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AquaBioTech Group

Impact of Reverse Osmosis discharges on the marine environment

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Final Report

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Contents

1. EXECUTIVE SUMMARY 9

2. INTRODUCTION 11

2.1 Expected Results 12

3. LITERATURE REVIEW 14

3.1 Introduction 14

3.1.1 Overview 15
3.1.2 Current state of Reverse Osmosis 16

3.2 Existing methodologies 18

3.2.1 Water parameters 19
3.2.2 Bio-indicators 20

3.3 Case Study 21

3.3.1 Malta Case 23

4. METHODOLOGY 25

4.1 Proposed Strategy 25

4.1.1 Study Areas 25

4.2 Water Monitoring Methodology 26

4.2.1 Physical Analysis 28
4.2.2 Chemical Analysis 30
4.2.3 Laboratory Analysis 31

4.3 Diving Surveys Methodology 32

4.3.1 Posidonia oceanica meadows 36
4.3.2 Echinoderms 37

5. RESULTS 38

5.1 Water Monitoring 38

5.1.1 Physical Water Parameters 38
5.1.2 Chemical Water Parameters 48

5.2 Dive Surveys 61

6. DISCUSSION 76

6.1 Physical Water Parameters 76

6.2 Chemical Water Parameters 81

6.3 Dive Transect Survey 85

7. CONCLUSION 94

8. MONITORING PROGRAMME 96

8.1 Monitor Brine Distribution/Dispersion Pattern 98

8.2 Monitor Brine Discharge Composition 100

8.2.1 Laboratory Analysis 101

8.3 Monitoring of bioindicator species 101

9. PROGRAMME OF WORKS 105

9.1 Reporting 105

9.2 Programme of Works 105

10. REFERENCES 108

11. DECLARATION 112

12. CONTACT INFORMATION 113

Table of Figures:

Figure 1. Location of the 3 RO plants in Malta, as made available by the Water Services Corporation. 26
Figure 2. Survey location of water monitoring stations at the Pembroke RO site. The distance between stations is 200 m. 27
Figure 3. Survey location of water monitoring stations at the Ċirkewwa RO site. The distance between stations is 100 m. 27
Figure 4. Survey location of water monitoring stations at the Għar Lapsi RO site. The distance between stations is 150 m. 28
Figure 5. Diving transects (T) locations for seabed monitoring at Ċirkewwa. The prefix 'C' denotes the site name, Ċirkewwa, with 'I' for Impact point and 'R' for Reference point. The red dot is the direct brine discharge point. 34
Figure 6. Diving transects (T) locations for seabed monitoring at Pembroke. The prefix 'P' denotes the site name, Pembroke, with 'I' for Impact point and 'R' for Reference point. The red dot is the direct brine discharge point. 35
Figure 7. Diving transects (T) locations for seabed monitoring at Għar Lapsi. The prefix 'L' denotes the site name, Lapsi, with 'I' for Impact point and 'R' for Reference point. The red dot is the direct brine discharge point. 35
Figure 8. Bathymetry map in Ċirkewwa according to sonde readings. 39
Figure 9. Bathymetry map in Għar Lapsi according to sonde readings. 40
Figure 10. Bathymetry map in Pembroke according to sonde readings. 41
Figure 11. Conductivity (µS/ cm) dispersion in Ċirkewwa across 4 seasons in surface, mid and deep waters. 44
Figure 12. Conductivity (µS/ cm) dispersion in Għar Lapsi across 4 seasons in surface, mid and deep waters. 45
Figure 13. Conductivity (µS/ cm) dispersion in Pembroke across all 4 seasons in surface, mid and deep waters. 46
Figure 14. Average boron concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons. 56
Figure 15. Average calcium concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons. 56

Figure 16. Average magnesium concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons. 57
Figure 17. Average sodium concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons. 57
Figure 18. Average potassium concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons. 58
Figure 19. Average sulphates concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons. 58
Figure 20. Average chlorides concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons. 59
Figure 21. Principal Component Analysis (PCA) of chemical water quality parameters at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons. 60
Figure 22. Average substrate percent cover at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C) across seasons. 63
Figure 23. Average shoot density (/ m2) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C) across seasons. 63
Figure 24. Average leaf length (cm) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C) across seasons. 64
Figure 25. Average substrate percent cover at the impact (IT) and reference transects (RT1 and RT2) in Għar Lapsi (L) across seasons. 66
Figure 26. Average shoot density (/ m2) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Għar Lapsi (L) across seasons. 66
Figure 27. Average leaf length (cm) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Għar Lapsi (L) across seasons. 67
Figure 28. Average substrate percent cover at the impact (IT) and reference transects (RT1 and RT2) in Pembroke (P) across seasons. 69
Figure 29. Average shoot density (/ m^2) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Pembroke (P) across seasons. 69

Figure 30. Average leaf length (cm) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Pembroke (P) across seasons. 70
Figure 31. Overall habitat map at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P). 72
Figure 32. Gantt Chart showing preliminary programme. 107

List of Tables:

Table 1. Chemical parameters analysed in the laboratory to investigate impacts of brine discharge at. 31
Table 2. Points in Interest with highest measurements of conductivity (µS/ cm) across all three RO planta, and all 4 seasons. 47
Table 3. The average of average turbidity at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P), using a Secchi disk (m). 48
Table 4. Number of Echinoderms sighted at impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L) and Pembroke (P) across all 4 seasons. 73

Context

This is a final report, for seasonal activities (summer, autumn, winter and spring) conducted according to reporting requirements of project Ref WSC/T/80/2021 to study the effect of brine discharge on the environment for the **Water Services Corporation.**

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Project Background: The Water Services Corporation produces and distributes nearly 31 million cubic metres of potable water per year and distributes it through a 2,100-kilometre network, to supply industrial, commercial and domestic consumers throughout the Maltese Islands. The Corporation has 3 Reverse Osmosis Plants namely, Pembroke, Ċirkewwa and Għar Lapsi from which the bulk of the water is produced. The remaining water is groundwater extracted through boreholes and pumping stations.

Project Scope: Given the insufficient natural water availability, to satisfy the national water demand, Malta is very dependent on the production of potable water through 3 seawater Reverse Osmosis (SWRO) plants. These plants are operated at a recovery of 42%, with brine (58%) discharged in coastal areas close to each plant.

Project Objectives: The subject of this project is the provision of a study to have a clear picture of the impact the discharge from SWRO plants is having on the marine environment. The following are the specific objectives:

1) To map the area of influence of brine discharges from the SWRO plants.

2) To investigate and identify any impact that brine discharges from the SWRO plants may have on the marine environment.

3) To develop monitoring programmes to ensure that the marine habitat is protected.

Survey Area: The surveys will be performed around the 3 SWRO plants, namely in Pembroke, Ċirkewwa and Għar Lapsi. Precise survey areas are defined in the following chapters.

1. Executive Summary

This report explores the impact of brine discharge on the marine environment at the Reverse Osmosis (RO) power plants in Ċirkewwa, Għar Lapsi, and Pembroke, under the jurisdiction of the Water Services Corporation (WSC). The survey, conducted over 4 seasons spanning two years, aims to establish baseline data. The results indicate variations in the volume of brine discharge, bathymetry, and benthic community composition across the surveyed sites, although similar species were observed at each location. Salinity levels generally conform to observed standards in the Mediterranean Sea, and the distribution patterns of the primary bioindicator species, *Posidonia oceanica*, appear to be influenced by the proximity to the brine discharge point, seabed bathymetry, depth, and currents. The report suggests that the impact sites will likely remain unchanged if the volume of discharge remains constant, contingent upon factors such as chemical composition, physical characteristics, and bioindicator species, as well as external influences such as climate change and other anthropogenic effluents. Long-term environmental monitoring is recommended to detect changes over a five-year period, facilitating the implementation of appropriate mitigation and management measures to ensure sustainable brine discharge practices while safeguarding the marine environment. It is proposed to apply a similar monitoring plan across all RO plants, considering minor variations in statistical significance between impact and reference points at each location. This approach offers an opportunity to assess the efficacy of a uniform monitoring methodology across plants of varying sizes and discharge volumes while sharing similar benthic environmental structures. Overall, this report aims to enhance understanding of the impact of brine discharge on surrounding seawater and the seabed.

The completion of a series of activities, comprising i) physical water parameter sampling, ii) chemical water parameter sampling, and iii) dive survey transects, was carried out at each RO plant during 4 survey seasons. The objective was to gather data on the condition of the water surrounding the brine discharge points and the overall health of the marine environment, with a focus on bioindicator species such as *Posidonia oceanica* and echinoderms. Additionally, observations were made on other habitats, including biogenic habitat such as algae communities, and bare substrate such as sand and rock.

A summary of the recent study's findings indicates notable spatial trends, with the highest average conductivity values observed during summer in Għar Lapsi and Pembroke, particularly near the impact point. Additionally, seasonal variations revealed significant fluctuations in chemical parameters such as boron, sulphates, and chloride during winter, with variations observed in both depth and distance from the brine source at all 3 locations. Notably, Għar Lapsi showed substantial differences in the growth of *Posidonia oceanica* between impacted and reference transects, particularly in winter, suggesting a more pronounced impact from brine discharge. Conversely, Ċirkewwa and Pembroke did not exhibit seagrass at their impact transects. Moreover, echinoderms, specifically the Hacelia attenuata species, were infrequently observed, with the majority recorded at Pembroke. General observations also noted the widespread occurrence of schools of fish in the vicinity of each brine discharge outfall point.

Subsequently, the results of this study recommends ongoing monitoring with appropriate modifications compared to the preceding survey. The suggested monitoring program entails conducting 2 seasonal surveys (winter and summer) instead of 4, for the next 5 years, while employing the same monitoring approach as the recent survey at each RO plant. The physical water parameters would be measured in a smaller area, while chemical water sampling and dive surveys would continue to be carried out at each RO plant's two reference and one impact transect, in order to monitor bioindicator species. This monitoring frequency can be adjusted in accordance with pertinent environmental regulatory guidance and based on campaign results that identify the range of ongoing potential impacts, particularly regarding their effect on the marine environment. The proposed strategy would hopefully enable the acquisition of data from both short-term and long-term monitoring. Short-term monitoring facilitates the identification of immediate impacts, while long-term monitoring is essential for evaluating trends and changes over an extended duration. This approach would facilitate informed decision-making and adaptive management to mitigate environmental impacts stemming from brine discharge, in alignment with local and international environmental regulations (Malta, EU).

2. Introduction

Malta, like most Mediterranean countries, is facing freshwater scarcity due to its climate and the lack of natural freshwater resources such as surface freshwater bodies. Therefore, Reverse Osmosis (RO) plants play a significant role in the production of potable water on the Maltese Islands. In 2015, just under 18 million cubic meters of water was produced by RO plants which account for 58% of all produced potable water in Malta, while the remainder was extracted from the ground. Especially in times of climate change, increasing summer heat waves and decreasing rainfall, this type of water production becomes increasingly important.

There are currently 3 Maltese RO plants, located in Ċirkewwa, Għar Lapsi and Pembroke. As part of the desalination process, seawater undergoes a complex treatment that requires high energy consumption to take away mineral components from saline water to produce drinkable freshwater. The resulting by-product of the desalination process is brine, which is released back into the sea at the RO's discharge points. In the case of the Maltese RO plants, they are operated at a recovery of 42%, with the remaining 58% discharged in coastal areas. The average brine discharges of the plant in Ċirkewwa, Għar Lapsi and Pembroke are 443,040 m^3, 725,413 m^3 and 1,363,879 m^3 respectively. The brine discharge levels fluctuate throughout the year with higher discharge levels during the summer months due to higher water demands.

Discharged brine shows changes in chemical and physical characteristics when compared to the intake of seawater. According to a variety of studies such as Fernandez-Torquemada & Sanchez-Lizaso (2007), Robert *et al.* (2010) and Fernandez-Torquemada *et al.* (2019), the higher saline content, as well as other changed parameters, can have impacts on marine ecosystems, mainly affecting the marine benthic communities, i.e., sessile organisms.

2.1 Expected Results

The anticipated outcome of this project was to enhance comprehension of the effects of Reverse Osmosis brine discharge on the marine environment. This understanding was derived from the data obtained during baseline investigations and was subsequently utilised to create a site-specific, long-term monitoring plan for each of the 3 study sites. In detail, and as described in Section 4.2 of the tender document, the following results were expected per site:

- Determine and evaluate the concentration dispersion rate and area of influence.
- Plot the salinity profile illustrating the vertical brine distribution.
- Map the spatial representation of the salinity distribution.
  NB: Other physical and chemical parameters deemed critical (e.g., pH) for the vertical profile and spatial representation of distribution were to be included in the profile.
- Determine the spatial extent of habitats, species composition and state of health.
- Identify the bioindicator species for effective monitoring.
- Quantify and measure the population of species identified.
- Establish monitoring and control/reference geolocations.
- Carry out the study/assessment in line with the Water Framework Directive and national environmental standards.

All the above results were aimed to feed into the development of a long-term monitoring programme for each site, which included the geolocation of recommended sampling points; the frequency of the sampling events to ensure seasonal coverage; and all available information on the marine habitats at the selected locations, including state of health.

Moreover, the results obtained during the implementation of this study period were consequently used as a benchmark.

The sampling program covered a period of 2 years with all activities collectively performed between June 2022 and September 2023. This ensured that it would capture any seasonal variations in the quantity of brine being discharged. Due consideration was given to the changing hydrodynamic conditions and how these may impinge upon brine and seawater mixing and consequently status of both water compositing and the benthic marine configuration.

3. Literature Review

3.1 Introduction

The percentage of freshwater (underground and surface) present on Earth represents only 2.5% of Earth's total water; 80% of this water, however, is stored on glaciers making only 0.5% accessible as a potable source (Ibrahim *et al.*, 2017). On the other hand, the remaining 97.5% stored in the ocean represents an almost unlimited resource: the amount of seawater extracted for desalination purposes is negligible compared to naturally occurring evaporation (Lattemann & Höpner, 2008).

Global water demand rose about 6 times over the last century in order to sustain the global growth and the higher consumes: the water use has soared from approximately 500 to 4000 km^3 yr^{-1} between 1900-2010 (Wada *et al.*, 2016). Water security – defined as the capacity of a population to safeguard access to water in acceptable quality and quantity – is getting harder and harder to guarantee. Indeed, at present time, nearly half of the world population live in areas which are potentially water-scarce at least one month per year: 3.6 billion people according to UN (WWAP, 2018) while Mekonnen and Hoekstra (2016) estimated 4.0 billion people. This number is expected to rise to 4.8 - 5.7 billion by 2050 (WWAP, 2018). Half a billion people live in areas which are water-scarce all year round (Mekonnen & Hoekstra, 2016). Concurrent factors such as climate change, unequal population growth, depletion and pollution of water sources will jeopardise water security over the following decades (Boretti & Rosa, 2019). For this reason, the idea of sourcing water from the ocean is a viable and attractive option.

By 2050, desalination plants will provide 192 x 10^6 m^3 day^{-1} of potable water (Darre & Toor, 2018); even though this estimate is not promising (water demand is expected to rise to 5,500-6,000 km^3 yr^{-1} (Burek *et al.*, 2016)), there are already some countries which heavily rely on the desalination process as a main drinking water source, especially in the GCC states (Gulf Cooperation Council). Saudi Arabia, for instance, fulfils 61% of their domestic water demand through the desalination of seawater (Alnajdi *et al.*, 2020) and Kuwait satisfies about 90% of their water demand through desalinating seawater (Hamoda, 2001).

Different processes can be employed to convert seawater into freshwater. The main ones are thermal-based and membrane-based technologies. Thermal-based processes rely on thermal energy supply to separate the water from its salts in vapour state; the vapour is then condensed to obtain potable water. This technology may result suitable in regions, such as the GCC, where water is particularly saline and energy is cheaper. Multi-stage Flash (MSF), multi-effect distillation (MED) and vapour compression distillation are the most widespread thermal techniques. State-of-the-art developments in membrane-based technologies caused them to become more popular due to lower energy consumption and environmental footprint (Darre & Toor, 2018). Ultrafiltration, electrodialysis and Reverse Osmosis (RO) are all examples of membrane-based techniques with the latter being the most widespread globally (including Malta) – it accounts for 61% of the world desalination technology (Nair & Kumar, 2013).

The purpose of this literature review is to examine the feasibility and the limitations of desalination RO plants currently in operation around the world in order to discern factors such as those likely to influence the marine environment. Ultimately, the WSC project will tackle the RO brine discharge effects on marine environment around the Maltese island; therefore, an outline of these plants is essential to better quantify the environmental impact of desalination and to conduct a pilot study in Malta to establish a monitoring protocol.

3.1.1 Overview

The process by which salts are removed from saline water is referred to as desalination. It is primarily a natural process which has continuously been occurring on Earth for millions of years: seawater evaporation as well as ice crystal formation near polar regions are natural processes which spontaneously exclude the mineral component of seawater. The advent of steam power and the advancement in thermodynamics, in the mid-eighteenth century, significantly enhanced the desalination development: although the early 1900s showed a success of the evaporation and condensation technique, it was the invention of asymmetrical membrane in the 1960s which led to a rapid growth of this industry (Nair & Kumar, 2013).

Over the following decades, new developments in membrane processes led to the commercialization of reverse osmosis. Even though a variety of membrane materials have been developed, spiral wound composite polyamide membranes are almost exclusively used today (Lattemann & Höpner, 2008). To date, water desalination has become a potentially feasible solution for water supply especially for arid countries; it is therefore crucial to address the main issues involved with this process to optimize it and ensure water security in the production process.

3.1.2 Current state of Reverse Osmosis

The term ‘osmosis’ is a spontaneous chemical phenomenon in which water ‘moves’ from lower to higher solute concentration. Reverse Osmosis makes use of high-pressure pumps to revert this process and squeeze the water from high (saline water) to low solute concentrations (potable water). The salts cannot pass through the semi-permeable membrane and the water is desalinised. The Seawater Reverse Osmosis plants (SWRO) are made of five major components:

1) **Seawater intake system.** This involves water being taken directly from the sea and passed through screens to remove larger debris, including but not limited to seaweed and fish.

2) **Feed pre-treatment facility.** The incoming water requires treatment in order to reduce the concentration of microorganisms and chemicals which may cause fouling on the membrane.

3) **High pressure pumps.** It supplies enough pressure to push the water through the membrane.

4) **RO membrane system.** This is a crucial post-treatment system which adjusts the water’s pH levels, remineralizes and disinfects it if necessary, ensuring that water meets the necessary and desired quality standards for safe consumption.

5) **Brine disposal and post-treatment facilities.** The concentrated brine is discharged back into the sea, integrating proper dilution to decrease the impact it has on the marine environment.

Reverse Osmosis presents several critical factors which could undermine their sustainability and, therefore, their feasibility in large scale water production.

Energy Costs-Effectiveness

RO plants consume between 3.5 kWh to 4.2 kWh of energy per m^3 of water (Darre & Toor, 2018). This energy consumption results in a high demand of energy which is usually obtained from fossil-fuel combustion. Feeding those plants with renewable energy sources could potentially minimise the impact of the desalination process; in Adelaide (South Australia) a RO desalination plant is run on wind and solar energy (Kämpf & Clarke, 2013). Furthermore, RO plants work with a relatively low efficiency; their recovery rate – which is the ratio of the volume of desalinated water produced over the feed water volume – can reach a maximum of approximately 60% (in the 1980s it could not exceed 25%). In particular, when the feed water is highly saline (such as Mediterranean Sea, Red Sea) the recovery efficiency does not usually exceed 30% (Ghaffour *et al.*, 2013).

Membrane Fouling

The accumulation of fouling on the membrane surface is a significant maintenance challenge. It is particularly difficult to address because if bacteria and microorganisms are not completely removed, they will continue to multiply and consequently block the membrane. The most common practices to prevent this are proper pre-treatment of the feed water, a periodical cleaning of the membrane, additional antifouling acids, disinfectants, scale inhibitors to the water and modification of membrane materials (Jiang *et al.*, 2017).

Environmental Concerns

A major environmental concern related to RO plants is the brine discharge. Brine is a hyper saline solution, obtained as a by-product of the RO desalination process, which, as observed in Malta, gets discharged at sea. Furthermore, brine usually contains higher concentrations (up to 4-10 times higher) of nitrates, phosphates, radioactive materials, toxic chemicals used as antiscalants for the pre-treatment process, as well as heavy metals (Xu *et al.*, 2013). The brine's physical properties make it sink and settle as a bottom layer in the sea. This layer of brine is long-lasting and doesn't spread out easily, thus potentially creating pressure on organisms living on the sea floor, particularly those that are benthic. High-salinity brine plumes were recorded to range from 10 metres (Gacia *et al.*, 2007) to several kilometres (Fernández-Torquemada *et al.*, 2005). Oxygen depletion, coastal eutrophication, reduced growth of marine organisms, and nervous system damages are all well-established consequences of long-term exposure to brine (Darre & Toor, 2018). Moreover, in areas where the intake water already contains organic pollutants, the brine discharged back at sea brings the same contaminants in higher concentration, unless it undergoes a treatment prior to discharge: in this case, discharge at sea could make bioaccumulation in marine organisms more severe (Xu *et al.*, 2013).

Increasing the speed of brine's diffusion after discharging represents the most common approach to mitigate its effects. A diffuser, for instance, was installed at the RO plant in San Pedro del Pinatar (SE, Spain) at the outlet of the pipeline (Del-Pilar-Ruso *et al.*, 2015). However, several options have been designed: devaporation, membrane distillation, forward osmosis, vibratory shear enhanced membrane filtration process and other hybrid processes (Xu *et al.*, 2013). In other cases, brine has been turned into an exploitable resource. The Israeli Mekorot Water Company created a SWRO plant which both produces water and table salt: the brine passes through a series of ponds so that there is no need to discharge it (Ravizky & Nadav, 2007). The same purpose is achieved in Japan through electro dialysis (ED) (Xu *et al.*, 2013). The project “Agua Doce” – in the semi-arid region of Brazil – developed an integrated production scheme which exploits brine to provide fishes for human consumption and feed for the cattle. Brine is used to farm Tilapia (*Oreochromis niloticus*) and/or for *Spirulina* cultivation. This first stage is followed by an enrichment of the brine with organic matter from local agricultural wastes; the brine is finally used to irrigate halophyte forage shrub plantations (Sánchez *et al.*, 2015).

3.2 Existing methodologies

This project aims to evaluate the environmental effects of brine discharges from the SWRO plants in Malta. It represents a pilot study around the Maltese island and, therefore, it will include the establishment of a monitoring program to ensure periodical assessment of the brine discharge impact. In this section, a review of previous studies on brine effects will be taken into consideration to highlight applied methodologies and to select the procedures which best suit this project according to different factors:

- Similarity of conditions with previous studies conducted.
- Specific benthic habitat assemblages of Malta.
- Specific brine parameters of Maltese SWRO plants.

3.2.1 Water parameters

SWRO requires seawater distillation which can consist of a thermal process involving the use of multistage flash (MSF). Here, water is pumped to a higher pressure and heated to its near boiling point thus producing thermal pollution. This form of pollution potentially increases seawater temperature, salinity, water current and turbidity (Al-Mutaz, 1991). For the above reason, measuring and monitoring of certain water parameters will be essential in order to observe pollutant occurrence from SWRO water discharge, among seasonal discharge sessions. If the concentrate flow differs significantly from that of the ambient seawater, then the possible implications could be identified (Dweiri and Badran, 2002).

Furthermore, RO uses membranes as part of the water purification process. These membranes are susceptible to fouling and scaling and as such they need to be cleaned with chemicals regularly that may be toxic to receiving waters. Moreover, pre and post treatment of seawater for parameters such as pH, coagulants, Chlorine (Cl), Copper (Cu), organics, Carbon dioxide (CO_2), Hydrogen sulfide (H_2S) and hypoxia can induce brine concentration almost twice that of local seawater. Because the discharge of brine is often accomplished via lengthy piping running deep into the sea or near coastline, marine benthos can be affected, and if leakages in the piping occurs, marine ecosystems at different levels of the water column equally remain exposed to bio-physical and chemical changes (Tularam & Ilahee, 2007).

Brine is a highly saline effluent and – due to its high salinity – it sinks as a bottom layer creating a plume which could cover several kilometres ranges. With time it contributes to oxygen depletion which, together with the salinity, exert negative effects on the local marine communities. Additionally, brine can contain products for pre-treatment processes (antiscalants and coagulants) and natural occurring radioactive material in 4 to 10 times higher concentrations (Darre & Toor, 2018). Studies which focus on brine, generally employ a CTD (conductivity, temperature, and depth) to obtain profiles of salinity and detect the brine plumes. The CTD is a package of electronic instruments which measures conductivity, temperature and depth by the means of pressure. Travelling down through the column water, it takes multiple measurements creating a profile of the parameter opposed to depth.

3.2.2 Bio-indicators

Marine benthic fauna and flora such as fish and seagrass are at risk of impingement and entrainment during open-ocean intake system operations. In Malta, SWRO concentrate can also cause significant damage on various benthic organisms such as *Posidonia oceanica*, a protected seagrass species in the Maltese islands.

- ***Posidonia oceanica***. It is an endemic seagrass species of the Mediterranean Sea which belongs to the Angiosperm division (Marine Phanerogam). *P. oceanica* forms extensive meadows on soft as well as hard substrata, from sea level down to a depth of 25 - 40m (sunlight and nutrient availability dependent). These meadows are 'climax' communities in the coastal seabed and play an essential ecological role providing valuable services – such as protection against coastal erosion, contribution to fisheries by supporting food webs or counteraction to marine plastic pollution (Sanchez-Vidal *et al.*, 2021). It is sensitive to low salinities, so that it is restricted to the open sea and does not occur in the vicinity of river mouths (Boudouresque *et al.*, 2009). Field experiments highlighted that *P. oceanica* is also very sensitive to high-salinity conditions. Effects include changes to seagrass structure and vitality at salinities of 39.1 PSU and 38.4 PSU respectively (Sánchez-Lizaso *et al.*, 2008). Other studies verified further damages such as necrosis in leaves, greater leaf loss, high mortality (at 50 PSU 100% mortality, at 45 PSU 50% mortality) and reduced growth pace (Latorre, 2005). *Posidonia oceanica* has been extensively used as a bioindicator species for brine impact studies which generally make use of quadrats to monitor shoot density (Fernández-Torquemada *et al.*, 2005), foliar growth (length and width), dead matte, and rhizome production (Gacia *et al.*, 2007).

- **Echinoderms**. The term Echinoderm indicates a large group (*phylum*) of marine invertebrates. It includes five classes: Asteroidea (starfish), Echinoidea (sea urchins), Holoturoidea (sea cucumbers), Ophiuroidea (brittle stars) and Crinoidea (feather stars). Most of the species are stenohaline (Santos-gouvea & Freire, 2007): that is, they tolerate a narrow range of salinity values being sensitive to fluctuations. Indeed, they lack an osmoregulatory organ, their body tissues are highly permeable to water and salts, and therefore their coelomic fluid is isosmotic to the environment (Diehl, 1986). Echinoderms could be selected as useful bioindicators due to their abundance, wide distribution and rapid response to environmental conditions (Fernández-Torquemada *et al.*, 2013). Previous studies monitored them to assess brine discharge impacts observing a disappearance of echinoderms in conjunction with plant operations by mean of visual census or transects (Fernández-Torquemada *et al.*, 2005).
- **Polychaetes**. They are a class of Annelids which includes marine worms. Polychaetes assemblage has been previously employed as a bioindicator: their wide geographical range and the presence of both sensitive and tolerant species make them a suitable bioindicator. Parameters which are usually accounted for are total abundance, family richness and diversity which could be estimated through common indexes (i.e. Shannon-Wiener index) (Del-Pilar-Ruso *et al.*, 2015).

3.3 Case Study

Hereafter, a brief paragraph retraces studies which tackled the same issue: brine effect on marine environment nearby the outlet of RO plants. While numerous studies have been undertaken, it is important to take into account the particular location conditions and the specific marine communities of these findings. The studies mentioned below were conducted in the Mediterranean Sea to assess the effects of plants with similar capacity to Pembroke SWRO in Malta.

1. Fernández-Torquemada *et al.*, 2005 conducted a study on the effect of brine discharge produced by the Alicante SWRO plant (Spain). Its capacity reaches 50,000

m^3/day with a conversion factor of 40% and a 68 PSU brine. The physico-chemical analysis showed a low dilution of brine: a plume was detected through three CTD (RBR) surveys – repeated in February, April and August (2004) – in a grid of more than 100 stations located in front of the discharge point (16 to 20 meters depth). To monitor the biological communities, *Posidonia oceanica* and echinoderms species were selected as indicators due to the ecological importance of the former and the low salinity tolerance of the latter. A total of 3 stations were identified, two of which were chosen as a control: twelve quadrats (40 X 40 cm) were established at each monitoring station to mark *Posidonia* shoots. The authors concluded that the brine dispersal followed the bathymetry and they reported a decline of echinoderms. *Posidonia* showed a lower vitality in front of the plant discharge even though the overall shoot balance resulted positive. (Fernández-Torquemada *et al.*, 2005).

2. Del-Pilar-Ruso *et al.* (2015), addressed the impact of brine (~70 PSU) discharged by the SWRO in San Pedro del Pinatar (Spain) carrying out an eight-year study. In this case, the plant has been operating since 2006 with a capacity of 65,000 m^3/day. In 2010, a diffuser was installed to mitigate the brine effect. The vertical profile of salinity was obtained through CTD measurements (RBR XR-420) in 2 surveys – one before and one after the diffuser deployment. As a bioindicator of the benthic community, the authors chose to analyse the polychaete assemblage: total abundance, family richness and diversity were assessed by mean of the Shannon-Wiener index. Twelve stations were selected (from 29 to 38 meters depth) and sampled 6 times: 1 prior to desalination activities, 4 during activities and 3 after the diffuser installation. Results showed that the plant discharge exerted a negative effect on surrounding marine habitats, but the implementation of mitigation measures in 2010 led to remarkable improvements: with the diffuser, salinity close to discharge measured less than 38.5 PSU and polychaete richness and diversity reached levels similar to those before the discharge. (Del-Pilar-Ruso *et al.*, 2015).

3.3.1 Malta Case

Malta is one of the top ten water-scarce countries in the world (Sapiano, 2021) and the most water-scarce in Europe (Hallett *et al.*, 2017). This is mainly due to different overlapping factors: the semi-arid Mediterranean climate – which causes a poor mean annual rainfall (approximately 550 mm) – and high evapotranspiration rates (Sapiano *et al.*, 2006). Besides, the absence of exploitable surface water, the remarkable population density contributes to the low water (readily usable) availability.

Groundwater represents the only natural resource for water replenishment and, for decades, has been over-extracted leading to poor water quality: it became increasingly saline – with chloride levels exceeding 2,000 mg/L – threatening food security in Malta (Sapiano *et al.*, 2006). By 1980, the recording of an annual average salinity of 1,600 mg/L drove the transition towards artificial water production which took shape during the late seventies with new desalination technologies (Hartfiel *et al.*, 2020).

At the time of this literature review, there are currently 3 functioning Reverse Osmosis (RO) plants which produce water in Malta – located in Għar Lapsi, Ċirkewwa and Pembroke (a new one was recently inaugurated (November 2021) in Ħondoq ir-Rummien, Gozo). Their capacities are 20,000, 18,600 and 54,000 m^3/day with recovery rates of 33%, 42% and 45%, respectively. Desalination plants supply 60% of usable water in Malta (18 million m^3 of water out of 31.2 million m^3 in 2015) according to estimates (Water Service Corporation, 2015), mitigating the pressure on groundwater sources. The intake water of these plants is through beach wells drilled close to the shore with the advantage that it does not need to undergo a pre-treatment. However, SWRO discharge the brine effluents at sea leading to potential environmental damages. Pembroke facility discharges brine at a temperature between 20°C and 21°C; the brine is therefore warmer during winter and colder during summer. In March 2021, for instance, seawater temperature reached 14.8°C whereas brine temperature was 20.1°C in Pembroke, 21.2 °C in Ċirkewwa and 20.2°C in Għar Lapsi; in August 2021, seawater temperature reached 28.3°C while brines was 21.0°C in Pembroke, 22.5°C in Ċirkewwa and 25.5°C in Għar Lapsi. On average, brine temperature at Ċirkewwa and Għar Lapsi fluctuates more compared to Pembroke: the former discharge at a maximum temperature of 22.7°C in September and October and a minimum of 21.2°C in March and

April; the latter discharges brine at a minimum temperature of 20.2°C in March and a maximum of 25.5 °C in August and September. The discharge's pH is 7.14 at Ċirkewwa and 7.15 at the other two plants; brine is therefore more acidic than environmental seawater – which ranges between 7.8 - 8.5 (Panel *et al.*, 2012).

Finally, desalination also creates a strong dependency on fossil fuels as the process is energy intensive: for every cubic metre of water produced, up to 4.6 kWh of electricity is required. In fact, desalination accounts for 4% of all Maltese electricity (Spiteri *et al.*, 2015).

To date, no study has been conducted to investigate the impact of RO plants on the ecosystems surrounding Malta (Hartfiel et al. 2020). It is therefore crucial to investigate the effects that brine discharge exert on the marine environment and to establish a monitoring protocol which could guarantee the water security without being harmful for the marine environment.

The literature review highlighted certain potential drawbacks of SWRO plants. The benefits they provide are surely of great importance and therefore, major issues need to be addressed to make Reverse Osmosis a feasible large-scale solution for water security.

This project will focus on investigating the effects of brine discharged at sea. To reach this aim, previous studies with similar conditions were compared and analysed. The result of the literature review allowed for the selection of the most suitable methodology, which can be seen in the following section.

4. Methodology

4.1 Proposed Strategy

To ensure the effective culmination of this project, 3 main approaches with their related methodology were envisioned:

Literature review, to ensure all background information was considered as well as to validate our proposed strategy with existing programmes.

***In situ* water monitoring surveys**, to estimate the dispersion rate and area of influence of the brine discharge (saline plumes), as well as obtaining crucial baseline data and distribution for all relevant parameters.

***In situ* diving surveys**, to assess first-hand the potential impacts of the brine discharge on the marine environment.

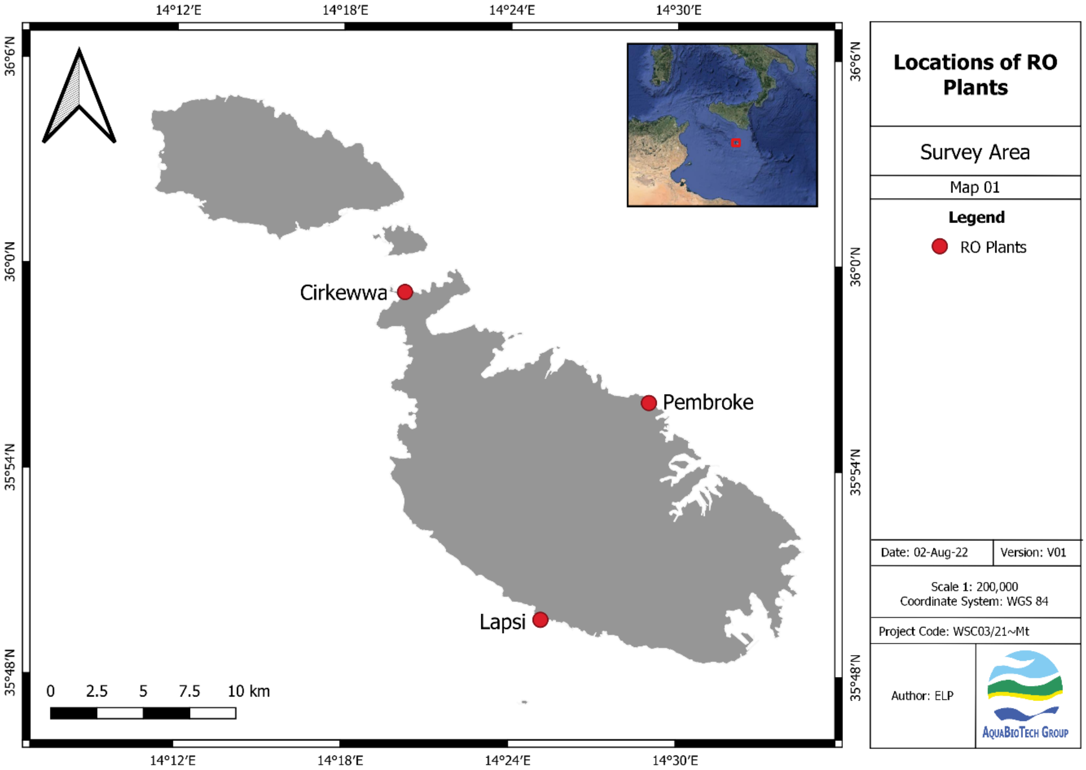
Each of the above-mentioned approaches is described in detail in the following subsections.

4.1.1 Study Areas

The study areas consisted of 3 locations (see Figure 1), corresponding to the 3 SWRO plants in Malta, namely:

- Pembroke RO plant
- Ċirkewwa RO plant
- Għar Lapsi RO plant

Figure 1. Location of the 3 RO plants in Malta, as made available by the Water Services Corporation.



For each of the 3 sites, the following described approaches were applied.

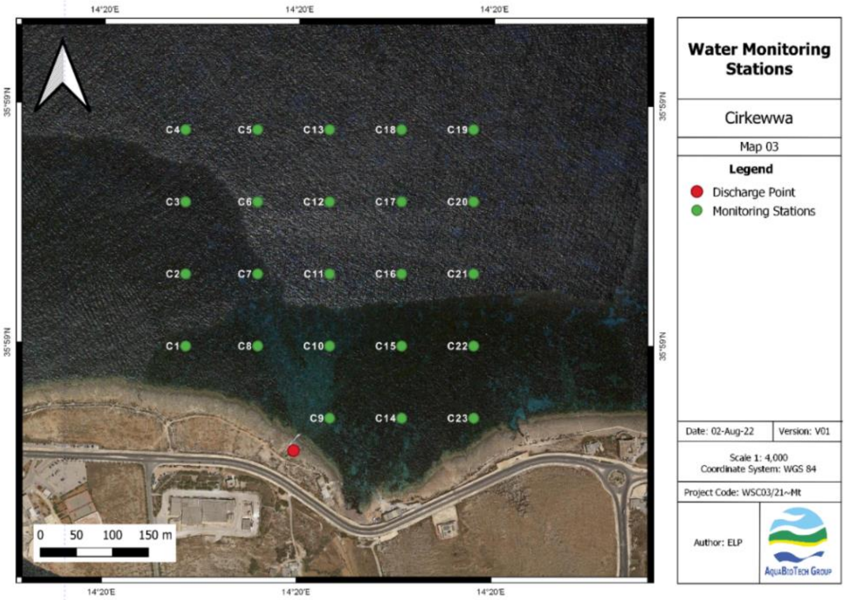
4.2 Water Monitoring Methodology

The water surrounding each of the discharge points was analysed to investigate potential deviations from the expected natural seawater parameters. For this, several water monitoring stations were selected (Figures 2, 3, 4), based on the volume of brine discharged at each station, as well as taking into consideration the location and particular bathymetric characteristics of each area. Water monitoring for chemical analysis was also performed at each dive location (Figures 5, 6 ,7). The number of monitoring stations surveyed, excluding those at reference dive sites, are illustrated below and distributed in a grid format.

Figure 2. Survey location of water monitoring stations at the Pembroke RO site. The distance between stations is 200 m.

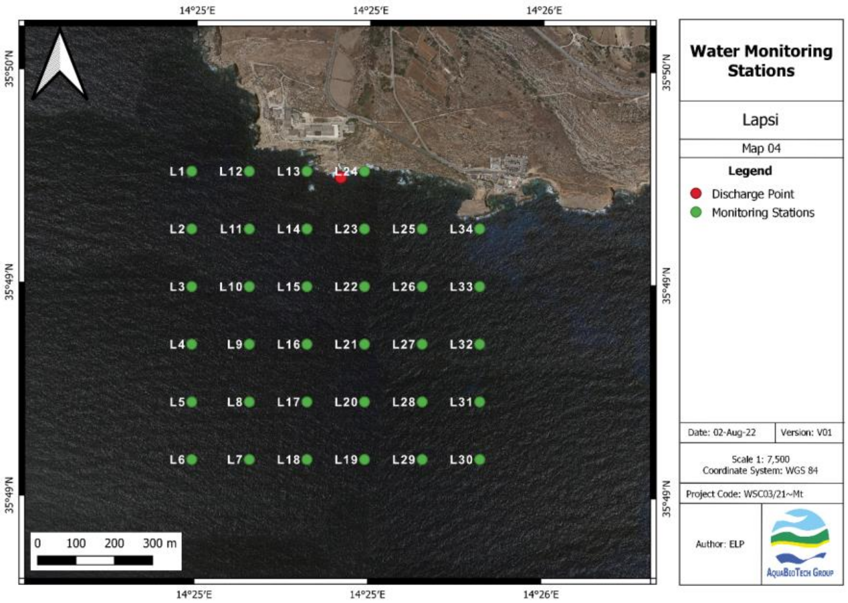
At Pembroke, a total of 65 stations in a grid, with 200 m equidistance between stations.

Figure 3. Survey location of water monitoring stations at the Ċirkewwa RO site. The distance between stations is 100 m.



At Ċirkewwa, a total of 23 stations in a grid, with 100 m equidistance between stations.

Figure 4. Survey location of water monitoring stations at the Għar Lapsi RO site. The distance between stations is 150 m.



At Għar Lapsi, a total of 34 stations in a grid, with 150 m equidistance between stations.

4.2.1 Physical Analysis

At each water sampling station, a multiparameter sonde was deployed from a vessel and lowered down the water column to the seabed. The coordinates at each monitoring station were validated using a Garmin handheld GPS (GPSMAP® 78s) and the monitoring depth at each location was measured using Navionics, a bathymetry GPS system on board the survey vessel. The primary parameters measured by the sonde included conductivity, temperature, and depth by means of pressure. These 3 parameters calculated salinity values. The sonde's features enable settings to take multiple measurements as it travels down the water column, which created the opportunity to establish vertical profiles including that of salinity, depth, pH and temperature. Continuous readings (second settings) can be taken by the sonde while being lowered manually through the water column. Owing to fluctuating weather conditions, careful consideration was given to adjusting the descent speed of the sonde to guarantee consistent and precise readings at each station. Monitoring the water near the seabed was crucial because hypersaline plumes sink to the sea bottom due to their high densities. The number of data points in the water column was regulated accordingly at each monitoring station to ensure its good representation, to allow for an accurate depiction of the vertical profile for each studied parameter, and eventually, to allow for the visualisation of dispersion rates and characterisations of the area of influence. The regulation of the data points was considered to allow for adaptation to an irregular bathymetry and variable seawater conditions at all 3 RO plants. Significantly strong swell, surface and underwater currents, and wave heights notably during autumn and winter, were carefully considered in order to stabilise both the survey vessel and survey equipment to ensure data was still collected with integrity. Owing to the demanding environmental conditions and technical constraints of the sonde, the data obtained at certain stations exhibited a lower degree of precision or reliability. For example, in Ċirkewwa, dominating strong westerly currents propelled the vessel in that direction, which in turn influenced precise readings of some data points. In such instances, when deemed necessary, average readings from nearby stations with more precise data were employed to offer a representative overview of the parameter readings at each location.

The data collected was analysed using specialised oceanographic software, Ocean Data View (ODV). This process included categorising the water column into 3 depth ranges (surface, medium, deep), based on the maximum depth at each monitoring station. Subsequently, the weighted average of parameters was computed within each column classification. This approach allowed for the visual examination and comparison of physical parameter trends across various depths within the water column, effectively illustrating a vertical dispersion plume.

Additional physical readings were obtained from reference points at each dive transect of all RO plants. However, this supplementary data was ultimately excluded from the final report due to the distant location from the discharge point and its limited value in determining the overall dispersion plume.

Apart from sonde measurements, water turbidity was assessed at the RO plants. This occurred at a total of 3 locations per plant, corresponding to the nearest point to shore along the dive survey transects. The process involved lowering a Secchi disk into the water on the shaded side of the vessel and recording the depths at which it disappeared and reappeared. The average of these depths was then recorded.

4.2.2 Chemical Analysis

The seawater's chemical parameters were analysed by taking samples. The specific water sampling stations were positioned along the diving survey transects, and the water samples were obtained at the point of the transect nearest to the shore. Each RO plant had one impact location directly at the discharge point, and 2 control locations were situated approximately 1 km on either side of the discharge point, making a total of 3 dive survey points. A total of 18 water samples were collected during each of the 4 survey seasons, with 6 samples per RO plant. It should be highlighted however that during the first survey, supplementary points, located along the extreme boundaries of the survey grid, furthest from the impact point, were surveyed at each RO plant and thus providing extra data. However, the consecutive 3 surveys did not perform sampling at the supplementary locations. This adaptation was incorporated because results from the first phase of surveys showed that acquiring additional water samples from sites beyond the 3 key survey points did not yield valuable data for drawing the necessary conclusions to assess the impacts of brine discharge in these areas. As a result, these additional water samples were ultimately excluded from the final results.

The seawater sampling was performed following relevant guiding standards of the ISO 5667 series, specifically ISO 5667-1:2006: Water quality – Sampling – Guidance on the design of sampling programmes and sampling techniques and ISO 5667-14: 1998 Water quality – Sampling – Guidance on quality assurance of environmental water sampling and handling.

The collection of water samples analysis carefully considered each RO plant's difference in volume brine discharge, rapidly changing depths of the seabed and dynamic hydrodynamics. To ensure methodological consistency, the first sample, in the shallow (S), was obtained at a depth of 1m below the surface. The second sample, in the deep (D), was determined on-site, taking into consideration the maximum water depth at each station. To obtain the water samples, a Niskin water sampler of 5 L volume was deployed over the side of the vessel. A total of 2 data points were therefore obtained at each sampling station: at 1 m below sea surface referred to as 'S' and 1 m above the seabed referred to as 'D'. The water was decanted into appropriate containers to be sent for water quality testing. The containers holding the sampled seawater were immediately labelled and stored away from direct sunlight inside a thermal box containing ice. They were preserved at ≤ 4° C and sent to the laboratory for analysis in the minimum amount of time possible. The handling of the samples was carried out in accordance with ISO 5667-3:2003: Water quality – Sampling – Part 3: Guidance on the preservation and handling of water samples.

To find out whether there was a statistically significant difference between chemical water parameters at the impact points, reference points and among seasons, parameters were first tested for normality followed by a two-way ANOVA. Statistical significance was performed on the data via Tukey's post-hoc or the Kruskal-Wallis test to inform in which factors the statistical significance occurred. A Principal Component Analysis (PCA) was then performed to enable visualisation of multidimensional data as it was a large set of data over wide areas.

4.2.3 Laboratory Analysis

Chemical analysis was carried out in the laboratory, in accordance with the relevant ISO or CEN standards, where applicable. The following parameters in Table 1 were analysed, ensuring the provision of valid and representative scientific data.

Table 1. Chemical parameters analysed in the laboratory to investigate impacts of brine discharge at.

Parameter	Units
Chlorides	ppm
Sodium	ppm
Boron	ppm
Potassium	ppm
Nitrates	ppm
Sulphate	ppm
Magnesium	ppm
Calcium	ppm

The chosen parameters are crucial for discerning the significant components of seawater at the brine discharge source.

4.3 Diving Surveys Methodology

Diving surveys were performed by experienced scientific divers to monitor the potential ecological impacts of brine discharge in the marine ecosystem at the 3 RO plants. At each RO plant, an impact location was defined, as well as 2 control locations ~1 km on either side of the discharge point, that share the same ecological characteristics as the impact location or rather, being sufficiently similar as the impacted location but remaining unaffected by the RO operation (Figures 6, 7 and 8). Following predetermined geo-coordinates, survey transect lines at each site were initially established by a diver laying out a measuring line reel on the seabed, using a wrist compass to maintain the correct bearing. The maximum length of each transect was set at 100 m, which represents a standard in underwater surveys, according to the compromise between dive safety and sufficient data acquisition. While laying the line on the outgoing route, underwater video footage was acquired to be used as a complimentary source of data to get visual footage of the entire line transect. Divers were positioned as closely as can centrally at the 50 cm mark (of a 1 m field of view) while recording. The transects started approximately 50 m from the shore edge at the discharge point because the fore areas were rocky and too shallow to conduct the dives. Moreover, the surf movement would have interfered with video recording and safety of both the diver and vessel. The returning route was used by divers to perform a high-level quadrat assessment of the benthos and the habitat. Within the survey quadrat (also photographed) the marine benthic habitat was characterized with a substrate percent cover method searching for the indicator species *Posidonia* meadows and echinoderms while other observed habitats falling into the quadrats where also accounted for including, macroalgae cover, and bare substrate, such as sand and or rock.

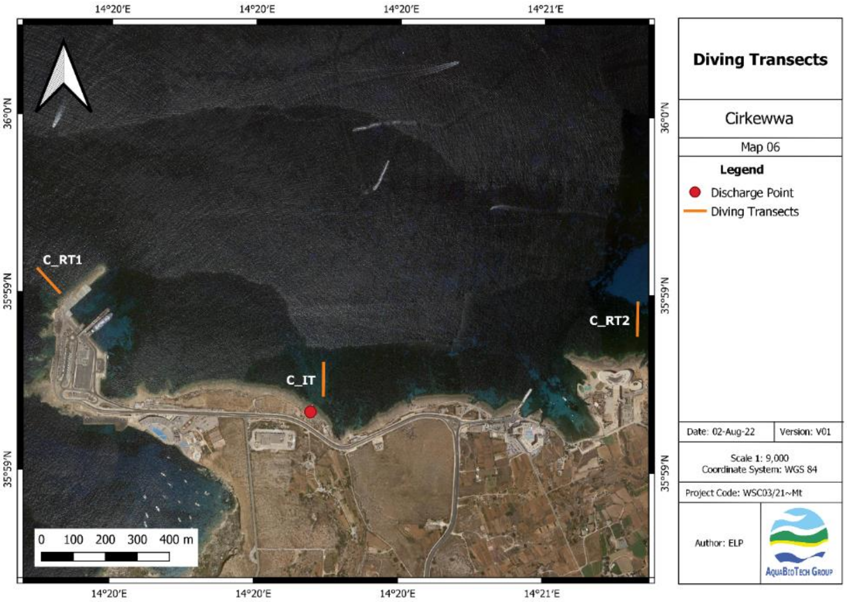
It is important to note that due to physical limitations, specific adaptations were implemented in the proposed quadrat methodology regarding the approach to data collection. Firstly, the survey quadrat size was initially planned to be 30 cm x 30 cm, however it was adjusted to

25 cm x 25 cm for all surveys, during the first set of survey activities. The modification demonstrated to be a more effective approach towards counting sparsely distributed benthic assemblages, as this was a common distribution type observed on the seabed. The determination of the quadrat's geo location was additionally established during the first survey. A random number generator was employed, generating 4 numbers between 0 and 100 since 4 quadrats were to be utilised per survey transect. The same 4 quadrat numbers were assigned to maintain consistency for each survey, in the same locations along the 100 m transect line.

Secondly, a more environmentally friendly and feasible approach than embedding permanent quadrats in the seabed along the survey transects was implemented. Instead, quadrat locations were geo referenced and these positions used for every survey with caution. This decision was prompted by the first survey where investigation demonstrated a highly uneven and unstable seabed structure (soft sand, very hard rocky platforms, delicate meadow) as well as strong water currents, particularly at Għar Lapsi, which rendered the initial methodology of quadrat embedment impractical.

Thirdly, to monitor echinoderms, the proposed plan was to have a total of 5 quadrats measuring 1 m^2 each for sampling on the seabed. The aim was to identify and count the echinoderms within these quadrats. However, due to practicality constraints involved in laying of quadrats regardless of absence of echinoderms during monitoring, the methodology was adjusted to sample a quadrat only when echinoderms were observed in the survey location (impact and 2 controls). This modification was necessary to align the methodology with the actual conditions and ensure the most efficient use of resources. By adapting the number of quadrats to the absence of echinoderms and focusing on a single quadrat, the study could still gather valuable data while maintaining feasibility and optimizing data collection efforts.

Figure 5. Diving transects (T) locations for seabed monitoring at Ċirkewwa. The prefix 'C' denotes the site name, Ċirkewwa, with 'I' for Impact point and 'R' for Reference point. The red dot is the direct brine discharge point.



At Ċirkewwa, transect C_RT1 (Figure 5) was repositioned to instead lay perpendicular to the other survey points after the first survey since making it parallel (as in the proposed plan) would have positioned the transect in the middle of active ferry activities between Malta and Gozo. This option had particular concern for dive safety and, for this reason, the transect was instead placed adjacent and behind the ferry terminal wall, far from boat traffic. This area still maintained survey integrity as a reference point, given that it is potentially not directly affected by brine water at the RO plant's discharge point as was the requirement of a reference location.

Figure 6. Diving transects (T) locations for seabed monitoring at Pembroke. The prefix ‘P’ denotes the site name, Pembroke, with ‘I’ for Impact point and ‘R’ for Reference point. The red dot is the direct brine discharge point.

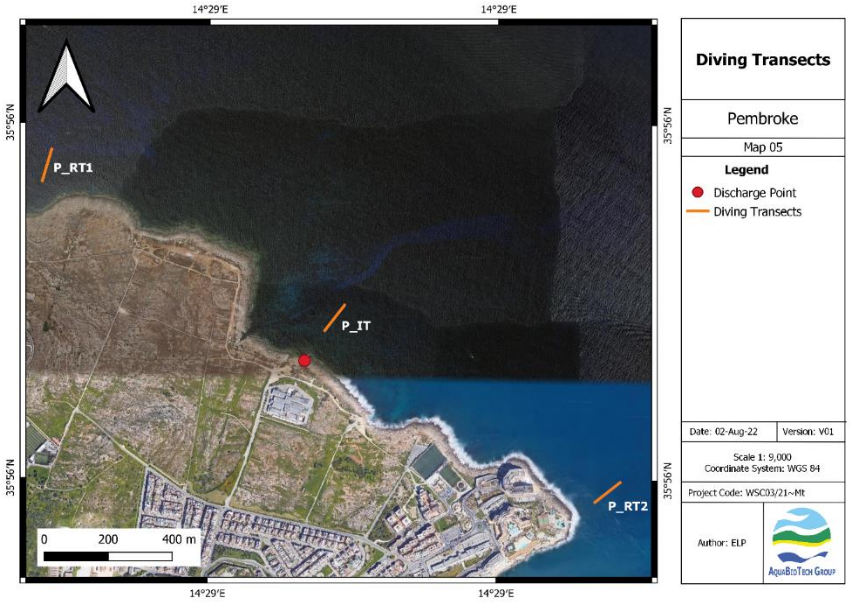
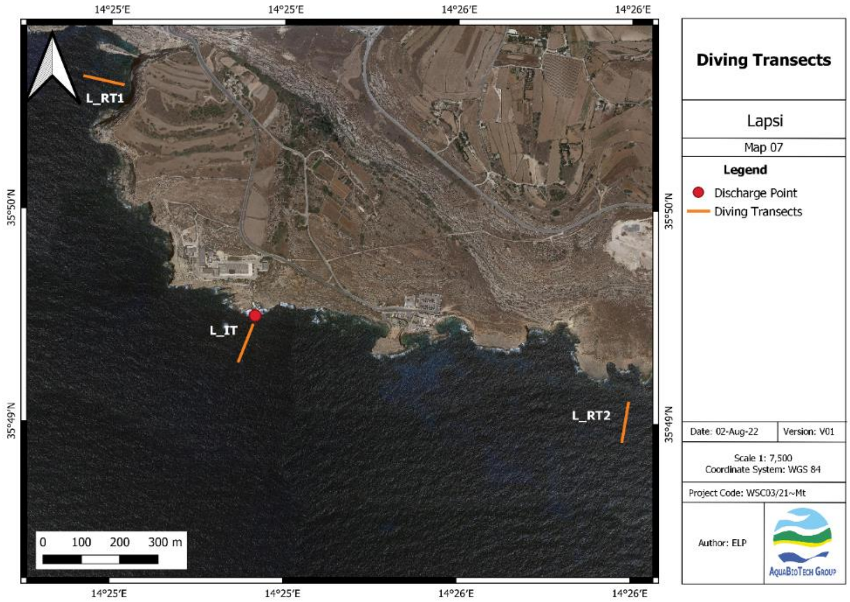


Figure 7. Diving transects (T) locations for seabed monitoring at Għar Lapsi. The prefix ‘L’ denotes the site name, Lapsi, with ‘I’ for Impact point and ‘R’ for Reference point. The red dot is the direct brine discharge point.



At Għar Lapsi, transect L_RT1 was repositioned perpendicular to the other survey points to ensure feasibility (after the first survey). If it had been left positioned parallel (as per proposed work plan), it would have required conducting the survey at depths that are too deep to be safely performed via SCUBA diving. After the first survey, it was discovered that the seabed depth in that area fluctuated rapidly, including reaching extreme depths that exceeded the safety limits for diving within the predefined 100 m length of the transect.

The rest of the methodology was followed as planned. During each of the dive surveys, the overall state of health of the marine ecosystem was assessed and the selected bioindicator species were monitored, quantified, and measured. Divers took pictures and video footage of all relevant seabed features, species and habitats, and carried the appropriate writing slates and any visual aids and tools for correct identification of species.

In all, the 2 key bioindicator species selected to investigate impacts from brine discharge are illustrated accordingly below.

4.3.1 *Posidonia oceanica* meadows

Posidonia is known to withstand high levels of salinity associated with brine discharge for a relatively short period of time, however longer exposures could cause irreversible damage to leaf tissues and eventually death of individuals and meadow regression. For the surveys, along an established 100 m survey line transect on the seabed, 4 quadrats measuring 25 cm x 25 cm were positioned at each location (transects recognised as one impact and 2 control). Each quadrat was placed in its respective geo location. Where *P. oceanica* was present, the indicators collected included (i) shoot density (number per square meter), (ii) leaf length (10 x random leaf lengths), and (iii) leaf number per shoot. Total percentage cover of *P. oceanica* was also counted to evaluate population dynamics. The quadrat positions, since as marked and selected areas of survey, were monitored each season with the objective to estimate shoot recruitment and mortality rates.

4.3.2 Echinoderms

Echinoderms, known for their high sensitivity to changes in seawater salinity, have been widely used as bioindicators for assessing the impacts of desalination (Torquemada et al., 2013). One quadrat measuring 1 m^2 was placed if echinoderms were spotted along the transect areas recognised as one impact and 2 control. The number of echinoderms within the quadrats were counted and identified. Other species that exhibited a significant presence on the seabed, were to be included as part of the survey using the form of percentage cover to help determine the population dynamics of echinoderms. Algae was a dominant habitat observed in the previous surveys where echinoderms where present.

5. Results

5.1 Water Monitoring

The physical and chemical parameters of water were analysed from each of the 3 RO plants to determine their values during surveys conducted 4 times over a span of 2 years.

5.1.1 Physical Water Parameters

The physical water parameters were demonstrated using dispersion plots, to visualise a brine discharge plume at each RO plant. The key parameters recorded comprised of conductivity (µS/ cm), salinity (PSU), temperature (°C), pH level, and dissolved oxygen (mg/l). Conductivity dispersion plots have been included in this report, while salinity, temperature, pH and dissolved oxygen (RDO) can be found in Appendix 1 – Appendix 22.

The variability in depth across the 3 RO plants was highlighted during surveys, potentially suggesting that bathymetry may have influenced pattern distribution of brine discharge.

As per sonde readings, the range of depths varied within the grid of monitoring stations at each plant as follows:

- Ċirkewwa: 3.6 m - 14.3 m (Figure 8).
- Għar Lapsi: 10.9 m - 86.8 m (Figure 9).
- Pembroke: 6.5 m - 41.9 m (Figure 10).

Among the 3 power plants, Ċirkewwa had the most gradual bathymetry (Figure 8), Għar Lapsi had the deepest survey points (Figure 9), whereas Pembroke had the widest variability of water depths (Figure 10).

Figure 8. Bathymetry map in Ċirkewwa according to sonde readings.

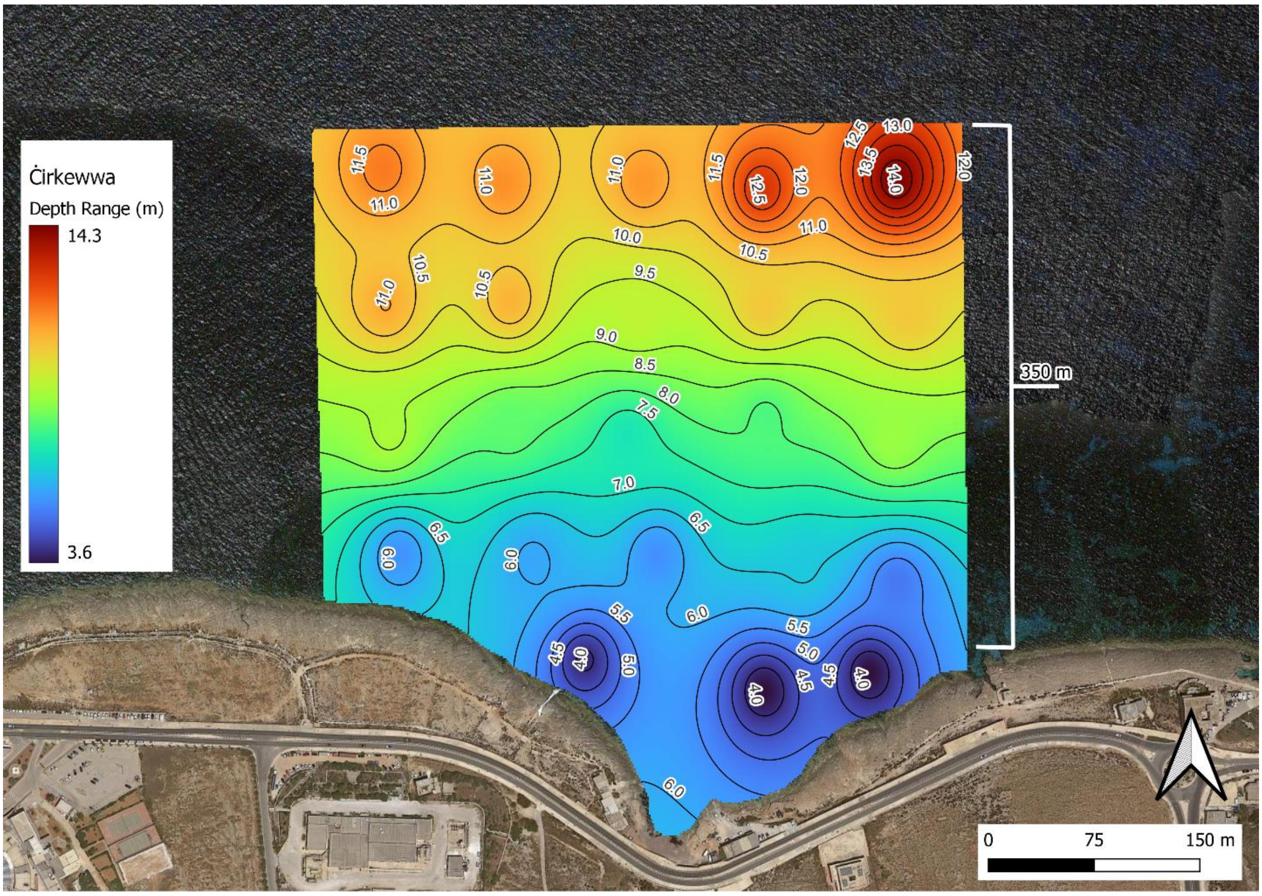


Figure 9. Bathymetry map in Għar Lapsi according to sonde readings.

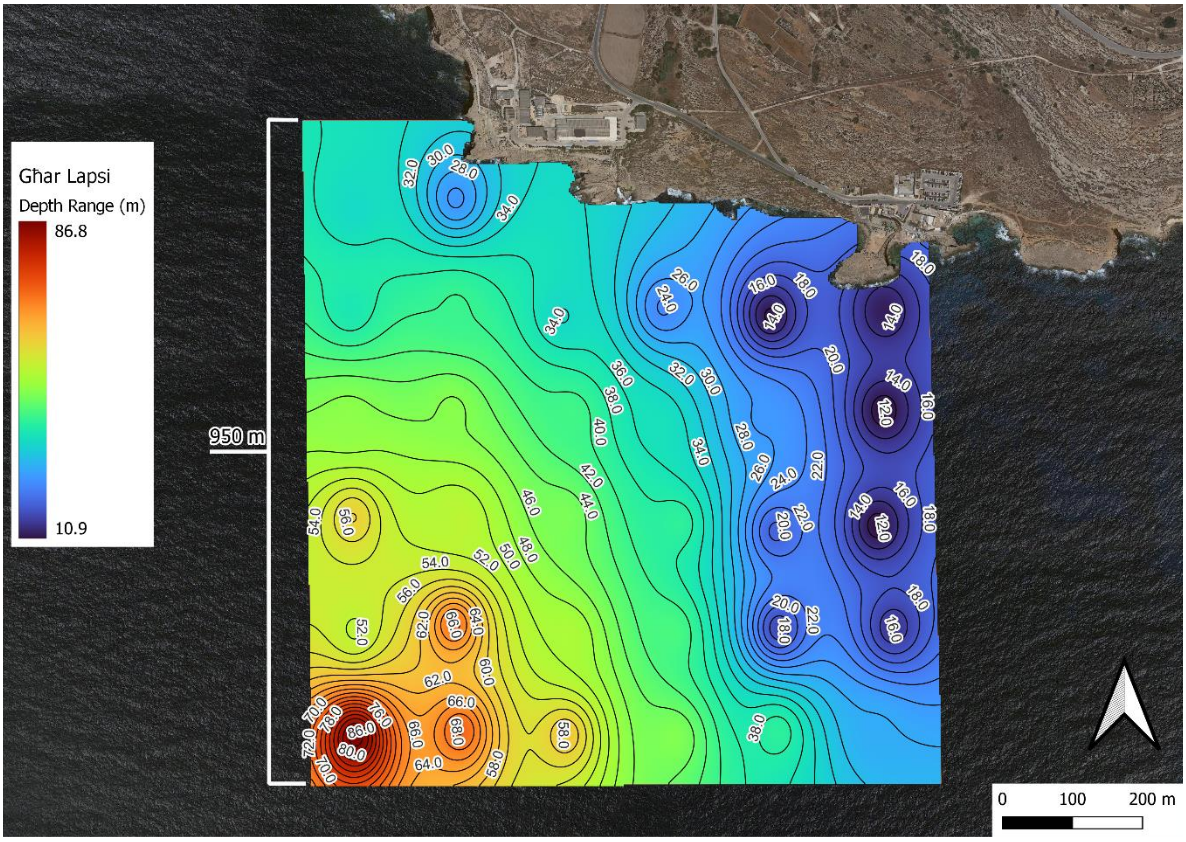
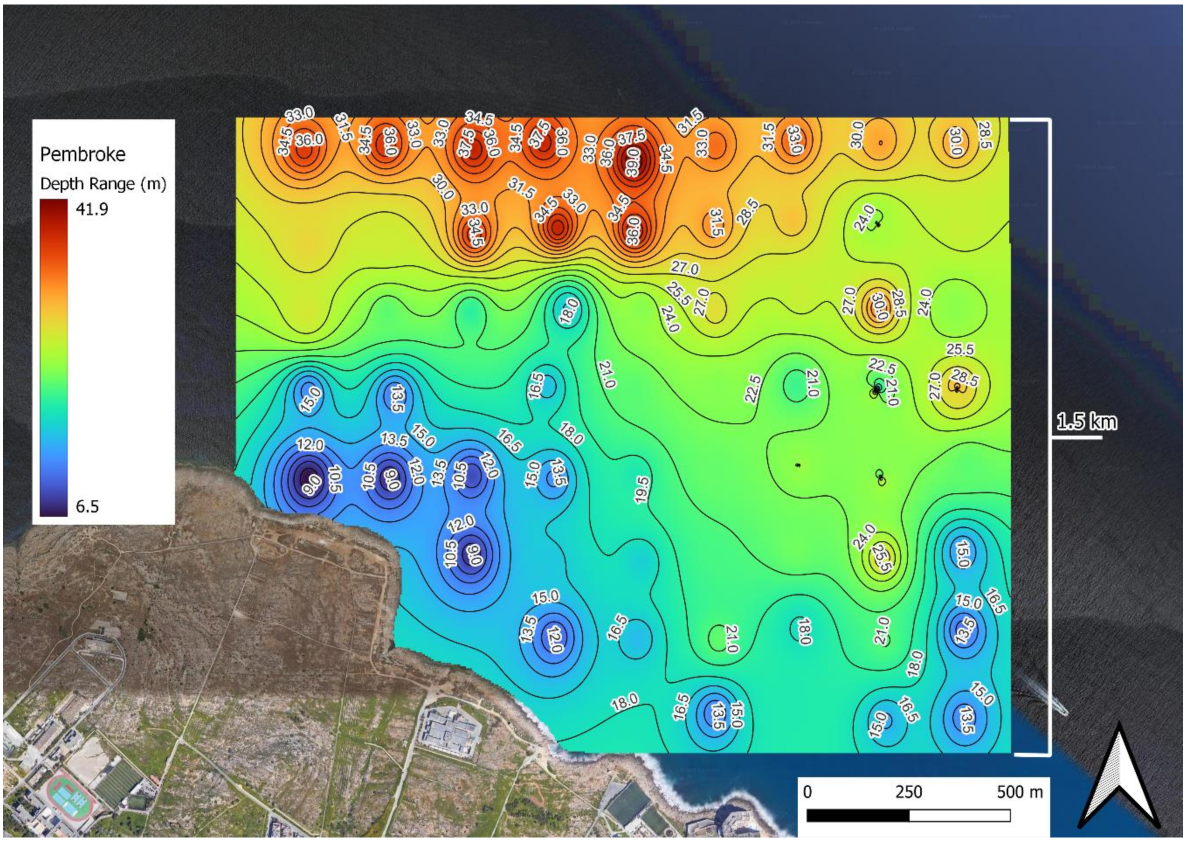


Figure 10. Bathymetry map in Pembroke according to sonde readings.



Bearing in mind that the volume of brine discharge, depth, and bathymetry were different at each RO plant, the following results were analysed accordingly. The primary physical water parameters selected to determine brine distribution ultimately was conductivity in relation to salinity, temperature, pH and RDO. Conductivity was selected as the main parameter for the purposes of this analysis because it is a reliable indicator of water quality, especially in relation to assessing brine discharge composition of RO plants.

In Ċirkewwa, at its 23 physical water parameter sampling stations (Figure 3), the varying depth of the site led to the categorisation of the water column into 3 depth ranges: surface level (0 – 5 m), midlevel (5 – 10 m), and deep level (10 – 15 m). This range was chosen as it was the most representative for facilitating comparisons across the 4 survey seasons. Additionally, a maximum conductivity threshold of 56,000 µS/ cm (Figure 11) was deemed necessary during data sorting to facilitate the visualisation of a substantial amount of conductivity data. Values above this threshold were sporadic and few and so this methodological approach removed outliers, therefore minimizing the occurrence of anomalies which can skew distribution patterns. Seasonality showed that the minimum, maximum and average conductivity (actual conductivity) in the survey grid varied per season as follows:

- Spring: 46,649 – 55,998 µS/ cm, (average 55,526 µS/ cm)
- Summer: 43,098 – 55,999 µS/ cm, (average 53,645 µS/ cm)
- Autumn: 43,201- 56,000 µS/ cm, (average 51,959 µS/ cm)
- Winter: 43,096 – 51,213 µS/ cm, (average 53,027 µS/ cm)

Autumn recorded the highest surface conductivity levels with the lowest in winter (respectively 56,000 µS/ cm and 43,096 µS/ cm) as per Figure 11. Near the discharge source, conductivity increased with depth from approximately 52,500 µS/ cm to 53,500 µS/ cm and 55,000 µS/ cm, corresponding to the surface, mid, and deep levels (Figure 11). Comparatively, the average values further away from the source, in the area north-west of the discharge source (looking out to sea) values are lower and increase with depth from 46,500 µS/ cm to 48,500 µS/ cm and 55,700 µS/ cm, corresponding to the surface, mid, and deep levels (Figure 11).

In Għar Lapsi, at its 34 physical water parameter sampling stations (Figure 4), the varying depth of the site led to the categorisation of the water column into 3 depth ranges: surface level (0 – 24 m), midlevel (24 – 48 m) and deep level (48 – 86 m). Seasonality showed that the minimum, maximum and average conductivity (actual conductivity) in the survey grid varied per season as follows:

- Spring 50,386 – 55,989 µS/ cm, (average 55,430 µS/ cm)
- Summer 43,435 – 56,000 µS/ cm, (average 53,300 µS/ cm)
- Autumn 43,295 – 55,949 µS/ cm, (average 50,932 µS/ cm)
- Winter 44,852 – 55,983 µS/ cm, (average 55,647 µS/ cm)

Summer recorded the highest surface conductivity levels with the lowest in autumn (respectively 56,000 µS/ cm and 43,295 µS/ cm) as per Figure 12. Near the discharge source, average conductivity increased with depth from approximately 50,500 µS/ cm to

51,750 µS/ cm and 55,700 µS/ cm (Figure 12), corresponding to the surface, mid, and deep levels. Comparatively, the average values further away from the source, in the area north-west of the discharge source (looking out to sea) values are lower and increase with depth from 46,500 µS/ cm to 48,500 µS/ cm and 55,700 µS/ cm, corresponding to the surface, mid, and deep levels (Figure 12).

In Pembroke, at its 65 physical water parameter sampling stations (Figure 5), the varying depth of the site led to the categorisation of the water column into 3 depth ranges: surface level (0 – 13 m), midlevel (13 – 27 m) and deep level (27 – 45 m). Seasonality showed that the minimum, maximum and average conductivity (actual conductivity) in the survey grid varied per season as follows:

- Spring 45,323 – 55,999 µS/ cm
- Summer 43,088 – 55,999 µS/ cm
- Autumn 43,846 – 55,995 µS/ cm
- Winter 43,000 – 55,912 µS/ cm

Both summer and spring recorded the highest surface conductivity levels with the lowest in winter (respectively 56,000 µS/ cm and 43,000 µS/ cm) as per Figure 13. Near the discharge source, conductivity increased with depth from approximately 48,000 µS/ cm to 55,100 µS/ cm and 55,600 µS/ cm (Figure 13), corresponding to the surface, mid, and deep levels. Comparatively, the average values further away from the source, in the area north-west of the discharge source (looking out to sea) values are relatively similar and also increase with depth from approximately 43,200 µS/ cm to 53,500 µS/ cm and 54,500 µS/ cm corresponding to the surface, mid, and deep levels (Figure 13).

Figure 11. Conductivity (µS/ cm) dispersion in Ċirkewwa across 4 seasons in surface, mid and deep waters.

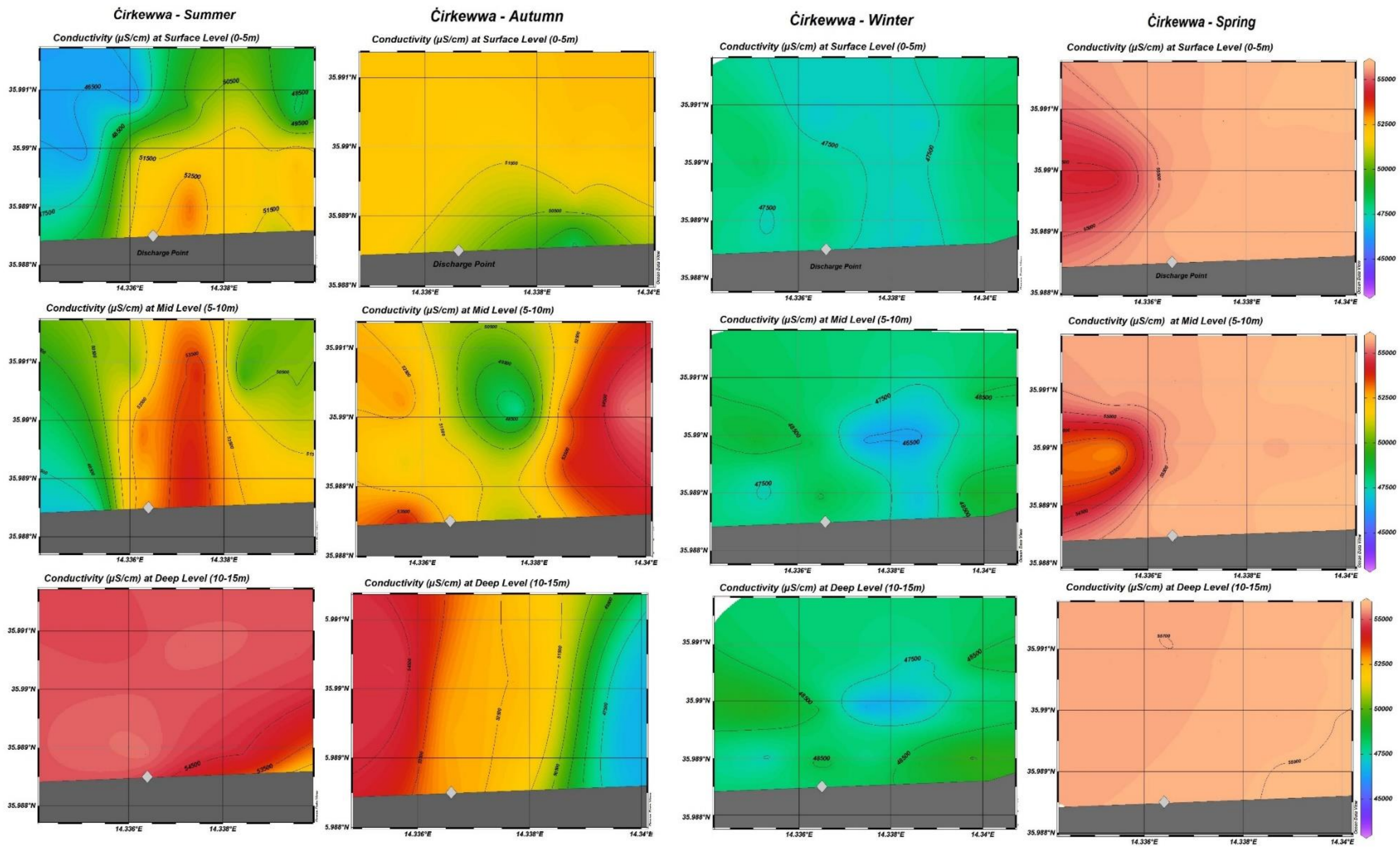


Figure 12. Conductivity (µS/ cm) dispersion in Għar Lapsi across 4 seasons in surface, mid and deep waters.

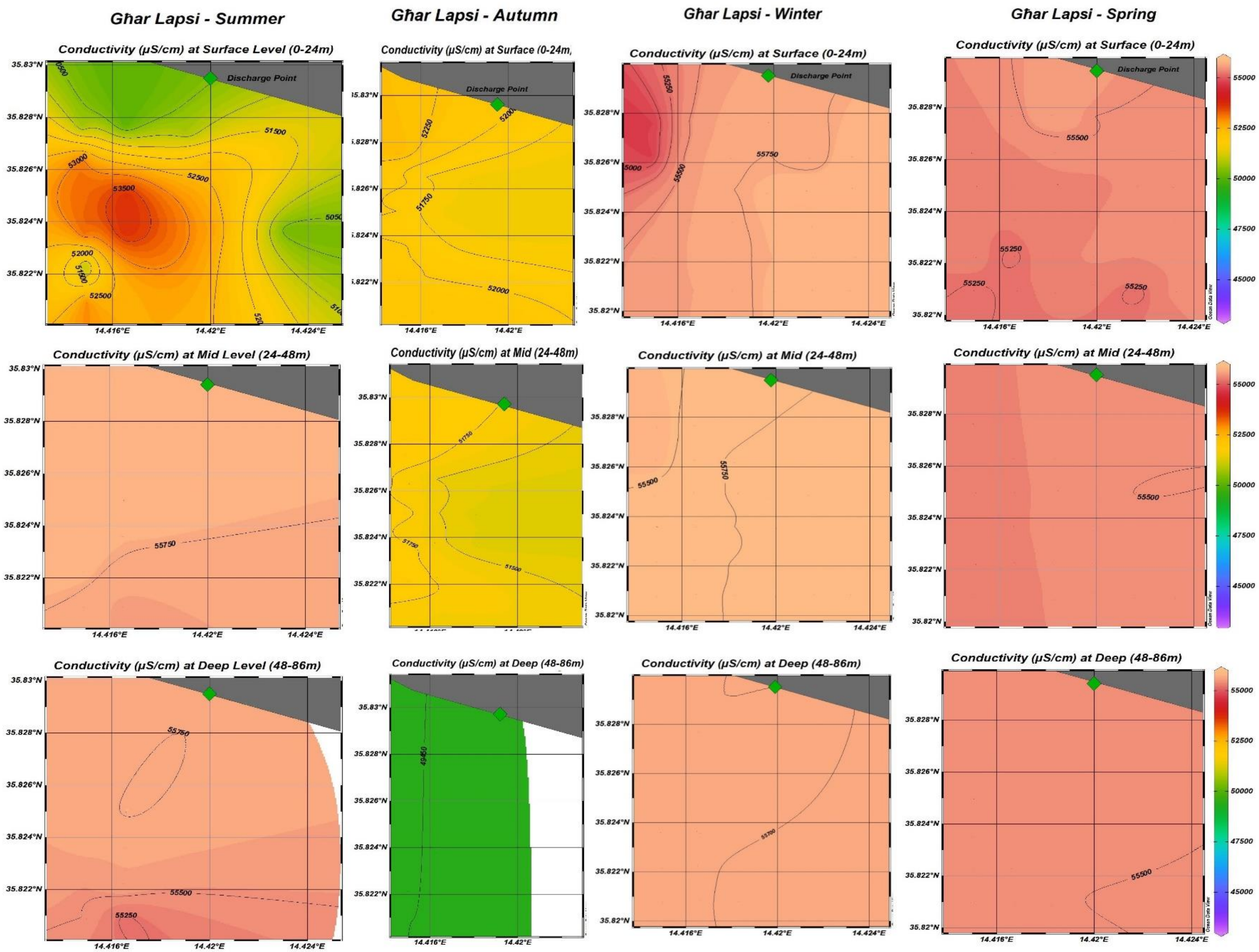
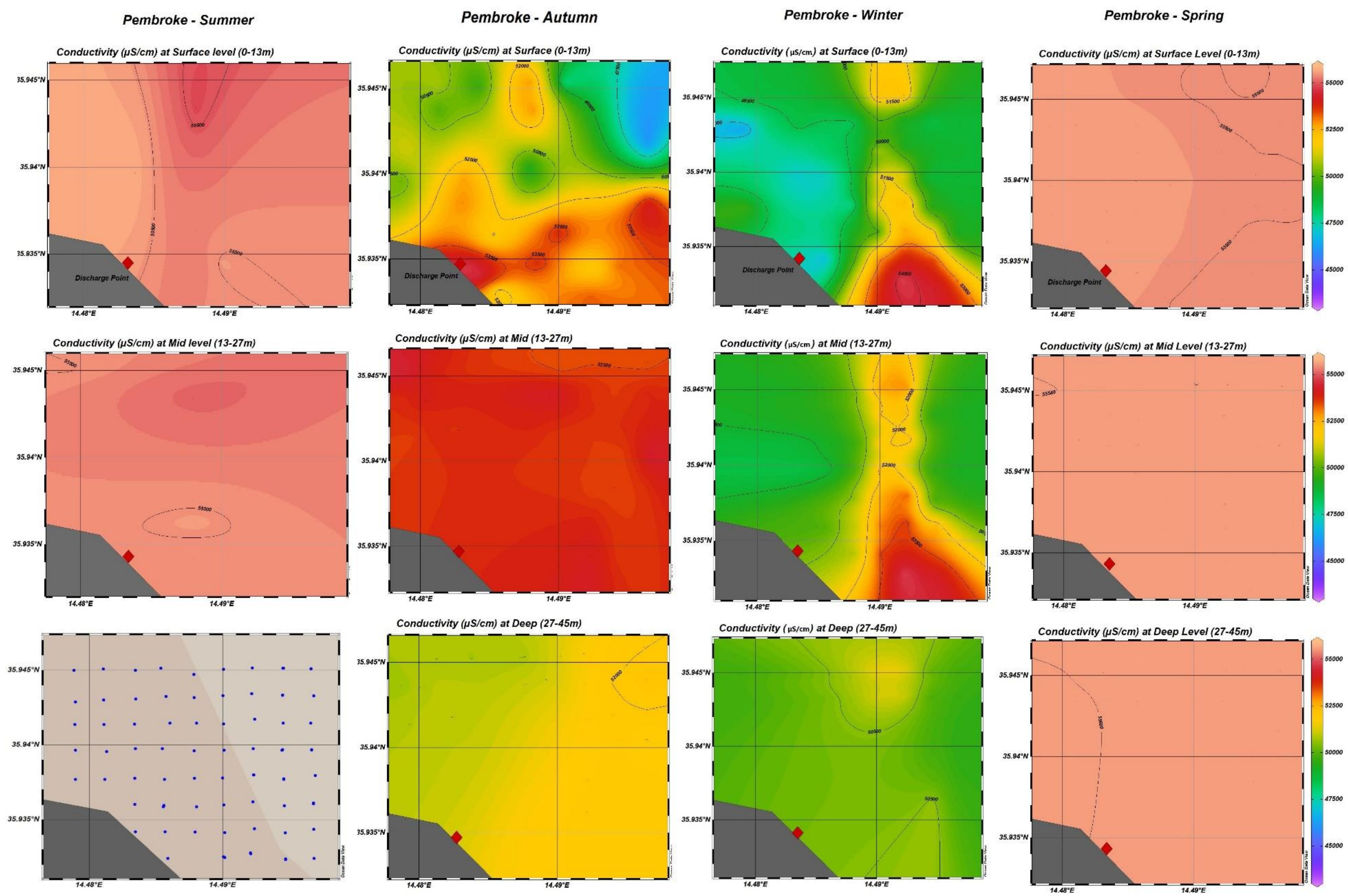


Figure 13. Conductivity (µS/ cm) dispersion in Pembroke across all 4 seasons in surface, mid and deep waters.



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Points of Interest

Following the results previously displayed in the dispersion graphs (Figure 11, Figure 12, Figure 13), it is also crucial to note that other areas of interest not previously visualised were also recorded. All recorded points with highest conductivity (µS/ cm) measurements across the 3 plants and 4 seasons can be seen in Table 2, with their respective sampling station points, latitude and longitudes, depth (m) and distance from discharge point.

Table 2. Points of Interest with highest measurements of conductivity (µS/ cm) across all 3 RO plants, and all 4 seasons.

Location	Season	Latitude	Longitude	Sampling Station	Maximum Conductivity (µS/ cm)	Depth (m)	Depth Level	Distance from discharge point
Pembroke	Autumn	35.93827	14.48305	P14	100,814	0.2	Surface	527m
Pembroke	Autumn	35.94518	14.48135	P5	74,902	0.4	Surface	1.3km
Pembroke	Autumn	35.93819	14.48125	P2	70,224	0.2	Surface	546m
Ċirkewwa	Autumn	35.99009	14.33660	C5	61,144	1.6	Surface	364m
Ċirkewwa	Autumn	35.98770	14.33684	C8	60,906	1.6	Surface	95m
Ċirkewwa	Autumn	35.98705	14.33729	C9	60,833	0.2	Surface	34m
Ċirkewwa	Autumn	35.98688	14.33866	C14	62,703	2.5	Surface	148m
Ċirkewwa	Autumn	35.98764	14.33897	C15	61,451	0.2	Surface	197m
Ghar Lapsi	Autumn	35.82230	14.41632	L8	63,603	0.2	Surface	653m
Ghar Lapsi	Autumn	35.82650	14.41625	L11	63,589	0.2	Surface	200m
Ghar Lapsi	Autumn	35.82623	14.41827	L14	63,156	0.2	Surface	186m
Ghar Lapsi	Autumn	35.82373	14.41825	L16	63,077	0.2	Surface	438m
Ghar Lapsi	Autumn	35.82506	14.41633	L10	62,876	0.2	Surface	375m

According to Table 2, Pembroke reached a maximum conductivity of 100,814 µS/ cm at sampling station P14, which is located 527 m away from the discharge point. This peak reading was a singular occurrence, followed by values of 74,902 µS/ cm at P5 (1.3 km distance from discharge point) and 70,224 µS/ cm at P2 (546 m distance from discharge point). All 3 values occurred at the surface within the first meter of the water column, in the season of Autumn.

Ċirkewwa had a peak conductivity of 62,703 µS/ cm at sampling station C14, located 364 m away from the discharge point. This value was followed by station C15 which recorded a conductivity of 61,451 µS/ cm (197 m distance from discharge point), and station C5 with a conductivity of 61,144 µS/ cm (364 m distance from discharge point). All values occurred at the surface level within the first 3 m of the water column, during the season of Autumn.

Ghar Lapsi had a peak conductivity of 63,603 µS/ cm at sampling station L8, located 653 m away from the discharge point. This value was followed by station L11 with a conductivity of 63,589 µS/ cm (200 m distance from discharge point) and station L14 with a conductivity of 63,156 µS/ cm (186 m distance from discharge point). All recorded values were observed at the surface level, within the first meter of the water column, during the season of Autumn.

Turbidity

An average of seasonal average turbidity (m) measurements for all impact and reference points in all 3 RO plants, across all 4 seasons can be seen in Table 3**.** The turbidity values in descending order at Ċirkewwa were 9 m, 5 m and 4 m (respectively at C_RT1, C_RT2 and C_IT). Meanwhile, at Għar Lapsi they were 9 m, 8 m, and 6 m (respectively at L_IT, L_RT2 and L_RT1), and Pembroke's values were 8 m, 5 m, and 5 m (respectively at P_RT2, P_RT1, and P_IT).

Table 3. The average of average turbidity at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P), using a Secchi disk (m).

Season	C_IT (m)	C_RT1 (m)	C_RT2 (m)	L_IT (m)	L_RT1 (m)	L_RT2 (m)	P_IT (m)	P_RT1 (m)	P_RT2 (m)
Autumn	4	9	4	7	5	8	5	4	8
Spring	3	10	4	9	5	7	5	3	7
Summer	4	10	5	10	6	8	5	7	10
Winter	4	8	5	9	8	8	5	4	8
Average of Average Turbidity (m)	**4**	**9**	**5**	**9**	**6**	**8**	**5**	**5**	**8**

5.1.2 Chemical Water Parameters

Graphs for the chemical water parameters are reported per parameter per season across all 3 RO plants, as can be seen in Figures 14 to 17.

Boron

Figure 14 displays the ranges in average boron concentrations across all 4 surveyed seasons at the impact point (IT) and 2 refence points (RT1 and RT2) of the 3 RO plants - Ċirkewwa (C), Għar Lapsi (L) and Pembroke (P). Seasonality concentrations showed that there was a variation of minimum and maximum average boron concentrations at each location:

- Ċirkewwa: Highest concentrations in autumn with 5.40 mg/l, 5.05 mg/l, 5.40 mg/l (respectively at IT, RT1 and RT2). Lowest in summer with 3.28 mg/l, 3.64 mg/l, 3.17 mg/l (respectively at IT, RT1 and RT2).

- Għar Lapsi: Highest concentrations in autumn with 4.65 mg/l, 4.50 mg/l, 5.25 mg/l (respectively at IT, RT1 and RT2). Lowest in winter 2.30 mg/l, 2.30 mg/l, 2.25 mg/l (respectively at IT, RT1 and RT2).

- Pembroke: Highest concentrations in autumn with 4.70 mg/l, 5.20 mg/l, 4.65 mg/l (respectively at IT, RT1 and RT2). Lowest in summer with 3.43 mg/l, 2.95 mg/l, 2.61 mg/l (respectively at IT, RT1 and RT2).

Comparisons across the 3 plants showed minimal differences between the impact and reference points (Figure 14). An average taken from all seasons combined showed that, both Ċirkewwa and Pembroke had higher average boron concentrations at the impact points compared to their reference points. Ċirkewwa had the highest set of average values with 4.19 mg/l vs 4.08 mg/l and 4.09 mg/l (respectively IT vs RT1 and RT2), followed by Pembroke with 3.98 mg/l vs 4.05 mg/l and 3.81 mg/l (respectively IT vs RT1 and RT2). Whereas Għar Lapsi had higher average concentrations at the reference points, RT1 and RT2 (respectively 3.39 mg/l and 3.47 mg/l compared to the impact point, IT (3.40 mg/l). Additionally, statistical analysis showed a significant difference between boron concentrations across the 4 seasons ($p < 0.05$). Seasonality showed that autumn retained the highest average boron concentrations with the lowest in summer at each of the 3 plants.

Calcium

Figure 15 displays the ranges in average calcium concentrations across all 4 surveyed seasons at the impact point (IT) and 2 refence points (RT1 and RT2) of the 3 RO plants - Ċirkewwa (C), Għar Lapsi (L) and Pembroke (P). Seasonality concentrations showed that there was a variation of collective minimum and maximum average calcium concentrations at each location:

- Ċirkewwa: Highest concentration in summer with 469.5 mg/l, 467.0 mg/l and 480.5 (respectively at IT, RT1 and RT2). Lowest in autumn with 248.5 mg/l, 282.5 mg/l and 140.5mg/l (respectively at IT, RT1 and RT2).

- Għar Lapsi: Highest concentration in winter with 579.5 mg/l (IT) and in autumn with 527.5 mg/l and 524.0 mg/l (respectively at RT1 and RT2). Lowest in summer with 425.0 mg/l (IT) and in spring with 416.5 mg/l and 426.0 mg/l (respectively at RT1 and RT2).

- Pembroke: Highest concentration in autumn with 514.0 mg/l, 515.5 mg/l, and 530.5 mg/ (respectively at IT, RT1 and RT2). Lowest in summer with 410.5 mg/l, 407.5 mg/l and 427.0 mg/l (respectively at IT, RT1 and RT2).

Comparisons across the 3 plants showed minimal differences between the impact and reference points. An average taken from all seasons combined showed that, both Ċirkewwa and Għar Lapsi had higher collective average calcium concentrations at the impact points compared to their reference points. Għar Lapsi had the highest set of average values with 492.9 mg/l vs 472.4 mg/l and 482.5 mg/l (respectively IT vs RT1 and RT2), followed by Ċirkewwa with 408.5 mg/l vs 402.3 mg/l and 377.5 mg/l (respectively IT vs RT1 and RT2) (Figure 15). Whereas Pembroke had higher average concentrations at the reference points, RT1 and RT2 (respectively 460.75 mg/l and 465.4mg/l compared to the impact point, IT (456.0 mg/l). Additionally, statistical analysis showed no significant difference between calcium concentrations across the 4 seasons ($p > 0.05$). However, the highest collective averages were in autumn for both Għar Lapsi and Pembroke whereas in summer at Ċirkewwa.

Magnesium

Figure 16 displays the ranges in average magnesium concentrations across all 4 surveyed seasons at the impact (IT) and 2 refence points (RT1 and RT2) of the 3 RO plants - Ċirkewwa (C), Għar Lapsi (L) and Pembroke (P). Seasonality concentrations showed that there was a variation of collective minimum and maximum average magnesium concentrations at each location:

- Ċirkewwa: Highest concentration in summer with 1,260.0 mg/l, 1,285.0 mg/l and 1,245.0 mg/l (respectively at IT, RT1 and RT2). Lowest in autumn with 756.5 mg/l, 886.0 mg/l, 465.5 mg/l (respectively at IT, RT1 and RT2).

- Għar Lapsi: Highest concentration in autumn with 1,607.5 mg/l, 1,584.0 mg/l and 1,571.5 mg/l (respectively IT, RT1 and RT2). Lowest in summer with 816.0 mg/l, 905.5 mg/l and 844.0 mg/l (respectively at RT1 and RT2).

- Pembroke: Highest concentration in autumn with 1,478.0 mg/l, 1,498.5 mg/l and 1,525.5 mg/l (respectively at IT, RT1 and RT2). Lowest in winter with 940.0 mg/l (IT) and summer with 941.5 mg/l and 946.5 mg/l (respectively at RT1 and RT2).

Comparisons across the 3 plants showed minimal differences between the impact and reference points. An average taken from all seasons combined showed that, Għar Lapsi had the highest collective set of average magnesium concentrations with 1,238.0 mg/l vs 1,238.8 mg/l and 1,232.6 mg/l (respectively IT vs RT1 and RT2), followed by Pembroke with 1,120.8 mg/l vs 1,118.6 mg/l and 1,135.6 mg/l (respectively IT vs RT1 and RT2) and finally Ċirkewwa with 1,029.5 mg/l vs 1,040.4 mg/l and 933.1 mg/l (respectively IT vs RT1 and RT2) (Figure 16). Additionally, statistical analysis showed no significant difference between magnesium concentrations across the 4 seasons ($p > 0.05$). However, the highest collective averages were in autumn for both Għar Lapsi and Pembroke whereas in summer at Ċirkewwa.

Sodium

Figure 17 displays the ranges in average sodium concentrations across all 4 surveyed seasons at the impact (IT) and 2 refence points (RT1 and RT2) of the 3 RO plants - Ċirkewwa (C), Għar Lapsi (L) and Pembroke (P). Seasonality concentrations showed that there was a variation of minimum and maximum average sodium concentrations at each location:

- Ċirkewwa: Highest concentration in autumn with 11,984.0 mg/l, 11,522.0 mg/l and 11,091.5 mg/l (respectively at IT, RT1 and RT2). Lowest in winter with 8,950.0 mg/l, 8,860.0 mg/l and 8,860.0 mg/l (respectively at IT, RT1 and RT2).

- Għar Lapsi: Highest concentration in autumn with 12,790.5 mg/l for IT, and winter with 12,916.5 mg/l and 12,643.5 mg/l (respectively at RT1 and RT2) noting that the autumn concentrations were relatively similar. The lowest were in summer 8,170.0 mg/l, 9,100.0 mg/l and 4,691.5 mg/l (respectively at IT, RT1 and RT2).

- Pembroke: Highest concentration in autumn with 12,296.5 mg/l,12,580.0 mg/l and 12,507.5 mg/l (respectively at IT, RT1 and RT2). Lowest in summer with 8,900.0 mg/l, 8,590.0 mg/l and 8,830.0 mg/l (respectively at IT, RT1 and RT2).

Comparisons across the 3 plants showed minimal differences between the impact and reference points. An average taken from all seasons combined showed that Ċirkewwa had the highest collective set of average sodium concentrations with 10,783.5 mg/l, 10,370.5 mg/l and 10,387.875 mg/l (respectively IT vs RT1 and RT2), followed by Għar Lapsi with 11,240.3 mg/l, 11,288.3 mg/l and 10,162.1 mg/l (respectively IT vs RT1 and RT2) and finally Pembroke with 10,052.9 mg/l, 10,152.5 mg/l and 10,303.1 mg/l (respectively IT vs RT1 and RT2) (Figure 17). Additionally, statistical analysis showed a significant difference in the potassium concentrations across the 4 seasons ($p < 0.05$) at Għar Lapsi only. Here, summer is significantly highlighted with the lowest concentrations compared to the rest of the seasons. Comparatively, autumn had the highest average concentrations at all 3 sites.

Potassium

Figure 18 displays the ranges in average potassium concentrations across all 4 surveyed seasons at the impact (IT) and 2 refence points (RT1 and RT2) of the 3 RO plants - Ċirkewwa (C), Għar Lapsi (L) and Pembroke (P). Seasonality concentrations showed that there was a variation of minimum and maximum average potassium concentrations at each location:

- Ċirkewwa: Highest concentration in summer with 453.5 mg/l, 435.5 mg/l and 465.5 mg/l (respectively at IT, RT1 and RT2). Lowest in winter with 311.0 mg/l (IT) and in autumn with 303.0 mg/l and 191.5 mg/l (respectively at RT1 and RT2).

- Għar Lapsi: Highest concentration in autumn with 652.5 mg/l, 636.0 mg/l and 624.5 mg/l (respectively at IT, RT1 and RT2). Lowest in summer with 326.5 mg/l, 364.5 mg/l and 347.5 mg/l (respectively at IT, RT1 and RT2).

- Pembroke: Highest concentration in autumn with 606.5 mg/l, 619.0 mg/l and 662.0 mg/l (respectively at IT, RT1 and RT2). Lowest in winter with 302.5 mg/l, 312 mg/l and 313 mg/l.

Comparisons across the 3 plants showed minimal differences between the impact and reference points. An average taken from all seasons combined showed that, Għar Lapsi had the highest collective set of average potassium concentrations with 460.5 mg/l vs 477.6 mg/l and 481.5 mg/l (respectively IT vs RT1 and RT2), followed by Pembroke with 412.5 mg/l vs 422.4 mg/l and 442.6 mg/l (respectively IT vs RT1 and RT2) and finally Ċirkewwa with 368.0 mg/l vs 348.5 mg/l and 332.1 mg/l (respectively IT vs RT1 and RT2) (Figure 18). Additionally, statistical analysis showed that there was no significant difference between potassium concentrations across the 4 seasons ($p < 0.05$) but highlighted that seasonality showed that autumn retained the highest average potassium concentrations at Għar Lapsi and Pembroke, while in summer at Ċirkewwa. The lowest concentrations were in winter at both Pembroke and Ċirkewwa whereas in summer at Għar Lapsi.

Sulphates

Figure 19 displays the ranges in average sulphate concentrations across all 4 surveyed seasons at the impact (IT) and 2 refence points (RT1 and RT2) of the 3 RO plants - Ċirkewwa (C), Għar Lapsi (L) and Pembroke (P). Seasonality concentrations showed that there was a variation of minimum and maximum average sulphate concentrations at each location:

- Ċirkewwa: Values were notably similar across each season. However, the highest concentration was in autumn with 2,942.0 mg/l, 3,067.0 mg/l and 2,930.5 mg/l (respectively at IT, RT1 and RT2). Lowest in winter with 2,850.0 mg/l, 3,100.0 mg/l and 2,800.0 mg/l (respectively at IT, RT1 and RT2).

- Għar Lapsi: Highest concentration in winter with 3,008.5 mg/l, 3,008.0 mg/l and 3,001.5 mg/l (respectively at IT, RT1 and RT2) noting that the spring concentrations were relatively similar. The lowest were in summer 2,600.0 mg/l, 2,800.0 mg/l and 2,800.0 mg/l (respectively at IT, RT1 and RT2).

- Pembroke: Highest concentration in autumn with 2,988.0 mg/l, 2,994.5 mg/l and 3,015.5 mg/l (respectively at IT, RT1 and RT2). Similar concentrations were recorded in the other 3 seasons with 2,900.0 mg/l, 2,900.0 mg/l and 2,950.0 mg/l (respectively at IT, RT1 and RT2).

Comparisons across the 3 plants showed minimal differences between the impact and reference points. An average taken from all seasons combined showed that, Ċirkewwa had a collective set of average sulphate concentrations with 2,898.0 mg/l, 3,029.3 mg/l, 2,845.1 mg/l (respectively IT vs RT1 and RT2), Għar Lapsi with 2,876.8 mg/l, 2,902.4 mg/l, 2,898.9

mg/l (respectively IT vs RT1 and RT2) and finally Pembroke with 2,922.0 mg/l, 2,923.6 mg/l, 2,941.4 mg/l (respectively IT vs RT1 and RT2) (Figure 19). Additionally, statistical analysis showed no significant difference in the sulphate concentrations across the 4 seasons ($p < 0.05$). Concentrations were relatively consistent between the impact and reference locations and seasonally, at each RO plant.

Chlorides

Figure 20 displays the ranges in average chloride concentrations across all 4 surveyed seasons at the impact (IT) and 2 refence points (RT1 and RT2) of the 3 RO plants- Ċirkewwa (C), Għar Lapsi (L) and Pembroke (P). Seasonality concentrations showed that there was a variation of minimum and maximum average chloride concentrations at each location:

- Ċirkewwa: Values were notably similar across each season. However, the highest concentration was in autumn with 23,843.5 mg/l, 23,689.5 mg/l and 24,390 mg/l (respectively at IT, RT1 and RT2). Lowest in spring with 22,000.0 mg/l, 23,000.0 mg/l and 20,500.0 mg/l (respectively at IT, RT1 and RT2).

- Għar Lapsi: Highest concentration in spring with 22,500.0 mg/l, 22,000.0 mg/l and 22,000.0 mg/l (respectively at IT, RT1 and RT2) noting that the winter concentrations were relatively similar. The lowest were in autumn 21,349.0 mg/l, 20,438.0 mg/l and 20,438.0 mg/l (respectively at IT, RT1 and RT2).

- Pembroke: Highest concentration in winter with 23,000.0 mg/l, 27,000.0 mg/l and 23,500.0 mg/l (respectively at IT, RT1 and RT2). Lowest in spring with 20,887.5 mg/l, 20,791.5 mg/l and 20,937.0 mg/l (respectively at IT, RT1 and RT2).

Comparisons across the 3 plants showed minimal differences between the impact and reference points. An average taken from all seasons combined showed that, Ċirkewwa had a collective set of average chloride concentrations with 22,960.9 mg/l, 23,672.4 mg/l and 22,597.5 mg/l (respectively IT vs RT1 and RT2), Għar Lapsi with 21607.5 mg/l, 21,818.1 mg/l, 21,373.6 mg/l (respectively IT vs RT1 and RT2) and finally Pembroke with 22,221.9 mg/l, 23,197.9 mg/l and 22,234.3 mg/l (respectively IT vs RT1 and RT2) (Figure 20). Additionally, statistical analysis showed no significant difference in the sulphate concentrations across the 4 seasons ($p < 0.05$). Concentrations were relatively uniform between the impact and reference locations and seasonally, at each RO plant. The autumn values in Pembroke showed a minimal difference with its values being the only ones below 21,000.0 mg/l in comparison to both locations within the site and against other RO plants.

Nitrates

The nitrate concentration across all points in Ċirkewwa and Pembroke was rendered as of <25 mg/l, and that of Għar Lapsi was <20 mg/l (Appendix 23). This means to they fell below the limit of detection under laboratory analysis. Therefore, no statistical analysis could be carried out.

Principal Component Analysis

Figure 21 displays the distribution pattern of the concentration readings from the water chemical analysis (at surface and deep readings per water column of survey location), using a Principal Component Analysis (PCA). The cluster of points in the figure represents a distribution where the first 2 principal components (PC1 and PC2) account for 99.8% of the total variance observed across all 3 RO plants, per season.

The concentration values of chemical parameters in spring are closely clustered, indicating minimal differences in seasonality between the impact and reference transects within each RO plant. In general, the smallest variance in concentration seems to occur in spring for each plant. The largest variance in chemical parameter concentration appears to have occurred in winter, with Ċirkewwa and Pembroke exhibiting a similar range of values compared to Għar Lapsi, whose concentration readings were collectively higher than the other RO plants. Similarly, a large variance occurred in summer, following an inverse pattern of winter concentration values. Here, Pembroke and Għar Lapsi had a similar range in concentrations compared to Ċirkewwa, highlighting the highest concentrations recorded. Furthermore, the scatter pattern in autumn illustrates that all 3 RO plants fell within a similar range of concentration values, with Ċirkewwa having a lower set of concentrations compared to a higher range at Għar Lapsi and Pembroke, which are clustered closer together. It is also worth noting that some sampling points resulted in anomalies, notably at Għar Lapsi and Pembroke. Moreover, among all the chemical parameters that were analysed, chloride (Cl) and potassium (Na) are illustrated in the PCA graph as the most prevalent chemicals detected from the water sampling.

Figure 14. Average boron concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons.

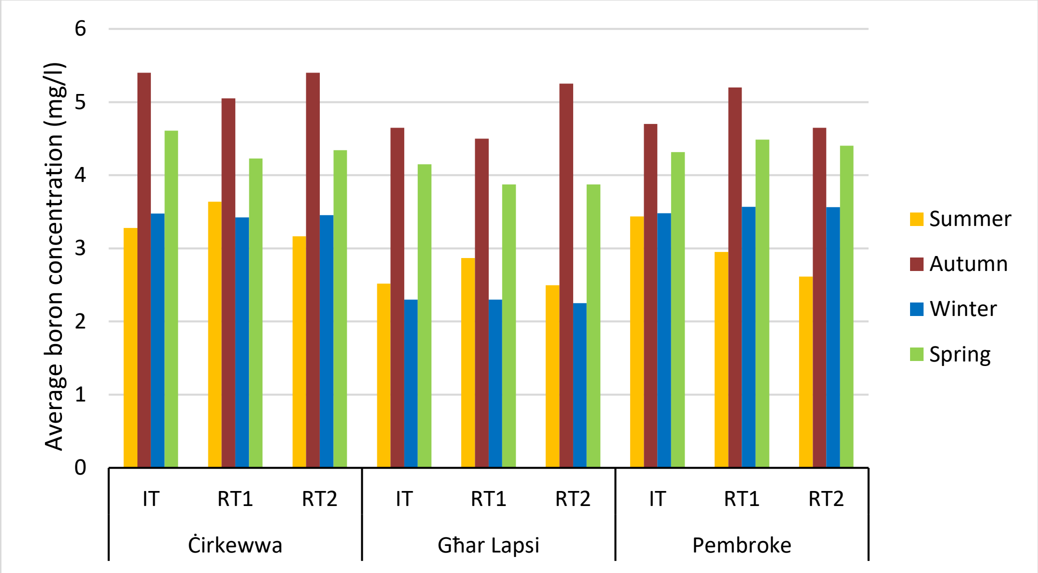


Figure 15. Average calcium concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons.

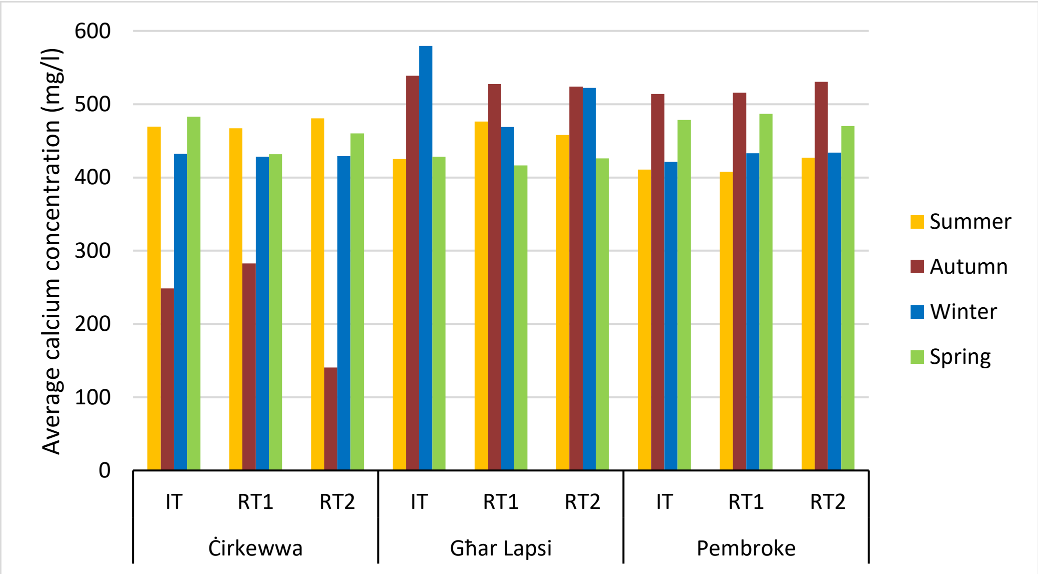


Figure 16. Average magnesium concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons.

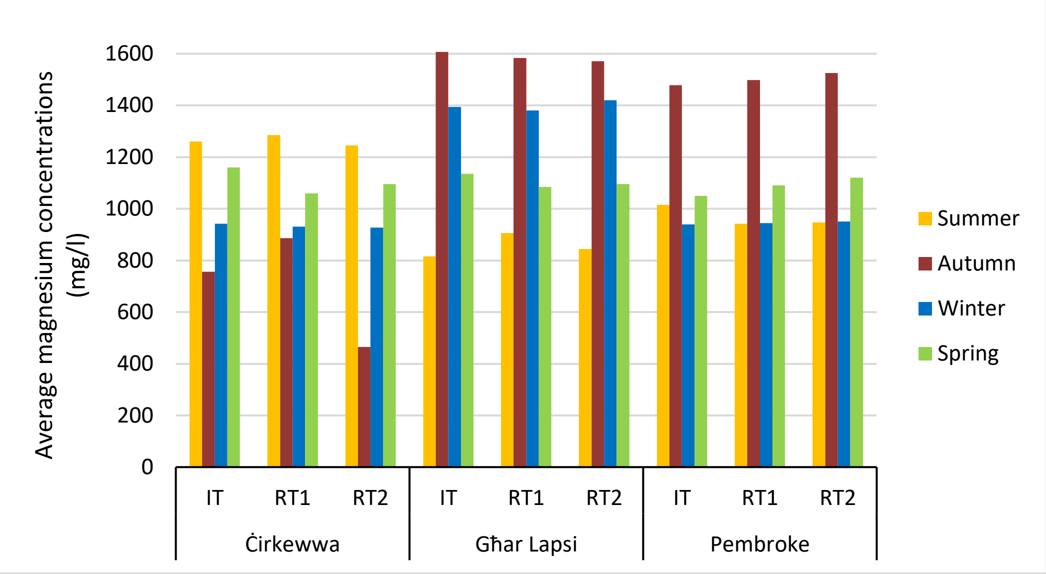


Figure 17. Average sodium concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons.

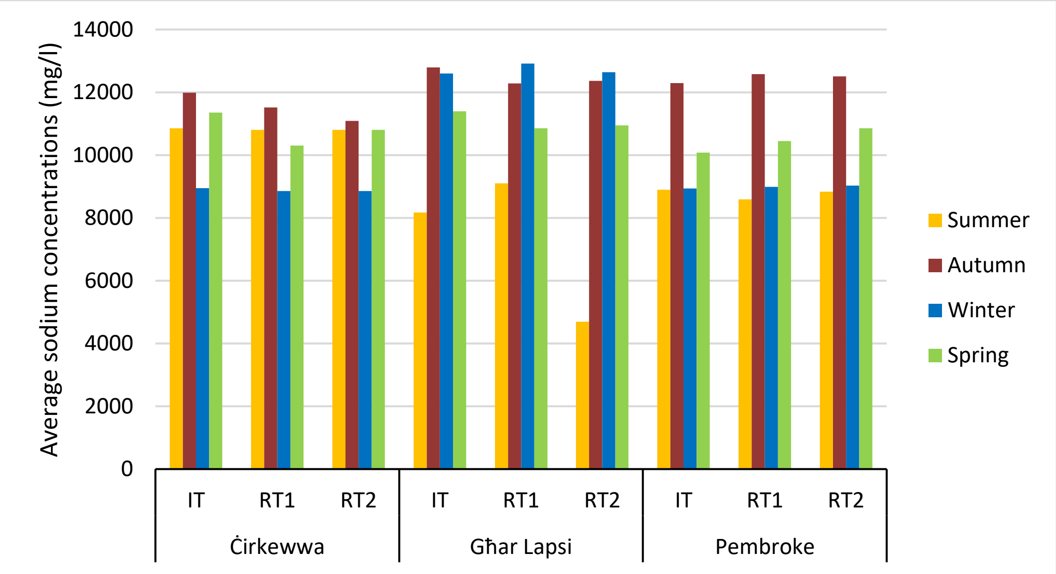


Figure 18. Average potassium concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons.

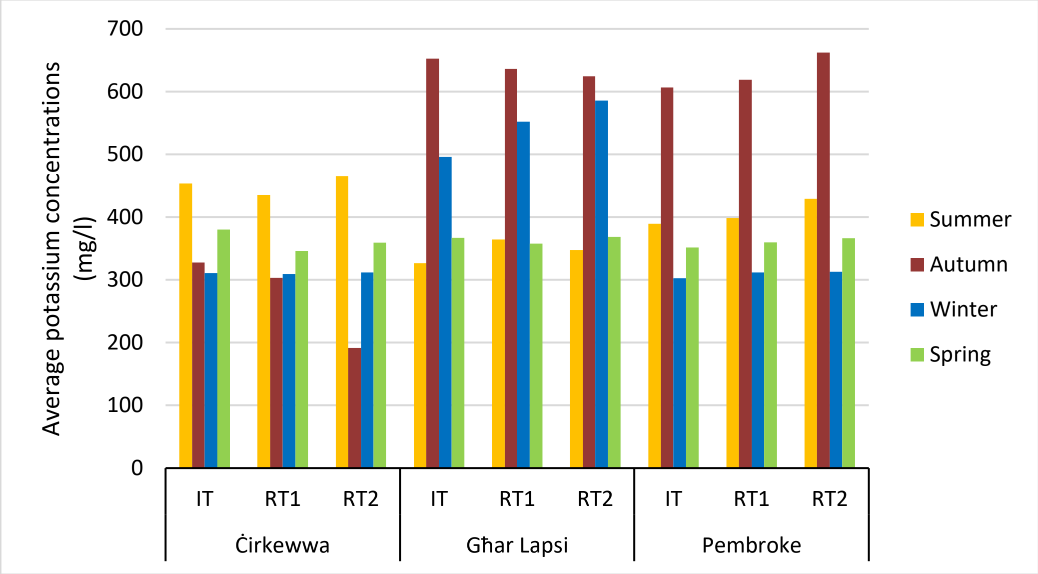


Figure 19. Average sulphates concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons.

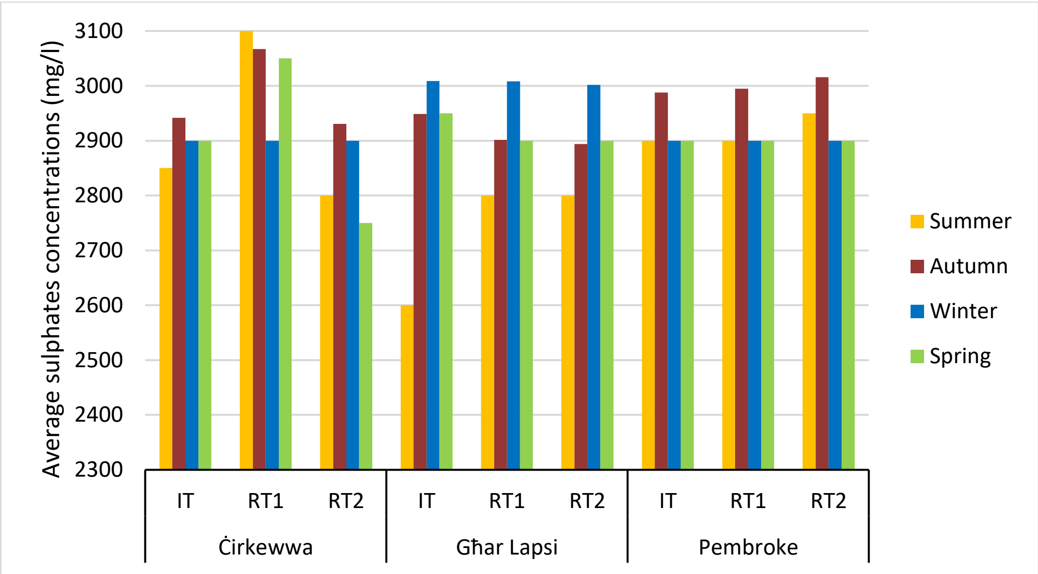


Figure 20. Average chlorides concentrations (mg/l) at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons.

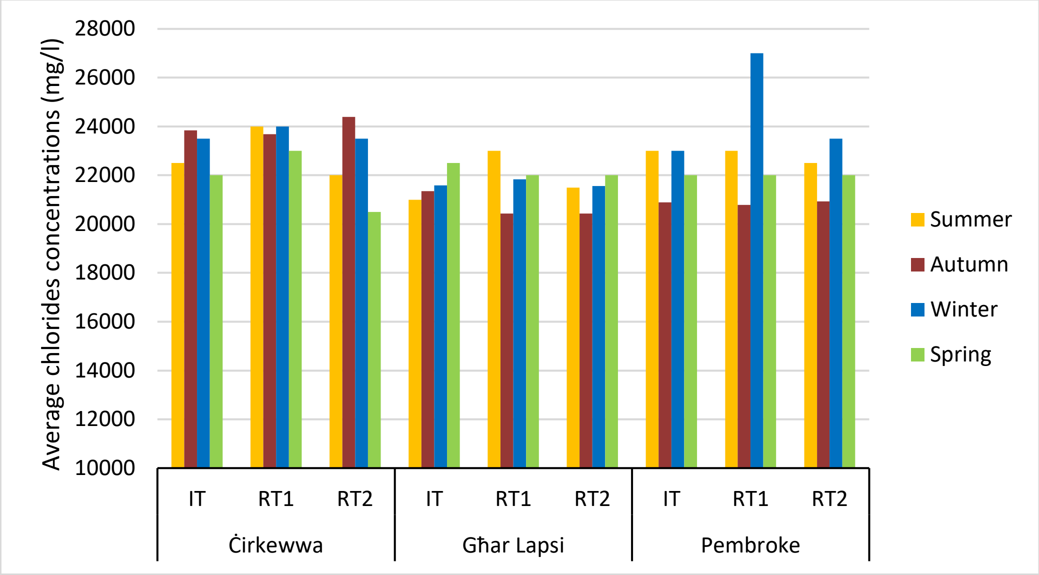
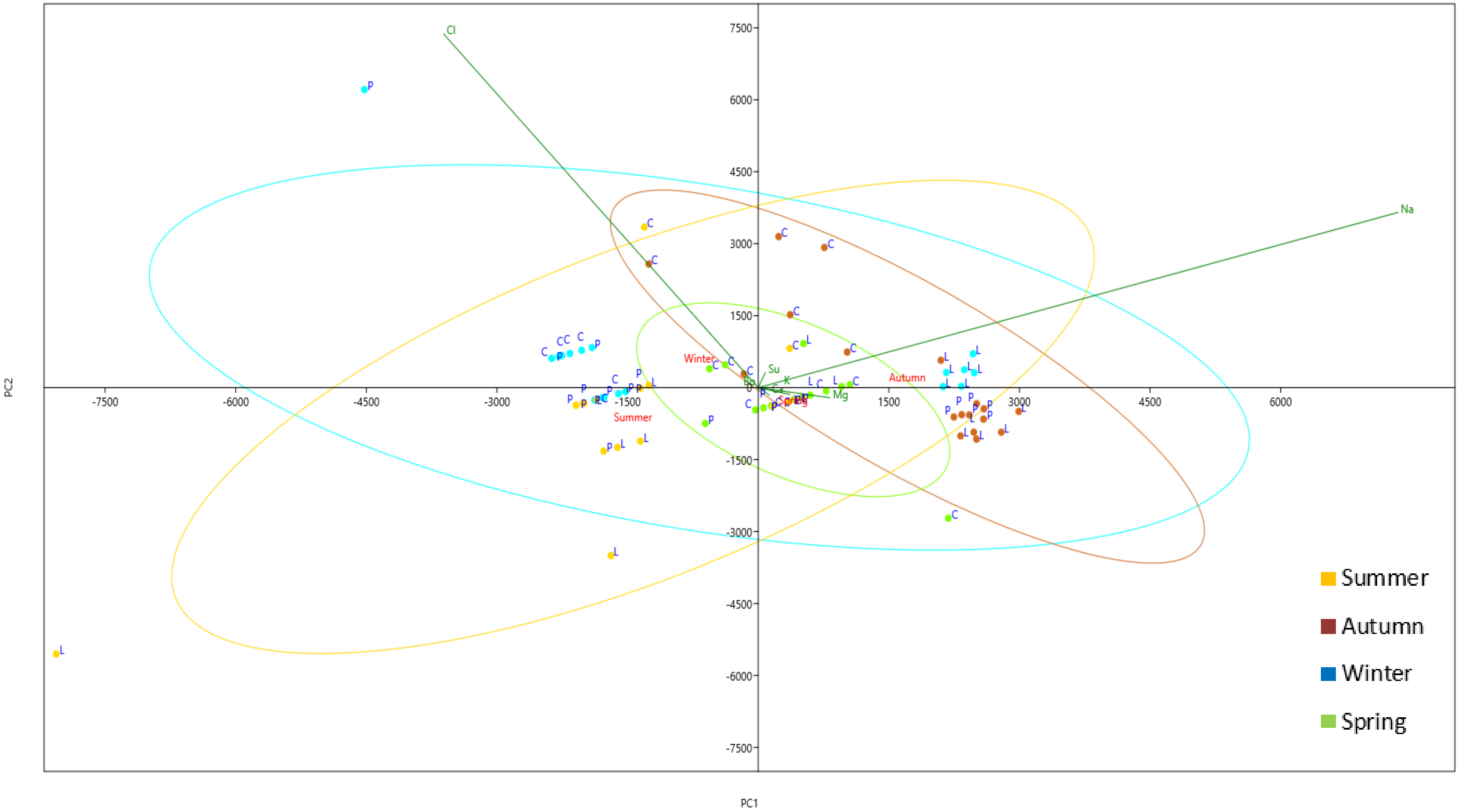


Figure 21. Principal Component Analysis (PCA) of chemical water quality parameters at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P) across 4 seasons.



5.2 Dive Surveys

A total of 6 line transects combined, were assessed during the dive transect surveys carried out across 4 seasons (Figures 6, 7, 8). Each of the transects at the RO plants had 1 impact and 2 reference transects that were surveyed 4 times over a span of 2 years. Observed species and benthic composition were recorded during quadrat data analysis of the dive transect. The significant substrate features encountered were *Posidonia oceanica*, algae, dead matte, sand and rock and echinoderms. Even though other substrate values were recorded, *Posidonia oceanica* and *echinoderms* were the bioindicator species for investigating the impacts of brine discharge on the environment. The impact points at the brine discharge source at all 3 RO plants showed large schools of fish in the water column. It's important to note that the presence of fish was inferred from general observations during the survey, as it was not included as a bioindicator or target species in this project's activities and thus no particular data collection was made in this regard.

Ċirkewwa

The survey transect depths were 2.5 m - 6.1 m, 5.1 m - 22.0 m, 5.5 m – 9.5 m (respectively IT, RT1, RT2).

The *Posidonia* average percent cover, shoot density and leaf length did not show any statistical significance ($p > 0.05$) between the impact (average of 36% / quadrat, 328 shoots / m^2 and 24 cm) and the 2 reference sites (respectively average of 38% / quadrat, 415 shoots / m^2, and 27 cm and 59% / quadrat, 663 shoots / m^2 and 26 cm for RT1 and RT2), disregarding the seasonality (Figures 22, 23 and 24).

When seasonality is considered, statistical tests showed only significant differences between autumn and the other 3 seasons ($p < 0.001$) for the variable averages of *Posidonia* percent cover, the shoot density and leaf length.

Autumn showed the highest average *Posidonia* percent cover (respectively 100%, 74% and 100% for IT, RT1 and RT2) against spring (respectively 0%, 53% and 45%), summer (respectively 45%, 0% and 33%), and winter (respectively 0%, 28% and 58%) (Figure 23).

The same pattern applied to the *Posidonia* average shoot density, with autumn recording the highest number of shoots / m^2 (respectively 832, 848, 1012 shoots for IT, RT1 and RT2), against spring (respectively 0, 616, and 560 shoots), summer (respectively 480, 0, and 360 shoots), and winter (respectively 0, 196, and 720 shoots) (Figure 23). Additionally, the number of visible leaves counted for each *Posidonia* shoot observation remained constant at 4 - 6 leaves per shoot.

The *Posidonia* average leaf length showed the longest occurred during autumn (respectively 66 cm, 54 cm and 50 cm for IT, RT1 and RT2) against spring (respectively 0 cm, 34 cm and 27 cm), summer (respectively 29 cm, 0 cm, and 17 cm), and winter (respectively 0 cm, 21 cm, and 8 cm) (Figure 24).

The algae average percent cover did not show any statistical significance between the impact (40%) and the 2 reference sites, (respectively 53% and 25% for RT1 and RT2) disregarding seasonality. However, when seasonality is considered, there was a statistical significance, especially highlighted between summer and autumn ($p < 0.001$). Summer showed the highest average algae percent cover (respectively 55%, 100% and 40% for IT, RT1 and RT2) against autumn (respectively 0%, 25% and 0%), spring (respectively 45%, 43% and 48%), and winter (respectively 59%, 45% and 11%) (Figure 23).

The sand average percent cover did not show any statistical significance between the impact (24%) and the 2 reference sites, (respectively 8% and 16% for RT1 and RT2) disregarding seasonality. However, when seasonality is considered, there was a statistical significance between winter and the other 3 seasons ($p < 0.001$). Winter showed the highest average algae percent cover (respectively 40%, 26% and 30% for IT, RT1 and RT2) against spring (respectively 56%, 5% and 8%), summer (respectively 0%, 0% and 0%), and autumn (respectively 59%, 45% and 11%) (Figure 22).

The percent cover of both dead matte and rock did not show any statistical significance between impact and the 2 reference sites. Considering seasonality did not show any statistical significance either.

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Figure 22. Average substrate percent cover at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C) across seasons.

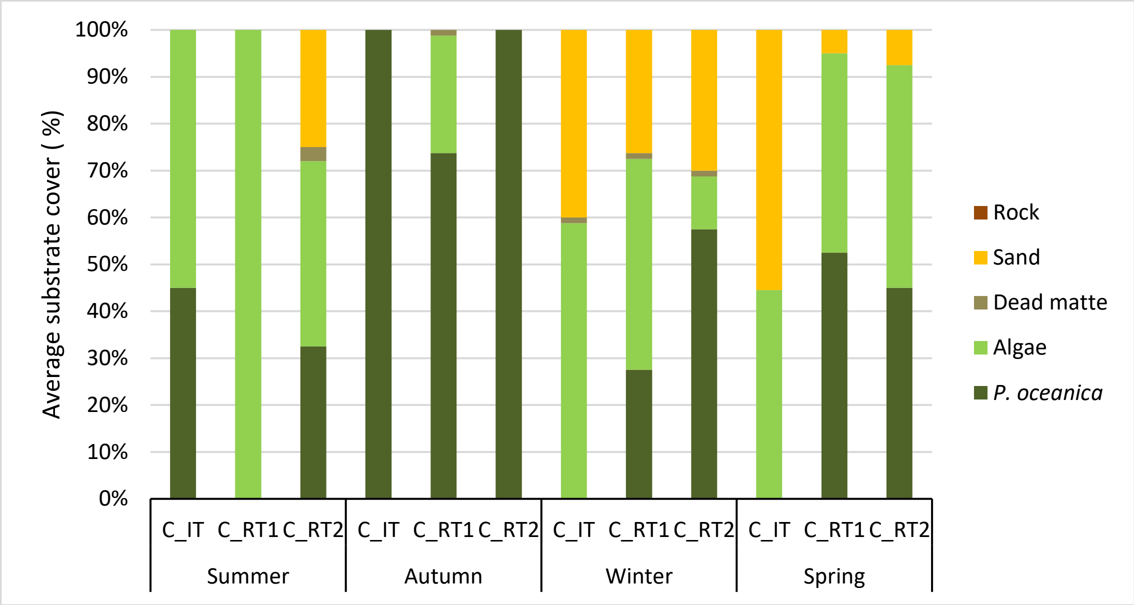


Figure 23. Average shoot density (/ m2) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C) across seasons.

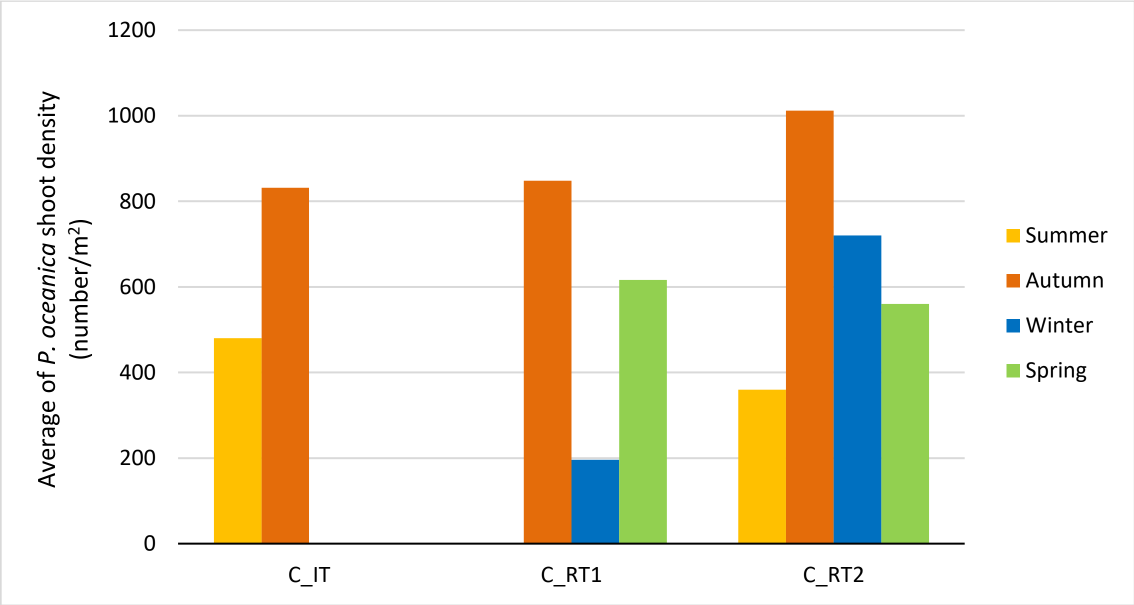
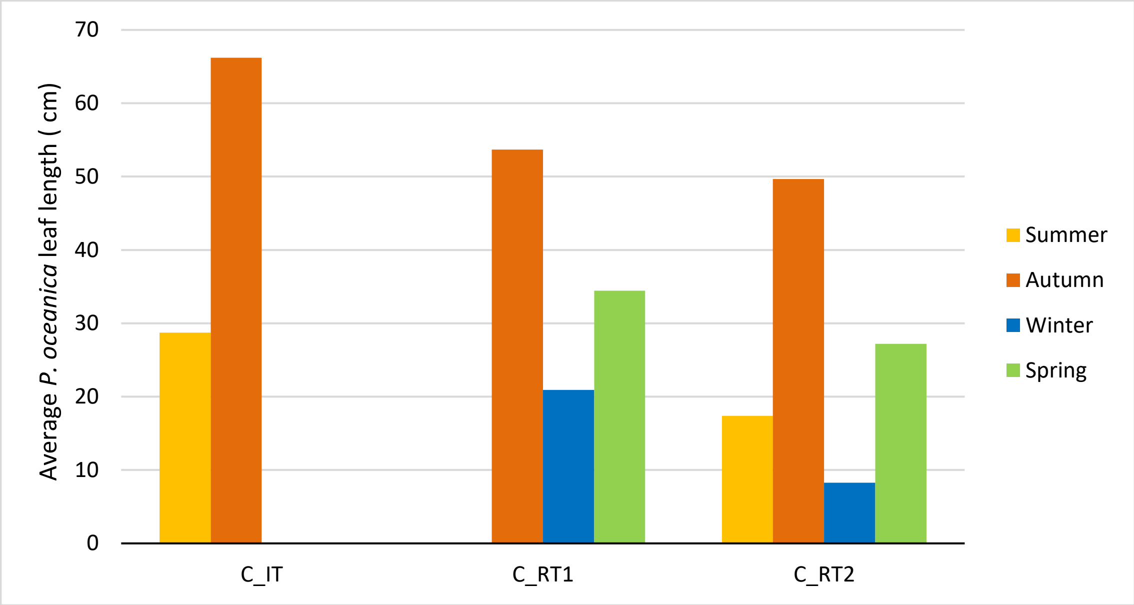


Figure 24. Average leaf length (cm) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C) across seasons.



Għar Lapsi

The survey transect depths were 7.8 m – 24.0 m, 6.0 m - 9.6 m, 7.3 m – 12.4 m (respectively IT, RT1, RT2).

The *Posidonia* average percent cover, shoot density and leaf length showed a statistical significance ($p < 0.05$) between the impact (average of 50% / quadrat, 483 shoots / m^2 and 34 cm) and the 2 reference sites (respectively average of 5% / quadrat, 35 shoots / m^2, and 3 cm and 46% / quadrat, 411 shoots / m^2 and 33 cm for RT1 and RT2), disregarding the seasonality (Figures 25, 26 and 27).

When seasonality is considered, statistical tests showed no significant differences among all 4 seasons ($p > 0.05$) for the variable averages of *Posidonia* percent cover, the shoot density and leaf length.

Nevertheless, relative values suggest that autumn showed the highest *Posidonia* average percent cover (respectively 73%, 20% and 75% for IT, RT1 and RT2) against spring (respectively 50%, 0% and 33%), summer (respectively 50%, 0% and 35%), and winter (respectively 26%, 0% and 43%) (Figure 25).

The same pattern applied to the average *Posidonia* shoot density, with autumn recording the highest number of shoots / m^2 (respectively 676, 140, 652 shoots for IT, RT1 and RT2), against spring (respectively 400, 0, and 272 shoots), summer (respectively 640, 0, and 400 shoots), and winter (respectively 216, 0, and 320 shoots) (Figure 26). Additionally, the number of visible leaves counted for each *Posidonia* shoot observation remained constant at 4 - 6 leaves per shoot.

The *Posidonia* average leaf length shows the longest during autumn (respectively 42 cm, 12 cm and 51 cm for IT, RT1 and RT2) against spring (respectively 33 cm, 0 cm and 19 cm), summer (respectively 39 cm, 0 cm, and 42 cm), and winter (respectively 20 cm, 0 cm, and 18 cm) (Figure 27).

The algae average percent cover did not show any statistical significance between the impact (42%) and the 2 reference sites, (respectively 70% and 41% for RT1 and RT2) disregarding seasonality. Similarly, when seasonality is considered, there was no statistical significance found. Regardless spring showed an average algae percent cover of (respectively 50%, 83% and 50% for IT, RT1 and RT2) like summer (respectively 50%, 85% and 43%), winter (respectively 43%, 50% and 45%), and autumn (respectively 26%, 63% and 25%) (Figure 25).

The sand average percent cover showed a statistical significance ($p < 0.001$) between the impact (3%) and the 2 reference sites, (respectively 24% and 10% for RT1 and RT2) disregarding seasonality. However, when seasonality is considered, there was no statistical significance found. Regardless winter showed an average sand percent cover of (respectively 9%, 50% and 10% for IT, RT1 and RT2) against spring (respectively 0%, 18% and 15%), summer (respectively 0%, 15% and 15%), and autumn (respectively 26%, 63% and 25%) (Figure 25).

The percent cover of both dead matte and rock did not show any statistical significance between impact and the 2 reference sites. Considering seasonality did not show any statistical significance either (Figure 25).

Figure 25. Average substrate percent cover at the impact (IT) and reference transects (RT1 and RT2) in Għar Lapsi (L) across seasons.

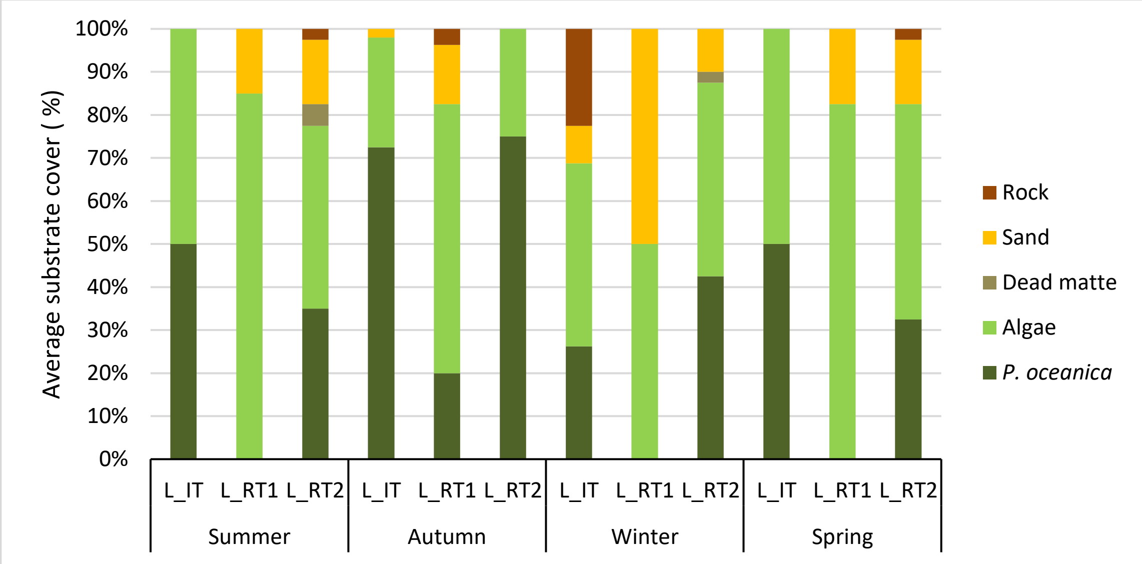


Figure 26. Average shoot density (/ m2) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Għar Lapsi (L) across seasons.

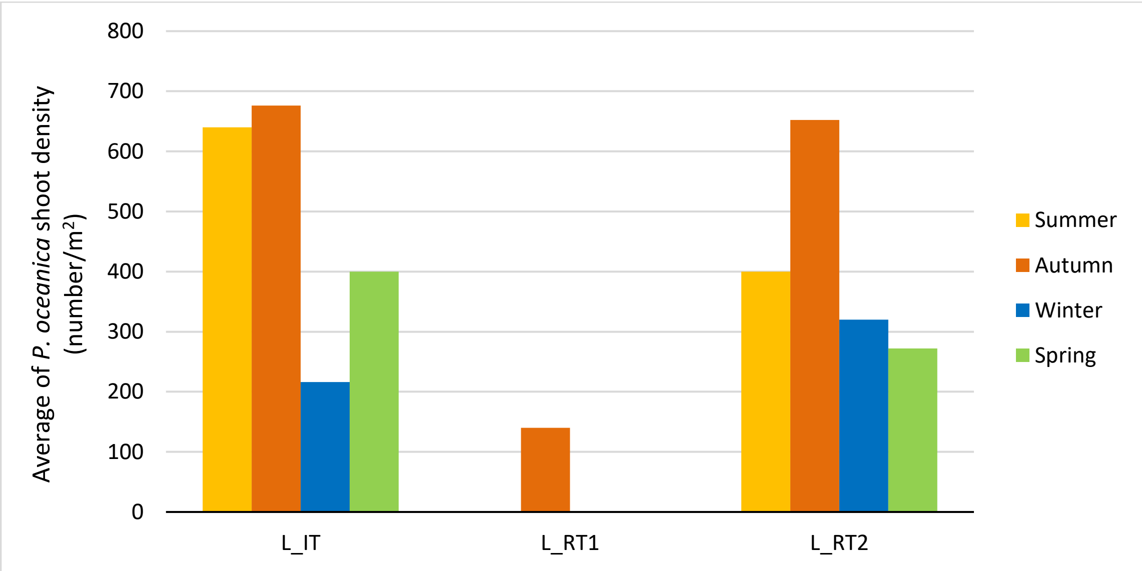
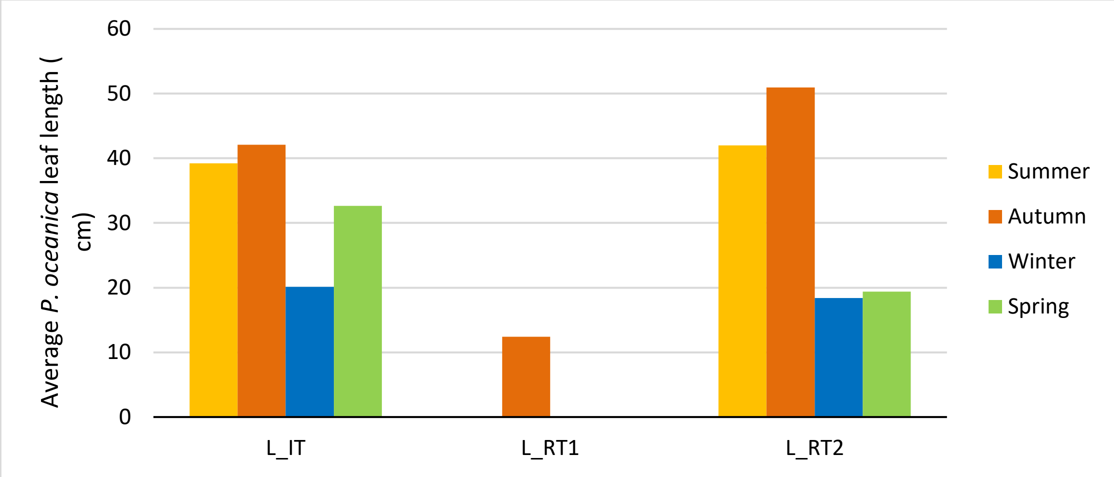


Figure 27. Average leaf length (cm) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Għar Lapsi (L) across seasons.



Pembroke

The survey transect depths were 3.7 m – 8.8 m, 7.9 m – 22.0 m, 5.5 m – 20.0 m (respectively IT, RT1, RT2).

The *Posidonia* average percent cover, shoot density and leaf length showed a statistical significance between the impact (average of 0% / quadrat, 0 shoots / m^2 and 0 cm) and the 2 reference sites (respectively average of 47% / quadrat, 392 shoots / m^2, and 26 cm and 38% / quadrat, 387 shoots / m^2 and 22 cm for RT1 and RT2), disregarding the seasonality (Figures 28, 29 and 30).

When seasonality is considered, statistical tests showed no significant differences among all 4 seasons ($p > 0.05$) for the variable averages of *Posidonia* percent cover, the shoot density and leaf length.

Nevertheless, relative values suggest that autumn showed the highest *Posidonia* average percent cover (respectively 0%, 25% and 100% for IT, RT1 and RT2) against spring (respectively 0%, 65% and 0%), summer (respectively 0%, 58% and 5%), and winter (respectively 0%, 39% and 49%) (Figure 28).

The same pattern applied to the *Posidonia* average shoot density, with autumn recording the highest number of shoots / m^2 (respectively 0, 176, 1036 shoots for IT, RT1 and RT2), against spring (respectively 0, 872, and 0 shoots), summer (respectively 0, 320, and 80 shoots), and winter (respectively 0, 200, and 432 shoots) (Figure 29). Additionally, the number of visible leaves counted for each *Posidonia* shoot observation remained constant at 4 - 6 leaves per shoot.

The *Posidonia* average leaf length shows the longest during autumn (respectively 0 cm, 12 cm and 62 cm for IT, RT1 and RT2) against spring (respectively 0 cm, 43 cm and 0 cm), summer (respectively 0 cm, 36 cm, and 11 cm), and winter (respectively 0 cm, 12 cm, and 15 cm) (Figure 30).

The algae average percent cover showed a statistical significance ($p < 0.001$) between the impact (82%) and the 2 reference sites, (respectively 24% and 59% for RT1 and RT2) disregarding seasonality. However, when seasonality is considered, there was no statistical significance found). Regardless, spring showed an average algae percent cover (respectively 94%, 13% and 100% for IT, RT1 and RT2) with summer (respectively 90%, 3% and 88%), winter (respectively 83%, 8% and 50%), and autumn (respectively 60%, 75% and 0%) (Figure 28).

The sand average percent cover showed a highly statistical significance between the impact (10%) and the 2 reference sites, (respectively 23% and 2% for RT1 and RT2) disregarding seasonality. Likewise, when seasonality is considered, there was also a statistical significance between winter and the other 3 seasons ($p < 0.001$). Winter showed the highest average algae percent cover (respectively 18%, 51% and 1% for IT, RT1 and RT2) against summer (respectively 10%, 18% and 5%), spring (respectively 6%, 23% and 0%), and autumn (respectively 6%, 0% and 0%) (Figure 28).

The percent cover of both dead matte and rock did not show any statistical significance between impact and the 2 reference sites. Considering seasonality did not show any statistical significance either (Figure 28).

Figure 28. Average substrate percent cover at the impact (IT) and reference transects (RT1 and RT2) in Pembroke (P) across seasons.

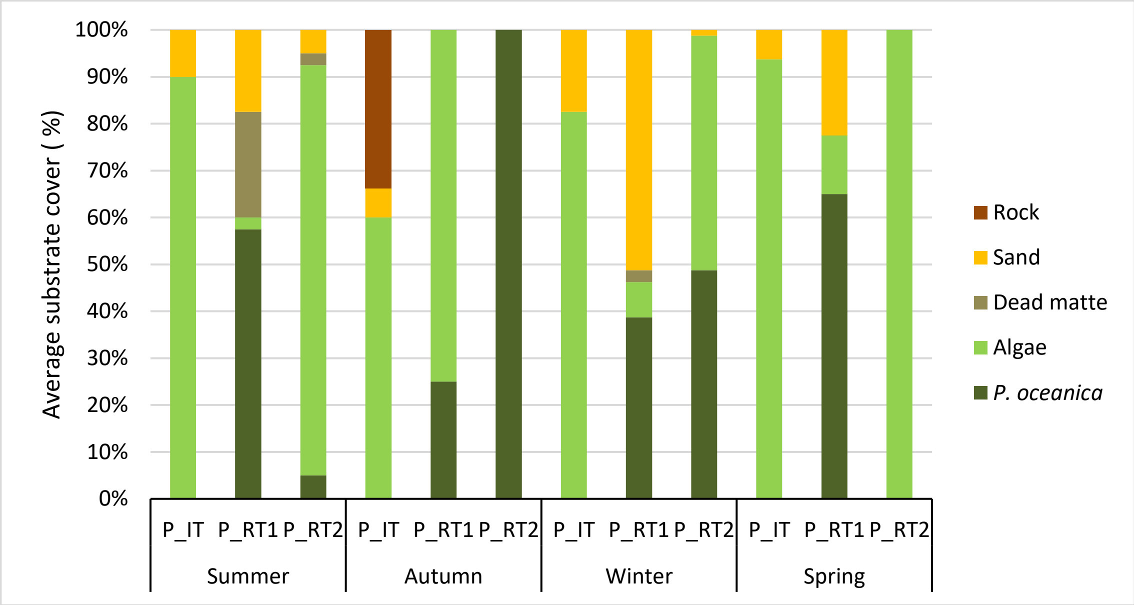


Figure 29. Average shoot density (/ m^2) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Pembroke (P) across seasons.

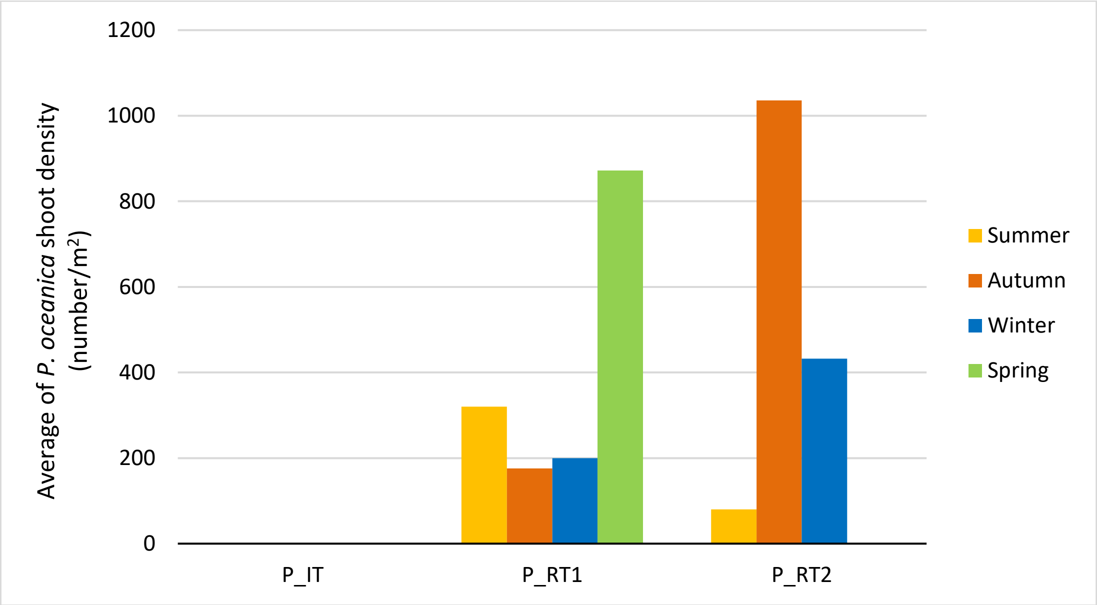
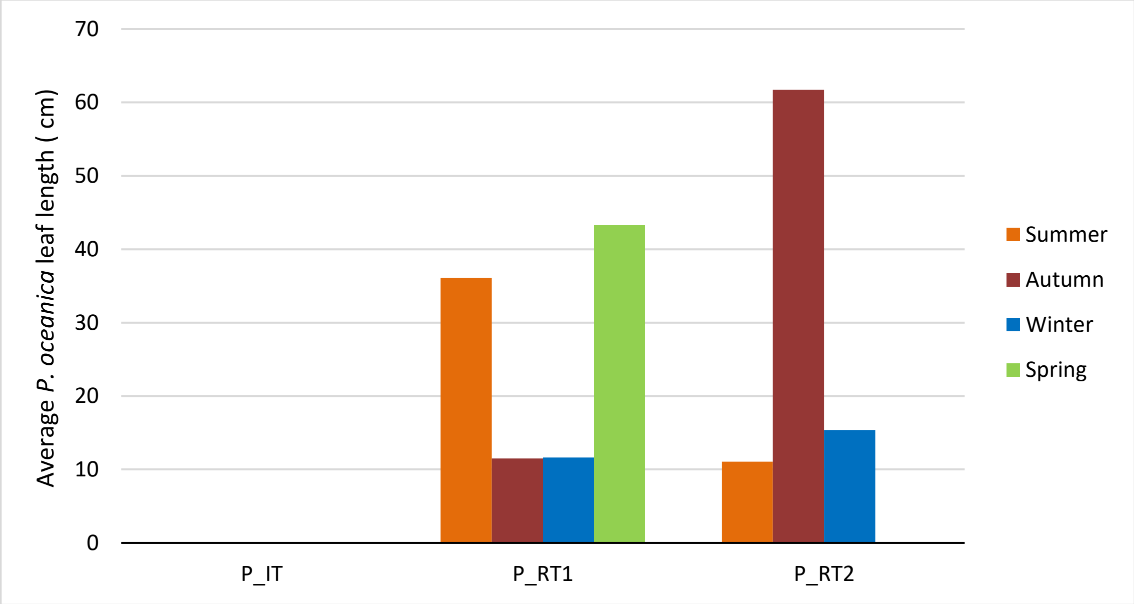


Figure 30. Average leaf length (cm) of *P. oceanica* at the impact (IT) and reference transects (RT1 and RT2) in Pembroke (P) across seasons.



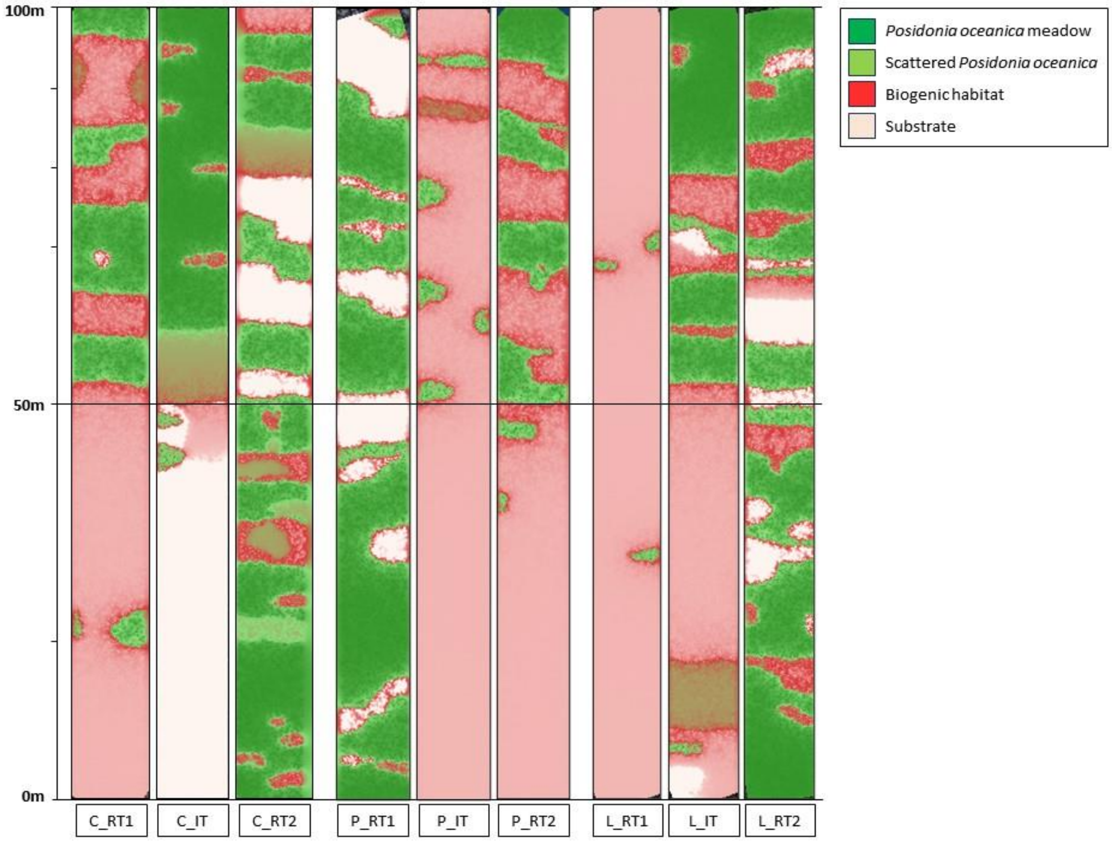
A habitat map was created to provide a visual representation of the distribution and location of different habitats within the dive transect in each of the 3 RO plants. It showed the majorly observed habitat types of the seabed as seen in Figure 31 - substrate, biogenic habitat (includes algae communities), scattered *P. oceanica*, and *P. oceanica* meadows.

At the Ċirkewwa impact transect (C_IT), the predominant habitat types identified included substrate, biogenic habitat, scattered *P. oceanica*, and *P. oceanica* meadows, accounting for approximately 40%, 15%, 10%, and 35% of the area, respectively. The initial 50 m of the transect were primarily characterised by substrate, encompassing a variety of sediment types (mud, sand) and rock. Subsequently, the area was increasingly occupied by scattered *P. oceanica*, with the remaining segment (70 m to 100 m) covered by established *P. oceanica* meadows intermixed with sporadic patches of biogenic habitat. Conversely, the reference transects exhibited similar habitats but with different proportions. For instance, transect C_RT1 consisted mainly of biogenic habitat and scattered *P. oceanica*, accounting for approximately 70% and 30%, respectively. The initial 50 m of this transect were dominated by biogenic habitat, with a smaller area of scattered *P. oceanica* at around 20 m. Moving further along the transect (55 m to 100 m), the seabed was characterised by alternating presence of scattered *P. oceanica* and biogenic habitat. Transect C_RT2, on the other hand, featured substrate, biogenic habitat, scattered *P. oceanica*, and *P. oceanica* meadows, constituting approximately 15%, 20%, 40%, and 25% of the area, respectively. The first 50 m of this transect were predominantly covered by *P. oceanica* meadows intermixed with biogenic habitat and scattered *P. oceanica*, while the remaining 50 m displayed a mix of substrate, biogenic habitat, and scattered *P. oceanica*.

At the Pembroke impact transect (P_IT), the primary habitat types identified included biogenic habitat and scattered *P. oceanica*, accounting for approximately 80% and 20% of the area, respectively. Throughout the entire transect, the predominant habitat was biogenic habitat with interspersed patches of scattered *P. oceanica* beginning at 50 m and continuing for the rest of the transect. In contrast, the reference transects exhibited similar habitats, but with a higher presence of Posidonia. For instance, transect P_RT1 was predominantly comprised of *P. oceanica* meadows (approximately 60%), interspersed with patches of substrate (25%), scattered *P. oceanica* (10%), and biogenic habitat (5%). The entire transect was characterised by *P. oceanica* meadows, with substrate becoming more prevalent in the final 50 m. Conversely, transect P_RT2 featured biogenic habitat, scattered *P. oceanica*, and *P. oceanica* meadows, constituting approximately 75%, 15%, and 10% of the area, respectively.

At the Għar Lapsi impact transect (L_IT), the observed habitat types included substrate, biogenic habitat, scattered *P. oceanica*, and *P. oceanica* meadows, accounting for approximately 5%, 40%, 35%, and 20% of the area, respectively. The initial 50 m of the transect were primarily characterised by biogenic habitat with scattered presence of *P. oceanica*, while the remainder of the transect featured a mix of scattered *P. oceanica* and biogenic habitat, with the last 30 m highlighting the presence of *P. oceanica* meadows. In comparison, the reference transects exhibited a similar habitat structure. For example, in transect L_RT1, the entire area was dominated by biogenic habitat (85%) with patches of scattered *P. oceanica* (10%) and fringes of biogenic habitat (5%). Conversely, transect L_RT2 displayed a more varied habitat composition with different habitats intermingled. The transect was dominated by *P. oceanica* meadows (60%), substrate (15%), biogenic habitat (15%), and scattered *P. oceanica* (10%). The first and last 20 m of the transect were covered with *P. oceanica* meadows, while the middle areas highlighted the presence of substrate and biogenic habitat.

Figure 31. Overall habitat map at the impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L), Pembroke (P).



Echinoderms

Echinoderm sightings showed no statistical significance between the impact and reference transects ($p > 0.05$) at all 3 RO plants. The same applied to seasonality. Moreover, observations of the identified species *Hacelia attenuata*, were only present at the reference transects of Pembroke and Għar Lapsi and none at Ċirkewwa (Table 4). The depths and locations along the dive transect varied for each sighting. A total of 4 echinoderms, located at different depths and transects, were observed at Pembroke. The sightings occurred in autumn (total of 2 in RT1 at 4 m) and winter (total of 1 at in RT1 at 13 m and, total of 1 in RT2 at 8 m). All sightings lay between 50 m and 96 m along the survey transect line. While at Għar Lapsi, a total of 2 echinoderms were observed during winter (total of 1 in RT2 at 9.7 m) and spring (total of 1 in RT1 at 9 m). All sightings lay between 86 m and 94 m along the survey transect line.

Table 4. Number of Echinoderms sighted at impact (IT) and reference transects (RT1 and RT2) in Ċirkewwa (C), Għar Lapsi (L) and Pembroke (P) across all 4 seasons.

Season	Site	Transect	Depth(m)	Replicate	Transect mark (m)	Quantity	Species
Autumn	Pembroke	P_RT1	4	Q1	50	1	*Hacelia attenuata*
Autumn	Pembroke	P_RT1	4	Q2	96	1	*Hacelia attenuata*
Winter	Għar Lapsi	L_RT2	9.7	Q1	94	1	*Hacelia attenuata*
Winter	Pembroke	P_RT1	13	Q1	86	1	*Hacelia attenuata*
Winter	Pembroke	P_RT2	8	Q1	50	1	*Hacelia attenuata*
Summer	Għar Lapsi	L_RT1	9	Q1	86	1	*Hacelia attenuata*

Comparison among the 3 RO plants

At Ċirkewwa and Għar Lapsi, there was no statistically significant difference ($p > 0.05$) observed in seasonal variability of *Posidonia* average percent cover, shoot density, and leaf length between the impact and reference transects. Conversely, Pembroke had a statistically significant difference ($p < 0.05$) in seasonal variance, where all 3 variables appeared higher in values in the reference transects compared to the impact transects.

Correlation was observed between a high *Posidonia* average percent cover and a high shoot density. The highest seasonal average percentage cover and shoot densities were recorded at reference transects in Ċirkewwa, RT2 (59%, 663 shoots / m^2) followed by Pembroke, RT1 (47%, 392 shoots / m^2) then Għar Lapsi, RT2 (46%, 411 shoots / m^2) (Figures 22, 25,28). The *Posidonia* average percent cover and shoot density at the impact transects were only recorded at Għar Lapsi, IT (50%, 483 shoots / m^2) and Ċirkewwa, IT (36%, 328 shoots / m^2) with none at Pembroke. Overall, the presence of *Posidonia* was observed across all sites with the highest combined coverage at Ċirkewwa, followed by Għar Lapsi and Pembroke (respectively 44%, 34%, 28%) (Figures 22, 25, 28).

The seasonal *Posidonia* average leaf length between impact and control transects varied per location. The highest average leaf lengths occurred at the reference sites of Għar Lapsi, RT2 then Ċirkewwa, RT1 and Pembroke, RT1 (respectively 33 cm, 27 cm, 26 cm). The lowest average leaf length was 50 cm at Għar Lapsi's RT1 (Figure 27). Then again, the average leaf length was also high at the impact transects of Għar Lapsi and then Ċirkewwa, and no presence was observed at Pembroke (respectively, 34 cm, 24 cm, and 0 cm). Għar Lapsi contained the highest lengths overall among all locations.

The algal diversity (represented by biogenic category in Figure 31) consisted of several species of various forms and sized, and included *Padina pavonica*, *Derbesia* sp., *Cystoseria* sp., *Halopteris* sp., *Sargassum vulgare* and few more unidentified species. The algae percent cover at the impact transects ranged from 40%, 42%, 82% (respectively at Ċirkewwa, Għar Lapsi and Pembroke). *Posidonia* epiphytic algae were notably observed but only among the meadows in reference sites at both Pembroke and Għar Lapsi. Overall, the presence of algae was significantly present across all sites with all impact transects exhibiting high values while one reference transect (RT2), at Ċirkewwa had the highest value (100%) overall in the surveys (Figures 22, 25, 28).

The average percentage cover of bare substrate (largely represented by sand and rock) was observed in each season. As sand, the impact sites highlighted the largest cover in winter at 24%, 3% and 10% (respectively at Ċirkewwa, Għar Lapsi and Pembroke). The average percent cover of bare rock was highest at the impact transects (IT) of Għar Lapsi and Pembroke (respectively 2.5% and 34%) (Figures 22, 25, 28)

Dead matte was present across all sites in summer and winter but notably in summer at Pembroke's reference transect (RT1) at 23%.

Overall, habitat map (Figure 31) indicates that the 3 RO plants shared similarities in terms of habitat types, featuring substrate, biogenic habitat (includes algae), scattered *P. oceanica*, and *P. oceanica* meadows at both impact and reference transects. At each RO plant's impact transects (C_IT, P_IT, L_IT), the initial 50 m of the survey transect were characterised by substrate, while the remaining 50 m exhibited substantial coverage of *P. oceanica* meadows and biogenic habitat. Notably, Ċirkewwa displayed the highest percentage cover of Posidonia, followed by Għar Lapsi and then Pembroke. Each RO plant illustrated that the predominant presence of scattered *P. oceanica* and *P. oceanica* meadows was observed at their respective reference transects (C_RT2, P_RT1, and L_RT2). In contrast, while Ċirkewwa showcased Posidonia at both its impact and reference transects, Għar Lapsi (L_RT1) was predominantly characterised by biogenic habitat, and Pembroke demonstrated significant presence of scattered *P. oceanica*.

The survey transects' depths varied across each RO plant with Ċirkewwa (2.5m, IT) having the shallowest depths and Għar Lapsi (24.0 m, IT) with the deepest while those at Pembroke ranged between 3.7 m to 22.0 m.

Additionally, Sightings of echinoderms did not show any seasonal statistical significance between the impact and reference transects at any of the 3 RO plants. The highest number (4) was observed at Pembroke during autumn and winter within its reference transects, compared to 2 sightings in winter and summer at Għar Lapsi's reference transects, while Ċirkewwa logged none (Table 4).

General observations

During the survey activities, large schools of fish, potentially endemic to Malta, were observed in the immediate vicinity of the brine discharge outfall at all three RO plants. This area was also encompassed by the halocline formed by the mixing of brine and seawater. Supplementary underwater images as points of interest (POI) were included in the submission of data files for reference.

6. Discussion

Although informative data has been provided, evaluating the environmental impacts of brine discharge was a complex and challenging task. The study has limitations because it lacks baseline data for making comparisons. As a result, the data collected only reflects the marine conditions observed during the 4 seasons surveyed over a span of 2 years.

The surveys illustrated that it was more feasible to establish seasonality relationships through the collection of water samples for chemical analysis, the measurement of physical water parameters as well as conducting benthic dive surveys. However, despite majority of the locations showing no statistical significance when comparison of impact transects against reference transects was made, notable visual differences have been discussed to infer localised potential impacts of brine discharge.

6.1 Physical Water Parameters

The information given on the distribution of brine at the Ċirkewwa, Għar Lapsi, and Pembroke RO plants, utilising conductivity measurements as a critical physical parameter, enables us to assess potential consequences on the development and reproductive processes of *Posidonia oceanica* (seagrass) and echinoderms. This evaluation spans both impact and reference stations, considering various water column depths throughout different seasons. Discussion of these results considers the knowledge gap for what can be defined as the normal range of seawater physical parameters in Malta.

Conductivity can indicate changes in the concentration of dissolved solids, such as salts and nutrients, which can affect the overall health and balance of marine ecosystems, while salinity can influence the distribution of marine organisms, and the behaviour of nutrients and gases in the water column. Following a linkage, higher conductivity leads to higher salinity. Furthermore, dissolved oxygen is associated with indicating good water quality, temperature can heavily influence water characteristics including chemical reactions, while pH plays a key role in creating a functional environment for marine life metabolism and which can also determine the solubility of chemicals in the water including oxygen.

At Ċirkewwa, the highest surface conductivity levels (56,000 µS/ cm) were recorded in autumn, and this could potentially stress *Posidonia* during a critical growth period, impacting its health and reproduction. The depth-related patterns suggest that near the discharge source, conductivity increased with depth. This depth-related increase may affect echinoderms, influencing their behaviour and larval development. Additionally, spatial trends show that average conductivity values further away from the impact point were lower. This spatial variability could result in uneven distribution and health of *Posidonia* and echinoderm populations.

Seasonal variability at Għar Lapsi, showed that here summer recorded the highest surface conductivity levels (56,000 µS/cm). Elevated conductivity during this season might pose challenges for *Posidonia* and echinoderms, affecting their growth and reproduction. The depth-related changes are similar to Ċirkewwa, where conductivity near the discharge source also increased with depth. Not only can this affect *Posidonia* community structure health, but echinoderms at different depths may experience varying levels of stress, potentially impacting their life cycles. Conductivity levels during seasons with higher values may influence the larval development of echinoderms, with potential repercussions for the population dynamics thus potentially explaining the few numbers observed during the surveys.

Furthermore, at Pembroke, both summer and spring recorded the highest surface conductivity levels (56,000 µS/ cm). This consistent pattern may indicate persistent stress on *Posidonia* and echinoderms during critical periods. The depth-related trends here also indicate that conductivity increased with depth near the discharge source, similar to the other RO plants. This depth-related impact may have implications for the spatial distribution of marine organisms on the seabed. Additionally, spatial consistency illustrates that average conductivity values further away from the impact point, those in the area north-west (looking out to sea), were relatively similar to those at the discharge source. This spatial consistency suggests a potential influence on the distribution and health of *Posidonia* and echinoderms across a broader area.

Moreover, Għar Lapsi exhibited the greatest depth among the 3 RO plants, reaching a depth of 93.2 m, followed by 40 m for Pembroke and 15 m for Ċirkewwa. This substantial difference in depth likely influenced the dispersion and concentration of conductivity. The increased depth at Għar Lapsi provided more space for the dispersion of conductivity down the water column, resulting in decreased salinity levels at middle and surface layers.

Pembroke and Għar Lapsi demonstrated more consistent ranges of conductivity across the middle and deep layers. In contrast, Ċirkewwa displayed a similar pattern of fluctuations throughout the entire water column. One significant factor influencing this distribution could be the limited survey depth of Ċirkewwa, reaching only up to 15 m. This restricted depth range leaves less room for the dispersion of salinity compared to the other 2 plants.

It is crucial to acknowledge that various factors, including bathymetry, depth, weather conditions, and subsurface currents, play pivotal roles in determining the distribution of physical parameters. These factors also influence the positioning of bioindicator species, contributing to the overall understanding of the complex interactions within the marine environment.

The variability in conductivity levels at the RO plants may have significant implications for the seabed ecosystem. Fluctuations in the populations of Posidonia oceanica and echinoderms could potentially impact the overall biodiversity and ecological dynamics of the area. Consequently, ongoing monitoring of conductivity levels and their ecological effects is imperative, particularly considering the sensitivity of Posidonia and echinoderms to such variations.

It is noteworthy that Posidonia demonstrates a marked tolerance to salinity, enabling it to withstand fluctuations between 38 - 51 PSU. The upper tolerance threshold of 51 PSU corresponds to a conductivity of approximately 73,000 µS/cm. Notably, at one specific location (Pembroke, Autumn), conductivity levels exceeding 73,000 µS/cm were recorded, ranging from above 70,000 µS/cm to 100,000 µS/cm in the surface water column within the first meter, as indicated by the raw multiparameter sonde data from water sampling station P7, coordinates 35.938273, 14.483053 (Table 2). It is crucial to emphasise that the detected elevated conductivity levels were not in close proximity to the discharge/impact point, which was approximately 0.5 km away. Consequently, it can be inferred that the observed conductivity levels are within the range of conditions conducive to Posidonia growth a tall 3 RO plants.

In addition, the absence of P. oceanica near the impact point is likely attributable to the naturally occurring substrate type (primarily biogenic habitat) observed during dive surveys, rather than the brine discharge or conductivity levels, as biogenic habitat does not support root system development as sandy platforms do. Nevertheless, continued monitoring of brine dispersion around Pembroke, particularly in light of the heightened conductivity levels observed in Autumn, remains essential.

Furthermore, it is essential to note that the extreme conductivity values were not included in the final conductivity dispersion graphs due to their intermittent nature and concentration at a few sampling stations, which could have skewed the overall dataset. These values were instead made available in the raw data files and summarised in Table 2.

Echinoderms, in contrast, have a lower tolerance level than Posidonia, typically withstanding marine conductivity ranges of approximately 40,000 to 45,000 µS/cm. The recorded conductivity levels slightly exceeded this spectrum at both the impact and reference areas of all the RO plants, indicating that both natural and brine-induced conductivity levels had an influence on the presence of echinoderms in the survey area.

However, this potential influence from brine discharge is supported by the absence of recorded echinoderms near the impact area, with only a limited number observed in the reference transects of Pembroke and Għar Lapsi.

To safeguard both Posidonia and echinoderms, it is essential to adopt effective management practices that reduce the impact of brine discharge. Regular environmental assessments play a crucial role in this effort. Additionally, it’s important to recognize that if the conductivity surpasses a critical threshold, it may jeopardise the health of Posidonia and hinder echinoderm reproduction.

In conclusion, the conductivity variations in brine discharge near *Posidonia oceanica* and echinoderms have the potential to impact their growth, reproduction, and overall health. Sustainable practices, informed by ongoing monitoring and research, are necessary to preserve the ecological balance of the seabed ecosystem near each RO plant discharge sources.

Turbidity

Turbidity is caused by suspended or dissolved particles in water that scatter light making water appear cloudy. Particulate matter can include sediment, fine inorganic and organic matter and soluble organic compounds. The measure of water clarity offers insight into its impact on the marine environment across the 3 RO plants.

At Ċirkewwa the descending order of turbidity values (9 m, 5 m, and 4 m) at reference points (C_RT1 and C_RT2) and the impact point (C_IT) suggests a gradient of decreasing turbidity away from the impact point. Lower turbidity values at the impact point imply clearer water in the vicinity, indicating that brine discharge may have had a relatively limited impact on water clarity at the seabed near *Posidonia* and echinoderms.

Whereas at Għar Lapsi, turbidity values (9 m, 8 m, and 6 m) at impact (L_IT) and reference transects (L_RT2 and L_RT1) in Għar Lapsi indicate a similar trend. The descending order suggests that the impact point has the lowest turbidity, potentially indicating a lesser impact of brine discharge on water clarity. This plant also had the most dynamic sea conditions which could have attributed to stirring of bottom sediment at especially the reference points.

Finally at Pembroke, turbidity values (8 m, 5 m, and 5 m) also follow a similar pattern. The impact point (P_IT) has the lowest turbidity, suggesting that brine discharge may have a relatively minor impact on water clarity at the seabed near *Posidonia* and echinoderms. Clearer water conditions are generally favourable for the growth and well-being of marine species.

There are potential impacts on marine environment under observed turbidity values. However, the rather lower turbidity at impact points across all RO plants suggests that the brine discharge may not be significantly affecting the water clarity around *Posidonia.* Clearer water conditions are generally favourable for the growth and health of seagrasses. Therefore, the lack of significant meadows at the impact locations is due to other environmental factors. Moreover, echinoderms, inhabiting the seabed, may benefit from lower turbidity as it enhances visibility. Clearer water conditions support various ecological processes, including feeding and reproduction, for these marine invertebrates.

Overall, the sequential ordering of turbidity values suggests a pattern of increasing turbidity moving away from the impact points. The clearer water conditions at the impact points may indicate a localised and relatively limited influence of brine discharge on water clarity. Moreover, depth increases as you move further away from the impact point thus making water less clear through the water column. However, it is important to note that general observations revealed the presence of haloclines at each impact point of the 3 RO plants, extending to approximately 60 cm from the discharge point. The absence or scarcity of Posidonia and echinoderms in this vicinity therefore appears to be influenced by a combination of brine discharge’s chemical and physical parameters (above the threshold favourable for echinoderms), depth and substrate type (results showed biogenic habitat dominating this area which is not ideal for Posidonia growth)

In summary, the turbidity results indicate that brine discharge may have a localised and relatively minor impact on water clarity near the designated impact points in the vicinity of Posidonia and echinoderms. Continuous monitoring of these trends remains essential to ensure the ongoing health of the marine ecosystem, taking into consideration the differences in bathymetry and hydrodynamic sea conditions at each plant.

6.2 Chemical Water Parameters

For the purposes of the final report, due to the large data set acquired across the 2 year surveys, i) the chemical parameters values of the shallow (s) and deep (d) were averaged in order discern concentrations at each sampling station ii) there was no statistically significant difference in concentration between the depth even though it is important to note that concentration did increase with depth by a minimal amount (mg/l) as can be seen in the laboratory analysis reports.

The lab analysis results illustrated that chemical concentrations are higher at the surface when compared to results obtained from depth, however this difference appeared to be minimal and thus seasonality and proximity to the discharge source provided more interpretation of the results. Discussion of these results considers the knowledge gap in what can be defined as the normal range of chemical parameters in Malta.

Boron

High levels of boron can be toxic to marine plants and animals, potentially leading to growth and reproductive issues. The data shows the highest concentrations in autumn, particularly at Ċirkewwa. Such elevated levels could adversely affect *Posidonia oceanica*, which is sensitive to environmental changes, and potentially disrupt echinoderm larval development.

Calcium

Calcium is vital for marine life, especially for organisms that build calcium carbonate structures like echinoderm skeletons. Fluctuations in calcium, as seen in the data, could impact these processes. For example, the high concentrations in summer at Ċirkewwa could benefit calcifying organisms, but the lower levels in autumn might challenge them.

Magnesium

Magnesium works in concert with calcium and is crucial for many biological functions in marine life. The highest concentrations were seen in autumn at Għar Lapsi and Pembroke, which might influence the osmotic balance and other physiological functions in marine organisms.

Potassium

Potassium is necessary for cell function in marine organisms. However, high levels, like those observed in autumn at Għar Lapsi and Pembroke, could disrupt ionic balance and affect sensitive species. The low concentration values on the other hand can limit their growth and productivity, affecting the overall health of the marine ecosystem.

Sodium

Sodium is a major ion in seawater and is critical for osmoregulation in marine organisms. The variations observed, especially the high concentrations in autumn, could impact marine life if the changes are abrupt or outside the normal range they can tolerate.

Sulphates

Sulphates are generally not toxic and are a part of the natural ocean chemistry. The data indicates relatively stable sulphate concentrations, which is unlikely to have significant negative impacts on marine environments.

Chlorides

Like sodium, chlorides are a major component of seawater and are essential for osmoregulation. The concentrations were fairly consistent, suggesting minimal impact from the RO plants' discharge.

Nitrates

Nitrates were below the detection limit, indicating low levels that are unlikely to cause eutrophication, which could otherwise lead to harmful algal blooms affecting the entire marine ecosystem.

Examples how these concentrations directly apply to each RO Plants are as follows:

- Ċirkewwa: Higher boron concentrations in autumn could stress local ecosystems during this season. However, the calcium and magnesium concentrations were within a beneficial range for calcifying organisms in summer. It is also worth noting that compared to other plants, Ċirkewwa exhibited relatively low potassium levels. Several potential factors could have contributed to this observation, including high dilution and mixing of brine discharge during sampling, potentially exacerbated by dynamic sea conditions such as currents. Additionally, the high biological uptake of potassium by aquatic organisms as part of their metabolic processes may have resulted in lower potassium levels in the water. Moreover, high absorption rates by surrounding sediment could have played a role in the observed low potassium levels.

- Għar Lapsi: The higher autumn potassium levels could pose a risk to marine life, while the stable sulphate and chloride levels suggest a minimal impact on the marine ecosystem's osmotic balance.

- Pembroke: The lowest sodium concentrations in summer compared to the other stations suggest a lesser impact on osmoregulation during this season.

Impact vs Reference Points

Overall, at Ċirkewwa and Pembroke, impact points tended to have higher average concentrations of boron, suggesting a localised effect of brine discharge. However, Għar Lapsi showed higher concentrations at reference points for some parameters, indicating the influence of other environmental factors.

An example of seasonal differences in certain chemical water composition at the impact points (IT) for all 3 RO plants can be summarised as follows:

- Pembroke had the highest average concentrations for:
    - Boron (3.45 mg/l) in autumn
    - Chlorides (23,000.0 mg/l) in winter
    - Sulphate (2,988.0 mg/l) in autumn
- Għar Lapsi had the highest average concentrations for:
    - Boron (4.65 mg/l) in autumn
    - Chlorides (22,500.0 mg/l) in spring
    - Sulphate (3,008.5 mg/l) in winter
- Ċirkewwa had the highest average concentrations for:
    - Boron (5.40 mg/l) in autumn
    - Chlorides (23,843.5 mg/l) similar across all seasons
    - Sulphate (2,942.0 mg/l) similar across all seasons

The complex interactions between the reverse osmosis plant, the marine environment, and seasonal variations makes it challenging to determine the factors contributing to the elevated boron, chloride, and sulphate levels. The high concentrations observed above could therefore, be due to reasons encompassing both natural and anthropogenic factors – besides inferring brine discharge as the primary cause.

Elevated boron levels observed during autumn may be attributed to multiple factors. Seasonal variations in water chemistry, encompassing temperature fluctuations, precipitation, and shifts in dissolved oxygen levels, are likely to have exerted a substantial influence on the solubility and concentration of boron in the seawater adjacent to the plant.

Furthermore, environmental conditions, such as heightened biological activity and alterations in water flow patterns, may have played a role in shaping the distribution and concentration of boron in the sampled areas. It is noteworthy that autumn marked a period characterised by dynamic water conditions, including strong currents and turbulent waves, potentially contributing to the observed boron levels. These conditions were prevalent at Ċirkewwa, where additionally high recreational and commercial boat activities (Ferry terminal and channel) had the potential to influence the composition of the surrounding seawater's chemical parameters.

Moreover, operational parameters, maintenance schedules, and potential fluctuations in the volume or composition of brine discharge from the reverse osmosis plant during autumn could have contributed to the variations in boron levels in the discharged water.

Similarly, the possible explanations for high chloride levels in winter, could have potentially been due to seasonal weather conditions where weather patterns, such as reduced rainfall and increased evaporation, can lead to higher chloride concentrations in the seawater due to the concentration of salts in the marine environment during this season – this was potentially the case during spring values at Għar Lapsi. During winter, as was the case in Pembroke, specific hydrodynamic conditions, such as water mixing processes, tidal patterns, and currents, could impact the dispersion and dilution of the discharged brine, potentially leading to elevated chloride levels in the vicinity of the discharge area.

Additionally, variations in the volume and concentration of brine discharge from the reverse osmosis plants during winter may have contributed to higher chloride levels in the surrounding waters. Factors such as increased brine production or changes in the salinity of the discharged brine could have potentially influenced chloride concentrations although this seems negligeable, especially at Ċirkewwa where the values remained constant throughout all 4 seasons.

The high concentration of sulphate in the results could be attributed to various factors. Firstly, naturally occurring biological processes, such as the decomposition of organic matter, can lead to the release of sulphates into the water. Additionally, natural geological formations and processes, such as the weathering of rocks and minerals rich in sulphur, can introduce sulphates into the seawater. At the brine discharge source in both Pembroke and Għar Lapsi, prevalent hard seabed structure and obvious rocky shores were observed. Moreover, the release of concentrated brine from the reverse osmosis plant at the time of sampling activities could have led to elevated levels of sulphates in the immediate vicinity due to the high concentration of salts in the discharged brine.

In conclusion, all 3 RO plants' brine discharge showed variations in chemical parameters across seasons, with some concentrations falling within similar ranges between plants as well as, individually, between the impact and reference points, as seen by the PCA analysis. The distribution of survey points for each plant within the seasonal ellipses indicated how the chemical parameters vary not just seasonally but also by location. Where points for a specific plant were clustered tightly within a season such as at Pembroke and Għar Lapsi in autumn, this suggested less variation in the chemical parameters for the plants during that season. Regardless, these observed fluctuation in chemical concentrations at each survey point can potentially affect marine organisms, particularly those sensitive to changes in water chemistry like *Posidonia oceanica* and echinoderms. Continuous monitoring and management strategies are essential to mitigate potential adverse effects on these important marine ecosystems.

6.3 Dive Transect Survey

Ċirkewwa

The results of the study on *Posidonia oceanica* showed that there were no statistically significant differences in the percent cover, shoot density, and leaf length between the impact site and the 2 reference sites. This suggested that the impact site did not experience any significant negative effects from brine discharge compared to the reference sites.

However, when seasonality was taken into account, significant differences were observed. Autumn was found to have the highest average percent cover, shoot density, and leaf length. This indicated that autumn was a favourable season for the growth and development of *Posidonia*. The presence of Posidonia was also observed to occur in the deeper areas of the survey transect compared to the shallow waters suggesting that deep water conditions are more stable and suitable environment for *Posidonia* with calm waters preventing continuous disturbance, suitable substrate to anchor in and cooler temperatures suitable for growth and reproduction. The shallow areas potentially had environmental stressors for

Posidonia growth both influenced by brine discharge components and seasonal variability in water conditions.

In contrast, spring showed a percent cover of 0% for both the impact site and the reference sites. This could have been due to various factors such as unfavourable environmental conditions or natural fluctuations in the growth pattern of *Posidonia* during this season.

Furthermore, the average shoot density was highest in autumn, with 832 shoots / m^2 for the impact site and 1,012 shoots / m^2 for reference site 2. This indicated that autumn was a period of increased shoot production for *Posidonia* with the area offering ample nutrients to support strong root growth and a suitable substrate for anchoring.

Similarly, the longest average leaf length was recorded during autumn, with 66 cm for the impact site and 54 cm for reference site, RT1. This suggested that autumn provided optimal conditions for leaf growth in *Posidonia* with the area offering calm water to protect leaf structure and nutrients to sustain growth.

Algae was a prevalent substrate in the area, being found in both impact and reference transects. The average percent cover at the impact location (55%) was consistent throughout the 4 seasons compared to both reference locations (RT1 and RT2) (45%). The slight difference suggested that that both types of areas offered ideal conditions for growth, including warm water temperatures, optimal salinity and pH levels, and ample sunlight in shallow areas to support photosynthesis. Moreover, during the summer, algae showed the highest overall percent cover at both impact and reference locations, where all the mentioned environmental conditions are optimal. These optimal conditions can potentially be attributed to high adaptability to brine discharge and or seasonal conditions exacerbated by brine.

Sand, referred to as bare substrate, was the third most prevalent substrate being also found in both impact and reference transects. Highly observed in winter, this season is concurrent with high water movement hence exposing the seabed creating little opportunity for marine organisms to securely attach to substrate. Concurrently, the largest sand cover was observed in the impact transect (56%) during spring so as well as hydrodynamic conditions occurring at the tail end of winter that exposing the seabed, it is also likely a naturally occurring feature of the location.

Although dead matte was present in small proportion, it was observed during both summer (3% in RT2) and winter (1% in all transects). Summer is influenced by higher temperatures, resulting in reduced oxygen levels, which contribute to the decomposition of marine life. Additionally, turbulent waters in winter can lead to the dislodging of marine substrates, further contributing to the presence of dead matte.

Furthermore, echinoderms were not observed at the site. They are osmoconformer organisms and are highly sensitive to brine discharges from desalination plants. Brine discharges contain high concentrations of salts and other chemicals that can disrupt the osmotic balance of echinoderms, leading to their disappearance from affected areas. The potential reasons could be applied to the impact transect area while at reference transects, the physical conditions such as bathymetry and hydrodynamics – including unfavourable water depth, strong currents, could be attributing factors.

Overall, these results highlighted the importance of considering seasonality when studying the ecological characteristics of *Posidonia oceanica*. The findings suggested that autumn is a critical period for the growth and development of this seagrass species. Further research is needed to understand the underlying factors responsible for the observed seasonal differences and their implications for the long-term sustainability of *Posidonia oceanica* population dynamics. Similarly, for echinoderms, understanding the specific factors contributing to their absence needs further research to understand their pattern distribution.

Għar Lapsi

The study found statistically significant differences in *Posidonia* percent cover, shoot density, and leaf length between the impact site and the 2 reference sites hence inferring that brine discharge had an impact on its status. The study also showed that growth and development of *Posidonia* is influenced by seasonality. Statistically significant differences were observed, with autumn showing the highest average percent cover, shoot density, and leaf length. This implied that autumn provides the most favourable growth conditions. *Posidonia oceanica*, as a seagrass species, is an important ecosystem engineer that provides essential ecological services such as carbon sequestration, nutrient cycling, and habitat provision for a wide range of marine organisms. The aforementioned implies the extent to which brine discharge has influenced *Posidonia* growth in the location but this does not go without mentioning the bathymetry at Għar Lapsi. Depth here was non uniform, drastically changing between a shallow (7.8m) and deep (24.0m) seabed and this factor also heavily influences *Posidonia* growth – deep-water plants store more carbon which enables a higher growth potential. A suitable amount of light availability and nutrients is likely a characteristic of the supporting.

Autumn had a percent cover of 100% for the impact site and reference site 2, while spring had 0%. The average shoot density was highest in autumn, and the longest average leaf length was recorded during autumn. These results suggest that autumn is a critical period for the growth and development of *Posidonia*.

Algae ranked as the second most prevalent substrate in the area, being found in both impact and reference transects. The average percent cover at the impact location (42%) compared to both reference locations (70% and 42%, respectively at RT1 and RT2) highlights a difference that suggested reference locations (significantly RT1) offered more ideal conditions for growth, including warm water temperatures, optimal salinity and pH levels, and ample sunlight in shallow areas to support photosynthesis. Moreover, summer and spring, showed the highest overall percent cover at both impact (around 50%) compared to reference locations (around 83% and 43%, respectively at RT1 and RT2), where all the mentioned environmental conditions were potentially optimal for algae growth.

Sand was the third most prevalent substrate largely observed in reference transects compared to the impact. Highly observed in winter, this season is concurrent with high water movement hence exposing the seabed creating little opportunity for marine organisms to securely attach to substrate. Concurrently, the largest sand cover was observed in reference transect RT1 (50%) during winter so as well as hydrodynamic conditions exposing the seabed, sand is likely a naturally occurring feature of the location.

Rock another bare substrate was largely observed in the impact transect (IT) during winter (23%) and this can be attributed to both hydrodynamic conditions exposing its surface and natural occurrence of the seabed.

Although dead matte was present in small proportion, it was observed during both summer (5%) and winter (3%) both times at reference transect, RT2. The summer season is influenced by higher temperatures, resulting in reduced oxygen levels, which contribute to the decomposition of marine life. Additionally, turbulent waters in winter can lead to the dislodging of marine substrates, further contributing to the presence of dead matte.

Furthermore, a small number of echinoderms, *Hacelia attenuata,* were observed at the site even though no statistical significance was found when considering both the elements of impact against reference transects and seasonality. The sightings were only recorded in winter and summer thus suggesting these seasons and position situated, provided the most favourable growth conditions – good water quality; suitable temperatures for metabolic processes; stable salinity, adequate nutrients to support growth and reproduction; favourable substrate such as sandy or rocky for attachment and burrowing.

Overall, further research is needed to understand the underlying factors responsible for the observed seasonal differences and their implications for the long-term sustainability of *Posidonia oceanica* populations. This information is critical for the development of effective conservation and management strategies for this important seagrass species. Overall, the study highlights the importance of considering seasonality in understanding the ecology and dynamics of *Posidonia*. Additionally, the extended research is needed to determine the factors influencing the distribution of echinoderms.

Pembroke

In trying to analyse the dynamics of *Posidonia oceanica*, because of effects of brine discharge, the studies examined the percent cover, shoot density, and leaf length of the seagrass at an impact site and 2 reference sites. The findings revealed significant differences in these parameters between the impact site and the reference sites, indicating the potential effects of external factors on the seagrass population.

However, the study went further to investigate the influence of seasonality on the observed differences. It was discovered that there were significant seasonal variations in the percent cover, shoot density, and leaf length of *Posidonia oceanica*. Autumn emerged as the season with the highest average values for these parameters. The impact site and reference site 2 both exhibited a percent cover of 100% in autumn, while spring recorded 0%.

Moreover, autumn also showed the highest average shoot density and the longest average leaf length. These findings suggest that autumn plays a crucial role in the growth and development of *Posidonia oceanica*. Understanding the specific factors that contribute to this seasonal variation is essential for the long-term sustainability of seagrass populations.

Algae ranked as the most prevalent substrate in the area, being found in both impact and reference transects. The average percent cover at the impact location (82%) compared to both reference locations (24% and 59%, respectively at RT1 and RT2) highlights a difference that suggested the impact location created more ideal conditions for growth, including warm water temperatures, optimal salinity and pH levels, and ample sunlight in shallow areas to support photosynthesis. Moreover, summer and spring, showed the highest overall percent cover at both impact (max 94%) compared to reference location (max 100% at RT2), where all the mentioned environmental conditions were potentially optimal for algae growth. Reference transect RT1 experience a high algae percent cover (75%) during autumn potentially due to localised optimal growth conditions during this period.

Sand was the third most prevalent substrate largely observed in reference transects compared to the impact. Highly observed in winter (18% and 51%, respectively at IT and RT1) this season is concurrent with high water movement hence exposing the seabed which creates little opportunity for marine organisms to securely attach to substrate.

Rock another bare substrate was only observed in the impact transect (IT) during winter (34%). This can potentially be attributed to both hydrodynamic conditions exposing its surface and natural occurrence of the seabed.

Although dead matte was present in small proportion, it was only significant during summer (23%) reference transect, RT1. The summer season is influenced by higher temperatures, resulting in reduced oxygen levels, which contribute to the decomposition of marine life. Localised seabed conditions potentially don't allow dead matte to settle and collect on the seabed and thus not observed elsewhere.

In conclusion, this study emphasised the significance of considering seasonality in studying the ecology and dynamics of *Posidonia*. It is important to note that this study has limitations. First, the research was limited to surveying each season therefore, the findings may not account for climate change variability. Additionally, the study eventually primarily focused on *Posidonia oceanica* because echinoderms numbers did not provide for comprehensive analysis due to natural lack of sufficient observations. Further research is needed to gain a thorough understanding of indicator species and dynamics under the long-term exposure to brine discharge. Nevertheless, the individualised results highlighted the critical role of autumn in the growth and development of *Posidonia oceanica*, particularly. The presence of algae was a significant substrate in summer, comprising of common species to the Maltese islands. Its presence potentially explains opportunistic take over where seagrass is less competitive and environmental conditions such as water chemistry and physical parameters favour algae growth instead. The presence of epiphytic algae on Posidonia could further attest to suitable water conditions for algae. Yet, more research is required to unravel the underlying factors behind these seasonal differences and their implications for conservation and management strategies. Ultimately, this knowledge will contribute to the effective protection and sustainability of seagrass populations.

Comparison among the 3 RO plants

Results from the dive survey indicated a lack of *P. oceanica* in the impact transects of Ċirkewwa and Pembroke, whereas Għar Lapsi reported a higher *Posidonia* percent and shoot density when compared to the only reference transect where *Posidonia* was recorded (RT2). The impact transect (IT) at Għar Lapsi is situated in an area characterized by greater depths. In fact, approximately 30 m away from the discharge point and the start of the survey transect, there is a notable depth change from 7 m to 15 m, resulting in a sudden steep drop in the seabed bathymetry. This change in bathymetry could potentially create favourable conditions for the growth of *Posidonia* meadows. The wider vertical area allows for the dispersion of saline discharge, coupled with exposure to faster flowing water and mixing with deeper water. This can facilitate the distribution of excess surface salinity by the deeper water, thereby reducing negative impacts on *Posidonia* growth. In contrast, the impact transects at Ċirkewwa, and Pembroke are located in relatively shallow sites with a consistent depth range of 4 m to 8 m. This shallow environment is more likely to contain a higher salinity, which can create a restricted environment where *Posidonia* growth is inhibited. *Posidonia* generally prefers conditions with lower salinity and cooler temperatures, and the presence of high saline conditions in these shallow sites may hinder its growth. The physical water parameter results show that salinity and temperature values were substantial at these locations ca 37.5 PSU, which is within the mortality range of *Posidonia* meadows. Then again, these salinity temperatures are within the average values observed in the Mediterranean region (35.0 PSU - 41.0 PSU).

A noticeable trend was observed among increased leaf length of *Posidonia*, higher percentage of coverage, and greater shoot density across the sites. Generally large areas within *Posidonia* meadows were characterized by a high shoot density per / m^2, occurring in autumn and to some extent summer, across the sites. Għar Lapsi and Ċirkewwa were the only sites with higher *Posidonia* presence along the impact transect, although growing patchy with epiphytes. And here too, the high percentage cover of seagrass corresponded to its high shoot density. Both these locations include the deepest area surveyed where ambient and bathymetric conditions supporting seagrass growth. Furthermore, dead matte was mainly observed during summer at Pembroke and Għar Lapsi, an observation which could be potentially concurrent with shedding after winter and calm conditions preventing wash away of the debris.

Despite their apparent density, the observed meadows of *Posidonia* exhibited sporadic clustering on the seabed. Most of the *Posidonia* was recorded at Ċirkewwa percent cover followed by Għar Lapsi then Pembroke. Visual observation of the meadows also displayed discolouration in their leaf blades and had some growth of epiphytic macroalgae (*Cladophora* spp*.,* and *Hydrolithon* sp.). This was most visible in summer and autumn. Furthermore, although there were sporadic patches of *Posidonia* leaves reaching 80 cm in length, the average range of leaf length was lower, measuring approximately 65 cm. This is notable considering that *Posidonia* leaf length typically ranges from 20 cm to 1m. The seagrass leaves also showed signs of physical deterioration, indicated by a dull white to brown coloration on the blade tips. This physical deterioration could potentially be associated with high salinity environments, nutrient deficiencies, and temperature fluctuations (Capo et al., 2020). However, it's worth considering that primary effects of increased global temperatures on seagrass can also alter growth rates and physiological functions thus climate change is a factor to consider (Short and Neckles, 1999).

Substrate percentage cover by algae was significant at all RO plants with Pembroke exhibiting the most followed by a similar range in Ċirkewwa and Għar Lapsi. The identified as algal diversity (*Padina pavonica, Cystoseira* sp*., Dictyota* sp*., Cladophora* spp*., Hydrolithon* sp*.)* common to the Maltese marine environment. The major growth form of algae (turf/mat) suggested it was potentially opportunistic algae that often occupies areas where there is abundant light and poor nutrients conditions less ideal for *Posidonia* growth. The algae formations didn't appear to follow a particular trend and were recorded at a range of depths in both the impact and reference sites, thus distance from the brine discharge source potentially plays a minor role in their distribution pattern.

Additionally, echinoderms are highly sensitive to brine discharges and play a role in controlling algae growth when present. However, few counts of *Hacelia attenuata* species were observed during the surveys. The most were recorded at Pembroke potentially due to favourable ambient and substrate conditions. Their poor status could potentially indicate poor water quality, including high saline conditions, which have chemical properties that inhibit the calcium carbonate structures essential for echinoderms' locomotion, support, and defence mechanisms. Echinoderms also serve as filter feeders, and their access to food can be affected by poor water quality, thereby posing a threat to their abundance and survival.

Supplementary observation included the high presence of schools of fish, in the areas immediately surrounding the brine discharge out fall at all 3 RO plant. Fish are found at brine discharge sources for several reasons. Firstly, brine discharge can create a unique environment with high salinity levels, which some fish species are adapted to tolerate or even thrive in. Additionally, the discharge of brine can introduce nutrients into the water, attracting smaller organisms that serve as food for fish. Furthermore, some fish may be attracted to the warmer water near brine discharge sources, as the process of desalination can raise the temperature of the discharged water. Moreover, brine discharge can create turbulence and currents, which can attract fish seeking shelter or opportunities for feeding. Lastly, in some cases, fish may be unintentionally drawn to brine discharge sources due to the disruption of their natural habitats caused by the discharge.

Overall, *Posidonia* emerged as the significant bioindicator species at Ċirkewwa, Għar Lapsi, and Pembroke. It can be concluded that the survey found statistically significant differences in the percent cover, shoot density, and leaf length between the impact site and the 2 reference sites at each location. However, when considering seasonality, significant differences were not observed among the 4 seasons. Nevertheless, relative values suggest that autumn consistently showed the highest average percent cover, shoot density, and leaf length across all 3 sites. These results indicate that autumn is a critical period for the growth and development of *Posidonia* oceanica. Further research is needed to understand the underlying factors responsible for the observed seasonal differences and their implications for the long-term sustainability of *Posidonia* oceanica populations, emphasizing the importance of considering seasonality in understanding the ecology and dynamics of seagrass species.

7. Conclusion

The study evaluates the impact of brine discharge on the marine environments of Ċirkewwa, Għar Lapsi, and Pembroke. Individualised conclusions for each RO plant regarding the impact of brine discharge on the marine environment are as follows:

- Ċirkewwa: Brine discharge impact at Ċirkewwa is variable, with *Posidonia oceanica* showing no significant growth differences between impacted and reference sites. Seasonal fluctuations, particularly in autumn and factors like depth contribute to a moderately affected marine ecosystem. Chemical water compositions such as Boron were the highest in this plant and attributed to the brine volume due to size of plant, precipitation, and increased solubility during autumn conditions. It had low potassium values attributed to high dilution of water and natural geological process during sampling. Conductivity readings rested around (56,000 µS/ cm) at the impact point and reduced further away from the source. Values exceeding this threshold were uncommon. It had the shallowest depths of all the RO plants.

- Għar Lapsi: Għar Lapsi experiences significant differences in *Posidonia oceanica* growth between the impact and reference sites, indicating a more pronounced brine discharge impact. However, bathymetry and depth mitigate the effects, potentially reducing negative impacts. The marine environment is more affected, influenced by seasonal variation and depth. The highest chloride concentration values were at its impact location potentially owing to evaporation levels in spring leading to high concentration of slats left behind in the water, in addition to presence of brine discharge. Conductivity readings values were highest in summer at the impact point (55,700 µS/ cm) and similar reduced further away from the source – followed the direction of persisting strong currents - north-west (looking out to sea). The site had the deepest areas of the 3 RO plants.

- Pembroke: Pembroke shows a notable difference in *Posidonia oceanica* growth between the impact and reference sites, suggesting a significant brine discharge impact. The impact appears more localised, influenced by consistent depth and certain seasonal conditions. The highest number (total of 4) echinoderms were recorded here. Pembroke had the lowest chemical compositions of Boron (3.45 mg/l) in autumn, of the 3 RO plants, attributed to the size of the plant and potential dilution from dynamic weather patterns.

Generally, conclusions, depth and distance from brine discharge points were major influences on physical and chemical water parameters. Natural environmental factors-influence by external factors including water currents and prevailing winds and, discharge characteristics – influence occurrence of halocline and high-water density, both play a crucial role in the distribution of physical parameters seen for example by conductivity elevating with increasing depth and its concentration values higher closer to the discharge source. Furthermore, winter dataset comparisons reveal noticeable changes in chemical concentrations. Pembroke had the highest readings, followed by Ċirkewwa and Għar Lapsi. Magnesium and sodium show significant variations between impact and reference points.
Regarding the bioindicator species, *Posidonia* is absent in impact transects, except in Għar Lapsi. Reference transects in Pembroke and Ċirkewwa have higher seagrass presence. Healthier seagrass growth occurs farther from brine discharge points. Echinoderms show higher counts in autumn and summer, absent in winter surveys. The benthic characteristics at all the 3 RO plants also highlighted the significant presence of algae species common to the Maltese island. They appeared by large both closer to the impact points and in the sallow areas of all survey points, and thus potentially suggesting that they are opportunistic communities whose growth and reproduction is favoured by brine discharge conditions and may easily adapt to future ambient conditions compared to *Posidonia*.

Overall seasonality, bathymetric structure, strong dynamic sea conditions and less evidently brine discharge-induced conductivity influence the distribution of water chemical and physical parameters and marine organisms. If volumes of brine discharge remain at similar rates, the study suggests that the current environmental status at the 3 RO plants may remain unchanged.

8. Monitoring Programme

Long-Term Environmental Monitoring of The Effects of Reverse Osmosis Brine Discharge in the Marine Environment, in Malta.

The following monitoring program is designed to study the impacts of brine discharge on the marine environment, particularly focusing on benthic composition with *Posidonia oceanica* as the major bioindicator specie, the chemical composition of waters immediately subject to brine effluent, and multi parameter physical water characteristics. The study will be based in Malta at the reverse osmosis plants located at Ċirkewwa, Għar Lapsi, Pembroke.

The above precedes the recent baseline study at the aforementioned RO plants which demonstrated present in situ conditions (as observed during surveys carried out between June 2023 and September 2023) and whose status will subsequently be monitored based on a long- term monitoring strategy to assess ongoing changes or impacts.

Long-term monitoring programmes are essential for understanding the behaviour and potential impacts of brine discharge in the marine environment. Typical monitoring programmes consist of the repeated observation of a system with the purpose of detecting a change (Kingsford, 1998; Short et al., 2002). These programmes should be designed to identify any potential signs of disturbance in an ecosystem at an early stage; therefore, they need regular sampling in time with an adequate replication at more than one location. Environmental monitoring should focus on data collected from relevant biological or physical parameters that are considered as useful targets for assessing the studied impact, and acceptable ranges of variation for these parameters must be established. The selection of bioindicator species and a correct experimental design able to deal with the natural spatial and temporal variability of these organisms is crucial.

The development of simple techniques and tools to detect and evaluate such potential impacts should aim to facilitate interpretation by stakeholders. The proposed methodologies are intended to be easily applied, simply understood and not costly.

It is recommended that a similar monitoring plan is applied across each of the 3 RO plants to enable future comparison of effectiveness at each site if needed, and also because the same parameters would be monitored. This recommendation takes into consideration that the plants each differ in size, brine volume discharge, bathymetry and yet have similar characteristics in their benthic cover – seagrass and biogenic reef.

Objectives:
Applicable to all the reverse osmosis plants located at Ċirkewwa, Għar Lapsi, Pembroke:

- To analyse the chemical composition of seawater (including chlorides, sodium) in the immediate area influenced by brine discharge to understand brine dispersion rate and potential effects on the marine environment.

- To monitor physical water parameters such as conductivity, pH, temperature, salinity, and turbidity to further evaluate the overall environmental impact.

- To assess the impact of brine discharge on bioindicator species. *Posidonia* oceanica is the selected species following not only its endemic protective status, it prevents coastal erosion, forms a highly productive ecosystem and biodiversity hotspot while remaining susceptibility to brine discharge.

Proposed overall strategy:

- Surveys should be carried out through the year to consider the seasonal variability (different winds, calms, breezes, etc.) and to include the possible formation of thermoclines and haloclines. It is recommended to achieve at least 2 campaigns per year, one in summer, with calm sea conditions, which could mean a lower dilution of the brine discharge, and the other one at the end of winter, with stronger hydrodynamic conditions and a more elevated brine dilution. It is advisable to maintain the 2 seasonal campaigns per year for a period of 5 years. Indeed, the recommended monitoring frequency for seagrass and seawater parameters near a brine discharge source of a reverse osmosis plant may vary based on specific regulatory requirements, environmental impact assessments, and the potential risks associated with the discharge. Typically, monitoring activities associated with baseline studies, such as the recently completed surveys, are conducted at regular intervals, often monthly or quarterly, to evaluate the potential impact on seagrass and seawater quality over time.

  In light of the above, the recommended 2 seasonal campaigns per year for 5 years may appropriately adjusted to implement longer survey intervals, such as every 2 years or representatively more. This adjustment could potentially be implemented in accordance with pertinent environmental regulatory guidance and based on campaign results that identify the range of ongoing potential impacts, particularly regarding their effect on the marine environment. When considering the implementation of longer intervals between surveys for such campaigns, a cautious approach is necessary, as extended timeframes /infrequent survey activities, may overlook critical events - both natural and anthropogenic - resulting in, for example, sudden loss of seagrass, adverse changes in the chemical composition and physical parameters of seawater, which are all factors likely to significantly influence the condition of marine habitat health and presence and thus important to gather data of this time periods. In all, implementing recommended constant monitoring at shorter intervals would facilitate the collection of a more detailed dataset at a higher resolution, thereby offering the potential to capture subtle changes potentially influenced by brine discharge.

- For chemical and physical water parameters survey, the grid of sampling stations (Figures 2, 3 and 4) would be established at their appropriate distances with the brine discharge area. The process would include the collection of water samples at appropriate depths to assess the vertical dispersion of the effluent and, the measurement of physical water characteristics at appropriate intervals in the water column.
- For benthic surveys via scuba, a total of 3 locations per site would be established (Figure 5, 6 and 7). An impact point directly influenced by brine discharge and 2 reference points, believed to not be directly influenced by runoff. This would provide a platform for comparison of the health status of the marine environment between the area types.

8.1 Monitor Brine Distribution/Dispersion Pattern

During each survey, a network of sampling stations needs to be established close to the brine discharge location at the reverse osmosis plants located at Ċirkewwa, Għar Lapsi, Pembroke (Figures 2, 3 and 4) .The size of the surveyed area and the spacing of the sampling grid varies to take into account factors such as the desalination plant's output, the discharge type, the receiving environment's bathymetry (significantly depth), hydrodynamic patterns and benthic species composition.

For the long-term monitoring, it is recommended that physical composition of seawater surrounding the brine discharge source is analysed for:

- Salinity (a product of conductivity) can affect the behaviour of aquatic organisms, influence water quality, and impact the suitability of water for various purposes. It's essential to consider the specific conditions of the water being tested including exposure to currents and depth in water column.

- pH Level: This indicates the acidity or alkalinity of the water. Fluctuations in pH can disrupt the balance of the marine ecosystem and impact overall biodiversity. If pH of discharge brine is lower than the natural seawater pH, it can lead to acidification of the surrounding water thus affecting survival and growth of benthic communities including *Posidonia* which thrives better in alkaline conditions (pH range 8 to 9).

-Temperature: The density of water changes with temperature, rising as it gets colder and decreasing as it gets warmer. When water contains a high concentration of brine, the byproduct of desalination, it suggests that the brine is more likely to sink and gather at the bottom of the sea. This accumulation of brine at the seafloor has the potential to cause adverse effects on marine life and ecosystems.

These samplings necessitate specialised equipment capable of capturing the parameters above. This facilitates the delineation of the brine plume and its diffusion across the area. Equipment like a multiparameter sonde or equivalent such as CTD instrument (conductivity, temperature, and depth) to attain physical measurements. Calibration of instruments with known standards is crucial for accurate and reliable measurements.

Furthermore, the below techniques are further recommended be incorporated in monitoring program, although not utilised in the baseline study. They have the capacity to provide and elaborate data collection and results:

- Incorporate the use of tracer studies as an additional means of gathering data on brine discharge patterns. This approach involves releasing a harmless tracer substance alongside the brine discharge. By tracking the spread of the tracer over time, valuable insights can be gained regarding the dispersion pattern of the brine. Including empirical measurements through tracer studies would provide supplementary information for the analysis of brine discharge patterns.

- Include Buoy or Drifter Studies. This approach involves releasing buoys or drifters at the discharge point and tracking their movement as this helps understand the spreading pattern / plume. Weather conditions especially wind and currents would be relevant factors to consider for this technique.

8.2 Monitor Brine Discharge Composition

To analyse the chemical composition of the water, it is necessary to collect water samples and send them to the laboratory for analysis. It is advisable to collect these samples from specific stations located at the dive survey sites.

These samplings necessitate specialised equipment capable of collecting the water samples with integrity. Equipment like a Niskin water sampler is recommended for its integrity and ease of use.

The proposed water sampling process is:

- At each RO plant, there are 3 dive survey transects: one impact location near the discharge source, and 2 control locations. For the purpose of chemical analysis, water samples should be collected from the starting point of each dive transect, closest to the shore, based on ease of access and proximity to brine runoff especially at the impact point. A total of 6 water samples per RO plant (18 in total) would be collected per survey campaign, with 2 samples taken at each location (Figures 5, 6 and 7).

- Using predefined geo-locations from the baseline studies, a total of 2 data points at each sampling station are designated: at 1m below sea surface and 1m above the seabed or (avoiding disturbance of seabed/stir up of sediment). Once collected, the water needs decanting into appropriate containers to be sent for water quality testing. The containers holding the sampled seawater need stored away from direct sunlight inside a thermal box containing ice. The recommended preservation temperature is ≤ 4°C while in transit to the laboratory.

The seawater sampling should be performed following relevant guiding standards of the ISO 5667 series, specifically ISO 5667-1:2006: Water quality – Sampling – Guidance on the design of sampling programmes and sampling techniques; ISO 5667-14: 1998 Water quality

– Sampling – Guidance on quality assurance of environmental water sampling and handling and, ISO 5667-3:2003: Water quality – Sampling – Part 3: Guidance on the preservation and handling of water samples.

- Boron: This metal in high concentrations can lead to changes in water chemistry which can further impact physiological processes related to growth and survival of seagrass.

8.2.1 Laboratory Analysis

It is recommended that the same chemical parameters (Table 1) are investigated for in the baseline study are used for the monitoring programme. The chemical analysis carried out in the laboratory would also be carried out in accordance with the relevant ISO or CEN standards, where applicable.

The chemical parameters for analysis are selected based on the composition of brine discharge where concentrations could potentially impact the marine environment especially sensitive species such as *Posidonia.*

8.3 Monitoring of bioindicator species

Effective environmental monitoring programmes involve the identification of biological indicators or bioindicator species. These species must be sedentary (since high mobility species do not necessarily reflect the local conditions), of ecological relevance, have a broad distribution, be extensively studied and be particularly sensitive to environmental variations (Molfetas and Blandin, 1981). The presence and abundance of a bioindicator can be considered as a representative and integrative measures of changes to the environmental conditions. Additionally, the recent baseline study suggests that combined use of different organisms and descriptors makes it possible to integrate the complexity of the ecological system and may allow a rapid detection of potential impacts. The selection of bioindicators must be adapted to local conditions, although a wide distribution of the bioindicators is always preferable.

For the monitoring plan, the bioindicator species recommended is *Posidonia oceanica* meadows, which are highly susceptible to the impact of desalination effluents due to their location in the littoral zone and their high sensitivity to changes in environmental quality. In addition to the recent baseline study, external research has shown that this species has low tolerance to increases in salinity levels (Buceta et al., 2003; Fernández-Torquemada and Sánchez-Lizaso, 2005).

The proposed assessment process:

- It is recommended to perform the long-term monitoring at the same sites investigated during baseline study. This would be beneficial for continuity, the opportunity to perform comparison. Besides, the sites include locations near the brine discharge sources and control sites are in unaffected areas, accounting for various environmental conditions.

- There would be 3 dive transect surveys at each RO plant, as established during baseline study (Figures 5, 6 and 7). Each line transect runs 100m long on the seabed. A total of 4 quadrats measuring 30 cm x 30 cm are positioned at previously established intervals. A video is recorded of the line transect and photos of each quadrat is captured. The line transects start closest to the shore, towards the open sea.

- Where *P. oceanica* is present, the recommended parameters to be measured are (i) shoot density (number per square meter), (ii) leaf length (10x random leaves lengths), and (iii) leaf number per shoot. Total percentage cover of *P. oceanica* is also counted to evaluate population dynamics.

- The same line transects, and quadrat positions would be monitored each season, with the objective to estimate shoot recruitment and mortality rates. This would be to identify any seasonal changes in *Posidonia oceanica* health, distribution, and population dynamics using parameters investigated considering it is a slow growing seagrass.

Furthermore, the below techniques are recommended be incorporated in monitoring program, although not strictly integrated in the baseline study:

- Monitor epiphytic algae: perform visual observation to calculate percentage cover on leaves on *Posidonia.*

- Algae diversity: perform both quadrat (using same method recommended for *Posidonia* surveys) and visual observations to identity species type, percent cover and establishment (filamentous algae, encrusting algae).

The above structure would provide an effective yet detailed approach to assess *Posidonia oceanica* meadows and associated benthic communities.

Overall, by implementing this long-term monitoring program, the aim would be to gain a comprehensive understanding of the impact of brine discharge from the reverse osmosis plants on the marine environment in Malta. The findings would hopefully provide valuable insights for environmental management and mitigation strategies to minimise potential adverse effects surpassing the present status. By including both chemical and physical parameters of seawater in the monitoring plan, we can more comprehensively assess the impact of brine discharge at impact and reference sites, providing a holistic understanding of the ecosystem's health, especially *Posidonia oceanica*, and aiding in effective conservation measures. Overall, the plan focuses on preserving the health and integrity of this sensitive and crucial marine ecosystem by evaluating both chemical and physical parameters of seawater mixing with brine discharge. All the above would factor in the different characteristics of Ċirkewwa, Għar Lapsi, Pembroke (brine discharge volume, plant size, bathymetry, hydrodynamics, and composition of benthic habitat species).

Monitoring studies should bear in mind immediate factors such as wave action/water flow/current that can affect the distribution brine discharge and thus halocline as well as status of sediment and location of seagrass meadows. Seagrass growth is optimal in deeper waters and in summer but unpredictable change in weather and thus climate dynamics has the potential to shift seasonal conditions thus marine species adapting to this fluctuation. Sediment characteristics (grain size, organic matter content, and mineral content) also influence seagrass growth and survival and finally, anthropogenic factors such as coastal development, pollution, and disturbances can impact the growth rate. These factors can also attribute to the ambient chemical and physical characteristic of seawater and should be accounted for during monitoring. For instance, dilution of brine during strong currents or seasonal temperature variations, light variations during summer thus influencing turbidity and nutrient levels in the water and water depth, also important to detect vertical distribution of salinity.

Weather conditions were the major impediment to perform baseline studies, and this should be considered when scheduling activities. Ċirkewwa and Għar Lapsi experienced the strongest surface currents while Ċirkewwa also had strong underwater surge. Għar Lapsi's bathymetry had the deepest sea bottom and the most significant non uniform seabed drastically changing between shallow and deep waters. Pembroke's seabed remained uniformly distributed.

Nevertheless, regular report findings to relevant stakeholders and communication once the plan is put into action, would be essential towards providing for instance, annual reports summarising findings and recommending actions where necessary. Reporting would include data collection and analysis with the aim to; establish a comprehensive database for all collected data and, utilise relevant statistical methods and data visualisation to analyse trends and identify patterns. Furthermore, implementing an adaptive management by means of monitoring results to adjust brine discharge practices if needed by relevant authorities, would also come highly recommended. This would provide the opportunity to informatively increase or decrease volume discharge suiting the size and capacity of each RO plant while potentially implementing measures to mitigate impacts on *Posidonia oceanica* and its associated habitats susceptible to brine influence.

Additionally, it is recommended that the duration of the long-term monitoring plan should continue indefinitely to ensure sustained protection of *Posidonia oceanica* and identified benthic habitats equally necessitating preservation from continuous exposure to brine discharge. The plan would also safeguard that water parameters are maintained within sustainable outputs of brine discharge volumes.

The proposed plan, as a whole, aims to enable the acquisition of data from both short-term and long-term monitoring. Short-term monitoring facilitates the identification of immediate impacts, while long-term monitoring is essential for evaluating trends and changes over an extended duration. This approach would facilitate informed decision-making and adaptive management to mitigate environmental impacts stemming from brine discharge, in alignment with local and international environmental regulations (Malta, EU).

9. Programme of Works

This was divided between fieldwork and written reports.

9.1 Reporting

Throughout the duration of the contract, a series of interim progress reports (IPR) for each site was prepared and submitted every 6 months. Each interim report was accompanied by a presentation with slides summarising progress and results obtained. On completion of the final studies, a final report was prepared and submitted including all data and results expected out of the contract, as per the tender document.

In summary, the following reports were submitted:

- **Interim Progress Report I (M6)**
    - IPR Pembroke I
    - IPR Ċirkewwa I
    - IPR Lapsi I
- **Interim Progress Report II (M12)**
    - IPR Pembroke II
    - IPR Ċirkewwa II
    - IPR Lapsi II
- **Interim Progress Report III (M18)**
    - IPR Pembroke III
    - IPR Ċirkewwa III
    - IPR Lapsi III
- **Final Report (M23),** including all sites.

9.2 Programme of Works

The preliminary programme of works was divided into 4 main activity types, as observed in the Gantt Chart (Figure 33). As reported during activities, certain initially scheduled events were adapted when fieldwork couldn't proceed as planned due to weather conditions, which posed safety concerns and could have impacted the quality of the data to be gathered. The contracting authority was informed where adaptations occurred.

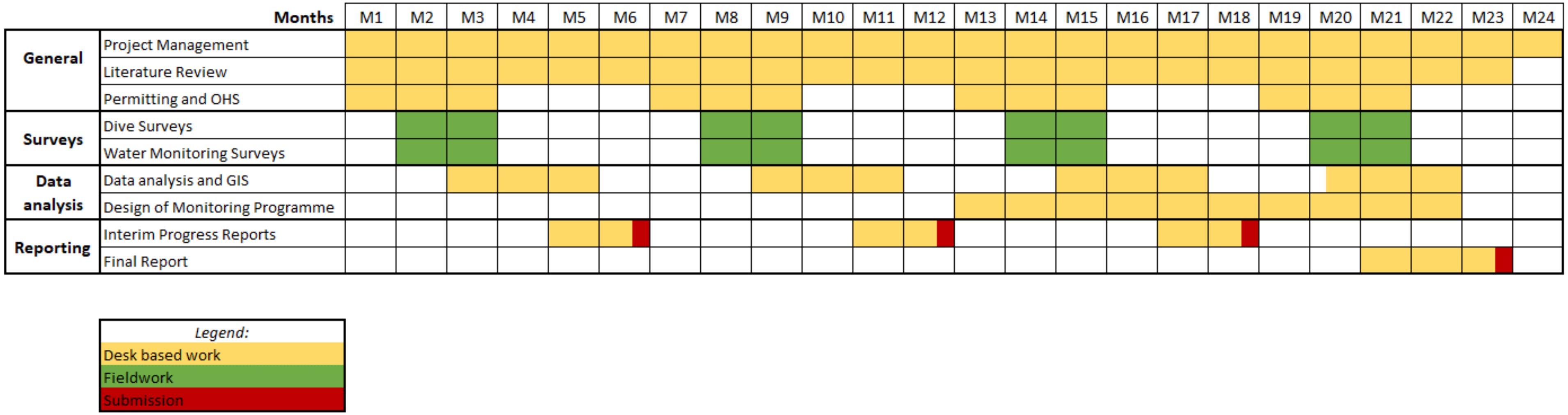
General includes all project management tasks, including internal and external meetings with the Authorities, and any site visits to the RO plants. Project management was expected to be a continuous process for the 2 years duration of the project and occurred as such. Permitting and health and safety considerations took place accordingly prior to the insitu surveys and throughout their implementation. The literature review was included in this category, as a continuous process to ensure knowledge on the matter was kept updated and any relevant new findings considered.

Surveys included all in situ surveys that wre conducted: the water monitoring surveys and the scientific diving. These surveys were conducted seasonally, and in a total of 4 events.

Data analysis referred to the processing of all obtained data, both from the field and any literature reviews. It included all GIS and plotting work, as well as the final design of a monitoring programme.

Reporting was dedicated to the actual compilation of the 3 interim reports (M6, M12, M18) and the final report (M23), including the corresponding presentations with slides summarising findings and results of studies.

Figure 32. Gantt Chart showing preliminary programme.



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AquaBioTech Group

www.abtmarine.com

11. Declaration

The enclosed document is prepared by the **AquaBioTech Group** and signed by the legal representatives of the company **AquaBioTech Limited**, specifically, stating that the contents of this document are considered to be factual and true, to the best of our collective knowledge.

Signed,

....

George D. Mantas
Business Development Director

…for and on behalf of…

ABT Marine
www.abtmarine.com

AquaBioTech Group
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16th February 2024

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