QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

IMPACTS ASSESSMENT OF DESALINATION BRINE DISCHARGE ON THE MARINE MICROBIAL BIODIVERSITY: A METAGENOMIC APPROACH

BY

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A Thesis Submitted to

the College of Arts and Sciences

in Partial Fulfillment of the Requirements for the Degree of

Masters of Science in Environmental Sciences

January 2022

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ABSTRACT

HOSSEINI, HODA, A., Masters of Science: January: 2022, Environmental Sciences

Title: Impacts Assessment of Desalination Brine Discharge on The Marine Microbial Biodiversity: A Metagenomic Approach

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Being a hotspot of desalination, the health of the marine ecosystem of the Arabian Gulf is facing a serious threat. Many studies are suggesting that desalination activities could potentially harm the marine life, however, little experimental data are being provided in this regard. The current study is dedicated to assessing the effect of discharging desalination brine into the sea on the marine microbiome. Umm Al Houl desalination plant in Al Wakra was chosen as a case study, where spatiotemporal investigation of sea microbiome diversity was carried out. The novelty in this study is the utilization of the metagenomic approach, where the microbiome sensitivity will be assessed passively by studying the environmental DNA of seawater. The result of the study suggests that the physico-chemical characteristics of the seawater receiving the brine were not altered, neither spatially nor temporally. Slight differences were noted between different locations; however, the changes were not correlated with distance from brine outfall. The resulting differences were likely the result of tides, waves, and other seawater dynamics. Similarly, when measuring the biodiversity of the microbiome from different samples, random differences were existing that could not be explained as a response of sample's proximity to the brine outfall. Which also indicates the effect seawater dynamics. However, the existence of seasonal differences was observed as summer season had higher diversity compared to the winter. Further

investigations and long-time monitoring are required to understand the effect of discharged desalination brine on the marine microbiome. Nevertheless, this study provides a foundation for future research to be conducted on the Arabian Gulf's microbial diversity.

DEDICATION

In dedication to my beloved family and supportive friends. First and foremost, to my father and mother whose faith and belief in me was the main source of power. A special feeling of gratitude to my sisters Amal, Hajar, and Zainab who were there for me during my best and worst. I will always appreciate the support of my husband Saeed who stood by my side throughout the process.

I am grateful for having all of you in my life.

ACKNOWLEDGMENTS

I would like to thank Qatar University and the Department of Biological and Environmental Sciences for providing me the opportunity to pursue my Graduate studies.

Special thanks to the Algal Technologies Program, CSD in Qatar University for hosting and supporting the research activities.

This report was made possible by the NPRP award [NPRP11S-0110-180248] from the Qatar National Research Fund (a member of The Qatar Foundation).

Specific appreciation to Umm Al Houl Power for the fruitful collaboration.

I would like to thank my supervisors and committee members who have been supporting me in my research. Expressing my deep gratitude to Dr. Imen Saadaoui for her continuous guidance and immense support.

Much appreciation to my friends and lab partners for their care and assistance.

The findings achieved herein are solely the responsibility of the authors.

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CHAPTER 1: INTRODUCTION

Marine pollution is a significant global environmental issue as pollutants change the physicochemical and biological properties of the sea, which in turn alters the functioning of marine ecosystems affecting the health and diversity of organisms. The Arabian Gulf is one of the most adversely affected marine environments worldwide, due to the combined impacts of pollution, climate change, oil and gas activities, and coastal anthropogenic disturbances. Recently, desalination activities are frequently added to the list of marine pollution drivers regionally and internationally. Regionally, Arabian Gulf countries represent a hotspot of desalination activities, responsible for nearly 50% of global desalination capacity.

Historically, very few studies regarding this issue have been conducted in the Arabian Gulf, and the present research project is intended to examine and characterize the potential impacts of desalination activity and brine discharge on the marine biodiversity. The adopted approach in this study is the metagenomic analysis of the water and soil samples from areas around the discharge point of Umm-Al Houl desalination plant.

The general aim of this research project is to assess the impact of desalination activities and brine discharge on the health of the receiving marine environment and especially on the marine biodiversity. Thus, this project has been designed to:

Objective 1: Characterize the physico-chemical characteristics of the seawater receiving brine discharge.

Objective 2: Investigate the spatiotemporal variability of microorganisms' diversity surrounding the brine discharge point using a metagenomics approach

Objective 3: Identify sensitivity of natural seawater microbiome to changes in abiotic conditions.

CHAPTER 2: LITERATURE REVIEW

1. Introduction

Marine environments are highly valuable as they host productive ecosystems. These ecosystems provide important goods (e.g. consumable fish, water, and raw materials) and services (e.g. tourism, flood and pollution control, and transportation) for humans (Barbier, 2017). However, marine environments are being continuously altered under the action of different stressors, such as climate change, anthropogenic disturbance, and chemical contamination (Chapman, 2017). Marine pollution and mainly the introduction of exogenous substances have the potential to affect water quality, harm various marine organisms, and affect human health (Beiras, 2018). Therefore, marine ecosystems are currently highly threatened because of marine pollution (Baztan et al., 2016).

The concerns with marine pollution are highly significant because different forms of pollution input to the marine environment lead to changes in the physicochemical and biological properties of the sea, which consequently alter its ecosystems affecting the health and diversity of organisms (Wowk, 2013). A recent study revealed that both biomass-specific primary production and chlorophyll content were significantly reduced due to heavy fuel oil pollution (Lemcke et al., 2019). Furthermore, increasing nutrient concentration in the Gulf of Mexico promoted eutrophication followed by acidification affecting marine ecosystems (Laurent et al., 2017).

Desalination activities are important drivers of marine pollution especially in the Arabian Gulf owing to the high dependency on desalination processes to produce freshwater (Sharifinia et al., 2019). According to Ibrahim and Eltahir (2019), nearly 50% of worldwide seawater desalination is processed in the countries surrounding the

Arabian Gulf including Kingdom of Saudi Arabia, Emirates, Kuwait, Qatar, and Bahrain. Desalination processes harm marine habitats owing to the construction of plants, water intake, and brine discharge altering community composition and loss of biodiversity (Sharifinia et al., 2019). Desalination activity in the Arabian Gulf is an emerging issue that must be investigated. Thus, we herein reviewed the most important drivers of pollution in the Arabian Gulf with particular emphasis on desalination plants and their effect on marine health. We also reviewed the different methods that can be used to assess marine health. Finally, several management approaches were proposed to minimise the negative effects of desalination plants in the region.

2. Drivers of pollution affecting marine health in the Arabian Gulf

The continuous variation in sea temperature and high salinity exerts natural stress on the marine environment of the Arabian Gulf (Naser, 2017). According to Joydas et al. (2015), water temperature increases to as high as 36°C during hot periods of summer and as low as 15°C in winter, with a salinity that could surpass 43 ppt. Human activities exert an even higher level of stress on the marine environment. In fact, the Arabian Gulf is one of the most anthropogenically affected regions (Halpern et al., 2008). The increasing pollution in the Arabian Gulf poses a great threat to marine habitats and aquatic biodiversity in the region (Sharifinia et al., 2019). The drivers of pollution in the Arabian Gulf are diverse, but climate change, oil and gas industry, coastal anthropogenic disturbance, and desalination plants are considered to be the most important sources of pollution (Figure 1).

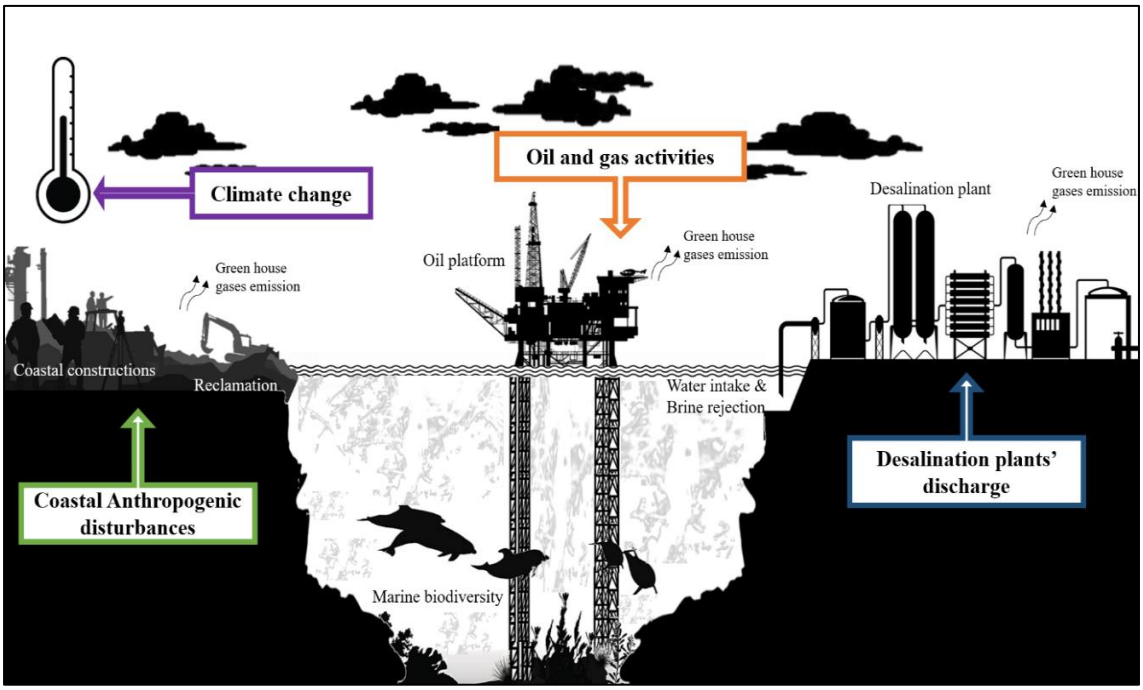

Figure 1: Drivers of pollution in the Arabian Gulf.

2.1 Climate change

Climate change is a global issue threatening aquatic ecosystems (Henson et al., 2017). It is an undeniable phenomenon altering the biogeochemistry of seas, which is triggered by increased temperature (Gattuso et al., 2015). Ocean warming is coupled with increasing stratification of the sea, which prevents nutrients from moving to the upper photic layers to support photosynthetic microorganisms (Steinacher et al., 2010). Climate change also induces the solubility of CO_2 into the seas due to its high concentration in the air (Henson et al., 2017). This results in decreasing the pH of oceans and negatively affecting calcareous organisms (Doney et al., 2012). Besides, oxygen solubility in the water will decrease as water temperature increases affecting aquatic organisms (Vaquer-Sunyer & Duarte, 2008).

Marine organisms are able to cope with such changes to some extent through physiological and phenological adaptation, as well as shifting population distribution and dynamics (Wabnitz et al., 2018). The result of these changes is reflected in species

richness (Jones & Cheung, 2015), and also affects the structure of marine communities (MacNeil et al., 2010). According to Wabnitz et al. (2018), climate change is increasing the pressure on the Arabian Gulf, resulting in the survival of certain organisms that tolerate extreme temperature and high salinity. Despite the high adaptability of some organisms, climate change still exerts negative effects on them. For instance, increased sea temperature in the Arabian Gulf resulting from climate change caused enormous bleaching followed by death in corals in 1996 and 1998 (Naser, 2014).

2.2 Desalination plant discharges

Arabian Gulf countries are characterised by an arid climate with a scarcity of freshwater (Elasha, 2010). Supplying the needed freshwater to the region is achieved by depending on desalination plants. Even though desalination processes are beneficial as they provide an important source of freshwater, they have a significant negative environmental effect on the marine chemistry and health with increasing the salinity, temperature, heavy metal concentration which ultimately alter the marine biodiversity (Roberts et al., 2010). With increasing populations and economies, desalination plants, as well as combined water and power production plants, are increasing in coastal areas of the Gulf. In fact, coastal infrastructure for water, energy, and food supply is becoming increasingly coupled with some mega plants supplying major cities in the region, thus posing several risks to coastal populations and marine ecosystems (Al-Saidi & Saliba, 2019). This is an emerging issue threatening the health of the Arabian Gulf.

2.3 Oil and gas activities

Arabian Gulf countries are the largest producers of oil and gas globally, and it is estimated that 65% of proven crude oil reserves belong to Arabian Gulf littoral countries (Modarress et al., 2016). By 2035, oil exports from the region are expected to

exceed 43 million barrels per day (Modarress et al., 2016). The development of oil and gas industry has caused destruction in the Arabian Gulf's marine environment. This is especially true when considering the activities associated with such developments, such as the construction of platforms, extraction of oil and gas, refining, and transportation.

As many spills and oil-related accidents go undocumented, it is difficult to quantify the effects of these events (Meshkati et al., 2016). Nonetheless, one of the largest oil spills happened in 1991 when 10 million barrels of oil were spilled during the Gulf War resulting in a long oil slick covering the Kuwaiti and Saudi coasts (Alhanaee et al., 2017). Oil pollution is believed to have an important effect on biota and biodiversity (Azevedo-Santos et al., 2016). Acute oil pollution causes mass mortality of organisms (McGenity et al., 2012) as well as substantial decreases in species richness (De La Huz et al., 2005). In their review, Pashaei et al. (2015) indicated that oil pollution and spills in the Arabian Gulf severely damaged mangrove forests, killing more than 500 sea turtles, decreasing 25% of shrimp fisheries, and polluting sediments.

2.4 Coastal anthropogenic disturbances

Coastal anthropogenic disturbances are a set of all human activities that induce damage to the aquatic habitats. They include coastal modification, dredging, land reclamation, and other activities that result in water pollution and habitat destruction (Naser, 2017). Arabian Gulf countries have experienced substantial development in economic and industrial sectors, which have resulted in considerable modification of the Gulf coastline to accommodate ports, artificial islands, marinas, coastal hotels, and even maritime cities, all of which increased marine pollution (Naser, 2015). For instance, high concentrations of heavy metals and hydrocarbons were reported in areas of active shipping in the Arabian Gulf (de Mora et al., 2010). Another study done in

Saudi Arabian coasts of the Arabian Gulf revealed that the concentrations of heavy metals including zinc, copper, chromium, and lead were enriched due to anthropogenic activities (Almasoud et al., 2015). Also, studying the PAHs concentrations in Qatari coastal sediments revealed moderate to high pollution in Al-wakrah port harbour (Soliman et al., 2014).

The introduction of such toxicants into coasts often promotes losing richness and biodiversity of marine species (Johnston & Roberts, 2009). Abd El-Wahab and Al-Rashed (2014) indicated that coastal habitats of Kuwait are negatively affected by human activities as both species composition and diversity of plants were considerably altered over the last five decades. In addition, based on Loughland et al. (2012), around 90% of native saltmarshes in the Arabian Gulf were lost due to coastal development and urbanisation. Baby et al. (2014) suggested that the carrying capacities of Kuwaiti's coastal habitats are decreasing due to urbanisation and industrialisation.

3. Desalination plants in the Arabian Gulf

Freshwater is a finite resource. Owing to the rapid increase in human population, unsustainable consumption, and the global changes in climatic conditions, water scarcity threatens many parts of the world (Odhiambo, 2017). The Arabian Peninsula has some of the scarcest freshwater resources worldwide (Elasha, 2010). This increased demand compared to the limited supply of freshwater drove the Arabian Gulf countries and especially the Gulf Cooperation Council (GCC) to rely on seawater desalination. It is estimated that the Arabian Gulf countries have a desalination capacity of 11 million m^3 per day. The region accommodates 213 actively operating desalination plants, and 51 others are expected to be commissioned in the near future (Sharifinia et al., 2019). Saudi Arabia (45%) and the United Arab Emirates (22%) are the two biggest contributors to desalination activities in the region.

3.1 Types of desalination plants

Desalination plants differ based on the separation technique used. There are two main classifications of desalination plants, namely thermal-based or membrane-based separation (Table 1). Globally, the most widely used desalination systems are based on reverse osmosis (RO) followed by multi-effect distillation (Miller et al., 2015). RO alone accounts for more than 60% of globally produced desalinated water. There are only three types of desalination plants present in the Arabian Gulf. These are RO, multi-effect distillation, and multi-stage flash. Multi-stage flash distillation accounts for 81% of water desalination, multi-effect distillation accounts for 13%, and RO accounts for only 6% (Sharifinia et al., 2019). Thermal desalination is the predominant technology due to the abundance of fossil fuels in GCC countries, making fossil-fuel-dependent powerplants the most economically attractive method for generating energy to drive desalination processes (Dawoud & Al Mulla, 2012).

Table 1: Types of desalination technologies adopted by the desalination plants.

Desalination Technology	Principles of the Desalination Technology	References
	Thermal-based desalination	
Vapor compression distillation (VC)	- Based on evaporating the incoming water using heat that comes from vapor compression. - Mechanical compressors are often utilised for heat generation. - Usually have small capacities and they could be coupled with multi-effect distillation (MED).	(Krishna, 2004)

Desalination Technology	Principles of the Desalination Technology	References
Multi-Stage Flash distillation (MSF)	- The influent water is heated to 120°C under high pressure. Heated water passes through successive flash units. - Each unit has a lower pressure compared to the one before allowing the hot water to evaporate as it flows through the flash units. - The evaporated portion is cooled and condensed on heat exchanger tubes. - Condensed water is collected as freshwater while the brine leaves the flash units to be discharged.	(Khoshrou et al., 2017)
Multi-Effect Distillation (MED)	- Based on passing heated water through successive effect units with low pressures. - It resembles the MSF except that it functions at lower temperatures reaching a maximum of only 75°C. - Condensation of vapor occurs as a result of exchanging heat with liquid. - Both MSF and MED produce freshwater of high quality with total dissolved solids of less than 10 mg/L and are efficient in brine treatment.	(Elsayed et al., 2018) (Chua & Rahimi, 2017) (Mabrouk & Fath, 2015)

Desalination Technology	Principles of the Desalination Technology	References
	Membrane-based desalination	
Reverse osmosis (RO)	- RO is the most commonly used membrane-based desalination processes. - It is a filtration process in which water is forced into a membrane that separates freshwater from brine. - In definition, osmosis is the movement of water through a semi-permeable membrane from a solution having fewer salts to a solution having more salts. - In reverse osmosis, pressure is applied to reverse the direction of water flow, which produces freshwater and concentrated brine.	(Miller et al., 2015) (Krishna, 2004)
Electrodialysis (ED)	- Based on the fact that dissolved salts are ions bearing either a negative or a positive charge. - ED utilises selective membranes that allows positively charged ions or negatively charged ions to pass through upon receiving electric currents. - Anion selective membranes and cation selective membranes are arranged alternatively in the desalination plant to separate all salts from freshwater.	(Krishna, 2004)

Desalination Technology	Principles of the Desalination Technology	References
Electrodialysis Reversal (EDR)	- EDR process was launched after the ED. - It is very similar to EDR in terms of operation principle. - The main difference is that the polarity of the direct current is reversed several times an hour. - This attracts ions to the opposite direction of the membrane. Afterwards, the freshwater is collected. Both ED and EDR are mostly used for brackish water rather than seawater.	(Buros, 2000) (Valero & Arbós, 2010) (Krishna, 2004)

3.2 Effect of desalination on marine environments

The effect of desalination activities on the marine environment has not been widely studied. In fact, according to Kress et al. (2020), the majority of the existing publications present predicted, potential impacts which are not based on observed or experimental data. In spite of that, based on the existing data, the effects could be categorised into two categories based on the intake of seawater and discharge of brine. Generally, desalination activities alter the structure and function of marine ecosystems, which is visualized by the affected marine communities and changes in the trophic interactions (Grossowicz et al., 2020).

3.2.1 Intake of seawater

Desalination plants rely on water from different sources, e.g. cooling water used in power plants, aquifers, ground water, and most commonly, open seas. When desalination plants are being constructed, pipes are installed to transport water from the

sea. This step disrupts the seabed causing resuspension of sedimented particles, including pollutants (Dawoud & Al Mulla, 2012). Disturbance and alteration of the seabed leads to habitat destruction, death of marine species, release of toxic pollutants from sediments, and increase in water turbidity.

Once plants are operating, massive volumes of water are pumped into the plants directly from the sea. These volumes are estimated to be double the amounts being produced (Kress et al., 2018). Along with water, many organisms are taken into the system that either get impinged (crash into the screens of the intake pipes) or entrained (travel with water reaching the plant) (Dawoud & Al Mulla, 2012). Entrainment and impingement result in severe injury and death of marine organisms (National Research Council, 2008). According to Missimer and Maliva (2018), assessing the effect on the marine environment resulting from entrainment and impingement is difficult. Parameters such as screen mesh size, pipe size, and volume of water intake, should be considered when designing the plant to reduce entrainment and impingement (National Research Council, 2008). Subsurface water intake reflects another potential solution (Missimer et al., 2013).

3.2.2 Discharge of the brine

Desalination processes result in the production of brine, which is a waste fluid characterised by high salinity and dissolved minerals (Danoun, 2007). The fate of the brine is usually disposal into the sea, considering it as one of the least costly disposal approaches(Fernández-Torquemada et al., 2019) . The desalination process goes through various phases; thus, the produced brine contains different chemicals and agents. The first important aspect to consider is the high salinity of the brine, which is at least 1.6–2 times higher than that of seawater (35 g/L average) (Panagopoulos et al., 2019). Furthermore, depending on the technology used (i.e. thermal technologies) brine

can exceed seawater ambient temperature by 1.37 to 1.82 times (Missimer & Maliva, 2018). The brine can also contain different chemicals, such as chlorine, cationic and anionic coagulants, acids, anti-scalants, heavy metals, and anti-foaming agents that are added during the desalination process (Alameddine & El-Fadel, 2007; Frank et al., 2019). Once discharged into the sea, these chemicals are considered to be toxic pollutants. Furthermore, the brine is highly alkaline as a result of calcium carbonates and sulfates (Danoun, 2007). The discharge of brine with these characteristics into the sea leads to significant changes in the physicochemical and biological parameters of the sea which ultimately affect marine life.

High temperature and salinity inhibit the growth of aquatic organisms (Wiltshire et al., 2010). Salinity elevations affect marine organisms such as plankton, microbes, and benthic species (Wood et al., 2020).In addition studies have shown that increasing the salinity of aquatic environments slightly above ambient conditions disrupts the osmotic regulatory abilities of some marine organisms resulting in dehydration and consequently death (Al-Shammari & Ali, 2018; Matsumoto & Martin, 2008). In addition, increasing the temperature of seawater increases the toxicity of some chemicals and metals, which adversely affects aquatic life (Uddin, 2014). Research has focused on seagrasses given the importance of seagrass habitats, which comprise diverse organisms and are sensitive to fluctuations in environmental conditions (Kress et al., 2018). Table 2 summarises research on the effect of increased salinity and temperature as a result of brine discharge into the sea.

Brine disposal also causes hypoxia resulting from decreasing concentrations of dissolved oxygen, which affect all marine organisms (Ahmed & Anwar, 2012). Increasing salinity is inversely proportional to the concentration of dissolved oxygen in the sea (Krayer et al., 2017). In addition, increasing water temperature through input of

hot brine decreases oxygen solubility (Ahlgren et al., 2017). Oxygen-depleted water bodies experience mass mortality of mussels, bivalves, and fish, and also disruption to coral reef functionality, invasion of opportunistic jellyfishes, and loss of biodiversity (Isensee & Valdes, 2018). The significance of brine discharge is arguably high in the Arabian Gulf considering that the Gulf is shallow and semi-enclosed with weak water circulation and limited freshwater input (Uddin et al., 2011). Such conditions accommodate adapted native species, which are resistant to fluctuations in the physicochemical parameters of the sea. Therefore, the extensive desalination activity in the region greatly threatens sensitive species, possibly leading to their extinction.

Table 2: The effect of increased salinity and temperature as a result of brine discharge.

Cause	**Study duration and location**	**Desalination Technique**	**Affected organism/ parameter**	**Result**	**Reference**
Increased salinity, 68 psu discharged brine	From June 2003 to August 2004 Alicante Spain near RO desalination plant	RO	seagrass *Posidonia oceanica*	Decline in the growth of the leaves. Increasing necrosis and mortality	(Fernández-Torquemada et al., 2005)
Increased salinity, 37, 39, 41 and 43 psu	Lab simulation for 47 days, mimicking the Mediterranean natural conditions when exposed to brine discharge.	Not Applicable	seagrass *Cymodocea nodosa*	Weakening photosynthetic rates	(Sandoval-Gil et al., 2012)

Cause	Study duration and location	Desalination Technique	Affected organism/ parameter	Result	Reference
Increased salinity, 40, and 46 psu	June 2015 and December 2015. Near Hadera desalination plant, Israel	RO	Benthic bacteria	Reduction of 60% in the abundance of bacterial species	(Frank et al., 2017)
Increased salinity, 55.6 and 54.7 ppt discharged brine	Near Marsa Humira and Shalateen desalination plants, Egypt.	RO	Coral reefs	Coral bleaching and death	(Nasr et al., 2019)
Increased salinity by 10% and anti-sealants	June 2016. Northern Gulf of Aqaba, Israel	RO	Corals *Stylophora pistillata*, *Acropora tenuis* and *Pocillopora verrucosa*	Partial bleaching of corals. Reduction in the abundance of bacteria and symbiotic algae	(Petersen et al., 2018)
Increased temperature 5-6℃ above ambient	June 2016 to April 2017. Ashkelon, Isreal	RO	Benthic foraminifera	Low species abundance and richness	(Kenigsberg et al., 2020)
Increased salinity, 43.45 ± 0.40 psu	August 2015. Bousfer plant located in Oran Bay	RO	Marine gastropod mollusc *Patella rustica*	Increased activity of antioxidant defense enzymes, as well as molecular damage of the tissue	(Benaissa et al., 2017)

4. Methods for assessing marine ecosystem response to stressors

Increased population and associated activities are exerting huge amounts of pressure on the marine environment (Halpern et al., 2015). Despite realising the importance and fragility of marine resources, we continue to exploit, destroy, and pollute the oceans, which leads to losses in functionality and biodiversity of aquatic ecosystems (Claudet & Fraschetti, 2010; McCauley et al., 2015). As a counter measure, many laws and regulations have been implemented globally aiming to protect the marine environment and conserve its ecosystems. Such regulations rely on our ability to assess marine health using different interconnected tools (Boyes & Elliott, 2014).

Assessing the health of the marine environment requires combining different parameters to reach a realistic conclusion. It is essential to adopt different assessment approaches including incorporating physical, chemical, and biological parameters. Borja et al. (2016) insisted on the significance of including biotic and abiotic factors, as well as human and social intervention when assessing environmental status. To develop a complete perspective of diversity indicators, biotic components should be incorporated into ecosystem assessments at various levels, including genus, species, population, and community levels (Haase et al., 2018). Considering that the assessment of each level serves a different objective, combining more than one would increase the objectivity of judgments of ecosystem health. In this context, various tools are implemented to assess marine health, most of which are oriented towards studying the diversity of marine organisms. There are two main categories of assessment tool comprising conventional methods and innovative recently developed strategies (Figure 2).

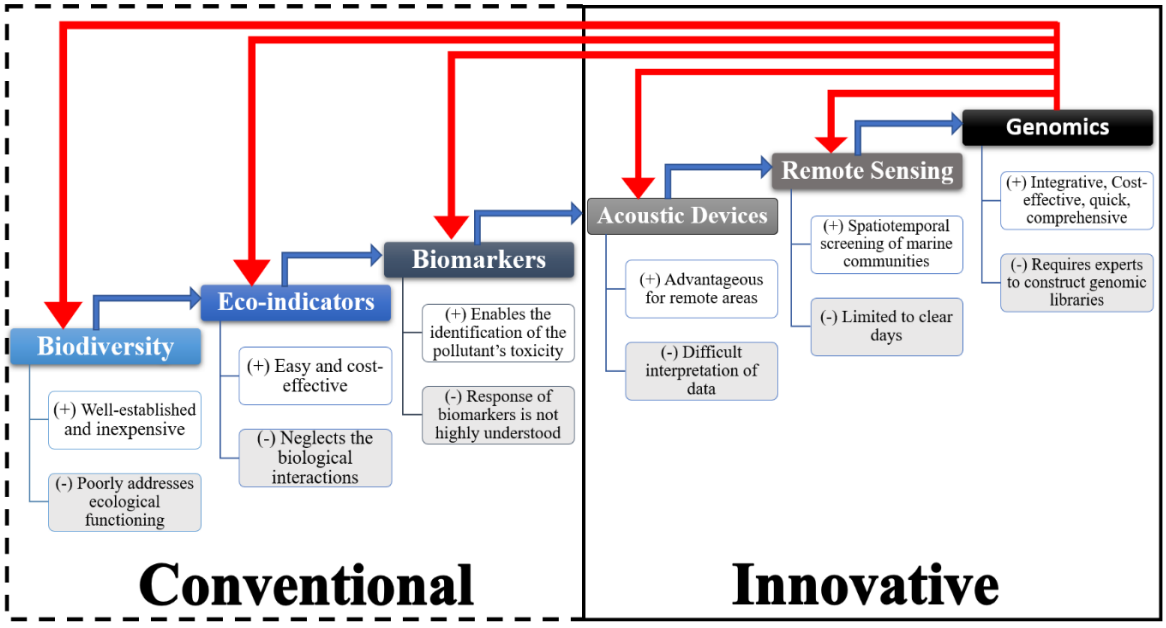

Figure 2: Different approaches adopted for the assessment of environmental effect of desalination on marine health.

4.1 Conventional tools for the assessment of marine health

Conventional tools are those that were historically used to monitor environmental health. They include several methods and approaches that were extensively used until the beginning of the 21st century. Thereafter, such approaches were amended by innovative methods owing to the development of methods to avoid the limitations of conventional techniques.

4.1.1 Biodiversity and abundance of faunal communities

Diversity is one of the most important parameters for examining the effect of human activities on marine ecosystems. Many diversity indices have been developed to interpret the data collected and relate these to assessments (Yoccoz et al., 2001). Indices are mostly based on the relative abundance of each species (Yoccoz et al., 2001). Shannon-Wiener and Simpson are the most widely used biodiversity indices

(Mendes et al., 2008) even though they are highly influenced by sample size, making them biased and, thus, less reliable (Hewitt et al., 2005).

Determining the biomass and abundance of faunal species has been widely used to monitor the marine environment. According to Pagola-Carte and Saiz-Salinas (2001), studying benthos can reveal important information about environmental conditions. Analysing the biomass and abundance of benthic species depends on primitive and simple protocols which involve divers collecting all benthos that fall within a quadrant of known area and quantifying and identifying the benthos (e.g. Barnes and Brockington, 2003). Strong et al. (2015) considered that these structural indicator studies are widely used for environmental monitoring because they are well established and inexpensive. However, indices are not yet highly informative regarding the functionality of the ecosystems. Similarly, Bremner et al. (2003) indicated that such studies can provide information on human effects on marine ecosystems at the community level, even though, they poorly address ecological functioning.

4.1.2 Ecological indicators

Ecological indicators are species that are either highly sensitive or tolerant to changes in an ecosystem. Studying the richness and abundance of indicator species provides rich information on marine health and response to pollution (Aguirre & Tabor, 2004; Parmar et al., 2016). On the one hand, sensitive species are usually dominant in marine systems and a reduction in their abundance indicates an altered ecosystem. On the other hand, tolerant species are opportunistic and highly resistant to environmental changes. Increase in the number of tolerant species is an indication of an altered ecosystem (Simboura & Zenetos, 2002). Fish are informative indicators as they are mobile, long lived, and they are present in all aquatic environments (Whitfield & Elliott, 2002). Indicator species are used to study the effect of human activities on marine

ecosystems. For example, phytoplankton are informative bioindicators of eutrophicated waterbodies, where they tend to grow rapidly (Singh et al., 2013). As indicated by Anttila et al. (2018), blooms of cyanobacteria in the Baltic Sea always indicate anthropogenic increase in the nutrient's inputs leading to eutrophication.

Furthermore, as described by Hosmani (2014), zooplankton are sensitive to changes in the physicochemical conditions of water (e.g. chemical composition, dissolved oxygen, pH, and temperature), which is why they are used as bioindicators. Algae were also used as indicators of organic pollution in lakes and freshwater bodies (Hosmani, 2013). Even though relying on indicator species for the assessment of environmental health is relatively easy and cost-effective, there are many shortcomings associated with these methods (Siddig et al., 2016). Firstly, considering a single population would not sufficiently represent the effect on the ecosystem as a whole. Secondly, using this approach neglects the biological interactions and its influence on the population of the indicator species. Finally, environmental factors other than the studied pollution (e.g. global warming) might influence the indicator species.

4.1.3 Biomarkers

In definition, biomarkers are changes that occur biologically, biochemically, or physiologically to organisms as a result of exposure to xenobiotic compounds (Hahn, 2002). Biomarkers have been incorporated in the ecotoxicological tests, where the existence of pollutants in a certain environment could be measured at the molecular level of affected organisms (Moore et al., 2004). This method enables the identification of pollutant toxicity in exposed organisms (Galloway et al., 2002). It is necessary to develop standards and define norms of biomarkers for every organism (Viarengo et al., 2000) to be able to compare experimental data with reference values. It is more advantageous to study multi-biomarkers to understand the effect of stressors on

organisms at the molecular level (Downs et al., 2002). This technique is not routinely used due to the difficulties associated with interpreting the acquired data because the response of biomarkers is not completely understood at different biological levels (Brown et al., 2004). Furthermore, biomarkers tend to be influenced by different factors other than pollution, e.g. organism age, salinity, and water temperature (Brown et al., 2004). Therefore, it can be difficult and complicated to link the pollution with its effect on biomarkers (Viarengo et al., 2000). Additionally, there are difficulties associated with selecting the biomarker that is representative of the changes occurring.

4.2 Innovative and novel methods for the assessment of marine health

Marine monitoring has always been challenging owing to the difficulties associated with providing impartial data, knowing that marine ecosystems are highly complex structurally and functionally (de Jonge et al., 2006). Traditional methods of monitoring are widely used as they are cost effective, widely accepted, and well established, although they still have many limitations (Bourlat et al., 2013; Strong et al., 2015). Conventional monitoring strategies are usually restricted by the sampling site, and are usually limited by the fact that the monitoring focuses on specific organisms that are easily monitored, which leads to inaccurate and biased results (Bourlat et al., 2013). When using conventional methods, marine environment health is assessed by targeting a certain taxonomic group during a certain life stage. Other interactions are mostly neglected, thus causing a lack in understanding of ecosystem interactions, and consequently, a poor understanding of the effect of the studied stressor on the marine ecosystem as a whole. Consequently, innovative strategies for monitoring and assessing the health of marine environments had to be developed. There have been different marine assessment tools emerging recently, and they are very promising as they overcome the shortcomings of conventional approaches. The main methods that

fall under this category are acoustic devices, remote sensing, and genomics (Borja et al., 2016).

4.2.1 Acoustic devices

An advanced method for monitoring marine ecosystems involves utilising acoustic devices to detect changes caused by human activity. Marine mammals are known for their acoustic specialisation where they rely on sound waves to communicate and navigate (Sousa-Lima et al., 2013). Owing to technological developments, we now have devices that can record underwater sounds produced by marine mammals. Following these developments, passive acoustic monitoring (PAM) devices were developed as non-invasive tools for better understanding marine mammals and the sounds they produce (Kalan et al., 2015). PAM is used to monitor animals by utilising remote acoustic tools such as hydrophones, stereo-phones, microphone arrays, and other auto-recording technologies (Marques et al., 2013). Species richness and community composition could be determined using PAM (Blumstein et al., 2011). PAM has proven to be highly beneficial when it comes to marine mammals because they are especially difficult to monitor optically because light cannot penetrate oceans for long distances. PAM systems can detect and classify marine mammals (Bittle & Duncan, 2013), which is why they are useful for studying the effect of human activities on marine health. Even though they are highly useful, PAM devices comprise continuous real-time monitoring systems that are associated with the long-term recording of vast amounts of data (Lammers et al., 2008). This is considered to be a major limitation as it requires extensive inspection and analysis of these data, which is usually unfeasible (Swiston & Mennill, 2009).

As an alternative, underwater fixed autonomous, sound recorders were developed in the early 1990s which addressed the limitations of PAM. These new

systems are characterised by lower costs, and they do not require experts for continuous sound monitoring (Sousa-Lima et al., 2013; Wiggins et al., 2012). Autonomous sound recorders record soundwaves and store the collected data within the device without being connected to reception stations (Wiggins et al., 2012). They do not require running and monitoring in person as they are installed on buoys or fixed to the sea floor where they record continuously. They could also be incorporated into autonomous underwater vehicles like ocean gliders (Fucile et al., 2006). After a defined period of time, the devices must be retrieved to analyse the acquired data (Sousa-Lima et al., 2013).

Using autonomous sound recorders could be highly advantageous for remote areas (e.g. polar regions) or when access to the location is difficult (e.g. deep sea or harsh weather) (Širović et al., 2009; Soldevilla et al., 2010). Utilizing these acoustic devices could be an efficient means to indirectly measure the biodiversity of acoustic mammals to assess ecosystem health (Sueur et al., 2008). Acoustic data could be analysed and interpreted through a variety of ways serving different purposes. For example, biodiversity could be assessed by quantifying spectral and temporal entropy H (Sueur et al., 2008). Parks et al. (2014) discovered that using noise compensated entropy (H_N) was the most representative index that reflects biological patterns and diversity in the marine environment. Despite the advantages of acoustic assessment, there remain issues concerning the interpretation of complex data. Besides, it is an indirect measure of biodiversity which is not always able to provide representative results. In addition, it is mainly used for marine mammals and not other organisms.

4.2.2 Remote sensing

Remote sensing is an innovative approach recently used to assess marine health. Remote sensing, including optical, thermal, and radar sensors, provides new prospects

for studying species, habitat distribution, and biodiversity (Pettorelli et al., 2014; Turner et al., 2003). This approach was previously used to monitor terrestrial ecosystems (Gross et al., 2009). However, recently, this technology was developed to include monitoring aquatic environments and was successfully implied for studying, monitoring, and managing mangrove (Kuenzer et al., 2011), coral reef (Hamel & Andréfouët, 2010), and even seagrass (Dekker et al., 2006) ecosystems.

According to van der Wal and Herman (2007), radar imaging is efficient at determining the composition of saltmarshes and intertidal habitats. Remote sensing proved to be cost effective for in-situ sampling, which provides data based on spatial and temporal screening of marine communities enabling scientists to understand the effect of human activities on the dynamics of marine communities (Rivas et al., 2006). According to Blondeau-Patissier et al. (2004), the development of remote sensing enabled spanning temporal and spatial recordings of sites, which overcomes the disadvantages of conventional in-situ marine monitoring techniques.

4.2.2.1 Assessing productivity

Owing to the large size of the seas and oceans, it is difficult to rely only on buoys and ships for monitoring purposes. Remote sensing is more informative, especially when it comes to assessing primary productivity. Since chlorophyll a is the main pigment for photosynthesis, its concentration could reflect primary productivity. The concentration of the pigment is determined using different remote sensing techniques, which operate in the visible region of the light spectrum (Klemas, 2010). Multispectral and hyperspectral imagers are used to measure chlorophyll concentration and thereby assess primary productivity using sensors like SeaWiFS and MODIS (M. J. Oliver et al., 2004). Chlorophyll is measured based on atmospheric spectral radiance, which is used to derive the spectral radiance of ocean surfaces (Bagheri et al., 2002).

Derived surface radiance is then used to calculate the reflectance which is important for chlorophyll identification and measurement (Philpot, 2007). According to Klemas (2010), obtaining accurate and calibrated data requires coupling the remote sensing data with data collected using ocean gliders, ships, and buoys.

4.2.3.2 Assessing the health of coral reefs

Remote sensing has been efficiently used to monitor coral reefs. Since coral reefs are highly fragile environments, it is important to monitor the effects of human activities on such ecosystems (Klemas, 2010). Coral studies using remote sensing could be direct in which data are related to the reef itself (Wabnitz et al., 2010), or indirect in which data represent the environmental conditions of the reef. Direct measurements include the location of reefs, patchiness, cover, and diversity of the habitat (Hamel & Andréfouët, 2010). Conversely, indirect measurements refer to temperature, turbidity, chlorophyll concentration, and organic matter concentration of the oceans, and also wind, rain, and cloud cover in the atmosphere (Hamel & Andréfouët, 2010). Coral reefs and submerged vegetation can be mapped by both hyperspectral and multispectral imagers (Akins et al., 2009).

Even though remote sensing represents a promising technique, there are still limitations that prevent such techniques from operating at full efficiency. Firstly, remote sensing observations and data are restricted to clear days because the presence of clouds restricts the collection of data especially in tropical and high-latitude areas (Peters et al., 2005). Furthermore, measuring the concentration of chlorophyll a to estimate the biomass of phytoplankton for productivity assessment is based on conversion factors which could be misleading (Rivas et al., 2006).

4.2.3 Genomics

Genomic assessment of marine environments provides information on marine

ecosystems at the cellular and microbial levels. This type of assessment provides information about micro-communities, their interactions, and the involved metabolic pathways, which allows a comprehensive evaluation of the functionality of an ecosystem (Bourlat et al., 2013), and provides a representation of the ecosystem's current and future response to stressors in terms of the effect on organisms and their interactions. Consequently, this will represent changes in populations and communities (Borja et al., 2016). Genomics techniques are considered to be an emerging tool and have shown promise for environmental monitoring. They essentially provide cost-effective and reliable measurements, which is why they are expected to substitute traditional methods (Bourlat et al., 2013).

As genomic approaches are becoming more widely applied owing to the technological advancement of sequencing tools (Mardis, 2008), genetic information for different species and habitats has become widely available in databases (Bik et al., 2012; Hajibabaei et al., 2011), which has facilitated the analysis of genetic data acquired from different ecosystems, including the marine environment. Following these developments, the methodologies of molecular analysis are constantly being refined, developing novel methods for different purposes (Leese et al., 2016).

4.2.3.1 DNA barcoding and meta barcoding

DNA barcoding involves sequencing and analysing standard short fragments of DNA known as DNA barcodes to identify the species of an unknown biological sample (Hebert et al., 2003). This type of genomic analysis is simple and does not require taxonomic experts as the sequenced barcodes are simply compared with other barcode sequences in a database to identify the species of the unknown sample. This technique is advantageous as it could be applied not only to biological specimens and tissues but also environmental samples for identifying all taxa in an area by metabarcoding

(Bourlat et al., 2013). With metabarcoding, it is possible to analyse DNA present in water, biofilms, and even sediment samples, which is referred to as environmental DNA (eDNA) (Leese et al., 2016).

Using this approach allows the assessment ofecosystem's biodiversity through monitoring multiple communities at once (Zhang et al., 2020). DNA barcoding and metabarcoding require a DNA barcode library that contains information about different species. Constructing such reference libraries requires expert taxonomists and extensive effort. In their review, Taylor and Harris (2012) mentioned that the main resources for DNA barcodes are Consortium for the Barcode of Life (CBOL), International Barcode of Life (iBOL), and the Barcode of Life Data System (BOLD).

4.2.3.2 Metagenomics

The conventional study of microbial species is restricted to culturable species, which are in the minority in the microbial world. Metagenomics offer an innovative approach to study culturing-recalcitrant microbial fractions through the DNA sequencing of any environmental sample (Culligan & Sleator, 2016). This approach is promising especially with the development of next-generation sequencing (DiBattista et al., 2020a; Schuster, 2008). It allows rapid environmental monitoring through relative abundance estimation (Günther et al., 2018). There are mainly two types of metagenomic method involving either 16S rRNA amplicons or whole genome shotgun sequencing.

- 16S rRNA amplicons

Combining the phylogenetic diversity approach with metagenomics would simplify assessments of the biodiversity of environmental samples using marker genes. According to McDonald et al. (2013), determining phylogenetic diversity depends on studying marker genes such as 16S rRNA to know the similarity between microbial

communities. As with DNA barcoding, metagenomics relies on reference libraries of genetic data of species. 16S rRNA techniques have been recently developed following the advancements in next-generation sequencing strategies that utilise 16S rRNA primers, such as the Illumina sequencer and 454 pyro-sequencer (Shah et al., 2011). Despite its efficiency in studying microbial diversity, the results provided by 16S rRNA metagenomic studies might not always be reliable since DNA extraction and PCR amplification procedures can produce biased data (Brooks et al., 2015).

- Whole-genome shotgun

This type of metagenomic technique sequences eDNA using random primers which results in overlapping genomic sequences (Ranjan et al., 2016). Such studies provide information about the genomic and metabolic characteristics of environmental samples (Kalyuzhnaya et al., 2008) Whole-genome shotgun sequencing could be favoured over amplicon sequencing because it is more objective and less biased. However, it is not widely used because it is expensive and requires analysis of vast amounts of data (Luo et al., 2014; Sims et al., 2014). It is important to mention that whole-genome shotgun sequencing has other limitations, such as lacking the ability to detect rare species (Kalyuzhnaya et al., 2008).

4.2.3.3 Microarrays

Microarrays are chips that contain a collection of labelled DNA probes, each probe representing a different species (Bourlat et al., 2013). DNA from environmental samples hybridises with a probe forming a complex, which will fluoresce upon subjecting it to UV radiation. Such techniques represent an innovative strategy for assessing environmental health (He et al., 2007), and allow for the detection of harmful microbes, or can infer the absence of locally dominant species in environmental samples, which could occur as a result of stressors. For example, studies have used

microarrays as efficient and rapid ways for identifying toxic algal species that are responsible for harmful algal blooms (Bricker et al., 2008; Doucette et al., 2009).

4.2.3.4 Quantitative real-time PCR

This method is based on quantifying a certain gene sequence belonging to a specific organism. DNA is quantified by comparing it to known values from standards curves and then it is correlated with the number of individuals of that species (Bourlat et al., 2013). This is a simple assessment tool for marine health, where knowing the abundance of a species allows us to determine whether that species is being affected by pollution. This technique can also be used to assess the genetic diversity in the affected environment (Smith & Osborn, 2009). Real-time PCR assays have high sensitivity and quantification power, making it a reliable marine monitoring tool (LeBlanc et al., 2020) However, even though this method is helpful for assessing marine health, it can only be applied to unicellular organisms with a known number of copies of the gene being quantified (Bourlat et al., 2013).

4.2.3.5 Transcriptomics

Transcriptomics is the study of gene expression. In the context of environmental monitoring, this study provides a comprehensive view of organisms' responses to stress at the molecular level (Devens et al., 2020). Gene expression can be studied through different techniques, such as using real-time PCR to quantify the concentration of RNA or through RNAseq (Bourlat et al., 2013).

In the Arabian Gulf, out of all of the assessment tools reviewed, genomics seems to be the most promising approach because it overcomes many of the limitations associated with the conventional assessment tools, complexity of interpreting acoustic data, and restrictions of remote sensing. Genomics approaches will provide an overall evaluation of marine ecosystems including their functionality. It will reveal the current

status of the Arabian Gulf and possible future responses through DNA analysis.

5. Management approaches for future coastal development

Since marine environments comprise, complex interacting ecosystems that provide extensive goods and services (Barbier, 2017), it is important to protect them. The extensive desalination activity in the Arabian Gulf is a potential threat to marine health. The effects of desalination plants can be limited by applying effective mitigation measures. Some of the important management approaches are presented in this section.

5.1 Optimisation of plant design

The negative effect of the desalination process could be reduced by optimising the design of the plant. Using membrane-based desalination could be less destructive to marine habitats since thermal-based desalination produces hot brine that adversely affects marine life when discharged. Besides, the inlet of the intake pipes should be located in places with low species abundance, avoiding productive areas to reduce the negative effect. Furthermore, the entrainment and impingement of organisms could be minimised by optimising the velocity of water flowing through the intake pipes and by optimising the mesh size of the screens in the intake pipes (Sharifinia et al., 2019). In addition, developing and using environmentally friendly desalination processes should be emphasised. For instance, solar-based desalination processes are being developed to increase their efficiency and applicability. Palenzuela et al. (2015) proposed coupling concentrated solar power plants with desalination plants in the Arabian Gulf to produce electric power and freshwater.

5.2 Treatment of brine before discharge

To minimise the negative effect of brine discharge, it is possible to treat the brine to remove hazardous chemicals used during the desalination process. Chlorine is an example of a hazardous chemical used during desalination since it has a biocide

activity. Lattemann and Höpner (2008) indicated that chlorine could be removed using sodium bisulfite for RO plant effluents, and hydrogen peroxide for thermal plant effluents. Source control is even more effective where hazardous chemicals are not used during the desalination process. Such chemicals have substitutes that are less environmentally destructive. In addition, many studies have focused on treating produced brine by enhancing water recovery. As reviewed by Panagopoulos et al. (2019), brine treatment technologies could be membrane based, thermal based, or zero liquid discharge based. These technologies work principally on producing pure water and compressed solids, which reduces brine volume.

5.3 National regulation

National monitoring and regulation are required for assessing the health of ecosystems and setting guidelines and rules for plant design and discharge parameters. For example, in response to the increasing pollution in the Arabian Gulf region, Kuwait established the Kuwait Environment Public Authority (KEPA). KEPA is an independent organisation responsible for maintaining the health of the environment and actively participates in enforcing legislation and setting standards. As a regulatory measure, KEPA monitors the quality of Kuwait's territorial water through continuous collection of data from 13 different stations. The quality of water is assessed based on different parameters to make sure it meets the local standards (Al-Mutairi et al., 2014). To control the increase in salinity of the sea resulting from desalination activities, KEPA set a salinity limit of 42 ppt (Uddin et al., 2011). This is an effective initiative for controlling the direct dumping of brine into the sea.

5.4 Regional cooperation

Since the Gulf waters are shared among several littoral countries, a regional approach to the control of marine pollution is indispensable. In this sense, Gulf littoral

countries adopted a legal instrument in 1979, namely the Kuwait Regional Convention for Cooperation on the Protection of the Marine Environment from Pollution (short Kuwait Convention). Based on this convention, the Regional Organisation for the Protection of Marine Environment (ROPME) was established in 1979 as an intergovernmental organisation encompassing all Gulf littoral countries. A cornerstone of ROPME policy includes an action plan (the Kuwait Action Plan) for monitoring and assessing the health of the Gulf. Furthermore, several additional protocols to the Kuwait Convention have been signed over the years, targeting pollution control from sources such as oil, exploration activities, land-based activities, shipping, and the disposal of hazardous materials. An additional protocol for biodiversity and protected areas has been discussed since the early 2000s but has not yet been implemented.

ROMPE has been important for promoting cooperation on marine pollution and initiating joint action, e.g. through ROPME Marine Emergency Mutual Aid Center (ROMPE/MEMAC) in Bahrain focusing on coordinating the Gulf's response to oil spills. The issue of brine discharge is part of the additional protocol on pollution from land-based sources adopted in the 1990s, which stipulates that regional regulations, programs, timetables, and measures need to be implemented to address pollution. However, regional action through ROPME has been limited to monitoring and analyses supported by international organisations. Integrating the increasingly important issue of brine discharge into future cooperative frameworks is an important step to effectively address this issue at the Gulf-wide scale. This also means shifting focus from analysing pollution and promoting protection to adopting more comprehensive measures including joint monitoring and regulation (Van Lavieren & Klaus, 2013). Further broader approaches that incorporate biodiversity protection and ecosystem

management have been part of the original mandate of ROPME but are not yet reflected in the practice of regional cooperation (Hamza & Munawar, 2009; Khan, 2008).

6. Final remarks

The Arabian Gulf is considered one of the most anthropogenically affected seas. It is facing different types of stressors inducing marine pollution, which affect the health of the marine environment. It is undeniable that climate change, oil and gas activities, and coastal reclamation are major contributors to this problem. However, recent desalination has been a debatable contributor to marine pollution. Owing to the scarcity of freshwater resources, Arabian Gulf countries rely on desalinating seawater to produce freshwater, which is used for drinking, agriculture, and other purposes. For the longest time, desalination was thought to be a solution for the water scarcity issue in the region. However, recently, negative environmental effects of extensive desalination activity have been revealed. The Arabian Gulf mostly uses thermal-based desalination processes, which result in discharging huge amounts of hot, salt-concentrated brine directly into the sea. Discharge of the brine became a forefront of policy debates after it was neglected, especially in the Gulf area where desalination is extensive, and the natural environmental conditions are harsh.

Considerable effects of brine discharge have been reported worldwide necessitating the adoption of efficient monitoring systems in the Arabian Gulf. Monitoring should develop beyond simple measurements into more integrative, adaptive, and multivariate technologies. For that, genomic monitoring is the most efficient in terms of ease, applicability, and objective representation. Since the Arabian Gulf is considered a hot spot of desalination activities, it is important to implement management approaches at different levels to reduce the negative effect on marine ecosystems. Firstly, optimisation of plant design is a promising approach where many

engineering parameters should be considered. Secondly, instead of direct discharge, brine should be treated pre-discharge. Finally, following in the footsteps of KEPA, laws and regulations for desalination activities should be implemented to prevent further damage to the Gulf's environment. Monitoring tools and assessment of the desalination activities should be incorporated into the regulations and policies to prevent future destruction of marine ecosystems.

CHAPTER 3: METHODOLOGY

1. Sampling site and sampling design

The study considered the Umm Al Houl desalination plant, located South of Al-Wakra, as a case study for this research.

Seawater samples were collected from stations aligned along transects placed in three directions surrounding the brine discharge point (Figure 3 and Table 3). From each transect, seawater samples were collected on two different depths (sub-surface and near seabed) on triplicates at different distances away from the discharge point. In addition, reference sample (negative control) was collected 2 km northward from the desalination plant brine discharge point for comparison purposes. The sampling was carried out on two seasons, summer 2020 (June) and winter 2021 (Feb).

Figure 3: Sampling stations around the Umm Al Houl desalination plant discharge outfall.

Table 3: Global Positioning System coordinates of the sampling points.

Transect	Distance from discharge point (m)	GPS Coordinates	
North	50	25° 6'29.70"N	51°38'10.09"E
North	100	25° 6'31.28"N	51°38'10.64"E
North	500	25° 6'43.62"N	51°38'14.95"E
North	1000	25° 6'59.15"N	51°38'20.39"E
North	2000	25° 7'30.36"N	51°38'31.32"E
East	0	25° 6'25.40"N	51°38'12.9"E
East	100	25° 6'24.50"N	51°38'17.34"E
East	500	25° 6'21.08"N	51°38'31.11"E
East	1000	25° 6'16.82"N	51°38'48.32"E
East	2000	25° 6'8.28"N	51°39'22.79"E
South	50	25° 6'23.89"N	51°38'8.65"E
South	100	25° 6'22.27"N	51°38'8.68"E
South	500	25° 6'9.27"N	51°38'8.88"E
South	1000	25° 5'53.00"N	51°38'9.13"E
South	2000	25° 5'20.49"N	51°38'9.63"E

2. Collection of seawater sample

Seawater samples were collected using a previously sterilised Neskin bottle deployed from a speedboat (all samples were collected on 2 litres). Then water was transferred to sterile Nalgene bottles (Thermo Scientific, USA). Seawater samples were stored in a cooled icebox during the transportation from the collection site to the laboratory at Qatar University.

3. Assessment of the physico-chemical characteristics of seawater samples

Physico-chemical characterization of the receiving seawater is an important indicator of the effects of discharged brine on the receiving environment.

3.1 On site physico-chemical characterization of seawater

Temperature, salinity, dissolved oxygen (DO) and pH were recorded directly at the sampling location using YSI ProPlus handheld multiparameter probe (Xylem Inc. OH, USA).

3.2 Ion Chromatography of collected water samples.

Collected seawater samples were diluted and then directly analysed by Ion Chromatography 850 Professional IC (Metrohm, Turkey) to determine the concentration of the cations (e.g Sodium, Calcium, Magnesium) and anions (e.g. Chloride, Bromide, Sulphate) present in the sample.

3.3 ICP-OES of water samples

15 ml seawater samples were analysed in terms of trace metals using ICP-OES (model iCAP-6500, Thermo Scientific).

4. Total DNA extraction and metagenomic analysis of the samples

At the laboratory, seawater samples were prefiltered, then filtered using a sterile 0.2 µm mixed-cellulose easter (MCE) filter (I.W. Tremont LabExact, USA). The filter was then used to perform total DNA extraction using DNeasy PowerWater kit (QIAGEN, Germany) following the manufacturer's instructions.

After extraction, NanoDrop 2000 spectrometer (Thermoscientific, US) was used to determine the quantity and purity of the DNA. Then primers were used to amplify ~ 300 bp of ribosomal DNA genes such as 16S rDNA for bacteria and 18S rDNA for eukaryotes including diatoms and algae (Table 4). The Platinum™ SuperFi II PCR Master Mix (Thermofisher Scientific, US) was used to undergo the Polymerase Chain Reaction (PCR). Then, PCR products were visualised on 1.5% agarose gel, and the band was excised then purified using GeneJet Gel Extraction Kit (Thermoscientific, USA) following the manufacturer's protocol. Purified PCR products were quality checked for size and quantity. Next, the NGS libraries were made using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs, USA) using the manufacturers recommendations. Briefly, the amplicons were each end-repaired,

indexed-adaptor ligated, amplified for 5 cycles, and cleaned. The resulting Illumina NGS libraries were then quality, and quantity checked, normalized, and pooled. The pooled NGS libraries were pair-end sequenced using the Illumina MiSeq V2 reagent kit (2x150) with ~5% PhiX control.

Table 4: Primers for the amplification of rDNA for metagenomic sequencing.

Primers	Sequence	Specificity	Fragment size	PCR condition	Reference
806RB **515FB**	5'GGACTACNVGGGTWTCTAAT3' 5'GTGYCAGCMGCCGCGGTAA3'	Bacteria and Archea and Cyanobacteria	~300 bp	Initial denaturation 94°C for 3 min; followed by 35 cycles of 94°C for 45 s, 54°C * for 60 s, and 72°C for 90s; with a final extension at 72°C for 10 min, and then a 4°C hold	(Walters et al., 2016)
Fish16sF/D **16s2R (degenerate)**	5′GACCCTATGGAGCTTTAGAC 3′ 5′CGCTGTTATCCCTADRGTAACT 3′	16S rRNA Fish	~200 bp	Initial denaturation 94°C for 3 min; followed by 35 cycles of 94°C for 45 s, 54°C * for 60 s, and 72°C for 90s; with a final extension at 72°C for 10 min, and then a 4°C hold	(Berry et al., 2017)

Primers	Sequence	Specificity	Fragment size	PCR condition	Reference
18S_uni_1F	5′GCCAGTAGTCATATGCTTGTCT 3’	universal primer V1-3 Perfect for eukaryotic marine biodiversity	~340-420 bp	Initial denaturation at 95 °C for 5 min, followed by 45 cycles for 30 s at 95 °C, 30 s at 54 °C*, and 45 s at 72 °C, with a final extension for 10 min at 72 °C	(DiBattista et al., 2020b)
18S_uni_400R	5′GCCTGCTGCCTTCCTT3′				

** Annealing temperature was optimized.*

5. Bioinformatics

The genomic data assembly was performed as it was described by Won et al. (2017). The genomic data was then trimmed using Trimmomatic v0.35 (Bolger et al., 2014) followed by filtration of the row data to generate reads of quality scores <30 using FastQC v0.11.4 (Andrews, 2010). The high quality paired-end reads were merged using FLASH v1.2.11 (Magoc & Salzberg, 2011) to generate longer sequences that will be analyzed using Quantitative Insights into Microbial Ecology (QIIME) 1.9.1(Cardoso et al., 2010). Then, open-reference OTU assignment step will be performed to generate operational taxonomic units (OTUs) (DeSantis et al., 2006; Edgar, 2010). Finally, OTUs of all reads were identified using BLAST search (>97% identical, E-value <10 7) based on 16srRNA and 18srDNA databases, and each OTU number was normalized and provided through clustering according to each taxonomical standard as it was described by Won et al. (2017).

The comparative analysis of the species identified from the different sampling stations with the positive control will aid in analysing the environmental impact of the brine discharge on the marine microbiome.

6. Statistical analysis

The data of the physico-chemical properties were analysed using R to produce PCA biplots and heatmaps. For the metagenomics part, all resulting data was analysed using dada2, phyloseq, and DESeq2. Then plots and graphs were generated using phyloseq and ggplot in R. Alpha diversity was represented by Chao1 diversity. Beta diversity was determined through UniFrac distance analysis, through which UPGMA Clustering (unweighted pair group method with arithmetic mean) and PCoA plots (principal coordinates analysis) were generated. Differential abundance analysis was represented by volcano plots that were generated using log2 fold change.

7. Bioprospecting of novel marine microorganisms

To enhance the growth of microbial species, the collected water samples from five directions were initially enriched using BG-11(Stanier et al., 1971), F/2 (Guillard & Ryther, 1962), and F/2 with silica medium. The enriched samples were incubated in growth chambers (Sanyo, Japan) at 28 °C, a photon flux density of 100 mol photons m^{-2} s^{-1} and a 12:12 h dark:light cycle, over a duration of 12 to 20 days. Later, serial dilution was performed on BG-11, f/2, and f/2 with silica agar media. Subculturing on agar media was continued subsequentially to get pure colonies of cyanobacteria, diatoms, and microalgae. Morphological identification of the strains was carried out using light-microscopy (40 ×, Primo Star HAL Microscope, full Köhler, stage drive R, FOV 20, Carl Zeiss, Germany).

Then, the identification of the novel isolates was confirmed by molecular biology. For that purpose, total genomic DNA of the pure isolates was extracted using Purelink

Plant total DNA purification kit (Invitrogen by Thermofisher Scientific, US) following the manufacturer's instructions. Then, the extracted genomic DNA was used as a template to undergo PCR using primers in Table 5, which amplifies the ribosomal rDNA such as 16SrDNA for cyanobacteria and 18S rDNA for microalgae. PCR products were visualised on 1% agarose gel, then the targeted band was excised and purified using GeneJet Gel Extraction Kit (Thermoscientific, US) following the manufacture's protocol. NanoDrop 2000 spectrometer (Thermoscientific, US) was subsequently used to determine the concentration of the DNA. Later, Sanger sequencing of the DNA fragments was conducted to identify the isolates. Genetic Analyzer 3500 (Applied Biosystems, California, USA) was used for the sequencing process using the same primers used for the PCR amplification. The resulting sequences were processed using the local alignment algorithm BLASTN. Then, the sequences presented high similarity with our sequence of interest were selected and used to perform multiple alignment using Clustal2 multiple sequence alignment program was (Larkin et al., 2007; Saitou & Nei, 1987).

Table 5: Primers used for the identification of purified isolates.

Primer	Sequence	Specificity	Fragment size	PCR condition	Reference
BS1F	5'GATCCTKGCTCAGGATKAACGCTGGC3'	Cyanobacteria	~1.8 kb	Initial denaturation step of 5 min at 95°C, followed by 35 cycles of 30s at 95°C, 45s at 54°C and 1min 30s at 72°C and a final extension step of 10min at 72 °C.	(Saadaoui et al., 2016)
BL9R	5'TTTGCGGCCGCTCTGTGTGCCTAGGTATCC3'				
EAF3	5'TCGACAATCTGGTTGATCCTGCCAG3'	Algae	~1.2 kb		
N1200R	5'AACATCTAAGGGCATCACAGACCTG3'				
D51218 S F	5' ATT CCA GCT CCA ATA GCG 3'	Diatoms	~400 bp	Initial denaturation step of 5 min at 95°C, followed by 35 cycles of 30s at 95°C, 45s at 61.8°C and 1min 30s at 72°C and a final extension step of 10min at 72 °C.	(Zimmermann et al., 2011)
D978rev 18S	5'GAC TAC GAT GGT ATC TAATC 3'				

CHAPTER 4: RESULTS AND DISCUSSION

1. Spatial and temporal characterization of the physico-chemical properties of the seawater receiving brine discharge

Throughout the whole desalination process, many chemicals are added directly or indirectly to the seawater that is being fed to the plant. Feed seawater gets indirectly contaminated with chemicals such as hydrocarbons and trace metals upon contact with pipes, tanks, heat exchangers and other plant's constituents. For instance, chromium, iron, copper, and nickel find their way into the brine as corrosion products from alloys used in the plants (Green et al., 2018). While other chemicals are deliberately added to the seawater as it has roles in preventing minerals from forming scales in the pipes as well as preventing the undesirable growth of microorganisms (Saud Bali Al-Shammari & Lulwa Ali, 2018). The fate of such chemicals is usually discharged into the sea as part of the brine that potentially has high temperatures and high salt concentrations. Therefore, it is important to check the physical properties as well as the chemical properties of the water that receives the brine discharge to verify if its characteristics are being altered.

This alteration in the physico-chemical characteristics of the sea is believed to affect the microbiome negatively. Thus, to have better understanding of the effect of brine discharge and to assess the sensitivity of the microbiome, it is required to characterize the receiving water physically and chemically.

Studied physical properties of the water included temperature (℃), salinity (ppt), pH, and dissolved oxygen DO (%) (Table A1). Temporally, temperatures recorded in the summer (37.14 ± 1.44) were generally higher than those recorded in winter (21.69 ± 1.18), however, no significant spatial differences were recorded in neither summer nor winter along the transects away from the brine discharge point. As for salinity, the

values averaged 35.22 ± 1.18 ppt and 35.97 ± 1.31 ppt for summer and winter respectively, displaying no significant differences among different seasons and complying with the salinity average of oceans being 33-37. Even along different distances neither summer nor winter showed significant variability between different sampling points. Similarly, pH and DO values did not differ significantly, neither spatially nor temporally.

The tested chemical properties of the receiving seawater samples included common ions using IC, and trace metals using ICP-OES, total carbon TC, and total organic carbon TOC. The generated raw data sets did not seem to show any trends or notable behaviours neither temporally nor spatially (Table A2, Table A3, Table A4). For instance, for the measured trace metals in the summer, cadmium, iron, cupper, and zinc were all under detection limit, while all other detected trace metals were within the oceans' average concentrations (Table A2). Further, none of the detected trace metals exceeded the levels set be the United States Environmental Protection Agency (USEPA, 2009) found in Table A5. Since all the measured trace metals in this study had concentrations lower that the criterion maximum concentration (CMC) it means that none of the quantified trace metals will cause an unacceptable effect on the aquatic communities. In addition, the concentration of common ions such as chloride, bromide, sulfate, calcium, sodium potassium, and magnesium were all within the detected ranges in the reference point. This also goes for the carbon content, which did not vary considerably along the distance from the discharge point and was within the range detected in the reference point. This is why, all physico-chemical analyses data were combined, normalized, then principal component analysis was performed to have an overall view of the changes in the properties of the receiving seawater if any.

The resulting PCA biplot of the summer samples given in Figure 4 shows that principal components one and two together explain 49.1% of the total variability of the data. Based on that, we can see that the concentrations of chloride, sodium, magnesium, potassium, bromide, and sulfate form a group of variables that are strongly correlated with each other. Temperature, salinity, pH, and DO form another group of variables that are strongly related to one another. A third group of variables is composed of the trace elements Barium, Vanadium, Boron, Strontium, and Chromium. The clustering of the samples did not represent a distance-dependent pattern. For instance, each cluster of samples contains some of the closest and some of the furthest samples relative to the discharge point. For such spatially variable samples to be in one cluster, it indicates that they share similar values of the tested variables. In other words, distance from the discharge point is not providing a visible discrimination in the physico-chemical properties of the samples.

As for winter samples, the PCA biplot in Figure 5 presents the first two principal components which explain 53.2% of the total variability of the data. From the figure it can be seen that total inorganic carbon and DO form a group of variables that are strongly correlated. Other than that, there is no clear grouping of the variables for the winter samples. In addition, similar to the summer samples, winter samples did not show distance-dependent pattern, as samples were randomly correlated with the tested physico-chemical properties.

Another representation of the data was carried out by constructing a heatmap that allows the clustering of the samples and the tested properties of the seawater. Figure 6 and Figure 7 are representing the clustering of the summer and winter analyses respectively. Unfortunately, even with the heatmaps, there seems to be no conclusive

evidences on the effect of brine discharge on the physico-chemical properties of the seawater.

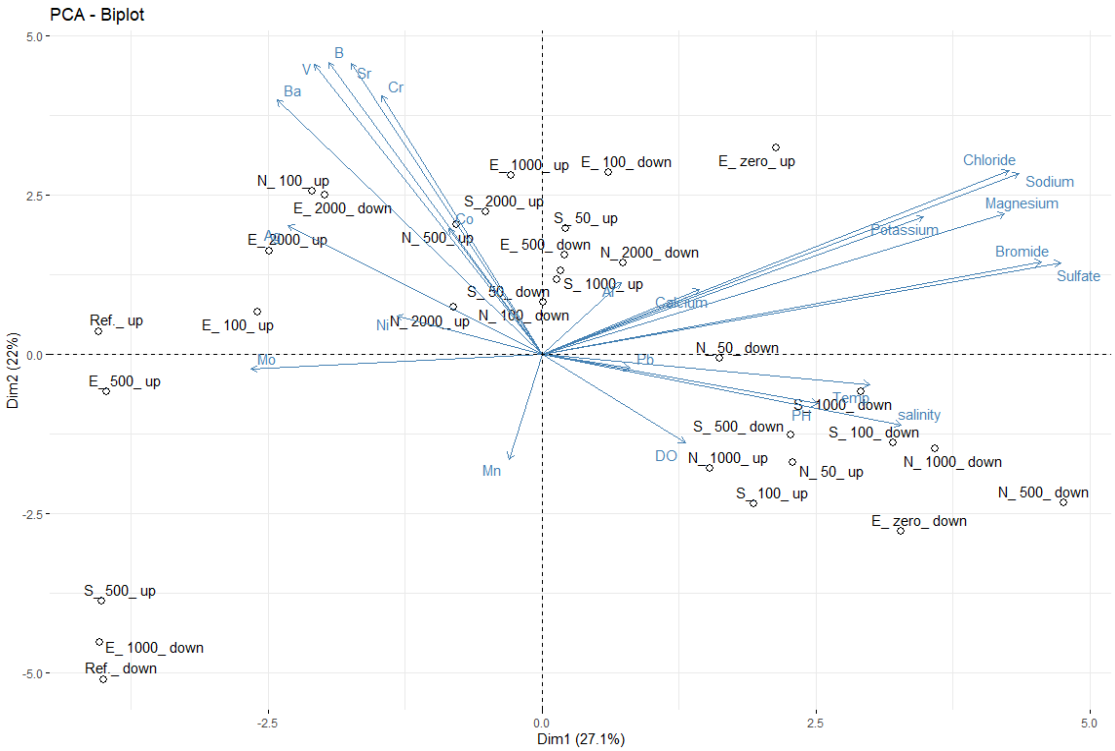

Figure 4: PCA Biplot for the physico-chemical properties of summer samples.

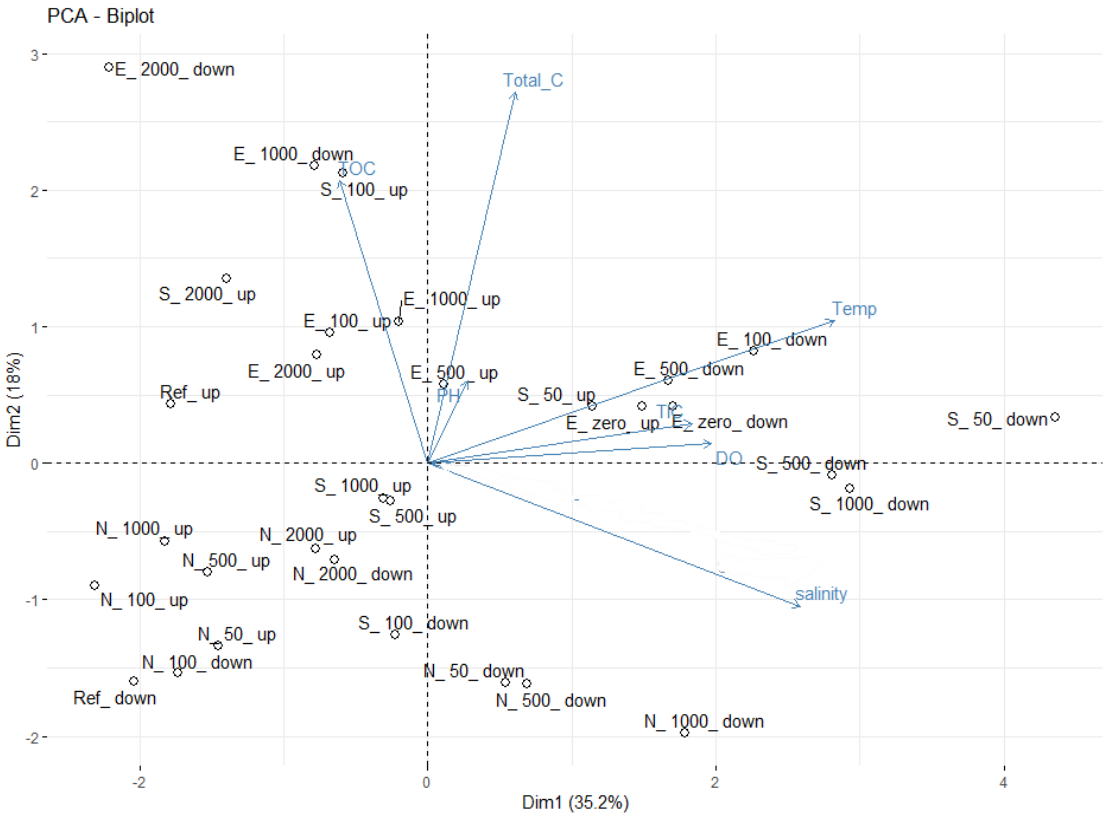

Figure 5: PCA Biplot for the physico-chemical properties of winter samples.

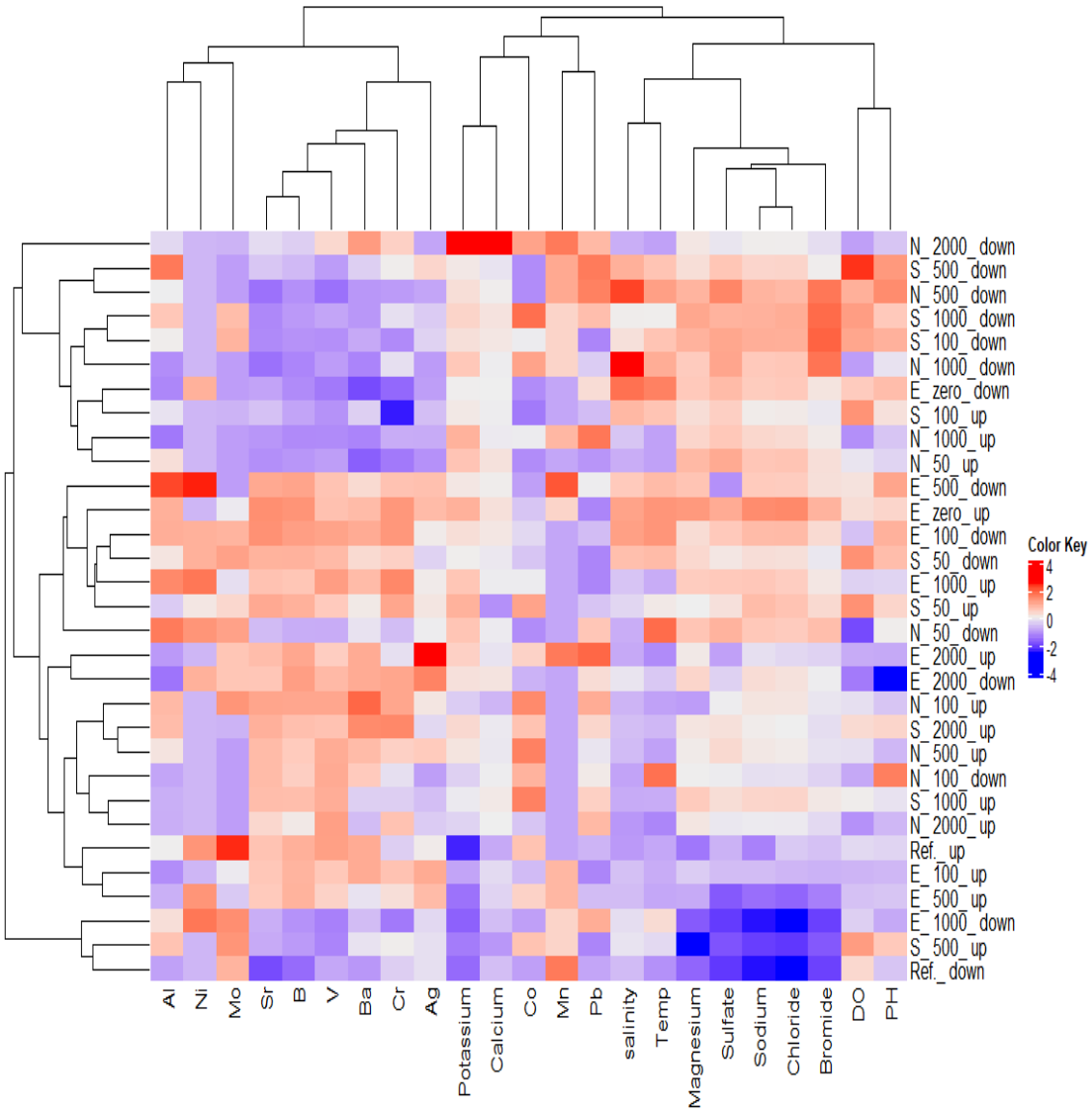

Figure 6: Heatmap showing the relationships among the summer samples and the physico-chemical parameters. The rows and columns are reordered so that similar parameters and similar samples are closer to one another. Color key represented as diverging palette base

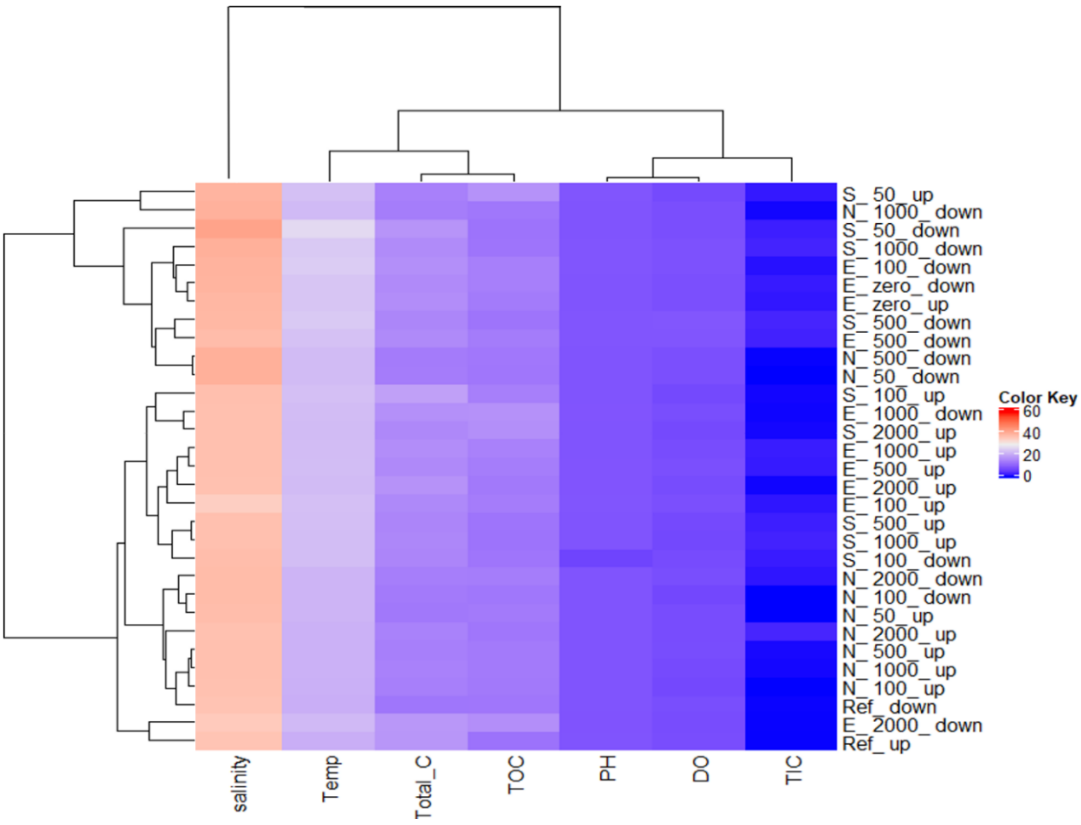

Figure 7: Heatmap showing the relationships among the winter samples and the physico-chemical parameters. The rows and columns are reordered so that similar parameters and similar samples are closer to one another. Color key is indicated as sequential palette ba

In parallel with our findings, a recent study that was conducted in Saudi Arabia revealed that the trace metal concentrations in the discharge of three different desalination plants were very low and none of them exceeded the ambient concentrations of the Gulf water (Saeed et al., 2019). The authors even enhanced their findings with toxicity test on brine shrimp and accumulation assessment in fish tissues and found that there was no significant effect of trace metals in both. In addition, when analysing the sediment samples surrounding Az-zour desalination plant in Kuwait, Al-Shammari & Ali (2018) found that the concentrations of trace metals in the samples where low and they fall within the standards of unpolluted areas.

On the contrary, in their study to investigate the concentrations of trace metals in the seawater of Gulf of Aqaba in Saudi Arabia, Al-Taani et al. (2014) attributed the high concentrations of cadmium, copper, arsenic, nickel, chromium, iron, and molybdenum to the existence of desalination plants in the region. Another study that investigated the trace metals in sediments surrounding Alkhobar desalination plant in Saudi Arabia found high concentrations of iron, aluminium, manganese, lead, chromium, copper, and arsenic (Alshahri, 2017). These high concentrations were explained by the deposition of those metals from the seawater that receives the rejected brine.

In fact, not being able to detect the presence of trace metals in the seawater samples does not exactly mean that it is not there in the environment. Trace metals tend to have different solubility properties, which make it either suspended in the water column or settled in the sediments. In their study Dimitrakakis et al. (2014), stated that significant amount of trace metals ends up in the sediments of the sea in a short time. This might explain why the results of the current study showed very low levels of trace metals in the seawater samples.

Furthermore, it is very likely for the concentrations of trace metals that are introduced into the seawater to be immediately diluted, where it will be detected anymore. However, it is more important to consider the load and the daily input in the marine environment. As a matter of fact, estimating the loading rate of trace metals during a certain time period will provide better assessment on the effect of the pollutant on seawater quality. In toxicology, when doing risk characterization, one should consider both dose-response assessment and exposure assessment.

2. Investigation of the spatial and temporal variability of microorganisms' diversity surrounding the brine discharge point using a metagenomics approach

2.1 Abundance of microorganisms and alpha diversity

Understanding the microorganisms' response to the changes in their surrounding environment starts by studying their occurrence in the affected areas and comparing it to unaffected areas. Since the work of metagenomic profiling of the Qatari marine environment is a new field of research, there are no previous data on the microbial composition of this environment. Which is why, the obtained results were compared to the reference point which is not affected by desalination brine discharge.

Figure 8 represents the raw abundance of species in different transects (a) and at different sampling points (b) in summer and winter. Generally, Summer samples showed much higher species abundance compared to winter samples. Direction-wise, samples collected from the east transect showed the highest abundance, followed by south, then north (Figure 8a). As for the different distances along each transect, the abundances varied for each distance (Figure 8b). However, there was absence of clear pattern in the microbial abundance along the distance gradient from the brine discharge point. Notably, the abundance of species in the putatively affected areas was higher than the reference point for both summer and winter.

Digging further into the microbial communities profiling, Figure 9 shows the contribution of different microorganisms in the abundance at phylum level. Overall, it can be seen that Mollusca is most abundant phylum, followed by Chlorophyta, Annelida, Diatomea, then Dinoflagellata. Mollusca were found at the highest abundance in summer. The potentially affected areas seemed to have more than 10 folds higher abundances compared to the reference. Most of which were belonging to the south and north direction, whereas less were found in the east direction. Similarly, the

abundance of Annelida was peaking in the summer, with higher abundance in the potentially affected areas compared to reference point. Additionally, north and south directions had higher abundance compared to east direction. Chlorophyta on the other hand was found more abundant in winter comparatively to the summer. The east direction had the highest abundance of the Chlorophyta, while north and south directions had less. Diatomea were found in both winter and summer seasons with slight preference to the summer over winter. Further, similar to the Chlorophyta, the Diatomea were found mostly in the east direction.

For further understanding of the data, alpha diversity was assessed to have an idea of the richness of each sample and to know the different organisms present in each sample. Chao 1 diversity which is an abundance-based estimation of species richness was measured for all samples based on the 16S rDNA and 18S rDNA OTUs along the three transects on two different seasons (Figure 10). Generally, the summer season showed higher Chao1 diversity compared to winter samples. The bacterial communities (16S rDNA) showed similar Chao1 diversity trends in summer and winter, where the diversity was decreasing along distance from discharge point for both east and south transects, while it was increasing for the north transect. As for the Eukaryotic communities (18S rDNA), in the summer season they experienced a slight increase in Chao1 diversity along the distance from the discharge point for both east and south transect whereas there was a notable decrease in the diversity along the north transect. For the same communities in winter, there was a decline in the diversity along the distance from the discharge point for both north and east transect. While the diversity along the south transect slightly increased. From this result we can interpret that there are changes in the diversity of the bacterial communities (16S rDNA) as well as eukaryotic communities (18S rDNA), however, the patterns of those changes are not

clearly visible and further investigations need to be carried out to better understand the results.

In agreement with our findings about the seasonal differences in microbial abundance, in their seasonal marine microbiome study, Kim et al. (2018) found that *Synechococcus* blooms were detected in the summer season. Further, studying the marine particulate matter continuum, (Mestre et al., 2020) found that the bacterial diversity increases in warmer seasons. However, other studies report higher microbial diversities colder seasons. For instance, the studies done by Gilbert et al. (2012) and Ladau et al. (2013) showed higher marine microbial diversity in winter. Another study reported, highest marine bacterial diversity in spring (Suh et al., 2015). Further, (Ward et al., 2017) reported diversity peaks of marine microbial communities in fall and spring.

Looking into the composition of the microbial communities, it was noted that many microbial phyla were found in higher abundance in areas receiving brine discharge compared to the reference point. In addition to the instances where species diversity was decreasing along the transects away from the discharge point. A potential reasoning for such observation is that brine discharge is acting as a intermediate disturbance that causes increase in species richness and diversity. This theory was described in (Townsend et al., 1997) as "the intermediate disturbance hypothesis (IDH)" and it can be even traced back to earlier studies. This theory suggests that equilibrium of ecological communities rarely happens. At low levels of disturbance, superior species are more competitive, and they eliminate other species which reduces diversity, while at high levels of disturbance, only tolerant species will survive reducing the biodiversity as well. On the other hand, moderate levels of disturbance are not too low as to allow competitive exclusion of species, and not too high as to eliminate non-

tolerant species. Thus, the intermediate level of the disturbance creates a state of nonequilibrium that promotes coexistence of species, which increases the species richness and species diversity.

This was detected in the study done on sediment microbial communities, where in was found that alpha diversity was higher in slightly disturbed areas (Ellis et al., 2017). Further, another study revealed that the highest bacterial diversity was found in soils receiving moderate to low levels of carbon and ultraviolet radiation, as well as high levels of pH, which was reasoned by the IDH (Delgado-Baquerizo & Eldridge, 2019). In addition, Frühe et al. (2021) found significantly higher bacterial richness, evenness, and diversity in moderately to low impacted areas with anthropogenic organic enrichment compared to highly impacted areas.

Despite that, understanding the effect of environmental stressors on microbial communities is complex and often very hard to predict (Kashtan et al., 2014). It involves complicated involvement of wide variety of parameters that act on the microbial diversity in the environment (Hornick & Buschmann, 2018). For instance, a recent study done by Cordier et al. (2019) on the areas surrounding a gas platform in Italy revealed the absence of apparent pattern in eukaryotic species richness along the distance away from the platform.

As discussed in DiBattista et al. (2020), the increase in species richness does not always reflect high environmental quality, and the variations in diversity are multidimensional which makes it hard to be directly correlated to environmental stressors. Besides the variations that are likely to result from the differences in methodologies and techniques adopted for the research.

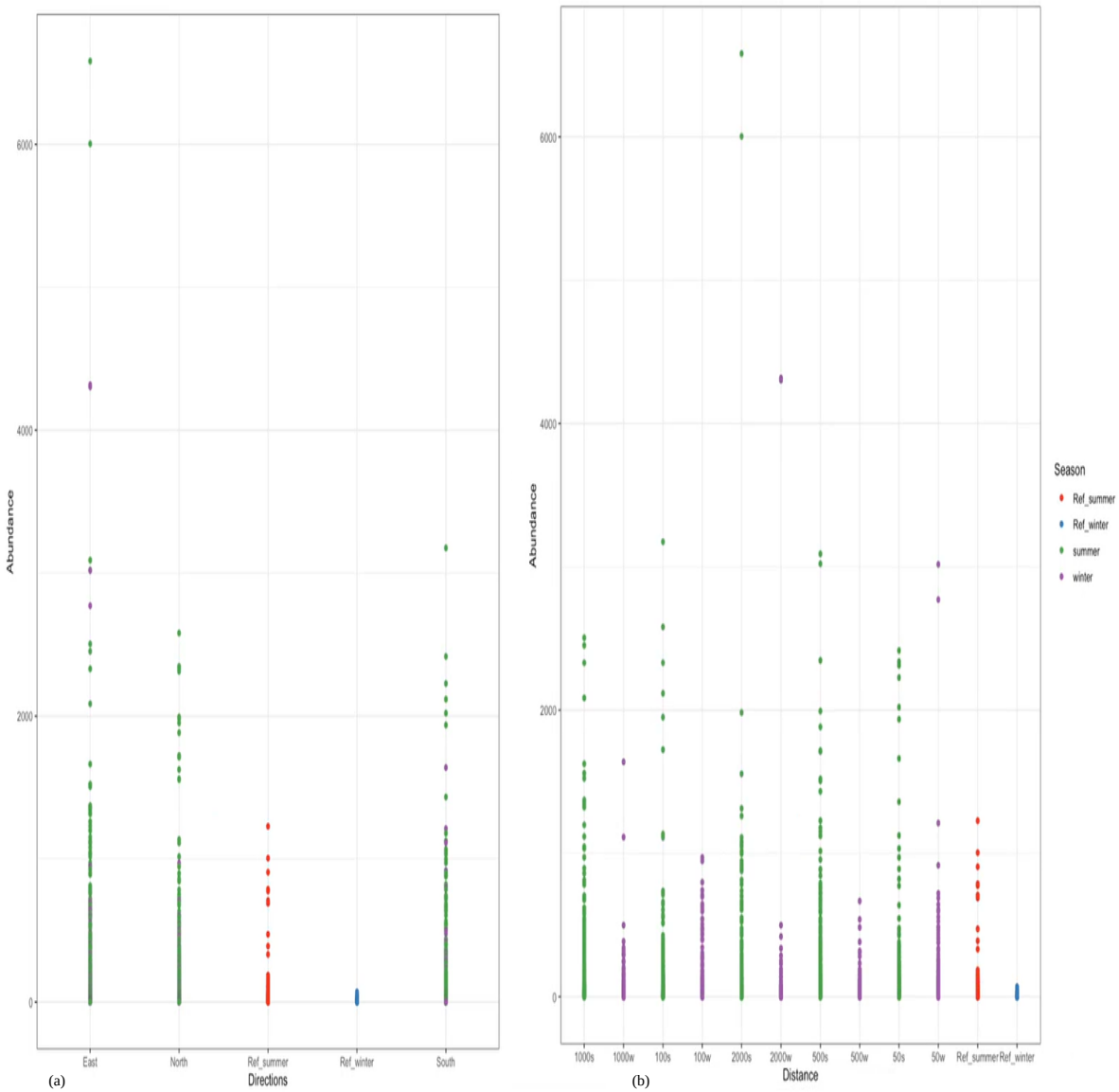

Figure 8: Abundance as a function of direction (a) and distance (b).

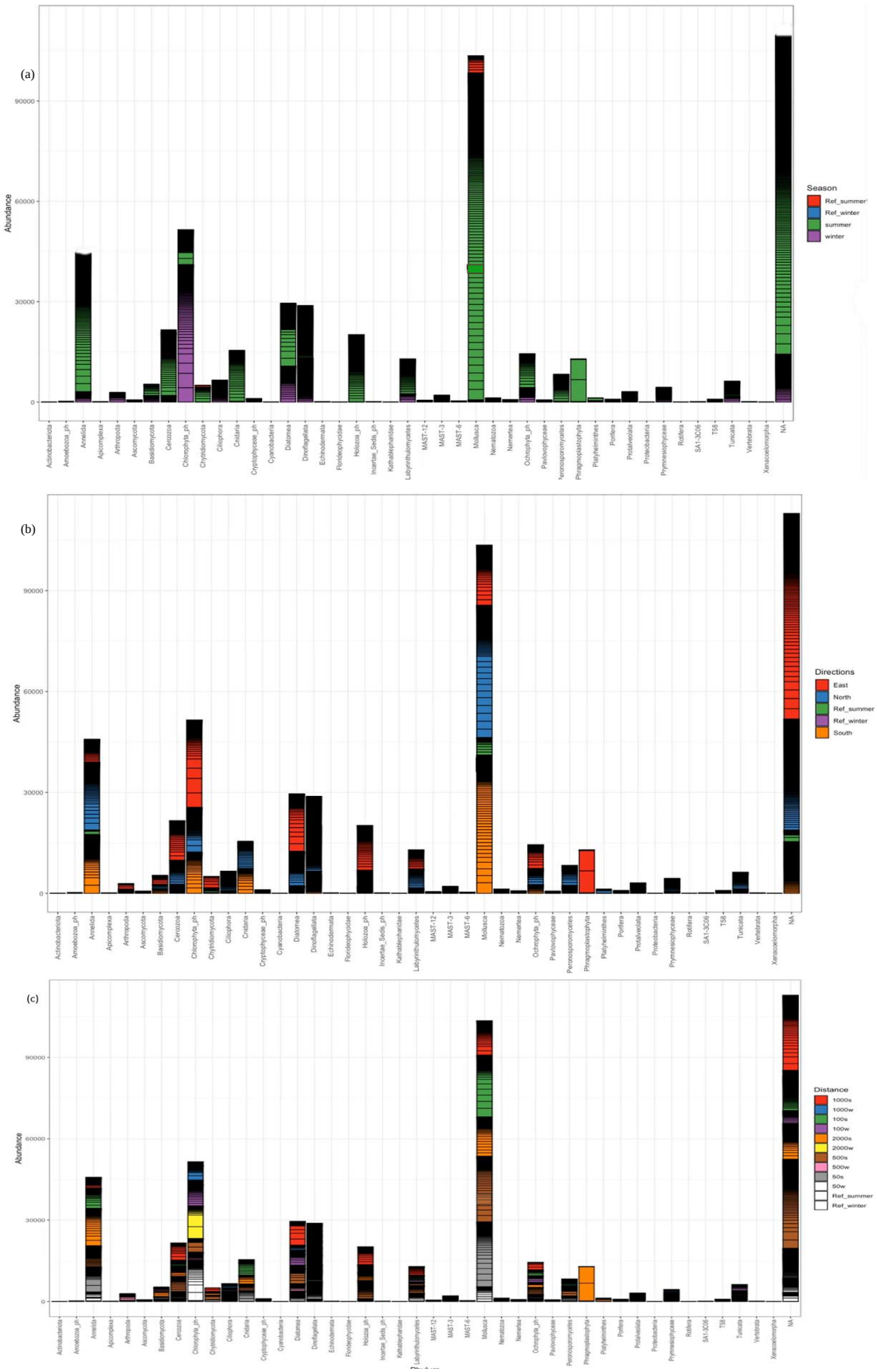

Figure 9: Abundance of microorganisms as a function of season (a), Direction (b), and distance (c) at the phylum level.

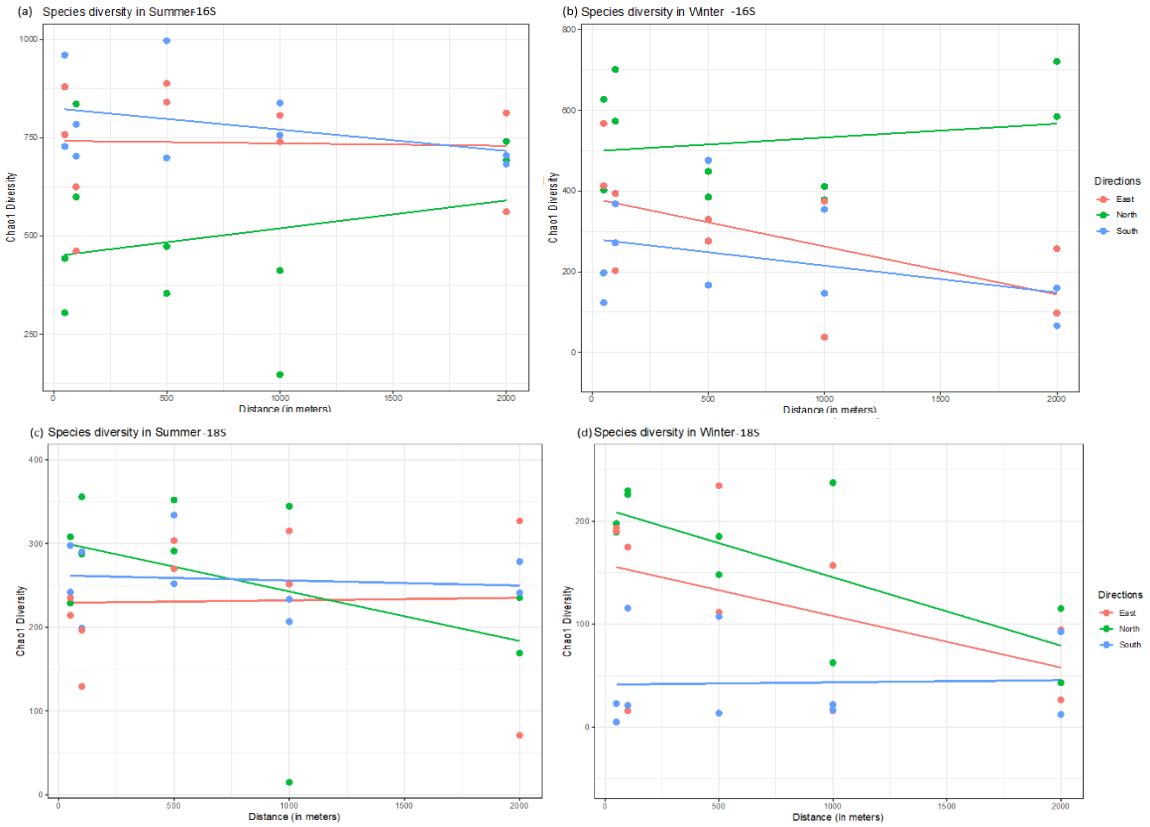

Figure 10: Chao1 diversity of the species in seawater receiving desalination brine discharge.

2.2 Beta diversity and ordination plots

Beta diversity is a mean of understanding the diversity differences that exists between different samples. This can be done by measuring the ecological distance between the samples through different analysis such as Bray-Curtis, and UniFrac. In this study UniFrac distances (unique fraction metric) were measured to provide clustering of the samples based on similarity between 16S rDNA OTUs and 18S rDNA OTUs shown in Figure 11 and Figure 12 respectively. Since the number of samples is big, it would not be easy to understand the clustering through UniFrac alone. Thus, ordination of the beta diversity results is required to visualize the clustering more clearly. In Figure 13 and Figure 14 the PCoA plot of the 16S rDNA

and 18S rDNA show that the summer samples are clustered together and that they are all close to one another in terms of UniFrac distances. The Cluster of winter samples on the other hand is more dispersed indicating less similarities in terms of UniFrac

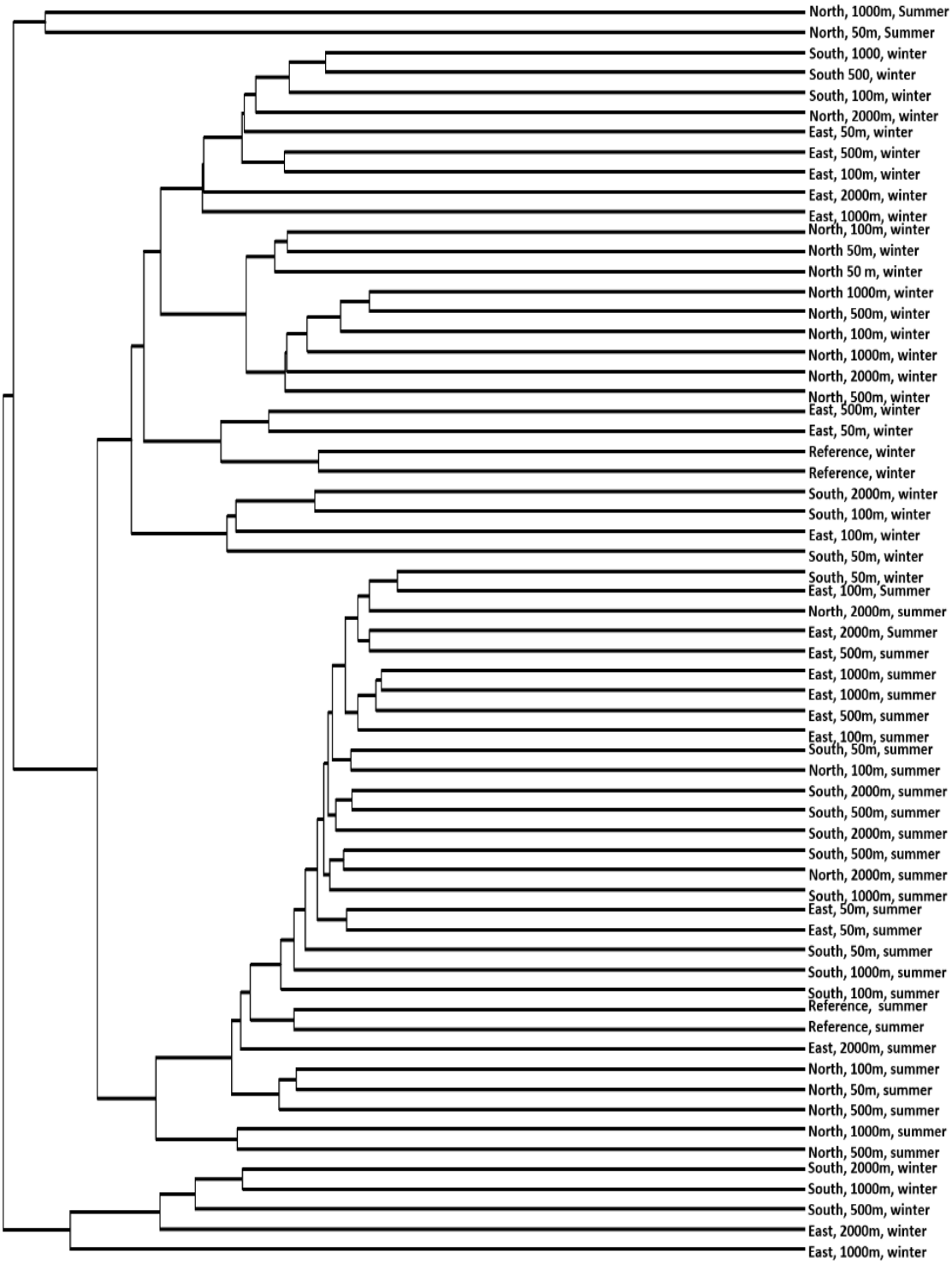

Figure 11: UPGMA Clustering based on UniFrac distance analysis of the 16 S rDNA reads.

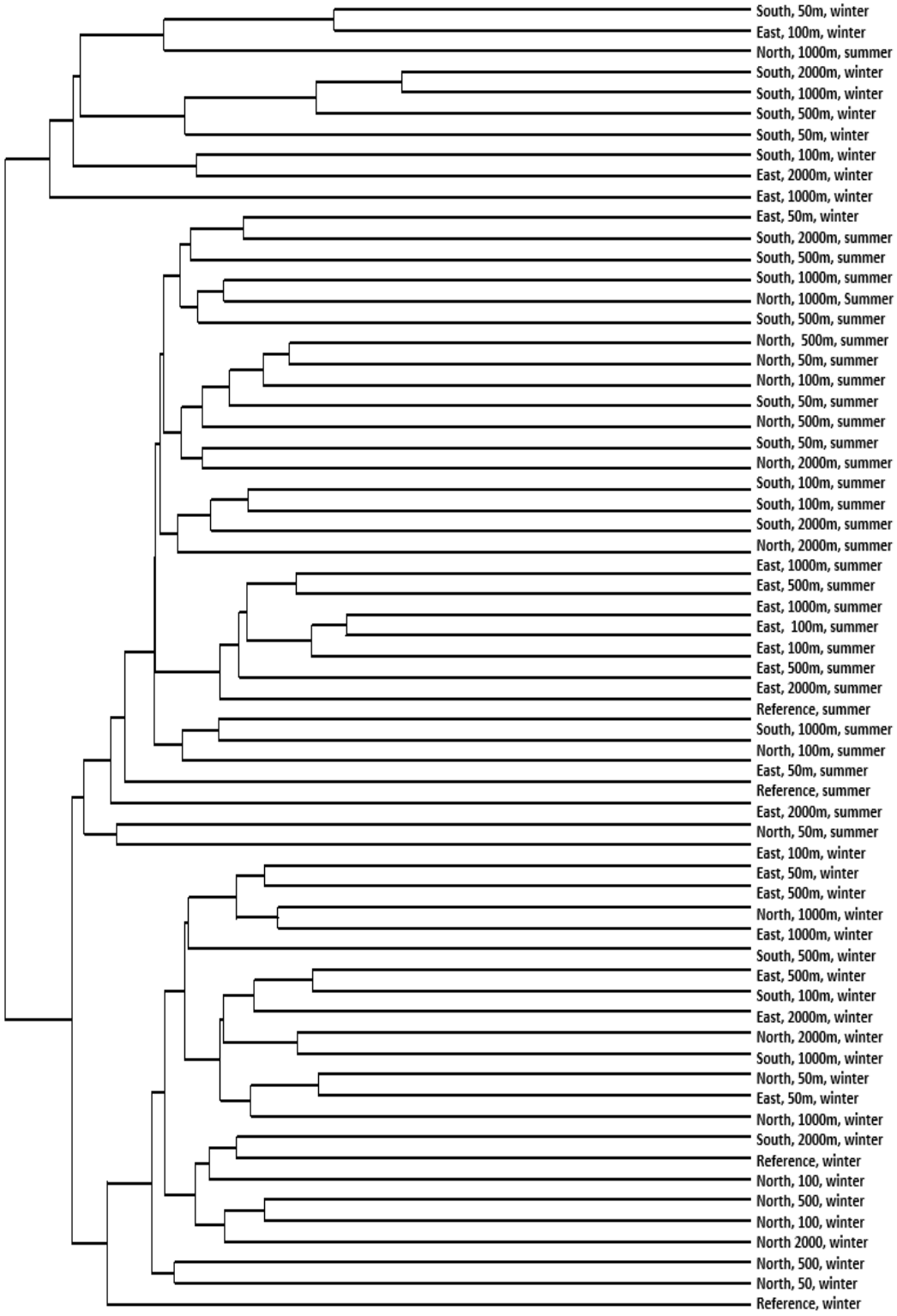

Figure 12: UPGMA Clustering based on UniFrac distance analysis of the 18 S rDNA reads.

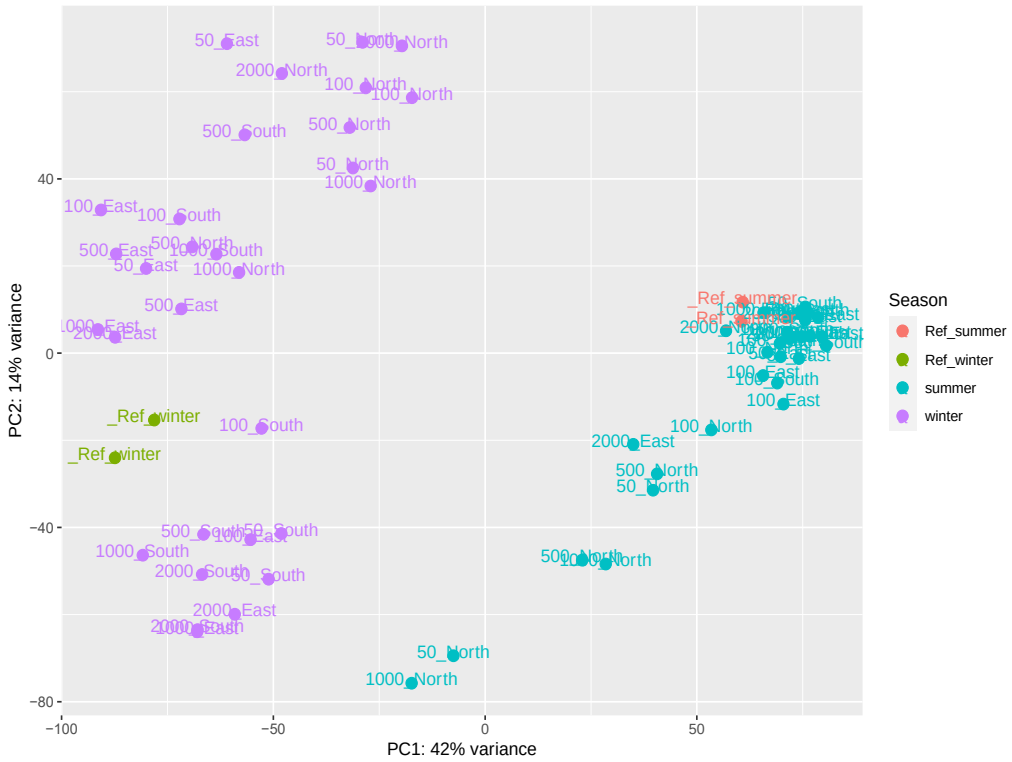

Figure 13: Ordination of beta diversity in principal coordinates analysis of the 16 S rDNA reads using UniFrac distances.

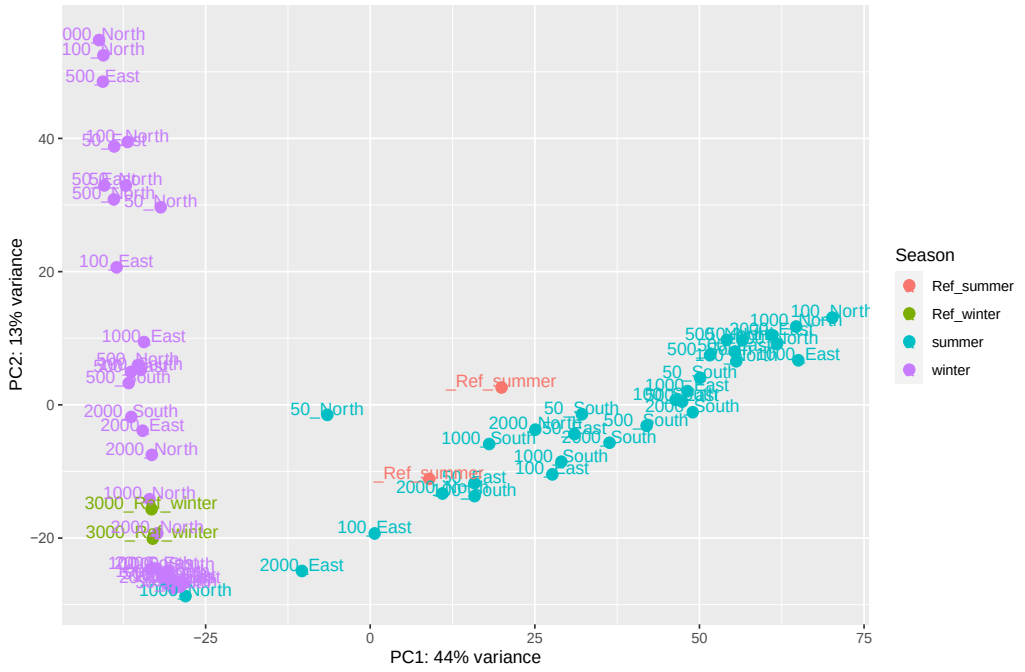

Figure 14: Ordination of beta diversity in principal coordinates analysis of the 18 S rDNA reads using UniFrac distances.

2.3 Microbiome statistical inference: Differential abundance analysis

Differential abundance analysis was carried out by calculating the log 2-fold change in abundance, in which it measures the up-regulated and down-regulated phyla when compared to the reference point. It is represented by volcano plot (Figure 15), where the up-regulated phyla get positive values, while the down-regulated ones get negative values. In the Summer season, Bacteriodota, Cyanobacteria, Mollusca, Diatomea, and Holozoa were among the mostly upregulated phyla compared to the summer reference. On the other hand, planctomycetota, Verrucomicrobiota, Proteobacteria, Annelida, Nematozoa, and Dinoflagellata were all down regulated. In winter, Proteobacteria and Dinoflagellata were among the most commonly up-regulated phyla.

According to Zheng et al. (2014), compared to species richness and diversity, the composition of bacterial communities may be a better representative of the sensitivity

of an ecosystem to stressors. Hence, looking into the differential abundance of microbial communities will provide better understanding on the effect of discharging brine into the sea.

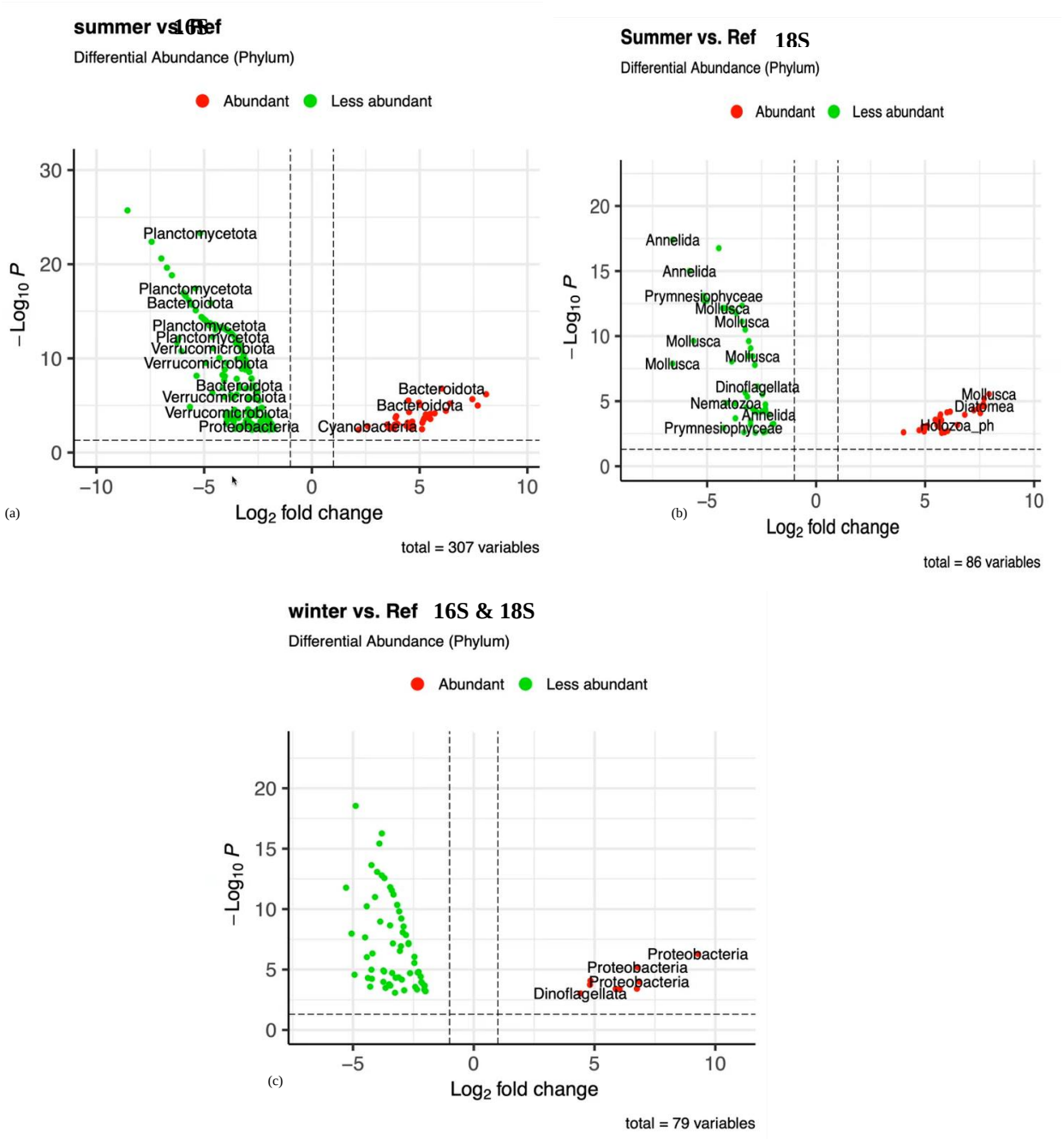

Figure 15: Differential abundance at the phylum level of organisms present in different seasons versus the reference.

3. Bioprospecting of novel marine microorganisms

Marine biotechnology provides opportunities in discovering and developing chemical compounds, pharmaceuticals, nutraceuticals, enzymes, and bioactive molecules through bioprospecting (Cox & King, 2013). Bioprospecting is an organized search of beneficial products derived from living organisms (e.g., plants, animals, microorganisms) to improve human life (Oyemitan, 2017). Given the assumption that the discharged brine from Umm Al Houl desalination plant has high temperature, high salt concentrations, and chemical contamination, it would be predicted that the microorganisms that are able to live in waters close from the discharge pipeline are extremophiles or have adaptation mechanisms that allows them to cope with such unfavourable conditions. Hence, water samples collected from points close to the discharge point were investigated in terms of culturable interesting microorganisms. The water was enriched with three different growth media which are BG-11, F/2, and F/2 with silica. The enriched water samples were kept in shaking incubator for 2-3 weeks until microorganisms started to grow. The growing microorganisms in liquid media were observed using light microscopy (Figure 16). Some of strains were then isolated and purified by repeated streaking on agar media (Figure 17). The DNA of the purified isolates was extracted and the 16S rDNA for cyanobacteria, and 18S rDNA for diatoms and algae were amplified by PCR. Then Sanger sequencing of the purified BLAST, the phylogenetic trees were constructed by neighbour-joining to identify the isolates (Figure 18).

From the phylogenetic trees it can be concluded that Diatom 1 is most likely *Fallacia* sp., Diatom 2 is very likely *Amphora* sp., while Diatom 3 turned out to be *Nitzschia inconspicua*. The isolated Cyanobacterium 1 is likely *Synechococcus elongatus*, Cyanobacterium 2 is *Xenococcus* sp., and Cyanobacterium 3 is

Marileptolyngbya sina. The isolated alga is believed to be *Chlorocystis* sp. All of the isolated species have high importance in the bioindustry.

For instance, *Amphora* species are known to have high fatty acid and lipid content, making it an excellent candidate for the production of biodiesel (Hogan et al., 2021). In addition, the fatty acids produced by this diatom has antiviral activity, anti-inflammatory, anti-oxidant, and anti-microbial activity (Khumaidi et al., 2020). *Nitzschia* diatoms are also commonly used for the production of bioethanol. They are characterised by their fast growth and their adaptive capabilities which makes them favourable for the production of biomass on large scale (Abdel-Hamid et al., 2013; A. Oliver et al., 2021). *Synechococcus* species are gaining a lot of interest lately, as they are being used in bioengineering. For instance, Lin et al. (2020) succeeded in bioengineering *Synechococcus* sp. to produce more photosynthetic sugar. Further, the species of *Leptolyngbya* are known for their high biomass production, and they proved to be effectively used for mitigation of carbon dioxide by bioconversion (Ganta et al., 2019). Lastly, Saadaoui et al., (2018) enhanced the performance of *Chlorocystis* sp. to produce more omega 3 fatty acids and other fatty acids suitable for biodiesel production.

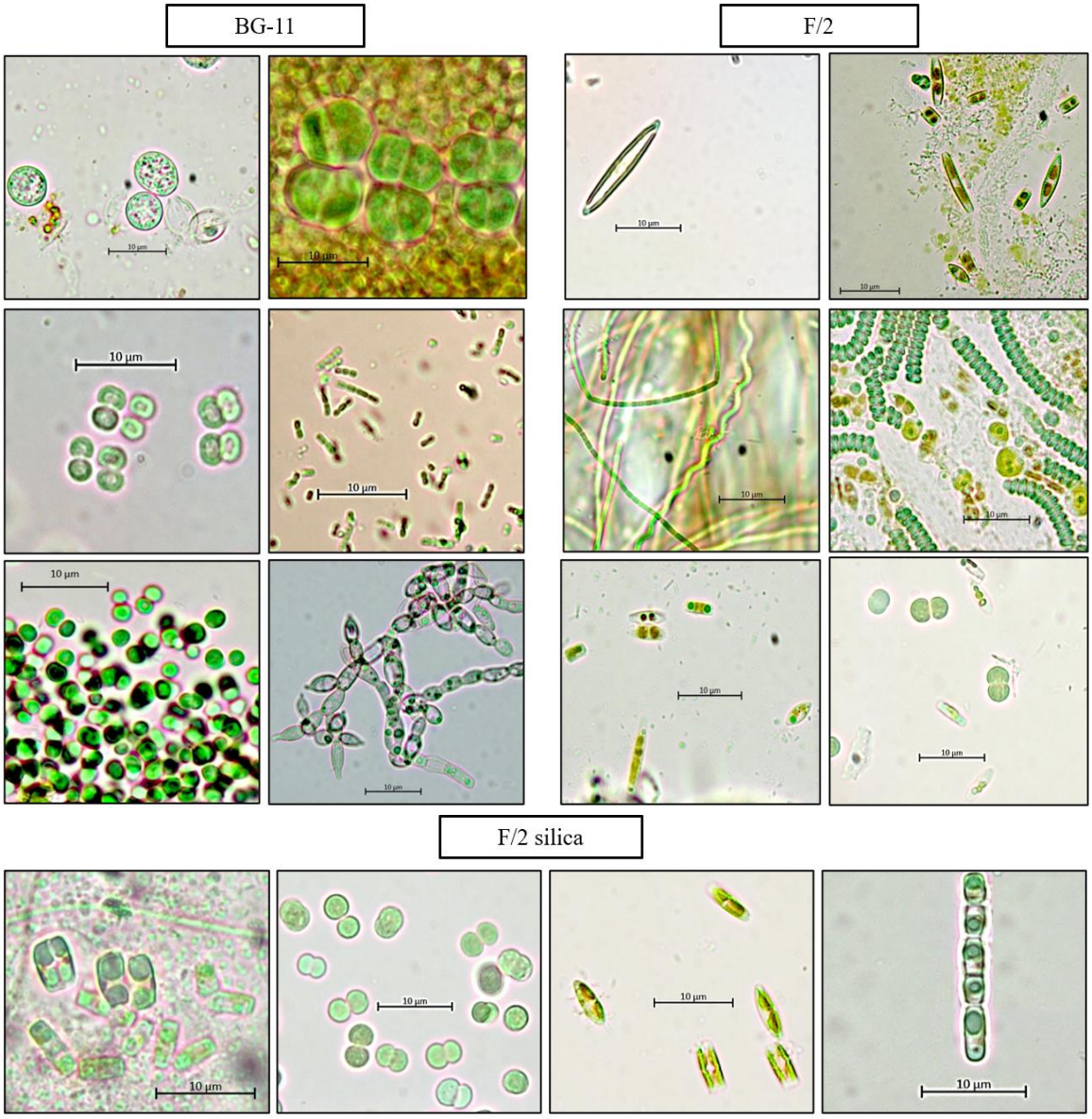

Figure 16: Morphological investigation by light microscopy 100× magnification of the culturable microorganisms found in seawater samples collected from the closest points to the discharge.

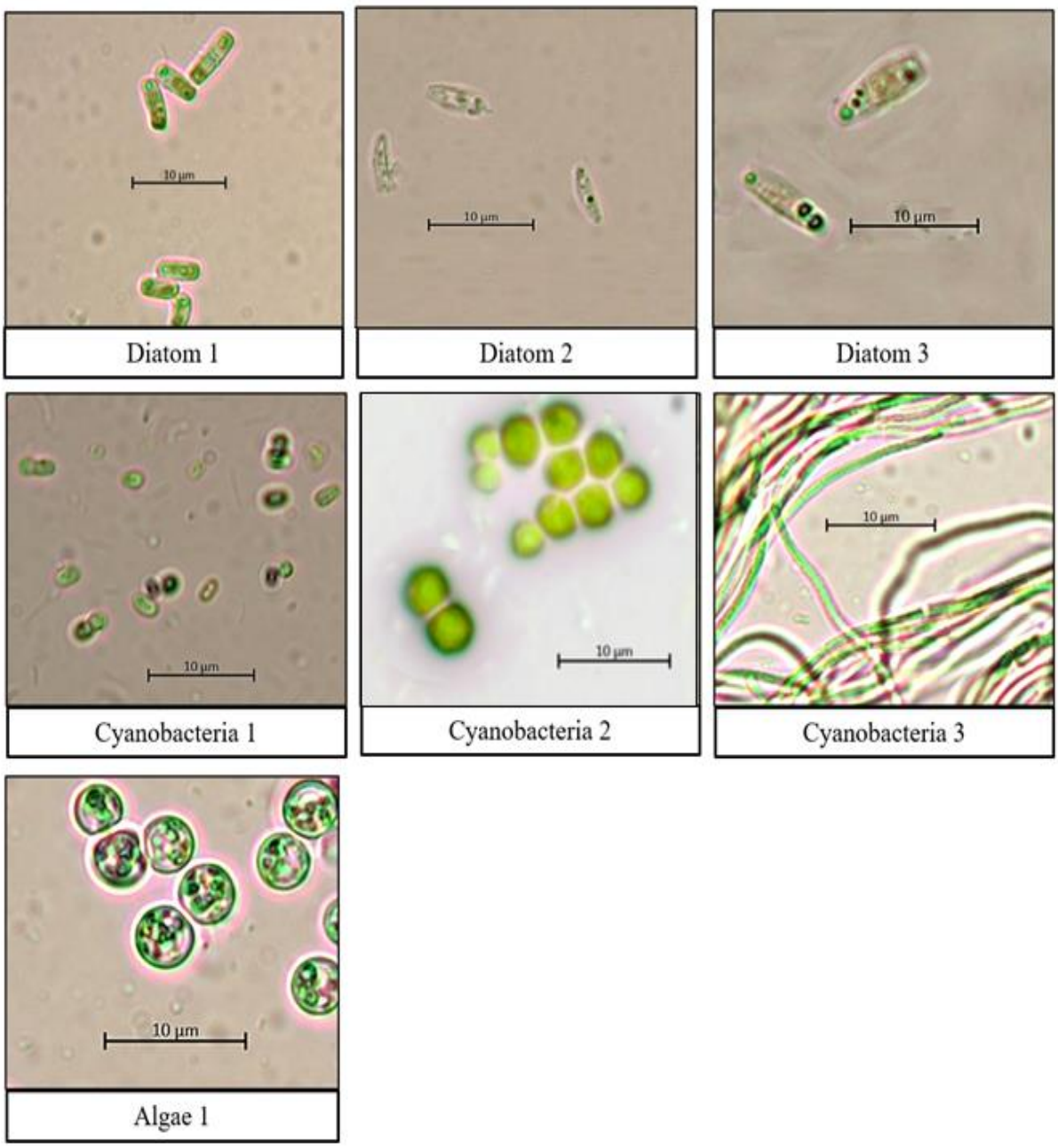

Figure 17: Purified diatoms, alga and cyanobacteria under light microscopy 100x.

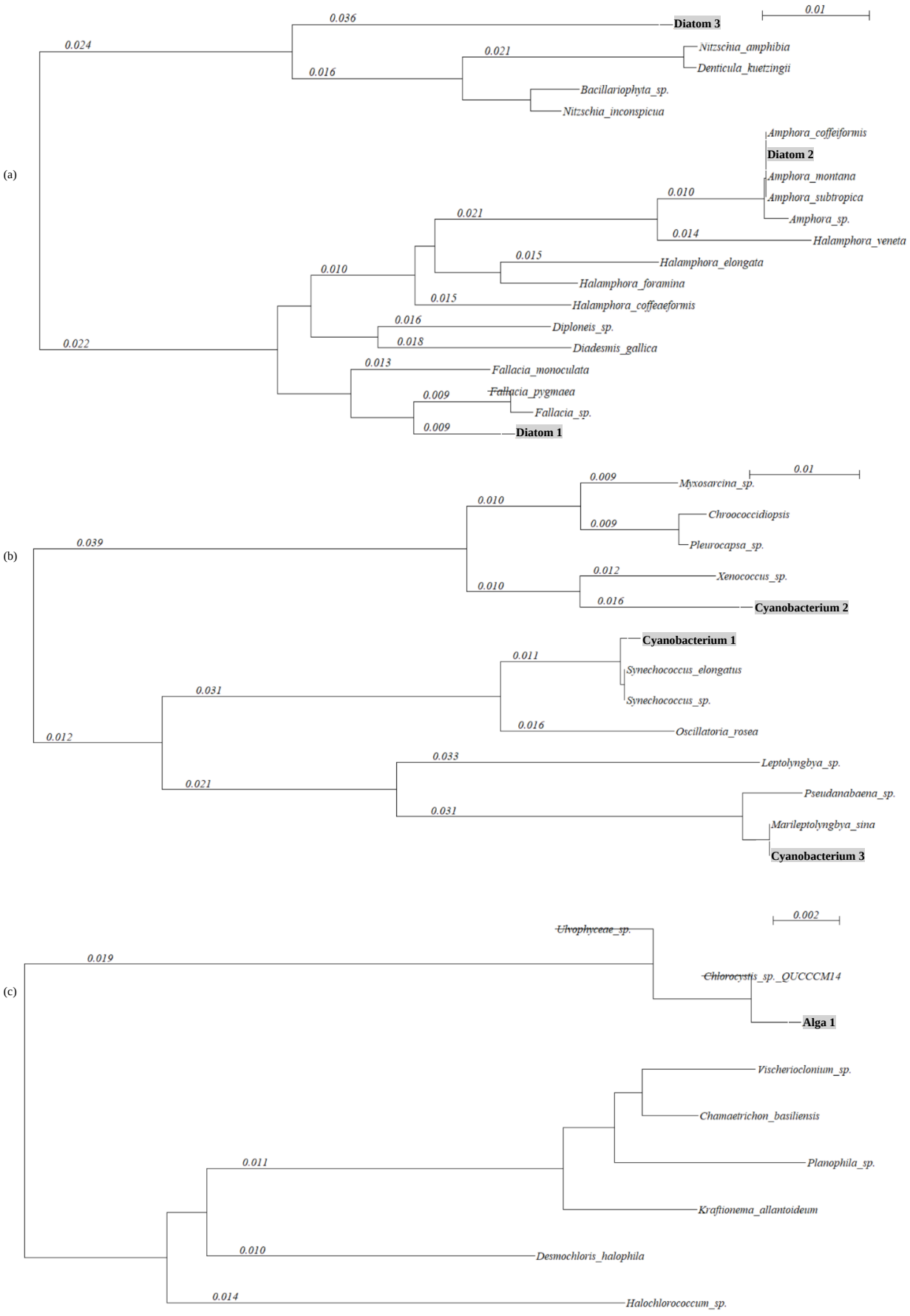

Figure 18: Phylogenetic tree for the identification of purified (a) diatoms, (b) cyanobacteria, and (c) alga.

4. Research relevance to Qatar's development

The international efforts towards environmental protection are guided by the United Nations Sustainable Development Goals (UN-SDGs). The Goal 14 of the SDGs emphasizes the necessity of preserving oceans and seas, which stresses on the protection of the marine biodiversity. In compliance with the international environmental protection standards, Qatar has set environmental development as one of the main pillars in the Qatar National Vision QNV 2030 which is going to be implemented through Qatar's National Development Strategy QNDS. Indeed, desalination has become the main source of providing freshwater to the Qatari community. Which is why this research was oriented towards studying the impacts of discharging desalination brine into the sea on the microbial diversity of the Arabian Gulf.

The research methodology developed for this work provides a reference for utilizing an innovative monitoring approach that has been optimized to suit the Qatari ecological conditions. The metagenomic approach used in this research provides a foundation that could be extended to a wide variety of studies to understand the effect of disturbances on different environments. Driving such innovation will facilitate research in Qatar specifically, and the region generally.

Additionally, this research presented the microbial profiling of the Arabian Gulf which is determined for the first time. Such information provides a strong database which opens the door for further investigations related to the microbial diversity of the Arabian Gulf. Future studies will be able to rely on our findings as reference and better comparisons will even be applicable. This will derive better conclusions which will ultimately aid in the improvement of the Qatar's environmental health and ensure its sustainability.

CHAPTER 5: CONCLUSION

Being the host of nearly 50% of the desalination capacity worldwide, the Arabian Gulf is facing a serious threat that result from the cumulative effects of all the desalination processes that are taking place in the region. Studies are suggesting that the marine ecosystems could be adversely affected from desalination plants and associated activities such as building the desalination plants, up-taking seawater, and discharging untreated or partially treated brine back into the sea.

The present study was conducted to assess the sensitivity of the microbiome in the Qatari coastal waters to desalination brine discharge from Umm Al Houl desalination plant in Al Wakra. The study was based on spatiotemporal investigation of sea microbiome diversity. Firstly, the physico-chemical characteristics of the seawater receiving the brine was investigated at different distances from the outfall, and on two different seasons. The results showed that all tested physical and chemical properties fall within the acceptable range of the regional and international waters and that they do not vary significantly from the reference point. In addition, the tested propertied would slightly differ between one station to another, however, the differences were random and did not follow a distance-dependent manner.

Secondly, the diversity of the microbiome was differing between one sample to the other in the same season. However, these differences were not related to the location of the sample and its proximity from the brine outfall. These small differences in the properties of the seawater or the diversity between different sampling locations could be attributed to the natural variability of the seawater, due to seawater dynamics (e.g. waves, tides, turbulence, and currents). Nevertheless, it was noted that there are seasonal differences, were summer samples had much more abundance and diversity compared to the winter samples.

It is important to note that Umm Al Houl Power plant is a new facility that started operating recently in 2019. Which might mean that more time is required until cumulative effects start appearing. In other words, when assessing a certain risk, it is important to consider both the concentrations and the exposure time. Hence, assessing the sensitivity of the Arabian Gulf's microbiome requires further investigation and long-time monitoring.

For the first time, this study provides the microbial community profiling of the Arabian Gulf which opens the door for further investigations. The findings of this study establish a solid foundation for future research to be conducted in Qatar and the region. Not only that, it also presents an innovative monitoring tool that could be actively applied in different research fields in the region and internationally.

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APPENDIX: PHYSICO-CHEMICAL PROPPERTIES OF THE SEAWATER RECEIVING BRINE DISCHARGE- RAW DATA

Table A 1: Physical properties of the seawater receiving brine discharge in summer and winter.

	Physical properties							
	Summer Season				Winter Season			
Location	**Temp. (°C)**	**Salinity (ppt)**	**pH**	**DO (%)**	**Temp. (°C)**	**Salinity (ppt)**	**pH**	**DO (%)**
Ref., up	35.90	34.00	8.12	94.10	19.90	34.74	8.27	100.40
Ref., down	35.50	34.50	8.11	102.10	19.90	34.89	8.28	101.00
N, 50, up	35.80	34.30	8.12	96.00	20.70	35.75	8.28	99.30
N, 50, down	39.90	34.30	8.14	70.80	21.60	37.76	8.30	108.20
N, 100, up	35.80	34.40	8.11	95.10	20.10	35.20	8.27	93.00
N, 100, down	39.80	34.15	8.23	86.20	20.70	35.98	8.29	92.80
N, 500, up	35.80	34.50	8.10	95.20	20.30	35.35	8.28	100.20
N, 500, down	38.90	38.00	8.22	110.00	21.60	37.79	8.32	108.60
N, 1000, up	35.80	34.65	8.11	82.00	20.30	35.28	8.28	96.60
N, 1000, down	38.60	38.60	8.13	84.30	21.40	37.62	8.32	105.80
N, 2000, up	35.30	33.97	8.10	82.40	20.30	35.25	8.27	98.90
N, 2000, down	35.80	34.31	8.11	84.70	20.60	36.03	8.31	102.90
E, zero, up	39.10	36.60	8.16	101.10	22.80	36.75	8.31	109.90
E, zero, down	39.50	37.40	8.18	104.60	22.80	37.17	8.31	110.70
E, 100, up	36.00	34.58	8.10	88.10	22.10	33.13	8.34	107.30
E, 100, down	39.10	36.65	8.19	90.30	23.70	37.36	8.35	113.70

	Physical properties							
	Summer Season				Winter Season			
Location	**Temp. (℃)**	**Salinity (ppt)**	**pH**	**DO (%)**	**Temp. (℃)**	**Salinity (ppt)**	**pH**	**DO (%)**
E, 500, up	35.90	34.52	8.11	90.30	21.80	35.34	8.33	106.80
E, 500, down	38.30	35.90	8.20	100.10	22.30	35.95	8.39	117.90
E, 1000, up	36.00	34.63	8.12	92.80	21.70	35.32	8.32	101.40
E, 1000, down	37.60	35.00	8.09	92.60	21.70	35.32	8.33	104.90
E, 2000, up	35.40	34.24	8.09	86.60	21.60	35.25	8.33	100.40
E, 2000, down	36.50	35.10	7.92	78.50	21.30	33.74	8.21	100.90
S, 50, up	37.30	34.90	8.16	116.00	22.20	37.13	8.40	102.20
S, 50, down	38.30	36.10	8.18	116.00	25.40	39.80	8.26	107.00
S, 100, up	38.10	36.23	8.15	115.70	22.10	35.42	8.29	97.20
S, 100, down	38.10	35.50	8.19	112.00	21.80	35.83	6.31	100.80
S, 500, up	36.80	35.09	8.17	114.00	21.90	35.31	8.29	97.30
S, 500, down	38.10	36.37	8.21	130.00	23.30	36.60	8.37	122.80
S, 1000, up	36.00	34.27	8.13	98.00	21.80	35.33	8.29	94.20
S, 1000, down	37.20	35.28	8.17	113.70	23.30	37.84	8.36	114.10
S, 2000, up	36.20	34.55	8.16	101.70	21.60	35.30	8.25	97.10
S, 2000, down	36.20	34.60	8.18	96.60	21.50	35.39	8.28	98.70
AVG ± STDV	37.14 ± 1.44	35.22 ± 1.18	8.14 ± 0.06	97.55 ± 13.08	21.69 ± 1.18	35.97 ± 1.18	8.24 ± 0.35	103.53 ± 7.02

Table A 2: Chemical properties- heavy metal composition of the seawater receiving brine discharge in summer.

ICP-OES of summer water samples (mg/L)																
Location	**Al**	**Ba**	**Cd**	**Cr**	**Co**	**Cu**	**Fe**	**Pb**	**Mn**	**Mo**	**Ni**	**Ag**	**Sr**	**V**	**Zn**	**B**
Ref., up	0.031	0.006	<dl	0.005	0.005	<dl	<dl	0.0006	<dl	0.013	0.003	0.007	9.786	0.608	<dl	5.276
Ref., down	0.018	0.003	<dl	0.005	0.003	<dl	<dl	0.004	0.002	0.007	<dl	0.006	7.384	0.455	<dl	3.858
N, 50, up	0.034	0.002	<dl	0.004	0.003	<dl	<dl	0.002	<dl	<dl	<dl	0.002	8.136	0.447	<dl	4.157
N, 50, down	0.052	0.004	<dl	0.005	0.003	<dl	<dl	0.0019	<dl	0.008	0.003	0.007	8.635	0.460	<dl	4.319
N, 100, up	0.041	0.007	<dl	0.008	0.007	<dl	<dl	0.0023	<dl	0.009	<dl	0.007	10.183	0.601	<dl	5.357
N, 100, down	0.019	0.005	<dl	0.006	0.006	<dl	<dl	0.0014	<dl	<dl	<dl	0.003	9.894	0.597	<dl	5.029
N, 500, up	0.033	0.006	<dl	0.007	0.007	<dl	<dl	0.0012	<dl	<dl	<dl	0.009	9.995	0.595	<dl	5.056
N, 500, down	0.030	0.003	<dl	0.004	0.003	<dl	<dl	0.0030	0.002	<dl	<dl	0.003	7.802	0.405	<dl	4.011
N, 1000, up	0.012	0.003	<dl	0.005	0.004	<dl	<dl	0.0031	0.001	<dl	<dl	0.003	8.212	0.429	<dl	4.078
N, 1000, down	0.015	0.003	<dl	0.006	0.006	<dl	<dl	0.0009	0.001	<dl	<dl	0.003	7.794	0.445	<dl	4.040
N, 2000, up	0.020	0.004	<dl	0.007	0.004	<dl	<dl	0.0021	<dl	<dl	<dl	0.005	9.510	0.609	<dl	4.805
N, 2000, down	0.027	0.006	<dl	0.007	0.006	<dl	<dl	0.0021	0.002	0.001	<dl	0.003	8.987	0.546	<dl	4.545
E, zero, up	0.043	0.006	<dl	0.008	0.004	<dl	<dl	<dl	0.001	0.004	<dl	0.010	10.470	0.572	<dl	5.503
E, zero, down	0.014	0.004	<dl	0.003	0.003	<dl	<dl	0.0016	<dl	<dl	0.002	0.003	8.366	0.412	<dl	4.081

	ICP-OES of summer water samples (mg/L)															
Location	**Al**	**Ba**	**Cd**	**Cr**	**Co**	**Cu**	**Fe**	**Pb**	**Mn**	**Mo**	**Ni**	**Ag**	**Sr**	**V**	**Zn**	**B**
E, 100, up	0.015	0.006	<dl	0.007	0.004	<dl	<dl	<dl	0.001	0.004	<dl	0.011	9.733	0.564	<dl	5.244
E, 100, down	0.043	0.006	<dl	0.008	0.004	<dl	<dl	0.007	<dl	0.007	0.002	0.007	10.440	0.605	<dl	5.422
E, 500, up	0.021	0.004	<dl	0.006	0.005	<dl	<dl	0.007	0.001	0.002	0.003	0.010	9.688	0.553	<dl	5.257
E, 500, down	0.060	0.005	<dl	0.007	0.003	<dl	<dl	0.013	0.002	<dl	0.004	0.010	10.135	0.569	<dl	5.376
E, 1000, up	0.050	0.006	<dl	0.008	0.004	<dl	<dl	<dl	<dl	0.003	0.003	0.007	9.794	0.611	<dl	5.099
E, 1000, down	0.034	0.004	<dl	0.004	0.003	<dl	<dl	0.023	0.001	0.009	0.003	0.006	8.479	0.419	<dl	4.128
E, 2000, up	0.017	0.006	<dl	0.006	0.005	<dl	<dl	0.033	0.002	0.006	<dl	0.024	9.865	0.558	<dl	5.348
E, 2000, down	0.011	0.006	<dl	0.008	0.003	<dl	<dl	0.016	<dl	0.006	0.002	0.013	9.741	0.580	<dl	5.420
S, 50, up	0.025	0.005	<dl	0.008	0.006	<dl	<dl	0.008	<dl	0.005	0.001	0.007	10.127	0.561	<dl	5.270

ICP-OES of summer water samples (mg/L)															
Location	**Al**	**Ba**	**Cd**	**Cr**	**Co**	**Cu**	**Fe**	**Pb**	**Mn**	**Mo**	**Ni**	**Ag**	**Sr**	**V**	**Zn**	**B**
S, 50, down	0.033	0.0005	<dl	0.007	0.004	<dl	<dl	<dl	<dl	0.008	0.002	0.005	10.005	0.579	<dl	5.262
S, 100, up	0.029	0.004	<dl	0.002	0.002	<dl	<dl	0.007	<dl	0.001	<dl	0.004	8.693	0.436	<dl	4.252
S, 100, down	0.031	0.003	<dl	0.004	0.004	<dl	<dl	<dl	0.001	0.007	<dl	0.005	8.079	0.433	<dl	4.130
S, 500, up	0.039	0.004	<dl	0.006	0.005	<dl	<dl	<dl	0.001	0.009	<dl	0.006	8.444	0.423	<dl	4.185
S, 500, down	0.053	0.004	<dl	0.006	0.003	<dl	<dl	0.031	0.002	<dl	<dl	0.008	8.751	0.445	<dl	4.400
S, 1000, up	0.021	0.004	<dl	0.005	0.007	<dl	<dl	0.018	<dl	<dl	<dl	0.004	9.866	0.593	<dl	5.163
S, 1000, down	0.038	0.003	<dl	0.006	0.007	<dl	<dl	0.021	0.001	0.007	<dl	0.005	8.052	0.453	<dl	4.180
S, 2000, up	0.040	0.007	<dl	0.008	0.005	<dl	<dl	0.017	<dl	0.001	<dl	0.006	10.034	0.570	<dl	5.154
S, 2000, down	0.073	0.000	<dl	<dl	0.005	<dl	<dl	0.025	0.001	<dl	<dl	<dl	0.008	<dl	<dl	0.042

ICP-OES of summer water samples (mg/L)															
Location	**Al**	**Ba**	**Cd**	**Cr**	**Co**	**Cu**	**Fe**	**Pb**	**Mn**	**Mo**	**Ni**	**Ag**	**Sr**	**V**	**Zn**	**B**
AVG ± STDV	0.03 ± 0.01	0.004 ± 0.002	<dl	0.006 ± 0.002	0.004 ± 0.001	<dl	<dl	0.017 ± 0.009	0.001 ± 0.0004	0.006 ± 0.003	0.002 ± 0.001	0.007 ± 0.004	8.906 ± 1.828	0.520 ± 0.075	<dl	4.611 ± 0.985

Note: dl' is detection limit

Table A 3: Chemical properties- common ions composition of the seawater receiving brine discharge in summer.

Ion chromatography of water sample (ppm)							
Location	**Chloride**	**Bromide**	**Sulfate**	**Sodium**	**Potassium**	**Calcium**	**Magnesium**
Ref., up	21813.42	53.83	3100.83	11547.50	357.67	446.75	1308.92
Ref., down	18685.58	46.00	2759.50	10520.42	390.25	493.08	1280.83
N, 50, up	23382.00	57.83	3507.50	13180.25	482.08	632.92	1603.50
N, 50, down	23227.67	60.33	3475.67	13160.08	481.92	589.42	1580.50
N, 100, up	22683.00	56.08	3266.08	12804.25	439.42	473.67	1368.50
N, 100, down	22225.67	54.92	3261.08	12522.33	441.42	592.92	1500.58
N, 500, up	22560.00	55.83	3346.42	12765.17	462.83	582.17	1507.17
N, 500, down	23610.83	65.33	3623.75	13406.42	466.42	602.92	1613.08
N, 1000, up	22898.42	57.00	3404.58	12972.00	493.33	588.08	1549.25
N, 1000, down	23321.25	65.50	3523.00	13144.83	480.17	593.83	1567.50
N, 2000, up	22382.25	55.33	3250.33	12630.08	442.75	597.67	1516.75

	Ion chromatography of water sample (ppm)						
Location	**Chloride**	**Bromide**	**Sulfate**	**Sodium**	**Potassium**	**Calcium**	**Magnesium**
N, 2000, down	22498.42	55.58	3245.00	12697.50	577.58	1514.33	1514.33
E, zero, up	24611.33	61.08	3502.25	13888.42	493.75	636.42	1663.17
E, zero, down	23272.42	57.42	3448.92	13110.83	456.92	597.83	1571.92
E, 100, up	21469.00	52.92	3128.67	12160.92	419.33	553.92	1444.83
E, 100, down	23593.25	58.17	3401.25	13325.17	466.58	620.25	1535.67
E, 500, up	20044.67	49.75	2847.42	11347.92	393.92	539.92	1391.25
E, 500, down	23212.08	57.75	3002.42	13124.67	461.25	603.67	1582.67
E, 1000, up	23179.50	57.25	3401.25	13164.42	481.25	591.25	1565.83
E, 1000, down	18655.83	45.92	2765.42	10530.08	385.67	490.08	1263.50
E, 2000, up	22067.75	54.92	3050.42	12523.42	474.83	573.92	1510.50
E, 2000, down	22752.92	56.50	3188.00	12896.83	464.83	628.25	1548.92
S, 50, up	23405.33	58.25	3319.17	13313.75	493.42	389.25	1498.50
S, 50, down	22785.75	56.17	3299.08	12884.58	457.00	583.92	1542.67
S, 100, up	22566.08	56.17	3381.00	12716.17	460.25	594.75	1532.50
S, 100, down	23823.33	66.58	3518.17	13475.92	465.50	619.58	1616.25
S, 500, up	19275.00	47.33	2830.17	10870.00	399.08	406.75	1033.92
S, 500, down	23028.25	56.75	3412.50	12987.67	460.50	574.08	1531.25
S, 1000, up	23029.00	57.17	3332.00	12994.75	453.67	614.67	1568.25
S, 1000, down	23868.08	66.08	3475.50	13438.92	472.67	630.08	1638.00
S, 2000, up	22463.67	55.58	3320.67	12704.42	469.83	585.75	1518.92
AVG ± STDV	22464.25 ± 1429.08	56.62 ± 5.02	3270.58 ± 230.13	12671.28 ± 835.90	456.33 ± 40.54	598.13 ± 179.07	1499.01 ± 128.96

Table A 4: Chemical properties- carbon content and common ions composition of the seawater receiving brine discharge in winter.

Location	Carbon content of water samples (Wt %)			Ion chromatography of water sample (ppm)						
	Total C	TOC	TIC	Chloride	Bromide	Sulfate	Sodium	Potassium	Calcium	Magnesium
Ref., up	16.720	12.113	0.727	24584.83	41.67	402.50	14004.58	474.00	600.42	1719.83
Ref., down	12.560	12.663	0.770	24760.92	805 8.25	411 5.25	14171.92	456.17	583.42	1727.00
N, 50, up	12.840	13.100	0.630	27645.50	67.25	412 5.42	15390.50	499.33	690.00	1908.00
N, 50, down	13.433	12.667	0.407	29014.33	71.17	414 6.25	16078.58	508.92	720.42	2005.42
N, 100, up	13.730	13.053	0.677	26503.08	62.33	407 3.75	14693.58	529.17	649.75	1823.42
N, 100, down	13.073	12.700	0.533	28105.33	68.50	422 7.08	15670.42	548.17	662.33	1906.33
N, 500, up	13.730	13.093	1.000	27074.75	62.25	394 1.92	15102.25	492.75	648.58	1847.92
N, 500, down	13.233	12.793	0.733	33541.33	77.25	475 5.58	18693.08	632.33	781.83	2280.00
N, 1000, up	14.093	13.187	0.917	26914.83	63.17	383 0.75	15066.75	507.83	642.67	1824.92
N, 1000, down	13.527	12.720	0.883	27218.25	64.67	391 9.17	15171.42	491.08	665.92	1838.33
N, 2000, up	14.103	12.623	2.953	25771.83	58.58	386 6.83	14324.58	454.50	583.42	1730.42
N, 2000, down	13.603	13.517	1.693	26842.25	61.08	402 0.58	14951.33	494.17	637.00	1809.83

Location	Carbon content of water samples (Wt %)			Ion chromatography of water sample (ppm)						
	Total C	TOC	TIC	Chloride	Bromide	Sulfate	Sodium	Potassium	Calcium	Magnesium
E, zero, up	15.577	13.207	1.807	25586.92	58.83	3048.58	14167.75	464.33	683.92	1802.83
E, zero, down	15.210	13.750	2.057	26648.08	61.58	3212.33	14804.42	517.83	640.92	1825.33
E, 100, up	15.013	13.427	1.700	25843.67	59.00	3346.75	14318.08	467.58	650.33	1736.67
E, 100, down	15.807	13.767	1.410	25727.25	58.92	3363.75	14227.75	459.42	627.33	1733.17
E, 500, up	15.127	13.530	2.033	24976.58	56.83	2932.08	13956.92	502.25	619.42	1724.08
E, 500, down	15.177	13.380	2.657	25160.33	57.58	3174.83	14064.08	456.42	615.67	1716.33
E, 1000, up	15.563	14.043	2.197	25408.75	61.00	3456.42	14241.25	465.75	645.58	1782.17
E, 1000, down	16.037	16.127	0.820	26171.42	61.08	3518.92	14602.75	512.75	589.00	1739.42
E, 2000, up	16.240	13.023	0.853	25926.75	59.75	3272.33	14357.83	469.75	625.92	1766.33
E, 2000, down	16.947	15.780	0.743	24996.92	58.25	2913.33	13899.00	466.92	632.75	1745.17
S, 50, up	13.877	16.177	1.917	28252.08	65.00	3699.17	15777.92	532.75	684.33	1918.33
S, 50, down	16.523	12.240	2.373	26295.92	60.33	3516.50	14696.92	500.83	641.83	1828.25
S, 100, up	18.093	13.710	0.883	26520.33	60.83	3464.67	14652.75	487.75	718.42	1838.50
S, 100, down	14.613	12.500	2.177	25725.50	59.00	3564.00	14244.92	475.75	606.17	1706.92

Location	Carbon content of water samples (Wt %)			Ion chromatography of water sample (ppm)						
	Total C	TOC	TIC	Chloride	Bromide	Sulfate	Sodium	Potassium	Calcium	Magnesium
S, 500, up	14.593	12.390	2.393	25509.50	44.00	3752.75	14589.17	472.25	680.42	1817.17
S, 500, down	14.677	12.367	2.933	27224.75	47.25	4361.08	15585.08	521.58	671.42	1926.00
S, 1000, up	14.783	12.227	2.733	25985.58	54.42	3804.42	14334.50	482.33	644.67	1771.75
S, 1000, down	15.300	12.323	2.810	30290.08	63.17	4163.67	16825.67	588.50	760.42	2078.42
S, 2000, up	14.960	15.810	0.910	27456.42	63.00	3893.42	15127.92	533.67	629.08	1808.17
S, 2000, down	15.133	0.623	11.937	25421.75	58.42	3628.83	13909.42	457.00	602.17	1701.67
AVG ± STDV	14.81 ± 1.28	13.36 ± 1.13	1.53 ± 0.82	26659.56 ± 1760.20	310.14 ± 1391.62	3723.53 ± 429.87	14865.72 ± 969.78	497.62 ± 39.21	651.11 ± 46.27	1824.63 ± 119.50

Table A 5: Criterion of maximum concentration (CMC) of trace metals in seawater set by the US EPA

Element	Criterion of maximum concentration CMC (µg/L)
Cadmium (Cd)	40.0
Copper (Cu)	4.8
Lead (Pb)	210.0
Nickel (Ni)	74.0
Chromium (Cr (VI))	1,100.0
Zinc (Zn)	90.0
Mercury (Hg)	1.8
Arsenic (As)	69