Scerri, R-M. & Borg, J. A. (2021). *Xjenza Online*, 9:2–13.

Xjenza Online - Science Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2021.1.01

Research Article

Influence of Desalination Plant Effluent on Shore Macroinvertebrate Assemblages

R-M. Scerri*[1], **J. A. Borg**[1]
[1] *Department of Biology, Faculty of Science, University of Malta, Msida, Malta*

Abstract. The present study was aimed to establish whether effluent from two desalination plants at different locations in Malta has an influence on biological attributes of rocky shore assemblages, and if so to determine the spatial extent of such influence, and whether the magnitude and extent of effects differs between the two plants. Samples of biota were collected from the Lower Mediolittoral Zone (LMZ) and Upper Mediolittoral Zone (UMZ) using a 20 × 20 cm quadrat, and from the Supralittoral Zone (SZ) using a 30 × 30 cm quadrat, at distances of 0 m, 15 m, 30 m, 80 m and 150 m away from the effluent outfall on either side of it. The collected biota were then sorted and identified in the laboratory. The results indicated that the influence of the desalination effluent on shore macroinvertebrate assemblages was localized at both study localities. Within the LMZ and UMZ, the influence was most evident 15 m away from the outfall, decreased beyond 30 m and was almost negligible 150 m away. In the case of the SZ, the influence was evident in the immediate vicinity of the outfall. Differences in the magnitude of the effect of effluent between the two study localities were attributed to the exposure of the shore to different flow regimes of the effluent discharge.

Keywords: reverse osmosis, hypersaline discharge, shore assemblages, macrofauna, Mediterranean

Acknowledgements. We thank Ing David Sacco [Manager, Water Production at Malta Water Services Corporation] and the Water Services Corporation for providing information on operations at the Ċirkewwa and Pembroke desalination plants. This work was supported by a grant awarded to JAB by the University of Malta's Research Support Services Directorate.

1 Introduction

Rapid global population growth, misuse of water resources and the effects of climate change all contribute to the issue of global water scarcity (Cisneros et al., 2014). One way to circumvent this problem is desalination; the process by which input water from sources such as seawater, brackish water, river water or wastewater is separated into two components: (i) water that may be used for human consumption, industry, irrigation for crop production, amongst others; and (ii) water with a high concentration of salts (Desaldata, 2018 as cited by Jones et al., 2019). The hypersaline effluent is usually discharged back into the same body of water (used as source) through an effluent outfall (Khordagui, 2015). Seawater desalination is regarded as a promising alternative to water extraction as it does not rely on climate-based water sources, and therefore does not exploit natural sources of freshwater. Additionally, the resultant freshwater output from the process is of high quality and can be provided continuously (Elimelech et al., 2011). Water treatment by Reverse Osmosis (RO) is the most widely used desalination process worldwide. The procedure increased in popularity in the 1980s following gradual transition from use of thermal technologies, namely Multi-Stage Flash (MSF) and Multiple-Effect Distillation (MED), to membrane-based ones (Jones et al., 2019).

Although desalination plants counteract water scarcity, their operation may have negative impacts on the environment. Desalination is an energy-intensive process, which uses an average of 4.0kWh/m^3; and most plants are currently reliant on burning fossil fuels, thus augmenting greenhouse gas emissions (Cooley et al., 2013). The marine environment is susceptible to negative impacts of desalination where plants are located in coastal areas, as it serves as the receiving body for

**Correspondence to*: R-M. Scerri (rebecca-maria.scerri.16@um.edu.mt)

the hypersaline discharge (Khordagui, 2015), which may have a salinity that is twice that of the receiving water (Tularam et al., 2007). In the absence of adequate mixing, the high density gradient between seawater and hypersaline water causes the latter to sink to the seabed, thus imposing hypersaline stress on benthic stenohaline biota (Khordagui, 2015). For example, in the Mediterranean, hypersaline effluent from RO desalination may have an adverse impact on seagrass *Posidonia oceanica* (Linnaeus) Delile habitat (Sanchez-Lizaso et al., 2008; Sandoval-Gil et al., 2012). Low tolerance to hypersaline stress has been shown in faunal groups such as polychaetes and echinoderms. For example, Del-Pilar-Ruso et al. (2008) noted that hypersaline effluent caused a decrease in polychaete species richness, abundance and diversity. Fernández-Torquemada et al. (2005) reported that hypersaline effluent caused the disappearance of echinoderm populations in the vicinity of an effluent outfall. Although studies of the influence of hypersaline effluent from desalination operations on sublittoral benthic assemblages are available (e.g. Del-Pilar-Ruso et al., 2008; Fernández-Torquemada et al., 2005; Sanchez-Lizaso et al., 2008; Sandoval-Gil et al., 2012), none appear to have been made to assess the influence of such brine discharge on rocky shore biotic assemblages.

Hypersaline discharge may contain chemical additives that are required for pre- and post-treatment in the plant equipment, and which may also have a negative impact on marine ecology (Tularam et al., 2007). The chemical additives may include anti-scalants, coagulants, biocides, cleaning chemicals and nutrients. The toxicity of some chemical additives such as anti-scalants are considered minimal. However, other additives such as heavy metals and nutrients may pose a threat to marine ecosystems if discharged at high concentrations. Heavy metals such as copper, nickel, chromium and molybdenum may accumulate in sediments and algae and persist in the marine environment in the vicinity of a desalination plant (Khordagui, 2015; Tularam et al., 2007). Algae are effective bioaccumulators of heavy metals, and high levels of these chemicals can negatively influence the abundance, feeding and survival rate of algal-associated fauna (Roberts et al., 2006). Nutrients such as nitrogen and iron, which are limited in marine systems, can affect primary production as high levels of these may result in eutrophication (RPS, 2009).

At present, desalination in Malta is carried out by three large plants, all of which use RO technology: one is located at Għar Lapsi on the southwestern coast and two are located on the northeastern coast at Ċirkewwa and Pembroke. The present study was aimed at establishing whether effluent from two desalination plants at different locations in Malta has an influence on biological attributes of shore assemblages, and if so the spatial extent of such influence, and whether the magnitude and extent of effects differs between the two plants.

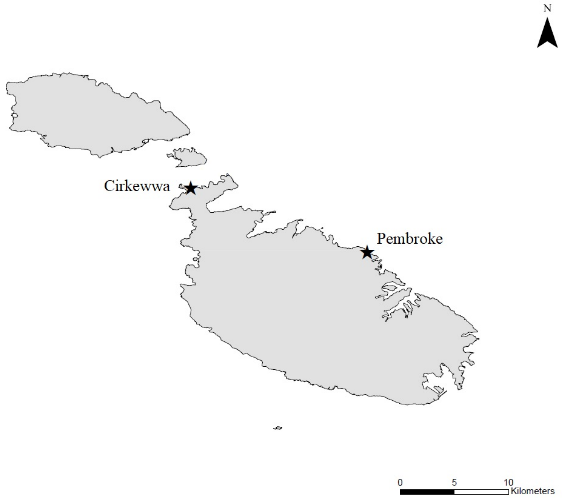

Figure 1: Map of the Maltese Islands showing the locations of the effluent outfall at Ċirkewwa and Pembroke

2 Materials and Methods

2.1 Study sites

The study localities where the two desalination plants are sited are Ċirkewwa and Pembroke (figure 1). Information provided by the Water Services Corporation (Ing D. Sacco, personal communication, 12th June 2020) indicates that the desalination plant at Ċirkewwa has a daily capacity of 8,400 m^3 between October and June, and increases to 11,600 m^3 between July and September. The corresponding daily production of hypersaline effluent is 12,600 m^3 and 17,400 m^3 respectively. The desalination plant at Pembroke has a daily capacity of 29,000 m^3 between October and June, and increases to 35,000 m^3 between July and September. The corresponding daily production of hypersaline effluent is 43,500 m^3 and 52,500 m^3 respectively. At both desalination plants, the chemical composition of the hypersaline effluent consists predominantly of chloride, sodium, magnesium, calcium, potassium, bicarbonate as well as total dissolved solids. The pH of the effluent is slightly acidic to neutral, ranging between 6.8 and 7.0. On average, the temperature of the effluent is between 19°C and 19.5°C and peaks at 21°C between August and October. The hypersaline discharge at the two desalination plants has a brine loading of around 70.2 g/L to 76 g/L. This means that over a 24 hour period between October and June, some 884,520 to 957,600 kg and 3,053,700 to 3,306,000 kg of brine are released to the marine environment at Ċirkewwa and Pembroke, respectively. Over a 24 hour period between July and September, some 1,221,480 to 1,322,400 kg and

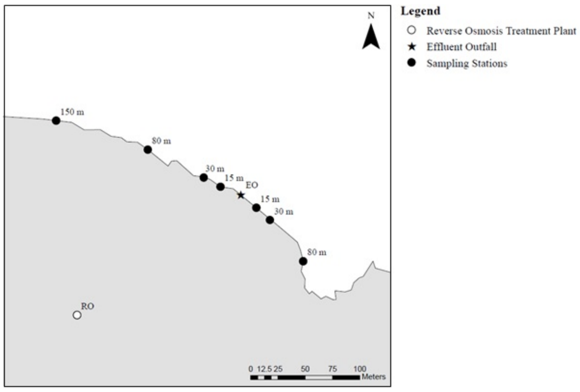

Figure 2: Map of the Ċirkewwa study area showing the locations of sampling stations and their respective distance (number in m) from the outfall. The location of the Ċirkewwa Reverse Osmosis Treatment Plant is also shown

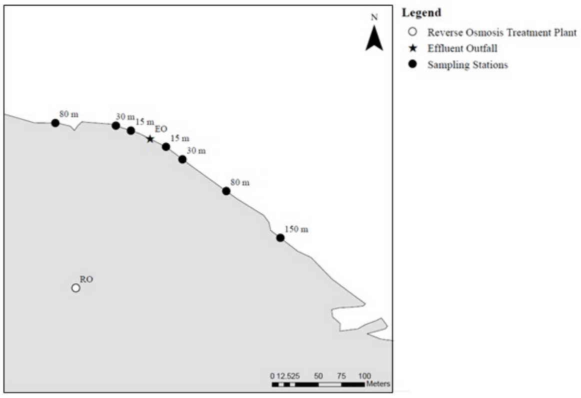

Figure 3: Map of the Pembroke study area showing the locations of sampling stations and their respective distance (number in m) from the outfall. The location of the Pembroke Reverse Osmosis Treatment Plant is also shown

3,685,500 to 3,990,000 kg of brine are released to the marine environment at Ċirkewwa and Pembroke, respectively.

At each of these two localities, the effluent originates from a desalination plant located behind the rocky shore. At both localities, the rocky shore faces north and the substratum, which comprises Coralline Limestone (Continental Shelf Department, 2016), is gently sloping with an inclination of 12° to 15° (Borg Axisa et al., 2013 as cited by Cassar et al., 2007).

2.2 Sample Collection and Processing

At each study area, sampling was carried out at stations located in the vicinity of the outfall and at several distances from it, on either side (east and west); these were: 15 m, 30 m and 80 m east and 15 m, 30 m, 80 m and 150 m west at Ċirkewwa, and 15 m, 30 m, 80 m and 150 m east and 15 m, 30 m and 80 m west at Pembroke; see figures 2 and 3. At each study area, samples of benthic macrobiota (fauna and flora) were taken from each sampling station. Two replicate samples were taken from the Lower Mediolittoral Zone (LMZ) and Upper Mediolittoral Zone (UMZ) using a 20 × 20 cm quadrat, and five replicate samples were taken from the Supralittoral Zone (SZ) using a 30 × 30 cm quadrat. The collected samples were preserved in 5% formal saline, and subsequently sorted to separate the macrofauna from algal material. The fauna were then identified to the lowest taxon possible; for the purpose of the present study all fauna were grouped at the family level, while algae were identified at least to the genus level. Following identification, the algal samples were dried at 70°C for 24 hours, following which the species biomass was recorded to the nearest 1/1000 g using an electronic balance.

2.3 Data Analysis

Mean values of total macrofaunal abundance in each of the three zones i.e. LMZ, UMZ and SZ, total number of macrofaunal families, and total algal biomass within the LMZ and UMZ, were used to show variation of these attributes amongst sampling stations and between the two study areas. Only two families were recorded from the SZ.

The Similarity Percentages analysis (SIMPER) was used to identify and select the three most important macrofaunal families in terms of the highest contribution to dissimilarity between different sampling stations at each of the two study areas. Bray–Curtis similarity was calculated on square-root-transformed data. The analysis was carried out using family abundance data for the LMZ and UMZ.

To test for significant differences in biological attributes of the shore biotic assemblages between sampling stations in the LMZ and UMZ at the two study areas, univariate one-way PERMANOVA tests were carried out on the data for number of macrofaunal families, for Pielou's evenness and Shannon-Wiener Diversity using the macrofaunal abundance, and for algal biomass. One-way PERMANOVA tests were also carried out using data for abundance of taxa which SIMPER analysis indicated as having the highest contribution to dissimilarity amongst sampling stations. Euclidean distance was used as a resemblance measure, alpha was set at 0.05, and the permutation number set to 9999. A multivariate one-way PERMANOVA was carried out using data for family abundance from the LMZ and UMZ from the two study areas. The data were square-root transformed to downweigh the abundant families, and to account for the less abundant and rare families (Clarke et al., 2001). The Bray-Curtis similarity measure was used to construct the similarity matrix, alpha was set at 0.05, and the permutation number set to 9999. Due to low values of possible permutations obtained for most of the tests, for both univariate and multivariate one-way PERMANOVA, Monte Carlo tests were carried out. In the event of a significant difference indicated by the PERMANOVA, a pair-wise PERMANOVA test was carried out to identify the source of significant difference for a given attribute between sampling stations. A principal coordinate analysis (PCO) was carried out to identify similarities or dissimilarities in the data indicated by the PERMANOVA analyses (Anderson et al., 2008).

All analyses were carried out using the PRIMER v7 software with PERMANOVA+ add-on.

3 Results

A total of 13,375 individuals belonging to 67 macrofaunal families were recorded; of these, 2,601 individuals belonging to 42 families were recorded from the Ċirkewwa study area, and 10,774 individuals belonging to 63 families were recorded from the Pembroke study area. A total of twelve species of algae, and two species of cyanobacteria were recorded, which together had a total biomass of 273.665 g.

The mean total macrofaunal abundance recorded from each sampling station from all three zones was higher at Pembroke than at Ċirkewwa for most (LMZ and UMZ) or all (SZ) stations. Macrofaunal abundance was low in one or both directions 15 m away from the effluent outfall in the LMZ and UMZ (figure 4A and B respectively) and nil at 0 m in the SZ (figure 4C) at Pembroke. Overall, an increase in mean total number of macrofaunal families recorded from the mediolittoral zone (LMZ + UMZ) was evident between the 15 m station and stations located further away from the effluent outfall, at both study areas (figure 5A and B respectively). Values of algal biomass were low, particularly at Ċirkewwa; however, a peak in biomass was evident at stations located at a distance of 30 m on either side of the effluent outfall at Pembroke (figure 6).

Univariate one-way PERMANOVA for the LMZ at Ċirkewwa indicated a significant difference [$p < 0.05$] for evenness, which the pair-wise tests indicated to result from a difference in evenness between the station located at 15 m and the station located at a distance of 80 m from the outfall. At Pembroke, a significant difference [$p < 0.05$] was indicated for number of macrofaunal families, Pielou's evenness and Shannon–Wiener diversity. For number of macrofaunal families, the pair-wise tests indicated significant differences between the stations located at a distance of 15 m and stations located at a distance of 30 m from the outfall. For diversity, the pair-wise tests indicated significant differences between the stations located at 15 m and stations located at 80 m and 150 m from the outfall. Univariate one-way PERMANOVA for the UMZ at Ċirkewwa indicated a significant difference [$p < 0.05$] for evenness

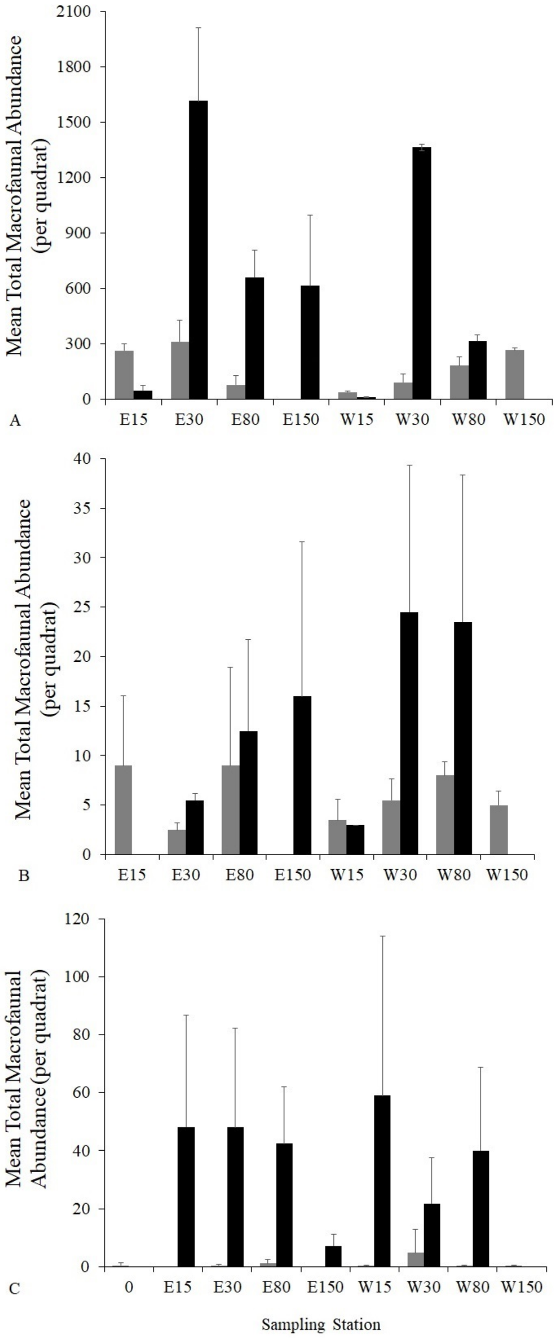

Figure 4: Mean total macrofaunal abundance per quadrat recorded from: A) Lower Mediolittoral Zone, B) Upper Mediolittoral Zone, C) Supralittoral Zone recorded from Cirkewwa (grey) and Pembroke (black). Error bars represent ±1 Standard Deviation. E = east side of the effluent outfall; W = west side of the effluent outfall; 15, 30, 80 and 150 represent the distance (m) from the effluent outfall

and diversity. For both these attributes, the pair-wise tests indicated a significant difference between the station located at 15 m and the station located at 150 m from the outfall. A significant difference was also indicated by the pair-wise tests for evenness between the station located at 30 m and the station located at 80 m from the outfall; and for diversity between the station located at 30 m and the station located at 150 m from the outfall. No significant differences were detected for any of the considered attributes at Pembroke (table 1).

The macrofaunal families identified by the SIMPER analysis as contributing most to dissimilarity between stations at Ċirkewwa were Ampithoidae, Hyalidae and Sabellidae. Results from univariate one-way PERMANOVA showed a significant difference [$p < 0.05$] for abundance of Ampithoidae and Hyalidae (table 2). For both families, the pair-wise tests indicated a significant difference between the station located at 15 m and all other stations located at a greater distance from the outfall. A significant difference [$p < 0.05$] for abundance of Ampithoidae was also indicated between the eastern station located at a distance of 30 m and stations located at a distance of 80 m on the eastern side and 150 m on the western side from the outfall. The macrofaunal families identified by SIMPER analysis as contributing most to dissimilarity between stations in the LMZ at Pembroke were Ampithoidae, Sabellidae and Tanaididae. A significant difference [$p < 0.05$] was indicated by univariate one-way PERMANOVA for the abundance of Ampithoidae and Tanaididae (table 2). For both families, the pair-wise tests indicated a significant difference in abundance between the stations located at 15 m and the station located at 80 m on the western side (Ampithoidae) and that at 30 m on the eastern side (Tanaididae). A significant difference [$p < 0.05$] was also indicated by the pair-wise tests for abundance of Ampithoidae between the station located at a distance of 30 m on the eastern side and the station located on the western side at a distance of 80 m from the outfall. Significant differences were indicated by the pair-wise tests in abundance of Tanaididae between the station on the eastern side at a distance of 30 m from the outfall and stations located at a distance of: (i) 80 m from the outfall on both eastern and western sides; and (ii) 150 m on the eastern side, from the outfall.

The macrofaunal families identified by SIMPER analysis for the UMZ data from Ċirkewwa and Pembroke were Chthamalidae, Patellidae and Trochidae. At Ċirkewwa, a significant difference [$p < 0.05$] in abundance was detected by univariate one-way PERMANOVA only for Trochidae. The pair-wise tests showed that the source of the significant difference was between: (i) stations located at a distance of 15 m and stations located at a distance of 80 m from the outfall, on both western

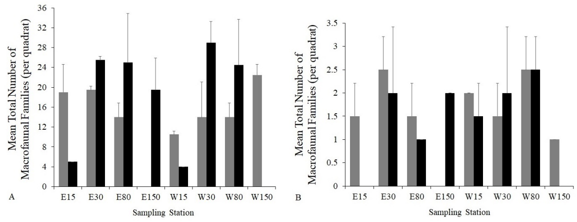

Figure 5: Mean total number of macrofaunal families per quadrat recorded from: A) Lower Mediolittoral Zone, B) Upper Mediolittoral Zone recorded from Cirkewwa (grey) and Pembroke (black). Error bars represent ±1 Standard Deviation. E = east side of the effluent outfall; W = west side of the effluent outfall; 15, 30, 80 and 150 represent the distance (m) from the effluent outfall

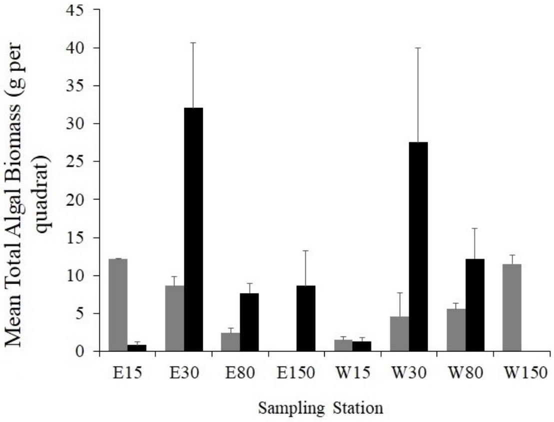

Figure 6: Mean total algal biomass in g per quadrat recorded from Ċirkewwa (grey) and Pembroke (black). Error bars represent ±1 Standard Deviation. E = east side of the effluent outfall; W = west side of the effluent outfall; 15, 30, 80 and 150 represent the distance (m) from the effluent outfall

Source of Variation	df			Number of Macrofaunal Families		Pielou's Evenness		Shannon–Wiener Diversity	
				Pseudo-F	p-value	Pseudo-F	p-value	Pseudo-F	p-value
Study area	6	Ċirkewwa	LMZ	2.3768	0.1451	6.7074	**0.0124**	3.6903	0.057
			UMZ	1.8	0.2241	4.4464	**0.0361**	4.7687	**0.0306**
		Pembroke	LMZ	6.0842	**0.0168**	4.3478	**0.0362**	8.1175	**0.0069**
			UMZ	1.9667	0.1975	5.0512	0.0842	1.7045	0.2492
Residual	7								
Total	13								

Table 1: Results of univariate one-way PERMANOVA for number of macrofaunal families, Pielou's evenness and Shannon–Wiener diversity from analyses of Lower Mediolittoral Zone (LMZ) and Upper Mediolittoral Zone (UMZ) macrofaunal data for Ċirkewwa and Pembroke. df = degrees of freedom. Significant p-values ($p < 0.05$) are indicated in bold.

Source of Variation	df		Ampithoidae		Hyalidae		Sabellidae		Tanaididae	
			Pseudo-F	p-value	Pseudo-F	p-value	Pseudo-F	p-value	Pseudo-F	p-value
Study area	6	Ċirkewwa	10.957	**0.0038**	5.3105	**0.0236**	2.4771	0.1284		
		Pembroke	4.2593	**0.038**			0.84167	0.577	32.703	**0.0002**
Residual	7									
Total	13									

Table 2: Results of univariate one-way PERMANOVA for abundance of Ampithoidae, Hyalidae, Sabellidae and Tanaididae from SIMPER analyses of Lower Mediolittoral Zone (LMZ) data for Ċirkewwa and Pembroke. df = degrees of freedom. Significant p-values ($p < 0.05$) are indicated in bold.

and eastern sides; and (ii) the station located at a distance of 30 m from the outfall on the western side and the station located at a distance of 80 m from the outfall on both western and eastern sides. At Pembroke, univariate one-way PERMANOVA indicated a significant difference [$p < 0.05$] in abundance only for Patellidae. The pair-wise tests showed that the source of this difference was between the station located at 15 m on the eastern side and (i) stations located at a distance of 30 m on both western and eastern sides and (ii) 80 m from the outfall on the western side (table 3).

Univariate one-way PERMANOVA indicated a significant difference [$p < 0.05$] for number of algal species and total algal biomass in the LMZ at both Ċirkewwa and Pembroke (table 4). For both study areas, the pair-wise tests did not indicate a significant difference between pairs of stations for number of algal species but the procedure indicated a significant difference for total algal biomass. For Ċirkewwa, the source of this difference was between: (i) the station located at a distance of 15 m on the western side and stations at 30 m on the eastern side, 80 m and 150 m from the outfall on the western side; and (ii) the station located at a distance of 15 m on the eastern side and stations at 80 m from the outfall on both western and eastern sides. The pair-wise tests also indicated a significant difference between: (i) the station at 80 m from the outfall on the eastern side and stations at 30 m and 150 m on the eastern and western sides respectively; and between: (ii) the station at 80 m on the western side and the station at 150 m from the outfall on the western side. For Pembroke, the source of this significance was between: (i) the station at a distance of 15 m from the outfall on the western side and stations at 30 m and 80 m from the outfall on the eastern side; and (ii) the station at a distance of 15 m from the outfall on the eastern side and stations at 30 m and 80 m from the outfall on the eastern side. The detected differences in biological attributes between the station located at a distance of 15 m and stations located further away from the outfall was corroborated by the results of multivariate analysis, in particular for the Pembroke study area. The results of multivariate one-way PERMANOVA (table 5) and pair-wise tests for the macrofaunal data from the LMZ at this study area indicated a significant difference [$p < 0.05$] in total abundance of macrofauna; the source of this difference was between: (i) the station located at a distance of 15 m from the outfall on the western side and stations at 30 m, 80 m and 150 m on the eastern side and 30 m on the western side; and (ii) the station located at a distance of 15 m from the outfall on the eastern side and stations at 30 m from the outfall on both western and eastern sides. At Ċirkewwa, the results of multivariate one-way PERMANOVA did not indicate a significant difference in total abundance of macrofauna between stations in the LMZ. The results of multivariate one-way PERMANOVA for macrofaunal data from the UMZ at Ċirkewwa indicated a significant difference [$p < 0.05$] between stations in total abundance of macrofauna; the pair-wise tests indicated that the source of this difference was between the station located at a distance of 30 m from the outfall on the eastern side and the station located at a distance of 150 m from the outfall on the western side. A significant difference [$p < 0.05$] between stations was also indicated for total abundance of macrofauna in the UMZ at Pembroke but the pair-wise tests did not identify the source of this difference. The plots from the PCO analyses for abundance data from the LMZ and UMZ at Pembroke (figure 7 A and B respectively) showed a separation between the station located at a distance of 15 m and stations located at a greater distance from the outfall on both western and eastern sides. These results corroborate those from the multivariate one-way PERMANOVA tests. The plot for macrofaunal data from the LMZ at Pembroke also indicates that stations located at a distance of 30 m from the outfall were grouped separately from rest of the stations. The respective plots for the LMZ and UMZ at Ċirkewwa (figure 7C and D respectively) indicated less separation of samples.

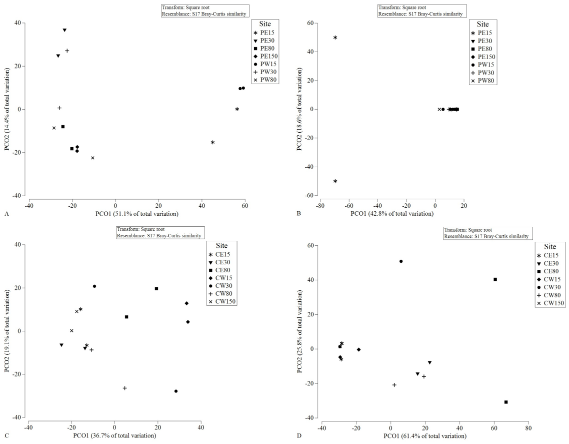

Figure 7: Plots from Principal Coordinates Analysis based on square-root transformed abundance data from: A) Lower Mediolittoral Zone at Pembroke, B) Upper Mediolittoral Zone at Pembroke, C) Lower Mediolittoral Zone at Cirkewwa and D) Upper Mediolittoral Zone at Cirkewwa. PE = Pembroke east; PW = Pembroke west; CE = Cirkewwa east; CW = Cirkewwa west. The numbers in the station codes represent the distance (in m) from the outfall

4 Discussion

Differences in macrofaunal abundance, number of macrofaunal families and algal biomass between the two study areas indicate that the influence of the hyper-

Source of Variation	df		Chthamalidae		Patellidae		Trochidae	
			Pseudo-F	p-value	Pseudo-F	p-value	Pseudo-F	p-value
Study area	6	Ċirkewwa	0.87577	0.5564	3.0406	0.084	19.093	**0.0002**
		Pembroke	0.98154	0.5072	4.8885	**0.029**	1.5	0.3084
Residual	7							
Total	13							

Table 3: Results of univariate one-way PERMANOVA for abundance of Chthamalidae, Patellidae and Trochidae from SIMPER analysis of Upper Mediolittoral Zone (UMZ) data for Ċirkewwa and Pembroke. df = degrees of freedom. Significant p-values ($p < 0.05$) are indicated in bold.

Source of Variation	df		No. of Algal Species		Total Algal Biomass	
			Pseudo-F	p-value	Pseudo-F	p-value
Study area	6	Ċirkewwa	7.5	**0.0099**	19.163	**0.0009**
		Pembroke	1.2778	0.3726	7.9191	**0.0083**
Residual	7					
Total	13					

Table 4: Results of univariate one-way PERMANOVA for number of algal species and total algal biomass from analysis of Lower Mediolittoral Zone (LMZ) algal data for Ċirkewwa and Pembroke. df = degrees of freedom. Significant p-values ($p < 0.05$) are indicated in bold.

Source of Variation	df			Pseudo-F	p-value
Study area	6	Ċirkewwa	LMZ	1.7348	0.0569
			UMZ	3.4257	**0.0132**
		Pembroke	LMZ	4.707	**0.0001**
			UMZ	2.0748	**0.0397**
Residual	7				
Total	13				

Table 5: Results of multivariate one-way PERMANOVA from analyses of Lower Mediolittoral Zone (LMZ) and Upper Mediolittoral Zone (UMZ) macrofaunal data for Ċirkewwa and Pembroke. df = degrees of freedom.

saline effluent was greater at Ċirkewwa compared to Pembroke. This may be attributed to exposure of the shore to different flow regimes of the effluent discharge; at Ċirkewwa, the effluent flows down a single channel directly into the open sea, whereas at Pembroke the effluent flows into several diverging channels before entering the open sea. Some of the latter channels terminate into rockpools, which receive the discharged effluent, and eventually overflow. Therefore, although the shore biotic assemblages at Pembroke appear to be influenced by the hypersaline effluent, while the volume of effluent released there is greater, the magnitude of this influence may be lower at this study area due to dispersal of the effluent into smaller bodies of water before it enters the sea. Another difference in results between the two study localities is that macrofaunal abundance and number of faunal families in the LMZ at Cirkewwa decreased on moving eastwards from the effluent outfall and increased on moving westwards, while such trend was not evident at Pembroke (figures 4 and 5). This could possibly be attributed to the higher exposure of the shore at Ċirkewwa to the locally-predominant northwesterly wind (Galdies, 2012) which would generate wave action and water movement that is expected to displace the effluent eastward, thereby influencing the shore biota present east of the discharge point to a greater extent than that present west of it. On the other hand, such influence is less at Pembroke which is less exposed (figures 4 and 5) to the northwesterly wind and related wave action.

The present results indicate that the influence of hypersaline effluent on shore biotic assemblages in the vicinity of the outfall at both desalination plants is highest within the stretch of shore located within a distance of 15 m from the discharge point. In the LMZ and UMZ at both Ċirkewwa and Pembroke, the influence of the hypersaline discharge was manifested by low values of abundance and number of families of macrofauna at the station located 15 m from the outfall, while an increase in values of these attributes occurred with increasing distance from the discharge point. The results of univariate and multivariate one-way PERMANOVA tests indicated differences in biological attributes; namely number of macrofaunal families, Pielou's evenness, Shannon-Wiener diversity and algal biomass between the station located at a distance of 15 m from the outfall and stations located at a greater distance from it, and that the observed differences with increasing distance from the discharge point were more evident at Pembroke compared to Ċirkewwa. These results were corroborated by the output from the PCO analysis of biological data from Pembroke (figure 7A and B) which clearly indicated that the stations at 15 m are distinguishable from the other stations.

Results from univariate one-way PERMANOVA tests for the LMZ indicated a significant difference in abundance of Ampithoidae between sampling stations at both study areas. Most of the pairs of stations that were significantly different included the station located at a distance of 15 m and stations located at a greater distance from the outfall. De-La-Ossa-Carretero et al. (2016) showed that amphipod abundance and diversity decreased in response to elevated salinity of brine effluent, especially in the vicinity of a hypersaline outfall. Results from univariate one-way PERMANOVA tests for the UMZ indicated a significant difference for Trochidae at Ċirkewwa between stations at 15 m and 80 m and between those at 30 m and 80 m and for Patellidae at Pembroke between stations at 15 m and 30 m and between those at 15 m and 80 m. *Phorcus turbinatus* (Born, 1778), the most abundant member of Trochidae recorded from the UMZ in the present study, is sensitive to changes in salinity (Menzies et al., 1992), hence decreased abundance of this species with decreased distance from the outfall may be due to the high salinity levels to which the shore habitat is exposed. Information on tolerance of Patellidae to above-ambient salinity levels appears to be lacking, but one would expect that species from this family will be as affected adversely by high salinity levels, particularly during their juvenile stages. As a consequence of external fertilization, the larval stages of marine gastropods, such as patellids, are planktonic. Mortality rates of the planktonic larval stage would be expected to be higher when exposed to unfavourably high salinity levels that lead to desiccation (Denny et al., 2007).

The results from univariate one-way PERMANOVA tests for algal biomass indicated a significant difference between the station located at a distance of 15 m and stations located at a greater distance away from the outfall on both western and eastern sides. A concurrent increase in algal structural complexity with increasing algal biomass would be expected as one moves further away from the effluent discharge point. At the station located at a distance of 15 m from the outfall, *Ulva* sp. and Cyanobacteria were the dominant flora; these are characterised by low structural complexity, which in turn would be expected to support a lower associated macrofaunal abundance (Hacker et al., 1990) and diversity (Hicks, 1985 as cited by Hauser et al., 2006). The genus *Ulva* comprises euryhaline species (Black et al., 1972), which have a high tolerance to elevated salinity compared with other macroalgae exposed to the same environmental conditions (Einav et al., 1995). Similarly to *Ulva* sp., some cyanobacteria species have a high tolerance to salinity levels. One of the two cyanobacterial species identified in the present study is *Symploca* sp., which is capable of tolerating salinity levels ranging between 48 PSU and 62 PSU (Nagasathya et al., 2008). At stations located at a distance of 30 m and more from the outfall, the influence of the hypersaline effluent is less pronounced and environmental conditions are more similar to background ones. This would allow the presence of algae that are more characteristic of natural ambient conditions, and which would have a higher biomass, as noted from the present results, and hence higher structural complexity that would be expected to support a higher diversity of associated macrofauna. The flora which dominated the stations located at a distance of 30 m and greater away from the outfall included *Jania rubens*, *Gelidium* sp., *Titanoderma* sp. and *Padina pavonica*, all of which are more typical of the rocky shore LMZ around the Maltese Islands

The results of the present study also show that the hypersaline discharge influenced shore biotic assemblages within the mediolittoral zone at a distance of 30 m away from the outfall but such influence was less than 15 m away from the discharge. This suggests that the influence of the effluent is localized, being most evident at a distance of 15 m from the outfall, and is lower at a distance of 30 m, and becomes negligible further away.

The supralittoral macrofauna comprised members of the families Littorinidae and Chthamalidae. Chthamalidae individuals were only recorded at Pembroke in one replicate sample. The abundance of the littorinid *Melarhaphe neritoides* (Linnaeus, 1758) was higher at Pembroke than at Ċirkewwa, and contrary to the obtained results for macrofaunal abundance in the LMZ and UMZ, the abundance of this species at stations located at a distance of 15 m from the outfall was comparable to that recorded from stations located at a greater distance from the discharge point; only in the immediate vicinity of the effluent outfall, i.e. at 0 m, was this species absent and hence its abundance was '0'. Thus, the influence of the effluent on littorinids within this zone may not be so large compared to that on other macrofaunal groups present in the LMZ and UMZ; this is probably due to the presence of ecotypes, i.e. a population adapted to the specific environmental conditions provided by the effluent (Brewer, 1994), the behavioural strategies of species from this family, and the lower exposure of the zone to wave action. *M. neritoides* typically shelters itself from harsh physical conditions such as wave action by occurring in dense aggregates within crevices on the rocky shore, as well as in pits and under overhangs, where the microclimate is benign. Moreover, the supralittoral zone receives sea-spray but is not submerged and it is only during strong wave action that this zone is wetted by the sea (Grech et al., 1989). Since the hypersaline effluent directly enters the sea, the supralittoral zone which is further inland is less affected than the mediolittoral zone further down. Therefore, exposure of *M. neritoides* to the effluent is minimal, especially due to the reduced wave action during the summer months.

5 Conclusions

Overall, the present results indicate a similar pattern of influence of hypersaline effluent on shore biotic assemblages in the vicinity of the discharge point, at both desalination plants under study. However, some differences in macrofaunal abundance, number of macrofaunal families and algal biomass were noted between the two study areas. This is to be expected and would result from differences in environmental factors between the two study localities, which would influence attributes of the shore biotic assemblages, as was noted in the present study wherein a different pattern of change in abundance and number of families of macrofauna between Ċirkewwa and Pembroke, on moving away from the effluent source, was evident. The influence of the hypersaline discharge was greater at Ċirkewwa compared to Pembroke; this was attributed to exposure of the shore to different flow regimes of the effluent discharge between the two desalination plants. Results for the LMZ and UMZ infer the largest influence of the hypersaline effluent on the shore biotic assemblages occurred within the stretch of shore located within 15 m away from the effluent outfall, and this observation was common to both study areas. The influence decreased beyond a distance of 30 m and was almost negligible at a distance of 150 m. In the SZ, the influence of the effluent on the shore biota was evident in the immediate vicinity of the outfall, at 0 m, where no species were recorded, while at a distance of 15 m from the outfall, the abundance of Littorinidae was comparable with that recorded from stations located at a greater distance from the discharge point. As far as the present authors are aware, this is the first time that the influence of hypersaline effluent from desalination operations on rocky shore biotic assemblages, specifically in the Mediterranean, has been assessed. The results from the present study, which should be considered preliminary especially given the limited effort adopted during sampling of the biota, suggest that dispersal of desalination effluent at the discharge point may decrease the level of adverse influence on shore biotic assemblages, while the overall findings should help coastal managers and planners in decision-making processes that concern site selection for desalination plants and aspects of the effluent discharge to the sea. Further investigations, which could include assessment of water quality, hydrodynamic aspects and other physico-chemical environmental factors, as well as more extensive sampling of biota associated with the different littoral and sublittoral zones, would be very useful as further evaluation of the influence of desalination effluent on shore and shallow water marine ecology.

Declarations

The authors have no relevant financial or non-financial interests to disclose. Conflict of Interest: The authors declare that they have no conflict of interest.

References

Anderson, M. J., Gorley, R. N. & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to software and statistical methods.* Devon: PRIMER-E Ltd.

Black, D. R. & Weeks, D. C. (1972). Ionic relationships of *Enteromorpha Intestinalis*. *New Phytologist*, *71*, 119–127.

Borg Axisa, G., Sciberras, J., Sciberras, A., Pisani, L., Sultana, J. et al. (2013). Visitors' site guide Pembroke Heritage Project [Retrieved April 26, 2019, from European Regional Development Fund Malta 2007–2013].

Brewer, R. (1994). *The science of ecology* (second ed.). Saunders College Publishing.

Cassar, L. F., Lanfranco, S. & Schembri, P. J. (2007). An ecological appraisal of the special area of conservation – candidate site of international importance, in the Pembroke Area, Malta. *Report prepared by Ecoserv Ltd for Nature Trust (Malta)*, *38*.

Cisneros, J. B., Oki, T., Arnell, N. W., Benito, G., Cogley, J. G. et al. (2014). Freshwater resources. In C. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea et al. (Eds.), *Climate change 2014: Impacts, adaptation, and vulnerability. part a: Global and sectoral aspects. contribution of working group ii to the fifth assessment report of the intergovernmental panel on climate change* (pp. 229–269). Cambridge University Press.

Clarke, K. R. & Warwick, R. M. (2001). *Change in marine communities: An approach to statistical analysis and interpretation* (second ed.). PRIMER-E Plymouth.

Continental Shelf Department. (2016). Geological map of the maltese islands [Retrieved April 26, 2019, URL: https://malta-contshelf.maps.arcgis.com/apps/MapTools/index.html?appid=79ad35031ff4449ea92df25a2e2e505e].

Cooley, H. & Heberger, M. (2013). *Key issues for seawater desalination in California: Energy and greenhouse gas emissions.* Oakland, CA: Pacific Institute.

De-La-Ossa-Carretero, J. A., Del-Pilar-Ruso, Y., Loya-Fernandez, A., Ferrero-Vicente, L. M., Marco-Mendez, C. et al. (2016). Response of amphipod assemblages to desalination brine discharge: Impact and recovery. *Estuarine, Coastal and Shelf Science*, *172*, 13–23.

Del-Pilar-Ruso, Y., De-la-Ossa-Carretero, J. A., Gimenez-Casalduero, F. & Sanchez-Lizaso, J. L. (2008). Effects of a brine discharge over soft bottom polychaeta assemblage. *Environmental Pollution*, *156*, 240–250.

Denny, M. W. & Gaines, S. D. (2007). *Encyclopedia of tidepools and rocky shores.* University of California Press.

Einav, R., Breckle, S. & Beer, S. (1995). Ecophysiological adaptation strategies of some intertidal marine macroalgae of the israeli mediterranean coast. *Marine Ecology Progress Series*, *125*, 219–228.

Elimelech, M. & Phillip, W. A. (2011). The future of seawater desalination: Energy, technology, and the environment. *Science*, *333*(6043), 712–717.

Fernández-Torquemada, Y., Sánchez-Lizaso, J. & González-Correa, J. (2005). Preliminary results of the monitoring of the brine discharge produced by the SWRO desalination plant of Alicante (SE Spain). *Desalination*, *182*(1), 395–402.

Galdies, C. (2012). The climate of the maltese islands. *Sustainable Energy 2012: The ISE Annual Conference Proceedings*, 65–69.

Grech, M. & Schembri, P. J. (1989). A laboratory study of the behavioural responses of littorina neritoides (mollusca: Gastropoda) in relation to its zonation on Maltese shores (central Mediterranean). *Marine Behaviour and Physiology*, *15*, 123–135.

Hacker, S. D. & Steneck, R. S. (1990). Habitat architecture and the abundance and body-size-dependent habitat selection of a phytal amphipod. *Ecology*, *71*(6), 2269–2285.

Hauser, A., Cotton, P. A. & Attrill, M. (2006). Effects of habitat complexity on the diversity and abundance of macrofauna colonising artificial kelp holdfasts. *Marine Ecology Progress Series*, *325*, 93–100.

Hicks, G. R. (1985). Meiofauna associated with rocky shore algae. In P. G. Moore & R. Seed (Eds.), *Ecology of rocky coasts.* Hodder & Stoughton.

Jones, E., Qadir, M., van Vliet, M. T., Smakhtin, V. & Kang, S. (2019). The state of desalination and brine production: A global outlook. *Science of the Total Environment*, *657*, 1343–1356.

Khordagui, H. (2015). Final report assessment of potential cumulative environmental impacts of desalination plants around the Mediterranean sea. *Sustainable Water Integrated Management (SWIM) - Support Mechanism*, *Activity 1.3.2.1.*

Menzies, R., Cohen, Y., Lavie, B. & Nevo, E. (1992). Niche adaptation in two marine gastropods, Monodonta turbiformis and M. turbinata. *Bollettino di zoologia*, *59*(3), 297–302.

Nagasathya, A. & Thajuddin, N. (2008). Cyanobacterial diversity in the hypersaline environment of the saltpans of southeastern coast of India. *Asian Journal of Plant Sciences*, *7*(5), 473–478.

Roberts, D. A., Poore, A. G. & Johnston, E. L. (2006). Ecological consequences of copper contamination in macroalgae: Effects on epifauna and associated herbivores. *Environmental Toxicology and Chemistry*, *25*(9), 2470–2479.

RPS. (2009). Effects of a desalination plant discharge on the marine environment of Barrow island.

Sanchez-Lizaso, J., Romero, J., Ruiz, J., Gacia, E. & Buceta, J. (2008). Salinity tolerance of the mediterranean seagrass *Posidonia oceanica*: Recommendations to minimize the impact of brine discharges from desalination plants. *Desalination*, *221*, 602–607.

Sandoval-Gil, J., Marín-Guirao, L. & Ruiz, J. (2012). Tolerance of Mediterranean seagrasses (*Posidonia oceanica* and *Cymodocea nodosa*) to hypersaline stress: Water relations and osmolyte concentrations. *Marine Biology*, *159*(5), 1129–1141.

Tularam, G. A. & Ilahee, M. (2007). Environmental concerns of desalinating seawater using reverse osmosis. *Journal of Environmental Monitoring*, *9*, 805–813.