Aquaculture 543 (2021) 736971

Contents lists available at ScienceDirect

Aquaculture

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

Sarcocornia neei: A novel halophyte species for bioremediation of marine aquaculture wastewater and production diversification in integrated systems

Clemens Pascal Beyer[a,b,1], Silvia Gómez[a,*,1], Gabriele Lara[a], Juan Pablo Monsalve[a], Jaime Orellana[c], Carlos Felipe Hurtado[a]

[a] *Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*
[b] *Escuela de Agronomía, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*
[c] *Erwin Sander Elektroapparatebau GmbH, 31311 Uetze-Eltze, Germany*

ARTICLE INFO

Keywords:
Integrated multi-trophic aquaculture (IMTA)
Aquaponics
Marine recirculating aquaculture system
Nitrate
Sustainable aquaculture

ABSTRACT

The integration of bioremediation systems is one of the most promising techniques to mitigate the environmental impact of aquaculture effluents. Also, it allows nutrient recycling, production diversification, and the creation of high-value by-products. In marine aquaculture, where the implementation of salt-tolerant extractive species is essential, halophyte plants have demonstrated to be optimal candidates for bioremediation of saline aquaculture wastewater.

This study aimed to evaluate for the first time the efficiency of *Sarcocornia neei* (Lag.), a halophyte plant with high adaptability, salinity tolerance and growth potential when irrigated with seawater, in removing nutrients from marine fish aquaculture wastewater and artificial effluents. Two experiments were carried out. In the first one, the growth rate, removal of inorganic nutrients, and accumulation of organic compounds in deep-water hydroponics and sand-substrate systems were evaluated in artificial effluents with different nitrogen and phosphate loads during 70 days. In the second, due to the better performance achieved by *S. neei* in deep-water systems, its nitrogen removal efficiency and productivity rates were evaluated in deep-water aquaponics with marine aquaculture wastewater and artificial effluents at higher nitrogen loads during 61 days.

The highest productivity rates achieved by *S. neei* (14.41 ± 0.78 kg m^{-2}) were obtained in deep-water culture units, reaching 100% plant survival, suggesting that this species is more suitable for its implementation in this type of system. Significant total ammonia nitrogen removal rates were obtained by the plants, achieving a maximum in sand-substrate systems (0.68 ± 0.41 g m^{-2} day^{-1}). The results of nitrate removal rates obtained by *S. neei* (11.25 ± 31.38 g m^{-2} day^{-1}) make this species an ideal potential candidate for the removal of this compound present in marine RAS effluents. Accumulation of organic compounds was corroborated by obtaining a significant increase ($p < 0.05$) in organic N (31.2 ± 0.1 mg g dry $weight^{-1}$) and organic P (4.0 ± 0.6 mg g dry $weight^{-1}$) content in plant biomass at the end of the trials.

These results indicated that *S. neei* is a good candidate for its use as a biofilter for marine aquaculture wastewater. Further investigations should be done to analyze possible influences on growth rates and nutrient removal efficiency by adding essential micronutrients, adjusting effluent salinity, and implementing different plant densities. Also, further studies could be interesting to determine the feasibility of long-term integration of a bioremediation system with *S. neei* associated with marine aquaculture effluents, approaching its application to industrial-scale production systems.

---

* Corresponding author at: Avda. Altamirano 1480, Valparaíso, Chile.
E-mail addresses: clemenspbeyer@gmail.com (C.P. Beyer), silvia.gomez@pucv.cl (S. Gómez), gabriele.rodrigues@pucv.cl (G. Lara), juan.monsalve@pucv.cl (J.P. Monsalve), jaime.orellana@aqua-sander.de (J. Orellana), felipe.hurtado@pucv.cl (C.F. Hurtado).

[1] Both authors have equally contributed to count as first author.

https://doi.org/10.1016/j.aquaculture.2021.736971
Received 2 December 2020; Received in revised form 23 May 2021; Accepted 24 May 2021

1. Introduction

With over 110 million tons produced in 2018, aquaculture has become the most developing food industry in recent decades, surpassing fishing in 2016 (FAO, 2018). The reduction of wild fisheries, the rising human population and its demand for seafood are the main causes of this huge expansion of aquaculture (Ottinger et al., 2016). To ensure the sustainable development of this industry, many production systems and techniques considering the right balance between the environmental, social and economic spheres have been improved in recent years (Henares et al., 2020).

Recirculating aquaculture systems (RAS) are high-tech systems based on the reuse and treatment of water, through the application of mechanical, chemical and biological processes. They are closed or semi-closed systems on land that reduce water consumption and the release of nutrients and pollutants into the environment (Zhang et al., 2011; Orellana et al., 2014). It is estimated that by 2030, more than 40% of the world's aquaculture production will be generated in RAS, which will diversify and intensify the aquaculture industry (del Campo et al., 2010; Badiola et al., 2012). Due to the higher control of their effluents compared to open water systems, RAS have become powerful candidates to combine with integrated systems (Martins et al., 2010; Li et al., 2019; Pedersen and Wik, 2020).

Integrated production systems are one of the most promising techniques to increase the long-term sustainability and profitability of aquaculture (Biswas et al., 2020). The concept of integrated multi-trophic aquaculture systems (IMTA) includes the co-cultivation of species from different trophic levels, where the nutrients discarded by the main fed species are removed *in situ* by other organisms (extractive species) (Buck et al., 2018). Invertebrates as sea cucumbers, sea urchins, molluscs and polychaetes are used in IMTA for the removal of organic compounds (MacDonald et al., 2011; Nelson et al., 2012; Montalto et al., 2017; Gómez et al., 2019; Stabili et al., 2019; Omont et al., 2020). Aquatic plants, macroalgae and microalgae are implemented for bioremediation of inorganic components (Skriptsova and Miroshnikova, 2011; Samocha et al., 2015; Milhazes-Cunha and Otero, 2017; Li et al., 2019; Neori et al., 2019; Sari and Adharini, 2020).

In marine aquaculture effluents, the integration of salt-tolerant extractive species is mandatory (Webb et al., 2012; Buhmann et al., 2015). Halophyte plants, tolerant to at least 200 mM NaCl (11.7 g l^{-1}), have demonstrated to be optimal candidates for bioremediation of saline aquaculture effluents (Custódio et al., 2017). The efficiency of halophytes as biofilters has been proven in aquaponics and sand-substrate systems, obtaining significant removals of the main nutrients contained in aquaculture wastewater, as ammonium (NH_4^+-N), nitrate (NO_3^--N), nitrite (NO_2^--N), phosphate (PO_4^{-3}-P), total suspended solids (TSS), total Kjeldahl nitrogen (TKN) and total phosphorus (TP) (Calheiros et al., 2012; Webb et al., 2012; Buhmann and Papenbrock, 2013; Buhmann et al., 2015; Quintã et al., 2015; Waller et al., 2015; Custódio et al., 2017; Marques et al., 2017; Pinheiro et al., 2020). Furthermore, its implementation in aquaculture activities as integrated systems allows the production of valuable by-products, biomass for human consumption and contributes to the preservation of freshwater, promoting marine agriculture (Ventura et al., 2011; Buhmann et al., 2015; Oliveira et al., 2020).

Aster tripolium L., and the genera *Salicornia* and *Sarcocornia spp* (seawort, glasswort, sea asparagus) are the most promising halophytes used in the bioremediation of marine aquaculture effluents (Custódio et al., 2017). However, knowledge is still limited and more investigation is needed to determine their optimal culture parameters, micronutrient needs, efficiency of nutrient absorption and their valorization to produce valuable components under the concept of sustainable integrated systems (Alonso et al., 2017; Doncato and Costa, 2020; Maciel et al., 2020).

Sarcocornia spp (family *Chenopodiaceae*, subfamily *Salicornioideae*) are halophyte plants with an extreme salt tolerance growing in coastal, estuarine and salty desert ecosystems (Ventura et al., 2011; Alonso et al., 2017). Previous studies revealed that *Sarcocornia fruticosa* can purify high-salinity industrial wastewater (Calheiros et al., 2012; Orlofsky et al., 2020) and *Sarcocornia ambigua* can be an efficient biofilter absorbing nutrients from marine aquaculture effluents (Pinheiro et al., 2017; Doncato and Costa, 2018; Pinheiro et al., 2020). Also, *Sarcocornia fruticosa* has been defined as producing lipids and omega-3 when grown with irrigation of seawater, giving additional value to its coupling in integrated aquaculture systems (Ventura et al., 2011).

Sarcocornia neei (Lag.), recently merged with *Salicornia neei*, is a perennial plant distributed along the Pacific coast of South America and in Argentina (Alonso and Crespo, 2008; Piirainen et al., 2017). This species has been catalogued as a strong candidate for marine agriculture in desert zones (Alonso et al., 2017). Also, due to its nutritional characteristics, *S. neei* has a high potential for its use in human and animal consumption (Arce et al., 2016; Riquelme et al., 2016). The adaptability of this species to different environmental conditions and its high growth rates when irrigated with seawater make it an optimal candidate for connecting to marine aquaculture wastewater remediation systems (Arce et al., 2016; Aquilino et al., 2020).

The objective of this study was to evaluate for the first time the efficiency of *S. neei* in removing nutrients from marine fish aquaculture wastewater and artificial effluents simulating different nitrogen loads. Plant productivity and growth rates, nitrate, ammonia and phosphate system removals and uptake efficiency as organic biomass by the plants were analyzed in deep-water and sand-substrate culture systems.

2. Materials and methods

Two different experiments were carried out. In the first one, the removal of inorganic components by *S. neei* and the accumulation in the plants were evaluated in deep-water hydroponics against sand-substrate systems in artificial effluents with different nitrogen and phosphate loads. In the second one, and according to the best results obtained by the halophyte plant in deep-water hydroponics systems, its nitrogen removal efficiency was evaluated in deep-water aquaponics with marine aquaculture wastewater and artificial effluents with higher nitrogen loads.

2.1. Experiment 1: Removal efficiency of inorganic nitrogen and phosphate compounds and accumulation of organic compounds in S. neei in deep-water vs. sand-substrate systems with different nutrient levels.

2.1.1. System design and management

The experiment consisted of evaluating four different treatments in the cultivation of *S. neei* with artificial effluents. Two of them with the implementation of sand substrate (SL: sand low nutrients; SH: sand high nutrients) and two other treatments in deep-water hydroponics (WL: water low nutrients; WH: water high nutrients). In this first trial (70 days), the accumulation of C, N and P in *S. neei,* the growth rate of the plants and the removal of inorganic nitrogen (NH_4^+-N and NO_3^--N) and phosphate (PO_4^{-3}-P) were studied for each treatment.

The experimental set up consisted of four independent systems operated with seawater. Each treatment had three replicates, in total twelve experimental units were implemented in a 30 m^2 greenhouse located outside the building. Seawater was extracted from the sea by a pumping system (Vogt AM-751), filtered using sand filters (Jacuzzi TP-30) and cartridge filters (Vigaflow 20, 5 and 1 μm) and treated with an ultraviolet system (Red Base 40w UV-C T5).

The details of the sand-substrate and deep-water experimental systems are shown in Fig. 1. The deep-water hydroponics (WL and WH) experimental units (area = 0.3 m^2, useful volume = 120 l) were aerated (HT-500 air pump, Sunsun, China) to maintain oxygen saturation. Air ventilation stones were placed on the bottom of each tank working in a 30 min on/off cycle (Buhmann et al., 2015). A floating raft (expanded polystyrene) was placed on each unit to keep the roots of *S. neei* inside

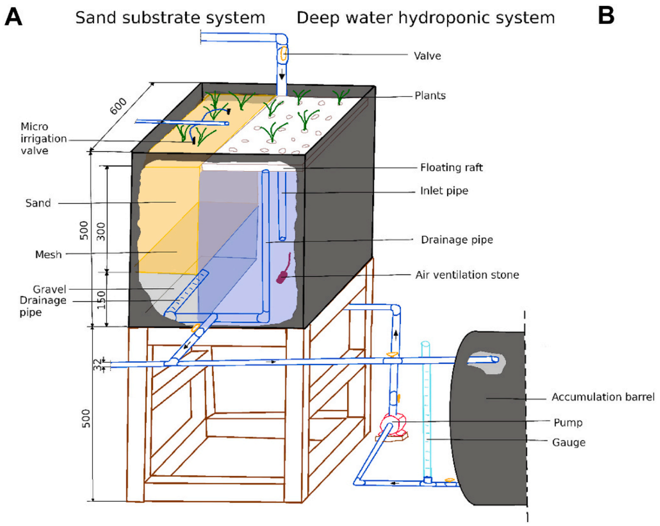

Fig. 1. Detail of system engineering: A) sand-substrate and B) deep-water experimental units used in the experiments. The outflow and inflow tubes to the reservoir go to the other replicates of each treatment that were irrigated by the pump. All dimensions are in mm.

the water (Quintã et al., 2015; Jesus et al., 2017). For water recirculation, a reservoir (190 l) and a pumping system (0.5 HP, TPm-60, Humboldt, China) were implemented in each series. To achieve full water circulation, overflow and inlet drainage pipes (to the bottom of the units) were implemented.

In sand-substrate systems (SL and SH), two horizontal drain pipes were implemented at the bottom of the experimental units, which topsides were perforated for even water drainage (Buhmann et al., 2015; Marques et al., 2017). All the units were filled up with a 15 cm-layer of washed gravel (grain size: 5–30 mm) followed by a 30 cm-layer of washed sand (grain size 0.5–8 mm) (Calheiros et al., 2012; Jesus et al., 2017). The two layers were separated by a perforable fabric of plastic mesh (Webb et al., 2012). Four microdispersion irrigation valves were implemented in each unit for irrigation. The design of the sand-substrate units and the circulation of the water was made according to Buhmann and Papenbrock (2013). In both types of systems, the water was returned by gravity to the reservoir after irrigation.

To simulate typical concentrations of these compounds in marine RAS wastewater, all the treatments were fertilized daily with NH_4Cl, KH_2PO_4 and $NaNO_3$ (Merck, Darmstadt, Germany) (Buhmann et al., 2015; Quintã et al., 2015; Jesus et al., 2017; Aquilino et al., 2020). WL and SL systems (low nutrient) consisted of maintaining the following concentrations of typical RAS effluents in the irrigation water: (NO_3^--N: 50 mg l^{-1}; PO_4^{-3}-P: 6 mg l^{-1}; NH_4^+-N: 0.1–0.2 mg l^{-1}). WH and SH systems (high nutrient) consisted of: (NO_3^--N: 250 mg l^{-1}; PO_4^{-3}-P: 30 mg l^{-1}; NH_4^+-N: 0.5–1 mg l^{-1}) (Orellana et al., 2014; Fuchs et al., 2015; Buhmann et al., 2015). Fertilization was performed daily until the desired concentrations were reached (30 days). Levels were maintained for 40 more days. The fertilized nutrient amounts were calculated through the daily nutrient deficit of the systems. Micronutrients were applied three times a week (Waller et al., 2015). The system removals of nitrate, ammonia and phosphate were calculated through the amount of daily fertilized nutrients in the 40 days.

To compensate water losses due to evapotranspiration and evaporation, tap water was used to refill the units (Webb et al., 2012; Buhmann et al., 2015). A theoretic hydraulic retention time of 60.75 min and a water flow of 2.22 l min^{-1} were implemented to each unit (Pinheiro et al., 2017). Water circulation was performed for one hour eight times a day.

To ensure a 14 h photoperiod of light, an artificial light system with two mercury vapor lamps (H-MET 250 W Granlight, Brazil) was implemented. An intensity of 8.2 ± 3.1 μmol m^{-2} s^{-1} was established to prevent the reproductive stage of plants and support the growth phase (Ventura et al., 2011; Buhmann et al., 2015). The light intensity was measured daily with a radiometer (HD2102.1, Delta Ohm, Italy) at 12:00 pm. The minimum and maximum air temperature and relative humidity were measured daily (44,550 EXTECH sensor, USA).

2.1.2. *Plant material*

S. neei (n = 132) plants were collected in *Salinas de Pullally* (32°24′50.6"S 71°24′33.3"W), Region of Valparaíso, Chile (Fig. 2). Plants (initial wet weight: 7.6 ± 3.4 g) were excavated from their sand soil with a spade without cutting the roots (Jesus et al., 2017). A cleaning with reverse osmosis filtered water was performed to eliminate residual soil upon arrival at the School of Marine Sciences (PUCV, Valparaíso, Chile).

120 plants were implemented in the experimental system (n = 30 per treatment, n = 10 per experimental unit, density = 253 ± 5 g m^{-2}, 33 plants m^{-2}) (Fig. 2). The initial (day 1) and final (day 105) wet weight of the plants were determined with a precision scale (Pedro et al., 2013). For the determination of the water and organic nitrogen, phosphorus and carbon content, 12 complete plants (with roots and shoots) were used in the beginning of the experiment. At the end, three random plants were taken from every unit (n = 36) and dried in a muffle furnace (353.15 K) until constant weight (Ventura et al., 2011).

A 35-days period for acclimation was established (Calheiros et al., 2012; Pinheiro et al., 2020). After that, the 30-days period of nutrients increasing and the 40-days of cultivation with the described nutrient concentrations (section 2.1.1) were carried out.

2.1.3. *Water quality analysis*

Water samples were taken daily at 10:00 am. Conductivity, pH, oxygen concentration and temperature were measured with a HQ40D Multi Parameter-Meter (Hach-Lange GmbH, Germany). Nutrient levels (ammonia, nitrate, nitrite and phosphate) were determined with a spectrophotometer (DR-2800, Hach-Lange GmbH, Germany) and the corresponding analysis kits of Hach (Ammonia: Method 8038 (Nessler Method), Nitrite: Method 8507 (Diazotization Method), Nitrate: Method 8039 (Cadmium Reduction Method), Phosphate: Method 8178 (Amino Acid Method)).

2.1.4. *Concentration of organic N, C and P in plants*

To determine the nutrient accumulation in plant tissue by *S. neei*, the concentration of organic nitrogen (N), carbon (C) and phosphorus (P) (mg g dry weight^{-1}) were analyzed in the plants at the beginning of the trial (n = 12) and compared with three random plants from every unit (n = 36) at the end of the experiments. Roots and shoots were separated. Dry plants were processed to fine powder with an electric mortar (RM 100, Retsch, Germany) (Buhmann et al., 2015). The dried samples were stored at 253.15 K until analysis.

Organic nutrient analyses were done in the Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven, Germany (AWI). Organic phosphate was hydrolyzed to orthophosphate and measured with the stannous chloride method. Organic nitrogen and organic carbon were analyzed with a CN-Analyzer. Daily average uptake was calculated by dividing the total accumulation by the 70-days period.

2.2. *Experiment 2: Nitrogen removal efficiency of S. neei in marine aquaculture wastewater vs. artificial effluents.*

2.2.1. *Experimental design and system management*

The experiments consisted of evaluating four different treatments in the cultivation of the halophyte *S. neei* in deep-water hydroponics: (AQE) Aquaculture effluent - aquaponics; (C) Control (seawater); (AEL) Artificial effluent low nitrate (100 mg l^{-1}) and (AEH) Artificial effluent high nitrate (500 mg l^{-1}). The growth rate and nitrogen removal efficiency by *S. neei* were studied in this second trial (61 days) for each

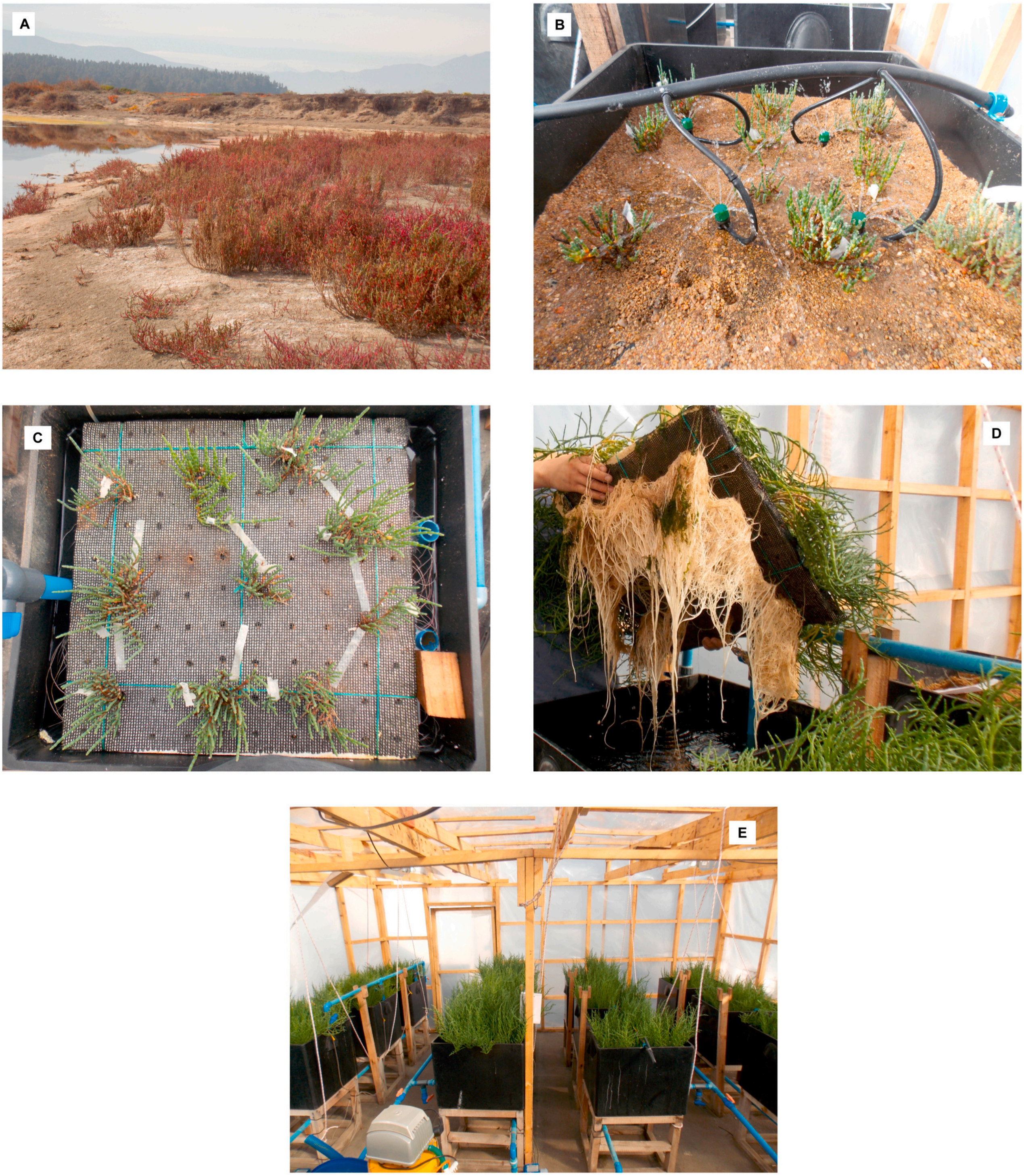

Fig. 2. A) Plant extraction site (salinas de Pullally). B) Sand-substrate system with microdispersion irrigation valves. C) Deep-water hydroponic system with floating raft. D) Floating raft before harvest. E) Experimental system.

treatment.

The aquaculture wastewater came from a *Seriola lalandi Seriola lalandi* (Valenciennes, 1833) culture in marine RAS ($n = 55$, mean weight $= 79.78 \pm 14.34$ g). The effluent used was extracted from the outlet of the cultivation tanks and pumped to the reservoir (190 l) of the experimental system, where it was aerated with air stones at the bottom of the tank (Webb et al., 2012; Marques et al., 2017). The control series consisted of the addition of filtered seawater extracted from the sea, to compare the survival and growth rates by the plants without nutrient addition. AEL and AEH treatments were elaborated by adding $NaNO_3$ (Dilaco, Chile) to the system to achieve a nitrate concentration of 100 mg l^{-1} and 500 mg l^{-1}, respectively. This concentration was chosen to determine the efficiency of *S. neei* in deep-water systems as a possible biofilter for long-term RAS with high nitrate concentrations (van Bussel et al., 2012; Buhmann et al., 2015). The extraction of seawater was carried out with the same procedure as in the previous experiment (section 2.1.1). Also, the experimental system was the same but, in this case, all of the units were implemented with deep-water hydroponics and with natural photoperiod (summer season, approx. 14 h of day light).

2.2.2. *Sarcocornia neei*

The location, the method of extraction and previous cleaning of the plants followed the same procedure as in experiment 1 (section 2.1.2). The *S. neei* cuttings ($n = 120$, length = 10 cm) were planted in hydroponics to strengthen the rooting (21 days) (Jesus et al., 2017; Pinheiro et al., 2017). After that, they were transferred to the experimental system.

Ten *S. neei* cuttings were implemented in each experimental unit (30 plants per treatment, 120 in total) corresponding to a density of 33 plants m^{-2}. The initial wet weight of *S. neei* cuttings implemented in the experimental system was 44.49 ± 8.79 g with a length of 13.21 ± 1.53 cm (mean ± SD, n = 120). The average final weight (g), biomass production (kg m^{-2}) and the gain in weight and length of *S. neei* were calculated for each treatment. Mortality was calculated like the proportion of dead plants at the end of the trials, expressed as a percentage.

2.2.3. *Water quality analysis*

The physicochemical parameters of water quality were measured in each treatment at the beginning and the end of the experiment. Dissolved oxygen, temperature and pH were measured with a portable multiparameter probe (Hach HQ40D) and salinity was determined using a refractometer (RHS-10ATC).

2.2.4. *Nitrate and ammonia removals*

Measurements of inorganic nitrogen components were made daily in each treatment. Ammonia (NH_3^+-N) and nitrate (NO_3^--N) concentrations were determined as specified in 2.1.3 section. The removal trend for nitrate concentration was evaluated until it approached zero, at which time it was returned to the initial concentration by the addition of $NaNO_3$ (AEL and AEH), aquaculture effluent (AQE) or seawater (control). No adjustments were made to the ammonia concentration for any treatment.

2.3. *Statistical analysis*

The analyses were carried out using GraphPad-Prism version 8.4.3. All data were tested for normality and homogeneity of variance using the Shapiro-Wilk test. The differences between initial and final biomass (length and weight) of *S. neei* and the initial and final concentration of ammonia and nitrate in all the experimental series were determined by independent samples *t*-test ($p < 0.05$). The differences in removal rates of nitrate and ammonia and the growth rates of *S. neei* between treatments were determined by two-way ANOVA analysis (experiment 1) and one-way ANOVA analysis (experiment 2). The significant differences were analyzed using the Tukey test ($p < 0.05$).

3. Results

3.1. *Experiment 1: Removal efficiency of inorganic nitrogen and phosphate compounds and accumulation of organic compounds by S. neei in deep-water vs. sand-substrate systems*

3.1.1. *Growth and biomass of S. neei*

Weight and length growth of the plants during this first trial are shown in Fig. 3. No length growth was observed over the acclimation period. With the addition of nutrients, the plants grew near to linear (R^2: 0.94–0.97). There were significant differences between system types and nutrient levels. The maximum growth in length was obtained in the deep-water system with low level of nutrients (WL final length: 48.63 ± 6.60 cm, $n = 30$). Biomass gain was significantly higher in deep-water systems (WL = 426.19 ± 198.69 g; WH = 377.37 ± 181.16 g, n = 30). The nutrient levels didn't influence the plant growth rates and no significant differences were found between WL and WH. The minimum growth in biomass by *S. neei* was obtained in sand-substrate low nutrient system (SL = 222.25 ± 107.66 g). No mortality was observed during experiment 1. Biomass production (fresh weight) was significantly higher in WL (14.41 ± 0.78 kg m^{-2}) and WH (12.64 ± 1.91 kg m^{-2}) treatments, compared to SL (7.48 ± 0.89 kg m^{-2}) and SH (9.87 ± 0.89 kg m^{-2}).

3.1.2. *Nitrogen and phosphate system removal efficiencies*

Nitrate (NO_3^--N), total ammonia nitrogen (TAN), phosphate (P) and total inorganic nitrogen (TIN) systems removals are shown in Fig. 4. The

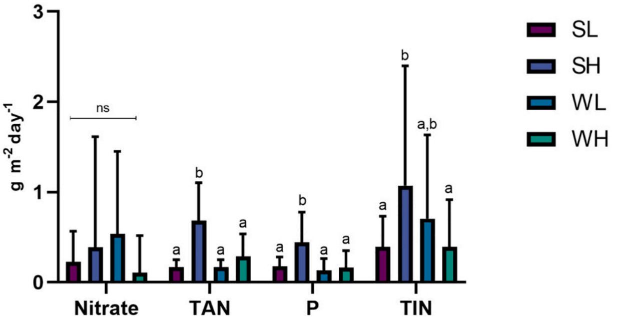

Fig. 4. Nitrate (NO_3^--N), total ammonia nitrogen (TAN), phosphate (P) and total inorganic nitrogen (TIN) removals achieved by the different systems with *S. neei* during the experiment 1. Treatments: SL (Sand low nutrients); SH (Sand high nutrients); WL (Water low nutrients); WH (Water high nutrients). Mean values ± SD (40 days). Significant differences ($p < 0.05$) are expressed with different letters. No significant differences in nitrate removal were found between treatments.

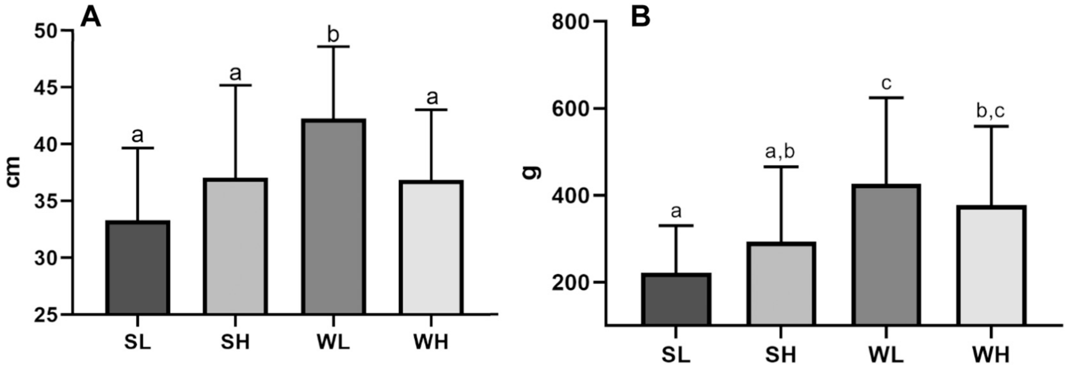

Fig. 3. Growth in lenght (A) and growth in weight (B) achieved by *S. neei* during the experiment 1 (105 days). Treatments: SL (Sand low nutrients): 33.31 ± 6.37 cm, 222.25 ± 107.66 g; SH (Sand high nutrients): 37.05 ± 8.14 cm, 293.09 ± 172.14 g; WL (Water low nutrients): 42.26 ± 6.33 cm, 426.19 ± 198.69 g; WH (Water high nutrients): 36.87 ± 6.17 cm, 377.37 ± 181.16 g. Mean values ± SD ($n = 30$). Significant differences ($p < 0.05$) are expressed with different letters.

highest removal efficiencies of TAN (0.68 ± 0.41 g m^{-2} day^{-1}) and P (0.44 ± 0.34 g m^{-2} day^{-1}) were obtained in SH treatment. No significant differences were observed in nitrate removal between treatments, with the highest value in WL (0.54 ± 0.91 g m^{-2} day^{-1}). TIN removal was higher in SH (1.07 ± 1.32 g m^{-2} day^{-1}), being similar to WL (0.70 ± 0.92 g m^{-2} day^{-1}). In WH, an accumulation peak of TAN (3 mg l^{-1}) and nitrite ($N\text{-}NO_2^- = 4.3$ mg l^{-1}) accompanied by the yellow coloring of the plants was observed during this trial.

3.1.3. *Organic nitrogen, phosphorus and carbon accumulation in plants*

Possible differences between the initial and final content of organic compounds in plant biomass were evaluated. The details of the initial and final organic content and the root-shoot ratio of *S. neei* are shown in Table 1. There were significant differences between organic content (N, P and C) in the plants before transplanting and after harvest. There was an increase in organic N and P content in all treatments. The highest values achieved were in *S. neei* shoots in SL treatment (31.2 ± 0.1 mg g dry $weight^{-1}$) for organic N, and in the plant roots in WH treatment (4.0 ± 0.6 mg g dry $weight^{-1}$) for organic P. Organic C content decreased significantly in all treatments. The root biomass decreased in all treatments, compared to the initial percentage (10.6 ± 2.5%).

At the end of the trial, the accumulation of organic compounds achieved by the plants was also compared between the different treatments. Organic nitrogen (N), phosphorus (P) and carbon (C) accumulation by *S. neei* are shown in Fig. 5. N, P and C plant uptakes were significantly higher in deep-water hydroponic systems. Organic carbon was the most accumulated element by the plants. The highest accumulations were obtained in WL (5.00 ± 0.49 g m^{-2} day^{-1}) and WH (4.44 ± 0.67 g m^{-2} day^{-1}) treatments. Organic P accumulation was higher in deep-water systems (WL = 0.068 ± 0.004 g m^{-2} day^{-1}; WH = 0.064 ± 0.009 g m^{-2} day^{-1}). Also, the accumulation of organic N was higher in WL (0.56 ± 0.038 g m^{-2} day^{-1}) and WH (0.52 ± 0.077 g m^{-2} day^{-1}) treatments.

3.1.4. *Water quality analysis and physicochemical parameters*

The physicochemical parameters of water in the different treatments are shown in Table 2. Oxygen saturation was maintained around 100% (96.8 ± 8.4% - 103.2 ± 8.8%). The maximum average ammonia (1.26 ± 1.07 mg l^{-1}) and nitrite (1.42 ± 1.50 mg l^{-1}) concentrations were obtained in WH. Salinity remained between 34.5 ± 0.9 and 35.7 ± 0.8 g l^{-1} (40 days). The maximum and minimum average daily air temperature in the greenhouse were 306.25 ± 4.0 K and 288.85 ± 1.8 K, respectively (70 days). The maximum and minimum average daily relative humidity were 70 ± 7% and 35 ± 10%. Average daily radiation measured inside the greenhouse was 638.2 ± 293.4 μmol m^{-2} s^{-1}.

3.2. *Experiment 2: Nitrogen removal efficiency of S. neei in marine aquaculture wastewater vs. artificial effluents.*

3.2.1. *Aquaculture wastewater and artificial effluents*

The results of physicochemical parameters of the aquaculture wastewater and artificial effluents implemented in the experimental system are shown in Table 3. The highest concentrations of ammonia were observed in AQE (initial = 0.49 ± 0.31 mg l^{-1}; final = 0.38 ± 0.54 mg l^{-1}). The initial concentration of ammonia in the rest of the series was significantly lower (C = 0.07 ± 0.02 mg l^{-1}; AEL = 0.04 ± 0.02 mg l^{-1}; AEH = 0.05 ± 0.01 mg l^{-1}). The initial nitrate concentration was 19.67 ± 1.30 mg l^{-1} (AQE) and 3.72 ± 0.50 mg l^{-1} (control). AEL and AEH were adjusted to the desired initial nitrate concentrations (99.28 ± 0.49 mg l^{-1} and 501.87 ± 0.75 mg l^{-1}, respectively). The initial average temperature in AQE was higher than the other treatments (294.45 ± 0.36 K). In all the treatments, the initial oxygen saturation was higher than 100% (105.68 ± 2.14%, $n = 12$). The initial pH in AQE (7.53 ± 0.07, $n = 3$) and in the rest of treatments (7.56 ± 0.13, $n = 9$) were similar. Salinity concentration was higher in AQE (36.00 ± 0.00 g l^{-1}) than in the rest of the series (35.00 ± 0.00 g l^{-1}).

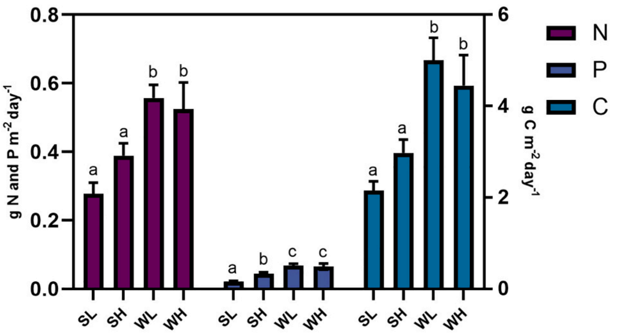

Fig. 5. Organic nitrogen (N), phosphorus (P) and carbon (C) accumulation in *S. neei* during the experiment 1. Treatments: SL (Sand low nutrients); SH (Sand high nutrients); WL (Water low nutrients); WH (Water high nutrients). Mean values ± SD (70 days, n = 120). N and P accumulation are related to the left axis. C accumulation is related to the right axis. Significant differences ($p < 0.05$) are expressed with different letters.

3.2.2. *Growth and biomass of S. neei*

The details of growth and production rates of *S. neei* during this second trial are shown in Table 4. Significant weight and length growth were obtained by *S. neei* in all treatments. The highest growth rate was obtained in AQE treatment (2.80 ± 0.30 g m^{-2} day^{-1}, final weight = 198.28 ± 12.29 g), where the biomass productivity (0.17 ± 0.02 kg m^{-2}) was also the highest achieved in 61 days ($n = 30$). In AQE treatment, flowering was observed in the 80% of the plants between day 16 and day 40 of the experiment. The lowest growth rates in weight were obtained by *S. neei* in the control (0.90 ± 0.11 g m^{-2} day^{-1}) and AEH (0.49 ± 0.05 g m^{-2} day^{-1}) systems. In this trial, plant mortality was observed in all treatments, achieving the maximum in AEH (50.56 ± 10.55%). No significant differences were observed in the final length of the plants between treatments (47.52 ± 11.63 cm, $n = 120$).

The biomass gain in weight and length of *S. neei* during this second trial are shown in Fig. 6. The maximum weight gain was obtained in the

Table 1
Initial and final characterization of the organic content of *S. neei* implemented in the different systems and additions of nutrient levels (experiment 1).

	Initial (wild)			SL			SH			WL			WH		
Organic N shoot [mg g DW^{-1}]	15.4	±	0.1^{a}	31.2	±	0.1^{b}	29.1	±	0.1^{b}	29.2	±	0.2^{b}	31.0	±	0.4^{b}
Organic N root [mg g DW^{-1}]	13.0	±	0.1^{a}	15.4	±	0.4^{b}	17.8	±	0.9^{b}	21.3	±	0.2^{b}	21.3	±	0.7^{b}
Organic P shoot [mg g DW^{-1}]	1.1	±	0.1^{a}	2.2	±	0.0^{b}	3.3	±	0.1^{b}	3.5	±	0.4^{b}	3.6	±	0.4^{b}
Organic P root [mg g DW^{-1}]	2.2	±	0.0^{a}	3.7	±	0.1^{b}	2.9	±	0.1^{b}	3.0	±	0.4^{b}	4.0	±	0.6^{b}
Organic C shoot [mg g DW^{-1}]	316.3	±	1.2^{a}	240.9	±	3.6^{b}	226.9	±	1.1^{b}	247.3	±	0.4^{b}	241.8	±	3.1^{b}
Organic C root [mg g DW^{-1}]	423.5	±	1.9^{a}	355.7	±	3.5^{b}	360.8	±	3.4^{b}	384.1	±	1.1^{b}	363.5	±	3.3^{b}
Dry mass shoot [%]	18.3	±	2.8^{a}	8.4	±	0.5^{b}	8.5	±	0.4^{b}	9.6	±	0.8^{b}	9.0	±	0.6^{b}
Dry mass root [%]	47.8	±	2.7^{a}	30.3	±	3.7^{b}	35.6	±	4.8^{b}	30.9	±	9.0^{b}	30.5	±	12.8^{b}
Root biomass [%]	10.6	±	2.5^{a}	2.4	±	1.4^{b}	1.0	±	0.4^{b}	3.5	±	2.5^{b}	5.0	±	4.1^{b}

SL: Sand system low nutrients, SH: Sand system high nutrients, WL: Water system low nutrients, WH: Water system high nutrients. Values are mean ± SD of 3 determinations. Values with different letters are significantly different from each other ($p < 0.05$). DW: dry weight.

Table 2
Characterization of physicochemical parameters of artificial effluents (experiment 1).

	SL			SH			WL			WH		
pH	7.7	±	0.2	6.9	±	0.4	7.6	±	0.2	6.4	±	1.0
T (K)	292.65	±	2.0	293.25	±	1.9	294.65	±	2.0	294.65	±	2.0
O_2 (% saturation)	103.2	±	8.8	99.1	±	6.4	97.3	±	8.1	96.8	±	8.4
O_2 (mg l^{-1})	9.3	±	0.7	8.9	±	0.6	8.6	±	0.7	8.6	±	0.4
Ammonia (NH_3–N)	0.14	±	0.11	0.47	±	0.8	0.18	±	0.07	1.26	±	1.07
Nitrate (NO_3^--N)	50.3	±	4.2	282.8	±	43	49.8	±	4.1	263.1	±	24.1
Nitrite (NO_2^--N)	0.03	±	0.03	0.62	±	0.61	0.09	±	0.06	1.42	±	1.50
Orthophosphate (PO_4^{3-})	4.9	±	0.96	27.0	±	3.8	5.9	±	0.5	30.1	±	1.1
Salinity (g l^{-1})	34.8	±	0.9	34.5	±	0.9	35.7	±	0.8	35.3	±	0.7

SL: Sand system low nutrients, SH: Sand system high nutrients, WL: Water system low nutrients, WH: Water system high nutrients. Values are mean ± SD (40 days). Ammonia, nitrate, nitrite and phosphate concentrations are shown in mg l^{-1}.

Table 3
Characterization of physicochemical parameters of marine aquaculture wastewater and artificial effluents (experiment 2).

	C		AQE		AEL		AEH	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
pH	7.70 ± 0.15	7.66 ± 0.12	7.53 ± 0.07	7.81 ± 0.02	7.53 ± 0.08	7.70 ± 0.04	7.45 ± 0.09	7.72 ± 0.09
T (K)	291.58 ± 0.06	294.92 ± 0.35	294.45 ± 0.36	295.92 ± 0.38	291.62 ± 0.06	295.58 ± 0.06	291.22 ± 0.12	296.05 ± 0.17
O_2 (% saturation)	106.97 ± 0.29	99.36 ± 0.75	102.6 ± 2.95	106.97 ± 0.29	105.83 ± 0.31	105.33 ± 2.44	107.30 ± 0.50	106.30 ± 1.01
O_2 (mg l^{-1})	8.16 ± 0.04	7.59 ± 0.13	7.94 ± 0.19	7.96 ± 0.08	8.29 ± 0.06	7.94 ± 0.26	8.21 ± 0.08	7.98 ± 0.12
Ammonia (NH_3-N)	0.07 ± 0.02	0.03 ± 0.01	0.49 ± 0.31	0.38 ± 0.54	0.04 ± 0.02	0.03 ± 0.00	0.05 ± 0.01	0.02 ± 0.00
Nitrate (NO_3^--N)	3.72 ± 0.50	0.83 ± 0.87	19.67 ± 1.30	3.31 ± 3.15	99.28 ± 0.49	8.35 ± 0.78	501.87 ± 0.75	150.45 ± 149.88
Salinity (g l^{-1})	35.00 ± 0.00	37.33 ± 0.58	36.00 ± 0.00	39.67 ± 1.15	35.00 ± 0.00	37.67 ± 0.58	35.00 ± 0.00	40.00 ± 1.00

Experiment 2: C: Control (seawater); AQE: Aquaculture effluent; AEL: Artificial effluent low nitrate; AEH: Artificial effluent high nitrate. Values are mean ± SD of 3 determinations (61 days). Ammonia, nitrate, nitrite and phosphate concentrations are shown in mg l^{-1}.

Table 4
Growth and production rates of *S. neei* in the deep-water hydroponics system (experiment 2).

	C			AQE			AEL			AEH		
Final weight (g)	90.59	±	$1.00^{b,c}$	198.28	±	12.29^{a}	119.11	±	21.53^{b}	70.05	±	5.99^{c}
Growth rate (g m^{-2} day^{-1})	0.90	±	$0.11^{b,c}$	2.80	±	0.30^{a}	1.27	±	0.27^{b}	0.49	±	0.05^{c}
Final length (cm)	45.77	±	2.59^{a}	58.17	±	17.79^{a}	50.03	±	4.68^{a}	36.11	±	4.44^{a}
Mortality (%)	26.67	±	6.82^{a}	35.00	±	$4.64^{a,b}$	32.78	±	$5.09^{a,b}$	50.56	±	10.55^{b}
Productivity (kg m^{-2})	0.06	±	0.01	0.17	±	0.02	0.08	±	0.02	0.03	±	0.00

AQE: C: Control (seawater); Aquaculture effluent; AEL: Artificial effluent low nitrate; AEH: Artificial effluent high nitrate. Values are mean ± SD (n = 120, 61 days). Values with different letters are significantly different from each other ($p < 0.05$).

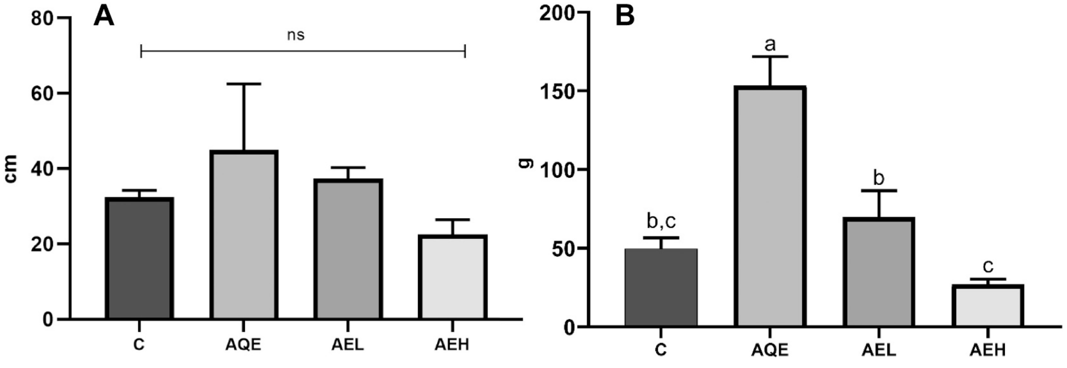

Fig. 6. Growth in lenght (A) and weight (B) achieved by *S. neei* during the experiment 2 (61 days). Treatments: C (Control): 32.40 ± 1.82 cm, 49.69 ± 6.9 g; AQE (Aquaculture effluent): 44.99 ± 17.49 cm, 153.54 ± 18.3 g; AEL (Artificial effluent low nitrate): 37.37 ± 2.96 cm, 69.73 ± 16.88 g; AEH (Artificial effluent high nitrate): 22.48 ± 3.96 cm, 27.12 ± 3.23 g. Mean values ± SD (n = 120). Significant differences ($p < 0.05$) are expressed with different letters. No significant differences in lenght growth were found between treatments.

plants implemented in the aquaculture wastewater (AQE = 153.54 ± 18.3 g, final weight = 198.28 ± 12.29 g, n = 30). The lowest weight increases were obtained in control (49.69 ± 6.90 g) and AEH (27.12 ± 3.23 g) systems. No differences were observed in the growth length of the plants between treatments ($p < 0.05$), obtaining the highest values in AQE system (growth length = 44.99 ± 17.49 cm, final length = 58.17 ± 17.79 cm).

3.2.3. Water quality analysis

The physicochemical parameters of water in the different treatments during this trial are shown in Table 3. To observe possible differences affecting plant growth and survival, they were characterized in all treatments and experimental units at the beginning and at the end of the experiment. The oxygen saturation remained close to 100% in all treatments (99.36 ± 0.75% - 106.97 ± 0.29%). The pH did not vary significantly in any treatment during the experiment (remaining between 7.45 ± 0.09 and 7.81 ± 0.02) achieving this maximum in the AQE series (day 61). Significant increases in water temperature were obtained in the control, AEL and AEH series (with lower initial temperatures). Initial salinity concentrations were increased in all treatments, with a final average of 38.66 ± 1.36 g l^{-1} ($n = 12$), and a maximum of 40.0 ± 1.0 g l^{-1} in AEH at the end of the experiences.

3.2.4. Nitrate and ammonia removal efficiency

The details of the final concentrations of ammonia (NH_3^+-N) and nitrate (NO_3^--N) achieved in the different treatments are shown in Table 3. The final nitrate concentration in AEH was taken on day 48 prior to the new $NaNO_3$ addition, when this concentration was closest to the minimum (44.2 mg l^{-1}).

The comparison of ammonia removal trends between the different treatments is shown in Fig. 7. The daily removal rate was significantly higher in AQE (0.078 ± 0.08 g m^{-2} day^{-1}) compared to AEL and AEH. In terms of percentage, this means the removal of 22.45% of the initial ammonia concentration.

There was significant removal of nitrate in all treatments. The nitrate removal rates achieved by *S. neei* in the different systems are shown in Fig. 8. The daily removal rate was significantly higher in AEH (11.25 ± 31.38 g m^{-2} day^{-1}) compared to other treatments. In this series, the plants needed 48 days to reach the minimum nitrate concentration achieved (44.2 mg l^{-1}), compared to AQE (25 days, 1.2 mg l^{-1}), control series (26 days, 0.26 mg l^{-1}) and AEL (28 days, 7.8 mg l^{-1}). Comparing the percentage of nitrate removal in the different treatments according to the initial concentration, the highest removal was obtained in AEL (91.59%), followed by AQE (83.17%), control (77.69%) and AEH (70.02%) series at the end of the trial (day 61).

4. Discussion

The results of this study demonstrated that *S. neei* is an efficient biofilter for reduction of inorganic compounds contained in marine RAS aquaculture wastewater and artificial effluents. Also, the growth rates obtained by the plants and the tolerance to high salinity concentrations make this species an ideal candidate for diversification of marine aquaculture production in integrated systems.

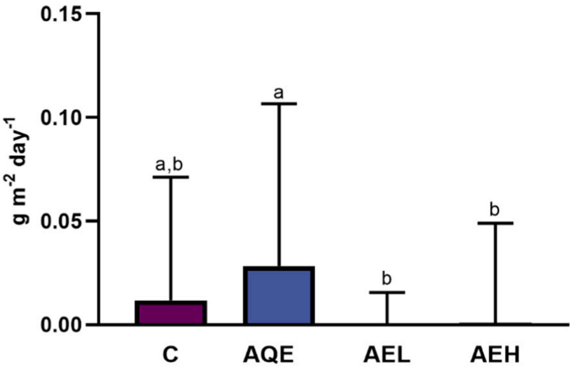

Fig. 7. Ammonia removal achieved by *S. neei* during the experiment 2. Treatments: C (Control): 0.033 ± 0.06 g m^{-2} day^{-1}; AQE (Aquaculture effluent): 0.078 ± 0.08 g m^{-2} day^{-1}; AEL (Artificial effluent low nitrate): 0.0018 ± 0.01 g m^{-2} day^{-1}; AEH (Artificial effluent high nitrate): 0.0027 ± 0.05 g m^{-2} day^{-1}. Mean values ± SD (61 days). Significant differences ($p < 0.05$) are expressed with different letters.

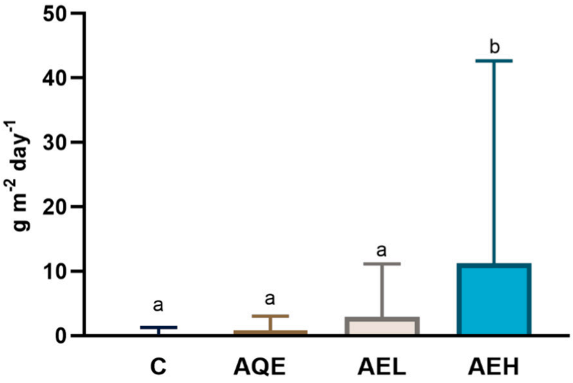

Fig. 8. Nitrate removal achieved by *S. neei* during the experiment 2. C (Control): 0.14 ± 1.13 g m^{-2} day^{-1}; AQE (Aquaculture effluent): 0.82 ± 2.19 g m^{-2} day^{-1}; AEL (Artificial effluent low nitrate): 2.90 ± 8.25 g m^{-2} day^{-1}; AEH (Artificial effluent high nitrate): 11.25 ± 31.38 g m^{-2} day^{-1}. Mean values ± SD (61 days). Significant differences ($p < 0.05$) are expressed with different letters.

4.1. Aquaculture wastewater and artificial effluents

The characterization of aquaculture wastewater (pH, temperature, salinity and ammonia concentration) was similar to previous studies with *Seriola lalandi* cultured in recirculating systems (Mota et al., 2014). Lower nitrate concentrations presented at this study (19.67 ± 1.30 mg l^{-1}) could be attributed to lower biomass of organisms and cultivation time in the moment of effluent extraction. Similar nitrate concentrations (21.4 ± 17 mg l^{-1}) were obtained from the culture of *Litopenaeus vannamei* to evaluate the efficiency of *Sarcocornia ambigua* when implemented in an integrated aquaponic bioremediation system (Pinheiro et al., 2017).

4.2. Sarcocornia neei: growth rates and productivity

The integration of *S. neei* in the bioremediation system resulted in all plants obtaining significant growth rates at the end of the experiments, achieving marketable size in all treatments (Ventura et al., 2011; Waller et al., 2015).

The maximum growth rates obtained by *S. neei* in this study were achieved in deep-water culture systems (experiment 1), where the plants reached a maximum productivity of 14.41 ± 0.78 kg m^{-2} and an individual biomass gain of 426.19 ± 198.69 g (WL treatment) in 105 days of cultivation. Some species of halophyte plants can achieve similar growth rates when implemented in different culture systems. This is the case of *Tripolium pannonicum*, which obtained a similar final biomass gain between deep-water hydroponics, sand and expanded clay treatments when implemented as a biofilter for saline water in bioremediation systems (Buhmann et al., 2015). In this study, *S. neei* achieved significantly different growth and productivity rates between treatments, suggesting that this species could be more suitable for implementation in deep-water culture systems (Custódio et al., 2017).

Nutrient levels had no influence on plant productivity between the WL and WH treatments. This is not consistent with previous studies, where higher concentrations of nutrients achieved higher growth rates of *Salicornia europaea* implemented in filter beds for saline wastewater treatment (Webb et al., 2012). In the present study, this could be due to the peak observed in the concentration of total ammonia nitrogen (TAN) and nitrite (WH treatment) accompanied by a yellowish color in the plants, suggesting a possible chlorosis and an excess of nutrients that could damage the health of the plants (Buhmann et al., 2015).

The productivity obtained by *S. neei* in this trial was much higher than that observed in previous studies with halophyte plants. *Sarcocornia spp* showed productivities of 3–4 kg m^{-2} in five consecutive harvests during a growing cycle of six months with total seawater irrigation (100%) (Ventura et al., 2011). *Tripolium pannonicum, Plantago coronopus* and *Salicornia dolichostachya* obtained 1.64 kg m^{-2} in hydroponics culture (35 days), when implemented in a marine RAS effluent of *D. labrax* (Waller et al., 2015). *Sarcocornia ambigua* also achieved lower productivity (0.61 ± 0.12 kg m^{-2}) used in aquaculture effluent bioremediation during eight weeks (Pinheiro et al., 2020). A halophyte biomass yield study with seawater irrigation was conducted with *S. bigelovii* for 6 years for seed oil production, obtaining an average annual yield of 1.7 kg m^{-2} of dry biomass (Glenn et al., 1998).

The growth and productivity rates of halophyte plants depend on the species and the salinity of the effluent. The genera *Salicornia* and *Sarcocornia* have been classified within the most tolerant to high concentrations of salinity (76 to 100% seawater) (Buhmann and Papenbrock, 2013; Ventura and Sagi, 2013). However, previous studies have shown that salinity is a growth inhibiting factor and lower growth rates are obtained by some species when irrigated with full marine water (Ventura et al., 2011; Buhmann and Papenbrock, 2013). On the other hand, the productivity of some perennial halophyte plants may not be affected by this parameter and their growth could be stimulated by the presence of NaCl (Ventura et al., 2011). In fact, concentrations between 8 and 17 g l^{-1} of NaCl stimulates the growth of halophytes of the *Amaranthaceae* family, such as *S. neei* (Pinheiro et al., 2020).

The use of brackish water and the adjustment of salinity of the effluent used can make the halophyte plants obtain higher growth rates (Ventura and Sagi, 2013; De Souza et al., 2018). *S. ambigua* obtains significantly higher growth rates when irrigated with aquaculture effluent with 16 g l^{-1} of NaCl compared to 32 g l^{-1} (Pinheiro et al., 2020). *Tripolium pannonicum* report higher biomass gain by adjusting the salinity of irrigation water to 15 g l^{-1}, compared to 22.5 and 30 g l^{-1}, but not affecting the plant health (Buhmann et al., 2015). The halophyte *Halimione portulacoides* achieved productivity of 17.2 kg m^{-2} (similar to this study) with an average salinity of 20 g l^{-1} in five months implemented as a biofilter in a super-intensive fish farm effluent (Marques et al., 2017). *Sarcocornia ambigua* obtained productivity of 8.2 kg m^{-2} in 73 days of experiments when irrigated with L. *vannamei* effluent at 36.2 ± 1.6 g l^{-1} NaCl, suggesting that *S. neei* could have faster growth rates (Pinheiro et al., 2017). On the other hand, halophytes of the genera *Atriplex* and *Salicornia* reported lower productivities (0.27 ± 0.15 kg m^{-2}) when irrigated with saline wastewater (500 mM NaCl) in two months of experiments, similar than obtained by *S. neei* in experiment 2 (Ventura and Sagi, 2013). Further studies with *S. neei* should be done to evaluate the possibility of obtaining higher growth rates and productivity at lower effluent salinities.

In experiment 2, the highest growth rates of *S. neei* were obtained in the series irrigated with aquaculture effluent (AQE). In previous studies, halophyte plants normally obtain higher productivity rates when higher nitrate concentrations are present in the water, suggesting that micronutrients contained in aquaculture effluent could play an important role (Doncato and Costa, 2020). Also, this could be due to the higher ammonia concentration present in AQE treatment. This compound is the most favorable for the growth of other halophytes under conditions of low N and high salinity, reporting higher growth rates by *S. bigelovii* with irrigation of NH_4^+-N compared to NO_3^--N (Kudo and Fujiyama, 2010). The growth results of *S. neei* obtained in experiment 2 suggest that this species obtains higher growth rates when NH_4^+-N is added to the irrigation water. In contrast, *S. europaea* reports lower results with NH_4^+-N supply (Ventura et al., 2010; Quintã et al., 2015). The differences could be related to culture parameters, tested nutrient concentrations or to the species used itself.

Flowering process took place in the plants irrigated with the marine RAS wastewater. This phenomenon was not expected due to the summertime photoperiod (14 h of light) (Waller et al., 2015). However, this has also been observed in previous studies with *S. bigelovii*, where flowering seems to be affected not only by day length but also by plant nutrition (Ventura and Sagi, 2013). These results suggest that some type of micronutrients present in the aquaculture effluent could induce this process.

The highest growth rates obtained by *S. neei* were not expected in the experimental series where flowering was observed. Halophytes of the genera *Salicornia* and *Sarcocornia* report that this should be prevented for a higher biomass production (Ventura and Sagi, 2013; Waller et al., 2015). Therefore, these results suggest that the presence of certain macro and micronutrients and even a more suitable concentration of nitrate (about 10 mg l^{-1}) could be the cause of the increased growth rates obtained in AQE treatment (Buhmann et al., 2015).

The growth achieved by *S. neei* was significantly lower in experiment 2 compared to experiment 1. This could be due to several factors. The lowest growth rates in weight were obtained in AEH (initial NO_3^--N: 501.87 ± 0.75 mg l^{-1}) and control (initial NO_3^--N: 3.72 ± 0.50 mg l^{-1}) treatments. This is consistent with the results obtained by *Tripolium pannonicum*, which growth rates were affected by nitrate concentrations higher than 100 mg l^{-1} and requiring a minimum nitrate concentration of 10 mg l^{-1} to obtain biomass production (Buhmann et al., 2015). Also, the higher mortalities obtained by *S. neei* in experiment 2 may have influenced the productivity results obtained. Previous studies with *S. neei* integrated to an affluent of *Litopenaeus vannamei* during 56 days achieved productivity rates of 0.14 kg m^{-2}, similar than the obtained in this trial, also due to the high plant mortalities (Schardong et al., 2020). The mortality rates were also similar to those obtained with *Sarcocornia ambigua* implemented in saline shrimp effluent (33.3–38.9%) during 57 days (Pinheiro et al., 2020).

In this study, the differences obtained in mortality rates among experiments could be due to the implementation of cuttings (experiment 2) directly in seawater without previous irrigation with tap water (Pinheiro et al., 2020). Furthermore, the higher mortalities obtained could also be due to the increase of water salinity during the trial, since tap water was not added daily to compensate evapotranspiration losses (as in experiment 1), being only added when nitrate concentrations were close to zero (Jesus et al., 2017). Another possible reason is the stress generated by the higher air temperatures inside the greenhouse in experiment 2, affecting the survival and growth rates of *S. neei* (Waller et al., 2015).

The similar growth rates in length obtained by *S. neei* between experiment 1 (42.26 ± 6.33 cm) and 2 (44.49 ± 17.49 cm) suggest that the use of artificial light may not be a requirement in bioremediation systems with this species during the summer season, but maybe an important factor in preventing the occurrence of flowering processes.

The time interval between harvests is also an important factor in the biomass yield obtained by halophyte plants. It has been determined that for *Salicornia spp* the highest biomass production is obtained with 3-week extraction intervals, due to the capacity of regeneration of this species (Ventura and Sagi, 2013). In this study, the harvest periods were 105 and 61 days, respectively. More studies should be carried out with *S. neei* to determine if a similar harvest method can increase the productivity obtained by this species.

Previous studies with halophyte plants have shown that there are differences in C assimilation through photosynthesis between indoor and outdoor systems, which can affect growth rates obtained by the plants (Liu et al., 2020). In this study, both experiments were carried out in indoor cultivation (greenhouse). It would be interesting to conduct future studies evaluating possible differences in the growth rates achieved by *S. neei* plants placed in outdoor cultivation systems.

4.3. Inorganic compounds removal rates

S. neei obtained significant removals of inorganic nitrogen (N) and phosphorus (P) compounds in both experiments, demonstrating that is a good candidate for its use as a biofilter for saline wastewater.

Nitrogen uptake by halophytes depends on salinity, nutrient concentration present in the effluent, hydraulic loading rate, the species used and the age of the plants (Calheiros et al., 2012; Pinheiro et al., 2020). Plants of the genera *Salicornia* and *Sarcocornia* can use both ammonia and nitrate as a source of nitrogen (Quintã et al., 2015).

However, in conditions of high salinity, some halophytes and macroalgae can promote the absorption of ammonia, inhibiting nitrate absorption (Pinheiro et al., 2017; Aquilino et al., 2020; Pinheiro et al., 2020). This is the case of *S. ambigua*, which presents a preference for ammonia absorption at salinities higher than its isosmotic point (16 g l^{-1}) (Pinheiro et al., 2020). Halophyte plants can also absorb nitrate from sediment or water through nitrate-reductase mechanisms. When plants absorb N in the form of nitrate, they have to reduce it to ammonia form before it becomes part of the organic compounds (Stewart et al., 1973).

In experiment 1, the maximum absorption rate of ammonia was obtained in SH (0.68 ± 0.41 g m^{-2} day^{-1}), while the highest nitrate absorption rate was achieved in WL (0.54 ± 0.91 g m^{-2} day^{-1}). The highest ammonia absorption obtained in sand-substrate units was possibly influenced by nitrification processes in the substrate of the experimental units. The sand can act as a biofilter and the ammonia is metabolized to nitrate, increasing its availability for the plants (Kudo and Fujiyama, 2010). Also, in saline effluents, ammonia uptakes by halophytes plants can predominate by inhibiting the absorption of nitrate in this type of substrate-based systems (Pinheiro et al., 2020).

Comparing with other studies, higher removals of ammonia were obtained in the experiment 1 by *S. neei* than other halophytes with the irrigation of saline industrial effluents (Calheiros et al., 2012). This may be due to, in this study, the plants were implemented in a high-aerated bioremediation system (promoting nitrification) in contrast to subsurface constructed wetlands (CWs). In these systems, anaerobic conditions are predominated and nitrification is limited to a thin oxygenated layer at the top of the wetland, playing a minor role in total system removal (Buhmann and Papenbrock, 2013).

These results suggest that the implementation of integrated systems with *S. neei* could be used for the reduction of both compounds, obtaining higher ammonia removal rates in sand-substrate systems and higher nitrate removal rates in deep-water systems. Further studies should be carried out to determine if these differences are maintained when integrating systems with *S. neei* into larger scale industrial aquaculture effluents.

In experiment 2, where all the treatments were implemented in deep-water systems, *S. neei* was more efficient in absorbing nitrate compared to ammonia. In this trial, different concentrations of nitrate were added to the experimental units, approx. 4 mg l^{-1} (control), 20 mg l^{-1} (AQE), 100 mg l^{-1} (AEL) and 500 mg l^{-1} (AEH), but there was no addition of ammonia.

The nitrate removal rates achieved in AQE treatment (0.82 ± 2.19 g m^{-2} day^{-1}) makes this species an ideal candidate for biofilter of marine RAS effluents, where nitrate concentrations higher than 20 mg l^{-1} may induce problems in cultured organisms (Buhmann and Papenbrock, 2013). The results obtained in this trial are consistent with previous reports by *S. bigelovii*, where ammonia can result in symptoms of toxicity when is contributed as the only form of N (Kudo and Fujiyama, 2010). Also, nitrate is the preferred form of N for *Salicornia spp*, producing higher biomass yields than plants irrigated with ammonia (Ventura and Sagi, 2013). *Salicornia persica* implemented in a constructed wetland for three months and irrigated with marine aquaculture effluent also achieved significantly higher removals of nitrate and nitrite (77–100%) compared to ammonia (0–3%) (Shpigel et al., 2013).

The daily nitrate removal rate in experiment 2 was significantly higher in AEH treatment (11.25 ± 31.38 g m^{-2} day^{-1}) compared to the rest. These results are consistent with other studies where better removal and absorption efficiencies were achieved by halophyte plants when higher N levels were available in the irrigation water (Lymbery et al., 2006; Kudo and Fujiyama, 2010; Buhmann and Papenbrock, 2013). In deep-water hydroponic systems with high levels of dissolved inorganic nutrients, plants have more access to dissolved nutrients and there is lower competition with soil microorganisms compared to sand-substrate systems (Boxman et al., 2017).

Even so, nitrate removal in AEH treatment was surprisingly high compared to experiment 1. The higher pH (above 7) could have favored the precipitation of nutrients in the hydroponic units (Buhmann et al., 2015). However, this phenomenon was not observed at any time during the experiments. This could also be due to the microorganisms present in the system. There is an enrichment of bacterial taxa in halophyte cultured in deep-water aquaponics, such as those involved in nitrogen absorption and denitrification processes (Oliveira et al., 2020).

Nutrient removals by denitrification increase under high nutrient loads in these type of bioremediation systems (Shpigel et al., 2013). In experiment 2, it is likely that nitrogen removal due to denitrification processes in the water column by anaerobic bacteria has been underestimated, and that total nitrogen removal has been taken as nitrate absorbed by plants (Buhmann et al., 2015). In this study, aeration systems were implemented in all experimental units to ensure high oxygen concentrations in the effluent water. However, in this type of systems, denitrification can take place in the reservoirs during periods of non-recirculation water or even in the lower water layers of the deep-water experimental units (Buhmann and Papenbrock, 2013; Shpigel et al., 2013). The differences in absorption of inorganic N compounds between the two experiments should be further evaluated in future studies. The concentration of nutrients and micronutrients, the characteristics of the aquaculture effluent or the seasonality may have influenced the disparity between the results obtained.

In experiment 2, the maximum percentage of nitrate absorption (83.17%) achieved by *S. neei* was lower than that reported by *Salicornia bigelovii*, removing 95.79% of the inorganic N components present in a saline effluent (35 g l^{-1}) in 86 days of trials (Brown et al., 1999). On the other hand, a similar dissolved nitrogen removal rate (7.9 g m^{-2} day^{-1}) was obtained by *Salicornia persica* implemented in gravel stone and irrigated with *Sparus aurata* aquaculture effluent at 35 g l^{-1} in 90 days (Shpigel et al., 2013).

The removal efficiency of nitrate and ammonia by *S. neei* should be further studied when both compounds are simultaneously present in the marine effluent with similar concentrations. A two-step bioremediation system could be implemented, according to Aquilino et al. (2020), to evaluate the preference for the absorption of these compounds by *S. neei*.

The maximum phosphate removal by *S. neei* in this study was obtained in SH treatment (0.44 ± 0.34 g m^{-2} day^{-1}). This is higher than reported by *Salicornia europaea,* obtaining a P absorption of 0.028 g m^{-2} day^{-1} in sand-substrate units similar to this study (Webb et al., 2012).

The fact that the maximum phosphate absorption has been obtained in sand-substrate *vs* deep-water systems is consistent with previous studies. In sand-substrate systems, processes of absorption to substrate particles and phosphate precipitation can occur. These phenomena can reduce the availability of this compound for the plants, while increasing the removal rates obtained by the entire system (Buhmann et al., 2015). Also, heterotrophic bacteria present in the sediment can contribute as phosphate consumers, increasing the removal rates obtained in this type of integrated systems (Buhmann and Papenbrock, 2013).

Comparing phosphate removal rates between sand-substrate systems (SL and SH), the efficiency was significantly higher in SH. This is consistent with other studies where the uptake of P compounds by halophytes is dependent on nitrogen supplementation (Webb et al., 2012). It is possible that the higher concentration of nitrate and ammonia added to SH treatment influenced the higher P removal rates obtained by the plants. On the other hand, previous studies report that higher P removals are obtained by halophytes when higher concentrations of the compound itself are added to the effluent (Buhmann and Papenbrock, 2013). Possibly, in this study, the higher addition of P to SH treatment also influenced *S. neei* to obtain higher P removals.

Previous studies with halophytes report higher removal efficiencies of N and P inorganic compounds by adjusting the salinity of aquaculture wastewater to 10 g l^{-1} compared to 35 g l^{-1} (Buhmann and Papenbrock, 2013). *S. ambigua* reports higher nutrient removals at salinities between 16 and 24 g l^{-1} (Pinheiro et al., 2020). Also, *Tripolium pannonicum* showed a decrease in absorption of N and P components by increasing salinity (Buhmann et al., 2015). On the other hand, studies with *Juncus kraussii* reported that salinity has not negative effects on nitrate removal, but on phosphate removal (Lymbery et al., 2006).

More studies should be done to estimate the maximum potential of *S. neei* removal of inorganic N and P in effluents with lower salinity values. However, this is interesting to implement this halophyte plant in a typical marine aquaculture effluent without the need to adjust salinity.

4.4. *Organic accumulation in plants*

S. neei obtained significant accumulation of organic C, N and P compounds, main components of aquaculture effluents (Buhmann and Papenbrock, 2013). The highest accumulation rates were obtained for organic C (5.00 ± 0.49 g m^{-2} day^{-1}), followed by organic N (0.56 ± 0.038 g m^{-2} day^{-1}) in WL treatment, without significant differences between WL and WH treatments.

Organic C was the most accumulated nutrient by plants. The absorption of this compound by halophytes in bioremediation systems comes mainly from the process of photosynthesis, lowering its absorption from the effluents (Marques et al., 2017). It has less impact in terms of biofilter activity but is very interesting in points of carbon sequestration and energy usability.

The significant accumulation of organic N obtained by *S. neei* is in line with previous studies, where the absorption of this compound as amino acids and peptides has been observed (Webb et al., 2012). The absorption and increase of N in the plants could be due to a physiological adaptation of halophytes to salinity stress, since the accumulation of these compounds helps osmoregulation processes (Buhmann and Papenbrock, 2013).

The higher contents of organic N and P obtained in plant tissues at the end of the experiments are results very promising due to the high loads of organic nutrients present in aquaculture effluents (Gómez et al., 2019). The maximum organic N content was obtained in SL shoots (31.2 ± 0.1 mg g dry $weight^{-1}$) and the maximum organic P (4.0 ± 0.6 mg g dry $weight^{-1}$) in WH roots. Other studies with *Sarcocornia fruticosa* and *Arundo donax* also obtained significant removals of TP and TKN from high salinity tannery wastewater (Calheiros et al., 2012). Higher absorptions are expected in bioremediation systems implemented in deep-water and sand-substrate systems, as this study, and not in constructed wetlands (CWs) where most of the organic removal is due to microbial processes (Webb et al., 2012).

The highest accumulations of organic phosphorus by *S. neei* (0.068 ± 0.038 g m^{-2} day^{-1}) were obtained in the roots of the plants in deep-water hydroponic systems (WH and WL treatments). This is consistent with other studies conducted with *Salicornia dolichostachya*, where the high increase in P content of plants was likely caused by an adsorption of P to the root surface (Waller et al., 2015). Higher removals of this compound in deep-water systems can be explained by the fact that, in substrate systems, phosphorus is accumulated in the sediment at a rate of 47–54% (with only 2–4% being released back into the water column) (Buhmann and Papenbrock, 2013). This may have decreased the availability of P to plants in sand-substrate systems.

Previous studies with *Sarcocornia spp* showed that these halophytes can be an alternative source of omega-3 polyunsaturated fatty acids and minerals for human or livestock consumption and commercial fish diets when irrigated with seawater (Ventura et al., 2011; Doncato and Costa, 2018). *S. neei* obtained interesting results as a source of dietary fiber and antioxidant potential (Riquelme et al., 2016). New analyses to study possibilities for the final use of the biomass produced would be interesting to add value to its implementation as a bioremediation species of saline effluents.

4.5. *Water quality parameters*

The implementation of air injection systems and aeration stones in the reservoirs and at the bottom of deep-water experimental units were effective in both experiments and in all treatments to ensure a correct dissolved oxygen concentration for the plants (experiment 1 = 96.8–103.2%; experiment 2 = 99.36–106.97%).

pH remained without significant differences among treatments and between initial and final concentrations, according to other studies using halophytes as biofilters in aquaculture effluents (Calheiros et al., 2012; Pinheiro et al., 2017). The pH remained above the optimum for plants grown in hydroponics (5.5–6.5) but obtaining good growth and survival results by *S. neei*, suggesting that the integrated bioremediation system could be used at this pH in order not to affect the cultured fish (Buhmann et al., 2015).

The highest salinity values were obtained in experiment 2 (final average = 38.66 g l^{-1}; maximum = 40.00 g l^{-1} in AEH). Probably due to the increase in environmental temperatures and the lack of freshwater replenishment to compensate the evapotranspiration processes, showing the tolerance of *S. neei* to high salt concentrations.

5. Conclusion

Integrated systems with halophyte plants is one of the most promising techniques to increase the sustainability of marine recirculating aquaculture systems. The bioremediation capacity of these plants allows the reduction of nutrient discharges into the aquatic environments, diversifying production and creating high value by-products.

In this study, the bioremediation efficiency of the halophyte *S. neei* was evaluated in a marine RAS effluent and in artificial effluents with different nutrient loads. The results obtained demonstrated that it is a promising candidate for marine aquaculture wastewater bioremediation. The obtained growth and productivity rates, the ammonia, phosphate and nitrate removal efficiencies, the accumulation of organic C, N and P compounds and its adaptability to high salt concentrations make it ideal to be used as a biofilter of marine aquaculture effluents.

Further investigations should be done to analyze possible influences on the growth rate, removal efficiency and nutrient accumulation by *S. neei* due to changes in effluent salinity, plant density, hydraulic loading rate or macro and micronutrient addition. The possibility to improve efficiency by breeding programs should be considered. Also, the long-term use of such a system integrated into an aquaculture facility needs to be investigated in terms of feasibility, to get closer to its application in industrial-scale production systems.

Funding

This work was supported by INNOVA-CORFO Project code: 14IDL2–29941.

Declaration of Competing Interest

None.

Acknowledgements

Special thanks to José Gallardo for supporting us with the equipment of the Laboratory of Applied Genetics (PUCV), and to Constanza Low for her help during the experiments in the Laboratory of Experimental Aquaculture. Further thanks to Rajko Thiele, Christiane Lorenzen and Bela H Buck from the AWI for their help and collaboration.

References

Alonso, M.Á., Crespo, M.B., 2008. Taxonomic and nomenclatural notes on south American taxa of *Sarcocornia* (*Chenopodiaceae*). Ann. Bot. Fenn. 45, 241–254. https://doi.org/10.5735/085.045.0401.

Alonso, M.F., Orellana, C., Valdes, S., Diaz, F.J., 2017. Effect of salinity on the germination of *Sarcocornia neei* (*Chenopodiaceae*) from two contrasting habitats. Seed Sci. Technol. 45 (1), 252–258. https://doi.org/10.15258/sst.2017.45.1.20.

Aquilino, F., Paradiso, A., Trani, R., Longo, C., Pierri, C., Corriero, G., de Pinto, M.C., 2020. *Chaetomorpha linum* in the bioremediation of aquaculture wastewater: optimization of nutrient removal efficiency at the laboratory scale. Aquaculture 523, 735133. https://doi.org/10.1016/j.aquaculture.2020.735133.

Arce, M.E., Bianciotto, O., Stronati, M.S., Yepes, M.S., Blessio, A.Y., Aras, F.M., 2016. Agriculture and sheep production on Patagonian sabkahs with *Sarcocornia neei* irrigated with seawater (Chubut–Argentina). In: Sabkha Ecosystems, Vol. 48. Springer, Cham, pp. 275–285. https://doi.org/10.1007/978-3-319-27093-7_15.

Badiola, M., Mendiola, D., Bostock, J., 2012. Recirculating aquaculture systems (RAS) analysis: Main issues on management and future challenges. Aquac. Eng. 51, 26–35. https://doi.org/10.1016/j.aquaeng.2012.07.004.

Biswas, G., Kumar, P., Ghoshal, T.K., Kailasam, M., De, D., Bera, A., Mandal, B., Sukumaran, K., Vijayan, K.K., 2020. Integrated multi-trophic aquaculture (IMTA) outperforms conventional polyculture with respect to environmental remediation, productivity and economic return in brackishwater ponds. Aquaculture 516, 734626. https://doi.org/10.1016/j.aquaculture.2019.734626.

Boxman, S.E., Nystrom, M., Capodice, J.C., Ergas, S.J., Main, K.L., Trotz, M.A., 2017. Effect of support medium, hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic systems. Aquac. Res. 48 (5), 2463–2477. https://doi.org/10.1111/are.13083.

Brown, J.J., Glenn, E.P., Fitzsimmons, K.M., Smith, S.E., 1999. Halophytes for the treatment of saline aquaculture effluent. Aquaculture 175 (3–4), 255–268. https://doi.org/10.1016/S0044-8486(99)00084-8.

Buck, B.H., Troell, M.F., Krause, G., Angel, D.L., Grote, B., Chopin, T., 2018. State of the art and challenges for offshore integrated multi-trophic aquaculture (IMTA). Front. Mar. Sci. 5, 165. https://doi.org/10.3389/fmars.2018.00165.

Buhmann, A., Papenbrock, J., 2013. Biofiltering of aquaculture effluents by halophytic plants: basic principles, current uses and future perspectives. Environ. Exp. Bot. 92, 122–133. https://doi.org/10.1016/j.envexpbot.2012.07.005.

Buhmann, A.K., Waller, U., Wecker, B., Papenbrock, J., 2015. Optimization of culturing conditions and selection of species for the use of halophytes as biofilter for nutrient-rich saline water. Agr Water Manage 149, 102–114. https://doi.org/10.1016/j.agwat.2014.11.001.

Calheiros, C.S., Quitério, P.V., Silva, G., Crispim, L.F., Brix, H., Moura, S.C., Castro, P.M., 2012. Use of constructed wetland systems with *Arundo* and *Sarcocornia* for polishing high salinity tannery wastewater. J. Environ. Manag. 95 (1), 66–71. https://doi.org/10.1016/j.jenvman.2011.10.003.

Custódio, M., Villasante, S., Cremades, J., Calado, R., Lillebø, A.I., 2017. Unravelling the potential of halophytes for marine integrated multi-trophic aquaculture (IMTA) a perspective on performance, opportunities and challenges. Aquac Environ Interact 9, 445–460. https://doi.org/10.3354/aei00244.

De Souza, M.M., Mendes, C.R., Doncato, K.B., Badiale-Furlong, E., Costa, C.S., 2018. Growth, phenolics, photosynthetic pigments, and antioxidant response of two new genotypes of sea asparagus (*Salicornia neei* lag.) to salinity under greenhouse and field conditions. Agriculture 8 (7), 115. https://doi.org/10.3390/agriculture8070115.

del Campo, L.M., Ibarra, P., Gutiérrez, X., Takle, H., 2010. Utilization of sludge from recirculation aquaculture systems. In: Nofima Marin AS report, innovation Norway. Report 9/2010, 73pp.

Doncato, K.B., Costa, C.S.B., 2018. Growth and mineral composition of two lineages of the sea asparagus *Sarcocornia ambigua* irrigated with shrimp farm saline effluent. Exp. Agric. 54 (3), 399. https://doi.org/10.1017/S0014479717000096.

Doncato, K.B., Costa, C.S.B., 2020. Micronutrient supplementation needs for halophytes in saline aquaponics with BFT system water. Aquaculture 735815. https://doi.org/10.1016/j.aquaculture.2020.735815.

FAO, Food and Agriculture Organization of the United Nations, 2018. The state of world fisheries and aquaculture. In: Meeting the Sustainable Development Goals. FAO, Rome, Italy, p. 2018.

Fuchs, V.I., Schmidt, J., Slater, M.J., Zentek, J., Buck, B.H., Steinhagen, D., 2015. The effect of supplementation with polysaccharides, nucleotides, acidifiers and *Bacillus* strains in fish meal and soy bean-based diets on growth performance in juvenile turbot (*Scophthalmus maximus*). Aquaculture 437, 243–251. https://doi.org/10.1016/j.aquaculture.2014.12.007.

Glenn, E.P., Brown, J.J., O'Leary, J.W., 1998. Irrigating crops with seawater. Sci. Am. 279 (2), 76–81. https://doi.org/10.2307/26070601.

Gómez, S., Hurtado, C.F., Orellana, J., 2019. Bioremediation of organic sludge from a marine recirculating aquaculture system using the polychaete *Abarenicola pusilla* (Quatrefages, 1866). Aquaculture 507, 377–384. https://doi.org/10.1016/j.aquaculture.2019.04.033.

Henares, M.N., Medeiros, M.V., Camargo, A.F., 2020. Overview of strategies that contribute to the environmental sustainability of pond aquaculture: rearing systems, residue treatment, and environmental assessment tools. Rev. Aquac. 12 (1), 453–470. https://doi.org/10.1111/raq.12327.

Jesus, J.M., Cassoni, A.C., Danko, A.S., Fiúza, A., Borges, M.T., 2017. Role of three different plants on simultaneous salt and nutrient reduction from saline synthetic wastewater in lab-scale constructed wetlands. Sci. Total Environ. 579, 447–455. https://doi.org/10.1016/j.scitotenv.2016.11.074.

Kudo, N., Fujiyama, H., 2010. Responses of halophyte *Salicornia bigelovii* to different forms of nitrogen source. Pedosphere 20 (3), 311–317. https://doi.org/10.1016/S1002-0160(10)60019-7.

Li, M., Callier, M.D., Blancheton, J.P., Galès, A., Nahon, S., Triplet, S., Roque D'Orbcastel, E., 2019. Bioremediation of fishpond effluent and production of microalgae for an oyster farm in an innovative recirculating integrated multi-trophic aquaculture system. Aquaculture 504, 314–325. https://doi.org/10.1016/j.aquaculture.2019.02.013.

Liu, Y., Maimaitijiang, T., Zhang, J., Ma, Y., Lan, H., 2020. The developmental enhancement of a C4 system with non-typical C4 physiological characteristics in *Salsola ferganica* (Kranz anatomy), an annual desert halophyte. Front. Plant Sci. 11, 152. https://doi.org/10.3389/fpls.2020.00152.

Lymbery, A.J., Doupé, R.G., Bennett, T., Starcevich, M.R., 2006. Efficacy of a subsurface-flow wetland using the estuarine sedge *Juncus kraussii* to treat effluent from inland saline aquaculture. Aquac. Eng. 34 (1), 1–7. https://doi.org/10.1016/j.aquaeng.2005.03.004.

MacDonald, B.A., Robinson, S.M., Barrington, K.A., 2011. Feeding activity of mussels (*Mytilus edulis*) held in the field at an integrated multi-trophic aquaculture (IMTA) site (*Salmo salar*) and exposed to fish food in the laboratory. Aquaculture 314 (1–4), 244–251. https://doi.org/10.1016/j.aquaculture.2011.01.045.

Maciel, E., Domingues, P., Domingues, M.R.M., Calado, R., Lillebø, A., 2020. Halophyte planxts from sustainable marine aquaponics are a valuable source of omega-3 polar lipids. Food Chem. 320, 126560. https://doi.org/10.1016/j.foodchem.2020.126560.

Marques, B., Calado, R., Lillebø, A.I., 2017. New species for the biomitigation of a super-intensive marine fish farm effluent: combined use of polychaete-assisted sand filters and halophyte aquaponics. Sci. Total Environ. 599, 1922–1928. https://doi.org/10.1016/j.scitotenv.2017.05.121.

Martins, C.I.M., Eding, E.H., Verdegem, M.C., Heinsbroek, L.T., Schneider, O., Blancheton, J.P., Roque d'Orbcastel & Verreth, J. A. J., 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquac. Eng. 43 (3), 83–93. https://doi.org/10.1016/j.aquaeng.2010.09.002.

Milhazes-Cunha, H., Otero, A., 2017. Valorisation of aquaculture effluents with microalgae: the integrated multi-trophic aquaculture concept. Algal Res. 24, 416–424. https://doi.org/10.1016/j.algal.2016.12.011.

Montalto, V., Martinez, M., Rinaldi, A., Sarà, G., Mirto, S., 2017. The effect of the quality of diet on the functional response of *Mytilus galloprovincialis* (Lamarck, 1819): implications for integrated multitrophic aquaculture (IMTA) and marine spatial planning. Aquaculture 468, 371–377. https://doi.org/10.1016/j.aquaculture.2016.10.030.

Mota, V.C., Martins, C.I., Eding, E.H., Canário, A.V., Verreth, J.A., 2014. Steroids accumulate in the rearing water of commercial recirculating aquaculture systems. Aquac. Eng. 62, 9–16. https://doi.org/10.1016/j.aquaeng.2014.07.004.

Nelson, E.J., MacDonald, B.A., Robinson, S.M.C., 2012. The absorption efficiency of the suspension-feeding sea cucumber, *Cucumaria frondosa*, and its potential as an extractive integrated multi-trophic aquaculture (IMTA) species. Aquaculture 370, 19–25. https://doi.org/10.1016/j.aquaculture.2012.09.029.

Neori, A., Guttman, L., Israel, A., Shpigel, M., 2019. Israeli-developed models of marine integrated multi trophic aquaculture (IMTA). J. Coast. Res. 86 (SI), 11–20. https://doi.org/10.2112/SI86-003.1.

Oliveira, V., Martins, P., Marques, B., Cleary, D.F., Lillebø, A.I., Calado, R., 2020. Aquaponics using a fish farm effluent shifts bacterial communities profile in halophytes rhizosphere and endosphere. Sci. Rep. 10 (1), 1–11. https://doi.org/10.1038/s41598-020-66093-8.

Omont, A., Elizondo-González, R., Quiroz-Guzmán, E., Escobedo-Fregoso, C., Hernández-Herrera, R., Peña-Rodríguez, A., 2020. Digestive microbiota of shrimp *Penaeus vannamei* and oyster *Crassostrea gigas* co-cultured in integrated multi-trophic aquaculture system. Aquaculture 521, 735059. https://doi.org/10.1016/j.aquaculture.2020.735059.

Orellana, J., Waller, U., Wecker, B., 2014. Culture of yellowtail kingfish (*Seriola lalandi*) in a marine recirculating aquaculture system (RAS) with artificial seawater. Aquac. Eng. 58, 20–28. https://doi.org/10.1016/j.aquaeng.2013.09.004.

Orlofsky, E., Chernoivanov, S., Asiag, A., Maor, I., Levi, N., Litaor, M.I., 2020. Biological reconditioning of sodium enriched zeolite by halophytes: case study of dairy farm effluent treatment. Int J Phytoremediat. 1–12. https://doi.org/10.1080/15226514.2020.1799932.

Ottinger, M., Clauss, K., Kuenzer, C., 2016. Aquaculture: relevance, distribution, impacts and spatial assessments–A review. Ocean Coast. Manag. 119, 244–266. https://doi.org/10.1016/j.ocecoaman.2015.10.015.

Pedersen, S., Wik, T., 2020. A comparison of topologies in recirculating aquaculture systems using simulation and optimization. Aquac. Eng. 89, 102059. https://doi.org/10.1016/j.aquaeng.2020.102059.

Pedro, C.A., Santos, M.S., Ferreira, S.M., Gonçalves, S.C., 2013. The influence of cadmium contamination and salinity on the survival, growth and phytoremediation capacity of the saltmarsh plant *Salicornia ramosissima*. Mar. Environ. Res. 92, 197–205. https://doi.org/10.1016/j.marenvres.2013.09.018.

Piirainen, M., Liebisch, O., Kadereit, G., 2017. Phylogeny, biogeography, systematics and taxonomy of *Salicornioideae* (*Amaranthaceae/Chenopodiaceae*) – A cosmopolitan, highly specialized hygrohalophyte lineage dating back to the Oligocene. Taxon 66 (1), 109–132. https://doi.org/10.12705/661.6.

Pinheiro, I., Arantes, R., do Espírito Santo, C.M., do Nascimento Vieira, F., Lapa, K.R., Gonzaga, L.V., Fett, R., Barcelos-Oliveira, J.L., Seiffert, W.Q., 2017. Production of the halophyte *Sarcocornia ambigua* and Pacific white shrimp in an aquaponic system with biofloc technology. Ecol. Eng. 100, 261–267. https://doi.org/10.1016/j.ecoleng.2016.12.024.

Pinheiro, I., Carneiro, R.F.S., do Nascimento Vieira, F., Gonzaga, L.V., Fett, R., de Oliveira Costa, A.C., Magallón-Barajas, F.J., Seiffert, W.Q., 2020. Aquaponic production of *Sarcocornia ambigua* and Pacific white shrimp in biofloc system at different salinities. Aquaculture 519, 734918. https://doi.org/10.1016/j.aquaculture.2019.734918.

Quintã, R., Santos, R., Thomas, D.N., Le Vay, L., 2015. Growth and nitrogen uptake by *Salicornia europaea* and *Aster tripolium* in nutrient conditions typical of aquaculture wastewater. Chemosphere 120, 414–421. https://doi.org/10.1016/j.chemosphere.2014.08.017.

Riquelme, J., Olaeta, J.A., Gálvez, L., Undurraga, P., Fuentealba, C., Osses, A., Orellana, J., Gallardo, J., Pedreschi, R., 2016. Nutritional and functional characterization of wild and cultivated *Sarcocornia neei* grown in Chile. Cien. Inv. Agr. 43 (2), 283–293. https://doi.org/10.4067/S0718-16202016000200011.

Samocha, T.M., Fricker, J., Ali, A.M., Shpigel, M., Neori, A., 2015. Growth and nutrient uptake of the macroalga *Gracilaria tikvahiae* cultured with the shrimp *Litopenaeus vannamei* in an integrated multi-trophic aquaculture (IMTA) system. Aquaculture 446, 263–271. https://doi.org/10.1016/j.aquaculture.2015.05.008.

Sari, D.R., Adharini, R.I., 2020. The utilization of *Gracilaria verrucosa* as fish processing wastewater biofilter. In: In E3S Web of Conferences (Vol. 147, p. 02022). EDP Sciences. https://doi.org/10.1051/e3sconf/202014702022.

Schardong, R.M.F., Moro, M.F., Bonilla, O.H., 2020. Aquaponic system with white shrimp *Litopenaeus vannamei* rearing and production of the plants *Batis maritima, Sarcocornia neei* and *Sporobolus virginicus*. Braz. Arch. Biol. Technol. 63 https://doi.org/10.1590/1678-4324-2020190118.

Shpigel, M., Ben-Ezra, D., Shauli, L., Sagi, M., Ventura, Y., Samocha, T., Lee, J.J., 2013. Constructed wetland with *Salicornia* as a biofilter for mariculture effluents. Aquaculture 412, 52–63. https://doi.org/10.1016/j.aquaculture.2013.06.038.

Skriptsova, A.V., Miroshnikova, N.V., 2011. Laboratory experiment to determine the potential of two macroalgae from the Russian far-east as biofilters for integrated multi-trophic aquaculture (IMTA). Bioresour. Technol. 102 (3), 3149–3154. https://doi.org/10.1016/j.biortech.2010.10.093.

Stabili, L., Cecere, E., Licciano, M., Petrocelli, A., Sicuro, B., Giangrande, A., 2019. Integrated multitrophic aquaculture by-products with added value: the Polychaete *Sabella spallanzanii* and the seaweed *Chaetomorpha linum* as potential dietary ingredients. Mar Drugs 17 (12), 677. https://doi.org/10.3390/md17120677.

Stewart, G.R., Lee, J.A., Orebamjo, T.O., 1973. Nitrogen metabolism of halophytes II. Nitrate availability and utilization. New Phytol. 72 (3), 539–546. https://doi.org/10.1111/j.1469-8137.1973.tb04405.x.

van Bussel, C.G., Schroeder, J.P., Wuertz, S., Schulz, C., 2012. The chronic effect of nitrate on production performance and health status of juvenile turbot (*Psetta maxima*). Aquaculture 326, 163–167. https://doi.org/10.1016/j.aquaculture.2011.11.019.

Ventura, Y., Sagi, M., 2013. Halophyte crop cultivation: the case for *Salicornia* and *Sarcocornia*. Environ. Exp. Bot. 92, 144–153. https://doi.org/10.1016/j.envexpbot.2012.07.010.

Ventura, Y., Wuddineh, W.A., Ephrath, Y., Shpigel, M., Sagi, M., 2010. Molybdenum as an essential element for improving total yield in seawater-grown *Salicornia europaea* L. Sci. Hortic. 126 (3), 395–401. https://doi.org/10.1016/j.scienta.2010.07.015.

Ventura, Y., Wuddineh, W.A., Shpigel, M., Samocha, T.M., Klim, B.C., Cohen, S., Shemer, Z., Santos, R., Sagi, M., 2011. Effects of day length on flowering and yield production of *Salicornia* and *Sarcocornia* species. Sci. Hortic. 130 (3), 510–516. https://doi.org/10.1016/j.scienta.2011.08.008.

Waller, U., Buhmann, A.K., Ernst, A., Hanke, V., Kulakowski, A., Wecker, B., Orellana, J., Papenbrock, J., 2015. Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. Aquac. Int. 23 (6), 1473–1489. https://doi.org/10.1007/s10499-015-9898-3.

Webb, J.M., Quintã, R., Papadimitriou, S., Norman, L., Rigby, M., Thomas, D.N., Le Vay, L., 2012. Halophyte filter beds for treatment of saline wastewater from aquaculture. Water Res. 46 (16), 5102–5114. https://doi.org/10.1016/j.watres.2012.06.034-.

Zhang, S.Y., Li, G., Wu, H.B., Liu, X.G., Yao, Y.H., Tao, L., Liu, H., 2011. An integrated recirculating aquaculture system (RAS) for land-based fish farming: the effects on water quality and fish production. Aquac. Eng. 45 (3), 93–102. https://doi.org/10.1016/j.aquaeng.2011.08.001.