QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

SEASONAL VARIATIONS OF DINOPHYSIS SPECIES AND THEIR TOXINS IN QATARI WATERS, ARABIAN GULF

BY

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ABSTRACT

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Title: Seasonal Variations Of Dinophysis Species And Their Toxins In Qatari Waters, Arabian Gulf.

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The results of investigations from several cruises carried out in Qatari waters between 2017 and 2018 were reported. The species composition, distribution and toxicity of *Dinophysis* community were examined. A total number of five taxa were recorded with dominancy of *Dinophysis caudata* followed by *Dinophysis miles.* Of these, two taxa are the first reports from Qatari waters: *Dinophysis mitra* and *D. acuminata*. The cell count revealed that the number of cells rose up from October 2017 toward the end of April 2018. Thirty-two freeze-dried samples were analyzed for Diarrhetic Shellfish Poison (DSP), confirming the presence of Pectenotoxin (PTXs) and Okadaic acid (OA), where toxin concentrations ranged from 0.0080 Ng/mg to 0.52 Ng/mg, respectfully.

A number of physical and chemical parameters were measured. The salinity of water collected ranged between 42.00 ‰ and 38.97 ‰. Throughout the area investigated the temperature ranged from in excess of 34 °C in summer to 20 °C in winter. Nutrient concentrations recorded were relatively low, except in samples collected from industrial effusion. The statistical analysis on the physical parameters, nutrients, and cell counts and species number showed a significant difference between seasons and stations at $P < 0.05$.

DEDICATION

To my Father (may God have mercy on his soul) and my Family

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CHAPTER 1: INTRODUCTION

Marine environments sustain a variety of living organisms starting from microorganisms up to large macro-organisms. This support comes with the diversity of marine habitats that are created naturally to provide certain conditions for each single organism. However, the presence of planktons depends on the natural conditions of the environment. So, planktonic growth and survival is limited by three major factors: light, nutrients and depth. In general, the primary producers are Phytoplankton, which are considered the base of all of the food chains in marine life. Planktonic presence is restricted mainly to the epipelagic zone, which is the zone that receives most of the sunlight. However, these planktons can sometimes impose natural adverse impacts when the conditions are favorable to them. For Example, when there is enough nutrients, optimal temperature and salinity; some species of this phytoplankton's start to bloom and found in patchiness. These patches of large cell numbers will start to create some difficulties to other species: higher oxygen demand, more decomposition rate, blocking of sunlight, production of some toxins, and sophistication of some marine creatures (ex. Fishes). Such events would also affect us as humans. On other hand, the microscopic planktonic algae of the world's oceans are a critical food source for filter feeding bivalves, shellfish (oyster, mussels, scallop and clams), as well as larva of commercially important crustaceans and finfish. In most areas, the proliferation of planktonic algae is beneficial for aquaculture, recreational and commercial fisheries. However, World Health Organization (2019) have stated that phytoplankton are intoxicating more than 60000 person / year with 1.5 % mortality rate. For instance, a bloom of 8 months occurred in the Arabian Gulf (Qatar University Biodiversity newsletter 2009) that extended from the

shores of United Arab Emirates up to Kuwaiti waters. This bloom have killed more than 70% of the coral reefs in the UAE were killed, many sea creatures died and most of the desalination plants were closed (ROPME, 1997). The international oceanography commission of UNESCO (2019) indicated that, there are two types of harmful algal bloom species (HABs): the toxin producers and high biomass producers, and both occur in Arabian Gulf region, with damages leading to huge economic losses. The frequency and severity of HAB events are increasing both in Qatar and around the globe, and furthermore, their distribution within the region appears to be expanding. In this situation, the importance of exchange of information and cooperative research has become obvious among scientists working in Arabian Gulf. Thus, this research will focus on the Dinophysis species, their distribution, produced toxins and the physical and chemical parameters effecting them, based on seasonal variation.

CHAPTER 2: LITERATURE REVIEW

Geography, Ecology and Marine Status of Arabian Gulf

The Arabian Gulf is located at subtropical, hyper-arid region with a latitude of 23.9º30.25º N and longitude 48.56 º.2 E, with a surface area 2390 km^2 with a semi-enclosed topography that connects through Strait of Hormuz to the Gulf of Oman at the southeast part (Al-Muftah, 1991; Al-Harbi, 2005). The Arabian Gulf ends at the northwest close to the shores of Iraq with a formation of a delta by Tigris and Euphrates rivers. The Arabian Gulf is shallow with depth ranging between 10 – 100 m, making almost whole Arabian Gulf within the photic zone (Al-Harbi, 2005). However, its photic zone generally extends to 6–15 m only. The landmasses surrounding the Arabian Gulf are very arid. The rainfall is low throughout the year, and, as a result, the loss of water from the Arabian Gulf by evaporation exceeds the input from rivers and run-off (Al-Muftah 1991). The shores of Arabian Gulf are mostly sedimentary with a gradual slope (El-Sorogy et al, 2018). Some reefs and limestone domes give a relief to the sedimentary and flat seabed, which support non-accreting coral and coral reefs communities, algal beds and seagrasses, most of which integrate together in many places (Sheppard et al, 2010 and 2012). Water temperature ranges between 20 °C (winter) to 34 °C (summer), and a maximum salinity of 48 ‰, with an average of 40 ‰, and an extreme of 70 ‰ in lagoons (Saudi Arabia, Salwa Bay) (Wabnitz et al 2018). Physical factors of the Arabian Gulf exert stressors on marine biota of the Arabian Gulf. The organisms in many cases are living under extreme limits and at their maximum of tolerance, in addition to the synthetic stresses arising (Al-Yamani et al, 2009). Moreover, Arabian Gulf bounded to many wealthy countries undergoing a rapid economic growth with the involvement of

extensive construction along the coasts and offshores, supported by gas and oil industries (El-Sorogy et. al, 2018). The aquatic environment of Arabian Gulf is changing rapidly with major developments at coastal zone (Sheppard. et al, 2010). Coastal changes are rapid; however, are overtaken by heavy construction that causes habitat loss, coastline alterations, seabed shifting, sedimentation, salinity and temperature variations in controlled water flow along the coast, in addition to climate change (Hamzehei et al, 2013; Sheppard et al, 2010; Quigg et al, 2013).

Geography and Environmental condition of Qatar

Qatar protruding in the middle of the Arabian Gulf has a unique position by being a peninsula between Strait of Hormuz and Shatt Al-Arab. The location of Qatar has a great influence on marine currents and the pattern of sedimentation along southeastern side of the Arabian Gulf. The anti-clock currents that enter the Arabian Gulf as cold dense water through the strait of Hurmuz being encountered by the western coast of Qatar, which has very shallow water with an average salinity of 60 ‰ (Al-Muftah et al, 2016; Al Mamoon et al, 2016). These currents in addition to the inputs of nutrients from shut Al-Arab and some Iranian rivers made the coastlines little productive. However, the Arabian Gulf known to be one of the oligotrophic areas that is extremely harsh in terms of its physical and chemical conditions (Sahu et al, 2018; Quigg et al, 2013; John et al, 2003; Al-Ansi et.al, 2002; Al-Khayat, 1998; Reynolds, 1993). Furthermore, a checklist of phytoplankton of the area prepared and introduced for the first time by Dorgham and Al-Muftah in (1986), followed by many recent studies, such as Al-Muftah (1991), Al-Muftah et al (2016), Al Shehhi et al, (2014), Quigg et al. (2013), Al-Yamani et al (2009), Al-Harbi, (2005), Glibert et al, (2002) and Subba (1998).

Physical Conditions

The surface water and coastal shallows of Qatar subjected to a strong temperature fluctuation, as it changes in response to seasonal and daily cycles of cooling and heating. These variations are considered in most Qatari waters, which are caused by the exchange of large water masses with the strait of Hurmuz. Also, strong north winds "Al-Shamal winds" result in frequent and comprehensive mixing of the entire water column while the vertical temperature gradients is almost constant with small changes during summer, it is not the case as density causes vertical stratification. As a result, most of Qatari marine water organisms are subjected to temperature fluctuation, specifically on seasonal basis (Quigg et al, 2013; Sheppard et al, 2010). Moreover, most of lands surrounding the Arabian Gulf are very arid as they receive a minimal amount of rain throughout the year, which makes the evaporation rate exceeds the rate of the water compensation by rivers and run offs. Furthermore, summer months are regularly hot with some periodic north winds with a very low precipitation. Geopolicity (2010) reported that; a total discharge of 180 /year of the river water of Tigris Mountain in April were reduced to be 22/year in October, whereas Isaev et.al (2009) reported a further reduction in the discharge rates to be 5 /year from Shatt Al-Arab as a result, the local circulation of water is very weak. The interchange of the gulf water with the Indian Ocean is restricted due to the narrow passage of the strait of Hurmuz. Additionally, Kämpf (2006), Al-Muftah (1991) and Hunter (1986) concluded that the gulf has two main types of water motion; residual and tidal. The residual tides are mainly driven by the change in water density and the change in winds direction. The density of the water that enters from the Gulf of Oman controls the residual tides by making the inflow currents to dominate on the Iranian side, while the

outflow do dominate the western side. The wind forces are controlled by seasonality. Although, the physical factors, such as the local eddies that occur with variability are acting as a complex and exists in the area between Qatar and Strait of Hurmuz.

Surface water temperature ranges from 36 °C in summer to 14.1 °C in winter in Qatar with an average of 21.9 °C. Maximum temperature variation reached at shallow lagoons and embayments that are isolated from the main body of water (Quigg et al, 2013, Sheppard et al, 2010, Al-Muftah 1998; Al-Muftah 1991). As a consequence, surface salinity at central parts (close to Qatar) has an average of 37 - 42 ‰, whereas close to the coast it has an average of 50 – 70 ‰ and in some local embayment and shallow lagoons it reaches as high as 70 ‰ around Qatar (Khor Aloodad and Salwa bay) (Sheppard et.al. 2010, Al-Muftah 1991; Dorgham and Al-Muftah (1986). Thus, the high salinity and temperature fluctuations is one of the major factors that affect, control, and limit the presence of marine organisms (Gedaria et al, 2007, Cembella, 1999). Additionally, aquatic biota found to be restricted and some does not exist in the area; making the diversity low (Sheppard et al, 2010, Bassem etal, 1977). However, Quigg et al (2013) found that Qatari environmental parameter plays a role in the gross production rates and the relatively low phytoplankton biomass while having high species diversity.

Marine Ecosystem of the Arabian Gulf

The major seawater nutrients are inorganic nitrogen compounds such as NH_4^{+}, NO_3^{-}, NO_2^{-}, silicate (SiO_4^{-3}), and phosphate (PO_4^{-3}). Seawater elements are trace compounds and the results of their analysis depend on various sources of contamination of seawater. Dorgham and Al- Muftah (1986), recorded different ranges of Qatari waters nutrients: PO_4^{3-} = 0.03 – 1.23 µg / L, NH_4^{+} =0.00 – 0.23 µg / L, NO_2^{-} = 0.00 – 0.16 µg / L, NO_3^{-}

= 0.12 – 0.90 μg / L.

Al-Muftah, (1991) stated that, nutrients concentrations recorded were relatively low. Quigg et al. (2013) recorded variable nutrients concentration. Nitrate and nitrite were rarely > 0.2 μM, while phosphate had an average of 0.48 μM and silicate had 1.93 μM. Al-Ansari et al. (2015) found that nitrate and nitrite ranged between 0.15 μg / L – 0.50 μg / L at the Exclusive Economic Zone (EEZ) of Qatar during summer season. Recently, considerable number of studies conducted biological surveys in the Arabian Gulf. Some taxonomical and ecological studies focused on plankton with the production of different lists of species (Al-Muftah et al, 2016; Al Shehhi et al, 2014; Quigg et al, 2013; Al-Muftah 2008; El-Din & Al-Khayat 2007; Al-Harbi 2005; Al-Muftah 1991; Dorgham & Al-Muftah 1986; Dorgham et al, 1987; Dorgham and Al-Muftah 1989 and Alkandri et al, 2009). The first investigator of the Phytoplankton of Arabian Gulf was Bohm in 1931. He focused on dinoflagellate species and listed 66 Taxa. Wood et al. (1963) published the first checklist of Phytoplanktons in the Indian Ocean with only two dinoflagellate species from Arabian Gulf. Another study by Halim (1970) dealt with planktonic composition between the Red Sea and the Arabian Gulf. The results showed that the Arabian Gulf had more restricted species diversity and abundancy. Hendy (1970) described benthic Diatoms of Kuwaiti waters where he reported 205 species of littoral diatoms. Enomoto (1971) studied the distribution and abundance of phytoplankton species that occur in Kuwaiti waters and reported 39 diatoms and 4 dinoflagellates. Al-Harbi (2005) identified 124 species, consisting of 80 diatoms, 43 dinoflagellates, and 1 silicoflagellates. Al-Kandri et al. (2009) conducted a study and listed 323 phytoplankton species on morphological features. Al-Yamani & Saburova (2011) identified a total of 272 Diatom

and 80 flagellate species, where some of which were described for the first time and some were algae. Dorgham & Al-Muftah (1986) registered 345 phytoplankton species with a major constitutes of diatoms and dinoflagellate. Diatoms were represented by 175 species, whereas dinoflagellate by 124 species. They published the first phytoplankton checklist of Qatari waters that constituted mainly of 225 diatoms, 152 dinoflagellates, 2 silico-flagellates and 11 blue-green algae. Dorgham et al. (1987) described 223 species in the northern part of Arabian Gulf including 134 diatoms and 86 dinoflagellates. Dorgham and Al-Muftah (1989) identified 345 species from southern part of the Arabian Gulf, where they listed 175 diatom and 124 dinoflagellate. Al-Muftah (1991) reported 255 dinoflagellate species from entire Qatari water. El-Din & Al-Khayat (2005) listed a total number of 92 phytoplankton species was identified belonging to 22 dinoflagellate, 68 Diatoms, and 2 cyanobacteria. Al-Muftah & Al-Nasr (2016) reported 44 dinoflagellate species belonging to nine different genras. The most prominent Harmful algal blooms (HAB) happened between 2008 and 2009, when 8-month bloom of toxic dinoflagellate species *Cochlodinium polykrikoides* occurred at the seashore of Qatar, UAE, and Oman, resulting in enormous kills of fish, marine creatures, and coral reefs. The event also resulted in the shutting off desalination plants (Al-Azri et al, 2014; Richlen et al, 2010; Zhao and Ghedira, 2014). Although, many potentially toxic diatom and dinoflagellate species and their blooms were reported in Arabian Gulf. Glibert et al. (2002) was the first to measure algal toxins. Al-Muftah et al. (2016) listed 32 toxic algae that belong to five different groups based on the toxins they produced including diarrhetic shellfish toxin (DSTs), paralytic shellfish toxins (PSTs), amnesic shellfish toxin (AST), polyether toxins, and cyclic imines (CIs). Culture isolations and qualitative algal identification

conducted to help elucidate the source of the toxins detected in the Gulf. There was a positive result for most of the toxins produced by *Dinophysis* including PSTs, DSTs, and OA.

Dinophysis has a small to medium sized theca dinoflagellates (25 – 150 micrometer). Its thecal plates is smooth to more or less ornament. The genus *Dinophysis* is differentiated from *Phalacroma* by the much-reduced size of the epitheca. *Dinophysis* blooms very rarely reaching the cellular concentration to cause discoloration of the surface water. Multi-specific blooms observed, making it difficult for identification unless isolating and culturing is involved. These blooms are problematic to filter feeders because of contamination caused by DSPs (Lassus et. al, 2016). Reguera et al. (2012) mentioned that at least 10 *Dinophysis* and 2 *Phaalcroma* species produce pectenotoxins or dinophysistoxins and okadic acid.

Dinophysis is commonly arising in plankton surveys in Arabian Gulf, including species stated in Table 12 – 16 (Al-Muftah 2016, Quigg et al, 2013; Al-Yamani et al, 2012, Al-Kandari et al, 2009; Heil et al, 2001, Dorgham & Al-Muftah, 1989). All of these species have been associated with toxin production from diverse part of the world. *Dinophysis caudata* usually distributed in subtropical/tropical seas and during warm periods at temperate seas. Single picked cells of *D. caudata* from the Philippines (Marasigan et al, 2001); Spain (Luisa et al 2006) and Singapore (Holmes et al, 1999) discovered strains that producing DTX 1, PTX 2, and/or OA. The outbreaks of DSPs related to the blooms of *D.caudata* are often accompanied by another dominant *Dinophysis* species. The epidemics were reported from Asia, Australia, America, and Europe (IOC-UNESCO, 2019) (Table 1). *Dinophysis miles* commonly occurred in the Arabian Sea, while they are

also detected in the Mediterranean and different regions of West Pacific and Indian Oceans. Individual cells of *D. miles* in Philippines contained DTX 1 and OA (Marasigan et al., 2001). *Dinophysis rotundata* (= *Phalacroma rotundatum*) was known to produce PTX2 and DTX1 (Suzuki et al., 2009). *Dinophysis acuminata* is distributed from warm to cold waters. However, it is present in the Mediterranean Sea but was not been strongly demonstrated (IOC-UNESCO, 2019). It is the major cause of DSP outbreaks in European Atlantic coasts, as well as outbreaks in New Zealand and Northeast Japan (Esenkulova & Haigh, 2012). *Dinophysis mitra* (= *Phalacroma mitra*) is broadly spread in warm and tropical waters (IOC-UNESCO, 2019).

The Genus of Dinophysis

Currently, 120 species have been recognized taxonomically in the genus of *Dinophysis* (Worms, 2017: Guiry, M.D. & Guiry, G.M., 2018, Liu J.Y., 2008, Tomas, C.R., 1997, Fukuyo Y, 1990, Moestrup et. al., 2009). However, OAs and PTXs (DST) up to date have been found recognizably in ten species of *Dinophysis* that occur in coastal waters. Most reported DSP occurrences in the world were initiated by only six species of *Dinophysis* (Reguera et al, 2014). Although, based on IOC- UNESCO (2017) Liu (2008), Gómez (2005), Guiry & Guiry, (2018). 10 *Dinophysis* species have been identified as toxic HAB species worldwide including *Dinophysis acuminate, D. acuta, D. caudata, D. fortii, D. infundibulum, D. miles, D. norvegica, D. ovum, D. sacculus* and *D. tripos.* One of the identified and recorded toxic species of dinoflagellate was found in the order of Dinophysiales. In Qatar, 22 *Dinophysis* species were recorded and identified in different researches and years (Al-Muftah & Al-Nasr 2016, Al-Muftah et al 2016, Dorgham & Al-Muftah 1986, 1989, Al-Muftah 1991).

Research Emphasis on Dinophysis

A study in New Zealand (MacKenzie et al, 2002) reported that a bloom of *Dinophysis acuta* and *D. acuminate* in some coastal zones resulted in a high level of DSP-toxin contamination.

Farah et.al (2018) conducted a study to survey toxic dinoflagellates assemblages from Karachi – Pakistan to Northern Arabian Sea. Seventy-two dinoflagellates taxa were identified, 42 were toxic species and 30 were potentially toxic species. *Dinophysis caudata* was the dominant species.

Al-Kandari et al (2009), conducted a study to examine phytoplankton species composition in Kuwait water. In their study, 323 phytoplankton species were observed and listed based on their morphological features. Out of the 323, 108 dinoflagellate species were identified representing 38 genera that belong to 17 families. However during their study, six *Dinophysis* species were recorded, mainly *Dinophysis acuta, D. caudata, D. miles, D. mitra, D. norvegica* and *D. rotunadta.*

Al-Muftah (1991) has described 21 *Dinophysis* species from Qatari waters with three dominated species, *D. caudata*, *D. miles,* and *D. rotundata*. Al-Muftah et.al, (2016) conducted a study in 2012 and 2013 that is considered one of the recent extensive studies about the toxin producing algal species in Qatari waters. In this study, 32 toxic algae belonging to five different groups were analyzed based on their toxins production. Results confirmed for the first time the existence of diarrheic shellfish toxin (DST), paralytic shellfish toxins (PSTs), cyclic imines (CIs), polyether-lactone toxins, and amnesic shellfish toxin (AST). Though, three toxin producers of *Dinophysis* occurred in Qatar including *Dinophysis caudata, D. miles* and *D. rotundata*. *Dinophysis caudata,*

and *D. miles* were the most observed species through the two years of sampling.

The Arabian Gulf, however, have been neglected in this context of studying *Dinophysis* species and their produced toxins The only extensive work on *Dinophysis* carried out in the region was along the central part (Bohm, 1931, Dorgham & Al-Muftah 1986,1989, Dorgham et al, 1987, Al-Muftah 1991 & 2016). Unfortunately, no through systematic studies was carried out along the Western side of the Arabian Gulf. Although routine monitoring of phytoplankton and HABs does not presently exist in most of Arabian Gulf. While only scattered short-term studies provide some figures on the spatial and temporal distribution of phytoplankton. This prospect has carried out in Qatari waters in an attempt to provide a more comprehensive picture. This work, therefore, forms the first extensive study on the *Dinophysis* of Qatari waters representing the central part of the Arabian Gulf.

Health Effects of HABS

HAB species are known to cause many health adverse effects such as Diarrhea, nausea, vomiting and cramps. These symptoms differ from one person to the other, as it can occur 3 hours to 12 hours after ingesting these toxins. The major source of HAB toxin are the filter feeders such as Mollusks that depend mainly on Phytoplankton as their food source. According to the World health Organization (2019) 60000 person around the world are being intoxicated by HABs with 1.5% mortality rate. According to Van Dolah, (2000) the lethal dose of DSPs of mics is 200 µg/kg. Based on the tests of the Panel on Contaminants in the Food Chain (2019), the least lethal level for human illness is in the region of 50 µg OA equivalents/person. HAB toxins can also cause an inhibition of the protein serine/threonine phosphatases (Van Dolah, 2000).

Table 1: The list of toxin producing and red tide causing agents of Dinophysis.

Species	Red tide	Produced toxin	Different Studies
*Dinophysis acuminate**	YES	Dinophysistoxin Okadaic acid	IOC-UNESCO 2017: Liu J.Y., 2008, Gómez, F., 2005, Guiry, M.D. & Guiry, G.M., 2018, Al-Kandari et. al 2010, Faust et al. 2002;2005, Farah et. al. 2018
D. caudata	Yes	Pectenotoxin 2 Pectenotoxin 2 SAa Okadaic acid Dinophysistoxin	IOC-UNESCO 2017: Liu J.Y., 2008, Gómez, F., 2005, Guiry, M.D. & Guiry, G.M., 2018, Al-Muftah et. al 2016,, Al-Kandari et. al 2010, Faust et al. 2002;2005, Farah et. al. 2018
D. miles	No	Dinophysistoxin Okadaic acid	IOC-UNESCO 2017: Liu J.Y., 2008, Gómez, F., 2005, Guiry, M.D. & Guiry, G.M., 2018, Al-Muftah et. al 2016

Table 1 (cont.): The list of toxin producing and red tide causing agents of Dinophysis.

Species	Red tide	Produced toxin	Different Studies
*D. mitra**	No	Dinophysistoxin	IOC-UNESCO 2017: Liu J.Y., 2008, Gómez, F., 2005, Guiry, M.D. & Guiry, G.M., 2018
D. rotundata	No	Dinophysistoxin	IOC-UNESCO 2017: Liu J.Y., 2008, Gómez, F., 2005, Guiry, M.D. & Guiry, G.M., 2018 Lee et al. 1989;2014 Al-Muftah et. al 2016

Note: (*) identified in current study

Blooms of DSP producing agents started to occur frequently worldwide. For instance, a massive fish kill incidence observed in Kuwaiti waters (Glibert, 2002, Al-Qabs news, 2017). Another red tide observed in Qatar along the coasts of Al-Khor, the causative agent for was alexandrium minutm (Al-Muftah and Al-Nasr 2016). These events are registered under the Dinophysis and prorocentrum HAB species. Another example is the massive marine mammals, fish, and coral reefs kills that lasted for 8 months through the year of 2008 – 2009 (Al-Muftah, 2016, Qatar Biodiversity Newsletter, 2008). However, such causative agents of the red tide events are poorly studied in Qatari waters and wide coastal areas are still unexplored. Only three periodical studies have conducted in Qatar (Al- Muftah, 1986, 1991 & 2016, Al-Muftah and Al-Nasr 2016). However, outbreaks of neither DSP nor PSP have been recorded in Qatari water.

CHAPTER 3: RESEARCH OBJECTIVES

Dinoflagellates constitute a very important group of marine phytoplankton. They are next to the diatoms, the most numerous in number of species and total abundance. As primary producers, they are of great ecological significance. Nevertheless, unlike diatoms they can also cause fish mortality on a large scale. In such instances, they occur in various numbers changing the color of water, this discoloration is always referred to as "red tides "(Wood, 1954). Thus**,** the following are the main goals of this project:

- To investigate the relationships between the physical and chemical parameters and *Dinophysis* species.
- To investigate the spatial distributions of *Dinophysis* in the area.
- To study the diversity of *Dinophysis* species
- To analyze the range of toxin profiles of *Dinophysis* along the eastern coast of Qatari waters.

CHAPTER 4: MATERIALS AND METHODS

Study Area

During the years 2017 – 2018, samples were collected by the use of R/V "Janan" of Environmental Research Center (Qatar University) along two lines transect located perpendicularly to the eastern coasts of Qatar. Eight hydrographic stations covering these two lines transects were established. These stations extended from the southern east of Qatar at station A & B to the Northern East close to station G & H (Figure 1). The first station (A) was 25 miles away from the coast and the distance between each station is 15 miles. Offshore stations are 45 miles away with 15 miles between each station. Samples were collected from all stations and from different depths, noting that the depth at some stations differed from one cruise to the other based on the season. In total, data obtained from eight stations during four cruises, as the following:

Cruise No. 1: This cruise lasted for two days (5th – 6th October 2017) and covered eight stations. The depth ranged between 21 to 38 m

Cruise No. 2: The cruise occupied two days (21st – 22nd December 2017) and covered eight stations with a depth ranging from 21 m to 42 m.

Cruise No. 3: Lasted for two days (22nd – 23rd February 2018) and covered eight stations with a depth ranged from 15 m to 44 m.

Cruise No. 4: This cruise took two days (27th – 28th April 2018) and covered eight stations with a depth ranging from 18 m to 38 m.

Figure. 1: Sampling stations.

The coordinates of the stations:

A: 25.288452 - 51.863400.

B: 25.288825 - 52.267986.

C: 25.431673 - 51.821469.

D: 25.434835 - 52.226673.

E: 25.665594 - 51.822547.

F: 25.668618 - 52.228657.

G: 25.999166 - 51.842171.

H: 25.990973 - 52.249742.

Sample Collection

Water samples for qualitative phytoplankton analysis were collected by means of fine net (50-micron size, 50 cm diameter) towed in the first meter of the surface water. Qualitative hauls were made 8 time per cruise by towing a phytoplankton net for 10 minutes behind the R/V at the speed of 2 nautical miles/h. The samples were preserved in 4% neutralized formalin (PROLABO). Quantitative samples were obtained by Nisken bottles (5 liter) from surface at 10, 20, 30 and 40 depths. These samples were preserved in Lugol solution (J.Crow's). The examinations of net samples were carried out under Zeiss light Microscope attached to laptop for picture acquisition.

Biological Analysis

Onboard of the R/V, initial examination of the collected live samples was conducted by the use of a light microscopy. Samples were then preserved in 4% neutralized formalin for later analysis. Simple slide preparation method was used at Qatar University laboratory to examine the samples under a light microscopy. Slides were observed using 4 x, 10x, and 40x objects with an ocular of 12.5 x eye pieces. Axiovision software was used for the acquisition of *Dinophysis* pictures. *Dinophysis* samples identified and listed by the use of different taxonomy catalogues and data bases (Al-Yamani, 2009, Fukuyo et al, 2014, IOC-UNESCO, 2019, WoRMS, 2017, Al-Muftah, 1991) by the use of USEPA (1994). Moreover, some of these samples were isolated and identified by the use of a scanning electron microscope (SEM). Several washing and separation steps were carried out to get the best results under SEM (Al-Muftah, 2000, Lee et al., 1985, 2000). For the best results for scanning electron microscope (SEM) several washing, and separation steps have been done:

- The samples were rinsed with distilled water and concentrated by centrifugation (5 – 7 times).
    - 50% absolute alcohol was added and left overnight.
    - The same procedure was repeated, for 70%, 90% and 100%, and stored in 100%.
    - After a through washing with alcohol a suspension of cells was dried onto glass slide preferable by natural evaporation at room temperature or over hotplate.
- A small cover glass (13 mm) cleaned and used for the chosen specimen.

- The coverglass was stuck face upward on SEM stub (13 mm) using adhesive tape and left overnight at room temperature or heated at 50 °C for 1 hour.
- Stubs were sputter with gold or gold-palladim in the usual way and then examined in a Hitashi E520 field Emission Scanning Electron Microscope, fitted with a stage which tilt to 54 degrees.
- Cell count analysis**:** Samples (1L) preserved in Lugol solution were kept overnight inside the laboratory to settle. These samples were siphoned by the use of a U-Shaped pipette to concentrate the samples into a 100 mL level. After that, 1 mL of sample was added on a Cell counting slide (Sedgewick Rafter) to count the cells under a light microscopy. Noting that, each concentrated sample was counted for three times to get an average and to make sure that those samples are statistically valid. The counts were entered into the following formula:

$$N = n \times v \times 1000$$

N: Number of cells / liters of seawater.

n: Average of the counts.

V: Concentrated volume of the sample.

1000: the initial volume of sample (before concentration).

Physical, Chemical and Toxin Analysis

Nutrients analysis and physical records: Salinity and temperature were recorded by the use of Conductivity temperature and depth rotary with the collection of Nutrient samples. Samples were collected by protected rotary CTD and Nisken bottles. Five nutrients were

determined; Nitrate, Nitrite, Phosphate, Ammonia and Silicate. EasyChem Plus spectrophotometer and USEPA 365.1 were used to determined nutrient concentrations. Different reference materials used to have a detection limit that can create different peaks for the desired nutrient (USEPA 365.1, ESC – QP – VPR – Rev.02, 2016).

Freeze-dried plankton samples analyzed for 48 different algal toxins including Okadaic acid (OA), Pectenotoxin (PTXs) and Dinophysistoxin (DTXs) which are of a main interest using liquid chromatography tandem mass spectrometry (LC/MS-MS) as described by Al Muftah et al. (2016).

- Algal samples were analyzed for domoic acid (JAOAC (1995), 78(2), 543-554 (modified), lipophilic toxins (JAOAC (2005), Int 88:761-772 (modified) and paralytic shellfish toxins (JAOAC (2015), Int 98(3) 609-621).
- The method uses two mass filters arranged sequentially with a collision cell between them. The filters can be used in static or scanning mode to select a mass-to-charge (m/z) ratio or m/z range. In the collision cell, the precursor ions collide with gas molecules and are fragmented into smaller ions referred to as product ions.

STATISTICAL ANALYSIS

The data was analyzed by the use of Analysis of Variance ANOVA and Tukey comparison in order to understand differences between different seasons, depths, station and their interaction. The analysis conducted to validate the results of the study and to prove the differences in chemical, physical and biological samples through the different seasons and depths.

CHAPTER 5: RESULTS

Salinity

During the four cruises, salinity showed small fluctuations in the range of 38.00 ‰ in the offshore water (station H - 10 meter) to greater than 42.00 ‰ at inshore stations (station A – 20 meter). Some stations had unusual increase and decrease patterns depending on the ample collection depth.

The salinity increased with depth at all stations vertically in October cruise 2017 (Table 9). The salinity values at station A and C show an increase in level toward northern part, while station E and G shows the opposite. The values at station B, D, F and H show a lower range of salinity moving near the northern area.

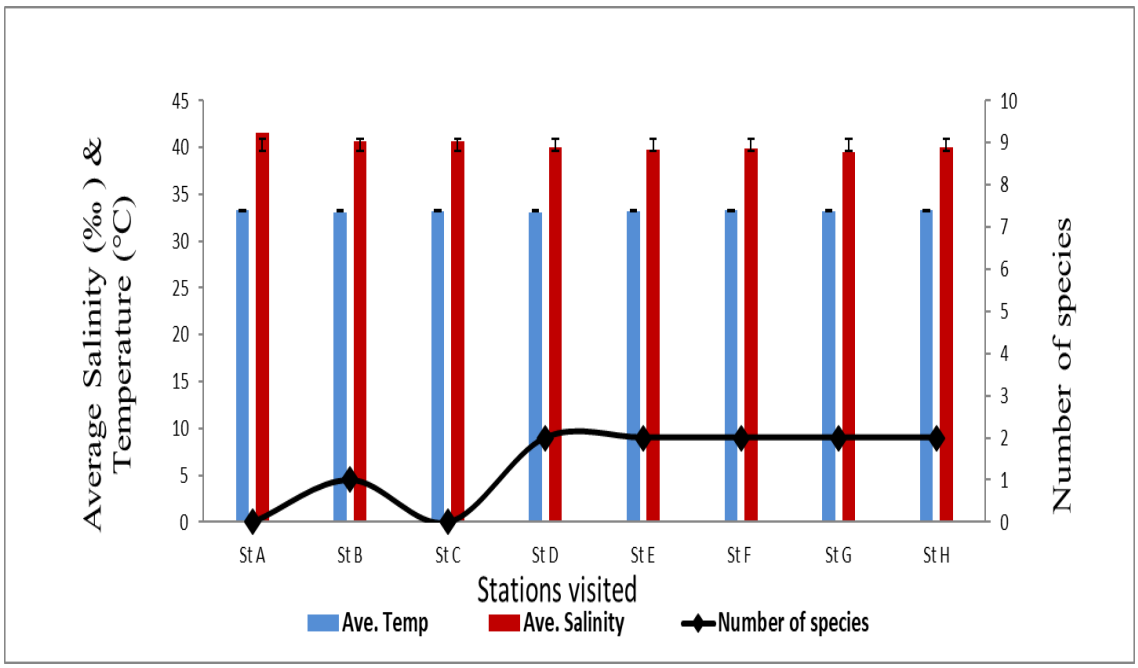

Figure 2: correlation between the Salinity &Temperature vs Number of species determined in October 2017.

During December 2017 cruise, the salinity shows gradual increase vertically with depth at all stations except for station B, where reading of less than 40.83 ‰ was recorded (Table 10). The salinity decreased horizontally and toward the northern direction.

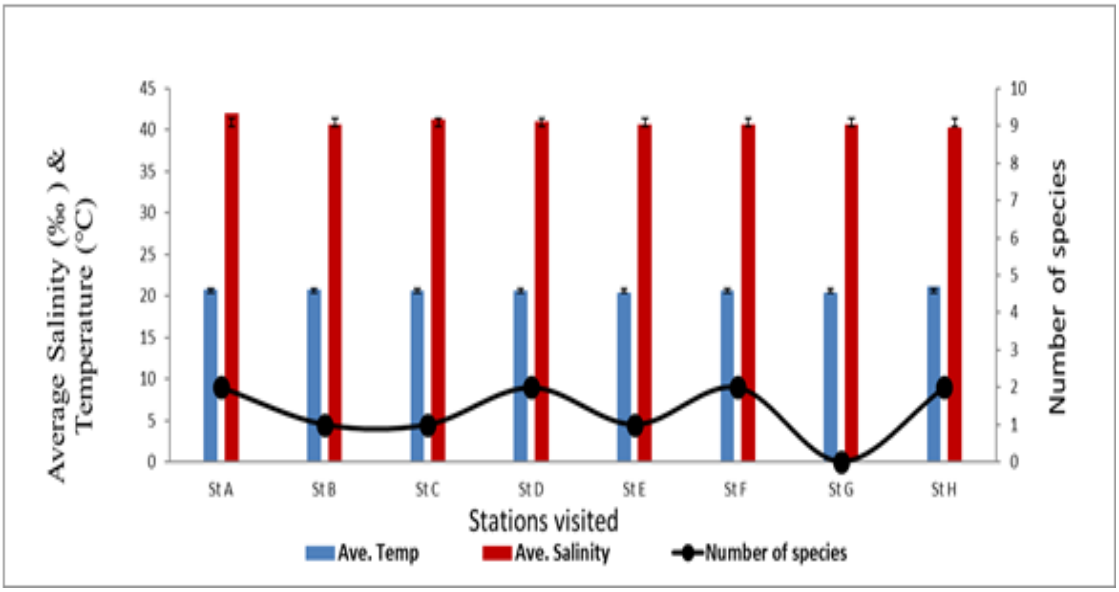

Figure 3: correlation between the Salinity &Temperature vs Number of species determined in December 2017.

In February 2018, the salinity has the same vertical pattern as the previous two cruises where it increased with depth at all stations (Table 11) and it reached more than 42.24 ‰ at station A -14 meter. Stations A and C have an increase in salinity, while decreased in station E, and then increased again in station G. However, stations B, D, F and H showed a decrease in salinity to the northern direction.

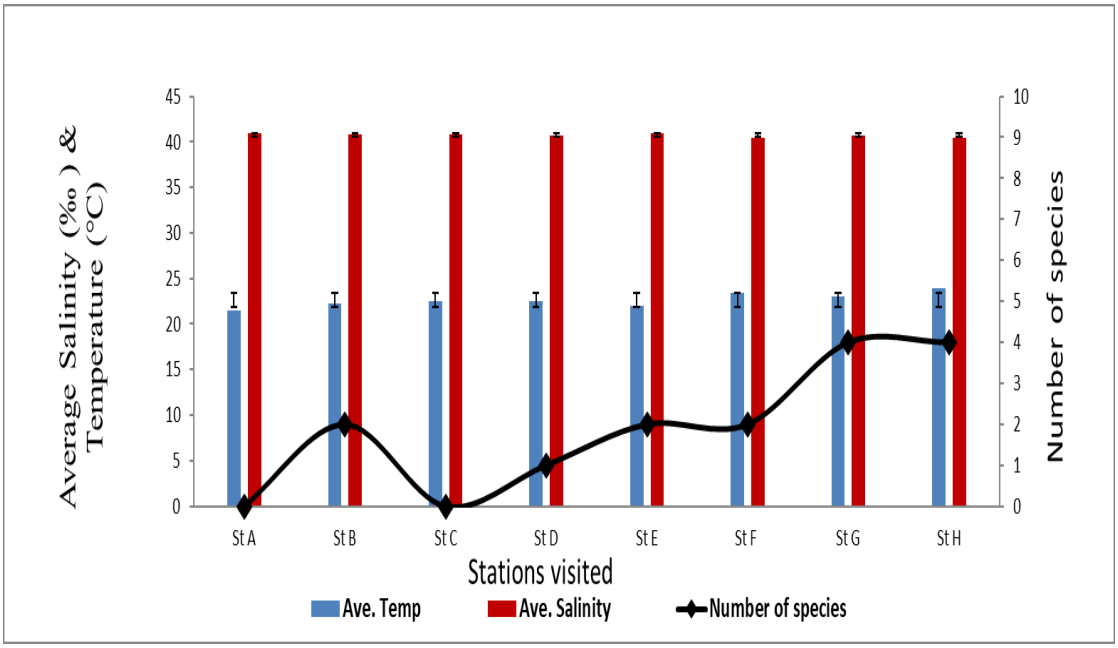

Figure 4: correlation between the Salinity &Temperature vs Number of species determined in February 2018.

During April 2018, the salinity increased with depth at stations A and C, while it declined in station E and then increased again in station G to reach 39.00 ‰ (Table 12). Offshore stations showed increase in salinity with depth as we move to the northern area and decreased horizontally at all stations towards the northern area with 39.56 ‰ at station H – 5 m.

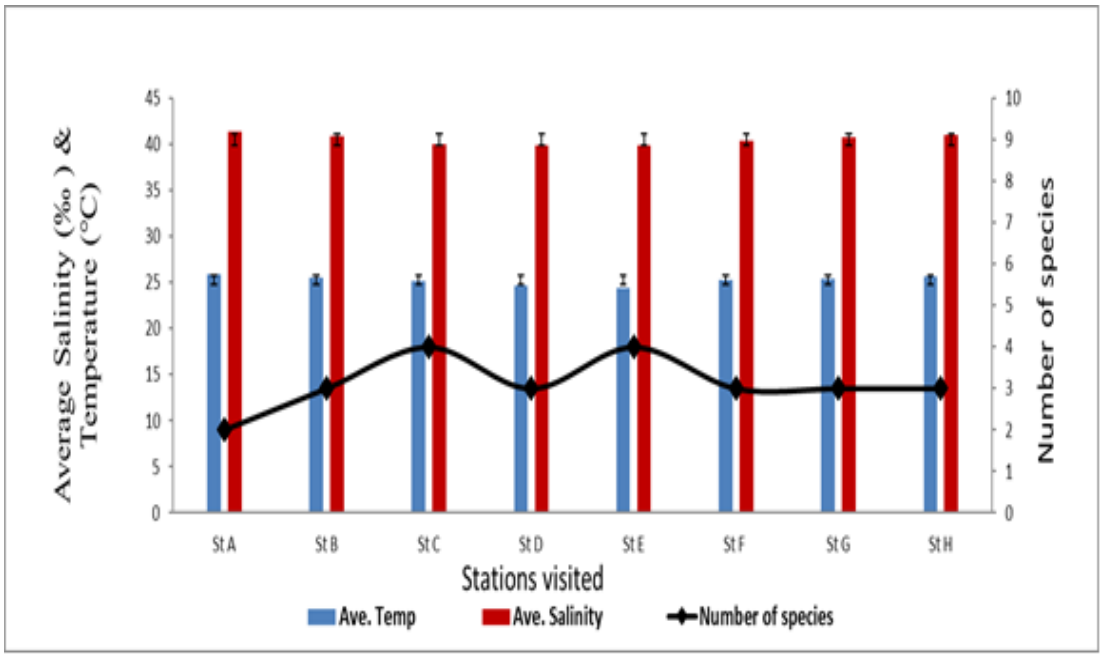

Figure 5: correlation between the Salinity &Temperature vs Number of species determined in April 2018**.**

Salinity strongly varied through seasons with P-Value: 0.00 with a 100% confidence level, while depth had a P-Value of 0.002 with a 98% confidence. However, the effect of season and depth on the ranges of salinity through the whole stud was insignificant with a P-Value of 0.657. Correspondingly, the records of salinity during the study assured by statistical analysis as it gives the same results, as salinity varied with season and with depth if looked individually on these two factors. However, season 2 and season 4 had almost the same ranges of salinity (Table 2).

Table 2: Tukey Pairwise Comparisons, Salinity in Depth and Season.

Depth / Season	1	2	3	4	Mean
1	39.82	40.63	40.71	40.39	40.39 (b)
2	40.12	40.67	40.79	40.44	40.51 (ab)
3	40.40	40.81	41.09	40.56	40.71 (a)
Mean	40.12 (c)	40.70 (ab)	40.86 (a)	40.46 (b)	40.54

Note: Means that do not share a letter are significantly different.

Table 2a: Analysis of variance for Salinity in Rep, Season & Depth

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Rep	7	22.0265	3.14664	25.15	0.000
Season	3	7.5757	2.52523	20.18	0.000
Depth	2	1.7361	0.86807	6.94	0.002
Season*Depth	6	0.5188	0.08647	0.69	0.657
Error	77	9.6352	0.12513		
Total	95	41.4923			

Temperature

Throughout the survey, the temperature showed a wide range of variation between 20.17 °C at station H (February 2018) to greater than 34.04 °C at station A (October 2017). Variations between the cruises were large, while differences within stations were small. Stations H and F, where the depth exceeded 40 meters, have a wide range of temperature fluctuations. In October has at station H the temperature was recorded at 32.75 °C at the surface, which dropped to less than 31.08 °C at station H 40 meter.

During December 2017, the surface temperature ranged between 21.50 °C (station A) to 24.32 °C (station H) and showed increase in northern direction. Vertically, the temperature showed small variations at deeper waters (Table 10).

In February 2018, the temperature exhibited the same characteristics as the data reported in December 2017 (Table 11). Lower temperature values were recorded from inshore stations, while offshore stations had higher values (21.24 °C). Toward the northern area, temperature values reduced in inshore stations, while they increased at offshore stations.

During the last monitoring (April 2018), temperature range between 23.00 °C to 26.00 °C (Table 12). A decrease with depth at stations A, B, D, E, F and G was observed, whereas at station C the temperature slightly inclined.

Temperature has significantly varied through different seasons (P-Value: 0.00) with a 100% confidence level. While, depth and depth and season had no statistical difference as their p-values were higher than 0.05. Although, temperature readings during the whole survey assure that the difference is only in season, and depth has a negligible effect on records. In addition, Tukey experiment showed that the significant difference only found between seasons, which represented by different letter (Table 3).

Table 3: Tukey Pairwise Comparisons, Temperature in Depth and Season.

Depth / Season	1	2	3	4	Mean
1	33.10	22.86	20.96	25.42	25.58 (a)
2	33.07	22.73	20.73	25.36	25.47 (a)
3	33.17	22.44	20.56	25.25	25.35 (a)
Mean	33.11 (a)	22.67 (c)	20.75 (d)	25.34 (b)	25.47

Note: Means that do not share a letter are significantly different.

Table 3a: Analysis of variance for Temperature versus Rep, Season & Depth

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Rep	7	2.90	0.414	1.62	0.142
Season	3	2122.97	707.658	2774.22	0.000
Depth	2	0.85	0.423	1.66	0.197
Season*Depth	6	0.70	0.116	0.46	0.839
Error	77	19.64	0.255		

Nutrients

Ammonia

Throughout the study, the levels of ammonia – nitrogen varied reaching the maximum level of 43.75 μg / L. The lowest ammonia – nitrogen concentrations, less than (0.11 μg / L) were recorded in December 2017 (Table 10), followed by 0.72 μg / L in October 2017 (Table 9), then 7.65 μg / L in April 2018 (Table 12) and 13.34 μg / L in February 2018 (Table 11), with different distributional patterns, either increased or decreased with depth. The concentrations of NH4 were high through the water column in February 2018, while there was a slight change in the inshore regions.

High ammonia – nitrogen levels, greater than 3.58 μg / L were recorded only at 3 stations (B, C & F) (Table 9) in October 2017. The concertation remained the same at all stations until April 2018. The highest overall NH4 levels throughout the study were determined in the offshore waters. At stations A and H, the concentrations of NH4 were high in February and April 2018, but in October and December 2017 (Figure 6 – 9) the concertation lowered.

Ammonia changed significantly through the study as the results showed, and proved by having a p-value of 0.00 considering the effect of season, while depth had no effect on the level with a p-value of 0.337. Moreover, the interaction of season and depth had no significant difference. Tukey comparison showed that all of the four seasons had a significant difference while season 1 and 4 (October & April) had almost the same levels of NH4 (Table 4).

Table 4: Tukey Pairwise Comparisons, Ammonia in Depth and Season.

Depth / Season	1	2	3	4	Mean
1	1.84	1.21	17.91	12.13	8.27 (a)
2	1.28	0.35	18.86	7.82	7.08 (a)
3	0.59	1.13	31.93	13.36	11.75 (a)
Mean	1.24 (bc)	0.89 (c)	22.90 (a)	11.10 (b)	9.03

Note: Means that do not share a letter are significantly different

Table 4a: Analysis of variance for Ammonia versus Rep, Season & Depth

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Rep	7	1358.4	194.1	1.14	0.350
Season	3	7767.8	2589.3	15.15	0.000
Depth	2	377.3	188.6	1.10	0.337
Season*Depth	6	749.9	125.0	0.73	0.626
Error	77	13156.6	170.9		
Total	95	23410.			

Nitrite

The distribution of nitrite varied between 0 to maximum of 7.37 μg / L. Low concertation of NO2 as observed in February and April 2018 (Tables 4 and 5) with an overall low concertation of (0.00 – 0.04 μg / L at station F). In October and December 2017, the same distributional patterns of NO2 levels were encountered in February and April 2018.

At offshore stations of F and H, on October and December sampling maximum of 20.00 μg / L of NO2 at station H of (30 meters) in both seasons was recorded (Table 9 – 10). In February 2018, the levels of NO2 increased with depth at inshore stations, except for station A that had zero NO2. The NO2 measurement at offshore stations followed the same pattern of increment with depth and mostly above the detection limits. In April 2018, there was no NO2 at station A and G, while at stations C and E was not detected. During the four sampling seasons concentrations fluctuated insignificantly (P-Value = 0.11) Moreover, NO2 was not detected at station A throughout the study.

Nitrite didn't change through the study as shown in the results, as well as the results of the statistical analysis that has a p-value of 0.111 for the effect of season and 0.581 for depth, which increased to 0.936 for the interaction of both. Tukey comparison also proved that there was no significant difference between seasons and depths (Table 5).

Table 5: Tukey Pairwise Comparisons, Nitrite in Depth and Season.

Depth / Season	1	2	3	4	Mean
1	0.74	0.74	0.15	0.00	0.41 (a)
2	0.92	0.92	0.25	0.00	0.52 (a)
3	0.82	0.82	1.05	0.21	0.73 (a)
Mean	0.83 (a)	0.83 (a)	0.48 (a)	0.07 (a)	0.55

Note: Means that do not share a letter are significantly different

Table 5a: Analysis of variance for Nitrite versus Rep, Season & Depth

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Rep	7	53.072	7.5817	5.02	0.000
Season	3	9.373	3.1245	2.07	0.111
Depth	2	1.654	0.8268	0.55	0.581
Season*Depth	6	2.701	0.4501	0.30	0.936
Error	77	116.310	1.5105		

Nitrate

The values of nitrate in Qatari waters varied mostly between 0.38 μg / L and 17.00 μg / L. In October 2017, the values ranged between 0.86 – 8.79 μg / L, the levels increased in a northerly direction and with depth in both offshore and inshore stations (Table 9). Undetectable concentrations of Nitrate was characterized in the whole water body in December, except of station H (40 meters), where the recorded level was 0.38 ug/L (Table 10).

In February 2018, the range of NO3 in inshore stations did not diverge much as it is found between 3.70 – 4.90 μg / L (Table 11) except for stations C and E, where levels exceeded 5.50 μg / L. In contrast, offshore stations had larger NO3 concertation, ranging between 3.20 – 8.20 μg / L. All stations had an increased NO3 levels with depth and toward the northern direction except for station G and E. Furthermore, values fluctuated largely ranging between 4.00 – 17.31 μg / L in April 2018 (Table 12). The levels of NO3 followed an unusual pattern of increase and decrease levels at inshore stations, while station A had no detectable NO3. Conversely, offshore stations had a decline in the level of NO3 with depth, except at station H. Levels of NO3 increased toward the northern parts to reach a maximum of 17.31 μg / L in station G – (10 meters).

During the study, NO3 levels recorded its minimum values in December 2017 as it occurred only at station H – 40 meters. In February and April 2018, the levels started to enormously increase and cross the detection limit as it reached 17.30 μg / L at some stations. Most of the high values found at station C, E and G which are close to the coastal, and station H that is close to Ras Lafan area.

Nitrate levels varied strongly through the study if considering the effect of seasonal

changes, which had a p-value of 0.00 with a 100% confidence level. The results of the chemical analysis confirm the same as values of NO3 differed between seasons. However, depth didn't show a significant difference on the level of NO3 as well as the interaction of depth and season which had a p-value above 0.05. Season 1 and 2 (October and December) have almost the same levels of NO and season 3 and 4 (February and April) share the same conditions of NO3 (Table 6).

Table 6: Tukey Pairwise Comparisons, Nitrate in Depth and Season

Depth / Season	1	2	3	4	Mean
1	4.49	0.00	4.16	9.41	4.52 (a)
2	0.14	0.00	5.39	7.01	3.13 (a)
3	2.40	0.00	10.82	7.39	5.15 (a)
Mean	2.34 (bc)	0.00 (c)	6.79 (ab)	7.94 (a)	4.27

Note: Means that do not share a letter are significantly different

Table 6a: Analysis of variance for Nitrate versus Rep, Season & Depth

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Rep	7	422.50	60.36	1.44	0.203
Season	3	1001.46	333.82	7.94	0.000
Depth	2	68.13	34.06	0.81	0.448
Season*Depth	6	235.08	39.18	0.93	0.477
Error	77	3236.48	42.03		

Phosphate

The values of reactive phosphate in Qatari waters varied generally between undetectable (0.00) and an absolute maximum of 47.73 μg / L. During October 2017, values ranged between 1.28 – 47.73 μg / L and higher concentration were recorded from inshore stations (Table 9). December cruise values fluctuated between 1.99 ug/L to 5.50 ug/L in the offshore stations (Table3). During February 2018, Phosphate concentrations increased with depth in inshore stations (Table 11) except for station A and G. However, the level increased generally towered the northern region. The level at Station B and F (offshore) rose up with depth, but fell at station D and H. The concentration in April 2018, varied between (0.00) and an absolute maximum of 6.18 (station F). Generally, the level of PO4 declined with depth in April 2018 (Table 12). Phosphate has no significant difference that represented in the analysis and in statistical analysis: Season has a P-Value of 0.110, Depth P-Value 0.678 and the interaction of Depth*season has a P-Value of 0.468. Tukey comparison also proved that there was no significant variance in the level of PO4 between seasons and depths (Table 7).

Table 7: Tukey Pairwise Comparisons, Phosphate in Depth and Season

Depth / Season	1	2	3	4	Mean
1	7.69	3.40	3.62	1.74	4.11 (a)
2	2.39	3.58	6.05	1.58	3.40 (a)
3	2.37	2.26	5.98	1.06	2.92 (a)
Mean	4.15 (a)	3.08 (a)	5.21 (a)	1.46 (a)	3.48

Note: Means that do not share a letter are significantly different

Table 7a: Analysis of variance for Phosphate versus Rep, Season & Depth

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Rep	7	218.03	31.15	1.05	0.403
Season	3	184.52	61.51	2.08	0.110
Depth	2	23.14	11.57	0.39	0.678
Season*Depth	6	167.91	27.98	0.94	0.468
Error	77	2281.16	29.63		
Total	95	2874.75			

Silicate

Throughout the survey, the distribution of reactive silicate in Qatari waters showed important differences on seasonal basis, as the level found in the range of undetectable 0.0 to 186.29 µg / L. During October 2017, the highest concentration of silicate (186.29 µg / L) as recorded in the offshore region (station H – 40 meter) with general increase toward the northern area and with depth. Vertical variation of silicate was small/moderate from surface to the bottom, while at station H the concentration increased dramatically from 32.21 µg / L at the surface to 180.99 at 40 meters. In December 2017, a remarkable decrease was observed (Table 10), as most of the area showed values in the range of 18.41 – 83.03 µg / L while the highest recorded value was at station F (10 meters). Vertically, the reactive silicate increased with depth only at station A and C, while decreased at all the other stations. Moreover, the level of SIO2 increased toward the northern region, while it decreases at the offshore stations except for station H. During February 2018, silicate levels dropped sharply to 5.00 µg / L, with the highest of 44.28 at station H – 30 meters (Table 11). In April 2018, the level raised up again at all stations with a general increase with depth and toward the northern direction (Table 12) (except for station - A which had 0.00 µg / L SIO2).

Silicate level was significantly different through different seasons, with a P-Value of 0.00. While Depth has a P-Value of 0.339 and the interaction of depth and season has a P-Value of 0.631. Tukey comparison showed stated a significant difference in SIO2 between seasons (Table 8).

Table 8: Tukey Pairwise Comparisons, Silicate in Depth and Season

Depth / Season	1	2	3	4	Mean
1	58.32	35.06	10.37	25.59	32.34 (a)
2	76.43	44.74	14.01	23.36	39.64 (a)
3	70.05	32.06	22.30	25.25	37.41 (a)
Mean	68.27 (a)	37.29 (b)	15.56 (c)	24.73 (bc)	36.46

Note: Means that do not share a letter are significantly different

Table 8a: Analysis of variance for Silicate versus Rep, Season & Depth

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Rep	7	6352.7	907.5	2.22	0.041
Season	3	38087.4	12695.8	31.07	0.000
Depth	2	896.2	448.1	1.10	0.339
Season*Depth	6	1777.4	296.2	0.72	0.631
Error	77	31466.6	408.7		
Total	95	78580.3			

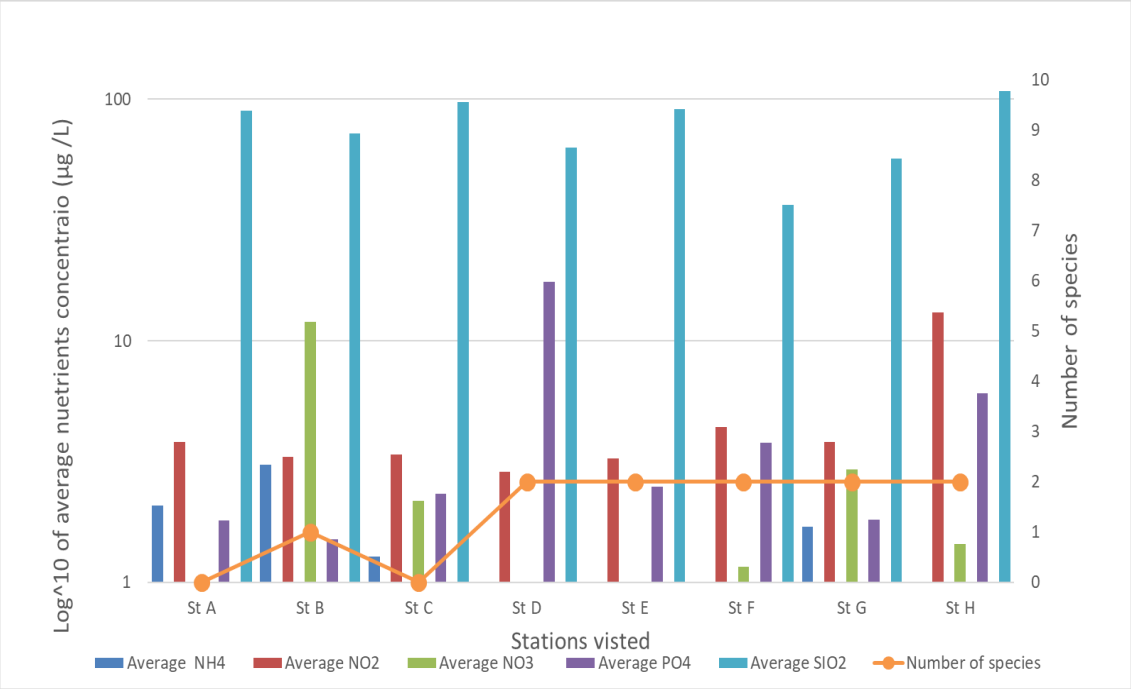

Figure 6: correlation between Nutrients and Number of species determined in October 2017.

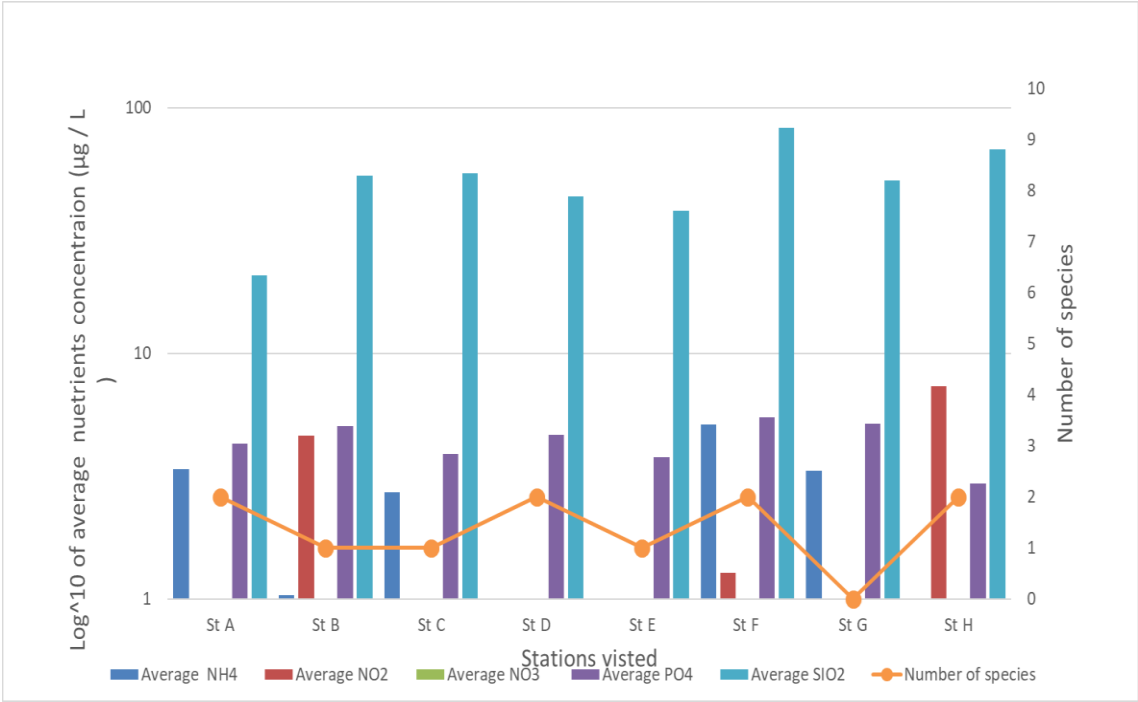

Figure 7: correlation between Nutrients and Number of species determined in December 2017.

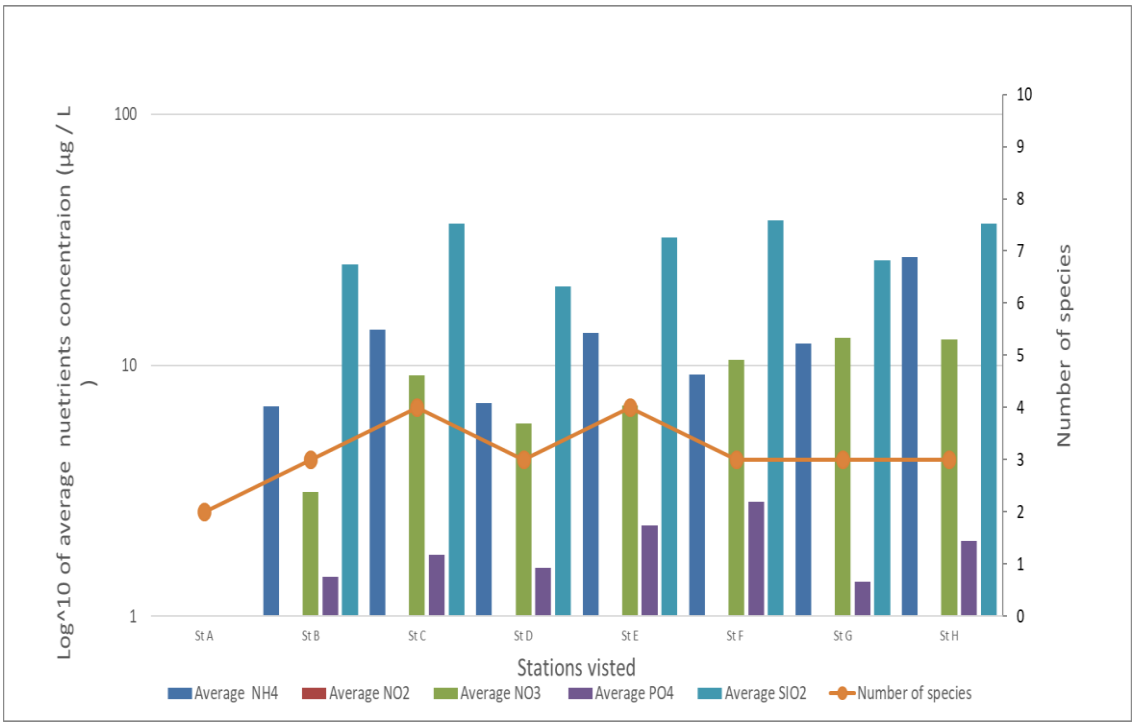

Figure 8: correlation between Nutrients and Number of species determined in February 2018**.**

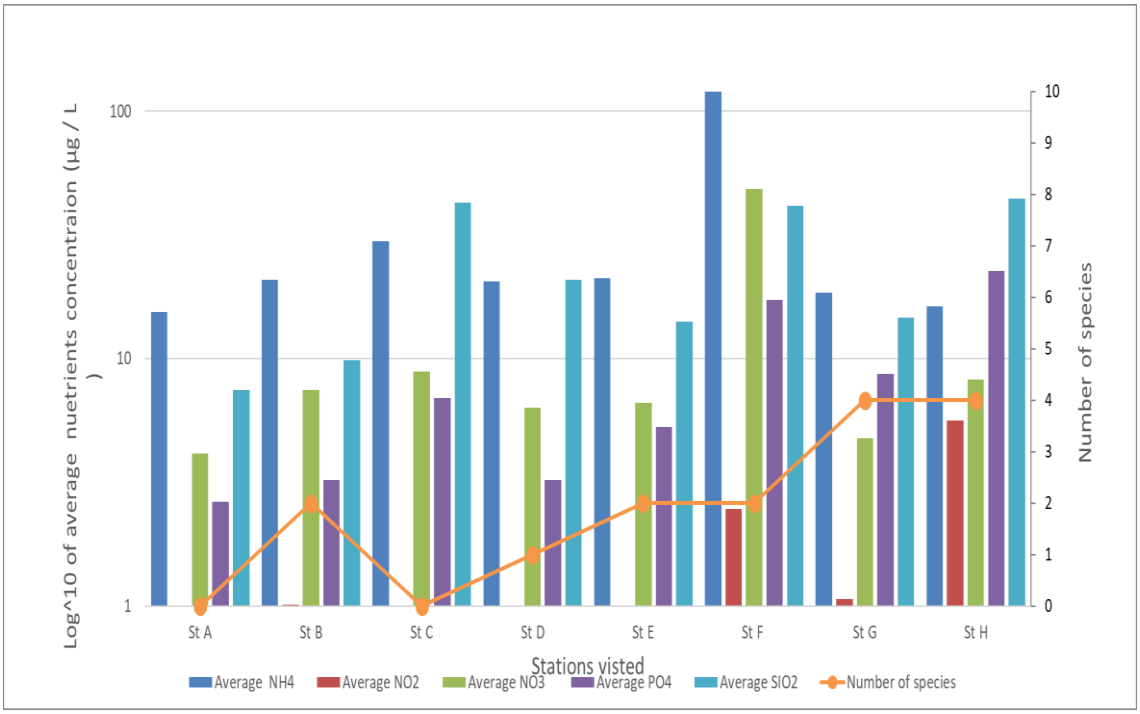

Figure 9: correlation between Nutrients and Number of species determined in April 2018.

Table 9: Physical and Chemical composition of Qatari waters (October 2017).

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg/ L)
A	0	33.07	41.08	3.09	3.62	0	1.93	82.65
	10	33.32	41.60	3.16	4.60	0	1.66	88.02
	20	33.51	41.88	0	3.26	1.12	1.83	98.25
B	0	33.18	40.41	3.84	3.29	35.91	1.49	64.30
	10	33.05	40.5	5.39	3.55	0	1.49	69.00
	20	33.29	40.87	0	3.08	0	1.56	83.40
C	0	33.34	40.18	0	2.72	0	1.49	49.82
	10	33.04	40.59	0	2.61	1.08	3.01	77.88
	20	33.72	41.81	3.84	4.81	5.43	2.47	163.76
D	0	32.88	39.56	0	2.76	0	47.73	50.49
	10	32.83	39.56	0.72	2.72	0	2.54	74.44
	20	32.82	39.62	0	3.14	0	2.27	63.85

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg/ L)
E	0	33.32	39.28	0	3.36	0	3.45	94.44
	10	33.16	39.84	0	2.98	0	1.56	86.08
	20	33.11	39.96	0	3.43	0	2.47	92.73
F	0	32.90	39.39	3.84	2.94	0	1.93	40.92
	10	32.90	39.39	0	3.65	0	1.66	46.39
	20	32.93	39.41	0	3.18	0.86	5.88	39.75
	30	33.93	40.67	0	7.85	3.77	5.71	186.29
	0	33.28	39.50	3.21	4.34	0	2.20	51.76
G	10	33.28	39.51	0.99	3.26	0	1.49	54.45
	20	33.15	39.53	0.91	3.86	8.79	1.76	64.07

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg/ L)
	0	32.75	39.10	0.77	3.29	0	1.29	32.21
	10	32.93	39.90	0	15.37	0	5.71	115.18
H	20	32.78	40.11	0	6.19	3.01	2.54	52.88
	30	31.98	40.21	0	20.17	4.21	8.35	159.73
	40	31.08	40.50	0	20.56	0	12.50	0

Table 10: Physical and Chemical composition of Qatari waters (December 2017).

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg /L)
A	0	21.50	40.95	3.38	0	0	1.99	19.45
	10	21.38	40.95	0.86	0	0	4.28	20.72
B	0	22.42	40.83	1.03	4.62	0	5.04	52.95
	10	22.15	40.80	0.25	0	0	2.68	19.52
	20	22.06	40.83	0.65	0	0	2.68	18.41
C	0	22.65	40.81	0.61	0	0	3.59	20.35
	10	22.48	40.86	0.57	0	0	3.90	54.29
	20	22.40	40.88	2.72	0	0	2.75	46.24
D	0	22.71	40.52	0.23	0	0	4.66	43.48
	10	22.57	40.65	0.74	0	0	3.90	32.06
	20	22.24	40.90	0	0	0	2.68	33.55

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg /L)
	0	22.08	40.94	0.12	0.01	0	3.78	38.10
E	10	22.05	40.97	0.12	0	0	3.06	29.75
	20	21.96	41.02	0.11	0	0	2.75	26.99
	0	23.55	40.37	0.39	0	0	2.68	24.97
	10	23.57	40.37	0.11	0.39	0	2.68	83.05
F	20	23.33	40.42	5.13	0.66	0	2.37	38.92
	30	23.29	40.49	0.59	1.28	0	5.50	50.71
	0	23.62	40.34	3.33	1.00	0	2.87	44.59
G	10	23.35	40.41	0.11	0.86	0	5.15	50.71
	20	22.41	41.07	0.19	0.32	0	2.37	39.82

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg /L)
	0	24.32	40.27	0.53	0.32	0	2.56	36.54
	10	24.22	40.28	0	6.11	0	2.94	67.80
H	20	23.82	40.34	0.18	5.62	0	2.49	52.50
	30	23.85	40.46	0.30	7.37	0	2.29	64.37
	40	23.80	40.53	0.66	7.15	0.38	2.18	57.50

Table 11: Physical and Chemical composition of Qatari waters (February 2018).

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg / L)
A	0	21.24	41.79	14.66	0	3.7	2.63	7.48
	10	20.65	42.11	15.41	0	4.13	2.25	6.03
B	0	21.01	40.66	15.81	0.08	4.13	1.88	6.19
	10	20.81	40.67	20.40	1.00	7.46	2.14	9.82
	20	20.56	40.82	20.82	1.01	7.07	3.24	9.42
C	0	21.11	40.88	21.07	0.08	4.05	1.08	5.3
	10	20.57	41.06	22.03	0.40	5.93	3.35	11.52
	20	20.55	41.82	29.78	0.72	8.9	6.93	42.67
D	0	20.95	40.50	18.74	0	4.17	3.24	13.94
	10	20.78	40.51	20.55	0.10	4.68	1.84	18.18
	20	20.49	41.40	20.21	0.11	6.32	2.74	20.88

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg / L)
	0	20.64	40.61	18.85	0	5.12	2.82	10.14
E	10	20.51	40.62	19.35	0	6.64	5.31	14.10
	20	20.29	40.69	21.14	0.21	3.75	1.73	12.00
	0	21.10	40.36	20.51	0	4.4	1.95	18.13
	10	20.86	40.45	20.62	0.47	4.97	4.18	23.46
F	20	20.54	40.73	13.34	2.46	4.86	1.73	41.46
	30	20.21	41.12	28.08	0.10	4.71	3.01	17.41
	0	20.43	40.63	18.54	1.07	4.49	8.67	12.65
G	10	20.44	40.63	16.22	0	4.77	6.67	14.66
	20	20.28	40.73	15.68	0	4.17	7.72	12.81

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg / L)
	0	21.24	40.25	15.13	0	3.23	6.67	9.09
	10	21.24	40.25	16.33	0	4.54	2.26	14.34
H	20	21.14	40.24	14.41	3.862	7.69	8.22	39.12
	30	21.07	40.25	15.25	5.629	8.26	6.14	44.28
	40	20.73	40.45	0	0	0	0	0

Table 12: Physical and Chemical composition of Qatari waters (April 2018).

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg / L)
A	0	25.94	41.38	0	0	0	0	0
	10	25.90	41.43	0	0	0	0	0
B	0	25.80	40.99	11.62	0	5.69	1.73	34.84
	10	25.60	41.05	0	0	0	0	0
	20	25.48	41.09	8.92	0	3.70	2.56	40.89
C	0	25.50	40.68	10.69	0.08	9.58	2.14	18.3
	10	25.56	40.73	10.56	0	8.52	1.88	27.34
	20	25.71	41.38	20.24	1.27	9.24	1.24	64.46
D	0	25.68	40.48	11.85	0	9.44	2.22	27.5
	10	25.47	40.56	9.31	0	8.09	2.44	34.52
	20	25.27	40.71	0	0	0	0	0

STATION NO.	DEPTH (M)	TEMP. °C	SALINITY ‰	NH4 (µg / L)	NO2 (µg / L)	NO3 (µg / L)	PO4 (µg / L)	SI (µg / L)
	0	25.17	39.99	13.53	0	4.10	1.73	36.86
E	10	25.17	39.99	7.65	0	5.93	2.93	30.08
	20	24.98	39.97	19.04	0.04	10.67	2.22	30.24
	0	25.36	40.17	12.73	0	16.51	2.52	42.59
	10	25.35	40.32	10.19	0	8.52	2.74	45.25
F	20	25.22	40.37	0	0.07	16.88	0	0
	30	25.03	40.68	13.78	0	0	6.18	63.17
	0	24.74	39.84	12.50	0	9.60	1.88	24.83
G	10	24.75	39.84	9.26	0	17.31	1.16	23.95
	20	24.60	39.84	14.89	0	11.63	1.08	29.51
	0	25.15	39.57	24.13	0	2.38	1.73	19.83
	10	25.06	39.63	15.57	0	7.68	1.46	25.72
H	20	24.89	39.66	43.75	0.31	6.97	1.39	36.86
	30	23.56	40.19	24.81	1.42	11.03	2.74	49.85
	40	0	0	26.65	0.69	17.06	2.67	50.66

Species composition

Dinophysis population of Qatari waters was characterized as moderate with the species of *Dinopyhsis caudata* and *D. miles* (Figure 10 - 11) being the dominant. During the whole survey, a total of five *Dinopyhsis* species were identified from surface waters, derived from 32 sample collections. The dominant species were defined as such when these appeared in the range of 60 – 100%, less dominant 20 - 60%, and occasional (rare) less than 20% of the total visited species. As has been noted, *D. caudata* was the most abundant species and was encountered 26 times (81.25 % of the total collection sites). While *D. miles* occurred 20 times (62.50 % of the total collection sites). The next common species was *D. rotundata*, which was present 7 times (21.88 %), followed by *D. mitra* 5 times (15.63%) and *D. acuminata* 3 times (9.38%).

October 2017

A total number of 2 species as recorded, *D. caudata* and *D. miles* where both occurred at 5 stations D, E, F, G and H out of the 8 stations monitored (Table 13). They both were dominant and encountered at the surface water. Both inshore and offshore line transects had a higher dominancy of these two species toward the northern side.

December 2017

The same 2 species were also identified in December sampling (Table 14). *D. caudata* encountered at 7 stations except at station G, while the main bulk was found at stations A and D. *D. miles* was observed only at 4 stations, A, D, F and H, and the majority were also found at stations A and D. Moreover, at the in-shore line transect, both species occurred more toward the northern stations, while decreased at offshore line transect at surface waters.

February 2018

The number of species encountered at this cruise was 4, namely *D. accuminata, D. caudata, D. miles* and *D. rotundata* (Table 15). These species had a different distributional pattern along the visited stations: *D. rotundata* was found at 2 stations, *D. accuminata* existed only at 3 stations, *D. miles* at 5 stations and *D. caudata* at 6 stations. Moreover, both *D. caudata* and *D. miles* formed the main bulk during this cruise. Additionally, less species encountered at in-shore stations, while more species were observed at offshore stations. The level of species determined increased (based on researcher observation) toward the northern direction at surface water.

April 2018

During this cruise, four species were identified: *D. Caudata, D. miles, D. mitra* and *D. rotundata* (Table 16). Both *D. caudata* and *D. miles* showed the main bulk as they occurred mostly at all stations, while both *D. mitra* and *D. routndata* encountered only at five stations. In addition, In-shore stations had a decreased level of existence of these species toward the norther parts of Qatari waters, whereas offshore stations had more *Dinophysis* species identified at northern directions.

Table 13: Species composition of Qatari waters in October 2017.

STATION NO. / SPECIES COMP.	A	B	C	D	E	F	G	H
Dinophysis acuminata	-	-	-	-	-	-	-	-
D. caudata	-	-	-	+	+	+	+	+
D. miles	-	-	-	+	+	+	+	+
D. mitra	-	-	-	-	-	-	-	-
D. rotundata	-	-	-	-	-	-	-	-

Note: Absent (-), present (+).

Table 14: Species composition of Qatari waters in December 2017.

STATION NO. / SPECIES COMP.	A	B	C	D	E	F	G	H
Dinophysis acuminata	-	-	-	-	-	-	-	-
D. caudata	+	+	+	+	+	+	-	+
D. miles	+	-	-	+	-	+	-	+
D. mitra	-	-	-	-	-	-	-	-
D. rotundata	-	-	-	-	-	-	-	-

Note: Absent (-), present (+).

Table 15: Species composition of Qatari waters in February 2018.

STATION NO. / SPECIES COMP.	A	B	C	D	E	F	G	H
Dinophysis acuminata	-	-	-	-	+	-	+	+
D. caudata	-	+	-	+	+	+	+	+
D. miles	-	+	-	-	+	+	+	+
D. mitra	-	-	-	-	-	-	-	-
D. rotundata	-	-	-	-	-	-	+	+

Note: Absent (-), present (+).

Table 16: Species composition of Qatari waters in April 2018.

STATION NO. / SPECIES COMP.	A	B	C	D	E	F	G	H
Dinophysis acuminata	-	-	-	-	-	-	-	-
D. caudata	+	+	+	+	+	+	+	+
D. miles	-	+	+	+	+	+	+	+
D. mitra	+	+	+	+	+	-	-	-
D. rotundata	-	-	+	-	+	+	+	+

Note: Absent (-), present (+).

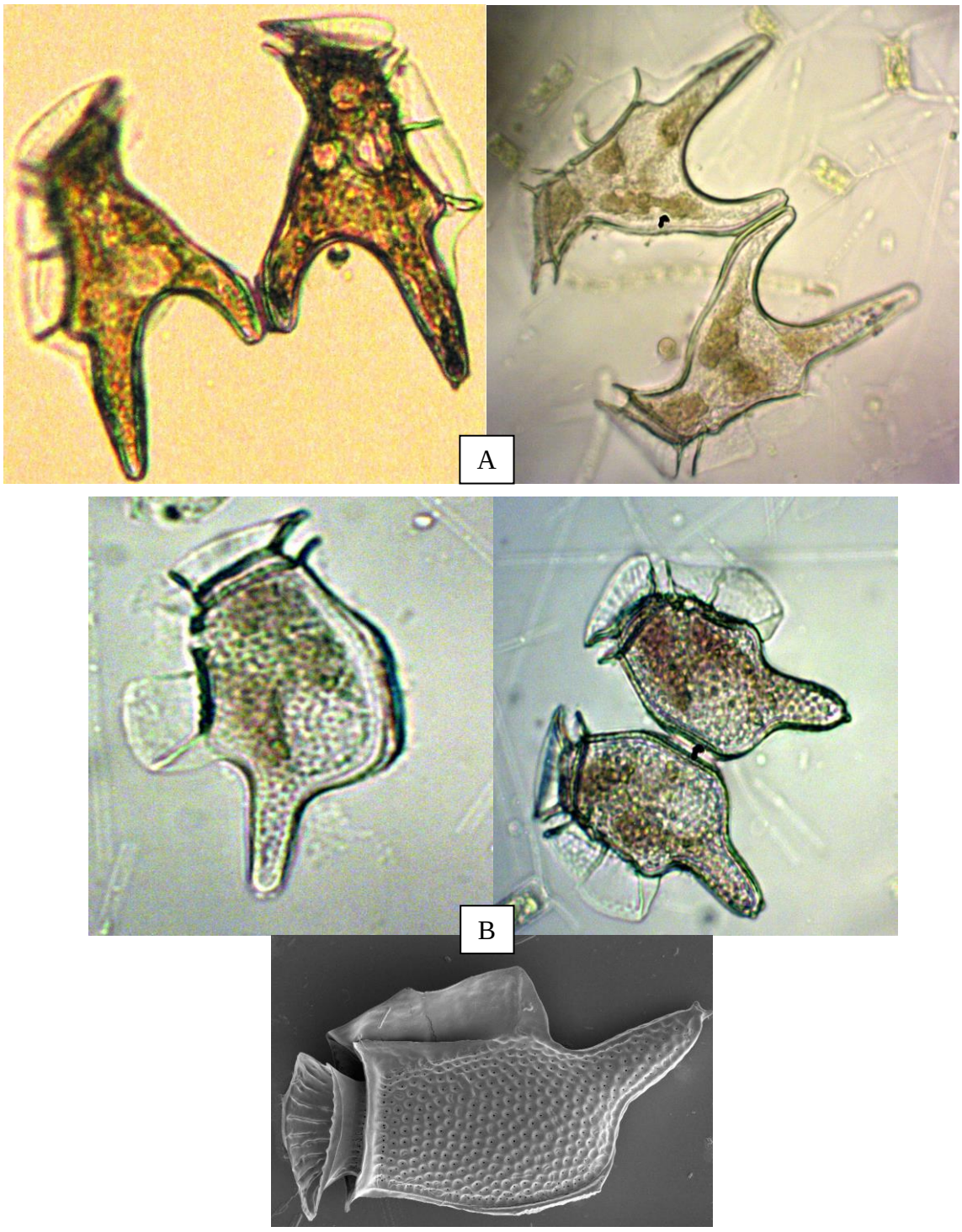

Figure (10): (A) *Dinophysis miles*, (B) *Dinophysis caudate* (SEM).

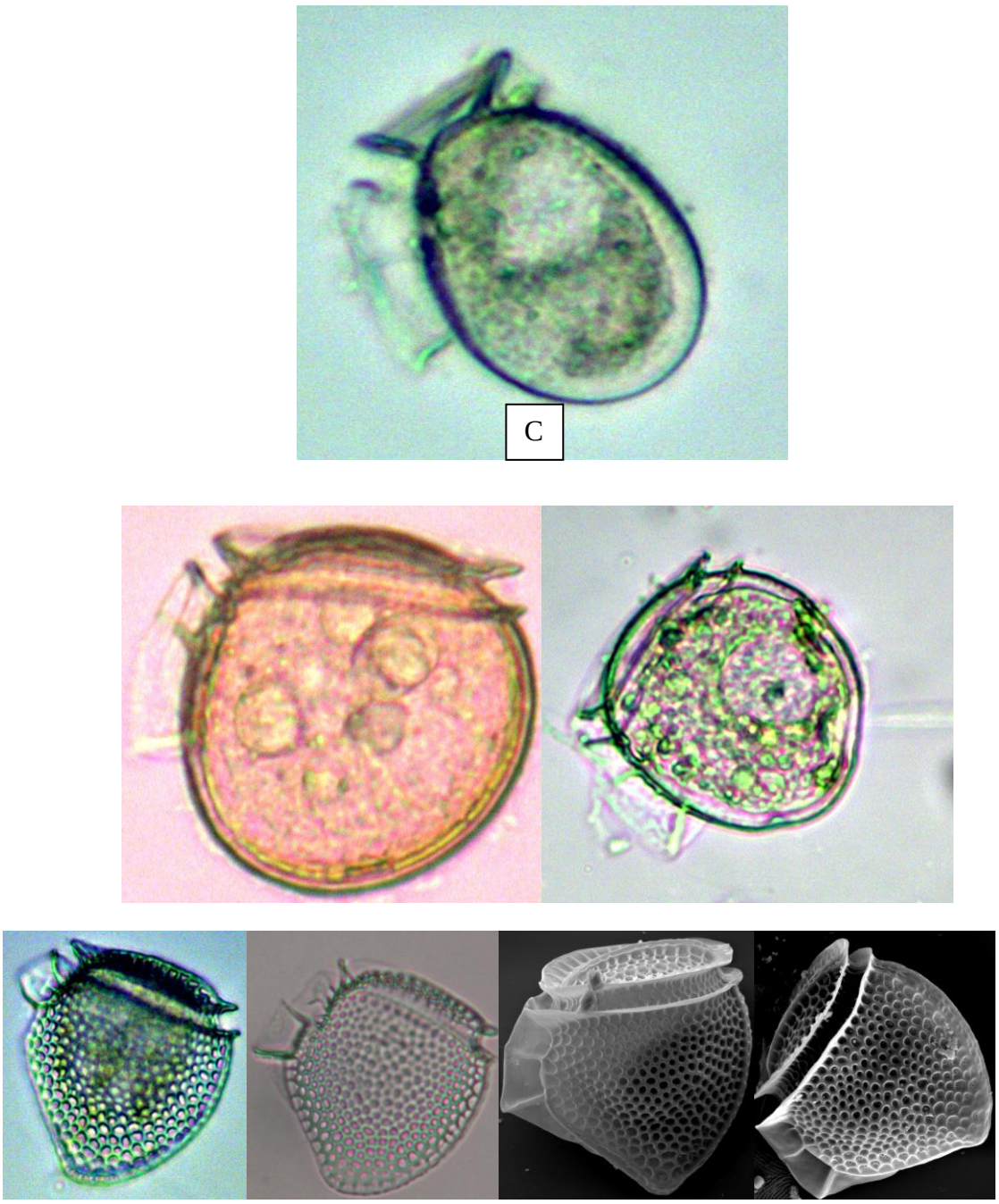

Figure (11): (C) *Dinophysis acuminata*, (D) *Dinophysis routndata*, (E) *Dinophysis mitra* (SEM).

Cell count

The counting analysis showed an average cell number of 66666.67 cells / L along the studied area in October 2017. A maximum of 300,000 cells / L was found at station F (0 meter) and a minimum of 33,333.33 encountered at station B, D, F and H mostly at the mid depths. In-shore line transect, an increase in cell numbers per liter with depth was observed except at stations A and C, which had no *Dinophysis* at all. Offshore line transects showed the same pattern as inshore line transect and no *Dinophysis* were encountered at stations B (0 meter), D (20 meter) and H (0 meter).

December 2017: An average cell number of 8.2 X 10^4 Cells / L was observed during this cruise. A maximum of 1 X 10^6 cells / L was counted at station D (10 meter) and a minimum of 3.3 X 10^4 cells/ L was encountered at stations B, E, F, G and H, mostly at the mid depths (10 meter). In in-shore line transect, a decrease in the number of cells per liter with depth was recorded. Offshore line transects showed the same pattern as inshore line transect and no Dinophysis where encountered at stations B (0 meter), D (20 meter) and C (0 meter).

February 2018**:** A maximum of 4.1 x 10^6 cells/L was determined at station E – 20 meters, with an average of 2.8 x 10^5 cells/ L (Figure 12). Although, the number of cells decreased with depth at inshore line transect, an overall increase in the number of cells in the ranging between 3.3 x 10^4 cells/L and 4.1 x 10^6 cells/L was observed. Both lines transect had an increased number of cells toward northern stations (Table 17).

During the April 2018 cruise, the number of cells/L increased dramatically to have a maximum of 2.7 x 10^7 cells/L at station D – 0 meter, with an average of 6.7 x 10^6 cells/L. Both lines show an increase in the number of cells with depth and toward the

northern parts of Qatari coasts.

Table 17: Number of *Dinophysis* cells / L determined at different stations during the study.

STATION NO.	DEPTH (M)	October 2017 Cell count (Cells / L)	December 2017 Cell count (Cells / L)	February 2018 Cell count (Cells / L)	April 2018 Cell count (Cells / L)
	0	0	1.6 x 10^5	0	1.5 x 10^6
A	10	0	0	0	1.5 x 10^6
	20	0	-	-	-
	0	0	0	3.6 x 10^5	2.3 x 10^5
B	10	3.3 x 10^4	6.6 x 10^4	6.6 x 10^5	1.2 x 10^6
	20	6.6 x 10^4	3.3 x 10^4	3.6 x 10^5	3.3 x 10^4
	0	0	0	0	1.4 x 10^6
C	10	0	0	0	1.7 x 10^6
	20	0	0	0	4.7 x 10^6
	0	1.3 x 10^5	0	5 x 10^5	2.8 x 10^6
D	10	3.3 x 10^4	1.0 x 10^6	5 x 10^5	1.4 x 10^6
	20	0	0	0	7.3 x 10^5

STATION NO.	DEPTH (M)	October 2017 Cell count (Cells / L)	December 2017 Cell count (Cells / L)	February 2018 Cell count (Cells / L)	April 2018 Cell count (Cells / L)
E	0	1.0 x 10^5	0	1.3 x 10^6	6.0 x 10^5
	10	2.6 x 10^5	3.3 x 10^4	1.2 x 10^6	1.7 x 10^5
	20	6.6 x 10^4	0	4.1 x 10^6	1.0 x 10^5
F	0	3.0 x 10^5	3.0 x 10^5	1.4 x 10^6	1.2 x 10^6
	10	1.3 x 10^5	0	7.0 x 10^5	1.3 x 10^6
	20	3.3 x 10^4	3.3 x 10^4	6.6 x 10^4	3.3 x 10^4
	30	3.3 x 10^4	0	3.3 x 10^4	0
G	0	1.0 x 10^5	1.0 x 10^5	2.0 x10^5	1.7 x 10^5
	10	6.6 x 10^4	3.3 x 10^4	1.6 x 10^5	1.0 x 10^5
	20	6.6 x 10^4	0	0	1.0 x 10^5
H	0	0	2.6 x 10^5	1.0 x 10^5	3.3 x 10^4
	10	3.3 x 10^4	3.3 x 10^4	1.0 x 10^5	1.1 x 10^6
	20	1.3 x 10^5	0	6.6 x 10^4	4.0 x 10^5
	30	1.0 x 10^5	0	0	0
	40	1.0 x 10^5	3.3 x 10^4	0	0

Cell count analysis showed significant difference in all of the comparisons and interactions (Table 18). P-Value was 0.00 in all of the factors used which indicate a 100% confidence of the difference in the number of Dinophysis cells in through stations, Depths and seasons (Table 23a). However, Tukey comparison shows some similarity based on different factors. For example, stations 4 (D) and 6 (F) has the same number of species, while station 5 (D) and station 3 (C) had different cell number. Although, depth 1 (0 meter) and depth 2 (10 meter) had the same number of cells while depth 3 (20 meter) is significantly different from the first two depths at all of the visited stations.

Table 18: Analysis of variance for Cell count versus Rep, Station, Depth, and Season

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Rep	2	0.26	0.128	0.02	0.978
Station	7	662.88	94.696	16.50	0.000
Depth	2	560.30	280.149	48.82	0.000
Season	3	2173.26	724.421	126.23	0.000
Station*Depth	14	370.65	26.475	4.61	0.000
Station*Season	21	1795.74	85.511	14.90	0.000
Depth*Season	6	581.09	96.848	16.88	0.000
Station*Depth*Season	42	1341.74	31.946	5.57	0.000
Error	190	1090.41	5.739		
Total	287	8576.32			

Toxin analysis

During the study, Pectenotoxin 2 (PTX 2), Pectenotoxin 2 SAa (PTX 2 – Saa), Okadaic acid (OA) and Dinophysistoxin 1 (DTX 1) were monitored. These toxic chemicals are known to produce by some *Dinophysis* caudata, *D. miles* and *D. accuminata*. The level of these toxins varied from being untraceable to more than 0.52 Ng/mg (PTX 2 – Saa) at Station D in April 2018. Moreover, toxin levels increased generally toward the northern region in October 2017, December 2017 and February 2018. While in April 2018, the level declined toward the north and the highest levels identified mostly at mid depths (10 – 20 meters).

October 2017, most of the stations have a below detection levels of Okadiac acid (Table 19). For instance, stations A and C have a below detection levels of Dinophysistoxin (DTXs: DTX 1), Pectenotoxin (PTXs: PTX 2, PTX 2 – Saa), and Okadiac acid (OA). However, PTX-2 occured in other stations to reaching a maximum of 0.44 Ng/mg at station D. While, DTXs and OA were not present during this cruise. The level of PTX 2 and PTX 2 – Saa increased at the mid stations (D, E, F, G, H) and toward the north areas.

Table 19: Toxin concentrations for each *Dinophysis* species identified in October 2017.

Species	Analyte	Ng/mg							
		A	B	C	D	E	F	G	H
Dinophysis caudata	Pectenotoxin 2 (PTX 2)	BD	0.0090	BD	0.44	0.067	BD	BD	0.024
	Pectenotoxin 2SAa (PTX 2 – Saa)	BD	BD	BD	0.21	0.021	0.033	0.0080	0.061
	Okadaic acid (OA)	BD	BD	BD	BD	BD	BD	BD	BD
	Dinophysistoxin 1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD
Dinophysis rotundata	Dinophysistoxin 1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD

Note: BD is below detection limit.

Species	Analyte	Ng/mg							
		A	B	C	D	E	F	G	H
Dinophysis	Okadaic acid (OA)	BD	BD	BD	BD	BD	BD	BD	BD
miles	Dinophysistoxin 1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD
Dinophysis	Pectenotoxin 2 (PTX 2)	BD	0.0090	BD	0.44	0.067	BD	BD	0.024
acuminata	Okadaic acid (OA)	BD	BD	BD	BD	BD	BD	BD	BD

Note: BD is below detection limit.

The concentration of *Dinophysis* toxins ranged between 0 in most stations to 0.44 Ng/mg in December 2017 (Table 20). PTX 2 – Saa and PTX-2 were the only two toxic species recorded in this cruise, with a maximum of 0.13 Ng/mg at station D and a minimum of 0.018 Ng/mg at station G. PTX-2 has been determined from all stations with a maximum of 0.44 Ng/mg and a minimum of 0.024 Ng/mg except at station F, C and A. The level of PTX 2 and PTX 2 –Saa increased toward the northern areas at both lines transect.

Table 20: Toxin concentrations for each *Dinophysis* species identified in December 2017.

Species	Analyte	Ng/mg							
		A	B	C	D	E	F	G	H
Dinophysis caudata	Pectenotoxin 2 (PTX 2)	BD	BD	BD	BD	BD	BD	BD	BD
	Pectenotoxin 2 SAa (PTX 2 – Saa)	0.064	0.024	0.044	0.13	0.039	0.0060	0.018	BD
	Okadaic acid (OA)	BD	BD	BD	BD	BD	BD	BD	BD
	Dinophysistoxin 1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD
Dinophysis rotundata	Dinophysistoxin 1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD

Note: BD is below detection limit.

Species	Analyte	Ng/mg							
		A	B	C	D	E	F	G	H
Dinophysis	Okadaic acid (OA)	BD	BD	BD	BD	BD	BD	BD	BD
miles	Dinophysistoxin 1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD
Dinophysis	Pectenotoxin 2 (PTX 2)	BD	0.0090	BD	0.44	0.067	BD	0.0050	0.024
acuminata	Okadaic acid (OA)	BD	BD	BD	BD	BD	BD	BD	BD

Note: BD is below detection limit

During February 2018 cruise, the level of toxins started to rise up (Table 21). The maximum level of Pectenotoxin 2 SAa (PTX 2 – Saa) with 0.11 Ng/mg was detected at station F, while the lowest reading for Pectenotoxin 2 (PTX 2) <0.001 Ng/mg was recorded at all stations. The level of toxins rose toward northern directions of offshore stations.

Table 21: Toxin concentrations for each Dinophysis species identified in February 2018.

Species	Analyte	Ng/mg							
		A	B	C	D	E	F	G	H
Dinophysis caudata	Pectenotoxin 2 (PTX 2)	BD	0.0017	BD	0.010	0.035	0.046	0.0037	0.011
	Pectenotoxin 2 SAa (PTX 2 – Saa)	BD	0.0085	0.0014	0.039	0.082	0.11	0.051	0.036
	Okadaic acid (OA)	BD	BD	BD	0.012	BD	BD	BD	0.010
	Dinophysisto1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD
Dinophysis rotundata	Dinophysisto1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD

Note: BD is below detection limit.

Species	Analyte	Ng/mg							
		A	B	C	D	E	F	G	H
Dinophysis	Okadaic acid (OA)	BD	BD	BD	0.012	BD	BD	BD	0.010
miles	Dinophysisto1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD
Dinophysis	Pectenotoxin 2 (PTX 2)	BD	0.0017	BD	0.010	0.035	0.046	0.0037	0.011
acuminata	Okadaic acid (OA)	BD	BD	BD	0.012	BD	BD	BD	0.010

Note: BD is below detection limit.

The maximum level recorded for Pectenotoxin 2 SAa (PTX 2 – Saa) was 0.52 Ng/mg at station D and 0.30 Ng/mg at station C in April 2018 (Table 22). The level of Pectenotoxin 2 (PTX 2) increased largely to reach 0.23 Ng/mg at station F. While, the minimum values of Okadaic acid (OA) have been detected at all stations (<0.003 Ng/mg). The highest level of toxins was found at mid stations (C, D, E, F, G, H), while toxin level declined toward the northern region.

Table 22: Toxin ranges for each Dinophysis species identified in April 2018.

Species	Analyte	Ng/mg							
		A	B	C	D	E	F	G	H
Dinophysis caudata	Pectenotoxin 2 (PTX 2)	0.040	0.0015	0.015	0.035	0.0013	0.23	0.043	0.0085
	Pectenotoxin 2 SAa (PTX2 – Saa)	0.15	0.038	0.30	0.52	0.013	0.13	0.027	0.011
	Okadaic acid (OA)	BD	BD	BD	BD	BD	0.0038	BD	BD
	Dinophysistoxin 1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD
Dinophysis rotundata	Dinophysistoxin 1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD

Note: BD is below detection limit.

Species	Analyte	Ng/mg							
		A	B	C	D	E	F	G	H
Dinophysis miles	Okadaic acid (OA)	BD	BD	BD	BD	BD	0.0038	BD	BD
	Dinophysistoxin 1 (DTX 1)	BD	BD	BD	BD	BD	BD	BD	BD
Dinophysis acuminata	Pectenotoxin 2 (PTX 2)	0.040	0.0015	0.015	0.035	0.0013	0.23	0.043	0.0085
	Okadaic acid (OA)	BD	BD	BD	BD	BD	BD	BD	BD

Note: BD is below detection limit.

CHAPTER 6: DISCUSSION

The factors usually supporting the development of harmful algal blooms are classified into two categories: 1) natural origins depending regularly on hydrological or weather patterns and specific data related to the biology of species involved in the event and causes that are directly/indirectly related to anthropogenic factors (e.g. human activities). Correlations between some hydrological and climatic events (e.g. El Nino) and the proliferation of some species of toxic dinoflagellate on a large scale found in the late 1980s (Lassus et.al, 2016).

The cruise of October 2017 was characterized by moderate surface salinity and rich nutrient content. Surface salinity values at the studied area covered water of low salinity (39.00 – 41.00 ‰). Surface temperature was almost constant (~30.00 ^{o} C). Vertical homogeneity of water temperature and salinity is evident, indicating weak water mixing. Previous work conducted in the Arabian Gulf and in Qatari waters (Quigg et al., 2013; Al-Muftah, 1991; El-Deeb and El- Samara, 1987; Mahmoud and Hassan, 1985) showed that waters of low salinity (38.00 – 39.00 ‰) and nutrient rich waters penetrate the gulf through the strait of Hurmuz. This inflowing water according to Al-Muftah (1991) could reach the northern coasts of Qatar. According to the present data, the inflowing nutrient rich and low salinity water was detected very near the Qatari coast during October 2017 (at all of the visited stations). During October 2017 the water column in this area was characterized by a stratification into two layers. The upper layer, down to 10 meters depth, had a lower salinity values (39.00 ‰), than the underlying one, where salinity values of greater than 40.00 ‰ was recorded. During December 2017, February 2018 and April 2018 the same visited station (area) did not show any type of stratification and

agreed with data presented by Quigg et al. (2013), Kämpf and Sadrinasab, (2006) and Al-Muftah (1991). Therefore, it is predicted that the upper layer in October cruise originated mainly from the advection of water of relatively low salinity and high nutrients from the Strait of Hurmuz toward the northeastern coasts of the Qatari peninsula.

In December 2017 results indicated higher salinity values (40.00 – 41.00 ‰) and an above detection limit of nutrients mostly at all of the stations except for NO3, which was not detected at all. The vertical salinity distribution confirms the vertical mixing conditions, with salinity vertically homogeneous. Vertical homogeneity of the water temperature (~21.00 °C – 23.00 ^{o} C) is also evident. Several previous studies (Al-Muftah 1991; Deeb and El- Samara, 1987; Mahmoud and Hassan, 1985) showed that water of high salinity and low temperature is characteristic of this month (season) of the year. However, they mentioned that this month has low nutrient concentration that was not determined in this study as the level of nutrients was mostly above the detection limits. The flowing two cruises (February & April 2018) followed the same trends of high salinity and lower temperature with high nutrients concentration.

Generally, the depth of the studied area increased from station 1 to station 8 (20 m to 45 m). The vertical profiles of salinity did not change much, whereas temperature varied but stayed similar for stations within the same season (Table 9 – 12). A slight increase in salinity (~1 ‰) was observed within the depth of 0 m to 20 m for some of the visited stations (e.g. station H of October 2017). The salinity (~42 ‰) was almost similar at all stations visited during the study. The temperature showed a uniform distribution within the depths of a season but differed from season to another. The temperatures detected in

October 2017 (31 – 33 °C) were higher than in December 2017 (21 – 24 °C), February 2018 (20 – 21 °C), and April 2018 (24 – 25 °C). Field records suggested a vertical mixing of the water column at some stations approximately at a depth of 10 m (e.g. station F in December 2017). Quigg et al. (2013) suggested also the mixing of the whole water column for some stations sampled at the eastern coasts of Qatar (February, May and July of 2010 & 2011).

Nutrients varied within stations and between seasons. Ammonia concentration changed largely and was mainly below detection limit (3.58 µg / L**)** for the first two cruises (October & December 2017: 0 µg / L – 5.00 µg / L), except for some stations (e.g. station B at 10 m of October 2017). February and April 2018 samples had an above detection limit values of NH4 (10.00 µg / L – 28.00 µg / L) at almost all of the stations, except station A of April 2018 which had no NH4. Nitrite levels dramatically varied and were mostly above detection limit (0.15 µg / L) during the whole study. The highest values recorded in October 2017 (3.00 µg / L – 21.00 µg / L). Nitrate in December 2017 not recorded while it varied among the other three seasons above detection limit (6.28 µg / L) (e.g. station B at 0 m of October 2017). Phosphate detected (2.27 µg / L) almost during the whole survey with the highest readings in April 2018 (3.00 µg / L – 17.00 µg / L). Silica considered, as the major constitute of nutrients in this study where its values were above detection limits (6.24 µg / L) at all of the stations (10.00 µg / L – 160.00 µg / L) except for few stations (e.g. station A of April 2018). The results of nutrients of this study were intensely higher than the previous studies conducted by Dorgham and Al-Muftah (1986), Al- Muftah (1991), A. Quigg et.al (2013) and Al-Ansari et al (2015).

Little is known about the species composition, distribution and the taxonomy of

Dinophysis in the Arabian Gulf and those in Qatari waters in particular. Based on the literature, dinoflagellates are not able to survive the harsh conditions of high salinity and temperature values of the Arabian Gulf. However, Al-Muftah (1991) proposed that continues transfer of these species with the inflowing water will make it possible for some to survive and acclimatize to the new environment in the Arabian Gulf. As a result, *Dinophysis* flora of Qatari waters is composed of cosmopolitan, warm water, tropical and, subtropical species. All of the species recorded are common in some countries and regions such as Philippines, Spain, Denmark, Singapore, Asia, Australia, America, Europe, and Mediterranean to different regions of the West Pacific and Indian Oceans (Guiry, 2018; Esenkulova & Haigh 2012; Suzuki et al, 2009; Spatharis et al, 2009; Moestrup et al, 2009; Liu 2008; Fernandez et al, 2006; Gómez 2005; Marasigan et al. 2001; Holmes et al. 1999; Tomas 1997; Fukuyo 1990).

Published works, however, an increase in numbers of all phytoplankton species reported in the Arabian Gulf due to their transportation from the Arabian sea and Gulf of Oman (Al-Muftah 1991; Dorgham and Muftah 1989; A. Quigg et.al. 2013 and Al-Muftah et.al 2016, Al-Nasr & Al-Muftah 2016). The distribution of the *Dinophysis* species recorded in the study area shows that this community is not rich in species composition. Al-Muftah (2016) found 3 species of Dinophysis, Quigg et.al. (2013) 1 species, Al-Harbi (2005) identified 2 *Dinophysis* species, Al-Kandri et al (2009) found 6 species, Al-Yamani & Saburova (2011) recorded no Dinophysis, Dorgham & Al-Muftah (1986) identified 3 species, Al-Muftah (1991) recorded 13 *Dinophysis* species. El-Din & Al-Khayat (2005) identified 2 Dinophsis species, and Al-Muftah & Al-Nasr (2016) reported 2 dinopyhsis.

The present study has identified five Dinopyhsis species from surface waters. In comparison to the published literature, this study recorded more European and South Asian *Dinophysis* species. The number of new records considered high in this study regardless of the low number of species identified. Two newly identified species from Arabian Gulf were *Dinophysis accuminata* and *D. mitra* (Table 7 – 10). The absence of these taxa from previous records may be because most of these are either shade and/or rare tropical forms.

The diversity is best shown by the two most prominent species: *Dinopyhsis caudata* and *D. mile* which were almost present at the four seasons in different stations. However, *D. accuminata,* and *D. rotundata* occurred only in February and April 2018, while *D.mitra* were common only for the first time in Qatar in April 2018. Al-Kandari et al (2009) identified all of the previous species from Kuwait waters except for *D. accuminata* identified for the first time by Farah et.al (2018) from Pakistani waters. Dorgham and Al- Muftah (1986), Al-Muftah (1991), Quigg et al, (2013) and Al-Muftah et al, (2016) covered most of the previous *Dinophysis* species in their studies except for *D. accuminata* and *D. mitra,* which encountered for the first time in published literature of Qatari waters.

Cell count analysis of total *Dinophysis* species showed a wide range of variations among seasons and within stations. The highest number of cells/liter (2.8 x 10^6 cells/L) counted in April 2018 at station D surface water. However, the ranges at each season was 0.00 cells/L - 3.0 x 10^5 in October, 0.00 cells/L - 3.0 x 10^5 cells/L in December 2017, 0.00 cells/L - 4.1 x 10^6 cells/L in February 2018 and 0.00 cells/L - 2.8 x 10^6 cells/L in April 2018. Dorgham and Al-Muftah in 1986 found the total number of cells

of Dinophlagelate species per liter in the range of 279 cells/L – 13200 cells/L. Al-Harbi (2005) mentioned that the number of cells of total dinoflagellate species counted in their study was 9.29% of the total phytoplankton sample.

The present research has shown the high salinity can be tolerated by *Dinophysis* species. While, changeable temperature values along with the moderate to high levels of nutrients of Qatari waters can control the number of *Dinophysis* species. The difference in species composition of the different seasons are significant. In general, the number of species was highest in February 2018 and April 2018, while the lowest in October 2017 and December 2017. During February and April 2018 temperature dropped (21.00 – 24.00 °C) and two *Dinophysis* species appeared (*D. acuminate and D. mitra*), and toxic readings increased because of lower temperature.

All seasons and some stations had detectable levels of Dinophysistoxin (DTXs: DTX 1), Pectenotoxin (PTXs: PTX 2, PTX 2 – Saa) and Okadiac acid (OA) based on UPLC-MS/MS analysis. October and December 2017 samples had the highest ranges (0.0080 Ng/mg – 0.45 Ng/mg). The highest level (0.44 Ng/mg) was found for Pectenotoxin 2 (PTX 2) at station D (October 2017) and station D (December 2017). These ranges dropped in February 2018 (0.010 Ng/mg - 0.046 Ng/mg). However, several stations in April 2018 started to have an elevated level of some toxins (e.g. Pectenotoxin 2 SAa (PTX 2 – Saa), 0.52 Ng/mg, at station D). Al-Muftah et al (2016) did not record any levels of OA and DTX1 in all of the stations visited except for station 4 (November 2013: 0.006 Ng/mg of OA), while an above detection limit of PTX2 and PTX2SA was recorded (e.g. station 4 0.38 Ng/mg & 0.041 Ng/mg respectively). *Dinophysis* species that were observed in this study are identified in different research as toxin producers

(Al-Muftah et al, 2016, Liu, 2008, Gómez, 2005, Guiry, 2018, Tomas, 1997, Fukuyo, 1990, Moestrup et al, 2009) and they are found in different parts of the world (Philippines, Spain, Mediterranean, Indian and West Pacific Oceans)

It is observed that there was an absence of regular distributional pattern of the chemical and biological parameters. Farah et al, (2018); Al-Muftah (2016); Quigg et al. (2013); Al-Muftah (1991); Wood (1968) noticed the same and attributed it to the complex pattern of water movements in tropical and subtropical areas of the Atlantic and to the similarity of the types of water; such conditions are most probably applicable to the Arabian Gulf.

The Arabian Gulf is facing an elevated occurrence of HABs, either due to environmental deterioration or/and to the increased monitoring and awareness efforts in the area. The introduction of ballast waters discharges along with high maritime traffic suggests that; exotic algae have been introduced (Al-Muftah, 2016, Quigg et al, 2013, Subba and Al-Yamani, 1998). The establishment of toxic *Dinophysis acuminate* and *D. mitra* in Qatar, as reported in this study, provides an example. With the occurrence of optimum conditions (ex. nutrient enrichment); these new species will start to blooming with a disturbing impact on the region. The existence of potentially toxic phytoplankton is known for the Arabian region, and this study is considered as one of the rare, as it demonstrates the presence of OA, DSTs and PTXs. The Arabian Gulf needs an intensive remediation measures and routine monitoring of phytoplankton biomass and toxins in general; because seawater is the major source of drinking water in the Arabian Gulf, as well as Arabian Gulf is the main source of seafood.

The present study has shown to some extent a similar pattern to those of other studies on

Harmful Algae of tropical and subtropical areas. However, much work remains to be conducted before we can say that we have a clear and full understanding of the behavior and distribution of *Dinopyhsis* in Qatari waters. It would be useful to have a long-term monitoring program. The diversity, distribution and abundance of *Dinophysis* and the derivatives of toxins produced are influenced by the physical parameters such as salinity, temperature, water circulation and shallowness of water column. In addition to the effect of nutrient variability during different seasons. The current data would provide a baseline for future researches that deal with HABs and their effect on marine environment and living creatures.

CHAPTER 7: CONCLUSION

This study confirmed the presence of DSPs in Qatari waters including OA, PTX2 and PTX 2 Saa. DTX 1 was below detection limits during the whole study. Some *Dinophysis* species such as *D. accuminata* and *D. mitra* were observed for the first time from Qatari waters and the Arabian Gulf. The cruises were dominated by *D. caudate* and *D. miles*. Temperature varied significantly between seasons but not much within seasons and depths. For the last decade, there was an agreement among scientists that validate the hypothesis of a global increase in harmful algal blooms or in toxic events in the world. This increase considered to have resulted in a geographical extension and expansion of these phenomena, together with an increase in the number of toxins producing agents and the number of produced toxin. However, this hypothesis was opposed with a counter argument that the increase in the awareness of governments, regulatory agencies, and the enhancement of techniques used for toxin detection and characterization of harmful species. The Arabian Gulf as a whole is located in a highly arid zone. The sum of precipitation and land drainage has no significant effects on its physical and chemical environment, except in the immediate vicinity of Shatt Al-Arab, and the few small rivers fed by precipitation in Zagros Mountains and discharging from the Iranian Coast. Moreover, the manner of water exchange between the Gulf of Oman (Salinity 36.5 ‰) and Arabian Gulf (Salinity 40 - 43 ‰) leads to significant variations in the physical and chemical characteristics of the Arabian Gulf (Al-Muftah, 1991, Shapper et al 2000). Low salinity water from Gulf of Oman penetrating the Arabian Gulf causes a decrease of the surface salinity along the Iranian coast, up to 39% and also reaches significant parts of the Emirates coast and northern Qatari offshore water (Brewer and Dyrssen,

1985; Hassan and Mahmoud 1985; Al-Deeb and El samra 1987; El samra 1988, Dorgham and Al-Muftah 1989; Al-Muftah 1991, Reynolds 1993). The physical and chemical characteristics of Qatari waters show that the whole area has unstable conditions changing among seasons, within stations and depths. The region divided based on the conditions encountered through the four seasons. Thus, much work must be done to understand and have a clear idea about the distribution and abundance of Dinophysis species in Qatari. Their toxins must be studied in order to avoid any future intoxication by humans and to avoid the collapse of our fish stocks and filter feeders.

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