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DEPARTMENT OF MARINE SCIENCES

EXPLORING THE EFFECTS OF SALINITY AND NUTRIENT INTERACTIONS ON *ULVA* METABOLISM

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Image: „Typical seaweed habitat of the Atlantic-Baltic Sea highlighting Ulva fenestrata*", Sophie Steinhagen – Seaweed Research, accessed May 16, 2025, https://seaweed-research-steinhagen.com/2023/06/14/crypticity-range-margins-and-invasive-species-new-publication-on-the-ulva-biodiversity-in-the-baltic-sea/*

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Sarah Verhoeven ***Popular scientific summary***

Salt, waste, and seaweed: farming *Ulva* for a sustainable future

By 2050, nearly 10 billion people will need food, far more than current agriculture can sustainably provide. Conventional farming consumes water, depletes soil, and contributes significantly to climate change. But what if we could grow high-protein food without heavy freshwater or land use? What if we could even farm the sea and turn nutrient-rich process water into a resource?

This study explores these questions by looking at how *Ulva*, a type of green seaweed, grows under different salinity and nutrient conditions, including process water from herring production.

Two species native to the Swedish west coast were investigated: *Ulva fenestrata* and *Ulva lacinulata*. Although similar in appearance, they responded differently to the tested salinity-nutrient treatments, especially in low-salinity waters (5 PSU, Practical Salinity Unit, a standard measure for seawater salinity) combined with the herring production process water. This process water comes from storing freshly caught herring in saltwater for up to three days. During this time, the water accumulates nutrients, including ammonium, which the seaweed can absorb and convert into valuable compounds like protein. But if the ammonium levels get too high, it becomes toxic and stresses the seaweed by limiting its growth, especially in low salinity waters. This effect was clear in the present study: *U. lacinulata* only showed poor or even negative growth in waters with 5 PSU and added process water. *U. fenestrata*, on the other hand, proved greater tolerance and robust growth under these challenging conditions. By contrast, both species grew well in moderate and high salinities (15 and 30 PSU). Besides faster growth, protein levels increased sharply when the process water was used. In some cases, even up to 40% of the seaweed's dry weight. This positions *Ulva* as a serious contender for the green protein food market. Still, the differences between the species matter and balancing growth and protein yield is essential to determine how and where specific *Ulva* species should be farmed. Ultimately, this study points to a future in which seaweed farms in low-salinity waters fertilised by industrial waste could help feed the planet.

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Abstract

Ulva species have recently attracted attention in aquaculture as an alternative sustainable protein source due to their high productivity and valuable biochemical composition. These traits can be influenced by environmental factors, including salinity and nutrient availability, which allows their adjustments to different industries or needs. This study investigates the interactive effects of salinity and nutrients on growth and biochemical properties in two *Ulva* species native to the Skagerrak, *U. fenestrata* and *U. lacinulata*. A specific focus was given to herring production process water as an alternative nutrient source to support sustainable aquaculture. Each species was cultivated in 21 treatment combinations, covering three salinities (5, 15, and 30 PSU) and three nutrient sources: synthetic nitrate-based medium (PES), synthetic ammonium-based medium (BC; 25µM, 75µM, and 150µM) and ammonium-based process water (HPPW; 25µM, 75µM, and 150µM). The results revealed species-specific responses: *U. fenestrata* showed high tolerance to low salinity and robust growth in ammonium concentrations derived from HPPW, while *U. lacinulata* was more salinity-dependent and sensitive to ammonium (-stress) in hyposaline conditions. Across treatments, the process water significantly enhanced protein and amino acid profiles, approximately doubling concentrations compared to levels under PES (positive control medium), especially at low to moderate salinities. Moreover, evidence for metabolic trade-offs concerning pigment and phenolic content as well as species-specific osmoregulatory strategies became apparent. Hence, this study underscores the potential of process water as an alternative nutrient source promoting circular aquaculture while optimising *Ulva* farming.

Keywords: *Ulva* cultivation, process water, sustainable aquaculture, protein, physiological responses

Contents

1. Introduction

By 2050, the global population is expected to reach 9.7 billion, significantly intensifying pressure on the food system and increasing competition for land and freshwater resources (FAO, 2022, 2018; Godfray et al., 2010; Lal, 2016). Meeting this demand will require producing nearly 50% more food compared to levels in 2012 (FAO, 2022). At the same time, agriculture faces increasing challenges from climate change, including droughts, salinisation and land degradation, while conventional food systems continue to strain natural resources and contribute significantly to greenhouse gas emissions (Mukhopadhyay et al., 2021; Poore and Nemecek, 2018; Ray and Foley, 2013). Ensuring food security in the future will require a transition towards sustainable, nutrient-rich and low-input alternatives, especially new high-quality protein sources (FAO, 2013; Pérez-Escamilla, 2017).

Edible macroalgae offer a promising solution (Blikra et al., 2021; Duarte et al., 2009; Stedt et al., 2022a, 2022c; Steinhagen et al., 2022, 2025; Van Den Burg et al., 2021). They are fast-growing organisms that require neither arable land nor freshwater and provide valuable nutrients with a low environmental footprint (Duarte et al., 2017, 2009; Lüning, 1992; Stedt et al., 2022e; Steinhagen et al., 2025, 2022). While brown (Phaeophyta) and red algae (Rhodophyta) dominate the industry, green algae (Chlorophyta) account for less than 0.1% of the European production, with approximately 17.000 tons harvested in 2019 (Junning Cia & Galli, 2021). Despite this small market share, the green macroalgae *Ulva* (Ulvales), commonly known as sea lettuce, is gaining increasing attention within aquaculture due to its high adaptability, fast growth rates and nutritional value (Bolton et al., 2016; Simon et al., 2022; Steinhagen et al., 2021).

Ulva is a cosmopolitan genus that thrives in diverse environments, from entirely marine to freshwater habitats (Rybak, 2018; Steinhagen et al., 2023). Its resilience is attributed to its physiological plasticity, high tolerance to stocking densities, and ability to assimilate nutrients rapidly and thereby help mitigate eutrophication (Al-Hafedh et al., 2015; Nardelli et al., 2019; Simon et al., 2022). In addition to its ecological resilience, the biomass of *Ulva* is nutritionally rich. It contains high levels of proteins, essential amino acids, fatty acids, minerals, vitamins, and antioxidants (Fort et al., 2024; Simon et al., 2022; Stedt et al., 2022a, 2022e; Steinhagen et al., 2025). This nutrient-dense composition supports its use across various industries, including food and feed, functional foods, nutraceuticals, cosmeceuticals, and pharmaceuticals (Bikker et

al., 2016; Ruslan et al., 2021). Beyond macronutrients, *Ulva* also contains various bioactive compounds that exhibit antioxidant properties like phenolics, carotenoids, and chlorophylls. Carotenoids and chlorophylls are also promising as natural food colourants (Martins et al., 2021). In addition to its nutritional and functional potential, *Ulva* is increasingly gaining attention for its potential in non-food sectors (Mata et al., 2016; Raikova et al., 2017).

The physiological resilience of *Ulva* supports diverse cultivation methods, including offshore farming (Jansen et al., 2016; Steinhagen et al., 2021; Zollmann et al., 2023) and land-based tank cultures (Dinesh Kumar et al., 2023; Laramore et al., 2022). Today, most *Ulva* cultivation occurs in coastal nearshore or offshore environments, where natural habitats offer a cost-effective production approach (Araújo et al., 2021). However, seasonal variability and reliance on natural conditions limit its consistency and expansion (Araújo et al., 2021; Steinhagen et al., 2022). While large-scale farming has traditionally focused on high-salinity marine waters, low-salinity environments, such as the Baltic Sea, estuaries, and rivers, remain mostly unexplored despite their potential for innovative farming methods. Expanding cultivation into these areas could support the European seaweed market, which is projected to grow by 7%-10% annually (Mendes et al., 2022). In contrast to open water systems, onshore tank cultivation allows for complete environmental control, independent of seasons, which enables precise regulation of growth parameters to optimise yield and nutritional quality. Hence, the accumulation of valuable bioactive compounds can be modulated (Hafting et al., 2012).

Environmental factors like salinity and nutrients are among the most influential factors regulating *Ulva* metabolism, affecting growth, biochemical composition, and protein content through physiological responses shaped by genotype-environment interactions (Fort et al., 2024; Steinhagen et al., 2025). A recent field study across the Atlantic-Baltic Sea transect has revealed consistent declines in crude protein and pigment concentrations in four *Ulva* species with decreasing salinity (Steinhagen et al., unpublished). However, contrasting results have been observed under controlled laboratory conditions, where short- and long-term exposure to low salinity has been shown to enhance crude protein levels, possibly due to increased nitrogen uptake and reallocation of carbon-to-nitrogen resources (Fort et al., 2024; Steinhagen et al., 2025).

While salinity plays a central role, nutrient availability, particularly in bioactive form, is equally important in driving metabolic performance (Li et al., 2019; Sun et al., 2024). Hence, using nutrient sources such as herring production process water for macroalgae cultivation purposes

(HPPW) has gained attention (Stedt et al., 2022b). HPPW is a nutrient-rich byproduct of herring processing that aligns with circular economy principles while providing highly bioavailable nitrogen (Stedt et al., 2022a, 2022b, 2022c). Cultivation of *U. fenestrata* in HPPW has been shown to sustainably increase biomass yield and elevate crude protein content to levels exceeding 30% of the dry weight (DW)(Stedt et al., 2022a).

While salinity and nitrogen enrichment each independently affect the physiology of *Ulva*, their interactive effects are not well understood. Previous studies suggest that increased nutrient availability may buffer against salinity-induced stress by supporting photosynthesis and nitrogen assimilation under brackish conditions (Choi et al., 2010; Zheng et al., 2019). However, whether nutrient-rich environments such as HPPW can synergistically amplify the positive effects of low salinities on protein and biochemical composition while protecting against the negative effects is currently unknown. This knowledge gap is particularly relevant in the context of sustainable aquaculture. In low-salinity offshore systems (Baltic Sea), optimising stress adaptation is essential, whereas in onshore systems, combining low-salinity cultivation with targeted nutrient supplementation (e.g. HPPW) could offer a powerful strategy to enhance biomass quality.

Addressing this gap, the present study investigates the interaction effect of salinity and nutrients on growth rate, protein content, and bioactive compound accumulation in two distinct *Ulva* species native to the Skagerrak, *Ulva lacinulata* and *Ulva fenestrata*, to evaluate their cultivation potential. The thesis follows three primary objectives: 1) Assess the potential of low-salinity environments (5 PSU, 15 PSU) as novel farming environments. 2) Investigate the interactive effects of salinity and nutrient availability on growth and biochemical properties, focusing on nitrogen assimilation, crude protein content, osmolyte accumulation, pigment composition, and amino acid profiles. 3) Evaluate the viability of HPPW as a nutrient source in these different salinity environments by determining its potential to promote growth and improve biochemical properties.

2. Materials and methods

2.1 Algal material and genetic identification

Two *Ulva* species were selected for this thesis: *Ulva fenestrata* Postels and Ruprecht, 1840 and *Ulva lacinulata* (Kützing) Wittrock, 1882. *U. fenestrata* is a well-established model organism in aquaculture research in the northern hemisphere due to its robust growth rates and farming suitability (Stedt, et al., 2022a, 2022b; Steinhagen et al., 2021, 2022). Specimens were sourced from long-term indoor cultivation tanks at the Tjärnö Marine Laboratory (TML). *U. lacinulata* individuals were hand-collected in January 2025 near the TML (Figure 1, Table 1), transported in seawater-filled jars, and rinsed upon arrival. The biomass was acclimatised for at least seven days in a controlled seawater system using filtered seawater (0.2µm) from 40m depth, supplemented with 1xProvasoli Enriched Seawater (PES) medium (Provasoli, 1968).

Taxonomic identification of *U. fenestrata* specimens followed previous work by Toth et al. (2020)(GenBank accession number: MN240309, MN2403110, MN240311), while hand-collected *U. lacinulata* individuals were morphologically identified in the field and genetically verified in the lab using the chloroplast *tufA* gene marker according to the protocol refined by Steinhagen et al. (2019). The DNA was extracted from three freeze-dried individuals using the DNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions: Approximately 20mg of the freeze-dried sample was ground using a pestle and lysed in 400µl Buffer AP1 with 4µl RNase. After vortexing and 10-minute incubation at 65°C with regular inversion, ensuring complete lysis, the Buffer P3 was added, and the mixture was incubated on ice for 5 min. A centrifugation at 14,000 rmp followed. The supernatant was passed through a QIAshredder column, mixed with the buffer AW1, and loaded onto a DNeasy Mini spin column. The DNA was washed and eluted in 100µl Buffer AE. A subsequent Polymerase Chain Reaction (PCR)(Mullis, 1986) was used to amplify the plastid elongation factor tufA using the primers tufGF4 (5′ GGNGC NGCNCAAATGGAYGG 3′)(Saunders and Kucera, 2010) and tufAR (5′ CCTTCNCGAATMGCRAAWCGC 3′)(Famà et al., 2002). Each PCR-tube contained the reaction components listed in Table 2. The amplification protocol involved an initial denaturation at 95°C for 5 min, followed by ten cycles of 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds. Subsequently, final extension cycles (25x) of 94°C for 30 seconds and 55°C for 30 seconds were performed. The success of the PCR was confirmed via gel electrophoresis.

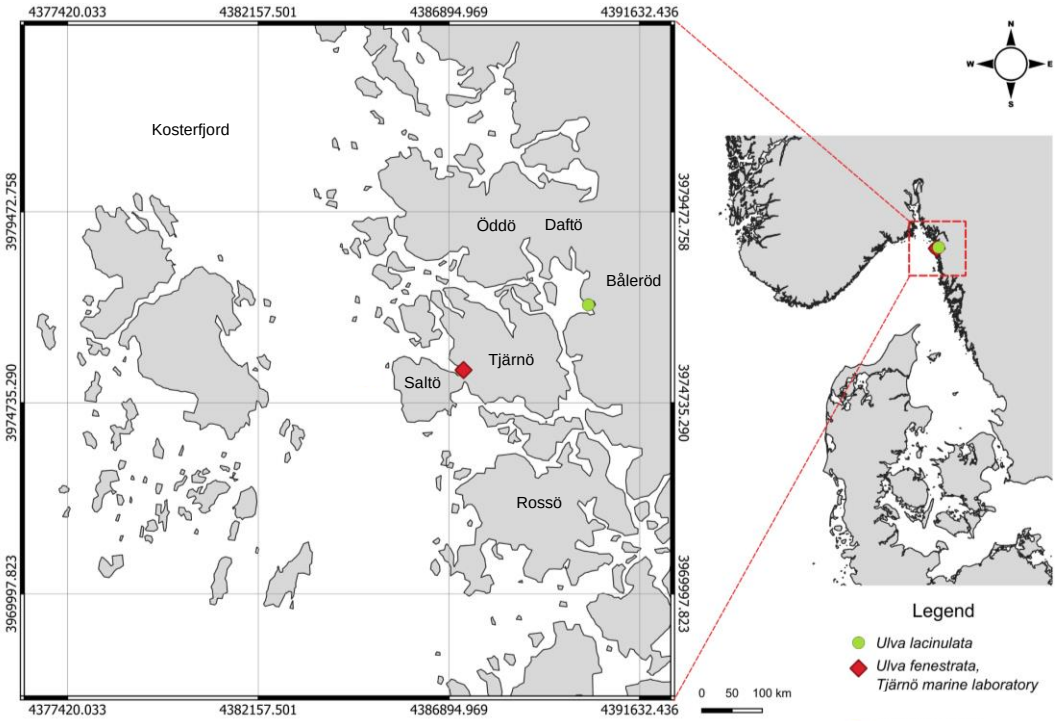

Figure. 1. Sample locations in the Kosterfjord area on the Swedish west coast. The map was made using QGIS (version 3.34.12).

Table. 1. *Ulva* species collected for the experiment

Species	Collection location	Coordinates	Date
U. fenestrata	Cultivation, Tjärnö Marine Laboratory, Sweden	58°52′33.7″ N 11°08′44.9″ E	05.11.24
U. lacinulata	Båleröd, Sweden	58°53'24.61"N 11°12'2.23"E	15.01.25

Table. 2. Composition of the PCR reaction mixture per PCR-tube.

	Components	volume
Master mix	H_20	25.4 µl
	Buffer	8 µl
	Primer tufGF4	2 µl
	Primer TufAR	2 µl
	dNTPs	0.8 µl
	Polymerase	0.8 µl
DNA	Extracted DNA	1µl
Total volume		40 µl

2.2 Herring production process water

The herring production process water (HPPW) used in the experiments originated from Sweden Pelagic AB (Hallgrens väg 1A, 47431 Ellös), a primary herring processing facility. Their process water TUB is generated from indoor storage tubs containing whole herrings in 3% NaCl-enriched water, resulting in a nutrient-rich byproduct after storage (Stedt et al., 2022b). According to Stedt et al. (2022a), TUB significantly stimulates growth and crude protein content in *U. fenestrata*.

The TUB used in this thesis was collected in October 2024, filtered (320µm) and stored at - 60°C in plastic containers to preserve its compound integrity. Preliminary analysis of this batch revealed nutrient concentrations of ammonium (NH_4^+, 47.3511 mg/L) and inorganic phosphorus (PO_4^{3-}, 233.2 mg/L), which were used as nutrient benchmarks for the experimental setup.

2.3 Experimental setup

Two sequential six-week trials were conducted to investigate the interaction effects of varying salinities and nutrient sources on *U. fenestrata* and *U. lacinulata*. Both trials were carried out under identical experimental conditions, using the same setup and protocols. Each trial involved controlled cultivation experiments in which salinity and nutrient conditions were manipulated. Salinity treatments were applied at three levels: 5 PSU (brackish), 15 PSU (moderately brackish), and 30 PSU (marine, used as the control). The nutrient manipulation was based on two different nutrient sources: TUB as herring production process water (HPPW) and a boosted control (BC). Both nutrient sources were tested at three dilutions based on the ammonium concentration (25 µmol/L, 75 µmol/L, and 150 µmol/L NH_4^+). For the BC, a 1M ammonium chloride solution served as the ammonium source. To ensure comparability of the nutrient sources, the BC treatments were enriched with additional PO_4^{3-} at the same concentrations found in the TUB (73.8 µmol/L, 221.5 µmol/L, and 442.9 µmol/L, 1M potassium dihydrogen phosphate solution). An additional culture medium supplemented with 1xPES served as the positive control, as it is a widely used cultivation medium for macroalgae. All salinity-nutrient treatment combinations were conducted with four replicates (see Table 3).

Before the experiment, ten randomly selected specimens of *U. fenestrata* and *U. lacinulata* were photographed (Canon 1100D; 1/25, F22, ISO400). Further photographic documentation

occurred at mid-time (week 3) and end (week 6) of the experiment. Excess water was removed by spinning the biomass 20 times in a salad spinner. A random 10g fresh weight (FW) sample was taken as the time zero reference (t_0), and another 10g FW was transferred into 1L cultivation units filled with the respective treatments (Table 3). All treatment combinations were enriched with 1ml of germanium oxide (GeO_2; 250mg/L) to prevent diatom growth and incubated in fridges at 12°C with a 16:8 light regime and light intensity of 100 photons m-2 s-1 with constant aeration. Water changes occurred every third day to maintain stable conditions and prevent bacterial growth. If the biomass exceeded 10g FW during weekly weight measurements (see 2.4), the excess was harvested to ensure consistent growth and prevent overcrowding. Final biomass recordings were conducted at the end of week six, after which samples were stored at -80°C before being lyophilised and homogenised into a fine powder for subsequent biochemical analysis.

Table. 3. The 21 tested salinity-nutrient treatment combinations. Salinity was manipulated on three levels (5, 15 and 30 PSU), while the nutrients were based on two ammonium-based sources: herring production process water (HPPW) and the boosted control (BC), each comprising three levels. S3 (30 PSU) and the nitrate based nutrient source PES (1ml, following the concentration of Provasoli (1968)) served as positive control. Each treatment combination had four replicates.

Salinity			**Nutrients**	
4x	**S1**	5 PSU	**HPPW1**	25 µmol/L NH_4^+, 73.8 µmol/L PO_4^{3-}
			HPPW2	75 µmol/L NH_4^+, 221.5 µmol/L PO_4^{3-}
			HPPW3	150 µmol/L NH_4^+, 442.9 µmol/L PO_4^{3-}
			(nutrient controls) **BC1**	25 µmol/L NH_4^+, 73.8 µmol/L PO_4^{3-}
			(nutrient controls) **BC2**	75 µmol/L NH_4^+, 221.5 µmol/L PO_4^{3-}
			(nutrient controls) **BC3**	150 µmol/L NH_4^+, 442.9 µmol/L PO_4^{3-}
			(positive control) **PES**	1ml (~329 µmol/L NO^{3-}, ~18.5 µmol/L as glycerophosphate)
4x	**S2**	15 PSU	**HPPW1**	25 µmol/L NH_4^+, 73.8 µmol/L PO_4^{3-}
			HPPW2	75 µmol/L NH_4^+, 221.5 µmol/L PO_4^{3-}
			HPPW3	150 µmol/L NH_4^+, 442.9 µmol/L PO_4^{3-}
			(nutrient controls) **BC1**	25 µmol/L NH_4^+, 73.8 µmol/L PO_4^{3-}
			(nutrient controls) **BC2**	75 µmol/L NH_4^+, 221.5 µmol/L PO_4^{3-}
			(nutrient controls) **BC3**	150 µmol/L NH_4^+, 442.92 µmol/L PO_4^{3-}
			(positive control) **PES**	1ml (~329 µmol/L NO^{3-}, ~18.5 µmol/L as glycerophosphate)
4x	**S3**	30 PSU (control)	**HPPW1**	25 µmol/L NH_4^+, 73.8 µmol/L PO_4^{3-}
			HPPW2	75 µmol/L NH_4^+, 221.5 µmol/L PO_4^{3-}
			HPPW3	150 µmol/L NH_4^+, 442.9 µmol/L PO_4^{3-}
			(nutrient controls) **BC1**	25 µmol/L NH_4^+, 73.8 µmol/L PO_4^{3-}
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			(nutrient controls) **BC3**	150 µmol/L NH_4^+, 442.9 µmol/L PO_4^{3-}
			(positive control) **PES**	1ml (~329 µmol/L NO^{3-}, ~18.5 µmol/L as glycerophosphate)

2.4 Growth measurements

To assess the specific growth rate (SGR), the FW of all *Ulva* cultivation units was measured weekly over the six-week experimental period. The SGR was calculated as daily growth based on changes in FW, using Equation 1. Before weighing, the excess surface water was removed using the standardised salad spinner method to ensure consistency. The weekly SGR values (Figure 2, App. 1) were calculated for each replicate and averaged by treatment to obtain the weekly mean and standard deviation (SD).

$$SGR\ [\%] = \frac{(Ln(FW_0) - Ln(FW_1))}{Days} * 100$$

Equation. 1. Calculation of the specific growth rate (SGR). FW_0: initial fresh weight; FW_1: fresh weight at the end time point. Days: number of days between measurements (in this study 7). SRG is expressed as a percentage by multiplying the result by 100.

2.5 Tensile strength

Visual structural differences between the treatments (App. 2.) initiated the assessment of the *Ulva* blade integrity under different cultivation conditions. The tensile strength was measured using a texture analyser (TVT, 6700, PerkinElmer (former Perten Instruments), Shelton, CT, USA). Each *Ulva* blade was clamped between two rubber grips attached to a lower and upper metal plate. Both plates featured a central hole for a probe to move through. The probe approached the blade with a speed of 1.6 mm/s until 10% extension or breakage of the blade, and the resulting force was recorded. The tensile strength could only be measured for *U. fenestrata*, as this analysis required fully intact blades (8 x 2.5 cm) to ensure reliable measurements without bias from pre-existing structural damage. In contrast, the *U. lacinulata* specimen collected in January displayed naturally perforated thalli.

2.6 Biochemical analysis

2.6.1 Pigment (chlorophylls a *and* b*, carotenoids) and phenolic content*

At the end of the experiment, 60 mg tissue samples were taken from homogenised *Ulva* material across all treatments and replicates for the pigment analysis, following the protocol described by Steinhagen et al. (2021). Each sample was extracted with 5 mL of 90% acetone, placed in an ultrasonic bath for 10 minutes, and shaken in the dark for 50 minutes to ensure thorough cell disruption. The extracts were subsequently centrifuged at 4,000 rpm for 10 minutes to separate

residual biomass from the acetone phase. The supernatant was transferred to cuvettes for spectrophotometric measurement at 664 nm, 647 nm, 480 nm, and 510 nm using a Lambda XLS+ spectrophotometer (Perkin Elmer, Waltham, MA, USA). Concentrations of chlorophyll *a* and *b* were calculated using the equations by Jeffrey and Humphrey (1975), while total carotenoids were estimated according to Parsons et al. (1984). Phenolic compounds were quantified using a slightly modified protocol involving a one-hour extraction of 65 mg homogenised *Ulva* in 70% ethanol in the dark on a shaker. The Folin–Ciocalteu assay (mixture of 8 mL dH20, 0.5 mL Foline-Ciocalteus reagent, 1.5 mL sodium carbonate (Na2CO3, 20mg/100ml) and 0.1 mL sample extract) was then used to determine the phenolic concentration, with gallic acid (Sigma-Aldrich, St. Louis, MO, USA) serving as the standard. The absorbance was measured at 765 nm, and total phenolic content was estimated using Equation 4.

$$Chl\ a\ [\mu g/ml] = 11.93\ E_{664} - 1.93\ E_{647} \qquad Chl\ b\ [\mu g/ml] = 20.36\ E_{647} - 5.50\ E_{664}$$

Equation. 2. Chlorophylls *a* and *b* estimations after Jeffrey and Humphrey (1975)

$$Total\ Carotinoid\ [\mu g/ml] = 7.6\ (E_{480} - 1.49\ E_{510})$$

Equation. 3. Total Carotenoid estimations after Parsons et al. (1984)

$$Penolic\ Content\ [mg/g] = \frac{C * 1.5}{DW}$$

Equation. 4. Phenolic content estimations in percentage: C: concentration according to the gallic acid standard curve measured with the photo spectrometer at 765 nm after Folin-Ciocalteus assay; DW: dry weight of respective biomass sample

2.6.2 Nitrogen, carbon, and crude protein content

Samples were taken from homogenised *Ulva* material. Four milligrams of each replicate was sealed in tin capsules and analysed for total nitrogen and carbon content via high temperature (~1150-1200°C) combustion in an oxygen-rich environment using a vario MICRO cube elemental analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany). The crude protein content was calculated based on the total nitrogen values, using a conversion factor 5 for macroalgae (Angell et al., 2016).

2.6.3 Amino acid properties and osmolytes

The amino acid analysis was performed by hydrolysing 100 mg of all samples across all treatments with 6M hydrochloric acid (HCl) at 110°C for 24 hours in a heating block. Before, the reaction tubes were flushed with nitrogen gas to minimise oxidative degradation. Following this hydrolysis, the samples were filtered to remove particles, and the remaining extract was stored at –20°C. 50µl of the hydrolysate was diluted 20-fold in acetic acid (HOAc) and analysed using liquid chromatography-mass spectrometry (6120 Quadrupole LC/MS, Agilent Technologies, Santa Clara, CA, USA). To ensure data quality, two blank samples and a series of calibration standards (dilution ratios: 1:10 to 1:320) were run after every 12th sample. Amino acid concentrations were quantified using Agilent's MassHunter Workstation software (Version B.09.00, Agilent Technologies, Santa Clara, CA, USA) based on calibration curves generated from the standard solutions.

A special focus was on proline, an amino acid with osmoprotective functions. To assess its potential role as an osmolyte across treatments, its relative abundance was calculated as the percentage of proline in relation to the total quantified amino acid content.

2.6.4 Water quotient

To determine the water content, all samples were weighed before and after freeze-drying. The water quotient (WQ) was then calculated as the percentage of water loss relative to the FW using Equation 5.

$$Water\ Quotient\ [\%] = \left(\frac{FW - DW}{FW}\right) * 100$$

Equation. 5. Calculation of the water quotient (WQ). FW: fresh weight. DW: dry weight. WQ is expressed as a percentage by multiplying the result by 100.

2.7 Statistical analysis

All data on growth, crude protein, pigments, amino acids, water quotient and relative proline were analysed using R (version 4.5.0)(R Core Team, 2014) via the RStudio interface (version 2025.12.1)(RStudio Team, 2020). Residual diagnostic plots were used to visually assess model assumptions, including normality of residuals and homogeneity of variances.

To analyse the SGR over time in *U. fenestrata* and *U. lacinulata*, a linear mixed-effect model (LMM) was fitted, including a random intercept for week to account for repeated measurements across time within replicates. A type III ANOVA was applied to assess the significance of the fixed effects (salinity and nutrients and their interaction). Where significant effects were found, a post hoc pairwise comparison was performed using the emmeans package (Lenth et al., 2025) with a Tukey adjustment.

Furthermore, either standard two-way ANOVAs (variable ~ Salinity*Nutrients) followed by Tukey's HSD post hoc test or Welch ANOVAs (variable ~ Salinity*Nutrients) with Games-Howell test (robust to variance heterogeneity and unequal sample size (Games and Howell, 1976) were performed to assess differences among treatments. When the model assumptions of normality or homogeneity of variances were violated, the data were log transformed to meet the ANOVA requirements (specific cases are indicated in the corresponding figure captions). Groups with fewer than three replicates due to insufficient biomass were excluded from comparative statistical analyses to ensure reliable estimation of within-group variance and to maintain the validity of parametric testing assumptions.

All statistical tests were considered significant at $p < 0.05$.

3. Results

3.1 Growth rate and biomass

3.1.1 Specific growth rate over time

For *U. fenestrata*, a linear mixed-effect model (LMM; Table 4) with a random intercept for week, evaluated using a Type III ANOVA, revealed a significant main effect of nutrient treatment on the specific growth rate ($F(6, 100) = 9.46$, $p < 0.001$). In contrast, neither salinity ($F(2, 100) = 0.55$, $p = 0.58$) nor time ($F(1, 19.6) = 5.42$, $p = 0.067$) showed significant independent effects. There was no significant interaction between salinity and nutrient treatment ($F(12, 100) = 0.11$, $p = 0.999$) or between either factor and time (all $p > 0.19$). The results are presented in Figure 2A. In the HPPW treatments panel (Figure 2A, middle), HPPW3 at 30 PSU (S3) displays the highest growth rate overall, peaking around week four, and is significantly greater than the reference group PES at 30 PSU (left panel; $p < 0.003$). Also notable is the sharp biomass crash in HPPW3 at 5 PSU after week four. Other treatment lines show less pronounced or overlapping growth, which aligns with their non-significance in the model. The pairwise comparison in week 6 (Table 4D) also revealed significant contrasts. For instance, growth under HPPW2 remained significantly higher than in BC1 and 2, PES, and HPPW1 treatments (all $p < 0.0002$).

Conversely, for *U. lacinulata*, the ANOVA (Table 4C) indicated that both salinity ($F(2, 100) = 9.04$, $p < 0.001$) and nutrient treatment ($F(6, 100) = 2.85$, $p = 0.013$) significantly influence the growth rate. Although time itself was not a significant main factor ($F(1, 14.4) = 0.16$, $p = 0.707$), the model identified strong interaction effects between salinity and time ($F(2, 100) = 46.74$, $p < 0.0001$), nutrients and time ($F(6, 100) = 4.62$, $p < 0.001$), and a significant three-way interaction between salinity, nutrient treatment, and time ($F(12, 100) = 2.17$, $p = 0.019$). This complex interaction pattern is visualised in Figure 2B, where several growth response curves diverge markedly across treatments, particularly in the HPPW and BC panels. The most extreme case is observed in BC3 at 5 PSU, which dropped to a negative SGR below -20% between weeks three and six ($p < 0.0001$), along with more moderate but significant declines in HPPW1-3 at 5 PSU (all $p < 0.05$). As recorded in *U. fenestrata*, several treatments in *U. lacinulata* differed significantly from the low-performing BC treatments (all $p < 0.027$; Table 6D). Additionally, salinity contrasts in week 6

were significant, with 5 PSU yielding different outcomes compared to 15 and 30 PSU ($p < 0.0001$), stating a time-dependent influence of salinity on the SGR.

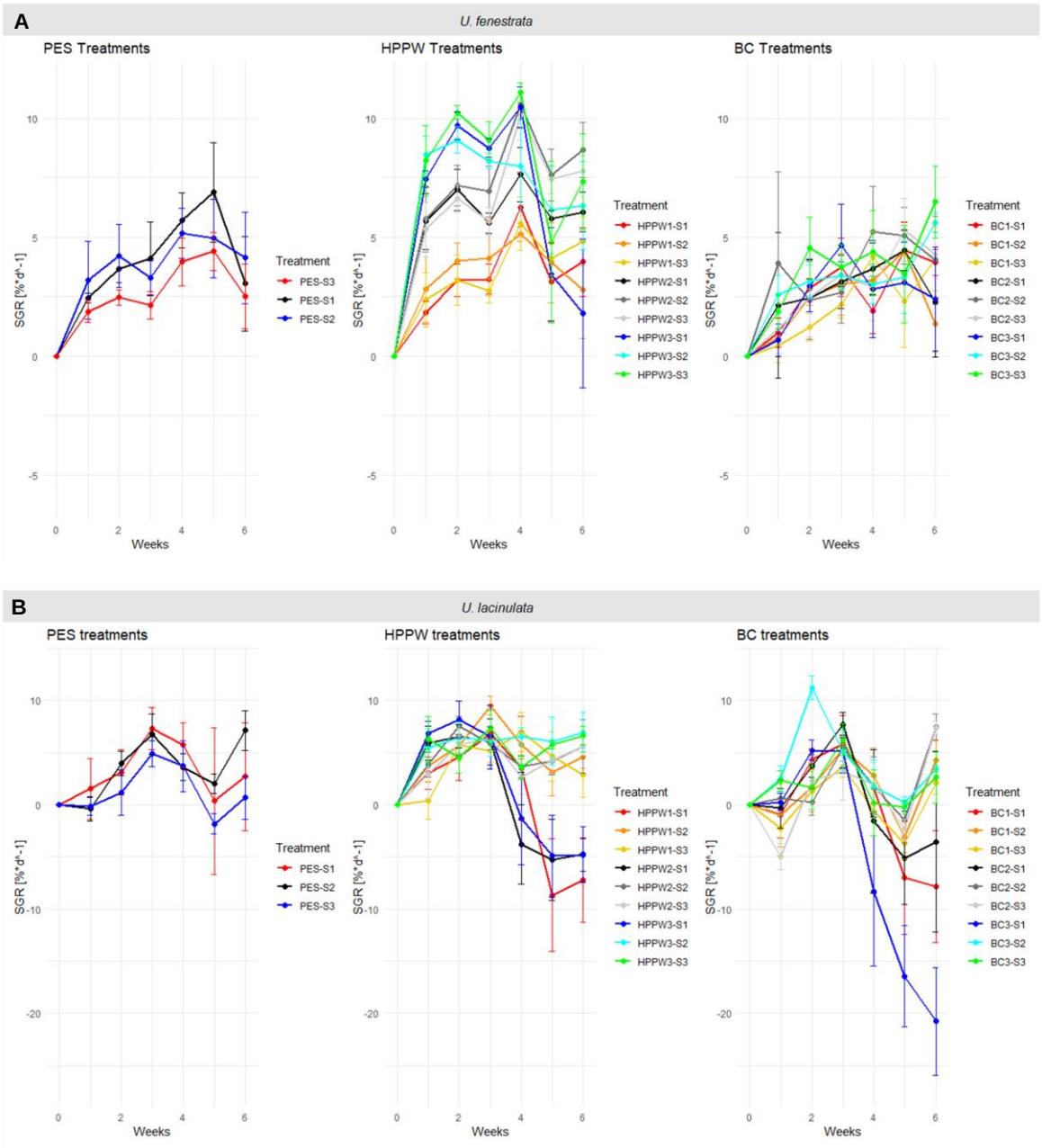

Figure. 2. Growth measurements over six weeks in (A) *U. fenestrata* and (B) *U. lacinulata*. For clarity, both plots were grouped by nutrient source: Provasoli enriched seawater (PES), herring production process water (HPPW), and boosted control (BC). Within all groups, the treatments are further differentiated by salinity levels (S1 = 5 PSU, S2 = 15 PSU, S3 = 30 PSU) and within HPPW and BC also by concentration levels (1 = 25µM, 2 = 75µM, 3 = 150µM NH_4^+). The growth performance is presented for each treatment as specific growth rate [%] per day in each week. Error bars indicate the standard deviation (n = 4).

Table. 4. Results of the linear mixed-effect model (LMM) assessing the specific growth rate of *U. fenestrata and U. lacinulata.* The model includes fixed effects (salinity, nutrients, and their interaction) and a random intercept for Week to account for repeated measures. Salinity was tested at three levels (5, 15 and 30 PSU), and nutrients at seven levels: Provasoli enriched seawater (PES); boosted control (BC), and the herring productions process water (HPPW) at 25, 75 and 150 µM NH_4^+. The reference treatment is the positive control (PES) at 30 PSU. A Type III ANOVA was applied to the LMM to evaluate the significance of the main effects and interactions. Where significant effects were detected, post hoc comparisons were conducted using Tukey-adjusted estimates. Significant *p*-values are indicated with stars (below 0.05 = *, 0.01 = **, 0.001 = ***). The results in (A) present the random effects. Panel (B) shows the fixed effects, with estimates, standard errors, degrees of freedom (df), t-values and *p*-values. Panel (C) shows the ANOVA (type III) results. Panel (D) presents the significant post hoc comparisons in week 6

A

	U. fenestrata		***U. lacinulata***	
Random effect	Variance	Std. Dev.	Variance	Std. Dev.
Week (Intercept)	1.883	1.372	7.339	2.709
Residual	2.148	1.465	5.752	2.398

B

	U. fenestrata					***U. lacinulata***				
	Estimate	SE	df	t value	*p*-value	Estimate	SE	df	t value	*p*-value
(Intercept)	0.97	1.37	19.57	0.71	0.49	1.09	2.47	14.41	0.44	0.67
5 PSU	0.57	1.41	100	0.41	0.69	0.97	2.31	100	0.42	0.68
15 PSU	0.78	1.41	100	0.56	0.58	-0.56	2.31	100	-0.24	0.81
BC1	-0.94	1.41	100	-0.67	0.51	-1.15	2.31	100	-0.50	0.62
BC2	-0.49	1.41	100	-0.35	0.73	-2.91	2.31	100	-1.26	0.22
BC3	0.12	1.41	100	0.09	0.93	0.59	2.31	100	0.26	0.80
HPPW1	0.11	1.41	100	0.08	0.94	0.64	2.31	100	0.28	0.78
HPPW2	1.84	1.41	100	1.30	0.20	1.11	2.31	100	0.48	0.63
HPPW3	4.57	1.41	100	3.24	*0.003* **	1.89	2.31	100	0.82	0.42
Week	0.50	0.38	19.57	1.34	0.20	0.04	0.68	14.41	0.06	0.95
5 PSU*BC2*Week	-0.33	0.58	100	-0.58	0.57	-2.13	0.91	100	-2.35	*0.021* *
5 PSU*BC3*Week	-0.32	0.58	100	-0.53	0.58	-4.21	0.91	100	-4.65	*< 0.0001* ***
5 PSU*HPPW1*Week	-0.05	0.58	100	-0.08	0.94	-2.57	0.91	100	-2.833	*0.006* **
5 PSU*HPPW2*Week	-0.38	0.58	100	-0.65	0.52	-2.52	0.91	100	-2.78	*0.006* **
5 PSU*HPPW3*Week	-0.78	0.58	100	-1.35	0.18	-2.58	0.91	100	-2.85	*0.005* **

C

	U. fenestrata				***U. lacinulata***			
	SS	df	*F* value	*p*-value	SS	df	*F* value	*p*-value
Salinity	2.35	100	0.55	0.58	103.95	100	9.04	*< 0.0001* ***
Nutrients	121.9	100	9.46	*< 0.001* ***	98.47	100	2.85	*0.013* *
Week	11.63	5	5.42	0.067	0.91	5	0.16	0.71
Salinity*Nutrients	2.78	100	0.11	0.99	13.55	100	0.20	0.20
Salinity*Week	5.74	100	1.33	0.27	537.72	100	46.74	*< 0.0001* ***
Nutrients*Week	19.09	100	1.49	0.19	159.30	100	4.62	*< 0.0001* ***
Salinity*Nutrients*Week	11.45	100	0.44	0.94	149.64	100	2.17	*0.019* *

D

Week 6			Estimate	SE	df	t value	*p*-value
U. fenestrata	Nutrients	BC1 – HPPW2	-5.02	0.85	100	-5.91	*< 0.0001* ***
		BC1 - HPPW3	-3.27	0.85	100	-3.27	*0.004* **
		BC2 - HPPW2	-4.38	0.85	100	-5.16	*< 0.0001* ***
		BC2 - HPPW3	-2.63	0.85	100	-3.1	*0.04* *
		HPPW1 - HPPW2	-4.07	0.85	100	-4.8	*0.0001* ***
		HPPW2 - PES	3.97	0.85	100	4.68	*0.0002* ***
U. lacinulata	Nutrients	PES - BC1	4.42	1.33	100	3.31	*0.021* *
		PES – BC3	7.65	1.33	100	5.73	*< 0.0001* ***
		BC1 – HPPW3	-4.31	1.33	100	-3.23	*0.027* *
		BC3 – HPPW1	-6.22	1.33	100	-4.66	*0.0002* ***
		BC3 – HPPW2	-6.28	1.33	100	-4.71	*0.0002* ***
		BC3 – HPPW3	-7.54	1.33	100	-5.65	*< 0.0001* ***
		BC2 – BC3	5.73	1.33	100	4.29	*0.0008* ***
	Salinity	5 PSU – 30 PSU	8.35	0.87	100	9.55	*< 0.0001* ***
		5 PSU – 15 PSU	-9.73	0.87	100	-11.14	*< 0.0001* ***

3.1.2 Tensile strength and morphological observations (Appendix 2)

U. fenestrata blades from three representative treatments (PES-S3, HPPW3-S3, and BC3-S3) were compared with respect to tensile strength (measured in week 5) and carbon-to-nitrogen (C/N) ratio (measured in week 6). A Welch-ANOVA revealed significant differences in both parameters (tensile strength: $F = 601.81$, $p < 0.0001$; C/N ratio: $F = 577.89$, $p < 0.0001$; see App. 2A, B). HPPW3 at 30 PSU (S3) showed the lowest tensile strength and C/N ratio, significantly lower than both BC3 and PES at 30 PSU (all $p < 0.04$). Visual inspection of the blade morphology (App. 2C-E) supports these findings: blades from HPPW3 were fragmented and degraded, while blades from BC3 and PES appeared more intact and structurally robust. It is also apparent that blades exposed to BC treatments appear lighter in colour than blades grown in HPPW and PES, still exhibiting a rich green colouration.

3.2 Crude protein and amino acids

After the six-week exposure, a significant interaction effect between salinity and nutrient treatments was identified for both crude protein content (*U. fenestrata*: two-way ANOVA, $F = 2.829$, $p = 0.00308$; *U. lacinulata*: two-way ANOVA, $F = 13.22$; $p < 0.0001$) and total amino acid (TAA) content (*U. fenestrata*: two-way ANOVA, $F = 8.508$, $p < 0.003$; *U. lacinulata*: two-way ANOVA, $F = 2.977$, $p < 0.0001$).

In *U. fenestrata*, the highest crude protein content (40.3% ± 3.7% dry weight) and TAA (38.9% ± 4.1%) were recorded at the lowest salinity (5 PSU) in combination with the highest process water treatment (HPPW3). Under these conditions, the crude protein and TAA content were significantly higher compared to PES (protein: 18% ± 2%, $p < 0.0001$; TAA: 17.4% ± 1.8%, $p < 0.0001$) and the corresponding ammonium-based BC3 treatment (protein: 10.3% ± 0.9%, $p < 0.0001$, TAA: 10.5% ± 0.9%, $p = 0.008$). The crude protein and TAA levels generally declined with increasing salinity across all salinities. HPPW treatments (1-3) consistently resulted in higher values than the control treatments, PES and BC, with BC performing the poorest.

A similar pattern was observed in *U. lacinulata*, with peak crude protein (45.4 % ± 1.3%) and TAA content (41.6% ± 2.9%) also occurring at 5 PSU under HPPW3. The observed nutrient-driven increase under HPPW treatments in *U. fenestrata* was less distinct in *U. lacinulata*, especially at higher salinities. At 5 PSU, only HPPW2 and 3 led to significantly higher crude protein values compared to the control PES (28.7% ± 6.4%, $p < 0.0001$), while neither of the HPPW treatments exhibited significantly pronounced increases at 30 PSU ($p > 0.05$).

Both species responded positively to HPPW treatments, with protein and TAA profiles increasing alongside nutrient concentration. Notable exceptions occurred in *U. lacinulata*, where PES occasionally surpassed HPPW1 at 30 PSU, and BC3 approached PES in crude protein content at 5 PSU.

For all treatments and species, the crude protein content estimated by the factor of five (Angel et al., 2016) adapted in this report corresponds well to the average conversion factor calculated from the TAA (4.62 ± 0.51).

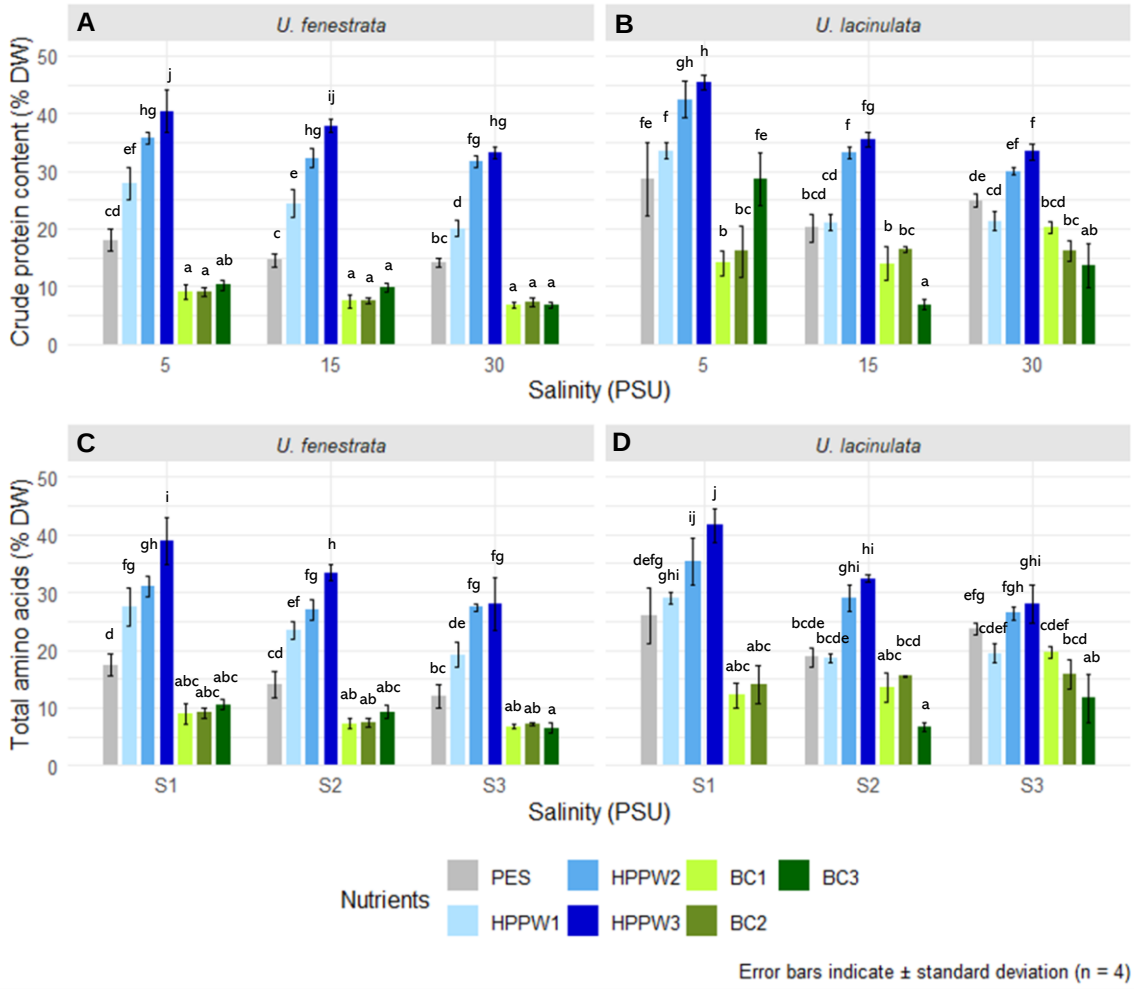

Figure. 3. (A, B) Crude protein content (% DW) and (C, D) total amino acids (% DW) in *U. fenestrata* and *U. lacinulata* cultivated under 21 combinations of nutrient sources and salinity levels (see Table 3). The total amino acid content for BC3 at 5 PSU in *U. lacinulata* could not be assessed due to insufficient biomass. Significant differences were assessed using a two-way factorial ANOVA with interaction effects, followed by Tukey's HSD post hoc test. The letters show statistically significant differences between treatment combinations.

3.3 Osmotic stress response

3.3.1 Proline content

While no interaction between salinity and nutrients was detected (*U. fenestrata*: $F = 0.710$, $p = 0.736$; *U. lacinulata*: $F = 1.222$, $p = 0.293$), salinity revealed a strong main effect in both species (two-way ANOVA, *U. fenestrata*: $F = 13.537$, $p < 0.0001$; *U. lacinulata*: $F = 40.042$, $p < 0.0001$), exhibiting increased relative proline levels with rising salinity (Figure 4C, D). The detected nutrient effect was more pronounced in *U. lacinulata* ($F = 6.619$, $p < 0.0001$)

compared to *U. fenestrata* (F =2.393, p = 0.038). BC2 and BC3 correlated with elevated proline concentrations, while HPPW2 and HPPW3 showed lower values.

Interestingly, the total proline content (Figure 4A, B) did not align with the observed relative proline trends, rather resembling crude protein. In *U. fenestrata*, the total proline content was stable across salinity, slightly decreasing under HPPW3. Although no interaction was observed, salinity and nutrient effects were significant ($p < 0.04$). Conversely, *U. lacinulata* showed a significant interaction between salinity and nutrient treatment (two-way ANOVA: F = 4.044, $p < 0.0002$), with BC treatments experiencing a salinity-related increase in total proline levels, while HPPW led to slight decreases.

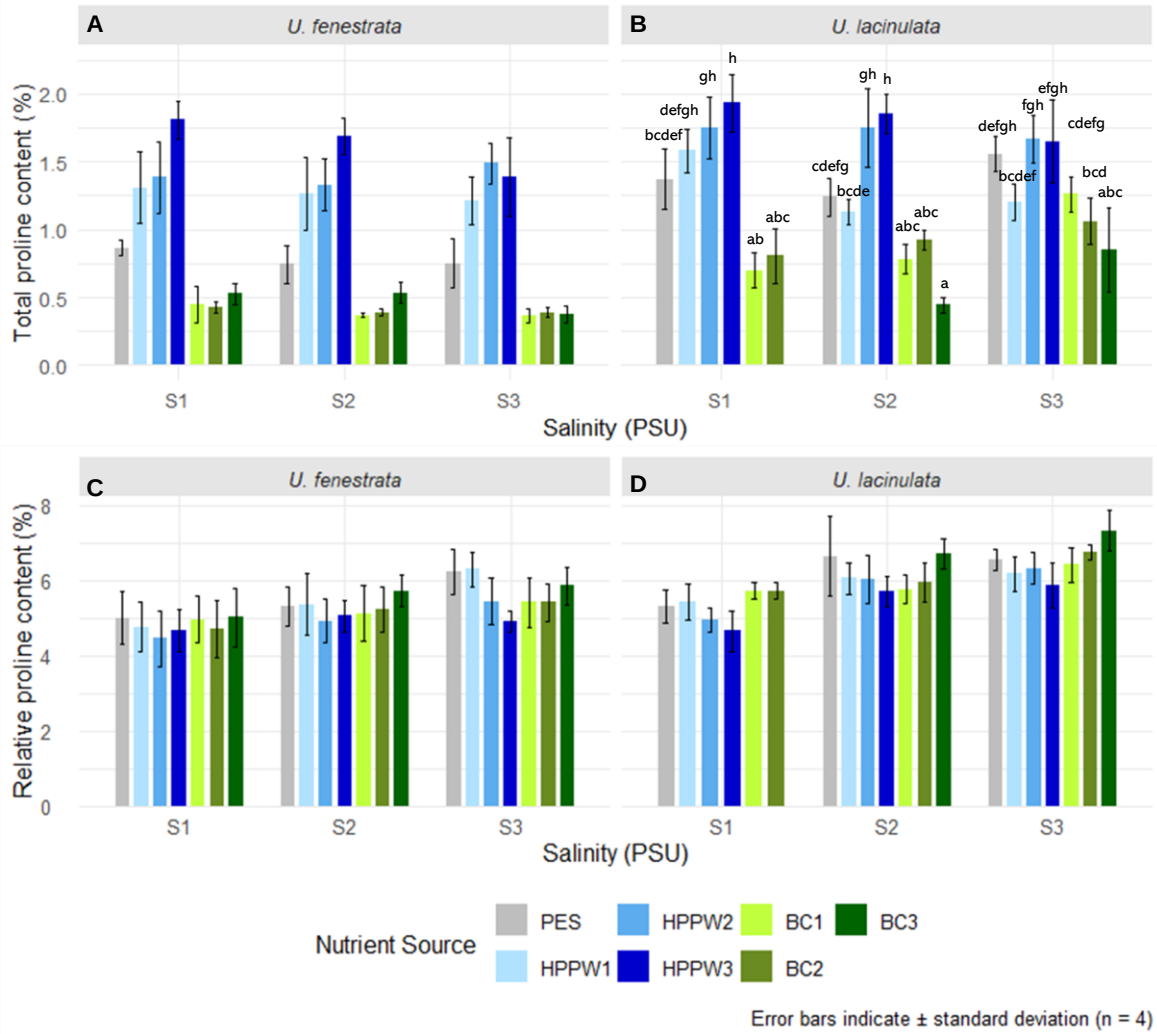

Figure. 4. Total and relative proline content (%) in (A, C) *U. fenestrata* and (B, D) *U. lacinulata* across all treatments (see Table 3). The relative proline content represents its portion of the total amount of amino acids in each sample measured. Significant differences were assessed using a two-way factorial ANOVA with interaction effects followed by Tukey's HSD post hoc test on each species separately. The letters show statistically significant differences between treatment groups.

3.3.2 Water quotient

A noteworthy interaction effect on water quotient (WQ) was identified in *U. fenestrata* (two-way ANOVA, F =5.947, p < 0.0001) and *U. lacinulata* (Welch-ANOVA, F = 29.631, p < 0.0001). In *U. fenestrata*, salinity alone also demonstrated a significant main effect (F = 110.889, p < 0.0001), revealing a clear trend of decreasing WQ with increasing salinity levels (Figure 5A). For *U. lacinulata*, the Welch ANOVA design did not permit a formal evaluation of the salinity main effect due to assumption violations; however, visual inspection suggests a slight downward trend in WQ as salinity rose, particularly under the BC3 treatment (Figure 5B). The lowest WQ values for *U. fenestrata* were observed at 30 PSU under BC and PES, while *U. lacinulata* exhibited its lowest values exclusively under BC3. Conversely, the HPPW treatments, especially HPPW3, consistently correlated with higher WQ values across salinity levels in *U. fenestrata*. Meanwhile, the PES control consistently showed intermediate WQ values. Overall, *U. lacinulata* displayed a more stable WQ response across different nutrient treatments.

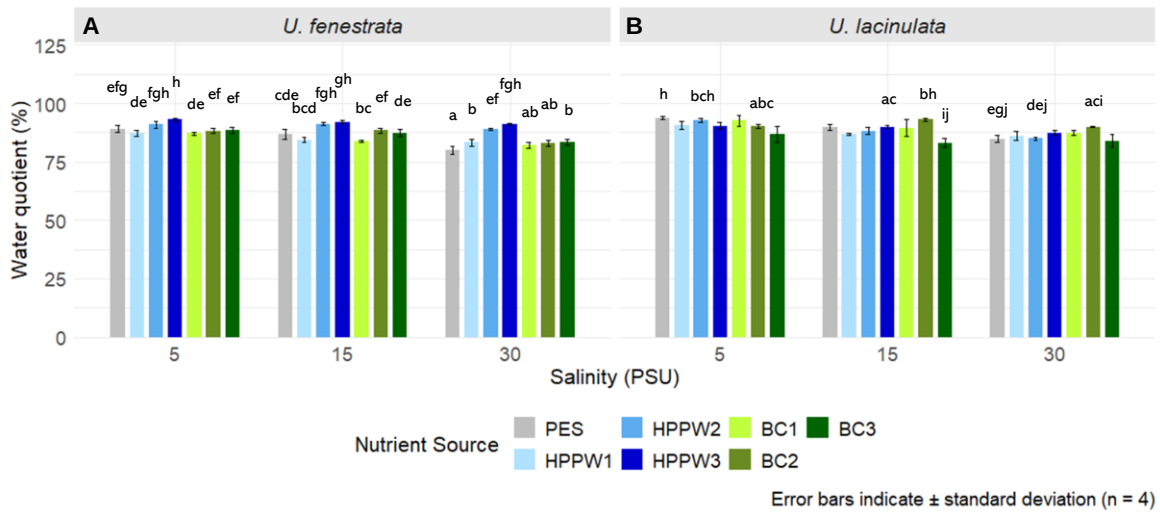

Figure. 5. Water quotient (% of fresh weight) in (A) *U. fenestrata* and (B) *U. lacinulata* across all treatments (see Table 3). The data for *U. fenestrata* was analysed using a two-way ANOVA followed by a Tukey HSD post hoc test. To account for unequal variances in *U. lacinulata,* a Welch's ANOVA with Games-Howell post hoc was performed. The letters show statistically significant differences between the treatments. In (B), only a subset of post hoc letters is presented. Letters were displayed selectively to highlight comparisons with broader statistical relevance, while groupings with significance limited to a single pairwise comparison were omitted for clarity.

3.4 Pigment and phenolic content

All pigments and phenolic parameters in *U. fenestrata* and *U. lacinulata* were significantly influenced by the interaction of salinity and nutrient treatments ($p < 0.05$), except for chlorophyll *b* in *U. fenestrata* ($p = 0.357$). Overall, concentrations peaked at low salinity (5 PSU), particularly under PES and HPPW1 treatments.

In *U. fenestrata*, chlorophyll *a* demonstrated a clear interaction effect (two-way ANOVA; $F = 2.496$, $p = 0.00956$), with peak values under HPPW1 at 5 PSU (1.76 mg/g DW) and a consistent decline as salinity increased. The BC treatments produced the lowest concentrations across all salinities (0.43 - 0.79 mg/g DW), significantly lower than PES and HPPW1/2 (all $p < 0.0001$). Under HPPW, the chlorophyll *a* content declined significantly with nutrient load (all $p < 0.2$), except between HPPW1 and 2 at 5 PSU ($p = 0.930$) (Figure 6A). Chlorophyll *a* levels in *U. lacinulata* were comparable in magnitude to those of *U. fenestrata* and also significantly influenced by salinity and nutrients (Welch ANOVA; $F = 67.709$, $p < 0.0001$). The highest and most stable concentrations occurred under PES across all salinities (1.34 - 1.7 mg/g DW). While HPPW values declined with increasing salinity, as in *U. fenestrata*, BC treatment values rose with salinity and surpassed HPPW at 30 PSU. The lowest overall value was found under HPPW3 at 30 PSU (0.51 mg/g DW). The Games-Howell post hoc comparison confirmed that several pairwise differences were statistically significant (Figure 6B).

Chlorophyll *b* followed similar patterns to chlorophyll *a* in both species but occurred at lower concentrations. While no significant interaction was detected in *U. fenestrata* (two-way ANOVA; $F = 1.125$, $p = 0.357$), the values still demonstrated a slight decline across salinities. The highest concentrations were under HPPW1, with lower values in HPPW3 and BC treatments (Figure 6C). In contrast, *U. lacinulata* showed a significant interaction (two-way ANOVA; $F = 16.18$, $p < 0.0001$) with chlorophyll *b* levels overall higher than in *U. fenestrata* and reflecting the trends observed for chlorophyll *a*. PES yielded the highest concentrations (1,82 – 2.35 mg/g DW), while HPPW treatments declined significantly with increasing nutrient load across all salinities (HPPW1 vs HPPW3: all $p < 0.04$). As observed for chlorophyll *a*, the BC treatments in *U. lacinulata* increased with salinity (Figure 6D).

Carotenoid concentrations mirrored the trends observed for chlorophylls and were significantly affected by the interaction of salinity nutrient treatment (*U. fenestrata*: two-way ANOVA, $F = 2.694$, $p = 0.00542$; *U. lacinulata*: Welch ANOVA, $F = 56.341$, $p < 0.0001$) (Figure 6E, F).

Both species recorded the highest carotenoid content under PES at 5 PSU (*U. fenestrata*: 1.16 mg/g DW; *U. lacinulata*: 1.08 gm/g DW). For *U. fenestrata*, levels declined with salinity in PES and HPPW, except for HPPW1, which remained stable. BC and HPPW3 consistently produced the lowest carotenoid values without significant differences (all $p > 0.05$). In *U. lacinulata*, however, these treatments led to increasing values with salinity, consistent with the chlorophyll patterns. However, due to high standard errors and missing data at 5 PSU, most salinity comparisons were non-significant, except for BC1 ($p = 0.019$).

Phenolic content in both species was also significantly affected by the salinity-nutrients interaction (*U. fenestrata*: two-way ANOVA, $F = 4.202$, $p = 0.0001$; *U. lacinulata*: Welch ANOVA, $F = 13.229$, $p < 0.0001$). In *U. fenestrata*, the highest phenolic levels were measured under HPPW treatments, while BC treatments yielded the lowest across all salinities. PES produced intermediate levels that differed significantly from both within each salinity (all $p < 0.01$; Figure 6G). In *U. lacinulata*, PES led to the highest phenolic content, particularly at 15 and 30 PSU. HPPW1 and HPPW2 showed modest increases at 5 PSU but were not statistically distinct from PES ($p > 0.05$). As salinity increased, treatment differences diminished. Due to biomass limitations, some data points were based on single replicates and excluded from statistical analysis (Figure 6H).

Across all pigment parameters, *U. lacinulata* generally exhibited higher total pigment concentrations than *U. fenestrata*, particularly for chlorophyll *b* (mean chlorophyll *b* across treatments: *U. lacinulata* = 1.121 mg/g of DW; *U. fenestrata* = 0.911 mg/g of DW).

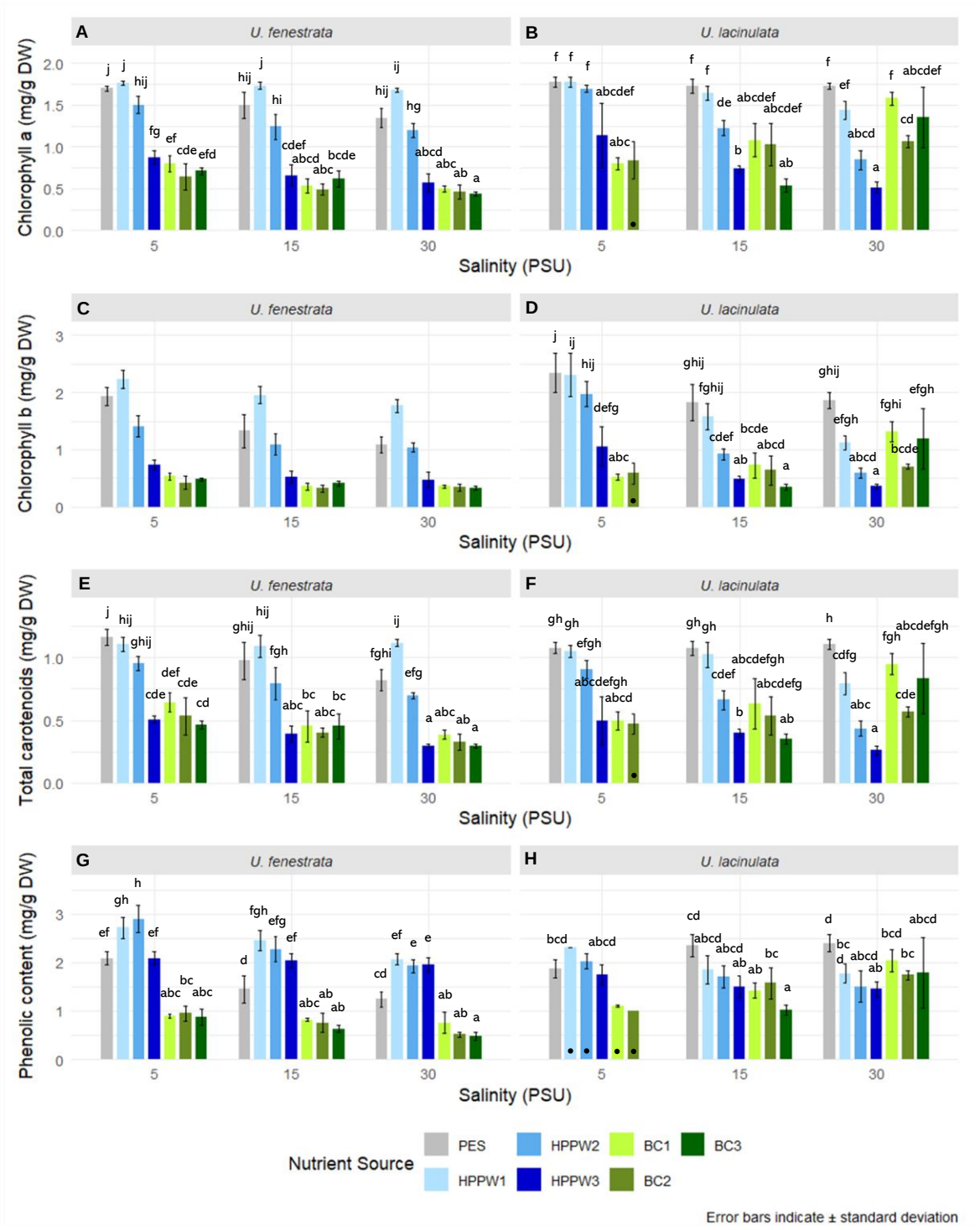

Figure. 6. Pigment content (chlorophylls *a* and *b*, and carotenoids) and total phenolic content across all treatments (see Table 3). Treatments with fewer than three replicates (marked with a black dot) were excluded from statistical analysis to ensure robust variance estimates. For pigments in *U. fenestrata* and *U. lacinulata* (A, C, E, D), the data was log-transformed and analysed using a two-way ANOVA with Tukey post hoc test. For *U. lacinulata* (B, D, F), a log transformation was performed prior to Welch's ANOVA with Games-Howell post hoc. The phenolic content (G, H) required no transformation. Statistical analysis for *U. fenestrata* used a two-way ANOVA with Tukey's post hoc. In contrast, the *U. lacinulata* data used Welch's ANOVA with the Games-Howell post hoc test.

4. Discussion

This study explored how salinity and nutrient interactions influence growth and biochemical traits in two *Ulva* species. By testing herring production process water (HPPW) under varying salinities, species-specific responses relevant to sustainable *Ulva* farming were identified.

4.1 *Ulva* growth potential in low salinities with different nutrient sources

The growth responses of *U. fenestrata* and *U. lacinulata* revealed distinct physiological strategies. *U. fenestrata* displayed a nutrient-driven growth pattern, with minimal sensitivity to salinity, while *U. lacinulata* showed a clear salinity dependence, with nutrient effects only becoming evident at moderate to high salinities.

As expected, increased nutrient availability correlated with elevated growth rates (Reidenbach et al., 2017). Earlier studies indicated that *Ulva* species generally prefer ammonium over nitrate as nitrogen source (Ale et al., 2011; Reidenbach et al., 2017), which aligns with the enhanced growth observed in the ammonium-rich HPPW treatments compared to the nitrate-based PES. However, the poor performance in all BC treatments, despite also being ammonium-based, suggests that factors beyond the nitrogen form may influence growth. The process water could contain a preferred overall nutrient composition or higher bioavailability.

This pattern was particularly conspicuous in *U. fenestrata*, sustaining fast growth rates under low-salinity conditions when exposed to highly concentrated process water treatments. Thus it suggests greater osmotic tolerance, than typically described for foliose *Ulva* species (high salinity species)(Rybak, 2018; Steinhagen et al., 2023). However, this plasticity appears to have limits: under the highest process water treatment (150µM NH_4^+) at 5 PSU, growth initially increased rapidly but collapsed by week four, likely due to ammonium toxicity. Such stress responses are often associated with an imbalance between uptake and assimilation, leading to energy and oxidative stress, as well as disrupted cellular pH homeostasis (Bittsánszky et al., 2015). Notably, the recorded growth recovery at higher salinities (15 and 30 PSU) under the same process water conditions points to a salinity-related threshold for ammonium stress tolerance.

In contrast, *U. lacinulata* experienced growth inhibition at 5 PSU under all ammonium-based nutrient treatments (HPPW, BC), although it is naturally found in mesohaline conditions (15.3 - 34 PSU)(Steinhagen et al., 2023). Only under nitrate-based PES media did the species

avoid negative growth rates (App. 1). This sensitivity may be amplified by its high nitrogen uptake rate, a common trait for green tide species (Fort et al., 2020), which can lead to toxic ammonium accumulation when ammonium cannot be efficiently assimilated. Absorbed nitrate, on the other hand, requires an enzymatic reduction to ammonium, which provides a regulatory buffer preventing its accumulation (Hurd et al., 1995; Phillips and Hurd, 2003) and might explain why *U. lacinulata* under PES maintained its growth.

While both species exhibit signs of ammonium-induced stress at low salinities, *U. fenestrata* sustained growth longer than *U. lacinulata* under the same conditions, indicating a more robust metabolic strategy and a higher tolerance to environmental fluctuations.

Growth declines observed in both species may also reflect the onset of reproduction (fertilisation or swarmer release), which diverts energy from vegetative growth (Balar and Mantri, 2020; Hiraoka, 2021). Moreover, low salinity itself may influence biochemical allocation, as Fort et al. (2024) and Xiao et al. (2016) have reported.

4.2 Salinity-nutrient interaction on physiology

For *Ulva* to establish a viable role in the future food market as a green protein alternative, its biochemical profile must be competitive with conventional protein crops. A favoured strategy is to increase and optimise the quality of the total protein content and essential amino acids (Stedt et al., 2022c, 2022e; Steinhagen et al., 2025).

Findings from this study show that *U. fenestrata* and *U. lacinulata* exhibit elevated crude protein and amino acids in response to high nutrient availability, particularly under ammonium-based process water treatments. The protein concentrations measured up to ~40% of dry weight, surpassing earlier reports of *Ulva* grown under similar nutrient conditions (~30% DW)(Stedt et al., 2022a) and salinity conditions (~28%)(Steinhagen et al., 2025), highlighting a synergistic amplification. This increase is especially noteworthy considering that protein levels in HPPW-grown *Ulva* approach those of soy (35-40%)(Grieshop and Fahey, 2001; Michelfelder, 2009) and reinforces that the culture media composition directly influences the protein accumulation (Stedt et al., 2022b, 2022e). Across salinity gradients, proteins generally increased under lower salinity and higher nutrient conditions, consistent with findings from Fort et al. (2024) and Steinhagen et al. (2025), who reported enhanced protein levels in various *Ulva* species at reduced salinities. In this study, *U. fenestrata* showed a particularly weak response to BC treatments across all salinities, while *U. lacinulata* displayed more salinity-dependent

responses, with generally higher BC values at 30 PSU, suggesting species-specific nutrient uptake efficiencies and salinity tolerance thresholds. Luo et al. (2012) reported distinct nitrate and ammonium uptake rates in *U. prolifera* and *U. linza*, supporting this hypothesis. In *U. fenestrata*, the lower protein levels under BC, despite equivalent ammonium and phosphate concentrations to HPPW, point to either differences in nutrient bioavailability (e.g., additional nitrogenous compounds in HPPW available after microbial breakdown) or uptake kinetics, which can be influenced by physical, biological and chemical aspects (Harrison and Hurd, 2001).

The amino acid profiles reflected the same overall trend. Both species showed enhanced total amino acid contents when grown in high concentrations of process water. Notably, the use of the nitrogen-to-protein conversion factor of 5 proved appropriate, as there was only a minor discrepancy between crude protein values calculated via the nitrogen content and those derived from direct amino acid quantification, confirming the reliability of this factor also discussed by Angell et al. (2016).

In addition to proteins and amino acids, pigments and phenolics play an essential role in the biochemical and physiological responses of *Ulva* species. Chlorophylls (*a*, *b*) are important for capturing light energy during photosynthesis (Rabinowitch and Govindjee, 1965), while carotenoids contribute to photoprotection by quenching reactive oxygen species (Burtin, 2003). Phenolic compounds, as secondary metabolites, are involved in several defence-related functions, including UV protection, herbivore deterrence, modulation of cell wall rigidity, and antioxidant activity (Burtin, 2003; Eismann et al., 2020). Together, they reflect photosynthetic efficiency and antioxidant defence.

In this study, the pigment and phenolic concentrations also demonstrated species-specific differences, which were significantly influenced by salinity and nutrient availability. At low salinity (5 PSU), they peaked in PES and HPPW1 treatments, suggesting that a moderate nitrogen supply (slow assimilation of nitrate or low bioavailable ammonium) supports a physiological balance between growth and photoprotection. These findings align with reports from *U. pertusa* and *U. fenestrata*, observing increased pigment and phenolic levels in response to short-term and long-term low salinity stress (Kakinuma et al., 2006; Steinhagen et al., 2025).

However, under high nutrient conditions (HPPW3), pigment concentrations decreased sharply despite significant increases in protein levels, which may indicate a metabolic trade-off, where

cellular resources are redirected towards primary metabolism (e.g., protein synthesis) at the expense of pigment synthesis. Secondary metabolites, including pigments, are typically synthesised during or close to the stationary phase of growth (Dhanarasu, 2012; Leandro et al., 2020), and the sustained exponential growth under nutrient-rich process water conditions may have suppressed this shift. These observations were evident in *U. fenestrata* and *U. lacinulata*, where the highest protein and amino acid concentrations (HPPW3) coincided with notably low pigment values, indicating metabolic prioritisation of growth over photoprotection. This pattern aligns with findings from other macroalgae. In the brown algae *Dictyota ciliolata*, nutrient enrichment stimulated growth without consistently increasing secondary metabolite production (Cronin and Hay, 1996). However, unlike *Dictyota*, where secondary metabolites decreased with nitrogen-fertilised growth, *Ulva* displayed increased phenolic content. This high-phenolic-low-pigment contradiction may be explained by ammonium-induced oxidative stress. High intracellular ammonium concentrations can elevate reactive oxygen species (ROS) production or disrupt homeostasis (Bittsánszky et al., 2015), leading to an upregulation of phenolics as an antioxidant defence.

U. lacinulata generally maintained higher pigment levels across all tested treatments than *U. fenestrata*, particularly under PES, suggesting a more conservative metabolic strategy or higher sensitivity to oxidative stress. The lower pigment and protein values in BC-grown *U. fenestrata* may reflect restricted nutrient uptake or stress, possibly due to the synthetic form of the ammonium, as discussed for the protein.

The principal component analysis (PCA; Figure 7) illustrates the impact of all tested nutrient sources on the species' metabolism, displaying distinct trait groupings and treatment separations. *U. fenestrata* under HPPW clusters strongly with protein and amino acid variables, while *U. lacinulata* exhibits more dispersed groupings, with pigment and phenolic traits playing a larger role across all nutrient sources, confirming that nutrient and salinity interactions not only affect biomass accumulation but also drive metabolic differences between the species.

Interestingly, although chlorophyll molecules contain nitrogen, and previous studies have linked blade colour intensity to tissue nitrogen content (Nissen et al., 2024; Robertson-Andersson et al., 2009; Stedt et al., 2022d), no strong correlation between crude protein and chlorophyll concentrations was visible in this study under HPPW. While PES- and BC-treated samples showed an alignment of protein and pigment levels, as previously reported in these studies, their relationship collapsed under HPPW. Possible explanations could be suppressed

pigment production with higher process water concentrations, regardless of nitrogen availability, or potential treatment residues diluting the pigment (e.g., deposits on the thallus surface of process water components).

The phenolic content also varied by treatment, in *U. fenestrata*, elevated phenolics were maintained under HPPW, generally within reported ranges (0.13 and 0.33% of DW) (Steinhagen et al., 2025, 2021; Toth et al., 2020), while BC treatments resulted in lower values. This could indicate the prioritisation of phenolic production over pigment synthesis as a protective strategy against oxidative stress in HPPW-grown *Ulva*. *U. lacinulata*, on the other hand, exhibited a more uniform phenolic profile across treatments, especially at 30 PSU, reinforcing the hypothesis of metabolic trade-offs between growth and stress defence driven by environmental conditions. This is particularly relevant when aiming for biomass with specific functional compounds for use in pharmaceuticals, cosmetics or functional foods. It should also be considered that, despite their antioxidative roles, phenolic compounds may act as anti-nutritional factors by forming complexes with proteins and minerals, thereby reducing their bioavailability (Bora, 2014).

Proline dynamics offer additional insight into osmotic and oxidative stress responses. *Ulva* is known for its salinity tolerance, maintaining cell turgor by adjusting osmotic potential (Kirst, 1990). This involves altering the concentration of internal inorganic ions and organic osmolytes, predominantly proline, in response to increasing salinity (Angell et al., 2015; Kakinuma et al., 2006; Lee and Liu, 1999). This aligns with the observed positive trend of relative proline under rising salinity in this study. However, total proline content appears strongly influenced by overall metabolic investment in growth and protein synthesis under different nutrient conditions. Moreover, in hyposaline conditions, intracellular water accumulates due to osmotic imbalance (Parida and Das, 2005). This is reflected in the higher salinity water content observed at lower salinities, consistent with earlier findings in *U. prolifera* (Zheng et al., 2019).

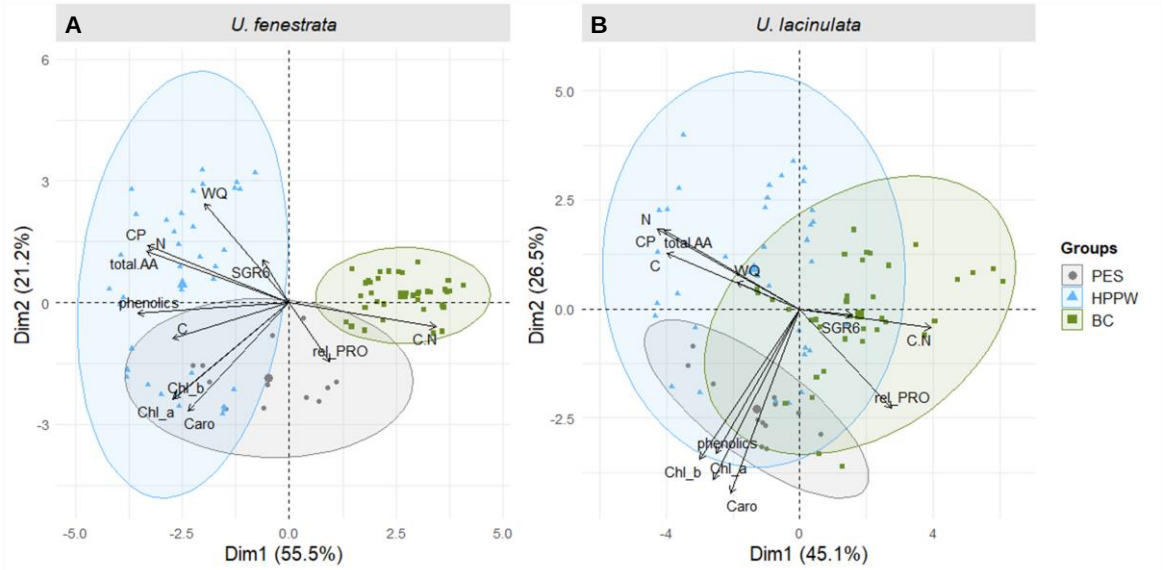

Figure. 7. Principal component analysis (PCA) illustrating the effects of the three nutrient sources (Provasoli enriched seawater (PES), herring production process water (HPPW) and boosted control (BC)) on the measured biochemical traits in (A) *U. fenestrata* and (B) *U. lacinulata*. The vectors represent metabolic parameters, including the carbon content (C), the carbon-to-nitrogen ratio (C/N), chlorophylls *a* and *b* (Chl_a, Chl_b), carotenoids (Caro), phenolics, relative proline content (rel_PRO), crude protein (CP), nitrogen (N), total amino acids (total.AA), the specific growth rate in week 6 (SGR6) and the water quotient (WQ)

4.3 Evaluation of HPPW as a nutrient source

Herring production process waters (HPPW) present a promising strategy for circular nutrient reuse in *Ulva* cultivation. Rich in ammonium, protein, micronutrients and fatty acids, these process waters provide essential nutrients and contribute to sustainable waste valorisation by reducing disposal costs and environmental impact (Osman et al., 2015; Westgate and Park, 2010). Its use aligns with integrated aquaculture systems, where macroalgae have been successfully cultivated alongside fish farms to remediate nitrogenous waste (e.g., Broch et al., 2013; Handå et al., 2013; Zhou et al., 2006). Studies concerning on-land tank cultivation have also reported significantly enhanced *Ulva* species' growth and protein content through exposure to process waters (Ashkenazi et al., 2019; Sebök and Hanelt, 2023; Stedt et al., 2022a). Compared to alternative media (PES: nitrate-based; BC: synthetic ammonium), HPPW offers a complex nutrient matrix that can boost growth and protein consistently, yet its composition is less controllable and varies between batches. In this study, moderate ammonium levels (75 µM) in HPPW maximised consistent growth, while higher concentrations (150µM) induced collapses in *U. lacinulata* and a temporary crash in *U. fenestrata* at the same salinity (5 PSU). These species-specific salinity-dependent toxicity thresholds mirror earlier work where

ammonium concentrations above 80µM became inhibitory for *Enteromorpha linza* and *E. compressa* (Taylor et al., 2001). While Stedt et al. (2022e) reported optimal growth of *U. fenestrata* at 20µM rather than 200µM, present findings suggest that moderate to high concentrations (75 µM and 150µM) can be effective (latter in 15 -30 PSU). By contrast, PES avoids toxicity by supplying nitrate, whose enzymatic reduction buffers intracellular ammonium levels but delivers lower overall growth rates and protein yields. For real-world applications, it is important to know that the HPPW composition can vary depending on the industrial process applied (Stedt et al., 2022e), and standardisations or pre-treatments (for example dilutions and microbial stabilisation) may be needed to ensure consistent nutrient delivery.

4.4 Implications and outlook

This study underlines the importance of matching species, salinity, and nutrient conditions to optimise biomass yield and biochemical quality in *Ulva* cultivation. Given the growing interest in low-salinity cultivation environments (e.g., the Baltic Sea), the results support previous work (Steinhagen et al., 2025), indicating that *Ulva* strains can thrive in brackish conditions. *U. fenestrata* appears especially suitable for such environments, especially when provided with appropriate nutrient sources. *U. lacinulata*, on the other hand, showed more limited potential at 5 PSU but performed well at 15 PSU, indicating moderate plasticity.

From a food system perspective, increasing protein levels through process water reuse presents a resource-efficient strategy that goes beyond yield, improving food quality per unit input. Moreover, observed biochemical changes in the tensile strength and carbon-to-nitrogen ratio of HPPW-grown *Ulva* may improve digestibility and protein extraction, which is relevant for the food and functional food industry. *Ulva* species typically contain high amounts of carbohydrates, up to 65% of DW, including structural polysaccharides like ulvan and cellulose (Juul et al., 2024; Kraan, 2012). These fibers are resistant to human digestive enzymes and can act as a physical barrier blocking the enzymes to access intracellular proteins (Juul et al., 2024). Thus, lower tensile strength may indicate reduced structural carbohydrates, potentially increasing digestibility and extractability. The low C/N ratio observed in HPPW treatments supports this hypothesis and suggests lower fiber content and higher crude protein levels. Interestingly, the composition of individual amino acids remained relatively stable, especially between PES and HPPW treatments, although the total amount of TAA increased (see App. 3).

This is contradicting with earlier research, reporting a reduction in relative total essential amino acids in *U. fenestrata* cultivated in HPPW compared to seawater (Stedt et al., 2022a). For a more conclusive interpretation, however, in-depth analysis of the dataset would be necessary. Nevertheless, practical implementation of HPPW as a cultivation medium requires attention to species-specific stress tolerances, especially ammonium concentrations and salinity thresholds. While HPPW shows promise, its variable composition and potential to induce stress in high concentrations call for careful management.

Despite robust trends, this study was limited by a small sample size and occasional lack of biomass in some treatments, restricting statistical power for certain variables in *U. lacinulata*. Furthermore, laboratory conditions do not fully reflect field dynamics (Steinhagen et al., unpublished). Future trials should extend over longer timeframes, including more replicates. In addition, investigating more stress-related metabolites, including sugar alcohols and dimethylsulfoniopropionate (DMSP), could deepen the understanding of osmotic responses and may also influence sensory properties relevant to food applications.

5. Conclusion

This study demonstrates that *Ulva* species are suitable for low-salinity farming, including brackish water coast environments like the Baltic Sea. *U. fenestrata* exhibited high growth rates and biochemical performance across all salinities, indicating high plasticity, while *U. lacinulata* showed salinity sensitivity. The interactive effects of salinity and nutrient source were evident in growth and biochemical composition. High nutrients, especially from ammonium-rich process waters, boosted protein and amino acid content, though extreme concentrations led to stress under hyposaline conditions. The differences in pigment and phenolic content further reflected species-specific metabolic strategies under varying cultivation media. Integrating the herring production process water as a nutrient source effectively promoted protein content and overall growth rates and demonstrated potential as a circular input for sustainable aquaculture. However, species-specific stress responses at high ammonium concentrations highlight the need for dose and exposure time optimisation. Overall, these findings support the feasibility of *Ulva* in low-salinity environments using nutrient-rich process waters, especially when matched to the right species and environmental conditions. Future work should expand on these results under field-relevant scales and conditions to validate their applicability for sustainable food systems.

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7. References

Ale, M.T., Mikkelsen, J.D., Meyer, A.S., 2011. Differential growth response of *Ulva lactuca* to ammonium and nitrate assimilation. J. Appl. Phycol. 23, 345–351. https://doi.org/10.1007/s10811-010-9546-2

Al-Hafedh, Y.S., Alam, A., Buschmann, A.H., 2015. Bioremediation potential, growth and biomass yield of the green seaweed, *Ulva lactuca* in an integrated marine aquaculture system at the Red Sea coast of Saudi Arabia at different stocking densities and effluent flow rates. Rev. Aquac. 7, 161–171. https://doi.org/10.1111/raq.12060

Angell, A.R., Mata, L., De Nys, R., Paul, N.A., 2016. The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. J. Appl. Phycol. 28, 511–524. https://doi.org/10.1007/s10811-015-0650-1

Angell, A.R., Mata, L., de Nys, R., Paul, N.A., 2015. Indirect and direct effects of salinity on the quantity and quality of total amino acids in *Ulva ohnoi* (Chlorophyta). J. Phycol. 51, 536–545. https://doi.org/10.1111/jpy.12300

Araújo, R., Vázquez Calderón, F., Sánchez López, J., Azevedo, I.C., Bruhn, A., Fluch, S., Garcia Tasende, M., Ghaderiardakani, F., Ilmjärv, T., Laurans, M., Mac Monagail, M., Mangini, S., Peteiro, C., Rebours, C., Stefansson, T., Ullmann, J., 2021. Current Status of the Algae Production Industry in Europe: An Emerging Sector of the Blue Bioeconomy. Front. Mar. Sci. 7. https://doi.org/10.3389/fmars.2020.626389

Ashkenazi, D.Y., Israel, A., Abelson, A., 2019. A novel two-stage seaweed integrated multi-trophic aquaculture. Rev. Aquac. 11, 246–262. https://doi.org/10.1111/raq.12238

Balar, N.B., Mantri, V.A., 2020. Insights into life cycle patterns, spore formation, induction of reproduction, biochemical and molecular aspects of sporulation in green algal genus *Ulva*: implications for commercial cultivation. J. Appl. Phycol. 32, 473–484. https://doi.org/10.1007/s10811-019-01959-7

Bikker, P., van Krimpen, M.M., van Wikselaar, P., Houweling-Tan, B., Scaccia, N., van Hal, J.W., Huijgen, W.J.J., Cone, J.W., López-Contreras, A.M., 2016. Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. J. Appl. Phycol. 28, 3511–3525. https://doi.org/10.1007/s10811-016-0842-3

Bittsánszky, A., Pilinszky, K., Gyulai, G., Komives, T., 2015. Overcoming ammonium toxicity. Plant Sci. 231, 184–190. https://doi.org/10.1016/j.plantsci.2014.12.005

Blikra, M.J., Altintzoglou, T., Løvdal, T., Rognså, G., Skipnes, D., Skåra, T., Sivertsvik, M., Noriega Fernández, E., 2021. Seaweed products for the future: Using current tools to develop a sustainable food industry. Trends Food Sci. Technol. 118, 765–776. https://doi.org/10.1016/j.tifs.2021.11.002

Bolton, J.J., Cyrus, M.D., Brand, M.J., Joubert, M., Macey, B.M., 2016. Why grow *Ulva*? Its potential role in the future of aquaculture. Perspect. Phycol. 3, 113–120. https://doi.org/10.1127/pip/2016/0058

Bora, P., 2014. Anti-Nutritional Factors in Foods and their Effects. J. Acad. Ind. Res. 3, 285–290.

Broch, O.J., Ellingsen, I.H., Forbord, S., Wang, X., Volent, Z., Alver, M.O., Handå, A., Andresen, K., Slagstad, D., Reitan, K.I., Olsen, Y., Skjermo, J., 2013. Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway. Aquac. Environ. Interact. 4, 187–206. https://doi.org/10.3354/aei00080

Burtin, P., 2003. NUTRITIONAL VALUE OF SEAWEEDS. Electron. J. Environ. Agric. Food Chem. 2, 498–503.

Choi, T.-S., Kang, E.-J., Kim, J.-H., Kim, K.-Y., 2010. Effect of salinity on growth and nutrient uptake of *Ulva pertusa* (Chlorophyta) from an eelgrass bed. ALGAE 25, 17–26. https://doi.org/10.4490/algae.2010.25.1.017

Cronin, G., Hay, M.E., 1996. Effects of Light and Nutrient Availability on the Growth, Secondary Chemistry, and Resistance to Herbivory of Two Brown Seaweeds. Oikos 77, 93–106. https://doi.org/10.2307/3545589

Dhanarasu, S. (Ed.), 2012. Chromatography and Its Applications. IntechOpen, Croatia.
Dinesh Kumar, S., Malar Vizhi, J., Abantika, M., Dhanya, N., Nadukkattu Nayagi, N., Satish, L., 2023. Production of *Ulva fasciata* biomass through optimization of culture condition for viable tank-based edible seaweed farming. Biomass Convers. Biorefinery 1–12. https://doi.org/10.1007/s13399-023-03966-8
Duarte, C.M., Holmer, M., Olsen, Y., Soto, D., Marbà, N., Guiu, J., Black, K., Karakassis, I., 2009. Will the Oceans Help Feed Humanity? BioScience 59, 967–976. https://doi.org/10.1525/bio.2009.59.11.8
Duarte, C.M., Wu, J., Xiao, X., Bruhn, A., Krause-Jensen, D., 2017. Can Seaweed Farming Play a Role in Climate Change Mitigation and Adaptation? Front. Mar. Sci. 4, 100. https://doi.org/10.3389/fmars.2017.00100
Eismann, A.I., Perpetuo Reis, R., Ferreira Da Silva, A., Negrão Cavalcanti, D., 2020. *Ulva* spp. carotenoids: Responses to environmental conditions. Algal Res. 48, 101916. https://doi.org/10.1016/j.algal.2020.101916
Famà, P., Wysor, B., Kooistra, W.H.C.F., Zuccarello, G.C., 2002. MOLECULAR PHYLOGENY OF THE GENUS *CAULERPA* (CAULERPALES, CHLOROPHYTA) INFERRED FROM CHLOROPLAST tufA GENE1. J. Phycol. 38, 1040–1050. https://doi.org/10.1046/j.1529-8817.2002.t01-1-01237.x
FAO, 2022. The State of the World's Land and Water Resources for Food and Agriculture – Systems at breaking point. Main report, 0 ed. Routledge, Rome. https://doi.org/10.4324/9780203142837
FAO, 2018. The future of food and agriculture – Alternative pathways to 2050 224pp.
FAO, 2013. Dietary protein quality evaluation in human nutrition. FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy.
Fort, A., Mannion, C., Fariñas-Franco, J.M., Sulpice, R., 2020. Green tides select for fast expanding *Ulva* strains. Sci. Total Environ. 698, 134337. https://doi.org/10.1016/j.scitotenv.2019.134337
Fort, A., Monteiro, J.P., Simon, C., Rosário Domingues, M., Sulpice, R., 2024. Short term decreases in salinity, combined with the right choice of species, can allow for a more nutritious *sea lettuce* lipid profile. Food Chem. 437, 137865. https://doi.org/10.1016/j.foodchem.2023.137865
Games, P.A., Howell, J.F., 1976. Pairwise Multiple Comparison Procedures with Unequal N's and/or Variances: A Monte Carlo Study. J. Educ. Stat. 1, 113–125. https://doi.org/10.3102/10769986001002113
Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C., 2010. Food Security: The Challenge of Feeding 9 Billion People. Science 327, 812–818. https://doi.org/10.1126/science.1185383
Grieshop, C.M., Fahey, G.C., 2001. Comparison of Quality Characteristics of Soybeans from Brazil, China, and the United States. J. Agric. Food Chem. 49, 2669–2673. https://doi.org/10.1021/jf0014009
Hafting, J.T., Critchley, A.T., Cornish, M.L., Hubley, S.A., Archibald, A.F., 2012. On-land cultivation of functional seaweed products for human usage. J. Appl. Phycol. 24, 385–392. https://doi.org/10.1007/s10811-011-9720-1
Handå, A., Forbord, S., Wang, X., Broch, O.J., Dahle, S.W., Størseth, T.R., Reitan, K.I., Olsen, Y., Skjermo, J., 2013. Seasonal- and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway. Aquaculture 414–415, 191–201. https://doi.org/10.1016/j.aquaculture.2013.08.006
Harrison, P.J., Hurd, C.L., 2001. Nutrient physiology of seaweeds: Application of concepts to aquaculture. Cah Biol Mar 42, 71–82.
Hiraoka, M., 2021. Massive *Ulva* Green Tides Caused by Inhibition of Biomass Allocation to Sporulation. Plants 10, 2482. https://doi.org/10.3390/plants10112482
Hurd, C.L., Berges, J.A., Osborne, J., Harrison, P.J., 1995. An in Vitro Nitrate Reductase Assay for Marine Macroalgae: Optimization and Characterization of the Enzyme for *Fucus Gardneri* (phaeophyta). J. Phycol. 31, 835–843. https://doi.org/10.1111/j.0022-3646.1995.00835.x
Jansen, H.M., Van Den Burg, S., Bolman, B., Jak, R.G., Kamermans, P., Poelman, M., Stuiver, M., 2016. The feasibility of offshore aquaculture and its potential for multi-use in the North Sea. Aquac. Int. 24, 735–756. https://doi.org/10.1007/s10499-016-9987-y

Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*1 and *c*2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz. 167, 191–194. https://doi.org/10.1016/S0015-3796(17)30778-3

Junning Cai, Galli, G., 2021. Global seaweeds and microalgae production, 1950-2019. https://doi.org/10.13140/RG.2.2.13736.03841

Juul, L., Nissen, S.H., Bruhn, A., Alexi, N., Jensen, S.K., Hammershøj, M., Dalsgaard, T.K., 2024. *Ulva* species: A critical review on the green seaweed as a source of food protein. Trends Food Sci. Technol. 149, 104534. https://doi.org/10.1016/j.tifs.2024.104534

Kakinuma, M., Coury, D.A., Kuno, Y., Itoh, S., Kozawa, Y., Inagaki, E., Yoshiura, Y., Amano, H., 2006. Physiological and biochemical responses to thermal and salinity stresses in a sterile mutant of *Ulva pertusa* (Ulvales, Chlorophyta). Mar. Biol. 149, 97–106. https://doi.org/10.1007/s00227-005-0215-y

Kirst, G.O., 1990. Salinity Tolerance of Eukaryotic Marine Algae. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 21–53. https://doi.org/10.1146/annurev.pp.41.060190.000321

Kraan, S., 2012. Algal Polysaccharides, Novel Applications and Outlook, in: Chang, C.-F. (Ed.), Carbohydrates - Comprehensive Studies on Glycobiology and Glycotechnology. InTech. https://doi.org/10.5772/51572

Lal, R., 2016. Feeding 11 billion on 0.5 billion hectare of area under cereal crops. Food Energy Secur. 5, 239–251. https://doi.org/10.1002/fes3.99

Laramore, S.E., Wills, P.S., Hanisak, M.D., 2022. Seasonal variation in the nutritional profile of *Ulva lactuca* produced in a land-based IMTA system. Aquac. Int. 30, 3067–3079. https://doi.org/10.1007/s10499-022-00950-3

Leandro, A., Pereira, L., Gonçalves, A.M.M., 2020. Diverse Applications of Marine Macroalgae. Mar. Drugs 18, 17. https://doi.org/10.3390/md18010017

Lee, T.-M., Liu, C.-H., 1999. Regulation of NaCl-induced proline accumulation by calmodulin via modification of proline dehydrogenase activity in *Ulva fasciata* (Chlorophyta). Funct. Plant Biol. 26, 595–600. https://doi.org/10.1071/pp99025

Lenth, R.V., Banfai, B., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Piaskowski, J., Riebl, H., Singmann, H., 2025. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package.

Li, H., Zhang, Y., Chen, J., Zheng, X., Liu, F., Jiao, N., 2019. Nitrogen uptake and assimilation preferences of the main green tide alga *Ulva prolifera* in the Yellow Sea, China. J. Appl. Phycol. 31, 625–635. https://doi.org/10.1007/s10811-018-1575-2

Lüning, K., 1992. Day and Night Kinetics of Growth Rate in Green, Brown, and Red Seaweeds. J. Phycol. 28, 794–803. https://doi.org/10.1111/j.0022-3646.1992.00794.x

Luo, M.B., Liu, F., Xu, Z.L., 2012. Growth and nutrient uptake capacity of two co-occurring species, *Ulva prolifera* and *Ulva linza*. Aquat. Bot. 100, 18–24. https://doi.org/10.1016/j.aquabot.2012.03.006

Martins, M., Oliveira, R., Coutinho, J.A.P., Faustino, M.A.F., Neves, M.G.P.M.S., Pinto, D.C.G.A., Ventura, S.P.M., 2021. Recovery of pigments from *Ulva rigida*. Sep. Purif. Technol. 255, 117723. https://doi.org/10.1016/j.seppur.2020.117723

Mata, L., Magnusson, M., Paul, N.A., de Nys, R., 2016. The intensive land-based production of the green seaweeds *Derbesia tenuissima* and *Ulva ohnoi*: biomass and bioproducts. J. Appl. Phycol. 28, 365–375. https://doi.org/10.1007/s10811-015-0561-1

Mendes, M.C., Navalho, S., Ferreira, A., Paulino, C., Figueiredo, D., Silva, D., Gao, F., Gama, F., Bombo, G., Jacinto, R., Aveiro, S.S., Schulze, P.S.C., Gonçalves, A.T., Pereira, H., Gouveia, L., Patarra, R.F., Abreu, M.H., Silva, J.L., Navalho, J., Varela, J.C.S., Speranza, L.G., 2022. Algae as Food in Europe: An Overview of Species Diversity and Their Application. Foods 11, 1871. https://doi.org/10.3390/foods11131871

Michelfelder, A.J., 2009. Soy: A Complete Source of Protein. Am. Fam. Physician 79, 43–47.

Mukhopadhyay, R., Sarkar, B., Jat, H.S., Sharma, P.C., Bolan, N.S., 2021. Soil salinity under climate change: Challenges for sustainable agriculture and food security. J. Environ. Manage. 280, 111736. https://doi.org/10.1016/j.jenvman.2020.111736

Mullis, K.B., 1986. The Polymerase Chain Reaction (Nobel Lecture). Angew. Chem. Int. Ed. 1209–1213. https://doi.org/10.1002/anie.199412091
Nardelli, A.E., Chiozzini, V.G., Braga, E.S., Chow, F., 2019. Integrated multi-trophic farming system between the green seaweed *Ulva lactuca*, mussel, and fish: a production and bioremediation solution. J. Appl. Phycol. 31, 847–856. https://doi.org/10.1007/s10811-018-1581-4
Nissen, S.H., Juul, L., Bruhn, A., Søndergaard, J., Dalsgaard, T.K., 2024. The biochemical composition and its relation to color of *Ulva* spp. upon harvest time. J. Appl. Phycol. 36, 2095–2107. https://doi.org/10.1007/s10811-024-03207-z
Osman, A., Gringer, N., Svendsen, T., Yuan, L., Hosseini, S.V., Baron, C.P., Undeland, I., 2015. Quantification of biomolecules in herring (*Clupea harengus*) industry processing waters and their recovery using electroflocculation and ultrafiltration. Food Bioprod. Process. 96, 198–210. https://doi.org/10.1016/j.fbp.2015.08.002
Parida, A.K., Das, A.B., 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicol. Environ. Saf. 60, 324–349. https://doi.org/10.1016/j.ecoenv.2004.06.010
Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical & Biological Methods for Seawater Analysis. Elsevier.
Pérez-Escamilla, R., 2017. Food Security and the 2015–2030 Sustainable Development Goals: From Human to Planetary Health. Curr. Dev. Nutr. 1, e000513. https://doi.org/10.3945/cdn.117.000513
Phillips, J.C., Hurd, C.L., 2003. Nitrogen ecophysiology of intertidal seaweeds from New Zealand: N uptake, storage and utilisation in relation to shore position and season. Mar. Ecol. Prog. Ser. 264, 31–48. https://doi.org/10.3354/meps264031
Poore, J., Nemecek, T., 2018. Reducing food's environmental impacts through producers and consumers 360, 987–992. https://doi.org/10.1126/science.aaq0216
Provasoli, L., 1968. Media and prospects of the cultivation of marine algea, in: Cultures and Collections of Algae: Proceedings of the 1968 U.S. - Japan Conference Society of Plant Physiology. Hakone, pp. 12–15.
R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing.
Rabinowitch, E.I., Govindjee, 1965. The Role of Chlorophyll in Photosynthesis. Sci. Am. 213, 74–83.
Raikova, S., Le, C.D., Beacham, T.A., Jenkins, R.W., Allen, M.J., Chuck, C.J., 2017. Towards a marine biorefinery through the hydrothermal liquefaction of macroalgae native to the United Kingdom. Biomass Bioenergy 107, 244–253. https://doi.org/10.1016/j.biombioe.2017.10.010
Ray, D.K., Foley, J.A., 2013. Increasing global crop harvest frequency: recent trends and future directions. Environ. Res. Lett. 8, 044041. https://doi.org/10.1088/1748-9326/8/4/044041
Reidenbach, L.B., Fernandez, P.A., Leal, P.P., Noisette, F., McGraw, C.M., Revill, A.T., Hurd, C.L., Kübler, J.E., 2017. Growth, ammonium metabolism, and photosynthetic properties of *Ulva australis* (Chlorophyta) under decreasing pH and ammonium enrichment. PLOS ONE 12, e0188389. https://doi.org/10.1371/journal.pone.0188389
Robertson-Andersson, D.V., Wilson ,D T, Bolton ,J J, Anderson ,R J, and Maneveldt, G.W., 2009. Rapid assessment of tissue nitrogen in cultivated *Gracilaria gracilis* (Rhodophyta) and *Ulva lactuca* (Chlorophyta). Afr. J. Aquat. Sci. 34, 169–172. https://doi.org/10.2989/AJAS.2009.34.2.7.894
RStudio Team, 2020. RStudio. RStudio.Integrted Development for R. RStudio.
Ruslan, F.S., Susanti, D., Mohammad Noor, N., Aminudin, N.I., Taher, M., 2021. Bioactive Compounds, Cosmeceutical And Nutraceutical Applications of Green Seaweed Species (Chlorophyta). Squalen Bull. Mar. Fish. Postharvest Biotechnol. 16, 41–55. https://doi.org/10.15578/squalen.514
Rybak, A.S., 2018. Species of *Ulva* (Ulvophyceae, Chlorophyta) as indicators of salinity. Ecol. Indic. 85, 253–261. https://doi.org/10.1016/j.ecolind.2017.10.061
Saunders, G.W., Kucera, H., 2010. An evaluation of rbcL, tufA, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. Cryptogam. Algol. 31, 487–528.
Sebök, S., Hanelt, D., 2023. Cultivation of the brackish-water macroalga *Ulva lactuca* in wastewater from land-based fish and shrimp aquacultures in Germany. Aquaculture 571, 739463. https://doi.org/10.1016/j.aquaculture.2023.739463

Simon, C., McHale, M., Sulpice, R., 2022. Applications of *Ulva* Biomass and Strategies to Improve Its Yield and Composition: A Perspective for *Ulva* Aquaculture. Biology 11, 1593. https://doi.org/10.3390/biology11111593

Stedt, K., Gustavsson, O., Kollander, B., Undeland, I., Toth, G.B., Pavia, H., 2022a. Cultivation of *Ulva fenestrata* using herring production process waters increases biomass yield and protein content. Front. Mar. Sci. 9, 988523. https://doi.org/10.3389/fmars.2022.988523

Stedt, K., Pavia, H., Toth, G.B., 2022b. Cultivation in wastewater increases growth and nitrogen content of seaweeds: A meta-analysis. Algal Res. 61, 102573. https://doi.org/10.1016/j.algal.2021.102573

Stedt, K., Steinhagen, S., Trigo, J.P., Kollander, B., Undeland, I., Toth, G.B., Wendin, K., Pavia, H., 2022c. Post-harvest cultivation with seafood process waters improves protein levels of *Ulva fenestrata* while retaining important food sensory attributes. Front. Mar. Sci. 9, 991359. https://doi.org/10.3389/fmars.2022.991359

Stedt, K., Toth, G.B., Davegård, J., Pavia, H., Steinhagen, S., 2022d. Determination of nitrogen content in Ulva fenestrata by color image analysis – a rapid and cost-efficient method to estimate nitrogen content in seaweeds. Front. Mar. Sci. 9, 1081870. https://doi.org/10.3389/fmars.2022.1081870

Stedt, K., Trigo, J.P., Steinhagen, S., Nylund, G.M., Forghani, B., Pavia, H., Undeland, I., 2022e. Cultivation of seaweeds in food production process waters: Evaluation of growth and crude protein content. Algal Res. 63, 102647. https://doi.org/10.1016/j.algal.2022.102647

Steinhagen, S., Enge, S., Cervin, G., Larsson, K., Edlund, U., Schmidt, A.E.M., Wahlström, N., Kollander, B., Pavia, H., Undeland, I., Toth, G.B., 2022. Harvest Time Can Affect the Optimal Yield and Quality of Sea Lettuce (*Ulva fenestrata*) in a Sustainable Sea-Based Cultivation. Front. Mar. Sci. 9. https://doi.org/10.3389/fmars.2022.816890

Steinhagen, S., Enge, S., Larsson, K., Olsson, J., Nylund, G.M., Albers, E., Pavia, H., Undeland, I., Toth, G.B., 2021. Sustainable Large-Scale Aquaculture of the Northern Hemisphere Sea Lettuce, *Ulva fenestrata*, in an Off-Shore Seafarm. J. Mar. Sci. Eng. 9, 615. https://doi.org/10.3390/jmse9060615

Steinhagen, S., Hoffmann, S., Pavia, H., Toth, G.B., 2023. Molecular identification of the ubiquitous green algae *Ulva* reveals high biodiversity, crypticity, and invasive species in the Atlantic-Baltic Sea region. Algal Res. 73, 103132. https://doi.org/10.1016/j.algal.2023.103132

Steinhagen, S., Johansson, I., Specht, J., Enge, S., Larsson, K., Undeland, I., Toth, G.B., 2025. Unlocking economic potential of the *Ulva* crop for low salinity environments: exploring the effect of salinity gradients on the performance and valuable compounds of Baltic Sea strains. Bot. Mar. 68, 65–82. https://doi.org/10.1515/bot-2024-0079

Steinhagen, S., Karez, R., Weinberger, F., 2019. Cryptic, alien and lost species: molecular diversity of *Ulva sensu lato* along the German coasts of the North and Baltic Seas. Eur. J. Phycol. 54, 466–483. https://doi.org/10.1080/09670262.2019.1597925

Steinhagen, S., Schrofner-Brunner, E., Schrofner-Brunner, B., Larsson, K., Undeland, I., unpublished. Green Gold Rush in the Baltic Sea: Investigating Sea Lettuce´s performance by distribution mapping of valuable compounds in a fluctuating environment.

Sun, B., Zhao, X., Qu, T., Zhong, Y., Guan, C., Hou, C., Tang, L., Tang, X., Wang, Y., 2024. The causal link between nitrogen structure and physiological processes of *Ulva prolifera* as the causative species of green tides. Sci. Total Environ. 953, 176170. https://doi.org/10.1016/j.scitotenv.2024.176170

Taylor, R., Fletcher, R.L., Raven, J.A., 2001. Preliminary Studies on the Growth of Selected 'Green Tide' Algae in Laboratory Culture: Effects of Irradiance, Temperature, Salinity and Nutrients on Growth Rate 44, 327–336. https://doi.org/10.1515/BOT.2001.042

Toth, G.B., Harrysson, H., Wahlström, N., Olsson, J., Oerbekke, A., Steinhagen, S., Kinnby, A., White, J., Albers, E., Edlund, U., Undeland, I., Pavia, H., 2020. Effects of irradiance, temperature, nutrients, and pCO2 on the growth and biochemical composition of cultivated *Ulva fenestrata*. J. Appl. Phycol. 32, 3243–3254. https://doi.org/10.1007/s10811-020-02155-8

Van Den Burg, S.W.K., Dagevos, H., Helmes, R.J.K., 2021. Towards sustainable European seaweed value chains: a triple P perspective. ICES J. Mar. Sci. 78, 443–450. https://doi.org/10.1093/icesjms/fsz183

Westgate, P.J., Park, C., 2010. Evaluation of Proteins and Organic Nitrogen in Wastewater Treatment Effluents. Environ. Sci. Technol. 44, 5352–5357. https://doi.org/10.1021/es100244s

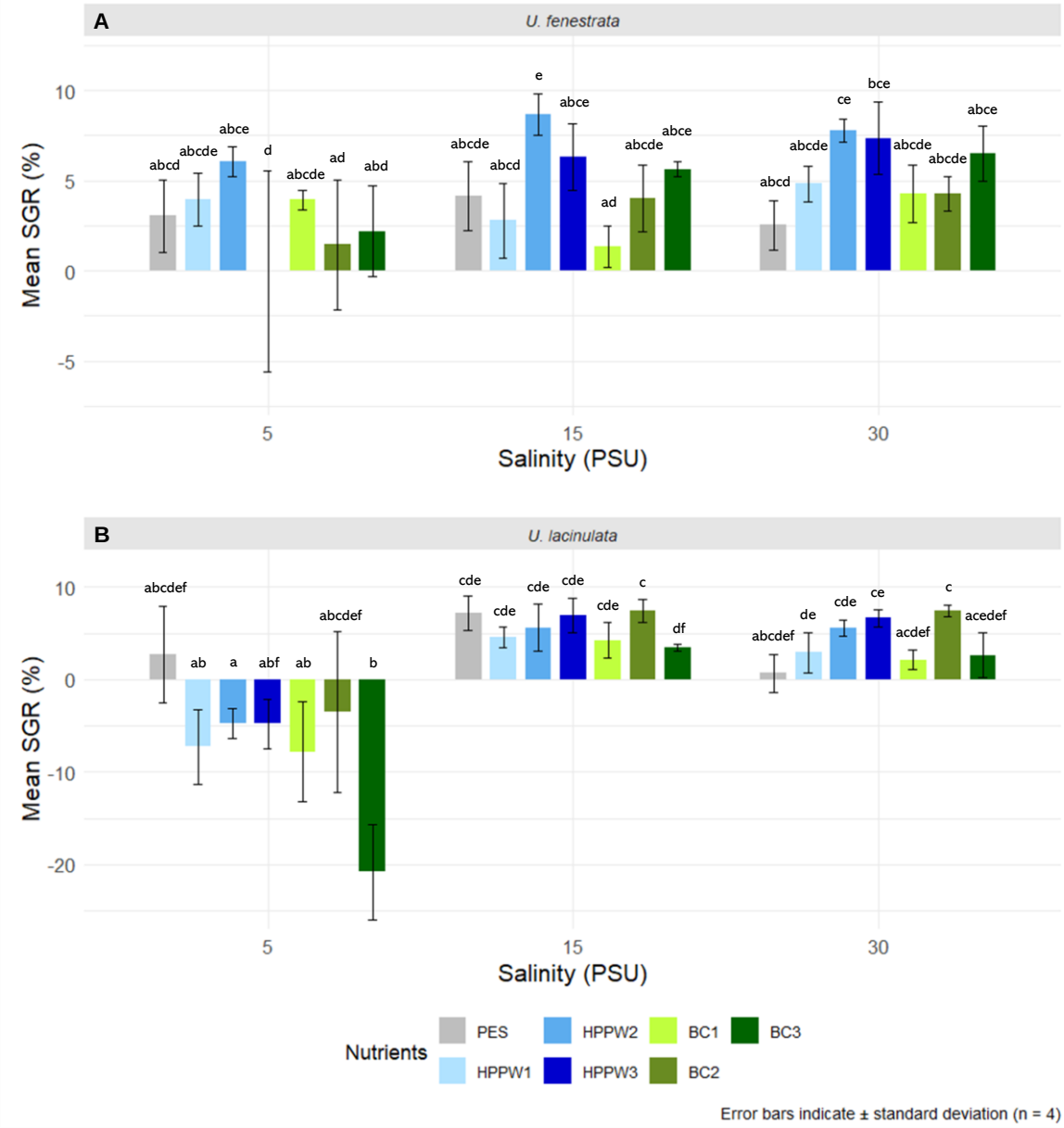
Xiao, J., Zhang, Xiaohong, Gao, C., Jiang, M., Li, R., Wang, Z., Li, Y., Fan, S., Zhang, Xuelei, 2016. Effect of temperature, salinity and irradiance on growth and photosynthesis of *Ulva prolifera*. Acta Oceanol. Sin. 35, 114–121. https://doi.org/10.1007/s13131-016-0891-0

Zheng, M., Lin, J., Zhou, S., Zhong, J., Li, Y., Xu, N., 2019. Salinity mediates the effects of nitrogen enrichment on the growth, photosynthesis, and biochemical composition of *Ulva prolifera*. Environ. Sci. Pollut. Res. 26, 19982–19990. https://doi.org/10.1007/s11356-019-05364-y

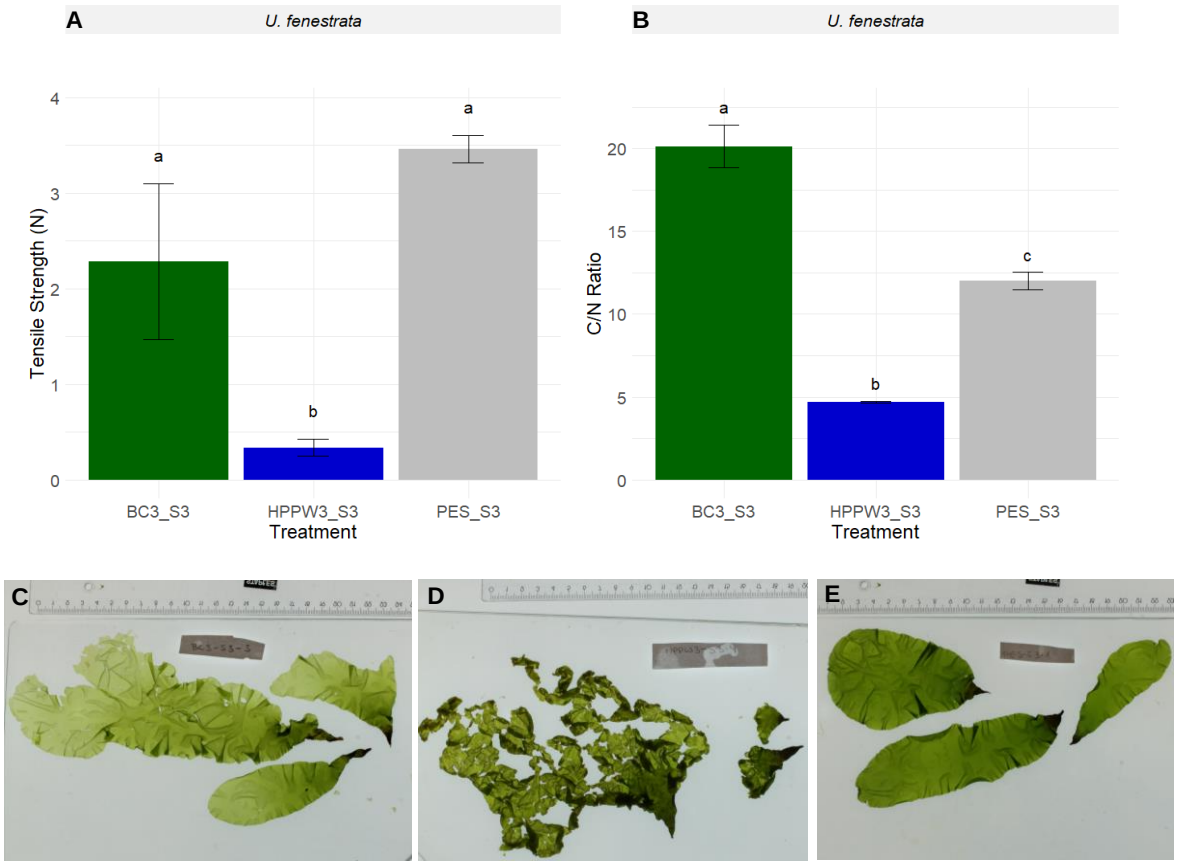
Zhou, Y., Yang, H., Hu, H., Liu, Y., Mao, Y., Zhou, H., Xu, X., Zhang, F., 2006. Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. Aquaculture 252, 264–276. https://doi.org/10.1016/j.aquaculture.2005.06.046

Zollmann, M., Liberzon, A., Palatnik, R.R., Zilberman, D., Golberg, A., 2023. Effects of season, depth and pre-cultivation fertilizing on *Ulva* growth dynamics offshore the Eastern Mediterranean Sea. Sci. Rep. 13, 14784. https://doi.org/10.1038/s41598-023-41605-4

8. Appendix



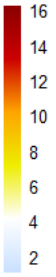
App. 1. Effect of salinity and nutrient treatments (see table 3) on growth in week 6 (endpoint of experiment) in (A) *U. fenestrata* and (B) *U. lacinulata*. Growth is displayed as the specific growth rate in percentage. Significant differences were assessed (A) using a two-way factorial ANOVA with interaction effects (Salinity × Nutrient), followed by Tukey's HSD post hoc test (α = 0.05) and (B) a log transformation was applied prior to welch-Anova to meet normality assumption followed by a Games-Howell post hoc. The letters show statistically significant differences between treatment combinations.



App. 2. (A) Tensile strength (measured in week 5) and (B) C/N ratio (measured in week 6; endpoint) of *U. fenestrata* exposed to three distinct treatments. A Welch-ANOVA was used to analyse the statistical differences in (A) and (B), with letters indicating significant differences between the treatments. (C – E) displays exemplary *Ulva* blades cultivated at 30 PSU under boosted control (BC3-S3), herring production process water (HPPW3-S3) and provasoli enriched seawater (PES3-S3) treatments respectively.

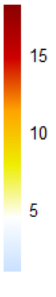
A

ARG	PHE	GLY	SER	THR	VAL	LYS	PRO	HIS	MET	TYR	ILE	ALA	LEU	ASP	GLU	
4.20	5.27	6.09	5.98	7.02	6.58	4.21	4.96	1.40	1.80	2.40	3.99	10.26	7.50	13.22	15.12	PES_S1
3.74	5.44	6.16	6.13	7.22	6.52	3.88	5.31	1.28	1.87	2.37	4.12	10.25	7.61	13.65	14.44	PES_S2
3.65	5.64	6.09	6.22	7.13	6.41	3.76	6.27	1.40	1.81	2.53	4.08	10.35	7.72	13.23	13.70	PES_S3
4.19	5.20	6.03	6.15	6.56	6.34	4.39	4.76	1.19	1.67	2.40	3.92	10.17	7.47	13.33	16.23	HPPW1_S1
3.99	5.45	5.94	6.26	6.56	5.89	4.54	5.41	1.25	1.73	2.59	3.90	9.73	7.69	13.27	15.81	HPPW1_S2
3.59	5.55	5.94	6.33	6.67	5.99	4.57	6.32	1.26	1.72	2.58	3.92	9.67	7.56	13.30	15.04	HPPW1_S3
4.51	4.98	6.44	6.53	6.46	6.50	4.75	4.47	1.12	1.75	2.17	3.89	10.28	7.15	13.66	15.35	HPPW2_S1
4.16	5.09	6.18	6.23	6.40	6.01	4.97	4.93	1.14	1.94	2.66	4.10	9.41	7.35	13.86	15.58	HPPW2_S2
3.90	5.10	5.80	6.20	6.40	5.90	4.94	5.44	1.08	1.88	2.99	4.01	9.34	7.17	13.59	16.25	HPPW2_S3
4.01	4.93	5.99	6.48	6.38	6.40	5.37	4.65	1.05	1.93	2.67	4.25	10.46	7.53	13.16	14.73	HPPW3_S1
3.95	5.09	5.98	6.04	6.30	6.14	5.28	5.05	1.07	2.01	3.00	4.34	9.65	7.61	13.44	15.04	HPPW3_S2
3.64	5.10	5.67	5.89	6.40	6.13	5.23	4.95	1.08	2.09	3.37	4.48	9.38	7.48	13.36	15.75	HPPW3_S3
3.74	5.61	6.03	6.96	7.44	6.92	3.37	5.03	1.57	2.01	2.60	4.29	10.86	7.16	13.79	12.62	BC1_S1
3.57	5.73	6.09	6.97	7.76	6.66	3.37	5.08	1.53	2.06	2.91	4.18	10.90	7.16	13.64	12.41	BC1_S2
3.33	5.90	6.15	7.21	7.74	6.69	3.40	5.42	1.60	2.02	2.83	4.18	10.96	7.19	13.62	11.79	BC1_S3
3.48	5.46	6.08	6.88	7.60	7.07	3.30	4.68	1.47	1.98	2.46	4.39	11.06	6.95	14.02	13.11	BC2_S1
3.44	5.77	6.22	6.79	7.77	6.66	3.54	5.19	1.49	2.08	2.65	4.21	10.67	7.24	13.56	12.70	BC2_S2
3.27	5.83	6.19	7.03	7.69	6.63	3.43	5.41	1.54	2.02	2.68	4.14	11.11	7.12	13.68	12.26	BC2_S3
3.44	5.45	6.19	6.80	7.51	6.75	3.56	5.00	1.40	1.84	2.30	4.18	11.07	7.04	13.92	13.54	BC3_S1
3.08	5.66	6.10	6.83	7.56	6.72	3.49	5.74	1.31	1.84	2.33	4.32	10.74	7.15	13.76	13.39	BC3_S2
3.26	6.22	6.19	6.76	7.63	6.73	3.31	5.87	1.65	2.02	2.89	4.33	10.59	7.63	12.82	12.10	BC3_S3



B

ARG	PHE	GLY	SER	THR	VAL	LYS	PRO	HIS	MET	TYR	ILE	ALA	LEU	ASP	GLU	
4.31	6.12	5.83	6.20	6.11	6.31	5.40	5.28	1.20	1.52	2.23	4.00	11.07	7.58	13.02	13.84	PES_S1
4.08	5.94	5.88	6.10	5.86	5.39	5.78	6.60	1.21	1.55	2.72	3.80	9.77	7.72	14.08	13.51	PES_S2
4.85	5.90	5.72	5.93	5.69	5.28	5.37	6.57	1.04	1.38	2.39	3.66	9.54	6.79	14.52	15.36	PES_S3
5.69	5.70	5.64	5.99	5.88	5.81	5.98	5.43	1.27	1.67	2.75	4.02	10.03	7.42	12.59	14.12	HPPW1_S1
5.52	5.28	5.61	5.40	5.39	5.39	5.92	6.05	1.39	1.67	2.94	3.61	8.58	7.62	13.83	15.80	HPPW1_S2
5.43	5.21	5.63	5.52	5.42	5.04	5.78	6.18	1.18	1.59	2.69	3.51	8.93	7.15	14.36	16.36	HPPW1_S3
4.15	5.47	5.87	5.84	6.31	6.17	6.10	4.96	1.24	1.96	2.83	4.32	10.18	7.91	12.73	13.97	HPPW2_S1
6.51	4.72	5.64	5.63	5.46	5.17	6.40	6.06	1.11	1.48	2.55	3.50	8.89	6.77	13.40	16.73	HPPW2_S2
6.69	4.61	5.45	5.43	5.35	5.18	6.39	6.34	1.04	1.42	2.46	3.47	8.42	6.52	13.03	18.19	HPPW2_S3
3.46	5.28	5.78	5.66	6.28	6.38	6.27	4.65	1.21	2.26	3.07	4.61	10.13	8.24	12.75	13.97	HPPW3_S1
5.91	4.65	5.47	5.48	5.69	5.42	6.53	5.72	1.12	1.64	2.69	3.80	8.66	7.00	13.08	17.16	HPPW3_S2
5.80	4.62	5.59	5.40	5.61	5.35	6.58	5.90	1.12	1.62	2.62	3.74	8.29	6.85	12.98	17.93	HPPW3_S3
4.34	6.82	5.66	6.30	6.28	6.10	5.54	5.73	1.51	2.00	3.82	4.45	10.83	7.35	11.23	12.02	BC1_S1
3.11	7.05	6.07	6.30	6.41	6.87	4.97	5.73	1.26	1.77	2.51	4.40	11.47	7.47	12.45	12.17	BC1_S2
3.70	6.72	5.88	6.51	6.24	5.70	5.53	6.43	0.95	1.36	2.33	4.01	10.75	6.52	13.48	13.92	BC1_S3
4.37	6.20	5.53	6.13	6.27	5.75	5.97	5.76	1.24	1.76	2.81	4.36	10.52	7.33	11.75	14.25	BC2_S1
2.99	6.86	6.01	6.38	6.60	6.37	5.17	5.95	1.07	1.72	2.40	4.41	11.20	7.35	12.76	12.76	BC2_S2
3.34	6.57	5.99	6.30	6.23	5.78	5.57	6.73	1.02	1.56	2.67	4.15	10.41	7.11	12.98	13.57	BC2_S3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	BC3_S1
4.37	6.27	6.05	5.99	5.77	6.27	5.52	6.73	1.91	2.34	4.08	4.23	9.51	8.16	10.94	11.88	BC3_S2
4.10	6.18	5.93	6.23	5.92	5.57	5.94	7.31	1.31	1.78	3.19	4.07	9.68	7.75	12.45	12.60	BC3_S3



App. 3. Heatmap showing the relative amino acid profiles of (A) *U. fenestrata* and (B) *U. lacinulata*. Each cell represents the mean proportion of a specific amino acid as a percentage of the total amino acid content (% of total), averaged across replicates per treatment. Darker shades indicate higher relative concentrations. The profile includes data from 21 treatments for each species (Table 3), excluding BC3-S1 in *U. lacinulata* due to missing data. All amino acid abbreviations: ARG: arginine (conditionally essential), PHE: phenylalanine (essential), GLY: glycine, SER: serine, THR: threonine (essential), VAL: valine (essential), LYS: lysine (essential), PRO: proline, HIS: histidine (essential), MET: methionine (essential), TYR: tyrosine, ILE: isoleucine (essential), ALA: alanine, LEU: leucine (essential), ASP: aspartic acid, GLU: glutamic acid