Water Research 144 (2018) 183–191

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Impact of brine and antiscalants on reef-building corals in the Gulf of Aqaba – Potential effects from desalination plants

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ARTICLE INFO

Article history:
Received 6 February 2018
Received in revised form
3 July 2018
Accepted 4 July 2018
Available online 4 July 2018

Keywords:
SWRO desalination
Brine discharge
Polyphosphonate-based antiscalants
Osmotic stress
Coral reefs
Gulf of Aqaba

ABSTRACT

Seawater reverse osmosis (SWRO) is becoming an increasingly important source of potable water in arid and semi-arid regions worldwide. Discharge of brine-effluent from desalination facilities has been shown to significantly impact coastal marine ecosystems ranging from seagrass meadows to microbial communities. In this study, we examined the impacts of increased salinity (10% above ambient) and presence of antiscalants (0.2 mg L^{-1}, polyphosphonate-based) on three reef-building coral species; *Stylophora pistillata*, *Acropora tenuis* and *Pocillopora verrucosa*, from the Gulf of Aqaba (northern Red-Sea). Our results indicate that the corals, as well as associated bacteria and algae, were significantly impaired by the elevated salinity and antiscalants, leading to partial bleaching. Specifically, the abundance of bacteria and symbiotic algae as well as calcification rates were typically lower (20–85%, 50–90% and 40–50%, respectively) following incubations with both amendments. However, the impact of desalination brine was often species-specific. Thus, we propose that the ecotoxicological criteria used for hard corals should be determined based on the sensitivity of key species in the community dominating the area affected by desalination discharge.

1. Introduction

Seawater desalination via reverse osmosis (SWRO) is becoming a frequent solution for supplying large volumes of potable water in arid and semi-arid countries worldwide (Grady et al., 2014; UN-WWAP, 2015). Large-scale SWRO plants draw coastal water as feed source and produce high salinity brine-effluent as a by-product, which is typically discharged back into the coastal environment. Salinity measured in the mixing zone of SWRO discharge outfalls is reported to be 1–10% above ambient and in rare occasions >25% above ambient levels (Kress et al., 2014; Petersen et al., 2018). Concomitant with higher salinity, SWRO effluent often contains chemicals that are used during the desalination process such as antiscalants and coagulants (Höpner and Lattemann, 2003; Missimer and Maliva, 2017; Roberts et al., 2010). Antiscalants are added in concentrations dictated by environmental regulations set by individual countries and are therefore not consistent between desalination plants (Greenlee et al., 2009; Jenkins et al., 2012). In Israel, environmental legislation instructed a maximum antiscalant concentration of 0.2 mg L^{-1} to the feedwater (Belkin et al., 2017; Israel Ministry of Environmental Protection, 2013). These compounds are assumed to discharge with the brine into the environment at similar concentrations, but are generally considered unavailable for biological processes (refractory) (Höpner and Lattemann, 2003).

Although desalination capacity has increased substantially worldwide, the environmental impacts of brine effluent on coastal ecosystems have received little attention (Lattemann, 2009; Petersen et al., 2018; Roberts et al., 2010). Previous studies focused on the effects of brine effluent on seagrass meadows, benthic

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https://doi.org/10.1016/j.watres.2018.07.009
0043-1354/

copepods, pelagic phytoplankton, and benthic bacteria (Belkin et al., 2017, 2015; Del-Pilar-Ruso et al., 2015; Frank et al., 2017; Sánchez-Lizaso et al., 2008). These studies demonstrated that the response and sensitivity to brine exposure varies considerably between species, sites, brine concentration and exposure time. To date, only a handful of studies have investigated the impact of desalination brine on corals, despite the fact that large scale desalination facilities are often situated near coral reefs (Lattemann and Höpner, 2008; Mabrook, 1994; Muthiga and Szmant, 1987; Röthig et al., 2016; van der Merwe et al., 2014). Since coral reefs are highly productive ecosystems with high biodiversity and biomass, loss of coverage may have cascading effects on higher trophic levels. In addition, coral reefs support local economies through fishing and tourisms, and are therefore considered to be important assets for coastal communities around such reefs including the northern Gulf of Aqaba (GoA), Red Sea, which borders Israel, Jordan and Egypt. Currently, several large-scale desalination facilities are planned to be constructed on the north shores of the Gulf of Aqaba; yet impacts on native corals following brine discharge are unknown.

Scleractinian corals (hermatypic) are a diverse group of marine invertebrates which are the key ecosystem engineers of coral reefs worldwide (Birkeland, 2015; Dubinsky and Stambler, 2011). Scleractinians are essentially holobiomes including the coral itself, endosymbiotic dinoflagellates (*Symbiodinium*), and various prokaryotes that colonize the coral's tissue and mucus (Muller-Parker et al., 2015). *Symbiodinium* have a key biochemical role in coral physiology, providing carbohydrates via photosynthesis in exchange for inorganic nutrients (Dubinsky and Stambler, 2011; Muller-Parker et al., 2015). Heterotrophic and phototrophic bacteria associated with the coral play fundamental roles in the function, fitness, and resilience of reef-building corals (Ritchie, 2006; Shnit-Orland and Kushmaro, 2009).

Changing environmental conditions such as elevated water temperatures (Clausen and Roth, 1975; Coles and Jokiel, 1978; Marshall and Clode, 2004) and eutrophication (Ferrier-Pages et al., 2000; Koop et al., 2001; Marubini and Davies, 1996) can significantly alter the physiology of scleractinian corals and their symbionts. These stressors may cause coral bleaching, *i.e.* loss of *Symbiodinium* from the coral's tissue (Brown, 1997; Mayfield and Gates, 2007; Muller-Parker et al., 2015), resulting, in many cases, in coral host mortality and subsequent reduction in coral coverage. Loss of coral coverage has previously been reported in the Red Sea in direct proximity of desalination plants (Mabrook, 1994). Yet, a recent *in situ* study reported no mortality or measurable impact on the photosynthetic efficiency of the coral, *F. granulosa* in that region (van der Merwe et al., 2014). However, major restructuring of the tissue-related microbiome of *F. granulosa* has been observed in a related study following controlled incubations with elevated salinities (Röthig et al., 2016). To date, very little data are available on the various possible effects of SWRO desalination brine, including the effects of higher salinity and specific chemicals that are associated with the brine, on the physiology of reef-building corals.

In this study, we investigated the physiological responses of three key reef-building coral species (*Stylophora pistillata, Acropora tenuis*, and *Pocillopora verrucosa*) in the GoA, where construction and operation of several desalination facilities is expected in the near future, to simulated brine discharge. To this end, collected coral specimens were grown for 2–4 weeks under elevated salinities (10% above ambient) and elevated salinity with 0.2 mg L^{-1} polyphosphonate antiscalant added, thus mimicking the expected exposure of corals to desalination brine in close proximity to an outfall. These experiments were conducted in open-aquaria and corals grown under ambient seawater conditions were used for comparison (unamended controls). Our results demonstrate the potential effects of desalination brine discharge on hard corals that are major builders of the coral reefs in the GoA.

2. Material and methods

2.1. Collection of corals and experimental setup

Colonies of *Stylophora pistillata, Acropora tenuis*, and *Pocillopora verrucosa* were selected as model organisms for reef building corals. Samples were collected in early June 2016 from the GoA (29°30′07.3 N, 34°55′00.9 E), Israel. The colonies were carefully sectioned into nubbins (3–4 cm height) and 72 nubbins of each species were randomly placed in nine aquariums (30 L) with flow through seawater and allowed to acclimate for 4 weeks under ambient conditions. The aquariums were shaded to simulate radiation equivalent to ~3 m water depth (~90 μmol quanta m^{-2} s^{-1} at midday, measured with a LI-CORLI-250A). Detailed description of the experimental setup is provided in Figure S1. The following treatments were initiated in triplicates after the acclimation period: 1) salinity of 10% above ambient (Sal), and 2) elevated salinity + antiscalants (0.2 mg L^{-1}) (Sal+Ant). Corals grown under ambient conditions were used as control. The salinity used in the experiment (e.g. 10% above ambient) was determined based on reports on the footprint of discharge brine plumes from various large-scale facilities (Kress et al., 2015, 2014, 2013). These values represent a worst-case scenario with limited dilution of the brine prior to discharge.

Antiscalant used was based on polyphosphonate ($C_9H_{21}N_3O_{15}P_5Na_7$, Diethylenetriamine pentamethylene phosphonic acid – DTPMP, Sigma-Aldrich) with a final concentration of 0.2 mg L^{-1}. This concentration was chosen based on the allowable use according to the Israel ministry of environmental protection permit regulation 19/6/2013 for the oligotrophic Mediterranean Sea (Belkin et al., 2017; Israel Ministry of Environmental Protection, 2013).

Treatment water used in the experiment (Sal and Sal+Ant) was prepared in 1 m^3 tanks using ambient seawater to which salt and antiscalant were added. The water in the tanks was stirred to ensure dissolution and homogenization of the salt and antiscalant; tanks were kept in a large, temperature-controlled container and water in the tanks was replenished every 5 days (Figure S1 and S2). Water flow (Sal, Sal+Ant and control) from the tanks to the aquaria was maintained at ~2 L h^{-1} using peristaltic pumps, resulting in replacement of the aquaria water volume at least twice a day.

Salinity, temperature and pH were measured daily around 9 a.m. and 3 p.m. with a multi-parameter water quality probe (WTW Multi 3500i) (Table 1). The Sal and Sal+Ant treatments were maintained for 4 and 2 weeks respectively, at which point visible changes in coral health were observed. Net production of dissolve inorganic carbon (DIC) and oxygen as well as calcification rates were also measured. Images of all the corals were taken at the end of the incubation. The tissue of the coral nubbins was then extracted and analyzed for heterotrophic bacteria, *Symbiodinium* abundance, bacterial production and total protein content. Detailed description of all the methods is provided below.

2.2. Sample analyses

2.2.1. Coral tissue removal and surface area measurement

Coral tissue was removed from the nubbins with an airbrush using filtered seawater (0.22 μm). All tissue samples were diluted to 25 ml and homogenized in a sonication bath for 10 min at >20 kHz. Tissue samples were then separated to aliquots for the different analyses. Surface area for each nubbin was calculated by the hot wax approach as described by Stimson and Kinzie (1991).

Table 1
Minimum, maximum, and mean temperature, salinity and pH measured during the experiment. pH measurements are adjusted to 25 °C according to Gieskes (1969).

Treatment	Temperature, °C			Salinity			pH at 25 °C		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Control	25.1	30.8	26.8 ± 1.3	40.6	42.6	41.2 ± 0.4	8.18	8.51	8.23 ± 0.05
Sal	24.7	29.8	26.4 ± 1.1	45.2	46.5	45.4 ± 0.6	8.10	8.46	8.19 ± 0.06
Sal+Ant	24.8	29.7	26.4 ± 1.3	43.6	47.4	44.7 ± 0.7	8.17	8.44	8.23 ± 0.05

2.2.2. Bacterial production (BP)

Bacterial production was measured using the ^{3}H-leucine incorporation technique (Kirchman et al., 1985). Subsamples of coral fragments (~0.5 cm) were incubated with 7 ml of filtered seawater (0.22 μm) and 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 tubes were sonicated (in a sonicator bath, Symphony, VWR) and water subsamples (1 ml) were divided into triplicate Eppendorf tubes and amended with cold 100% trichloroacetic acid (TCA) to stop the reaction. Samples treated with TCA at time zero were used as a blank. The samples were processed following the micro-centrifugation technique (Simon et al., 1990). 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 (Simon and Azam, 1989). Total carbon uptake rates were normalized to surface area of the incubated coral pieces.

2.2.3. Heterotrophic bacteria and Symbiodinium abundance

Tissue samples were immediately fixed with 50% glutaraldehyde solution (final concentration 0.15% vol/vol), frozen in liquid nitrogen, and kept at −80 °C. Upon analysis, samples were fast-thawed in a 37 °C water bath and analyzed with an Attune Nxt Acoustic Focusing Cytometer. One-micron beads served as a size standard. Taxonomic discrimination of *Symbiodinium* cells was done using side-scatter, forward-scatter and red fluorescence (630 nm). Heterotrophic bacteria were first stained with SYTO9 and determined based on green fluorescence at 488 nm against side-scatter (Bar-Zeev and Rahav, 2015).

2.2.4. Protein content

Frozen (−20 °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 30 s 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 divided into triplicates and 200 μL of the reagent mixture from a Pierce BCA Protein Assay Kit was added. The samples were incubated for 30 min at 37 °C before analysis on an Infinite M200 Tecan instrument. Concentration was normalized to coral surface area.

2.2.5. Net production and calcification

Coral nubbins of *S. pistillata* and *A. tenuis* from each treatment were incubated for 1.5–2 h in 800 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) analyses. Samples collected for DIC were immediately poisoned with 0.05% vol/vol saturated $HgCl_2$ solution and kept refrigerated until analysis, within 1–2 weeks of sampling. TA samples were kept refrigerated until analysis by potentiometric Gran titration of ~22 g of 0.45 μm filtered subsamples (Whatman GFF) using a Metrohm Titrino 785 Titrameter with a temperature corrected pH probe and titration solution of 0.05 N HCl. Calculation of TA employed the method described by Sass and Ben-Yaakov (1977). 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 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 in line with a LiCor 6252 IR CO_2 analyzer. Measurements were calibrated using seawater A. Dickson CRMs (Batch #155). The repeatability of the measurements was 1.5 ± 1.0.

Daytime net production (photosynthesis minus respiration) was 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} cm^{-2}, $P_{net\text{-}DIC}$, μmol DIC L^{-1} h^{-1} cm^{-2}, Equations (1) and (3)). Net production of $CaCO_3$ (G, μmol $CaCO_3$ kg SW^{-1} hr^{-1} cm^{-2}) was calculated as a function of TA uptake during the incubation period (Equation (2)). Subsequently all rates were normalized to surface area of the coral nubbins that were used in the incubations. In equations (1)–(3), the i and f indices refer to initial and final concentrations at the beginning and end of each incubation period and A_{nubbin} is the measured surface area of each nubbin (Schneider and Erez, 2006).

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

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

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

2.3. Data and statistical analysis

The physiological responses of the corals and corresponding symbionts and bacteria to the treatments were measured at the beginning and at the end of the experiment (week 2 and 4 for the Sal+Ant and Sal treatment, respectively). Herein, we present and discuss the results at the end of the experiment comparing them to corals grown under control conditions. Results were compared using ANOVA followed by a Fisher (LSD) post-hoc analysis with significance of $p < 0.05$. Values and significance are reported in supporting information Table S1.

3. Results and discussion

3.1. Changes in the appearance of reef-building corals following exposure to experimental treatments

Images of *S. pistillata*, *A. tenuis* and *P. verrucosa* demonstrate clear signs of bleaching following incubation with elevated salinity and elevated salinity with the addition of antiscalants for four and two weeks, respectively (Fig. 1). Concurrently, coral tissue loss was recorded, and the polyps of all three coral species in both treatments were highly retracted. This negative response was mostly notable for *P. verrucosa* and *A. tenuis*, and to a lesser extent for *S. pistillata* (Fig. 1). We attribute these responses to osmotic stress induced by the brine and note that the impact on corals as a holobiome is species-specific. Severe impacts on coral health due to increased salinities (10%) have been shown before (Ferrier-Pagès et al., 1999; Lirman and Manzello, 2009; Muthiga and Szmant, 1987). However, some studies suggest that corals may adapt and recover after weeks of exposure to enhanced salinity. Retraction of polyps is the first line of defense of corals to osmotic shock and since the polyps also allocate most of the energy needed for calcification (Anthony et al., 2002; Mayfield and Gates, 2007) a prolonged retraction can cause a decrease in coral growth.

3.2. Impact of experimental treatments on coral-associated bacteria

Diverse microbial communities, including heterotrophic bacteria, archaea and cyanobacteria are associated with corals (Koren and Rosenberg, 2006; Reshef et al., 2006). In this study we focused mainly on the impact of brine on heterotrophic bacteria that are found in the coral tissue and mucus. Following incubations with elevated salinity and antiscalants, the abundance of heterotrophic bacteria associated with *S. pistillata* was decreased by up to 4-fold (Fig. 2A). Heterotrophic bacterial abundance of *A. tenuis* decreased moderately following the Sal and Sal+Ant treatments (~25% and ~85%, respectively) (Fig. 2B). In contrast, bacterial abundance associate with *P. verrucosa* was higher following the Sal incubation, and lower in the Sal+Ant treatment (70%) (Fig. 2C). Bacterial production rates were higher (2–25-fold) for all three species following incubation with antiscalants compared to the control (Fig. 2D–F). However, for all three species no changes in bacterial production rates were found following incubation with elevated salinity alone. This suggests that the polyphosphonate-based antiscalant provided limiting nutrient/s (possibly P) for the heterotrophic bacteria. Results from a recent study indicated that bacteria can utilize phosphonates, resulting in enhanced activity (Dyhrman, 2016).

Coral-associated bacterial communities undergo restructuring following events of coral bleaching and rapid environmental changes (Brown and Bythell, 2005; Ritchie, 2006; Shnit-Orland and Kushmaro, 2009). Previous research show that upon prolonged exposure to high salinity the microbiome of *F. granulosa*, was significantly restructured to encompass bacterial species with higher osmolyte production (Röthig et al., 2016). This change enables the coral to adapt to hyper-saline conditions by up-regulating genes related to osmotic-stress (Röthig et al., 2016). In addition, it has also been shown that short-term exposure to high salinities can lead to a reduction in benthic bacterial abundance, possibly due to osmotic shock (Frank et al., 2017). Yet, under the same conditions specific bacterial activity (*e.g.,* bacterial production per cell) increased significantly. It has been suggested that bacterial metabolism is increased to produce various osmoprotectant molecules (Frank et al., 2017). In the past decade, phosphonate has been recognized as an important source of phosphorous for marine bacteria (Tseng and Tang, 2014; Xiaomin et al., 2013). Indeed, a recent mesocosm study indicated that high concentrations of phosphonate-based antiscalants can enhance planktonic bacterial productivity (Belkin et al., 2017).

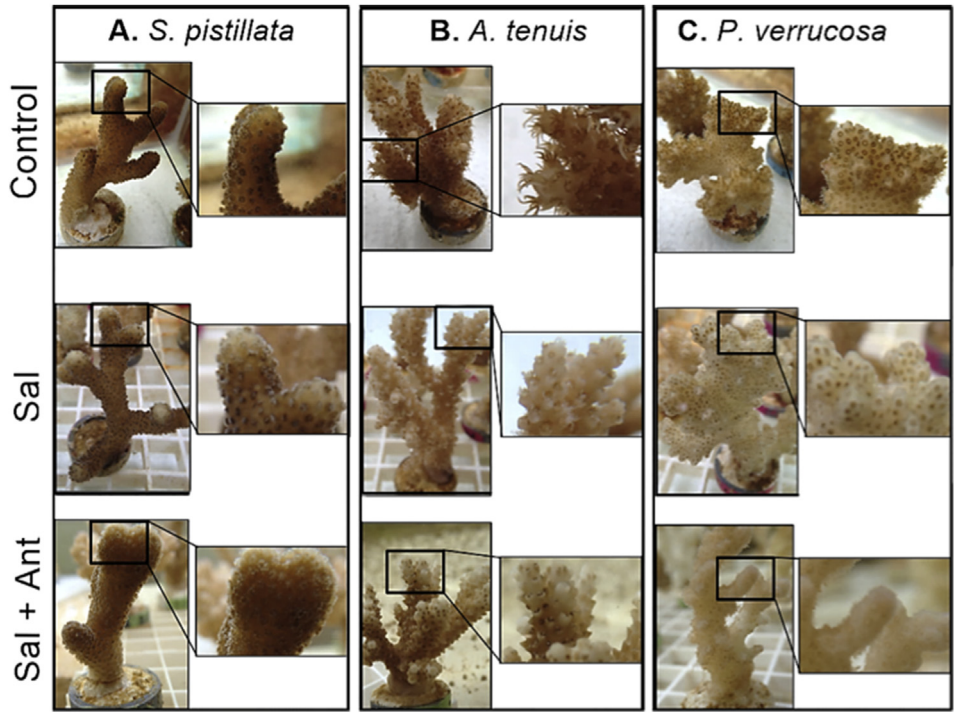

Fig. 1. Representative *in situ* images of coral nubbins, (A) S. *pistillata*, (B) *A. tenuis* and (C) *P. verrucosa* following 2–4 weeks of incubation. Top panels represent the coral species under control conditions, while middle and bottom panels show corals that were grown with salinity 10% above ambient (Sal) and with antiscalants in addition to the salinity increase (Sal+Ant).

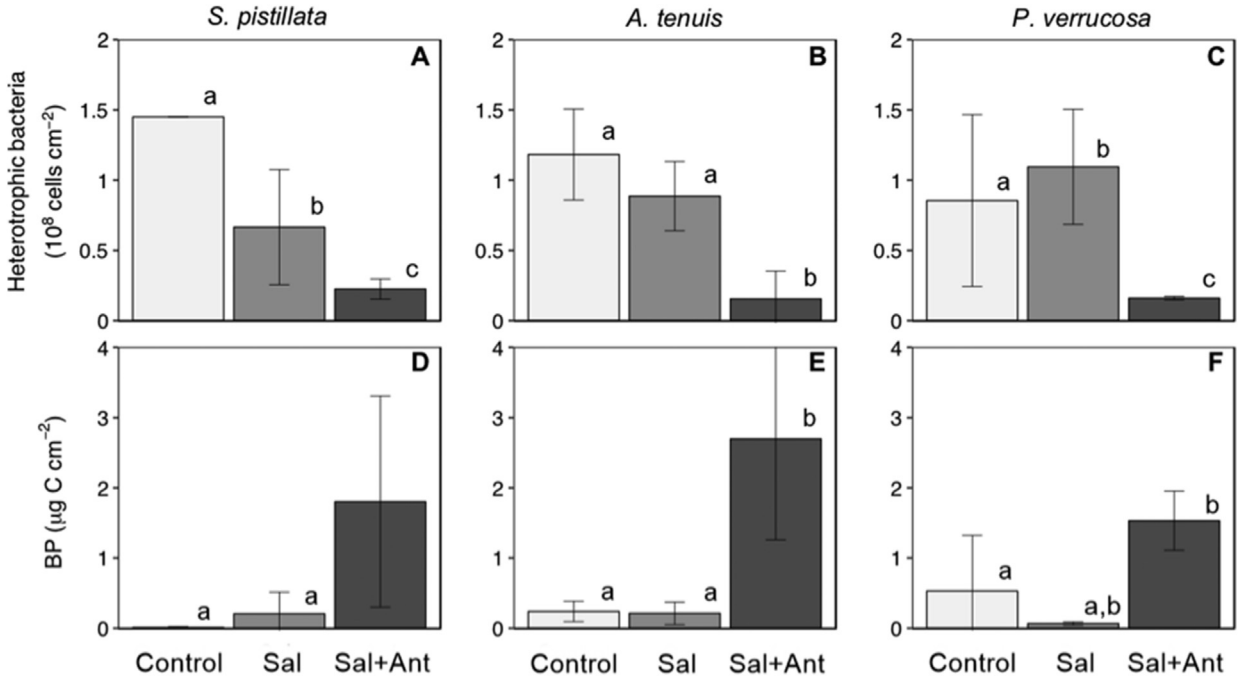

Fig. 2. Impact of increased salinity (Sal) and increased salinity + phosphonate-based antiscalant (Sal+Ant) on (A–C) heterotrophic bacterial abundance and (D–F) bacterial production (BP) following 2–4 weeks of incubation. Measurements were compared to the control samples that were grown for the same length of time under ambient conditions. Symbiotic bacteria and corresponding BP were measured in (A,D) *S. pistillata* (B,E) *A. tenuis* and (C,F) *P. verrucosa*. Letters above the bars refer to ANOVA followed by a Fisher (LSD) post-hoc analysis with significance of $p < 0.05$.

Thus, we surmise that the coral-associated bacteria in our study expressed osmotic shock in response to the short-term exposure to salinity higher (10%) than ambient, and that this may have resulted in lower bacterial abundance. Many bacterial phylotypes may increase the metabolic rates and produce various osmo-protective molecules. Concurrently, it is likely that bacteria, which can biodegrade phosphonate-based antiscalants, provide nutrients otherwise unavailable to the coral and other bacteria, contributing to increased bacterial activity. We suggest that addition of bioavailable phosphate via degradation of the polyphosphonate antiscalant could be an important nutrition source for microbial communities that are associated with corals in ultra-oligotrophic environments such as the GoA when such compounds are present in the water column.

3.3. Effects of experimental treatments on coral-symbiotic algae

Symbiodinium abundance in *S. pistillata* was significantly reduced following incubation in both the Sal and Sal+Ant treatments (by 65% and 95%, respectively) compared to the control samples (Fig. 3A). For the other two coral species, the response differed between treatments: *Symbiodinium* abundance decreased for *A. tenuis* by ~90% in the Sal treatment, while for *P. verrucosa* the decrease was maximal in the Sal+Ant treatment (by 90%) (Fig. 3B and C).

Based on these results we suggest that the impact of desalination brine, namely increased salinity and/or antiscalants concentrations on *Symbiodinium* differs between coral species (*i.e.* species-specific). Species of *Symbiodinium* are identified in several genetic clades (Rowan, 1998) which have been shown to vary between coral species and individuals on various temporal, spatial and environmental scales (Baker et al., 2013; LaJeunesse et al., 2010). It has further been suggested that corals may be able to incorporate several different clades of *Symbiodinium* in their tissue, thus providing strategic advantage in changing environmental conditions (Byler et al., 2013). Therefore, each species and individual represented in this study, likely had a unique assembly of *Symbiodinium* clades. This may explain the variability in *Symbiodinium* abundances following the treatments (Fig. 3A–C) as some corals could have an assembly of *Symbiodinium* with a greater tolerance for high salinity. Alternative explanation may be related to the host tissue thickness and polyp microenvironment that can differ between the coral species and which may lead to different resilience within the time framework of this study (Wangpraseurt et al., 2015).

3.4. Response of the coral-holobiome following exposure to experimental treatments

Total protein measured in this study orientated from a combination of the host coral and symbiotic algal cells, as well as the bacteria and archea consortium that make up the holobiome associated with the coral (Achituv et al., 1994; Rosenberg et al., 2007). A linear correlation ($R^2 = 0.6$ and P_{val} of 0.001) was found between total protein and *Symbiodinium* abundance (Figure S3), but not between total protein and bacterial abundance. Thus, we surmise that total protein, extracted from the corals' tissue and mucus, mostly originated from the algal cells and the coral host tissue.

Protein extracted from *S. pistillata* in both the enhanced salinity and antiscalants treatments was significantly lower (by 50% and 75%, respectively) than the control (Fig. 4A). Protein extract from *A. tenuis* was also lower (~30% and ~50%, respectively) for both treatments (Fig. 4B) compared to the control. In contrast, a significant increase in protein was measured for *P. verrucosa* in the Sal treatment but not in the Sal+Ant treatment (Fig. 4C) compared to the control.

Correlation between protein content and *Symbiodinium*

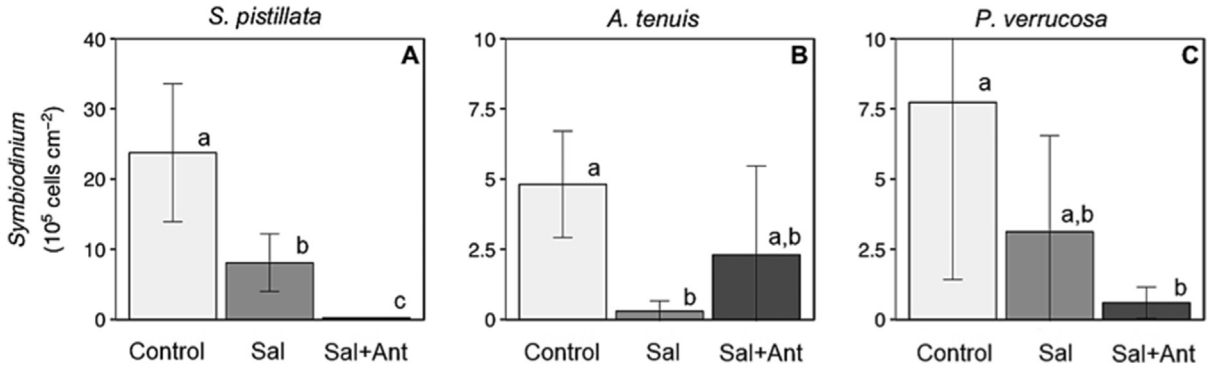

Fig. 3. Impact of increased salinity (Sal) and increased salinity with the addition of phosphonate-based antiscalant (Sal+Ant) following 2–4 weeks of incubation on (A–C) *Symbiodinium* abundance. Measurements were compared to the control samples that were grown under ambient conditions. *Symbiodinium* abundances were measured from (A) *S. pistillata*, (B) *A. tenuis* and (C) *P. verrucosa*. Letters above the bars refer to ANOVA followed by a Fisher (LSD) post-hoc analysis with significance of $p < 0.05$.

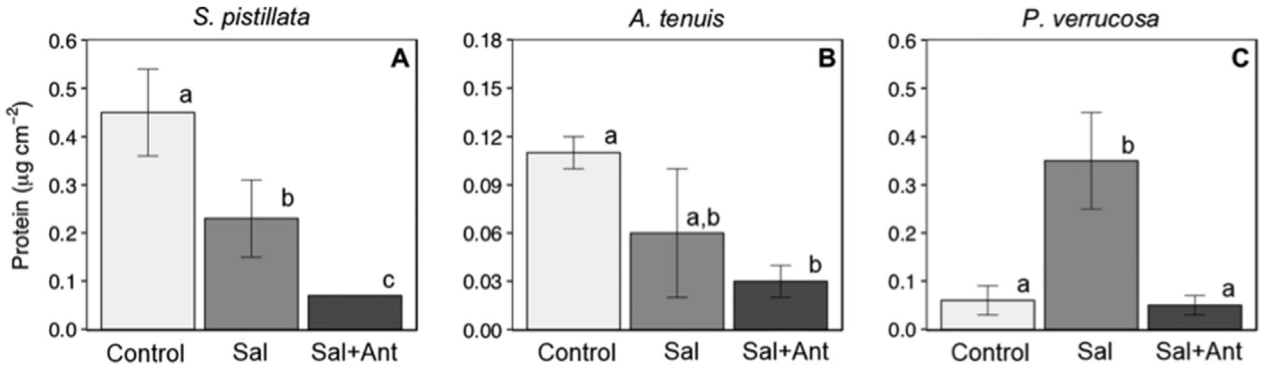

Fig. 4. Impact of increased salinity (Sal) and increased salinity and phosphonate-based antiscalant (Sal+Ant) on total protein content following 2–4 weeks of incubation. Measurements were compared to the control samples that were grown under ambient conditions. Total protein contents were measured from (A) *S. pistillata* (B) *A. tenuis* and (C) *P. verrucosa*. Letters above the bars refer to ANOVA followed by a Fisher (LSD) post-hoc analysis with significance of $p < 0.05$.

abundance has been documented numerous times (Achituv et al., 1994; Fabricius, 2005) although a fraction of total protein is associated with the coral tissue as well (Achituv et al., 1994). It can therefore be assumed, that both the loss of coral tissue (Fig. 1) and decrease in *Symbiodinium* abundance (Fig. 3) contributed to the protein biomass loss in our study. The relation between coral tissue mass and protein content has been suggested as a sensitive indicator of coral health, as the tissue (e.g. coral polyps) allocates energy reserves for calcification (Anthony et al., 2002; Houlbreque, 2004). Hence, the combination of loss of coral tissue, reduction of *Symbiodinium* abundances and of protein biomass, indicate a significant decrease of coral health in response to the increase in salinity and/or antiscalant tested in this study.

Net O_2 production, CO_2 uptake and calcification rates measured for *S. pistillata* and *A. tenuis* decreased with time throughout the study period in all treatments including the unamended control. However, the average net O_2 production rates in *S. pistillata* were lower (40%) in the experimental treatments compared to the control (Fig. 5A). In contrast, there was only a minor reduction in the overall average net O_2 production rates in *A. tenuis* in the experimental treatments compared to the control (Fig. 5B). Overall average CO_2 uptake rates were lower (30–50%) in the experimental treatments compared to the control in *A. tenuis* as well as in *S. pistillata* (Fig. 5C and D). Addition of antiscalants reduced the overall average net CO_2 uptake by 50–55% for both coral species. The average calcification rates in *S. pistillata* following incubation with increased salinity exhibited no apparent response relative to the control (Fig. 5E). However, incubation of *S. pistillata* with antiscalants caused nearly a 50% reduction relative to the control (Fig. 5E). Average calcification rates in *A. tenuis* decreased by ca. 30% in the Sal treatment, while in the antiscalants treatment calcification decreased by ca. 50% relative to the control (Fig. 5F). Values are reported in Table S2. These results suggest that polyphosphonate antiscalants affect the coral calcification independently of increased salinity, perhaps by poisoning crystal growth at the site of calcification (Dunn et al., 2012; Simkiss, 1964a, 1964b).

Net rates of O_2 production and CO_2 uptake measured in this study reflect the balance between photosynthetic activity and aerobic respiration by the coral holobiome. Low net production rates, that were measured in previous studies following incubation with high salinities, were attributed to loss of *Symbiodinium* and/or a retraction of the coral polyps due to osmotic shock (Ferrier-Pagès et al., 1999; Muthiga and Szmant, 1987). Similarly, a significant drop in net production was shown for the Red Sea coral *F. granulosa* in higher than ambient salinity; however the coral appeared to be able to acclimate to these conditions after 29 days of exposure (Röthig et al., 2016). We assume that the decreases in net production measured in this study also occurred as a response to the significant decrease in *Symbiodinium* abundance and, to a certain extent, the retraction of the coral polyps. Concurrently, the low abundance of heterotrophic bacteria and the loss of protein, indicate that the catabolic rates of the coral were highly impaired (Anthony et al., 2002).

Taking all of the above into consideration, although corals of the GoA are adapted to high salinity (up to 41) (Fine et al., 2013), our results indicate that 10% salinity increase above ambient levels may

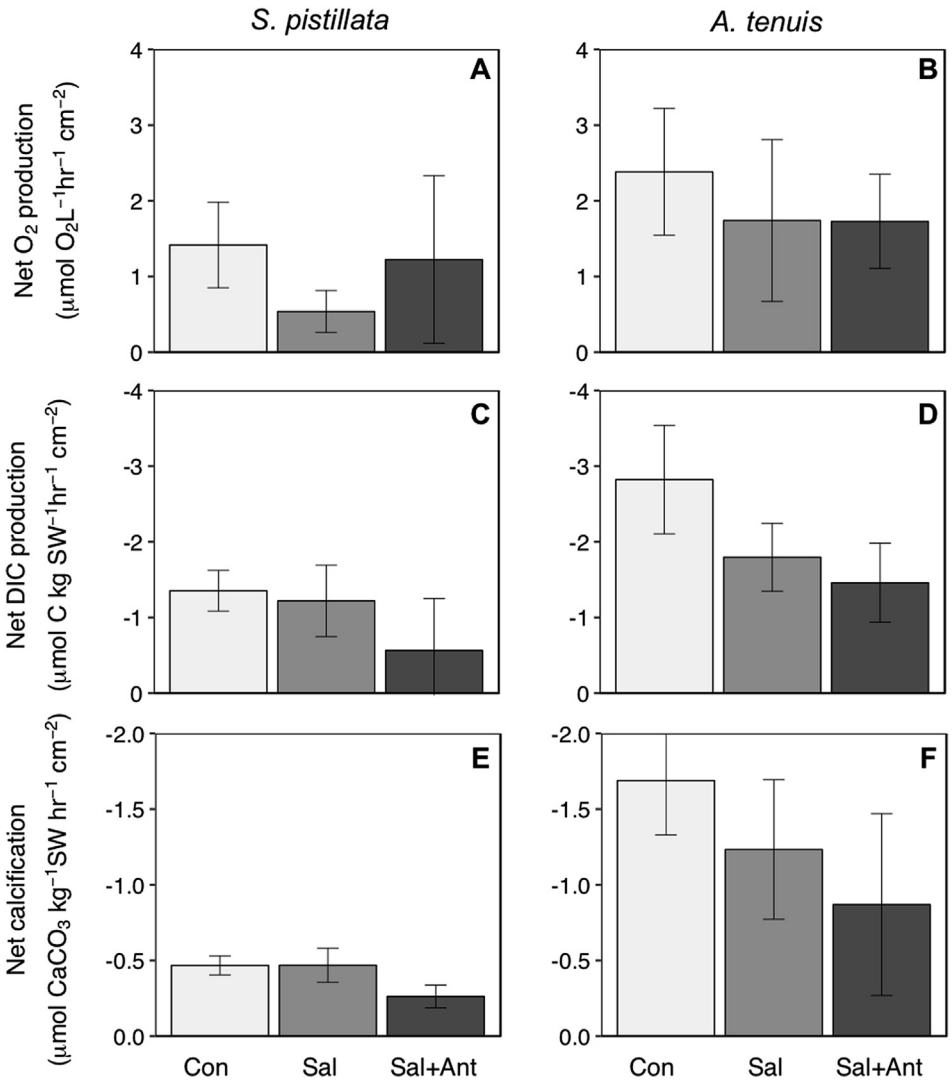

Fig. 5. Net O_2 production (A, B), respiration (C, D) and calcification rates (E, F) for *A. tenuis* and *S. pistillata* with increased salinity (Sal) and increased salinity and polyphosphonate-based antiscalant (Sal+Ant). Values represent the average and standard deviations gained over the course of the experiment and normalized to the surface area of *S. pistillata* and *A. tenuis* nubbins.

cause osmotic stress as this reaches the limit of the physiological tolerance for corals without adaptation (Mayfield and Gates, 2007). However, 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 (Barshis et al., 2013; Hughes et al., 2003; Palumbi et al., 2014). This is mostly attributed to genetic variation in both the corals and their corresponding *Symbiodinium* (Baums et al., 2014), and could be an explanation to the large variability seen in our results.

To the best of our knowledge, this is the first study published on the effects of polyphosphonate antiscalant on corals. Accordingly, we compare our results to studies where increases in phosphate concentration were investigated, assuming phosphonate could serve as a phosphate source (Tseng and Tang, 2014; Van Mooy et al., 2009). Previous studies on elevated phosphate concentrations around corals have not established any consistent trends in *Symbiodinium* abundance and protein contents, but have found reduced calcification and impaired reproduction (Fabricius, 2005; Harrison and Ward, 2001; Johnson et al., 1979; Muller-Parker et al., 2015; Walker and Ormond, 1982). Other studies have also shown no impacts or only slight decreases in calcification and *Symbiodinium* abundances due to increased phosphate concentrations (Fabricius, 2005; Holcomb et al., 2010). Our results, similarly to other studies, indicate that the responses of hard corals to increased salinity and phosphate (from antiscalants) are species-specific.

4. Conclusion

Water scarcity and the operation of large-scale desalination facilities coincide with many regions where coral reefs are found. Yet, only sparse and limited research has been carried out with focus on the impact of seawater desalination brine on reef-building corals. Our results clearly indicate that desalination brine may negatively impact the scleractinian hard corals holobiont, namely the coral and associated *Symbiodinium* and microbial community. We found that elevated salinity (10% above ambient) altered coral physiology and visual appearance. Concurrently, increased salinity together with the addition of polyphosphonate-based antiscalant often resulted in a greater impact on all three coral species tested. We suggest that this enhancement is due to a multi-stressor effect, namely osmotic-shock and increased eutrophication. Moreover, our data demonstrate that the impact of desalination brine on hard

corals is species-specific. Thus, we suggest that the ecotoxicological criteria adapted for hard corals should be determined based on tests done on key organisms forming the local coral communities that dominate the area at which desalination discharge is expected.

Taken together, we suggest that special attention should be given to the discharge of SWRO brine from current and future desalination facilities in the GoA, Red Sea. We propose that the brine from these facilities will be discharged via a diffuser system to increase brine dilution and minimize possible effects. Moreover, it is possible that the impact of multi-stressors such as osmotic shock and eutrophication would be greater in GoA than in other aquatic environments due to the confined basin and oligotrophic conditions of this water body. Since coral reefs around the GoA have considerable economic value to communities that reside in the area, it is central to maintain the wellbeing of this unique ecosystem. Finally, we recommend that future research on the effects of SWRO desalination on hard corals will also include changes in temperature and coagulants (which are often used in SWRO desalination) that were not investigated in this study.

Acknowledgement

Support was received from the NSF Coastal Sees program, #1325649, and NSF-OISE IRES #1358134 for AP and the Ministry of Infrastructures, Energy and Water Resources (Israel) (grant #: 214-17002) to E.B-Z and E.R. Partial student support for KLP was received from The Geological Society of America (grant #: 11215-16), The Weigel Scholarship for Coastal Studies, Myers Oceanographic and Marine Trust and The Explorers Club Youth Activity Fund (grant #: 83402). We would also like to thank the students, faculty and staff of Interuniversity of Marine Sciences in Eilat, espcially prof. Maoz Fine for use of his temperature controlled container, Ben-Gurion University of the Negev, Anne Bogler and Hila Frank, and UC Santa Cruz, Dan Killiam and Michele Markowitz, for help and support in completing this work.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.07.009.

References

Achituv, Y., Ben-Zion, M., Mizrahi, L., 1994. Carbohydrate, lipid, and protein composition of zooxanthellae and animal fractions of the coral *Pocillopora damicornis* exposed to ammonium enrichment. Pac. Sci. 48, 224–233.

Anthony, K.R.N., Connolly, S.R., Willis, B.L., 2002. Comparative analysis of energy allocation to tissue and skeletal growth in corals. Limnol. Oceanogr. 47, 1417–1429. https://doi.org/10.4319/lo.2002.47.5.1417.

Baker, D.M., Andras, J.P., Jordán-Garza, A.G., Fogel, M.L., 2013. Nitrate competition in a coral symbiosis varies with temperature among *Symbiodinium* clades. ISME J. 7, 1248–1251. https://doi.org/10.1038/ismej.2013.12.

Bar-Zeev, E., Rahav, E., 2015. Microbial metabolism of transparent exopolymer particles during the summer months along a eutrophic estuary system. Front. Microbiol. 6, 1–13. https://doi.org/10.3389/fmicb.2015.00403.

Barshis, D.J., Ladner, J.T., Oliver, T.A., Seneca, F.O., Traylor-Knowles, N., Palumbi, S.R., 2013. Genomic basis for coral resilience to climate change. Proc. Natl. Acad. Sci. U. S. A 110, 1387–1392. https://doi.org/10.1073/pnas.1210224110.

Baums, I.B., Delvin-Durante, M.K., Lajeuness, T.C., 2014. New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. Mol. Ecol. 23, 4203–4215. https://doi.org/10.1111/mec.12788.

Belkin, N., Rahav, E., Elifantz, H., Kress, N., Berman-Frank, I., 2017. The effect of coagulants and antiscalants discharged with seawater desalination brines on coastal microbial communities: a laboratory and *in situ* study from the southeastern Mediterranean. Water Res. 110, 321–331. https://doi.org/10.1016/j.watres.2016.12.013.

Belkin, N., Rahav, E., Elifantz, H., Kress, N., Berman-Frank, I., 2015. Enhanced salinities, as a proxy of seawater desalination discharges, impact coastal microbial communities of the eastern Mediterranean Sea. Environ. Microbiol. 17, 4105–4120. https://doi.org/10.1111/1462-2920.12979.

Birkeland, C., 2015. Coral Reefs in the Anthropocene, Coral Reefs in the Anthropocene. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-7249-5.

Brown, B.E., 1997. Coral bleaching: causes and consequences. Coral Reefs 16, 129–138. https://doi.org/10.1007/s003380050249.

Brown, B.E., Bythell, J.C., 2005. Perspectives on mucus secretion in reef corals. Mar. Ecol. Prog. Ser. 296, 291–309.

Byler, K.A., Carmi-Veal, M., Fine, M., Goulet, T.L., 2013. Multiple symbiont acquisition strategies as an adaptive mechanism in the coral *Stylophora pistillata*. PLoS One 8, 1–7. https://doi.org/10.1371/journal.pone.0059596.

Clausen, C.D., Roth, A.A., 1975. Effect of temperature and temperature adaption on calcification rate in the hermatypic coral *Pocillopora damicornis*. Mar. Biol. 24, 529–536. https://doi.org/10.1126/science.1152509.

Coles, S.L., Jokiel, P.L., 1978. Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. Mar. Biol. 49, 187–195. https://doi.org/10.1007/BF00391130.

Del-Pilar-Ruso, Y., Martinez-Garcia, E., Giménez-Casalduero, F., Loya-Fernández, A., Ferrero-Vicente, L.M., Marco-Méndez, C., de-la-Ossa-Carretero, J.A., Sánchez-Lizaso, J.L., 2015. Benthic community recovery from brine impact after the implementation of mitigation measures. Water Res. 70, 325–336. https://doi.org/10.1016/j.watres.2014.11.036.

Dubinsky, Z., Stambler, N., 2011. Coral Reefs: an Ecosystem in Transition. Springer, New York.

Dunn, J.G., Sammarco, P.W., LaFleur, G., 2012. Effects of phosphate on growth and skeletal density in the scleractinian coral *Acropora muricata*: a controlled experimental approach. J. Exp. Mar. Biol. Ecol. 411, 34–44. https://doi.org/https://doi.org/10.1016/j.jembe.2011.10.013.

Dyhrman, S.T., 2016. Nutrients and their acquisition: phosphorus physiology in microalgae. In: Borowitzka, M.A., Beardall, J., Raven, J.A. (Eds.), The Physiology of Microalgae. Springer International Publishing, Cham, pp. 155–183. https://doi.org/10.1007/978-3-319-24945-2_8.

Fabricius, K.E., 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Mar. Pollut. Bull. 50, 125–146. https://doi.org/10.1016/j.marpolbul.2004.11.028.

Ferrier-Pages, C., Gattuso, J.-P., Dallot, S., Jaubert, J., 2000. Effect of nutrient enrichment on growth and photosynthesis of the zooxanthellate coral *Stylophora pistillata*. Coral Reefs 19, 103–113. https://doi.org/10.1007/s003380000078.

Ferrier-Pagès, C., Gattuso, J.P., Jaubert, J., 1999. Effect of small variations in salinity on the rates of photosynthesis and respiration of the zooxanthellate coral *Stylophora pistillata*. Mar. Ecol. Prog. Ser. 181, 309–314. https://doi.org/10.3354/meps181309.

Fine, M., Gildor, H., Genin, A., 2013. A coral reef refuge in the Red Sea. Global Change Biol. 19, 3640–3647. https://doi.org/10.1111/gcb.12356.

Frank, H., Rahav, E., Bar-Zeev, E., 2017. Short-term effects of SWRO desalination brine on benthic heterotrophic microbial communities. Desalination 417, 52–59. https://doi.org/10.1016/j.desal.2017.04.031.

Gieskes, J.M., 1969. Effect of temperature on the pH of seawater. Limnol. Oceanogr. 14, 679–685. https://doi.org/10.4319/lo.1969.14.5.0679.

Grady, C.A., Weng, S.-C., Blatchley, E.R., 2014. Global potable water: current status, critical problems, and future perspectives. In: Younos, T., Grady, C.A. (Eds.), Potable Water: Emerging Global Problems and Solutions. Springer International Publishing, Cham, pp. 37–59. https://doi.org/10.1007/978-3-319-06563-2_2.

Greenlee, L.F., Lawler, D.F., Freeman, B.D., Marrot, B., Moulin, P., 2009. Reverse osmosis desalination: water sources, technology, and today's challenges. Water Res. 43, 2317–2348. https://doi.org/10.1016/j.watres.2009.03.010.

Harrison, P.L., Ward, S., 2001. Elevated levels of nitrogen and phosphorus reduce fertilisation success of gametes from scleractinian reef corals. Mar. Biol. 139, 1057–1068. https://doi.org/10.1007/s002270100668.

Holcomb, M., Mccorkle, D.C., Cohen, A.L., 2010. Long-term effects of nutrient and CO2 enrichment on the temperate coral *Astrangia poculata*. J. Exp. Mar. Biol. Ecol. 386, 27–33. https://doi.org/10.1016/j.jembe.2010.02.007.

Höpner, T., Lattemann, S., 2003. Chemical impacts from seawater desalination plants — a case study of the northern Red Sea. Desalination 152, 133–140. https://doi.org/10.1016/S0011-9164(02)01056-1.

Houlbreque, F., 2004. Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral *Stylophora pistillata*. J. Exp. Biol. 207, 1461–1469. https://doi.org/10.1242/jeb.00911.

Hughes, T.P., Baird, A., Bellwood, M., Card, M., Connolly, S., Folke, C., Jackson, J.B.C., Kleypas, J., Lough, J.M., Marshall, P., Palumbi, S.R., Pandolfi, J.M., Rosen, B., Roughgarden, J., 2003. Climate change, human impacts and the resilience of coral reefs. Science 301, 929–933.

Israel Ministry of Environmental Protection, July 2013. Israel Environmental Bulletin, vol. 39, pp. 8–10. Jerusalem.

Jenkins, S., Roberts, P., Schlenk, D., Weis, J., 2012. Management of Brine Discharges to Coastal Waters; Recommendations of a Science Advisory Panel, vol. 694. California Water Resources, Costa Mesa, CA, pp. 1–45. Technical report.

Johnson, K.M., Burney, C.M., Smetacek, V., 1979. Effects of elevated nitrogen and phosphorus on coral reef growth. Limnol. Oceanogr. 24, 935–940.

Kirchman, D., K'nees, E., Hodson, R., 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49, 599–607.

Koop, K., Booth, D., Broadbent, A., Brodie, J., Bucher, D., Capone, D., Coll, J., Dennison, W., Erdmann, M., Harrison, P., Hoegh-Guldberg, O., Hutchings, P., Jones, G.B., Larkum, A.W.D., O'Neil, J., Steven, A., Tentori, E., Ward, S., Williamson, J., Yellowlees, D., 2001. ENCORE: the effect of nutrient enrichment

on coral reefs. Synthesis of results and conclusions. Mar. Pollut. Bull. 42, 91–120. https://doi.org/10.1016/S0025-326X(00)00181-8.
Koren, O., Rosenberg, E., 2006. Bacteria associated with mucus and tissues of the coral *Oculina patagonica* in summer and winter. Appl. Environ. Microbiol. 72, 5254–5259. https://doi.org/10.1128/AEM.00554-06.
Kress, N., Shoham-Frider, E., Lubinevsky, H., 2015. Marine monitoring Around the Outfalls of Palmachim and Soreq Desalination Facilities. Israel Oceanographic & Limnological Research Ltd.
Kress, N., Shoham-Frider, E., Lubinevsky, H., 2014. Marine monitoring Around the Outfalls of Palmachim and Soreq Desalination Facilities. Israel Oceanographic & Limnological Research Ltd.
Kress, N., Shoham-Frider, E., Lubinevsky, H., 2013. Marine monitoring Around the Outfalls of Palmachim and Soreq Desalination Facilities. Israel Oceanographic & Limnological Research Ltd.
LaJeunesse, T.C., Pettay, D.T., Sampayo, E.M., Phongsuwan, N., Brown, B., Obura, D.O., Hoegh-Guldberg, O., Fitt, W.K., 2010. Long-standing environmental conditions, geographic isolation and host-symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium*. J. Biogeogr. 37, 785–800. https://doi.org/10.1111/j.1365-2699.2010.02273.x.
Lattemann, S., 2009. Protecting the marine environment. In: Micale, G., Rizzuti, L., Cipollina, A. (Eds.), Seawater Desalination. Springer, pp. 273–299. https://doi.org/10.1007/978-3-642-01150-4.
Lattemann, S., Höpner, T., 2008. Environmental impact and impact assessment of seawater desalination. Desalination 220, 1–15. https://doi.org/10.1016/j.desal.2007.03.009.
Lirman, D., Manzello, D., 2009. Patterns of resistance and resilience of the stress-tolerant coral *Siderastrea radians* (Pallas) to sub-optimal salinity and sediment burial. J. Exp. Mar. Biol. Ecol. 369, 72–77. https://doi.org/10.1016/j.jembe.2008.10.024.
Mabrook, B., 1994. Environmental impact of waste brine disposal of desalination plants, Red Sea, Egypt. Desalination 97, 453–465. https://doi.org/10.1016/0011-9164(94)00108-1.
Marshall, A.T., Clode, P., 2004. Calcification rate and the effect of temperature in a zooxanthellate and an azooxanthellate scleractinian reef coral. Coral Reefs 23, 218–224. https://doi.org/10.1007/s00338-004-0369-y.
Marubini, F., Davies, P.S., 1996. Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Mar. Biol. 127, 319–328. https://doi.org/10.1007/BF00942117.
Mayfield, A.B., Gates, R.D., 2007. Osmoregulation in anthozoan-dinoflagellate symbiosis. Comp. Biochem. Physiol. Mol. Integr. Physiol. 147, 1–10. https://doi.org/10.1016/j.cbpa.2006.12.042.
Missimer, T.M., Maliva, R.G., 2017. Environmental issues in seawater reverse osmosis desalination: intakes and outfalls. Desalination 434, 198–215. https://doi.org/10.1016/j.desal.2017.07.012.
Muller-Parker, G., D'Elia, C.F., Cook, C.B., 2015. Interactions between corals and their symbiotic algae. In: Birkeland, C. (Ed.), Coral Reefs in the Anthropocene. Springer, pp. 1–271. https://doi.org/10.1007/978-94-017-7249-5.
Muthiga, N.A., Szmant, A.M., 1987. The effects of salinity stress on the rates of aerobic respiration and photosynthesis in the hermatypic coral *Siderastrea siderea*. Biol. Bull. 173, 539–551. https://doi.org/10.2307/1541699.
Palumbi, S.R., Barshis, D.J., Bay, R.A., 2014. Mechanisms of reef coral resistance to future climate change. Science 344, 895–899.
Petersen, K.L., Frank, H., Paytan, A., Bar-Zeev, E., 2018. Impacts of seawater desalination on coastal environments. In: Gude, V.G. (Ed.), Sustainable Desalination Handbook. Butterworth-Heinemann, pp. 437–463. https://doi.org/https://doi.org/10.1016/B978-0-12-809240-8.00011-3.
Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I., Rosenberg, E., 2006. The coral probiotic hypothesis. Environ. Microbiol. 8, 2068–2073. https://doi.org/10.1111/j.1462-2920.2006.01148.x.
Ritchie, K.B., 2006. Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar. Ecol. Prog. Ser. 322, 1–14.
Roberts, D.A., Johnston, E.L., Knott, N.A., 2010. Impacts of desalination plant discharges on the marine environment: a critical review of published studies. Water Res. 44, 5117–5128. https://doi.org/10.1016/j.watres.2010.04.036.
Rosenberg, E., Koren, O., Reshef, L., Efrony, R., Zilber-Rosenberg, I., 2007. The role of microorganisms in coral health, disease and evolution. Nat. Rev. Microbiol. 5, 355–362. https://doi.org/10.1038/nrmicro1635.
Röthig, T., Ochsenkühn, M.A., Roik, A., Van Der Merwe, R., Voolstra, C.R., 2016. Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. Mol. Ecol. 25, 1308–1323. https://doi.org/10.1111/mec.13567.
Rowan, R., 1998. Diversity and ecology of zooxanthellae on coral reefs. J. Phycol. 34, 407–417. https://doi.org/10.1046/j.1529-8817.1998.340407.x.
Sánchez-Lizaso, J.L., Romero, J., Ruiz, J., Gacia, E., Buceta, J.L., Invers, O., Fernández Torquemada, Y., Mas, J., Ruiz-Mateo, A., Manzanera, M., 2008. Salinity tolerance of the Mediterranean seagrass *Posidonia oceanica*: recommendations to minimize the impact of brine discharges from desalination plants. Desalination 221, 602–607. https://doi.org/10.1016/j.desal.2007.01.119.
Sass, E., Ben-Yaakov, S., 1977. The carbonate system in hypersaline solutions: dead Sea brines. Mar. Chem. 5, 183–199. https://doi.org/10.1016/0304-4203(77)90006-8.
Schneider, K., Erez, J., 2006. The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*. Limnol. Oceanogr. 51, 1284–1293. https://doi.org/10.4319/lo.2006.51.3.1284.
Shnit-Orland, M., Kushmaro, A., 2009. Coral mucus-associated bacteria: a possible first line of defense. FEMS Microbiol. Ecol. 67, 371–380. https://doi.org/10.1111/j.1574-6941.2008.00644.x.
Simkiss, K., 1964a. Possible effects of zooxanthellae on coral growth. Experientia 20, 140. https://doi.org/10.1007/BF02150699.
Simkiss, K., 1964b. Phosphates as crystal poisons of calcification. Biol. Rev. 39, 487–504. https://doi.org/10.1111/j.1469-185X.1964.tb01166.x.
Simon, M., Alldredge, A.L., Azam, F., 1990. Bacterial carbon dynamics on marine snow. JSTOR 65, 205–211.
Simon, M., Azam, F., 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51, 201–213.
Stimson, J., Kinzie III, R.a, 1991. Temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linneaus) under nitrogen-enrichment and control conditions. J. Exp. Mar. Biol. Ecol. 153, 63–74.
Tseng, C.H., Tang, S.L., 2014. Marine microbial metagenomics: from individual to the environment. Int. J. Mol. Sci. 15, 8878–8892. https://doi.org/10.3390/ijms15058878.
UN-WWAP, 2015. The United Nations World Water Development Report 2015: Water for a Sustainable World. Paris. https://doi.org/978-92-3-100071-3.
van der Merwe, R., Rothig, T., Voolstra, C.R., Ochsenkuhn, M.a, Lattemann, S., Amy, G.L., 2014. High salinity tolerance of the Red Sea coral *Fungia granulosa* under desalination concentrate discharge conditions: an in situ photophysiology experiment. Front. Mar. Sci. 1, 1–8. https://doi.org/10.3389/fmars.2014.00058.
Van Mooy, B.a.S., Fredricks, H.F., Pedler, B.E., Dyhrman, S.T., Karl, D.M., Koblízek, M., Lomas, M.W., Mincer, T.J., Moore, L.R., Moutin, T., Rappé, M.S., Webb, E. a, 2009. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature 458, 69–72. https://doi.org/10.1038/nature07659.
Walker, D.I., Ormond, R.F.G., 1982. Coral death from sewage and phosphate pollution at Aqaba, Red Sea. Mar. Pollut. Bull. 13, 21–25.
Wangpraseurt, D., Pernice, M., Guagliardo, P., Kilburn, M.R., Clode, P.L., Polerecky, L., Kühl, M., 2015. Light microenvironment and single-cell gradients of carbon fixation in tissues of symbiont-bearing corals. ISME J. 10, 788–792.
Xiaomin, Y., Doroghazi, J.R., Janga, S.C., Zhang, J.K., Circello, B., Griffin, B.M., Labeda, D.P., Metcalf, W.W., 2013. Diversity and abundance of phosphonate biosynthetic genes in nature. Proc. Natl. Acad. Sci. Unit. States Am. 110, 20759–20764. https://doi.org/10.1073/pnas.