

Ecophysiology of Kuwaiti macroalgae with special emphasis on temperature and salinity tolerance related to the conditions at desalination plant outfalls

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Abstract

Brine discharged from seawater desalination plants impacts marine life by exposure to increased salinity and, in some cases, temperature. However, the responses of individual species to such stress remains poorly known yet their understanding is essential for assessing and predicting the impacts of seawater desalination plants. In this study, unialgal cultures obtained by the Germling Emergence Method of 34 taxa representative of the Rhodophyta, Chlorophyta and Phaeophyceae seaweeds in the Arabian Gulf, and isolated from the vicinity of two large desalination plants in Kuwait, were subjected to increased temperature and salinity under experimental conditions in the laboratory. The dataset is complemented by measurements of seawater temperature and salinity obtained at increasing distances from the outfalls of desalination plants and along the Kuwaiti coastline including from two pristine areas, Boubiyan and Fintas. Chlorophyta, especially *Ulva* spp., and Phaeophyceae displayed remarkable tolerance against hypersaline and thermal stress, suggesting that this group can cope better with adverse environmental conditions. Members of the Rhodophyta were considerably more sensitive to temperature increases.

Keywords

Brine; desalination plants; Germling Emergence Method; hypersaline and thermal stress tolerance, unialgal culture.

Running Head

Temperature and salinity tolerance of Kuwaiti seaweeds in the context of seawater desalination plants

1 Introduction

The Arabian Gulf is one of the harshest marine environments globally, due to marked temperature fluctuations and summer sea temperatures around 36°C (Naser 2011). Furthermore, the Gulf is one of the world's most saline water masses, with an average salinity of 40-50 (contrasting with the world ocean average of 35; Durack 2015). Salinity may rise considerably higher in enclosed bays, e.g., Sulaibikhat Bay (Doha study site) or intertidal pools due to evaporation, with 60 being commonly recorded at low tide at the western end of Kuwait Bay (Jones 1986). Also, higher salinities occur in Kuwait Bay due to discharges from desalination plants, as well as the effect of industrial and mining processes on the marine environment (Ahmad and Baddour 2014; Alosairi et al. 2018). Generally, in Kuwait, salinity decreases gradually from southern (44) to northern waters of the region (Al-Subbiya, close to Boubiyan Island, 36, this study) because of the diluting influence of freshwater inflow from the rivers of Iraq (Al-Yamani 2021).

There is an urgent need for fresh water in several parts of the world, which has meant that historically, especially in the Arabian Peninsula, which is one of the most arid regions of the world and chronically short of potable water (Moossa et al. 2022), marine environmental issues

associated with desalination have been considered secondary concerns (Safrai and Zask 2008). In the Gulf, rapid population growth resulting in increased demand for fresh water has led to an increasing number of seawater desalination plants being constructed in coastal areas (Hamoda 2001; Abdul-Wahab 2007). Kuwait alone accounts for approximately 15% of the world's total installed desalination capacity and 19% of the Gulf Cooperation Council (GCC) countries' total installed capacity (Al-Wazzan and Al-Modaf 2001). Demand for desalinated water in Kuwait is expected to grow further; in 2020, the population of Kuwait was 5 million, but is predicted to reach 7 million in 2030 (Gulseven 2016).

Desalination plants contribute to the thermal and hypersaline pollution of the surrounding environment (Abdul Wahab 2007; Ma et al. 1998). The magnitude of this impact depends on the technological characteristics of a given desalination plant - i.e., whether a plant operates by reverse osmosis (RO) or a distillation-based technique such as multi-stage flash distillation (MSFD) or multi-effect distillation (MED; Fernández-Torquemada and Sánchez-Lizaso 2005). It is also related to the physical and biological conditions of the marine environment receiving the effluents (e.g., bathymetry and currents) – for example, the northern Arabian Gulf, especially Kuwait Bay where one of the plants studied here is located, is extremely shallow and has weak hydrodynamics (Uddin et al. 2011). Typically, during the desalination process the salt concentration of desalination brine discharged directly into the near-shore environment (Hoepner and Lattemann 2003) is up to 2.5 times that of seawater, ranging from 44 to 90 (Fernández-Torquemada and Sánchez-Lizaso 2005). In addition, desalination brine from MSFD and MED – which applies for the plants in Kuwait investigated here – is accompanied by much elevated temperature, which for MSFD can vary from 3°C up to 20°C above ambient temperature (Al Barwani and Purnama 2008; Hashim and Hajjaj 2005). In contrast, brine from RO-based

desalination plants usually has ambient, or only marginally, increased temperature. Both salinity and temperature extremes are locally exacerbated by the highly saline and hot brine discharge of seawater desalination plants. The harmful impacts of brine on marine organisms can be further exacerbated by locally reduced dissolved oxygen levels and toxic compounds such as heavy metals stemming from corrosion inside the plants (which is especially associated with MSFD and MED plants), but also corrosion inhibitors and anti-scalants (Panagopoulos and Haralambous 2020).

Macroalgae are primary producers and habitat engineers, forming nursery communities for a variety of marine organisms. Sessile native species can be particularly sensitive to environmental changes such as exposure to desalination brine, resulting in changes in species diversity and abundance near brine disposal (Lattemann and Höpner 2008; Naser 2013; Smyth and Elliott 2013). Although macroalgal species can typically adapt to minor stresses and might even tolerate extreme situations temporarily, potentially they can be impacted when exposed over a long period of time (van der Merwe et al. 2013, 2014a).

Macrophytes in the Arabian Gulf are characterized by low species richness due to harsh environmental conditions such as high salinity and temperature levels (Naser 2013; Price et al. 2006; Sheppard et al. 2010). It can be assumed, therefore, that organisms inhabiting the coasts of Kuwait live at the limits of their physiological tolerance and must be adapted to survive despite the wide thermal fluctuations and salt content increasing (Uddin et al. 2011). This problem applies especially to poorly flushed and sensitive environments, such as that used in this work, a study area in Doha, which is situated in the innermost part of Kuwait Bay (Hashim and Hajjaj 2005; Uddin et al. 2011). Understanding changes in seaweed communities requires accurate knowledge of the diversity in local species, which may help to predict the ecophysiological

responses to environmental impacts such as hypersaline/thermal stress and climate change, which often result in changes in species diversity (Kokabi et al. 2016; Raffo et al. 2014).

Scientific studies that aim to understand possible impacts of desalination plants on marine macrophytes are inconsistent in their conclusions (Roberts et al. 2010). In some cases, studies conclude that desalination plants have a strong potential for detrimental impacts of brine disposal on the diversity of floral communities (Lattemann and Höpner 2008; Mauguin and Corsin 2005), especially on seagrass meadows (Xevgenos et al. 2021). Other studies, however, concluded that there is a negligible impact on macroalgal distribution through desalination brine discharge (Areqat and Mohamed 2005; Hashim and Hajjaj 2005). In fact, understanding of the tolerance and ecophysiology of key benthic macroalgal species in the Gulf, including Kuwait, is poor (Kim and Jeong 2013; Missimer and Maliva 2018).

In the work described here, a range of juvenile and microscopic stages of local benthic macroalgae were examined *in vitro* for physiological responses under controlled conditions in order to estimate tolerance to two abiotic stressors associated with brine outfalls (temperature and salinity), with the aim of increasing understanding of how the natural diversity of intertidal macroalgae changes at various distances from brine outfalls at typical desalination plants. The two locations of Doha East (DE) and Al Zour South (ZS) were representative of the environmental diversity in Kuwait and the Arabian Gulf. Based on observations, it was hypothesized that the diversity of macroalgae, assessed by cultivation of cryptic algae from natural substrata collected at various distances from brine outfalls, was negatively affected by the thermal and hypersaline conditions associated with desalination plant effluents. Also, it was hypothesized that the desalination plants located on the open seacoast (ZS) have less influence on algal diversity than those in the semi-enclosed Kuwait Bay (DE), due to differences in

oceanographic, topographic and bathymetric features. This study will help to evaluate and predict the future of macroalgal diversity around desalination plants in the Gulf Region in general and in the areas of DE and ZS in particular, in response to both desalination activities and the expected future increase in salinity and temperature due to climate change.

2 Materials and methods

2.1 Description of study sites

Surveys were conducted during April 2019, when triplicate samples of natural substrata, including small rocks, pebbles, mollusc shells and sediment material, were collected in 50-ml Falcon tubes from the upper intertidal zone and rocky shore at each location during low tide. From Doha ('b' in Figure 1), 66 samples were collected from shallow tide pools and 161 isolates at various distances (13 waypoints) away from the DE outfall: (0, 6, 50, 70, 90, 180, 300, 800, 1500 and 1700 m; Figure S1). At Al-Zour ('d' in Figure 1), 14 waypoints (54 samples yielding 191 isolates) were collected at different distances away from the ZS outfall (28, 34, 50, 80, 100, 200, 600, 800 and 950 m), including the reference sites of Fintas and Boubyan Island ('a' and 'c' in Figure 1). During February 2019, additional sampling locations were also included to ensure better representation of the algal diversity of Kuwait; cryptic stages of seaweeds were isolated from randomly collected samples from Northern and Southern Provinces of Kuwait coast including offshore islands. Samples were transported to Bezhin Rosko (Santec, France) to isolate and cultivate the microscopic stages of the diverse cryptic macroalgae contained in them. There, samples were processed using the Germling Emergence Method, following standard methods (Peters et al. 2015). In summary, during collection approximately 0.5 cm³-sized rock fragments, pebbles, and old shells were placed in Falcon tubes (15 or 50 ml) to avoid any contamination

with spores from surface water. Isolates exhibiting different morphologies were further cultivated and identified morphologically before confirming the identification with molecular methods; laboratory culture followed standard methods (Coelho et al. 2012). Each isolate was given a code number, details of the collection site (including site name, location coordinates, topographical details and date of sampling; Supplementary Table S1). The complete list of strains employed in this study is provided in Table S2. Salinity was measured *in situ* at high tide using a portable refractometer (National Industrial Supply, Temecula, California, USA), whilst a multi-parameter water Quality Meter (HORIBA U10, Ltd, Kyoto, Japan) was used to measure the temperature.

2.1.1 Doha East Power Desalination Plant (DE)

The DE site occupies the southwestern extremity of Kuwait Bay, which is situated along the northern coastline of Kuwait. This site is a shallow, tide-dominated embayment with a tidal mud flat basin (~20 km²), and a maximum depth of approx. 8 m (Al-Sarawi et al. 2002; Figure S1). The Bay is a semi-enclosed, hypersaline water body with low wave and current energy and a flushing time of 65 days (Pokavanich et al. 2013). In this area, there are major socioeconomic activities that could be regarded as point sources of pollution and eutrophication, but the Bay represents a unique ecosystem and a significant nursery ground for many fish and shrimp species (Al-Mutairi et al. 2014; Al-Yamani et al. 2004). This desalination plant, which at the time of sampling was operating an MSFD-based system, has a net production of desalinated water of 56.04 Mm³ yr⁻¹ (million cubic meters per year) (<https://water.fanack.com/kuwait/water-infrastructure-in-kuwait/>).

The reference area for Doha was Boubiyan Island ('a' in Figure 1), because it is away from heavy urbanization and eutrophication sources on Kuwait Bay. Boubiyan is one of the largest islands in the Kuwaiti coastal chain, situated in the north-western corner of the Arabian Gulf, with an area of 863 km². It is close to the Shatt Al-Arab Delta and has a flat, low topography with salt marshes, especially on the northwest coast, which is deeply indented swampland (Smith et al. 2022).

2.1.2 Al-Zour South Power Desalination plant (ZS)

The ZS power desalination plant is approximately 90 km south of Kuwait City, near the border with Saudi Arabia, in the southern province of Kuwait (Figure S1). The marine environment there is characterized by sandy and rocky coastal intertidal flats, or wave-cut cliffs carved into the old coastal ridges in open waters (Al-Said et al. 2017). Wave and current energy are moderate to high and the mean tidal range is approx. 2 m (Al-Sarawi et al. 1998). This plant operates as an MSFD-based system; however, during high demand, ZS also operates RO units capable of producing a total of 101.79 Mm³ yr⁻¹ of fresh drinking water, equivalent to 20% of Kuwait's installed water treatment capacity (<https://water.fanack.com/kuwait/water-infrastructure-in-kuwait>). The Fintas beach reference site ('c' in Figure 1), which is located at open water on the middle-southern shore of Kuwait, is not impacted by desalination brine. In general, this shoreline is characterized by a hard rocky substratum.

2.2 Salinity and thermal stress experiment

The isolates used in all experiments were in the juvenile stages of thallus development obtained from substratum samples Germling Emergence (GE technique; Hasan et al. 2023b) and

were transferred to Petri dishes containing sterile Provasoli-enriched natural sea water (PES) (Provasoli 1966) medium with different salinities (35, 45, 60, or 80). The salinities were calculated using an established formula (Lewis and Perkin 1978), where salinity $S = \text{mass of salt in grams} / \text{mass of seawater in grams} \times 1000$. Short-term salinity treatments were conducted by adding commercially available sodium chloride (99.5% purity) to seawater (Fisher Scientific, Belgium). Throughout the duration of the experiments, salinities were monitored using a portable conductivity meter (Mettler Toledo FE30\EL30 conductivity meter; Leicester, UK). Controls were grown at 33 based on the lowest salinity recorded in the northern province of Kuwait, in particular Boubiyah Island (Al-Yamani et al. 2004). A constant temperature of 25°C ($\pm 1^\circ\text{C}$) was maintained in a 240-l SANYO CO₂ incubator (MIR -253 CFC FREE- UK). To examine the temperature tolerance range, unialgal isolates were incubated with a constant salinity of 33 (resembling ambient conditions) and an average irradiance of 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ measured with a Skye Instruments SKP 200 light meter (Skye Instruments Ltd, UK) in a 12:12 h light–dark regime at temperatures of 3°, 10°, 25°, 30°, 35°, and 40° C ($\pm 1^\circ\text{C}$). The cultures were incubated for 14 days, then returned to 25°C for an additional 2 weeks to determine if they were able to recover their ability to grow. At the end of the incubation period, isolate survival, morphology and ability to generate new growth were observed under a stereomicroscope (Leica S6E with 10x eyepieces; Morrisville, US) (Bolton 1983).

Survival over the experimental period was scored by assessing the extent of thallus bleaching under each salinity or temperature treatment. For both experiments, cultures of all shoots were photographed to record any detrimental effects, such as the presence of dead cells and percentage of bleached surface (Cambridge et al. 2017). The observed stress symptoms were

determined visually as indicated by percentage of thalli bleached, and grouped into 5 categories based on the extent of loss of pigmentation in the thalli:

- Isolates with no loss in pigments were grouped as Not Affected (NA) and given a value of 1.
- Those showing a loss of pigmentation of no more than 25% of their thalli were assigned as Some Bleaching (SB) and given a value of 0.75.
- Those showing loss in pigmentation of between 25% and 50% of the thalli were assigned as Partial Bleaching (PB) and were given a value of 0.5.
- Bleaching between 50% and 80% was assigned as Mostly Bleached (MB) and given a ratio of 0.25.
- Thalli that were completely Bleached (B) (80-99%) were assigned a value of 0 (Legendre et al. 2005).

Further, mortality rates were estimated as the percentage of thalli that did not re-grow when returned to normal salinity (33) or temperature ($25 \pm 1^\circ \text{C}$). Average survival value was calculated for each species. Species were then grouped according to phylum, and the percentage survival of each phylum was calculated by summing all species survival averages, dividing by

the number of species under that phylum, and multiplying by 100, $\%s = \frac{Av_{sP}}{nsp} \times 100$.

2.3 DNA extraction, PCR amplification and sequencing

DNA was extracted from 5-15 mg wet weight of algal cultures using CTAB and the GENEJET Plant Genomic DNA Purification Kit (Thermo Scientific, Vilnius, Lithuania)

following the manufacturer's protocol (Gachon et al. 2009). Extracted DNA from the unialgal isolates was amplified by polymerase chain reaction (PCR). The primers used were specific to the nuclear ribosomal markers SSU (White et al. 1990), ITS2 (Lane et al. 2006) and the plastid-encoded gene for elongation factor 1-alpha (*tufA*) locus (Famà Patrizia et al. 2002) used for Chlorophytes, the partial mitochondrial gene regions (5'COI; Saunders 2005) to examine Phaeophyceae, and plastid-encoded *psaA*, *psbA* gene (Yoon et al. 2002) and plastid locus, such as Rubisco spacer region *rbcL* (Kawai et al. 2007; Peters and Ramírez 2001) were used for Rhodophyta. PCR products were sequenced by a commercial Sanger sequencing service (Source Biosciences, Cambridge. UK). Consensus sequences were aligned using the software BioEdit Editor (Hall et al. 2010) and sequences compared to published data using NCBI BLAST searches (<http://www.ncbi.nlm.nih.gov>) (Altschul et al. 1997). The sequences were deposited in GenBank/NCBI (Table S2). Further details of the cultured isolates related to this study are published separately (Hasan et al. 2023b).

3 Results

3.1 Physical-chemical parameters for DE and ZS desalination plants

Salinity data distributions were plotted on maps (Figure 2 a and c) and ranged between 40 and 43.5, and sea water temperature (SST) ranged between 36°C and 38.4°C (Figure 2b and d, Table S3). At Boubiyan, the reference site for Doha, the average salinity was 36, and sea surface temperature (SST) values ranged from 23.1° to 37°C (Table S3). Al-Zour samples showed variation in salinity between 40 and 43.5; Figure 3a and c), while temperature (SST) ranged from 35.86 to 39.43°C (Fig. 3 b, and d, Table S3). The salinity and temperature (SST) values of the reference site (Fintas) for Al-Zour were 40 and 21.6 °C, respectively (Table S3).

3.2 Substratum samples and molecular analysis obtained from Germling Emergence

A total of 345 unialgal macroalgal clones were obtained from 308 substrate samples (Hasan et al. 2023b). The work was focused on 47 algal isolates that were tested for salinity and temperature tolerance. Of these, 32 isolates (68%) were obtained using the GE method from sediment samples, while 14 strains (32%) were obtained from macroscopic field material or as mature individuals that were cultivated directly; 1 strain was obtained and cultivated by fertilization of fertile cells *in vitro* (Table S1). Amongst the 47 isolates, 31 (66%) were collected in the vicinity of the DE and ZS desalination plants (April 2019) and the other 16 (34%) were collected from different sites on the Kuwait coast (February-April- 2019). Overall, 35 (74%) of the strains were collected from the Southern Province, including Fintas, whereas 12 (26%) strains were collected from the Northern Province, including Boubiyan. The strains selected for ecophysiological experiments covered a wide range of taxa across the 3 major phyla of macroalgae. Also, the aforementioned study in Kuwait revealed one new genus and species in the red algal order Bangiales (Hasan et al. 2022).

Sequences were divided into three groups. Using molecular methods, 19 isolates of Chlorophyta were identified (40% of total, 13 species), along with 15 isolates of Phaeophyceae (32%, 11 species) and 13 isolates of Rhodophyta (28%, 10 species). Based on the DNA barcoding results (Table S2), 12 sequences indicated species-level identity, 15 enabled genus-level identification, and 5 clustered with higher-level taxonomic entities in each phylum.

3.3 Effect of salinity on algal viability

The viability of 44 selected strains (only for salinity treatment) was tested under varying salinity levels. When incubated for 14 days in seawater with a salinity of either 35 or 45 at a constant temperature of 25°C, the cultures showed no negative response in terms of viability (100% at both salinities). Furthermore, none of the tested algal isolates had any visible stress signs at those values. At salinity levels of 60 and 80, however, cultures showed variable responses (Figure 4). Members of the phylum Chlorophyta were the least affected by incubation with a salinity of 60, in which 96% of Chlorophyta species survived (Table S4). Two species (*Ulva ohnoi* [K55] and *Cladophora laetevirens* [K56]) out of a total of 18 tested species of green algae had some bleaching (5-20%). *Cladophora laetevirens* was the only species with some bleaching which was able to regain growth when returned to normal seawater salinity. The Phaeophyceae species were the most affected algae when incubated at 60, developing degrees of bleaching between 20 and 100%, with an overall species viability of 58% (Figure S2). In particular, strain *Sphacelaria* sp. (K17) was seriously affected by the 60 and 80 salinity treatments. Additionally, among 4 strains of *Feldmannia mitchelliae* (K47, K48, K198 and K216), only isolate K216 was not affected; K47 and K198 had some degree of bleaching, between 0-20%, and K48 exhibited bleaching ranging between 5 and 80% (Table S4). Among the Rhodophyta, only five out of 12 tested strains (*Porphyrostromium* sp. [K36], *Polysiphonia* sp. [K61], *Ceramium* sp. [K62] and *Spyridia* sp. [K66, K78]) exhibited some visible stress signs (Table S4). The species survival rate for the red algal species was 82% (Figure S2).

When incubated in a salinity of 80, cultures of Phaeophyceae and Rhodophyta tested here showed varying degrees of bleaching, with the majority exceeding 50%, and some thalli also becoming visibly distorted as wrinkling. The general species viability of Rhodophyta and

Phaeophyceae was 29% and 30%, respectively. Only *Porphyrostromium* sp. (K36) was able to regain growth after partial bleaching (20%) when returned to normal (control) seawater salinity (Table S4). A total 4 out of 18 tested strains of Chlorophyta (*Bryopsis* sp. [K49], *U. ohnoi* [K55], *C. laetevirens* [K56] and *Rhizoclonium* sp. [K58]) were showing 50% or more bleaching. The general species survival rate for the Chlorophyta was 71% (Figure S2). Overall, the survival rate of Chlorophyta was considerably less affected by increased salinity, especially in the 80 salinity treatment (Figure S2). Chlorophyta showed notably greater tolerance to hypersaline treatments at all concentrations (35, 45, 60 and 80). At moderately increased salinity (60), members of the Phaeophyceae exhibit loss of pigmentation/bleaching to a higher degree than Rhodophyta and Chlorophyta. However, when salinity levels up to 80 were applied, Rhodophyta showed the highest degrees of bleaching with lethal effects against all three algal phyla.

3.4 Temperature tolerance range of isolated strains

Algal strains isolated from samples collected at various locations from Kuwait using the GE method were incubated at temperatures ranging from 3 to 40 °C. None of the 47 isolated strains responded negatively when incubated for 14 days at temperatures between 25 °C and 30 °C with a constant salinity of 33. Indeed, this cultivation temperature seemed to be optimal for all isolated strains, with the highest growth observed at 25 °C and 30 °C (Figure S3) and there were no visible signs of stress, such as loss of pigmentation or bleaching. At an incubation temperature of 35°C, the least affected species were Chlorophyta, with 96% viability. Among the Chlorophyta, only one strain (*Bryopsis* sp. [K49]) of 19 tested isolates showed some bleaching (<20%; Table S5). In the Rhodophyta, out of 13 strains tested, a species of Acrochaetiales (K34)

displayed 50% or more bleaching signs, while *Ceramium* sp. (K62), exhibited <20% bleaching. The survival rate for Rhodophyta species was 93%. Isolates of Phaeophyceae were more affected by increasing thermal stress – in particular, *Sphacelaria* sp. (K17, K71), *Sphacelaria tribuloides* (K72), a species of Acinetosporaceae (K19) and *Iyengaria stellata* (K24) - showed various degrees of bleaching (between 20% and 50%), with a species survival rate of 91% (Figure 5). None of the strains examined for 35°C treatment and showing various degrees of beaching were capable of recovering when returned to normal control temperature.

Isolates subjected to a temperature of 40°C, as compared to 35°C, displayed a reduced survival rate. At 40°C, 31 of 47 (66%) isolates showed 50% or more bleaching of the thallus. At 40°C, Chlorophyta were least affected, with 4 of 19 (21%) tested strains showing no bleaching at this higher temperature, namely *U. tepida* (K52), two strains of Ulvellaceae (K43, K45) and *Rhizoclonium* sp. (K58). Only 10 Chlorophyta isolates - *U. tepida* (K4, K25, K54), *Ulvella* sp. (K39, K40), a strain of Planophilaceae (K41), *Bryopsis* sp. (K49), *U. ohnoi* (K55), *Cladophora laetevirens* (K56) and one species of *Chaetomorpha* Kützinger sp. (K73) - displayed 50% or more bleaching. The overall viability rate for the Chlorophyta group was 54% (Figure 5). Among the Rhodophyta, all 13 strains showed a significant degree of bleaching (50-100% of thalli) at 40°C. The exception was *Polysiphonia* sp. (K61), which showed less than 20% bleaching. The survival rate for Rhodophyta species was 21%. Similarly, 11 of 15 species of Phaeophyceae tested showed bleaching in the 50-100% range, with the exception of a strain of Chordariaceae (K22), *Elachista stellaris* (K26), two species of *F. mitchelliae* (K198, K216), *Bachelotia* sp. (K70) and *Colpomenia sinuosa* (K87), each of which exhibited <20% bleaching. Relative viability of Phaeophyceae was 52%, approximately similar to the Chlorophyta (54%). Chlorophyta showed

high tolerance to thermal stress at the increased temperatures tested (35, 40°C), with less loss in pigmentation/bleaching than algae in the other two phyla.

At 40°C, species of Rhodophyta showed the highest degree of bleaching, compared to the other algal phyla. Table S5 shows the entire dataset from the ecophysiological experiments, displayed as survival rates of isolated strains during the temperature stress experiments (35°C, 40°C). Among the 43 strains showing effects of stress, seven strains of Chlorophyta and two of Phaeophyceae were able to recover when returned to 25°C, whereas no Rhodophyta showed any recovery.

The temperature tolerance limit of the isolates in the cooler range was also wide, down to 3°C. Among the Rhodophyta, 96% of the taxa incubated for 14 days at 3°C survived, and only one species of Acrochaetiales sp. (K34) and *Spyridia* sp.(K78) of the 13 tested exhibited some bleaching and visible signs of stress (< 20%). Of the Chlorophyta, 96% of taxa also showed high viability under the cool conditions, with only two isolates (Ulvellaceae [K43] and *Bryopsis* sp. [K49]) of the 19 tested exhibiting bleaching (<20%). Isolates of Phaeophyceae were the most affected in cold water, showing between 20% and 100% bleaching, with a general species viability of 89%. Two strains (*Bachelotia* sp. [K70] and *Sphacelaria tribuloides* [K72]) showed over 50% bleaching, while *Sphacelaria* sp. (K17) showed beaching symptoms of less than 20%. Seven of 47 tested strains that showed some sign of bleaching at 3°C were able to re-grow when returned to ambient temperature (25°C).

During incubation at 10°C, Rhodophyta and Chlorophyta showed optimal growth; viability was 100% for Chlorophyta and Rhodophyta species at this temperature. Among brown algae, *Bachelotia* sp. (K70) showed the most pronounced bleaching (50-80%) in response to incubation

at 10 °C. Viability of brown algae at 10°C was 93%. Only *Bachelotia* sp. (K70) fully recovered when subsequently incubated at the control temperature.

When comparing species viability of isolates of the three algal phyla, the Rhodophyta were the most sensitive (21%) at a temperature of 40°C, while both green and brown algae showed higher viabilities of 54% and 52%, respectively.

3.5 Algal diversity at different distances from the DE and ZS brine discharges

Using the Germling Emergence Method, seven strains of Chlorophyta were isolated from samples collected at the Doha site (Figure 6). When examining the morphospecies diversity at different distances from outfalls at the Al-Doha site, Chlorophyta species started to appear at 800 m distance, suggesting that macroalgal species diversity increased with distance from the outfalls (Figure 7a). At Boubiyan Island, the reference site for Doha, two Phaeophyceae taxa were collected (Figure 6). The highest diversity was clearly at the Al-Zour site, with 18 different species identified (8 green, 5 red and 5 brown). At Al-Zour, two species of *Chaetomorpha* sp. were obtained from samples collected at 27 m (waypoint sampling 3) from the discharge outfall, and a further 16 species, including red and brown taxa, were found at 950 m from the outfall (wpt 13; Figure 7b). At Fintas, the reference site for Al-Zour, six isolates, four red and two brown species were collected and identified at this site (Figure 6). Overall, diversity at the Doha site was low compared to Al-Zour. Regarding the diversity of the algal groups in relation to distance (Figure 8), increased diversity with increased distance away from the brine outfall ($R^2=0.79$) of Al-Zour was observed. Further isolates used in this study were obtained from other locations along the Kuwait coastline (Table S6).

4 Discussion and conclusions

4.1 Environmental parameters near desalination plants

Studies addressing how physical and biological factors influence seaweed community dynamics in the Arabian Gulf are few, particularly in Kuwait. This young body of water is under extreme temperature and salinity conditions compared to other bodies of water globally, including the Indian Ocean, from which it extends (Saeed et al. 2019; Uddin 2014). Temperature and salinity conditions are further exacerbated in the vicinity of brine outfalls of the continuously increasing desalination plant facilities along the coast. In the present research, unialgal isolates obtained by the GE technique were assessed *in vitro* for their tolerance to different salinity and temperature regimes. Subsequently, these findings were compared to the seaweed diversity in relation to temperature fluctuations and salinity near two brine outfalls. Salinity of the Gulf waters around the Kuwait coast ranges broadly from 38-42, and a salinity limit of 42 was set by the Kuwait Environmental Public Authority (KEPA) for Kuwait marine waters as safe for the biota and ecological balance in the marine environment (Al-Yamani et al. 2004; Uddin 2014).

With regard to sea water temperatures in Kuwait, the sea surface temperatures (SST) regularly exceed 36°C in August with a high seasonal temperature amplitude of over 25°C between summer and winter (Alosairi et al. 2011; Reynolds 1993). In Kuwait Bay, the average SST recorded was 10°C during January and 36°C in August, while at Al-Zour, the average SST in summer was 33.9°C and 15.1°C in winter (Al-Yamani et al. 2004; Lee and Kim 2019). According to KEPA standards (2002), 23.52°C was recorded as a mean for the annual SST for the coastal waters of Kuwait, with a range of 10.77 to 33.21°C. All salinity and temperature measurements in the present study (Table S3), which were obtained from discharge points of the

two desalination plants, were higher than the KEPA standards and decreased with increasing distance from the brine discharge points. The salinity nearest to the DE and ZS brine outfalls was as high as 43.5 (at both sites), decreasing to approximately 40 near Doha at a distance of 190 m (wpt 9) and 39.5 at Al-Zour at a distance 950 m (wpt 13). According to an observation by Saeed et al. (2019), the mean increases in salinity values at both outfalls (compared to surrounding seawater at the sites) were almost 4. These values are similar to those described by Al Dousari (2009), who demonstrated that seawater desalination by the MSFD process (operational at both DE and ZS) generally results in an increase in salinity of the discharge flow of between 2-4 above ambient mean levels for Kuwait seawater. This result agrees with the fact that mixing of the brine with sea water occurs in the study areas within a short distance. These findings were in line with other studies (Roberts et al. 2010; van der Merwe et al. 2014b). This problem is compounded by the topographic nature of the site - especially Doha - with shallow tidal pools and slow water flush circulation (Al-Mutairi et al. 2014), together with fine-grained muddy river sediments deposited from the nearby Shatt-Al-Arab estuary. These properties may contribute to the increased capacity of finer particles to trap pollutants, heat and salt within muddy sediments in the area (Al-Sarawi et al. 2002). At Boubiyan (control site for DE), salinity was 36, which was the lowest among all the study locations in this work. This finding was in line with a recent study (Alosairi and Pokavanich 2017), and could suggest that the influence of freshwater discharge minimizes the occurrence of high salinity in northern Kuwait, which remains a serious issue in the south. In comparison, at Fintas (control site to Al-Zour), salinity was 40.

The SST at Al-Zour (39.43°C) was higher than at DE (38.4°C), with the values for both DS and ZS exceeding mean KEPA standards for SST in coastal waters of Kuwait by 4-6° C (Al-Mutairi et al. 2014). These findings of a temperature between 4 and 6°C above ambient and

mean levels for Kuwait seawater reflect some of the potential impacts of MSFD-based seawater desalination (Al-Shammri and Ali 2018). In former studies and the present work, all the recorded temperatures were below 40°C. However, intertidal seaweeds can be exposed to air temperatures considerably higher than 40°C during summer at low tide. It can be assumed that intertidal seaweeds have developed physiological adaptations to such a broad range of daily fluctuations in temperature and salinity caused by tidal changes (Einav et al. 1995). While marine life can temporarily adapt to minor changes in temperature for short durations, extended long-term temperature change can be fatal (Lattemann and Höpner 2008). Indeed, since 1985, the seawater temperature of Kuwait Bay and the northern Arabian Gulf has increased at an average of 0.6 (± 0.3) °C per decade (Al-Banna and Rakha 2009; Al-Rashidi et al. 2009) which is about three times faster than the global average rate. The differences are due to regional and local effects (Kim and Jeong 2013). Smith et al. (2007) showed that, around the typical desalination plants operating in the Gulf Region, salinity can be up to 44 and temperature can be 4-6°C above ambient levels. These findings concur with other studies (Al Dousari 2009; Al-Shammari and Ali 2018; Roberts et al. 2010).

4.2 GE diversity in Kuwait

The diversity and abundance of seaweeds found in Kuwait varies at different times of the year in response to the wide seasonal changes in temperature. Increased irradiance and photosynthetic rates during increasingly long days, in combination with increasing though not extreme temperatures starting in the spring season, provide optimal growth conditions for intertidal macroalgae (Arenas and Fernández 2000). During the extremely hot summer, many of the macroscopic seaweed thalli disappear, with most species surviving as cryptic stages.

Consequently, the structure and composition of macroalgal assemblages fluctuates both in time and space in response to the extreme conditions in the Arabian Gulf (John 2012; Kokabi et al. 2016; Saeed et al. 2019).

The GE method is adapted to capturing much of the cryptic macroalgal diversity at a given site (Peters et al. 2015). In the present study, a total of 47 GE isolates representing 34 species were tested for salinity and temperature tolerance under *in vitro* conditions. These isolates were identified using morphological and molecular techniques (Hasan et al. 2023b).

At the time of sampling, 7 isolates were identified at Doha. This area is commonly densely covered with dominant Chlorophyta species. The diversity revealed by GE was low compared to Al-Zour, with only members of the phylum Chlorophyta being detected at approx. 806 m (wpt 10) from the outfall until 1770 m (wpt 7) distance.

The marine environment of Kuwait is also strongly influenced by the discharge of the Shatt Al-Arab estuary with a maximum peak of discharging fresh water from March to July (Al-Said et al. 2017). This freshwater input is associated with a high nutrient content, which contributes to noticeably higher nutrient concentrations at Doha, where nutrients accumulate due to low wave action and the bathymetry of this bay (Al-Yamani et al. 2004; Martínez et al. 2012; Villacorte et al. 2015). Thus, in this study, opportunistic green algal species of the genus *Ulva* were abundant at Doha during winter and early spring and are among the fastest growing macroalgae under conditions of high nutrient and light availability (Phillips and Hurd 2003). Competition among thalli of green algae for space at Doha is intense, in particular when light levels increase in spring and *Ulva* species generally have high levels of desiccation resistance (Nybakken 1993). These observations are consistent with results of Al-Hasan and Jones (1989), Al-Yamani et al. (2014) and Hasan et al. (2023b), who mentioned that the genus *Ulva* grows extensively in blanket-like

communities in the Doha area, where there is less competition with other species. Overall, this province contains low species diversity, most of which occurs in rock pools among the aforementioned platforms, where several grazers of macroalgae, such as sea urchins and demersal fish, are commonly observed and have a permanent grazing impact upon the diversity of macroscopic algae (Castro et al. 2008; Fatemi et al. 2012).

At the Boubiyan reference site, two brown algal isolates, *Ectocarpus subulatus* (K23) and *Colpomenia sinuosa* (K87) were identified; however, these are clearly adapted to unstable substrata. The limited occurrence of brown and red algae at Doha can also be linked to the muddy substrates (John 1986). The soft nature of these substrata can also explain why the Northern Province is mostly unsuitable for attachment and growth of several macroalgae particularly large brown macroalgae (Hasan et al. 2023a), which agrees with our observations.

In general, our observations showed that the Southern Province was rich in Rhodophyte taxa, with 13 isolates representing 10 taxa; see Hasan et al. (2023b). This finding was emphasized by Al-Hasan and Jones (1989) and Al-Yamani et al. (2014), who also mention this pattern in their previous publications on diversity of the macroalgal flora of Kuwait. At the Fintas reference site, the flora includes four red algal species, *Sahlingia subintegra* (K28), *Stylomena alsidii* (K31), *Erythrotrichia* sp. (K32) and *Porphyrostromium* sp. (K36), together with two brown algal species, *Iyengaria stellata* (K24) and *Feldmannia mitchelliae* (K48). These observations are consistent with those of Al-Yamani *et al.* (2014), who indicated that taxa such as *Sahlingia subintegra*, *Erythrotrichia* sp. and *Iyengaria stellata* were common in the intertidal zone or washed up on the beach in the Fintas area. At the Al-Zour site, 18 species (out of a total of 47 isolated strains) were found, with Chlorophyta being the most frequently encountered phylum close to the outfall. Isolates of *Chaetomorpha* sp. (K73, K74), *U. tepida*

(K4) and one brown alga (Acinetosporaceae, K19) were found at around 27-28 m from the desalination plant outfall (wpt 2,3). The first red algal species (*Acrochaetium* sp., K13) appeared at wpt 4 (49 m). With increasing distance from the Al-Zour outfall, phylogenetically more diverse germling isolates were obtained, until wpt 11 and 12 at 640 and 800 m, respectively. Wpt 12, at 800 m, was the richest point in terms of phylogenetic diversity of algal isolates (Table S6). Overall, the GE results from Al-Zour showed increased algal phylogenetic diversity with greater distance from the outfall. This finding may be attributed to several factors, such as the lesser anthropogenic impact on the open sea in the south, along with the higher hydrodynamic energy in this region (2 m tidal range; Al-Yamani et al. 2004). This conclusion is in line with the notion that enclosed and shallow sites with abundant marine life can generally be assumed to be more sensitive to desalination plant discharges than exposed, high energy, open-sea locations, which are more capable of diluting and dispersing the discharges (Lattemann and Höpner 2008). Also in line with these observations, our results show that, at Al-Zour, hydrodynamics, together with an abundance of suitable substrata for seaweed attachment (Alghunaim et al. 2019), likely contribute to increased species diversity (Puente et al. 2017). This contrasts with the Doha region, where calm waters with low currents favour a community with low diversity. This suggestion is consistent with Uddin and Al Ghadban (2011), who mentioned that the southern waters of Kuwait have coral reefs that support a rich diversity of marine macrophytes.

4.3 Salinity and temperature tolerance treatments

The eco-physiological experiment findings showed that *Ulvelia*, *Chaetomorpha* sp. and *Ulva* sp. were the genera least affected by treatment with increased salinities (60-80) with 4 strains showing 50% or more bleaching. Chlorophyta, especially *Ulva* (Ulvaceae), are capable of

survival and growth in marine environments with wide fluctuations in salinity, from freshwater to brine, and are able to tolerate wide ranges of temperature and desiccation (Macler 1988). In field conditions, according to Russell (1987), intertidal algae generally are able to tolerate seawater salinities of 10-100, while subtidal seaweeds are less tolerant at withstanding increased salinities within a range of 18-52. Also, intertidal algae typically only grow in a species-specific zone of the shore/depth profile, and would not survive for long above or below the normal zonation for the species (Chu et al. 2012; Schonbeck and Norton 1980). For example, *Ulva intestinalis* individuals were only opportunistically observed at salinities over such a wide range (Tsutsui et al. 2015). Another study found that *Ulva flexuosa* populations had a very wide range of occurrence at various degrees of salinity (Rybak 2015). *Ulva flexuosa* has been observed in freshwater, brackish waters and hyperhaline waters (Rybak 2018).

Interestingly, *Chaetomorpha*, which occupies the uppermost level of the intertidal zone at Al-Zour, was able to grow at 40° C with no visible symptoms of bleaching in the field but, under laboratory conditions in the 40°C treatment, two isolates of the genus exhibited differing responses: *Chaetomorpha* sp. (K73) showed more than 80% bleaching, while *Chaetomorpha* sp. (K74) had less than 20% bleaching, again supporting the notion of significant ecophysiological plasticity among closely related isolates. The K52 strain of *Ulva tepida* was not affected by heat stress, whereas K51 isolates showed 20% bleaching, *U. tepida* isolates K4 and K54 had bleaching in the range of 50-80%, and K25 showed 99% bleaching. A study in Thailand indicated that *Chaetomorpha* spp. occurred in salinities of 3.4–90.0 and water temperatures between 20.1 and 40.9° C. According to Lawton et al. (2013), filamentous *Ulva* species, such as *U. ohnoi*, exhibited high survival rates for over 3 months under laboratory conditions, and were highly resilient under a wide range of temperature treatments. *Ulva ohnoi* is an opportunistic alga

with high growth rates and broad environmental tolerance. Work in Australia showed that *Cladophora albida* was able to survive at temperatures up to 40°C (Gordon et al. 1980). In this respect, most intertidal marine algae can tolerate temperatures largely in the upper range of those experienced in the natural environment. In addition, specimens of the same species acclimated to different areas of the shoreline demonstrated different temperature responses. Temperature tolerance affects the position of species habitable zones. For example, *Enteromorpha linza* from the high and low-intertidal zones in Langstone Harbour on the south coast of England shows different temperature responses (Innes 1988; Taylor et al. 2001). Consistent with these findings, a study in Israel (Einav et al. 1995) found that *Ulva lactuca* had a tolerance to high temperatures which is remarkable in that it showed positive photosynthetic rates at 37°C for more than 3 h. In addition, the same authors mentioned that thalli of *Enteromorpha compressa* growing in the uppermost of intertidal zone were much more resistant to high, low temperature, desiccation, and varying salinity levels (Einav et al. 1995).

Based on the results reported here and those in previous publications, it is apparent that *Chaetomorpha* sp. and *Ulva* sp. can readily grow, survive and monopolize habitats in stagnant waters where salinity and temperature greatly fluctuate because of their euryhaline and eurythermal nature, as well as the high growth rates of these taxa (Parida and Das 2005; Rybak 2018). In Kuwait, intertidal algae of the warm temperate shores are typically eurythermal species, which can survive fluctuations of low to high temperature (Al-Hasan and Jones 1989).

In the present study, interestingly, under controlled incubation conditions, Phaeophyceae proved the most sensitive, with a survival rate of 89% when incubated at 3°C (low temperature stress), compared to isolates of Rhodophyta and Chlorophyta (96%). In the temperature stress experiment, Phaeophyceae had a survival rate of 52% at 40 °C and, in combination with

salinities of 60 or 80, survival was 58% and 30%, respectively. Among the particularly resilient strains was *Ectocarpus subulatus* (K23), collected from Boubiyan. The genus *Ectocarpus* is particularly well suited for such comparative studies because it comprises a wide range of morphologically and physiologically diverse species that have adapted to different marine and brackish water environments over a wide latitudinal range (Dittami et al. 2020a, b). Peters et al. (2015) showed that isolates of *E. subulatus* were highly resistant to elevated temperature and low salinity. The optimal temperature ranges for growth and the maximum temperatures for long-term survival of isolates of *E. subulatus* can be correlated with the temperature regimes at the original collection sites (Klein Jan et al. 2017). The isolate of *E. subulatus* (K23) tested was eurythermal (maximum survival temperature of 33° C), with no signs of bleaching at 35° C. According to Bolton (1983), Port Aransas is the southernmost population of *Ectocarpus* known in North America, where there is a large temperature amplitude of 17 °C between winter (13°) and summer (30°). It is interesting that the same *Ectocarpus* species appears to be present in Kuwait, where the summer temperature is even higher. Supporting the notion of brown algae being more sensitive to temperature than green algae, none of the Phaeophyceae in this study were isolated from sites within 100 m of desalination plant outfalls, except for a species of Acinetosporaceae (K19) at 28 m (wpt 2) from the Al-Zour site. In this work, the most tolerant isolates among the Phaeophyceae were a species of Chordariaceae (K22), *Elachista stellaris* (K26), *Feldmannia mitchelliae* (K198, K216), *Bachelotia* sp. (K70), *Ectocarpus subulatus* (K23) and *Colpomenia sinuosa* (K87).

In the experiments reported here, Rhodophyta showed high mortality or bleaching by day 14 of incubation, when incubated at 40° C, with a survival rate of 21%. Therefore, red algae were less tolerant to higher temperatures, compared to green and brown algae. Rhodophyta

dominating the middle and lower parts of the intertidal zone showed a narrower temperature response, could not tolerate exposure to high temperatures for prolonged time periods and were more sensitive to desiccation and salinity changes (Einav et al. 1995). It appears that red algae growing in the stressful intertidal zone are more frequently confined to a single zone, when compared to Chlorophyta and Phaeophyceae. They are usually limited to the lowermost zone sampled (i.e. the lower intertidal, having the least air exposure) and only poorly represented at the mid-tide level (John 2012; Kokabi et al. 2016). Fralick and Mathieson (1975) separated 4 *Polysiphonia* species based on their temperature optima, revealing cold-water and warm-water affinities. This study showed that *Polysiphonia* species (cold and warm affinities) could survive at 27 to 30°C and were not affected by temperatures as low as 5°C. This notion is consistent with observations in this study that *Polysiphonia* sp. (K61) showed 20% bleaching when incubated at 40°C. Kübler and Davison (1993) observed that red algae may continue to grow for several hours while maintaining high rates of photosynthesis at $\geq 35^\circ\text{C}$, but did not survive for eight days. Thus, our results suggest that this phylum can potentially be used as a bioindicator for thermal pollution caused by the desalination plants.

In general, temperature and salinity are considered the most important abiotic factors that determine the local and/or regional distribution of macroalgae (Pereira et al. 2017), as these factors are critical for survival, germination and growth of the vegetative cells (Imai and Itakura, 1999). In this study, 41 (87%) isolates investigated at low temperature (3°C) had a broad range of physiological responses to stressful conditions, meaning that the extreme temperatures tested negatively affected survival. This finding is with line with our isolated samples collected in winter/spring, when these algae are acclimated to seawater temperature down to 10 °C and even down to 0 °C (winter) for intertidal populations exposed to air on Kuwait coasts (Alosairi and

Pokavanich 2017). Without exception, all unialgal cultures in this study thrived at 25°C and 30°C (optimal temperature).

According to Hoepner and Lattemann (2003), increasing the salinity from brine discharge points may not be tolerated by organisms in habitats that are in other respects adapted to high values, such as those that tolerate salinities of 42.5 or more in the northern parts of the Red Sea. Ambient salinity in the Red Sea may already be close to the physiological limit of endemic species, and salinity has been stressed as an important environmental factor in survival, even for relatively hardy marine species (Hoepner and Lattemann 2003). The Arabian Gulf, with higher salinity than the adjacent Indian Ocean, is particularly vulnerable to salinity increases, which has been, and will be, exacerbated by decreasing freshwater inflows and warmer temperatures due to climate change (Missimer and Maliva 2018).

In conclusion, this study showed that the intertidal algal diversity observed within 30 m from the outfall of the ZS desalination plant in Kuwait was well adapted to variable salinities and temperatures, based on *in vitro* experiments. As a group, the green algae were remarkably resilient and least affected by increased salinity and temperatures, exhibiting high tolerance. It is worth noting that small discoid green members of the order Ulvales were only isolated at 800-1000 m distance from the DE outfall, probably because these algae require surface attachment to other marine plants, which are absent in the vicinity of desalination brine effluents. It is clear that brine discharge from desalination plants has a local impact on intertidal macroalgal communities in Kuwait. However, further detailed studies are warranted to assess the ecophysiological consequences of these discharges into the marine environment of Kuwait (Al-Said et al. 2017). A recent study on the Mediterranean seagrass *Cymodocea nodosa* (Tsioli et al. 2022) provided details of cellular and molecular responses to temperature and salinity stress, as well as the

interactions between both stressors – arguably, this approach should also be applied to a representative subset of seaweed species explored in the present study, ideally including both tolerant and sensitive species.

In general, the algal species encountered in the present study were euryhaline and/or eurythermal. It is tempting to speculate that with the predicted increase in temperature and salinity in the Arabian Gulf due to the increasing scale of seawater desalination (Al-Rashidi 2009; Alosairi and Pokavanich 2017; Alosairi et al. 2018), the best adapted among intertidal algae will be the Chlorophyta and Phaeophyceae, which due to their thermal tolerance will have a better chance of survival. In contrast, Rhodophyta were the most susceptible to increasing stressors. In summary, the evidence from this study supports the hypothesis that salinity and temperature-related environmental stress do not result in a decrease in species diversity within the vicinity of desalination and power plants in the Gulf.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Identification, habitat and collection sites in Kuwait of the 47 algal cultures used in ecophysiological experiments in the present study.

Table S2. List of isolated algal strains collected from Kuwait, grouped according to the closest sequence match in GenBank and relevance to desalination brine outfalls of Doha and Al-Zour.

Table S3. Coordinates of way points, mean temperature (°C) and salinity (psu) of sampling distances for the outfall of the desalination plant (Doha East and Al-Zour South) including their references sites.

Table S4. Survival rates of representatives of each phylum at different salinities (35, 45, 60, and 80 psu).

Table S5. Survival rates for each phylum at different temperatures (3, 10, 25, 30, 35 and 40°C).

Table S6. Diversity of algal isolates obtained by GE at increasing distances from outfall at DE, ZS and reference sites (Boubiyan and Fintas).

Figure S1. Map of Kuwait showing way points sampling at the Doha (top right) and at Al-Zour desalination plant (bottom right), respectively. (GIS maps produced using ArcGIS software; National Geographic, Esri, Garmin, USA).

Figure S2. Viability of tested species of Chlorophyta, Phaeophyceae and Rhodophyta algae under different salinities.

Figure S3. Viability of tested species of Chlorophyta, Phaeophyceae and Rhodophyta incubated at different temperatures.

Figure legends

Figure 1. Map of Kuwait with the locations of (a) Boubiyan (pristine site), (b) Doha East desalination plant (North), (c) Fintas (pristine site), and (d) Al Zour South power (South)-desalination plant (GIS maps produced using ArcGIS software; National Geographic, Esri, Garmin, USA).

Figure 2. Map of the Doha site showing the distribution of salinity (a) and surface water temperature (b) near the Doha East desalination sampling site (red arrow indicates outfall discharge point). Mean temperature (c) and salinity (d) at different distances from the Doha East desalination plant outfall. (GIS maps produced using ArcGIS software; National Geographic, Esri, Garmin, USA).

Figure 3. Map of the Al-Zour site showing the distribution of salinity (a), and surface water temperature (b) near Al-Zour South desalination sampling site (red arrow indicates outfall discharge point). Mean temperature (c) and salinity (d) at different distances from the Al-Zour

South desalination plant. (GIS maps produced using ArcGIS software; National Geographic, Esri, Garmin, USA).

Figure 4. The results of salinity tolerance experiments with cultures of green algae (a), brown algae (b) and red algae (c) grown in salinities of 35, 45, 60 and 80. Stress and vitality symptoms developed in culture over a period of 14 days were measured as follows: some bleaching of thalli (SB) = 75%, partial bleaching (PB)=50%, mostly bleached (MB) = 25%, completely bleached thalli (B)= 0%. Blue, orange, grey, yellow indicate the salinities used. Symbols before isolates names indicate where they were found: *, 0-100 m from outfalls; ~, 200-700 m from outfalls; ^, 800-1000 m from outfalls; ’’, found at reference sites. Isolates without symbols represent different maritime regions from the Northern and Southern Provinces in Kuwait including offshore islands.

Figure 5. The results of temperature tolerance experiments with cultures of green algae (a), brown algae (b) and red algae (c) grown in salinities of 35, 45, 60 and 80. Stress and vitality symptoms developed in culture over a period of 14 days were measured as follows: some bleached cells (SB) = 75%, partially bleached (PB) = 50%, mostly bleached (MB) =25%, completely bleached thalli (B) = 0%). Isolates marked * were obtained from close to outfalls (0-100 m); isolates marked ^ were from the far sector (800-1000 m), and isolates marked ~ originated from the middle sector (200-700 m). The symbol ’’ corresponds to isolates from reference sites. Isolates without any symbol represent different maritime regions from Northern and Southern Provinces in Kuwait including offshore islands.

Figure 6. Algal diversity at different locations: Boubiyan, Doha, Fintas, and Al-Zour. A total of 47 algal isolates were obtained from four study sites (focusing on desalination plant outfall sites and references sites) by cultivation from cryptic stages in sediment samples. Doha harbored the lowest diversity, with a total of 7 identified species, the majority of which were green algae compared to a total of 18 different collected species of macroalgae identified at Al-Zour.

Figure 7. Morphospecies diversity at different distances from the outfalls at Doha (a) and Al-Zour (b). The isolation of macroalgae from cryptic stages in sediment samples revealed the presence of additional species that were not detected macroscopically in the field e.g., in the Far and Far Away sectors at Doha, 7 species were identified among the isolates [Ulvellaceae sp. (K43), *Ulvella* sp. (K44), *Rhizoclonium* sp.(K58), *Ulvella* sp. (K40), *Ulva ohnoi* (K55), *Ulvella* sp. (K39), and Ulvellaceae sp. (K45)]. At Al-Zour, species diversity increased with distance with 1 brown, 5 green and 1 red algal species in the Close sector. But within 640-950 m (i.e. Far sector), 4 species of brown algae, 4 red algae, and 3 green alga were observed.

Figure 8. Phylogenetic diversity at Al-Zour as a function of distance away from the outfall displayed by regression value (R). There is a positive correlation between diversity and distance from the outfall with $R^2 = 0.7931$ for Al-Zour.

Ecophysiology of Kuwait macroalgae with special emphasis on temperature and salinity tolerance.

Supplementary Material

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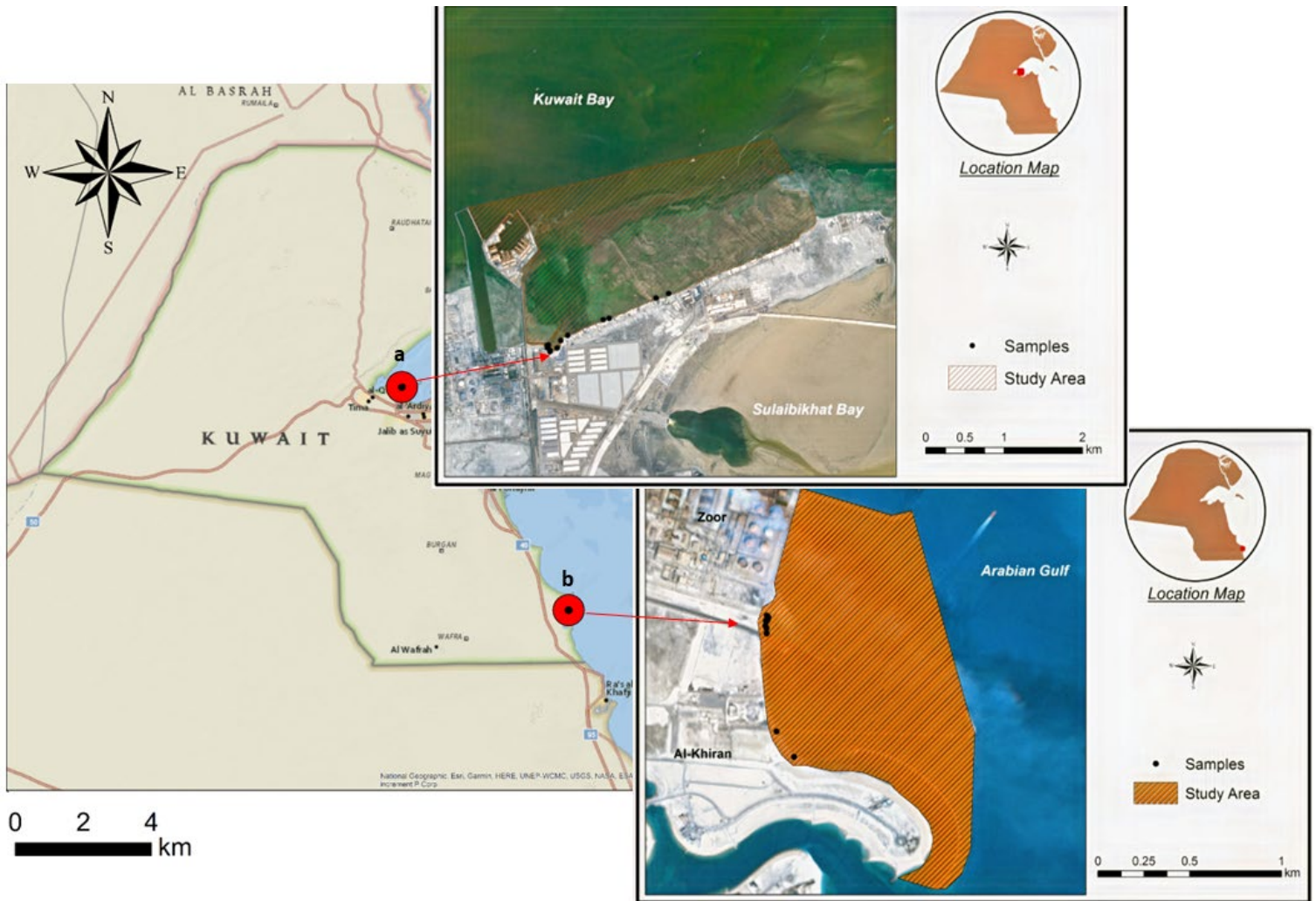


Figure S1. Map of Kuwait showing waypoints sampling at the Doha desalination plant (top right) and at Al-Zour desalination plant (bottom right). (GIS maps produced using ArcGIS software; National Geographic, Esri, Garmin, USA).

Table S1. Identification, habitat and collection sites in Kuwait of the 47 algal cultures used in ecophysiological experiments in the present study. Type of sample: Germling emergence - GE; Field-collected macroalgal thalli – F; Settled zoids – S.

Culture code	Code K	Collection date	Locality Site	Habitat or Substratum	Type of sample	Region of Kuwait	Coordinates
K4	K19-4-67-1	6/4/2019	Al-Zour	Pebbles, shells among rocks in low intertidal, wave-swept	GE	South	28° 41' 53.4" N, 48° 22' 43.6" E
K13	K19-4-83-1	6/4/2019	Al-Zour	Pebbles and shells among rocks, low intertidal, wave-swept	GE	South	28° 41' 52.7" N, 48° 22' 43.3" E
K17	K(19-2-)33-1	5/2/2019	Khiran	Rope densely covered with different filamentous algae	F	South	28°40'32.1"N, 48°21'19.1E
K19	K19-4-72-2	6/4/2019	Al-Zour	Pebbles, shells among rocks in low intertidal, wave-swept	GE	South	28° 41' 53.4" N, 48° 22' 43.6" E
K21	K19-4-97-3	6/4/2019	Al-Zour	Pebbles, shells near bridge foot in low intertidal, wave-swept	GE	South	28° 41' 33.1" N, 48° 22' 45.12" E
K22	K19-4-107	6/4/2019	Al-Zour	Substratum among rocks in low intertidal, wave-swept	GE	South	28° 41' 28.5864" N, 48° 22' 48.63" E
K23	K19-4-130-1	8/4/2019	Subbiya	Sheltered tide pool	F	North	29° 43' 57.17" N, 48° 6' 20.96" E
K24	K19-4-159-1	9/4/2019	Fintas	Exposed rocky platform in low intertidal	S	Middle	29° 10' 35.27" N, 48° 7' 23.56" E
K25	K19-4-93-1	6/4/2019	Al-Zour	Pebbles, shells among rocks in low intertidal, wave-swept	GE	South	28° 41' 50.53" N, 48° 22' 43.23" E
K26	K(19-2-)28-2	5/2/2019	Khiran	Blade of large knife thrown into water, sheltered but with current in lowermost intertidal	GE	South	28°40'32.1"N, 48°21'19.1E
K28	K19-4-148-5	9/4/2019	Fintas	<i>Cladophora</i> sp. on exposed rocky platform at upper subtidal	GE	Middle	29° 10' 35.2668" N, 48° 7' 23.5632" E
K30	K19-4-110-1	6/4/2019	Al-Zour	Substratum from upper subtidal	GE	South	28° 41' 25.872" N, 48° 23' 0.0744" E
K31	K19-4-138-1	9/4/2019	Fintas	Stone exposed rocky platform in upper subtidal	GE	Middle	29° 10' 35.267" N, 48° 7' 23.56" E
K32	K19-4-161-5	9/4/2019	Fintas	Drifting	GE	Middle	29° 10' 35.2668" N, 48° 7' 23.56" E
K33	K19-4-121-1	6/4/2019	Al-Zour	Boulder in mid intertidal, densely covered by <i>Enteromorpha</i> sp. and brown algae	GE	South	28° 41' 28.59" N, 48° 22' 48.63" E
K34	K19-4-112-5	6/4/2019	Al-Zour	Substratum from upper subtidal	GE	South	28° 41' 25.87" N, 48° 23' 0.07" E
K36	K19-4-143-4	9/4/2019	Fintas	Pebbles, shells in upper subtidal	GE	Middle	29° 10' 35.27" N, 48° 7' 23.56" E
K38	K19-4-93-3	6/4/2019	Al-Zour	Substratum among rocks in low intertidal, wave-swept	GE	South	28° 41' 50.53" N, 48° 22' 43.23" E
K39	K19-4-59-2	5/4/2019	Doha	Shallow tide pool on tidal flat	GE	North	29° 22' 36.61" N, 47° 49' 3.7" E
K40	K19-4-45-2	5/4/2019	Doha	Shallow tide pool on tidal flat	GE	North	29° 22' 28.21" N, 47° 48' 41.4" E
K41	K19-4-108-3	6/4/2019	Al-Zour	Substratum among rocks in low intertidal, wave-swept	GE	South	28° 41' 28.5864" N, 48° 22' 48.63" E
K42	K19-4-103-4	6/4/2019	Al-Zour	Substratum among rocks in low intertidal, wave-swept	GE	South	28° 41' 28.5864" N, 48° 22' 48.63" E
K43	K19-4-37-3	5/4/2019	Doha	Upper subtidal	GE	North	29° 22' 27.56" N, 47° 48' 38.73" E
K44	K19-4-1-3	5/4/2019	Doha	Upper subtidal	GE	North	29° 22' 27.56" N, 47° 48' 38.73" E
K45	K19-4-66-2	5/4/2019	Doha	Shallow tide pool on tidal flat	GE	North	29° 22' 38.5" N, 47° 49' 9.62" E

K47	K19-4-121-2	6/4/2019	Al-Zour	Bolder in mid intertidal, densely covered by <i>Enteromorpha</i> sp. and brown algae	GE	South	28° 41' 28.59" N, 48° 22' 48.63" E
K48	K19-4-144-1	9/4/2019	Fintas	Pebbles and shells in upper subtidal	GE	Middle	29° 10' 35.27" N, 48° 7' 23.56" E
K49	K(19-2)18-1	5/2/2019	Khiran	Shallow subtidal (collected by snorkeling), polychaete tube	F	South	28°39'48"N, 48°22'10"E
K51	K(19-2)72-3	7/2/2019	Qaruh	Subtidal from coral at 6 m depth	GE	South	28°49'N, 48°46'30"E
K52	K(19-2) 66-1	7/2/2019	Qaruh	Low intertidal, north-facing vertical surface, artificial hard substratum (old landing)	F	South	28°49'04.6N, 48°46'32.3"E
K54	K(19-2) 187-3	18/2/2019	Failaka	Drifting from rocky beach, sand-colored rocks	F	North	29°25'47.9"N 48°18'01.7"E
K55	K(19-2) 192-2	19/2/2019	Doha	Drifting substratum from base of <i>Ulva</i> sp.	GE	North	29°22'41.4"N 47°49'19.2"E
K56	K19-4-117-2	6/4/2019	Al-Zour	Scraped from concrete, also cyanobacteria from upper subtidal	GE	South	28° 41' 33.1" N, 48° 22' 45.13" E
K58	K19-4-137-1	8/4/2019	Doha	Sheltered, dead shells from small oysters from tide-pool	GE	North	29° 22' 15.89" N, 47° 48' 16.9" E
K61	K(19-2) 35-4	5/2/2019	Khiran	Rope densely covered with different filamentous algae, subtidal	F	South	28°40'32.1"N, 48°21'19.1E
K62	K(19-2) 51-1	6/2/2019	Al Nuwaiseeb	Beach drift, rocky shore	F	South	28°35'00"N, 48°23'56.8"E
K66	K(19-2)169-1	18/2/2019	Failaka	Loose shell from rocky shore (black rocks)	F	North	29°28'03.8"N 48°17'10.1"E
K68	K19-4-109-2	6/4/2019	Al-Zour	Substratum from upper subtidal	GE	South	28° 41' 25.87" N, 48° 23' 0.074" E
K70	K (19-2) 81-1	7/2/2019	Umm al-Maradim	Rock from intertidal, small pool	F	South	28°40'49.8"N, 48°39'00"E
K71	K(19-2)176-1	18/2/2019	Failaka	Rocky tidepool (black rocks)	F	North	29°28'03.8"N 48°17'10.1"E
K72	K19-4-123-1	6/4/2019	Al-Zour	Bolder in mid intertidal, densely covered by <i>Enteromorpha</i> sp. and brown algae	GE	South	28° 41' 28.59" N, 48° 22' 48.63" E
K73	K19-4-76-2	6/4/2019	Al-Zour	Substratum among rocks in low intertidal, wave-swept	GE	South	28° 41' 53.7" N, 48° 22' 43.3" E
K74	K19-4-78-2	6/4/2019	Al-Zour	Substratum among rocks in low intertidal, wave-swept	GE	South	28° 41' 53.7" N, 48° 22' 43.3" E
K78	K(19-2)35-2	5/2/2019	Khiran	Rope densely covered with different filamentous algae in subtidal	F	South	28°40'32.1"N, 48°21'19.1E
K87	K(19-2)245-1	23/2/2019	Bubiyah	Drifting dark material	F	North	29°36'09.1"N 48°09'22.9"E
K198	K(19-2)198-1	21/2/2019	Kubbar	Muddy sediment, deep subtidal at 35 m depth	GE	South	29°04'18"N, 48°29'30"E
K216	K(19-2)216-1	21/2/2019	Kubbar	Collected by diving at 10 m depth	F	South	29°04'18"N, 48°29'30"E

Table S2. List of isolated algal strains collected from Kuwait, grouped according to the closest sequence match in GenBank and relevance to desalination brine outfalls of Doha East and Al-Zour South.

DNA Strain ID	Taxonomy (Phylum, Order, Family)	Taxon identification according to GenBank	Relevance to desalination brine outfalls	Target DNA	Primers	Coverage %	% ID	Accession no. of closest match	GenBank accession no.	Locality and Reference
K4 (K19-4-67-1)	Chlorophyta, Ulvales, Ulvaceae	<i>Ulva tepida</i> Y.Masakiyo <i>et S.Shimada</i>	C-Z	ITS2	KP5F, KG4R	94	99.68	KT374006	OP221966	Australia, Phillips <i>et al.</i> (2016)
K25 (K19-4-93-1)	Chlorophyta, Ulvales, Ulvaceae	<i>Ulva tepida</i> Y.Masakiyo <i>et S.Shimada</i>	C-Z	ITS2	KP5F, KG4R	100	99.7	KT374006	OP221968	Australia, Phillips <i>et al.</i> (2016)
				<i>tufA</i>	<i>tufAF</i> , <i>tufAR</i>	76	99.78	MZ870658	OP235394	New Caledonia: South Province, Lagourgue <i>et al.</i> (2022)
K51(K19-2-72-3)	Chlorophyta, Ulvales, Ulvaceae	<i>Ulva tepida</i> Y.Masakiyo <i>et S.Shimada</i>		ITS2	KP5F, KG4R	94	100	KT374006	OP219456	Australia, Phillips <i>et al.</i> (2016)
K52 (K19-2-66-1)	Chlorophyta, Ulvales, Ulvaceae	<i>Ulva tepida</i> Y.Masakiyo <i>et S.Shimada</i>		ITS2	KP5F, KG4R	97	99.72	KT374006	OP219457	Australia, Phillips <i>et al.</i> (2016)
K54 (K19-2-187-3)	Chlorophyta, Ulvales, Ulvaceae	<i>Ulva tepida</i> Y.Masakiyo <i>et S.Shimada</i>		ITS2	KP5F, KG4R	78	98.9	KT374006	OP219458	Australia, Phillips <i>et al.</i> (2016)
K55 (K19-2-192-2)	Chlorophyta, Ulvales, Ulvaceae	<i>Ulva ohnoi</i> M.Hiraoka <i>et S.Shimada</i>	F-D	<i>tufA</i>	<i>tufAF</i> , <i>tufAR</i>	96	100	KF195523	OP235395	Australia, Lawton <i>et al.</i> (2013)
K45 (K19-4-66-2)	Chlorophyta, Ulvales, Ulvellaceae	Ulvellaceae Schmidle sp.	FA-D	<i>tufA</i>	<i>tufAF</i> , <i>tufAR</i>	89	99.29	KU362068	OP235400	Japan: Akashi, Sauvage <i>et al.</i> (2016)
K38 (K19-4-93-3)	Chlorophyta, Ulotrichales, Planophilaceae	Planophilaceae Škaloud <i>et Leliaert</i> sp.	C-Z	ITS2	KP5F, KG4R	90	82.61	FR865755	OP221972	Switzerland:Basel, Gachon <i>et al.</i> (2011)
K39 (K19-4-59-2)	Chlorophyta, Ulvales, Ulvellaceae	<i>Ulvella</i> P.Crouan <i>et H.Crouan</i> sp.	FA-D	<i>tufA</i>	<i>tufAF</i> , <i>tufAR</i>	95	95.48	MF547414	OP235397	South Korea, Kim and Kim (2017)
K40 (K19-4-45-2)	Chlorophyta, Ulvales, Ulvellaceae	<i>Ulvella</i> P.Crouan <i>et H.Crouan</i> sp.	F-D	<i>tufA</i>	<i>tufAF</i> , <i>tufAR</i>	99	95.43	MF547414	OP235398	South Korea, Kim and Kim (2017)
K41 (K19-4-108-3)	Chlorophyta, Ulotrichales, Planophilaceae	Planophilaceae Škaloud <i>et Leliaert</i> sp.	F-Z	ITS2	KP5F, KG4R	67	88.29	MT991545	OP221976	Germany: Saxony-Anhalt, Sommer <i>et al.</i> (2020)
K42 (K19-4-	Chlorophyta,	Ulvellaceae Schmidle sp	F-Z	ITS2	KP5F, KG4R	94	93.65	EF595452	OP221973	United Kingdom:

103-4)	Ulvales, Ulvellaceae									Angus, Rinkel <i>et al.</i> (2012)
K43(K19-4-37-3)	Chlorophyta, Ulvales, Ulvellaceae	Ulvellaceae Schmidle sp.	F-D	ITS2	KP5F, KG4R	59	84.78	EF595459	OP221974	Iceland: Sudar-Bar, Rinkel <i>et al.</i> (2012)
K44 (K19-4-1-3)	Chlorophyta, Ulvales, Ulvellaceae	<i>Acrochaete</i> N.Pringsheim sp.	F-D	ITS2	KP5F, KG4R	81	97.24	EF595452	OP221975	United Kingdom: Angus, Rinkel <i>et al.</i> (2012)
		<i>Ulvella</i> P.Crouan <i>et</i> H.Crouan sp.		<i>tufA</i>	<i>tufAF, tufAR</i>	93	97.55	ON526874	OP235399	Malta: Mediterranean Sea, Bartolo <i>et al.</i> (2022)
K49 (K19-2-18-1)	Chlorophyta, Bryopsidales, Bryopsidaceae	<i>Bryopsis</i> J.V.Lamouroux sp.		<i>tufA</i>	<i>tufAF, tufAR</i>	91	92.81	FJ432653	OP235396	France: Pas de Calais, Verbruggen <i>et al.</i> (2009)
K73 (19-4-76-2)	Chlorophyta, Cladophorales, Cladophoraceae	<i>Chaetomorpha</i> Kützing sp.	C-Z	ITS2	KP5F, KG4R	94	97.22	MT991611	OP221970	China; Chen <i>et al.</i> (2020)
K74 (19-4-78-2)	Chlorophyta, Cladophorales, Cladophoraceae	<i>Chaetomorpha</i> Kützing sp.	C-Z	ITS2	KP5F, KG4R	94	95.27	MT991611	OP221971	China; Chen <i>et al.</i> (2020)
K58 (K19-4-137-1)	Chlorophyta, Cladophorales, Cladophoraceae	<i>Rhizoclonium</i> Kützing sp.	R-B	ITS2	KP5F, KG4R	25	97.37	LS990761	OP221980	Indonesia, Leliaert (2018)
K56 (K19-4-121-3)	Chlorophyta, Cladophorales, Cladophoraceae	<i>Cladophora laetevirens</i> (Dillwyn) Kützing	M-Z	ITS2	KP5F, KG4R	89	98.44	AB665564	OP221978	Japan, Hayakawa <i>et al.</i> (2012)
K17 (K 19-2-33-1)	Ochrophyta, Sphacelariales, Sphacelariaceae	<i>Sphacelaria</i> Lyngbye sp.		<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	36	96.3	AJ287890	OQ630967	Japan: Sado Island, Draisma <i>et al.</i> (2002)
K19 (K19-4-72-2)	Ochrophyta, Ectocarpales, Acinetosporaceae	Acinetosporaceae G.Hamel ex Feldmann sp.	C-Z	COI	Gaz2F, Gaz2R	100	91.95	LM995326	OQ615883	Italy, Peter <i>et al.</i> (2015)
K21 (K19-4-97-3)	Ochrophyta, Ectocarpales, Chordariaceae	Chordariaceae Greville 1830 sp.	M-Z	<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	91	92.12	AY079434	OP491541	Pohang, eastern coast of Korea, Lee <i>et al.</i> (2002)
K22 (K19-4-107-5)	Ochrophyta, Ectocarpales, Chordariaceae	Chordariaceae Greville 1830 sp.	F-Z	<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	68	91.23	AF207812	NA	Siemer and Pedersen (1999)
K23 (K19-4-130-1)	Ochrophyta, Ectocarpales, Ectocarpaceae	<i>Ectocarpus subulatus</i> Kützing	R-B	<i>rbcL</i>	<i>rbcL1273F-rbcS139R</i>	95	100	ESU38750	OP491536	USA: Port Aransas, Texas, Stache-Crain <i>et al.</i> (1997)

K24 (K19-4-159-1)	Ochrophyta, Ectocarpales, Scytosiphonaceae	<i>Iyengaria stellata</i> (Børgesen) Børgesen	R-F	COI <i>psa</i> A	Gaz2F, Gaz2R <i>psa</i> A130F, <i>psa</i> A970R	96 96	100 100	MN598603 MN587733	OQ615882 OP235416	Kuwait, Al-Bader and Variyam (2019) Kuwait, Al-Bader and Variyam (2019)
K26 (K 19-2-28-2)	Ochrophyta, Ectocarpales, Chordariaceae	<i>Elachista stellaris</i> Areschoug		<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	99	98.87	LC016514	OP491560	Japan, Kawai <i>et al.</i> (2016)
K47 (K19-4-121-2)	Ochrophyta, Ectocarpales, Acinetosporaceae	<i>Feldmannia mitchelliae</i> (Harvey) H.-S. Kim 2010	F-Z	<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	75	99.39	AB302306	OP491537	Japan: Fukuoka, Tanaka <i>et al.</i> (2010)
K48 (K19-4-144-1)	Ochrophyta, Ectocarpales, Acinetosporaceae	<i>Feldmannia mitchelliae</i> (Harvey) H.-S. Kim 2011	R-F	<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	76	98.92	AB302306	OP491538	Japan: Fukuoka, Tanaka <i>et al.</i> (2010)
K198 (K19-2-198-1)	Ochrophyta, Ectocarpales, Acinetosporaceae	<i>Feldmannia mitchelliae</i> (Harvey) H.-S. Kim 2012		<i>rbcL</i>	<i>rbcL</i> P2F, <i>rbcL</i> 952R	96	99.53	AB302306	OP491555	Japan: Fukuoka, Tanaka <i>et al.</i> (2010)
K216 (K19-2-216-1)	Ochrophyta, Ectocarpales, Acinetosporaceae	<i>Feldmannia mitchelliae</i> (Harvey) H.-S. Kim 2013		<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	78	99.15	AB302306	OP491556	Japan: Fukuoka, Tanaka <i>et al.</i> (2010)
K70 (K19-2-81-1)	Ochrophyta, Scythamiales, Bachelotiaceae	<i>Bachelotia</i> (Bornet) Kuckuck ex Hamel sp.		<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	97	97.91	AF207797	OP491558	Ban NZ strain, Siemer and Pedersen (1999)
K71 (K19-2-176-1)	Ochrophyta, Sphacelariales, Sphacelariaceae	<i>Sphacelaria Lyngbye</i> sp.		<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	66	97.43	AJ287891	OQ630966	Japan, Draisma <i>et al.</i> (2002)
K72 (K19-4-123-1)	Ochrophyta, Sphacelariales, Sphacelariaceae	<i>Sphacelaria tribuloides</i> Meneghin	F-Z	<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	54	98.70	AJ287892	OP491539	Japan, Draisma <i>et al.</i> (2002)
K87(19-2-245-1)	Ochrophyta, Ectocarpales, Scytosiphonaceae	<i>Colpomenia sinuosa</i> Derbes et Solier	R-B	<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	74	98.93	AB578988	OP491559	USA: HI, Oahu, Kogame <i>et al.</i> (2011)
K13 (K19-4-83-1)	Rhodophyta, Acrochaetiales, Acrochaetiaceae	<i>Arcochaetium</i> NägeliCramer sp.	C-Z	<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	50	98.01	MH414967	OP491543	Taiwan, Lee (2018)
K34 (K19-4-112-5)	Rhodophyta, Acrochaetiales	Acrochaetiales Feldmann sp.	M-Z	<i>rbcL</i>	<i>rbcLRH3F-rbcS139R</i>	99	92.48	MH414967	OP491545	Taiwan, Lee (2018)
K28 (K19-4-148-5)	Rhodophyta, Erythropeltales, Erythrotrichiaceae	<i>Sahlingia subintegra</i> (Rosenvinge) Kornmann	R-F	<i>psbA</i> <i>rbcL</i>	<i>psbA1F, psbA1R</i> <i>rbcL</i> kitoF-	92 93	99.89 100	EF660256 JF292603	OP235411 OP491547	USA: James Island, Zuccarello <i>et al.</i> (2008) India, Zuccarello <i>et al.</i>

			JrSR				(2011)			
K30 (K19-4-110-1)	Rhodophyta, Stylonematales, Stylonemataceae	<i>Stylonema alsidii</i> (Zanardini) K. Drew	F-Z	<i>psbA</i>	<i>psbA1F</i> , <i>psbA1R</i>	92	100	EF660251	OP235409	Japan, Zuccarello <i>et al.</i> (2008)
K31 (K19-4-138-1)	Rhodophyta, Stylonematales, Stylonemataceae	<i>Stylonema alsidii</i> (Zanardini) K. Drew	R-F	<i>psbA</i>	<i>psbA1F</i> , <i>psbA1R</i>	92	100	EF660251	OP235410	Japan, Zuccarello <i>et al.</i> (2008)
K32 (K19-4-161-5)	Rhodophyta, Erythropeltidales, Erythrotrichiaceae	<i>Erythrotrichia</i> Areschoug sp.	R-F	<i>psbA</i>	<i>psbA1F</i> , <i>psbA1R</i>	95	98.7	GQ280892	OP235406	Australia: Mallacoota, VIC, Zuccarello <i>et al.</i> (2010)
K33 (K19-4-121-1)	Rhodophyta, Erythropeltidales, Erythrotrichiaceae	<i>Erythrotrichia</i> Areschoug sp.	F-Z	<i>psbA</i>	<i>psbA1F</i> , <i>psbA1R</i>	89	98.6	JF292675	OP235407	New Caledoni, Zuccarello <i>et al.</i> (2011)
K36 (K19-4-143-4)	Rhodophyta, Erythropeltidales, Erythrotrichiaceae	<i>Porphyrostromium</i> Trevisan, V.B.A. sp.	R-F	<i>psbA</i>	<i>psbA1F</i> , <i>psbA1R</i>	77	95.71	JF292699	OP235408	USA: Bioler Bay, Oregon, Zuccarello <i>et al.</i> (2011)
K66 (K19-2-169-1)	Rhodophyta, Ceramiales, Spyridiaceae	<i>Spyridia</i> Harvey sp.		<i>rbcL</i>	<i>rbcLRH3F</i> - <i>rbcS139R</i>	89	99.47	MT975955	OP491535	India, Kundu and Bast (2020)
K78 (K19-2-35-2)	Rhodophyta, Ceramiales, Spyridiaceae	<i>Spyridia</i> Harvey sp.		<i>rbcL</i>	<i>rbcLRH3F</i> - <i>rbcS139R</i>	74	99.6	MT975955	OQ630968	India, Kundu and Bast (2020)
K62 (K19-2-51-1)	Rhodophyta, Ceramiales, Ceramiaceae	<i>Ceramium</i> Roth sp.		<i>rbcL</i>	<i>rbcLRH3F</i> - <i>rbcS139R</i>	97	95.63	AF521798	OP491533	South Korea, Cho <i>et al.</i> (2003)
K61 (K19-2-35-4)	Rhodophyta, Ceramiales, Rhodomelaceae	<i>Polysiphonia</i> Greville sp.		<i>rbcL</i>	<i>rbcLRH3F</i> - <i>rbcS139R</i>	71	93.52	MT676278	OP491532	Australia, Diaz-Tapia <i>et al.</i> (2020)
K68 (K19-4-109-2)	Rhodophyta, Ceramiales, Delesseriaceae	<i>Dasya caraibica</i> Børgesen	F-Z	<i>rbcL</i>	<i>rbcL765F</i> - <i>rbcL1381R</i>	100	99.33	ON002437	OP491551	Bermuda, Cassidy <i>et al.</i> (2022)

C: Close samples, collected within 100 m of the outfall or reference site; M: Middle samples, collected 200-700 m from brine outfall or reference site; F: Far samples, collected 800-1000 m from brine outfall or reference site. FA: Very Far samples, collected from

more than 1000 m from brine outfall or reference site. D: Doha; Z: Al-Zour; R: reference site; B: Boubiyan; FI: Fintas. F-D: Samples from Doha, collected from a distance more than 1000 m. Isolates without any symbol were from locations with no desalination plants. Letter combinations indicate in which sector of a given site an isolate was collected: E.g. F-Z means that isolates were collected in the far sector from the Al-Zour site.

Table S3. Coordinates of waypoints, mean temperature (°C) and salinity at different sampling distances from the outfall of the desalination plant (Doha East and Al-Zour South) including their reference sites.

Location sites	Sampling Way points	Distance from fallout (m)	Coordinates (GPS)	Salinity psu	Temperature (°C)	Comments
Boubiyan island (reference site for DE).	Wpt 1		29°43'57.2"N 48°06'21.0"E	36	25	
	Wpt 2		29°48'08.2"N 48°03'57.5"E	37	24.5	
	Wpt 3		29°48'08.9"N 48°04'05.3"E	37	25.6	
	Wpt 4		29°52'52.8"N 48°07'16.5"E	37	23.7	
	Wpt 5		29°55'05.5"N 48°08'18.7"E	36	23.9	
	Wpt 6		29°56'14.8"N 48°05'54.5"E	37	23.1	
Doha						
	Wpt1		29° 22' 14.3" N, 47° 48' 14.2" E			Middle of the outlet.
	Wpt 2	76.04	29° 22' 16.81" N, 47° 48' 12.017" E	43.5	38.16	Last point away in cement wall
	Wpt 3	51.59	29° 22' 15.93" N, 47° 47' 48' 12.2" E	43.5	38.4	cement wall
	Wpt 4	79.12	29° 22' 17.13" N, 47° 48' 12.6504" E	43.5	38.25	Near small sewage outfall
	Wpt 5	0	29° 22' 14.3" N, 47° 48' 13.45" E	43	37.7	Main fall discharge and small sewage outfall
	Wpt 6	6.32	29° 22' 14.95" N, 47° 48' 13.5" E	43	38.11	Main out fall discharge
	Wpt 7	1770	29° 22' 38.5" N, 47° 49' 9.62" E	42	37.21	
	Wpt 8	1540	29° 22' 36.61" N, 47° 49' 3.67" E	40.5	36.97	
	Wpt 9	882.45	29° 22' 28.22" N, 47° 48' 41.44" E	40	36	
	Wpt 10	806.13	29° 22' 27.56" N, 47° 48' 38.73" E	40	36.98	Away beside the chalet
	Wpt 11	83.75	29° 22' 15.8052" N, 47° 48' 16.88" E	42	36.3	Near the Emergency Rain fall outlet
	Wpt 12	188.64	29° 22' 18.99" N, 47° 48' 18.6" E	40.5	36.7	beside the chalet
	Wpt 13	303.68	29° 22' 21.1" N, 47° 48' 21.924" E	41.5	37.07	beside the chalet
Fintas (reference site for ZS).						
	Wpt 1		29° 10' 22.89" N, 48° 7' 27.83" E	40	21.8	
	Wpt 2		29° 10' 22.21" N, 48° 7' 30.5" E	40	21.3	
	Wpt 3		29° 10' 35.267" N, 48° 7' 23.6" E	40	21.6	
Al-Zour						
	Wpt 1	51.07	28° 41' 52.8252" N, 48° 22' 43.0104" E	41	39.39	
	Wpt 2	28.16	28° 41' 53.3472" N, 48° 22' 43.5684" E	42.5	39.38	Outfall discharge
	Wpt 3	27.39	28° 41' 53.6676" N, 48° 22' 43.266" E	42	39.25	
	Wpt 4	49.72	28° 41' 52.7244" N, 48° 22' 43.3128" E	43	39.32	
	Wpt 5	53.11	28° 41' 52.674" N, 48° 22' 43.2264" E	43	39.34	
	Wpt 6	33.03	28° 41' 53.0664" N, 48° 22' 43.5288" E	43	39.42	
	Wpt 7	34.74	28° 41' 53.0844" N, 48° 22' 43.4892" E	43	39.42	

Wpt 8	80.7	28° 41' 51.8784" N, 48° 22' 42.9492" E	43.5	39.4
Wpt 9	85.38	28° 41' 51.45" N, 48° 22' 43.158" E	43	39.4
Wpt 10	108.56	28° 41' 50.5284" N, 48° 22' 43.2264" E	42.5	39.43
Wpt 11	640.32	28° 41' 33.1044" N, 48° 22' 45.1236" E	40	36.06
Wpt 12	800	28° 41' 28.5864" N, 48° 22' 48.6192" E	40	36.41
Wpt 13	949.39	28° 41' 25.8648" N, 48° 23' 0.0744" E	39.5	36.41
Wpt 14	194.91	28° 41' 47.9" N, 48° 22' 43.252" E	40	35.86

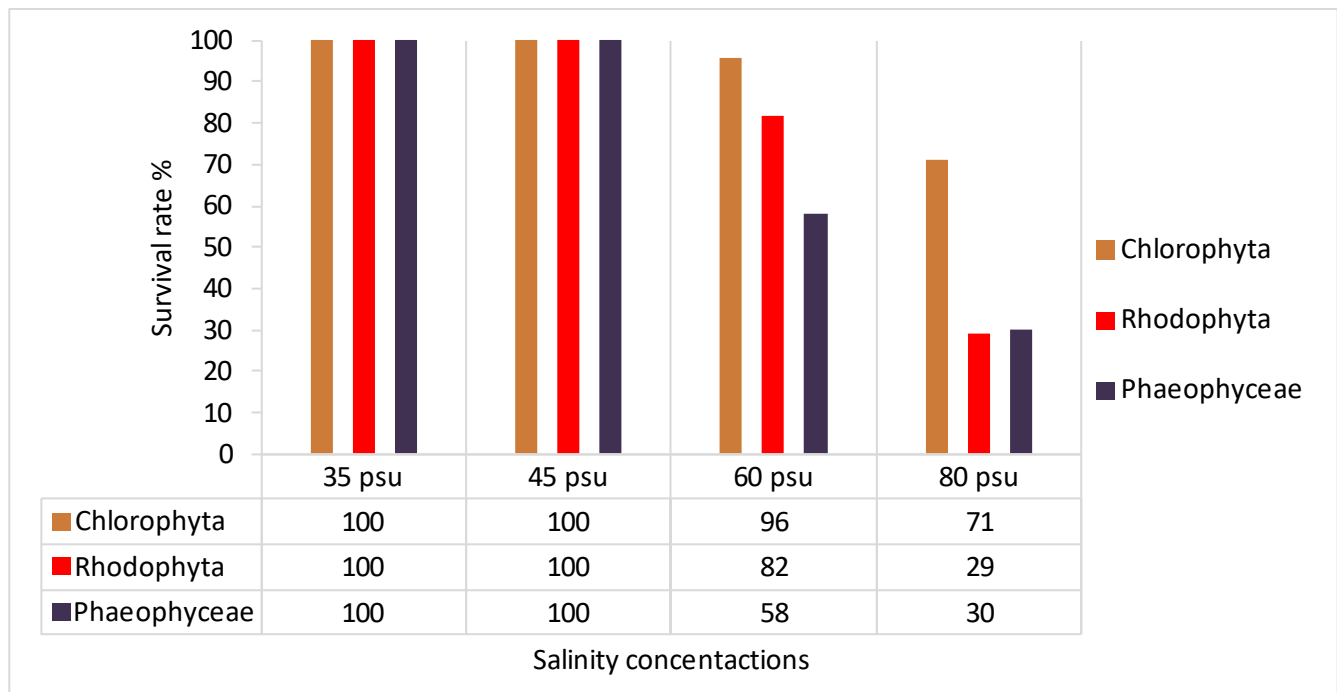


Fig S2. Viability of tested species of Chlorophyta, Phaeophyceae and Rhodophyta under different salinities.

Table S4. Survival rates of algal isolates at different salinities (35, 45, 60, and 80). Degree of vitality (estimated percentage) which represents the extent of bleached thalli is estimated as a result of stress over a given period (14 days) x100. Degree of vitality: not affected (NA); some bleaching (Sb) = 75%; partial bleaching (Pb) = 50%; mostly bleached (Mb) = 25%; bleached thalli (B)=0%. Symbols: * species revived after return to 25°C; ^^ species showed further deterioration after return to 25°C.

Strain Code	Strain ID code	Genotype ID	35 psu	45 psu	60 psu	Onset 33psu (14d)	80 psu	Onset 33psu (14d)
Chlorophyta		18 isolates; 12 species				11.5/12=96%		8.19/12=67%
K4	K19-4-67-1	<i>Ulva tepida</i> OP221966	NA	NA	NA	NA	NA	NA
K25	K19-4-93-1	<i>Ulva tepida</i> OP221968	NA	NA	NA	NA	Sb	Sb
K51	K (19-2-)72-3	<i>Ulva tepida</i> OP219456	NA	NA	NA	NA	NA	NA
K52	K (19-2-)66-1	<i>Ulva tepida</i> OP219457	NA	NA	NA	NA	NA	NA
K54	K (19-2)187-3	<i>Ulva tepida</i> OP219458	NA	NA	NA	NA	NA	NA
K55	K (19-2)192-2	<i>Ulva ohnoi</i> OP235395	NA	NA	Sb	Sb^	Mb	Mb
K38	K19-4-93-3	Planophilaceae sp. OP221972	NA	NA	NA	NA	NA	NA
K41	K19-4-108-3	Planophilaceae sp. OP221976	NA	NA	NA	NA	NA	NA
K39	K19-4-59-2	<i>Ulvella</i> sp. OP235397	NA	NA	NA	NA	NA	NA
K40	K19-4-45-2	<i>Ulvella</i> sp. OP235398	NA	NA	NA	NA	NA	NA
K44	K19-4-1-3	<i>Ulvella</i> sp. OP235399	NA	NA	NA	NA	NA	NA
K45	K19-4-66-2	Ulvellaceae sp. OP235400	NA	NA	NA	NA	NA	NA
K42	K19-4-103-4	Ulvellaceae sp. OP221973	NA	NA	NA	NA	Sb	Sb
K49	K (19-2)18-1	<i>Bryopsis</i> sp. OP235396	NA	NA	NA	NA	B	B
K56	K19-4-121-3	<i>Cladophora laetevirens</i> OP221978	NA	NA	Pb*	Sb	B	B
K58	K19-4-137-1	<i>Rhizoclonium</i> sp. OP221980	NA	NA	NA	NA	Sb^	Mb
K73	K19-4-78-2	<i>Chaetomorpha</i> sp. OP221970	NA	NA	NA	NA	NA	NA
K74	K19-4-76-2	<i>Chaetomorpha</i> sp. OP221971	NA	NA	NA	NA	NA	NA

Phaeophyceae			14 isolates; 10 species		5.82/10=58%		3/10=30%	
K17	K (19-2-)33-1	<i>Sphacelaria</i> sp. OQ630967	NA	NA	B	B	B	B
K71	K (19-2)176-1	<i>Sphacelaria</i> sp. OQ630966	NA	NA	NA	NA	B	B
K72	K19-4-123-1	<i>Sphacelaria tribuloides</i> OP491539	NA	NA	Mb	Mb	Mb	Mb
K19	K19-4-72-2	Acinetosporaceae sp. OQ615883	NA	NA	Pb	Pb^	Mb	Mb
K26	K (19-2)28-2	<i>Elachista stellaris</i> OP491560	NA	NA	NA	NA	Sb	Sb
K21	K19-4-97-3	Chordariaceae sp. OP491541	NA	NA	Mb	Mb^	B	B
K23	K19-2-130-1	<i>Ectocarpus subulatus</i> OP491536	NA	NA	Sb	Sb^	B	B
K24	K19-4-159-1	<i>Iyengaria stellata</i> OP235416	NA	NA	Sb	Sb	Sb	Sb
K47	K19-4-121-2	<i>Feldmannia mitchelliae</i> OP491537	NA	NA	Sb	Sb^	Mb	Mb
K198	K (19-2)198-1	<i>Feldmannia mitchelliae</i> OP491538	NA	NA	Sb	Sb^	B	B
K216	K (19-2)216-1	<i>Feldmannia mitchelliae</i> OP491555	NA	NA	NA	NA	Mb^	B
K48	K19-4-144-1	<i>Feldmannia mitchelliae</i> OP491556	NA	NA	Mb	Mb^	B	B
K70	K (19-2)81-1	<i>Bachelotia</i> sp. OP491558	NA	NA	NA	NA	Pb	Pb
K87	K (19-2)245-1	<i>Colpomenia sinuosa</i> OP491559	NA	NA	Sb	Sb^	Pb	Pb
Rhodophyta			12 isolates; 9 species		7.38/9= 82%		2.63 /9 =29%	
K34	K19-4-112-5	Acrochaetiales sp. OP491545	NA	NA	NA	NA	Pb	Pb
K13	K19-4-83-1	<i>Arcochaetium</i> sp. OP491543	NA	NA	NA	NA	Sb^	Pb
K28	K19-4-148-5	<i>Sahlingia subintegra</i> OP491547	NA	NA	NA	NA	B	B
K30	K19-4-110-1	<i>Stylonema alsidii</i> OP235409	NA	NA	NA	NA	B	B
K31	K19-4-138-1	<i>Stylonema alsidii</i> OP235410	NA	NA	NA	NA	B	B
K32	K19-4-161-5	<i>Erythrotrichia</i> sp. OP235406	NA	NA	NA	NA	Sb	Sb
K33	K19-4-121-1	<i>Erythrotrichia</i> sp. OP235407	NA	NA	NA	NA	Mb	Mb
K36	K19-4-143-4	<i>Porphyrostromium</i> sp. OP235408	NA	NA	NA	Sb^	Pb*	Sb
K61	K (19-2)35-4	<i>Polysiphonia</i> sp. OP491532	NA	NA	NA	Sb^	Pb^	Mb
K62	K (19-2)169-1	<i>Ceramium</i> sp. OP491533	NA	NA	Pb	Pb^	B	B
K66	K (19-2)51-1	<i>Spyridia</i> sp. OP491535	NA	NA	B	B	B	B

K78	K (19-2) 35-2	<i>Spyridia</i> sp. OQ630968	NA	NA	NA	Sb^	Mb	Mb
	0%	Not affected	1 NA	Species survival %				
	80-99%	Bleached	0 B	Salinity	35	45	60	80
	50-80%	Mostly bleached	0.25 Mb					
	20-50%	Partially bleached	0.5 Pb	Chlorophyta	100%	100%	11.5/13=9 6%	9.19/13=71%
	5-20%	Some bleaching	0.75 Sb	Phaeophyceae	100%	100%	5.82/10=5 8%	3/10 = 30%
				Rhodophyta	100%	100%	7.38/9= 82%	2.63 /9 =29%

Note: In this experiment only 44 isolates were used. Yellow highlighted rows correspond to similar species.

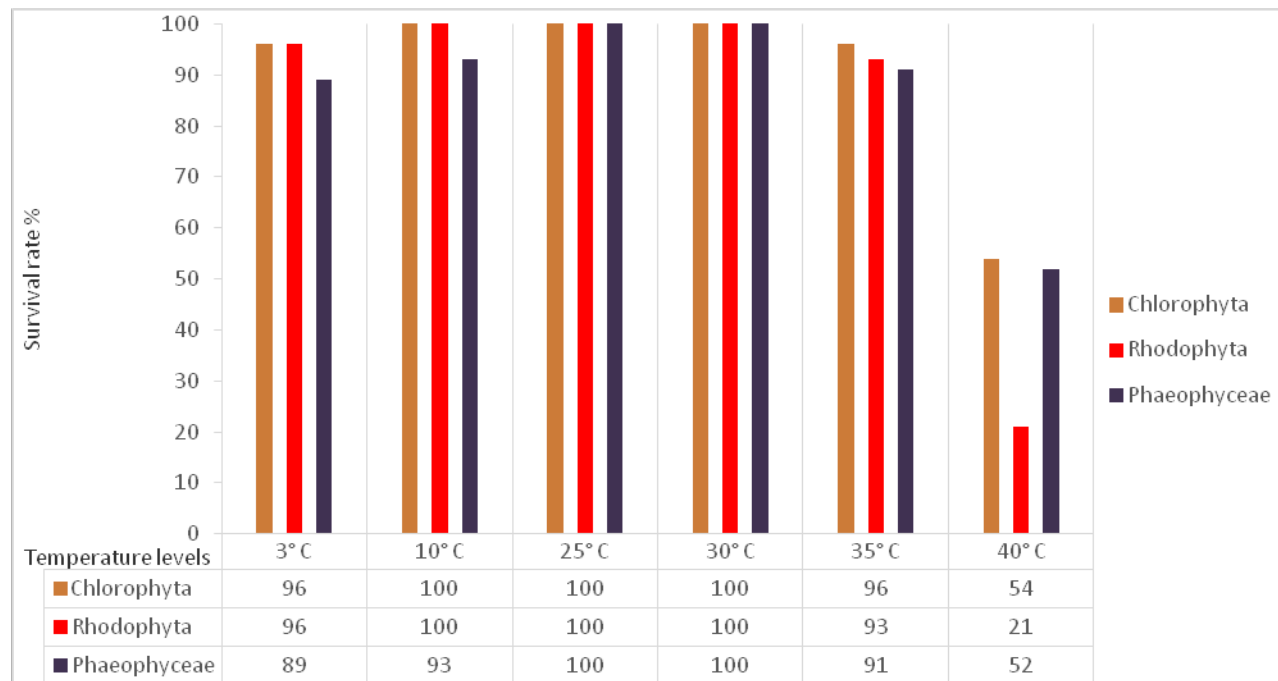


Fig. S3. Viability of species of Chlorophyta, Phaeophyceae and Rhodophyta incubated at different temperatures treatments.

Table S5. Survival rates of each phylum at different temperatures (3, 10, 25, 30, 35 and 40°C).

Degree of vitality (estimated percentage) which represents the extent of bleached thalli is estimated as a result of stress over a given period (14 days) x100. Degree of vitality: not affected (NA); some bleaching (SB) = 75%; partial bleaching (PB) = 50%; mostly bleached (MB) = 25%; bleached thalli (B)=0%. Symbols: * species revived after return to 25°C; ^^ species showed further deterioration after return to 25°C. In this experiment only 47 isolates were used. Yellow highlighting indicates closely related species.

Strain code	Strain ID code	Closest GenBank match	3°C	10°C	25°C	30°C	35°C	40°C
Chlorophyta		19 isolates; 13 species	12.5/13=96%	100%	100%	100%	13.5/13=96%	6.96/13=54%
K4	K19-4-67-1	<i>Ulva tepida</i> OP221966	NA	NA	NA	NA	NA	MB*
K25	K19-4-93-1	<i>Ulva tepida</i> OP221968	NA	NA	NA	NA	NA	B
K51	K (19-2-)72-3	<i>Ulva tepida</i> OP219456	NA	NA	NA	NA	NA	SB*
K52	K (19-2-)66-1	<i>Ulva tepida</i> OP219457	NA	NA	NA	NA	NA	NA^
K54	K (19-2)187-3	<i>Ulva tepida</i> OP219458	NA	NA	NA	NA	NA	MB*
K55	K (19-2)192-2	<i>Ulva ohnoi</i> OP235395	NA	NA	NA	NA	NA	B
K38	K19-4-93-3	Planophilaceae sp. OP221972	NA	NA	NA	NA	NA	SB*
K39	K19-4-59-2	<i>Ulvella</i> sp. OP235397	NA	NA	NA	NA	NA	MB
K40	K19-4-45-2	<i>Ulvella</i> sp. OP235398	NA	NA	NA	NA	NA	PB
K44	K19-4-1-3	<i>Ulvella</i> sp. OP235399	NA	NA	NA	NA	NA	SB*
K41	K19-4-108-3	Planophilaceae sp. OP221976	NA	NA	NA	NA	NA	PB^
K42	K19-4-103-4	Ulvellaceae sp. OP221973	NA	NA	NA	NA	NA	SB*
K43	(K19-4-37-3)	Ulvellaceae sp. OP221974	SB*	NA	NA	NA	NA	NA
K45	K19-4-66-2	Ulvellaceae sp. OP235400	NA	NA	NA	NA	NA	NA
K49	K (19-2)18-1	<i>Bryopsis</i> sp. OP235396	SB*	NA	NA	NA	PB^	B
K56	K19-4-121-3	<i>Cladophora laetevirens</i> OP221978	NA	NA	NA	NA	NA^	B
K58	K19-4-137-1	<i>Rhizoclonium</i> sp. OP221980	NA	NA	NA	NA	NA	NA^
K73	K19-4-76-2	<i>Chaetomorpha</i> sp. OP221970	NA	NA	NA	NA	NA	B*
K74	K19-4-78-2	<i>Chaetomorpha</i> sp. OP221971	NA	NA	NA	NA	NA	SB

Phaeophyceae			15 isolates; 11 species	9.75/11=89%	10.25/11=93%	100%	100%	10/11=91%	5.75/11=52%
K17	K (19-2-)33-1	<i>Sphacelaria</i> sp. OQ630967	SB*	NA	NA	NA	NA	SB^	MB^
K71	K (19-2)176-1	<i>Sphacelaria</i> sp. OQ630966	NA	NA	NA	NA	NA	SB^	PB
K72	K19-4-123-1	<i>Sphacelaria tribuloides</i> OP491539	PB*	NA	NA	NA	NA	SB^	PB*
K19	K19-4-72-2	Acinetosporaceae sp. OQ615883	NA	NA	NA	NA	NA	SB^	MB^
K21	K19-4-97-3	Chordariaceae sp. OP491541	NA	NA	NA	NA	NA	NA	PB^
K22	K19-4-107-5	Chordariaceae sp. AF207812	NA	NA	NA	NA	NA	NA	SB^
K23	K19-2-130-1	<i>Ectocarpus subulatus</i> OP491536	NA	NA	NA	NA	NA	NA	MB^
K24	K19-4-159-1	<i>Iyengaria stellata</i> OP235416	NA	NA	NA	NA	NA	SB^	PB^
K26	K (19-2)28-2	<i>Elachista stellaris</i> OP491560	NA	NA	NA	NA	NA	NA	SB^
K47	K19-4-121-2	<i>Feldmannia mitchelliae</i> OP491537	NA	NA	NA	NA	NA	NA	MB^
K48	K19-4-144-1	<i>Feldmannia mitchelliae</i> OP491538	NA	NA	NA	NA	NA	NA	MB*
K198	K (19-2)198-1	<i>Feldmannia mitchelliae</i> OP491555	NA	NA	NA	NA	NA	NA	SB^
K216	K (19-2)216-1	<i>Feldmannia mitchelliae</i> OP491556	NA	NA	NA	NA	NA	NA	SB^
K70	K (19-2)81-1	<i>Bachelotia</i> sp. OP491558	MB	MB*	NA	NA	NA	NA	SB^
K87	K (19-2)245-1	<i>Colpomenia sinuosa</i> OP491559	NA	NA	NA	NA	NA	NA	SB^
Rhodophyta			13 isolates; 10 species	9.63/10=96%	100%	100%	100%	9.25/10=93%	2.05/10=21%
K34	K19-4-112-5	Acrochaetiales sp. OP491545	SB*	NA	NA	NA	NA	PB^	MB^
K13	K19-4-83-1	<i>Arcochaetium</i> sp. OP491543	NA	NA	NA	NA	NA	NA	MB^
K28	K19-4-148-5	<i>Sahlingia subintegra</i> OP491547	NA	NA	NA	NA	NA	NA^	B
K30	K19-4-110-1	<i>Stylonema alsidii</i> OP235409	NA	NA	NA	NA	NA	NA	PB^
K31	K19-4-138-1	<i>Stylonema alsidii</i> OP235410	NA	NA	NA	NA	NA	NA	PB^
K32	K19-4-161-5	<i>Erythrotrichia</i> sp. OP235406	NA	NA	NA	NA	NA	NA	B
K33	K19-4-121-1	<i>Erythrotrichia</i> sp. OP235407	NA	NA	NA	NA	NA	NA	B
K36	K19-4-143-4	<i>Porphyrostromium</i> sp. OP235408	NA	NA	NA	NA	NA	NA	B
K61	K (19-2)35-4	<i>Polysiphonia</i> sp. OP491532	NA	NA	NA	NA	NA	NA	SB^
K62	K (19-2)169-1	<i>Ceramium</i> sp. OP491533	NA	NA	NA	NA	NA	SB^	B
K68	K19-4-109-2	<i>Dasya caraibica</i> OP491551	NA	NA	NA	NA	NA	NA	B
K66	K (19-2)51-1	<i>Spyridia</i> sp. OP491535	NA	NA	NA	NA	NA	NA	B

K78	K (19-2)35-2	<i>Spyridia</i> sp. OQ630968	SB*	NA	NA	NA	NA	PB^	
Key words	survival	value	3°C	10°C	25°C	30°C	35°C	40°C	
Bleach	80-99%	0 B	Chlorophyta	96%	100%	100%	100%	96%	54%
Mostly bleach	50-80%	0.25 Mb	Phaeophyceae	89%	93%	100%	100%	91%	52%
Partially bleached	20-50%	0.5 Pb	Rhodophyta	96%	100%	100%	100%	93%	21%
Some bleach	5-20%	0.75 Sb	Sb						
Not affected	0%	1 NA	NA						

Table S6. Diversity of algal isolates obtained by Germling Emergence at increasing distances (indicated by waypoint, wpt) from outfall discharges at Doha East and Al-Zour South desalination plants and their reference sites (Boubiyan and Fintas). Additional sampling locations were also included to ensure better representation of the algal diversity of Kuwait.

Location sites		Taxa		
Doha	Distance/m	Chlorophyta	Rhodophyta	Phaeophyceae
wpt1				
wpt 2	76.04			
wpt 3	51.59			
wpt 4	79.12			
wpt 5	0			
wpt 6	6.32			
wpt 7	1770	Ulvellaceae sp. (K45)		
wpt 8	1540	<i>Ulvella</i> sp. (K39)		
wpt 9	882.45	<i>Ulvella</i> sp. (K40) <i>Ulva ohnoi</i> (K55)		
wpt 10	806.13	Ulvellaceae sp. (K43), <i>Ulvella</i> sp. (K44), <i>Rhizoclonium</i> sp.(K58)		
wpt 11	83.75			
wpt 12	188.64			
wpt 13	303.68			
Fintas				
3	Site3		<i>Sahlingia subintegra</i> (K28) <i>Stylonema alsidii</i> (K31) <i>Erythrotrichia</i> sp. (K32) <i>Porphyrostromium</i> sp. (K36)	<i>Iyengaria stellata</i> (K24) <i>Feldmannia mitchelliae</i> (K48)
Al-Zour				
wpt 1	51.07			
wpt 2	28.16	<i>Ulva tepida</i> (K4)		Acinetosporaceae sp. (K19)
wpt 3	27.39	<i>Chaetomorpha</i> sp. (K73), <i>Chaetomorpha</i> sp. (K74)		
wpt 4	49.72		<i>Arcochaetium</i> sp. (K13)	

wpt 5	53.11			
wpt 6	33.03			
wpt 7	34.74			
wpt 8	80.7			
wpt 9	85.38			
wpt 10	108.56	<i>Ulva tepida</i> (K25) Planophilaceae sp.(K38)		
wpt 11	640.32	<i>Cladophora laetevirens</i> (K56)		Chordariaceae sp.(K21)
wpt 12	800	Planophilaceae sp. (K41) Ulvellaceae sp. (K42)	<i>Erythrotrichia</i> sp. (K33)	<i>Feldmannia mitchelliae</i> (K47) Chordariaceae sp.(K22) <i>Sphacelaria tribuloides</i> (K72)
wpt 13	949.39		<i>Stylonema alsidii</i> (K30) <i>Dasya</i> <i>caraibica</i> (K68) Acrochaetiales sp.(K34)	
wpt 14	194.91			<i>Ectocarpus subulatus</i> (K23) <i>Colpomenia sinuosa</i> (K87)
Boubiyan & Subbiya Failaka Kubbar Island		<i>Ulva tepida</i> (K54)	<i>Spyridia</i> sp. (K66)	<i>Sphacelaria</i> sp.(K71) <i>Feldmannia mitchelliae</i> (K198) <i>Feldmannia mitchelliae</i> (K216)
Qaruh Island		<i>Ulva tepida</i> (K51) <i>Ulva tepida</i> (K52)		
Umm al-Maradim Island				<i>Bachelotia</i> sp. (K70)
Khiran		<i>Bryopsis</i> sp. (K49)	<i>Polysiphonia</i> sp. (K61) <i>Spyridia</i> sp. (K78)	<i>Sphacelaria</i> sp. (K17) <i>Elachista stellaris</i> (K26)
Nuwiseeb			<i>Ceramium</i> sp. (K62)	

