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Effect of salinity on the composition of a seawater-adapted strain of *Scenedesmus almeriensis*

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ABSTRACT

The main objective of this study was to evaluate the potential adaptation to seawater of the freshwater strain *Scenedesmus almeriensis* and to evaluate the effect of salinity on its growth, morphology, and biochemical composition. Incorporating low seawater concentrations into the culture medium (up to 103 mM NaCl) resulted in an increase in biomass productivity from 0.15 to 0.22 $g{\cdot}L^{-1}{\cdot}day^{-1}$ and an increase in the maximum specific growth rate from 0.14 to 0.26 day^{-1}. This was attributed to the presence of micronutrients in the seawater and adaptive responses to stress. Despite a lower biomass productivity (0.11 $g{\cdot}L^{-1}{\cdot}day^{-1}$), *Scenedesmus almeriensis* was able to grow well (0.15 day^{-1}) in a medium formulated with only seawater and commercial fertilisers. Cell morphology was significantly affected, with a 150 % increase in cell perimeter and an increase in roundness from 61.5 % (freshwater) to 95.8 % (seawater). The use of seawater also affected the chemical composition of the biomass. Seawater favoured the synthesis of specific fatty acids that have nutritional and industrial value, including polyunsaturated fatty acids. The protein content was slightly reduced under saline conditions but remained at 40 % (which is high compared to other biomasses). The results highlight the potential of seawater as a sustainable and cost-effective substitute for freshwater to produce *Scenedesmus almeriensis*. Future studies will validate the production of these strains at the large scale and identify potential industrial uses for the biomass produced.

1. Introduction

Raceway reactors, which are responsible for approximately 90 % of microalgae biomass production, are the most cost-effective option due to their low capital and operating costs [1,2]. Although sustainable, microalgae production in raceway reactors poses certain challenges, including high water requirements. The control and automation of microalgal photobioreactors has improved significantly over the last decade, allowing reductions in water losses and nutrient input. However, to maximise the sustainability of microalgae production, further efforts are needed to reduce the water requirements still more along with the consumption of non-renewable resources (e.g., nutrients and energy).

Using different types of water to formulate culture media (e.g., wastewater or seawater) has proven to be a suitable strategy for producing microalgae with a lower water input. The use of wastewater reduces costs and simultaneously contributes to environmental remediation, as microalgae can efficiently recover nutrients such as nitrogen and phosphorus, which are responsible for eutrophication [3–5]. Microalgae can also be produced using seawater. The production of microalgae using seawater represents an advantageous alternative to wastewater - when using wastewater, the resulting biomass cannot be utilised for high-value applications or human consumption due to contamination risks [6]. Seawater not only enables the cultivation of safe biomass suitable for multiple industries but also stimulates the synthesis of valuable metabolites such as carotenoids and

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polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acids (EPA) and docosahexaenoic acid (DHA) [7]. One of the limitations of seawater is that not all strains can cope with the high salinity. Indeed, most strains of commercial interest are naturally present in freshwater environments.

The microalga *Scenedesmus almeriensis* is a microalga of commercial importance. It is interesting because of its high growth rate and robustness and because it can be produced year-round in regions with a moderate climate, such as the south of Spain. To date, this microalga has been produced using freshwater [8] and wastewater [9]. It has shown potential for use as a plant biostimulant [10] and as a source of lutein for producing human food [11]. However, to the best of the authors' knowledge, seawater cultivation of *Scenedesmus almeriensis* has not yet been investigated. For this reason, the aim of this work was to evaluate the potential adaptation of *Scenedesmus almeriensis* to seawater and to assess the effect of salinity on its growth and biochemical composition. This is the first time that this microalga has been gradually adapted to a culture medium formulated with seawater only. In addition, both the original strain and the seawater-adapted strain were fully characterised in terms of their morphology and chemical composition.

2. Materials and methods

2.1. Biomass production

The strain used was *Scenedesmus almeriensis* (CCAP 276/24). The culture medium consisted of 0.90 $g{\cdot}L^{-1}$ $NaNO_3$, 0.14 $g{\cdot}L^{-1}$ $KH2PO_4$, 0.18 $g{\cdot}L^{-1}$ $MgSO_4$, and 0.02 $g{\cdot}L^{-1}$ Karentol® Mix Super (Kenogard, Barcelona, Spain), a commercial micronutrient mixture that includes Mo, Mn, B, Fe, Cu and Zn. The inocula were produced using 1 L spherical flasks at a constant aeration of 0.2 v/v/min and a PAR of 350 μmol photons$\cdot m^{-2}\cdot s^{-1}$.

Cells were grown using 300 mL bubble columns (the working volume was 250 mL), as previously described [12]. Production was maintained for 12–13 days. Then, approximately 80–90 % of the biomass was harvested by centrifugation. The remaining 10–20 % of the culture was used as an inoculum for the next production run. The culture medium was prepared using the same nutrients described above and increasing concentrations of seawater. The cells were gradually adapted to a seawater content in the culture medium from 0 % to 100 % (from 0 to 515 mM NaCl). Biomass production was carried out using nine independent photobioreactors.

2.1.1. Determination of the culture performance

The biomass concentration was measured by dry weight. For this, 20 mL of the culture were filtered using 0.2 μm filters that had previously been weighed and dried. The filtered samples along with the filters were dried in an oven at 80 °C for 24 h. To remove excess salts, the biomass was washed twice with an equal volume of distilled water and then filtered again with the same filter. The chlorophyll fluorescence ratio (Fv/Fm) was determined using an AquaPen AP 100 fluorimeter (Photon System Instruments, Czech Republic) following 15 min of storage in the dark. Absorbance was measured daily at 400–700 nm using a Genesys 150 UV–vis spectrophotometer (Thermo Fisher Scientific, Spain).

2.2. Microscopic determinations

Culture micrographs were taken using a Leica DM 750 microscope coupled to a Flexacam i5 camera (Leica Microsistemas, Barcelona, Spain) and using Enersight software (Leica Microsistemas, Barcelona, Spain). The images were analysed using Fiji software (ImageJ ver. 1.54 g, Java 1.8.0_345 [64 bit]). Briefly, the images were first calibrated by adding a scale to convert the pixel dimensions to micrometres. Then, a threshold was applied to restrict the measurements to the highlighted pixels corresponding to cells. The threshold regions of interest were subsequently added to the ROI (region of interest) Manager to optimise the analysis, ensuring that only the cells were analysed. The selected ROIs were used to determine the radius, area, perimeter, volume, colour parameters, and roundness of the cells. The last of these was calculated using the equation:

$$Roundness(\%) = \frac{Area}{\pi \cdot Major\ axis^2}$$

where the major axis represents the primary axis of the best-fitting ellipse. The results presented are the mean value of 50 cells per technical replicate. The cells were randomly selected.

2.3. Analytical determinations

2.3.1. Macromolecular composition

The total protein content was determined using the Kjeldahl method (Nx5.95) [13]. The total lipid content was determined gravimetrically after extraction using a mixture of chloroform:methanol (2:1; v/v) [14]. Lastly, the ash content was calculated gravimetrically after calcination in a muffle furnace at 600 ºC for 12 h [15]. The carbohydrate content was determined by difference. The chlorophyll content and the total carotenoid content were estimated spectrophotometrically, as described in a previous work [16].

2.3.2. Amino acid composition

Amino acid determination was performed on 100 mg of freeze-dried biomass hydrolysed in 10 mL of 6 N HCl under vacuum at a temperature of 110 °C for 24 h [17]. The hydrolysate was passed through 0.45 μm filters, washed and evaporated to dryness under a nitrogen stream. The resulting dry residue was dissolved in distilled water and amino acid analysis was performed using a Perkin Elmer Series 200 HPLC system (MA, USA) with a Perkin Elmer Altus A-10 fluorescence detector (MA, USA). The determination conditions are described elsewhere [18]. The amino acid determination was carried out in duplicate per natural replicate.

2.3.3. Composition of fatty acids

The preparation of fatty acid methyl esters (FAMEs) was performed using a MARS 6 Express 40 microwave system (CEM Corporation, Matthews, NC, USA), as described in a previous study [19]. Briefly, 1.5 g of biomass, 100 μL of internal standard solution and 10 mL of potassium hydroxide in methanol (2.5 %, w/v) were saponified in a microwave reaction vessel by heating the system to 130 ºC for 4 min and maintaining this temperature for 4 min. After cooling at room temperature, the methyl esterification was carried out by adding 15 mL of an acetyl chloride solution in methanol (5 %, w/v) and heating it to 120 ºC for 4 min and maintaining this temperature for 2 min. After cooling, the FAMEs were extracted by adding 10 mL of pentane and 20 mL of saturated salt solution and shaking. The FAME quantification was performed on the top pentane layer, as described elsewhere [18]. A Perkin Elmer Clarus 580 gas chromatograph (MA, USA) equipped with a flame ionisation detector and an Agilent CP-Sil 88 capillary column (100 m × 0.25 mm, 0.2 μm; CA, USA) was used for the analysis. The determination of fatty acid methyl esters (FAMEs) was performed in duplicate for each natural replicate.

2.3.4. Determination of volatile organic compounds

Volatile organic compounds (VOCs) were determined using a 7890 B gas chromatograph (GC) coupled to a 5977 A mass spectrometer (MS), both from Agilent (Santa Clara, USA), equipped with a HP-FFAP capillary column (50 m x 200 μm x 0.33 μm, Agilent, Santa Clara, USA) having a column flow of 1.44 $mL{\cdot}min^{-1}$. Samples of freeze-dried microalgae powder were prepared according to a previously described methodology [20]. Briefly, 25 mg of the freeze-dried microalgae powder were weighed into 20 mL headspace vials, sealed with a crimp cap and

stored at −20°C. After a 10-min incubation period, the gas phase of the samples was extracted at 80°C for 10 min at a stirring rate of 800 rpm. For the desorption of the VOCs into the GC, the in-tube extraction trap was heated to 300 °C with a desorption flow of 150 mL·min^{-1} for 4 min into the cooled injection system equipped with a Tenax filled liner (Gerstel, Sursee, Switzerland). VOC identification from the MS data was conducted by comparing the MS spectra with the NIST database spectra (Version NIST17, National Institute of Standards and Technology, Gaithersburg, USA), as recommended by the Metabolomics Standard Initiative [21]. The detection of sulphur compounds was performed using a Pulsed Flame Photometric detector (PFPD, 5380, OI Analytical, College Station, USA) and sulphur compound standards (Merck, Buchs, Switzerland) were measured for identification. More detailed information on the VOC analyses and equipment used can be found in the Supplementary Material section.

2.4. Statistical analysis

Data were analysed using a one-way analysis of variance (ANOVA) followed by Fisher's LSD post-hoc test. A p-value less than 0.05 was considered statistically significant. All statistical analyses were performed using Statgraphics Centurion v18 (Statgraphics Technologies Inc., VA, USA).

3. Results and discussion

3.1. Biomass production

Previous studies have revealed that a variation in the salinity of the culture medium strongly influences *Arthrospira platensis* growth, reflecting the sensitivity of the microalgae to high salt concentrations [22]. Fig. 1A shows the effect of salinity on the growth of *Scenedesmus almeriensis*. The results showed that the specific growth rate of the microalgae produced in fresh water (0.0 mM NaCl) was 0.13 ± 0.01 day^{-1}, with a maximum biomass concentration of 2.2 g·L^{-1}. The strain has efficient mechanisms to maintain its osmotic balance in the absence of salt stress. As the salinity of the medium increased to 51.5 mM NaCl (10 % seawater, 90 % freshwater), growth remained rapid and consistent, with no effect on the maximum specific growth rate (0.15 ± 0.03 day^{-1}). In turn, when the concentration increased to 103 mM (20 % seawater, 80 % freshwater), the maximum specific growth increased to 0.26 day^{-1} ($p < 0.05$). This agrees with a previous work in which the authors revealed that *Scenedesmus almeriensis* tolerates low salinity levels well [23]. The effect of salinity on growth was more evident at concentrations of 309 and 412 mM NaCl (60 and 80 % of seawater in the culture medium, respectively). In these cases, the maximum biomass concentration reached was lower ($p < 0.05$) as well as the specific growth rates, which were determined as 0.11 ± 0.02 and 0.09 ± 0.02 day^{-1}, respectively ($p < 0.05$). The osmotic stress caused by adding seawater hinders cellular water regulation and photosynthesis, essential processes for microalgal growth and survival in high salinity environments [24]. This might have limited the maximum biomass concentration reached when the seawater content was higher. When the cells were produced using just seawater (515 mM NaCl), growth was not negatively affected, reaching a biomass concentration of 1.9 g·L^{-1} and a maximum specific growth rate of 0.14 ± 0.01 day^{-1}, comparable to that of the control. This suggests that the cells adapted well to the gradual increase in salinity. However, the cells needed a longer lag period to adapt to the increased salinity.

Fig. 2A shows that the Scenedesmus almeriensis biomass productivity varied considerably with the seawater concentration in the culture medium, reflecting the influence of salinity on growth. When produced using freshwater, biomass productivity was 0.15 g·L^{-1}·day^{-1}. On adding seawater at a concentration of 103 mM NaCl (20 % seawater), the biomass productivity was higher (0.22 g·L^{-1}·day^{-1}), suggesting that seawater might contain micronutrients that promote *Scenedesmus almeriensis* growth. This result is in line with the maximum specific growth rate at this salinity, which was the highest ($p < 0.05$). Moreover, it is also possible that moderate stress levels might have activated certain biochemical mechanisms that promote growth [25]. At higher salinity concentrations, biomass productivity decreased and was lower when the medium was produced using seawater only. The main reason for the lower bioactivity observed was the increased lag phase coming from increasing salinity. Unlike the *Scenedesmus almeriensis* investigated in this study, most freshwater strains present reduced growth and survival in high salinity environments. All microalgae are unable to grow beyond a certain threshold, which is strain dependent. For example, while *Chlorella* sp. exhibited a high resistance to osmotic stress (600 mM), *Coelastrella* sp. and *Chlamydomonas reinhardtii* exhibited only moderate resistance to increased sea salt concentrations [26].

The maximum quantum efficiency of photosystem II, namely the Fv/Fm ratio, has been widely used to assess the impact of environmental stress factors, such as salinity, on microalgal cells [27]. Fig. 2B shows the effect of seawater on the Fv/Fm ratio of *Scenedesmus almeriensis*. The Fv/Fm values were not affected in the lower salinity media, with values of around 0.65. According to a previous study, the optimal value for this microalga produced using freshwater is around 0.6 [1]. This is an indication that the cells were not under stress. No negative effects were observed compared to the freshwater control. This is in line with the biomass productivity results, with salinity values of 51–103 mM NaCl (10–20 % seawater) even having a positive effect on growth. However, when the culture medium was formulated using 60 % seawater or more,

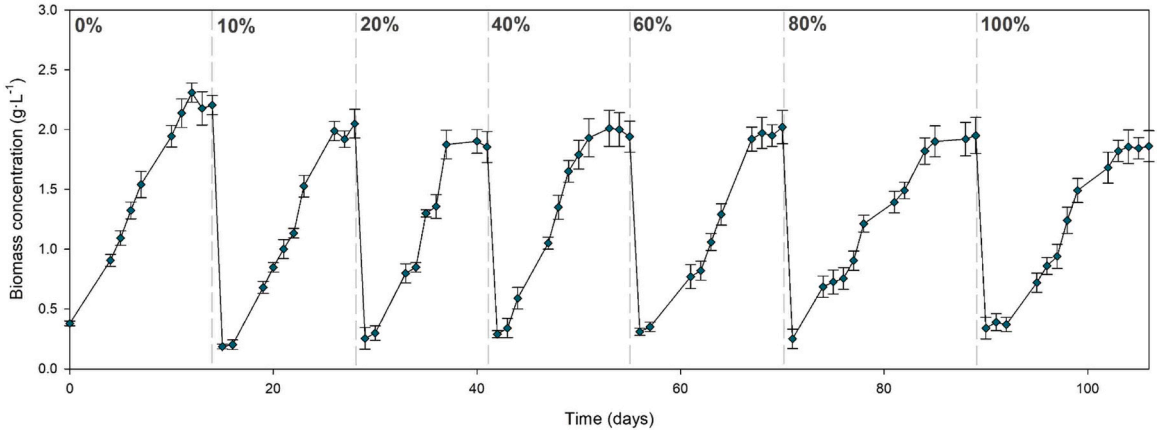

Fig. 1. Effect on the growth of *Scenedesmus almeriensis* of gradually replacing freshwater with seawater. The different percentages refer to the seawater percentage (30 g·L^{-1}) in the culture medium.

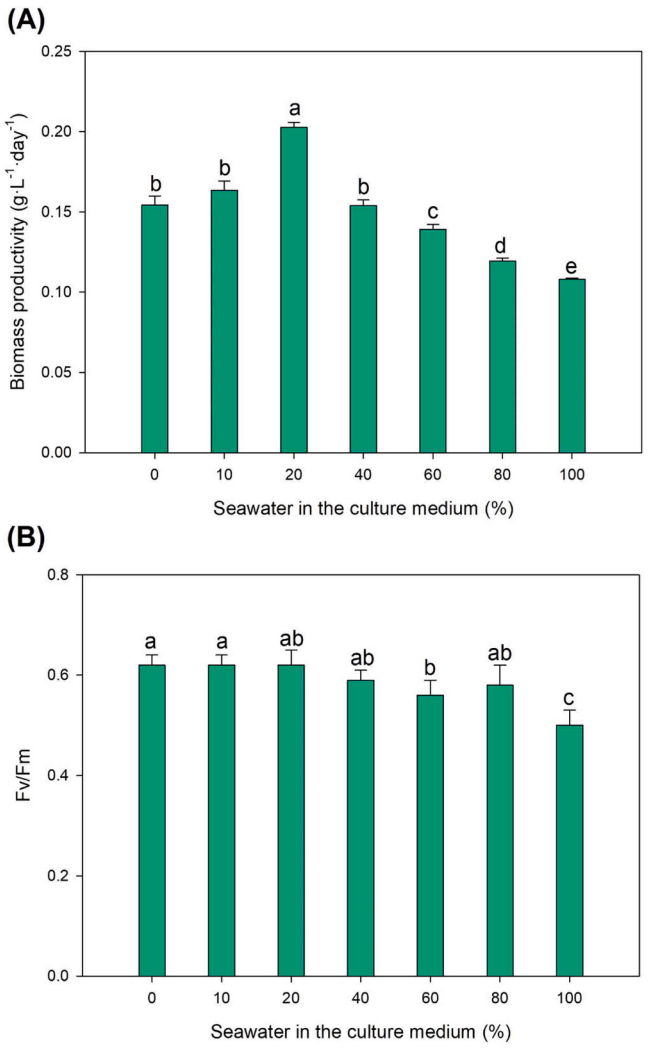

Fig. 2. Effect of seawater on (A) biomass productivity and (B) the Fv / Fm value. Different letters indicate significant differences ($p < 0.05$).

a significant reduction in the Fv/Fm values was observed. The lowest Fv/Fm value was observed when the cells were produced using 100 % seawater, suggesting that the cells experienced stress because of the medium's elevated salinity. These results are consistent with those for biomass productivity, which was lower compared to media containing freshwater only.

Microscopic observations revealed that the cells changed their morphology as a result of the increased salinity. Table 1 lists the effects of salinity on cell morphology. Briefly, the cells increased significantly in size as they adapted to seawater ($p < 0.05$). The average radius increased from 8.4 μm when produced using freshwater to 21.7 μm when produced using only seawater, representing a 158.7 % increase ($p < 0.05$). Similarly, the perimeter of the cells produced using seawater increased almost 3-fold when compared to the ones produced using freshwater ($p < 0.05$). These results are in line with previous studies that reported a significant increase in the size of microalgal cells because of salinity. For example, a previous study observed a 150 % increase in the perimeter of *Chlorella* cells produced using TAP medium or TAP medium enriched with NaCl at a concentration of 600 mM (37.9 g·L^{-1}) [26]. Another work observed an increase in the length of *Dunaliella tertiolecta* cells from 9.4 to 12.3 μm [28]. It has been hypothesised that the observed increase in size is caused by a limiting in cell division and because osmosensing and osmoregulation might induce metabolic changes that influence volume as well as biochemical composition [29]. The cells might accumulate osmolytes and inorganic ions inside their cells to balance the osmotic pressure, and this, in turn, can lead to a water influx, thus increasing the cells' volume. An increase in size may also help to reduce the concentration of salts inside the cells, thereby mitigating the toxic effects. This is reflected in a study on the adaptability of *Dunaliella salina* during osmotic stress [30]. Not only the cell size was affected by salinity but also their shape. The roundness of the cells increased from 61.5 % in the freshwater-produced cells to 95.8 % in the seawater-produced cells ($p < 0.05$). A perfect circle has a roundness value of 100 %. This variation in shape can be attributed to the smaller surface area relative to the spherical shape's volume, as confirmed by the study on the static allometry of unicellular green algae and cell surface area scaling [31]. Indeed, a spherical shape has the smallest surface area for a given volume, thus helping to reduce water loss from osmotic stress. Moreover, a spherical shape distributes osmotic pressure more evenly across the cell surface.

Table 1
Morphological characteristics of *Scenedesmus almeriensis* cells produced using freshwater or seawater. Values represent mean values of three independent determinations ± SD.

	Freshwater	Seawater
Cell radius (μm)	8.39 ± 0.88^{b}	21.71 ± 1.72^{a}
Cell area (μm^2)	226.80 ± 46.31^{b}	1489.20 ± 140.23^{a}
Perimeter (μm)	58.11 ± 5.77^{b}	152.02 ± 16.82^{a}
Roundness (%)	61.50 ± 7.52^{b}	95.82 ± 2.11^{a}
*L**	63.49 ± 1.80^{a}	51.16 ± 1.39^{b}
*a**	16.36 ± 0.28^{b}	18.81 ± 0.64^{a}
*b**	42.47 ± 0.66^{b}	47.21 ± 0.30^{a}

* Significant differences are indicated by different letters in the same row ($p < 0.05$)

3.2. Effect of salinity on the biomass composition

3.2.1. Macromolecules

It is known that the culture medium composition has a marked effect on the biochemical composition of microalgal biomass [18]. The results for the biomass macromolecular composition produced using either freshwater or seawater are presented in Table 2. The results indicate that the biomass protein content was 44.6 g·100 g^{-1} when produced using freshwater and 39.1 g·100 g^{-1} when produced using seawater ($p < 0.05$). The change in the culture medium composition affected the cells' metabolism, decreasing protein synthesis under increasingly saline conditions. This reduction could be an adaptive strategy that allows microalgae to balance osmotic pressure in high salinity environments [32]. Previous studies using other strains have shown similar results [22]. In a study on *Tetraselmis chuii* grown at salinities of 30 and 40 g·L^{-1}, the protein content increased significantly to 34.4 and 40.5 %, respectively. This was accompanied by concurrent increases in lipid and carbohydrate levels. Similarly, *Chlorella vulgaris* subjected to saline concentrations of 10, 24, and 30 g·L^{-1} showed a significant increase in the carbohydrate content (13.1, 15.1, and 13.4 %, respectively) and an increase in the protein proportion [33]. Despite the decrease observed in the protein concentration, the protein content of the seawater-produced

Table 2
Effect of seawater on the macromolecular composition of *Scenedesmus almeriensis*. Values represent mean values of three independent determinations ± SD and are expressed on a dry weight basis.

Compound	Freshwater (g·100 g^{-1})	Seawater (g·100 g^{-1})
Proteins	44.59 ± 0.90^{a}	39.10 ± 0.95^{b}
Lipids	14.66 ± 1.88^{a}	16.17 ± 1.15^{a}
Ash	9.47 ± 0.75^{a}	11.50 ± 1.57^{a}
Carbohydrates	31.27 ± 2.08^{a}	33.23 ± 2.06^{a}

*Significant differences are indicated by different letters in the same row ($p < 0.05$)

biomass was still quite high compared to other natural sources [34]. Table 2 shows the effect of seawater on the lipid content of the biomass. The results indicate that salinity did not affect lipid production and accumulation. These contrast with previous studies indicating that salinity might promote lipid production in microalgae. For example, a study published in 2015 showed that *Scenedesmus* sp. increased lipid production by 31 % under saline conditions [32] while another, published in 2021, revealed that the lipid content increased by 7–25 % when *Scenedesmus quadricauda* was produced in a 400 mM NaCl medium [35]. It is possible that the effect of salinity on lipid production is strain-dependant or that higher seawater concentrations are required to observe a significant effect. Table 2 also presents the ash and carbohydrate contents. Overall, the use of seawater did not affect the ash or carbohydrate contents in the biomass, despite the medium's greater mineral content. It is important to note that the biomass was washed twice with distilled water to remove excess salts from the surface of the cells. Previous studies suggested that the osmotic stress associated with high salinity leads to carbohydrate accumulation in several microalgae species (Shetty et al., 2019). Overall, the results indicate that *Scenedesmus almeriensis* is a robust and adaptable microalga capable of thriving in a seawater culture medium without significant changes in biomass composition (except for a reduction in the protein content when cultured with seawater).

3.2.2. Amino acids, fatty acids, and volatile organic compounds

Table 3 shows the effect on the amino acid profile of *Scenedesmus almeriensis* when using seawater. Both arginine and proline were found at higher concentrations in the microalgae produced using seawater ($p < 0.05$). The higher concentration of proline is interesting because of its protective role: it helps to mitigate environmental stress. It also has potential applications in agricultural supplements (Ghosh et al., 2022). Arginine is known for its important role in the urea cycle and its usefulness as a supplement for animals, for example, to help alleviate sepsis [36]. Histidine, isoleucine, valine, leucine, tryptophan, and phenylalanine were more abundant in the biomass produced using freshwater ($p < 0.05$). The primary reason for the high protein content is the elevated protein concentration in the biomass cultivated in freshwater. In contrast, the reduction in protein content observed when microalgae are grown in seawater is attributed to metabolic changes. These changes trigger the production of low-molecular-weight carbohydrates, likely serving as an adaptive mechanism to regulate osmotic pressure in high-salinity environments.

Table 3
Effect of seawater on the amino acid profile of *Scenedesmus almeriensis*. Values represent mean values of three independent determinations ± SD and are expressed on a dry weight basis.

Compound	Freshwater (g·100 g^{-1})	Seawater (g·100 g^{-1})
Asp	3.73 ± 0.08^{a}	2.86 ± 0.04^{b}
Glu	5.44 ± 0.12^{a}	4.68 ± 0.07^{b}
Ser	2.23 ± 0.05^{a}	1.56 ± 0.02^{b}
His	1.25 ± 0.03^{a}	0.94 ± 0.01^{b}
Gly	2.14 ± 0.05^{a}	1.31 ± 0.02^{b}
Thr	1.87 ± 0.04^{a}	1.30 ± 0.02^{b}
Arg	2.84 ± 0.06^{b}	3.79 ± 0.05^{a}
Ala	2.69 ± 0.06^{a}	2.00 ± 0.03^{b}
Tyr	2.17 ± 0.05^{a}	1.63 ± 0.02^{b}
Val	2.79 ± 0.08^{a}	2.05 ± 0.04^{b}
Met	1.56 ± 0.04^{a}	1.25 ± 0.03^{b}
Trp	5.05 ± 0.14^{a}	3.14 ± 0.07^{b}
Phe	2.04 ± 0.06^{a}	1.33 ± 0.03^{b}
Ile	2.90 ± 0.08^{a}	2.02 ± 0.03^{b}
Leu	2.01 ± 0.06^{a}	1.49 ± 0.04^{b}
Lys	1.22 ± 0.03^{a}	1.24 ± 0.03^{a}
Pro	2.09 ± 0.06^{b}	3.76 ± 0.08^{a}
Total	44.03 ± 0.02^{a}	36.25 ± 0.06^{b}

*Significant differences are indicated by different letters in the same row ($p < 0.05$)

The fatty acid profile of both biomasses is listed in Table 4. As mentioned previously, the use of seawater generally favours the accumulation of lipids. In this study, there was an effect on fatty acid composition although the lipid content was not statistically higher. Some of the compounds that showed significant differences, and were found at higher levels when the biomass was produced using seawater, include myristic acid ME, lauric acid ME, palmitic acid ME, linoleic acid ME, oleic acid ME, and stearic acid ME ($p < 0.05$). Others such as caproic acid ME, caprylic acid ME, and capric acid ME were not detected in the biomass produced using freshwater but were present in the biomass produced using seawater. The lipid content can be modulated by various culture conditions and nutrients in the medium. Other factors include temperature and salt concentration. This suggests that a saline environment acts as a stimulus for synthesising or accumulating certain fatty acids. This is probably a biochemical mechanism for adapting to the demands of the marine environment [37,38]. Some fatty acids were present in higher concentrations when the biomass was produced using freshwater. For example, alpha-linolenic acid ME was present at higher concentrations in the freshwater-produced biomass whilst others, such as vaccenic acid ME, were only identified in the freshwater-produced

Table 4
Effect of seawater on the fatty acid profile of *Scenedesmus almeriensis*. Values represent mean values of three independent determinations ± SD and are expressed on a dry weight basis.

Compound	Freshwater (mg·100 g^{-1})	Seawater (mg·100 g^{-1})
C6:0; Caproic acid ME	0.00 ± 0.00^{b}	2.87 ± 0.30^{a}
C8:0; Caprylic acid ME	0.00 ± 0.00^{b}	0.40 ± 0.57^{a}
C10:0; Capric acid ME	0.00 ± 0.00^{b}	2.64 ± 0.39^{a}
C11:0; Undecanoic acid ME	0.00 ± 0.00	0.00 ± 0.00
C12:0; Lauric acid ME	12.19 ± 2.59^{b}	67.39 ± 0.23^{a}
C13:0; Tridecanoic acid ME	69.04 ± 0.18^{a}	50.76 ± 0.05^{b}
C14:0; Myristic acid ME	27.00 ± 0.26^{b}	70.78 ± 0.69^{a}
C14:1n5c; Myristoleic acid ME	0.00 ± 0.00	0.00 ± 0.00
C15:0; Pentadecanoic acid ME	11.70 ± 1.21^{a}	1.80 ± 0.17^{b}
C15:1n5c; Pentadecenoic acid ME	0.47 ± 0.16^{b}	5.77 ± 0.13^{a}
C16:0; Palmitic acid ME	1185.38 ± 3.18^{b}	3404.35 ± 7.22^{a}
C16:1n7c; Palmitoleic acid ME	104.94 ± 0.14^{b}	151.74 ± 1.15^{a}
C17:0; Heptadecanoic acid ME	16.71 ± 0.03^{b}	31.77 ± 0.20^{a}
C17:1n7c; Heptadecenoic acid ME	6.19 ± 0.53^{a}	0.77 ± 0.07^{b}
C18:0; Stearic acid ME	37.51 ± 0.55^{b}	265.88 ± 0.72^{a}
C18:1n9t; Elaidic acid ME	6.77 ± 1.33^{b}	15.07 ± 1.15^{a}
C18:1n7t; Vaccenic acid ME	1.45 ± 0.40^{a}	0.00 ± 0.00
C18:1n9c; Oleic Acid ME	489.44 ± 0.75^{b}	1958.20 ± 3.15^{a}
C18:1n7c; Vaccenic acid ME	325.66 ± 0.54^{b}	801.29 ± 2.45^{a}
C18:2n6t; Linolelaidic acid ME	3.40 ± 0.11^{a}	2.50 ± 0.31
C18:2n6c; Linoleic acid ME	697.04 ± 0.13^{b}	3678.64 ± 7.06^{a}
C20:0; Arachidic acid ME	4.17 ± 0.03^{b}	30.68 ± 0.49^{a}
C18:3n6c; gamma-Linolenic acid ME	40.97 ± 0.15^{a}	16.96 ± 0.22^{b}
C20:1n9c; 11-Eicosenoic acid ME	5.28 ± 2.46^{b}	13.39 ± 0.26^{a}
C18:3n3; alpha-Linolenic acid ME	2374.74 ± 1.21^{a}	836.42 ± 0.31^{b}
C21:0; Heneicosanoic acid ME	0.00 ± 0.00	0.00 ± 0.00
C20:2n6c; 11,14-Eisodienoic acid ME	0.00 ± 0.00	0.00 ± 0.00
C22:0; Behenic acid ME	27.78 ± 0.06^{a}	14.83 ± 1.20^{b}
C20:3n6c; 8,11,14-Eicosatrienoic acid ME	3.81 ± 0.24^{a}	3.06 ± 1.50^{a}
C22:1n9c; Eruic acid ME	0.00 ± 0.00^{b}	6.11 ± 0.07^{a}
C20:3n3c; 11,14,17-Eicosatrienoic acid ME	2.15 ± 0.04^{a}	0.00 ± 0.00^{b}
C20:4n6c; Arachidonic acid ME	22.55 ± 0.63^{a}	7.36 ± 0.08^{b}
C22:2n6c; 13,16-Docosadienoic acid ME	0.00 ± 0.00	0.00 ± 0.00
C24:0; Lignoceric acid ME	8.39 ± 0.22^{b}	22.45 ± 0.16^{a}
C20:5n3c; 5,8,11,14,17-Eisopentaenoic acid ME	40.67 ± 2.20^{a}	18.54 ± 2.05^{b}
C24:1n9c; Nervonic acid ME	0.00 ± 0.00^{b}	18.51 ± 1.68^{a}
C22:5n3c; 7,10,13,16,19 Docosapentaenoic acid ME	1.65 ± 0.33^{a}	1.43 ± 2.03^{a}
C22:6n3c; 4,7,10,13,16,19 Docosahexaenoic acid ME	0.00 ± 0.00	0.00 ± 0.00
Total	5527.08 ± 28.66^{b}	11502.36 ± 35.54^{a}

biomass. In both samples, the most abundant unsaturated fatty acid MEs were linoleic acid and oleic acid, while the most common saturated fatty acid ME was palmitic acid. The fatty acid composition of microalgal cells depends both on the physiological state of the cells and the salt concentration in the culture medium [39]. The results show that the total fatty acid ME content was higher in the biomass produced with seawater than that produced using freshwater. A previous study indicated that the fatty acid composition of *Scenedesmus obliquus* depends on the salt concentration (and the strain used), achieving 41 % oleic acid at a concentration of 50 mM NaCl (Salama et al., 2013).

Volatile organic compounds in microalgae remain relatively unexplored even though they may significantly contribute to the organoleptic characteristics of the algal biomass and determine its final application. The information available mainly comprises descriptive analyses and a limited number of scientific investigations [40]. To the best of the authors' knowledge, this is the first study to carry out analyses on the acclimation of microalgae to increasing salt concentrations in the growth medium. When cultivated in seawater, the profile of volatile organic compounds in *Scenedesmus almeriensis* indicated an increase in aldehydes, ketones, acids, and sulphur compounds along with a more pronounced decrease in hydrocarbons and esters (Table 5). Additional data are provided as Supplementary Material. Some general trends, such as the higher abundance of sulphur compounds in seawater microalgae and hydrocarbons in freshwater species, were found in this study for the same species acclimated to different environments [41]. These molecules derive mainly from lipids, and particularly from polyunsaturated fatty acids, so this variation can be strongly correlated to the changes in lipid composition described above. From a sensorial perspective, the changes in the volatile organic compound profile may lead to significant changes in the biomass aroma, given the low odour thresholds of aldehydes, ketones, and alcohols. The compounds presenting the most evident variation as a function of salt concentration were ethyl acetate and heptadecane, with higher values in seawater, followed by hexanal, acetic acid, and 3,5-octadien-2-one, that had higher values in freshwater (Table 5). Interestingly, most of these compounds were also identified as biomarkers for identifying growth phases in six marine microalgae [42]. The authors of that study reported that ethyl acetate and heptadecane varied between the exponential, stationary and declining phases. This suggests that some of the variations observed in this study may be due to differences in growth performance rather than salt stress per se. Moreover, ethyl acetate is a short-chain ester widely used in the food and chemical sectors. From a biotechnological perspective, the accumulation of ethyl acetate in a freshwater microalga grown in seawater is interesting as it suggests an alternative, sustainable way to produce this molecule [43]. Ethyl acetate is produced *in vivo* through the reaction of acetyl-CoA and ethanol catalysed by alcohol acetyltransferases [44]. Under osmotic stress, eukaryotic green algae produce small organic solutes with a neutral charge and low toxicity for osmoregulation, typically glycerol or simple sugars. The rearrangement in the sugar metabolism may be the source of ethyl acetate overproduction. Further research is required to elucidate the metabolic mechanism involved.

Table 5
Relative abundance of volatile organic compounds (arbitrary area units) of *Scenedesmus almeriensis* produced using freshwater or seawater. Values represent mean values of three independent determinations ± SD.

Compound	Freshwater (AAU·10^3)*	Seawater (AAU·10^3)*
Aldehydes		
Hexanal	85.7 ± 5.1^b	1167.2 ± 48.4^a
Heptanal	14.5 ± 1.2^b	67.3 ± 2.4^a
Alkanes		
Decane	7.0 ± 1.2^a	13.2 ± 6.8^a
Dodecane	152.5 ± 3.9^b	181.9 ± 9.3^a
Pentadecane	249.1 ± 26.7^a	285.9 ± 9.8^a
Tetradecane	124.2 ± 6.6^b	158.9 ± 5.7^a
Hexadecane	564.7 ± 22.5^a	123.0 ± 1.3^b
Heptadecane	7809.1 ± 182.9^a	3290.4 ± 21.4^b
Alkenes		
1-Pentadecene	126.0 ± 1.9^b	173.0 ± 4.4^a
8-Heptadecene	691.1 ± 18.9^a	10.4 ± 1.0^b
1-Heptadecene	-	1.4 ± 0.0
Ketones		
3,5-Octadien−2-one	449.6 ± 12.6^b	1094.5 ± 128.0^a
alpha-Ionone	175.4 ± 0.9^b	306.9 ± 3.2^a
3-Buten−2-one, 4-(2,6,6-trimethyl−1-cyclohexen−1-yl)-	351.3 ± 0.1^b	459.7 ± 8.2^a
2-Pentadecanone, 6,10,14-trimethyl-	5.4 ± 0.3^a	5.2 ± 0.5^a
1H-Pyrrole−2,5-dione, 3-ethyl−4-methyl-	10.2 ± 0.6^b	197.9 ± 8.8^a
2(4 H)-Benzofuranone, 5,6,7,7a-tetrahydro−4,4,7a-trimethyl-, (R)-	133.3 ± 1.6^b	242.2 ± 4.3^a
Alcohols		
1-Penten−3-ol	395.3 ± 9.0^a	293.0 ± 20.1^b
1-Butanol, 3-methyl-	108.9 ± 6.6^b	187.7 ± 17.9^a
1-Hexen−3-ol	25.5 ± 2.3^a	36.9 ± 7.8^a
1-Hexanol	17.6 ± 2.2^b	25.5 ± 0.7^a
Cyclohexanol, 5-methyl−2-(1-methylethyl)-, (1 alpha,2 alpha,5 alpha)-	89.3 ± 9.8^a	135.2 ± 38.1^a
Acids		
Acetic acid	178.3 ± 24.2^b	998.0 ± 41.4^a
Hexanoic acid	1.8 ± 0.1^b	11.4 ± 3.3^a
Esters		
Ethyl Acetate	14536.2 ± 1162.9^b	1981.3 ± 198.6^a
Hexadecanoic acid, ethyl ester	2.8 ± 1.2^b	17.3 ± 3.3^a
Amides		
Acetamide	4.9 ± 0.9^a	5.1 ± 0.7^a
Sulphur compounds		
Dimethyl sulfoxide	32747 ± 3511^a	41436 ± 7257^a
Dimethyl sulfone	1068 ± 307^a	1622 ± 460^a
Benzothiazole	6389 ± 1.0^b	9429 ± 1314^a
Unknown S-compound	317 ± 3.2^b	1820 ± 231^a
Others		
Unknown 1, *m/z* 69	166.2 ± 6.4^a	145.4 ± 1.6^a
Unknown 2, *m/z* 67	2.8 ± 0.5^b	6.4 ± 1.8^a

Significant differences are indicated by different letters in the same row ($p < 0.05$). Abbreviations: AAU, arbitrary area units.

4. Conclusions

These results highlight the ability of *Scenedesmus almeriensis* to adapt or acclimatise to different environments, offering innovative solutions to problems related to water sustainability. The biochemical composition of this strain varies significantly depending on the type of water used in its production. The biomass produced with seawater was characterised by an increase in important fatty acids and amino acids such as proline and arginine, valuable components in food supplements and agricultural additives. This strain's tolerance to high salinity levels, with no significant impact on productivity (maintaining a biomass concentration of 1.9 g·L^{-1} at 515 mM and 0 mM NaCl) and the improved quality of its biomass under saline conditions favour its potential application in sectors such as agriculture, aquaculture, and biofuel production. On the other hand, the freshwater-produced biomass demonstrated an important advantage as it contained a high concentration of essential amino acids, making it an ideal alternative for developing functional foods and high-value nutritional products.

CRediT authorship contribution statement

Tomás Lafarga: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Lucie K. Tintrop:** Investigation. **Daniel Kurpan:** Formal analysis. **Francisco Javier Alarcón:** Formal analysis. **Garcia-Vaquero Marco:** Formal analysis. **Silvia Villaró-Cos:** Supervision, Investigation. **María Salinas-García:** Formal analysis. **Elia Rivera-Sánchez:** Writing – original draft, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.nbt.2025.09.008.

Data availability

Data will be made available on request.

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