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# Impacts of seawater desalination brine on coastal environments

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by
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# Abstract

**Impacts of seawater desalination brine on coastal environments**

By

**Karen Lykkebo Petersen**

Terrestrial water resources are scarce in arid and semi-arid regions of the world and increasing demands for water worldwide are adding additional pressures on limited water resources. Seawater desalination provides a reliable source of potable water. The process of desalination creates a high-salinity byproduct that is discharged back into the coastal environment by various methods (pipes, diffusers, channels). The brine effluent is often mixed with chemicals used at the desalination facilities and can, without dilution; reach salinities twice of the ambient feed-water. Despite a growing desalination capacity worldwide, the effects of that brine discharge on local coastal environments are not fully understood or constrained.

This thesis investigates the impacts of desalination brine discharge on coastal biology and chemistry, and is comprised of three chapters: **Chapter 1** investigates the in-situ impacts of desalination discharge measured before and after the Carlsbad Desalination Plant (California) became operational on both water chemical parameters and benthic macro fauna; **Chapter 2** investigates the impacts of desalination brine on corals from The Gulf of Aqaba in a 4-week incubation experiment with salinities 10% above ambient and addition of phosphonate-based antiscalants; and **Chapter 3** reviews current knowledge of the impacts of brine discharge on a wide range of coastal organisms.

The Carlsbad Desalination Plant became operational in December 2015, and water, sediment and biological samples were collected prior to operations (December 2014 and September 2015) and after operations began (May and November 2016). A distinct brine plume of salinity 34-37 (ambient salinity 33.3) extending 600m offshore from the discharge point was observed after the plant started discharging. This is despite the coastal area being “high-energy” and previous computer models predicting mixing of the brine to be better. The salinity plume does not have an immediate effect on the benthic environment: Macro fauna abundance (tube-forming Polychaetes) significantly increases compared to post-operation surveys, however the in-faunal organisms show no change. The benthic area around the outfall has same level of disturbance pre and post operations, possibly because of the

continuous discharge from the local power plant. A 5-week bioassay of brittle stars in brine treatments indicated a trend towards decreased growth and impaired agility in increased salinities but no significant change was observed. Finally, using mean wave height along the California coast as a proxy for coastal mixing, locations of proposed desalination facilities are compared to the location of Carlsbad Desalination Plant to evaluate the expected mixing properties of those areas.

In the brine incubation experiment of hard corals (*Stylophora pistillata, Acropora tenuis*, and *Pocillopora verrucosa*) there were an overall decrease in coral performance and changes in the corals' physiology. *Symbiodinium* abundance, protein content and heterotrophic abundances were significantly reduced at the end of the incubation in all three species. In particular, the addition of phosphonate-based antiscalant to a 10% salinity increase reduced the amount of corals tissue symbionts, calcification rates and oxygen production rates. These results suggest that coral reefs may be susceptible to exposure to SWRO discharge and calls for careful selection of locations of SWRO facilities around coral reefs.

Various marine species have various response and resilience to increased salinity and brine discharge. To increase SWRO facilities sustainability and minimize their footprint in the coastal environment, sufficient mixing of the brine effluent is important. Diffusor systems generally provide the best mixing, but co-location of SWRO facilities with power plants, already discharging cooling water, minimizes additional impacts of the brine. Local monitoring of the marine benthic environment is encouraged in locations of proposed desalination facilities.

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# Chapter 1

# Environmental impacts of brine discharge - an in situ case-study from Carlsbad Desalination Plant

## Abstract

Fresh water demand is increasing world wide as populations and agriculture expand and climate change intensify droughts and areal extent of arid regions. Seawater reverse osmosis desalination is an increasing solution for the supply of portable water in arid and semi-arid coastal regions, however the effects of brine-effluent discharge associated with this technology on the local coastal environment are not fully constrained. In this study, we use *in-situ* measurement of water chemistry and some biological indicators collected pre- and post-discharge in the coastal area immediately adjacent to the newly constructed Carlsbad Desalination Plant. Data quantify the impacts and changes occurring within the first year post discharge. A significant increase in salinity from an ambient 33.3 to 36 in coastal bottom water extending 600 m offshore of the discharge location. Opportunistic polychaetes is dominating the benthic substrate in the impacted area by. We tested desalination brine effects on brittle stars in a 5-week long incubation experiment and observed a trend of decreasing growth. Our work highlights the difficulties of effective mixing of high-density brine with coastal seawater even in a high wave area and suggests the need for implementation of more effective discharge methods. We further highly the need for comprehensive coastal monitoring in areas of proposed desalination facilities along with laboratory assessments of key coastal species.

## Introduction

Fresh water demand is increasing worldwide due to a variety of factors that includes population growth, agricultural expansion and environmental changes[1]. At the same time, natural fresh water sources are decreasing in amount and quality[2]. Specifically, in California, increasing agriculture activity and population growth have exhausted the natural groundwater reservoirs, resulting in substantial land subsidence and seawater intrusion[3]. Furthermore, droughts that drain water reservoirs state-wide are a reoccurring phenomenon in the region and are expected to increase in frequency in the future[4,5]. California is one of the largest economies in the world due to agriculture, and water security is of paramount concern in the state[4]. Seawater desalination has become a progressively more popular way to meet fresh water shortages in coastal arid and semi-arid regions, and in recent years, technological improvements and decreasing cost have increased the interest in seawater reverse osmosis (SWRO). Seawater desalination currently produces of ~70 million $m^3$ daily with estimates of a doubling of capacity in 2020[2,6]. In California, ten small seawater desalination facilities are currently operating, with a combined capacity of 51.5 MGD (million gallons $day^{-1}$)[7]. In 2015 a large-scale SWRO plant located at Carlsbad started operation, with a capacity of 50 MGD. Seven other large-scale plants have been proposed along the California coast with capacities of 10-150 MGD[7].

SWRO facilities typically draw coastal water as feed and continuously discharge high salinity brine effluent back to the coastal environment. The discharge in most cases occurs either directly at the shoreline through pipes or channels, or further from the coast through diffuser systems[8–10]. In California, the brine is diluted before discharge, as state regulation does not allow discharge to the ocean of water with salinities that are more than 2 units above ambient[11]. The discharging brine-effluent may contain chemicals used during the desalination process such as antiscalants, coagulants, trace metals and cleaning chemicals (*e.g.* detergents, oxidants and biocides)[12,13]. One of the important challenges facing SWRO plants is the need for the brine to effectively mix with the ambient seawater to reduce the area impacted by high salinity. If poorly mixed, the high-density brine sinks to the sea bottom potentially causing osmotic stress on the benthic community. However, the efficiency of water mixing is very site-specific and depends on the salinity of the discharging brine and local coastal conditions (waves, currents and bathymetry) as well as the design of the outfall. The California Coastal Plan (2015) specifies that salinity around the discharge area cannot exceed 2 salinity units above the ambient salinity, measured 100 m from the discharge

area[11]. The Carlsbad Desalination Plant is an exception to this rule with an allowed discharge zone of 200 m due to its capacity. Mixing in the discharge zone is usually assessed prior to operation using computer models. At the Carlsbad Plant, a hydrodynamic model assumed a starting salinity at 42 by the outfall, and the model predicted a decrease of salinity to 35.5 (ambient 33.3) at a distance of 196 m, therefore fulfilling the California Ocean Plan requirements[14].

Despite the increasing use of SWRO desalination, the impacts on the coastal environment due to brine-effluent discharge are ill-constrained and understudied[12,15]. Past research on pelagic phytoplankton and benthic microbes, seagrass, polychaetes and more recently corals, suggest that responses and tolerances are highly variable between species [15–21]. The productivity and growth rate of phyto- and zoo plankton, as well as benthic bacteria, did not have pronounced changes, community structure switched at salinity increases of 10% above ambient[16,17,22,23]. Seagrass meadows and corals seem to have lower thresholds, with ~5% salinity increases causing mortality and/or decreased growth[15–17,20,21,24]. For benthic organisms, the Benthic Opportunistic Polychaetes and Amphipods index (BOPA-index) has been used successfully as an indicator of the level of "disturbance" in areas impacted by pollution[25–28] and it has been shown to be useful in salinity disturbed areas as well[29]. The method is based on the antagonistic relationship between sensitive and opportunistic species; amphipods are more sensitive to pollution and brine than polychaetes, which are usually indifferent or resistant[25,27,28,30]. The index calculates a number between 0 and 0.3, where 0-0.04 is considered to be of high ecological status, 0.04-0.14 good, 0.14-0.19 moderate, 0.19-0.26 poor and 0.26-0.3 bad[25,28].

The coast of California is a highly productive zone supported by upwelling of nutrient rich sub-surface water. This manifest itself in large kelp beds and rocky reefs with high biodiversity and rich plankton communities that serve as feeding grounds for numerous whale and dolphin species. To protect this unique ecosystem a statewide system of 124 marine protected areas (MPA's) has been established[31,32]. Coastal kelp beds are particularly important as they support high biodiversity and the effects of salinity increase have, to the best of our knowledge, not been studied.

In this study, we collected samples around the outfall of the Carlsbad Desalination Plant before and after the plant became operational, to quantify changes in water chemistry and biology. We then compared the *in-situ* results to a controlled bioassay incubation experiment with brittle stars (*Ophiothrix spiculata*) in water collected at the discharge outfall. To put our data into a larger context for California, we prepared maps of wave height and benthic habitat

characteristics of two coastal areas with proposed desalination plants; the Southern California coast from San Clemente to San Diego, and the Monterey Bay region.

## Methods

### Study area

The Carlsbad Desalination plant (Poseidon Water) is the first (and currently only) large-scale SWRO desalination facility in California. Operations began in December 2015 with a daily capacity of 50 MGD providing 10% of San Diego County's water supply. The plant is located in Carlsbad in an industrial area in the southern end of Agua Hedionda Lagoon (Figure 1). Seawater enters the lagoon through a dredged channel at the north end, about ~1 km from an intake and is pumped by the Encina Power Station and used as cooling water (since 1954), the desalination plant channels a fraction (10%) of this water for SWRO (Figure 1B). The brine effluent (after SWRO-processing) is mixed with the remaining power plant cooling water in a 1:10 dilution before being discharged back through a wide (10-15 m) open channel (outfall) that extends ~75 m offshore from Carlsbad Beach [33]. The Carlsbad Beach is a relatively high-energy beach with wave heights of ~2.5 m in winter and ~1.5 m in summer [34–37]. The near-shore habitat at Carlsbad Beach is dominated by sandy bottom to the south with scattered small rocky reefs and seagrass patches at the northern end of the beach[38]. The near-shore is relatively shallow with depths of 5-10 m up to 800 m offshore and deeper water (~20 m) starting ~1 km offshore[35].

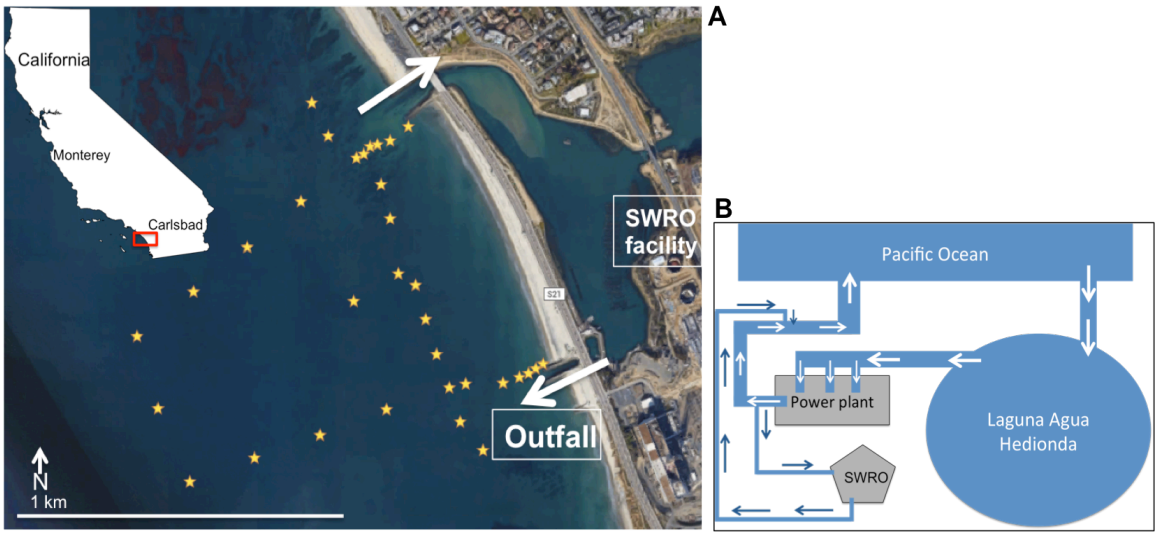



**Figure 1** Left (**A**): Aerial schematic of the study site with yellow stars indicating sampling stations. Bottom (**B**): Schematic of the water intake and use by the power plant and SWRO. Modified from Poseidon Water.

**Sample collection and biological surveys**

To assess the impacts of desalination brine discharge on coastal water chemistry and biology, water and sediment samples were collected and benthic surveys were conducted in two seasons prior to the start of the SWRO plant operation (pre-operation) and twice in the first year after discharge began (post-operation). The pre-operation samples were collected in December 2014 and September 2015, twelve and three months before operation began, and the post-operation samples were collected in May and November 2016, five and eleven months after operation began. Samples were collected by scuba divers from a small boat, starting ~100 m offshore (or closer if conditions allowed). Samples were taken on transects perpendicular to the beach every 25 m out to 200 m, and then at 250, 300, 400, 600, 800 and 1000 m from shore. The parallel transect was 200 m offshore, and samples were collected every 100 m from 200 m south of the outfall to 200 m north of the intake channel (Figure 1A). In addition, one sample was collected directly at the head of the discharge channel where Carlsbad Boulevard crosses the channel. Bottom and surface water samples were collected in 1 L acid cleaned, sample rinsed, Nalgene bottles. Sediment samples (from upper 10 cm) were collected in 250 ml plastic jars and frozen until analysis (section 0). The temperatures of water samples were measured on the boat, immediately after collection, with a YSI probe (YSI 30SCT). Water samples were kept in a cooler until they were filtered and processed, within 4 hours after collection (section 0).

Biological surveys were conducted along two continuous perpendicular transects, 1 m wide, from 100 to 200 m offshore, and in 1x1 m quadrats (1 $m^2$) at ~100 m intervals further offshore. Along continuous transects, a 100 m tape was laid on the sea floor and all visible benthic organisms within 0.5 m of each side of the tape were recorded for each 10 m long section (10 $m^2$). Visible benthic organisms were enumerated separately by two scuba divers, and counts were later averaged. At offshore (200 m offshore and beyond) and parallel stations, organisms were recorded in ten randomly distributed 1$m^2$ quadrats per station. Organisms were assigned to 10 categories: Porifera (sponges), Anthozoa, Gastropoda, Bivalvia, Cephalopoda, Polychaete, Echinodermata, Arthropoda, fish, macroalgae and seagrass.

**Water and sediment analyses**

Water samples were analyzed for salinity, chlorophyll *a*, nutrients ($PO_4$, $NO_3$ and $SiO_2$), particulate organic matter (POM), dissolved organic carbon (DOC) and trace metals. Salinity was determined using a Guideline Portasal salinometer.

*Chlorophyll* a: 250 ml of seawater were filtered onto GF/F filters and kept frozen in the dark until further analysis. The filters were added to vials with 7 ml of 90% acetone and left to extract for 12 hours before measurements. Chlorophyll *a* (Chl *a*) concentration was measured using a TD-700 fluorometer calibrated with Chl *a* according to the EPA 445 method, using CS-5-60 and CS-2-64 glass filters.

*Nutrient* concentration: Seawater samples were filtered through 0.2 µM filters and kept frozen until analysis. Nitrate, phosphorous and silicate was measured using a nutrient flow injection auto-analyzer. Total nitrogen and total dissolved phosphate were determined as nitrate and soluble reactive phosphate following oxidation with persulphate and UV radiation, and samples were analyzed as described above.

*Dissolved organic carbon*: Samples for DOC were collected in pre-ashed glass vials. The samples were acidified to pH 2 using concentrated HCl and were analyzed on an autosampler Shimadzu TOC-V with prepared standards of 2.125 g KHP $L^{-1}$. Detection limits were 0.3 µM for carbon with 2 ml injection.

*Particulate organic matter*: 400 ml seawater was filtered onto pre-ashed (450°C for four hours) GF/F filters and kept frozen. The filters were dried for 48 hr at 50$^{o}$ C, wrapped in tin foil and pressed. The carbon to nitrogen molar ratio (C:N) and C and N isotope ratios were measured using mass spectrometry (CE instruments NC2500). The samples were analyzed with standards of pugel and acetanilide to an accuracy of ± 0.11 ‰.

*Trace metals*: Trace metal samples were collected in acid-cleaned sample-rinsed LDPE bottles and acidified to pH 2 prior to analysis. Samples were diluted 500 fold in a trace metal clean laboratory. The samples were analyzed on an Element XR ICP-MS with standard curves prepared using NIST 1640a standard in high resolution parts per trillion (ppt). The average of two procedural blank values was used for blank correction. The samples were analyzed for concentrations of Li, Na, Mg, K, Ca, Mn, Fe, Sr and Ba.

*Benthic polychaetes and amphipods*: Sediment samples were thawed and washed with 90% ethanol. The ethanol rinse was collected and polychaetes and amphipods were counted immediately under a dissecting microscope. For polychaetes, individuals of family Paraonidae and Capitellidae were counted and the amphipods individuals of Gammarids were most abundant with some representatives from family Hyperiidae, Caprellidae and Corophiidae present as well. The BOPA-index was calculated for each sample using methods described by Dauvin et al. (2007) (eq. 1), where $fp_{op}$ is the proportion of polychaetes and $f_a$ is the proportion of amphipods[25]. The index gives a number from 0-0.3, where higher numbers indicate a higher fraction of polychaetes and a disturbed environment[25,28].

$$Eq\ 1: \quad BOPA = \log\left(\frac{fp_{op}}{f_a + 1} + 1\right)$$

*Sediment grain size*: The sediment was dried and sorted using twelve sieves from 0.5 Φ to 4.75 Φ and each fraction was weighed. Mean grain size and the sorting factor were determined using methods described by Folk et al. (1966)[39].

*Phytoplankton cell count*: 50 ml of unfiltered surface water were fixed with 2% formalin and kept cool. The samples were homogenized and 25 ml was left in settling chambers for a minimum of 12 hours. Samples was viewed under a Zeiss Axiovert 200 microscope with 20x/0.3 Ph1Var magnification and half of the settled chamber was counted for each sample and calculated to cells per liter (this data is not available for December 2014 due to poor sample preservation).

**Bioassay experiment**

To assess the impact of brine on a common marine organism, a laboratory bioassay exposed brittle stars (*Ophiothrix spiculata*) to brine collected in the field. The experiment consisted of two brine treatments and a control (ambient seawater) with replicates (n=20). Brine water was obtained from the outfall channel of Carlsbad Desalination plant, filtered through 0.2 µM pore filters and stored cool in the dark. The brine treatments were: 1) Undiluted discharge water of salinity 36 (referred to 100% hereafter); 2) 50% dilution of the discharge brine with

ambient seawater to salinity 34 (referred to 50% hereafter). The brittle stars were collected from Monterey Bay by Monterey Abalone Co. 400 ml of the relevant water was added to 20 glass culture dishes for each treatment. Two brittle stars were randomly assigned to each jar (40 brittle stars per treatment). The jars were placed in a water table with running seawater to maintain constant temperature, and placed out of direct light. Water was changed every 2-3 days and the incubation lasted 5 weeks. At the end of the incubation, all brittle stars were subjected to an agility test. Each animal was flipped onto its back, and the time taken to return to normal orientation was recorded. Measurements of growth and weight were used to compare effects of the treatments on the brittle stars.

Body measurements of the brittle stars were: Wet weight, dry weight, body diameter, arm lengths, skeleton weight and tissue weight. Pre-incubation wet-weight and arm length (estimating from photographs) were measured for all stars and a full body-part measurement was done at the end of the incubation: Wet-weight was determined by placing a star on a tissue paper for 2 seconds before recording its weight to an accuracy of ±0.001g. Body diameter and arm lengths were measured to the closest mm, and arm length was averaged over all 5 arms. The brittle stars were dried in a $50^{o}$ C oven for 48 hours before obtaining dry-weight. The tissue was dissolved using 50% bleach and the skeleton dried and weighed. Tissue weight was calculated by the difference between skeleton weight and total dry-weight.

### Statistics and geospatial analyses

All statistical analyses were done using software R. Water chemical data was analyzed by two-way ANOVA and Welch Two Sample t-test. The biological surveys results were pooled and compared using $Chi^2$-test. Differences between the brittle stars in the treatments were tested using one-way ANOVA. Salinity and temperature data was mapped in ArcGIS 10.2 using Inverse Distance Weighting (IDW) interpolation to visualize temperature and salinity variation. The mean wave height of Monterey Bay and Southern California were mapped in ArcGIS 10.2 using data compiled by Erikson et al. (2014)[36,37]. Coastal biology and habitat data available from California Department of Fish and Wildlife was used as well[38].

## Results and discussion

### Salinity and temperature of the discharging brine

The salinity of the discharging effluent in the outfall channel was ~37 after mixing with the power plant cooling water. This is significantly higher than the salinity of ~33.3 seen

throughout the mixed coastal zone before operation and post-operation away from the outfall. Around the outfall, the bottom water salinity after the desalination plant initiated operation was significantly higher (between 34-36) than pre-operation salinity (33.3) at the same locations ($p_{ANOVA} < 0.001$). As it discharges, the high-salinity effluent immediately sinks to the bottom and in the process it mixes with seawater which reduces the effluents' salinity from ~37 to about 35, with salinities of 34-36 extending 600 m offshore (Figure 2). The high density plume could easily be observed as a distinct halocline extending ~40 cm above the sea bottom. Despite the high wave intensity (1.5m to 2.5m on average) for much of the year at the discharge site mixing is limited.

Both surface and bottom water temperatures were 1-2 $^{o}$C warmer around the outfall than the intake area and further offshore during all four sampling trips. This shows that operation of the desalination plant did not alter the temperature imprint of the power plant. During September 2015, temperatures throughout the coastal area were high due to El Niño conditions with ocean temperatures above normal[40] (Figure 3).

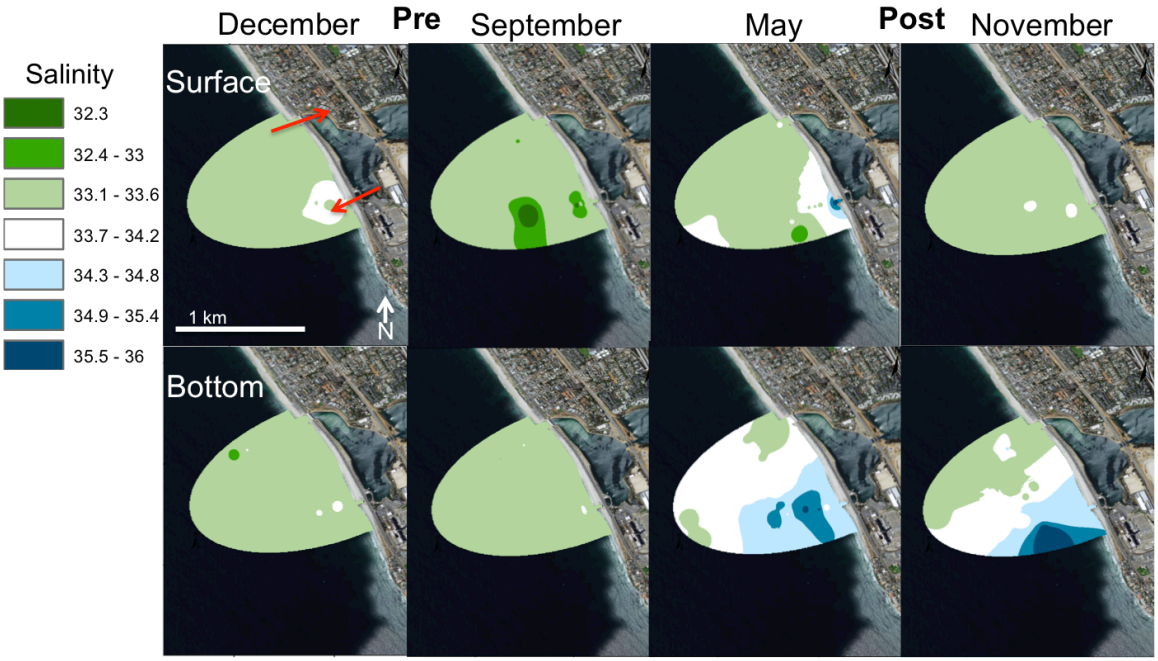



**Figure 2:** GIS-mapped salinity measurements for surface (top row) and bottom water (bottom row). Pre-discharge measurements (December and September) are shown in the two left-most columns and the post-discharge (May and November) in the right-most columns

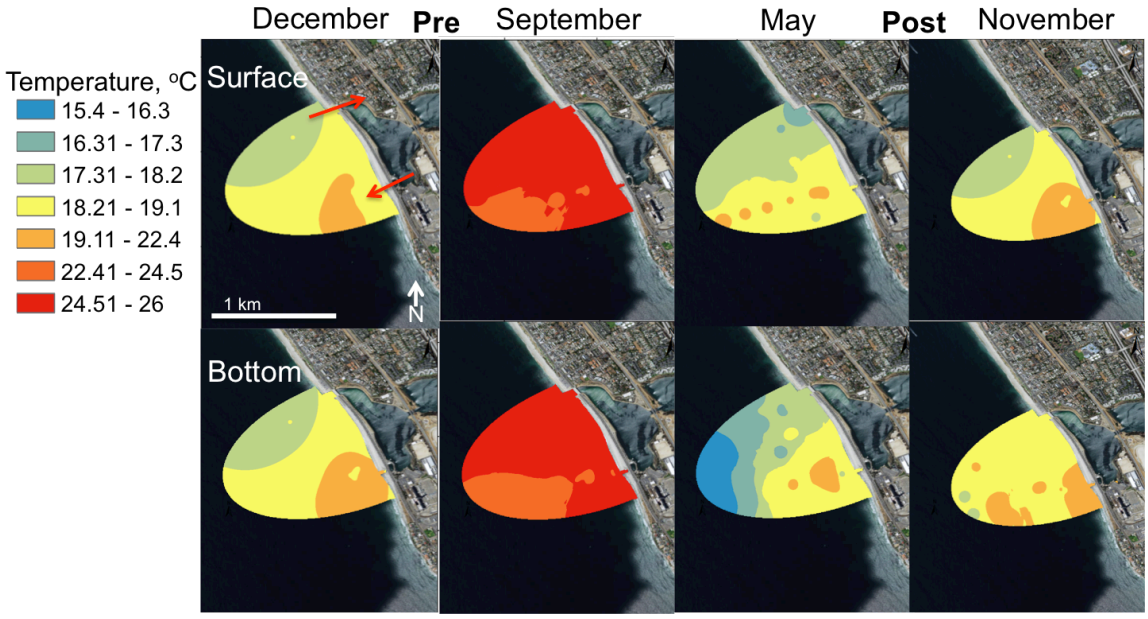



**Figure 3:** GIS-mapped temperature measurements for surface (top row) and bottom water (bottom row). Pre-discharge measurements (December and September) are shown in the two left-most columns and the post-discharge (May and November) in the right-most columns.

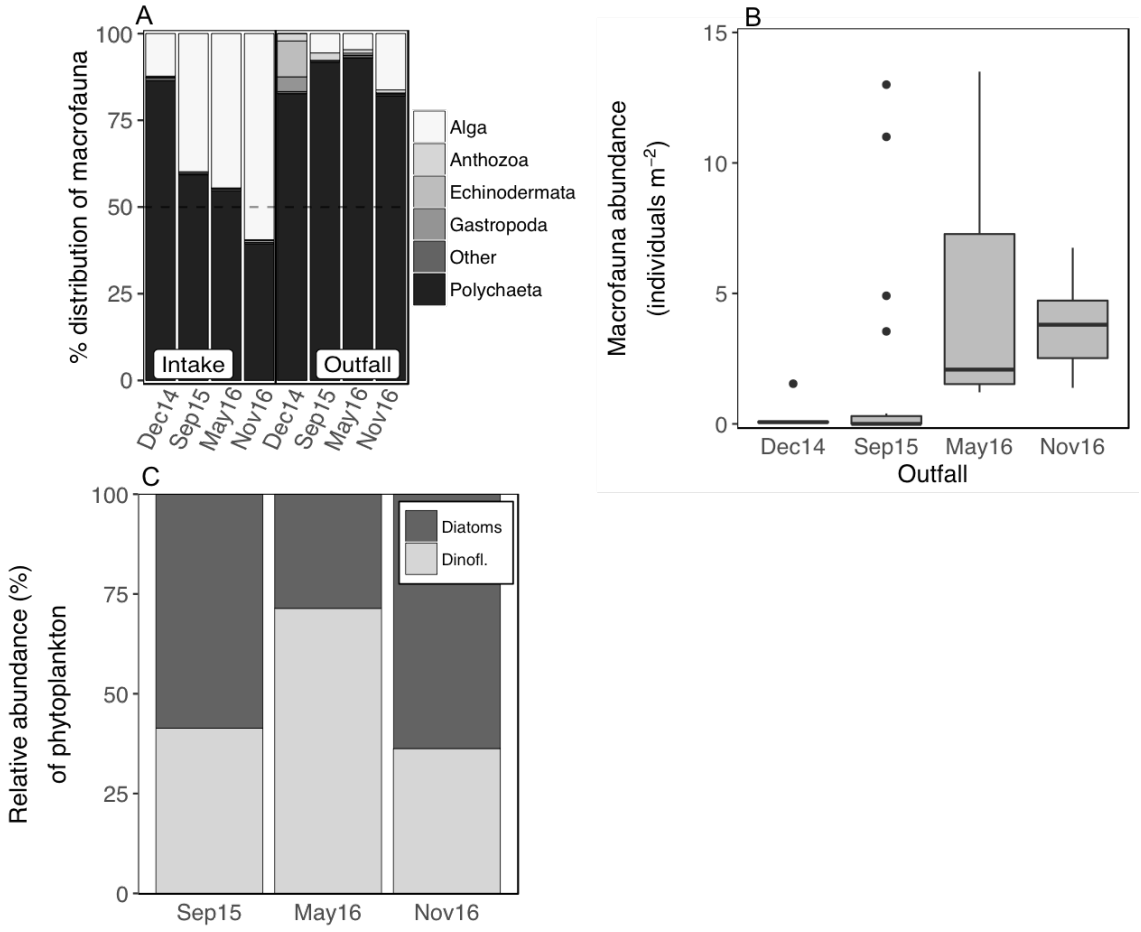
Previous models used to predict the impact area of the brine discharge plume that took into account the physical properties of Carlsbad Beach (shallow with high wave energy) indicated that conditions at the site are optimal for brine dilution through extensive mixing in the near-shore area[14,34,35]. Our data showing a discrete salinity plume extended from the outfall to at least 600 m offshore, raising questions of the validity of the mixing models and emphasizes the need for post-operation monitoring.

The spatial footprint of the brine plume at Carlsbad Beach differed slightly between May 2016 and November 2016 in its extent offshore, probably due to seasonal differences in tidal and wave mixing. During May it was 0.78 m with winds blowing from the west (W), whereas in November 2016, average wave height was 0.95 m with winds blowing from the west-south-west (WSW) [34]. WSW-winds have the same orientation as the outfall channel, whereas the W-wind blows at a slight angle on the channel. These minor differences in wave height and wind direction could cause differences in the mixing efficiency of water at the outfall-ocean boundary, with greater mixing in November when the wave height was greater. The California Ocean Plan restricts brine discharge from SWRO-plants to 2 salinity units above ambient at a distance of 100 m from the discharge point, but this was increased to 200 m for the Carlsbad Desalination Plant[41]. Our salinity measurements in both May and November 2016 show that this is not fulfilled as salinity of up to 36 (ambient 33.3) was measured 400-600 m from the discharge point at the end of the discharge channel. These salinity measurements were made during relatively high ocean-swell and it is possible to expect poorer mixing when the swell is reduced (*e.g.* summer months, Figure 7) and the brine effluent will cover a larger area. A comprehensive study on desalination plants in Spain and their brine discharge also showed large salinity plumes (5-10% above ambient) extending far from the discharge source (up to 3 km) and distinct patterns of seasonality in the mixing trends with summer mixing being far less[42,43]. DOC, POM, trace metals and Chl *a* concentrations did not differ significantly between sampling times. Nutrient levels were generally lower close to shore than offshore but did not differ significantly between pre- and post-operations (Supplemental material).

**Impacts on benthic organisms**

The benthic habitat around the outfall area is dominated by sand while the area around the intake channel also has a small rocky reefs and seagrass beds. The sorting value (σ) of sediment grains are greater by the outfall (σ= 0.8) than around the intake (σ = 0.4) likely as a result of the high flow rate of water from the channel removing finer sediment, giving significantly different species composition between the two areas ($p_{Chi^2} < 0.001$)(Figure 4A).

Near the intake the abundance of algae and sea grass was similar to that of polychaetes, whereas the outfall was dominated (80-90%) by tube-forming polychaetes (family Onuphidae). Because the organisms' distribution heavily depends on benthic substrate type, differences in abundance between the intake and outfall zones cannot be attributed to the




brine discharge. To assess the effect of the discharge, we compared pre- and post-operation abundances of organisms around the outfall, in the sandy environment.

**Figure 4: A**: Percent distribution of visible organisms counted at the intake and outfall at four sampling times and (**B**) total abundance of individuals at the outfall for four sampling times the 75% quantiles over median. **C:** The relative abundance of phytoplankton in the surface waters in pre-operation (Sep15) and post-operation (May16 and Nov16). Percentage of diatoms are shown in dark grey and dinoflagellates (Dinofl.) in light grey.

The abundance of visible macrofauna (mainly polychaetes) by the outfall was significantly higher post-operation compared to pre-operation ($p_{Chi^2} < 0.001$) (Figure 4B). The abundances of in faunal organisms in the surface sediments also follow this trend, but the differences are not statistically significant (Figure 5A). The BOPA-index for the surface sediment indicates higher disturbance (average index value of 0.23) in the outfall area compared to the surrounding benthic environment (average index value of 0.18). BOPA values were greater at the outfall both before and after the desalination plant began operations (Figure 5B). This suggests that the high flow rate of the outflow channel is causing this disturbance and it is not strictly limited to the brine discharge. Observations around other desalination facilities have linked the disturbances measured by higher BOPA to higher salinity and BOPA has been shown to decrease after implementation of diffusors to regulate brine discharge[44]. The most abundant phytoplankton were diatoms and dinoflagellates and their relative abundances differed seasonal, with fall samples (September and November) having more diatoms (~60%) and spring samples (May) being dominated by dinoflagellates (~75%). There was no difference between pre- and post-operation during fall season (Figure 4C).

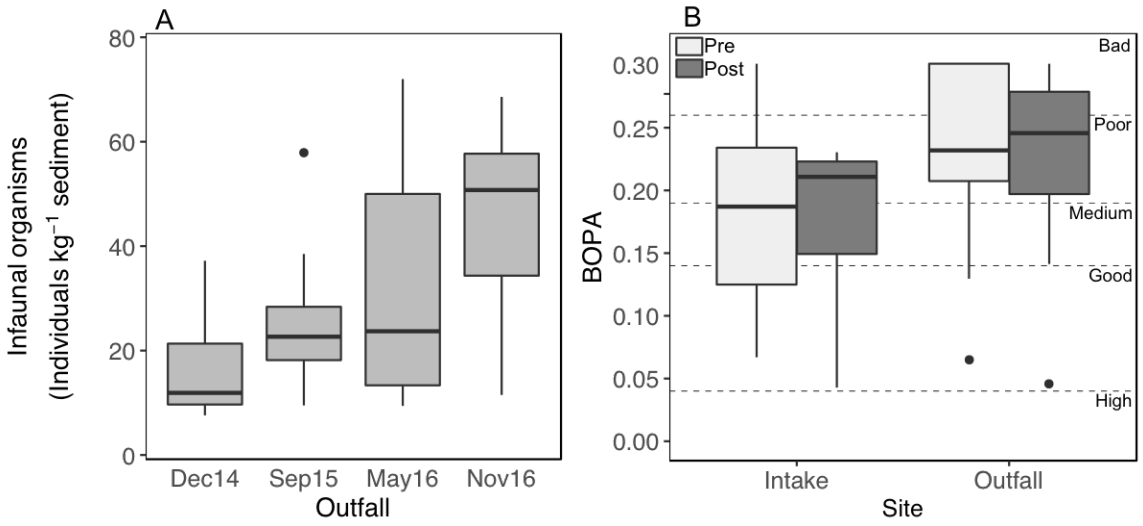



**Figure 5. A:** The abundance of infaunal organisms in the outfall area for four sampling times with 75% quantiles. **B:** BOPA-values for the intake and outfall at post- (dark grey) and pre-operation (light grey) with 75% qauntiles over median.

Previous work has shown rapid responses in productivity and changes in community structure of phytoplankton and pelagic heterotrophic bacteria exposed to conditions mimicking brine-discharge[16,23]. As the brine plume from Carlsbad Desalination plant sinks

and is restricted to the bottom water and sediment, we would expect limited impact on the phytoplankton as these organisms are mostly in the upper water column. Frank et al. (2016) recently showed a significant change in abundance and metabolic activity of benthic heterotrophic bacteria in salinity increases of 5%, suggesting these changes could affect higher trophic levels. High salinity has also been shown to significantly decrease the abundance of benthic amphipods[42].

The coastal zone is a highly dynamic environment where many factors contribute to changes in hydrography, water chemistry, and biology. Seasonal changes in wave height, tidal movement, currents and upwelling vary over short (days) and long (months-years) time scales causing dynamic changes in water temperature and chemistry, which affect the distribution and abundance of organisms[40,46,47]. Our results show a clear trend of greater abundance of polychaetes in the outfall area, which can be attributed to the discharge brine. There was also a slight increase in the relative abundance in the opportunistic fauna (BOPA-index) (Figure 5B). While we cannot completely rule out the possibility that the observed differences are related to seasonality, or that the 2015 El Niño affected the benthic communities, it is striking that the opportunistic polychaetes (Paraonidae and Onuphidae) dominate the outfall zone post operation while their abundance was much lower at pre-operation sampling. These opportunistic polychaetes have repeatedly been shown to dominate the benthic fauna under higher than ambient salinities[19,29,48]. The decrease in diversity and conditions favoring opportunistic species within the brine plume are likely community responses to osmotic stress or a response to some other (yet unidentified) stressor within the effluent. In the study conducted by de-la-Ossa-Carretero et al. (2016) implementation of a diffuser system at a desalination plant in Spain enhanced the mixing of the brine with the ambient seawater, which resulted in recovery of the diversity of the benthic fauna with a coinciding drop in BOPA-values[44].

**Impaired growth of brittle stars in effluent brine**

Survival of the brittle stars was not affected by the brine-effluent. Over the course of the experiment, the brittle stars in the control grew on average by 0.16±0.1 mm, while those the stars in the 50% and 100% treatments only grew 0.04±0.09 mm and 0.04±0.02 mm, respectively (Figure 6A). These differences were not statistically significant ($p_{ANOVA}$ = 0.06), probably because of the large variances and a few outliers. The brittle stars' agility, also appeared to be slightly impaired. The brittle stars in the 100% treatment took longer to turn over (on average 7.1 sec) than the controls. In comparison, the control and 50% the stars took an average of 5.3 sec and 4.9 sec to turn around (Figure 6B).

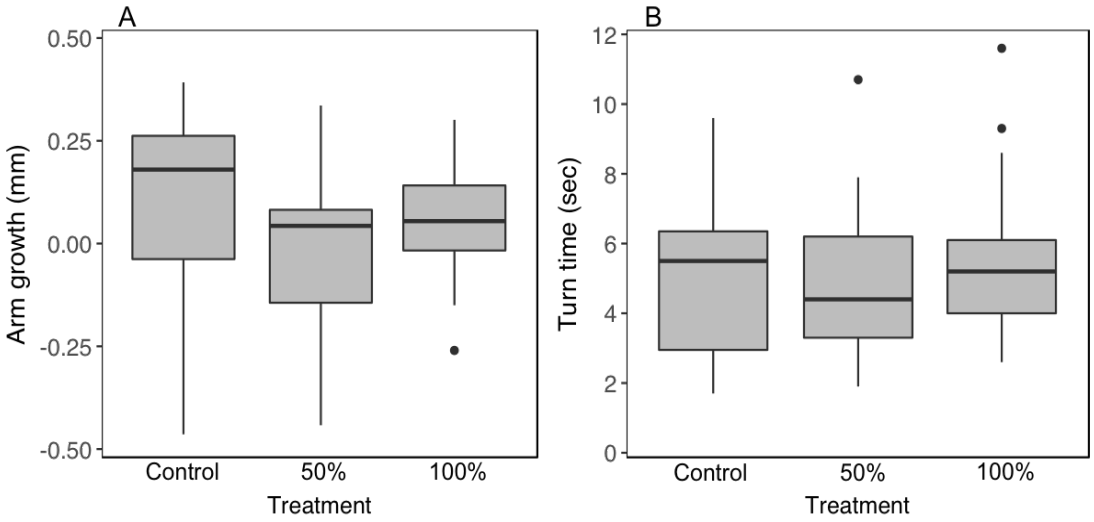



**Figure 6. A:** The relative arm-growth in mm, and **(B)** the turn time in seconds of brittle stars after 5 weeks of incubation.

While the differences in agility were not significantly different, the combination of impaired growth and lower agility suggests the stars are affected by the treatments to a certain degree. Longer experiments should be conducted to verify this trend. Most toxicity tests and studies of resilience of echinoderms have focused on urchins or sea cucumbers, with very few studies on brittle stars[49]. Despite their worldwide distribution, brittle stars have only recently received attention as a key organism for benthic environments[50]. Brittle stars, and echinorderms in general, are important organisms in marine benthic communities as they are considered to be at intermediate trophic levels where they both serve as prey and predator/scavenger and may also serve as both top-down and bottom-up regulators[49–51]. Brittle stars are occur naturally in every marine environment and studies on their adaptability to salinity changes (adult brittle stars) show a large tolerance for species in brackish conditions to marine (salinity ranges from 15 to 30)[49]. The minimal impacts on the stars in our experiment, suggest these adult brittle stars have great tolerance for increased salinity in the order of 5-10% increase. Larval and juvenile stages tend to be more vulnerable, and studies need to investigate the effect on these life stages[51]. Other echinoderms (example urchins) may be more sensitive to salinity changes, and since they are important components in many ecosystems, negative impacts on these organisms may have cascading effects in the locally[51,52].

## Summary

Rocky reefs, kelp beds and seagrass beds are widespread along the coast of California, and these highly productive ecosystems can be sensitive to small changes[52,53]. Several desalination plants have been proposed along the California coast, some in areas with extensive kelp beds. Therefore, it is important to understand the responses of kelp organisms (and the coastal ecosystems) as a whole to the brine effluent discharged from SWRO systems (Figure 7)[54,55]. Previous work has shown higher mortality rates and lower sporulation in the macroalga *Ulva pertusa* in controlled experiments with increased salinities 5-10% over ambient [22]. Similarly, seagrasses are particular sensitive to salinity increases[20,24,56]. We suggest to conduct comprehensive analyses of SWRO brine-effluent on giant kelp (*Macrocystis pyrifera*) and on key echinoderma species, for example purple urchins, that are abundant in coastal California. Our data suggest that the impact of SWRO discharge is relatively limited for organisms inhabiting sandy seafloors which suggests such sites may be preferable for new SWRO facilities, particularly where wave action and mixing are high. Moreover models of plume impact should be realistic using waves and tidal movement as well as hydrologic characteristics. At Carlsbad Beach despite relatively high average wave heights both in summer and winter (Figure 7A and B) mixing was not sufficient to maintain salinity at less than 2 units above ambient within 200 m from shore. In addition more effective discharge schemes (like diffusers) should be considered. Another consideration is proximity to existing operational power plants or wastewater treatment plants, which are already discharging water that could be used to dilute the SWRO brine prior to discharge. We encourage comprehensive modeling of mixing trends that take into account seasonal differences in tides, waves and wind at proposed desalination sites. In addition to reducing the footprint of brine plumes, alternative discharge technologies such as diffuser systems should be considered and the effect of such discharge scheme modeled. Both models and practice indicate that diffuser systems increase the mixing of discharge brine and ambient seawater[13,42,44,45].We further encourage long-term monitoring (ideally every month) of coastal areas within the vicinity of proposed plants both before and after construction and operation.

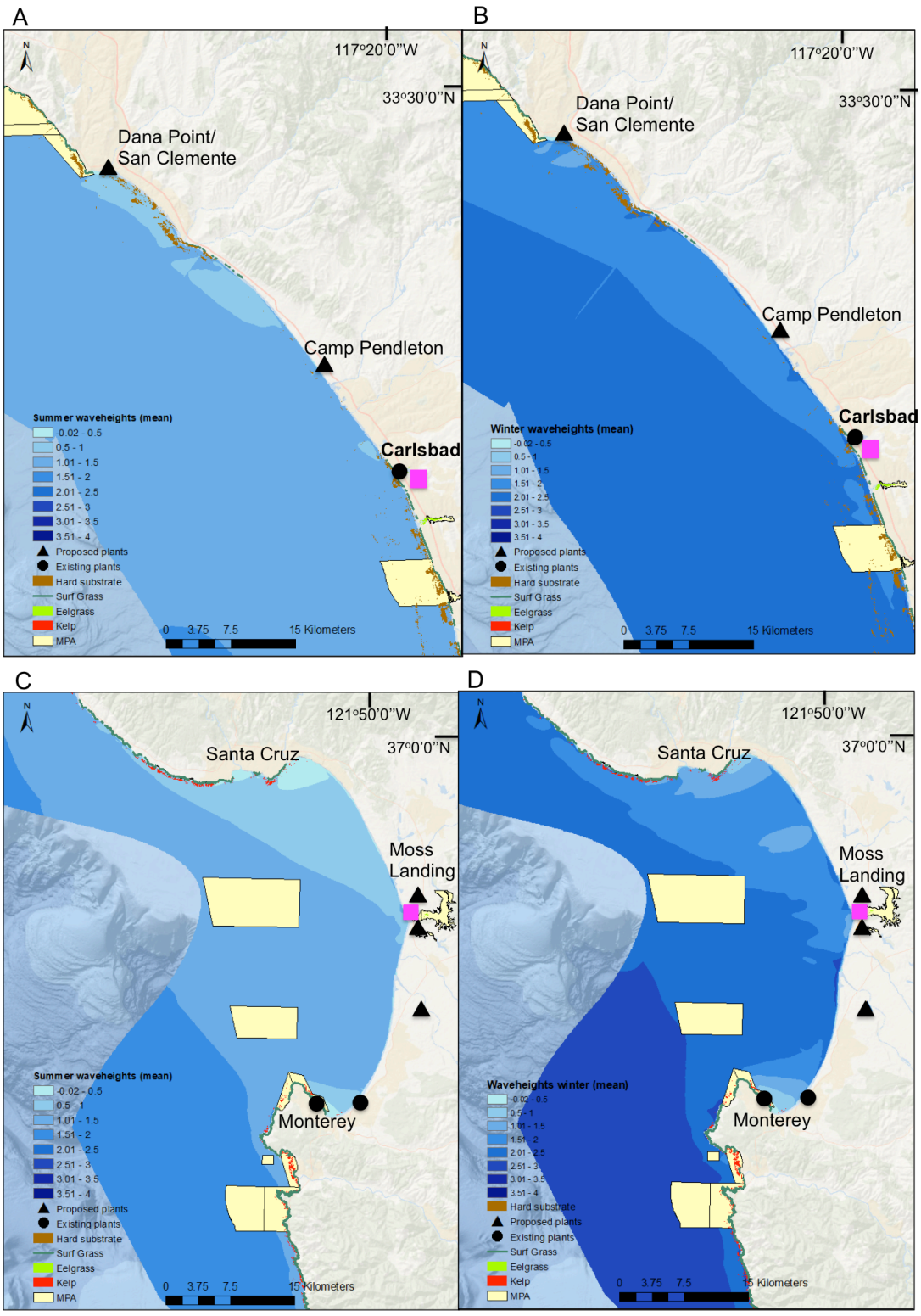



**Figure 1.** The mean wave height in summer (right column) and winter (left column) for Southern California (top row) and Monterey Bay (bottom row) along with major benthic communities. Dots indicate operational desalination facilities, triangles current proposed desalination facilities, pink square operating power plants with coastal discharge.

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# Chapter 2

# Effects of brine and antiscalants on Red Sea hard corals – Potential effects from desalination plants

## Abstract

Seawater reverse osmosis (SWRO) is an important source of potable water for arid and semi-arid regions; however the continuous discharge of brine-effluent from desalination facilities poses potential negative impacts on the local coastal environment. In this study, we examine the impacts of increased salinity and phosphonate (a common additive used in SWRO) addition on three reef-building coral species, *Stylophora pistillata*, *Acropora tenuis* and *Pocillopora verrucosa*, from the Northern Gulf of Aqaba, in the Red-Sea. Coral nubbins of each species were collected and incubated in triplicates in: 1) salinity of 10% (~ 44) above ambient (~ 40), 2) 10% salinity increase + phosphonate-based antiscalant (0.2 mg $L^{-1}$), and 3) unamended controls. Our results indicate that the physiology of all coral species tested was negatively affected by treatments that mimicked SWRO discharge. *Symbiodinium* abundance, protein content and corals-associated heterotrophic bacterial abundance were significantly lower in the treatments than in the control. Further, the net $O_2$ production and calcification rates were significantly reduced, resulting in reduced skeleton secretion and buildup. These results suggest that coral reefs may be susceptible to exposure to SWRO discharge and calls for careful selection of locations of SWRO facilities around coral reefs.

## Introduction

The demand for fresh water is increasing worldwide, mainly due to population growth and agricultural and industrial expansion[1]. In parallel, the amount and quality of fresh water resources are decreasing due to widespread pollution and eutrophication of terrestrial reservoirs, as well as local and regional changes in the water cycle following urbanization, and global change processes[2]. Desalination of seawater is becoming progressively popular as a potable water source and currently has a total capacity of 70 million $m^3$ $d^{-1}$ worldwide, with estimates of doubling that capacity by 2020[2,3]. In the last decade, advances in membrane technologies have favored seawater reverse osmosis desalination (SWRO), which is currently used in 80% of desalination facilities[4,5]. Large-scale SWRO plants draw coastal water as feed and continuously produce high salinity brine-effluent as a by-product, which is discharged back into the coastal environment. The brine can contain chemicals used during the production process such as antiscalants and coagulants[6,7]. Despite the increase in desalination capacity worldwide, the impacts of brine effluent on coastal ecology, biology and geochemistry remain understudied[6,8]. Previous work includes reports on the impacts of brine effluent on seagrasses, benthic copepods, pelagic phytoplankton, and benthic bacteria[9–12]. These studies suggest that response and sensitivity to brine exposure varies considerably between species. Seagrass meadows responded negatively to salinity increases of ~5% over ambient levels[11], whereas phytoplankton and zooplankton seem to be able to tolerate salinity increases of 10% or more above ambient[13,14]. To date, few studies have investigated the impact of desalination brines on corals[8,15,16]. Recent work found no measurable effects of brine discharge on the photosynthetic efficiency of the coral, *Fungia granulosai,* but a significant changes in the mircobiome of the coral[15,16].

Reef building corals (hermatypic) are a large and diverse group of marine invertebrates, making up the large coral reefs worldwide[17,18]. Deteriorating environmental conditions, including high temperatures[19–21], nutrients[22–26] and low pH[27,28] can impact calcification in hermatypic corals. Corals maintain their cellular osmotic balance by keeping their internal osmolyte concentration equivalent to that of the surrounding seawater[18,29]. Corals can encounter osmotic stress from changes in ion concentration both externally (*e.g.,* salinity increase or decrease) and internally (*e.g.,* stress related cellular build-up of reactive oxygen species)[29]. These stressors can cause corals to bleach[29–31]. Most hermatypic coral species have a symbiotic relationship with photosynthetic algae of the genus *Symbiodinium* embedded in the tissue of the coral host. *Symbiodinium* provide the corals with energy (sugar

by-product of photosynthesis) in exchange for inorganic nutrients and protection[31,18]. It is possible that the *Symbiodinium* may also be impacted directly by brine discharge with consequences for the coral host. Therefore the construction of large-scale coastal desalination facilities in proximity to coral reefs is a major concern.

The Gulf of Aqaba (GoA) is an inlet of the Red Sea bordered by Israel, Egypt, Jordan and Saudi Arabia. It is a deep (1825 m), long (180 km) and narrow (10-20 km) semi-enclosed basin that is connected to the Red Sea by a narrow (5 km) shallow sill (252 m) at the Straits of Tiran. The GoA is a relatively warm hypersaline water body with mean salinity of ~40.5-41 and average surface water temperatures varying from ~26 $^{o}$C in summer (August) to ~21 $^{o}$C in winter (February)[32]. The GoA sustains more than 200 coral species and may serve as a refuge for future reefs[33]. Currently, small-scale desalination facilities (capacities of 2000-8000 $m^3day^{-1}$) using multi-stage flash technology or brackish water reverse osmosis are present along the coast of Egypt, Israel and Jordan[34], and due to the extreme aridity of this region, large-scale SWRO desalinations facilities have been suggested in Eilat (IS) and Aqaba (JO)[35]. However, the brine discharge from such large-scale facilities could pose threats to the coastal ecosystem which, in turn, could affect coral reef well being and the economies and populations that depend on the reefs. It is therefore important to assess the potential impacts of brine-discharge on coastal coral reefs to ensure that best practices that reduce the impact are considered.

In this study, we investigated the effects of exposure to desalination brine (including phosphonates) on the physiology and growth of three common reef-building coral species in the GoA, *Stylophora pistillata, Acropora tenuis*, and *Pocillopora verrucosa*. Two treatments were tested: 1) 10% elevated salinity over the ambient levels; and 2) 10% above ambient salinity + 0.2 mg $L^{-1}$ antiscalants (poly-phosphonate). Results were compared to corals kept in control conditions (ambient seawater). These treatments simulate a scenario to mimic an area of discharge where coastal mixing with seawater is limited.

## Methods

### Experimental setup and sample collection

Five coral colonies of each species *Stylophora pistillata, Acropora tenuis*, and *Pocillopora verrucosa* (referred to as Sty, Acro, and Poc, respectively herein) were collected in early June 2016 at the underwater nursery in the Gulf of Aqaba near the Inter-University Institute for Marine Sciences (IUI) in Eilat, Israel. The colonies were broken into nubbins of 3-4 cm height,

glued onto standing bases, and placed randomly in nine 30 L aquaria, resulting in three replicates of each treatment. Each aquarium contained 8 nubbins of each species, resulting in 24 nubbins per species per treatment. The tanks were shaded to simulate radiation equivalent to ~3 m water depth (~90 µmol quanta $m^{-2}$ s in mid noon, LI-CORLI-250A), and the fragments were left to acclimate for 3 weeks in a flow-through ambient seawater tank. Following the acclimation period, the following treatments were initiated: 1) Saline (Sal): 10% increase over ambient salinity (*e.g.,* from a salinity of 41 to 45), 2) Antiscalant (Ant): 10% increase over ambient salinity and polyphosphonate ($C_9H_{21}N_3O_{15}P_5Na_7$, Diethylenetriamine pentamethylene phosphonic acid - DTPMP) at a final concentration of 0.2 mg $L^{-1}$. Three aquaria were selected as control with ambient seawater (salinity of ~41). The Sal and Ant treatments were prepared by adding 4 g $L^{-1}$ of Red Sea to two tanks of 1000 L of ambient seawater to increase the salinity to ~45 and the polyphosphate antiscalant was added to one of the tanks. Water flow to the aquaria was maintained at ~1 L $hr^{-1}$ using peristaltic pumps, resulting in replacement of the aquaria water volume at least twice daily. Salinity, temperature and pH were measured daily with a multi-parameter water quality probe (WTW Multi 3500i). Minimum, maximum, and mean temperature, salinity and pH are reported in Table 1. The incubations were maintain until clear visible changes in coral health were observed, which was after 4 weeks for the Sal treatment, and after 2 weeks for the Ant treatment.

**Table 1.** Minimum, maximum, and mean temperature, salinity and pH measured for three treatments during the experiment. pH measurements are normalized to 25 $^{o}$C.

| Treatment | Temperature, $^{o}$C | | | Salinity | | | pH at 25 $^{o}$C | | |
|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean |
| **Con** | 25.1 | 30.8 | 26.8 | 40.6 | 42.6 | 41.1 | 8.17 | 8.50 | 8.27 |
| **Sal** | 24.7 | 29.8 | 26.5 | 45.3 | 46.5 | 45.1 | 8.07 | 8.46 | 8.22 |
| **Ant** | 24.8 | 29.7 | 26.3 | 44.7 | 47.4 | 44.3 | 8.16 | 8.44 | 8.24 |

Respiration and photosynthetic activity were measured weekly (using Walz Imaging-PAM M-series), and every other week (1 day, 2 weeks and 4 weeks) a nubbin of each species from each aquarium was collected (3 nubbins per treatment per species) for additional physiological analyses. The coral's tissue was removed and analyzed for total protein content, and heterotrophic bacterial and *Symbiodinium* abundances. Bacterial production, coral calcification rates, and the combined coral and symbionts net production rate were also measured prior to tissue removal.

**Sample preparation and laboratory analysis**

Coral tissue was removed with an airbrush using filtered seawater. All tissue samples were diluted to 25 ml and homogenized by sonication for 10 min at >20 kHz before separation into aliquots that were prepared for various analyses (see below). Surface area was calculated using hot wax as described by Stimson & Kinzie (1991)[36].

*Bacteria and Symbiodinium abundance*

Tissue samples were immediately fixed with 50% glutaraldehyde solution, frozen in liquid nitrogen, and kept at -80 $^{o}$C. Upon analysis, samples were fast-thawed in a 37 $^{o}$C water bath and analyzed on an Attune Nxt Acoustic Focusing Cytometer with 1µm beads as standards. Taxonomic discrimination of *Symbiodinium* was done using cell side scatter, cell forward scatter and red fluorescence (630 nm). Heterotrophic bacteria were first stained with SYTO 9 Green Fluorescent Nucleic Acid Stain and determined based on green fluorescence at 488 nm against side scatter (Belkin et al., 2015).

*Protein content*

Frozen (-20 $^{o}$C) tissue samples were thawed at room temperature and 1 ml of 1:4 Lauber Buffer was added. The samples were sonicated 3 times for 30s at 50 kHz and centrifuged for 10 min at 12000 rpm. The supernatant was collected in a new tube and the pellet was discarded. The supernatant was then added in triplicates to sample wells, and 200 µL of reagent mixture (Pierce BCA Protein Assay Kit) was added. The plates were incubated for 30 min at 37 $^{o}$C before analysis on a Infinite M200 Tecan. Concentration was normalized to coral surface area.

*Net production and calcification*

Coral nubbins of *Stylophora* and *Acropora* from each treatment were incubated for 1.5-2 h in 700 ml beakers filled with water from the corresponding treatments. At the end of the incubation, dissolved oxygen (DO), pH, salinity, and temperature were measured (WTW Multi 3500i). Water samples were obtained for dissolved inorganic carbon (DIC) and total alkalinity (TA). Samples of DIC were immediately poisoned with 0.05% vol/vol saturated $HgCl_2$ solution and kept refrigerated with the TA samples until analysis, within 1-2 weeks of sampling. TA was determined by potentiometric Gran titration of ~ 22 g subsamples, filtered through Whatman GFF 0.45 µm filters using a Metrohm Titrino 785 Titrameter with a temperature corrected pH probe and titrated using HCl 0.05 N. Calculation of TA employed the method described by Sass & Ben-Yaakov (1977)[37]. Measurements were calibrated using seawater CRMs from A. Dickson's lab (Batch #155). The precision of these measurements was ±2 µmol kg$^{-1}$ (2 measurements per sample). DIC was extracted from the 1.6 mL sub-samples by acidifying them with phosphoric acid ($H_3PO_4$, 10%) using a custom, automated $CO_2$ extractor and delivery system (AERICA by MARIANDA) and high grade $N_2$ (99.999%) was used as a carrier gas connected on line with a LiCor 6252 IR $CO_2$ analyzer. Measurements were calibrated using seawater Dickson CRMs (Batch #155). The repeatability of the measurements was ±1.5±1.0 µmol kg$^{-1}$ (mean ± STD of all measurement errors, n=32).

Daytime net production due to photosynthesis and respiration, were calculated as a function of DO production and DIC consumption during the incubation period ($P_{net\text{-}DO}$, µmol $O_2$ L$^{-1}$ hr$^{-1}$, $P_{net\text{-}DIC}$, µmol DIC L$^{-1}$ h$^{-1}$, Equations 1, 3). Net production of $CaCO_3$ (G, µmol $CaCO_3$ kg SW$^{-1}$ hr$^{-1}$) was calculated as a function of TA uptake during the incubation period (Equation 2). In equation 1-3, the i and f indices refer to initial and final concentrations at the beginning and end of each incubation period [28].

$$(1)\quad P_{net-DO} = \frac{\left(DO_f - DO_i\right)}{\Delta t}$$

$$(2) \quad G = \frac{0.5 \cdot (TA_i - TA_f)}{\Delta t}$$

$$(3) \quad P_{net-DIC} = \frac{(DIC_i - DIC_f) - 0.5 \cdot (TA_i - TA_f)}{\Delta t}$$

*Bacterial production (BP)*

Bacterial production was measured using the $^{3}$H leucine incorporation technique[38]. Coral fragments (~0.5 cm) were cut and incubated with 4 ml of filtered seawater (0.2 µm) amended with Leucine-[4,5-3 $^{3}$H] (Perkin Elmer, specific activity: 160 Ci mmol$^{-1}$) at a final concentration of 100 nmol L$^{-1}$. The coral fragments were incubated for 4 h in the dark at ambient temperature. At the end of the incubation, the fragments were sonicated and subsamples of the water (1.7 ml) were transferred to Eppendorf tubes and amended with cold 100% trichloroacetic acid (TCA). Samples treated with TCA at time zero were used as a control. The samples were later processed following the micro-centrifugation technique[39]. After adding 1 mL of scintillation cocktail (Ultima-Gold) to each tube, the samples were counted using a TRI-CARB 2100 TR (Packard) liquid scintillation counter. A conversion factor of 3 kg C per mole leucine incorporated was used, assuming an isotopic dilution of 2.0[40]. Total carbon uptake rate was normalized to surface area of the coral piece.

*Photosynthetic efficiency*

Coral nubbins were dark-acclimated for a minimum of 15 minutes before transfer to a Walz Imaging-PAM M-series for analysis. The image of each nubbin was analyzed using Imaging-Win software (v. 2.00m; Walz Gmbh) to obtain the dark-level fluorescence yield ($F_0$) and the maximum fluorescence yield ($F_m$) used to calculate the maximum quantum yield $F_v/F_m$ [41].

**Data and statistical analysis**

We measured the corals' responses as changes through time, and we present our data as the difference from the control values measured at the end of the experiment (2 and 4 weeks for Ant and Sal treatment, respectively). *Symbiodinium* and heterotrophic bacteria abundance and protein content were measured 1 day after the incubation begun to evaluate short term responses. The photosynthetic efficiency ($F_v/F_m$) is presented as a time series for each coral in each treatment. One-way ANOVA was conducted to test for decipherable statistically significant trends between treatments and control, between both of species and time ($\alpha$=0.05). Table with values and significance levels is provided in the supplementary material.

Principal component analysis (PCA) was done on protein concentration, *Symbiodinium* and heterotrophic bacteria abundance, and bacterial production at the end of the experiment (2 or 4 weeks).

## Results and Discussion

This study examines the impacts of brine and antiscalants used in the SWRO desalination process on three key corals species in the GoA. Work was conducted in the GoA where unique coral reefs with large biodiversity benefit tourism and local economies, and where plans for new SWRO desalination facilities are underway to address water needs of the growing population in this arid region[42]. Concerns have been raised about the discharge of high-salinity brine from SWRO facilities and specifically their impact on corals. In most countries, regulation and discharge methods are implemented to prevent salinity increases of more than 10% above ambient[43–45]. However, 5-10% increases in salinity in the close vicinity of outfalls have been reported[8,46–49]. The treatments in our experiment, therefore, are environmentally relevant and represent a worst-case but possible scenario of SWRO brine-effluent discharge into a near-shore environment of limited tidal and wave mixing (such as in the Gulf of Aqaba).

### Changes in corals physiology

At the end of the incubation (2 and 4 weeks for Ant and Sal treatment, respectively), the corals in both treatments showed a general reduction in *Symbiodinium* abundance, protein content, $O_2$ production and calcification rates compared to the control (Figures 1-5). The corals in the Sal and in the Ant also showed signs of bleaching; their tissues were visibly paler and the polyps more retracted in corals from these treatments than in the corals in the control (Figure 1).

Photosynthetic efficiency ($F_v/F_m$) varied between the species and treatments (Figure 2). For all three species, $F_v/F_m$ was very variable relative to the control for the first 72 hours with values going from 0.55-0.65 for Acro, 0.5-0.6 for Poc and 0.52-0.62 for Sty, than later on. A week into the incubation, $F_v/F_m$ leveled out with means of 0.62 for Acro and 0.60 for Poc and Sty. Changes during the first days of incubation suggest that *Symbiodinium* are responding to an immediate stress and, as time progresses, they recover. There was no significant difference in the values. A similar response was observed by Kuanui et al. (2015) on cultivated *Symbiodinium* from the coral *Pocillopora damicornis*, *Acropora millepora* and *Platygyra sinensis* in response to increased salinities, where changes in $F_v/F_m$ were apparent

on the first day of exposure, but had stabilized after 30 days of exposure the $F_v/F_m$ [50]. Similarly, van der Merve et al. (2014) found no changes in $F_v/F_m$ in *in-situ* measurements of *Fungia granulosa* in a brine discharge area [15]. This indicates that *Symbiodinium* are able to adapt quickly to the condition.

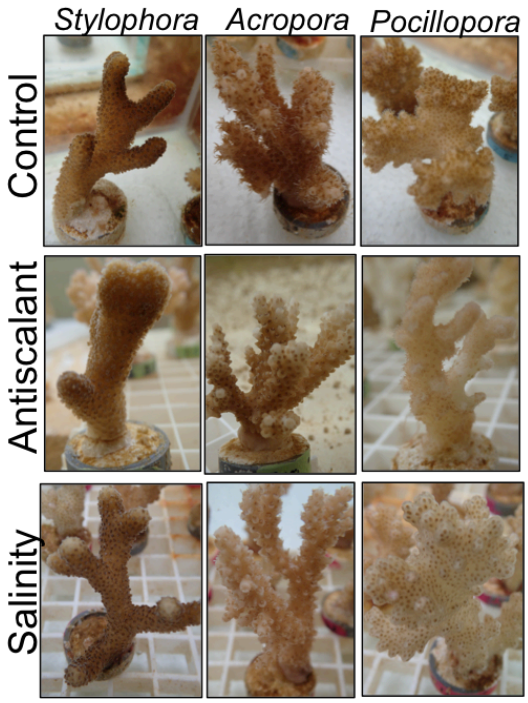


**Figure 1.** Coral fragments collected at the end of the incubations of *Stylophora* (left), *Acropora* (middle) and *Pocillopora* (right), in control (top), antiscalant (middle) and salinity (bottom) treatment.

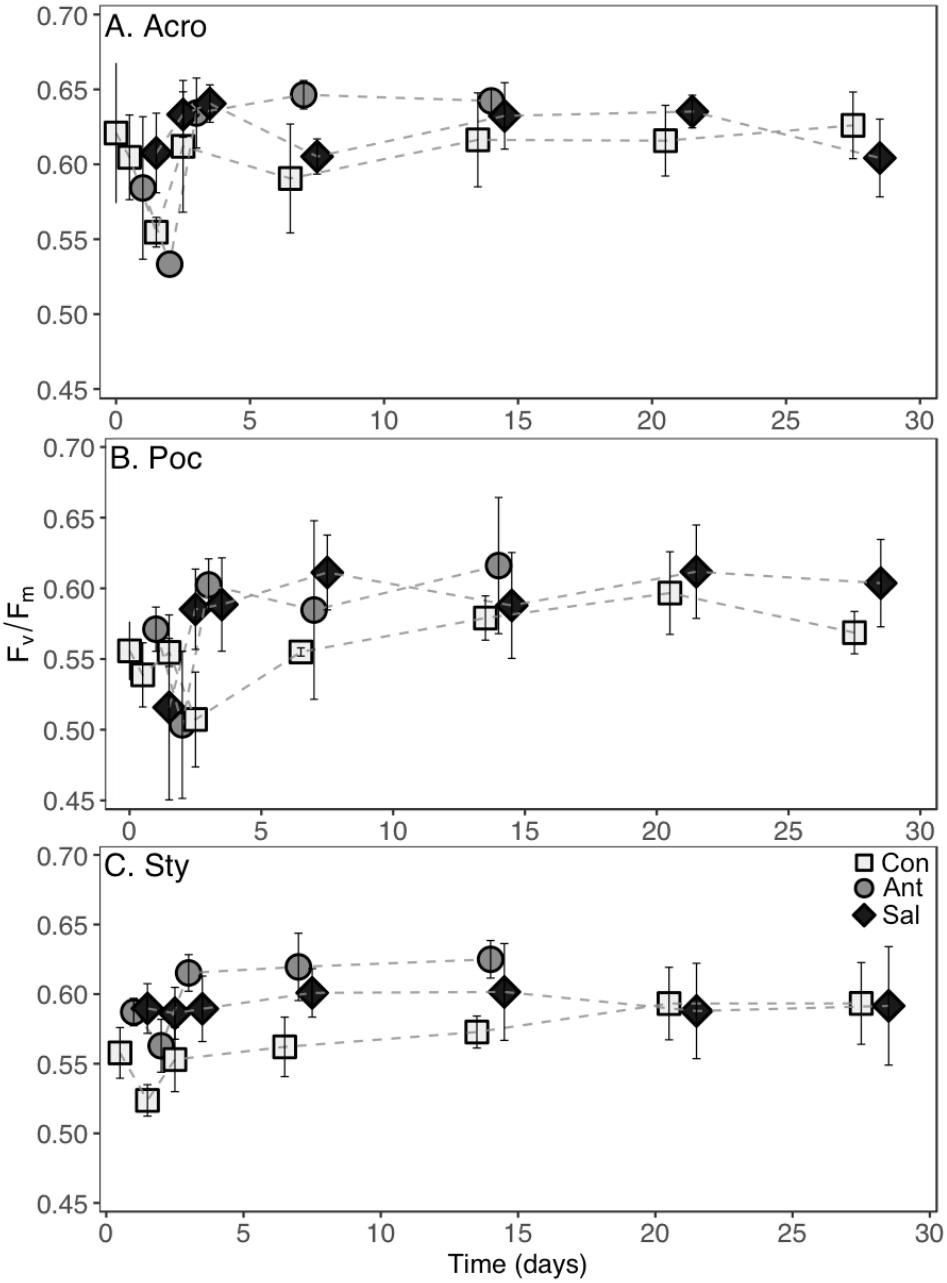


**Figure 2.** Photosynthetic efficiency ($F_v/F_m$) ±S.D for *Acropora* (**A**), *Pocillopora* (**B**) and *Acropora* (**C**) in control (light grey circles), Ant treatment (grey squares) and Sal treatment (black triangles) over the course of the experiment. Error bars corresponds to standard deviation.

In contrast, the *Symbiodinium* abundance was significantly reduced at the end of the incubation in both treatments, with decreases of 50-95% from control ($p < 0.001$) (Figure 3). This suggests that the remaining *Symbiodinium* are compensating for the reduced

abundance, and keep the photosynthetic efficiency at levels equal to the control. Comparing *Symbiodinium* abundances after 1 day and at the end of the incubation, there were different responses for each species and treatment: In the Sal treatment, *Symbiodinium* increased in all three species after 1 day (10%, 150% and 50% for Sty, Poc and Acro) (Figure 3A), whereas the *Symbiodinium* abundance in the corals in the Ant treatment decreased by 80%, 95% and 75% for Sty, Poc and Acro, respectively (Figure 3B). After 4 weeks, the *Symbiodinium* abundances in the corals in the Sal treatment were reduced by 90%, 80% and 50% for Acro, Poc and Sty. This indicates that the short term reduction of $F_v/F_m$ was accompanied by higher *Symbiodinium* numbers, possibly as a compensation mechanism. Over time as $F_v/F_m$ stabilized, the *Symbiodinium* abundances were lower than in the control, suggesting that the overall response is negative (photosynthetic efficiency stable but *Symbiodinium* density lower). The addition of phosphonate appeared to have reduced the *Symbiodinium* abundance already at day 1 while $F_v/F_m$ stabilized the abundances remain low.

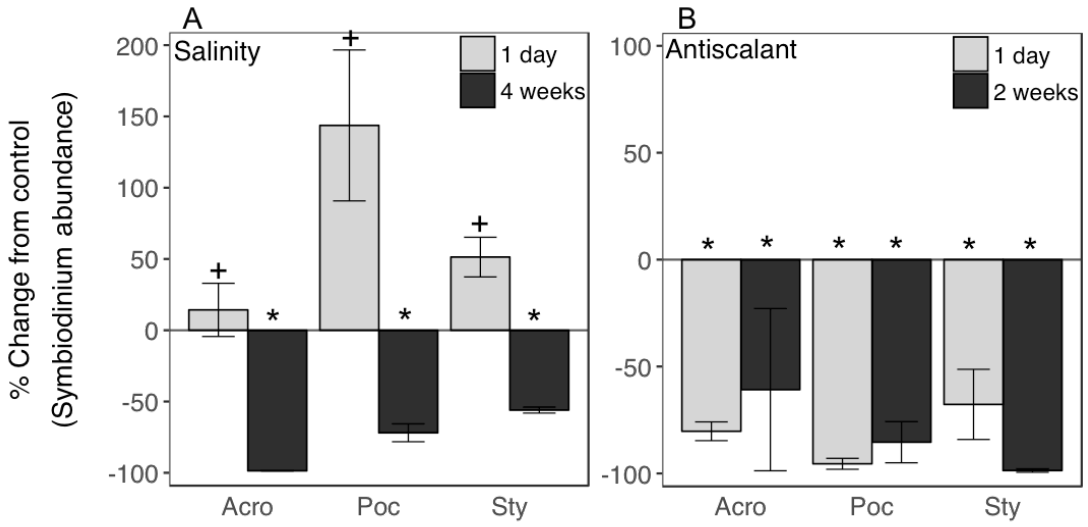


**Figure 3.** The percent change ±S.E from control in *Symbiodinium* abundance for three species in Sal treatment (**A**) and Ant treatment (**B**) at day 1 (light grey bars) and at the end of the experiment (4 or 2 weeks, dark grey bars). * $p < 0.001$, + $p < 0.05$ with.

Because of the symbiotic relationship between corals and *Symbiodinium*, a reduced symbionts abundance will result in less energy for the corals, which impacts growth and reproduction of the coral[31]. Previous studies confirm this species-specific response to salinity changes: In short-term exposure studies (5-7 days), *Porites* sp. and *Siderastrea* sp. had discolored tissue and retraction of polyps in salinities 10-15% above ambient seawater, along with decreased net productivity[51–53]. The protein content was generally lower in the Ant

treatment both after 1 day and at the end of the experiment compared to the changes seen in the Sal treatment: ~45% decrease for Acro and Poc and 25% increase for Sty at day 1, and a further decrease of 60%, 55%, 80% of control for Acro, Poc and Sty, respectively after 2 weeks (Figure 4). The Sal treatment had a less severe impact, with protein concentrations reduced by 45% for Acro at both 1 day and at the end of the incubation, a 180% increase after 1 day and 50% decrease at the end for Poc, and a 50% increase followed by a 50% decrease for Sty ($p < 0.05$). The large differences in response between the corals again indicate species-specific responses to the treatments. The combined effect of higher salinity and antiscalant resulted in larger differences than in the control (less *Symbiodinium* and protein) suggesting an additive effect of multiple stressors.

This is, to the best of our knowledge, the first study published on the effects of phosphonate on corals. Accordingly, we compare our results to studies of impacts of increased phosphate on corals assuming phosphonate could serve as a phosphate source[54,55]. Previous studies on elevated phosphate concentrations around corals have not established any consistent trends between to *Symbiodinium* abundance and protein contents, but have found reduced calcification and impaired reproduction[31,56–59]. Our results indicate that phosphonate can have severe impacts on coral physiology too.

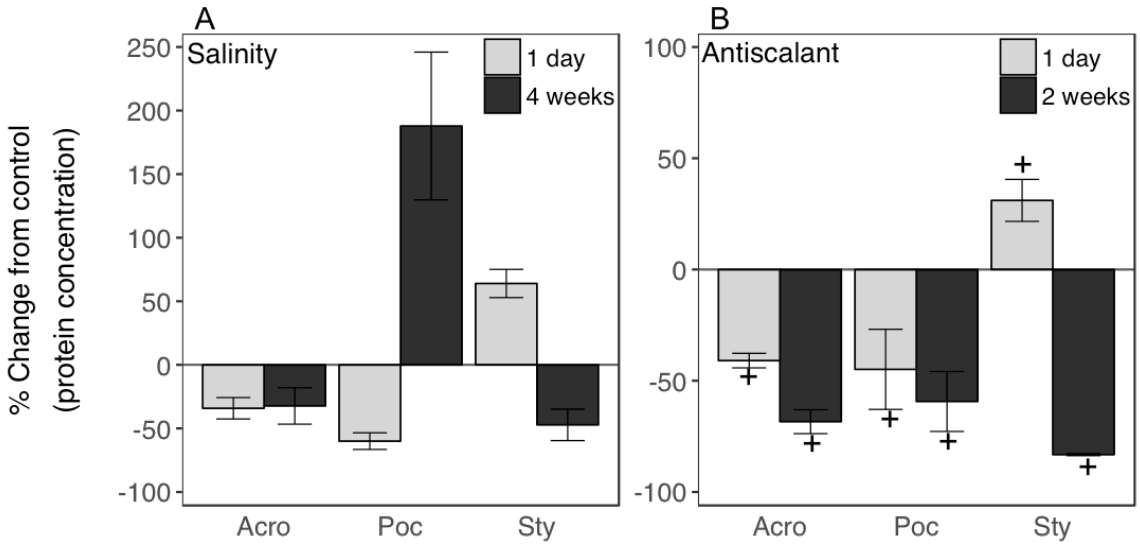


**Figure 4.** The percent change from control in protein concentration ±S.E for all three species in Sal treatment (**A**) and Ant treatment (**B**) at day 1 (light grey bars) and at the end of the experiment (4 or 2 weeks, dark grey bars). Significance level of $p < 0.05$ indicated with +.

Net $O_2$ production, respiration and calcification were significantly reduced in both the Sal and Ant treatments for Acro and Sty compared to control ($p < 0.05$) (Figure 5A and B). For production and respiration, the Sal treatment had a slightly greater impact on Acro (~60% reduced for production and respiration) compared to Sty (~50% reduced), however the Ant treatment had opposite responses with Acro reduced by ~45% and 55%, and Sty reduced 60% and 80% for production and respiration, respectively. In previous work high salinity decreased productivity, which could be attributed to retraction of polyps and/or the loss of *Symbiodinium*[51,60]. In our samples, *Symbiodinium* abundances were reduced and the polyps were more retracted in the treatments when compared to the control. In a short-term study (4 hr) of exposure to hypersaline water (40% above ambient) coral *Fungia granulosa* had a significant drop in calcification rates and oxygen production, however with long-term exposure (29 days) to salinities levels 15% above ambient, the corals appeared able to acclimate to the conditions and return to ambient oxygen production levels[16].

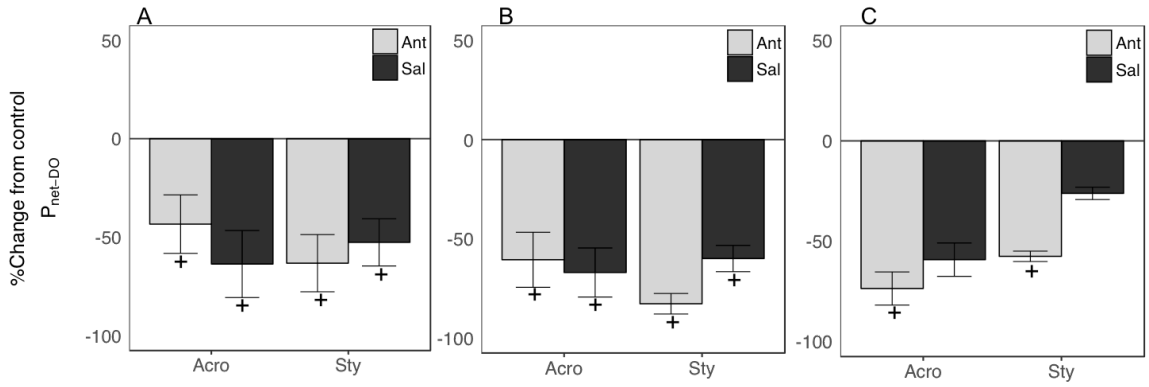


**Figure 5.** Percent changes from control at the end of the experiment for $O_2$ production ±S.D (**A**), respiration (**B**) and calcification (**C**) in the Ant and Sal treatment for *Acropora* and *Stylophora.* Significance level of $p < 0.05$ indicated with +.

The Ant treatment had greater impacts on calcification rates of Acro and Sty with reductions of 75% and 55%, compared to the Sal treatment, which had reduced calcification by 60% and 25% for Acro and Sty compared to control (Figure 5C). There are currently no published data on corals' responses to phosphonate, but in long-term (years) *in-situ* studies with increased phosphate concentrations, calcification increased in *Acropora* sp. and declined in *Pocillopora* sp. and *Stylophora* sp.[25]. Other studies have also shown no impacts or slight decreases in calcification and *Symbiodinium* abundance in increased phosphate concentrations[56,61]. These studies indicate species-specific responses to such stressors, increased salinity and phosphonate, as in our study.

For all measured parameters, most trends are generally similar in all species and both treatments (*e.g.* increase or decrease relative to control), but there was great variability in absolute changes both between and within species. This may be related to the environmental settings and growth conditions of the individual coral colonies used in the experiment. Coral colonies of the same species can have distinct responses to stressors, as seen in large bleaching events where responses of adjacent colonies of the same species are not identical[62–64]. This is mostly attributed to genetic variation in both the corals and their corresponding *Symbiodinium*[65].

In most studies, significant responses are documented only when salinities are at or greater than 10% above ambient seawater, indicating a generally high tolerance in changing salinity, and sufficient osmoregulation capabilities for many species of corals[51–53]. However, due to biological and environmental variability, physiological responses of hard corals to salinity, and possibly other stressors, are likely to be specific to the local coastal environment. Future studies should assess corals' salinity thresholds at individual reef sites where desalination plants are expected to operate. Our results do not show signs of acclimation, and respiration and production were lower than controls, even after weeks of exposure, suggests that a 10% salinity increase is sufficient to negatively impact coral calcification.

Coral recovery was not investigated in this experiment, and it is possible that the observed effects are transitory. However, as SWRO plants discharge brine continuously, any corals in the vicinity of a discharge pipe or outfall area are likely to be in constant contact with the discharge effluent with either opportunity for acclimation, competition interactions among species or reduction in coral cover.

## Changes in coral-associated microbial communities

The abundance of heterotrophic bacteria associated with corals had different responses in the Sal treatment compared to the Ant ($p < 0.05$) (Figure 6). In the Sal treatment, bacterial abundance did not change over time for Acro (~15% increase compared to control), for Poc there was a 25% reduction after 1 day and a ~50% increase after 4 weeks, and for Sty it was reduced by 50% after 4 weeks (Figure 6A). In the Ant treatment, the microbial activity associated with Acro did not seem to be severely impacted, as the initial response (1 day) was a slight increase in abundance (15% higher than control) that is then declined over the experiment to 25% less than control (Figure 6B). Poc and Sty abundances were reduced by

~75%, both after 1 day and at the end of the experiment. Sty experienced a greater change in abundance from 50% at day 1 to 95% reduction after 2 weeks.

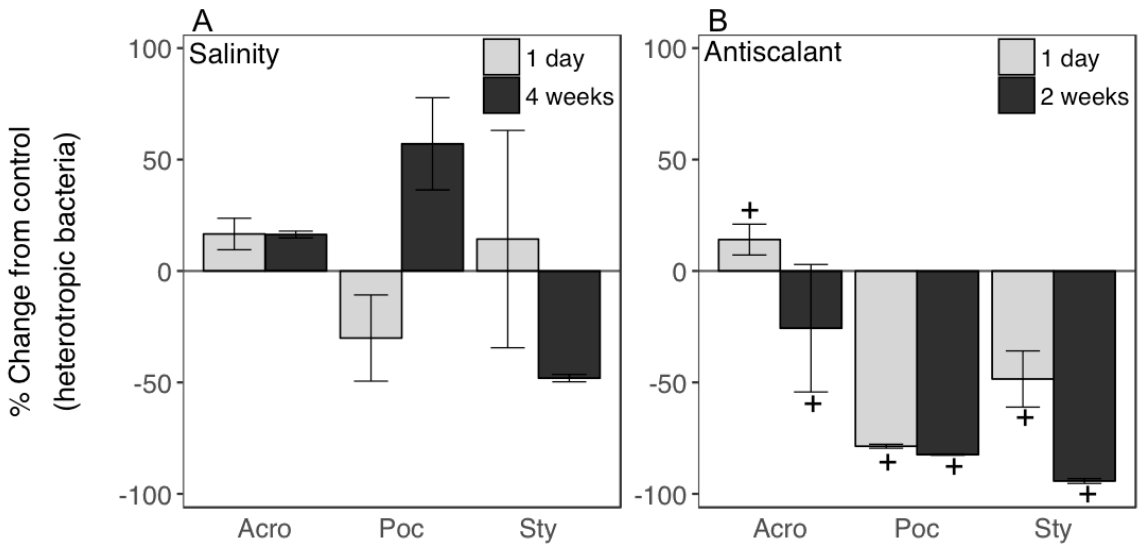


**Figure 6.** The percent change from control in heterotrophic bacterial abundance ±S.E for all three species in Sal treatment (**A**) and Ant treatment (**B**) at day 1 (light grey bars) and at the end of the experiment (4 or 2 weeks, dark grey bars). Significance level of $p < 0.05$ indicated with +.

The bacterial production however is significantly higher for all three species in the Ant treatment ($p_{ANOVA} < 0.001$). The Sal-treatment reduces bacterial production by 20% and 50% relative to control for Acro and Poc, respectively and increases the production for Sty by ~200% (Figure 7A). The Ant treatment increases the activity by 500% for Acro and orders of magnitude for both Poc and Sty (4500% and 1500% increase respectively).

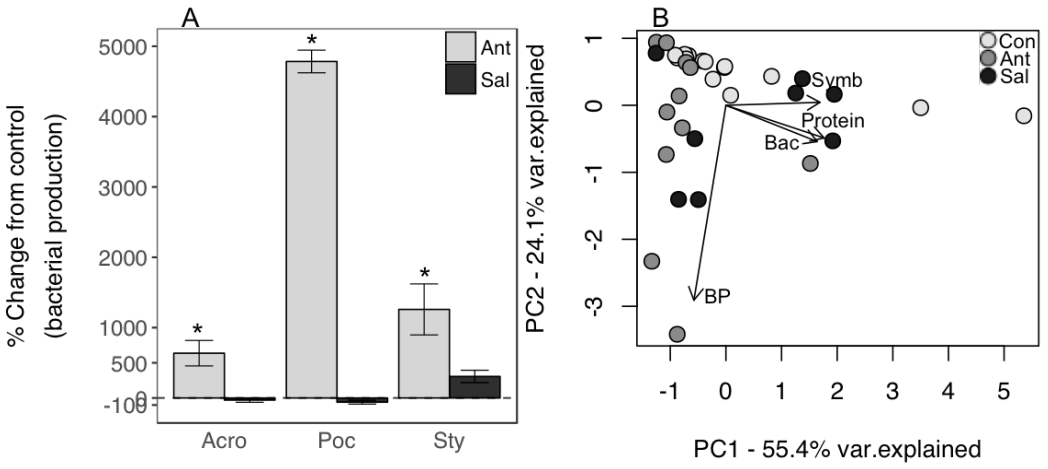


**Figure 7. A**: The percent change from control in bacterial production ±S.E at the end of the experiment for all three species of coral. Significance level $p < 0.001$ indicated with *. **B**: PCA plot of *Symbiodinium* abundance (Symb), protein concentration (Protein), bacterial abundance (bac) and bacterial production (BP)

In the past decade, phosphonate has been recognized as an important source of phosphorous for marine bacteria[54,66], and addition of a phosphorous source in the Ant treatment could support higher production of bacteria associated with the corals in this treatment, compared to bacteria in the Sal treatment. This could be especially true for microbial communities in an ultra-oligotrophic environment (as GoA) that is extremely P-limited during the summer. Bacterial production seems to be driven by the Ant treatment whereas the endo-symbiotic analysis (*Symbiodinium*, protein and bacterial abundance) shows stronger relation with the Sal treatment and controls (Figure 7B). The increased production likely corresponded to the epi-bacterial community being favored by the sudden increase in P, and not by microbes imbedded in the corals.

Coral-associated bacterial communities undergo restructuring following events of coral bleaching and rapid environmental changes, and this restructuring could increase the risk of coral diseases[16,67–71]. In a study of coral *Fungia granulosa*, there was a significant restructuring of the microbiome upon prolonged exposure of bacterial taxa to high salinity associated with increased osmolyte production[16]. The differences in bacterial abundance and production reported herein may be the result of a similar shift in microbiome communities. Bacterial community structure has also been shown to change in the water column, and in association with pelagic plankton, under increased salinities in mesocosm experiments[14].

Belkin et al (2017) also show that phosphonate-based antiscalants can enhance bacterial productivity as it relieves P-limitatio [10]. Our results show that coral microbiomes are affected by increased salinity and addition of antiscalants, however a more in-depth genetic analysis of changes in the species and diversity of bacterial taxa associated would provide greater insight into the drivers of changes.

## Summary and Conclusion

For almost all parameters measured in this study, we saw reductions in the high salinity treatments compared to controls. As corals live in symbiosis with *Symbiodinium*, and have a diverse microbiome surrounding their tissue[31], impacts on one component of the coral biome could be followed by impacts on other components. The different responses in our data can be attributed to species-specific responses to stressors.

This study confirms that high concentrations of desalination brine could be damaging to local coral reefs, and indicates a need for more research on the direct effects of antiscalants and the synergistic effects of temperature, salinity increase and antiscalants, and also coagulants used in SWRO but not investigating in this study. Monitoring reef health and adapting conservation management strategies in the vicinity of desalination plants can minimize impacts. Specifically, we show that increased salinity and antiscalants can be harmful to reef building corals, and strongly suggest monitoring and survey programs prior to installation of new SWRO facilities and implementing of proper discharge dilution systems (*e.g.,* diffusers).

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# Chapter 3

# Impacts of seawater desalination on coastal environments

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## Abstract

Seawater reverse-osmosis desalination facilities discharge brine-effluent with potential implications to the integrity of marine coastal environments. Typical desalination brine-effluent consists of hypersaline-seawater along with additional antiscalants and coagulants, which are often mixed with cooling-water of adjacent power-plants. The spatial distribution of the brine-plume, namely the perimeter, flow direction and buoyancy, vary according to the volume and rate of discharge as well as the dispersion technology. In this chapter, we will present a comprehensive overview highlighting the environmental effects of brine-effluent on various coastal species, including bacteria, zooplankton, seagrass, fish-larvae and corals. Recent studies have suggested that desalination brine-effluent may alter the activity and diversity of bacteria and microalgae, reduce the abundance of meiofauna, as well as impact the physiology of seagrass meadows around the outfall site. Following the above, we will discuss possible means and measures to monitor and minimize the interface of brine-effluent with marine coastal biota.

## Introduction

Arid and semi-arid regions of the world are under constant water stress as they attempt to accommodate the growing need for food and potable water [1–3]. Current limitations in freshwater availability have stimulated the use of desalination technologies worldwide, with desalinated water coming online at a rate of ~85 M $m^3$ $d^{-1}$ [4,5]. Advances in membrane technology during the last decades have favored reverse osmosis, which currently accounts for more than 80% of the global desalination industry [6,7]. Large-scale desalination facilities that are based on seawater reverse osmosis (SWRO) use coastal water as feed and continuously discharge similar volumes of concentrated brine-effluent back into the marine environment. The SWRO brine-effluent contains high concentration of salts, as well as various chemicals such as antiscalants and coagulants if used in the facility and discharged with the brine-effluent [8,9]. Brine-effluent from large-scale desalination facilities is discharged back to the coastal environment via direct surface discharge on the coastline or via diffuser systems away from the shore (up to 2 km) [9–11]. To maximize dilution and increase the buoyancy of the SWRO brine-effluent, it is often mixed with cooling water of adjacent power-plants resulting in a warm (up to 25% over ambient) and saline (up to 10% over ambient) brine-effluent plume. Nevertheless, the brine-effluent can be denser than ambient seawater (depending on the volume of cooling-water that is used for mixing) [12]. In such cases the brine-effluent tends to sink and flows as a concentrated stream on the seabed [12–14]. It has been previously pointed out that such dense saline plumes can extend further away from the discharge site along the seafloor (Table 1) and possibly accumulates within the sediment pore water. The brine-effluent plume is dynamic and dispersed along with the cooling-water outflow from the adjacent power-plant (in the case of surface-discharge that is diluted) or from the outflow-pipe in cases of direct dispersion by diffuser systems. The dispersion of the brine-effluent plume can also be affected by the bathymetry of the seabed at the outfall region. Although the overall footprint area of the brine-effluent plume can be estimated and modeled, the specific location of the brine-effluent on the seafloor often changes due to changing currents [13,15]. These dynamic conditions can result in episodic short term (hours or days) exposure of benthic organisms to the brine-effluent at the perimeters of the plume as well as long term chronic exposure (months or years) at the outfall or the plume center.

**Table 1.** Spatial dispersion of the brine-effluent plume at the seabed around desalination plants worldwide. Table was modified after Frank et al. 2017

| Desalination plant | Brine discharge flux ($M\ m^3\ y^{-1}$) | Discharge technology | 2%-5% salinity above ambient ($Km^2$) | 5%-10% salinity above ambient ($Km^2$) | Reference |
|---|---|---|---|---|---|
| * Alicante (Spain) | 66 | Beach-discharge | ~23 | 9.8 | 13 |
| Maspalomas II (Spain) | 22 | Beach-discharge | ~0.2 | ~0.04 | 16 |
| ** Ashkelon, (Israel) | 120 | Beach-discharge | 1.4 | 0.06 | 17,18 |
| ** Hadera (Israel) | 145 | Beach-discharge | ~2 | ~0.1 | 17,18 |
| *** Sorek/Palmachim (Israel) | 90-150 | Diffuser system | < 2 | <0.1 | 19 |
| Perth (Australia) | 0.14 | Diffuser system | ~0.11 | <0.1 | 20 |
| Monterey Bay regional water project (USA) | 30 | Diffuser system | ~0.3 | ~0.12 | 21 |

* Brine-effluent was not diluted prior to discharge. ** The dispersion area is linked to the volume of cooling-water from the adjacent power-plant that was mixed with the brine effluent. *** Two separate discharge points that are close to each other.

The possible impacts on coastal ecosystems related to SWRO desalination can be divided into three main categories [8,10,22–24]:

(i) Impingement and entrainment of marine organisms associated with seawater that is drawn to the desalination facility (typically related to surface intake) [23,25–27]. Impingement refers to adult marine organisms such as fish, crabs, etc. which are large enough to be retained by the intake screens. Entrainment is associated with smaller (often planktonic) organisms including larva and juveniles of different marine species that pass through the intake screens and are transported with feedwater into the desalination facility. The survival rates of entrained organisms are often considered to be nearly zero. Yet, the actual survival rates and significance of impingement and entrainment of organisms are different from site to site and are often not clear.

(ii) Constant release of brine-effluent that is a by-product of the desalination process. The disposal of brine-effluent may result in osmotic stress due to elevated salinities compared to those in the receiving environment. Thermal stresses are also associated with the desalination brine-effluent if it is mixed with coolant water of adjacent power-plants.

(iii) Discharge of different chemicals that are often used in the desalination process into the coastal environment along with the brine-effluent. These chemicals include different types of antiscalants and coagulants. Antiscalants may induce eutrophication of oligotrophic coastal environments by adding organic phosphorus (e.g. polyphosphates), while coagulants can enhance water turbidity and water coloration.

Comprehensive, long-term monitoring of large-scale SWRO desalination facilities along the Israeli coastline have reported that antiscalants, coagulants and heavy metals were not detected at the outfall area [17,19,28–31]. In addition, it was determined that antiscalants and coagulants did not accumulate around the outfall area of these specific desalination facilities. However, previous studies and surveys around other operational desalination facilities have shown that exposure to the brine-effluent (mainly to elevated salinities) could impact marine organisms, including vertebrates, invertebrates, seagrass and polychaeta as well as plankton and fish-larva, in a diameter of up to a several hundred meters from the outfall [8,17–19,25,30,32–35]. Moreover, it has been suggested that these impacts may be more significant in enclosed basins, nature reserves, rocky-shores and/or around other sensitive marine environments where water circulation is limited [36–38]. Nevertheless, to date the effects of brine-effluent discharge on coastal marine ecosystems are poorly understood, thereby merit further research via controlled bioassay experiments and long-term monitoring-campaigns around the outfall of operational desalination facilities.

In this book chapter we review the latest reports and studies focusing on the impact of SWRO desalination brine-effluent discharge on marine ecosystems, namely coastal flora and fauna. It should be noted that throughout the chapter we do not report on lifecycle assessments nor define impact as “positive” or “negative” implications but rather specify “impacts” as any deviation from the natural environmental conditions at the site prior to discharge. Finally, we describe the needs for dynamic site specific monitoring approaches and suggest possible means to minimize the effects of SWRO desalination on coastal marine organisms and ecosystems.

## The impact of desalination brine-effluent on zooplankton

Plankton are free-floating organisms that dependent on ocean currents for movement and consists of diverse groups including bacterioplankton, phytoplankton and zooplankton. Planktonic organisms range in size from picoplankton (0.2 µm and 2 µm) to large zooplankton (up to few mm long). In this chapter, we will focus on zooplankton while phytoplankton and bacterioplankton are covered in greater detail in another chapter (see Chapter by Nurit Kress et al.) of this book.

*Zooplankton* consists of a large and complex group of animals including crustacean, copepods, marine larvae and various worms. The life cycle of many zooplankton organisms can be complex and include egg, larvae and adult stages. The potential impacts of desalination brine-effluent may be different for these different life stages making assessment of the impacts on specific species harder to quantify [39]. Zooplankton can inhabit both the pelagic and benthic zones of the ocean. In this chapter we will focus on pelagic zooplankton those that live in the water column. Zooplankton grazes on micro-plankton, primarily phytoplankton and bacterioplankton, which is in turn dependent on seasonal changes such as temperature [40,41] that affect the growth rates, metabolism, and respiration rates [42,43].

To date, there have only been a handful of studies investigating the effects of desalination brine-effluent on pelagic zooplankton. These studies have found that the adult stages of zooplankton have a higher tolerance for salinity changes than larvae stage [44]. For adult zooplankton mortality is generally not observed until salinity is increased by about 40% over ambient salinity, whereas reproduction, and survival of eggs and juvenile can be affected by a salinity increase of about 20% above ambient [44–49]. However the response varies among species, for example an incubation experiment exposing rotifers to desalination brine-effluent where the salinity was 40% above ambient conditions, reported no significant mortality [50]. A

summary of reported mortality rates for different zooplankton species at elevated salinities is detailed in Table 2.

Increases in temperature (within an organisms tolerance range) typically enhance growth rates of larvae and increase respiration in adult copepods [39,51]. Increases in sea-surface temperature on a global scale have been connected with decreases in zooplankton abundance and shifts in zooplankton communities [43,52]. Accordingly, local temperature increases within the discharge plume of SWRO plants, at facilities in which the brine-effluent is diluted with power-plant cooling water, are expected to show a similar pattern on zooplankton communities.

Chemical additive used in some SWRO desalination facilities such as antiscalants or coagulants may also impact zooplankton if discharged with the brine-effluent. To the best of our knowledge, there are currently no studies published on the effects of antiscalants or coagulants on zooplankton. However, a study in the fresh water Lake Tahoe (California, USA) pointed to a significant decrease in reproduction among copepods cultivated with the addition of aluminum based coagulants [53].

The overall impacts of desalination brine-effluent discharge on zooplankton are vague and not well understood. Moreover, the possible effects of SWRO brine on the zooplankton food-web (related to phytoplankton and bacterioplankton) were not studied and are currently unknown. It is possible, that SWRO effluent will impact zooplankton species around the outfall, but at this point, there is not sufficient data to arrive at a satisfying conclusion.

**Table 2.** A summary of mortality of various zooplankton species in increased salinities. It should be noted that the salinity increase over the ambient coastal environment following the brine-effluent discharge of SWRO is often lower than 10%.

| Species | Location | Salinity increase from ambient seawater | Salinity measured | Mortality | Reference |
|---|---|---|---|---|---|
| Rotifer | South Korea | 30-40% | 40 | 7% | 50 |
| Mysid shrimp | Texas, USA | 60% | 45 | 40% | 49 |
| Copepod juvenile | Tungkang, Taiwan | 25% | 25 | 30% | 44 |
| Copepod adult | Tungkang, Taiwan | 25% | 25 | 0 | 44 |
| Copepod juvenile | Florida, USA | 30% | 45 | 40% | 48 |
| Copepod adult | Florida, USA | 30% | 45 | 15% | 48 |
| Copepod juvenile | Wukan Bay, Japan | 20% | 35 | 70% | 47 |

## Benthic bacteria around the outfall of desalination facilities

Marine sediments are biodiverse ecosystems playing a key role in different biogeochemical cycles such as nutrient recycling and organic matter decomposition [54,55]. Heterotrophic bacteria have a central role within the benthic fauna community as they regulate various biochemical processes such as decomposition of organic matter and nutrients remineralization in the sediment [56,57]. Organic matter and nutrients can then be released to the water column and/or assimilated by benthic bacteria. This bacterial biomass can then be consumed by bacterivores and utilized by higher trophic levels [57–61]. The growth efficiency

and community structure of benthic bacteria is often affected by environmental parameters such as temperature, salinity, oxygen concentrations, organic matter quantity and quality, as well as nutrients availability. Theses parameters impact the metabolic patterns of bacteria, for example by changing the rates of anabolic reactions (carbon assimilation) or catabolic processes (respiration) [62,63]. It should be noted that cyanobacteria (photosynthetic prokaryotes) also constitute an important component of the benthic microbial assemblage and they play an important role as primary producers [64].

Currently, studies or technical reports regarding the impact of SWRO brine-effluent discharge on benthic bacteria are highly scares. To the best of our knowledge there is also no published data on the effects of desalination brine-effluent on benthonic cyanobacteria. In the following section we will focus on benthic heterotrophic bacteria and review the possible effects of osmotic stress due to SWRO brine-effluent discharge.

**Short and long term impact of SWRO brine-effluent discharge on benthic bacteria**

Exposure of benthic bacteria to SWRO brine-effluent may impose short-term (days) to chronic (years) effects. It was recently shown in controlled microcosm experiments that short-term (2 days) exposure to elevated salinity (>5% above the ambient) resulted in a significant reduction (60%) in bacteria abundance [15]. In addition, the metabolic activity per bacterial cell was found to increase following these short term (days) exposures to SWRO brine-effluent. It has been proposed that under these higher salinity conditions osmotic shock has prompt production of osmoprotectants [15], namely carbon rich molecules that are used to adjust the osmotic pressure of the bacterial cell to allow survival [65–67].

Chronic effects of SWRO brine-effluent on benthic bacteria are of great importance due to long-term operation of desalination facilities. Environmental measurements were recently conducted near a large scale operating desalination facility with a surface discharge system (producing ~150 M m3 Y-1 of desalinated water) along the Israeli coastline. Sediments were sampled by the RV Mediterranean Explorer. Three locations were selected for each desalination facility: at the brine-effluent outfall, within the brine-effluent plume, and at reference stations that were not affected by the brine-effluent. The outfall station was chronically exposed to salinity of ~ 9% above ambient, while the “plume” station was exposed to variable conditions between 2% and 5% above ambient salinity. The chronic exposure to brine-effluent at the outfall resulted in higher bacterial abundance and production (52 % and 60%, respectively) compared to the reference station (Figure 1). Diversity analysis indicated that community structure at the outfall and reference stations where significantly different. The main families at the outfall station comprised primarily of Stramenopiles, Pirellulaceae,

Piscirickettsiaceae, while the reference station was colonized by Bacteriodaceae, Prevotelaceae, and Eterobacteriacea families (Frank et al. in Prep). We stress that additional environmental campaigns from the above sites as well as different coastal ecosystems are needed to establish a clear conclusion regarding the chronic effects of SWRO brine-effluent on benthic bacteria.

Changes in the community structure and metabolic traits of benthic bacteria can potentially affect ecosystem functionalities on long time scales 68. The effect of brine-effluent on benthic bacteria needs to be further assessed in regards to the size of the outfall area and habitat properties such as sediment type and bathymetry. It should be highlighted that the impact of water temperature and chemical discharge (such as antiscalants) on benthic microbial communities have been overlooked so far and therefore warrant further investigation.

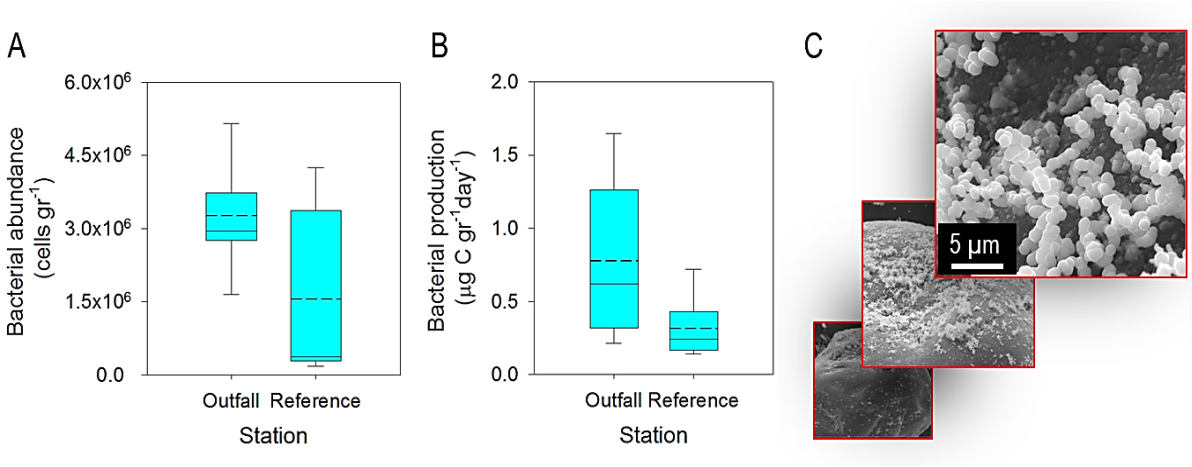


**Figure 1. A:** Benthic bacterial abundance and **B:** production near a large-scale SWRO desalination facility within the outfall and reference stations (Frank et al. in Prep). The figures represent the pooled data from four annual cruises. **C:** Representative micrograph of benthic heterotrophic bacteria attached to a sand grain.

## Impact of osmotic stress on benthic Meiofauna

Soft bottom meiofauna include various microscopic organisms that range from tens of micrometers to one mm. These organisms consist of invertebrates from different phyla such as Crustacea, Echinodermata, Mollusca, Annelida, Nematoda and Foramenifera [69,70].

Meiofauna could impact microbenthic abundances and shape the macrobenthic populations by grazing on their larvae [71]. In coastal environments the number of meiofaunal species dwelling within the sediment is high and often exceeds the number of species living on the seabed due to the turbulence conditions [70].

Previous reports have highlighted that benthic Meiofauna (specifically metazoans) are highly sensitive to different anthropogenic effects [33,69]. Therefore, metazoans are often used as bio-indicators to assess impacts on the meiofaunal community in coastal environments. Nematodes are the most abundant and diverse phylum within the meiofauna group [71–73]. As permanent dwellers of the interstitial coastal environment, they are constantly exposed to anthropogenic pollutants [72,74], and possibly also to osmotic stress due to brine-effluent from desalination discharge. Although Nematodes are used as bio-indicators for different pollutants [71,74] to date no studies were conducted to assess the impact of desalination on the activity and biodiversity of nematodes.

Crustaceans were also shown to be highly sensitive to many different anthropogenic pollutants [75,76] as well as to salinity increase due to SWRO brine-effluent discharge. For example, amphipods assemblages studied near the San Pedro desalination facility in SE Spain where they were exposed to high salinity levels of up to 53 near the brine-effluent outfall [77] show reduced abundance and diversity (Fig. 16, Shannon-Winner index). Moreover, salinity changed the biological traits and functionality of the community selecting for detrivorous and domicolous species, at the expense of carnivorous, omnivorous and fossorial species. It should be noted that once a diffuser system was deployed at the end of the discharge pipeline (4 years after operation initiation), amphipods abundance rapidly recovered.

Near the same desalination facility an investigation of brine-effluent effects on Polychaeta assemblages was also conducted [78]. Community parameters including: family richness, diversity and abundance were measured. Polychaeta assemblage was mainly disturbed close (0-300 m) to the discharge point, where abundance, richness and diversity were reduced. Following the deployment of the diffuser nearly full recovery of the Polychaeta diversity and richness was achieved. Similar findings were reported in a study that was conducted in Chabahar bay (Oman Sea, Iran), indicating a decrease in abundance, richness and diversity closest to the brine-effluent outfall [79]. Polychaeta were also investigated near the outfall of a large desalination facility in Alicante (NE Spain) discharging ~65,000 $m^3$ $d^{-1}$ of brine-effluent at a salinity of 68 (note, brine was not mixed prior to discharge). Polychaeta assemblages were different at the outfall, and composed of a homogenous group of several

families. Measurements at the two stations closest to the brine-effluent outfall (distance of ~660 m between them) showed a decrease in richness, diversity and abundance at all sampling dates (compared to un-impacted locations). It should be noted that a previous study has indicated that few Polychaeta families (Syllidae and Capitellidae) displayed short term resistance or tolerance (Paraonodae) to osmotic stress while the abundance of other families (Mpharetidae, Nephtyidae and Spionidae) sharply decreased [80].

Echinoderms such as, starfish, sea-urchins and sea-cucumbers are also ecologically important benthic macro fauna. These organisms function as filter-feeders and predators of benthic algae, benthic bacterial consortiums (termed also as biofilms) as well as other meiofauna such as bivalves and snails. It has been shown that this group of organisms can comprise more than 90% of the benthic biomass [81]. Echinoderms were reported to be strict marine phyla, and therefore are expected to be highly sensitives to changes in salinity [46]. A study conducted near the Alicante desalination plant pointed out a significant decrease in Echinoderms abundance (100 %) after the desalination plant started operating (2003) [82]. A significant recovery of Echinoderms abundance was noted after the initiation of mixing of the brine-effluent with seawater prior to discharge. A recent study [77] noted that the use of Echinoderms abundance and the benthic opportunistic polychaeta and amphipods (BOPA) indices were an effective and convenient measure for the determination of environmental degradation as a result of brine-effluent discharge. The BOPA (Eq. 1) indices measure the proportion of opportunistic Polychaeta families (Capitellidae, Eunicidae, Magelonidae, Nephtydae and Paraonidae) [78] in relation to the proportion of amphipods which are considered to be sensitive to environmental changes. It is calculated according to the equation:

$$BOPA = \log\left[\frac{fp_{op}}{f_a + 1} + 1\right] \qquad \text{Eq. 1}$$

Whereas $fp_{op}$ is the opportunistic Polychaeta proportion of all fauna (0 to 1) and $f_a$ is the amphipod proportion of all fauna (0 to 1) [83]. The maximum value of the index is 0.3013, and it indicates a highly disturbed area with only opportunistic Polychaeta families, while 0 indicates no opportunistic Polychaeta, or higher proportion of Amphipods in the benthic metazoan community.

During the last decade a series of comprehensive monitoring surveys for benthic meiofauna (abundance and community structure) were conducted adjacent to large-scale SWRO desalination facilities located on the Israeli coastline [17,19,29–31]. These facilities apply two

disposal approaches: surface discharge of brine which is diluted with cooling-water of an adjacent power plant (used at Hadera and Ashkelon desalination plants) or a diffuser system discharging the brine away from the shore (used at Sorek\Palmachim desalination plants). Significant changes to the community structure of benthic meiofauna near the brine\cooling-water outfall of Hadera and Ashkelon desalination facilities were reported [18,28]. In some cases there was also a reduction in the abundance of benthic meiofauna next to the outfall brine\cooling-water. It should be stressed that these effects were only observed within a few hundred of meters of the discharge point and could not be linked directly to osmotic/temperature stresses related to brine-effluent discharge [18,28]. Instead, it was suggested that these changes in benthic meiofauna resulted from physical disturbance of the sediment due to the strong water currents of the discharged brine/cooling water [18,28]. Monitoring reports from the Sorek\Palmachim desalination outfall list no significant impacts of brine-effluent on benthic meiofauna [19,30,31]. However, a report from 2014 noted that some local effects on the community structure of benthic meiofauna may be linked to the brine-effluent discharge [30]. These reports concluded that additional monitoring campaigns were recommended to determine the significance of these effects.

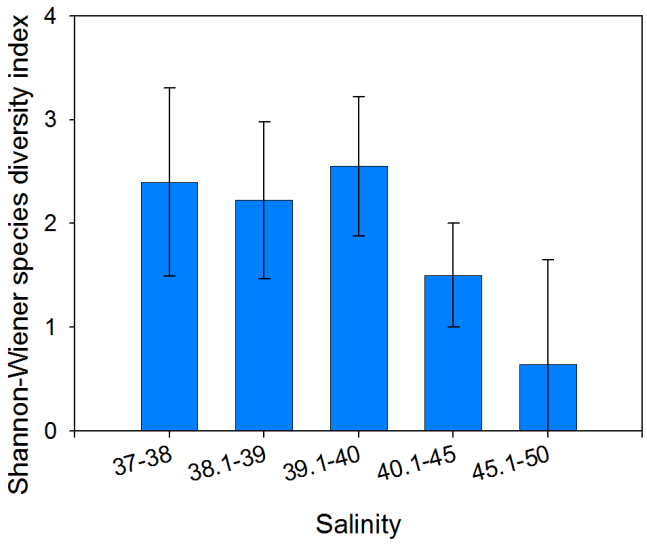


**Figure 2.** Meiofaunal diversity in different salinities measured along a brine-effluent plume from four operating—desalination facilities [77–80].

## The effects of desalination brine-effluent on seagrass

Seagrass meadows are highly productive habitats and form key ecosystems in costal environments worldwide [84,85]. Seagrass meadows export on average 24 % [86] of their net

production and serve as important trophic links to nearby ecosystems [84]. The contribution of seagrass meadows include sediment stabilization and improvement of water clarity, source of food to the coastal and adjacent ecosystems, provision of oxygen to the water and sediment as well as a habitat for various organisms [84,85]. Accordingly, seagrass meadows are estimated as one of the most valuable habitats in terms of ecosystem functionality [87]. SWRO desalination facilities have shown to impact the physiology and growth of seagrass meadows due to osmotic stress around the brine-effluent discharge point (Figure 3) [88–91].

*Posidonia oceanica* is an endemic specie to the Mediterranean Sea [92]. In a series of mesocosm experiments the susceptibility of *P. oceanica* to elevated salinities was tested by evaluating the mortality of leaves and their recovery after returning to ambient salinities [91]. It was found that the growth of the leaves was inhibited in salinities above 39 and overall mortality occurred at salinities above 53. A later study examined the effects of elevated salinities (37 to 43) on photosynthesis by *P. oceanica.* The results indicate that net and gross photosynthetic rates of *P. oceanica* were significantly reduced by 25%-33% and 13%-20%, respectively following exposure to higher salinities than the control. In addition, dark respiration of *P. oceanica* increased significantly (by 98%) when exposed to high salinities (41 and 43). It was suggested that the respiratory demand for osmoregulation reduces the photosynthetic rates and therefore inhibits growth [89]. An *in situ* study that was performed close to an operating desalination facility at the Balearic Islands of Spain found that chronic exposure to the brine-effluent resulted in *P. Oceanica* leaf necrosis [90]. Finally, it has been reported that no *P. Oceanica* were observed up to 25 m from the discharge point [90].

Cy*modocea nodosa* also showed sensitivity to exposure to SWRO brine-effluent [93]. *C. nodosa* is a relatively small, fast growing seagrass that can tolerate a broad range of environmental conditions. Nevertheless, exposure to brine-effluent for one month (near the Alicante desalination plant, NW Spain) caused reduction in growth rates and higher mortality of shoots closer to the brine-effluent discharge [93]. It has been suggested that the constant salinity increase following brine-effluent discharge resulted in higher energetic costs, due to a need for maintenance of a proper turgor pressure. It has also been speculated that *C. nodosa* plants were losing their inner-cellular water content and were accumulating ions from the environment to achieve the proper pressure to cope with the elevated salinities. Changes in water salinities and osmoregulation were possibly the reason for shoots deterioration following the chronic exposure to SWRO brine-effluent.

Considering the above, it has previously been suggested that seagrass species inhabiting soft bottom habitats may serve as bio-indicators for habitat degradation in response to brine-

effluent discharge 92,94. Criteria for seagrass species to be used as a bio-indicator for the impact of brine-effluent on the coastal environment were established by carrying controlled, laboratory experiments. The Seagrass Posidonia australis was incubated for 6 weeks at elevated salinities (37 to 54) and the following physiological parameters were monitored: survival, growth, photosynthesis, metabolism parameters, carbohydrate and amino acid concentration 95. Additional mesocosm experiments were conducted in order to evaluate the growth and survival of other seagrass species including C. nodosa and Zostrea noltii hornemann. These seagrass species where grown with salinities of up to 72 91. It should be noted that these salinities levels are not found next the outfall of operating desalination facilities. Nonetheless, in these studies survival and growth of shoots were significantly decreased compared to the control treatments. Additional physiological parameters of P. australis were found to be affected by the by high salinity concentrations after 6 weeks of incubation 95. Specifically, it was found that amino acids composition in rhizome and leaf tissues increased, and compatible solutes concentration in leaf tissue raised [95].

It has recently been reported that for large seagrass species necrosis had the shortest response time to hyper salinity, while in small species photosynthetic rate, necrosis, leaf growth and mortality all had a similarly short response time. In both cases response time ranged from 0-10 weeks [94]. We conclude that it is essential to test what are the proper physiological parameters (e.g. photosynthetic rate, leaf necrosis and meadow density) to be used in order to evaluate the extent of habitat deterioration. It should be highlighted that the seagrass species and the relevant brine-exposure time should also be considered before using seagrass as brine-effluent bio-indicators.

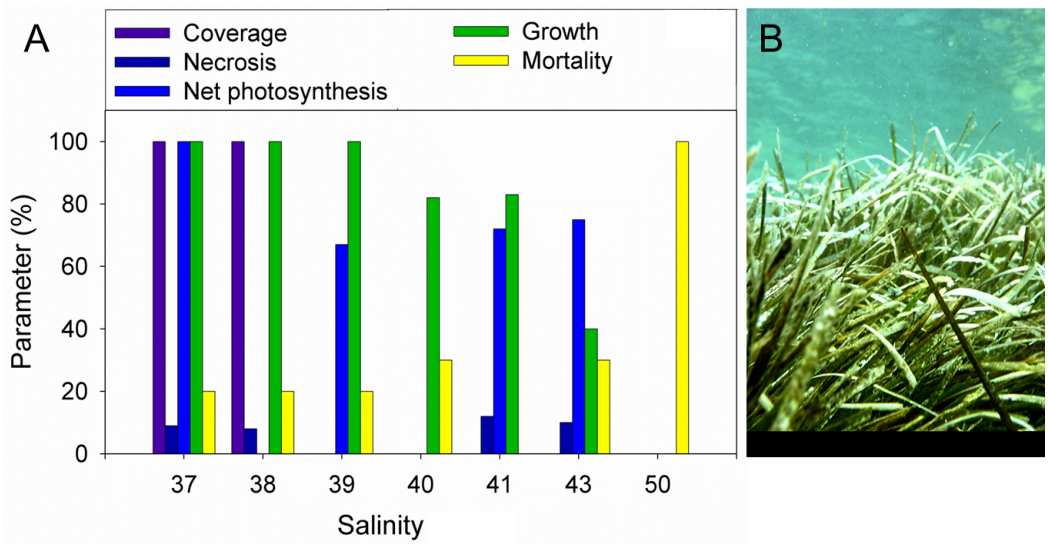


**Figure 3. A:** Seagrass ecological and physiological parameters that were altered due to elevated salinities in mesocosm experiments and following environmental samplings. All data represents percent change from the control in the specific study or experiment the data was taken from. **B:** An example of *Posidonia oceanica* meadow, Photo-credit (personal permission): Sagi Maayan. The data for the figure was adapted from [90,91,96].

## Desalination brine-effluent impacts on fish-larvae

Fish eggs and larvae are small (1mm to >10mm, species dependent) are plankton that drift in the upper photic zone of the water column. Fish eggs are completely dependent on ocean currents, buoyancy, and water density for their position in the water column, while the larval stage have some limited swimming ability [97–99]. The larvae are hatched with yolk sac still attached to their body which is consumed within the first few days after hatching (Figure 4). As larva grow, they start developing swim bladders which are used by adult fish to maintain buoyancy [99,100]. Temperature increase (within the species' tolerance range) tends to accelerate embryo and larvae development, which can increase the risk of larvae mortality since the development time is shortened (Blaxter 1988; Kestemont et al. 2003). At the early developmental stage, fish-larvae undergo significant morphological changes. Therefore, the larvae are more susceptible to environmental changes [101,102]. The development of the egg and larvae can vary from days to weeks depending on the species, therefore larvae can be affected by changes in their environment even if the exposure is on time scale of hours [99,100,103,104].

Larvae and eggs of different fish species appear to have great resilience to small changes in salinity. However, at salinity increases of ~50% or higher than ambient (which are rarely or ever encountered in modern desalination facilities), larvae and eggs show significant and acute mortality (after 24-48 hours of exposure) [105–107]. Increasing salinity to 5-15% above ambient (which could be encountered sometimes close to the outfall) lowers the larvae's survival rate, but the impact is rarely found to be significant. Figure 4 compares the survival of larvae of 7 species of fish when salinity is increased by 5-10% over ambient conditions [108–114]. In addition to increased mortality it has been shown that fish-larvae decrease in size and have higher degree of defected and/or inefficient inflation of swim bladders when cultivated in salinity of 10-15% higher than ambient [106,108,109,113,114].

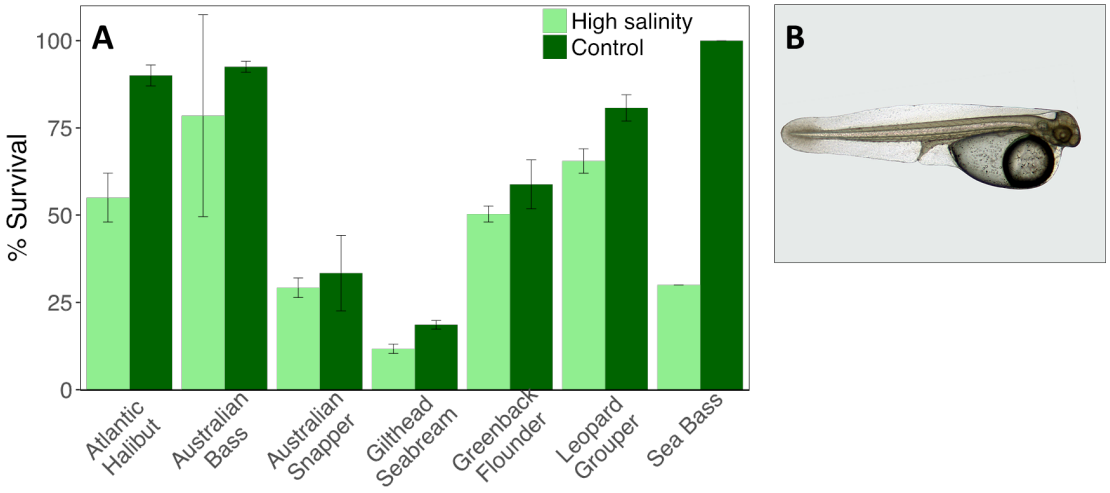


**Figure 4**. **A:** The percent survival of the larvae of 7 species of fish. Larvae were hatched in either "optimum salinity" (Control) or in salinity treatments ranging from 5% to 15% over optimum [108–114]. **B**: A newly hatched Sea Bass larvae, photo-credit (personal permission): David J Ostrach, Ph.D [115].

In laboratory experiments, higher temperatures (~5 $^{o}$C over ambient) lowered the survival of fish eggs and larvae [102,111,112]. Higher temperatures can also shorten the hatching time of eggs resulting in un-developed larvae or a quicker absorption of the yolk-sac of developed larvae [116]. Certain species have shown swim bladder defects at higher temperatures, and one species (Australian Snapper, *Pagrus auratus*) had a 100% mortality rate with a 30% temperature increase above maximum environmental temperatures for 9 days [109,111,117,118].

Larvae of different species of fish respond differently to various stressors. For some species, the combined effect of increasing salinity and temperature increased mortality and swim bladder deficiency [112,117], but for other species this synergy was not found and only temperature changes were found to have a significantly impact on the larvae [109,111].

At present, no studies have investigated the effects of chemicals used (antiscalants and coagulants) in SWRO desalination facilities on fish larvae or fish. However, studies on coagulants (aluminum based) in a fresh water setting have shown a negative impact on fish embryo development and fish larvae survival at high concentrations [53,119]. To the best of our knowledge there are currently also no published studies reporting on experiments with actual brine-effluent from desalination plants, hence the direct effects of SWRO brine-effluent discharge in on fish eggs or larva remain unknown. We stress that dedicated research as well as monitoring programs are highly needed to evaluate the impacts of desalination brine-effluent discharge on prominent species of fish eggs and larvae around the outfall of these facilities.

## The effects of desalination brine-effluent on coral physiology

Coral reefs comprise some of the richest habitats in the ocean, supporting great biodiversity, biomass and productivity [120]. In addition, coral reefs are important for local economies, fisheries and tourisms [120–122]. Corals are marine invertebrates (phylum: Cnidaria; class: Anthozoa) and the hermatypic coral species (reef-building) can consist of large colonies that span many kilometers. These corals have a mutualistic relationship with dinoflagellates of the genus *Symbiodinium* (referred to as Zooxanthellae) that are integrated within the tissue of the coral where they provide organic matter to the coral via photosynthesis in return of nutrients [123,124]. Corals are osmo-conformers and adjust their osmotic balance with intercellular osmolytes. The osmotic balance can be affected by many environmental stressor (temperature, nutrient levels, salinity changes) and an imbalance of osmolytes often leads to coral bleaching [125]. Coral reefs are often found around tropical, sub-tropical and even cold-water regions resulting in specific adaptions (e.g. to temperature, salinity, etc.) of species even within the same genus [126,127]. In the last decade, coral reefs have experienced an increase in the extend and frequency of bleaching events due to different environmental stressors such as rising ocean temperatures, seawater acidification as well as anthropogenic impacts such as coastal eutrophication [121,122,128–131]. In line with the above, the construction of new desalination facilities around coastal zones with coral reef ecosystems raises concerns over the possible impact on coral physiology and survivability [132,133].

Coral habitats can experience changes in salinity on diurnal and seasonal time scales as tides and rainfall/evaporation can impact local salinity [134]. Short-term exposure studies (5-7 days) with elevated salinities (~15% above ambient) have shown a similar response in different coral species (*Porites furcata, Siderastrea radians and S. siderea*): (i) decrease in

primary production within the first 6-12 hours following exposure, (ii) retraction of coral polyps, and (iii) discoloring of the tissue [135–137]. In these studies, corals were able to recover to normal primary productivity rates (post-incubation) within 36 hours to 1 week, and made a full recovery of polyps and tissue within one week to a month.

Previous reports have indicated that exposure of *Stylophora pistillata* for three weeks to increase salinity of ~10% above ambient resulted in 70% mortality [138]. However, in a recent study with *Stylophora pistillata* in 10% above ambient salinity, mortality was not detected, but a significant drops in protein content and zooxanthellae abundance were measured (Figure 5). A similar response was observed for *Acropora tenuis* and *Pocillopora verrucosa* both in salinity of 10% over ambient and in 10% increased salinity with addition of phosphanate (< 2 mg $L^{-1}$).

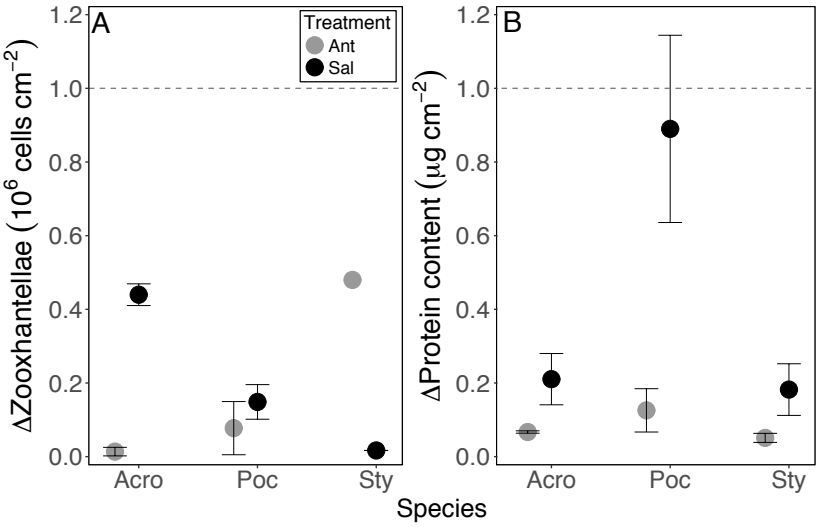


**Figure 5**. Data from 4 week-long coral incubation with 10% salinity increase over ambient (solid black) and 10% salinity increase with 6 μL $L^{-1}$ phosphanate addition (solid grey). **A:** The relative change between treatment and control zooxanthellae abundance for *Acropora* (Acro), *Pocillopora* (Poc) and *Stylophora* (Sty). **B:** Relative change in protein content between treatment and control.

The dramatic decrease in zooxanthellae and protein contents (Figure 5) and the mortality reported by Ferrier-Pagès (1999) stresses the need for monitoring of coral reefs in the vicinity of desalintion plants. A recent *in-situ* study (the only one to date) in the Red Sea has positioned the coral *Fungia granulosa* at the discharge channel of an operating SWRO plant and followed different parameters [133]. The corals showed no change in primary production and none of the specimens were significantly affected. Furthermore pure cultures of the symbiotic zooxanthellae (*S. microadriaticum*) were incubated in salinities between 25 and 55. Cell growth of *S. microadriaticum* were found to decrease only in salinities of 55 (which is not

likely to occur around the outfall of SWRO desalination facilities), indicating a high salinity tolerance of the zooxanthellae.

In general, antiscalants (phosphonate based) are not harmful in the doses used in desalination plants (~2 mg/L) [139], however recent observations pointed that these concentrations may impact the physiology of *S. pistillata, A. tenuis* and *P. verrucosa* (Lykkebo Petersen et al., in prep.). To the best of our knowledge, no published studies have reported on the effects of coagulants on corals.

Rising ocean temperatures is one of the main stressor causing coral bleaching [129,140]. The water temperature of desalination brine-effluent can be elevated compared to background ambient temperatures (by up to 25 % over the ambient) and this results in local plumes of warm seawater around the discharge area ([8,10,142], Frank et al. in prep). Exposure to temperature of as little as 3 $^{o}$C above mean maximum levels for several weeks have shown to cause bleaching in *Pocillopora sp.*, *Montastrea sp.*, *Acropora sp.* and other species spanning reefs in Hawaii, Caribbean and Australia [126,127,142–144]. Thermal stress was also positively correlated with coral disease outbreaks [126,140]. However the response varies based on location as *S. pistillata, P. damicornis,* and *A. eurystoma* that were collected in the Gulf of Aqaba (Red Sea), did not show any significant change after 4 weeks of incubation in temperatures of 10% above ambient. Only at a 40% temperature increase (from ~25 $^{o}$C to 34 $^{o}$C) the abundance of zooxanthellae was significantly reduced [145].

Corals exposed to multiple environmental stressors tend to have a higher risk of bleaching and a higher mortality rate [129,135,140]. This is because the efficiency in which the corals sustain energy-demanding processes becomes compromised under multiple stressors. This effect was shown by Lirman et al., (2009) who exposed corals to salinity of 25% above ambient and found that they were unable to clear their tissue of sediment [135]. The response to different stress factors, such as increased salinity and temperature, are variable between species of coral even in the same geographic location. The above underlines the difficulty of providing globally relevant guidelines for desalination management around coral reefs to minimize possible impacts, and emphasizes the need for local environmental studies prior to the construction of large-scale desalination facilities. At this point, published data on the direct effect of desalination brine-effluent discharge on coral reefs are extremely scarce and virtually non-existing. Therefore, we stress that coral mesocosm studies (short-term) as well as dedicated monitoring schemes (long-term), should be carried out to determine the local impact of brine-effluent discharge on coral reefs.

## Looking forward: Nexus of SWRO desalination and coastal environments

SWRO desalination industry is booming with a tight, bidirectional (intake and discharge) interfaces to the costal environment. Extensive information has been accumulating during the past decade with respect to the possible impacts imposed by the desalination industry on the marine environment [8,11,18,19,22,24,25,32,36,37,79,139,146]. These effects were suggested to impact different marine organisms, however were more pronounced in sessile organisms such as seagrass or benthic fauna. It should be stressed that the reported impacts of most SWRO desalination were highly local and restricted only to the vicinity of the intake port or the outfall area. Yet, we surmise that due to growing water scarcity, future concerns related to desalination expansion and possible impacts to coastal environments (specifically to enclosed and sensitive ecosystems) should be further evaluated. These impacts may include: (i) Construction of new SWRO desalination facilities at high densities. (ii) Designing increasingly larger SWRO desalination plants with greater production capacities (>300 M $m^3$ $y^{-1}$) and potential impacts. (iii) Drawing large volumes of feed-water from and discharging large amounts of brine-effluent to coastal environments with limited water circulation.

We suggest that mitigating the impacts of the SWRO desalination on the coastal environments in years to come could be optimized (together with standard provisions such as near and far-field modeling etc.) by the following measures:

(i) Carrying dedicated precursory environmental-impacts-assessments that are site specific. Namely, to determine the chemical concentrations at which adverse biological effects are apparent for endemic organisms prior to the contraction of any large-scale desalination facility. These values should be used as a reference point and set the criteria for future, long-term monitoring surveys.

(ii) Conducting long-term monitoring programs that are tailored to each desalination site (e.g. based on the biology and hydrography of the location) or region (such as the oligotrophic environment or semi-enclosed lagoons). Parameters that will be monitored should be pre-determined according to ranges of sensitivity of key organisms that are relevant to the coastal environment, similarly to Long et al. 1995 [147]. We stress that these monitoring programs should be dynamic and evaluated yearly to minimize over-sampling and reduce redundancy in term of the sampled parameters. Providing such data could contribute to better and more realistic regulation for the interface of the desalination industry and coastal environments.

(iii) Applying best available technology to reduce any possible effects on the coastal environment that may result from SWRO desalination brine-effluent. We propose that using diffuser systems as a favorable discharge approach will reduce the impact of brine on the coastal ecosystems. The advantages of the diffuser systems over surface discharge include: minimizing the dependency on power-plant cooling-water for dilution and setting the buoyancy of the brine-effluent plume prior to discharge. In addition, using diffuser system will enable to discharge the brine far from the shoreline and\or away from any ecologically-sensitive areas. Brine-effluent can be allocated via the diffuser system into pre-determined locations, thereby maximize dispersion by coastal currents. Finally, we urge that additional impetus should be allocated toward development of new approaches to minimize the use of chemicals in the desalination process [148] and eventually toward zero liquid discharge solutions.

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