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Evaluating physico-chemical and biological impacts of brine discharges for a sustainable desalination development on South America's Pacific coast

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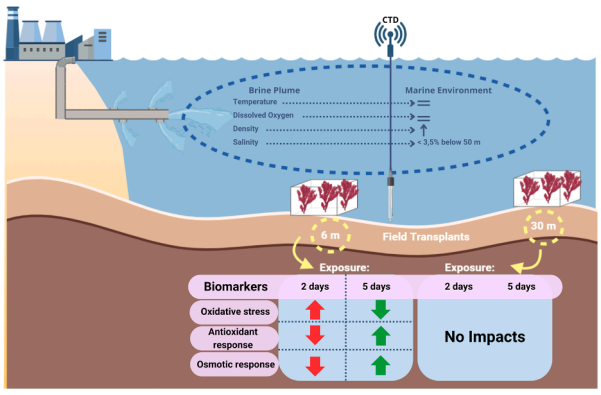
HIGHLIGHTS

- Maximum brine salinity increase was less than 3.5 % below 50 m from discharge point.
- Brine discharge increased density but did not alter temperature or dissolved oxygen.
- Brine discharge induced short-term (2 days) stress responses in *R. corallina*.
- R. *corallina* transplants showed no cellular damage after 5 days of brine exposure.
- Specific regulations to limit brine discharges salinity are highly recommended.

GRAPHICAL ABSTRACT



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ABSTRACT

The expansion of seawater desalination is presented as a new way to supply fresh water for many coastal regions as an effort to counteract the increasing water scarcity. However, brine discharges also pose significant environmental challenges regarding their potential environmental impacts of marine ecosystems. The main objective of this study was to assess the physico-chemical impact of the brine discharges from Seawater Reverse Osmosis (SWRO) desalination plants on South America pacific coastal ecosystems, assessing its potential physical-chemical impact (temperature, salinity, density and dissolved oxygen) on the receiving marine environment, and evaluating the oxidative and osmotic stress responses of the red macroalgae *Rhodymenia corallina* through diagnostic biomarkers in field-transplantation experiments. Our results showed that the increase over natural salinity in the affected area was less than 3.5 % in a radius of 50 m from the discharge point. Also, we

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demonstrated that the brine discharges increase the density but not significant affect the temperature and dissolved oxygen of the marine environment. In addition, diagnostic biomarkers showed a negative effect on oxidative, osmotic and antioxidant stress responses in *R. corallina* after two days of brine exposure, particularly at the nearest brine diffuser transplantation site. However, after five days, antioxidant and osmotic parameters exhibited full recovery, indicating the cessation of the redox imbalance. Based on the results obtained, we demonstrated that the use of appropriate mitigation measures combined with an appropriate oceanographic location of the submarine outfall, would ensure a sustainable desalination operation without generating significant environmental impacts on the coastal ecosystems.

1. Introduction

Fresh water availability has been strongly reduced by the increasing demand for water resources for different uses (e.g., human consumption, tourism, agriculture, industrial activities, among others), as well as by the effects of climate change and the poor management of these resources [1]. There are many factors that have affected the significant decrease in the availability of water resources. Firstly, the impact of climate change, which has strongly reduced the freshwater availability in many world regions [2]. Secondly, the high water demand for different uses, such as agricultural demand, water consumption, industrial activities, among others [3]. Therefore, it is urgent to seek new freshwater sources instead of the traditional ones, in order to overcome the global water scarcity problem. In this context, desalination emerges as a crucial alternative to transform salt water into quality freshwater, ensuring that the water meets the quality standards required for human consumption and/or for other purposes [1,4].

Desalination technology by reverse osmosis stands out for its higher efficiency and lower energy consumption compared to other technologies, which makes it the most used option worldwide [5,6]. This method involves the passage of pressurized salt water through a selective membrane allowing the passage of water and excluding salts and minerals [5]. From the seawater reverse osmosis (SWRO) desalination process, two main significant products are obtained, a high-quality permeated water and a rejection discharge called brine discharge. The last is mainly characterized by presenting a high concentration of salts that can double the salinity of the captured seawater, and may also contain certain chemical by-products employed during the pretreatments (e.g., antifouling, coagulants, flocculants, among others), also discarded along with the brine discharge into coastal ecosystems, which could affect the physical-chemical quality of the receiving environment [7-9].

There are numerous methods of discharge rejection, but the most widely used method globally due to its higher economic and logistical feasibility compared to other methods, is through a marine outfall or discharge on the coast [10]. Given the characteristics of brine discharges, when discharged into the sea, a plume of higher density is generated with respect to the receiving marine environment that moves forward following the bathymetric line of the seabed [11,12]. The dispersion behavior of saline plume depends on different factors, such as the plant production volume, brine salinity, technology employed, geomorphological conditions (bathymetry, type of seabed, among others), oceanographic conditions (e.g., currents, natural salinity), among others [13,14]. According to these characteristics, it has been observed that saline plumes can reach from tens of meters to several kilometers from the discharge point. For example, the saline plume from the San Pedro Del Pinatar SWRO plant in Spain was detected at a distance of 3 km, while the plume from the Palmachim SWRO plant in Israel extended for more than 4.4 km or the plume from the Minera Escondida in Chile extended for more than 700 m [15-18].

Saline plumes can generate environmental impacts by presenting high salinities compared to the receiving environment, producing osmotic shock in the benthic communities existing in the area of influence, affecting their abundance and richness. For example, impacts have been identified on echinoderms in the Mediterranean Sea (e.g., *Paracentrotus lividus*, *Echinaster sepositus*) or benthic communities that are particularly sensitive to salinity changes, due to their limited osmoregulatory capacity [19-22]. Likewise, negative impacts, measured using stress biomarkers, have been identified on seagrasses, such as *Posidonia oceanica* meadows in the Mediterranean Sea, or *Posidonia australis* in Australia, due to their low tolerance to natural salinity changes [23-26].

Biomarkers are defined as naturally occurring metabolites, expressed/repressed genes or other cellular processes in an organism, that can be measured upon a physiological or toxicological event. In ecotoxicology, biomarkers measurements can provide insights of the organism's exposure to different environmental stressors. These biomarkers serve as indicators of biological stress or damage caused by exposure to abiotic stressors, such as hypersaline conditions, metals, or temperature variations [27]. Specifically, biomarkers have been used to assess the overall health and fitness of organisms exposed to brine in both short- and mid-term scenarios ([28,29]). In the case of the Chilean Pacific coast, recent studies using stress biomarkers have highlighted the effect on photosynthetic rate, oxidative stress and damage, together with the antioxidant response in macroalgae (*Ectocarpus sp*. and *Dictyota kunthii*) that were transplanted in the discharge area of the SWRO of La Chimba (Antofagasta), when they were exposed to increases of more than 4.4 % and 7 % over the natural salinity of 34.4 psu [30,31]. Also, a recent study with Chilean seagrass (*Zostera chilensis*) under experimental laboratory conditions has also evidenced an effect on photosynthetic rate and an increase in oxidative stress caused by osmotic stress [32]. In addition, the South American Pacific Coast stands out as a region with marine upwellings of high intensity and frequency, which contribute to the recirculation of energy and nutrients through the food chain. This turns it into an area of great ecological potential, characterized by its high productivity and biological biodiversity. Besides from its exposure to ocean waves and its considerable depths, this coast counts on nearby submarine canyons, aspects that contribute to the diversity of marine habitats along its length, together with upwelling events and climate [33].

However, the environmental impacts of brine discharges can be minimized if appropriate and/or preventive measures are adopted in order to ensure a proper brine dilution. Therefore, it is essential to study the prospective area affected by brine discharges, in order to prevent their potential impact on marine ecosystems, and to adopt technological and logistical measures when it is necessary to minimize their area of impact [7,34]. In the absence of specific normative criteria to regulate brine discharges from desalination plants in Chile, projects are currently regulated by the "Servicio de Evaluación Ambiental" (Environmental Evaluation Service, SEA), where, projects are required to be submitted to the environmental evaluation process in order to assess the potential environmental impact they could generate during the construction and operation phase. Thus, including the necessary preventive and corrective measures to ensure that projects are developed without producing significant environmental impacts. Projects can be submitted through an Environmental Impact Assessment (EIA) or an Environmental Impact Statement (EIS), depending on the magnitude of their potential environmental impacts [35-37]. In addition, they must include an Environmental Monitoring Plan (EMP) that must include the necessary requirements in order to ensure that the projects are not producing significant environmental impacts, or failing that, if they are generating

significant impacts, mitigation measures must be adopted to reverse them [16,38,37].

Due their ecological relevance, macroalgae have been commonly used as bioindicators of marine pollution for the impact of numerous anthropogenic activities, such as the potential impact of brine discharges on marine ecosystems [28,31]. Among the macroalgae species, *Rhodymenia corallina* has been proposed as a suitable bioindicator species for assessing stress biomarkers of sessile marine organisms exposed to the impact of brine discharges, as it is an abundant species at the subtidal zones nearby brine discharges outfall of several desalination plants located in the north Pacific coast of Chile.

The main objective of this study was to assess the physico-chemical and biological impact of the brine discharges from SWRO desalination plants on South America pacific coastal ecosystems, using the Nueva Atacama SWRO plant located in Atacama Region (Chile) as a case study. For that, we evaluated: i) the physico-chemical quality of the receiving marine environment, studying the dispersion pattern of temperature, depth, dissolved oxygen (DO), density and salinity; and ii) the study of the oxidative and osmotic stress responses of *R.corallina* through field-transplantation experiments at different salinities following the results of the saline plume dispersion pattern.

2. Materials and methods

2.1. Study area

The study area includes the Punta Zorro sector situated in close proximity to Punta Padrones (Fig. 1), in Caldera city (Atacama Region, Chile). Here, the brine discharge from the Nueva Atacama SWRO plant is discharged through a marine outfall. Caldera bay exhibits depth variations, ranging from 5 m in shallow waters to 50 m in the deepest areas. The primary drivers of ocean currents in the area are wind and tides, with a mixed semidiurnal tidal cycle. Regarding salinity, there is evidence of uniformity in the water column, with an average sea surface salinity of 34.43 psu and 34.45 psu at 20 m depth, suggesting a homogeneous distribution of dissolved components in the water. Furthermore, this area is distinguished by the frequent occurrence of intense seasonal upwelling events.

2.2. Nueva Atacama desalination plant

The Nueva Atacama SWRO plant began operating in 2021 with a capacity of 300 L/s. The plant extracts seawater through an underwater pipe with an open intake at a depth of 25 m. The rejection system includes a 169 m submerged outfall connected to 14 m long Y-shaped diffusers, equipped with 8 nozzles of 250 mm diameter, located at about 20 m depth. The brine effluent has an average salinity of 65.3 psu, temperature of 13°C and density of 1049.62 kg/m3.

2.3. Experimental design

To estimate the dispersion area of the brine from the effluent discharge and the area affected by the saline plume, a grid of 60 sampling stations was designed in order to make measurements at stations located at different distances from the outfall (Fig. 2). The extension of the study area and the grid spacing between stations was defined according to the production volume of the desalination plant, the discharge method and the oceanographic characteristics of the receiving marine environment [18,39].

2.4. CTD (Conductivity, temperature, and depth) samples collection and data processing

In January 2023, a sampling campaign was conducted with 60 stations, including a salinity control point located more than 2 km outside the bay to compare salinity levels with the brine discharge area and natural values. A CTD instrument (Hydrolab HL7) was used to carry out continuous measurements of temperature, salinity and DO at different depths, recording data in surface and bottom water at each station. All stations were positioned using a GPS based on UTM coordinates, employing the WGS 84 geographic coordinate system.

Once data was obtained, a filtering to avoid outliers that could have

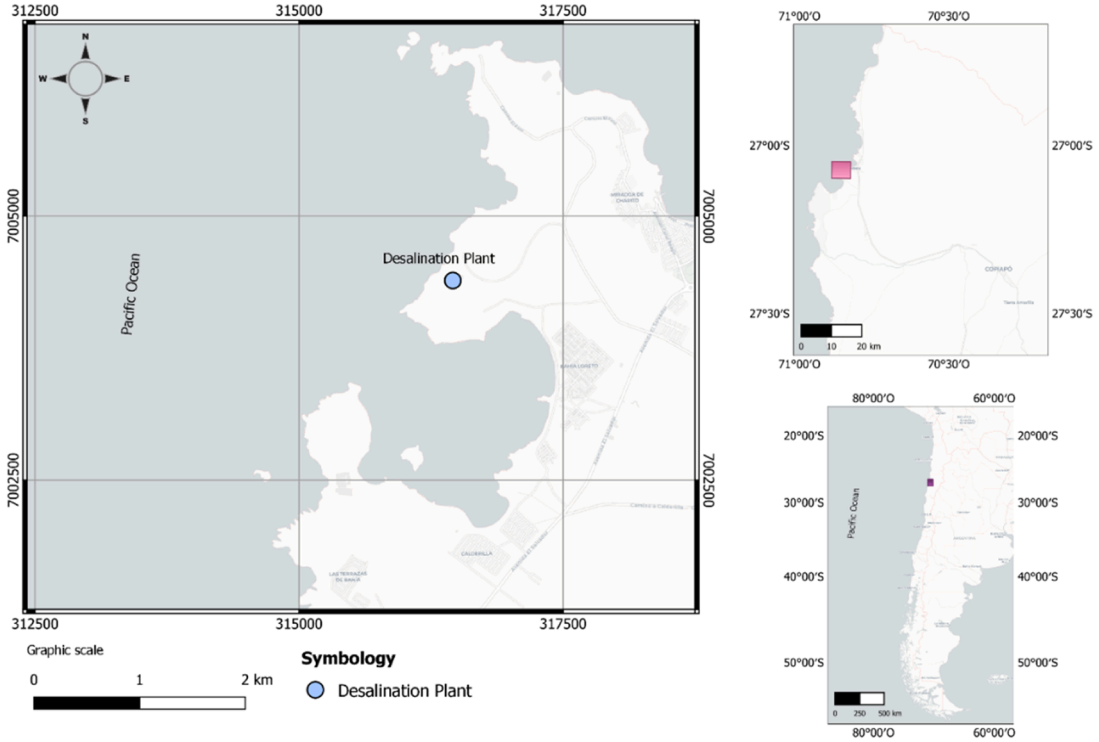

Fig. 1. Map of the study area included in the Punta Zorro sector where the Nueva Atacama desalination plant is located, in the Atacama Region.

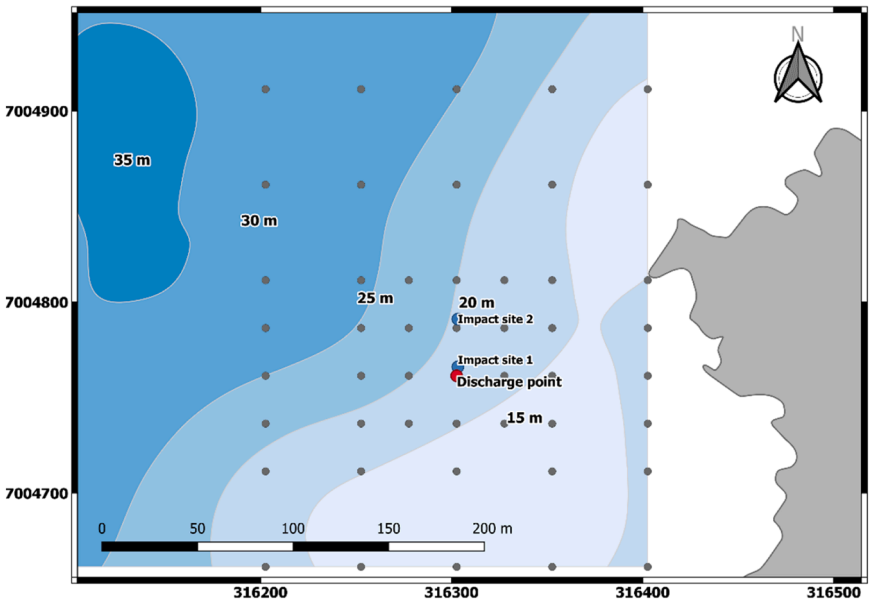

Fig. 2. Spatial representation of bathymetry and sampling stations employed to study the dispersion area of the brine discharge from the Nueva Atacama desalination plant.

been influenced by various factors, such as the presence of suspended material. Also, these values were particularly focused on the last meter of depth corresponding to the seabed, since the saline plume, being denser than seawater, is concentrated in the deepest layer [14,40]. In addition, to calculate the percentage increase of salinity with respect to natural salinity in the defined sampling stations, a salinity control point located more than 2 km outside the bay was used, which presented an average salinity of 34.4 psu. The absolute difference between the salinity measured at each sampling station and the salinity at the control point, was used to calculate the increase in salinity caused by the brine discharge in the study area. Subsequently, this difference was presented as a percentage rate to estimate the area with a greater increase of 1 %.

2.5. Field transplantations experimental design

R. corallina samples were collected by scuba divers at 12 m in Caldera Bay, Caldera, Atacama region, Chile (-27.04716, −70.81595), placed in plastic flasks with seawater and acclimated with natural light and continuous air flow for 24 h. After acclimation 100 g of *R. corallina* fronds were distributed inside the transplantation devices. Transplant devices consisted in transparent plastic boxes (17 ×15 x 6 cm), subdivided in four internal spaces (corresponding to 4 biological replicates) with 250 perforations to allow water flux freely (Fig. 3) which was based in a previously prototype [31]. Transplants were located at two sites nearby the Nueva Atacama desalination brine discharge pipe. First impact site (I1) and second impact site (I2) were located 6 m and 30 m perpendicular the brine diffuser at 12 m depth, respectively (Fig. 2). The control site was located in the same place where samples were collected within Caldera bay. Transplants devices were tied to 15 kg concrete anchors and installed by scuba divers at the different sites and collected after 2- and 5-days post installation against brine exposure. Salinity at the different transplantation sites were measured with the CTD sensor. Salinities averages obtained were 34.4, 35.5 and 35 psu for control site, I1 and I2 respectively. Upon collection, all samples were washed with distilled water, immediate frozen using liquid nitrogen and transported to the HUB-AMBIENTAL UPLA research center in Valparaíso, Chile, utilizing a dry shipper container. Subsequently, all samples were preserved at −80 °C to facilitate further biochemical analyses.

2.6. Quantification of total reactive oxygen species (ROS) content

ROS content was obtained using the Fluorometric Intracellular ROS

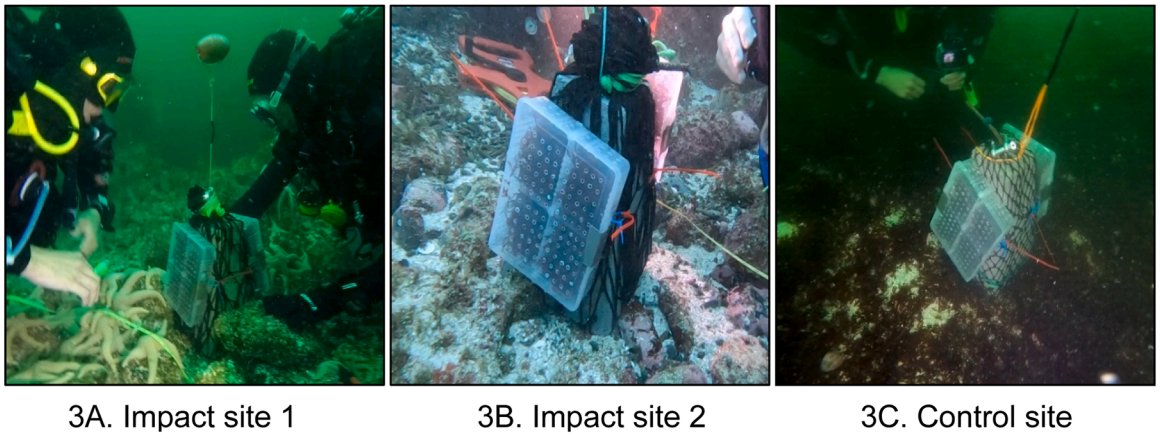

Fig. 3. Field-transplantation devices of *Rhodymenia corallina*. 3 A: Impact site (I1) located 6 m from the brine diffuser point (35.5 psu). 3B: Impact site (I2) located 30 m from the brine diffuser point (35 psu). 3 C: Control site located in the Caldera bay (34.4 psu).

Kit (orange) supplied by Sigma-Aldrich [29]. Briefly, 30 mg of the frozen biomass underwent homogenization into powder using a mortar and liquid nitrogen. Subsequently, this powder was combined with 300 µL of 0.5 M HCl and subjected to centrifugation at 7500 rpm for 5 minutes at 4 °C. Following centrifugation, 100 µL of the resulting supernatant was transferred to a black 96-well microplate with transparent bottom and mixed with 100 µL of 100 mM sodium phosphate buffer (pH 6.8). To this mixture, 0.5 µL of the provided fluorophore was added. The relative fluorescence units (RFU) were then measured at excitation and emission wavelengths of 540 nm and 570 nm, respectively, utilizing a fluorometric spectrophotometer Cytation 5 (Agilent Biotek Instruments).

2.7. Quantification of lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) were used as a proxy to quantify lipid oxidative damage [29,31], with modifications. First, 100 mg of frozen biomass was pulverized into powder using a mortar and liquid nitrogen. This powder was mixed with 600 µL of 20 % trichloroacetic acid (TCA), vortexed for 10 minutes, and then centrifuged at 13,000 xg for 10 minutes at 4 °C. Following centrifugation, 200 µL of the resulting supernatant was combined with 200 µL of 0.5 % thiobarbituric acid (TBA) solution (diluted in 20 % TCA) and incubated for 45 minutes at 80 °C. Subsequently, the mixture underwent an additional 5-minute incubation at room temperature for cooling down the sample. The absorbance of the final mixture was measured at 532 nm utilizing a SPECTROstar Microplate Reader (BMGlabtech). This measurement was performed in a 96-well microplate, with 200 µL of the mixture loaded per plate. A standard curve was constructed using a commercial malondialdehyde standard (Sigma-Aldrich).

2.8. Quantification of carbonylated protein

The quantification of carbonyl groups within proteins followed the procedure outlined by Cubillos et al. [41] and Pérez-Hernández et al. [29], with modifications. First, 50 mg of frozen biomass was homogenized into a fine powder using a mortar and liquid nitrogen. This powder was then mixed with 300 µL of 0.5 M HCl and 300 µL of FARPB buffer (from the Plant RNA extraction kit, Favorgen). The resulting mixture was vortexed for 10 minutes and centrifuged for 15 minutes at 10, 000 rpm and 4 °C. Following centrifugation, 300 µL of the supernatant was incubated with 60 µL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) for 30 minutes in darkness at room temperature. Then, 360 µL of cold 20 % trichloroacetic acid (TCA) was added to the mixture. This was incubated on ice for 15 minutes before undergoing centrifugation at 10,000 xg for 5 minutes at 4 °C to eliminate the supernatant. The resultant pellet was washed with 1 mL of ethanol:ethyl acetate (1:1) solution and centrifuged for 1 hour at 10,000 xg at 4 °C. Following washing, the pellet was air-dried for 20 min at room temperature. The pellet was dissolved in 500 µL of 6 M guanidine-HCl and incubated for 15 minutes at 37 °C, and vortexed every 5 minutes. Finally, 500 µL of supernatant was mixed with 150 µL of 6 M guanidine-HCl, and absorbance was measured at 366 nm using a 96-well UV microplate reader. The concentration of carbonyl groups was determined using the Lambert-Beer equation, utilizing a molar extinction coefficient of 22,000 M-1 cm-1. Results were normalized based on the total protein content, quantified using the Bradford method [42].

2.9. Quantification of reduced (ASC) and oxidized (DHA) ascorbate

The concentrations of ASC and DHA were assessed using the ferric tripyridyl triazine (FRAP reagent) method [31,43]. First, 100 mg of frozen biomass was homogenized into powder using a mortar and liquid nitrogen and lysed with 450 µL of 0.5 M HCl. This mixture was vortexed for 10 minutes and subsequent centrifugation at 15,000 xg for 15 min at 4 °C. For ASC quantification, 50 µL of the supernatant was mixed with 250 µL of FRAP solution containing 250 mM sodium acetate buffer (pH 3.6), 0.83 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ; Sigma-Aldrich), and 1.7 mM FeCl3 in a ratio of 10:1:1. The absorbance of the resulting mixture was immediately measured at 593 nm using a microplate reader. For the determination of total ascorbate (ASC + DHA), 250 µL of the supernatant was incubated with 2.5 µL of 100 mM dithiothreitol (DTT) for 1 h at room temperature, which effectively reduced all DHA to ASC. The reaction was halted by adding 2.5 µL of 5 % N-ethylmaleimide. Subsequently, 50 µL of this solution was mixed with 250 µL of FRAP solution, and the absorbance was measured at 593 nm. The DHA content was obtained by subtracting ASC from the total ascorbate. A standard curve was constructed using commercial L-ascorbate (Sigma-Aldrich).

2.10. Quantification of glutathione (GSH) and glutathione disulfide (GSSG) concentrations

To quantify GSH and GSSG concentrations, the protocol outlined by Sáez et al., [43] and Rodríguez-Rojas et al., [31] was employed with minor adjustments. For determination of the total glutathione pool (GSH + GSSG), 100 mg of liquid nitrogen-grounded samples were lysed using 500 µL of 0.1 M HCl and vortexed for 10 minutes at room temperature. Subsequently, 400 µL of supernatant was collected following centrifugation at 7400 xg for 15 min at 4 °C, and then neutralized with 400 µL of 500 mM sodium phosphate buffer (pH 7.5). Next, 50 µL of the neutralized supernatant was mixed with 250 µL of GR buffer, which comprised 100 mM sodium phosphate buffer (pH 7.5), 0.1 mM EDTA, 0.34 mM NADPH, 0.5 U of glutathione reductase, and 0.2 mM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB or Ellman's reagent). The absorbance at 412 nm was immediately recorded using a microplate reader every 20 seconds for 5 minutes. For measuring GSSG concentrations, 50 µL of neutralized supernatant was incubated with 1 µL of 1 M 4-vinylpiridine for 45 minutes at room temperature, followed by mixing with 250 µL of GR buffer. The GSH content was then calculated by subtracting the GSSG content from the total glutathione. A standard curve was generated using commercial GSH (Sigma-Aldrich).

2.11. Quantification of free amino acids and proline levels

The quantification of free amino acids concentration followed the ninhydrin positive substances (NPS) protocol [29] with modifications. The procedure began by homogenizing 100 mg of frozen biomass into a fine powder using a mortar and liquid nitrogen. Subsequently, the powdered biomass was mixed with 400 µL of 0.1 M HCl, vortexed for 10 min, and then centrifuged at 7000 xg for 15 minutes at 4 °C. After centrifugation, 350 µL of the resulting supernatant was mixed with 350 µL of 100 mM sodium citrate buffer (pH 4.8). Next, 290 µL of the extract was mixed with 10 µL of 2 % ninhydrin solution, followed by incubation for 20 minutes at 95 °C. Following incubation, the mixture was allowed to cool to room temperature over 10 min. Finally, 150 µL of the cooled sample was mixed with 150 µL of 100 mM sodium citrate buffer (pH 4.8). The absorbance of this final mixture was measured using a multiplate spectrophotometer reader at two wavelengths: 560 nm for free amino acids and 403 nm for proline/hydroxyproline content. Calibration curves were generated using a commercial free amino acids solution (Sigma-Aldrich).

2.12. Statistical analysis and spatial representation

Interpolation was carried out using the Kriging statistical method, a reliable and widely used technique to predict values of a variable at unsampled locations, based on a model. The objective of Kriging is to minimize the prediction error by finding an optimal weighing of the neighboring points for the estimation at each location. To do this, it seeks to find a combination of the neighboring values that best fits the spatial correlation structure, and, therefore, results in a smaller discrepancy between the estimates and the actual values. To perform the spatial interpolation analysis of the collected data, the ArcGIS Pro

software was used [18].

Regarding the results submission, the predictions obtained by Kriging were represented in contour maps form. These maps obtained show the spatial distribution of the study variables of interest (salinity, temperature, depth and DO). In addition, transects in 3D format were made for the salinity, temperature and DO variables in order to represent the dispersion pattern of the saline plume based on depth throughout the study area. For this purpose, the DIVA (Data-Interpolating Variational Analysis) method was employed in the open access program Ocean Data View (ODV).

For oxidative and osmotic stress biomarkers results, the normality was assessed using the Shapiro-Wilk test, while homoscedasticity was assessed using Bartlett's test. Following confirmation of normality and homoscedasticity, a one-way ANOVA was executed to identify significant differences. Subsequently, the Tukey's Test was conducted as a post-hoc test for multiple comparisons. A significance level of $\alpha = 0.05$ (p-value) was employed for all statistical analyses. The computations were carried out using R console version 4.2.1 in RStudio version 2023.12.0. Four biological replicates were considered for all transplant experiments and biochemical assays, while three replicates were utilized for chronically exposed samples.

3. Results

3.1. Physico-chemical dispersion pattern of the saline plume

The results showed that the discharge's saline plume presents a dispersion pattern with northwestward direction, following the bathymetric line of the seabed (Figs. 4A and 4B). The average salinity values obtained during the CTD campaign ranged from 34.1 to 35.2 psu. The maximum % increase in salinity with average values was 2.4 % compared to natural salinity, which was located at a distance of 25 m to the southwest with respect to the discharge point.

Regarding the maximum salinity values, they ranged between 34.1 and 35.5 psu. The maximum salinity value was identified at the point closest to the outfall, at 25 m from the discharge point, representing an increase of 3.34 % with respect to natural salinity (Fig. 4B).

Regarding the density values, it was observed that the density dispersion has a similar pattern to that of the salinity plume, showing a dispersion with northwestward direction, following the maximum slope line (Fig. 5). The density values ranged from 1025.6 to 1026.5 kg/m^3, with the highest density point being identified at 25 m, and the lowest density point at 55 m away from the discharge point. The maximum values ranged between 1025.7 and 1026.7 kg/m^3, similarly to the average values.

Fig. 6 represents the temperature map made with average temperature values. In the map, it is evident that the highest temperatures are located at shallow depths, while the lowest temperatures are located at greater depths. The values recorded ranged between 13.1°C and 14.75°C. The maximum average temperature value was observed at 180 m northeast from the discharge point, located closer to the coast and at a depth of 9 m. The minimum average temperature value was found at a distance of 223 m northwest from the discharge point, at a depth of 29.5 m. Regarding the maximum temperature values, values similar to those found with average values were observed.

Regarding the DO distribution, the results showed that there is no relationship with the saline plume dispersion, and it was observed that DO concentrations have a direct relationship with depth (Fig. 7). The results showed that with the increase in depth, a decrease in DO was observed. The average DO values ranged from 3.2 mg/L to 6.7 mg/L. Regarding the maximum DO values recorded, these ranged between 3.5 mg/L and 6.8 mg/L. The point with the maximum DO concentration was found at a distance of 180 m northeast from the discharge point at a depth of 9.3 m, while the minimum concentration recorded is 3.2 mg/L and is located 224 m northwest from the discharge point at a depth of 29.5 m.

Fig. 8 presents the results of transects of salinity, temperature and DO dispersion according to the water column. The results obtained with salinity indicate that the saline plume is dispersed mainly from the outfall point following the bathymetric line up to 20 and 30 m of depth, with complete dilution occurring at approximately 250 m from the discharge point in the seabed. The temperature distribution indicated the formation of an isotherm at the first 5 m of the surface, with a temperature of approximately 14.75 – 14 °C, and a subsequent decrease in temperature with depth, reaching approximately 13.1 °C at the seabed. Regarding DO distribution, a similar pattern to that observed for temperature was observed. A surface layer exhibiting elevated DO concentrations was observed, with a subsequent decline in DO content with increasing depth, reaching a minimum at a depth of 25 m.

3.2. Cellular diagnosis after brine exposure in R. corallina

To perform a diagnosis on the cellular stress upon brine exposure on *R. corallina*, we used stress biomarkers that included the quantification of reactive oxygen species (ROS) accumulation, thiobarbituric acid reactive substances (TBARS) and protein carbonylation as a marker for lipid peroxidation and protein oxidation, respectively. After 2 days of brine exposure *R. corallina* transplants displayed a significative accumulation on the total ROS content, compared to control site, in the nearest impact site solely (Fig. 9A). Whereas after 5 days, levels of total

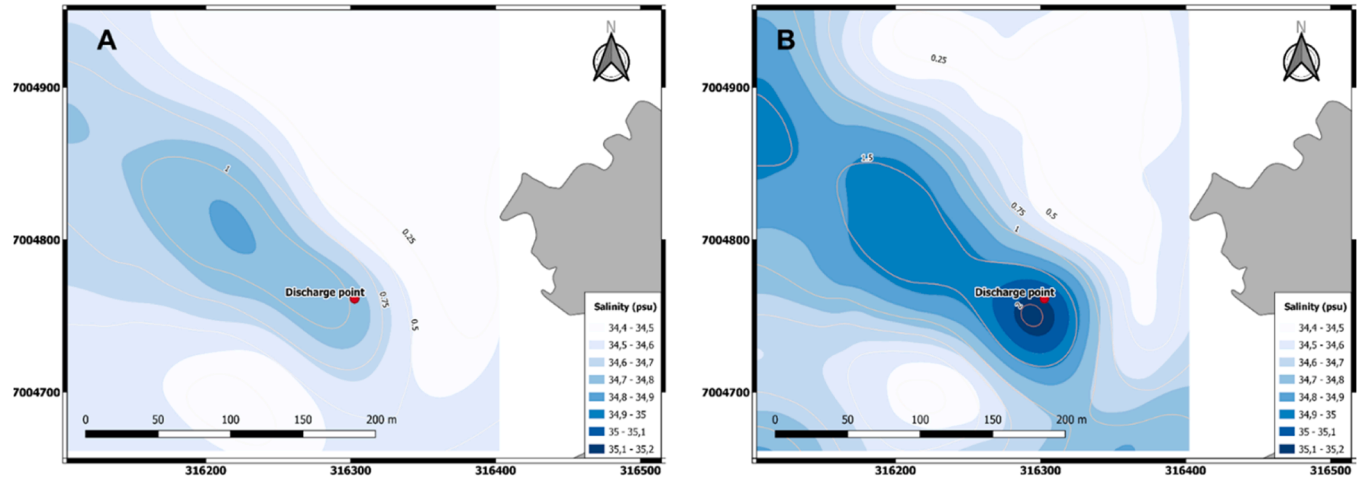

Fig. 4. Spatial representation of the saline plume in the seabed around the Nueva Atacama outfall area performed with average salinity values (4 A) and maximum salinity values (4B), apart from including the salinity percentage increases.

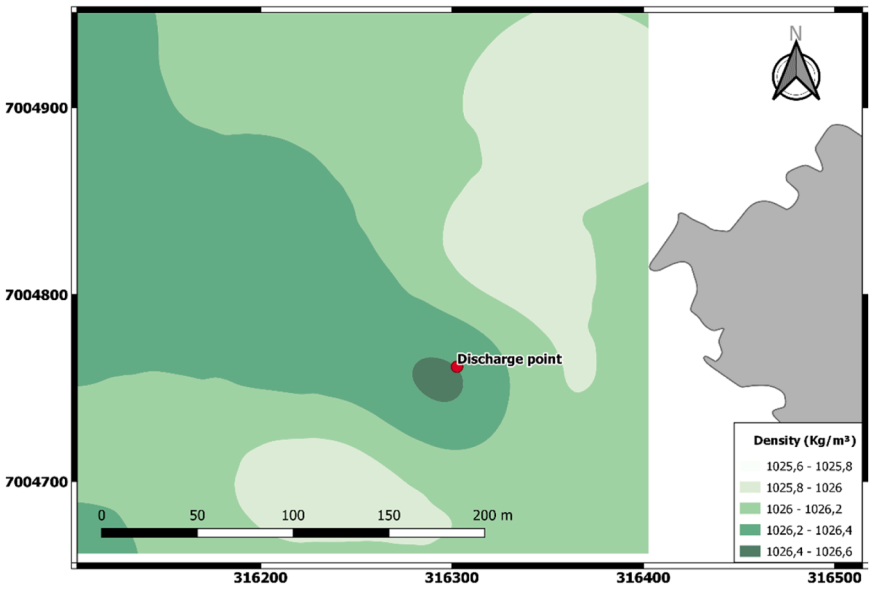

Fig. 5. Spatial representation of the saline plume in the seabed around the Nueva Atacama outfall area performed with maximum density values.

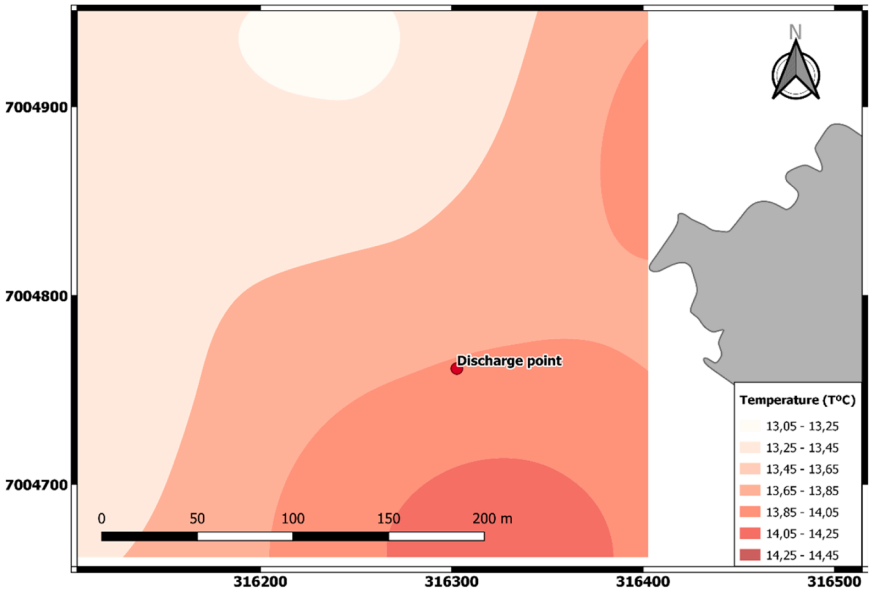

Fig. 6. Distribution map of temperature in the seabed near the Nueva Atacama outfall based on average values.

ROS in brine-impacted sites were similar to those of the control transplants. Regarding oxidative damage biomarkers, lipid peroxidation remained stable across all samples, while a slight but significative increase in carbonylated proteins was observed after 2 days of brine exposure (Figs. 9B and 9C). After 5 days, however, both biomarkers showed a significative decrease in transplants samples at brine-impacted site 1 (~30 %), suggesting a reduction in oxidative damage compared to control values (Figs. 9B and 9C).

To investigate the defensive responses of *R. corallina* against brine exposure, we evaluated its antioxidant and osmotic responses by measuring total ascorbate and glutathione content (antioxidants), along with the quantification of free amino acids and proline (osmolytes). Ascorbate accumulation showed no significant differences between transplants after 2 days of brine exposure and remained predominantly in its reduced form (~90 %), regardless of transplant site. However, after 5 days, a ~16 % increase was observed exclusively in transplants from impacted site 2 (Fig. 10A). In contrast, glutathione levels sharply decreased after 2 days of brine exposure at both impacted sites, particularly at impacted site 1 (~80 %) (Fig. 10B). Despite this, a rapid recovery in glutathione levels occurred after 5 days of brine exposure, with total glutathione at impacted site 1 reaching ~50 % higher than in control transplants, while remaining comparable to control levels at impacted site 2. Reduced glutathione (GSH) levels varied across all transplant samples but stayed within a range of 40–70 % (Fig. 10B). Regarding the osmotic response, free amino acids (NPS) and proline exhibited a similar pattern, with both showing a significant decrease (~30 %) in transplant samples at both impacted sites after 2 days, compared to control samples (Figs. 10C and 10D). However, by day 5, no significant differences in these osmotic parameters were observed when compared to control values.

4. Discussion

Seawater desalination is presented as an alternative to counteract the

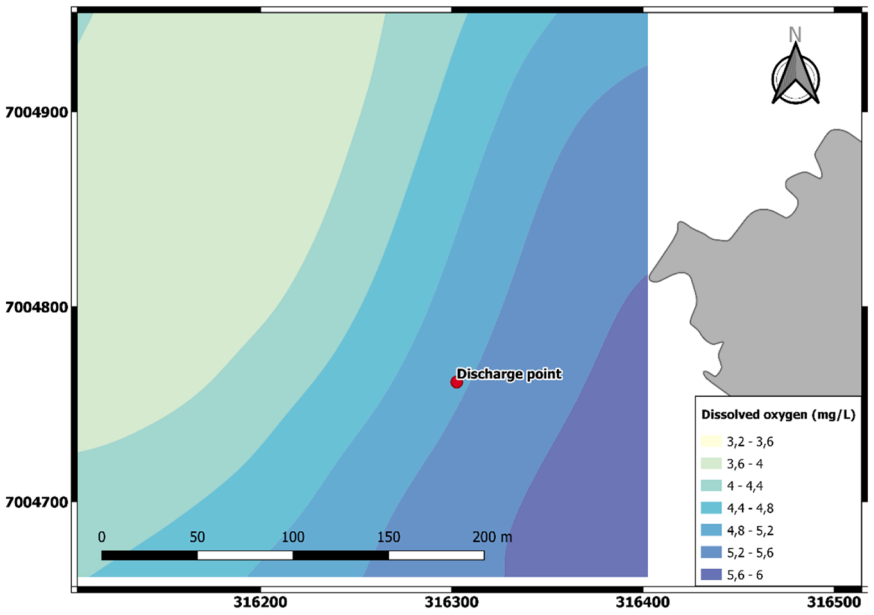

Fig. 7. Distribution map of dissolved oxygen in the seabed near the Nueva Atacama outfall based on average values.

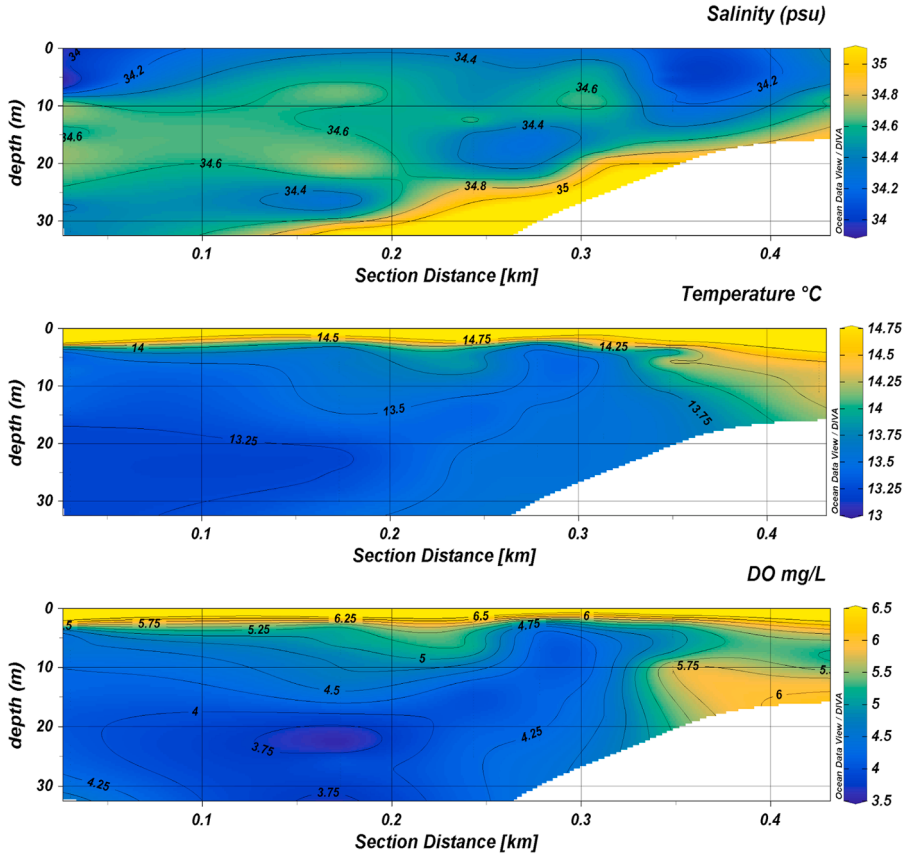

Fig. 8. Spatial distribution of oceanographic parameters in the study area (salinity, temperature and DO).

reduction of water availability due to climate change, thus promoting social and economic sustainability [44]. In the South American Pacific coast, a significant number of projects are expected to be implemented in the coming decades in countries such as Chile or Perú, raising socio-environmental concerns due to the potential impacts of brine discharges on marine ecosystems [45,46]. The uncertainty regarding these impacts highlights the necessity for specific scientific research for the coastal ecosystems, given that the majority of studies have focused on other regions, such as the Mediterranean, which have markedly different oceanographic and ecosystemic conditions, that are not extrapolatable to the characteristics of the South American Pacific coast [18]. The development of this study assessing the physico-chemical impact of the brine discharges, is crucial to understanding and mitigating the potential impact of brine discharges to the expected

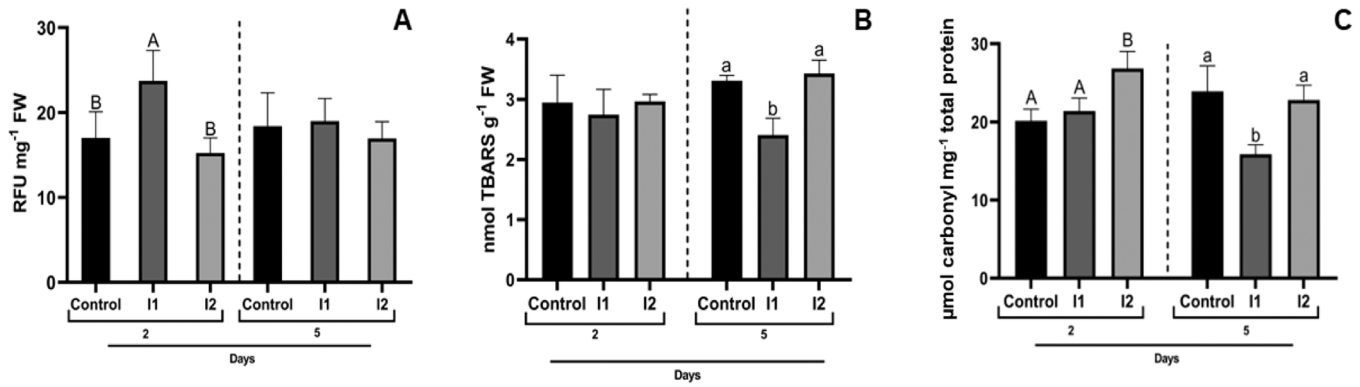

Fig. 9. Oxidative stress response and damage in transplants of *R. corallina* exposed to desalination brine. A) Effect of brine exposure on total ROS content. B) Effect of brine exposure on lipid peroxidation. C) Effect of brine exposure on protein carbonylation. The data is presented as mean ± SD, with $n = 4$ and a significance level of $p < 0.05$.

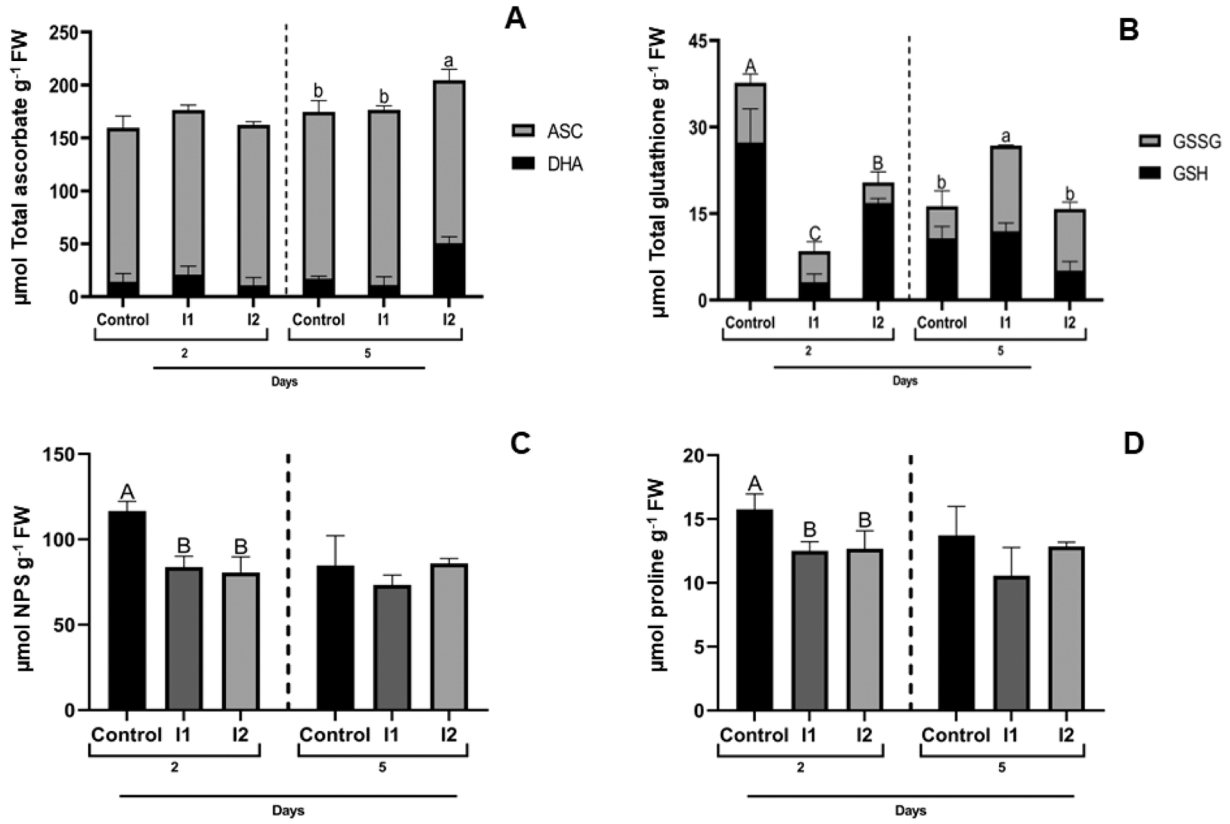

Fig. 10. Antioxidant and osmotic stress responses in *R. corallina* exposed to desalination brine. A) total ascorbate and B, total glutathione, as antioxidant biomarkers. C) free amino acids and D, proline as osmolytes biomarkers. The data is presented as mean ± SD, with $n = 4$ and a significance level of $p < 0.05$.

development of desalination [1,47].

The results demonstrated that the salinity increase exceeding 1.5 % with respect to the natural salinity of the sea (34.4 psu), was significantly reduced by approximately 150 m from the discharge point, employing the maximum values, and reduced to less than 50 m when the average values were used. Furthermore, the maximum salinity increase identified was 3.34 % at a distance of 25 m from the discharge point. This is attributed to the limited production capacity of the desalination plant (300 L/s), the favourable hydrodynamic conditions that facilitate the mixing of the effluent with the receiving marine environment and the efficient design of the diffusers employed in the rejection system [14, 48]. In comparison to studies conducted in other regions globally, the rapid dilution of the discharge from the Nueva Atacama SWRO plant is noteworthy, as salinity increases from 1.5 % to 15 % have been reported in other SWROs. For instance, in Ashkelon (Israel), a maximum salinity excess of 10 % was observed at a distance of 3 km from the outfall, while in Palmachim (Israel), an increase of 15 % and a dispersion of the saline plume of up to 1 km was recorded [17]. The aforementioned dilution data corroborate the finding of Sola et al. [18], which assessed three desalination plants in northern Chile with different production characteristics. The study identified that in the studied desalination plants, 5 % of the natural salinity was not exceeded within a radius of less than 100 m from the discharge point. Moreover, similar results were identified in the Candelaria's desalination plant, also located in Caldera, which has a similar production capacity (43,200 m^3/day), finding maximum salinity increase of 3.34 % in the first 20 m from the

discharge point, and a 1 % salinity increment was identified compared to the natural values (34.4 psu) in a radius below 50 m from the discharge point [18].

Likewise, the results showed that density distribution followed a direct pattern with the dispersion of the saline plume, with predominant northwestward direction and reaching the maximum density at the point of maximum salinity. This is because a salinity increase generates a direct density increase, therefore, the density distribution responds to the influence of brine discharge [13,40]. In addition, the results showed that the brine discharge did not cause significant changes in DO concentrations in the water column of the affected area, with a uniform decrease in concentration at higher depths. The results obtained coincide with literature, showing an uniform decrease in DO concentration towards deeper strata during the summer season in Caldera Bay, reaching values of 3.97 ± 0.07 mL L-1 (5.68 mg/L) at 20 m depth [49]. Regarding water temperature, it was observed that the discharge did not generate changes in the temperature values of the water column either, remaining in a range of 13–14.5°C, similar to natural conditions. This is because the reverse osmosis process does not generate a substantial heat exchange, so the sea temperature remains practically constant. The results show a similar trend to temperature decrease with depth, in line with what has been reported in other regions of the world, where it has been observed that brine discharges do not affect temperature and/or DO concentrations in marine ecosystems [12,50]. When any alteration in temperature values has been observed, it has been attributed to desalination plants employing thermal technology, and not to the reverse osmosis process [51].

In this study we investigated the biological effects of Nueva Atacama brine discharge on a non-model red macroalgae species, *R. corallina*, which inhabits within the subtidal zone of the coast of the Atacama region, near several desalination plants outfalls. As previously stated, brine impacts are both ecosystem- and species-specific, and should be carefully attended considering several physicochemical, oceanographic and other environmental factors. Stress biomarkers, as rapid short-term health status indicators, were measured to diagnose this local macroalgae cellular performance against potential osmotic and oxidative stress in transplanted individuals within the brine influence area. In this experimental approach, we selected a comprehensive set of biomarkers including general stress parameters, such as oxidative stress and damage, antioxidant-related molecules, and osmotic stress biomarkers. By combining these biomarkers, we aimed to provide a more accurate and holistic diagnosis of the physiological consequences on *R. corallina* tissue under hypersaline conditions. The overall cellular response of *R. corallina* to brine exposure suggests that its defensive mechanisms are sufficient to counteract the mild oxidative stress caused by brine in the short-term (i.e., after 2 days of exposure) at the nearest impact site (3.5 % above natural salinity), effectively protecting the cellular environment from oxidative damage and osmotic shock, as full acclimation after 5 days of brine exposure was evidenced. The use of in situ transplant experiments as a biomonitoring approach to assess brine impacts through stress biomarkers, has proven to be a novel biotechnological tool for exploring short-term detrimental effects on ecologically relevant benthic bioindicators. In this context, the cellular sensitivity of macroalgae species can vary based on their evolutionary genetic traits. For instance, transplants of the model brown macroalgae Ectocarpus spp. located at 10 m (7 % above natural salinity) and 30 m (4.4 % above natural salinity) from the brine discharge pipe of Aguas Antofagasta desalination plant, exhibited impaired photosynthetic performance, ROS overproduction and ascorbate accumulation after 7 days [31]. Thus, indicating persistent oxidative stress during a middle-term biomonitoring and potentially leading to a chronic cellular stress condition. Conversely, the local subtidal macroalgae species D. kunthii from Antofagasta bay, experienced brief oxidative stress condition followed by cellular redox state recovery during the same Ectocarpus spp. transplant experiment at the brine discharge zone of Aguas Antofagasta [30]. These findings highlight the importance of carefully establishing species-specific salinity thresholds, particularly for local benthic primary producers, in order mitigate brine impacts across other trophic levels.

In the regulatory context of Chile, no guidelines have been established that regulate the maximum salinity levels in the areas affected by brine discharges. This highlights the need to establish a specific regulation to properly prevent the potential environmental impact of future desalination projects [38]. Consequently, it is imperative to enhance the EMPs in Chile, incorporating descriptors that guarantee the appropriate monitoring of brine discharges into the marine environment in the absence of such monitoring, as well as the removal of descriptors that are not linked to the proper monitoring of the effects of brine discharges. In particular, desalination plants are currently required to comply with the Supreme Decree N°90 regulation (Chilean environmental law that regulates pollutant discharges into marine water courses). However, this regulation does not specify maximum salinity limits and includes irrelevant requirements, that rather respond to other anthropogenic impacts (e.g., wastewater). It is therefore imperative to improve the PVAs to enable the proper monitoring of brine discharges and the removal of irrelevant descriptors that generate additional economic costs without ensuring a greater protection of marine ecosystems [38]. Recent field combined with laboratory research has begun to demonstrate the responses of some benthic organisms from the Chilean coast to brine exposure, thereby highlighting the necessity for the development of specific biomonitoring tools to assess the potential negative impact on marine ecosystems [29,31].

Consequently, international regulations, such as the Australian, are occasionally employed during the development of environmental assessment processes [52]. In order to assess the extent of the impact of brine discharges, such guidelines suggest that, in the case of estuarine and coastal waters, it is recommended that changes in salinity of the receiving marine environment be kept below 5 % with respect to the natural salinity of the sea, or not exceed 1 psu maximum with respect to natural salinity, at a maximum distance of 100 m from the discharge point. In this study, the salinity increase levels were less than 3.5 % in the entire dispersion area of the saline plume, which would indicate that the project would comply with the Australian guidelines to properly address the environmental impact of brine discharges on coastal ecosystems. The results of this study are consistent with those obtained in Sola et al., [14], where other desalination plants in northern Chile that also complied with the Australian guidelines were assessed.

In summary, based on the results obtained and the scientific literature reviewed, it is crucial to adopt scientifically supported strategies, such as the use of diffusers and the correct location of the discharge point, to favor a rapid mixing process and thus reduce the extension area of the influence of brine discharges [34,53]. The results of this study will serve as a reference for future research where desalination is in the early stages of development and is facing similar challenges. However, further studies of a scientific nature are needed to assess the potential areas affected by other desalination plants with different production characteristics and different environmental oceanographic conditions in order to ensure a sustainable development desalination [44].

5. Conclusions

The results of this study indicate that the saline plumes from the SWRO plants exhibited a dispersion pattern following the bathymetric line of the seabed. Our results showed that saline plume exhibited a notable dilution at approximately 200 m, reaching a salinity below 4 % in less than 50 m from the discharge point. Additionally, it was observed that the density dispersion pattern of saline plumes matched the salinity dispersion pattern, as an increase in salinity resulted in an increase in the density of the saline plume. Conversely, it was observed that the brine discharges not result in significant alterations to the temperature and DO values within the water column of the receiving marine environment. Regarding biological effects, transplants of the local biomonitor

macroalgae *R. corallina* did not showed any detrimental effects on its cellular metabolisms after 5 days of brine exposure.

Our findings suggest that the use of diffusers, combined with an appropriate oceanographic location of the submarine outfall, would facilitate the comply with the environmental standards/regulations applied internationally, without producing significant environmental impacts on South American pacific coast ecosystems. Further, in order to accomplish a sustainable operation with marine environment, it is important to conduct rigorous studies to ascertain the most appropriate location for the brine discharges from SWRO plants, considering the natural dilution of discharges with the marine environment. Based on the results obtained, this will ensure that potential environmental impacts of brine discharges are adequately minimized on marine ecosystems.

Environmental implication

This study evaluates the environmental impacts of brine discharges from desalination plants on South America's Pacific coastal ecosystems, focusing on physico-chemical changes (salinity, density, temperature, dissolved oxygen) and stress biomarkers in *Rhodymenia corallina*. Brine discharge increased salinity and density locally, causing short-term oxidative and osmotic stress in *R. corallina* near discharge location. However, a full recovery and cessation of the redox imbalance was observed after five days. These results highlight the need for strategic outfall placement and effective mitigation measures to minimize environmental risks. The findings offer valuable insights for global sustainable desalination practices.

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CRediT authorship contribution statement

Pérez-Hernández Gabriela: Writing – review & editing, Conceptualization. **Silva-García Roderick:** Formal analysis, Data curation. **Blanco-Murillo Fabio:** Writing – review & editing, Conceptualization. **Pereira-Rojas Jeniffer:** Writing – review & editing, Conceptualization. **Santana-Anticoy Constanza:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Sola Iván:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Rodríguez-Rojas Fernanda:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Sáez Claudio:** Writing – review & editing, Conceptualization. **Díaz María José:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability

Data will be made available on request.

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