Initial environmental impacts of Piney Point wastewater discharge into Tampa Bay, Florida

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# Abstract

From March 30th to April 9th, 2021, 215 million gallons of legacy phosphate mining wastewater from the Piney Point facility were released into Tampa Bay (Florida, USA). An estimated 205 tons of total nitrogen were exported to Lower Tampa Bay, exceeding typical annual nitrogen load estimates in a matter of days. An initial phytoplankton response was observed in samples closest to the discharge site, with chlorophyll concentrations exceeding 50 ug/L. Macroalgae blooms of cyanobacteria (*Lyngbya* spp.) were observed beginning in May, with biomass estimated at 0.5 kg/m2 at some locations. Blooms of *Karenia brevis* were observed beginning in May and continuing through July. Reported fish kills tracked bloom concentrations, prompting local cleanup efforts to remove over 1700 tons of dead fish. Combined, these observations indicate abnormal conditions in Tampa Bay following release of wastewater from Piney Point, which is supported by comparison to the decades of baseline environmental monitoring data for the region.

*Key words*: nitrogen, phosphate mining, Tampa Bay, wastewater, water quality

# Introduction

Ecosystem management paradigms for estuaries of the Gulf Coast of Florida, USA are based primarily on the control of nutrient pollutants from stormwater and wastewater sources. The effects of nitrogen from source inputs are well understood as a limiting nutrient for the growth of algal blooms that can degrade water quality, having a negative effect on inter- and subtidal habitats ([Greening et al., 2014](#ref-Greening14); [Howarth and Marino, 2006](#ref-Howarth06); [Nixon, 1995](#ref-Nixon95); [Parker et al., 2012](#ref-Parker12)). Seagrasses in particular are a primary endpoint for assessing the impacts of nutrient pollution on water quality based on established relationships between nitrogen, phytoplankton growth, water clarity, and light requirements for seagrass species observed in nearshore environments ([Beck et al., 2018b](#ref-Beck18g); [Dixon and Leverone, 1995](#ref-Dixon95); [Greening and Janicki, 2006](#ref-Greening06); [Kenworthy and Fonseca, 1996](#ref-Kenworthy96)). Tampa Bay is the largest estuary in Florida located in a heavily urbanized watershed of nearly 3 million individuals. Historical gains in seagrass coverage in Tampa Bay have been achieved through public-private partnerships and consensus-based approaches to science applications that seek to limit the total nutrient loads into major bay segments ([Greening et al., 2016](#ref-Greening16); [Janicki and Wade, 1996](#ref-Janicki96)). Together, these efforts have resulted in the long-term recovery of Tampa Bay through a reduction in nitrogen loads, improvements in water clarity, and baywide expansion of seagrass coverage to benchmark targets established for the region ([Greening et al., 2014](#ref-Greening14); [Sherwood et al., 2017](#ref-Sherwood17)).

Ongoing threats and challenges to protecting water quality of Gulf Coast estuaries persist despite historical gains in environmental recovery. Although point-source inputs of nutrient loads from wastewater treatment plants into Tampa Bay have been reduced, non-point sources from wastewater and stormwater runoff contribute nutrients to the bay, particularly during the rainy season from June to September ([Janicki Environmental, Inc., 2017](#ref-Janicki17), [2008](#ref-Janicki08)). Atmospheric deposition of nutrients from coal-based power production and automobile traffic further contribute about one-quarter of the total nitrogen inputs to the bay ([Poor et al., 2013](#ref-Poor13)). Climate change stressors, such as sea level rise, changing rainfall patterns, and temperature alterations, may further perturb ecosystem dynamics and assimilative capacity by reducing system resilience to nutrient inputs ([Burke, 2017](#ref-Burke17); [Sherwood and Greening, 2014](#ref-Sherwood14)). Many of these challenges are addressed by ongoing efforts of the US EPA National Estuary Program to implement a science-based resource management plan for the Bay ([N. O’Hara, Shafer Consulting, Inc., 2017](#ref-Ohara17)). The Tampa Bay Estuary Program has been instrumental in coordinating efforts among local and regional partners to address legacy pollutants and current threats to the long-term protection of bay resources ([Greening et al., 2016](#ref-Greening16), [2014](#ref-Greening14)).

Wastewater byproducts from mining are a global threat to the quality of surface and groundwater resources worldwide ([Hudson-Edwards et al., 2011](#ref-Hudson11); [Tayibi et al., 2009](#ref-Tayibi09)). Fertilizer is produced through the “wet process” reaction to create phosphoric acid by treating mined phosphate rock with sulfuric acid ([Burnett and Elzerman, 2001](#ref-Burnett01); [Pérez-López et al., 2016](#ref-Perez16)). The process generates large amounts of waste, creating approximately one unit of phosphoric acid per five units of waste precipitate, or phosphogypsum (CaSO HO). Impurities, contaminants, and radionuclides exist in phosphogypsum, making it commercially invaluable and the resulting waste is typically stored on-site in large earthen stacks (gypstacks) or holding ponds ([Burnett and Elzerman, 2001](#ref-Burnett01)). The stacks are usually near distribution centers where fertilizer is shipped elsewhere, such as port facilities close to coastal resources or population centers ([Beck et al., 2018a](#ref-Beck18)). There are obvious environmental and human health risks associated with these stacks, primarily through controlled or uncontrolled discharge to surface waters or groundwater contamination through leaching from unlined or poorly maintained stacks. Examples exist worldwide demonstrating the potential harm of these facilities on the environment ([Beck et al., 2018a](#ref-Beck18); [El Zrelli et al., 2015](#ref-elzrelli15); [Pérez-López et al., 2016](#ref-Perez16); [Sanders et al., 2013](#ref-Sanders13); [Tayibi et al., 2009](#ref-Tayibi09)).

The geology of central Florida is rich in phosphates that have supported a multi-billion dollar mining industry for fertilizer used in food production ([Henderson, 2004](#ref-Henderson04)). By 2001, an estimated 40 million tons of phosphogypsum were created each year in northern and central Florida ([Burnett and Elzerman, 2001](#ref-Burnett01)). Currently, seventeen phosphogypsum stacks (two active, five inactive, ten closed, [Florida Department of Environmental Protection](https://geodata.dep.state.fl.us/datasets/6277c3b1eeae4a818f8683fc29e6b35b_0/about)) exist in the Tampa Bay watershed with no long-term plan for closure or disposal of waste to prevent impacts to the environment. The Piney Point facility located in Palmetto, Florida is a large phosphogypsum stack established located less than two miles from the shore of Tampa Bay and near two aquatic preserves ([Henderson, 2004](#ref-Henderson04)). Bankruptcy of the mining company responsible for the stack in 1999 transferred ownership to a third-party, with oversight by the Florida Departmental of Environmental Protection (FDEP). Decreasing holding capacity of the ponds with seasonal rain events, tropical storms, and storage of dredging material from nearby Port Manatee have contributed to degradation of the facility. Discharges of wastewater from the stacks occurred in the early 2000s and 2011 to nearby Bishop Harbor connected to Tampa Bay ([Garrett et al., 2011](#ref-Garrett11); [Switzer et al., 2011](#ref-Switzer11)). Recently, FDEP authorized an [emergency order](https://floridadep.gov/sites/default/files/21-0323.pdf) on March 30th, 2021 to release wastewater from the stacks directly into lower Tampa Bay to prevent catastrophic failure of the berms supporting the holding ponds.

This paper provides an initial assessment of environmental conditions in Tampa Bay over five months following the recent release of 215 million gallons of legacy phosphate mining wastewater in April, 2021. The goal is to describe the results of monitoring data of surface waters collected in response to the discharge event to assess deviation of current conditions relative to long-term, seasonal records of water quality, phytoplankton, and seagrass/macroalgae datasets available for the region. We provide a brief overview of the history of the Piney Point facility, including past wastewater releases and impacts observed in Tampa Bay. A timeline of events in 2021 is also provided, which is supported by the results from 2021 response-based monitoring of conditions in and around Piney Point. The results of this study provide an initial documentation of impacts to the natural resources of Tampa Bay that can be used to inform long-term assessments of acute wastewater discharge events on the environmental quality of the region. We focus primarily on the perspective of the Tampa Bay Estuary Program in its role in coordinating monitoring and evaluating short-term impacts, particularly in the context of long-term management goals that leverage resources from existing partnerships among local resource management institutions.

# Methods

## History of Piney Point

The Piney Point facility in Palmetto, Florida was established in 1966 by the now defunct Borden Chemicals company near Port Manatee on the southeast shore of lower Tampa Bay. Port operations were primarily for export of phosphate production by the plant. Numerous environmental issues were observed in these early years, including suspected wastewater contamination in nearby Bishop Harbor, groundwater contamination from industrial solvents, and air pollution from plant emissions ([Henderson, 2004](#ref-Henderson04)). Ownership of the facility was transferred to different companies over the course of operation and in 1993 the plant was acquired by Mulberry Phosphates, Inc., which also owned a mining facility in Mulberry, Florida to the north. In 1997, 54 million gallons of phosphate mining process water from the Mulberry plant spilled into the Alafia River, the second largest tributary to Tampa Bay, killing 1.3 million fishes and impacting 153 hectares of wetland habitat.

The Mulberry corporation filed for bankruptcy in 2001, transferring regulatory oversight of the Piney Point facility to FDEP. Although phosphate production no longer occurred at the site, focus over the next twenty years centered on containment and treatment of wastewater on-site to minimize environmental impacts. Despite these efforts, reduced holding capacities and degraded physical integrity of the holding ponds likely contributed to discharge events to surficial and ground waters. Tropical storm Gabrielle in 2001 produced 13 inches of rain, causing over 10 million gallons of wastewater from the stack to be released into Bishop Harbor, with an estimated 15.4 tons fo nitrogen (pers. comm. D. Eckenrod, USEPA). Species of phytoplankton associated with harmful algal blooms were observed around this time ([Garrett et al., 2011](#ref-Garrett11)). From November 2003 to October 2004, treated process water from Piney Point was discharged to Bishop Harbor to reduce the likelihood of an uncontrolled spill. [Switzer et al.](#ref-Switzer11) ([2011](#ref-Switzer11)) reported minimal impacts to nekton communities, although an increase in macroalgal blooms of *Ulva spp.* and *Gracilaria spp.* was observed as a potential indication of nutrient eutrophication. Around the same time, 248 million gallons of wastewater from Piney Point were barged 120 miles offshore to the Gulf of Mexico to reduce strain on holding capacity of storage ponds ([Hu and Muller-Karger, 2003](#ref-Hu03)). Efforts for onsite treatment were also increased during this period to increase pH, remove heavy metals, and reduce nutrient concentrations to minimize impacts of discharge to local areas.

HRK Holdings, LLC (hereafter, HRK) acquired Piney Point in August 2006 through an administrative agreement with FDEP. This agreement transferred responsibility of the site to HRK with the intention that any future uses must protect and be compatible with the integrity of stack closure and long-term care. In 2011, HRK agreed to the storage of 1.5 million cubic yards of dredged material and seawater from Port Manatee to improve shipping capacity at the port (i.e., Berth 12 construction). This material was added to the existing holding pond at Piney Point, further reducing holding capacity leading to an emergency discharge that released 169 million gallons of dredged saltwater slurry and 3.5 tons of nitrogen to Bishop Harbor. The dredging and deposit of slurry at Piney Point continued following structural fortifications to the holding stacks to ensure integrity with additional loadings. HRK maintains ownership and responsibility of the site to present day, with oversight by FDEP.

Discharges from Piney Point did not occur again until 2021. Leakages from a tear in the plastic liner of the southern holding pond (NGS-S) were suspected when water quality samples with a similar conductivity as the wastewater were detected at onsite seepage interceptor drains. The NGS-S holding pond held 480 million gallons of wastewater, as a mixture of remnant process water from phosphate production and seawater from port dredging operations. Water quality parameters of NGS-S measured in 2019 were well above baseline conditions typical of surface waters in Tampa Bay (Table 1), particularly for total phosphorus (160 mg/L) and total nitrogen (230 mg/L). Due to public safety and property concerns over catastrophic failure of the holding walls, an [emergency order](https://floridadep.gov/sites/default/files/21-0323.pdf) was issued by FDEP on March 29th for HRK to begin release of wastewater from the stack into Tampa Bay to reduce physical strain on the stacks. Unlike past discharges from the site, HRK was authorized to release wastewater through a stormwater management system (under NPDES permit) that emptied at Port Manatee. This was done with the assumption that backwater habitats (e.g., Bishop Harbor) may be spared the impacts of additional effluent from the site. From March 30th to April 9th, approximately 215 million gallons of wastewater were released to lower Tampa Bay. Over this ten day period, an estimated 205 tons of nitrogen were delivered to the bay, exceeding the annual assimilative nutrient capacity of lower Tampa Bay in a matter of days ([Tampa Bay Nitrogen Management Consortium, 2010](#ref-tbep03a10)).

## Monitoring response to the emergency discharge

Monitoring of the natural resources of Tampa Bay in response to the wastewater release at Piney Point began in April, 2021 and continued over the following months. These data were collected through a coordinated effort, facilitated in part by the TBEP. Monitoring agencies and local partners that collected data included FDEP, Environmental Protection Commission (EPC) of Hillsborough County, Parks and Natural Resources Department of Manatee County, Pinellas County Division of Environmental Management, Fish and Wildlife Research Institute of the Florida Fish and Wildlife Conservation Commission (FWC), City of St. Petersburg, TBEP, Sarasota Bay Estuary Program, Environmental Science Associates, University of South Florida, University of Florida, and New College of Florida. Monitoring efforts focused on a suite of parameters expected to respond to increased nutrient loads into the bay, which included water quality sampling (laboratory processing of discrete samples and *in situ* measurements), phytoplankton cell counts, and seagrass and macroalgae transect surveys (Figure 1). Additional samples for contaminants (e.g., heavy metals), benthic sediment, and nekton surveys were also conducted but they are not reported here.

Established laboratory and field sample protocols for all survey methods were based on an [Interagency Monitoring Project Plan](https://drive.google.com/drive/u/0/folders/1oBGvjdve-Gpo4Kn3Ovn8a8-yVoP25eec) maintained by the TBEP in agreement with USEPA standards and those of the inter-agency partners. To the extent possible, data quality objectives followed guidelines outlined in the TBEP Data Quality Management Plan ([E.T. Sherwood, G. Raulerson, M. Beck, M. Burke, 2020](#ref-tbep1620)). Many of the local partners also participate in the Southwest Florida [Regional Ambient Monitoring Program](https://tbep.org/our-work/boards-committees/technical-advisory-committee/#ramp) that ensures similar standards and protocols are followed in the collection of monitoring data, including routine cross-reference of samples between laboratories to check precision of measured values. Discrete water quality samples were taken primarily from surface grabs by boat and processed by the respective laboratories of each participating agency. For this paper, we focus on parameters related to the nutrient management paradigm for the bay and the expected phytoplankton response form inorganic nitrogen entering the day. This included evaluation of total nitrogen (mg/L), total ammonia nitrogen (NH + NH, mg/L), nitrate/nitrite (NO + NO, mg/L), total phosphorus (mg/L), orhophosphate (PO), and chlorophyll-a (ug/L) concentrations. Samples for pH, salinity (psu), temperature (C), and dissolved oxygen saturation (%) are also evaluated given the role these parameters can have as indicators of wastewater contamination (pH), physical drivers of primary production (salinity, temperature), and indicators of primary production and respiration (dissolved oxygen). Overall, sample effort was variable given agency resources at the time of the discharge event and over the next few months. As appropriate, water quality data were aggregated at the weekly scale and by major areas of interest (Figure 1a) given the hypothesized impacts of the discharge relative to Piney Point.

Phytoplankton samples were also collected by multiple partners and included a mix of quantitative samples enumerating major taxa by cell concentrations and qualitative presence/absence samples. Taxa were aggregated into major groups of interest for Tampa Bay, with a focus on diatoms (Bacillariophyta and other centric taxa) as common primary producers observed throughout the growing season and species associated with harmful algal blooms (HABs) as a potentially adverse outcome of these species outcompeting others in response to nutrient inputs from Piney Point. Evaluation of HABs data included specific focus on the red tide organism *Karenia brevis* and *Pyrodinium bahamense* that can occur in the bay depending on salinity and temperature conditions during the growing season. Occurrence of both these species has historically been spatially distinct, with *K. brevis* originating in the Gulf oc Mexico and occuring in higher salinity portions of the bay, whereas *P. bahamense* has been observed consistently each year since 2008 in Old Tampa Bay (northwest segment) during the summer. Data for *K. brevis* were also obtained from event-based monitoring samples collected by FWC and available from the Harmful Algal BloomS Observing System ([HABSOS](https://habsos.noaa.gov/)). Because of the increased occurrence of red tide samples in July following the emergency discharge, fish kill reports from FWC were also evaluated in relation to key municipalities (Tampa, St. Petersberg) impacted by the event. Fish kill reports were obtained from the FWC [online database](https://public.myfwc.com/fwri/FishKillReport/searchresults.aspx).

Seagrass and macroalgae transect samples were collected approximately biweekly at locations around Piney Point starting in April. Each year, the TBEP coordinates inter-agency sampling among regional partners at over sixty fixed locations throughout the bay. Because of the time-sensitive nature of the potential impacts of wastewater on seagrasses near Piney Point, the sampling protocol used at the routine monitoring locations was modified as a “rapid” design to sample seagrasses and macroalgae along a fifty meter transect at several of the long-term monitoring sites, as well as new locations selected along the shore and small subembayments (e.g., Bishop Harbor) to provide a more comprehensive coverage of the seagrass community near Piney Point. Seagrasses and macroalgae were identified and abundances were estimated using Braun-Blanquet coverage within a 50 cm quadrat at 10m distances along each transect. Dominant seagrass species in the bay include *Halodule wrightii*, *Syringodium filiforme*, and *Thalassia testudinum*. Other seagrass species (i.e., *Halophila spp.*, *Ruppia maritima*) were also observed but were present at much lower abundances and were not evaluated herein. Macroalgae taxa were aggregated by major group (i.e., red, green, and cyanobacteria) based on expected responses to nutrient pollution. Seagrasses and macroalgae abundances were converted to frequency occurrence estimates (i.e., number of locations present divided by total locations sampled) at the transect scale or within major areas (Figure 1a) depending on the analysis described below.

## Long-term monitoring data

Water quality data in Tampa Bay have been collected at fixed sampling sites since 1974 by the Environmental Protection Commission of Hillsborough County. These data include samples at 45 stations used by the TBEP to assess annual progress towards programmatic goals and regulatory thresholds applicable for each of the major segments in Tampa Bay. Discrete water samples are collected monthly at mid-depth and processed in the laboratory immediately after collection. Similarly, the Parks and Natural Resources Department of Manatee County have been collecting data in Tampa Bay at locations south of Piney Point and south of the main dredged channel of Tampa Bay (approximate longitudinal axis of the bay). The sampling design is similar to the EPC data, with the exception that sites are sampled approximately every three months but at a higher spatial density per unit area. Additionally, the period of record for monitoring data from Manatee County began in 1996.

Long-term water quality monitoring data from Hillsborough and Manatee counties were used to establish baseline conditions for the major areas of interest in Figure 1a to compare with the response monitoring data described above. Station data from Hillsborough County were obtained for the middle and lower segments of Tampa Bay. Station data from Manatee County were obtained for areas in lower Tampa Bay, Terra Ceia Bay, Anna Maria Sound, and northern Sarasota Bay. For the same water quality parameters noted above (i.e., nitrogen, phosphorus, pH, temperature, salinity), observations at each monitoring station were averaged for each month across years from 2006 to 2020. This period represents a “recovery” stage for Tampa Bay where water quality conditions were much improved from historical conditions during a more eutrophic period and when seagrass areal coverage was trending towards and above a 1950s benchmark target of 38,000 acres ([Greening et al., 2014](#ref-Greening14); [Sherwood et al., 2017](#ref-Sherwood17)). For each month, the mean values +/- 1 standard deviation for each parameter at each station were quantified and used as reference concentrations relative to results at the closest monitoring station that was sampled in response to Piney Point. This comparison was made to ensure that the response data were evaluated relative to stations that were spatially relevant (e.g., long-term conditions in Terra Ceia Bay are not the same as those in middle Tampa Bay) and seasonally-specific (e.g., historical conditions in April are not the same as historical conditions in August). Spatial matching of each response monitoring station relative to the long-term monitoring stations was accomplished using the “st\_nearest\_feature()” function from the sf R package ([Pebesma, 2018](#ref-Pebesma18)). In some cases, the nearest long-term station did not include data for a every monitoring parameter at a response location and the next closest station was used as reference. Long-term water quality data are available from the University of South Florida Water Atlas (<https://wateratlas.usf.edu/>).

## Data Analysis

The R statistical programming language (v4.0.2) was used to import, synthesize, and analyze all datasets provided by multiple partners ([R Core Team, 2020](#ref-RCT20)). Partner data were uploaded or entered manually as Google spreadsheets, where they were imported into R using the googlesheets4 ([Bryan, 2020](#ref-Bryan20)) and googledrive ([D’Agostino McGowan and Bryan, 2020](#ref-DAgostino20)) R packages. The suite of R packages available in the tidyverse ([Wickham et al., 2019](#ref-Wickham19)) were used to wrangle the data into an appropriate format for analysis. The tbeptools R package ([Beck et al., 2021](#ref-Beck21)) was used to import and summarize long-term monitoring data for Tampa Bay, specifically the EPC water quality data and seagrass transect database.

Quantitative assessments of trends included boxplot summaries, principal components analysis (PCA), Spearman rank correlations between pairs of variables, and multiple comparison tests to assess trends between months. Assessments were first evaluated only on total nitrogen, chlorophyll-a, and secchi disk depth as a general analysis of potential patterns in eutrophication following wastewater release. Further analyses were conducted to compare all data types together, including the entire suite of water quality data, seagrass frequency occurrences, and macroalgae frequency occurrences to identify potential mechanisms of change using seagrasses as an endpoint for evaluating potential impacts. Data describing *K. brevis* cell concentrations were only evaluated qualitatively because the data are from event-based sampling and generally do not represent a random sample appropriate for statistical testing. Observations for each data type were typically aggregated to the weekly or monthly scale given that sampling occurred at different days over the five month period. Spatial comparisons were based primarily on the areas identified in Figure 1a. For PCA, all variables were standardized to zero mean and unit variance so that the central tendencies and ranges of all variables were similar. Variables with log-normal distribution were log-transformed (i.e., nutrients, chlorophyll) prior to analysis.

The “PCA” function from the FactoMineR R package ([Lê et al., 2008](#ref-Le08)) was used for PCA and the “ggord” function from the ggord R package ([Beck, 2021](#ref-Beck21b)) was used to plot the results. For total nitrogen, chlorophyll-a, and secchi depth, differences in concentrations between months within each area (Figure 1a) were evaluated using a Kruskal-Wallis one-way analysis of variance (ANOVA) followed by multiple comparisons using 2-sided Mann-Whitney U tests ([Hollander et al., 2013](#ref-Hollander13)). Probability values were adjusted using the sequential Bonferroni method described in ([Holm, 1979](#ref-Holm79)) to account for the increased probability of Type I error rates with multiple comparisons. An adjusted p-value < 5% ( = 0.05) was considered a significant difference between months. For statistical tests using water quality data, only the monitoring results from FDEP were used for analysis given the consistency of sample location and collection date compared to the remainder of the data obtained from other partners.

# Results

## Timeline of events from April 2021

A general narrative of 2021 events in Tampa Bay following release of wastewater from Piney Point is shown in Figure 2. After the discharge stopped on April 9th, an initial phytoplankton response was observed near Piney Point peaking around mid-April (Area 1, Figure ??b). Taxa from the Bacillariophyta phylum (diatoms) were dominant in April, with a maximum chlorophyll concentration of 265 ug/L, although median concentrations for each week in April were less than 10 ug/L. The initial diatom bloom did not persist past April. On April 20th, *K. brevis* was first observed near Anna Maria Sound at the southern edge of the mouth of Tampa Bay and reached bloom concentrations (>10k cells/L) by May 23rd, although observations were limited to lower Tampa Bay. Also during May, *Lyngbya sp.* (cyanobacteria macroalgae) were observed at high abundances in Anna Maria Sound and near Port Manatee. *Lyngbya* were observed in large floating mats on the surface and covering benthic and seagrass habitat below the water column at these locationa. By June 27th, fish kill reports attributed to red tide were increasing as *K. brevis* increased in abundance in lower and middle Tampa Bay. The center of tropical storm Elsa passed to the west of Tampa Bay on July 5th, causing a shift in prevailing winds from the southeast. This shift contributed to an increase in fish kill reports by moving dead fish closer to heavily populated areas of Tampa Bay, specifically for the cities of St. Petersburg and Tampa. Concentrations of *K. brevis* in middle and lower Tampa Bay peaked in mid-July, with bloom conditions not observed in the bay by August. The remainder of this section quantifies these events with monitoring data collected in response to Piney Point.

## Water quality response

From April to August 2021, 6503 samples were collected for chl-a, dissolved oxygen, ammonia, nitrate/nitrite, pH, salinity, and secchi depth (Table 2). Of these samples, 9.9% were outside of the normal range defined by the long-term monthly monitoring data for the baseline period from 2006 to 2020 (below for Secchi depth, above for all others). The percent of observations outside of the normal range varied by location and parameter. For chl-a, over half of the observations were above the normal range for area 1, whereas only 9.1% and 24% were above for areas 2 and 3, respectively. Similarly, total nitrogen concentrations were above the normal range for 39.9% of observations in area 1, whereas concentrations were above for only 12.2% and 23.4% of observations in areas 2 and 3. Secchi observations for the period of observation were below the normal range for 46.4% of observations in area1 and for 24.2% and 43.5% of observations in areas 2 and 3. Notable differences were also observed for dissolved oxygen (e.g., 49.7% were above in area 1, 47.6% in area 2). Physical parameters (e.g., salinity, temperature) were generally within range over the five month period. Inorganic nitrogen (ammonia, nitrate/nitrite) were generally within range, although initial time series showed much higher concentrations in April near area 1. Spatial variation among the parameters showed that values were generally above the normal range (or below for Secchi) for many locations concentrated near Piney Point, Anna Maria Sound, and the northern mouth of Tampa Bay (Figure 3).

Boxplots of total nitrogen, chl-a, and secchi depth show the temporal progression of observations by area relative to the normal ranges from long-term monitoring data (Figure 4). For area 1, total nitrogen and chlorophyll concentrations were frequenlty above normal ranges beginning the week of April 4th and lasting through the month. Concentrations remained similar to baseline conditions until June and July when median values were often the baseline range. Secchi observations in area 1 were below baseline ranges in April and June. Observations in areas 2 and 3 were more often within the normal range, wiht some exceptions for total nitrogen and chl-a in area 3 during some weeks of May, June, and July. Statistical comparisons between months for total nitrogen, chl-a, and secchi depth (Table 3) supported the results in Figure 4. Kruskal-Wallis tests that assessed if at least one of the months had significantly different observations for each parameter and were significant for total nitrogen, chl-a, and secchi depth for area 1, total nitrogen and chl-a for area 2, and chl-a and secchi depth for area 3 (Table 3). Results of multiple comparison tests that evaluated differneces between pairs of months generally showed that April/May were different from June/July depending on area and parameter. Observations in the later months were generally higher (or lower for Secchi) corresponding to increasing *K. brevis* abundances.

Multivariate assessment of all water quality parameters that combined results across weeks by area showed significant associations between groups of variables (Figure 5). The first principal component explained 41%, 74%, and 47% of the variation among water quality parameters for areas 1, 2, and 3, respectively. These components generally described a positive association between nutrients and chlorophyll concentrations and a negative association with secchi depth and dissolved oxygen. The second and third principal components explained a minorit of the variation of among water quality parameters such that 36%, 20%, and 34% was explained by both axes for areas 1, 2, and 3, respectively. The patterns explained by these axes were less clear, although physical drivers were partially explained in some cases (e.g., component 2 for area 2, Figure 5b). Spearman rank correlations between the pairs of variables used for each of the PCAs showed significant assocations. Total nitrogen was positively associated with chlorophyll and negatively associated with secchi depth for areas 1 and 2 ( and , respectively). Dissolved oxygen was negatively associated with chl-a only in area 3, although the temporal progression likely changed over time in the other areas (e.g., increase followed by decrease). Temperature generally had a positive association with chlorophyll ( for area 2, for area 3). pH was positively associated with chl-a in areas 1 and 2 ( for both).

## Seagrass and macroalgae trends

## Red tide and fish kill reports

* Figure 2 timeline of events
* Figure 3 map of water quality observations
* Figure 4 boxplots by week with +/-1sd for monthly long-term
* Figure 5 pca and correlations of all wq variables, by area
* Figure 6 seagrass and macroalgae example transect
* Figure 7 seagrass, macroalgae frequency occurrence estimates
* Figure 8 seagrass, macralgae, wq pca and correlation matrix
* Figure 9 red tide historic and current, fish kill reports historic and current
* Table 1 Stack characteristics
* Table 2 water quality data collected and summarized
* Table 3 Multiple comparisons of water quality by month/area, with median/min/max
* Table 4 Macroalgae freq. occurrence summaries by month/area, with median/min/max
* Table 5 Seagrass freq. occurrence summaries by month/area, with median/min/max

Seagrass: note decline of HW and correlations in the matrix, these make sense.

Seagrass interp from DT:

First off, Marcus please correct me if any confusion on my part about how to read the chart, but it’s my take that the asterisks reflect levels of significance, and that a box without an asterisk – I didn’t pay much attention to that. Also, a negative number would be a “cool” color, reflecting an inverse relationship, while a positive number (warm color) would reflect a positive correlation?

If I have this correct, then what stood out to me was some of the following – seagrass distributions are highly inversely correlated between most of the species, which reflects seagrass being clonal organisms that tend not to grow interspersed with each other in a random manner. S. filiforme does not grow in shallow waters, because it’s blades can’t “lay down” during low tides, and it often is the deepest growing species in some of our cleaner areas, while H. wrightii can be both the shallowest and deepest growing species in areas like Hillsborough Bay.

More relevant here is that there is no significant correlation between the abundance of cyanobacteria and either chlorophyll-a or TN or TP. To me, that makes sense, since macroalgae like cyanos seem to reflect an alternative nutrient destination, compared to phytoplankton. The strongest correlations out there are the well expected ones between TN and Chl-a, but also between TP and NH3/4, which suggests overall nutrient availability tracks together, and phytoplankton continue to be a major destination of nitrogen availability.

But what about the positive correlation between cyanobacteria and S. filiforme? From my experience in Sarasota Bay, that makes sense, since the highest levels of cyanobacteria were found entangled within the manatee grass meadows in the fairly deeper waters farther offshore. Cyanos are strongly negatively correlated with red algae, since they occurred at different times, basically.

Finally, keep in mind that “Lyngbya” life cycles are such that most of the biomass produced does not stay where it was produced. Those clumps of Lyngbya growing on the bottom get lifted up off the bottom by the oxygen bubbles you can see in their blooms, and they then are carried along the shoreline to decompose on whatever shoreline that they’ve been blown to. Our photos (and others) clearly show that there is MUCH more Lyngbya in our bays than what we’ve sampled along the bottom of the bay – that’s a bit different than “traditional” red algae biomass.

# Discussion

* Explain results - what’s up with inorganic nutrients? Likely driving phyto response but concentrations are not a good indicator. Uptake is rapid…
* Comparison to other locations/past events - Grand Bay, Bishop Harbor, Huelva estuary ([Pérez-López et al., 2016](#ref-Perez16), [2010](#ref-Perez10)), Dillon report about Grand Bay [link](https://www.wrri.msstate.edu/pdf/2016dillon_finalreport.pdf)
* Analysis limitations: no smoking gun but 2021 is an anomaly, additional info (benthic diversity TBD, nekton diversity TBD, large mammals, etc.), response-based monitoring may be biased
* Long-term closure plan? NGS-N is treated with spray evaporation system, but concentrations of TN/TP higher than NGS-S (see EO)/
* Potential long-term impacts TBD
* Current challenges in TB/southwest FL - OTB, seagrass loss (possibly linked to 2018 red tide, effects of Hurricanes ([Tomasko et al., 2020](#ref-Tomasko20))), red tide, climate change
* Risk of decline (IRL ex.), regression of past progress

# Acknowledgments

# Figures

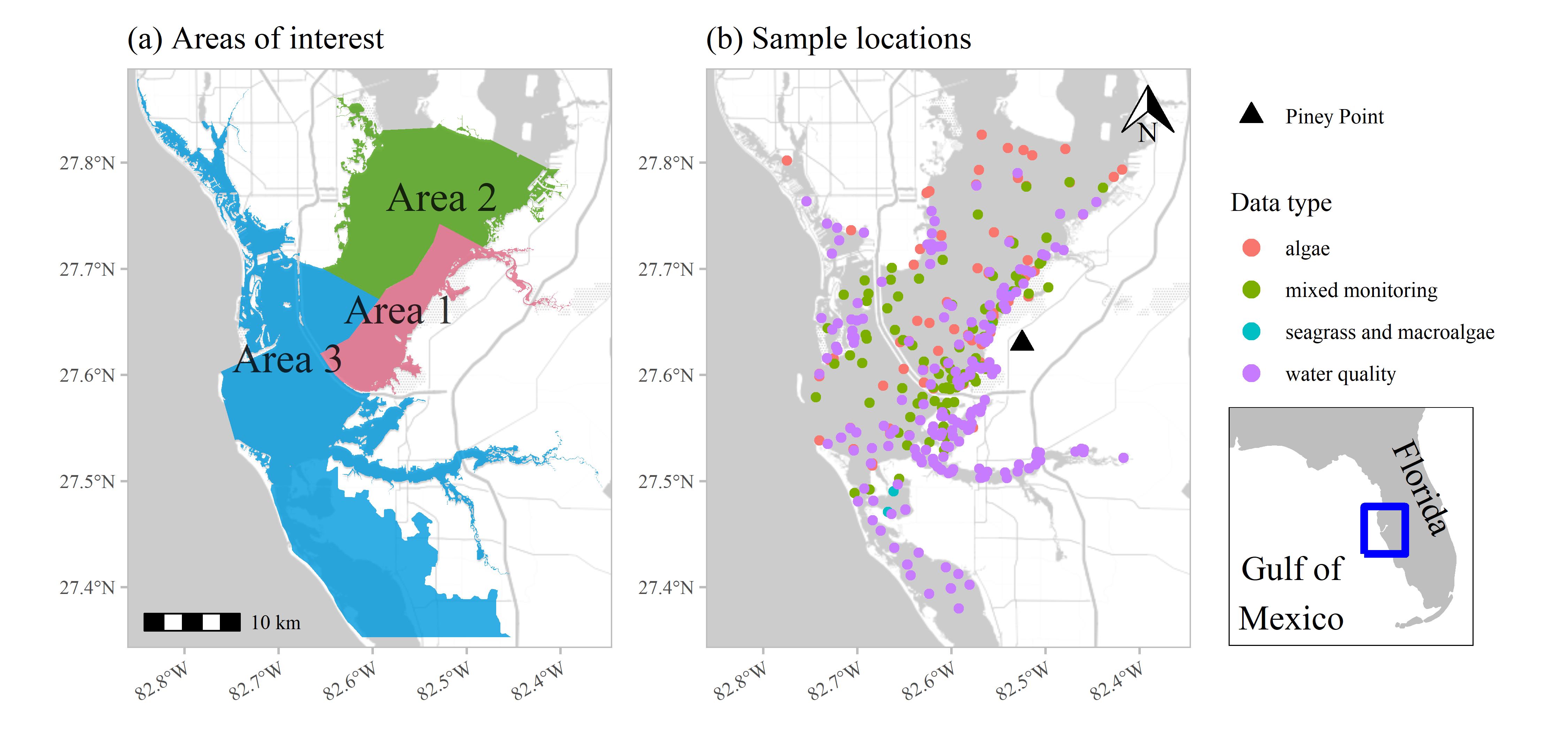


Figure 1: Areas of interest (a) for evaluating status and trends in response-based monitoring data and sample locations from March to July 2021 by monitoring data type (b) in response to wastewater discharge from Piney Point. Data types include algae sampling, seagrass and macroalgae, water quality (field-based and laboratory samples), and mixed monitoring (algae, seagrass and macroalgae, water quality). Inset shows location of Tampa Bay on the Gulf coast of Florida, USA.



Figure 2: Graphical timeline of events from the discharge of wastewater effluent at Piney Point starting Mrach 30th, 2021 through the end of July with the gradual decline of red tide in Tampa Bay.

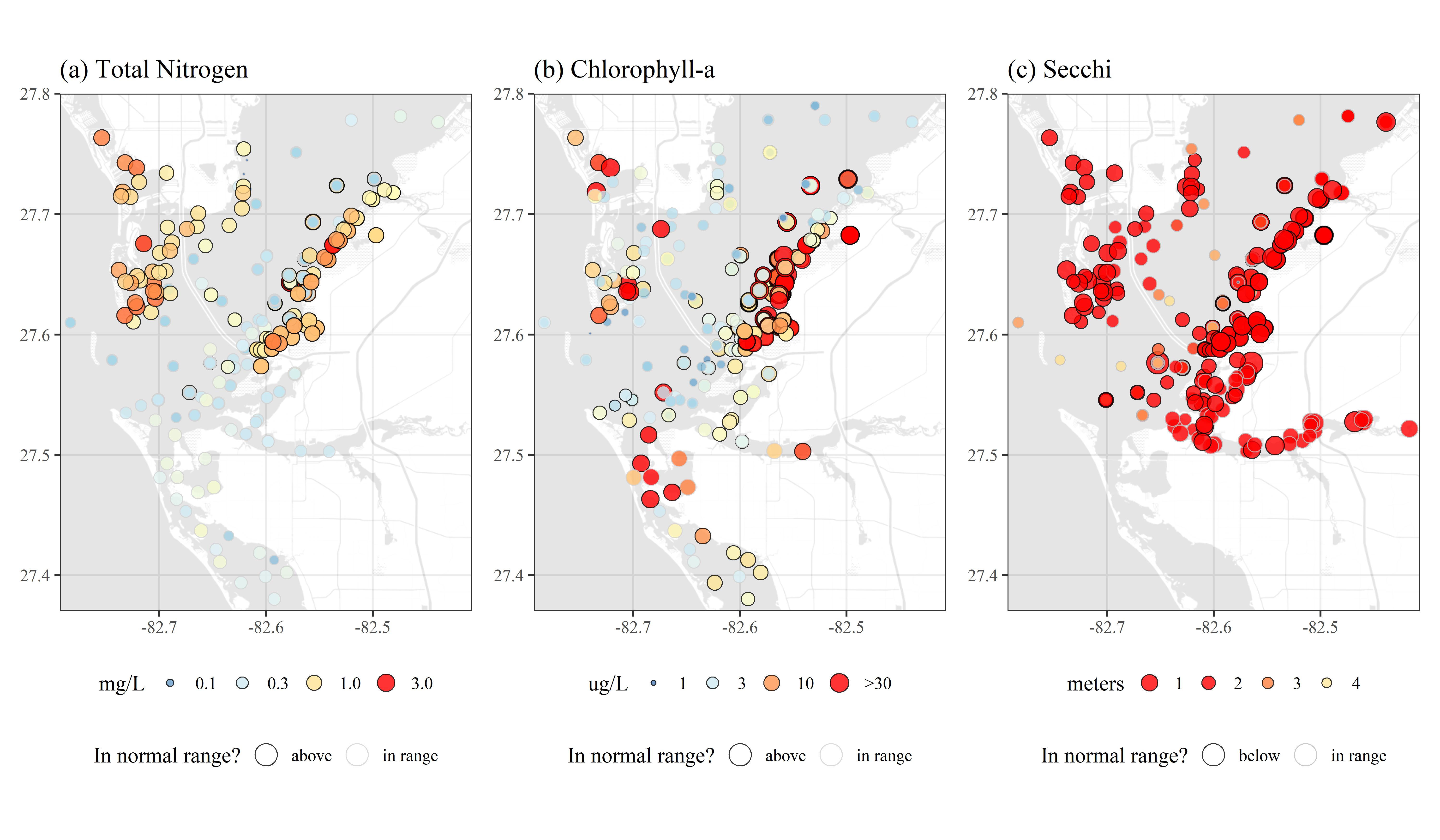


Figure 3: Sampled water quality data for April to July 2021 in response to wastewater discarge from Piney Point for (a) total nitrogen (mg/L), (b) chlorophyll-a (ug/L), and (c) secchi disk depth (meters). Values outside of the normal range (above for total nitrogen and chlorophyll, below for secchi) are outlined in black and those in normal range are outlined in light grey. Color ramps and point sizes show relative values (reversed for Secchi). Normal ranges are defined as within +/-1 standard deviation of the mean for the month of observation from 2006 to 2021 for values collected at the nearest long-term monitoring site to each sample location. Chlorophyll observations were truncated to a maximum of 30 ug/L to remove some outliers.

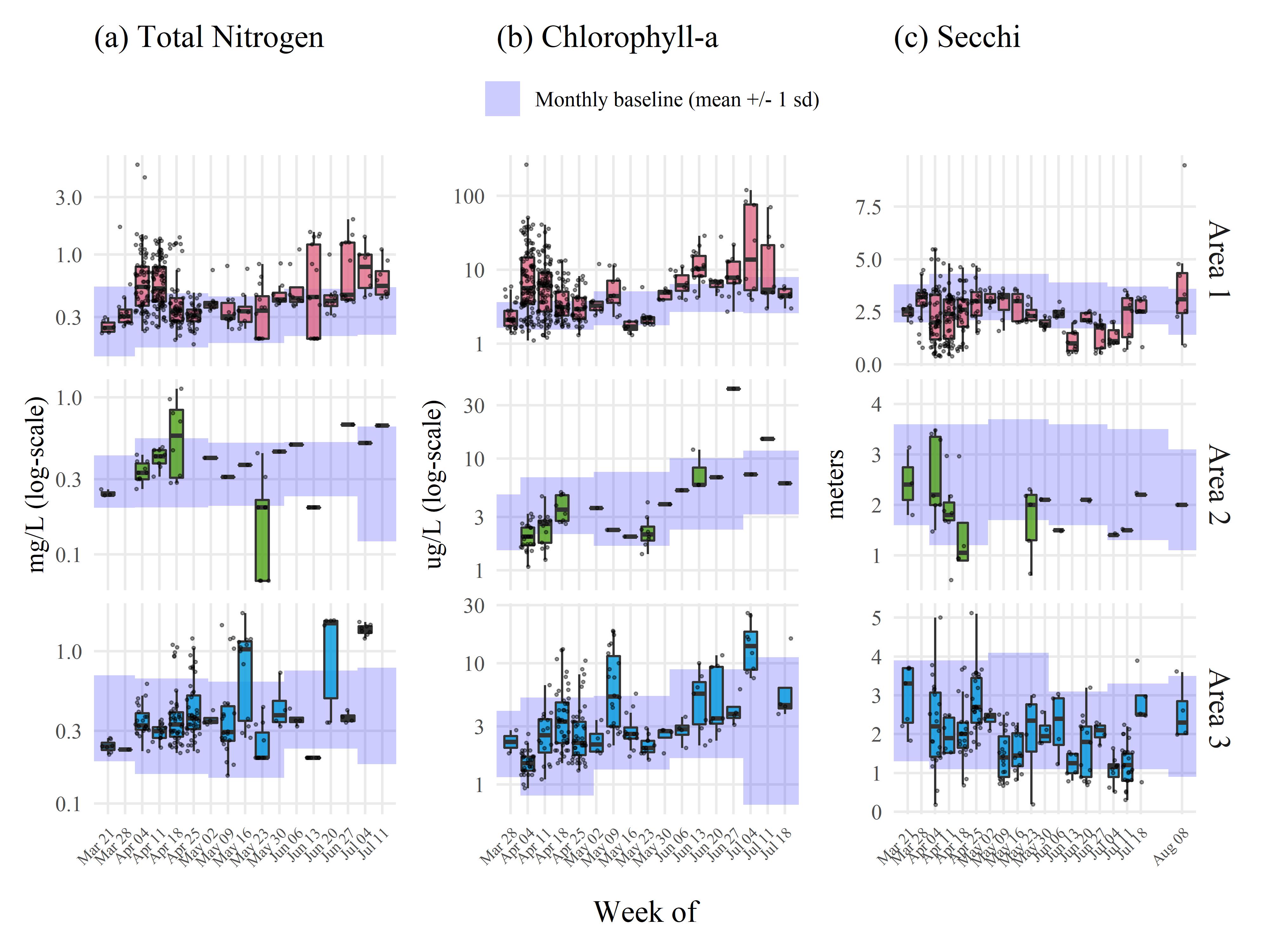


Figure 4: Sampled water quality data by week for April to July 2021 in response to wastewater discarge from Piney Point for (a) total nitrogen (mg/L), (b) chlorophyll-a (ug/L), and (c) secchi disk depth (meters). Observations are aggregated by week and within assessment areas shown in Figure 1a. Normal ranges for the month of observation (monthly baseline) and area are shown by the blue shaded areas. Normal ranges are defined as within +/-1 standard deviation of the mean for the month of observation from 2006 to 2021 for values collected at long-term monitoring sites within each area.

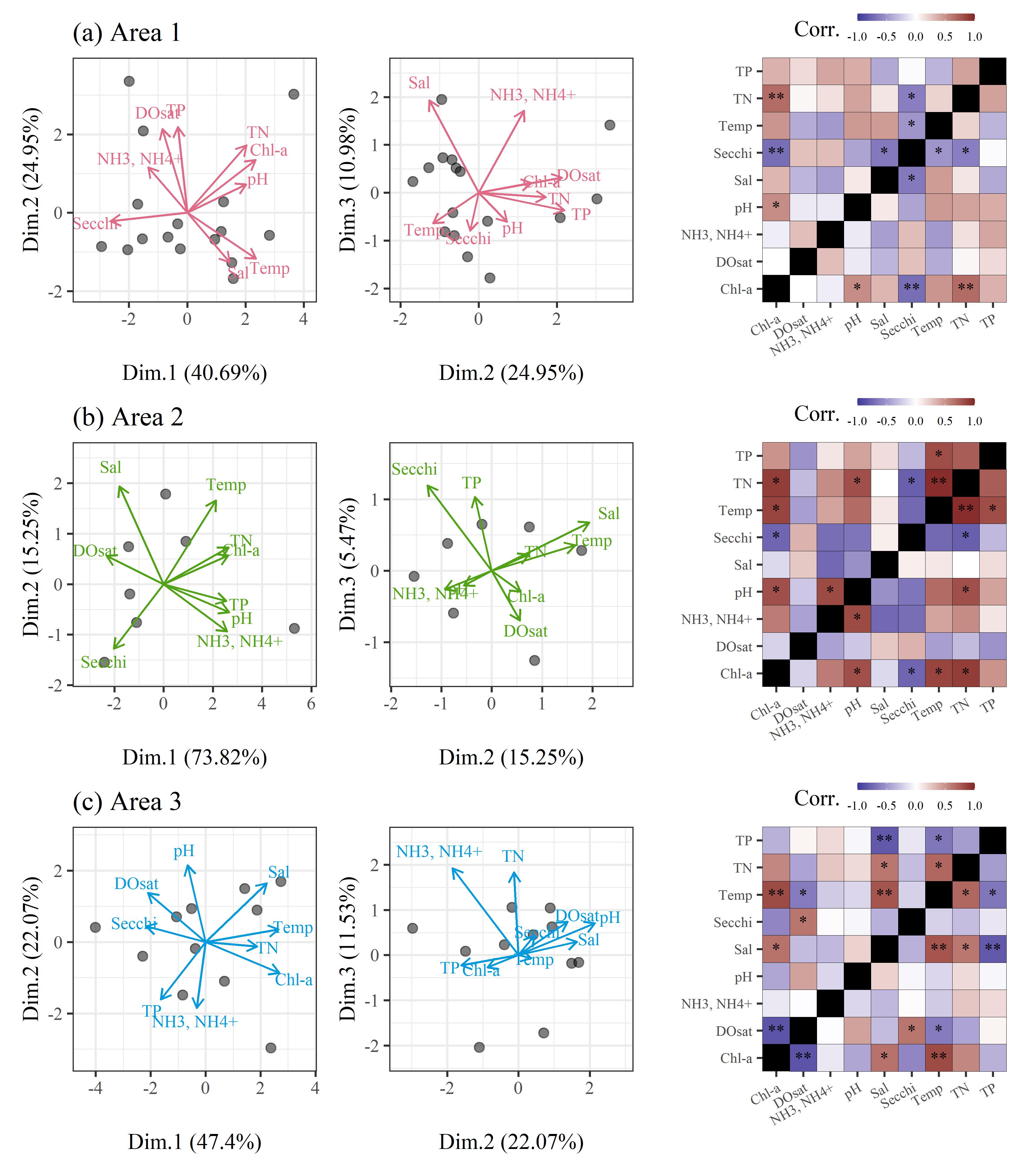


Figure 5: Principal components analyses (PCA) and correlation matrices for water quality variables by week for April to July 2021 in response to wastewater discharge from Piney Point for (a) area 1, (b) area 2, and (c) area 3 (Figure 1a). All variables were standardized to zero mean and unit variance prior to PCA. Variables with log-normal distribution were log-transformed prior to analysis. Pearson correlations indicate the linear strength of assocation between pairs of variables. \*\* p < 0.005, \* p < 0.05, blank is not signifcant at = 0.05.



Figure 6: Results for (a) seagrass and (b) macroalgae rapid response transect surveys at a site (S3T6, -82.55866 W longitude, 27.64483 N latitude) near Piney Point. Sample dates in 2021 are shown in rows with transect meter results shown in columns. Results show dominance of manatee grass (*Syringodium filiforme*) and red macroalgae groups, with abundances of *Lyngbya sp.* (cyanobacteria) peaking in June and green macroalgae (*Ulva sp.*) increasing in July. Abundances are Braun-Blanquet coverage estimates.



Figure 7: Frequency occurrence estimates for (a) area 1 and (b) area 2 (Figure 1a) for seagrass (top) and macroalgae (bottom) rapid response transect surveys across all transects (n = r nrow(rstrnpts)) near Piney Point. Estimates are grouped by sample months in 2021. Frequency occurrences are absolute for each taxa based on presence/absence.

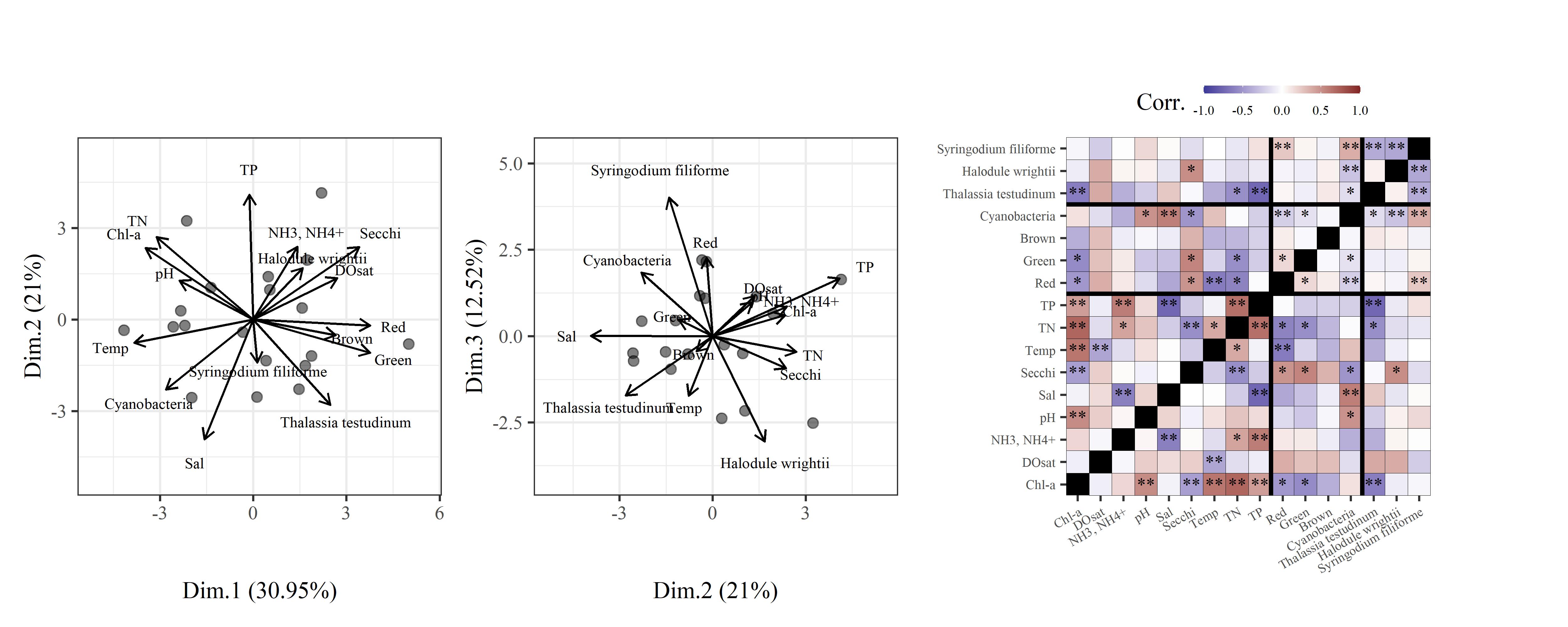


Figure 8: Principal components analysis (PCA) and correlation matrix for water quality variables, macroalgae, and seagrasses by week for April to July 2021 in response to wastewater discharge from Piney Point. Only areas 1 and 3 (Figure 1a) are included where seagrass transects were surveyed. All variables were standardized to zero mean and unit variance prior to PCA. Variables with log-normal distribution were log-transformed prior to analysis. Thick black lines in the correlation matrix separate water quality, macroalgae, and seagrass variables. Pearson correlations indicate the linear strength of assocation between pairs of variables. \*\* p < 0.005, \* p < 0.05, blank is not significant at = 0.05.

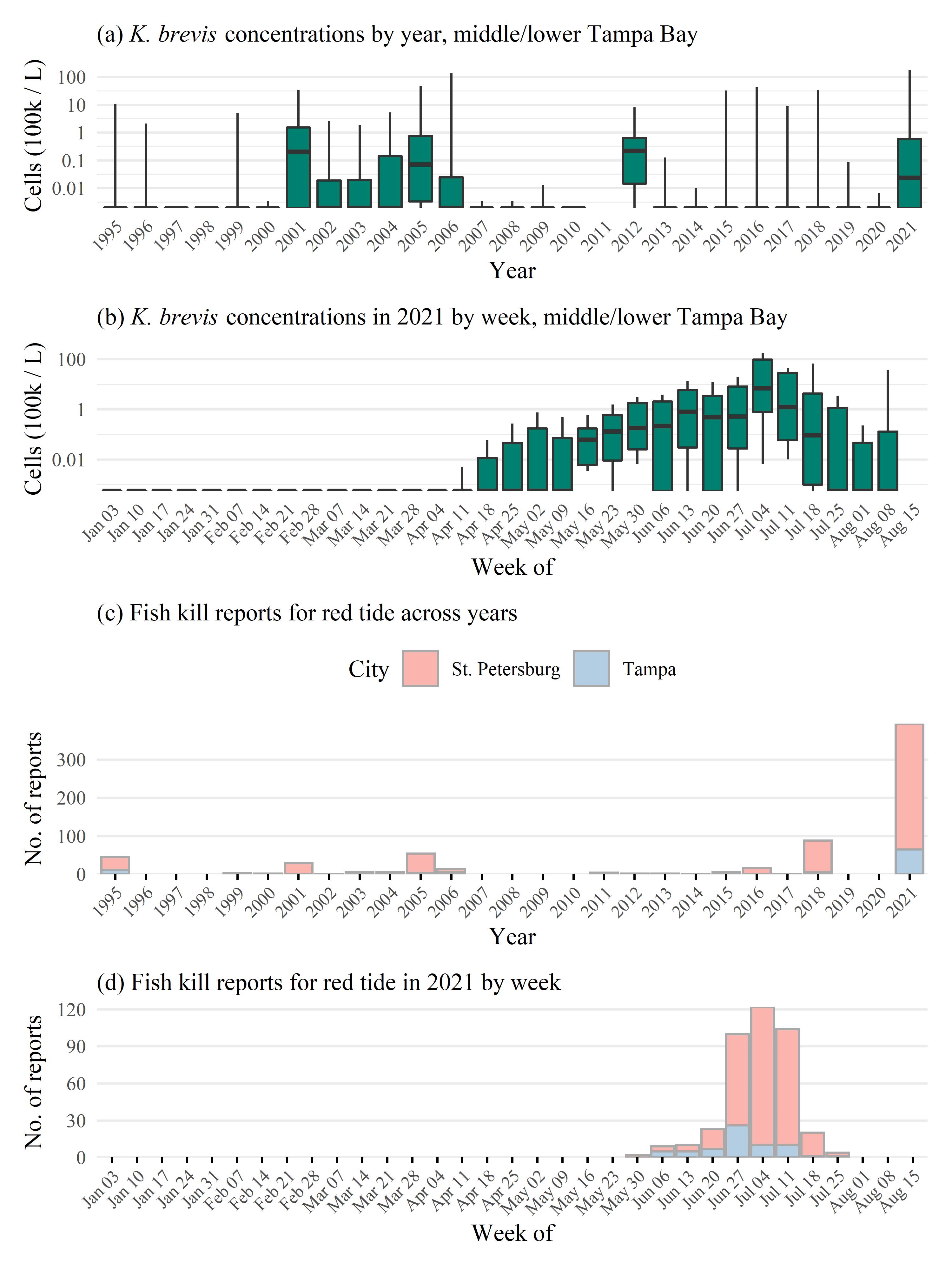


Figure 9: *Karenia brevis* concentrations (100k cells/L) and number of fish kill reports for the contiguous record showing cell concentrations (a) by year and (b) by week in 2021 and reported fish kills by city (Tampa, St. Petersburg) (c) by year and (d) by week in 2021. Red tide concentrations show minimum, tenth percentile, median, 90th percentile, and maximum for each year or week for middle and lower Tampa Bay. *K. brevis* cell counts are from NOAA Harmful Algal BloomS Observing System (HABSOS, <https://www.ncei.noaa.gov/maps/habsos>), Fish kill reports are from Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Insitute Fish Kill Database, attributed to *K. brevis* (<https://public.myfwc.com/FWRI/FishKillReport/>).

# Tables

Table 1: Measured concentrations from the phosphogypsum stack (NGS-S) at Piney Point from a 2019 sample and end-of-pipe samples from April 2021 for relevant water quality variables. Values are compared to normal annual medians (min, max) for concentrations in lower Tampa Bay. Normal medians are based on data for a baseline period from 2006 to 2020 from long-term monitoring stations in lower Tampa Bay collected monthly by the Environmental Protection Commission of Hillsborough County. Effluent concentrations were taken from two samples on April 6th and 13th at the outflow at Port Manatee. Averages were taken when two measured values were available from both effluent sample dates. Missing values were not measured in the effluent.

|  |  |  |  |
| --- | --- | --- | --- |
| Water quality variable | 2019 stack value | 2021 end-of-pipe value | Bay median (min, max) |
| Nitrate/Nitrite (mg/L) | 0.004 | 0.292 | 0.003 (0.003, 0.018) |
| NH3, NH4+ (mg/L) | 210 | 210 | 0.019 (0.006, 0.0395) |
| TN (mg/L) | 230 | 220 | 0.286 (0.199, 0.455) |
| TP (mg/L) | 160 | 150.5 | 0.085 (0.058, 0.146) |
| Ortho-P (mg/L) | 150 | 147.5 | 0.049 (0.009, 0.055) |
| DO (% sat.) | 107.5 | - | 91 (86, 92.2) |
| pH | 4 | - | 8.1 (8, 8.1) |
| Chl-a (ug/L) | - | 105 | 3.1 (2.3, 3.5) |

Table 2: Summary of water quality variables collected from March to July 2021 in response to wastewater discharge from Piney Point. Variables are grouped by major areas interest for evaluating status and trends shown in Figure 1a. Summaries are median, maximum, and minimum values. Total observations (N obs.) and the percentage of observations in range, above, or below normal ranges are also shown. Normal ranges are defined as within +/-1 standard deviation of the mean for the month of observation from 2006 to 2021 for values collected at the nearest long-term monitoring site to each sample location.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Area | Water quality variable | Med. (Min., Max.) | N obs. | in range | above | below |
| 1 | Chl-a (ug/L) | 4.5 (1.1, 265.01) | 440 | 40.9 | 50.7 | 8.4 |
|  | DO (% sat.) | 98.4 (28.3, 215.3) | 386 | 26.9 | 49.7 | 23.3 |
|  | NH3, NH4+ (mg/L) | 0.008 (0, 14.86) | 430 | 50.2 | 23.0 | 26.7 |
|  | Nitrate/Nitrite (mg/L) | 0.01 (0, 0.14352) | 219 | 47.5 | 10.0 | 42.5 |
|  | pH | 8.1 (7, 9.1) | 432 | 47.2 | 38.7 | 14.1 |
|  | Sal (ppt) | 30.4 (12.9, 34.6) | 397 | 84.1 | 4.8 | 11.1 |
|  | Secchi (m) | 2.4 (0.4, 9.5) | 289 | 38.4 | 15.2 | 46.4 |
|  | Temp (C) | 25.3 (19.6, 32.9) | 398 | 63.3 | 19.6 | 17.1 |
|  | TN (mg/L) | 0.42 (0.178, 5.6) | 371 | 56.6 | 39.9 | 3.5 |
|  | TP (mg/L) | 0.12 (0.019, 3.9) | 439 | 59.7 | 29.6 | 10.7 |
| 2 | Chl-a (ug/L) | 2.5 (1.08, 42) | 66 | 63.6 | 9.1 | 27.3 |
|  | DO (% sat.) | 96.8 (60.6, 153.3) | 63 | 39.7 | 47.6 | 12.7 |
|  | NH3, NH4+ (mg/L) | 0.005 (0.002, 0.035) | 62 | 74.2 | 0.0 | 25.8 |
|  | Nitrate/Nitrite (mg/L) | 0.01 (0.00078, 0.014) | 41 | 90.2 | 2.4 | 7.3 |
|  | pH | 8 (7.3, 8.5) | 82 | 67.1 | 18.3 | 14.6 |
|  | Sal (ppt) | 27.6 (22.3, 32.3) | 63 | 93.7 | 0.0 | 6.3 |
|  | Secchi (m) | 2 (0.5, 3.5) | 33 | 69.7 | 6.1 | 24.2 |
|  | Temp (C) | 25 (19.9, 31.6) | 63 | 69.8 | 7.9 | 22.2 |
|  | TN (mg/L) | 0.35 (0.068, 1.13) | 49 | 71.4 | 12.2 | 16.3 |
|  | TP (mg/L) | 0.096 (0.05, 0.235) | 55 | 20.0 | 18.2 | 61.8 |
| 3 | Chl-a (ug/L) | 2.7 (0.93, 25.9) | 221 | 66.1 | 24.0 | 10.0 |
|  | DO (% sat.) | 99 (42.4, 229.9) | 197 | 52.3 | 25.9 | 21.8 |
|  | NH3, NH4+ (mg/L) | 0.008 (0.002, 0.041) | 206 | 56.3 | 0.5 | 43.2 |
|  | Nitrate/Nitrite (mg/L) | 0.01 (0.00078, 0.043) | 146 | 59.6 | 6.2 | 34.2 |
|  | pH | 8.1 (6.2, 14.4) | 220 | 62.3 | 29.5 | 8.2 |
|  | Sal (ppt) | 32.1 (1.4, 36.5) | 255 | 81.6 | 8.6 | 9.8 |
|  | Secchi (m) | 2 (0.2, 5.1) | 193 | 43.0 | 13.5 | 43.5 |
|  | Temp (C) | 25.8 (19.6, 32.1) | 255 | 58.8 | 16.1 | 25.1 |
|  | TN (mg/L) | 0.334 (0.152, 1.78) | 209 | 73.2 | 23.4 | 3.3 |
|  | TP (mg/L) | 0.061 (0.019, 0.589) | 223 | 71.3 | 17.0 | 11.7 |

Table 3: Comparison of total nitrogen, chlorophyll-a, and secchi depth by areas of interest (Figure 1a) and month. Overall signifance of differences of concentrations between months for each water quality variable and area combination are shown with Chi-squared statistics based on Kruskall-Wallis rank sum tests. Multiple comparisons with Mann-Whitney U tests (Comp column) were used to evaluate pairwise monthly concentrations for each water quality variable in each area. Rows that share a letter within each area and water quality variable combination have concentrations that are not significantly different. Probability values were adjusted for the pairwise comparisons using the Bonferroni method in [Holm](#ref-Holm79) ([1979](#ref-Holm79)). \*\* p < 0.005, \* p < 0.05, blank is not signifcant at = 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Area | Water quality variable | Chi-Sq. | Comp. | Month | N obs. | Med. (Min., Max.) |
| 1 | TN (mg/L) | 26.1\*\* | a | Apr | 135 | 0.39 (0.22, 5.6) |
|  |  |  | a | May | 32 | 0.36 (0.24, 0.83) |
|  |  |  | b | Jun | 38 | 0.43 (0.31, 1.1) |
|  |  |  | c | Jul | 16 | 0.635 (0.43, 1.4) |
|  | Chl-a (ug/L) | 41.53\*\* | a | Apr | 144 | 3.3 (1.1, 41) |
|  |  |  | b | May | 32 | 2.4 (1.3, 12) |
|  |  |  | c | Jun | 38 | 6.6 (2.7, 28) |
|  |  |  | c | Jul | 24 | 5.5 (3, 120) |
|  | Secchi (m) | 35.74\*\* | a | Apr | 117 | 2.9 (0.8, 5.5) |
|  |  |  | a | May | 28 | 3 (1.6, 3.6) |
|  |  |  | b | Jun | 34 | 2 (0.5, 3) |
|  |  |  | b | Jul | 24 | 2.2 (0.7, 3.5) |
| 2 | TN (mg/L) | 10.29\* | a | Apr | 18 | 0.39 (0.26, 0.48) |
|  |  |  | a | May | 4 | 0.39 (0.31, 0.44) |
|  |  |  | a | Jun | 3 | 0.5 (0.45, 0.67) |
|  |  |  | a | Jul | 2 | 0.585 (0.51, 0.66) |
|  | Chl-a (ug/L) | 15.98\*\* | a | Apr | 22 | 2.5 (1.5, 4.6) |
|  |  |  | ab | May | 4 | 2.15 (1.9, 3.6) |
|  |  |  | b | Jun | 4 | 6 (3.9, 42) |
|  |  |  | b | Jul | 3 | 7.2 (6, 15) |
|  | Secchi (m) | 1.41 | a | Apr | 14 | 2 (0.5, 3.5) |
|  |  |  | a | May | 1 | 2 (2, 2) |
|  |  |  | a | Jun | 3 | 2.1 (1.5, 2.1) |
|  |  |  | a | Jul | 3 | 1.5 (1.4, 2.2) |
| 3 | TN (mg/L) | 5.64 | a | Apr | 48 | 0.33 (0.22, 0.48) |
|  |  |  | a | May | 16 | 0.335 (0.26, 0.43) |
|  |  |  | a | Jun | 10 | 0.35 (0.32, 0.72) |
|  |  |  | a | Jul | 4 | 0.36 (0.34, 0.41) |
|  | Chl-a (ug/L) | 28.4\*\* | a | Apr | 48 | 1.9 (1, 4.1) |
|  |  |  | ab | May | 16 | 2.35 (1.7, 3.4) |
|  |  |  | b | Jun | 12 | 2.8 (1.8, 3.6) |
|  |  |  | c | Jul | 8 | 4.15 (3.1, 16) |
|  | Secchi (m) | 12.24\* | a | Apr | 41 | 2.7 (1.5, 5.1) |
|  |  |  | b | May | 16 | 2.2 (0.2, 3) |
|  |  |  | ab | Jun | 12 | 2.2 (1.2, 3.2) |
|  |  |  | ab | Jul | 12 | 2.2 (1.4, 3.9) |

Table 4: Comparison of macroalgae frequency occurrence by areas of interest (Figure 1a) and month. Overall signifance of differences of frequency occurrence between months for macroalgae groups and area combination are shown with Chi-squared statistics based on Kruskall-Wallis rank sum tests. Multiple comparisons with Mann-Whitney U tests (Comp column) were used to evaluate pairwise monthly frequency occurrences for each macroalgae group in each area. Rows that share a letter within each area and macroalgae group combination have frequency occurrences that are not significantly different. Probability values were adjusted for the pairwise comparisons using the Bonferroni method in [Holm](#ref-Holm79) ([1979](#ref-Holm79)). \*\* p < 0.005, \* p < 0.05, blank is not signifcant at = 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Area | Macroalgae group | Chi-Sq. | Comp. | Month | N obs. | Med. (Min., Max.) |
| 1 | Red | 27.18\*\* | a | Apr | 23 | 1 (0, 1) |
|  |  |  | ab | May | 24 | 0.708 (0, 1) |
|  |  |  | c | Jun | 14 | 0.121 (0, 0.722) |
|  |  |  | bc | Jul | 14 | 0.321 (0, 0.882) |
|  |  |  | abc | Aug | 8 | 0.464 (0.222, 0.857) |
|  | Green | 5.13 | a | Apr | 23 | 0 (0, 0.75) |
|  |  |  | a | May | 24 | 0 (0, 0.429) |
|  |  |  | a | Jun | 14 | 0 (0, 0.167) |
|  |  |  | a | Jul | 14 | 0 (0, 0.333) |
|  |  |  | a | Aug | 8 | 0.083 (0, 0.667) |
|  | Cyanobacteria | 21.67\*\* | a | Apr | 23 | 0 (0, 0) |
|  |  |  | ab | May | 24 | 0 (0, 1) |
|  |  |  | b | Jun | 14 | 0.08 (0, 1) |
|  |  |  | ab | Jul | 14 | 0 (0, 0.417) |
|  |  |  | ab | Aug | 8 | 0 (0, 0) |
| 3 | Red | 18.49\*\* | a | Apr | 7 | 0.917 (0.917, 1) |
|  |  |  | ab | May | 12 | 0.917 (0.25, 1) |
|  |  |  | c | Jun | 12 | 0.333 (0, 0.833) |
|  |  |  | bc | Jul | 4 | 0.333 (0, 0.833) |
|  | Green | 2.27 | a | Apr | 7 | 0 (0, 0.667) |
|  |  |  | a | May | 12 | 0 (0, 0.833) |
|  |  |  | a | Jun | 12 | 0 (0, 0.833) |
|  |  |  | a | Jul | 4 | 0 (0, 0) |
|  | Cyanobacteria | 8.25\* | a | Apr | 7 | 0 (0, 0.083) |
|  |  |  | a | May | 12 | 0 (0, 0.833) |
|  |  |  | a | Jun | 12 | 0.167 (0, 1) |
|  |  |  | a | Jul | 4 | 0 (0, 0.4) |

Table 5: Comparison of seagrass species frequency occurrence by areas of interest (Figure 1a) and month. Overall signifance of differences of frequency occurrence between months for seagrass species and area combination are shown with Chi-squared statistics based on Kruskall-Wallis rank sum tests. Multiple comparisons with Mann-Whitney U tests (Comp column) were used to evaluate pairwise monthly frequency occurrences for each seagrass species in each area. Rows that share a letter within each area and seagrass species combination have frequency occurrences that are not significantly different. Probability values were adjusted for the pairwise comparisons using the Bonferroni method in [Holm](#ref-Holm79) ([1979](#ref-Holm79)). \*\* p < 0.005, \* p < 0.05, blank is not signifcant at = 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Area | Seagrass species | Chi-Sq. | Comp. | Month | N obs. | Med. (Min., Max.) |
| 1 | Thalassia testudinum | 0.95 | a | Apr | 23 | 0.444 (0, 1) |
|  |  |  | a | May | 24 | 0.5 (0, 1) |
|  |  |  | a | Jun | 14 | 0.5 (0, 0.778) |
|  |  |  | a | Jul | 14 | 0.5 (0, 1) |
|  |  |  | a | Aug | 8 | 0.417 (0, 0.833) |
|  | Halodule wrightii | 3.36 | a | Apr | 23 | 0.25 (0, 1) |
|  |  |  | a | May | 24 | 0.167 (0, 1) |
|  |  |  | a | Jun | 14 | 0.208 (0, 0.941) |
|  |  |  | a | Jul | 14 | 0.25 (0, 1) |
|  |  |  | a | Aug | 8 | 0.083 (0, 0.667) |
|  | Syringodium filiforme | 0.55 | a | Apr | 23 | 0 (0, 1) |
|  |  |  | a | May | 24 | 0.083 (0, 1) |
|  |  |  | a | Jun | 14 | 0 (0, 1) |
|  |  |  | a | Jul | 14 | 0.083 (0, 1) |
|  |  |  | a | Aug | 8 | 0.417 (0, 1) |
| 3 | Thalassia testudinum | 0.29 | a | Apr | 7 | 1 (0, 1) |
|  |  |  | a | May | 12 | 0.875 (0, 1) |
|  |  |  | a | Jun | 12 | 0.792 (0, 1) |
|  |  |  | a | Jul | 4 | 0.617 (0.333, 1) |
|  | Halodule wrightii | 2.53 | a | Apr | 7 | 0.417 (0, 1) |
|  |  |  | a | May | 12 | 0.292 (0, 1) |
|  |  |  | a | Jun | 12 | 0.216 (0, 1) |
|  |  |  | a | Jul | 4 | 0 (0, 0.667) |
|  | Syringodium filiforme | 1.17 | a | Apr | 7 | 0.417 (0, 0.833) |
|  |  |  | a | May | 12 | 0 (0, 1) |
|  |  |  | a | Jun | 12 | 0.167 (0, 0.833) |
|  |  |  | a | Jul | 4 | 0 (0, 0.667) |

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