In-situ marine monitoring and environmental management of SWRO concentrate discharge: A case study of the KAUST SWRO plant

Dissertation by
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In Partial Fulfillment of the Requirements
For the Degree of
Doctor of Philosophy

King Abdullah University of Science and Technology
Thuwal, Kingdom of Saudi Arabia

June 2014
EXAMINATION COMMITTEE APPROVALS FORM

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ABSTRACT

Marine monitoring and environmental management of SWRO concentrate discharge:
A case study of the KAUST SWRO plant

Riaan van der Merwe

Concentrate (when discharged to the ocean) may have chronic/acute impacts on marine ecosystems, particularly in the mixing zone around outfalls. The environmental impact of the desalination plant discharges is very site- and volumetric specific, and depends to a great extent on the salinity tolerance of the specific marine microbial communities as well as higher order organisms inhabiting the water column in and around this extreme discharge environment. Scientific studies that aim to grant insight into possible impacts of concentrate discharge are very important, in order to understand how this may affect different marine species when exposed to elevated salinity levels or residual chemicals from the treatment process in the discharge site.

The objective of this PhD research was to investigate the potential environmental effects of the concentrate discharge in the near-field area around the submerged discharge of the King Abdullah University of Science and Technology (KAUST) seawater reverse osmosis (SWRO) plant by a combination of biological and hydrological studies.

Possible changes in microbial abundance were assessed by using flow cytometric (FCM) analysis on a single-cell level in 107 samples, taken from the discharge area, the feed-water intake area and two control sites. Results indicate that changes in microbial abundance in the near-field area of the KAUST SWRO outfall are minor and appear to be the result of a dilution effect rather than a direct impact of the concentrate discharge.
In order to also investigate potential impacts on higher order organisms, a long-term in-situ salinity tolerance test at the discharge site was conducted on the coral *Fungia granulosa* and its photophysiology. The corals were exposed to elevated levels of salinity as a direct result of concentrate discharge. Their photosynthetic response after exposure to extreme salinity conditions around the full-scale operating SWRO desalination discharge was measured. A pulse amplitude modulated (PAM) fluorometer was used to assess photochemical energy conversion in photosystem II (PSII) measured under constant concentrate discharge conditions. Based on a literature review, we anticipated distinct impairment of photosynthetic characteristics as a response to elevated salinity levels. We also expected particularly quick indications of bleaching for the specimens exposed to the highest salinity levels. The hypothesis was strongly rejected as symbiotic dinoflagellates of *Fungia granulosa* demonstrated high tolerance to hyper saline stress as measured by effective quantum yield of PSII (ΔF/Fm’) during this study.

A series of propulsion driven autonomous underwater vehicle (AUV) missions with velocity and salinity measurements were used for possible plume detection and evaluation of the discharge. The Cornell Mixing Zone Expert System (CORMIX) was additionally utilized in order to assess discharge performance under different ambient velocity magnitudes. Results show that AUV missions could provide significant insight with regards to plume identification and effluent discharge environmental impact studies. Combined with robust in-situ field measurements, models and expert systems were used to evaluate possible impacts on the marine environment in comparison with regulatory mixing zones and dilution criteria.
Based on the findings and existing environmental governance (national and international), a revised regulatory framework for mixing zones within the Kingdom of Saudi Arabia is recommended.
ACKNOWLEDGEMENTS

This thesis is dedicated to Alfred.

I would like to thank the following individuals for their support and contributions:

My supervisor, Prof. Gary Amy. Thank for your straightforward advice and professional approach in assisting me as I progressed through my PhD. I also thank you for the freedom to choose an inimitable research path and your willingness to contribute greatly towards my career advancement, continuously, and without any reservations.

My co-supervisor, Dr. Sabine Lattemann. Your insights in all aspects of the research projects, your knowledge of marine environmental impacts and monitoring systems as well as your detailed comments have been invaluable. I am indebted for your advice and contributions during the last 3 years. Thank you for believing in me.

I acknowledge all my co-authors, contributing extensively in their areas of expertise. Their profound knowledge and assistance were fundamental to the completion of this PhD thesis.

I would like to thank King Abdullah University of Science and Technology (KAUST) for providing me the generous fellowship, which granted me the opportunity to immerse myself full-time into these research activities.

Thanks are due to Dave Pallett for his willing assistance during diving operations, many times in atrocious conditions. I sincerely appreciate your commitment to getting the job done—every time. It is a pleasure diving with you.

I would like to extend thanks to the KAUST SWRO plant and personnel for providing regular and robust data and their interest to assist in on-site sampling events.

Arauna, Layla and Benjamin. You define my existence. Thank you for your unconditional love, cheering me on, and sharing in moments of joy and success.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>KAUST</td>
<td>King Abdullah University of Science and Technology</td>
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<tr>
<td>WDRC</td>
<td>Water Desalination and Reuse Center</td>
</tr>
<tr>
<td>KSA</td>
<td>Kingdom of Saudi Arabia</td>
</tr>
<tr>
<td>MSF</td>
<td>Multi-stage flash distillation</td>
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<tr>
<td>SWRO</td>
<td>Seawater reverse osmosis</td>
</tr>
<tr>
<td>PME</td>
<td>Presidency for Meteorology and Environment</td>
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<tr>
<td>GER</td>
<td>General Environmental Regulations and Rules for Implementation</td>
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<tr>
<td>EIA</td>
<td>Environmental impact assessment</td>
</tr>
<tr>
<td>RMZ</td>
<td>Regulatory mixing zone</td>
</tr>
<tr>
<td>RCJY</td>
<td>Royal Commission for Jubail and Yanbu</td>
</tr>
<tr>
<td>BAT</td>
<td>Best available technologies</td>
</tr>
<tr>
<td>BEP</td>
<td>Best environmental practices</td>
</tr>
<tr>
<td>MOPM</td>
<td>Ministry of Petroleum and Mineral Resources</td>
</tr>
<tr>
<td>CORMIX</td>
<td>Cornell Mixing Zone Expert System</td>
</tr>
<tr>
<td>NFR</td>
<td>Near-field region</td>
</tr>
<tr>
<td>FCM</td>
<td>Flow cytometry</td>
</tr>
<tr>
<td>GPS</td>
<td>Global positioning system</td>
</tr>
<tr>
<td>ADV</td>
<td>Acoustic Doppler velocimeters</td>
</tr>
<tr>
<td>BD</td>
<td>Becton, Dickinson and Company</td>
</tr>
<tr>
<td>SG</td>
<td>SYBR Green I</td>
</tr>
<tr>
<td>SGPI</td>
<td>SYBR Green propodium iodide</td>
</tr>
<tr>
<td>NDL</td>
<td>No decompression limit</td>
</tr>
<tr>
<td>SDI</td>
<td>Silt density index</td>
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<tr>
<td>REMUS</td>
<td>Remote Environmental Measuring UnitS</td>
</tr>
<tr>
<td>AUV</td>
<td>Autonomous underwater vehicle</td>
</tr>
<tr>
<td>LBL</td>
<td>Long baseline</td>
</tr>
<tr>
<td>DR</td>
<td>Dead reckoning</td>
</tr>
<tr>
<td>DVL</td>
<td>Doppler velocity log</td>
</tr>
<tr>
<td>INS</td>
<td>Internal navigation system</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
<tr>
<td>MLWS</td>
<td>Mean low water spring</td>
</tr>
<tr>
<td>SSEI</td>
<td>Significant economic importance</td>
</tr>
<tr>
<td>PAM</td>
<td>Pulse amplitude modulated (fluorometer)</td>
</tr>
<tr>
<td>PSII</td>
<td>Photosystem II</td>
</tr>
<tr>
<td>WET</td>
<td>Whole effluent toxicity</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetically active radiation</td>
</tr>
<tr>
<td>LED</td>
<td>Light-emitting-diode</td>
</tr>
<tr>
<td>$\Phi_{\text{PSII}}$</td>
<td>Effective quantum yield of photosystem II</td>
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An increasing water demand and lack of renewable natural water resources in Saudi Arabia will result in a greater dependence on desalination and consequently amplify impacts on the marine environment, especially in near-field areas of desalination discharges. The discharge of concentrate from seawater reverse osmosis (SWRO) plant operations into the marine environment may adversely affect water quality in the near-field area surrounding the outfall. In order to assess such impacts, an extensive research project was conducted, involving three different modules or experiments of which the results are presented in this thesis.

The existing SWRO desalination plant at the King Abdullah University of Science and Technology (KAUST) was selected for the case studies. The plant is located on the KAUST campus (located approximately 90 km north of Jeddah). Under current operational conditions (January 2014), the raw water intake is on the order of 2,825 m$^3$/hour (67,800 m$^3$/day) with a recovery ratio of 39% (product water: 26,442 m$^3$/day), resulting in an average concentrate flow of 1,723.25 m$^3$/hour (41,358 m$^3$/day) that is discharged to the Red Sea. The facility is small compared to full-scale SWRO and thermal plants, which can reach capacities up to 500,000 m$^3$/day or more than 1,500,000 m$^3$/day in single locations, respectively. The submerged outfall (discharge structure) is located at 22$^\circ$ 17.780N, 39$^\circ$ 04.444E and sits at a water depth of 18 m, approximately 2.8 km offshore. This was also the study area for the research modules/experiments.

Notwithstanding the fact that environmental impacts on receptors as a result of concentrate discharges from desalination plants have been published in scientific literature, very few studies have demonstrated scientific monitoring data and results, based on *in-situ* experiments. Additionally, many datasets are vague with respect to sampling and statistical techniques applied. These deficient statistics and lack in supporting data are necessitating continued research into the ecological effects, mitigation measures and appropriate monitoring systems.

The Kingdom of Saudi Arabia (KSA) has acted with urgency to develop a comprehensive framework for measuring and monitoring activities that cause environmental degradation. The principle national environmental regulatory body within KSA, the Presidency for Meteorology and Environment (PME) has recently revised
measures to ensure efficient use of natural resources, prevention of depletion, and implementation of sustainable development. Saudi Arabia has adopted a comprehensive list of environmental standards and guidelines. The PME recently implemented (March 2012) the revised ambient water quality- and discharge standards with a prescribed salinity concentration of $\Delta 0\%$ above ambient salinity levels for high-value and marine classified areas, and $\Delta 2\%$ above ambient for industrial classified zones. The standards apply at the edge of a regulatory defined mixing zone (discussed in Chapter 3). The fact that the national regulator has adopted a number of vigorous regulations indicates that it is taking environmental issues more seriously, although the current regulations may need further adjustment.

It is widely accepted that SWRO concentrate discharge has the potential to cause significant impacts on the marine environment if not well designed and managed—whereas our results seem to reject this hypothesis—at least, for the near-field discharge area of the relatively small KAUST SWRO plant based on in-situ monitoring, utilizing state-of-the-art equipment and processes for marine environmental impact assessment.

**Research Objectives**

The main research objectives of this thesis were:

- To assess possible changes in microbial abundance as a result of concentrate discharge into the near-field area (<25 m) using flow cytometry (FCM);
- To conduct a long-term in-situ salinity tolerance test on higher order organisms native to the area, using the coral *Fungia granulosa* and its photophysiology;
- To combine a series of propulsion driven autonomous underwater vehicle (AUV) missions with stationary velocity and salinity measurements for the effective evaluation of the hydrological characteristics in the discharge area;
- To apply a mixing zone model to the discharge characteristics, the mixing characteristics of the site, and the exiting discharge technology to determine plume conditions at the boundary of the specified RMZ;
- To suggest recommendations for improved outfall designs and a revised regulatory framework with regards to concentrate discharge regulations and mixing zones within KSA.
Thesis presentation

This thesis contains 4 chapters, which are based on peer-reviewed scientific journal publications and international conference proceedings.

Chapter 1 is a condensed version of the original publication on environmental governance and its effects on concentrate discharge from desalination plants in the Kingdom of Saudi Arabia. Since the publication of this paper (June 2012) the Kingdom’s awareness of environmental issues has increased and Saudi Arabian environmental laws have notably been revised, showing Saudi Arabia’s urgency to develop a comprehensive framework for monitoring and regulating activities that may cause environmental degradation, also in the marine environment.

Chapter 2 deals with flow cytometric (FCM) analysis to assess possible changes in microbial abundance as a result of concentrate discharge from SWRO plants into the near-field area surrounding the outfall. As aquatic microbial communities respond very rapidly to changes in their environment, they can be used as indicators for monitoring ambient water quality.

Chapter 3 focuses on the combination of autonomous underwater vehicle (AUV) missions with stationary velocity and salinity measurements in order to evaluate how the existing SWRO desalination discharge may affect the hydrological conditions in the mixing zone. Based on the monitoring data, we calculated expected dilution ratios (using line plume equations) and also applied the Cornell Mixing Zone Expert System (CORMIX) to predict plume conditions at the boundary of the mixing zone. In order to assess likely environmental variability, we also conducted a sensitivity analysis to investigate mixing zone compliance over a range of ambient conditions.

Chapter 4 describes a long-term (29 days) salinity tolerance test, conducted in-situ at the KAUST SWRO outfall on the coral Fungia granulosa. The corals were exposed to elevated levels of salinity as a direct result of concentrate discharge. Their photosynthetic response in photosystem II (PSII) was assessed using a pulse amplitude modulated (PAM) fluorometer (DIVING-PAM, Walz) under constant concentrate discharge conditions.
Chapter 1

A review of environmental governance and its effects on concentrate discharge from desalination plants in the Kingdom of Saudi Arabia

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Abstract:

The likely environmental impacts of desalination plant concentrate discharges (in most instances twice the concentration of the ambient environment) on local marine ecosystems have been controversially discussed for many years. Additionally, an increasing water demand and lack of renewable natural water resources in Saudi Arabia will result in a greater dependence on desalination and consequently amplify impacts on the marine environment and multifactorial ecosystems in near-field areas of desalination discharges. Accurate scientific baseline data should address information on intake- and outfall locality, brine (concentrate) discharge and chemical characteristics (i.e., effluent concentration, mass flow rates (flux)), local effects, and even cumulative effects of desalination activities, at least to a regional and even national scale. Even if such data is available, it is in many cases non-transparent and not accessible, or it is overlooked as a result of ambiguous desalination related policies. This paper focuses on national environmental regulations in the Kingdom of Saudi Arabia (KSA) and how it impacts concentrate discharge into receiving waters.

Keywords: Environmental impact assessment; Seawater desalination; Environmental regulations; Concentrate discharge; Marine ecosystems.

This chapter is based on the following peer-reviewed published paper:

1.1 Introduction

The scarcity of available water resources along with inadequate rainfall or prolonged droughts has stimulated seawater desalination projects across numerous areas of the world [1]. However, desalination operations may lead to environmental impacts mainly as a result from the concentrate produced together with residual chemicals been discharged into the sea. This can contaminate aquatic ecosystems and may cause unfavorable impacts of far reaching ecological effect on the marine watery communities [2]. The acutest effects are localized in closeness to these concentrate discharges and may cause deteriorations of local hydrography and water quality of the receiving medium which interfere directly on the physical processes of the biotype, such as enzymatic activity, nutrition, reproduction, breathing and photosynthesis [3].

There are various methods for the disposal of the concentrate, but ocean brine disposal is considered to be the least expensive option [4], [5]. If the concentrate is discharged into the sea, the density difference between the concentrate and seawater induce the formation of a stratified water column which can affect previously stable salinity environments [6]. The magnitude of the impact will depend on the characteristics of the desalination plant and its reject effluent waste stream, physical and hydro geological factors, bathymetry, waves, currents, depth of water column, etc. These factors would determine the extent of the mixing zone and therefore the amplitude of impact [3], [7], [8].

Despite the fact that environmental impacts on receptors as a result of concentrate discharges from desalination plants have been published in scientific literature, little measured data of the concentrate characteristics have been published [9]. In-situ data of
marine environmental impacts is also intermittent or regarded as confidential information. Existing environmental policies are generally centered on broad-based principles and do not include environmental requirements and guidelines for desalination specific criteria, e.g. effluent- and ambient characteristics, pretreatment, intakes, outfalls, or compliance and monitoring programs.

The need for safer methodologies with regards to concentrate discharge was already highlighted more than two decades ago, as well as fair and pragmatic regulations concerning effluent- and ambient standards, for the application of desalination processes [10]. More recently, concentrate characteristics and their possible marine impacts have been mainly discussed by academia, ecologists and to some extent by the environmental regulators. In addition, monitoring data of dispersion and effects of the hyper-saline effluents originated by desalination plants are very scarce [9], [11]. In the majority of instances where data is made available, it generally indicates effluent standards only, with ambient standards entirely absent. Taking into consideration the role and importance of water for sustenance of humanity, any policy encompassing the issues of water must include a comprehensive coverage of desalination specific regulations [12], currently deficient in governance of the major desalination user countries.

1.2 Impact on the environment

The concentrate as waste stream is a high saline solution that must be disposed of, mainly by discharging it back to the marine environment. Most of the impact on the marine environment is a consequence of the positioning of both the intake- and discharge locations. If the operation requires submerged piping elements, the initial impact during the laying of pipes on the seabed is temporary and confined to the location of works, but
even this impact—if not mitigated—may still be significant. The severity of the impact is a function of the level of disturbance to the environment and of the natural sensitivity, which in turn is dependent on the specific nature of the habitat and specific communities.

The increased salinity (associated with membrane and thermal desalination technologies) and temperature (associated with thermal desalination technologies only) are not ‘pollutants’ in the classical sense, but salt concentrations and temperature values that deviate strongly from ambient levels can still be harmful to marine life. Changes in salinity, turbidity and the presence of chemicals are vital parameters that influence the distribution of marine species. Species can typically adapt to minor deviations from these conditions and might even tolerate extreme situations temporarily, but will not withstand harsh environmental conditions in the long term. Reject streams of desalination plants with high levels of ‘pollutants’ can be fatal to marine life and can cause a lasting change in species diversity and abundance in the discharge site. Marine organisms can be attracted or repelled by the new environmental conditions, and those more adapted to the new situation will eventually prevail in the discharge zone. This will result in a change in the biocenosis, however, will more likely be accompanied by an overall decline in biodiversity [8]. If the concentrate has a higher density than seawater, it will likely spread over the sea floor (unless it is dissipated by an adequate outfall system) where it might affect benthic habitats [13]. Consequently, adverse impacts may occur on the composition and distribution of the marine biota in the disturbed near-field areas especially when high levels of concentrate discharges coincide with the sensitive ecosystems. Previous ecological monitoring studies have found variable effects ranging from no significant impacts to benthic communities, through to widespread alterations to
community structure in other organisms, mainly if concentrate is released to poorly flushed environments [14]. Although environmental effects appear to be limited to close proximities of outfalls it must be noted that a large proportion of published work provides little quantitative data that could be assessed independently. Abrupt changes in ambient water quality as a result of concentrate discharge may be an important controlling factor for the distribution of marine species, which normally can be found in marine habitats that provide favorable environmental conditions for specific ecotypes [15].

1.3 Environmental regulators

Currently, the total global seawater desalination capacity is on the order of 65.5 million m$^3$/day$^1$. Saudi Arabia has the largest seawater desalination capacity and accounts for almost one fifth of the global seawater desalination capacity. Multi-stage flash (MSF) distillation and seawater reverse osmosis (SWRO) are the two major processes in the Kingdom, with MSF having around two thirds of the total installed desalination capacity [12]. SWRO is by far prevalent outside of the Middle East where membrane processes accounts for nearly two thirds of contracted capacity. Numerous desalination plants are attempting to conform to effluent discharge regulatory standards, but are lacking monitoring programs and corresponding datasets when it comes to the effect of concentrate discharge to the receiving water (ambient standards). Additionally, many datasets are vague with respect to sampling and statistical techniques applied. These deficient statistics and lack in supporting data are necessitating continued reports on ecological effects, mitigation measures and appropriate monitoring systems. The

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$^1$ International Desalination Association (2011).
impact on sensitive receptors necessitates continued emphasis and monitoring in (at least) near field areas of concentrate discharges.

### 1.3.1 The Presidency for Meteorology and Environment (PME)

The principal National Environmental Regulatory body within Saudi Arabia is the Presidency of Meteorology and Environment (PME). Protection of the environment is inscribed in the Kingdom’s Basic Law of Governance, which is effectively KSA’s constitution. Article 32 states that:

“The State shall endeavor to preserve, protect and improve the environment and prevent its pollution”.

Since the publication of this paper and as the Kingdom’s awareness of environmental issues increased, Saudi Arabian environmental laws have notably developed and have been revised, showing Saudi Arabia’s urgency to develop a comprehensive framework for measuring and monitoring activities that cause environmental degradation. The framework also includes revisions for the sustainable management of ambient water quality by protecting the water supply and the natural aquatic environment. The new regulations apply to all coastal and underground water and include any surface freshwater (permanent or temporarily). The National Ambient Water Quality Standards apply to all coastal and underground waters in KSA and include any surface freshwater that may be permanent or temporarily. This standard revises the General Standards for the Environment (specifically document number 1409-01) issued by the Presidency of Meteorology and Environment (PME) with effect on March 24, 2012. Desalination operators discharging concentrate to the marine environment are now
subject to the revised Wastewater Discharge Standard (as of March 24, 2012). It aims at enabling Saudi Arabia to reach its ambient water quality objectives and sets out use-related criteria and specific limits on individual discharges designed to protect water quality. Requirements for regulatory mixing zones (RMZ) as stipulated by the latest version of the National Ambient Water Quality Standard for KSA is discussed in Chapter 3 as part of this thesis.

It is however evident that a lot of the environmental legislation have been adopted on an ad-hoc basis which is demonstrated by the fact that environmental laws are either contained in laws relating to other substantive issues, or adopted by ratifying or signing international/regional conventions regarding certain specific environmental issues; e.g. Saudi Arabia is a signatory to the 1992 Regional Convention for the Conservation of the Red Sea and Gulf of Aden Environment (commonly referred to as the Jeddah Convention). The Jeddah Convention seeks, in broad terms, to preserve and protect the special hydrographic and ecological characteristics of the marine environment of the Red Sea and Gulf of Aden. Because a number of the revised environmental quality objectives for ambient water quality as well as the discharge limits for effluents have been ‘adopted’ elsewhere, some of the suggested concentrations and values might not be feasible—especially when centered on standards without requirements and guidelines for desalination specific criteria.

The two principal regulatory controls in relation to environmental law are as follows:

1.3.1.1 Public Environmental Law
The Public Environmental Law was enacted by Royal Decree No. M/34 dated 28/7/1422 Hejri (corresponding to 16 October 2001), and was published in the Official Gazette number 3868 dated 24/8/1422 Hejri (corresponding to 9 November 2001). The Public Environmental Law creates a general regulatory framework for the development and enforcement of environmental rules and regulations, and assigns general responsibility for this to the PME.

1.3.1.2 General Environmental Regulations (GER) and Rules for Implementation

These regulations were issued by the Minister of Defense and Aviation, and in addition to its responsibilities under the Public Environmental Law, the PME is made responsible for issuing or withholding its consent for projects so as to ensure compliance with the Public Environmental Law and the Implementing Regulations. Under the Implementing Regulations, any licensing authority (i.e., any other authority, other than PME, that is responsible for issuing a permit to projects that may have a negative impact on the environment) must ensure that an environmental impact assessment (EIA) is conducted by a PME registered environmental consultancy (at the expense of the client) during the feasibility study of any project.

Projects that may have a negative impact on the environment are separated into three categories. The GER stipulate the “Fundamentals and Standards for EIA of Industry and Development Projects” under Appendix 2 of the Rules for Implementation, whilst Appendix 2.1 dictates the “Guidelines for Classification of Industrial and Development Projects” under the three categories. The method of assessment will depend on the classification of the project based on the level of expected impacts.
• Category_1: This includes projects that are not expected to have significant environmental impacts;

• Category_2: This category covers projects that may have significant environmental impacts (normally, impacts are restricted to the site boundary and can be fully mitigated); and

• Category_3: These are projects whose construction or operation activities are likely to have significant adverse environmental impacts, which cannot be fully mitigated, will produce off-site emissions or discharges and will impact zones beyond the site boundary (the PMEs GER stipulate that desalination plants should be regarded as Category_3 projects).

1.3.2 The Royal Commission for Jubail and Yanbu

The Royal Commission for Jubail and Yanbu (RCJY) has a special status in the environmental legislative system of KSA. It is responsible for the planning, development, construction, operation and maintenance of the various infrastructure and services of Jubail (including Ras Al-Khair, located approximately 60 km north of Jubail) and Yanbu industrial cities, and in particular, the encouragement of downstream industries that utilize Saudi Arabia’s natural resources to produce value added products for local use and export. These ever-expanding industrial complexes and their industries require huge amounts of process water, in almost all instances acquired from the adjacent marine environment (Arabian Gulf and the Red Sea, respectively).

Established in 1975, the RCJY is responsible for providing the complete infrastructure, both physical and societal, needed to construct and operate the huge
industrial developments at Jubail, Ras Al-Khair and Yanbu. It is in charge of community and human resources development, environmental protection and the development of private-sector investments in these industrial cities. They have developed and adopted regulations, standards and guidelines to control substances emitted, discharged, or deposited, and noise generated within the industrial cities. The environmental regulations, standards and guidelines are specific to these industrial cities. These are intended to clearly state the environmental protection regulations and to formally define the requirements for adherence to them. They are solely responsible for overseeing and controlling pollution associated with the development and operation of these industrial complexes.

An optimistic objective of the RCJY has been industrialization coupled with environmental protection. Since inception, the RCJY has been determined that these ‘cities’ would be models of environmental planning and management in addition to being productive manufacturing complexes. The RCJY has realized that there must be a close cooperation between industries and environmental management personnel in order to achieve this goal. The RCJY has issued the Royal Commission Environmental Regulations (RCER) to be adopted by industries both in Jubail (including Ras Al-Khair) and Yanbu. Any facility operating or planning to operate on the Royal Commission property will be required to comply with these regulations\(^2\). They demonstrate their own environmental regulations under their Environmental Control Department (ECD) which monitors the industrial cities’ ambient air quality and emissions, water resources as well as marine monitoring, with particular focus on the cooling water discharge areas. In

\(^2\) Royal Commission Environmental Regulations (RCER-2010, Volume I, Regulations and Standards)
addition, the Royal Commission expects operators in the industrial cities to utilize Best Available Technologies (BAT) for environmental control. BAT must as a minimum achieve emission or discharge standards in RCER taking into account energy, environmental and economic impacts. BAT assessment is conducted for new, reconstructed and modified facilities. If an operator is not in compliance with RCER, the operator of a facility must provide an assessment of BAT to address environmental issues that were identified by the Royal Commission as posing a direct detrimental environmental or public health impact.

RCER states that the Royal Commission reserves the right to enter and access the facility, upon reasonable prior notice of at least 24 h, for the purpose of regular surveillance, monitoring, and inspection to verify compliance with regulations. The operator must also facilitate the Royal Commission, upon reasonable request, to review all environmental related records, methods and procedures to verify compliance with the RCER. Based on merit, this is the major dissimilarity between the RCER and the PME’s GER.

1.3.3 The Ministry of Petroleum and Mineral Resources

The Ministry of Petroleum and Mineral Resources (MOPM (located in Riyadh, Saudi Arabia)) was established in 1960 to execute the general policy related to oil, gas and minerals within the Kingdom of Saudi Arabia. MOPM is responsible for national planning in the area of energy and minerals, including petrochemicals by overseeing affiliate companies’ activities from exploration and development to refining and distribution. All oil, gas and petrochemical related EIAs are dealt with and approved or disapproved by the Ministry (including such projects within the RCJYs jurisdiction). All
the remaining sectors’ (outside of jurisdiction of RCJY) EIAs are sent to the PME for approval. Specifically, the Ministry monitors the activities of Saudi Aramco (together with the Supreme Council for Petroleum and Minerals), Saudi Texaco, Aramco Gulf Operations, the Saudi Arabian Mining Company (Ma’aden) and also oversees the Saudi Geological Survey.

1.4 The current status of the regulatory framework

The GER has to some extent standardized the EIA process, when compared to assessments prior to the release of these regulations. Although the GER consequently standardized the EIA process under the PME regulations, the standard however does not provide guidance on the depth of the assessment, which is a major issue of concern when it comes to the quality and scientific significance of the findings of the EIAs. A major inadequacy in the Kingdom’s environmental governance is—although legal requirements have been enacted (revised or not)—they are not enforced. The enforcement of environmental policies and procedures requires the availability of independent bodies with adequate power. The environmental legislative system in the Kingdom of Saudi Arabia would benefit from the establishment of an independent regulatory body, which is equipped with sufficient staff and trained personnel as well as institutional rights to enforce the various environmental policies and procedures. Although the PME, RCJY and MOPM are accepted as competent authorities for EIA and environmental acceptability, their role and responsibilities could further be strengthened.

The major shortcoming however is the non-existence of tailored desalination-specific regulations. Desalination-focused regulations based on scientific criteria should be generated and implemented. Notwithstanding the fact that the PME recently revised
the ambient water quality- and discharge standards, it might still be in need of amendment as the PMEs prescribed salinity concentration of $\Delta 0\%$ above ambient salinity levels for high-value and marine classified areas, and $\Delta 2\%$ above ambient for industrial classified zones is not feasible. A $\Delta 0\%$ increase above ambient is unachievable as this means there must be infinite dilution in the mixing zone, or you allow only a discharge with the ambient salinity value. This is significant, taking into consideration growing desalination capacities in the Kingdom and the appropriate environmental management associated with concentrate discharge. In addition to these deficiencies, there is also a lack of robust up-to-date scientific baseline data in order to support reports on ecological effects, mitigation measures and appropriate marine monitoring systems.

1.5  **Recommendations**

1.5.1  **Attaining sound environmental data**

- Data, which is currently scattered amongst numerous government and private sector institutions should be pooled in a central database to improve collaboration and assessment;
- The national focal point should be provided with full access to data that are required for national assessment and reporting;
- Data should be available to all agencies and shared among major stakeholders;
- Facilities should be required to cooperate in order to allow for assessment of cumulative effects in sea areas with high desalination densities;
- Resources (financial, trained personnel, etc.) of the environmental regulatory system should be improved.
1.5.2 Strengthening the assessment system

- The capability of the technical guidelines, responsibilities, EIA report content and data control should be improved;
- Explicit desalination regulations should be generated, adopted and enforced;
- Implementation of perpetual monitoring systems at desalination intake and discharge locations;
- Follow coherent legal requirements and the actual implementation thereof;
- There must be a holistic coverage of environmental impacts on receptors as part of the decision making process for locating and building new desalination plants, or expanding existing facilities;
- The establishment of a national task force can also improve environmental standards for desalination plants.

The responsibility belongs to the environmental regulatory bodies (PME and RCJY), desalination operators as well as academia, to be active participants in the process by collecting and maintaining data that is transparent and available for all to share. Research centers (e.g. KAUST WDRC) play a very important role in providing scientific data in minimizing the impacts of desalination plants, not only regionally, but also globally. It is a big challenge to develop and implement cost-effective desalination techniques with a minimum adverse impact on water quality and environment. It is recognized that implementation of increased environmental measures will have an impact on cost, which is a consequential consideration. These costs should be evaluated with respect to long-term costs associated with a ‘doing nothing’ approach, particularly with
regards to adopting appropriate environmental measures whether in the construction of new ‘greenfield’ plants or retrofitting ‘brownfield’ plants currently in operation.

A national task force could also initiate a dialogue and seek cooperation with bordering countries in order to develop regional standards for desalination plants in order to safeguard the environmental protection of the shared marine water bodies of the Arabian Gulf and the Red Sea. For example, the Water and Power Research Center of Abu Dhabi Water and Electricity Authority [15] has set up procedures to be followed on the study of environmental feasibility of building or extending the capacity of desalination plants. Sharing this information among the riparian states of the Arabian Gulf and the Red Sea could be a first step in developing regional guidelines. A summary of procedures below:

1.5.3 Baseline data collection and record keeping

Baseline field measurements should (at least) consist of:

- Hydrodynamic field measurements should be carried out and should include water levels, current flow velocities and directions of flow discharges. The hydrodynamic measurements must be used in understanding the flow pattern in the discharge vicinity and in the calibration of the hydrodynamic model;

- Water quality measurements should be carried out to evaluate the concentrations of the substances (residual chlorine, dissolved oxygen, ambient seawater temperature, salinity, pH, ammonia, etc.) and how the may affect the water quality and marine species;

- A biological survey should be carried out at the intake and discharge locations in order to evaluate the ecosystem in the area. A detailed sampling protocol
(grid) should be generated and the area thoroughly surveyed. Divers, utilizing adequate underwater cameras, should record photos and videos on the grid. This data should provide a detailed description of local habitats and species.

1.5.4 Development of numerical flow- and water quality models

Flow velocity and flow pattern is the main transport and dispersion mechanism of the concentrate from the discharge point. A numerical flow model (e.g. the Cornell Mixing Zone Expert System (CORMIX)) simulates the flow pattern and assists in the configuration of the intake and outfall of the plant. Deep submerged discharges are generally preferred. Numerous design practices favor a steep discharge angle of 60° above horizontal (however, laboratory experiments and supplemented data combined with a model tool (CorJet, a jet integral model within CORMIX), suggest that flatter discharge angles of about 30° to 45° above horizontal may have considerable design advantages (true for discharges in strongly inclined environments [16])). The goal of water quality modeling is to simulate the water quality of the waters around the discharge. The flow pattern from the hydrodynamic model could be used as an input for the water quality model as it is the main transport mechanism of the substances.

1.5.5 Habitat evaluation procedures

The effect of the water quality change as a result of the concentrate discharge must be evaluated against the nature of the habitat in the discharge vicinity (baseline). This can also be done by comparing baseline data vs. operational data and combining it with concentration measurements of substances and comparing this to toxicity data or species-specific thresholds. If the study shows that the plant discharge will affect receptors, measures should be taken to minimize the impact, e.g. changing discharge
configurations to redistribute the substances in the concentrate in such a way to reduce their concentrations to an acceptable level.

1.6 Conclusions

Current deficient statistics and lack in supporting data are necessitating continued reports on ecological effects, mitigation measures and appropriate monitoring systems. Regulators must impose stricter policies on marine- and effluent water quality monitoring programs at existing and new desalination facilities. The desalination process has a vital role in meeting the ever-increasing demand for water by various sectors in Saudi Arabia and also in the wider Middle Eastern region, where the use of conventional water resources has reached critical limits. The environmental agencies (PME, RCJY and MOPM (as well as Saudi Aramco as *de facto* regulator)) are in theory ultimately responsible for setting environmental regulations and standards. These responsibilities are in practice fragmented, which creates institutional overlaps and contradictory interpretations regarding environmental protection practices, implementation and enforcement. The existing environmental policies are generally centered on broad-based principles and do not include environmental requirements and guidelines for desalination specific criteria, e.g. effluent- and ambient characteristics, pretreatment, intakes, outfalls, or compliance and monitoring programs. There remains an inescapable need to strengthen the environmental legislation and regulatory framework within the Kingdom. This could be addressed through the establishment of a task force (for improvement and consistency of national practices, regulations and also to initiate adoption and implementation of cross-border desalination regulations.
References


Chapter 2

Flow cytometric assessment of microbial abundance in the near-field area of seawater reverse osmosis concentrate discharge

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Abstract:

The discharge of concentrate and other process waters from seawater reverse osmosis (SWRO) plant operations into the marine environment may adversely affect water quality in the near-field area surrounding the outfall. The main concerns are the increase in salt concentration in receiving waters, which results in a density increase and potential water stratification near the outfall, and possible increases in turbidity, e.g., due to the discharge of filter backwash waters. Changes in ambient water quality may affect microbial abundance in the area, for example by hindering the photosynthesis process or disrupting biogenesis. It is widely accepted that marine biodiversity is lower in more extreme conditions, such as high salinity environments. As aquatic microbial communities respond very rapidly to changes in their environment, they can be used as indicators for monitoring ambient water quality. The objective of this study was to assess possible changes in microbial abundance as a result of concentrate discharge into the near-field area (<25 m) surrounding the submerged offshore discharge of the King Abdullah University of Science and Technology (KAUST) SWRO plant. Flow cytometric (FCM) analysis was conducted in order to rapidly determine microbial abundance on a single-cell level in 107 samples, taken by diving, from the discharge area, the intake area and two control sites. FCM analysis combined the measurement of distinct scatter of cells and particles, autofluorescence of cyanobacteria and algae, and fluorescence after staining of nucleic acids with SYBR® Green for a total bacterial count. The results indicate that changes in microbial abundance in the near-field area of the KAUST SWRO outfall are minor and appear to be the result of a dilution effect rather than a direct impact of the concentrate discharge.
Keywords: Flow cytometry; Microbial abundance; Reverse osmosis; Concentrate; Near-field; Environmental impact.

This chapter is based on the following peer-reviewed paper:

2.1 Introduction

Seawater reverse osmosis (SWRO) desalination is expanding globally as a means to provide potable water [1]. While the societal benefits of desalination are well recognized, concerns are often raised over potential adverse environmental impacts of SWRO plants, especially with regards to the possible adverse effects of concentrate discharge (in this paper ‘concentrate’ refers to the brine of SWRO plants as waste stream and the process effluents discharged with it into a marine environment). It is widely suggested that the concentrate discharge may result in negative impacts on the marine environment, but what is stated about the issue is in many cases not based on actual monitoring in the discharge sites of SWRO plants [2]. Most important in this respect are the possible changes in species composition, species diversity and population density with the most acute effects in the vicinity of the discharge outlet (near-field) [3]. Salinity is generally considered to be a dominant environmental factor regulating aquatic community structure, but it is not well understood whether this is primarily due to the direct physiological effect of salinity stress [4]. Existing literature has postulated that microbial research in saline environments could contribute to the study of the broad scale distribution of microbial communities and help to discern the potential factors underlying microbial diversity patterns in extreme environments [5]. Thus, it is expected that microbial studies along salinity gradients could shed additional light on this topic. The majority of current studies investigated the influence of salinity on the distribution of microbial diversity on temporally dynamic saline systems [6], whereas virtually nothing has been reported on microbial abundance in the near-field areas of hyper saline concentrate discharges from desalination.
Flow cytometry (FCM) has previously been used as a monitoring methodology in aquatic microbial ecology, diversity and viability. Compared with other techniques, FCM facilitates rapid data acquisition and multi-parameter analysis, leading to increased popularity and widespread applications, including studies of bacterial cell cycles and microbial monitoring in seawater [12]. The use of FCM in seawater microbial analysis has important potential and marine scientists are only beginning to discover these possibilities. Microbial cells can be detected by FCM irrespective of their cultivability, which overcomes a major obstacle in the study of microbiology. In this study we discuss how FCM data can be used, based on the differentiating properties of photosynthetic and non-photosynthetic cell properties, count, size and fluorescence in order to evaluate relative changes in microbial abundance as a result of SWRO concentrate discharge.

2.2 Materials and methods

2.2.1 Case study

The existing SWRO facility at the King Abdullah University of Science and Technology (KAUST) was selected as a case study. The facility was designed to provide all potable water needs for the campus as well as the residential areas. The plant has a product-water capacity of 40,000 m³/day but is currently not operating at full load. On average, the raw water intake is 55,920 m³/day with a recovery ratio of 37%, resulting in an average concentrate flow of 35,230 m³/day (August 2012) that is discharged to the sea. The outfall (discharge structure) is located at 39° 04.444E, 22° 17.780N and sits at a water depth of just below 18 m, approximately 2.8 km from the pump station (Figure 2.1a). The concentrate is pumped through a 1,200 mm diameter pipeline to the offshore structure (Figure 2.1b) where the concentrate is pushed up in a concrete riser and
discharged horizontally through four discharge screens \((1,800 \text{ mm} \times 1,000 \text{ mm})\) approximately 6 m from the seafloor.

Figure 2.1 a: KAUST SWRO offshore discharge structure locality (source: Google earth). b: Schematic of the underwater discharge structure.
The study was done in three field campaigns which entailed sampling and FCM analysis for four 25 m transects (A, B, C, D) around the discharge structure (Figure 2.2a). The field sampling campaigns were conducted on 08/08/2012 (mm/dd/yyyy), 10/23/2012, 02/18/2013 and 02/19/2013 to represent seasonal variations. Samples were taken by diving at a water depth of 17.6 m (at seafloor level assuming the concentrate, due to its higher density than ambient seawater will spread along the seafloor) in 5 m intervals along each 25 m transect (0 m, 5 m, 10 m, 15 m, 20 m, 25 m) around the discharge structure (Figure 2.2a). Pre-sterilized 15 mL Axygen and Celltreat tubes were used to collect samples which were transported to the laboratory under cold storage and analyzed on the same day of sampling.

Diving as a means of sample collection was selected to ensure exactness of underwater sampling locations (collecting samples from the boat may have been problematic and in all probability inaccurate, taking into consideration wind, currents, drifting and quantifying defined sampling depths at precisely 5 m sampling interims). A maritime quality global position system (GPS) was used to define exact sampling locations as well as a fully integrated swath bathymetry and side scan sonar system (EdgeTech 4600) was utilized in order to determine accurate bathymetric conditions around the outfall (Figure 2.2b). With the deployment of two acoustic Doppler velocimeters (ADVs), consideration was also given to how fast water is moving across the water column, tidal variations and prevailing current direction around the discharge area. In order to account for salinity stratification, samples were also collected at water depths of 5 m (above the outlet structure) and 10 m (directly outside the outlet structure and shown in the results as ‘diffuser opening’). During each of the campaigns, a sample
was also collected at the KAUST SWRO intake for comparison with ambient conditions (‘intake control’).

Additionally, during the 3rd campaign, control samples were also collected at two different dive locations (control-1: 39° 04.343E, 22° 18.074N and control-2: 38° 57.580E, 22° 18.028N). The nearby control site was 583 m (heading: 342° relative to the discharge structure), in similar water conditions (but in the absence of the concentrate) and at the same depth as the discharge structure, while the far-field control was located in close proximity to Al-Fahal reef (North) about 13 km off the Saudi Arabian coast in the Red Sea. This was done in order to compare microbial communities in typical Red Sea water to those of the samples taken at the discharge site and the nearby control site, respectively. Seven samples were collected along a transect of 50 m at both of the control sites (nearby and far-field) and are representative of two opposite 25 m transects as performed around the discharge site. In total, 107 samples (81 at the discharge, 3 at the intake, 14 at the control sites and 9 at the KAUST SWRO plant) were collected and analyzed utilizing FCM.
2.2.2 FCM analysis

Microbial detection systems that rely on cell replication are limited by the requirement to grow bacteria in an artificial environment. Lack of symbiotic partners or an unsuitable micro-environment may result in non-representative plate counts from natural samples. Also, some cells will only grow aerobically, some only anaerobically,
and some under both conditions. Therefore representative counts, which ideally detect healthy, injured, dormant, and ‘viable but sometimes non-culturable’ as well as truly dead bacteria, can only be obtained by direct optical methods [13]. Contrary to microbial detection systems that rely on cell replication, and to FCM’s benefit (in combination with stains), it is capable of measuring all microbial cells, regardless of their physiological state.

FCM facilitates individual measurements of each cell by light scattering and/or fluorescence. Light scattering does not in itself allow discrimination of bacterial cells from other sub-micrometric particles and colloids, which are usually present in natural water samples and which scatter light similarly to bacteria. Light scattering may thus provide information about the particle or cell number and size, however, in order to distinguish bacterial cells from other particles, it is also necessary to measure fluorescence. Lasers are used to either excite the (unstained) autofluorescent organisms (cyanobacteria and algae) and/or the stained bacterial cells, which thereby become excitable by the blue laser. The fluorescence intensity is measured in both cases for each particle using sensitive photomultiplier tubes and is performed at rate of thousands of events per second.

For this study, the flow cytometry measurements were done using a BD Accuri® C6 flow cytometer (Ann Arbor, MI), with 488 nm excitation from a blue solid-state laser. Green emission was collected in the FL1 channel (533 ± 30 nm (with excitation at 488 nm)) and red fluorescence in the FL3 channel (>670 nm). All values were recorded on logarithmic scales. The run limit was set to 50 µL, fluidics on ‘medium’ (35 µL/min; core size 16 µm) and the thresholds were set to 900 nm for red fluorescence in the FL3
channel for measuring the (unstained) autofluorescent phytoplankton (cyanobacteria and algae) and with a threshold of 600 nm for green excitation in the FL1 channel for the total bacterial cell counts (labeled with Invitrogen SYBR® Green I nucleic acid gel stain (supplied as a 10,000× concentrate in dimethyl sulfoxide (DMSO))).

For the autofluorescence measurements, 200 µL of each sample were transferred to a standard 96-well plate (flat bottom) with laser excitation set on red fluorescence in the FL3 channel (>670 nm) and measured in triplicate.

For total bacterial counts, 700 µL of each sample were transferred to sterile Eppendorf tubes, incubated at 35 °C for 10 minutes, stained with SYBR® Green I (SG) nucleic acid gel (7 µL in 700 µL (1:100 dilution)), vortexed and incubated for a second time for a duration of 10 minutes. 200 µL were then transferred to a standard 96-well plate (flat bottom) with laser excitation set for green fluorescence and then measured (also in triplicate). For the SYBR Green Propodium Iodide (SGPI) staining protocol, 495 µL of the seawater samples were transferred to sterile Eppendorf tubes and incubated at room temperature for 15 minutes. 5 µL of 100× SG + 1000 µg/mL PI were added to the seawater samples at room temperature (final dye concentration: 1× SG + 10 µg/mL PI), vortexed and incubated at room temperature in a dark environment for a second time for a duration of 15 minutes. 200 µL were then transferred to a standard 96-well plate and analyzed (FL1, threshold set to 600 nm).

No dilutions were necessary as the events/second did not exceed the performance limit with regards to data acquisition rate (<4,000 events/second recommended). The procedures were quantified with a protocol from [14] and [15] as many stains/dyes are sensitive to the ionic strength of the medium. Also, bacteria living under saline
conditions are likely to have different membranes or membrane behavior than bacteria living in freshwater. Data acquisition was done with the BD Accuri CSampler software (version 1.0.264.21), sample analyses were performed in triplicate and the patterns were reproducible.

2.3 Results and discussions

2.3.1 Establishing the baseline

The data from the first campaign (conducted on 08/08/2012) suggested clear and measurable results in both the autofluorescent cyanobacteria and algae (Figure 2.3) as well as the total bacterial count stained with SYBR Green (Figure 2.4).

Figure 2.3: Abundance of autofluorescent phytoplankton (cyanobacteria and algae) during campaign1. Error bars indicate standard deviation on triplicate measurements.

Figure 2.3 shows that autofluorescent events vary between 107 – 155 events/µL, while significantly less (95 events/µL) were recorded directly at the diffuser opening.
Figure 2.4: Abundance of total bacterial count (stained with SYBR Green) during campaign 1. Error bars indicate standard deviation on triplicate measurements.

FCM, in combination with a nucleic acid stain was used for measuring the total microbial abundance in the different transects as shown in Figure 2.4. Numbers vary between 697 events/µL (transect A6 (25 m)) and 1,189 events/µL (transect D3 (10 m)) while 698 events/µL were recorded at the diffuser opening.

With respect to the FCM data, gating (Figure 2.5) was used to exclude the ‘background noise’. Figure 2.6a–h show FCM density plots of the autofluorescent events (cyanobacteria and algae) in each sample (50 µL) against the cell size as measured by light scattering for the different stations along transect D (Figure 2.6a–f) as well as directly at the diffuser opening (Figure 2.6g) and at a water depth of 5 m above the discharge structure (Figure 2.6h). In order to identify and quantify cell subsets, FCM density plots can be sequentially separated, based on fluorescence intensity, by creating a series of subset extractions (gates). Gating does not change the intensity value assigned to an event, but allows for decision on which data to view and which data to ignore or discard. Subsets can continue to be gated to generate further subsets, until a collection of
cells for which a graphic display or analyzed statistic is required. Note that the
‘proportional’ values (e.g., 36.5% for D6) represent the number of events within the gate
compared to the total (or in other words: 63.5% of events are background noise for D6).
The corresponding absolute values are shown in Table 2.1 (i.e., 36.5% equals 7,744
events for D6).

Figure 2.5: An example of a FCM density plot of autofluorescent events, illustrating the
most important terminologies and the gating strategy that was consistently applied
throughout all samples.

The results show an increase in autofluorescent microbial cell counts away from
the outfall in some transects. The ‘proportional’ values in Figure 2.6a–f increase from
just fewer than 33% (transect station D1 at 0 m distance from the outfall) to 36.5% (D6 at
25 m), corresponding to an increase from 6,496 (D0) to 7,744 (D6) events/50µL in Table
2.1. The sample from the diffuser opening (Figure 2.6g) clearly shows the lowest
number of events (4,746 events/50µL) and only a sample proportional value of 26.5%.
Table 2.1: Autofluorescent events (transect D). Relative standard deviation was calculated on triplicate measurements.

<table>
<thead>
<tr>
<th>Transect D Campaign1</th>
<th>Events/50µL</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6 (25 m)</td>
<td>7,744</td>
<td>1.0</td>
</tr>
<tr>
<td>D5 (20 m)</td>
<td>7,438</td>
<td>2.3</td>
</tr>
<tr>
<td>D4 (15 m)</td>
<td>7,329</td>
<td>2.3</td>
</tr>
<tr>
<td>D3 (10 m)</td>
<td>6,616</td>
<td>2.4</td>
</tr>
<tr>
<td>D2 (5 m)</td>
<td>6,374</td>
<td>4.9</td>
</tr>
<tr>
<td>D1 (0 m)</td>
<td>6,496</td>
<td>1.5</td>
</tr>
<tr>
<td>Diffuser Opening b</td>
<td>4,746</td>
<td>0.9</td>
</tr>
<tr>
<td>5 m Depth c</td>
<td>6,180</td>
<td>5.0</td>
</tr>
</tbody>
</table>

a D1 (0 m) is at seafloor level (depth of 17.6 m), directly at the bottom of the discharge structure.
b The diffuser opening samples were taken directly outside the discharge screen, approximately 6 m from the seafloor (11.6 m from the surface).
c The 5 m depth samples were collected directly above the discharge structure (5 m from the surface).

Figure 2.6: FCM density plots of autofluorescent events (cyanobacteria and algae) for the different sampling stations along transect D (Fig. 2.6a–f), as well as directly at the diffuser opening (Fig. 2.6g) and at a water depth of 5 m above the discharge structure (Fig. 2.6h).

2.3.2 Confirming the baseline

A second campaign was conducted on 10/23/2012 in order to corroborate results from campaign1. The campaign methodology was therefore similar to that of the first
campaign. Figure 2.7 shows the measured results for the phytoplankton, whereas Figure 8 illustrates the same for the total bacterial count after labeling the nucleic acids.

Figure 2.7: Abundance of autofluorescent phytoplankton (cyanobacteria and algae) during campaign2. Error bars indicate standard deviation on triplicate measurements.

The results of autofluorescent phytoplankton from transect D in Figure 2.7 again show an increase in events away from the discharge structure, varying from 96 (D1 (0 m)) to 124 (D6 (25 m)) events/µL. As seen in the first campaign, the lowest concentration (62 events/µL) was recorded at the diffuser opening. In contrast, the SWRO feedwater intake displayed the highest value of 133 events/µL (data not shown).
Figure 2.8: Abundance of total bacterial count (stained with SYBR Green) during campaign 2. Error bars indicate standard deviation of triplicate measurements.

Total cell concentrations were similar in all transects. The highest concentration was recorded at transect C6 (1,149 events/µL) with only 496 events/µL recorded at the diffuser opening. An even higher value of 1,329 events/µL was recorded at the feedwater intake (data not shown). Figure 2.9 shows the FCM density plots of autofluorescent events (cyanobacteria and algae) for the different stations along transect C (Figure 2.9a–f), as well as from the diffuser opening (Figure 2.9g) and at the SWRO intake (Figure 2.9h). ‘Gating’ was again used to exclude the ‘background noise’.

Table 2.2: Autofluorescent events (transect C). Relative standard deviation was calculated on triplicate measurements.
In Figure 2.9a–g, which corresponds to the data Table 2.2, it can be seen that there is a proportional increase from 48.6% (Figure 2.9f, transect station C1) corresponding to 4,810 events/50µL (Table 2.2) to just fewer than 54% (Figure 2.9b (C5) and Figure 2.9a (C6)) or 6,010 and 5,693 events/50µL, respectively. The analysis also confirms that samples collected at the diffuser opening distinctly have the lowest number of measured events (3,107 events/50µL), which only accounts for 41.9% of the proportional value (Figure 2.9g). The proportional fraction of the sample collected at the raw water intake was 62.1% (Fig. 9h), which is with 6,627 events/50µL, also much higher than the values measured at the end of transect (C5, C6).

Similar to the results of the first campaign (Figure 2.6, Table 2.1), an increase in autofluorescent microbial cell counts away from the outfall was also observed during the second campaign.

Figure 2.9: FCM density plots of autofluorescent events (cyanobacteria and algae) for the different stations along transect C (Fig. 2.9a–f), as well as from the diffuser opening (Fig. 2.9g) and at the SWRO intake (Fig. 2.9h).
We also considered a possible linear correlation between conductivity (mS/cm) and FCM events/µL (Figure 2.10). The data from transects A – D suggests that high conductivity levels are correlated with low microbial cell counts and that microbial cell counts increase as conductivity decreases. This trend could be attributed to a negative (toxic) effect of the concentrate discharge on the microorganisms in the discharge area. However, it could also be explained by a mere dilution effect: the concentrate, which has high conductivity and low bacterial numbers after the pretreatment and desalination process, is mixing with ambient seawater (with lower conductivity but higher bacterial numbers than the concentrate), thereby leading to an increase in bacterial numbers while conductivity decreases as mixing takes place with increasing distance from the outfall. To further investigate this hypothesis, sampling was additionally carried out at two control sites during the third campaign (see section 2.3.3).

Figure 2.10: Linear correlation between conductivity (mS/cm) vs. FCM events/µL (transects A, B, C, D (campaign2)).
2.3.3 Comparing the impact and control sites

The third campaign was conducted over two days, 02/18/2013 and 02/19/2013, respectively, the reason being dive safety. Sample collection was limited to three dives per day and within no decompression limits (NDL). The sampling methodologies were similar to the two previous campaigns with the addition of collecting samples from two control sites. Figure 2.11 shows the measured results for the autofluorescent cyanobacteria and algae, and Figure 2.12 the total bacterial count (stained with SYBR Green) from samples collected during campaign 3.

Figure 2.11: Abundance of autofluorescent phytoplankton (cyanobacteria and algae) during campaign 3.

The results in Figure 2.11 depict that data (events/µL) varied quite significantly from the first day (transects A and B) to the next (transects C and D), with counts ranging from 71 (A4 (15 m)) to 58 (A1 (0 m)) events/µL and from 137 events/µL (D6 (25 m)) to 95 events/µL (D1 (0 m)). However, samples taken at the diffuser opening over the two days showed very similar results (on the order of 64 events/µL).
Figure 2.12: Abundance of total bacterial count (stained with SYBR Green) during campaign3.

Highest total bacterial counts (Figure 2.12) were recorded furthest away (25m) from the discharge structure with 1,065 events/µL at transect D6 (day 2), with the lowest events/µL of 748 events/µL (D1) on the same transect and over the same period in time. Events/µL from the sample taken at the diffuser opening were 723 events/µL.

In order to further investigate the dilution hypothesis (Figure 2.10, section 2.3.2), we compared results from the near-field area around the discharge, to those of two control sites. The total bacterial cell counts for the discharge site (transect D) as well as for control-1 (nearby) and control-2 (far-field) are presented in Table 2.3 and Table 2.4.

Table 2.3: Total bacterial events (transect D).

<table>
<thead>
<tr>
<th>Transect D Campaign3</th>
<th>Events/50µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6 (25 m)</td>
<td>53,269</td>
</tr>
<tr>
<td>D5 (20 m)</td>
<td>48,094</td>
</tr>
<tr>
<td>D4 (15 m)</td>
<td>50,935</td>
</tr>
<tr>
<td>D3 (10 m)</td>
<td>44,440</td>
</tr>
<tr>
<td>D2 (5 m)</td>
<td>44,093</td>
</tr>
<tr>
<td>D1 (0 m)</td>
<td>37,408</td>
</tr>
<tr>
<td>Diffuser Opening</td>
<td>36,155</td>
</tr>
</tbody>
</table>
Table 2.4: Total bacterial events/50µL for control-1 (nearby) and control-2 (far-field).

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>Volume (µL)</th>
<th>Events/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-1 Transect W6 (25 m)</td>
<td>47,711</td>
<td>50</td>
<td>954</td>
</tr>
<tr>
<td>Control-1 Transect W4 (15 m)</td>
<td>49,883</td>
<td>50</td>
<td>998</td>
</tr>
<tr>
<td>Control-1 Transect W1 (5 m)</td>
<td>49,016</td>
<td>50</td>
<td>980</td>
</tr>
<tr>
<td>Control-1 Transect WX (0 m)</td>
<td>57,682</td>
<td>50</td>
<td>1153</td>
</tr>
<tr>
<td>Control-1 Transect X1 (5 m)</td>
<td>46,668</td>
<td>50</td>
<td>933</td>
</tr>
<tr>
<td>Control-1 Transect X4 (15 m)</td>
<td>45,913</td>
<td>50</td>
<td>918</td>
</tr>
<tr>
<td>Control-1 Transect X6 (25 m)</td>
<td>45,239</td>
<td>50</td>
<td>905</td>
</tr>
<tr>
<td>Control-2 Transect Y6 (25 m)</td>
<td>26,521</td>
<td>50</td>
<td>530</td>
</tr>
<tr>
<td>Control-2 Transect Y4 (15 m)</td>
<td>25,839</td>
<td>50</td>
<td>517</td>
</tr>
<tr>
<td>Control-2 Transect Y1 (5 m)</td>
<td>26,147</td>
<td>50</td>
<td>523</td>
</tr>
<tr>
<td>Control-2 Transect YZ (0 m)</td>
<td>28,334</td>
<td>50</td>
<td>567</td>
</tr>
<tr>
<td>Control-2 Transect Z1 (5 m)</td>
<td>29,430</td>
<td>50</td>
<td>589</td>
</tr>
<tr>
<td>Control-2 Transect Z4 (15 m)</td>
<td>28,655</td>
<td>50</td>
<td>573</td>
</tr>
<tr>
<td>Control-2 Transect Z6 (25 m)</td>
<td>29,504</td>
<td>50</td>
<td>590</td>
</tr>
</tbody>
</table>

*Samples from control-1 were collected 583 m northwest (heading: 342° from the discharge structure), in similar water conditions (but in the absence of the concentrate) and at the same depth as the samples from campaign3, transect D. The ‘nearby control’ transect was 50 m in length and is representative of two opposite 25 m transect as performed around the discharge site. 7 samples were collected and analyzed.

b The second control site (control-2) was in close proximity to Al-Falah reef about 13 km off the Saudi Arabian coast in the Red Sea. The ‘far-field control’ transect was also 50 m in length (representative of two opposite 25 m transect as performed around the discharge structure) where 7 samples were collected and analyzed.

The results from transect ‘D’ suggests a similar tendency as in the previous two campaigns with events/50µL increasing further away from the discharge structure. The analyses also re-confirm that samples collected at the diffuser opening repeatedly have the lowest number of measured events. Measured events from both the control sites (control-1 (nearby) and control-2 (far-field)) did not show any significant variance in counts or proportional values with regards to samples analyzed for their own respective transects (Table 2.4), whereas cell counts at transect D during campaign3 show considerable changes ranging from 53,269 events/50µL at D6 to 36,155 events/50µL at the diffuser opening which demonstrates a dilution effect around the discharge location.
In order to further support the hypothesis that the negative correlation between conductivity and cell counts (Figure 2.10) might be due to dilution rather than toxicity effects, we also collected and analyzed samples (06/06/2013) at various sampling points from the KAUST SWRO plant. The results (average for duplicate samples) are presented in
Table 2.5, while Figure 2.13 illustrates the microbial trends at the plant as measured using FCM. A Spruce Filter™ system, which consists of several layers of inert natural material, is being used as pre-treatment (running at approximately 60m³/m²/hour) to the reverse osmosis membranes and was installed as an alternative to the more traditional dual media filters. It does not require the addition of coagulant chemicals to achieve the required silt density index (SDI). The required backwash water volume varies between 0.1 and 1% of the filtered water. Currently, the plant operates a shock-chlorination (sodium hypochlorite) method once a week for a duration of approximately 2 – 3 hours (dosage: 10 mg/L). This is followed by de-chlorination (sodium bisulphate with a dosage of 0.3 – 0.5 mg/L) downstream of the cartridge filters. This SWRO facility also injects an anti-scalant (Berkosafe SW (phosphonate)) downstream of the Spruce- and upstream of the cartridge filters at a dosage of 3 – 5 mg/L. All sampling campaigns (either at the discharge location or at the plant) were conducted outside of the shock-chlorination time frame and also in the nonoccurrence of treated or untreated brackish water and neutralized chemical cleaning waste discharges and can be considered as ‘normal operational conditions’.
Table 2.5: KAUST SWRO sampling points.

<table>
<thead>
<tr>
<th>Sampling Point</th>
<th>Autofluorescent phytoplankton (events/µL)</th>
<th>Total bacteria (SYBR Green (events/µL))</th>
<th>Conductivity (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Water</td>
<td>29</td>
<td>473</td>
<td>54.3</td>
</tr>
<tr>
<td>Downstream of Spruce Filter (media filter)</td>
<td>9</td>
<td>171</td>
<td>54.4</td>
</tr>
<tr>
<td>Downstream of Cartridge Filter</td>
<td>1</td>
<td>90</td>
<td>51.9</td>
</tr>
<tr>
<td>Downstream of Cartridge Filter (after de-chlorination &amp; anti-scalant)</td>
<td>6</td>
<td>163</td>
<td>52.6</td>
</tr>
<tr>
<td>Downstream of Cartridge Filter (after high pressure pump)</td>
<td>5</td>
<td>173</td>
<td>52.6</td>
</tr>
<tr>
<td>Reject after 1st Pass</td>
<td>9</td>
<td>164</td>
<td>80.4</td>
</tr>
<tr>
<td>Feed Water to BWRO</td>
<td>1</td>
<td>5</td>
<td>1.205</td>
</tr>
<tr>
<td>Reject after 2nd Pass</td>
<td>0</td>
<td>2</td>
<td>8.75</td>
</tr>
<tr>
<td>Permeate to Distribution Network</td>
<td>0</td>
<td>2</td>
<td>0.183</td>
</tr>
</tbody>
</table>

Based on this, it can be seen that there are very clear reductions in both the autofluorescent events/µL as well as the total bacterial counts downstream of the Spruce- and cartridge filters (29 events/µL to only 1 event/µL for autofluorescent phytoplankton and from 473 events/µL to 90 events/µL for the total bacterial count (SYBR Green)). After dosing the feed water (downstream of the cartridge filters) with an anti-scalant (phosphonate) as well as sodium bisulfite for de-chlorination, the events/µL are increasing slightly, from a single autofluorescent event/µL to 6 events/µL and from 90 events/µL to 163 for SYBR Green. This could be explained as a result of microbial regrowth in the absence of chlorine (after de-chlorination) and likely availability of nutrients (certain bacteria have evolved the ability to metabolize phosphonates as nutrient sources [16]). Very similar numbers were recorded in the reject stream after the first pass (6 events/µL (autofluorescent phytoplankton)) and 163 events/µL (SYBR Green), respectively), regardless of a much higher conductivity (80.4 mS/cm). The concentrate is made up as a combination of the rejects from both the first- and second pass, which is
both low in bacterial numbers. This demonstrates why samples collected at the diffuser opening distinctly have the lowest number of measured events.

Figure 2.13: Changes in the concentration of total bacteria stained with SYBR Green and autofluorescent phytoplankton (algae & cyanobacteria) at different stages of the KAUST SWRO plant. Data points are average values of duplicate measurements (FW: feed water, SF: spruce filter effluent, CF: cartridge filter effluent, DC: after de-chlorination, HPP: after high pressure pumps, R1: reject after 1st pass, BWRO: feed water to BWRO, R2: reject after 2nd pass, PM: permeate to distribution network).

Additionally, we also considered the effect of elevated conductivity levels on the broad microbiological spectrum (autofluorescent phytoplankton (cyanobacteria and algae), total bacterial count (SYBR Green) and a viability analysis where samples were stained with SYBR Green Propidium Iodide (SGPI) for intact cell counting). Six samples, 1 as a control and 5 samples that were spiked in 60 mg/L increments with sea salts (Sigma-Aldrich (S9883)), were incubated for a duration of 48 hours at 4°C and analyzed using FCM. This was done in order to obtain similar conductivity variations representative of operational conditions at the plant. The data illustrated in Error!
Reference source not found. confirms only marginal variances in events/μL, with cyanobacteria the least affected.

This also supports the hypothesis that the observed increase in microbial cell counts away from the discharge structure may be the result of dilution rather than chronic or acute salinity and toxicity effects.

Figure 2.14: The effect of elevated conductivity on microbial abundance and viability during 48 hours incubation. All samples were measured in duplicate. Conductivity changes were affected by the addition of artificial sea salt in order to represent similar conductivity ranges during normal operational conditions at the KAUST SWRO plant.

2.4 Conclusions

Although it is widely accepted that SWRO concentrate has the potential to cause significant impacts on the marine environment, little understanding is based on actual monitoring involving state-of-the-art procedures for environmental impact assessment. Almost none of what is reported in literature depicts possible impacts on microbial abundance in the near-field area of concentrate discharge. A monitoring campaign was therefore conducted to investigate potential impacts on microbial abundance in the near-
field discharge area of the KAUST SWRO plant. Based on three sampling campaigns and up-to-date FCM analysis, it appears that the anticipated impacts on microbial abundance in the near-field area of the KAUST SWRO discharge zone are minor. The results of this research distinctly showed a change in microbial abundance as a function of distance away from the discharge structure. We also considered a possible negative correlation between conductivity (mS/cm) and FCM events/µL as well as the effect of elevated conductivity levels on microbial abundance and viability—which lead to the hypothesis that the observed changes might be a result of normal dilution, where the concentrate (high conductivity; low microbial numbers) is ‘pushed’ back into an already saline marine environment with preeminent bacterial abundance. However, this data does not exclude the possibility that changes in microbial diversity or viability could occur, which was not detected with the FCM analysis. Pyrosequencing would be a necessary analyzing tool to assess complete microbial richness, diversity, community structure and relative abundance in order to assess if these differences in a distance away from the discharge structure would be significantly different. Moreover, it is clear that the impact of increased salinity as a result of SWRO concentrate discharge is not limited to microorganisms, and future research will focus on an in-situ coral photosynthetic yield experiment for assessing effects of higher salinity on coral photosynthesis in near-field areas of seawater desalination plants.
References


Chapter 3

Combining autonomous underwater vehicle missions with velocity and salinity measurements for the evaluation of a submerged offshore SWRO concentrate discharge

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Abstract:

High salinity discharges from seawater reverse osmosis (SWRO) plants into the marine environment may adversely affect water quality in the area surrounding the outfall. In general, very little systematic information on the potential impacts from full-scale operations on marine biota is available and even less to quantify such impacts for regulatory purposes. Scientifically validated and efficient planning tools in the form of predictive models and expert systems are normally used to assist regulators with regards to possible impacts on the marine environment. Numerical modeling has always been an efficient tool for predicting wastewater discharges and also more recently for high salinity discharges into seawater. The purpose of this study was to combine a series of propulsion driven autonomous underwater vehicle (AUV) missions with velocity and salinity measurements for the effective evaluation of a submerged offshore seawater reverse osmosis (SWRO) concentrate discharge near the campus of the King Abdullah University of Science and Technology (KAUST). The Cornell Mixing Zone Expert System (CORMIX) was additionally utilized in order assess discharge performance under different ambient velocity magnitudes.

The paper therefore focuses on the evaluation of an existing SWRO desalination discharge with emphasis on the regulatory framework of the mixing zone (RMZ). The objective of this case study is to develop an approach that can be followed by SWRO plant operators and environmental competent agencies for establishing regulatory mixing
zones for SWRO plants in the Kingdom of Saudi Arabia and worldwide, based on robust
field monitoring.

*Keywords*: Desalination; Seawater reverse osmosis; Concentrate discharge; Autonomous
underwater vehicle; Modeling; Near-field; Regulatory mixing zone; Marine monitoring;
Environmental impact assessment.
3.1 Introduction

The main waste stream resulting from seawater reverse osmosis (SWRO) desalination is the brine or concentrate, which characteristically is more saline than the raw seawater. In the context of this paper ‘concentrate’ will refer to the waste stream as a whole including salinity as well as process chemicals which are discharged along with the concentrate into the marine environment. For SWRO desalination plants located in close proximity to the sea, ocean discharge is still generally and preferred as the least expensive concentrate disposal method.

Environmental regulators typically set the numeric limits or guidelines for different aquatic pollutants reflecting the nature of conventional type effluents such as wastewater or cooling water and expect that SWRO discharges would be capable of meeting these standards as well. A major shortcoming however is the non-existence of tailored desalination-specific regulations with regards to concentrate discharges into the marine environment, not to mention situations where the concentrate is blended with wastewater or cooling water before discharge [1]. A second shortcoming is that most numeric discharge values restrict the levels of aquatic pollutants at the discharge point (effluent standards) but not for the receiving waters (ambient standards). However, a combination of effluent and ambient standards with a regulatory mixing zone concept that takes dilution and degradation of pollutants into account provides the best approach for regulating multiple dischargers in a joint environment. This paper more specifically looks at the aspects of ambient standards and regulatory mixing zones for SWRO facilities and related compliance monitoring studies.
In SWRO, the discharge flow rate can be on the order of between 30–70% (recovery ratios may vary) of the feed water, which implies 1.3–1.7 times the seawater concentration. Either extremely low salinities or extremely high salinities may impact ocean biota if the biotas are exposed over a prolonged period of time \([2]–[4]\). For concentrate negatively buoyant or dense discharges, the benthic environment could be exposed to high salinities for as long as the effluent continues \([5]\). The significance of the impact will depend on the design criteria of the plant and discharge structure, and environmental and hydro-environmental factors of the discharge area, which may include bathymetry, tides, waves, currents, depth of the water column, etc. \([6]\). These factors help determine the extent of the mixing zone and therefore the amplitude of assumable impacts \([4], [7]–[9]\). From an environmental point of view, the design of the particular discharge structure must be adequate so that the effluent discharged adheres to the receiving water guidelines (ambient standards) beyond the mixing zone, and also to ensure that critical salinity limits will not be exceeded in areas of sensitive marine ecosystems.

Currently, monitoring studies of concentrate dispersion are very scarce and where data are made available, it often can be evaluated in the light of effluent standards only, with ambient standards in many cases nonexistent \([10]–[13]\). Additionally, discharge designs are often not optimized and regulations often lack clear guidance with regards to the monitoring of ambient standards \([1], [6], [14]\). Taking into account the effluent properties and particular discharge configuration, mathematical models can assist in predicting discharge dilution ratios and plume behavior under different conditions \([15]–[17]\). The combination of autonomous underwater vehicle (AUVs) measurements, fixed
sampling points, current meters and appropriate mathematical-function models can be valuable tools to analyze the near-, mid- and far-field dispersion of a concentrate plume so that likely environmental impacts on the marine environment can be controlled and mitigated. The objective is to produce a baseline that will provide SWRO plant operators and environmental regulators with a realistic and achievable regulatory monitoring framework to ensure sustainable concentrate discharge management.

3.2 Methods

The existing SWRO facility at the King Abdullah University of Science and Technology (KAUST) was selected as a case study. The plant is located on the KAUST campus (located approximately 90 km north of Jeddah, Saudi Arabia) and was designed to provide all potable water needs for the campus as well as the residential areas. The plant has a product-water capacity of 40,000 m$^3$/day but is currently not operating at full load (it may intermittently operate at full load during summer months when demand increases). On average, the raw water intake is 2,825 m$^3$/hour with a recovery ratio of 39% (26,442 m$^3$/day produced water), resulting in an average concentrate flow of 1,723 m$^3$/hour (41,352 m$^3$/day) that is discharged to the sea. The outfall (discharge structure) is located at 39° 04.444E, 22° 17.780N and sits at a water depth of 18 m, approximately 2.8 km from the pump station (Figure 3.1a). The concentrate is pumped through a 1,200 mm diameter pipeline to the offshore structure (Figure 3.1b) where the concentrate is pushed up in a concrete riser and discharged horizontally through four discharge screens (1,800 mm × 1,000 mm) approximately 6 m from the seafloor.
Figure 3.1 a: KAUST SWRO offshore discharge structure locality (source: Google earth).
b: Schematic of the underwater discharge structure.
3.2.1 Data collection

3.2.1.1 REMUS autonomous underwater vehicle (AUV)

We utilized a Remote Environmental Measuring UnitS (REMUS 100, Kongsberg Maritime) propulsion driven AUV to conduct our surveys in the area around the KAUST SWRO discharge structure. The REMUS used (Figure 3.2) was configured to meet our specific mission requirements and was fitted with a side-scan sonar (EdgeTech), acoustic Doppler current profiler (Teledyne RD Instruments), light scattering sensor (WET Labs), conductivity, temperature and depth (CTD) sensors (Neil Brown Ocean Sensors, Inc.) as well as an onboard video camera.

Table 3.1 lists the most important specifications and features of the REMUS 100.

![Figure 3.2: The REMUS 100 AUV ready for deployment. The labels show the standard sensors and payload of the AUV.](image)

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle diameter:</td>
<td>19 cm (7.5 in)</td>
</tr>
<tr>
<td>Vehicle length:</td>
<td>160 cm (63 in)</td>
</tr>
<tr>
<td>Weight in air:</td>
<td>38.5 kg (85 lbs)</td>
</tr>
<tr>
<td>Trim weight:</td>
<td>1 kg (2.2 lbs)</td>
</tr>
<tr>
<td>Maximum operating depth:</td>
<td>100 m (328 ft)</td>
</tr>
<tr>
<td>Endurance:</td>
<td>Typical mission endurance is 8-10 hours subject to speed</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Propulsion:</strong></td>
<td>Direct drive DC brushless motor to open 3-bladed propeller</td>
</tr>
<tr>
<td><strong>Velocity range:</strong></td>
<td>Up to 2.3 m/s (4.5 knots) variable over range</td>
</tr>
<tr>
<td><strong>Navigation:</strong></td>
<td>Long baseline; Doppler-assisted dead reckoning; Inertial navigation system; GPS</td>
</tr>
<tr>
<td><strong>Transponders:</strong></td>
<td>4 transponders are provided with 20-30 kHz operating frequency range</td>
</tr>
<tr>
<td><strong>Tracking:</strong></td>
<td>Emergency transponder, mission abort, and in-mission tracking capabilities</td>
</tr>
<tr>
<td><strong>Software:</strong></td>
<td>Hydroid’s Vehicle Interface Program (VIP) for programming, training, post-mission analysis, documentation, maintenance, and troubleshooting</td>
</tr>
<tr>
<td><strong>Data exporting and reporting:</strong></td>
<td>HTML report generator and ASCII text export</td>
</tr>
<tr>
<td><strong>Operations:</strong></td>
<td>Capable of operating 4 vehicles simultaneously in the same water space</td>
</tr>
</tbody>
</table>

REMUS uses multiple modes of navigation that are vehicle determined. REMUS 100 navigates during a mission using Long Baseline (LBL) and Dead Reckoning (DR). The on-board computer automatically determines the preferred method, and can vary it through the mission. Alternatively REMUS 100 can navigate combining dead reckoning (DR) assisted with Doppler velocity log (DVL) and surfacing at regular intervals for GPS fixes. Optionally, an inertial navigation system (INS) can be added enabling non-LBL navigation, eliminating the need to place bottom transponders. As the vehicle was operated within communication range of the acoustic transponders, the AUV operated in LBL navigation for both the missions. LBL navigation is based on the principles of triangulation. The latitude and longitude of each of the transponders was preprogrammed into the REMUS mission files, whereas the vehicle then calculated its position by computing its range to the acoustic transponders (with a maximum range of 2.5 km) [18]. Data is used in real time to calculate the speed of sound for distance calculations during navigation, while a pressure transducer at the bulkhead is also used in real time for the mission navigation to maintain preprogrammed depths.
Two separate REMUS missions were conducted on 09/18/2012 (mm/dd/yyyy (summer)) and 02/20/2013 (winter), which also represent possible seasonal variances. The AUV was deployed between 09:00 and 11:00 for both missions, under constant wind speeds and clear sky conditions. The first and second mission deployments are shown in Figure 3.3. The deployment approach was to focus on the discharge area to examine possible effects from a dense (negatively buoyant) plume as a result of the concentrate discharge from the KAUST SWRO facility. It was programmed to first run a series of seven 650 m lines in length spaced 50 m apart covering a width area of 300 m. The mission parameters (legs) were set so that the vehicle maintained depths between 3 m and 5 m above the seafloor (depending on bathymetry and obstructions, e.g. the 1,200 mm diameter pipeline or the actual discharge structure itself) between reefs in a water column ranging from 14 m – 20 m. The second mission (with a slightly different heading) was programmed to run a series of seven 500 m lines, also spaced 50 m apart in the same project area (mission parameters set marginally deeper so that the vehicle recorded measurements at depths between 1 m – 3 m above the seafloor). Exact vehicle depths for both the mission runs are presented in Figure 3.4a and Figure 3.4b.
Figure 3.3: REMUS deployment missions on 09/18/2012 (mm/dd/yyyy (yellow)) and 20/02/2013 (purple) with preprogrammed sampling areas of 650 m × 300 m (deployment 1) and 500 m × 300 m (deployment 2). All transects are spaced 50 m apart. Deployment locations of two acoustic Doppler velocimeters (ADVs) are also shown. ADV_1 was deployed in a water depth of 16 m, and ADV_2 in 18 m, respectively (source: Google earth).

Figure 3.4 a and b: Vehicle depths during the two separate deployment missions. Mission parameters were set to maintain preprogrammed depths above the seafloor (depending on bathymetry) between reefs in a water column ranging from 14 m – 20 m. A short video showing highlights of the separate missions can be viewed here: https://vimeo.com/88590619.
3.2.1.2 AQUADOPP acoustic Doppler velocimeters

Two acoustic Doppler velocimeters (ADV (Aquadopp 2000 m, Nortek AS)) were deployed for measuring current velocity and direction at two locations around the SWRO discharge structure (deployment locations are shown in Figure 3.3). The ADVs are designed for stationary applications and can be deployed on the bottom, on a mooring rig, on a buoy or on any other fixed structure. For this study, the ADVs are secured to two separate heavy-duty steel tripods and deployed via an electric winch from a research vessel (ADV_1: 16 m water depth; ADV_2: 18 m water depth). Data was recorded from 08/06/2012 (mm/dd/yyyy) to 01/14/2013 (just under 6 months) with measuring intervals set at 10 min. This deployment period is also representative of seasonal variations. The instrument can measure water velocities with a range of ±3 m/s (accuracy: 1% of measured value ±0.5 cm/s) and has a maximum sampling rate (output) of 1 Hz and an internal sampling rate of 23 Hz. We also utilized its temperature sensor (range: −4 °C to +40 °C, accuracy/resolution: 0.1 °C/0.01 °C) with a time response of 10 min.

The instrument works by transmitting highly pitched ‘pings’ of sound at a constant frequency into the water. As the sound waves travel, they ricochet off particles suspended in the moving water, and reflect back to the instrument. Due to the Doppler effect, sound waves bounce back from a particle moving away from the profiler have a slightly lowered frequency when they return. Particles moving toward the instrument send back higher frequency waves. The difference in frequency between the waves the instrument sends out, and the waves it receives is called the Doppler shift. The instrument uses this shift to calculate how fast the particle and the water around it are moving (velocity). Sound waves that hit particles far from the profiler take longer to
come back than waves that strike close by. By measuring the time it takes for the waves
to bounce back and the Doppler shift, the velocimeter can measure current speed with
each series of pings.

3.3 Results and discussions

3.3.1 Plume identification

Both REMUS AUV missions were performed to possibly detect the plume and
illustrate its direction, physical and bio-optical properties. The distribution of these
properties in the marine environment could therefore be influenced by the dense
discharge (an average concentrate flow of 41,352 m$^3$/day (1,723 m$^3$/hour (April 2014))),
given the likelihood that effects on certain properties might be very subtle. During the
first mission path (09/18/2012) the REMUS consistently measured very similar salinity
levels along the 7 transects with no significant signal variances recorded.
Table 3.2 shows the average (AVE), standard deviation (STDEV), minimum (MIN) and maximum (MAX) values for the parameters measured by the vehicle during the deployment missions. Figure 3.5 shows the exact REMUS mission path, illustrating the slight salinity variances. Figure 3.6a–c also shows only slight variances in temperature, chlorophyll $a$ and turbidity.
Table 3.2: Statistical data recorded for the parameters measured by the REMUS during the two missions. Records for the periods where the vehicle was still at the surface are not included. Statistical data is representative for a mission depth of ≥0.97 m (mission 1) and ≥-1.17 m (mission 2), respectively. Average concentrate salinities measured at the SWRO plant was 56.9 at a temperature of 32.1 (density: 1037.08 kg/m³).

<table>
<thead>
<tr>
<th>MISSION 1</th>
<th>Salinity (g/L)</th>
<th>Temperature (°C)</th>
<th>Density (kg/m³)</th>
<th>Chlorophyll a (µg/L)</th>
<th>Turbidity (NTU)</th>
<th>AUV Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVE</td>
<td>40.11</td>
<td>30.17</td>
<td>1025.15</td>
<td>0.59</td>
<td>0.9</td>
<td>-12.17</td>
</tr>
<tr>
<td>STDEV</td>
<td>0.13</td>
<td>0.13</td>
<td>-</td>
<td>0.11</td>
<td>0.71</td>
<td>2.87</td>
</tr>
<tr>
<td>MIN</td>
<td>39.63</td>
<td>28.18</td>
<td>1025.47</td>
<td>0.26</td>
<td>0.11</td>
<td>-0.97</td>
</tr>
<tr>
<td>MAX</td>
<td>41.82</td>
<td>30.52</td>
<td>1026.31</td>
<td>0.89</td>
<td>4.96</td>
<td>-15.46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MISSION 2</th>
<th>Salinity (g/L)</th>
<th>Temperature (°C)</th>
<th>Density (kg/m³)</th>
<th>Chlorophyll a (µg/L)</th>
<th>Turbidity (NTU)</th>
<th>AUV Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVE</td>
<td>39.71</td>
<td>24.56</td>
<td>1026.71</td>
<td>0.54</td>
<td>3.1</td>
<td>-15.45</td>
</tr>
<tr>
<td>STDEV</td>
<td>0.35</td>
<td>0.41</td>
<td>-</td>
<td>0.08</td>
<td>2.03</td>
<td>2.67</td>
</tr>
<tr>
<td>MIN</td>
<td>39.12</td>
<td>23.01</td>
<td>1026.74</td>
<td>0.29</td>
<td>0.46</td>
<td>-1.17</td>
</tr>
<tr>
<td>MAX</td>
<td>51.51</td>
<td>25.76</td>
<td>1035.23</td>
<td>1.02</td>
<td>9.18</td>
<td>-18.29</td>
</tr>
</tbody>
</table>

Figure 3.5: An illustration of the exact REMUS mission path (mission 1) measuring salinity along the 7 pre-determined transects around the KAUST SWRO discharge structure. No significant discernible salinity signals were recorded near the discharge structure albeit a minimal salinity range of only 1 g/L set for 39.5 – 40.5 g/L (black meaning lower salinity, white meaning higher salinity,). The red line across the seven transects is the vehicle returning to the boat at the surface (slightly lower measurements).
Figure 3.6: Data presented in the figure shows that no significant and/or discernible plume detection measurements with regards to any of the additional parameters (temperature, chlorophyll $a$ and turbidity) could be confirmed. Figure legends varied from 29 – 30 °C for temperature, 0.31 – 0.81 µg/L for chlorophyll $a$ and 0 – 5 NTU for turbidity. The bathymetry depth legend ranged from -20.83 to -9.11 m.

Based on the complete data set of measurement recorded during mission 1, the relative standard deviation (%RSD) for salinity and temperature is only 0.33% and 0.43%, respectively, and 18.16% for chlorophyll $a$. Turbidity measurements show a higher relative standard deviation of 78.42% (close to the reef bank, approximately 60 m west from the underwater discharge structure). This however is not a clear indication of plume proximity and might be the result of accumulated settling particles common in some reef areas [19].

The second mission path (20/02/2013) was pre-programmed to cover a slightly smaller rectangular area of 500 m × 300 m, also with transects spaced at 50 m apart. Primarily, mission parameters were set deeper so that the vehicle recorded measurements at depths between 1 m – 3 m above the seafloor could improve the likelihood of
recording a possible plume signal. The data from this mission suggested clear and measurable results in salinity variances (Figure 3.7a and Figure 3.7b).

Figure 3.7: The vehicle mission path measuring salinity (legend range: 39 – 41 g/L) along the pre-determined transects during the second deployment. b: A clear and measurable salinity signature for the range 39.7 – 40.3 g/L was recorded. The vehicle also recorded a maximum salinity measurement of 51.51 g/L, which is also considerably higher than the maximum value of the first mission (41.82 g/L).
As distance increases from the discharge structure, the concentrate in the study area naturally mixes with the receiving waters to the point of showing little to no discernible salinity variations within rather short distances. It is therefore interesting to observe a number of salinity signatures also at greater distances away from the discharge structure, albeit for a minor salinity range (39.7 – 40.3 g/L) in slightly deeper locations (Figure 3.7b). If these are to be associated with the plume, it may be explained by a very thin effluent plume spreading on the seafloor, accumulating in small channels or basins, resulting in the slightly higher signals in these regions. Although differences in the salinity field were observed, variations in background properties can cause ambiguity in identifying exact plume behavior. This suggests that monitoring only a single or even a pair of properties may not be adequate to unambiguously identify ‘discharge’ affected waters and it may be necessary to differentiate other components of the particulate field [20]. In addition, the marine environment surrounding the KAUST SWRO discharge location is physically and biological active, e.g., concentration of particles due to the processes of primary production and sediment re-suspension that might be much higher than in open ocean areas. Despite the high salinity characteristics of the concentrate, very rapid mixing combined with boundary interaction, entrainment processes, etc. can make accurate identification of the plume based on salinity alone very difficult. This can again be confirmed with by low %RSD values for salinity (0.87%) and temperature (1.65%) for the complete second mission (again, based on the complete statistical data set from mission 2). Chlorophyll \(a\) concentrations were homogenous with an average concentration of 0.54 µg/L throughout the transects.
3.3.2 Water current velocities

Exact deployment locations of the ADVs were formerly shown in Figure 3.3. Data were recorded for the period 08/06/2012 (mm/dd/yyyy) to 01/14/2013 (>5 months) with measuring intervals set at 10 min. Figure 3.8 provides both the ADVs recorded water temperature measurements whereas Figure 3.9 and Figure 3.10 illustrate the ADV velocity plots for the complete deployment period, broken down in monthly intervals. Water current velocities vs. wind speed are also provided in Table 3.3 (note: in order to make a meaningful comparison, we have trimmed the ADV data (with original measuring intervals set at 10 min) to the same data acquisition rate as the wind observations (one measurement every hour) for the same period. That is the reason why values in Table 3 differ slightly from the data presented in Figure 3.9 and Figure 3.10). Figure 3.11 shows principle component analysis for the ADVs, while Figure 12 provides an overview of the velocity magnitudes. Current velocities from ADV_1 showed a minimum mean velocity of 6.33 cm/s (September 2012) and a maximum mean of 11.85 cm/s for January 2013. A maximum combined current velocity of 30.99 cm/s was also recorded in January 2013 (01/11/2013, 20:45). Mean current velocities recorded from ADV_2 showed moderately higher deep current movements with a minimum mean velocity of 7.53 cm/s (September 2012) and a maximum mean of 18.89 cm/s measured for December 2012. A maximum combined current velocity of 32.2 cm/s was again recorded in January 2013 (01/10/2013, 20:35). Current velocities are predominantly in a westerly direction, clearly showing seasonal variances with an increase in magnitude from the relevant summer months (August, September and October (mean velocity of 7.22 cm/s)) into November, December and January (with a mean of 10.87 cm/s). Water temperatures ranged
from 28.84 °C – 33.64 °C (AVE 31.69 °C) in summer (August, September and October) and declined between November and January gradually to a value minimum value of only 21.72 °C (AVE 26.74 °C). Current velocities are predominantly in a westerly direction, clearly showing seasonal variations with an increase in magnitude from the summer months (August, September and October) into winter (November, December and January) (Figures 9, 10). Mean current velocities were higher during winter than during summer months and higher at ADV_2 (10.62 in summer, 18.50 cm/s in winter) than at ADV_1 (7.22 in summer, 10.87 cm/s in winter).

Figure 3.8: ADV_1 (16 m water depth) and ADV_2 (18 m water depth) recorded water temperature measurements.
Figure 3.9: ADV_1 velocity plots for the deployment period August 6\textsuperscript{th}, 2012 to January 14\textsuperscript{th}, 2013. The view represents a ‘top’ view angle (looking down on the ADV) as ‘up’ current velocities were insignificant (AVE: 1.67 cm/s). Velocity value limits (for the figures) are 0.3 m/s (30 cm/s (x-axis)) and 0.15 m/s (15 cm/s (y-axis)) respectively.
Figure 3.10: ADV_2 velocity plots for the (same) deployment period August 6th, 2012 to January 14th, 2013. The view again represents a ‘top’ view angle (looking down on the ADV) as ‘up’ current velocities were negligible (AVE: 0.68 cm/s). Current velocities are distinctly in a westerly direction, clearly showing similar seasonal variances as measured from ADV_1 with an increase in magnitude from the relevant summer months (August, September and October) into winter (November, December and January). Noticeable though is the consistently higher current velocity magnitudes for the winter months with an average of 18.5 cm/s and minimum mean of 7.03 cm/s for the same period; comparing this with August, September and October’s average current velocity of 10.62 cm/s and a minimum mean velocity of only 0.42 cm/s. Velocity value limits (for the figures) are 0.3 m/s (30 cm/s (x-axis)) and 0.15 m/s (15 cm/s (y-axis)) respectively.
Figure 3.11: ADV principal component analysis (PCA). The lengths of the principal components are based on the velocity magnitudes. For ADV_1 we have that the main direction explains only 68% of the variance of the data, while the secondary direction explains the other 32%. For ADV_2, the main direction supports 86% of the data and 14% the other direction.

Figure 3.12: An overview of the velocity magnitudes measured for the complete deployment period. ADV_1 show average velocities of 0.066 m/s (August 2012), 0.063 m/s (September 2012), 0.086 m/s (October 2012), 0.099 m/s (November 2012), 0.114 m/s (December 2012) and 0.118 m/s (January 2013). ADV_2 (for the same deployment period) suggest higher average velocities, particularly during the specific winter months. Average velocities were 0.101 m/s (August 2012), 0.075 m/s (September 2012), 0.14 m/s (October 2012), 0.18 m/s (November 2012), 0.189 m/s (December 2012) and 0.187 m/s (January 2013).
We also considered wind as a possible factor that might influence current velocity and direction. A fully instrumented shore-side tower located on the KAUST campus (equipped with an ASIMET Sonic Wind Module (sensor: Gill Instruments WindObserver II Ultrasonic Anemometer)) was utilized to collect data for wind speed and direction. Data were collected for the same deployment period as the ADVs (from 08/06/2012 (mm/dd/yyyy) to 01/14/2013)). As mentioned previously, in order to make a meaningful comparison, we have trimmed the ADV data (with original measuring intervals set at 10 min) to the same data acquisition rate as the wind observations (one measurement every hour) for the same period. The data are presented in Table 3.3.

Table 3.3: Water current velocity and direction vs. wind speed and direction for the period August 6th, 2012 – January 14th, 2013.

<table>
<thead>
<tr>
<th></th>
<th>ADV_1 (water depth of 16 m)</th>
<th>ADV_2 (water depth of 18 m)</th>
<th>WIND</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Magnitude (m/s)</td>
<td>Heading (degrees)</td>
<td>Magnitude (m/s)</td>
</tr>
<tr>
<td>AUG 2012</td>
<td>0.05</td>
<td>283.26</td>
<td>0.09</td>
</tr>
<tr>
<td>SEP 2012</td>
<td>0.04</td>
<td>274.42</td>
<td>0.06</td>
</tr>
<tr>
<td>OCT 2012</td>
<td>0.07</td>
<td>287.80</td>
<td>0.13</td>
</tr>
<tr>
<td>NOV 2012</td>
<td>0.08</td>
<td>290.27</td>
<td>0.18</td>
</tr>
<tr>
<td>DEC 2012</td>
<td>0.10</td>
<td>279.91</td>
<td>0.19</td>
</tr>
<tr>
<td>JAN 2013</td>
<td>0.11</td>
<td>271.47</td>
<td>0.19</td>
</tr>
<tr>
<td>AVE</td>
<td>0.07</td>
<td>282.29</td>
<td>0.13</td>
</tr>
</tbody>
</table>

To further visually explicate this, we compared the three datasets (ADV_1, ADV_2 and wind) in a data-rose (Figure 3.13).
Figure 3.13: The figure indicates frequency in a particular direction. Wind heading in green, and those of water currents for ADV_1 in blue, and ADV_2 in red respectively. We can observe a concentration of westerly headings for both the ADVs (just under 60% frequency at ADV_2 and a 40% frequency measured at ADV_1). Prevailing wind directions show a southeasterly aggregation with a mean heading of 148.01°, approximately 15% of the time.

Taking into consideration the locality, size and water depth of the study area, the data suggest no well-defined or comparative relation between prevailing winds and deep-water current direction. For the study area, deep-currents around the discharge structure were predominantly in a westerly direction with a mean velocity of 0.07 m/s. Wind headings showsouthwesterly and southeasterly aggregations with an average wind speed of 2.61 m/s.
3.3.3 In-situ salinity measurements

In order to analyze the correlation of the parameters obtained by the REMUS and the ADVs to the effluent discharge characteristics, additional near-field measurements have been taken. In order to establish near-field dilution ratios, we collected water samples at seafloor level (diving) along a 25 m transect in a distance away from the discharge structure. Samples were collected in duplicate (using Falcon 50 mL Conical Centrifuge tubes and Cubitainers, each with a 1 L capacity) and measured for salinity (with a WTW Cond 3310 meter and TetraCon 325 probe) at six points (discharge screen, 0 m, 2.5 m, 5 m, 15 m and 25 m respectively (dive dates: January 15-16\textsuperscript{th}, 19\textsuperscript{th}, 21\textsuperscript{st}, 23\textsuperscript{rd}, 30\textsuperscript{th} and February 13\textsuperscript{th} 2014). Results of the salinity measurements are presented in Figure 3.14. The results from this data are also comparable with the data measured by the AUV during the two separate missions.

Figure 3.14: In-situ salinity measurements in a distance away from the discharge structure. Average salinity values measured at the different sampling stations were 48.81 g/L (discharge screen), 41.87 g/L (0 m), 41.29 g/L (2.5 m), 41.29 g/L (5 m), 41.19 g/L (15 m) and 41.01 g/L (25 m) respectively.
3.4 Compliance analysis and modeling

3.4.1 Mixing processes

It is important to clearly differentiate between the physical aspects of hydrodynamic mixing processes that determine the effluent fate and distribution, and the administrative construct of mixing zone regulations that are intended to prevent any negative impacts of the concentrate on the marine environment and associated uses. Mixing processes are usually divided into two regions in which different physical mechanisms predominate. In the first, the mixing is intense; it results mainly from turbulence generated by the initial buoyancy and momentum of the discharge and their interactions with the ambient flow. This region is also known as the near-field. Beyond this region, the self-induced turbulence has decayed and mixing results primarily from ambient oceanic turbulence. In this region, called the far-field, dilution increases at a much slower rate than in the near-field. The ambient conditions of the receiving waters are described by the water body’s geometric and dynamic characteristics [5], [21]. In many cases, both these conditions (geometric and dynamic) can be taken as a ‘steady-state’ with little temporal variation because the spatial and time scales for the mixing processes are usually on the order of meters and minutes up to perhaps one hour, respectively. The far-field region is located further away from the discharge point. For dense discharges, this is the point where the concentrate turns into a gravity current, sinks to the bottom and spreads horizontally following the slope of the seafloor bathymetry [6]. In this case, flow and mixing characteristics are dominated by large scales (kilometers and hours) [15], [22].

Near-field processes are therefore intimately linked to the discharge parameters and under the control of the designer. Whatever these processes are, the turbulence
generated by the discharge eventually decays, and near-field mixing ceases to be significant. This marks the end of the near-field, and the dilution at this point is defined as the near-field dilution. In reality, the dilution asymptotically approaches its limiting value, with no sudden transition to the far-field. The end of the near-field is therefore considered as the location where dilution no longer changes significantly with distance [17] and/or where the source fluxes (momentum and buoyancy) do not have any influence on mixing. Accordingly, we can therefore affirm that near-field mixing characteristics are controlled by the source (discharge characteristics), whereas far-field characteristics will be controlled by ambient conditions.

3.4.2 Mixing zone regulations

Water quality policies in both Saudi Arabia (where the case study is located) and in the United States include the concept of a mixing zone—a limited area where initial dilution of an aqueous discharge occurs. The discharge of concentrate into the marine environment can be considered from two different points of view (with regards to its possible impacts on the ambient water quality). On a larger scale, seen over the entire receiving water body, care must be taken that water quality conditions that protect designated beneficial uses, habitats and species are achieved. This is the realm of the general waste load allocation (WLA) procedures and models. On a local scale, or in the immediate discharge vicinity, additional precautions must be taken to insure that high initial pollutant concentrations are minimized and limited to small zones, areas, or volumes. The generic definition of these zones, commonly referred to as “mixing zones”, is embodied in water quality regulations and often cited in the regulations of permit granting authorities. As stated previously, mixing zones are administrative constructs
that are independent of hydrodynamic mixing processes.

In summary, the RMZ is a definition that allows for the initial dilution of a discharge rather than imposing strict ‘end-of-pipe’ concentration requirements for conventional and toxic discharges. It may therefore allow for efficient natural pollution assimilation and can be used as long as the integrity of the water body as a whole is not impaired.

3.4.3 Requirements for regulatory mixing zones

As part of this paper, we summarized the most important requirements for RMZ as stipulated by the latest version of the National Ambient Water Quality Standard for the Kingdom of Saudi Arabia (KSA), and for comparison, the Mixing Zones Water Quality Standards (compilation of EPA Mixing Zone Documents http://water.epa.gov/scitech/swguidance/standards/mixingzones/) from the United States Environmental Protection Agency (USEPA). The National Ambient Water Quality Standards apply to all coastal and underground waters in KSA and include any surface freshwater that may be permanent or temporarily. This standard revises the General Standards for the Environment (specifically document number 1409-01) issued by the Presidency of Meteorology and Environment (PME) and entered into force on March 24, 2012. The standards also refer to the specifications of ambient waters including safety, aesthetics, and physical and chemical aspects.

3.4.3.1 RMZ requirements for KSA

The following requirements apply in relation to a mixing zone in a receiving water body:
• The zone of influence or (*regulatory*) mixing zone will be designated in order to minimize possible impacts on the environment. However, the absolute maximum size of the mixing zone will be determined on a case-by-case basis using the methodology in Table 3.4 and limited to a maximum 100 m radius;

• Acutely toxic limits should not be exceeded within the mixing zone. Analyzing methods must be in accordance with 40 CFR Part 136 (Guidelines Establishing Test Procedures for the Analysis of Pollutants)³;

• Mixing zones should not impinge on sensitive areas, such as coral reefs, recreational areas or important spawning or nursery areas for aquatic organisms;

• Nearby mixing zones (if any) should not merge or overlap;

• No mixing zone should intrude the mean low water spring (MLWS) shoreline;

• Effluents containing materials that can settle to form objectionable deposits (that may result in the growth of detrimental, invasive or nuisance species) should not be discharged;

• Alternative mixing zone areas may, on a case-by-case basis, be agreed on by the Competent Agency (Precedency of Meteorology and Environment (PME)) to represent areas that have been designated as sites of significant economic importance (SSEI). An official application (supported by evidence justifying the award of a temporary permit) for a SSEI must be submitted to the PME; and

• Should a facility determine that the prescribed methodology (calculating of mixing zones in the Red Sea and Arabian Gulf) is technically unachievable, they must produce a scientific study (approved by the PME) to confirm the best

³ Whole Effluent Toxicity Test Methods, USEPA
http://www.epa.gov/region9/qa/pdfs/40cfr136_03.pdf
achievable mixing zone dimensions. In order to ensure the proper dispersion and minimize possible effects on the marine environment, best available techniques (BAT) and best environmental practices (BEP) must be selected (suitable for the purpose).

Table 3.4: Calculation of RMZ for the Red Sea and Arabian Gulf.

<table>
<thead>
<tr>
<th>Water Depth (m)</th>
<th>Red Sea Significance Values ($SV$)</th>
<th>Water Depth (m)</th>
<th>Arabian Gulf Significance Values ($SV$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------------------------</td>
<td>----------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Horizontal extent of RMZ radius (m)</td>
<td></td>
<td>Horizontal extent of RMZ radius (m)</td>
</tr>
<tr>
<td>5 or less</td>
<td>10</td>
<td>25</td>
<td>40</td>
</tr>
</tbody>
</table>

4 Red Sea/Arabian Gulf Significance Values ($SV$): 2/4 high-value area (locally, nationally or internationally protected areas); 5/8 marine classified area (under the jurisdiction of KSA, i.e., territorial coastal waters being 12 international nautical miles of the shoreline); 8/12 classified as industrial through local or national planning regulations (including zones surrounding fixed offshore platforms).
<table>
<thead>
<tr>
<th>Water Depth (m)</th>
<th>Red Sea Significance Values (SV)²</th>
<th>Water Depth (m)</th>
<th>Arabian Gulf Significance Values (SV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arabian Gulf</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Horizontal extent of RMZ radius (m)</td>
<td>Horizontal extent of RMZ radius (m)</td>
<td></td>
</tr>
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The method in Table 4 represents the basic screening model for defining the maximum horizontal extend of the RMZ ($SD_{ave}$ where $S$ denotes the significance value of the habitat and $D_{ave}$ the average water depth at the discharge location). The maximum horizontal extent of the RMZ radius shall not exceed 100 m. For the KAUST case study, the RMZ will be 90 m, based on the significance value of 5 (marine classified area) and a water depth of 18 m ($5 \times 18 = 90$, hence RMZ$_{90}$). Additionally, the PME’s prescribed salinity concentration at the boundary of the RMZ is $\Delta 0\%$ above ambient salinity levels for high-value and marine classified areas, and $\Delta 2\%$ above ambient for industrial classified zones. For comparison, and to put the Kingdom’s RMZ requirements into perspective, we also call attention to the USEPA definitions and requirements for RMZ.

3.4.3.2 USEPA definitions and requirements for the RMZ
The USEPA defines the RMZ as an ‘allocated impact zone’ where numeric water quality criteria may be exceeded as long as acutely toxic conditions remain within limits. It can be thought of as a limited area or volume where the initial dilution of a discharge occurs. Water quality criteria apply at the boundary of the RMZ, not within the mixing zone itself. Furthermore, the area or volume of an individual RMZ or group of mixing zones must be limited to an area or volume as small as practicable and must not interfere with the designated use(s), habitats or marine species within the receiving water body. The mixing zones must also avoid impingement of biological important areas and “shore hugging” plumes should be prevented.

Within the RMZ, the USEPA requires that any allocated impact zone should be free from point or non-point sources related to:

- Material in concentrations that will cause acute toxicity to marine life;
- Material in concentrations that settle to form objectionable deposits;
- Floating debris, oil scum and any other matter in concentrations that may form nuisances;
- Substances in concentrations that could produce objectionable color, odor, taste or increase in turbidity; and
- Substances in concentrations that can produce undesirable marine life or result in a dominance of nuisance species.

The USEPA salinity limit for brine discharges is given as an increment of no more than 4 g/L relative to ambient (300 m compliance point (relative to discharge)). The RMZ could also be specified by length, area or volume around the discharge source.
Since these areas of impact, if disproportionately large, could potentially adversely impact the productivity of the water body and have unanticipated ecological consequences, they should be carefully evaluated and appropriately limited in size.

Although there is substantial variation in the specifics of the regulations, but both share two key elements: a salinity limit and a point of compliance expressed as a distance from the discharge.

3.4.4 Discharge dilution

SWRO concentrate is commonly discharged into coastal waters by means of diffusers which promote rapid dilution of the effluent, reducing the concentrations of salinity and its environmental impacts to very low levels. However, the existing KAUST SWRO discharge structure (Figure 1b) is more similar to an intake structure than to outfalls typically used for SWRO plants. The objective is to examine dilution performance of this particular design by using the results obtained by the measuring campaigns, AUV missions and ADVs, and complementing them by using mathematical dilution equations. Due to the fact that this discharge structure results in an uncommon initial mixing process, the following considerations apply: (i) the effluent leaves the discharge screens with very low velocities, thus does not induce considerable mixing by the usual velocity shear, as for jet-like designs, (ii) The negatively buoyant discharge tends to ‘burble’ out of the discharge screen with very low exit velocity and little discharge-induced mixing. It creates a mixing layer causing dilution by turbulence originating in the sinking plume, before impinging with the seafloor. This mixing behavior can be approximated by the line plume equation, (iii) the discharge, even though
rectangular is hereby approximated by a single falling line plume (b = the buoyancy flux per unit length; q = volume flux per unit length; H = plume’s centerline height above the seafloor). Previous researchers have reported different values of the constants in this equation depending on the diffuser geometry and the location of the dilution measurement. For a line plume, the measured coefficient, $C_1$, is 0.49. Rouse et al. (1952) and Kotsovinos (1975) measured centerline dilutions in a freely rising (or sinking) line plume and obtained $C_1 = 0.42$ (also used for our predictions). Koh and Brooks (1975) performed a theoretical entrainment analysis of a line plume in finite water depth. They assumed that the presence of the surface layer reduced dilution (contrary to literature) and estimated $C_1 = 0.38$.

$$S_n = 0.42 \times \frac{b^{1/3} H}{q}$$

Using the equation for a line plume, we predict a dilution of 7.16:1 at the end of the near-field region (contact/impinging with the seafloor). In order to assess the likely impact(s), we also calculated the required salinity dilution to verify that receiving water objectives will be met at the edge of the RMZ$_{90}$ (21.84:1 ($\Delta$2% above ambient in accordance with the National Ambient Water Quality Standards for Saudi Arabia)). A $\Delta$0% increase above ambient is unachievable as this means there must be infinite dilution in the mixing zone, or you allow only a discharge with the ambient salinity value. The required dilution criteria were then compared with calculated dilution ratios based on data from the water samples collected at seafloor (by diving), at the discharge screen and at seafloor level along a transect in distances of 0 m, 2.5 m, 5 m, 15 m and 25 m, respectively (Figure 3.15). Dilution ratios varied from 1.88:1 (discharge screen) to 12.27:1 at a distance of 25 m away from the discharge structure.
Comparing the regulatory requirement (RMZ\textsubscript{90}) (in this case, a dilution ratio of 21.84:1 (0.79 g/L above ambient salinity allowed) with the results from the ADV data, AUV missions as well as the in-situ salinity measurements and calculated dilution ratios, a expert system for mixing zone analysis will be required to confirm if the required dilution ratio (21.84:1) at RMZ\textsubscript{90} will be met. In this case, the discharge configuration is hydrodynamically ‘stable’—that is the discharge strength (measured by its momentum flux) is weak in relation to the layer depth and in relation to the stabilizing effect of the negative discharge buoyancy (measured by its buoyancy flux). Initial dilution is caused by turbulence originating in the sinking plume and the vortices. Farther away from the discharge structure it changes to a gravitational diffusion which creates a stable density profile that ultimately causes the turbulence generated in the near-field to collapse and mixing to cease. A bottom density current forms, where (in the absence of ambient stratification) the plume will proceed down the slope with very little turbulence or mixing. That is why we clearly observe only slight dilution ratio changes from 2.5 m (10.24:1) to 25 m (12.27:1).

The existing discharge design results in low dilution ratios (specifically in the near-field as a result of a very low discharge momentum flux). Although effluent concentrations are currently fairly “low” (on the order of 56.9 g/L (measured at the plant and an average of 48.81 g/L at the discharge screen)), effluent concentration will increase should the plant operate at a better recovery ratio (given that feed water intake flows remain similar). As part of this this study, an appropriate hydrodynamic model is used also used in order for the assessment of the regulatory mixing zone.
3.4.5 The CORMIX system for RMZ analysis

The plume model used in this study is the Cornell Mixing Zone Expert System (CORMIX\(^5\)) which consists of a series of software systems for analysis, prediction and design of marine discharges into receiving waters, with the emphasis on the geometry and dilution characteristics of the initial mixing zone, including the evaluation of regulatory requirements. The CORMIX methodology emphasizes the role of boundary interaction on mixing. Boundary interaction occurs when the flow contacts either the surface or bottom, or a terminal layer in a density-stratified ambient environment. Boundary interaction also determines if mixing is controlled by stable\(^6\) or unstable\(^7\) discharge source conditions and defines the transition from near-field to far-field mixing.

Simulation model selection in CORMIX is controlled by the graphical user interface (GUI) and mixing zone rule-base. Based upon input data, the model employs the rule-base to execute the appropriate hydrodynamic simulation model based upon the

\(^5\) http://www.cormix.info/
\(^6\) Usually occurs for a combination of strong buoyancy, weak momentum and deep water. Thus they are often referred to as ‘deep water’ conditions.
\(^7\) This occurs when a recirculation phenomenon appears in the discharge vicinity. This local recirculation leads to re-entrainment of already mixed water back into the buoyant jet region. Unstable may be considered synonymous to ‘shallow water’ conditions.
discharge and environment data specified. The code used (CORMIX v8.0GTH) has four core hydrodynamic simulation and visualization models in order to simulate diverse discharge situations. These hydrodynamic models are CORMIX 1,2,3 and DHYDRO for single-, multi-port, surface and dense discharges. DHYDRO is especially well suited for its ability to model negatively buoyant concentrate discharges from single- or multi-port diffusers in laterally unbounded coastal environments (with sloping bottoms) [23]. CORMIX is unique among environmental simulation models in that it is “data driven” where it allows users to enter and modify data interactively to describe the discharge and ambient conditions. Using its rule-based expert systems technology, it then automatically determines the most appropriate hydrodynamic model to simulate the conditions specified by the user. Finally, CORMIX assembles and executes a sequence of appropriate hydrodynamic simulation modules which, when executed together, predict the trajectory and dilution characteristics of a complex flow. It is designed to analyze water quality criteria within RMZ and has been successfully applied to the design and monitoring of ocean discharge systems and is also recognized by regulatory authorities for environmental impact assessment [21], [24]–[26].

As part of this study, we modeled three different base cases (average-, low- and high velocity case scenarios) in order to identify possible improvements for the outfall design of the KAUST SWRO plant. The high- and low velocity case scenarios are representative of seasonal variations, whereas “average” represents concentrate discharge, under what is to be considered normal conditions. The KAUST SWRO plant’s average concentrate discharge flow of 0.479 m$^3$/s was applicable for all the scenarios. For comparability, we assume the same concentrate salinity and temperature
as well as the same ambient salinity and temperature for the case scenarios to ensure an equal density difference for the different scenarios. Ambient velocity measurements are based on the data acquired from ADV_1 (as it was most closely located to the discharge structure). Very few assumptions were made, as all the necessary input parameters for the model were based on actual monitoring and fieldwork collected data.

3.4.5.1 Case scenarios

The low velocity case scenario is most likely to occur during the relevant summer months (August, September, October), hence using the minimum ambient mean velocity of 0.04 m/s (September 2012) and average wind speed of 1.68 m/s (October 2012 (Table 3)). The model simulation for a high velocity case scenario (most likely during winter) is based on a maximum ambient mean velocity of 0.11 m/s (January 2013) and average wind speed of 3.87 m/s (January 2013). Normal conditions were based on the average magnitudes of the data sets, with an average velocity magnitude of 0.07 m/s and a wind speed of 2.61 m/s. Figure 3.16 shows the CORMIX model flow visualizations for the case scenarios. Based on the discharge/environmental interaction and mixing behavior, CORMIX predicted the flow class for all three scenarios to be “NH_1”, which is characterized as a submerged negatively buoyant discharge horizontally or near-horizontally from the discharge port. The classification scheme places emphasis on the near-field behavior of the discharge and uses the length scale concept as a measure of the influence of each potential mixing process. Flow behavior in the far-field, after boundary interactions, is largely controlled by ambient conditions.
Figure 3.16: Flow visualizations for the case scenarios. (a) Low velocity scenario; (b) High velocity scenario; (c) Average velocity scenario (representative of concentrate discharge under ‘normal’ ambient conditions). The discharge density difference is -12.26 kg/m$^3$, which makes the effluent negatively buoyant, consequently sinking towards the seafloor. The red rectangle in each visualization shows the boundary of the regulatory mixing zone (for this instance, at a distance of 90 m).
The near-field region is the zone of strong initial mixing before boundary interaction with no direct regulatory implication. It is however important that the regulatory mixing zone will include the near-field. This near-field information might be useful for discharge designers because this region is sensitive to the discharge design conditions. The modeling focuses on the evaluation of an existing SWRO desalination discharge, with the emphasis on regulatory requirements at the boundary of the mixing zone. Based on the methodology in Table 4, the applicable RMZ for our case study is 90 m (significance value of 5 in a water depth of 18 m). In order to assess the likely impact(s) of the three different case-scenarios, we used the previously calculated required dilution ration for RMZ$_{90}$ (21.84:1 ($\Delta$2% above ambient in accordance with the National Ambient Water Quality Standards for Saudi Arabia)) to verify that receiving water objectives will be met at the edge of the mixing zone. Consequently, CORMIX was applied to the discharge characteristics, the mixing characteristics of the site, and the exiting discharge technology to determine plume conditions at the boundary of the specified RMZ (Table 3.5).

<table>
<thead>
<tr>
<th></th>
<th>Low-velocity scenario (0.04 m/s ambient velocity)</th>
<th>High-velocity scenario (0.11 m/s ambient velocity)</th>
<th>Average-velocity scenario (0.07 m/s ambient velocity)</th>
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<tbody>
<tr>
<td>Ambient salinity</td>
<td>39.60 g/L</td>
<td>39.60 g/L</td>
<td>39.60 g/L</td>
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<tr>
<td>Effluent salinity</td>
<td>56.90 g/L</td>
<td>56.90 g/L</td>
<td>56.90 g/L</td>
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<tr>
<td>$\Delta$2% of ambient salinity</td>
<td>0.79 g/L</td>
<td>0.79 g/L</td>
<td>0.79 g/L</td>
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<tr>
<td>Salinity limit at RMZ$_{90}$</td>
<td>40.39 g/L</td>
<td>40.39 g/L</td>
<td>40.39 g/L</td>
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<tr>
<td>Actual salinity above ambient</td>
<td>0.273 g/L (39.87 g/L at RMZ$_{90}$)</td>
<td>1.184 g/L (40.78 g/L at RMZ$_{90}$)</td>
<td>0.595 g/L (40.2 g/L at RMZ$_{90}$)</td>
</tr>
<tr>
<td>Actual salinity at RMZ$_{90}$</td>
<td>39.87 g/L</td>
<td>40.78 g/L</td>
<td>40.20</td>
</tr>
<tr>
<td>Corresponding dilution ((S)) ratio (21.84:1 required)</td>
<td>Low-velocity scenario (0.04 m/s ambient velocity)</td>
<td>High-velocity scenario (0.11 m/s ambient velocity)</td>
<td>Average-velocity scenario (0.07 m/s ambient velocity)</td>
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<tr>
<td>63.4:1</td>
<td>14.6:1</td>
<td>29.1:1</td>
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<tr>
<td>Plume location</td>
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</table>
| \[x = 90 \text{ m}\] \[
y = 37.95 \text{ m}\] \[
z = -18.44 \text{ m}\] | \[x = 90 \text{ m}\] \[
y = 8.04 \text{ m}\] \[
z = -18.25 \text{ m}\] | \[x = 90 \text{ m}\] \[
y = 16.56 \text{ m}\] \[
z = -18.31 \text{ m}\] |
| Plume dimensions                                |                                                 |                                                 |                                                 |
| Half-width \((bh)\) = 11.73 \text{ m}\)     | Half-width \((bh)\) = 13.59 \text{ m}\)      | Half-width \((bh)\) = 11.98 \text{ m}\)      |
| Thickness \((bv)\) = 5.39 \text{ m}\)     | Thickness \((bv)\) = 1.77 \text{ m}\)        | Thickness \((bv)\) = 3.06 \text{ m}\)        |
| Cumulative travel time \((TT)\) to the RMZ boundary | 2248.59 sec.                                      | 815.02 sec.                                      | 1283.52 sec.                                      |

CORMIX predicted dilutions of 4.1:1 at end of the near-field region, 9.2:1 at 15.19 m, and 14.7:1 at 28.1 m, which sits well with the line plume equation as well as calculated dilutions from the water samples collected in a transect away from the discharge structure, at seafloor level (7.6:1 at 0 m, 10.2:1 at 2.5 m and 5 m, 10.9:1 at 15 m and 12.3:1 at 25 m (Figure 15)), and is also representative of salinity measurements recorded by the AUV.

In order to assess likely environmental variability, we also conducted a sensitivity study (Table 3.6) using CorSens (a CORMIX post-processing tool). This was done to address possible issues relating to mixing zone compliance over a range of ambient conditions. The average velocity base case was “seeded” as source file for the sensitivity study and the parameter chosen was ambient velocity \((UA \text{ (m/s)})\) with the range set from 0.07 m/s to 0.34 m/s in ten increments. All other parameters were kept the same as for the case scenario model runs, with an average concentrate discharge flow of 0.479 m³/s,
discharge density of 1037.08 kg/m$^3$, ambient depth of 18.2 m, and surface and bottom densities of 1024.82 kg/m$^3$, respectively.

Table 3.6: CorSens results summary.

<table>
<thead>
<tr>
<th>Case number</th>
<th>Flow class</th>
<th>Sensitivity parameter (ambient velocity ($U/A$ (m/s)))</th>
<th>Plume location (x (m))</th>
<th>Plume location (y (m))</th>
<th>Plume location (z (m))</th>
<th>Dilution (S) ratio</th>
<th>Salinity concentration ($C$ (mg/L)) above ambient salinity levels</th>
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<tr>
<td>KAUST_S01</td>
<td>NH1</td>
<td>0.07</td>
<td>90</td>
<td>16.56</td>
<td>-18.31</td>
<td>29.10:1</td>
<td>595</td>
</tr>
<tr>
<td>KAUST_S02</td>
<td>NH1</td>
<td>0.10</td>
<td>90</td>
<td>9.36</td>
<td>-18.26</td>
<td>16.90:1</td>
<td>1020</td>
</tr>
<tr>
<td>KAUST_S03</td>
<td>NH1</td>
<td>0.13</td>
<td>90</td>
<td>6.20</td>
<td>-18.24</td>
<td>11.30:1</td>
<td>1540</td>
</tr>
<tr>
<td>KAUST_S04</td>
<td>NH1A2</td>
<td>0.16</td>
<td>90</td>
<td>5.48</td>
<td>-18.24</td>
<td>72.30:1</td>
<td>239</td>
</tr>
<tr>
<td>KAUST_S05</td>
<td>NH1A2</td>
<td>0.19</td>
<td>90</td>
<td>5.53</td>
<td>-18.24</td>
<td>84.80:1</td>
<td>204</td>
</tr>
<tr>
<td>KAUST_S06</td>
<td>NH1A2</td>
<td>0.22</td>
<td>90</td>
<td>5.57</td>
<td>-18.24</td>
<td>97.10:1</td>
<td>178</td>
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<tr>
<td>KAUST_S07</td>
<td>NH1A2</td>
<td>0.25</td>
<td>90</td>
<td>5.60</td>
<td>-18.24</td>
<td>109:1</td>
<td>158</td>
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<tr>
<td>KAUST_S08</td>
<td>NH1A2</td>
<td>0.28</td>
<td>90</td>
<td>5.62</td>
<td>-18.24</td>
<td>121:1</td>
<td>143</td>
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<tr>
<td>KAUST_S09</td>
<td>NH1A2</td>
<td>0.31</td>
<td>90</td>
<td>5.64</td>
<td>-18.24</td>
<td>133:1</td>
<td>130</td>
</tr>
<tr>
<td>KAUST_S10</td>
<td>NH1A2</td>
<td>0.34</td>
<td>90</td>
<td>5.66</td>
<td>-18.24</td>
<td>144:1</td>
<td>120</td>
</tr>
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</table>

The observed flow class change indicates a change in physical mixing processes that can effect plume trajectory and dilution. In this case there is a flow classification with a dynamic plume bottom attachment (NH1 A2) compared with a flow class without plume bottom attachment (NH1). This also explains the better dilution ratios in the (NH1 A2) flow class, as a result of plume/bottom interaction.

In order to achieve the $\Delta 2\%$ above ambient RMZ$_{90}$ requirement, salinity should be less or equal to 40.39 g/L at the 90 m regulatory mixing zone boundary in any distance away from the discharge. This will mark a dilution of 21.84:1 (40.39 g/L). Both the low- and average velocity scenarios achieved the regulatory requirement, whilst the high velocity scenario only achieved a dilution of 14.6:1 (40.78 g/L) at RMZ$_{90}$. The CorSens results summary also predicted sufficient dilution ratios above 21.84:1 with an increase in ambient velocity, except for velocities ranging between 0.10 m/s – 0.13 m/s. This also
validates the non-compliance for the high velocity case scenario, modeled at 0.11 m/s ambient velocity. CorSens similarly predicts that salinity concentrations for case numbers 2 (KAUST_S02) and 3 (KAUST_S03) will also be in non-compliance with salinity values of 40.62 g/L and 41.14 g/L respectively. It must be underlined that all salinity concentrations with regards to the velocity case scenarios, as well as for CorSens case numbers 1 – 10 would be in non-compliance with the PME’s prescribed salinity concentration of Δ0% above ambient salinity levels for marine classified areas.

3.4.6 Recommendations

AUV missions can provide significant insight with regards to plume identification and effluent discharge environmental impact studies. It is important to target appropriate environmental and biological parameters as variations in background properties can cause ambiguity in identifying exact plume behavior. This suggests that only a single or even a pair of properties may not be adequate to unambiguously identify “discharge” affected waters and to differentiate other components of the particulate field. Combined with robust in-situ field measurements, models and expert systems can be used to assist regulators with regards to possible impacts on the marine environment by defining appropriate mixing zones and dilution criteria.

Mixing zone allowances can increase the mass loadings of the pollutants to the water body and may adversely impact immobile species, such as benthic communities, in the immediate vicinity of the outfall. Because of these and other factors, regulatory mixing zones must be applied carefully, to ensure sustainable management of concentrate discharge and ambient water quality, protect the natural aquatic environment and provide a basis for the protection and restoration of waters within the Kingdom of Saudi Arabia.
This case study revealed problems when applying the PME’s regulatory discharge concept to a case study, especially a prescribed salinity concentration of $\Delta 0\%$ above ambient salinity levels for high-value and marine classified areas seems unrealistic, as this suggests infinite dilution in the mixing zone, which in reality is unrealistic and hydrodynamically not possible.

Recently, the Southern California Coastal Water Research Project (SCCWRP) proposed scientific based recommendations for concentrate discharge into coastal waters. Jenkins, et al. (2012) suggested a revised regulatory framework that at least should include defining the near-field characteristics of the discharge, which are used to evaluate the effectiveness of the discharge design for minimizing ecological impacts.

A monitoring program of both the effluent and the receiving environment should be required for all discharges having potential for environmental impacts. Laboratory toxicity testing of effluent using local species and sublethal endpoints should be included and for concentrate discharges, *in-situ* salinity tolerance tests around existing desalination discharges should also be conducted. For instance, appropriate scientific studies may indicate if coral species in the Red Sea would be able to retain their photosynthetic capacity after exposure to the short- and long-term extreme salinity conditions that are expected to occur in full-scale operations. Field monitoring should also include analysis of other benthic organisms to detect changes of concern. There is, however, no single discharge strategy that is optimum for all types of anticipated scenarios. Different discharge strategies should therefore be used, depending on site-specific conditions. Taking this into consideration, we suggest the following in order to improve higher initial dilutions in the near-field and minimizing possible impacts at the existing KAUST
SWRO plant:

• A multiport diffuser will generally provide the highest dilution ratio for dense discharges. We also recommend installing a cost effective unidirectional multiport diffuser in the place of the existing KAUST SWRO discharge structure in order to achieve higher initial dilutions in the near-field.

• Blending concentrate with existing discharges can be effective in achieving higher dilution of the discharge, but is not recommended. Pumping additional treated wastewater will result in higher energy costs. Additionally, when concentrate is blended with wastewater, chemical/physical interactions of the concentrate with wastewater constituents may produce toxic effects, possibly resulting in adverse environmental impacts. Also, in extremely water scarce countries like KSA, wastewater should be considered a resource and not used for blending SWRO concentrate.

• Other constituents may require monitoring far outside the regulatory mixing zone, such as dissolved oxygen (DO), in order to address long-term or cumulative effects [27].

• For negatively buoyant plumes, such as those arising from dedicated SWRO desalination concentrate discharges, a revised regulatory framework is needed. This framework should include a revised definition of the regulatory mixing zone and a field-monitoring component (monitoring should at least cover the area of the RMZ and must involve one or two control sites).

• Another element that may need revision is the requirements of the water quality (ambient) conditions to be met at the RMZ. Plume models could also be applied
to the discharge characteristics, the mixing characteristics of the site, and the proposed discharge technology to determine the initial dilution and verify that receiving water objectives will be met at the edge of the mixing zone.

Together with selecting the most suitable discharge location, necessary salinity reduction can be achieved through sufficient near field hydrodynamic mixing (which can be achieved through appropriate outfall/discharge design).

### 3.5 Conclusions

In this paper, we reported results from two separate AUV missions with velocimeters and hydrodynamic flow visualizations for the evaluation of a submerged offshore SWRO concentrate discharge. During the first AUV mission, no significant salinity signatures were recorded (MIN 39.63 g/L; MAX 41.82 g/L; 0.32 %RSD), whereas during the second AUV survey mission, a clear and measurable salinity signature was recorded with average salinity measurements of 39.71 g/L and a maximum measurement of 51.51 g/L close to the discharge structure. As distance increases from the discharge structure, the concentrate in the study area typically mixes with the receiving waters to the point of showing little to no discernible salinity abnormalities within rather short distances. This can again be confirmed with low %RSD values for salinity (0.87%) and temperature (1.65%) for the complete second mission. Chlorophyll a concentrations were also homogenous with an average concentration of 0.54 µg/L measured throughout the transects during the second REMUS mission.

Water current velocity data were also recorded utilizing two acoustic Doppler velocimeters (ADVs), deployed for just under a 6-month period. Results clearly show noticeable seasonal variations with higher mean velocities recorded during winter months.
and lower ambient flows during the summer months. ADV_1, which was deployed closest to the discharge structure, showed average velocities of 0.066 m/s (August 2012), 0.063 m/s (September 2012), 0.086 m/s (October 2012), 0.099 m/s (November 2012), 0.114 m/s (December 2012) and 0.118 m/s (January 2013). The data show that currents are predominantly in a westerly direction, however, the data do not suggest a relation between prevailing winds and deep-water current direction.

We also examined the existing SWRO discharge dilution performance using mathematical dilution equations. The most appropriate approximation for our case study was to predict near-field dilution using the equation for a line plume, which predicted a dilution of 7.16:1 at the end of the near field region. We also presented flow visualizations for three case scenarios (in low-, high- and average-ambient velocity) with emphasis on the regulatory framework of the mixing zone (RMZ) using the Cornell Mixing Zone Expert System (CORMIX). CORMIX predicted dilutions of 4.1:1 at end of the near-field region, 9.2:1 at 15.19 m, and 14.7:1 at 28.1 m, which sits well with the line plume equation as well as calculated dilutions from actual water samples collected in a transect away from the discharge structure, at seafloor level while diving (7.6:1 at 0 m; 10.2:1 at 2.5 m and 5 m; 10.9:1 at 15 m; 12.3:1 at 25 m (Figure 15)) and is also representative of salinity measurements recorded by the AUV. Based on our findings, a revised regulatory framework for mixing zones within the Kingdom of Saudi Arabia was recommended.
References


Chapter 4

Investigating the effects of elevated salinity from seawater reverse osmosis desalination concentrate on the coral *Fungia granulosa* and its photophysiology

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**Abstract:**

Concentrate (when discharged to the ocean) may have chronic/acute impacts on the marine ecosystems in the near-field area of the discharge. The environmental impact of the desalination plant discharge is very site- and volumetric specific, and depends to a great extent on the salinity tolerance of the specific marine microbial communities as well as higher order organisms (e.g. corals) inhabiting the water column in and around an extreme discharge environment. Scientific studies that aim to understand possible impacts of elevated salinity levels are very important, especially for species that have no known mechanisms of osmoregulation, such as corals, so that a change in salinity may affect their metabolism and survival/reproductive capacities.

The main purpose of this study was to conduct long-term (29 days) *in-situ* salinity tolerance tests at an offshore seawater reverse osmosis (SWRO) discharge on the coral *Fungia granulosa* and its photophysiology. The corals were exposed to elevated levels of salinity as a direct result of concentrate discharge. Their photosynthetic response after exposure to extreme salinity conditions around the full-scale operating SWRO desalination discharge was assessed. A pulse amplitude modulated (PAM) fluorometer (DIVING-PAM, Walz, Germany) was used to assess photochemical energy conversion in photosystem II (PSII) measured under constant concentrate discharge conditions.
Keywords: Desalination; Seawater reverse osmosis; Concentrate discharge; Salinity tolerance; Coral; *Fungia granulosa*; Photosynthesis; Marine monitoring; Environmental impact assessment.
4.1 Introduction

Environmentally safe disposal of the concentrate produced by seawater desalination plants is one of the key factors determining the environmental impacts of a desalination plant. The maximum salinity that can be tolerated by marine organisms in a desalination discharge area is defined as a salinity tolerance threshold and depends on the type of marine organisms inhabiting the area as well as the period of time which these organisms are exposed to elevated salinity levels [1].

Most commonly, the methodology used to evaluate the impact of desalination concentrate discharge on marine organisms is based on the United States Environmental Protection Agency’s (US EPA) whole effluent toxicity (WET) test. WET tests replicate the total effect and actual environmental exposure of aquatic life to toxic pollutants (e.g., salinity) as measured by an organism's response upon exposure to the sample (e.g., lethality, impaired growth, photosynthetic performance, reproduction, etc.). The test however only allows predicting levels above which marine organisms die based on controlled laboratory conditions.

*In-situ* affects of high salinity on marine species and in particular on corals (as a direct result of concentrate discharge) have not been thoroughly studied. Detrimental affects on reef-building (hermatypic corals) can occur due to physiological stress on the coral host or zooxanthellae (or both). Zooxanthellae (endosymbiotic photosynthetic dinoflagellates) are found in the endodermal tissues of reef-building corals. ‘Coral bleaching’ occurs when environmental conditions disrupt the symbiosis, leading to the degeneration and/or expulsion of zooxanthellae from the coral host [19] and is characterized by the loss of dinoflagellate symbionts (genus *Symbiodinium*) and/or
symbiont pigmentation loss [20]. As a result of photosynthetic pigment loss, the white skeleton becomes visible through the transparent coral tissue, giving the organism a ‘bleached’ white appearance. Bleaching can be fatal to the coral unless the symbiotic relationship is quickly re-established. Corals are considered stenohaline, osmoconformers [21]—yet only a limited number of studies have considered the in-situ quantitative impacts of hyper salinity on corals and the possible effect it might have on the dinoflagellate functionality within the host. Most literature on salinity as a potential stressor is also dated [2]–[4].

Previous work studied the effect of 10 – 20 g/L rapid change in salinity on the rates of respiration and photosynthesis of the hermatypic corals *Siderastrea siderea*, *Porites lutea* and *Pocillopora damicornis* [2], [5]. This research has mainly dealt with large variations in salinity over short incubation periods.

Ferrier-Pagès *et al* (1999) studied the effects of a long-term (3 weeks) change in salinity (increase or decrease of 2 to 4 g/L) on the rates of photosynthesis and respiration of the zooxanthellate of the coral *Stylophora pistillata*. Specimens were exposed to 4 levels of salinity (34, 36, 38 (control salinity) and 40 g/L). This study concluded that salinity had a significant effect on the protein concentration. The results also suggested a significant effect on the rates of photosynthesis, respiration and on the gross production to respiration ($P_g$:R) ratio. Gross maximal photosynthetic rates were 50% lower at 34, 36 and 40 g/L than at the control salinity (38 g/L). The $P_g$:R ratio was always higher at the control salinity, whereas most of the corals maintained at 40 g/L died [4].

A more recent study presented an empirically derived salinity threshold (responses of corals to low salinity) for sensitive *Acropora* spp. from the Keppel Islands.
in the southern inshore Great Barrier Reef, based on *in-situ* exposure to major flood events [3]. Hoegh-Guldenberg, *et al.* (1989) also studied the population density and export of *Symbiodinium* (zooxanthellae) under reduced salinity levels. Exposing *Stylophora pistillata* and *Seriatopora hystrix* to seawater at a reduced salinity of 30% and at several temperatures did not affect any biomass or metabolic variable measured in this study, whereas reducing salinity even further (to 23%) caused death, without any reduction in the population density of zooxanthellae within 48 h. The authors concluded that exposure to moderately reduced salinity for 4-10 days does not induce bleaching characteristics [6].

Clearly, very little has been done to study the possible *in-situ* effects of elevated salinity levels on physiological functions (such as photosynthesis) necessary for coral survival during long-term exposure, especially as a direct result of concentrate discharge. Though a number of studies have reported the effects of multiple stressors on coral species [6]–[10], similar information on the effects of salinity is lacking. Ecological monitoring studies as direct result of concentrate discharges have found variable effects on corals and other marine species, ranging from no significant impacts on microbial abundance [11], through to widespread alterations to community structure in coral, soft-sediment ecosystems and seagrass (*Posidonia oceanica*) when discharges are released to poorly flushed environments [12]–[16]. Similarly, elevated salinity may affect hermatypic corals due to physiological stress on the coral animal and/or the corals’ algal symbionts due to salinity stress [17] (if the symbiotic relationship between coral and algae is disrupted), affecting coral metabolism and photophysiology [18].
The objective of this experiment was to determine long-term effects (4 weeks) of a significant salinity increase (about 12 g/L or 30 % above ambient) on the Red Sea coral *Fungia granulosa* using pulse amplitude modulated (PAM) fluorometry.

PAM has become a commonly used tool to assess the photosynthetic performance of coral dinoflagellate symbionts under normal and stressed (bleaching) conditions. PAM fluorometry measures the efficiency with which light energy is converted into chemical energy at photosystem II (PSII) level. Any change in photosynthetic efficiency will affect all photosystem electron transport processes downstream of PSII. Consequently, PSII is considered to be the most stress-sensitive part of the photosynthetic pathway [19], [22]. Evaluating the chlorophyll fluorescence pathway can therefore indicate an organism’s photosynthetic efficiency under changing or stressful conditions, e.g. varying salinity regimes [18].

Many marine organisms have the natural ability to adapt to a wide range of salinity concentrations, as long as these changes occur gradually. Voutchkov (2009) stated that marine organisms exposed to increasing salinities in a series of small increments can tolerate higher salinities than organisms exposed directly to high salinities. Since our experiment includes direct exposure of *Fungia granulosa* to an extreme salinity environment without any incremental acclimation and adaption, this experiment is representative for a ‘worst-case’ scenario where the corals were exposed to an abrupt increase in salinity.

Based on the literature, we anticipated distinct impairment of photosynthetic characteristics as a response to elevated salinity levels. We additionally expected particularly quick indications of bleaching for the specimens exposed to the highest
salinity levels (on average, on the order of 9.5 g/L (24%) above ambient)), directly at the discharge screen.

4.2 Materials and methods

The existing SWRO facility (purposely, the submerged discharge location) at the King Abdullah University of Science and Technology (KAUST) was selected for the case study. The plant is located on the KAUST campus (located approximately 90 km north of Jeddah) and was designed to provide all potable water needs for the campus as well as the residential areas. Under current operational conditions, the raw water intake is on the order of 2,825 m$^3$/hour with a recovery ratio of 39%, resulting in an average concentrate flow of 1,723.25 m$^3$/hour (41,358 m$^3$/day) that is discharged to the Red Sea. The submerged outfall (discharge structure) is located at 22° 17.780N, 39° 04.444E and sits at a water depth of 18 m, approximately 2.8 km from the pump station (Figure 4.1a). The concentrate is pumped through a 1,200 mm diameter pipeline to the offshore structure (Figure 4.1b) where the concentrate is pushed up in a concrete riser and discharged horizontally through four discharge screens (1,800 mm × 1,000 mm) approximately 6 m above the seafloor.

4.2.1 Coral collection, relocation and setup

This research was conducted from January 15$^{th}$ – February 13$^{th}$ 2014 (29 days) and involved an in-situ salinity-stress experiment on the coral *Fungia granulosa* (Figure 4.2). All corals were collected from Fsar Reef (22° 13.945N, 39° 01.783E) approximately 9 km southwest (heading: 212°) from the study site. Ambient salinity was on the order of 39 g/L (measured at a water depth of 8 m. For a baseline, we also measured ambient
light conditions as well as effective PSII quantum yield for three *Fungia granulosa* specimens *in-situ* at the collection site before relocating the corals to the study area (ambient light conditions were on the order of 40 PAR (photosynthetically active radiation) measured at approximately 09:00) and the average $\Delta F/F_m$ yields were around of 0.7 (for the ‘control’ specimens (as a baseline)). Corals were handled with powder-free latex gloves and care was taken to ensure corals were maintained under ambient field conditions prior to relocation. Specimens were collected on January 14$^{th}$ (at depths between 16 m – 19 m (which is comparable to the depth at the study area)). They were then relocated to the study area, labeled (using nylon fishing line and under water paper tags), photographed and placed on the roof of the discharge structure (water depth of 10 m), in equivalent light conditions for an acclimation period of 20 hours. On the morning of the 15$^{th}$, the specimens were tied to the 6 monitoring stations (at the discharge screen (station 1) and along a 25 m transect (northwards (transect stations 2 – 6)). We also allocated a variety of specimen sizes randomly at each station to ensure a uniform distribution in triplicates.

First measurements (T0) were recorded between 11:00 and noon on the same day. Figure 4.3 illustrates the 6 underwater monitoring stations located in a distance away from the discharge structure (at the discharge screen (station 1), 0 m (station 2), 2.5 m (station 3), 5 m (station 4), 15 m (station 5) and 25m (station 6)). At monitoring station 1, specimens were secured horizontally and directly to the stainless steel discharge screen, whereas for monitoring stations 2 – 6, they were placed and secured on pre-deployed concrete blocks at 0 m, 2.5 m, 5 m, 15 m and 25 m, respectively. Figure 4.4 presents a general view of the experimental setup.
Figure 4.1 a: The KAUST SWRO offshore discharge (study area) and Inner Fsar (coral collection site (source: Google earth)).
b: Schematic of the underwater concentrate discharge structure.
Figure 4.2: The free-living mushroom coral *Fungia granulosa* (Klunzinger, 1879). The picture was taken at the site from which the corals were collected and shows the typically wavy appearance of the septa. Corals are circular, up to 135 mm in diameter, flat or with a central arch. Specimens are usually brown in color.

Figure 4.3: The 6 underwater monitoring stations located in a distance away from the discharge structure.
Figure 4.4: At monitoring station 1, specimens were secured horizontally and directly to the northwards-facing discharge screen.  b: An example of the concrete blocks before they were deployed at each of the underwater monitoring stations on the seafloor.  c: Shows a specific specimen, tied (with nylon fishing line) to one of the concrete blocks at station 6. The figure also points out 2 of 6 temperature data loggers as well as an example of the coral identification tags.

4.2.2 Data collection

4.2.2.1 Environmental data
As part of this study, we routinely collected data on temperature, dissolved oxygen (DO) and salinity. We also visually assessed all specimens for any evidence of bleaching during each of the 7 sampling dives (T0 – T6). For temperature, we utilized six HOBO Pendant® Temperature Data Loggers (one at each of the 6 monitoring stations). Temperature data at monitoring stations 1 – 5 was recorded from 09:00 on January 15th, 2014 to 11:00 on February 13th 2014, whereas temperature measurements at monitoring station 6 commenced at a later stage (11:30 on January 30th). Recording intervals for all the temperature loggers were set at 10 minutes. For dissolved oxygen and salinity measurements, water samples were collected during 7 dives (T0 – T6 (January 15th (T0), 16th (T1), 19th (T2), 21st (T3), 23rd (T4), 30th (T5) and February 13th (T6) of 2014)) at each of the monitoring stations. Water samples for dissolved oxygen and salinity measurements were collected using Falcon 50 mL Conical Centrifuge Tubes (for salinity measurements) and 1 L low-density polyethylene (LDPE) Cubitainers (for dissolved oxygen). Dissolved oxygen and salinity were measured immediately after finishing each dive. Measurements were conducted using a WTW 3500i Multi-Parameter Water Quality Meter with a CellOx® 325 DO electrode for dissolved oxygen and a WTW Cond 3310 Meter with TetraCon® 325 probe for salinity. All instruments were calibrated in accordance with manufacturer's specifications.

4.2.2.2 Effective quantum yield ($\Delta F/F'_{m'}$)

Together with collecting data on the environmental parameters, an underwater chlorophyll fluorometer (DIVING-PAM, WALZ, Germany) was used to assess the effective quantum yield ($\Delta F/F'_{m'}$), where $\Delta F$ is the increase of fluorescence yield, $F'_{m'}$ – $F$, induced by a saturation pulse; and $F'_{m'}$ the maximal fluorescence yield of an
illuminated sample with all PS II centers closed) of the symbiotic algae *in-situ* during each of the experiment’s sampling intervals (T0 – T6).

Photons, once they reach photosynthetic cells, are collected through the light-harvesting complex. Their energy is shuttled to the reaction center of the cell and finally reaches the photosystem II (PS II) in form of electrons. Schreiber *et al.* (1986) states that, depending on the light intensity, energy is processed in three competing pathways: (i) Dissipation as heat, also called non-photochemical quenching (ii) fluorescence, and (iii) photochemistry, called photochemical quenching, where energy enters the electron transport chain and is used for photosynthesis. While (i) and (ii) are photoprotective mechanisms, (iii) enables adenosine triphosphate (ATP) synthesis and carbon fixation in plants. PAM fluorometry allows differentiating between these pathways: Fluorescence is measured to assess the photosynthetic state of PSII of a photosynthetic organism [23].

In corals, symbiont pigments are excited by the instrument’s pulse modulated red light from a light-emitting-diode (LED (655 nm)). The LED-light is passed through a cut-off filter (Balzers DT Cyan, special) resulting in an excitation band peaking at 650 nm, with a very small ‘tail’ at wavelengths beyond 700 nm. Fluorescence is detected with a PIN-photodiode (type BPY 12, Siemens) at wavelengths beyond 700 nm, as defined by a long-pass filter (type RG 9, Schott). Measurements were taken by placing the end of the probe gently against the surface of each coral. Photosynthetically active radiation (PAR) reads were obtained via the PAR sensor (fiber quantum sensor, averaged over 15 sec) and a transparent rubber tube covering the probe kept a distance of approximately 3 mm (such that a normal sample at standard settings gives a signal of 300-500 units (in accordance with manufacture’s recommendations)) between the
fiberoptics and the coral surface. After a ~5 s period a weak pulse-modulated red measuring light (0.15 µmol photons m$^{-2}$s$^{-1}$) was applied to determine the steady-state fluorescence ($F$) under ambient light. To quantify light adapted maximum fluorescence ($F_{m'}$), a short pulse (800 ms) of saturating actinic light (3000 µmol photons m$^{-2}$s$^{-1}$, 650 nm) was applied [24]. The effective quantum yield ($\Delta F/F_{m'}$) of photochemical energy conversion in PSII of each measurement was calculated based on $F$ and $F_{m'}$:

$$\Phi_{PSII} = (F_{m'} - F)/F_{m'} = \Delta F/F_{m'} [25].$$

A high yield (greater difference between steady-state fluorescence and maximum fluorescence) indicates low photoprotection, a low yield shows a high degree of photoinhibition. Thus, the effective quantum yield provides a measure for the current state of photoinhibition (or photoprotection) on PSII level. Yields measured from the same organism or species at different times under similar (ideally identical) light conditions can provide information about stress on the PSII level. Changing ambient light conditions may influence the comparability of measurements: With higher light $F$ increases which decreases $\Delta F/F_{m'}$.

$F$ and $F_{m'}$ measurements of each specimen were conducted between 11:00 and 12:00 to ensure comparable daytime conditions. All $\Delta F/F_{m'}$ measurements were taken in triplicate for each coral. A short video showing one of the authors operating the DIVING-PAM as part of this experiment can be viewed here: [https://vimeo.com/95486530](https://vimeo.com/95486530) or [http://youtu.be/cc-monk1wDXI](http://youtu.be/cc-monk1wDXI).

### 4.3 Results
4.3.1 Ecological conditions

Changes in water temperature show a maximum range of 4.2 °C. A maximum temperature of 28.46 °C was recorded twice at monitoring station 1 (directly at the discharge screen) on 02/04/2014 (mm/dd/yyyy (00:50 and 01:50, respectively)), whereas a minimum temperature of 24.26 °C was recorded at monitoring station 6 on 02/11/2014 (01:10). Water temperatures recorded at the discharge screen were on average 0.33 °C higher compared to those at stations 2 – 6. The data also suggests a minor decrease (-0.47 °C) in average temperatures in a distance away from the discharge structure (26.3 °C (station 1) – 25.83 °C (station 6)). Table 4.1 shows the average (AVE), standard deviation (STDEV), minimum (MIN) and maximum (MAX) values for temperature recorded at all the underwater monitoring stations. Figure 4.5 provides the recorded water temperature measurements for the duration of the experiment.

Table 4.1: Temperature data recorded using six HOBO Pendant® Temperature Data Loggers (one at each of the 6 monitoring stations). Recording intervals for all the temperature loggers were set at 10 minutes.

<table>
<thead>
<tr>
<th></th>
<th>Station_1 (Discharge Screen)</th>
<th>Station_2 (0 m)</th>
<th>Station_3 (2.5 m)</th>
<th>Station_4 (5 m)</th>
<th>Station_5 (15 m)</th>
<th>Station_6 (25 m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAX</td>
<td>28.46</td>
<td>27.76</td>
<td>27.67</td>
<td>27.57</td>
<td>27.57</td>
<td>27.57</td>
</tr>
<tr>
<td>MIN</td>
<td>24.74</td>
<td>24.64</td>
<td>24.55</td>
<td>24.45</td>
<td>24.35</td>
<td>24.26</td>
</tr>
<tr>
<td>AVE</td>
<td>26.30</td>
<td>26.08</td>
<td>26.02</td>
<td>25.99</td>
<td>25.84</td>
<td>25.83</td>
</tr>
<tr>
<td>STDEV</td>
<td>0.78</td>
<td>0.63</td>
<td>0.62</td>
<td>0.64</td>
<td>0.73</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Recorded water temperature measurements for the duration of the experiment. Noticeable, the higher temperatures recorded at station 1. The figure also shows the minor decrease in temperatures in a distance away from the discharge structure (stations 2 – 6).

Dissolved oxygen (DO) varied from a high of 6.37 mg/L at monitoring station 6 (measured during T0 on 01/15/2014) to a low of 5.75 mg/L measured at the discharge screen during T2 on 01/19/2014. From the data, average dissolved oxygen levels (for T0 – T5) at each of the different monitoring stations were 6.02 mg/L (station 1; discharge screen), 6.07 mg/L (station 2; 0 m), 6.08 mg/L (station 3; 2.5 m), 6.07 mg/L (station 4; 5 m), 6.11 mg/L (station 5; 15 m) and 6.09 mg/L (station 6; 25 m), respectively. The average oxygen level for all measurements is 6.07 mg/L with a percent relative standard deviation (%RSD) of 2.88%. Dissolved oxygen recorded during T2 showed slightly lower levels compared to the other values, but is still considered to be normal. Oxygen levels were not measured for T6 (02/13/2014). Figure 4.6 shows an overview of the recorded measurements.
Salinity data from T0 – T6 suggests clear differences between stations. As distance increases from the discharge structure, the concentrate in the study area naturally mixes with the receiving waters to the point of showing little to no discernible salinity abnormalities within rather short distances. A maximum salinity of 51.2 g/L was recorded at the discharge screen (station 1) during T1 (01/16/2014) which is nearly 12 g/L or 30% higher than ambient (39.6 g/L) conditions. The average salinity for all measurements (T0 – T6) at the discharge screen (station 1) is 49.11 g/L with a %RSD of 2.83, compared to an average salinity of 41.45 g/L (1.85 g/L or 4.67% above ambient) for transect stations 2 – 6. Figure 4.7 clearly shows very rapid dilution of the concentrate as soon as it exits the discharge screen. Initial dilution is caused by turbulence originating from the sinking plume and the vortices, whereas further away from the discharge structure it changes to gravitational diffusion which creates a stable density profile that ultimately causes the near-field turbulence to collapse and mixing to cease. A bottom density current forms, where (in the absence of ambient stratification) the plume then proceeds down a slight slope with very little turbulence or mixing. That might be a
reason why highest salinity changes were observed when the plume sinks from the discharge screen (station 1) to the bottom (station 2) whereas only slight salinity changes were observed from the bottom of the discharge structure (station 2) to 25 m distance away (station 6).

Figure 4.7: In-situ salinity measurements in a distance away from the discharge structure. Average salinity values measured at the different monitoring stations were 49.11 g/L (discharge screen), 41.97 g/L (0 m), 41.46 g/L (2.5 m), 41.42 g/L (5 m), 41.31 g/L (15 m) and 41.06 g/L (25 m), respectively.

4.3.2 Photosynthetic efficiency

$\Delta F/F_{m}'$ was measured for the same 18 corals (1.1, 1.2, 1.3 (station 1); 2.1, 2.2, 2.3 (station 2); 3.1, 3.2, 3.3 (station 3); 4.1, 4.2, 4.3 (station 4); 5.1, 5.2, 5.3 (station 5); 6.1, 6.2, 6.3 (station 6)) in triplicates (per specimen) for T0 – T6 (results are presented in Figure 4.8). Average $\Delta F/F_{m}'$ yields were 0.705 (station 1), 0.694 (station 2), 0.687 (stations 3 and 4), 0.693 (station 5) and 0.690 (station 6), respectively. These results are on the order of the ‘baseline’ yields measured under natural conditions (average $\Delta F/F_{m}'$ yields were around 0.701) and suggest no lasting discernable effect on PS II level of the dinoflagellate symbionts and/or noticeable symbiont pigmentation loss (based on visual
observations). Noticeable are the lower yields during T1 at transect stations 3 – 6 which coincides with high(er) PAR measurements under extremely good visibility conditions in the surrounding water (Figure 4.8c, d, e, f). PAR measurements during T1 for monitoring stations 1 and 2 were much lower, since those stations are permanently shaded by the discharge structure (Figure 4.8a, b).

Figure 4.8: Stress-response of effective PSII quantum yield ($\Delta F/F_{m}'$) to elevated salinity.

### 4.4 Discussion

Based on the literature, we anticipated distinct impairment of photosynthetic characteristics as a response to elevated salinity levels. We also expected particularly quick indications of bleaching for the specimens exposed to the highest salinity levels, directly at the discharge screen. The hypothesis was strongly rejected as symbiotic
Dinoflagellates of *Fungia granulosa* demonstrated high tolerance to hyper saline stress as measured by effective quantum yield of PSII (ΔF/Δm') during this study. ΔF/Δm' indicated that symbiont photosynthetic efficiency was not affected by elevated salinities. This was also the case directly at the discharge screen (station 1), where the corals were exposed to salinity levels on average, 9.51 g/L (24.01%) above ambient conditions (39.6 g/L). A temporary decrease in ΔF/Δm' at T1 for at stations 3 – 6 is a result of high PAR levels (44 – 82 (Figure 4.9)). At the same time, stations 1 and 2 were shaded by the discharge structure (PAR 11 and 16) and did not show any reduced ΔF/Δm’ readings. The high standard deviation (±31) of PAR levels reflects the difference between stations 1 and 2, relative to stations 3 – 6. Underwater visibility at the study area during T1 on 01/16/2014 was remarkably good (in comparison with ‘normal’ conditions) and these temporal changes are therefore considered unrelated to salt-sensitivity. Overall, PAR readings at station 1 were higher than at all other stations, which cannot be used as an indication for the best photochemical efficiency of PSII since the corals were generally measured under the lowest light conditions as they were permanently ‘shaded’ by the discharge.
Although symbiont and pigment densities were not measured, changes in *Symbiodinium* and/or pigment density were visually assessed during each sampling event—with no visible bleaching evident. This may indicate higher salinity tolerance of *Fungia granulosa* compared to species where substantial loss of pigmentation and/or symbionts has been documented. So far, little attention has been given to understanding osmoregulation in corals and how osmotic shifts under salt-stress conditions can affect both the host and/or symbiont physiology. Chartrand *et al.* (2009) states that a threshold response is indicative of the coral maintaining and successfully regulating its internal osmotic balance. Coral health and survival largely depend on the interaction between the coral host and photosynthetic symbiont algae, as glycerol produced by the symbiont is transferred to the host where it may be rapidly respired as a major energy source or stored in cellular pools [18]. *Fungia granulosa* did not show any significant response to elevated salinities in this experiment. At station 1 (as a worst-case scenario), corals were exposed to an average salinity of 49.11 g/L (9.51 g/L (24.01%) higher than ambient
conditions) for a period of 29 days without any significant effect on photosynthetic performance. Thus, within environmentally realistic salinity ranges to be expected as a result of SWRO concentrate discharge, it is expected that this species would be able to tolerate prolonged exposure to fairly large changes in salinity. Additional studies will be necessary to determine whether *Fungia granulosa* is unique in this regard and how other corals will respond to similar conditions. This could also suggest that some coral species contain mixed populations of zooxanthellae with different degrees of (salinity) tolerance [26]; however this speculation required further detailed investigation.

### 4.5 Outlook

Based on our unexpected observations we suggest to further investigate the tolerance of Red Sea corals to high salinity levels without observable impacts on photosynthetic performance. We suggest several approaches: (i) To gain a broader picture a comparative study with other coral species within the Red Sea or the same species in another coral reef (Carribean, Indian Ocean, Great Barrier Reef, etc.) could prove/disprove this phenomenon to be specimen/Red Sea specific. (ii) To identify specific acclimation mechanisms of the coral *Fungia granulosa* a short-term response (within hours) could deliver further clues. (iii) Assessing the third compartment of the coral holobiont (besides coral host and algal symbiont). Microbial communities within the coral tissue could grant insight in the holobionts physiological acclimation [27], [28].
References


Extensive monitoring campaigns in the area around the KAUST SWRO discharge were conducted to investigate potential changes in the hydrological characteristics of the site as well as potential impacts on marine life at two different ecological levels.

The results of the microbial study using flow cytometry (FCM) distinctly showed a change in microbial abundance as a function of distance away from the discharge structure. Besides elevated salinity levels, a possible negative correlation between conductivity (mS/cm) and FCM results (events/µL) was considered as an explanation for this observed trend. This lead to the hypothesis that the observed changes might be a result of normal dilution, where the concentrate (high conductivity; low microbial numbers) is ‘pushed’ back into an already saline marine environment with preeminent bacterial abundance.

Furthermore, we also considered that possible effects of increased salinity as a result of SWRO concentrate discharge is not limited to microorganisms, and therefore turned our focus on an in-situ coral photosynthetic yield experiment on a higher order organism (*Fungia granulosa*) for assessing effects of higher salinity on photosynthetic performance, also in the near-field area of the submerged SWRO discharge. Based on the literature, we anticipated distinct impairment of photosynthetic characteristics as a response to elevated salinity levels. We also expected particularly quick indications of bleaching for the specimens exposed to the highest salinity levels (on average, on the order of 9.5 g/L (24%) above ambient), directly at the discharge screen. The hypothesis was rejected as symbiotic dinoflagellates of *Fungia granulosa* demonstrated high
tolerance to hyper saline stress as measured by effective quantum yield of PSII ($\Delta F/F_{m}'$) during the in-situ salinity-stress experiment.

In order to investigate the hydrological characteristics of the site, a state-of-the-art propulsion driven autonomous underwater vehicle (AUV) was utilized to conduct surveys in the area around the KAUST SWRO discharge structure. This was combined with continuous velocity measurements at two fixed monitoring stations using acoustic Doppler velocimeters (ADVs) as well as in-situ salinity measurements (collected in a transect away from the discharge structure, at seafloor level while diving). Based on the field data and results, it was evaluated whether or not the outfall is compliant with the regulatory requirements. The Cornell Mixing Zone Expert System (CORMIX) was used in order to confirm if the required dilution ratio of 21.84:1, corresponding to a salinity increase of 0.79 g/L above ambient, will be met in a distance of 90 m from the outfall which constitutes the edge of the regulatory mixing zone for the KAUST facility. CORMIX predicted dilutions of 4.1:1 at the end of the near-field region (2.2 m), 9.2:1 at 15.2 m, and 14.7:1 at 28.1 m, which sits well with the line plume equation as well as calculated dilutions from the water samples collected in a transect away from the discharge structure, at seafloor level (7.6:1 at 0 m, 10.2:1 at 2.5 m and 5 m, 10.9:1 at 15 m and 12.3:1 at 25 m), and is also representative of salinity measurements recorded by the AUV.

We should keep in mind that the KAUST outfall is unusual, as it seems more similar to an intake structure than to outfalls typically used for SWRO plants. The current design results in an uncommon initial mixing process (as demonstrated in Chapter 3). Mixing zone allowances can increase the mass loadings of the pollutants to the water
body and may adversely impact immobile species, such as benthic communities, in the immediate vicinity of the outfall (especially true for inappropriate discharge designs). Because of these and other factors, regulatory mixing zones must be applied carefully. Our results (from Chapter 3) revealed difficulties when applying the PME’s regulatory discharge concept, particularly with the revised prescribed salinity concentrations of $\Delta 0\%$ above ambient salinity levels for high-value and marine classified areas and $\Delta 2\%$ above ambient concentrations at the boundary of the RMZ for industrial classified areas. $\Delta 0\%$ suggests infinite dilution in the mixing zone, which in reality is unrealistic and hydrodynamically not possible. Based on the results of the in-situ field measurements and the fact that CORMIX predicted dilution ratios that was in non-compliance suggest that—under certain ambient conditions—the required dilution ratios might not be met at the boundary of the regulatory mixing zone. Taking into consideration the volumetric specifics of the case study, larger discharges (or similar volumes with higher salt concentration) will find it difficult to comply with the prescribed $\Delta 2\%$ above ambient concentrations at the boundary of the RMZ (for industrial classified areas). The results from the assessment on microbial abundance in the near-field discharge area (during 3 different campaigns), the effect of elevated conductivity on microbial abundance and viability (during 48 hours incubation) as well as a long-term (29 days) salinity tolerance experiment in-situ on the coral *Fungia granulosa* and its photophysiology rejected the hypothesis of possible significant impacts as a result of elevated salinity levels on microbial abundance and higher order organisms (*Fungia granulosa*). Results showed no significant impacts—which lead to the hypothesis that the observed changes in microbial abundance might be a result of normal dilution, where the concentrate (high conductivity;
low microbial numbers) is ‘pushed’ back into an already saline marine environment with preeminent bacterial abundance. The hypothesis of significant effects on photosynthetic performance as a result of elevated salinity levels was also rejected as symbiotic dinoflagellates of *Fungia granulosa* demonstrated high tolerance to hyper saline conditions.

We also reviewed regulatory standards that have been applied locally and internationally as well as a wide-ranging literature review on the toxic effects of concentrate discharges. The effects (or lack thereof) of desalination concentrate vary widely, depending on the organism, site, the biotic community at the site, the nature of the concentrate, and to what degree it is disperse. From an environmental point of view, the design of the particular discharge structure must be adequate so that the effluent discharged adheres to the receiving water guidelines (ambient standards) beyond the mixing zone, and also to ensure that critical salinity limits will not be exceeded at the boundary of the regulatory mixing zone. For KSA, limits apply at the boundary of the regulatory mixing zone, depending on the significance value and depth at the discharge area (to a maximum distance of 100 m). A depth-based approach to define the RMZ boundary can be considered best environmental practice (BEP). However, a prescribed salinity concentration of $\Delta 0\%$ above ambient for high-value and marine classified areas is hydrodynamically not possible. A more realistic approach would be to define a small incremental value above ambient. We therefore used $\Delta 2\%$ (for industrial zones) to evaluate discharge performance of the KAUST outfall. Our results showed that $\Delta 2\%$ could not be met under certain ambient velocities ($0.10 - 0.13$ m/s), which occurred during winter months (Nov – Jan, $0.11$ m/s on average) as measured by ADV_1. Taking
the existing volumetric discharge of the KAUST SWRO into consideration, bigger facilities could also find it difficult to comply with RMZ in KSA. A dilution of 21.84:1 (calculated to meet Δ2% at RMZ90 (specifically for the KAUST plant)) should be readily achievable through appropriate discharge design.

To achieve this, a combined monitoring and modeling approach should also be applied to other (industrial) desalination facilities in the Red Sea (and the whole of KSA) to evaluate their regulatory compliance. Effluent should be monitored for specified physical and chemical parameters and because of uncertainties in plume behavior and modeling (models should be validated based on situ-measurements from the given sites), we also recommend field monitoring programs in discharge areas (pre-discharge conditions and continued after discharge has begun in order to assess possible changes in the ecosystem (comparing it with one or two control sites). Further toxicity studies should be carried out to evaluate salinity tolerance levels of native species from the Red Sea (and the Arabian Gulf). The focus should be on corals and their symbionts and other benthic species, such as echinoderms and sea grasses. Based on the findings, a revision of the RMZ may be required.

We certainly can’t change ambient conditions, but together with selecting the most suitable discharge location, necessary salinity reduction can be achieved through sufficient near field hydrodynamic mixing, achievable through appropriate outfall/discharge design.

**APPLICABILITY TO OTHER FACILITIES**

It is widely accepted that concentrate discharge from desalination plans may have negative effects on the environment. Studies from Australia, California and Spain have
shown that slight salinity elevations due to concentrate discharge can affect the survival rates of marine species. Consequently, strict salinity thresholds have been adopted in all 3 countries, which apply to specific ecosystems or facilities, e.g. the salinity threshold for *Posidonia oceanica* seagrass meadows in Spain is 38.5 compared to an ambient salinity of 37-38, whereas the allowable salinity increase for the Perth SWRO plant in Western Australia is 1.2 units above ambient in 50 m distance. The regulatory framework in Sydney requires a salinity limit of $\leq 1$ ppt at 75 m (as a maximum), while the Gold Coast regulator enforce a maximum increment of $\leq 2$ ppt at a compliance point of 120 m from the discharge. Carlsbad and Huntington Beach (California) impose an absolute salinity limit of $\leq 40$ ppt at a compliance point of 1,000 feet relative to the discharge.

At the moment, very little is known from the Red Sea region about the salinity tolerance of marine species and possible impacts from desalination discharge, despite the high conservation value of its ecosystems and high desalination capacities. Data on compliance monitoring of existing desalination facilities with existing environmental regulations is also scarce. The results from this thesis underwrite the relevance of *in-situ* monitoring through appropriate methods and techniques and could also be applied at other facilities, on national as well as international level. The three different modules/experiments of which the results were presented, as well as the recommendations from the thesis could be used to strategize appropriate measuring and monitoring campaigns—on a much broader scale—and is not limited to the KAUST case study.
JOURNAL ARTICLES


CONFERENCE PROCEEDINGS (ORAL PRESENTATIONS)


2. International Conference on Desalination, Environment and Marine Outfall Systems (13-16 April 2014) | Sultan Qaboos University, Muscat, Sultanate of Oman | Title: Combining autonomous underwater vehicle missions with velocimeters and hydrodynamic flow visualizations for the evaluation of a submerged offshore SWRO concentrate discharge.


4. Workshop on Seawater Intakes and Desalination/Wastewater Outfalls: Coastal Environmental Impacts (7-8 October 2013) | Jointly hosted by KAUST’s Water Desalination and Reuse Center (WDRC) and Red Sea Research Center (RSRC) | KAUST, Saudi Arabia. Title: The effect of elevated conductivity levels on microbial abundance in the near-field discharge area & an introduction to regulatory mixing zones (RMZ).
5. 17th Annual Water Reuse & Desalination Research Conference (6-7 May 2013) | Phoenix, Arizona. Title: Flow cytometry (FCM) for assessing possible environmental impacts on microbial abundance in near-field areas of SWRO concentrate discharge.

6. 1st International Conference on Desalination using Membrane Technology (7-10 April 2013) | Sitges, Spain. Title: Changes in microbial communities in the near-field area of SWRO discharges.


10. Symposium on Environmental Problems in the Arab World – Meeting the Challenges of Sustainable Development (26-28 February 2012) | Sultan Qaboos University (SQU), Muscat, Sultanate of Oman.