

Low diversity triggers harmful algae bloom (HAB) occurrence adjacent to desalination plants along the Red Sea

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ABSTRACT

Rapid, large scale growth of microscopic planktonic algae, called harmful algal blooms (HABs) have afflicted many marine coastal regions around the world. Few studies of HABs have been conducted in the Red Sea where desalination plants along the Saudi Arabian Red Sea coast provide drinking water for millions of people. We hypothesized that desalination effluent near the outlet of these desalination plants may alter the phytoplankton species composition and contribute to the selection of HABs in these areas. To test this hypothesis, a 2-year study from 2014 to 2016 was conducted to determine the diversity structure, spatio-temporal distribution and seasonal succession of phytoplankton populations using monthly samples from three different sites along the Saudi Arabian coast near Jeddah, Al-Shoaiba and Al-Quonfuduah. The results found a total of 125 phytoplankton species belonging to 4 major groups: Cyanobacteria (Cyanoprokaryonta) (*Oscillatoria* sp. and *Anabaena* sp.), Dictyocophyceae (*Dictyocha* sp.), Dinophyceae (85 species) and Bacillariophyceae (37 species). We identified high population percentages of the toxigenic species *Dinophysis miles* and *D. caudata* as well as the cyanobacteria *Anabaena* sp. The lowest diversity index (0.02 bits cell⁻¹) was detected on September 2016 at the Al-Quonfuduah near shore station during a bloom of *Nitzschia* sp. (4.4×10^5 cells L⁻¹). The lowest diversity detected at Al-Shoaiba in June 2015 (0.6 bits cell⁻¹) was during the proliferation of *Dinophysis miles* (7.2×10^4 cells L⁻¹). The highest diversity index (2.88 bits cell⁻¹) was detected at Jeddah during April 2016 due to the proliferation of 57 different species with equal concentrations. During the study period, Al-Quonfuduah had the most favorable conditions for the proliferation of toxigenic species and for overall numbers of microalgae taxa. The autumn period and nearshore stations had the most favorable conditions for phytoplankton among the three sites. Our results conclude that low phytoplankton diversity at the nearshore stations triggering an HAB occurrence may due to the high salinity and temperature of the desalination plant effluent.

Keywords: Desalination plants; Phytoplankton; Harmful algal bloom; Seasonal succession; Toxigenic species

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1. Introduction

Harmful algal blooms (HABs) refer to high cell numbers of freshwater phytoplankton (cyanobacteria) and marine phytoplankton (eukaryotic flagellates and diatoms) that are toxin producers and/or cause other dangerous negative effects such as oxygen stress or biofouling [1]. In reference to interfering with the normal function of a desalination plant, it is the HAB effect from high biomass that is more important [2]. The actual risk to potable water from a desalination plant is minimized due to the reverse osmosis (RO) process used in the desalination process [3]. In this study, we designate HAB species as those that are known to be a problem from their high biomass and HAB toxigenic species as those phytoplankton that are known to produce potent toxins according to the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae [4].

Toxin production by some micro-algal species remains unclear. Algal toxins as secondary metabolites have no obvious function in the producer organism's internal metabolic budget but have very serious physiological effects in mammals. Toxin producers may be using their toxins as a defensive strategy against other organisms or as a toll for space completion [5].

Different species of dinoflagellates have been responsible for different HAB events in the Arabian Gulf. The dinoflagellate *Gymnodinium selliforme* was responsible for the red tide event that occurred in Kuwait during September to October of 1999. This event caused the death of 80,000 fish followed by another bloom of the non-toxic ciliate *Mesodinium rubrum* [6]. The species *Cochlodinium polykrikoides* Margalef was responsible for the catastrophic HAB event that lasted for 8 months during 2008–2009 along the Arabian Gulf shore near the United Arab Emirates [7].

These events highlight a problem for countries in the Arabian Gulf that depend mainly on seawater desalination as a source of freshwater. HAB disruption of desalination plant operations poses a problem to adequate and safe drinking water supplies in this part of the world [7]. Many regions in the Gulf of Aden and Arabian Gulf have experienced HAB events that trigger mass fish deaths. High fish mortality was detected along the Qatari coast and offshore during the summer of 1996 and 1998. Substantial amounts (about 40 tons) of dead fish were recorded off their eastern shoreline. Neurotoxic Shellfish Poisoning (NSP) by the dinoflagellate *Chattonella antiqua* caused an almost 80% fish mortality at aquaculture sites in this area [8].

In recent years, it has become clear that HABs pose a problem for seawater desalination using RO [2,9,10]. About 150 countries worldwide including Saudi Arabia depend on desalination plants to cover part or most of their needs for drinking water. It is estimated that the desalination capacity will only be increased in the future [11].

During HAB episodes, up to a 40% reduction in desalination plant production can occur along with a lower quality of desalinated drinking water as a result of taste and odor problems and even HAB toxin residues [11,12].

Operation disruptions at desalination plants have been reported due to HAB blockage of intake filters, fouling of filtration surfaces and damage to costly RO membranes. HABs have also led to water shortages and electricity shutdowns [13–15].

These problems from HABs demonstrate the necessity for synchronized HAB monitoring programs, improved protocols and/or innovations to avoid desalination plant shutdown during HAB events [12]. Therefore, it is important to understand and manage the biological and ecological phytoplankton dynamics of these coastal areas especially those adjacent to desalination plants. Although studies have investigated the composition and ecological circulation of phytoplankton near Jeddah on the east side of the Red Sea [16–21], few were focused on phytoplankton diversity and biomass at stations close to the desalination plant at Jeddah [2,9,22]. In addition, specific knowledge about the phytoplankton community and their ecological and biological dynamics adjacent to desalination plants at the Red Sea southern part has not been done.

Phytoplanktons are used as indicators of water quality [23]; however, in front of a desalination plant, it is also crucial to monitor phytoplankton spatio-temporal events and HAB occurrence in order to avoid disruption of these desalination plants. To address this, the objective of this study was to focus on four important questions: first, what type of phytoplankton taxa and species are likely to cause a threat to the desalination plants; second, what time of the year do the HABs occur; third, where is a water bloom likely to occur. Is it near the desalination plant (i.e., you will not have much time to avoid it) or farther from the shoreline water intake where you will have some time to manage the situation. The fourth question, is there any relation between diversity and HAB occurrence. We focused on information which would allow us to predict periods of microalgae proliferation, especially, HABs, which could contribute to clogging of desalination plant RO membrane systems. To accomplish our goal, we choose three different stations (increasing distances from the shore) near desalination plants (sites) at Jeddah and then heading south to Al-Shoaiba and Al-Quonfuduh.

2. Materials and methods

2.1. The study area

Three sites near the western coast of Saudi Arabia (east Red Sea coast) were selected for monitoring of algae (Fig. 1). The sites are offshore from desalination plants located at Jeddah (J), Al-Shoaiba (S) and Al-Qunfuduh (Q) (100 and 420 km south of Jeddah, respectively). Three replicates were sampled from three different depths (surface, 5 and 10 m) at three stations (150, 750 and 1,250 m from the desalination plants) at each site. In this study, the abbreviations J-1, S-1 and Q-1 stand for the first station at each site. The total samples collected from each trip were 81 water samples and 18 plankton net samples. This study covers the months of December 2014 to August 2016 with a total 21 field trips, 1,710 water samples and 378 net samples.

2.2. Sampling

For phytoplankton analyses, samples were collected using two L Niskin bottles. For qualitative and quantitative analysis of phytoplankton, aliquots (1 L) were preserved in amber-color bottles using a 4% iodine solution.



Fig. 1. Geographical map focusing the sampling sites. Notes: Jeddah (Latitude: N 21°32'58.7", Longitude: E 39°06'35.6"); Al-Shoiba (Latitude: N 20°39'57.0", Longitude: E 39°30'00.9"); Al-Quonfuduah (Latitude: N 19°04'20.1", Longitude: E 41°09'38.6").

2.3. Phytoplankton enumeration

Sedimentation in 1,000 mL graduated cylinders was used to concentrate samples [24]. After a 4- to 8-d settling period, the top 950 mL of the sample was siphoned off and the bottom 50 mL was retained. Using Utermöhl's method [25], the 50 mL concentrated sample was counted under an inverted microscope after a 24- to 48-h settling period. Phytoplankton net samples were also taken and used to assist identification.

2.4. Phytoplankton identification

Phytoplankton taxa were identified according to references [26–33].

2.5. HAB and HAB toxic species definitions

In this study, we designate HAB species as those that are known to be a problem due to their high biomass, and HAB toxic species are those phytoplankton that are known to be able to produce potent toxins [4].

In choosing a general toxicity test method (mouse bioassay) for the phytoplankton samples collected, we were guided by the following general methods for monitoring, detection and analysis of phycotoxins. These include (1) bioassay using small animal, microbial and cell receptor cultures, (2) biochemical assays mainly

immunoassay and enzyme assay with recent additions of genetic-based assays using polymerase chain reaction and (3) analytical methods especially column chromatography but increasingly mass spectrometry linked to column chromatography.

More importantly, our ability to thoroughly characterize HABs is complicated by the complex array of toxins produced by algae (9). Marine algal species produce a suite of toxic components and some remain to be described. In addition, most toxins are composed of families of closely related compounds. Slightly different structure forms of a toxin can exhibit different levels and types of toxicity, and may be characterized using different detection methods and analytical approaches. Such complexity and variability can sometimes yield vague or contradictory conclusions regarding the exact source of toxicity in a natural sample.

Finally, characterization of HAB events is complicated by inherent difficulties associated with linking specific toxins measured in natural water samples to a specific algal species in a complex, natural phytoplankton assemblage. Finally, the presence of a species known to produce a toxin does not necessarily indicate the presence of toxins. Despite these shortcomings, there is a good amount of information for many of the major algal toxins and their producers in coastal waters of the most important ones impacting desalination plants. For our 2-year study, we chose the mouse bioassay as it would give us an indication of general toxicity within a plankton sample and allow us to focus on what species in the sample might be producing a toxin(s).

Our study used the mouse bioassay [33–35] to detect toxicity from these possible toxins. Over the course of the study, 432 samples from the 50 μ plankton net concentrate were used for a mouse toxicity test. If mouse toxicity was found, defined as a rapid death (within minutes), we use the term "HAB Toxins" to define the result. However, we use the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae to refer to potential toxigenic specie(s) that may produce phycotoxins during water blooms and we refer to these as "HAB Toxigenic Species."

2.6. Diversity and dominance indexes

The species diversity index (H' ; bits cell⁻¹) was used to study the structure of the phytoplankton assemblages [36]. Phytoplankton species diversity index (H') was calculated using the following formulae:

$$H' = -\sum_{N_i} \frac{N_i}{N} \log_2 \frac{N_i}{N}$$

where N is the total number of individuals of all species and N_i is the frequency of species in the sample.

2.7. Chlorophyll-a determination

An YSI EXO2 sonde (Yellow Spring, OH, USA) was used to measure chlorophyll-a as an indication of total phytoplankton for the three depths (surface, 5 and 10 m) and three stations at each site.

2.8. Statistical analysis

Pearson correlation coefficients (r values) were estimated between all characteristics measured on ship cruises (across month, site, station and depth) using Excel Analysis Tool Pak. Means and standard error were computed using Means Procedure available in SAS software. Data were subjected to analysis of variance (ANOVA) using the ANOVA General Linear Model procedure in the SAS software [37].

3. Results

3.1. Phytoplankton structure identification

Throughout the study, 125 phytoplankton species were identified belonging to four major groups (Families): Cyanobacteria (Cyanoprokaryonta) (*Oscillatoria* sp. and *Anabaena* sp.), Dictyocophyceae (*Dictyocha* sp.), Dinophyceae (85 species) and Bacillariophyceae (37 species) (Table 1). A few silico-flagellates (Dictyocophyceae) were observed during this study. *Dictyocha* sp. was identified at the Jeddah and Al-Quonfuduh sites only during December 2014 and January 2015. The highest number of taxa (92) was recorded at Al-Shoaiba and Al-Quonfuduh, while 90 species were observed at Jeddah. However, in April 2016, the highest number of species (56) was recorded at Jeddah-1. In March 2015, the phytoplankton community was represented by 52 taxa at Al-Shoaiba-1. Al-Quonfuduh-1 had the highest number of taxa (49) in January 2015. The number of phytoplankton species identified was in the range of previous reports in the northern part of the Red Sea (110 spp.) [38], near Jeddah (124 spp.) [39] and in the western coast of the Gulf of Aqaba (127 spp.) [40]. Lower numbers (73 and 100 spp., respectively) were reported at Jeddah [20]. However, [41] and [42] recorded higher numbers (145 and 220 spp. respectively) at Jeddah.

The most common genera were: *Protoperdinium* and *Neoceratium* with 16 and 13 species, respectively (Table 1). No previous study has identified *Neoceratium* in Al-Shoaiba and Al-Quonfuduh, but it was reported at Jeddah [43]. In fact, *Neoceratium* spp. were responsible for the spring peak (21.6×10^3 cells L^{-1}) in Jeddah and (9×10^3 cells L^{-1}) in Al-Shoaiba. Another research group [20] found the same result which they concluded reflected the more eutrophic state of Jeddah and Al-Shoaiba ecosystems [43].

During the study period, and at the different sites, stations and depths, the phytoplankton community was dominated by dinoflagellates. The number of taxa, Dinophyceae (85 species) and Bacillariophyceae (37 species) identified during our study period, were less compared with those in the oceanic waters of the central Red Sea [18,44] and in the north western Red Sea [41]. However, our results are comparable with that found in the coastal waters of the Red Sea [45]. During our study period and for the different sites, stations and depths, the phytoplankton community was dominated by the dinoflagellates. This is due to the proliferation of the genus *Neoceratium*. This genus is the most diverse for Saudi Arabia [43]. In fact, 13 *Neoceratium* species were recorded during this study. This number is less than the number (23) recorded in the Red Sea [46]. *Neoceratium* is known to be a common genus in the Red Sea and in other tropical waters [46].

Table 1

List of phytoplankton species identified during the study period (December 2014 to November 2016)

List of species	Jeddah	Al-Shoaiba	Al-Quonfuduh
Diatoms			
<i>Amphora</i> sp. ^a	+	+	+
<i>Bacteriastrium</i> sp.	Ab	+	+
<i>Bellerochea</i> sp.	+	+	+
<i>Biddulphia pulchella</i>	Ab	Ab	+
<i>Biddulphia</i> sp.	+	+	+
<i>Chaetoceros</i> sp.	+	+	+
<i>Climacodinium frauenfeldianum</i>	+	+	+
<i>Cocconeis</i> sp.	Ab	Ab	+
<i>Coscinodiscus grani</i>	Ab	+	+
<i>Coscinodiscus radiates</i>	Ab	Ab	+
<i>Coscinodiscus</i> sp.	+	+	+
<i>Eucampia</i> sp.	+	+	+
<i>Fragilaria</i> sp.	Ab	Ab	+
<i>Grammatophora</i> sp.	Ab	Ab	+
<i>Guinardia</i> sp.	+	+	+
<i>Gyrosigma</i> sp.	Ab	+	+
<i>Hemiaulus hauckii</i>	+	+	+
<i>Leptocylindricus danicus</i>	+	+	+
<i>Leptocylindricus</i> sp.	Ab	Ab	+
<i>Licmophora</i> sp.	Ab	+	Ab
<i>Melosira</i> sp.	Ab	+	+
<i>Navicula</i> sp. ^a	+	+	+
<i>Nitzschia longissima</i> ^a	+	Ab	+
<i>Odontella</i> sp.	+	+	+
<i>Paralia</i> sp.	Ab	+	+
<i>Pseudo-nitzschia</i> sp.	+	Ab	Ab
<i>Pinnularia</i> sp.	+	+	+
<i>Pleurosigma</i> sp.	+	+	+
T+ <i>Pseudo-nitzschia</i> sp. ^b	+	Ab	+
<i>Rhabdonema</i> sp.	+	Ab	Ab
<i>Rhizosolenia</i> sp.	+	+	+
<i>Rhizosolenia styliformis</i>	+	+	+
<i>Skeletonema costatum</i>	+	+	+
<i>Striatella unipunctata</i>	+	+	+
<i>Synedra</i> sp.	+	+	+
<i>Thalassionema nitzschioides</i> ^a	+	+	+
<i>Thalassiosira</i> sp. ^a	+	+	+
Dinoflagellates			
T+ <i>Alexandrium minutum</i> ^b	Ab	+	Ab
T+ <i>Alexandrium polygramma</i> ^b	+	+	Ab
T+ <i>Alexandrium spinifera</i> ^b	+	+	+
T+ <i>Amphidinium carterae</i> ^b	Ab	Ab	+
T+ <i>Amphidinium</i> sp. ^b	+	Ab	+
<i>Amphisolenia bidentata</i>	+	+	+
<i>Amphisolenia globifera</i>	+	+	+

Continued

Table 1 Continued

List of species	Jeddah	Al-Shoaiba	Al-Qunfudhuh
<i>Blepharocysta</i> sp.	+	+	+
<i>Centrodinium</i> sp.	+	+	+
<i>Ceratocorys horrida</i>	+	+	+
T+ <i>Chatonella marina</i> ^b	Ab	+	+
<i>Citharistes</i> sp.	+	+	Ab
<i>Cochlodinium</i> sp.	Ab	Ab	+
T+ <i>Dinophysis caudata</i> ^b	+	+	+
T+ <i>Dinophysis miles</i> ^b	+	+	+
T+ <i>Dinophysis norvegica</i> ^b	+	+	+
<i>Dinophysis parva</i>	+	+	Ab
<i>Dinophysis rotundata</i>	+	+	+
T+ <i>Dinophysis schuettii</i> ^b	+	+	+
<i>Diplopsalis</i> sp.	+	+	+
<i>Goniodoma</i> sp.	+	+	+
<i>Goniodoma sphaericum</i>	+	Ab	Ab
T+ <i>Gymnodinium aurelome</i> ^b	+	Ab	Ab
<i>Gymnodinium</i> sp. ^a	+	+	+
<i>Gyrodinium fusiforme</i>	+	Ab	+
<i>Gyrodinium</i> sp.	+	+	+
<i>Gyrodinium spirale</i>	+	Ab	Ab
<i>Histioneis isseli</i>	+	Ab	Ab
T+ <i>Karlodinium</i> sp. ^b	+	Ab	+
T+ <i>Katodinium</i> sp. ^b	Ab	+	Ab
<i>Mesoporos</i> sp.	Ab	Ab	+
<i>Neoceratium candelabrum</i>	+	+	Ab
<i>Neoceratium furca</i>	+	+	+
<i>Neoceratium fusus</i>	+	+	+
<i>Neoceratium horridum</i>	Ab	Ab	+
<i>Neoceratium inflatum</i>	Ab	Ab	+
<i>Neoceratium lineatum</i>	+	+	+
<i>Neoceratium lunula</i>	+	+	+
<i>Neoceratium macroceros</i>	+	+	+
<i>Neoceratium pentagonum</i>	Ab	+	Ab
<i>Neoceratium ranipes</i>	+	+	Ab
<i>Neoceratium trichoceros</i>	+	+	+
<i>Neoceratium tripos</i>	+	+	+
<i>Neoceratium vulture</i>	+	+	+
<i>Ornithicercus magnificus</i>	+	+	+
T+ <i>Ostreopsis ovata</i> ^b	Ab	+	Ab
<i>Oxytoxum scolopax</i>	+	+	Ab
<i>Oxytoxum</i> sp.	+	Ab	Ab
<i>Oxytoxum tessellatum</i>	Ab	Ab	+
<i>Peridinium</i> sp.	+	+	+
<i>Phalacroma cuneus</i>	Ab	+	Ab
<i>Phalacroma rapa</i>	Ab	+	Ab
<i>Podolampas palmipes</i>	+	+	+

Continued

Table 1 Continued

List of species	Jeddah	Al-Shoaiba	Al-Qunfudhuh
<i>Polykrikos</i> sp.	+	+	+
<i>Prorocentrum compressum</i>	Ab	+	+
T+ <i>Prorocentrum concavum</i> ^b	+	+	+
<i>Prorocentrum gracile</i>	+	+	+
T+ <i>Prorocentrum lima</i> ^b	+	+	+
<i>Prorocentrum micans</i>	+	Ab	+
T+ <i>Prorocentrum rathymum</i> ^b	+	+	+
<i>Prorocentrum triestinum</i>	+	+	Ab
T+ <i>Protoceratium</i> sp. ^b	+	Ab	+
<i>Protoperidinium bipes</i> ^a	+	+	+
<i>Protoperidinium claudicans</i> ^a	+	+	+
<i>Protoperidinium conicum</i> ^a	+	+	+
<i>Protoperidinium curtipes</i> ^a	+	+	+
<i>Protoperidinium depressum</i> ^a	+	+	+
<i>Protoperidinium diabolium</i> ^a	+	+	+
<i>Protoperidinium divergens</i> ^a	+	+	+
<i>Protoperidinium elegans</i> ^a	+	+	+
<i>Protoperidinium grande</i> ^a	+	+	+
<i>Protoperidinium leonis</i> ^a	+	Ab	Ab
<i>Protoperidinium lineatum</i> ^a	Ab	+	+
<i>Protoperidinium oblongum</i> ^a	+	+	+
<i>Protoperidinium ovatum</i> ^a	+	+	+
<i>Protoperidinium ovum</i> ^a	+	+	+
<i>Protoperidinium pellucidum</i> ^a	Ab	+	Ab
<i>Protoperidinium pentagonum</i> ^a	Ab	+	Ab
<i>Protoperidinium roseum</i> ^a	+	+	Ab
<i>Protoperidinium steinii</i> ^a	+	+	+
<i>Pseudophalacroma</i> sp.	Ab	+	+
<i>Pyrophaccus</i> sp.	+	+	+
<i>Scriptiella</i> sp.	Ab	+	+
<i>Scriptiella trochoidae</i>	+	Ab	Ab
<i>Spiraulax</i> sp.	Ab	+	Ab
Dictyochophyceae			
T+ <i>Dictyocha</i> sp. ^b	Ab	+	Ab
Cyanophyceae			
T+ <i>Oscillatoria</i> sp. ^b	+	+	+
T+ <i>Anabaena</i> sp. ^b	+	+	+

Note: +, present; Ab, absent.

^aHAB species.^bT+ species that are toxigenic harmful algae bloom species according to the IOC-UNESCO taxonomic reference list of harmful micro algae.

3.2. Spatio-temporal phytoplankton densities distribution

During the study period, the phytoplankton densities decrease from the near shore (150 m) (2, 2.8 and 6.6 × 10⁴ cells L⁻¹) to the open sea stations (1,250 m) (1.2, 1.8 and 6.5 × 10⁴ cells L⁻¹), respectively at Jeddah, Al-Shoaiba and Al-Qunfudhuh (Fig. 2). In addition, total phytoplankton concentrations increased from Jeddah (1.8 ± 0.2 × 10⁴ cells L⁻¹)

to Al-Quonfuduah ($2.3 \pm 0.16 \times 10^4$ cells L^{-1}) (Fig. 3). The highest phytoplankton concentrations were detected on the surface and at 5 m while the lowest were detected at 10 m. This result is common for all stations and sites.

With regard to seasonal variations, the phytoplankton community showed the highest concentrations in autumn for all sites (Fig. 4). During this period, the highest concentrations ($0.8 \pm 0.02 \times 10^4$ cells L^{-1}), ($1.6 \pm 0.07 \times 10^4$ cells L^{-1}) and ($4.2 \pm 0.05 \times 10^4$ cells L^{-1}) were recorded in Jeddah, Al-Shoaiba and Al-Quonfuduah, respectively. For Jeddah, the same high concentration ($0.8 \pm 0.07 \times 10^4$ cells L^{-1}) was also observed in summer. For Jeddah and Al-Shoaiba sites, the lowest mean concentrations ($0.4 \pm 0.01 \times 10^4$ cells L^{-1}) and ($0.3 \pm 0.01 \times 10^4$ cells L^{-1}) were recorded, respectively, during the spring. However, the lowest concentrations ($0.9 \pm 0.08 \times 10^4$ cells L^{-1}) were recorded during winter at Al-Quonfuduah (Fig. 4).

Similar phytoplankton concentrations were observed in the Red Sea, and it was concluded that conditions of moderate temperature, salinity and nutrients as being the most favored parameters controlling phytoplankton proliferation [2]. This was confirmed by other studies conducted in the Red Sea [47–50]. However, another study for the Red Sea found that the highest chlorophyll-a concentrations were

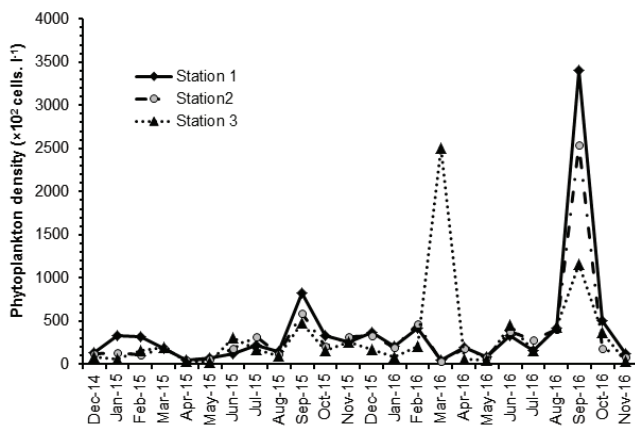


Fig. 2. Spatio-temporal repartition of the phytoplankton densities in the different studied stations regardless of their site.

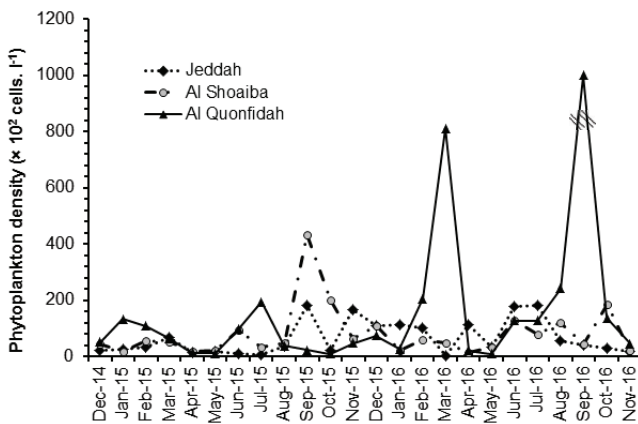


Fig. 3. Spatio-temporal repartition of the phytoplankton densities in different sites regardless of their stations.

present during the winter, whereas the lowest were found in the summer [51].

As little is known about the phytoplankton community structure and density at Al-Shoaiba and Al-Quonfuduah, our study is the first to provide specific information on the microalgae community structure over a 2-year time period for these areas of Saudi Arabia.

Chlorophyll-a seasonal variations (Fig. 5) using onsite sonde measurements showed a peak at Al-Quonfuduah during autumn matching the seasonal phytoplankton density peak at the same place and time (Fig. 4). A positive correlation was found between phytoplankton and chlorophyll-a at Al-Quonfuduh but not at the other sites. This discrepancy in chlorophyll-a concentration with phytoplankton count was due to the cell size variation of the different phytoplankton bloom species resulting in a variation between these two parameters [2]. This means that chlorophyll-a measurement should not be the sole parameter for phytoplankton monitoring programs.

To define a bloom using chlorophyll measurement, it has been concluded that for an aquatic ecosystem with a background chlorophyll concentration of $0.2 \mu g L^{-1}$, which is the case in the Red Sea, levels of chlorophyll that exceed $2\text{--}5 \mu g L^{-1}$ indicates the presence of an algal bloom [52]. In our study, the highest concentrations of chlorophyll-a appearing at Jeddah

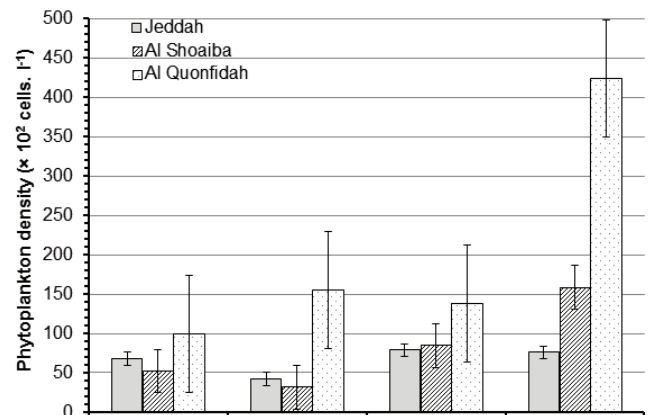


Fig. 4. Seasonal repartition of the total phytoplankton densities in different sites during the study period.

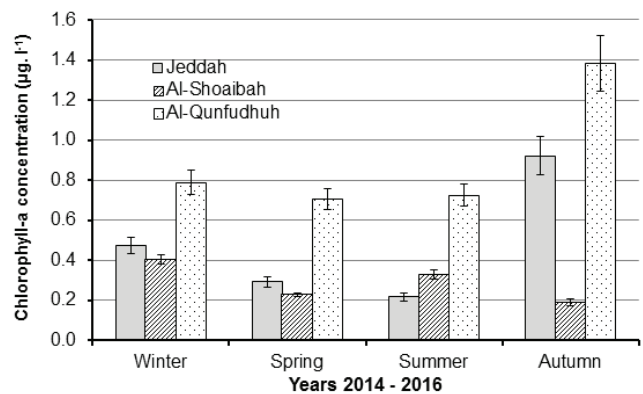


Fig. 5. Chlorophyll-a profile in $\mu g L^{-1}$ during the study period in different sites during the study period.

and Al-Shoaiba did not exceed these critical threshold levels of 2–5 $\mu\text{g L}^{-1}$, while it often exceeded this level at Al-Qunfudhuh.

3.3. HAB and HAB toxigenic species profile

According to IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae, the HAB species and potential toxin producers (HABs toxigenic) most recorded in our study were as follows: *Prorocentrum lima*, *Ostreopsis ovata*, *Chatonella marina*, *Amphidinium* sp., *Alexandrium spinifera*, *Alexandrium minutum*, *Dinophysis caudata*, *Dinophysis miles*, *Gymnodinium* sp., *Peridinium* sp., *Goniodoma* sp., *Protoperidinium depressum*, *P. divergens*, *P. curtipes*, *P. bipes*, *P. ovum* and *P. ovatum* (Table 1).

The cell count for HAB non-toxicogenic species was higher in all months compared with the HAB toxigenic species, except in January 2015, June 2015 and September 2016 (Fig. 6). This result is due to the proliferation of the HAB toxigenic species *Dinophysis caudata* (1.9×10^5 cells L^{-1}) at Al-Qunfudhuh during January 2015, *Dinophysis miles* (1.67×10^5 cells L^{-1}) at Al-Shoaiba during June 2015 and the bloom of HAB toxigenic species: *Alexandrium spinifera*, *Alexandrium polygramma*, *Dinophysis caudata* and *Gymnodinium* sp. at all stations during September 2016.

With regard to the relative weight of HAB toxigenic species to the non-toxicogenic, our results showed higher HAB toxigenic count only at Al-Qunfudhuh (Fig. 6). This site is distinguished by the relatively high concentration of HAB toxigenic species (*Dinophysis caudata*, *Dinophysis miles*, *Dinophysis rotundata*, *Gymnodinium aurelome*, etc.). For example, on January 2015, 4.3×10^4 cells L^{-1} of *Dinophysis caudata* was found at the near-shore surface station Al-Qunfudhuh. However, on March 2016, a bloom of *Dinophysis miles* (1.6×10^5 cells L^{-1}) was detected at a depth of 5 m at Al-Qunfudhuh-3.

The highest concentration of dinoflagellates was detected in March 2016 from *Dinophysis caudata* and *Dinophysis miles* (47 and 34×10^3 cells L^{-1} , respectively). In fact, *Dinophysis caudata* is one of the toxigenic species that causes diarrhetic shellfish poisoning and is characterized by a wide distribution in tropical and temperate coastal waters [53]. Even if no fish mortality was detected in our study areas during the bloom, it is important to point out the season and site for predicting a future HAB. Although, *D. caudata* and *D. miles* were reported from the Arabian Sea, no previous study has indicated their presence in the Red Sea [54,55]. It is therefore possible that

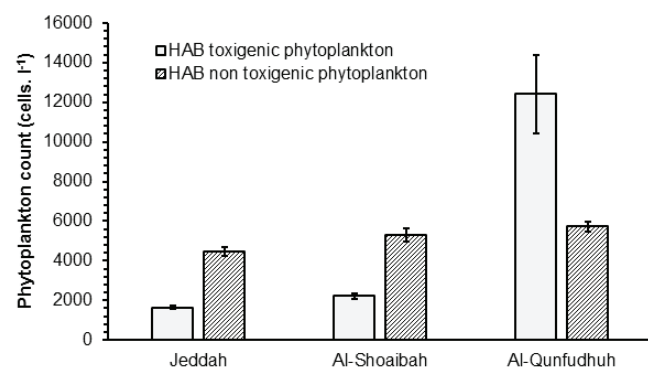


Fig. 6. Average of HAB toxigenic and non-toxicogenic phytoplankton for the three sites during the study period.

these two species are reported along the Saudi Arabian coast for the first time.

3.4. Toxicity detection

Table 2 shows the results of mouse toxicity tests done over the study period. Toxicity is described as mouse lethality (survival times) based upon replicate male Swiss albino mice 18–22 g were injected by the intraperitoneal route with 1.0 mL of concentrated plankton tow net sample. Signs of neurotoxicity and survival time were noted. A scale of 1–4 where (+++) equals lethality within 10 min to no signs of poisoning (–) was used. We chose the mouse bioassay as it would give us an indication of general toxicity from a mixed plankton sample (i.e., different toxin mixtures) and allow us to focus on what species in the sample might be producing a toxin(s).

During the highest HAB count (2.25×10^5 cells L^{-1}) for the Al-Qunfudhuh bloom in September 2016 no mouse toxicity was found as the organism (*Nitzschia longissima*) responsible for the bloom is a HAB non-toxicogenic species. Earlier in March 2016, another bloom with a lower cell count (8.5×10^4 cells L^{-1}) was recorded at Al-Qunfudhuh and the responsible organism (*Dinophysis caudata*) is listed as a HAB toxigenic species. However, mouse toxicity was not detected in this sample at this time. This phenomenon occurs when a toxigenic species occur, but no toxin is being produced. In other research, the mouse bioassay has been used to demonstrate the existence of toxic and non-toxic strains of *Coolia monotis* [56]. Other reports showed the effect of environmental factors that trigger the toxigenic strain to produce the toxin [57–60].

Among all the samples, only five at Al-Shoaiba-1 on December 2014, Jeddah-2 on June 2015 and the three samples on June 2016 for Al-Qunfudhuh-1, 2, 3 that showed high toxicity (+++) causing 5 min or less survival time in 20 g male mice (Table 2). Mouse toxicity in samples for Al-Shoaiba-1 on December 2014 was due to the presence of *Dinophysis miles* while the responsible organism for toxicity at Jeddah-2 on June 2015 was *Oscillatoria* sp. The toxicity for the three samples at Al-Qunfudhuh three samples of June of 2016 occurred as a result of the presence of *Dinophysis miles*. Only 29 samples showed moderate toxicity (++). The rest of the samples showed either low or no toxicity at all (Table 2).

The more important risk factor for the desalination plant is HAB occurrence regardless of toxicity. This is because the desalination process uses RO technique which removes toxins from the seawater [61]. This group reported that the health risk from low level HAB toxins in the desalinated drinking water produced by multi-stage flash distillation, multiple-effect distillation or seawater reverse osmosis methods would not be high enough to be a risk for humans. Therefore, this study focused on HAB species regardless of their toxicity as a hazard for operation of the desalination plant.

3.5. Descriptive statistics

An ANOVA was conducted on measured parameters to determine significance across month, site, station, depth and their interactions (Table 3). These results showed that variability across year, season and site (December 2014 to November 2016) was significant ($p < 0.001$) for all measured

Table 2
Toxicity detection of the phytoplankton samples collected by the 50 µm pore size net using mouse bioassay test during the study period

Sampling time	Site and stations								
	Jeddah			Al-Shoaiba			Al-Qunfudhuh		
	J-1	J-2	J-3	Sh-1	Sh-2	Sh-3	Q-1	Q-2	Q-3
Dec 2014	–	+	–	+++	–	+	+	+	–
Jan 2015	–	–	–	–	–	–	–	+	–
Feb	+	+	–	+	+	–	–	+	–
Mar	–	–	–	+	+	–	+	–	–
Apr	–	–	–	+	+	–	–	–	–
May	–	–	–	–	–	–	–	–	–
Jun	+	+++	+	+	+	+	+	+	+
Jul	–	–	–	–	–	–	+	+	+
Aug	+	–	–	+	+	–	+	+	+
Sep	++	+	+	–	+	+	+	–	+
Oct	–	–	–	–	–	–	–	–	–
Nov	–	+	+	–	–	++	+	+	+
Dec	+	++	++	–	+	+	+	+	++
Jan 2016	+	++	–	–	–	–	++	–	++
Feb	–	+	++	++	++	++	–	+	+
Mar	–	–	–	–	–	–	–	–	–
Apr	+	–	+	–	–	–	+	–	++
May	++	++	–	–	–	+	–	–	–
Jun	+	+	++	++	+	–	+++	+++	+++
Jul	+	–	–	–	++	+	++	+	+
Aug	–	++	++	+	–	–	–	–	++
Sep	–	++	++	++	++	++	++	–	–
Oct	–	–	–	–	–	–	–	–	–
Nov	–	+	+	–	–	++	+	+	+

Note: +++ represents high toxicity (death time less than 10 min); ++ represents moderate toxicity (death time more than 10 min to 1 h); + represents low toxicity (no death but neuro signs); – represents non-toxic (no signs at all).

Table 3
Level of significance from ANOVA for measured variables across year, season, sites, stations and depths

Variables	Y	Se	Si	St	D	Se-Si	Se-St	Se-D	SI-St	Si-D	St-D
1 Total phyto-plankton	***	***	***	**	***	***	NS	***	NS	NS	NS
2 Toxigenic phyto-plankton	***	***	***	NS	NS	***	NS	*	NS	NS	NS
3 Non-toxigenic phyto-plankton	NS	***	***	***	***	***	NS	***	NS	NS	NS
4 Chlorophyll a	***	***	***	NS	***	***	NS	NS	NS	NS	NS
5 Salinity	***	***	NS	NS	NS	***	NS	NS	NS	NS	NS
6 Temperature	***	***	***	NS	NS	***	NS	NS	NS	NS	NS

Notes: Y: years, Se: seasons, Si: sites, St: stations, D: depths. Levels of significance are indicated by *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. NS = not significant.

parameters except for non-toxigenic phytoplankton across year and for salinity across site. All interactions were not significant except for season-site which was highly significant for all parameters and for season-depth for all phytoplankton parameters with the three sites. The highly significance ($p < 0.001$) for seasons-sites interactions for all

traits is an indication that the data need to be examined within each site.

Pearson correlation coefficients (r values) between measured parameters for near shore surface at Jeddah, Al-Shoaiba and Al-Qunfudhuh are presented in Table 4. After we ran the statistics analysis for possible correlations at all sites, stations

Table 4
Pearson correlation coefficients (*r* values) between measured parameters average for the near shore surface stations over all months and sites

	Jeddah			Al-Shoaiba			Al-Quonfuduh		
	Total phytoplankton	Toxicogenic phytoplankton	Non-toxicogenic phytoplankton	Total phytoplankton	Toxicogenic phytoplankton	Non-toxicogenic phytoplankton	Total phytoplankton	Toxicogenic phytoplankton	Non-toxicogenic phytoplankton
Total phytoplankton	0.827**			0.658**			0.992**		
Toxicogenic phytoplankton		0.888**		0.966**	0.443**		0.009	-0.121	
Non-toxicogenic phytoplankton			0.070	0.397**	0.179	0.412**	0.094	0.113	-0.152
Temperature, °C			0.167	0.196	-0.010	0.238*	0.247*	0.233*	0.096
Salinity, psu			0.143	0.021	0.362**	-0.142	0.267*	0.205	0.465**

Notes: **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

and depths we found that the most useful and clearly interpreted results were those for the near shore surface stations. That is because we are dealing with different ecosystems at each plant station, as well as for the unique ecosystem of the near shore stations due to brine release from the RO process. Of most interest are correlations between phytoplankton (total, toxicogenic or non-toxicogenic) and other measured parameters. Correlations between temperature, salinity, chlorophyll a and total phytoplankton were positive.

3.6. Phytoplankton diversity profile

The highest diversity index (2.9 bits cell⁻¹) was recorded at Jeddah-1 in April 2016 (Fig. 7). This may be due to the proliferation of 57 different species with equal concentrations. The same observation may explain the highest diversity found at Al-Shoaiba-1 station during March 2015 (3 bits cell⁻¹) (Fig. 7). In fact, during this last period, we detected the highest number of species (60 species). Our study reveals that Jeddah has a diverse and uniform spatial and seasonal distribution of phytoplankton species compared with the other two sites (Fig. 7).

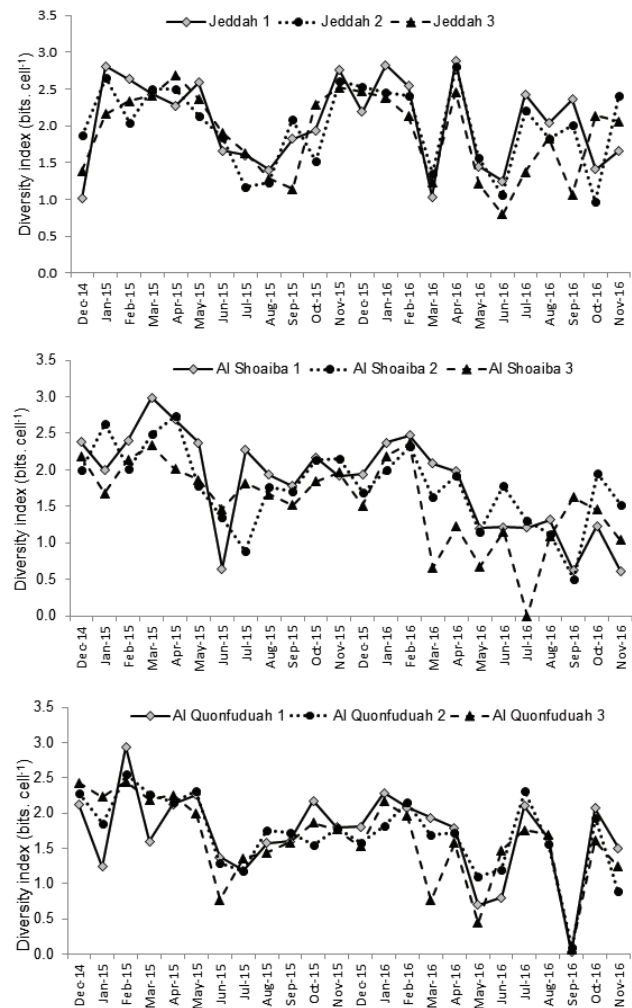


Fig. 7. Spatio-temporal repartition of the diversity index in different sites and stations.

The lowest diversity index ($0.02 \text{ bits cell}^{-1}$) was during the highest bloom of *Nitzschia* sp. ($4.4 \times 10^5 \text{ cells L}^{-1}$) in September 2016 at Al-Quonfuduah-1 (Figs. 2, 3 and 7). The lowest diversity detected at Al-Shoaiba in June 2015 ($0.6 \text{ bits cell}^{-1}$) was preceded by a proliferation of *Dinophysis miles* ($7.2 \times 10^4 \text{ cells L}^{-1}$) (Fig. 7).

In general the average highest diversity was during the winter season ($2.3 \text{ bits cell}^{-1}$) while the average lowest diversity ($0.9 \text{ bits cell}^{-1}$) was detected during summer and autumn. Diversity increased at the northern site (Jeddah) and decreased heading south to Al-Shoaiba and Al-Quonfuduah (Fig. 7). For the vertical distribution, the highest phytoplankton diversity was detected at 5 m for each station.

The lowest diversity coincided with the highest proliferation of microalgae cells at the near shore stations compared with the far shore stations regardless of their sites. This low diversity phenomena in the area adjacent to the desalination plant may be due to conditions of high salinity, temperature and pH as a result of desalination process. These conditions lower competition and may explain why we observed lower diversity and higher HAB species presence. Supporting this is our finding that higher temperature and salinity were recorded during summer and autumn seasons compared with winter and spring seasons (Figs. 8 and 9). This indicates

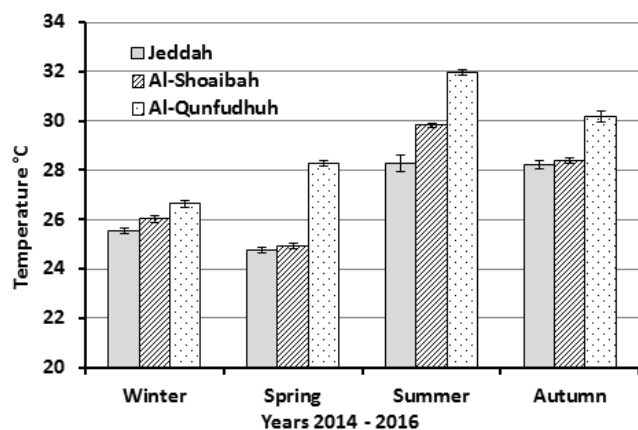


Fig. 8. Temperature profile in °C during the study period in Jeddah, Al-Shoaiba and Al-Quonfuduh stations.

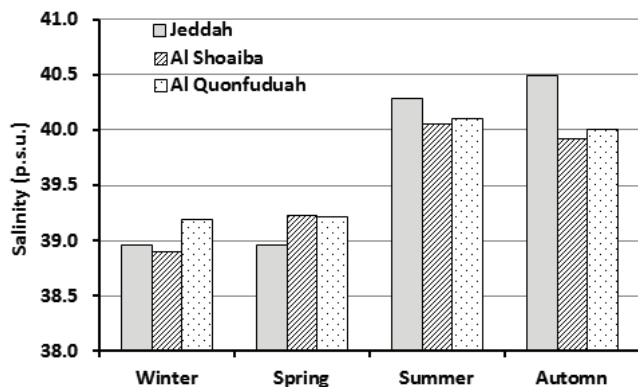


Fig. 9. Salinity profile in PSU during the study period in Jeddah, Al-Shoaiba and Al-Quonfuduh stations.

that the phytoplankton community in this particular ecosystem was exposed to more severe conditions before (summer) and during water bloom occurrence (in autumn). Desalination effluent is normally characterized as having higher salinity and temperature as well as higher amounts of dissolved minerals and particulates [62]. These desalination effluents can reach 75 ppt salinity. Within 20 m of the outlet, salinity can range between 2 to more than 5 ppt above background salinity and temperature can be as high as 10°C – 15°C warmer than intake seawaters [63]. This higher salinity in front of the desalination plant was also observed in the Arabian Gulf (Kuwait) where salinity of the water adjacent to the desalination plant ranged between 4 and 12 ppt above ambient salinity [64].

These higher salinity and temperatures can alter the phytoplankton community as well as other aquatic communities near seawater desalination plants on the northern Red Sea. The increased salinity and temperature of these coastal waters change the physiological conditions for organisms and lead to changes in the aquatic communities for the Red Sea [65]. A good example of this is the very low diversity ($0.02 \text{ bits cell}^{-1}$) at Al-Quonfuduah in station-1 adjacent to the desalination plant in September 2016 during a bloom of *Nitzschia* sp. (Figs. 2, 3 and 7).

Although similar low diversity profiles were observed at station-2 and station-3 (0.09 and $0.05 \text{ bits cell}^{-1}$, respectively) for Al-Quonfuduah in September 2016 (Fig. 7), the bloom occurred only at station-1, which means that low diversity is not the only factor that triggers the bloom in this area adjacent to the desalination plant. According to others, the effluent discharge presents a source of nitrogen and phosphorus which contributes to phytoplankton proliferation [41,66]. Moreover, the occurrence of anthropogenic inputs in the coastal zone will increase the loading of nutrients.

4. Conclusion

The phytoplankton community found during our study was dominated by dinoflagellates. The highest microalgae numbers, especially toxigenic species, were found at the southern site (Al-Quonfuduah) during autumn. The near shore stations showed the most favorable conditions for phytoplankton proliferation, especially HABs compared with the far shore stations regardless of site. The two HAB species were: *Dinophysis caudata* and *D. miles*. This is the first time these two species have been found at high concentrations along the southern part of the Saudi Arabian coasts. Our study results allow us to predict that HABs would most likely to occur in the autumn, especially during September.

During this study, the northern site (Jeddah) showed the highest phytoplankton diversity and lower concentrations compared with the southern site at Al-Quonfuduah. This supports our hypothesis that lower diversity is correlated with higher phytoplankton concentrations. The fact that there are stations of low diversity in the same place and time (e.g., Al-Quonfuduah in September 2016) without a bloom occurrence supports our hypothesis that the low diversity phenomenon is triggering the bloom. This is because if the bloom is the reason for low diversity, we would not see low diversity without the bloom. The three low diversities and one bloom at Al-Quonfuduah in 2016 also confirm that bloom occurrence needs other factors (nutrient availability,

presence of a bloom forming species, etc.) to help the initiation of a bloom.

We were also able to detect such relationships for different stations (near shore vs. far shore) where the lowest diversity and highest phytoplankton concentrations were detected at the near coastal stations compared with the open sea stations. This again confirms our hypothesis that lower diversity in the ecosystem adjacent to a desalination plant trigger HAB occurrence (i.e., higher cell numbers and higher biomass) due to the high salinity and high temperature effluent. Higher temperature and salinity from the plant effluent decreases diversity and increases biomass promoting a water bloom possibility.

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