Changes in the Phytoplankton Composition of Laguna Grande (Fajardo, Puerto Rico) following the 2015 *Sargassum* spp. Bloom[1]

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Abstract: Pelagic *Sargassum* beaching events are a global environmental issue that have caused increasing detrimental effects to coastal ecosystems since the 1970s. The 2015 pelagic *Sargassum* bloom in the Atlantic and the Caribbean caused significant impacts to coastal ecological communities in these areas. In enclosed systems, such as bays and lagoons, the *Sargassum* impacts could be particularly problematic due to the longer water residence times. An example of such an ecosystem is Laguna Grande bioluminescent lagoon, Puerto Rico. This lagoon slowly exchanges water with the ocean through a narrow channel (average about 5 m across), which results in the accumulation of the bioluminescent dinoflagellate *Pyrodinium bahamense* and other planktonic organisms. In this study we describe the 2015 pelagic *Sargassum* accumulation event in Laguna Grande, and report associated changes in the phytoplanktonic composition. Before this event, other studies conducted between 2003 and 2013 reported mean densities of *P. bahamense* ranging from 14,492 to 28,202 cells/L. However, the *P. bahamense* population was absent from the lagoon during the 2015 *Sargassum* influx. After the entrance of large quantities of pelagic *Sargassum* into the lagoon, accompanied with other marine algae and sea grasses, the macrophytes remained inside and began to decompose. Thirteen phytoplankton taxa not previously observed inside the lagoon were detected after the entrance of the macrophytes. We hypothesize that the changes in the phytoplanktonic composition in the lagoon were caused by altered water quality conditions following the entrance of the pelagic *Sargassum* and other macrophytes.

Key Words: phytoplankton composition, Laguna Grande, Puerto Rico, *Sargassum* influxes, *S. fluitans, S. natans,* bioluminescence, *Pyrodinium bahamense*, *Ceratium furca*, *Tripos furca,* diatoms, dinoflagellates, cyanobacteria

Introduction

Pelagic *Sargassum* spp. and other macroalgae beaching events have increased worldwide, affecting the shore and adjacent water since the 1970s

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(Smetacek and Zingone 2013). During the 2000s the number of seaweed (including *Sargassum)* beaching reports increased further, as did the magnitude of the beaching events, presenting a new environmental challenge (Smetacek and Zingone 2013, Fidai et al. 2020). Seaweed beaching events can impair shore-based activities due to their prominent physical mass. Large quantities of seaweed smothering the shoreline can deter tourists, and the dense, drifting seaweed can prevent swimmers and small boats from accessing the sea (The Caribbean Council 2019). Decomposing *Sargassum* releases hydrogen sulfide gas and ammonia, which, depending on concentration, could cause respiratory, skin, and neurocognitive symptoms in humans. Toxic exposure typically happens approximately 48 hours after it washes ashore (Resiere et al. 2023). Seaweed accumulations may also cause detrimental effects on other coastal ecosystems, such as coral reefs (Smetacek and Zingone 2013, Gaskill 2015) and seagrasses (van Tussenbroek et al. 2017), can interfere with sea turtle hatchling recruitment (Gavio and Santos-Martínez 2018, Schiariti and Salmon 2022), and can lead to hypoxic conditions in nearshore environments (Cabanillas-Terán et al. 2019) due to *Sargassum*'s spp. bacterial decomposition.

Several massive pelagic *Sargassum* influxes have been reported in the Caribbean Sea during the 2010 – 2020 decade (Johnson et al. 2012, Gavio et al. 2015, Schell et al. 2015, Louime et al. 2017, van Tussenbroek et al. 2017, Wang et al. 2019). These events, as well as those in Western Africa and in other regions, have received wide media attention, mostly due to their negative effects in the tourism industry and to the negative environmental issues associated with the large accumulations of *Sargassum* (Gaskill 2015). Satellite-derived information of *Sargassum* mats and hindcast models suggest that recent influxes are associated with blooms forming and flourishing in the North Equatorial Recirculation Region (NERR) of the Atlantic, under high ocean temperatures and high nutrient inputs, where they may have circulated for an extended time, eventually being released into the North Brazil Current, flowing into the Eastern Caribbean or drifting to the coast of Western Africa (Johnson et al. 2012, Smetacek and Zingone 2013, Wang et al. 2019). Floating *Sargassum* incoming from the Sargasso Sea could also enter the Caribbean through its various passages (e. g., Windward Passage, Mona Passage, Anegada Passage). The Caribbean Current, in concert with other oceanic and wind-driven currents, then transports the *Sargassum* westward through the Yucatán Passage into the Gulf of Mexico. The floating *Sargassum* is either washed ashore on the Gulf of Mexico coastlines or gets swept out the Florida Strait via the Gulf Stream. This drifting loop (Webster and Linton 2013) serves to reintroduce *Sargassum* back into the Sargasso Sea, perpetuating its existence (Frazier 2014, Hill 2016, SEAS 2018). Brooks et al. (2018) indicate that connectivity between the tropical Atlantic and the Sargasso Sea is mostly one-way with the Sargasso Sea acting as

a "dead end" for *Sargassum*. Recent *Sargassum* bloom events in the Atlantic show connections to nutrient enrichments and climatic variations. Under continued nutrient enrichment due to deforestation and fertilizer use in agriculture, along with the substantial mass of *Sargassum* seed populations lingering in the tropics, it is likely to observe recurrent accumulations of *Sargassum* and beaching events (Wang et al. 2019).

Sargassum influxes in the Caribbean Sea can be predicted by several months of lead time using MODIS (Moderate Resolution Imaging Spectroradiometer) satellite observations from bloom conditions in the Central West Atlantic (Wang and Hu 2017). Hu (2009) developed a Floating Algae Index (FAI) using the vegetation red-edge reflectance in the near-infrared to detect floating *Sargassum* mats using MODIS imagery. Due to the cloud-masking difficulty of FAI, Wang and Hu (2009) developed the Alternative Floating Algae Index (AFAI). The AFAI can effectively mask clouds through band combinations and has previously been applied to study *Sargassum* distributions. The AFAI was then used to develop the *Sargassum* Watch System (SaWS), which provides images of floating *Sargassum* within 4-6 hours of satellite overpass (University of South Florida, Optical Oceanography Laboratory 2018a, Hu et al. 2016).

Monitoring inundation events relies on a combination of in situ and remote-sensing data that have been specifically designed to detect *Sargassum.* Interoperable tools for data distribution, information management, and visualization are critical; and such a framework would benefit essential economic, social, and environmental domains and would define the baseline needed to coordinate future science-driven monitoring and evaluation efforts, including contributions toward eventual sustainable commercial exploitation/reuse of *Sargassum* (Triñanes et al. 2021).

Caribbean bioluminescent bays and lagoons are rare and unique coastal ecosystems. They usually have narrow and shallow mouths, restricting water flow; therefore, nutrients tend to accumulate and help to sustain the highly productive and persistent phytoplankton populations. Temperature, salinity, and watershed-derived nutrients are important environmental drivers for these populations (Soler-Figueroa and Otero 2015). These water bodies appear to be threatened by many factors, including poor water quality (e.g., Oyster Bay, Jamaica; Environmental Solutions, Ltd. 2005), habitat deterioration (e.g., Fire Lake Lagoon, Bahamas; Harvey 1952), light pollution (causes poor perception of bioluminescence, e.g., Bahía Fosforescente, Lajas, Puerto Rico; Soler-Figueroa and Otero 2016) and unwise management. The environmental impact of massive influxes of pelagic *Sargassum* entering bioluminescent bays and lagoons should be greater than in more open systems since they usually have longer water residence times and water quality should be more affected.

Laguna Grande is a coastal bioluminescent lagoon located on the north-east coast of Puerto Rico. Its bioluminescence is produced almost exclusively by the dinoflagellate *Pyrodinium bahamense* Plate 1906 var. *bahamense* (Steidinger and Tangen 1997), which can reach very high densities in bioluminescent water bodies in the Caribbean (Seliger et al. 1971, Sastre et al. 2013, Raimundi-Rivera 2015, Soler-Figueroa and Otero 2016). The dinoflagellate *Tripos furca* (Gómez 2013) [formerly known as *Ceratium furca* (Ehrenberg) Claparède and Lachmann 1859 var. *hircus* (Steidinger and Tangen 1997)] is not bioluminescent but can reach high densities in Laguna Grande (Sastre et al. 2013, 2015; Raimundi-Rivera 2015). Laguna Grande is also an important environment for many other marine organisms, including mangroves, seagrasses, macroalgae, fishes and benthic invertebrates (Weaver et al. 1999). During year 2015 Laguna Grande received a total of 84,396 visitors from kayak tour operators (Milagros Cartagena Haddock, Land Use and Forestry Permits Section, Puerto Rico Department of Natural and Environmental Resources). Also, an unrecorded number of people use Laguna Grande's ecosystem services every day, such as small-boat users, kayak users, and recreational fisherpersons.

The objective of this work is to describe the impact of the 2015 pelagic *Sargassum* accumulation event on the phytoplankton community in Laguna Grande, Puerto Rico. To perform this investigation, we used satellite images to detect and localize the presence of *Sargassum*, previously reported population densities of *P. bahamense* and *T. furca*, and our determinations on phytoplankton taxa composition.

Methods

Study Site

Laguna Grande, Puerto Rico, is bordered by *Rhizophora mangle* Linnaeus, 1753 (Red Mangrove) and situated within a basin-type mangrove forest (*sensu* Lugo and Snedaker 1974, Zayas 1979) (Figure 1). Its average depth is about 3 m and the maximum depth approximately 5 m. It occupies an area of about 50 ha and contains about 662,000 m^3 of water. The south-eastern portion of Laguna Grande is connected to Las Croabas Bay by a 1.5 km long tidal inlet/outlet channel that averages about 5 m across and 1 m deep (Soler-López and Santos 2010). Tidal fluctuations can significantly affect *P. bahamense* population densities in sites located in and near the inlet/outlet channel (Raimundi Rivera 2015). Laguna Grande is circumscribed by the Cabezas de San Juan Natural Reserve, managed by Para La Naturaleza (formerly known as the Conservation Trust of Puerto Rico).

During a three-year study (from 2003 to 2006) conducted on stations located in the central portion of Laguna Grande, Sastre et al. (2013) reported temperatures ranging from 25.7-34.0°C (mean = 29.1°C) and salinity

concentrations ranging from 17.8-42.2 psu (mean = 35.5 psu). Soluble reactive phosphorus ranged from 0.01-0.61 mg/L (mean = 0.08 mg/L) and nitrates ranged from 0.04-0.56 mg/L (mean = 0.12 mg/L). Sastre et al. (2015) reported oxygen concentrations ranging from 1.44-6.90 mg/L (mean = 4.7 mg/L) and pH ranging from 7.7–8.1 (mean = 7.9).

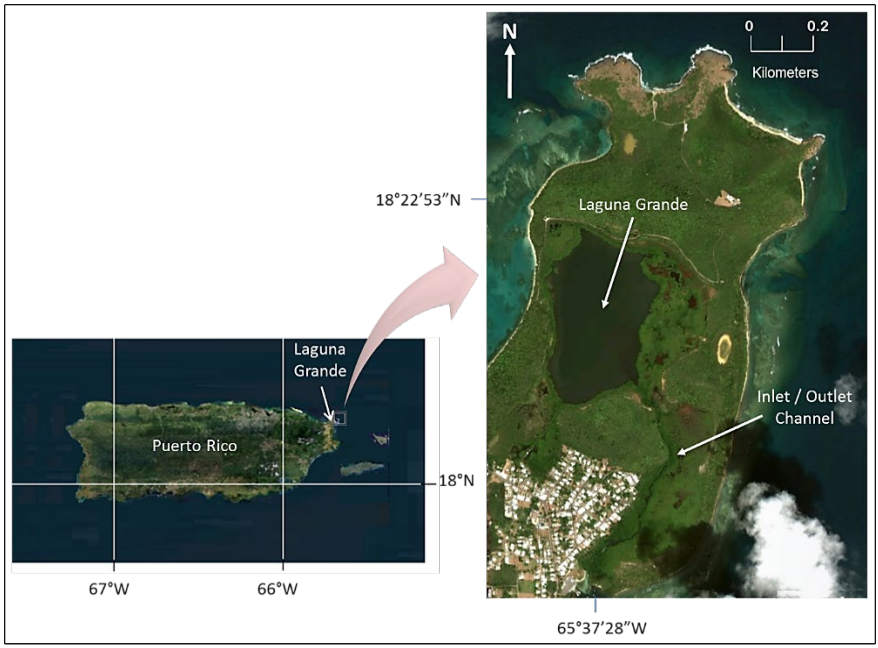

Figure 1. Satellite image of Puerto Rico (left), and a detailed image of Cabezas de San Juan Natural Reserve, showing the location of Laguna Grande and the inlet / outlet channel (right). Satellite images from Google Earth.

Densities of *Pyrodinium bahamense* and *Tripos furca*

To compare population densities of *Pyrodinium bahamense* and *Tripos furca*, before and after the entrance of *Sargassum* spp. into Laguna Grande, we used data from Sastre et al. (2013), who sampled at monthly intervals during a three-year period (three sampling stations); from Sastre et al. (2015), who sampled weekly during 38 sampling dates (two sampling stations); from Raimundi-Rivera (2015), who sampled during several tidal cycles on six occasions during three months (five sampling stations); from Antuna-Castillo et al. (2016), who sampled on five occasions during three months (four sampling stations); and from Arce (2016), who sampled twice during a three-week period (two sampling stations). The mean densities of *P. bahamense* and *T. furca* reported in these studies (op. cit.), in sites located inside the lagoon, were used

to construct Figure 4. The laboratory methods used in these studies for concentrating, counting, and calculating phytoplankton population densities are very similar and all procedures were performed in the same laboratory at the University of Puerto Rico at Humacao. Briefly, water samples were collected at the surface and at 2 m depth. The surface samples were collected at approximately 10 cm below the surface by grab sampling. The 2 m depth water samples were collected by suctioning water using a manual diaphragm water pump connected to a 2.5 cm diameter flexible tube, or with a sub-surface grab sampler (similar to the Wheaton Science Products Sub-Surface Grab Sampler I, model number 990250). The water sample volume among these studies ranged from 248.5 to 1,000.0 mL and replicates from zero to three. Water samples were immediately preserved in 2% formalin or 1% Lugol's solution.

The preserved water samples were mixed several times by gentle inversion and allowed to settle by gravity for three days (Wetzel and Likens 2000). Each sample was concentrated to 100 mL by carefully removing the supernatant with an automatic pipette. The supernatant was gravity filtered with a 20 µ Nitex® Nylon Bolt Cloth, and the cloth inspected for the presence of phytoplankton. In order to retain organisms, if any were observed in the cloth, these were returned to the concentrated sample. After hand-mixing the concentrated plankton sample, one mL subsample was obtained and transferred to a Sedgewick-Rafter (S-R) counting cell (Wetzel and Likens 2000). The S-R counting cell was covered with a cover glass and all *P. bahamense* and *T. furca* were counted under a microscope. Phytoplankton counts were used to calculate cell density.

Taxonomic Diversity

All samples were taken in the central portion of Laguna Grande (Figure 1). Taxonomic determinations of microalgae were carried out at least monthly, from January 2013 to February 2017, from the samples preserved in 2% formalin or 1% Lugol's solution (the former not adequate for naked dinoflagellates). To optimize taxonomic identifications, additional phytoplankton samples were analyzed while still alive. Individual phytoplankton cells were isolated following the technique of Andersen and Kawachi (2005). Identifications of isolated cells were carried out under a Nikon Eclipse E-600, a Leica CME, or an Olympus BH-2 microscope, using magnifications ranging from 100x to 400x. Taxonomic determinations were made according to Tomas (1997), and Hernández-Becerril and Navarro (1996), to the lowest possible taxa. The scientific nomenclature of the phytoplankton was based on the International Code of Nomenclature for Algae, Fungi and Plants (Turland et al. 2018).

Data Analyses

Data consisted of presence/absence information for most of the most recently observed algae (placed in 23 taxa, Table 1, denoted with an asterisk) for sampling dates, ranging from November 18, 2013 to January 27, 2017[6]. We performed an Exploratory Data Analysis (EDA) to gain a better understanding of the presence/absence patterns of the various taxa of microalgae and examined the difference in prevalence before and during the *Sargassum* presence in the lagoon. Another EDA was performed to examine the proportion of microalgae taxa present over time, before and during the presence of pelagic *Sargassum* in the lagoon. To visualize our results and have clearer picture of the possible effects of the *Sargassum* event, we constructed a presence/absence pattern chart over time of the different taxa and taxonomic groups (dinoflagellates, diatoms, and cyanobacteria) of microalgae.

Statistical Analyses

We conducted a series of randomization tests for each taxon of algae about whether the pelagic *Sargassum* event affected its presence/absence state. To formulate the question, the variable of interest is the presence/absence state of a certain microalgae at time point t, denoted by X(t), where t = 1,..T. The null hypothesis states that the distribution of X(t) is the same before and during the *Sargassum* event, while the alternative hypothesis states that the distribution of X(t) changes before and during this event. The randomization test is a nonparametric method, which does not require the observations to be independent or normally distributed. The procedures involve pooling the observed data from "before the event" and "during the event", shuffling the pooled data and reassigning them to "before" and "during" groups, calculating the test statistic for this certain permutation, and then repeating the procedure to get an empirical distribution of the test statistic under the null hypothesis. The p-value was calculated as the probability that the test statistic is equal to or more extreme than its observed value from real data according to its empirical distribution under the null hypothesis. Thus, if the test statistic observed from real data is extreme compared to its empirical distribution, we rejected the null hypothesis. The test statistic was chosen to be the mean difference, i.e., proportion for "during" minus proportion for "before", and when deriving its empirical distribution, we generated 10,000 random permutations. Since we

[6] The collection dates were: 18 and 26 November 2013; 03, 10, 18, and 23 December 2013; 07, 15, 22, and 28 January 2014; 03 and 11 February 2014; 18 and 25 March 2014; 04, 10, and 23 April 2014; 02, 08, 16, and 29 May 2014; 12, 17, and 25 June 2014; 09, 19, 24, and 31 July 2014; 12, 16, and 22 September 2014; 04, 10, 17, and 24 October 2014; 06 November 2014; 10 February 2015; 24 October 2015; 11 and 21 November 2015; 19 and 23 December 2015; 08 January 2016; 07 and 12 February 2016; 11 March 2016; 06 May 2016; 22 August 2016; 17 October 2016; 19 December 2016; 27 January 2017.

performed hypothesis tests for 23 types of microalgae and three taxonomic groups (diatoms, dinoflagellates, and cyanobacterua), we adjusted for multiple testing using the Bonferroni correction, and only rejected the null hypothesis when the p-value was less than 0.05 / (23+2) = 0.002. This gave an overall Type I error (false-positive) rate of 0.05 across all tests. Exploratory data analysis and statistical analysis was performed using R statistical software (R Core Team 2021).

Appendixes 1-3 contain the .csv files and the codes used for the statistical analyses. Those data are also available from the corresponding author.

Results

MODIS Aqua satellite, 1-km resolution images show rafts of *Sargassum* spp. mixed with other floating algae in the Caribbean Sea during July 27, 2015 (Figure 2). Because of their reflectance spectra characteristics, these algae are shown as brighter features (usually as green, yellow-green, and yellow pixels) on the AFAI images (Wang and Hu 2016). The long-curved lines (also called "image slicks") are very typical of *Sargassum* spp. blooms (Hu et al. 2015). During 2015 floating *Sargassum* spp. slicks were transported by the Brazil Current into the Caribbean Sea (Wang and Hu 2016, 2017). Very few floating algae were observed in the Atlantic Ocean, north of Puerto Rico (Figure 2). Larger scale 30-m resolution Landsat satellite images show the presence of floating *Sargassum* (as well as other floating algae) close to Laguna Grande during August 2015 (Figure 3) (red arrows indicate floating algae slicks). Large quantities of these autotrophs were transported by the flood-tidal currents into Laguna Grande, through the Laguna Grande Channel (Figure 1). The floating *Sargassum* that entered Laguna Grande consisted of *S. fluitans* (Børgesen) Børgesen 1914 and *S. natans*, (Linnaeus) Gaillon 1828, the only pelagic species within this genus (Taylor 1960, Wynne 2017, Guiry and Guiry 2021).

Population density of *Pyrodinium bahamense* and *Tripos furca,* before and during the 2015 *Sargassum* accumulation event, are shown in Figure 4 (Sastre et al. 2013, 2015; Raimundi-Rivera 2015; Antuna-Castillo 2016; Arce 2016). Before the event, these authors (op. cit.) reported mean densities of *P. bahamense* ranging from 14,492 to 28,202 cells/L. However, *P. bahamense* was absent from the lagoon during the 2015 *Sargassum* influx, until our last sampling date on November 11, 2015. Typically, seasonality does not cause the prolonged absence of *P. bahamense* (Sastre et al., 2013, 2015), as was observed in this study. *Tripos furca* was present in Laguna Grande in all studies, before and during the *Sargassum* influx. The reported mean densities of *T. furca* ranged from 476 to 2,602 cells/L.

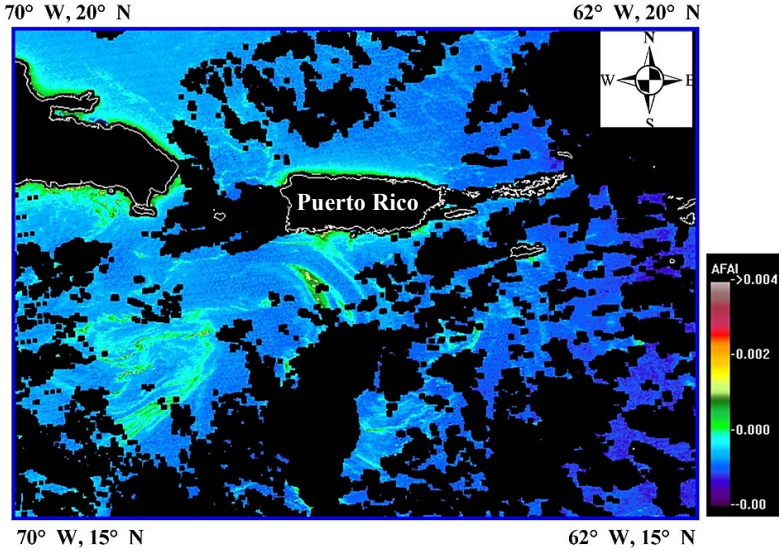

Figure. 2. Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua 1-km resolution satellite image showing rafts of floating algae (green, yellow-green, and yellow pixels) on the Caribbean Sea approaching Puerto Rico on July 27, 2015. Image from University of South Florida, Optical Oceanography Laboratory, *Sargassum* Watch System (University of South Florida, Optical Oceanography Laboratory 2018b). Alternative Floating Algae Index (AFAI) values indicated to the right.

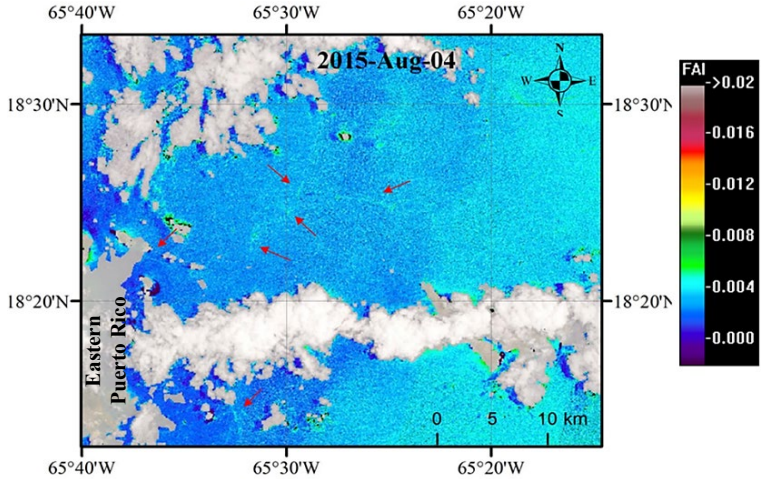

Figure 3. LANDSAT 30-m resolution satellite image showing rafts of floating algae (indicated by red arrows), during August 4, 2015, east of Laguna Grande, Puerto Rico. Floating Algae Index (FAI) values indicated to the right.

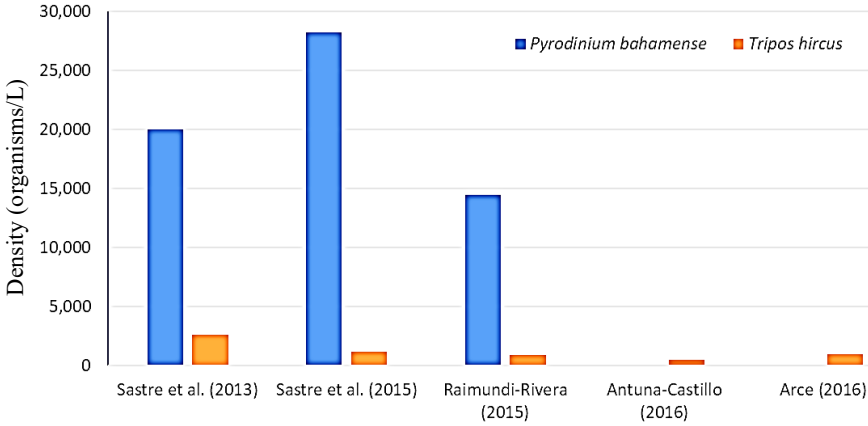

Figure 4. Densities of *Pyrodinium bahamense* and *Tripos furca* before the *Sargassum* event (left: Sastre et al. 2013, 2015; Raimundi-Rivera 2015) and during the event (right: Antuna-Castillo 2016, Arce 2016).

Twenty-four phytoplankton taxa were detected before the *Sargassum* accumulation event (all sampling dates included) in Laguna Grande and 17 during the event, out of 37 taxa (Table 1). Twenty (53%) taxa were observed only before the event, 13 (32%) only during the event and 4 (11%) before and during the event. Though more planktonic diversity samples were collected and analyzed prior to the *Sargassum* event in 2015, the presence/absence data suggests that the community composition was different before versus during the event. Unfortunately, we were not able to determine cell density in our samples.

Table 1. Phytoplankton taxa detected in Laguna Grande, Fajardo, Puerto Rico before and during the *Sargassum* event. The presence of a taxon is denoted with a 1; absence with a 0. Authority names are as in AlgaeBase (Guiry and Guiry, 2021). The nomenclature of phytoplankton was based on the International Code of Nomenclature for Algae, Fungi and Plants (Turland et al., 2018). The asterisk (*) indicates the taxa was detected from November 18, 2013 to January 27, 2017 that were included in the statistical analyses. Taxa without asterisk indicates it was detected before those dates.

TAXA **Phylum Miozoa, Superclass Dinoflagellata**	**Present before the *Sargassum* event**	**Present during the *Sargassum* event**
Pyrodinium bahamense L.Plate *	1	0
Tripos furca (Schröder) F.Gómez *	1	1
Tripos hircus (Ehrenberg) F.Gómez *	1	1
Ceratium cf. *trichoceros*	1	0
Protoperidinium spp. *	1	0

Protoperidinium pellucidum Bergh	1	0
Protoperidinium quinquecorne (Abé) Balech *	0	1
Gyrodinium sp.	1	0
Gyrodinium spirale (Bergh) Kofoid & Swezy *	0	1
Prorocentrum mexicanum Osorio-Tafall	1	0
Prorocentrum gracile F.Schütt	1	0
Gonyaulax cf. *digitalis* *	1	0
Gonyaulax polygramma F.Stein *	0	1
Gonyaulax verior Sournia	1	0
Cochlodinium polykrikoides Margalef	1	0
Lepidodinium sp. *	0	1
Gyrodinium estuariale E.M.Hulbert *	0	1
Levanderina fissa (Levander) Moestrup & al. *	1	0
Dinophysis caudata Kent *	1	0
Phylum Bacillariophyta		
Cylindrotheca sp.	1	0
Chaetoceros spp. *	1	0
Bacteriastrum sp.	1	0
Thalassionema spp. *	1	0
Melosira spp. *	1	0
Navicula spp. *	1	1
Cymbella sp. *	0	1
Coscinodiscus spp. *	1	0
Pleurosigma spp. *	1	1
Nitzschia longissima (Brébisson) Ralfs / *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C.Lewin	1	0
Pseudo-nitzschia sp. *	0	1
Tropidoneis sp.	1	0
Rhizosolenia sp. *	0	1
Phylum Chlorophyta		
Ulothrix sp.	0	1
Phylum Cryptophyta		
Chroomonas sp.	0	1
Phylum Cyanobacteria		
Aphanocapsa sp.	0	1
Microcystis sp. *	0	1
Spirulina sp. *	0	1
Number of taxa on each of the two phases of the event	24	17
Number of taxa	37	
Number of taxa unique to either phase of the event	20	13
Number of taxa common to each phase of the event	4	
Number of taxa included in analyses, *	23	

The Exploratory Data Analysis (Figure 5, page 215) shows that the proportion of presence of most taxa before the event (left hand side of the graph) is different to that observed during the event (right side of the graph). The taxonomic composition changed (before the event vs. during the event) and it seems most taxa were replaced by other taxa, possibly due to the presence and consequences of the entrance of pelagic *Sargassum* and other floating algae into Laguna Grande. As an example, we observed that *Pyrodinium bahamense, Chaetoceros* spp., and *Melosira* spp. were predominantly present before the pelagic *Sargassum* event, and *Gonyaulax polygramma, Lepidodinium* sp., and *Tripos furca* were mostly present during the event (Figure 5). *Tripos furca* was the only alga that showed a relatively high proportion both before the event and during the event.

Figure 6 (page 216) shows the proportion of present microalgae taxa over time, before the pelagic *Sargassum* event (left panel) and during the event (right panel). The proportion at any time point refers to the proportion of particular taxa of microalgae present out of the 23 total taxa observed. The proportion of present microalgae taxa was most of the time higher before the event than during the event. This lower proportion of microalgae observed during the event is a result of the lower number of taxa present in those samples. In general, there was an increasing tendency in the proportion of present alga types through time, during the *Sargassum* event.

The presence/absence pattern chart over time for the different taxa and taxonomic groups are shown in Figure 7 (page 217, left panel, before the *Sargassum* event; right panel, during the event). Most taxa displayed a consistent presence or absence throughout time before the *Sargassum* event. However, during the event the data is much noisier, mostly due to the emergence of new taxa. In general, results show a change in pattern and a drop in algae presence during the pelagic *Sargassum* event.

Figure 8 (page 219) shows the randomization test results, where the sample difference in proportion is plotted for all types of algae. Twelve types were found to have experienced a significant change before and during the *Sargassum* event. *Pyrodinium bahamense, Chaetoceros* spp., *Melosira* spp., *Coscinodiscus* spp., *Navicula* spp., *Thalassionema* spp., *Tripos furca, Gonyaulax digitalis* and *Protoperidinium* spp. observed negative significant change in proportion (proportion for "during" minus proportion for "before"), while *Gonyaulax poligramma, Lepidodinium* sp. and *Tripos hircus* observed a positive change in proportion. The negative significant change was observed in the taxa that were mostly present before the *Sargassum* event, while the positive significant change was observed in the algae that were mostly present during the *Sargassum* event.

All the hypothesis tests are two-sided, so we can conclude that there is either a significant difference, or not, but cannot infer directional conclusions (meaning significant increase/decrease).

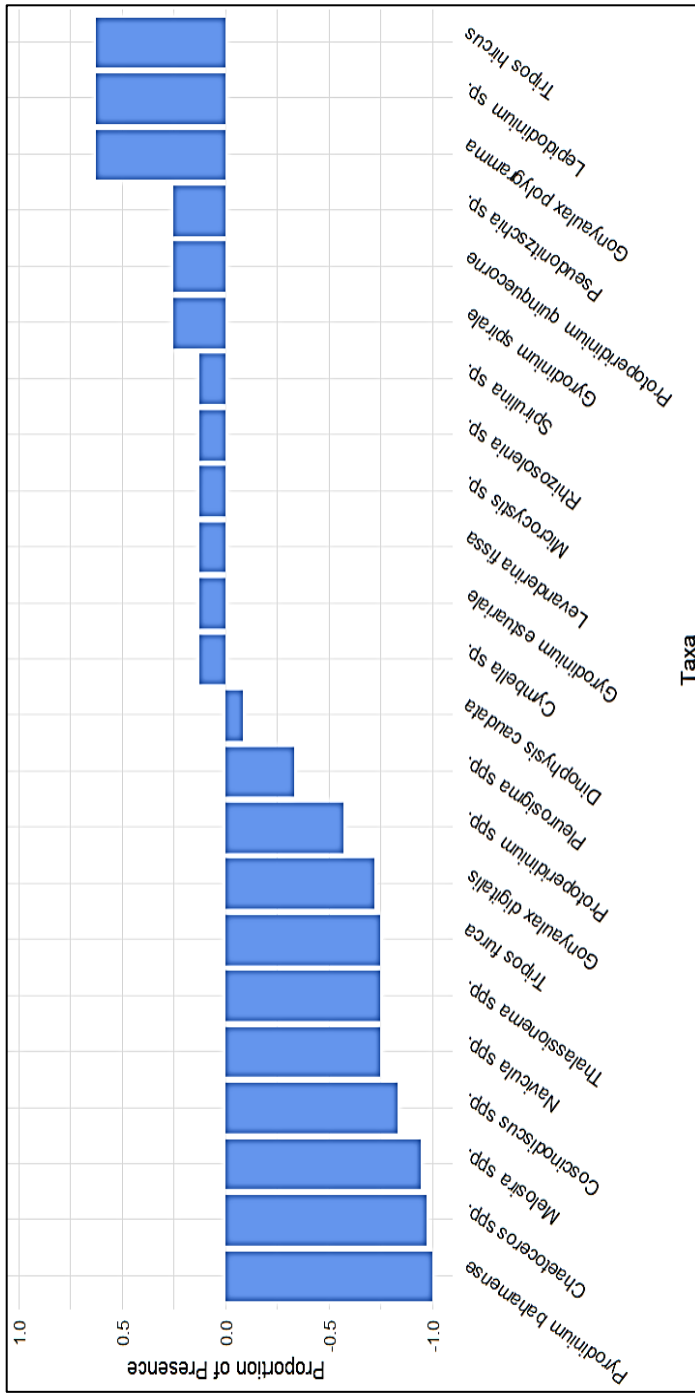

Figure 5. Proportion of microalgae present during - proportion of microalgae present before the entrance of pelagic *Sargassum* spp. into Laguna Grande (Fajardo, Puerto Rico). To calculate the proportion of presence for a time period (before/during), the count of times a taxon was measured as present (1) was divided by the number of data collection points during that time period (36 for before, or 8 for during). This figure shows the result of calculating the proportion of presence before the *Sargassum* event and subtracting it from the proportion of presence during the *Sargassum* event.

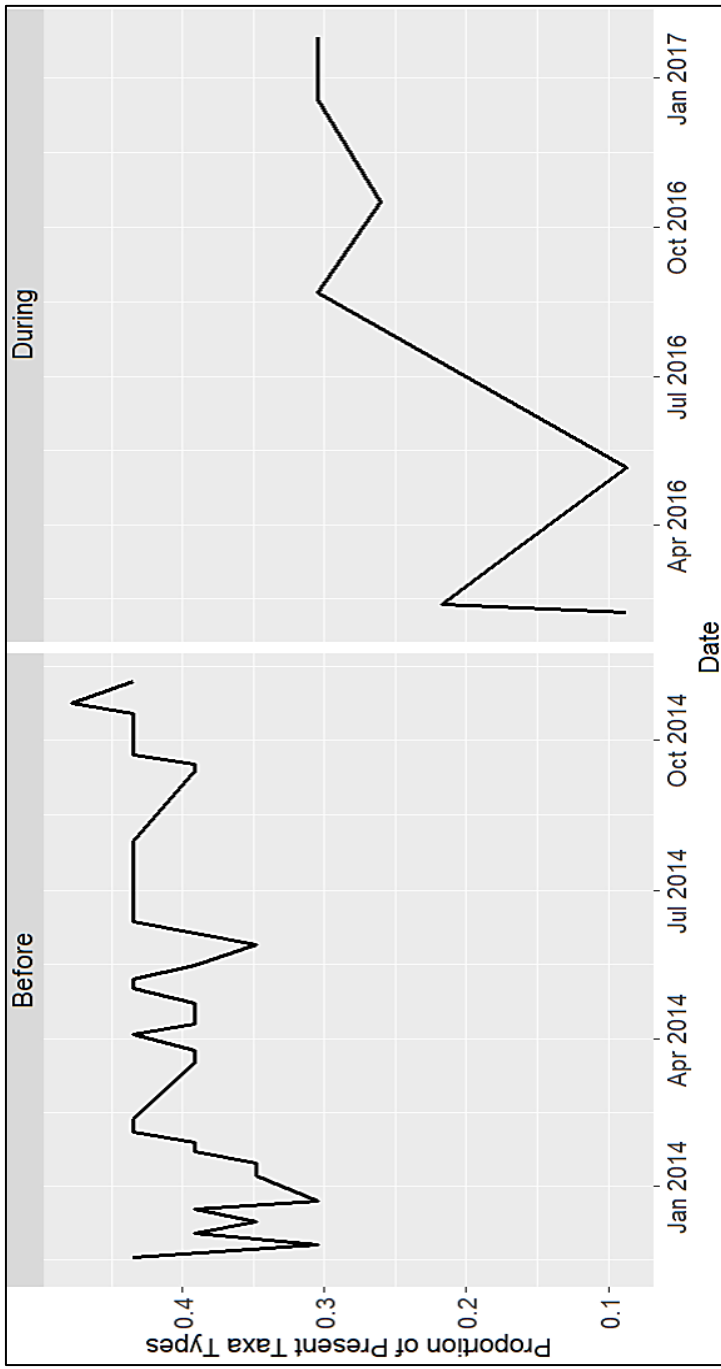

Figure 6. Proportion of microalgae presence over time before and during the *Sargassum* spp. event in Laguna Grande (Fajardo, Puerto Rico). Proportion at a particular sampling point refers to the proportion of unique species (or genera if the species was unidentified) present out of the 23 detected.

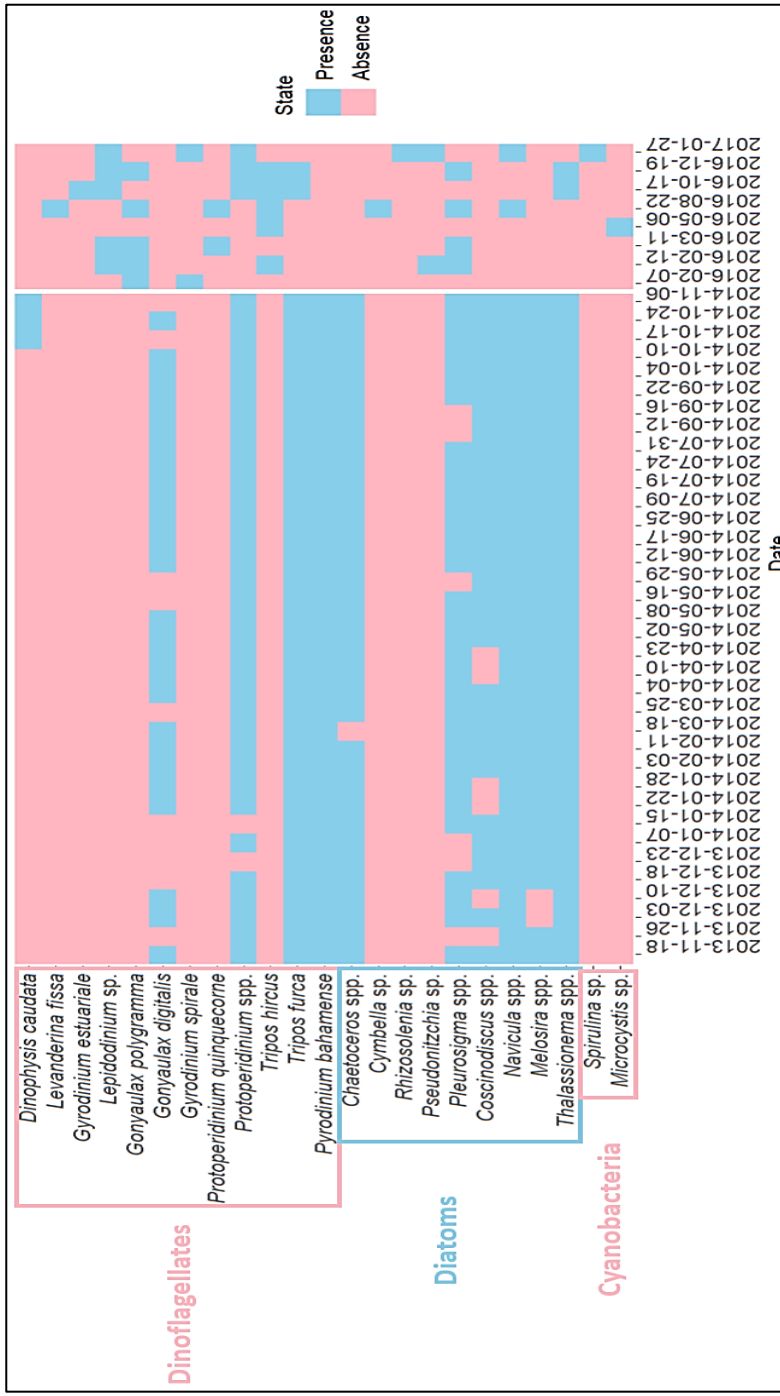

Figure 7. Presence/absence of the different microalgae over time. The white, vertical, dividing line denotes the difference between the before (to the left) and during (to the right) of the pelagic *Sargassum* spp. data collection periods.

Discussion

During 2015 pelagic *Sargassum* beaching events were first reported in Puerto Rico on April 28, in the Biosphere Reserve, Guánica (Rivera Arguinzoni 2015). On August 5, other *Sargassum* beaching events were reported on various sites in the western (Cabo Rojo) and southern (Lajas) coasts of Puerto Rico, and in the island of Vieques, Puerto Rico (Bosque Pérez 2015). On August 18, large quantities of dead fish were observed in Laguna Grande, which coincided with the entrance of *Sargassum, Syringodium*, and other primary producers into the lagoon (Departamento de Recursos Naturales y Ambientales de Puerto Rico 2015b). On this date and other later dates, massive amounts of pelagic *Sargassum*, and other floating primary producers in the area (e.g., *Syringodium*, *Thalassia*), were observed being transported into Laguna Grande (M. P. Sastre, personal observations, University of Puerto Rico at Humacao, Humacao, Puerto Rico). These organisms stayed confined in the Lagoon, due to the enclosed geomorphological characteristics of this water body, and unfavorable wind and/or water currents that did not promote their exit back into the ocean.

We hypothesize that the *Sargassum*, other marine algae, and sea grasses, that entered Laguna Grande decomposed and resulted in altered water quality. This, in part, facilitated a change in the phytoplankton community composition in the lagoon. During this process, the concentrations of organic and inorganic nutrients most likely increased. Also, oxygen levels most likely decreased (mainly due to the oxygen consumption that occurs during the bacterial decomposition of organic matter), as well as pH levels (mainly due to aerobic bacterial respiration which increases CO_2 levels in the water, shifting the aquatic carbonate system reactions toward the products, thus producing more hydrogen ions, lowering the pH of the water) and light levels.

As partial evidence of our hypothesis, oxygen concentrations in Laguna Grande, measured during the daytime by the Puerto Rico Department of Natural and Environmental Resources on August 18, 2015, were much lower (0.32-0.35mg/L, mean = 0.50 mg/L; Departamento de Recursos Naturales y Ambientales de Puerto Rico 2015a) than typical measurements that were reported before the event (mean = 4.7 mg/L, Sastre et al. 2015). From October 2015 to January 2016, Antuna-Castillo et al. (2016) reported oxygen concentrations ranging from 4.5–7.8 mg/L (mean = 6.2 mg/L) during the daytime in Laguna Grande, suggesting an increase in this parameter several months after the event.

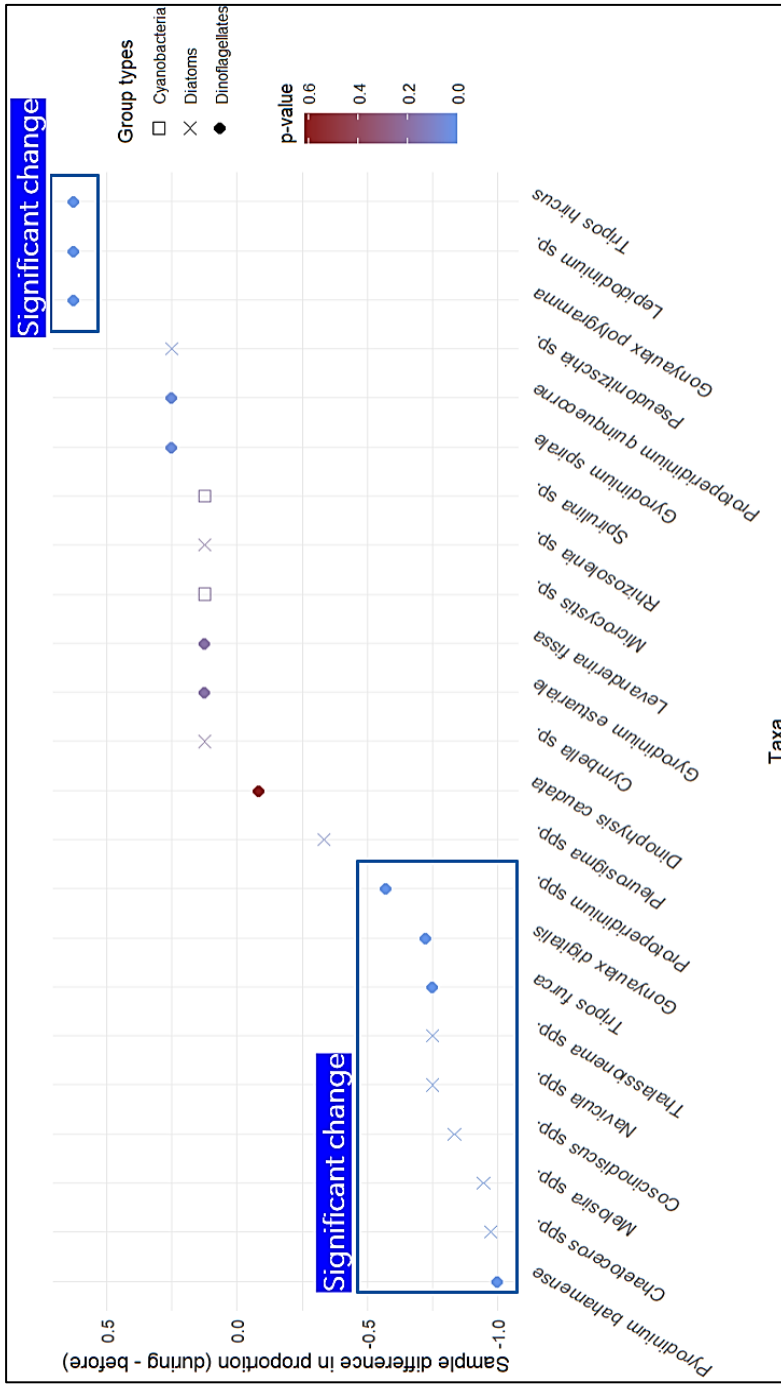

Figure 8. Randomization test results. shows the randomization test results, where the sample difference in proportion is plotted for all types of algae.

Even though watershed-derived nutrients are important environmental drivers for phytoplankton populations (Soler-Figueroa and Otero 2015), the decomposition of the large quantities of *Sargassum* (as well as other primary producers) that entered Laguna Grande most likely changed the overall nutrient profile in the lagoon and altered the typical concentrations. We hypothesize that the phytoplankton species composition changed (Table 1), since phytoplankton is particularly sensitive to nutrient concentrations (e.g., Goldman 1980, Reynolds 1997). We identified 13 taxa that were observed in Laguna Grande only during the *Sargassum* event (Table 1); and propose that at least some of these taxa entered Laguna Grande along with the pelagic *Sargassum*, since some diatoms (e.g., *Nitzchia*, *Navicula, Fragilaria, Pleurosigma, Thalassionema*) can live attached to floating *Sargassum* (Carpenter 1950, Maples 1984). It is possible that some of the organisms detected during the *Sargassum* event were present in Laguna Grande before but due to their very low densities they were never detected in the water samples. Also, that some taxa that were not detected during the event (e.g., *Pyrodinium bahamense*) were present but in very low quantities, and that some species that were not detected in our study sites were present and more abundant in other areas in the lagoon. Also, some species could have been present during other dates but not detected during the sampling dates. *Sargassum* spp. produces several compounds toxic to microalgae (Wang et al. 2012) which could have caused additional phytoplanktonic mortality and/or changes in the taxonomic composition in Laguna Grande.

Sastre et al. (2013, 2015) described *P. bahamense* population crashes due to different causes, but in both studies the population recovered in approximately three to four months to historically average (approximately 20,000 cells/L) densities. In this study, *P. bahamense* was not detected in the water samples even after approximately 1.5 years after the *Sargassum* started to enter the lagoon. This event could have affected both the planktonic stage, and benthic cyst stages of *P. bahamense*, hindering the recovery of this species. The population density of the dinoflagellate *Tripos furca*, the second most abundant phytoplankton species in Laguna Grande (Sastre et al. 2013, 2015; Raimundi-Rivera 2015), was not affected noticeably by the *Sargassum* event (Figure 4), suggesting it is a more robust and/or resilient species. This species has been reported to be mixotrophic, being able to photosynthesize but can also ingest, mainly small (10–40 µm) ciliates (e.g., *Strobilidium* spp., Smalley et al. 2003). The mixotrophic characteristic of this species could help its resilience since it can survive on various energy sources (photosynthesis and heterotrophy).

Our data analyses indicate there are various lines of evidence suggesting an effect of the pelagic *Sargassum* accumulation event in Laguna Grande on the phytoplankton community, including: 1. The proportional presence of the different taxa of algae is clearly different before the event versus during the

event (only four taxon in common out of 23 taxa); 2. The proportion of present microalgae taxa was generally much higher before the event; and during the event the proportion was generally smaller, especially at the beginning; 3. The presence/absence pattern of the observed taxa was clearly different before the event than during the event; 4. A randomization statistical test, about whether pelagic *Sargassum* affects the presence/absence state of microalgae, detected significant differences ($p < 0.05$) in proportion (proportion for "during" the event minus proportion for "before" the event) in 12 taxa of microalgae (9 taxa before the event and 3 during the event).

This is the first report about the possible detrimental effects of a major pelagic *Sargassum* influx on the phytoplankton taxa composition inside a tropical coastal lagoon. Local macrophytes, such as *Thalassia testudinum* and *Syringodium filiforme*, which could also accumulate in large quantities due to the detaching effects of large waves and/or high wind conditions, could also enter and accumulate in tropical lagoons, causing similar effects. However, in this report the effects caused by the pelagic *Sargassum* seem to prevail over those caused by the other macrophytes due to the visually larger mass and extent of the *Sargassum* accumulations.

According to quantitative visual observations of bioluminescence (based on 4 categories), performed on a regular basis by Para La Naturaleza in Laguna Grande, no bioluminescence or lower than normal levels were observed at least until the end of July, 2017 (Leonor Alicea, personal communication, *Para La Naturaleza*, May 4, 2022), suggesting the presence of few or no bioluminescent organisms, including *P. bahamense*, and the possible continuation of the effects generated by the *Sargassum* influx. On September 6, 2017, Hurricane Irma's center (category 5 hurricane) passed approximately 50 miles north of Laguna Grande; and on September 20, Hurricane María (category 4 hurricane, with maximum sustained winds of 155 miles per hour during landfall) passed over Puerto Rico, including Laguna Grande. Even though, to our knowledge, no data was obtained on the possible effects of these hurricanes in Laguna Grande, in our opinion, the water currents generated by Hurricane María flushed Laguna Grande and most likely affected the phytoplankton composition in the lagoon.

At the moment of this writing (May 12, 2022), sediment has continued to accumulate in the Laguna Grande inlet/outlet channel. This situation most likely has affected the flow and residence time of the water in Laguna Grande. It can also alter the physical-chemical characteristics of the lagoon (e.g., temperature, salinity, nutrient concentrations), which can affect the species composition of phytoplankton (e.g., Goldman 1980, Reynolds 1997). To maintain the ecosystem services presently provided by Laguna Grande as well as to maintain and perhaps improve its bioluminescence, careful dredging of the inlet/outlet channel while preventing or minimizing sediment transport, which could

damage nearby coral formations and other marine life, should be seriously considered.

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Appendix 1. The data: algae.csv file.

Obs	Date	Status	Pyrodiniu	Tripos.fur	Tripos.hir	Protoperi	Protoperi	Gyrodiniu	Gonyaula	Gonyaula	Lepidodin	Gyrodiniu	Levanderi	T
1	11/18/2013	Before	1	1	0	1	0	0	1	0	0	0	0	
2	11/26/2013	Before	1	1	0	1	0	0	0	0	0	0	0	
3	12/3/2013	Before	1	1	0	1	0	0	1	0	0	0	0	
4	12/10/2013	Before	1	1	0	1	0	0	1	0	0	0	0	
5	12/18/2013	Before	1	1	0	1	0	0	0	0	0	0	0	
6	12/23/2013	Before	1	1	0	0	0	0	0	0	0	0	0	
7	1/7/2014	Before	1	1	0	1	0	0	0	0	0	0	0	
8	1/15/2014	Before	1	1	0	0	0	0	0	0	0	0	0	
9	1/22/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
10	1/28/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
11	2/3/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
12	2/11/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
13	3/18/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
14	3/25/2014	Before	1	1	0	1	0	0	0	0	0	0	0	
15	4/4/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
16	4/10/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
17	4/23/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
18	5/2/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
19	5/8/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
20	5/16/2014	Before	1	1	0	1	0	0	0	0	0	0	0	
21	5/29/2014	Before	1	1	0	1	0	0	0	0	0	0	0	
22	6/12/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
23	6/17/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
24	6/25/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
25	7/9/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
26	7/19/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
27	7/24/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
28	7/31/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
29	9/12/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
30	9/16/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
31	9/22/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
32	10/4/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
33	10/10/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
34	10/17/2014	Before	1	1	0	1	0	0	0	0	0	0	0	
35	10/24/2014	Before	1	1	0	1	0	0	1	0	0	0	0	
36	11/6/2014	Before	1	1	0	1	0	0	0	0	0	0	0	
44	2/7/2016	During	0	0	0	0	0	1	0	1	0	0	0	
45	2/12/2016	During	0	0	1	0	0	0	0	1	1	0	0	
46	3/11/2016	During	0	0	0	0	1	0	0	1	1	0	0	
47	5/6/2016	During	0	0	1	0	0	0	0	0	0	0	0	
48	8/22/2016	During	0	0	1	0	1	0	0	1	0	0	1	
49	10/17/2016	During	0	1	1	1	0	0	0	0	1	1	0	
50	12/19/2016	During	0	1	1	1	0	0	0	1	1	0	0	
51	1/27/2017	During	0	0	0	1	0	1	0	0	1	0	0	

Thalassior	Melosira.s	Navicula.s	Coscinodi	Pleurosigr	Pseudo.ni	Rhizosole	Cymbella.	Chaetocer	Microcysti	Spirulina.s	Dinophysis.caudata
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	0	0	0	0	0	1	0	0	0
1	0	1	1	1	0	0	0	1	0	0	0
1	0	1	0	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	0	0	0	0	1	0	0	0
1	1	1	1	0	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	0	1	0	0	0	1	0	0	0
1	1	1	0	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	0	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	0	1	0	0	0	1	0	0	0
1	1	1	0	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	0	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	0	0	0	0	1	0	0	0
1	1	1	1	0	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	0
1	1	1	1	1	0	0	0	1	0	0	1
1	1	1	1	1	0	0	0	1	0	0	1
1	1	1	1	1	0	0	0	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0	0
0	0	1	0	1	0	0	1	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	1	0	0	0	0	0	0	0
0	0	1	0	0	1	1	0	0	0	1	0

Appendix 2. The groups.csv file.

Taxa	Type	Before	During	Overall
Chaetoceros spp.	DIATOM	97	0	decreased
Coscinodiscus spp.	DIATOM	83	0	decreased
Cymbella sp.	DIATOM	0	12.5	same
Dinophysis caudata Kent	DINO	8	0	same
Gonyaulax c.f. *digitalis*	DINO	72	0	decreased
Gonyaulax polygramma F.Stein	DINO	72	65	same
Gyrodinium estuariale E.M.Hulbert	DINO	0	88	increased
Gyrodinium spirale (Bergh) Kofoid & Swezy	DINO	0	75	increased
Lepidodinium sp.	DINO	0	63	increased
Levanderina fissa (Levander) Moestrup & al.	DINO	0	13	same
Melosira spp.	DIATOM	94	0	decreased
Microcystis sp.	CYANO	0	13	same
Navicula spp.	DIATOM	100	25	decreased
Pleurosigma spp.	DIATOM	83	50	decreased
Protoperidinium quinquecorne (Abé) Balech	DINO	0	25	increased
Protoperidinium spp.	DINO	34	3	decreased
Pseudo-nitzschia sp.	DIATOM	0	25	increased
Pyrodinium bahamense L.Plate	DINO	100	0	decreased
Rhizosolenia sp.	DIATOM	0	13	same
Spirulina sp.	CYANO	0	13	same
Thalassionema spp.	DIATOM	100	25	decreased
Tripos furca (Schröder) F.Gómez	DINO	100	53	decreased
Tripos hircus (Schröder) F.Gómez	DINO	0	63	increased

Appendix 3. Code for the statistical analyses and graphs presented herein.

This code, prepared in RStudio, is organized following the order of the statistical analyses. The colors represent different aspects of the code. For instance, the brown pound signs, #, signify comments; green fonts represent characters or text; blue characters represent columns, rows, or vectors; and **bold dark blue** characters represent user specified functions or for loops. Figures included in the paper are denoted in the comments.

Preprocessing and Data Organization

```
# Packages
library(reshape2)
library(tidyverse)
library(data.table)
library(astsa)
library(gginference)
library(purrr)
library(ggtext)

# Reading in algae data and grouping information data
algae <- read_csv("algae.csv")
groups <- read_csv("groups.csv")

# Fixing column names to only include the first part of the string
colnames(algae) <- str_extract(names(algae), "[^.]*.[^.]*")

########## Scientific grouping ###########
# Cleaning up grouping data
groups <- groups[c(1:23),c(1:2)] # getting rid of extraneous rows/columns
groups$Taxa <- str_replace_all(groups$Taxa, " ", ".") # converting all spaces to periods
groups$Taxa <- str_replace_all(groups$Taxa, "-", ".") # converting all hyphens to periods
groups$Taxa <- str_extract(groups$Taxa, "[^.]*.[^.]*") # getting rid of last part of string
to match the # Pulling out the diatoms and getting rowSums for count data
```

```
diatom_names <- groups %>% filter(Type == "DIATOM") %>% pull(Taxa)
diatom <- algae %>% select(diatom_names)
diatom.count <- rowSums(diatom)

# Pulling out the dinos and getting rowSums for count data
dino_names <- groups %>% filter(Type == "DINO") %>% pull(Taxa)
dino <- algae %>% select(dino_names)
dino.count <- rowSums(dino)

# Pulling out the cyanos and getting rowSums for count data
cyano_names <- groups %>% filter(Type == "CYANO") %>% pull(Taxa)
cyano <- algae %>% select(all_of(cyano_names))
cyano.count <- rowSums(cyano)
# Creating final summarized dataframe for export

algae_groups <- data.frame(algae[,1:3],diatom.count,dino.count,cyano.count)

# Exporting file as .csv file
# write_csv(algae_groups, file = "algae_groups.csv")
# transform the layout of the dataset (heatmap and randomization test)
algae_df <- melt(algae, id=1:3,value.name='State',variable.name = 'algae')
algae_df$State <- factor(algae_df$State,levels=1:0,labels=c('presence','absence'))
algae_df <- left_join(algae_df, groups[, 1:2], by = c('algae' = 'Taxa'))
algae_df <- algae_df[order(algae_df$Type), ]
algae_df$algae <- factor(algae_df$algae, levels = unique(algae_df$algae))
```

Exploratory Data Analyses

```
#------------------------------#
# #
# exploratory data analysis #
# #
```

```
#------------------------------#
################ First 3 Bar plots #################
#Remove invalid rows and columns
pieces <- algae[,-c(1,2,3)]

#Isolating before data
before = pieces[-c(37,38,39,40,41,42,43,44),]

#Isolating during data
during = pieces[c(37,38,39,40,41,42,43,44),]
#Taking proportion for before and during
Before <- as.numeric(colMeans(x = before))
During <- as.numeric(colMeans(x = during))

#Creating column of algae types to use in the barplot
Type <- c('*Pyrodinium bahamense*', '*Tripos furca*', '*Tripos hircus*',
'*Protoperidinium* spp.', '*Protoperidinium quinquecorne*', '*Gyrodinium spirale*',
'*Gonyaulax digitalis*', '*Gonyaulax polygramma*', '*Lepidodinium* sp.', '*Gyrodinium
estuariale*',
'*Levanderina fissa*', '*Thalassionema* spp.', '*Melosira* spp.', '*Navicula* spp.',
'*Coscinodiscus* spp.', '*Pleurosigma* spp.', '*Pseudonitzschia* sp.', '*Rhizosolenia*
sp.', '*Chaetoceros* spp.', '*Microcystis* sp.', '*Spirulina* sp.', '*Dinophysis caudata*')

#Creating the difference for during - before
Difference = During - Before

#Rounding the proportion values for before to the 3rd decimal place
Before = round(Before, 3)

#Combinding all 3 columns into a data frame
count <- data.frame(Before, During, Type)
```

```
#Bar plot for before
ggplot(data=count, aes(x = reorder(Type, Before), y = Before)) + geom_bar(stat = "identity", fill = "cornflowerblue", #Bar plot for during
ggplot(data=count, aes(x = reorder(Type, During), y = During)) + geom_bar(stat = "identity", fill = "cornflowerblue") #Bar plot for the difference between during - before (reordered from least to greatest)

######## Figure 5 in Manuscript #################
ggplot(data=count, aes(x = reorder(Type, Difference), y = Difference)) + geom_bar(stat = "identity", fill

############ Overall Line Plot ######################################
# Looking at overall presence of algae over time
algae$Total.Presence <- rowSums(algae[,4:26])
algae %>% ggplot(aes(x = Date, y = Total.Presence)) + geom_line() +
facet_wrap(~ Status, scales = "free_x") +
labs(y = "Total Count of Present Algae Types",
subtitle = "Count at a particular time point refers to the number of unique types of algae present ggtitle("Algae Presence over Time Before vs. During Sargassum Presence in Laguna Grande")

######## Figure 6 in Manuscript #################
algae %>% ggplot(aes(x = Date, y = Total.Presence/23)) + geom_line(linewidth = 1) +
facet_wrap(~ Status, scales = "free_x") +
labs(y = "Proportion of Present Taxa Types",
subtitle = "Proportion at a particular time point refers to the proportion of unique types of taxa ggtitle("Proportion of Taxa Presence over Time Before vs. During *Sargassum*

################ Clustering ###################################
# Restrict to before Sargassum
algae_before <- t(algae[1:36,-c(1:3,27)])
###############################
```

```
### Hierarchical Clustering ##############################
## average linkage ##
hier.av = hclust(dist(algae_before), method="average")

# dendrogram
plot(hier.av, xlab = "Algae Types", ylab = "Distance",
main = "Cluster Dendrogram of Algae Types Before Sargassum Presence")

# Create a dataframe for each resulting before cluster to do more EDA
algae_gp1 <- data.frame(algae[,1:3],algae$Dinophysis.caudata,algae$Spirulina.sp,
algae$Microcystis.sp,algae$Cymbella.sp,algae$Rhizosolenia.sp,
algae$Pseudo.nitzschia,algae$Levanderina.fissa,
algae$Gyrodinium.estuariale,algae$Lepidodinium.sp,
algae$Gonyaulax.polygramma,algae$Gyrodinium.spirale,
algae$Tripos.hircus,algae$Protoperidinium.quinquecorne)
colnames(algae_gp1)[4:16] <- sub(".*algae.", "", names(algae_gp1[4:16]))
algae_gp2 <- data.frame(algae[,1:3],algae$Gonyaulax.digitalis,algae$Pleurosigma.spp,
algae$Coscinodiscus.spp,algae$Melosira.spp,algae$Protoperidinium.spp,
algae$Chaetoceros.spp,algae$Navicula.spp,algae$Thalassionema.spp,
algae$Pyrodinium.bahamense,algae$Tripos.furca)
colnames(algae_gp2)[4:13] <- sub(".*algae.", "", names(algae_gp2[4:13]))

# EDA to examine behavior over time during Sargassum presence for each group
# Filter data to look at During only - group 1 (mostly absent before)
algae_gp1_filt <- algae_gp1 %>% filter(Status == "During")
par(mfrow = c(5,3), mai = c(0.3, 0.4, 0.5, 0.1))
for (i in 4:16) {plot(x=algae_gp1_filt$Date, y = algae_gp1_filt[,i], type = 'b',
xlab = "", ylab = "",
main = names(algae_gp1_filt[i]),
col = "blue", ylim = c(-0.1,1.1), yaxt = "n")
axis(2, at = c(0,1))}
mtext("Cluster Group 1 Presence/Absense During Sargassum (Feb. 2016- Jan. 2017)",
```

```
side = 3, line = -1.5, outer = T)

# Filter data to look at During only - group 2 (mostly present before)
algae_gp2_filt <- algae_gp2 %>% filter(Status == "During")
par(mfrow = c(4,3), mai = c(0.3, 0.4, 0.5, 0.1))
for (i in 4:13) {plot(x=algae_gp2_filt$Date, y = algae_gp2_filt[,i], type = 'b',
xlab = "", ylab = "",
main = names(algae_gp2_filt[i]),
col = "blue", ylim = c(-0.1,1.1), yaxt = "n")
axis(2, at = c(0,1))}
mtext("Cluster Group 2 Presence/Absense During Sargassum (Feb. 2016- Jan. 2017)",
side = 3, line = -1.5, outer = T)
par(mfrow = c(1,1))

############## Heatmaps ######################
# heatmap for all algae
######## Figure 7 in Manuscript #################
labels <- rev(c('*Dinophysis caudata*','*Levanderina fissa*','*Gyrodinium estuariale*',
'*Lepidodinium* sp.','*Gonyaulax polygramma*','*Gonyaulax digitalis*',
'*Gyrodinium spirale*','*Protoperidinium quinquecorne*','*Protoperidinium* spp.',
'*Tripos hircus*','*Tripos furca*','*Pyrodinium bahamense*','*Chaetoceros* spp.',
'*Cymbella* sp.','*Rhizosolenia* sp.','*Pseudonitzchia* sp.','*Pleurosigma* spp.',
'*Coscinodiscus* spp.','*Navicula* spp.','*Melosira* spp.','*Thalassionema* spp.',
'*Spirulina* sp.','*Microcystis* sp.'))
ggplot(algae_df, aes(x=Date, y=algae))+
geom_tile(aes(fill=State))+
labs(y='',title='Presence/absence of each type of taxa')+
theme(panel.background = element_rect(fill="white")) +
scale_fill_manual(values=c('skyblue','lightpink')) +
scale_y_discrete(labels = labels) +
theme(axis.text.x=element_text(angle=90, size = 11),
axis.text.y=element_markdown(size = 11),
```

```
legend.text=element_text(size=11))

# heatmap for two algae as an example
ggplot(algae_df[algae_df$algae %in% c('Microcystis.sp', 'Chaetoceros.spp'), ],
aes(x=Date, y=algae))+
geom_tile(aes(fill=State))+
labs(y='',title='Presence/absence of each type of algae')+
theme(panel.background = element_rect(fill="white")) +
scale_fill_manual(values=c('skyblue','lightpink')) +
theme(axis.text.x=element_text(angle=90))
algae_df$State <- ifelse(algae_df$State == 'presence', 1, 0)

############## Line plot with groups #############
# Looking at grouped presence of algae over time
algae_groups %>% ggplot() + geom_line(aes(x = Date, y = diatom.count/9, color =
"Diatoms"), linewidth = geom_line(aes(x = Date, y = dino.count/12, color =
"Dinoflagellates"), linewidth = 1) +
facet_wrap(~ Status, scales = "free_x") +
labs(y = "Proportion of Present Algae Types",
subtitle = "The proportion at a particular time point refers to the proportion of unique
types of color = "Legend", caption = "Note that there are presence/absence measurements
for 9 types of Diatoms, scale_color_manual(values = c("Diatoms" = "red",
"Dinoflagellates" = "blue")) +
ggtitle("Algae Presence over Time Before vs. During Sargassum Presence in Laguna
Grande by Group")

##############Barplot of proportion of Algae in Groups############
#Before
Diatoms.b = sum(algae_groups$diatom.count[1:36])/sum(algae_groups$diatom.count)
Dinoflagellates.b = sum(algae_groups$dino.count[1:36])/sum(algae_groups$dino.count)
#During
Diatoms.d = sum(algae_groups$diatom.count[37:44])/sum(algae_groups$diatom.count)
```

```
Dinoflagellates.d = sum(algae_groups$dino.count[37:44])/sum(algae_groups$dino.count)

#New data frame of algae proportion by group
prop=data.frame(Status=c("Before","During","Before","During"),
Groups_of_ALgae=c("Diatoms","Diatoms","Dinoflagellates","Dinoflagellates"),
Proportion_of_Presence=c(Diatoms.b, Diatoms.d,Dinoflagellates.b,Dinoflagellates.d) )
#Bar plot
ggplot(prop, aes(x=Groups_of_ALgae,y=Proportion_of_Presence,fill=Status)) +
geom_bar(stat="identity", position="dodge")+
labs(x="Groups of Algae",y="Proportion of Presence",title="Proportion of Algae
Presence Before/During scale_fill_brewer(palette="Paired")+
theme_minimal()
```

################################ Statistical Analyses

```
#----------------------#
# #
# randomization test #
# #
#----------------------#
# randomization test for Microcystis.sp
# reference: https://bookdown.org/curleyjp0/psy317l_guides5/randomization-testing.html
algae1 <- algae_df[algae_df$algae == 'Microcystis.sp', c(3, 5)]
results<-vector('list',10000)

# repeat the randomization for 10000 times
for(i in 1:10000){

# pool and shuffle the data
x <- split(sample(algae1$State), rep(c('Before', 'During'),
as.vector(table(algae1$Status))))

# calculate the observed test statistic for this randomization
```

```
results[[i]]<-mean(x[[2]]) - mean(x[[1]])
}
df <- data.frame(difs = unlist(results))

# the observed test statistic for raw data
(stat <- mean(algae1$State[algae1$Status == 'During']) -
mean(algae1$State[algae1$Status == 'Before']))

# the histogram that shows the empirical distribution of the test statistic under null hypothesis
ggplot(df, aes(x=difs)) +
geom_histogram(color="black", fill="lightpink", alpha=.4) +
geom_vline(color="navy",lwd=1,lty=2,xintercept = stat) +
theme_bw() +
xlab('Test statistic (mean difference between "during" and "before")') +
ggtitle("Mean Differences from 10000 randomizations of Raw Data\n Algae Type: Microcystis.sp")

# p value is the probability that the test statistic is as extreme as the value observed from raw data, (p.value <- mean(abs(df$difs) >= abs(stat)))
# randomization test for Chaetoceros.spp
algae1 <- algae_df[algae_df$algae == 'Chaetoceros.spp', c(3, 5)]
results<-vector('list',10000)
for(i in 1:10000){
x <- split(sample(algae1$State), rep(c('Before', 'During'),
as.vector(table(algae1$Status))))
results[[i]]<-mean(x[[2]]) - mean(x[[1]])
}
df <- data.frame(difs = unlist(results))
(stat <- mean(algae1$State[algae1$Status == 'During']) -
mean(algae1$State[algae1$Status == 'Before']))
ggplot(df, aes(x=difs)) +
```

```
geom_histogram(color="black", fill="skyblue", alpha=.4) +
geom_vline(color="navy",lwd=1,lty=2,xintercept = stat) +
theme_bw() +
xlab('Test statistic (mean difference between "during" and "before")') +
ggtitle("Mean Differences from 10000 randomizations of Raw Data\n Algae Type: Chaetoceros.spp")
(p.value <- mean(abs(df$difs) >= abs(stat)))

# randomization test for each type of algae, record the p-values
algae.names <- unique(algae_df$algae)
p.values <- rep(NA, length(algae.names))
for(j in 1:length(algae.names)){
algae1 <- algae_df[algae_df$algae == algae.names[j], c(3, 5)]
results <- vector('list',10000)
for(i in 1:10000){
x <- split(sample(algae1$State), rep(c('Before', 'During'), as.vector(table(algae1$Status))))
results[[i]]<-mean(x[[2]]) - mean(x[[1]])
}
df <- data.frame(difs = unlist(results))
stat <- mean(algae1$State[algae1$Status == 'During']) - mean(algae1$State[algae1$Status == 'Before'])
p.values[j] <- mean(abs(df$difs) >= abs(stat))
}

df <- data.frame(algae = algae.names, p.value = p.values) %>%
left_join(groups, by = c('algae' = 'Taxa'))
write.csv(df, 'p_values_for_randomization_test.csv')
algae0 <- aggregate(algae_df$State, list(algae_df$Date, algae_df$Status, algae_df$Type), sum)
colnames(algae0) <- c('Date', 'Status', 'Type', 'Count')
```

```
# randomization test for the group DINO
algae1 <- algae0[algae0$Type == 'DINO', c(2, 4)]
results<-vector('list',10000)
for(i in 1:10000){
x <- split(sample(algae1$Count), rep(c('Before', 'During'),
as.vector(table(algae1$Status))))
results[[i]]<-mean(x[[2]]) - mean(x[[1]])
}
df <- data.frame(difs = unlist(results))
(stat <- mean(algae1$Count[algae1$Status == 'During']) -
mean(algae1$Count[algae1$Status == 'Before']))
ggplot(df, aes(x=difs)) +
geom_histogram(color="black", fill="orange", alpha=.4) +
geom_vline(color="navy",lwd=1,lty=2,xintercept = stat) +
theme_bw() +
xlab('Test statistic (mean difference between "during" and "before")') +
ggtitle("Mean Differences from 10000 randomizations of Raw Data\n Dinoflagellate")
(p.value <- mean(abs(df$difs) >= abs(stat)))

# randomization test for the group DIATOM
algae1 <- algae0[algae0$Type == 'DIATOM', c(2, 4)]
results<-vector('list',10000)
for(i in 1:10000){
x <- split(sample(algae1$Count), rep(c('Before', 'During'),
as.vector(table(algae1$Status))))
results[[i]]<-mean(x[[2]]) - mean(x[[1]])
}
df <- data.frame(difs = unlist(results))
(stat <- mean(algae1$Count[algae1$Status == 'During']) -
mean(algae1$Count[algae1$Status == 'Before']))
ggplot(df, aes(x=difs)) +
geom_histogram(color="black", fill="red", alpha=.4) +
```

```
geom_vline(color="navy",lwd=1,lty=2,xintercept = stat) +
theme_bw() +
xlab('Test statistic (mean difference between "during" and "before")') +
ggtitle("Mean Differences from 10000 randomizations of Raw Data\n Diatom")
(p.value <- mean(abs(df$difs) >= abs(stat)))

###############P values plot###################
######## Figure 8 in Manuscript #################
name=c(
"*Pyrodinium bahamense*",
"*Tripos furca*","*Tripos hircus*" , "*Protoperidinium* spp.", "*Protoperidinium quinquecorne*",
"*Gyrodinium spirale*","*Gonyaulax digitalis*","*Gonyaulax polygramma*","*Lepidodinium* sp.",
"*Gyrodinium estuariale*","*Levanderina fissa*","*Thalassionema* spp.","*Melosira* spp.",
"*Navicula* spp.","*Coscinodiscus* spp.","*Pleurosigma* spp.","*Pseudonitzschia* sp.","*Rhizosolenia* "*Cymbella* sp.","*Chaetoceros* spp.","*Microcystis* sp.","*Spirulina* sp.","*Dinophysis caudata*")
algae_final <- read.csv("algae.csv")
meanfun <- function(z){
i <- mean(algae_final[37:44,z])-mean(algae_final[1:36,z])
return(i)
}
result <- map_dbl(4:26,meanfun) #Mean difference in proportion
pvalue=c(0,0,1e-04,9e-04,0.0291,
0.0288,1e-04,2e-04,0,0.1787,
0.1803,0,0,0,1e-04,0.0637,
0.0298,0.1808,0.1825,0,0.1856,0.1786,0.6162)
groupname = c(
"Dinoflagellates",
"Dinoflagellates","Dinoflagellates","Dinoflagellates","Dinoflagellates",
```

```
"Dinoflagellates","Dinoflagellates","Dinoflagellates","Dinoflagellates",
"Dinoflagellates","Dinoflagellates","Diatoms","Diatoms",
"Diatoms","Diatoms","Diatoms","Diatoms","Diatoms",
"Diatoms","Diatoms","Cyanobacteria","Cyanobacteria","Dinoflagellates")
change=as.data.frame(t(algae_final))
change=change[-(1:3),]
newalgae=cbind(name,groupname,change,mean=result,pvalue)
ggplot(newalgae,aes(x=reorder(name,mean),y=mean,label=name,shape=groupname))+
geom_point(aes(colour=pvalue),size=2.8)+
scale_color_gradient(low = "cornflowerblue", high = "firebrick4")+
labs(y="Sample difference in proportion (during - before)",x="Taxa",colour="p-
value",shape="Group types")+
theme_minimal()+
theme(axis.text.x = element_markdown(angle = 60,hjust=1,size=11),
axis.text.y = element_text(size = 10), axis.title = element_text(size = 12))+
scale_shape_manual(values=c(0,4,16))

#########################################################################
```