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# Testing the hydroponic performance of the edible halophyte *Halimione portulacoides*, a potential extractive species for coastal Integrated Multi-Trophic Aquaculture

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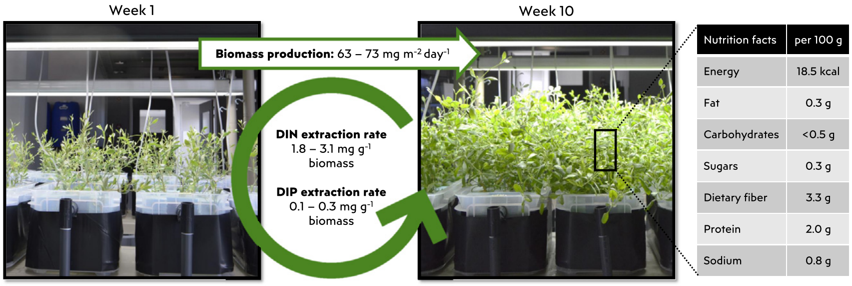
## HIGHLIGHTS

- *Halimione portulacoides* grew efficiently in saline hydroponic conditions.
- DIN-N extraction rates were 1.8–3.1 mg $g^{-1}$ of biomass produced.
- DIP-P extraction rates were 0.1–0.3 mg $g^{-1}$ of biomass produced.
- Yields under non-limited conditions of N and P were 63.0–73.0 g $m^{-2}$ $day^{-1}$.
- Leaves' nutritional profile was comparable to other halophytes and leafy greens.

## GRAPHICAL ABSTRACT



| Nutrition facts | per 100 g |
|---|---|
| Energy | 18.5 kcal |
| Fat | 0.3 g |
| Carbohydrates | <0.5 g |
| Sugars | 0.3 g |
| Dietary fiber | 3.3 g |
| Protein | 2.0 g |
| Sodium | 0.8 g |

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## ABSTRACT

Sea purslane *Halimione portulacoides* (L.) Aellen is a candidate extractive species for coastal Integrated Multi-Trophic Aquaculture (IMTA) to recycle the dissolved inorganic nitrogen (DIN) and phosphorus (DIP) wasted by excretive species. To test its suitability, saline aquaculture effluents were simulated in the laboratory using a hydroponics approach to cultivate the plants. Nutrient extraction efficiency, growth performance and nutritional profile were assessed under a range of DIN and DIP concentrations representing three different aquaculture intensification regimes and using Hoagland's solution as a control. Over a 10-week period, hydroponic units under non-limited N and P conditions displayed daily extraction rates between 1.5 and 2.8 mg DIN-N $L^{-1}$ $day^{-1}$ and 0.1–0.2 mg DIP-P $L^{-1}$ $day^{-1}$ and yielded between 63.0 and 73.0 g $m^{-2}$ $day^{-1}$ of *H. portulacoides* biomass. Relatively to biomass produced, *H. portulacoides* extracted between 2.6 and 4.2 mg DIN-N $g^{-1}$ and 0.1–0.4 mg DIP-P $g^{-1}$. The treatment with low-input of DIN and DIP (6.4 mg N $L^{-1}$ and 0.7 mg P $L^{-1}$) induced some degree of nutrient limitation, as suggested by the extremely high extraction efficiencies of DIN extraction (99%) in parallel with lower productivity. The nutritional profile of *H. portulacoides* leaves is comparable to that of other edible halophytes and leafy greens and could be a low-sodium alternative to salt in its lyophilized form. From the present study, we conclude that the edible halophyte *H. portulacoides* can be highly productive in hydroponics using saline water irrigation with non-limiting concentrations of DIN and DIP and is, therefore, a suitable extractive species for coastal IMTA in brackish waters.

## 1. Introduction

In the European Union, aquaculture must comply with different policies (e.g. Marine Strategy Framework Directive, Water Framework Directive, Birds Directive, Habitats Directive, Marine Spatial Planning

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Directive) that address broad environmental issues and aim at promoting sustainable economic development (Science for Environment Policy 2015). The need to respect environmental legislation led to the development of a more sustainable aquaculture production model known as Integrated Multi-Trophic Aquaculture (IMTA). IMTA entails the integrated production of low trophic organisms that recover and utilize the relatively high amounts of nutrients wasted in different physical (particulate and dissolved) and chemical forms (organic and inorganic) during the production cycle of artificially fed organisms (e.g. fish, shrimp) (Buck et al. 2018; Chopin et al. 2012; Granada et al. 2016). A recent more utilitarian definition of IMTA was proposed within the scope of the INTEGRATE Project, which states that IMTA is the "enhanced production of aquatic organisms (with or without terrestrial organisms) of two or more functional groups, that are trophically connected by demonstrated nutrient flows and whose biomass is fully or partially removed by harvesting to facilitate ecological balance" (Dunbar et al. 2020).

Commercially relevant fed-aquaculture species, such as the Atlantic salmon (*Salmo salar*), the rainbow trout (*Oncorhynchus mykiss*) and the Pacific white shrimp (*Litopenaeus vannamei*), are not that efficient at utilizing proteins from feeds as they normally display average protein retention efficiencies <30% (Fry et al. 2018; Ytrestøyl et al. 2015).Previous estimations suggest total nitrogen (N) losses in fish-farms can reach percentages as high as 60–80% of total N-input from aquafeeds, while total phosphorus (P) losses can reach 70–85% of total P-input (Islam 2005; Wang et al. 2012). Besides promoting eutrophication, the buildup of nutrients in the ecosystem can also shift benthic chemistry and disturb ecological interactions (Bannister et al. 2014; Sanz-Lázaro et al. 2011; Sarà et al. 2011; Troell et al. 2009; Valdemarsen et al. 2012), especially if the dilution/carrying capacity of the ecosystem is compromised (Guillen et al. 2019). These lost nutrients, in both particulate and dissolved forms, can be used by non-fed extractive organisms in IMTA settings: filter feeders (e.g. bivalves, sponges, small crustaceans) can filtrate the suspended particulate matter, the deposit feeders (e.g. sea cucumbers, polychaetas, sea urchins) can feed on deposited particulate matter and the primary producers (e.g. plants, algae) can extract dissolved nutrients from the water (Chopin et al. 2012; Troell et al. 2009). Several publications have already addressed IMTA from different scientific perspectives, demonstrating its environmental and economic benefits (e.g. Abreu et al. 2011; Barrington et al. 2010; Buck et al. 2018; Chopin 2015; Fang et al. 2016; Granada et al. 2016; Hughes and Black 2016; Kleitou et al. 2018; Knowler et al. 2020; Li et al. 2019).

Halophytes are a particularly interesting group of plants due to their ability to thrive in saline environments (Flowers et al. 1986; Flowers and Colmer 2008) and their proven agricultural uses (Panta et al. 2014), which make them potentially suitable extractive species for IMTA in brackish waters (Gunning et al. 2016). Previous research indicates consistent positive outcomes in terms of productivity and nutrients extraction capacity, using either constructed wetlands or hydroponics/aquaponics systems as halophytes extraction units for IMTA (Custódio et al. 2017). Even if mostly unknown to the general public, many halophyte species are in fact suitable for human consumption (Barreira et al. 2017; Loconsole et al. 2019) and are a rich source of bioactive secondary metabolites with commercial applications (e.g. nutra-, pharma- and cosmeceuticals) (Buhmann and Papenbrock 2013; Ksouri et al. 2012; Maciel et al. 2016; Patel et al. 2019; Rodrigues et al. 2014). These features indicate the existence of a rather untapped economic potential that can prompt the integration of the most suitable halophyte species into IMTA production frameworks.

The main objective of the present study is to evaluate the productivity, nutritional profile and nutrient extraction capacity of *Halimione portulacoides* (L.) Aellen, a common edible halophyte of European saltmarshes, in saline hydroponic conditions to understand its horticultural potential for IMTA in brackish waters. To mimic real aquaculture effluents, different combinations of dissolved inorganic N and P concentrations were selected based on values reported in the literature to represent semi-intensive (6.0 mg N $L^{-1}$; 0.8 mg P $L^{-1}$), intensive (20.0 mg N $L^{-1}$, 3.0 mg P $L^{-1}$) and super-intensive (100.0 mg N $L^{-1}$, 6.0 mg P $L^{-1}$) aquaculture effluents.

## 2. Materials and methods

### 2.1. Plant material

*Halimione portulacoides* stems were collected in Ria de Aveiro coastal lagoon (40°38′04.1″N 8°39′40.0″W) in April 2017. Stems were cut-off from healthy fully-grown plants and brought to the laboratory to produce grafts. Grafts with 4 nodes were placed in polyethylene containers with Hoagland's solution under natural conditions of light and temperature to promote root development. Elemental nutrient concentrations in the solution were as follows: 40.00 mg Ca $L^{-1}$, 60.00 mg K $L^{-1}$, 16.00 mg Mg $L^{-1}$, 56.00 mg N $L^{-1}$, 16.00 mg P $L^{-1}$, 0.28 mg B $L^{-1}$, 0.03 mg Cu $L^{-1}$, 1.12 mg Fe $L^{-1}$, 0.11 mg Mn $L^{-1}$, 0.34 mg Mo $L^{-1}$, 0.13 mg Zn $L^{-1}$.

After three months, on July 2017, rooted grafts underwent a week of acclimation to controlled indoor conditions and salinity prior to the start of the experiment. Plants were progressively adapted to a salinity of 20 ppt by adding 0.5, 1.0, 1.5 and 2.0% artificial sea salt to the Hoagland's solution consecutively and every two days. Grafts with similar weights were randomly selected and distributed across the experimental hydroponic units.

### 2.2. Experimental setup

Opaque polypropylene boxes (interior volume: 270 × 170 × 170 mm) were used for the hydroponic units and the indoor grow-out experiment lasted 10 weeks. The nutrient extraction efficiency was assessed for nine weeks (from week two to week nine). Experimental hydroponic units were designed to be a deep-water culture type hydroponics. Each unit had a 30 mm thick extruded polystyrene raft floating on the water column. Rafts were perforated with 10 holes (20 mm wide) to insert plants. Plants were fixed in place with natural cotton. An overflow inlet was created (at 110 mm from the bottom) to keep the water volume within five liters in each unit and the water column was continuously aerated by an air stone connected to a small aerator. Units were refilled with reverse osmosis water to compensate for evapotranspiration as needed. The basis for the nutrient solution was artificial seawater with a salinity of 20 ppt, prepared by dissolving commercial Red Sea salt (Red Sea, Cheddar, UK) in tap water purified by reverse osmosis (V2Pure 360 RO System, TMC, Hertfordshirem UK). The photoperiod was 14 h light: 10 h dark and hydroponic units were illuminated by tubular fluorescent white lamps (Philips 54 W/830 Min Bipin T5 HO ALTO UNP) delivering an average photosynthetically active radiation (PAR) of ~320 μmol $m^{-2}$ $s^{-1}$ (canopy top), checked twice a week with a spherical micro quantum sensor (US-SQS/L, Heinz Walz, Pfullingen, Germany). Water temperature and pH were measured with multi-parameter portable meter (ProfiLine pH/Cond 3320, WTW, Weilheim, Germany) and dissolved oxygen was measured with a portable oxygen meter (Oxi 3310, WTW, Weilheim, Germany), carefully following the operation and calibration instructions provided in the manufacturer's operating manuals.

The experimental design consisted of four nutrient solutions (including a control solution) and five replicates per treatment, in a total of twenty hydroponic units. From a total of 500 grafts produced, 220 were selected based on the average size, and 200 were randomly distributed across the experimental units, in a total of ten plants per unit (the remaining 20 grafts were used to measure initial aboveground and belowground biomass). The random distribution followed a randomized sequence of integers from 1 to 200, distributed throughout 20 columns (=hydroponic units) and 10 rows (=position in the floating raft), generated with a tool provided in https://www.random.org. Plant density was equivalent to 220 plants $m^{-2}$.

The control solution was the modified Hoagland's solution used for grafts development as described above, which guaranteed non-limited nutritional conditions (Control = 56.0 mg N $L^{-1}$ and 15.5 mg P $L^{-1}$). The three treatment solutions consisted of different combined concentrations of N and P to represent the wide range of values recorded across the fish-farming intensification continuum, i.e., super-intensive, intensive and semi-intensive land-based marine fish farms. Hoagland's solution, prepared with saline water, was used as control as it ensures that plants were not limited by nutrients, corresponding in this way to optimal nutritional conditions. The published literature on the remediation of saline aquaculture effluents by halophytes was consulted in order to select realistic N: P combinations corresponding to semi-intensive (low [N, P]), intensive (medium [N, P]) and super-intensive (high [N, P]) effluents (Buhmann et al. 2015; Lin et al. 2005; Quintã et al. 2015; Waller et al. 2015; Webb et al. 2013). Treatment labels and theoretical concentrations of N and P chosen as the treatment solutions were: $[N,P]_{low}$ = 6.0 mg N $L^{-1}$ and 0.8 mg P $L^{-1}$; $[N,P]_{med}$ = 20.0 mg N $L^{-1}$ and 3.0 mg P $L^{-1}$; $[N,P]_{high}$ = 100.0 mg N $L^{-1}$ and 6.0 mg P $L^{-1}$. All other macro- and micro-nutrients were kept equal across treatments. Detailed elemental composition of experimental treatments is presented in Table S1 as Supplementary Material.

### 2.3. Hydroponics media analysis

Retention times (RTs; the time wastewater remains in a remediation tank) used in remediation studies are highly variable, spanning from a couple of hours to several days, depending on the desired efficiencies (Toet et al. 2005). In constructed wetlands, higher RTs are positively correlated with higher N and P extraction efficiencies and time recommendations for significant extraction of contaminants are between 3 and 10 days (García et al. 2010; Vera et al. 2016; Wu et al. 2015). Nutrient extraction studies with halophytes in the context of IMTA have used a wide range of RTs, from 12 h (Marques et al. 2017) to five weeks (Buhmann et al. 2015). After taking into consideration the range of RTs used in previous studies and the above recommendations, one week (seven days) was considered a reasonable and operationalizable RT for IMTA to allow for substantial extraction efficiencies.

Hydroponic media samples were collected from the hydroponic units at the end of every extraction period to obtain final N and P concentrations. Initial media samples were collected from each treatment-solution batch to determine the initial N and P concentrations and calculate weekly mass-balances. Each sample was filtered (Whatman GF/C, 1.2 μm pore size) and stored at −20 °C prior to analysis.

A Skalar San$^{++}$ Continuous Flow Analyzer (Skalar Analytical, Breda, The Netherlands) was used to determine dissolved ammonium ($NH_4$-N), nitrogen oxides ($NO_x$-N) and orthophosphate ($PO_4$-P) concentrations in media samples, using Skalar's standard automated methods for $NH_4$-N (Modified Berthelot reaction for ammonia determination), $NO_x$-N (Total UV digestible nitrogen / nitrate + nitrite / nitrite) and $PO_4$-P (Total UV digestible phosphate / orthophosphate). Dissolved inorganic nitrogen (DIN-N) was calculated as the sum of $NH_4$-N and $NO_x$-N and dissolved inorganic phosphorus (DIP-P) corresponded to $PO_4$-P.

### 2.4. Growth parameters

Halophyte grafts were identified, individually photographed and weighed prior to being distributed throughout the hydroponic units. The remaining 20 rooted grafts from the 220 grafts selected were used to establish the initial weight condition for above- and belowground biomass. At the end of the experiment, plants were again individually photographed, separated into above- and belowground parts and weighed. Aboveground biomass was further separated into edible (leaves) and non-edible biomass (stems), since the edible biomass was to be analyzed for its nutritional profile. Leaves were pooled by experimental unit and stored at −80 °C until further analysis. Photos were analyzed with an image processing software (ImageJ 1.51) to measure the stems and count the leaves.

### 2.5. Nutritional profile analysis

A nutritional analysis was carried out on homogeneous samples of the pooled biomass of leaves from each experimental unit to determine the nutritional profile of *H. portulacoides* leaves and assess any potential changes promoted by the availability of N and P. The parameters analyzed were ash, carbohydrates, crude protein, dietary fiber, energy, fat, moisture, sodium and sugars. All values are presented in grams per 100 g of wet weight (WW), except for energy which is presented as kJ per 100 g of WW. Nutritional parameters were analyzed by a certified laboratory, following internal analytical procedures (Mérieux NutriSciences, Vila Nova de Gaia, Portugal).

### 2.6. Statistical analysis

Statistical analysis was performed using R v3.4.3 (64-bit) software in combination with R Studio v1.1. Data were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) to inform about the appropriate test. One-way ANOVA was used to compare average growth, nutritional and extraction measurements. *Post-hoc* Tukey's HSD test for individual means comparison was performed when significance was observed. Non-parametric Kruskal-Wallis test was used whenever data failed to meet ANOVA assumptions, followed by a Wilcoxon signed-rank test for pairwise comparison, if statistical significance was detected. Repeated measures ANOVA was used to assess changes in average extraction efficiencies (%) in time. Geenhouse-Geisser correction was employed when the sphericity assumption was violated. Bonferroni *post-hoc* test was used for pairwise comparison. Significant differences were considered at $p < 0.05$ in all statistical tests.

## 3. Results and discussion

### 3.1. Environmental conditions

Average water temperature in hydroponic units was 22.2 ± 1.3 °C, salinity was 20.3 ± 0.3 ppt, dissolved oxygen was 7.2 ± 0.5 mg $L^{-1}$ and PAR (measured at the top of the canopy) was 317.5 ± 52.9 μmol $m^{-2}$ $s^{-1}$. The average pH, measured at the end of the extraction period, was 7.7 ± 0.1 during the first five weeks. At the end of the week, the pH dropped to values between 6.6 and 6.8 in all treatments, which coincided with an abrupt increase in room temperature overnight due to a failure in the ventilation system. This event resulted in a consequent increase in water temperature of ~2.4 °C above the average values registered in the week prior (Table S2 as Supplementary Material). Plants were exposed to an increase in water temperature for a maximum of 16 h, since solutions were renewed in the following morning, corresponding to the end of a remediation period. From this point on, $[N,P]_{high}$ units consistently displayed an acidic pH ($6 < pH < 7$), contrary to the other treatments which displayed values > 7 in the following weeks (Table S2). Average pH values in each treatment condition during the entire experimental period were: $[N,P]_{low}$ = 7.5 ± 0.3; $[N,P]_{med}$ = 7.5 ± 0.3; $[N,P]_{high}$ = 6.9 ± 0.8 and Control = 7.4 ± 0.4.

### 3.2. DIN and DIP extraction efficiencies

The initial concentrations of DIN-N and DIP-P measured in each treatment solution were as follows: $[N,P]_{low}$ = 6.38 ± 0.15 mg DIN-N $L^{-1}$ and 0.68 ± 0.04 mg DIP-P $L^{-1}$; $[N,P]_{med}$ = 20.83 ± 0.53 mg DIN-N $L^{-1}$ and 2.76 ± 0.20 mg DIP-P $L^{-1}$; $[N,P]_{high}$ = 101.47 ± 2.66 mg DIN-N $L^{-1}$ and 5.08 ± 0.24 mg DIP-P $L^{-1}$; and Control (modified Hoagland's solution) = 55.58 ± 5.99 mg DIN-N $L^{-1}$ and 11.85 ± 0.94 mg DIP-P $L^{-1}$.

DIN-N extraction efficiencies in each hydroponic unit were measured at the end of each extraction period (Fig. 1A) and a repeated-measures ANOVA determined that 'treatment', 'time' and their interaction had a significant main effect ($p < 0.001$) in extraction efficiencies. *Post-hoc* tests revealed that units under $[N,P]_{low}$ and $[N,P]_{med}$ were significantly more efficient on average ($p < 0.004$) than both $[N,P]_{high}$ and Control, a direct result of the lower concentration of DIN-N present in the former treatments. Since an interaction effect of 'treatment' and 'time' was present, statistical analyses were performed to determine differences in efficiency i) between treatments at each time point and ii) within treatments across time-points. The non-parametric Kruskal-Wallis test was used for i) and, at all time-points, 'treatment' had a significant main effect in DIN-N extraction efficiency ($p < 0.01$). Pairwise comparisons revealed that plants under Control were less efficient ($p < 0.05$) than $[N,P]_{low}$ from weeks 2 to 5 and plants under $[N,P]_{high}$ were less efficient ($p < 0.05$) than both $[N,P]_{low}$ and $[N,P]_{med}$ from weeks 2 to 10. Moreover, plants under $[N,P]_{low}$ and Control did not display significant changes in their extraction efficiency over time ($p = 0.35$ and $p = 0.15$, respectively). On the other hand, the factor 'time' significantly affected ($p = 0.01$) the extraction efficiencies of plants under $[N,P]_{med}$ and $[N,P]_{high}$. A pairwise comparison revealed that $[N,P]_{med}$ units were more efficient ($p < 0.05$) in week 9 compared with week 2, and $[N,P]_{high}$ units were less efficient ($p < 0.05$) in week 6 compared with all other weeks, except week 5.

In terms of the total levels of DIN-N extracted (Fig. 2A), the Control units extracted the most, with a total of 882.4 ± 284.8 mg ($=2.8 \pm 0.9$ mg $L^{-1}$ $day^{-1}$) extracted, followed by $[N,P]_{med}$ units which extracted a total of 736.8 ± 125.9 mg ($=2.3 \pm 0.4$ mg $L^{-1}$ $day^{-1}$). The $[N,P]_{high}$ units extracted significantly less DIN-N than Control ($p = 0.01$), with a total of 483.8 ± 179.5 mg ($=1.5 \pm 0.6$ mg $L^{-1}$ $day^{-1}$). The $[N,P]_{low}$ units removed 284.3 ± 0.5 mg, essentially the full amount of supplied DIN-N, indicating the onset of N-limitation during the extraction period and cannot be compared with the other treatments. Overall $[N,P]_{low}$ units extracted, on average, 99% of the total N input,

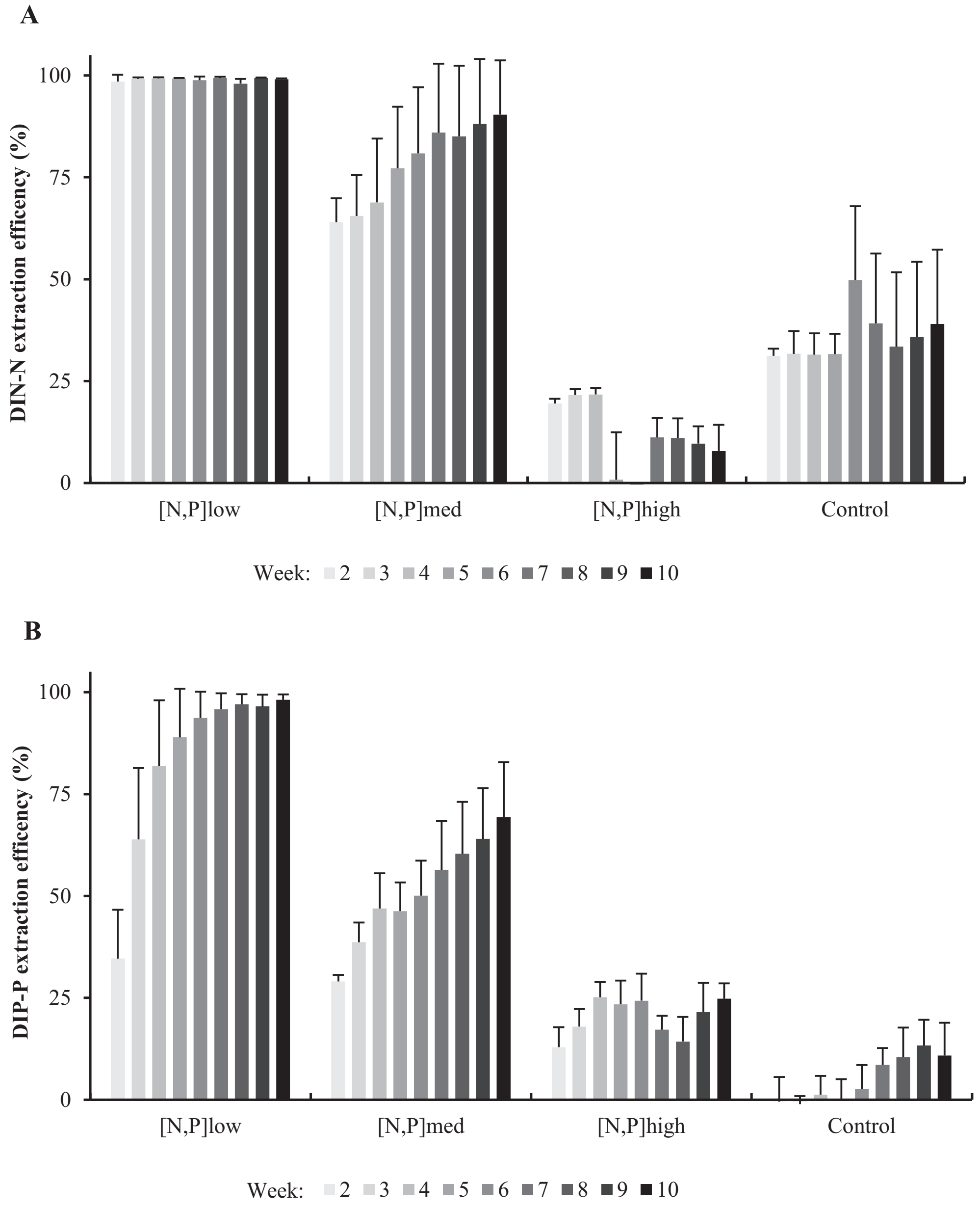

**Fig. 1.** Weekly extraction efficiencies of DIN-N (A) and DIP-P (B). Bars represent standard deviations ($n = 5$). Test-statistic: Repeated measures ANOVA (statistical results in 'Results' section). Treatments: $[N,P]_{low} = 6$ mg N $L^{-1}$ & 0.8 mg P $L^{-1}$; $[N,P]_{med} = 20$ mg N $L^{-1}$ & 3.0 mg P $L^{-1}$; $[N,P]_{high} = 100$ mg N $L^{-1}$ & 6.0 mg P $L^{-1}$; Control = 56 mg N $L^{-1}$ & 15.5 mg P $L^{-1}$.

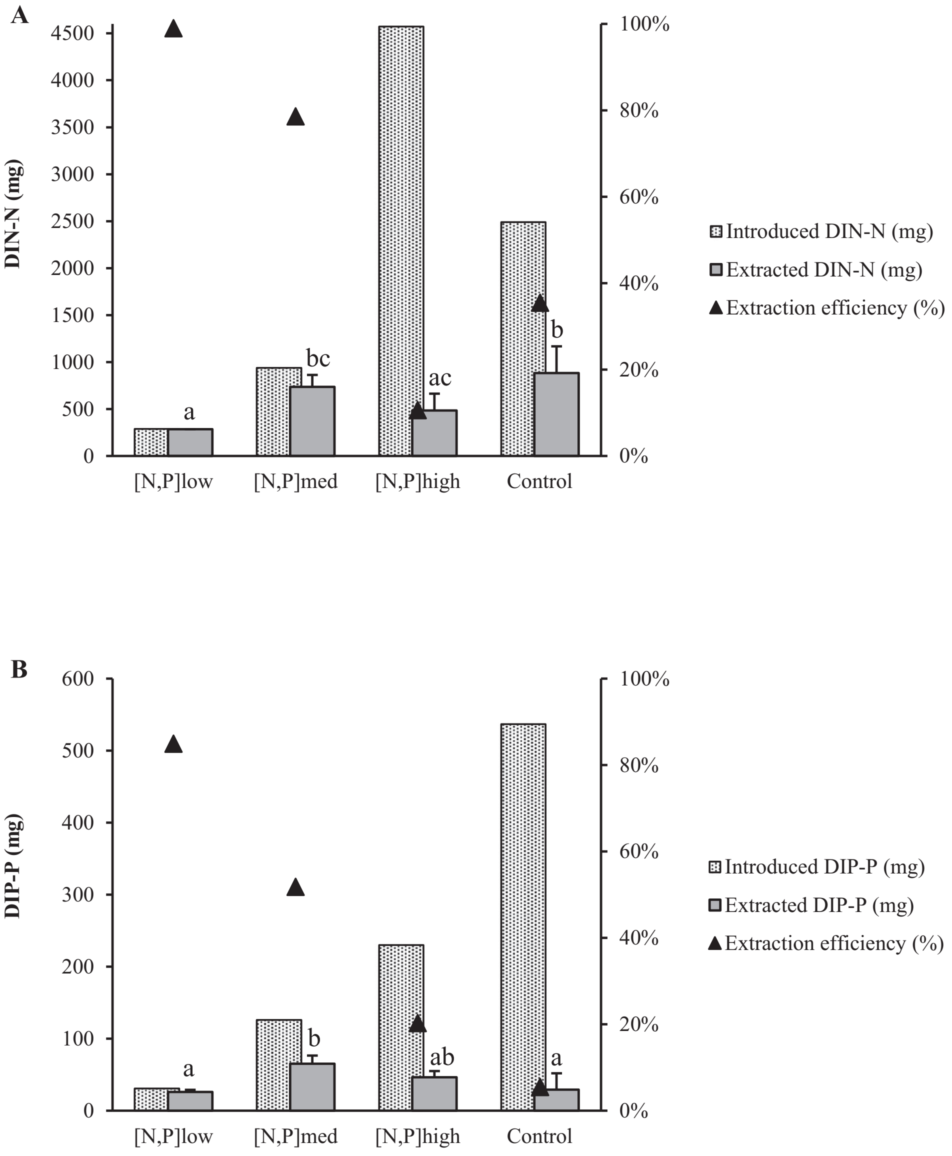

**Fig. 2.** Total extracted DIN-N (A) and DIP-P (B) from week-2 to week-10 (9 weeks). 'Extracted DIN-N' vertical bars represent the standard deviations. ($n = 5$). Test-statistic: One-way ANOVA & Tukey HSD's test for pairwise comparison, with different letters showing significant differences in 'Extracted DIN-N' between treatments ($p < 0.05$). Treatments: $[N,P]_{low} =$ 6.0 mg N $L^{-1}$ & 0.8 mg P $L^{-1}$; $[N,P]_{med} =$ 20.0 mg N $L^{-1}$ & 3.0 mg P $L^{-1}$; $[N,P]_{high} =$ 100.0 mg N $L^{-1}$ & 6.0 mg P $L^{-1}$; Control = 56.0 mg N $L^{-1}$ & 15.5 mg P $L^{-1}$.

$[N,P]_{med}$ units extracted 79%, $[N,P]_{high}$ units 11% and Control units 35%. The normalization of total DIN-N extracted by the total biomass produced (Fig. 3A) suggests that certain nutritional conditions might promote higher extraction rates per unit of biomass. $[N,P]_{med}$ and Control units extracted on average 4.2 (±0.3) and 4.2 (±0.6) mg DIN-N $g^{-1}$ of biomass respectively, which was significantly more ($p < 0.05$) than $[N,P]_{high}$ (2.5 ± 0.7 mg DIN-N $g^{-1}$ of plant). $[N,P]_{low}$ also displayed lower rates but as a result of the total depletion of DIN-N during the extraction period.

Regarding DIP-P extraction results, repeated measures ANOVA determined that 'treatment' and the interaction of 'treatment' with 'time' had a significant main effect ($p < 0.001$) in the extraction efficiencies of DIP-P (Fig. 1B). *Post-hoc* tests revealed that all treatments significantly differed from each other in terms of average extraction efficiency ($p < 0.04$), with $[N,P]_{low}$ and $[N,P]_{med}$ displaying the highest efficiencies (associated with the lower concentrations of DIP-P in those treatments compared with $[N,P]_{high}$ and Control). Kruskal-Wallis test, performed at each time-point, revealed that 'treatment' had a significant effect in the extraction efficiencies ($p < 0.01$) and pairwise comparisons showed that Control removed significantly less DIP-P ($p < 0.05$) than $[N,P]_{low}$ at all time-points and $[N,P]_{med}$ from weeks 2 to 6. Units under $[N,P]_{high}$ removed significantly less ($p < 0.05$) than $[N,P]_{low}$ from weeks 5 to 10. Moreover, 'time' had a significant main effect ($p < 0.02$) in DIP extraction efficiencies within all treatments. Pairwise comparisons revealed that the extraction efficiency in $[N,P]_{low}$ units was significantly lower ($p < 0.05$) only in week 2 compared with the other weeks, and $[N,P]_{med}$ did not display significant changes between extraction periods. $[N,P]_{high}$ units were significantly less efficient ($p < 0.05$) in week 8 compared with weeks 4 and 9, and Control units were less efficient ($p < 0.05$) in week 2 compared with week 9.

In terms of the total quantities of DIP-P extracted (Fig. 2B), the Control units extracted a total of 29.3 ± 22.6 mg (=0.09 ± 0.07 mg $L^{-1}$ $day^{-1}$), which was significantly less ($p < 0.01$) than $[N,P]_{med}$, with a total of 65.3 ± 11.2 mg (=0.21 ± 0.04 mg $L^{-1}$ $day^{-1}$) extracted. $[N,P]_{high}$ units extracted 46.6 ± 8.5 mg (0.15 ± 0.03 mg $L^{-1}$ $day^{-1}$) and $[N,P]_{low}$ extracted 26.1 ± 2.9 mg (0.08 ± 0.01 mg $L^{-1}$ $day^{-1}$), which was close to total input suggesting possible P-limitation during the experimental period. Overall $[N,P]_{low}$ units extracted, on average, 85% of the

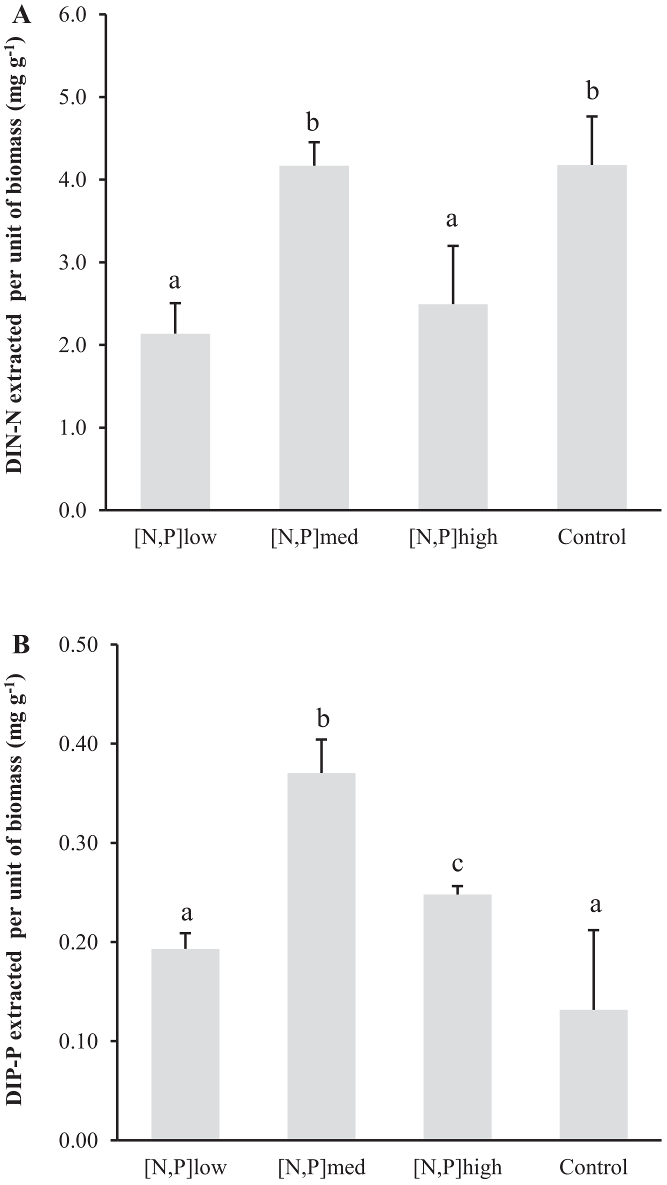

**Fig. 3.** Extracted DIN-N (A) and DIP-P (B) per unit of biomass produced. Vertical bars represent the standard deviations ($n = 5$). Test-statistic: One-way ANOVA & pairwise Tukey HSD's test (DIN-N) and non-parametric Kruskal-Wallis test & pairwise Wilcoxon signed-rank test (DIP-P), with different letters showing significant differences between treatments ($p < 0.05$). Treatments: $[N,P]_{low} = 6.0$ mg N $L^{-1}$ & 0.8 mg P $L^{-1}$; $[N,P]_{med} = 20.0$ mg N $L^{-1}$ & 3.0 mg P $L^{-1}$; $[N,P]_{high} = 100.0$ mg N $L^{-1}$ & 6.0 mg P $L^{-1}$; Control = 56.0 mg N $L^{-1}$ & 15.5 mg P $L^{-1}$.

total P input, $[N,P]_{med}$ units extracted 52%, $[N,P]_{high}$ units 20% and Control units 5%. After normalizing total DIP-P extracted by the total biomass produced (Fig. 3B), $[N,P]_{med}$ emerged as the condition with the highest rate of DIP-P extracted, 0.37 (±0.03) mg DIP-P $g^{-1}$, significantly higher ($p < 0.05$) than the other treatments.

By testing a wide range of N and P concentrations in this study, it was possible to estimate the extraction capacity of *H. portulacoides* hydroponic units and, excluding the $[N,P]_{low}$ results (due to evidence of nutrient limitation), daily extraction rates varied between 1.5–2.8 mg DIN-N $L^{-1}$ $day^{-1}$ and 0.1–0.2 mg DIP-P $L^{-1}$ $day^{-1}$. Relatively to biomass production, *H. portulacoides* extracted between 1.8–3.1 mg DIN-N $g^{-1}$ and 0.1–0.3 mg DIP-P $g^{-1}$ of biomass produced.

Previous studies have used *H. portulacoides* to extract N and P, displaying different outcomes as a results of different experimental conditions. Using hydroponic modules, Marques et al. (2017) exposed *H. portulacoides* to real aquaculture effluents with N and P concentrations very similar to those of $[N,P]_{low}$ conditions (salinity of 20 ppt; 9.0 mg DIN-N $L^{-1}$ and 0.3 mg DIP-P $L^{-1}$). However, extraction efficiencies were much lower, which can be partially explained by a much smaller RT of 12 h, resulting in only 65% reduction in DIN and a surprising 27% increase in DIP (probably due to the mineralization of organic matter). In another experiment, Buhmann et al. (2015) exposed *H. portulacoides* to N and P concentrations similar to the Control treatment but using a much longer RT of 5 weeks, achieving better extraction efficiencies than the present study (58% reduction in DIN and a 51% reduction in DIP). Waller et al. (2015) irrigated *Aster tripolium* L. and *Salicornia dolichostachya* Moss with an artificial effluent with 19.0 mg N $L^{-1}$ and 3.0 mg P $L^{-1}$ (similar to $[N,P]_{med}$) and employing an RT of 1 day. However, DIP extraction efficiencies were practically close to zero and only *S. dolichostachya* was capable of removing some of the DIN, with a 20% efficiency. These observations show the importance of choosing appropriate RTs, depending on the concentration of nutrients, in order to promote a substantial extraction of N and P.

In general, most studies available in the literature which assessed halophytes' extraction capacity in the context of IMTA, have been performed using constructed wetlands or aquaponics (using inert media) and only a few have used soilless hydroponic systems (Custódio et al. 2017). Nonetheless, a general observation (regardless of the plant extraction module employed) is the difficulty to reliably compare extraction efficiencies from different studies due to a great diversity of production variables potentially affecting performance.

### 3.3. Growth performance

Growth parameters were determined for each hydroponic unit by pooling measurements from each plant ($n = 10$). Each group started the experimental grow-out period with average initial biomass between 44.6 and 49.3 g per hydroponic unit (Table 1). At week 10, the Control displayed the highest total biomass (279.4 ± 44.7 g), which was significantly higher ($p = 0.02$) than $[N,P]_{low}$ (195.9 ± 28.7 g) (Table 1).

Vegetative development over the experimental period can be visualized in Fig. S1 (in the Supplementary Material). The $[N, P]_{low}$ units yielded a significantly lower aboveground biomass (155.2 ± 16.7 g) compared with Control (245.4 ± 40.4 g; $p = 0.003$), $[N, P]_{med}$ (216.3 ± 36.5; $p = 0.048$) as well as $[N, P]_{high}$ (228.6 ± 35.4 g; $p = 0.015$). The belowground biomass and the root: shoot ratio of plants growing under $[N, P]_{low}$ were higher than the other treatments, but only root: shoot differences statistically significant ($p < 0.0001$). Results suggest the higher ratio resulted from a lower aboveground development rather than a higher belowground development. The number of leaves was lowest in $[N,P]_{low,}$ but differences were not significant. The sum of all stems lengths per hydroponic unit was significantly lower in [N,P]low compared with Control ($p = 0.002$) and the other treatments ($p < 0.05$).

From these results, it follows that *H. portulacoides* was indeed affected by the availability of DIN and DIP, as differences in biomass allocation and a reduction in vegetative growth were observed in $[N,P]_{low}$ units, compared to the other conditions. Prioritizing the allocation of resources towards increasing root area at the expense of aerial growth is typically observed in plants under nutrient-limited conditions (Ågren and Franklin 2003; Bonifas et al. 2005; Gedroc et al. 1996; Levang-Brilz and Biondini 2003) and evidence from this study suggests plants in $[N.P]_{low}$ were nutrient-limited as they favored root development and showed a decline in total productivity. Because N and P are both essential elements involved in numerous biological and physiological processes in plants (e.g. genetic material, transfer of energy), the low availability of those nutrients will constrain plant development (Hopkins and Huner 2008). In the other experimental conditions, *H. portulacoides* displayed similar productivity and aerial development suggesting that plants were not nutrient-limited at concentrations of at least 20.0 mg DIN-N $L^{-1}$ and 3.0 mg DIP-P $L^{-1}$.

**Table 1**

Growth performance of *Halimione portulacoides* hydroponic units (mean ± standard deviation). Values presented are pooled measurements of the individual plants in each hydroponic unit. Test-statistic: One-way ANOVA & Tukey HSD's test for pairwise comparison, different letters indicate statistically significant differences between treatments: $p < 0.05$. Treatments: $[N,P]_{low}$ = 6.0 mg N L$^{-1}$ & 0.8 mg P L$^{-1}$; $[N,P]_{med}$ = 20.0 mg N L$^{-1}$ & 3.0 mg P L$^{-1}$; $[N,P]_{high}$ = 100.0 mg N L$^{-1}$ & 6.0 mg P L$^{-1}$; Control = 56.0 mg N L$^{-1}$ & 15.5 mg P L$^{-1}$.

| | Unit | $[N,P]_{low}$ | $[N,P]_{med}$ | $[N,P]_{high}$ | Control |
|---|---|---|---|---|---|
| Initial total biomass (per unit) | g | 44.6 ± 6.0 | 47.4 ± 6.3 | 49.3 ± 6.2 | 48.6 ± 2.1 |
| Final total biomass (per unit) | g | 195.9 ± 28.7$^{a}$ | 245.5 ± 39.7$^{ab}$ | 257.6 ± 40.8$^{ab}$ | 279.4 ± 44.7$^{b}$ |
| Final aboveground biomass (per unit) | g | 155.2 ± 16.7$^{a}$ | 216.3 ± 36.5$^{b}$ | 228.6 ± 35.4$^{b}$ | 245.4 ± 40.4$^{b}$ |
| Final belowground biomass (per unit) | g | 40.3 ± 12.9 | 28.9 ± 4.5 | 29.0 ± 5.5 | 32.0 ± 6.7 |
| Productivity | g m$^{-2}$ day$^{-1}$ | 48.0 ± 7.9$^{a}$ | 62.9 ± 13.7$^{ab}$ | 66.1 ± 11.2$^{ab}$ | 73.3 ± 14.5$^{b}$ |
| Root: shoot ratio | | 0.26 ± 0.06$^{a}$ | 0.13 ± 0.02$^{b}$ | 0.13 ± 0.01$^{b}$ | 0.13 ± 0.02$^{b}$ |
| Leaves count (per unit) | n | 1658 ± 167 | 1879 ± 103 | 2008 ± 190 | 1958 ± 277 |
| Stems length (sum per unit) | m | 1.83 ± 0.12$^{a}$ | 2.29 ± 0.26$^{b}$ | 2.39 ± 0.22$^{b}$ | 2.51 ± 0.32$^{b}$ |

$^{a,b}$Different letters indicate statistically significant differences between treatments ($p < 0.05$).
*g* - grams; *n*- count; *m* - meters.

Under non-limited nutrient conditions, *H. portulacoides* displayed a productivity range between 63.0 and 73.0 g m$^{-2}$ day$^{-1}$ (88% is above ground biomass). To compare the productivity of *H. portulacoides* with other studies, yields reported in other publications are converted to g m$^{-2}$ day$^{-1}$ using the data provided. In previous hydroponic studies, *H. portulacoides* displayed total productivity values either lower (<35.0 g m$^{-2}$ day$^{-1}$) (Buhmann et al. 2015) or higher (112.0 g m$^{-2}$ day$^{-1}$) (Marques et al. 2017) than the present study. Again, reliable comparisons are difficult to make since the experimental conditions in the different studies were considerably different, including the availability of nutrients, hydraulic factors (e.g. RTs), and light conditions (e.g. PAR). Overall, the productivity of *H. portulacoides* seems to fluctuate considerably, due to environmental variables such as nutrient availability and competition for resources (Emery et al. 2001; Morzaria-Luna and Zedler 2014).

The productivity of other halophytes have also been studied in the context of IMTA (Custódio et al. 2017). Boxman et al. (2017) tested the productivity of *Sesuvium portulacastrum* (L.) L. and *Batis maritima* L. irrigated with platyfish (*Xiphophorus* sp.) aquaculture effluents (~6.0–12.0 mg DIN-N L$^{-1}$) and obtained yields of 17.7 and 10.7 g m$^{-2}$ day$^{-1}$ respectively. Irrigated with a red drum (*Sciaenops ocellatus*) effluent (~10.0–70.0 mg DIN-N L$^{-1}$), *S. portulacastrum* and *B. maritima* displayed total productivity of 3.5 and 1.1 g m$^{-2}$ day$^{-1}$ of dry weight respectively (Boxman et al. 2018). These were substantially poorer performances compared with *H. portulacoides* in this study. *Aster tripolium* and *S. dolichostachya* irrigated with a European seabass (*Dicentrarchus labrax*) effluent (~19.0 mg DIN-N L$^{-1}$ and 3.0 mg DIP-P L$^{-1}$) displayed total productivities of 35.0 g m$^{-2}$ day$^{-1}$ for *A. tripolium* and 86.0 g m$^{-2}$ day$^{-1}$ for *S. dolichostachya* (Waller et al. 2015). *Sarcocornia ambigua* (Michx.) M.A. Alonso & M.B.Crespo irrigated with a Pacific white shrimp (*Litopenaeus vannamei*) effluent (~22.0 mg DIN-N L$^{-1}$ and 5.0 mg DIP-P L$^{-1}$) produced 112.3 g m$^{-2}$ day$^{-1}$ (Pinheiro et al., 2017). Besides the variability introduced by different experimental conditions (e.g. location, RT, PAR, salinity, planting density, space available for growth, grow-out time, etc.), differences in growth performance between studies using different species can also be associated with their different life-cycles and species-specific physiological adaptations which are modulated differently by the combination of biotic and abiotic factors (Crain et al. 2004; Silvestri et al. 2005; Veldkornet et al. 2016).

Deciding on the appropriate trade-off between nutrient extraction efficiency and productivity is paramount for an extraction unit to be effective. As observed in this study, an effluent with relatively low availability of nutrients, despite allowing for potentially high extraction efficiencies, will decrease total productivity under long RTs. The connectivity between IMTA functional groups (excretive species and extractive species) must be intentionally managed to optimize productivity while maximizing nutrient uptake which is the main purpose of multi-trophic integration. Moreover, the development of an IMTA technical standard is necessary for research and commercial purposes to allow for reliable comparisons between systems and enable the social and economic potential of IMTA.

### 3.4. Nutritional profile

To the authors' best knowledge, the nutritional profile of *H. portulacoides* edible leaves was analyzed for the first time in the present study. Results from the nutritional analysis are summarized in Table 2. The fresh leaves of *H. portulacoides* displayed a water content of 90% and their average nutritional profile (Control condition) was as follows: ash = 3.5 g 100 g$^{-1}$, carbohydrates ≤ 0.05 g 100 g$^{-1}$, dietary fibers = 3.3 g 100 g$^{-1}$, fat = 0.3 g 100 g$^{-1}$, protein = 2.0 g 100 g$^{-1}$, sodium = 0.8 g 100 g$^{-1}$ and sugars = 0.3 g 100 g$^{-1}$. Moreover, 100 g of fresh leaves yield 76.5 kJ (or 18.5 kcal) of energy.

Protein and sodium concentrations in the leaves of *H. portulacoides* were significantly affected by the experimental conditions. Protein was significantly lower ($p < 0.001$) in the leaves of $[N,P]_{low}$ treated

**Table 2**

Nutritional parameters from *Halimione portulacoides* leaves. Test-statistic: One-way ANOVA & Tukey HSD's test for pairwise comparison, different letters indicate statistically significant differences between treatments: $p < 0.05$. Treatments: $[N,P]_{low}$ = 6 mg N L$^{-1}$ & 0.8 mg P L$^{-1}$; $[N,P]_{med}$ = 20 mg N L$^{-1}$ & 3.0 mg P L$^{-1}$; $[N,P]_{high}$ = 100 mg N L$^{-1}$ & 6.0 mg P L$^{-1}$; Control = 56 mg N L$^{-1}$ & 15.5 mg P L$^{-1}$.

| | | $[N,P]_{low}$ | $[N,P]_{med}$ | $[N,P]_{high}$ | Control |
|---|---|---|---|---|---|
| Ash (inorganic matter) | g 100 g$^{-1}$ WW | 3.63 ± 0.15 | 3.64 ± 0.13 | 3.50 ± 0.13 | 3.53 ± 0.08 |
| Carbohydrates | g 100 g$^{-1}$ WW | <0.5$^{*}$ | <0.5$^{*}$ | <0.5$^{*}$ | <0.5$^{*}$ |
| Dietary fiber | g 100 g$^{-1}$ WW | 2.70 ± 0.68 | 2.76 ± 0.43 | 2.84 ± 0.54 | 3.30 ± 0.30 |
| Energy | kJ 100 g$^{-1}$ WW | 75.7 ± 14.6 | 73.4 ± 10.9 | 77.3 ± 13.4 | 76.5 ± 5.8 |
| Fat$^{\dagger}$ | g 100 g$^{-1}$ WW | 0.32 ± 0.05 | 0.26 ± 0.04 | 0.30 ± 0.14 | 0.33 ± 0.07 |
| Moisture | g 100 g$^{-1}$ WW | 90.7 ± 0.8 | 90.7 ± 0.4 | 90.5 ± 0.8 | 90.4 ± 0.2 |
| Protein | g 100 g$^{-1}$ WW | 1.50 ± 0.04$^{b}$ | 1.97 ± 0.13$^{a}$ | 2.09 ± 0.10$^{a}$ | 2.01 ± 0.12$^{a}$ |
| Sodium | g 100 g$^{-1}$ WW | 0.77 ± 0.10$^{a}$ | 0.76 ± 0.02$^{a}$ | 0.61 ± 0.08$^{b}$ | 0.75 ± 0.04$^{a}$ |
| Sugars | g 100 g$^{-1}$ WW | 0.30 ± 0.10 | 0.36 ± 0.05 | 0.36 ± 0.05 | 0.30 ± 0.07 |

WW – wet weight.
$^{a,b}$Different letters indicate statistically significant differences between treatments ($p < 0.05$).
$^{*}$ Below equipment detection limit.
$^{\dagger}$ Non-parametric Kruskal-Wallis test (normality assumption violated).

plants (1.5 g 100 $g^{-1}$ wet weight (WW)), compared to the Control and the other two treatments (2.0–2.1 g 100 $g^{-1}$ WW). Sodium concentration was significantly lower ($p < 0.02$) in the leaves of $[N,P]_{high}$ treated plants (0.6 g 100 $g^{-1}$ WW), compared with Control and the other treatments (0.8 g 100 $g^{-1}$ WW). The possibility of a dilution effect in the total edible aboveground biomass was assessed by calculating the total amount of protein and sodium in each unit. The total protein content in the fresh edible biomass collected from $[N,P]_{low}$ units (2.0 ± 0.3 g) was also significantly lower ($p < 0.01$) than Control (4.3 ± 0.9 g), $[N,P]_{med}$ (3.5 ± 0.6 g) and $[N,P]_{high}$ (4.0 ± 0.5 g). On the other hand, the total sodium content in the fresh edible biomass from $[N,P]_{high}$ units (1.1 ± 0.1 g) did not significantly differ from the other treatments, contrarily to its concentration values, suggesting a dilution of sodium in the biomass. Only $[N,P]_{low}$ units (1.0 ± 0.2 g) were significantly lower ($p = 0.01$) than Control (1.6 ± 0.4 g), due to their lower biomass. $[N,P]_{med}$ displayed a total of 1.4 ± 0.3 g of sodium in its edible biomass.

The nutritional profiling of plants under the different treatments demonstrates that the concentration of some nutritional compounds is affected by the availability of nutrients in the solution. Protein concentration was significantly lower in $[N,P]_{low}$ treated plants and a decrease in protein is a typical symptom of N-limitation (Geary et al. 2015; Hopkins and Huner 2008), which further confirms the state of nutrient limitation of *H. portulacoides* in that condition. Sodium content was found at lower concentrations in $[N,P]_{high}$ treated plants, but a dilution effect in the total aboveground biomass could partially explain this observation since the absolute values of sodium in $[N,P]_{high}$ were not significantly different from other treatments. Nonetheless, a lower accumulation of sodium in plant tissues when N is available at very high concentrations has been previously observed in glycophytes (rye grass and barley) and the halophyte *Spartina alterniflora* Loisel. (Hessini et al. 2009; Kant et al. 2007; Sagi et al. 1997).

*Halimione portulacoides* leaves can be consumed either as a fresh product or processed as biosalt, an approach already employed for other commercially available halophytes (Feng et al. 2013; Loconsole et al. 2019). The nutritional profile of leaves, both in their fresh and dried format, is described and compared with analogous products such as other halophyte species (*Salicornia* spp.), two leafy greens, a seaweed and regular table salt (Table 3). The reference nutritional composition for *H. portulacoides* is assumed to be the one resulting from plants irrigated with the control Hoagland's solution.

In its raw format, *H. portulacoides* leaves present the lowest carbohydrates content and the highest dietary fiber content compared with the other products. In all products, sugars and fat contents are <0.6% and protein content ranges between 1.5% (*S. bigelovii*) and 2.9% (kelp). In terms of sodium, *S. bigelovii* has the highest percentage (1%), *H. portulacoides* comes in second (0.8%) followed by kelp (0.2%). The remaining products have residual amounts of sodium. In its dry format, *H. portulacoides* is higher in inorganic matter (ash), lipids and protein contents than *Salicornia* spp. In terms of sodium, dried *S. ramosissima* has the highest amount (9.0%), followed by *H. portulacoides* (7.8%) and *S. perennis* (6.4%). Regular table salt content in sodium is 4 to 5 times higher than in dehydrated halophytes, therefore these plants could be used as low-sodium alternatives to salt for culinary purposes. Nonetheless, sodium is still present in relatively higher amounts in halophytes than other plants and, following the World Health Organization recommendation of <2 g $day^{-1}$ of sodium (WHO 2012), an healthy adult would have to consume 270 g of fresh *H. portulacoides* leaves per day (25 g dried) to reach that threshold.

A note should be made about the potential accumulation of noxious compounds in edible tissues, such as metals and prophylactic drugs present in aquaculture effluents (Cabrita et al. 2019; Rosa et al. 2020), since these can pose risks to human health (Rai et al. 2019). Species that have low rates of accumulation or accumulate

**Table 3**
Nutritional profile of *Halimione portulacoides* leaves and other comparable food items.

| | Ash | Carbohydrates | Dietary fiber | Energy | Fat | Moisture | Protein | Sodium | Sugars | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | g 100 $g^{-1}$ | g 100 $g^{-1}$ | g 100 $g^{-1}$ | kJ 100 $g^{-1}$ | g 100 $g^{-1}$ | g 100 $g^{-1}$ | g 100 $g^{-1}$ | g 100 $g^{-1}$ | g 100 $g^{-1}$ | |
| Salt (table) | – | – | – | – | – | – | – | 38.76 | – | USDA (2019a) |
| Fresh product | | | | | | | | | | |
| *Halimione portulacoides* | 3.53 ± 0.08 | <0.50 | 3.30 ± 0.30 | 76.50 ± 5.80 | 0.33 ± 0.07 | 90.40 ± 0.02 | 2.01 ± 0.12 | 0.75 ± 0.04 | 0.30 ± 0.07 | Present study |
| *Salicornia bigelovii* | 4.36 ± 0.37 | 4.48 ± 0.46 | 0.83 ± 0.13 (crude fiber) | – | 0.37 ± 0.01 | 88.4 ± 1.4 | 1.54 ± 0.10 | 1.00 ± 0.71 | – | Lu et al. (2010) |
| Kelp (seaweed) | – | 9.57 | 1.30 | 179.9 | 0.56 | 81.58 | 1.68 | 0.23 | 0.60 | USDA (2019b) |
| Spinach (*Spinacia oleracea*) | – | 3.63 | 2.20 | 96.2 | 0.39 | 91.4 | 2.86 | 0.08 | 0.42 | USDA (2019c) |
| Watercress (*Nasturtium officinale*) | 1.20 | 1.29 | 0.50 | 46.00 | 0.10 | 95.11 | 2.30 | 0.04 | 0.20 | USDA (2019d) |
| Dried product | | | | | | | | | | |
| *Halimione portulacoides* | 36.67 ± 1.05 | – | 34.22 ± 2.90 | 793.40 ± 59.90 | 3.40 ± 0.69 | 0 | 20.87 ± 1.14 | 7.82 ± 0.38 | 3.10 ± 0.69 | Present study |
| *Salicornia ramosissima* | 29.20 ± 0.60 | – | – | – | 1.87 ± 0.18 | 0 | 5.20 ± 0.29 | 8.99 ± 0.05 | – | Barreira et al. (2017) |
| *Sarcocornia perennis* | 23.30 ± 0.30 | – | – | – | 2.25 ± 0.05 | 0 | 6.90 ± 0.70 | 6.41 ± 0.09 | – | Barreira et al. (2017) |

mostly in non-edible tissues will be more appropriate extractive species from a product-safety perspective for IMTA. From this perspective, *H. portulacoides* is also a good candidate since previous studies showed higher retention of metals (>90%) in belowground organs compared with aboveground organs (Cabrita et al. 2019; Castro et al. 2009). However, additional studies are needed concerning the accumulation of other potentially toxic compounds.

Interestingly, *Halimione portulacoides* grown hydroponically indoors displayed a distinct visual phenotype compared to its wild counterparts (Fig. S2 in the Supplementary Material). Specimens of *H. portulacoides* grown indoors are greener than conspecific plants in the wild and both their leaves and stems show a more delicate phenotype, as they appear thinner and less lignified. Phenotypic plasticity might explain those differences, as plants must adapt to sometimes very different indoor conditions (Palacio-López et al. 2015). Since indoor conditions lack many of the natural environmental stimuli that shape plant's "natural" phenotype, indoor plants can feature distinct morphological and physiological adaptations, such as higher specific leaf area and higher leaf N concentration, compared with their wild counterparts (Poorter et al. 2016). For example, *Arabidopsis thaliana* (L.) Heynh. grown indoor displayed larger leaves with different shapes and longer petioles, as well as 25–35% more total chlorophyll content and 30% less xanthophyll pigments than field-grown plants (Mishra et al. 2012). A major environmental stimulus that greatly dictates the morphology of plants is wind, as it promotes shorter and thicker leaves and stems to reduce aerodynamic drag and increase mechanical strength (Onoda and Anten 2011; Wu et al. 2016). Lack of wind stimulation promotes longer, thinner leaves and stems in indoor plants, as observed in *H. portulacoides*. From a product development perspective, phenotypic plasticity of plants can be advantageous to producers, as it provides the possibility to tailor sensory and functional traits (e.g. color, texture, secondary metabolites) of cultured plants and, as such, contribute to add them value (Marondedze et al. 2018).

## 4. Conclusions

The capacity of *H. portulacoides* to extract substantial amounts of DIN and DIP from saline effluents was experimentally demonstrated in the present study. Moreover, *H. portulacoides* leaves present a nutritional profile very similar to that of some leafy greens and other commercial halophytes and with low amounts of sodium compared with regular table salt (80% less), making it a suitable vegetable for human use with economic potential. The integration of *H. portulacoides* in aquaculture systems can therefore promote the eco-intensification of coastal aquaculture in brackish waters, decreasing the loss of dissolved nutrients to the environment and increasing biomass production per unit of feed input with little additional production costs. Promoting halophytes production through IMTA can help make aquaculture enterprises cleaner and more productive, competitive and sustainable.

## Author's contribution

MC, RC and AL designed the experiment. MC conducted the grow-out experiment. MC and AL conducted the laboratory analysis. MC, AL and RC analyzed the data. MC performed statistical analysis. MC wrote the manuscript. PC, SV, RC and AL reviewed the manuscript.

## CRediT authorship contribution statement

**Marco Custódio:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Sebastián Villasante:** Writing – review & editing, Supervision. **Ricardo Calado:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Funding acquisition. **Ana I. Lillebø:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Funding acquisition.

## Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.144378.

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