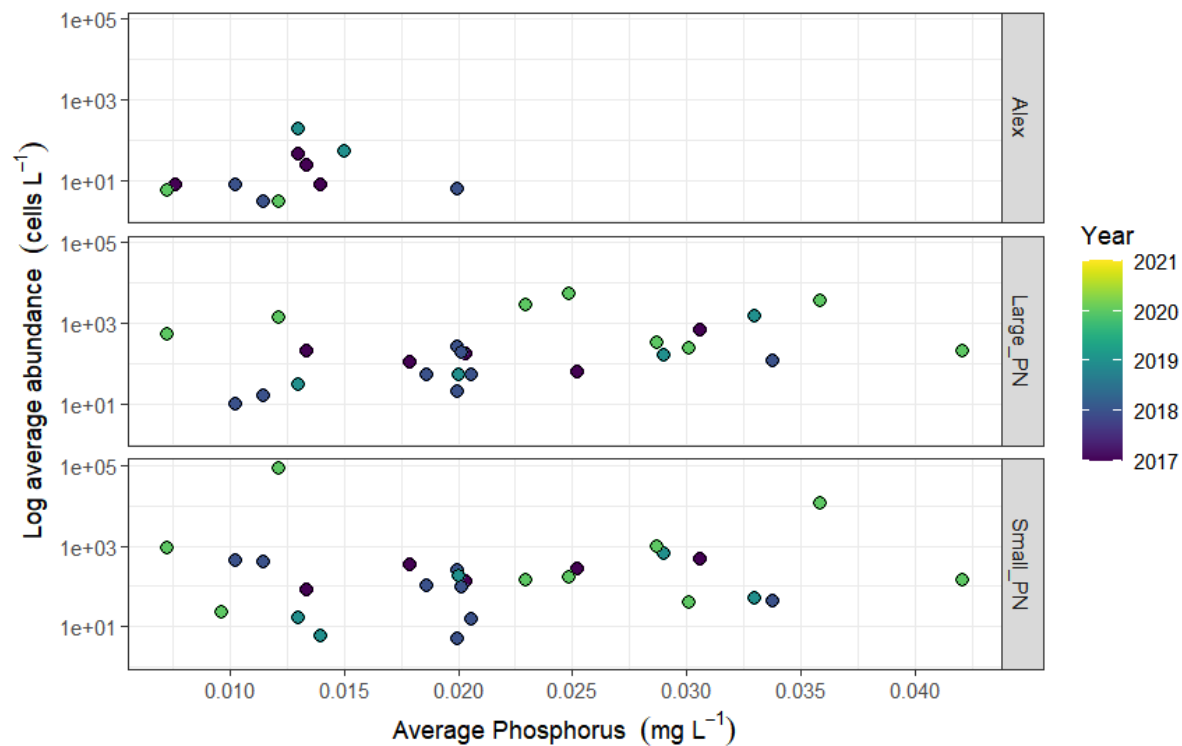


Figure 10. Abundance of *Alexandrium* spp. (cells L⁻¹) and *Pseudo-nitzschia* spp. (cells L⁻¹) from 2017 to 2021 in relation to phosphorus concentration (mg L⁻¹). *Alexandrium* spp. is not present above 0.020 mg L⁻¹ while *Pseudo-nitzschia* spp. is present far above this value.

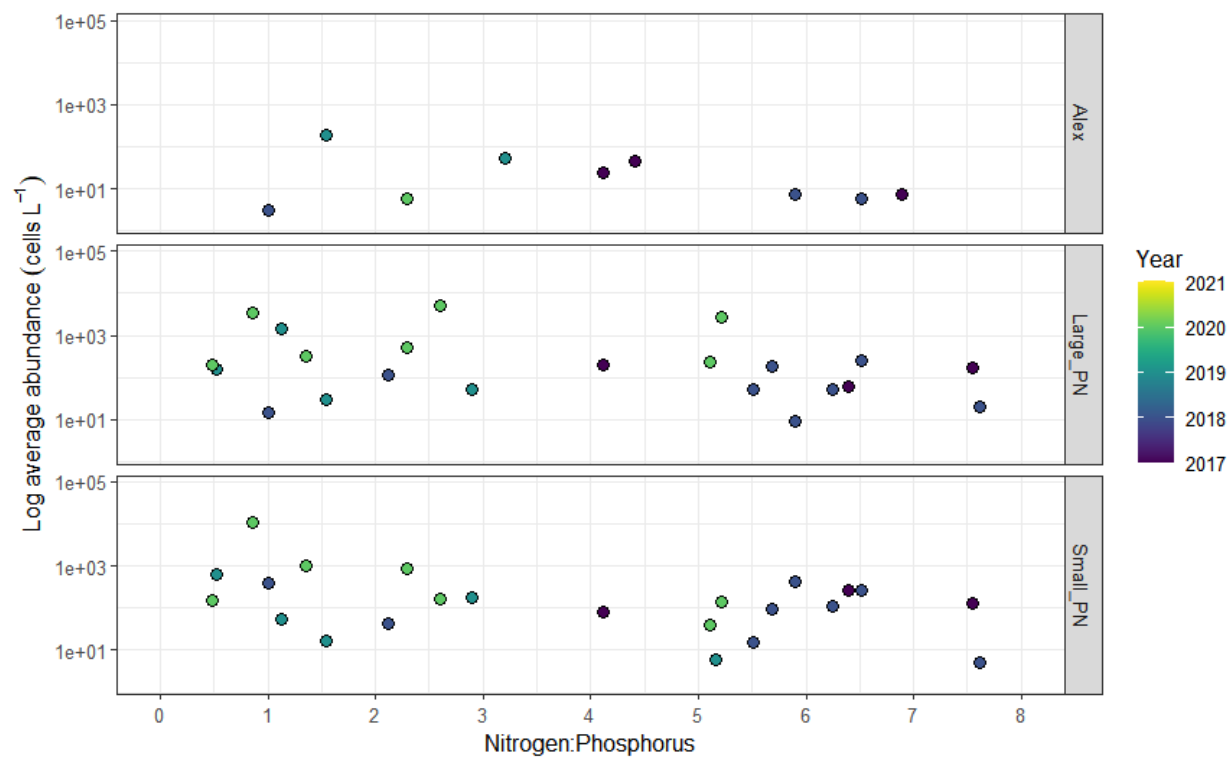


Figure 11. Abundance of *Alexandrium* spp. (cells L⁻¹) and *Pseudo-nitzschia* spp. (cells L⁻¹) in relation to the nitrogen:phosphorus ratio from 2017 to 2021. No trends or specific cut-offs were observed in this data.

Discussion

Cell Abundance Trends

There were clear seasonal patterns in the abundance of *Alexandrium* spp. and *Pseudo-nitzschia* spp., with *Alexandrium* spp. being more abundant during summer months, and *Pseudo-nitzschia* spp. being present in coastal New Hampshire throughout the year. In the Gulf of Maine, a seasonal succession of phytoplankton species is observable, including diatoms that typically dominate during the spring bloom, and dinoflagellates that increase starting in May (Kane, 2011). There is a myriad of factors that could be driving these observational differences, and many potential environmental drivers are explored in the next section.

Interestingly, there were no differences in the observational periods of the species between the UNH Pier and HHHR2 sampling locations. Except for *Alexandrium* spp. being observed one month earlier at the UNH Pier site than at the HHHR2 site. There are two potential explanations for the early appearance of *Alexandrium* spp. at UNH Pier before HHHR2. First, UNH Pier is located near the mouth of the Piscataqua River, which drains the Great Bay Estuary. The almost daily flushing of Great Bay Estuary contributes to high nutrient export (Flynn, 2008) thereby providing more nutrients to UNH Pier than the Hampton Estuary would provide to HHHR2. Second, it may be that local coastline currents cause *Alexandrium* spp. cells to be advected from UNH Pier down to HHHR2 causing a delay in when we see *Alexandrium* spp. accumulation at the HHHR2 location. In general, advection is found to distribute *Alexandrium* spp. from more northern waters to southern areas along the GOM coast (Zhang et al., 2020).

Comparing across time, high interannual variability in population abundance for both species was observed, however no increase in population abundance over time was observed for

either species. In general, HABs have been shown to increase in intensity with a warming ocean (Gobler et al., 2017). As the GOM is rapidly increasing in temperature, it may be expected that HAB species will follow suit by extending the length of blooms and expanding into new areas. While an increase over time is not observed for this study, it is prudent to highlight that this study only considered a time window between 2017 and 2022, which may be too short a time to clearly show annual trends in either species.

When the two species were observed to co-occur, there were also no trends between the abundance of *Alexandrium* spp. and *Pseudo-nitzschia* spp. This suggests that the presence of one species was not significantly beneficial or detrimental to the other. There are documented cases of both species having allelopathic capabilities (e.g. the production of harmful chemical compounds) that affect other species of phytoplankton. The production of allelochemicals may play an important role among HAB species in bloom formation (Legrand et al., 2003).

Alexandrium spp. has been shown to produce hemolytic toxins that inhibit the growth of various phytoplankton species (reviewed in Arzul et al., 1999; Fistarol et al., 2004; Yang et al., 2010). Interestingly, saxitoxin itself does not appear to cause allelopathic effects on other phytoplankton species, but its production could be correlated with allelopathic metabolites (Arzul et al., 1999; Fistarol et al., 2004).

Pseudo-nitzschia spp. has also demonstrated the ability to produce allelopathic compounds (Prince et al., 2013; Xu et al., 2015). It is unknown what exact secondary metabolite(s) cause the allelopathic effects but domoic acid does play a role in the allelopathic effects of *Pseudo-nitzschia* spp. Two different studies found that dissolved domoic acid has an allelopathic impact on co-occurring phytoplankton species, but the toxicity appears to be related to specific environmental conditions (Van Meerssche and Pinckney, 2017; Van Meerssche et al.,

2018). Therefore, the potential toxicity between these two species is likely more complicated than simple co-occurrence, and likely relies on environmental conditions that will mediate the interaction thereby influencing overall population abundance.

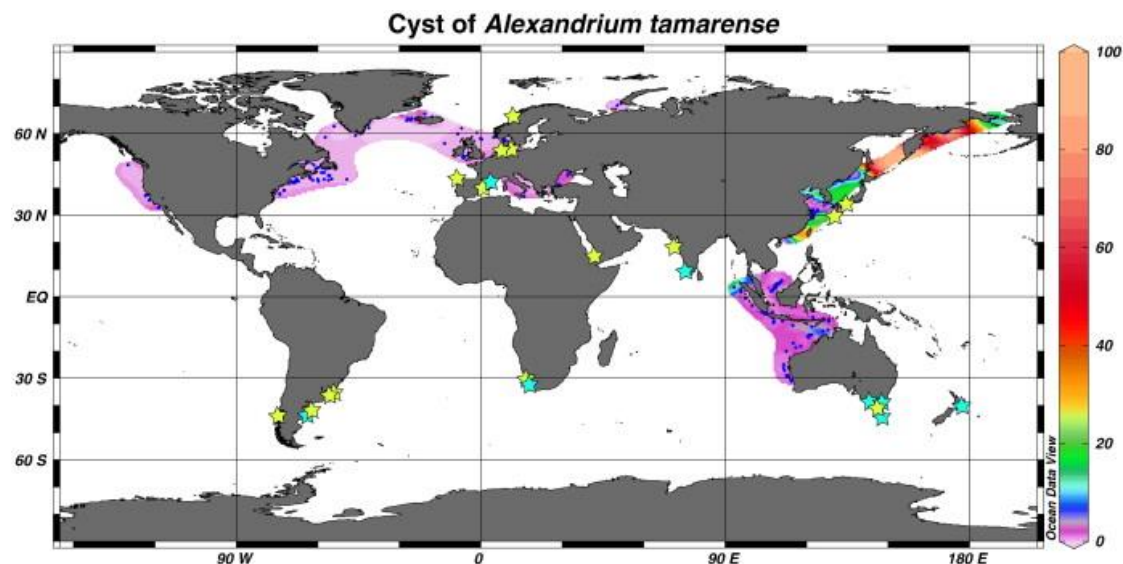


Figure 12. The above map was obtained from Zonneveld et al. (2013) and specifically displays the global distribution of *Alexandrium tamarens* and effectively demonstrates the ability of *Alexandrium* spp. species to grow worldwide.

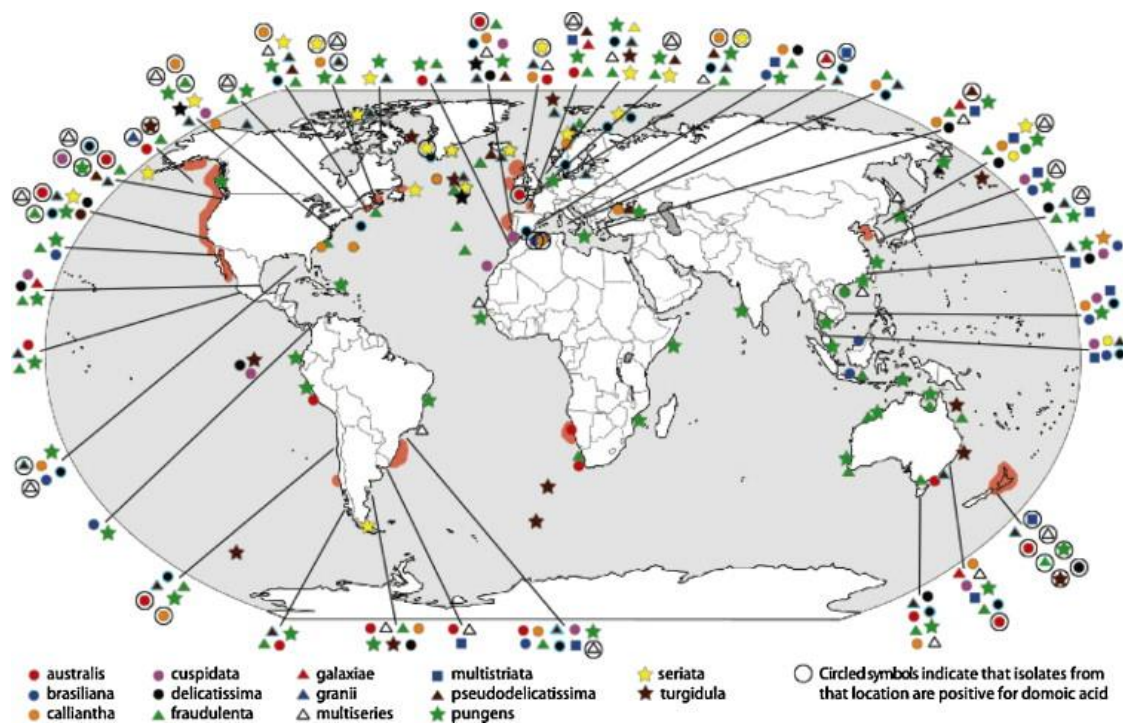


Figure 13. The above map was obtained from Trainer et al. (2012) and displays the global distribution of several species within the genus *Pseudo-nitzschia* spp. and whether they are toxic.

Environmental Trends

The observed patterns in *Alexandrium* spp. and *Pseudo-nitzschia* spp. were examined in relation to a variety of environmental factors. For both temperature and salinity, *Pseudo-nitzschia* spp. was observed at the lower extremes relative to *Alexandrium* spp. Previous laboratory work has also found that *Pseudo-nitzschia* spp. has a wider optimal growth temperature and salinities relative to *Alexandrium* spp. Bill et al. (2016) demonstrated that *Alexandrium* spp. can grow in temperatures ranging from 10-24°C and is only inhibited when temperatures are below 7.7°C or above 24°C. Additionally, *Alexandrium* spp. can grow in salinity ranges of 15-35 ppt with growth inhibition occurring outside of these ranges (Bill et al., 2016). On the other hand, *Pseudo-nitzschia* spp. has been documented in temperatures of -0.39°C, exceeding laboratory lows of 2°C (Almandoz et al., 2008). Similarly, in Louisiana-Texas coastal waters various species of *Pseudo-nitzschia* spp. have been found to grow in salinities ranging from 1 to >35ppt (Thessen et al., 2005). In the lab it has been observed that *Alexandrium* spp. decreases in growth rate as temperature increases but remains unaffected under higher salinity (Etheridge and Roesler, 2005). With the Gulf of Maine being predicted to increase in temperature by 4.5°C by the end of the century (Shin and Alexander, 2020) this could facilitate the takeover of *Pseudo-nitzschia* spp. as the dominant HAB in the GOM.

Like temperature and salinity, *Pseudo-nitzschia* spp. was present over a wider range of nitrogen and phosphorus concentrations relative to *Alexandrium* spp. In particular, *Alexandrium* spp. was only observed within a narrow phosphorus range ($>0.02 \text{ mg L}^{-1}$), whereas *Pseudo-nitzschia* spp. was observed in concentrations as high as 0.04 mg L^{-1} . Additionally, out of all the parameters examined using partial least squares analysis, phosphorus ranked as the most important factor influencing the distribution of each alga. These differences are also likely

attributable to the nutrient uptake kinetics of each species. (Seeyave et al., 2009). There is laboratory work investigating the response of these two algal species to a range of phosphorus concentrations. One previous study demonstrated that *Alexandrium* spp. is sensitive to low phosphorus concentrations and has relatively poor phosphorus uptake rates compared to other phytoplankton species (Jauzein et al., 2010). Similar research is not available for *Pseudo-nitzschia* spp., but previous studies have demonstrated that phosphorus limitation may be a cause for domoic acid production (reference: Terseleer et al., 2013).

Pseudo-nitzschia spp. has high affinity for nitrate and excels at nitrate uptake (Seeyave et al., 2009). In addition, *Pseudo-nitzschia* spp. has been shown to prefer nitrate (Cochlan et al., 2008), but is also able to grow well on other forms of inorganic and organic nitrogen (review: Auro and Cochlan, 2013) in comparison to *Alexandrium* spp. which does not grow on other forms (Seeyave et al., 2009). These two factors could allow *Pseudo-nitzschia* spp. to outcompete *Alexandrium* spp. when there is an influx of nitrogen to the system. During such times *Pseudo-nitzschia* spp. could quickly take up nitrate and then once depleted *Pseudo-nitzschia* spp. could switch to other forms of nitrogen to sustain the population (Seeyave et al., 2009). In another time series study looking at data from 1998 to 2018 in the GOM, it was shown that both dissolved inorganic nitrogen and dissolved inorganic phosphorus were very slightly increasing over time (Balch et al., 2022). If this continues then it may come down to previously mentioned factors to determine which species, if either, will outcompete the other.

Conclusion

Overall, *Alexandrium* spp. has a clear seasonal pattern while *Pseudo-nitzschia* spp. does not. This difference in observational window could be attributed to a myriad of factors, but one important distinction between these two HAB species that could impact when they are observed

in the field is their life cycles. Dinoflagellates, including *Alexandrium* spp., have an asexual and sexual phase consisting of continuous asexual reproduction until the population is triggered into undergoing sexual reproduction (Bravo and Figueroa, 2014). The environmental trigger is unknown, but sexual reproduction in *Alexandrium* spp. ends in the formation of a cyst. This cyst becomes negatively buoyant and sinks into the sediment allowing *Alexandrium* spp. to survive in less ideal environments. In the GOM, it has been documented that *Alexandrium* spp. forms cysts during a dormant period every winter, preventing it from being prevalent in the water column at that time (reviewed by Pilskaln et al., 2014). Additionally, research has found that when the cysts exit the sediment in the spring, they can fuel intense HABs (Anderson and Wall, 1978; Anderson et al., 2021; reviewed by Pilskaln et al., 2014). The life cycle of *Pseudo-nitzschia* spp. also includes an asexual and sexual phase, however at no point does it enter a resting phase.

Therefore, *Pseudo-nitzschia* spp. can proliferate through the water column during the resting cyst times of *Alexandrium* spp. When compared against nutrients, phosphorus appeared to be the most important nutrient within the data, and *Pseudo-nitzschia* spp. may have a competitive advantage over *Alexandrium* spp. in relation to nutrient uptake. However, *Alexandrium* spp. still may be able to sustain blooms due to the production and survival of resting cysts. These factors could allow *Pseudo-nitzschia* spp. and *Alexandrium* spp. to cohabitate in the GOM.

Within this study there were some limitations that had to be accepted for investigation of cohabitation between the species to be possible. The first was concerning the size classes of *Pseudo-nitzschia* spp. Background analysis was performed on the small and large size classes to determine if size was important. It was observed that there was a difference as to whether the size class was small or large leading to the size classes being kept separate. However, when looking at the small size class of *Pseudo-nitzschia* spp. it cannot be determined that all the

individuals belong within this size class. *Pseudo-nitzschia* spp. has a very wide range of sizes and over time, due to their reproduction cycle, the smaller half of the organism will continue to shrink and can cross over from the large to the small size class. To rectify this, it would be useful to be able to identify which species belong to each size class and which species are indeed producing HABs in the GOM. This would give us a better outlook as to what the state of HABs is for the future in the GOM.

Another limitation of the study is a long running problem in many areas of the world. This is lack of a cohesive dataset to study. The data used for analysis came from two different sources, NHDES and PREP. The NHDES has been collecting weekly since 2017 while PREP collects data intermittently at several different places along the coast and in estuaries. It was often impossible to align HAB count data and associated environmental parameter data. In addition, as can be seen in Figure 1, many of the PREP locations used for the UNH Pier sampling location of NHDES are further inland. The dataset was also short, only covering five years of time which may not be long enough to observe patterns and make predictions of HAB species. It would be beneficial to establish a long running data set that includes reliable environmental parameters, nutrients, and cell abundances of HAB species.

Along with the before mentioned limitations and ways to rectify them this study does leave open several questions about future trends for these two HAB species in the GOM. Not all species in a genus are toxic so it would be useful to ascertain which *Pseudo-nitzschia* spp. species are causing HABs here. The initial bloom in 2016 is said to have been caused by *P. australis* (Anderson et al., 2021) but it would be good to know if others are causing HABs and even if they are switching months/years in which each species blooms. We can then further study those species' interactions with *A. catenella* and see if one will dominate over the other. This can

also be expanded to include climate change data. For species such as *A. catenella* that do appear to be sensitive to higher temperatures this could make it possible for *Pseudo-nitzschia* spp. to push out *Alexandrium* spp. due to the extensive warming of the GOM. Having this information will allow for easier and more accurate model creation to better predict bloom formation in an ever-changing Gulf of Maine.

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