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Strain selection and temperature responses of *Ulva* and *Ulvaria* (Chlorophyta) for application in land-based cultivation systems

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ABSTRACT

The macroalgal family, *Ulvaceae*, holds promising candidates for cultivation in land-based Integrated Multi-trophic Recirculated Aquaculture Systems (IMRAS) due to their fast growth and nutrient uptake capabilities. Selection of appropriate strains, however, is crucial before implementation in IMRAS. In this study, an evaluation of *Ulvaceae* strains was conducted through an initial screening of in total nine strains, eight sourced from natural habitats and one commercial *Ulva* producer. The abiotic conditions were characterised by high nutrient concentrations (883 μM NO_3^--N) and were kept uniform for all strains during the screening. Following the initial screening, the effect of temperature on growth was investigated (10, 16, 22, and 28 °C) in two selected strains (*Ulva compressa* and *Ulvaria obscura*) under high nutrient conditions. This study demonstrated that four investigated *Ulvaceae* strains achieved high and stable growth rates (15–22% fresh weight d^{-1}) in indoor free-floating cultures. Further, there was consistent and significant nitrogen uptake potential of a single *Ulva compressa* strain across temperatures between 10 and 22 °C (0.03 g N L^{-1} $Week^{-1}$ corresponding to 74% of added dissolved inorganic nitrogen). *Ulva compressa* grew across all investigated temperatures with weekly variations in biomass yields (18–40 g dry weight m^{-2} day^{-1}) while *Ulvaria obscura* grew stably at 10, 16, and 22 °C (15–16 g dry weight m^{-2} day^{-1}). The findings of this study enhance our understanding of the potential uses of *Ulvaceae* strains in land-based cultivation and serves as a stepping stone for the integration of *Ulvaceae* cultivation into IMRAS on a commercial scale.

1. Introduction

The continued expansion of the aquaculture industry is constrained by its associated nutrient emissions and potential contribution to eutrophication [1–4]. Cultivating seaweeds from the opportunistic and fast-growing macroalgae family *Ulvaceae* is mentioned as a potential bioremediation strategy to mitigate nutrient emission [5,6]. As of 2020, aquaculture accounted for 50% of global aquatic animal production, emphasising the significant risk of nutrient emissions from this sector [7]. In this context, the integration and co-cultivation of *Ulvaceae* could have a potential to capture and reuse nutrients in current aquaculture practices, in both land and sea-based systems.

In land-based aquaculture systems, *Ulvaceae* cultivation can be implemented as an additional biofilter which provides control of effluent flow and composition [6,8,9]. In land-based integrated multi-trophic recirculated aquaculture systems (IMRAS), the cultivation of *Ulvaceae* absorbs nutrients from the effluents generated by i.e. finfish, crustacean or bivalve production. This approach has been studied over

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the past decades [9–14]. The produced algal biomass can subsequently be utilized for food, feed, or biochemical compounds [5,15–18]. However, not all *Ulvaceae* species are equally effective for IMRAS cultivation.

Certain *Ulvaceae* strains outperform others in free floating cultures. Recent studies have highlighted that *Ulvaceae* strains vary in growth rates and physiological responses both within and among species [19–21]. This is particularly relevant when selecting *Ulvaceae* strains for IMRAS, as it can affect production yields, marketable products, and bioremediation efficacy. Different land-based aquaculture systems may also be characterised by specific abiotic conditions depending on the primary cultivated species such as cold-water Atlantic salmon (*Salmo salar*) or tropical Pacific white shrimp (*Litopenaeus vannamei*). Consequently, screening species and assessing optimal culture conditions are crucial prior to implementing *Ulvaceae* in IMRAS.

While many *Ulvaceae* species demonstrate a high level of phenotypic buffering to varying abiotic conditions, optimal growth conditions often differ between species and strains [21–23]. For example, the effect of temperature has been correlated to growth of *Ulva lactuca* and to the kinetics of nitrate reductase of *Ulva prolifera*, where temperatures up to 15–20 °C was considered optimal for growth and nitrate reductase activity [23–25]. Additionally, the biochemical composition of various *Ulva* strains tissue has been shown to change with water temperature where protein yields decrease with temperature while carotenoid content increase with temperature [26,27]. However, given the extensive intraspecies variation in the growth responses and biochemical composition of *Ulva* strains from different locations, the effects of temperature may be highly strains specific [19,26]. This selection of species and optimal growth conditions is important for IMRAS cultivation, as it affects the effectiveness of *Ulvaceae* for bioremediation and production of marketable products such as protein and carotenoids, and therefore, it is essential to assess several *Ulvaceae* strains across a range of abiotic conditions that mimic realistic IMRAS environments before commercial implementation.

In Denmark, research on *Ulvaceae* cultivation over recent decades has mainly focused on its production potential and applications in energy, food and feed [17,28], as well as its use in bioremediating wastewater from agricultural sources, such as biogas plants and manure [29,30]. However, studies on the use of *Ulvaceae* for bioremediation of aquaculture emissions are increasingly relevant as the land-based production of *S. salar* and *L. vannamei* is expanding in Europe. Danish land-based aquaculture companies are often required to lead their wastewater through denitrification systems before emitting the effluent into the environment [31], suggesting that *Ulvaceae* cultivation may serve as an extra nutrient removal step after this process. Moreover, research on potential variation within Scandinavian *Ulvaceae* strains has been largely overlooked, unlike similar studies in other temperate regions [19–21]. A thorough screening of Danish *Ulvaceae* strains could not only support future *Ulvaceae* IMRAS cultivation across Northern Europe but also enhance the ecological understanding of green algae.

The primary aim of this study was to evaluate the suitability of Danish *Ulvaceae* species and strains as nutrient capture organisms in a variety of land-based IMRAS designs. In the first experiment, nine *Ulvaceae* strains were exposed to high-nutrient conditions, evaluating their growth potential, tissue nitrogen and pigment content, and photosynthetic rates. The second experiment examined the temperature tolerance (10–28 °C) of two strains selected based on the first experiment, assessing differences in their nutrient uptake potential, biomass production, and photosynthetic performance. These investigations offer insights into the practical application of Danish *Ulvaceae* strains in different types of IMRAS.

2. Material and methods

2.1. Algae collection

A total of nine different *Ulvaceae* strains were sampled in late September 2021. The term "strain" refers to algae sampled from different locations, representing different genotypic or ecotypic variants within the same species or different species, as defined by Fort et al. [19]. Eight *Ulvaceae* strains were sampled from their natural habitat at seven different locations in Denmark (Table 1, Fig. 1) and one *Ulva compressa* strain (UC3) was collected from Pure Algae Denmark (Grenaa, Denmark), where it had been cultivated in land-based cultures for at least one month prior to this study. The collection sites represented a range of environmental nutrient concentrations and previous recordings of *Ulvaceae* occurrence. Seven of the nine collected *Ulvaceae* strains (UA, UC1, UC2, UC3, UF, UL, and UO) had foliose morphology while the remaining two strains had tubular morphology (U and UI) (Table 1). The algae were transported to Aarhus University in 10 L plastic buckets with water from the collection site.

The surface water salinity, temperature, total dissolved inorganic nitrogen, and total phosphorus concentrations at the collection sites were obtained from the nearest monitoring stations of the Danish National Monitoring Program (NOVANA), using the September mean (2010–2020) (Table 2). Field measurements of environmental conditions were conducted at Virksund and Mariager Fjord due to lack of monitoring data. Pure Algae Denmark provided information on the abiotic conditions in their land-based *Ulva* cultivation system.

2.2. Species identification

DNA was extracted from silica-dried tissue from thallus pieces of the sampled algal strains (n = 3). Species identification was conducted using chloroplast-encoded *tuf*A sequences as a genetic marker for DNA barcoding following the methods described in Tran et al. [33]. Gene sequences have been deposited to GenBank under accession numbers (PQ658360-PQ658367, Table 1).

2.3. Acclimation

In the laboratory, the algae were rinsed of epiphytes, grazers, and sediment, using seawater from the algae collection site and artificial seawater (Instant Ocean® Sea Salt, Aquaria Systems). To reduce self-shading and ensure a uniform distribution of biomass, algae with large foliose thalli were cut to a diameter of approximately 5 cm. The algae were placed in cylindrical plexiglass tanks (diameter 13 cm, height 40 cm) (Pure Algae Denmark, Grenaa, Denmark) containing each 3.5 L of growth medium. The algae were added at a fresh weight (FW) stocking density of 7.1 g FW L^{-1} (except strain UO which was at 5.7 g FW L^{-1} due to low biomass at the collection site) and acclimated for one week at 15 °C, and an irradiance of 120 μmol photons m^{-2} s^{-1} (TL5 HO, 39 W, 830/840, Philips, Netherlands) with a 14-hours light and 10-hours dark cycle. The growth medium was artificial seawater (Instant Ocean® Sea Salt, Aquarium Systems) with a salinity of 20–22, enriched with nutrients (Cell-Hi F2P, Varicon Aqua Solutions) to provide 198 μM nitrate nitrogen (NO_3^--N) and 35 μM phosphate phosphorus (PO_4^--P). Nutrient concentrations were added to a concentration > 100 μM nitrate to ensure optimal growth conditions as according to Nielsen et al. [29] and Sode et al. [30]. The tanks were aerated with atmospheric air through silicon tubes at the bottom to keep the algae suspended and allow for gas exchange.

2.4. Experiment 1 - strain selection

Following one week of acclimation, the nine *Ulvaceae* strains were transferred to plexiglass tanks (diameter 13 cm, height 30 cm) (Pure Algae Denmark, Grenaa, Denmark) containing each 2.8 L artificial seawater (Instant Ocean® Sea Salt, Aquarium Systems) with a salinity of 20–22, and kept at 15 °C in a thermoregulated room. Each strain was distributed over five replicate tanks (biological replicates), except for *U. lacinulata*, which had only four due to low biomass. The foliose thalli

Table 1
Ulvaceae strain ID, species, Genbank accession numbers, collection sites (name, GPS coordinates) and morphology of the 9 *Ulvaceae* strains at date of collection and 1–3 weeks after start of the experiments. Strain morphologies included cluster [21], foliose and tubular. *Ulva compressa* was considered conspecific to *U. mutabilis* according to Steinhagen et al. [32].

Strain ID	Identified species	Accession no.	Collection site	GPS coordinates (North, East)	Morphology at collection	Morphology during experiments
U	Not identified	-	Nakkebølle Fjord	55.061219, 10.377758	Tubular	Tubular, cluster
UA	*U. australis*	PQ658361	Nykøbing Mors	56.794874, 8.871575	Foliose	Foliose, cluster
UC1	*Ulva compressa*	PQ658360	Seden Beach	55.441539, 10.449016	Foliose	Foliose
UC2	*U. compressa*	PQ658362	Skive Fjord	56.559324, 9.054899	Foliose	Foliose
UC3	*U. compressa*	PQ658367	Pure Algae Denmark	56.407860, 10.927283	Foliose	Foliose
UF	*Ulva fenestrata*	PQ658365	Fornæs	56.443133, 10.958914	Foliose	Foliose
UI	*U. intestinalis*	PQ658366	Fornæs	56.443133, 10.958914	Tubular	Tubular, cluster
UL	*Ulva lacinulata*	PQ658364	Mariager Fjord	56.690542, 10.058817	Foliose	Foliose, cluster
UO	*Ulvaria obscura*	PQ658363	Virksund	56.606361, 9.295726	Foliose	Foliose

Fig. 1. Map of Denmark showing the eight algae collection sites and the ID of strains collected at each site. For strain ID see Table 1.

were cut to a maximum diameter of 5 cm, and the initial stocking density was 1.5 g FW L^{-1} for all strains.

Nutrients (f/2, [34]) were added to the tanks corresponding to 883 μM NO_3^--N and 42 μM PO_4^--P. It was not expected that nitrogen addition to concentrations above 100 μM would influence algae growth and hence the increase from approximately 200 μM to 883 μM would not influence growth [29,30]. The nutrient concentration was used to mimic a typical non-specific aquaculture effluent, where nutrient concentrations exceed the algae requirements [29]. Nitrate was chosen as the nitrogen source instead of ammonia to mimic aquaculture effluent which has undergone denitrification prior to emission or algae cultivation. It was estimated that all nine *Ulvaceae* strains were acclimated to the high nutrient concentrations as their tissue nitrogen content were above 4% of DW at the beginning of Experiment 1 (except for UC1 where tissue nitrogen content was around 2.6% of DW), suggesting sufficient tissue nitrogen content for optimal growth [35]. *Ulvaria obscura* had a higher tissue nitrogen content than the other *Ulvaceae* strains (6.7% of DW) potentially caused by exposure to a higher nitrogen concentration (34.7 μM g^{-1} FW) during the initial acclimation than the other strains (27.9 μM g^{-1} FW). The tanks were aerated with atmospheric air using a single 4 mm silicone tube at the base of the tanks to keep the algae suspended and minimize self-shading.

Irradiance was supplied by a LED light source (Cosmorrow, LED 40 W 24 V Blooming, 6500 K - 2100 K - 660 nm, Secret Jardin, Manage, Belgium) positioned at the side of the tanks with a 14-hours light and 10-hours dark cycle. Surface irradiance was approx. 160 μmol photons m^{-2} s^{-1} and ranged from 91 to 169 μmol photons m^{-2} s^{-1} depending on whether measurements were taken at the bottom or the top of the tanks.

The experiment ran for three weeks, during which algae biomass was harvested, tanks were cleaned, and the growth medium renewed weekly. A salad spinner (OXO Good Grips, USA) was used to standardise the removal of excess water from the harvested biomass before

Table 2
Salinity, temperature, and nutrient concentrations at the algae collection sites, presented as September means ± SE (total range) (2010–2020) based on data from the Danish National Monitoring Program (NOVANA), measured during algae sampling, or provided by Pure Algae Denmark. Dissolved inorganic nitrogen included the concentrations of nitrate, ammonium, and nitrite. Phosphorus was the total concentration of orthophosphate in the water. Superscript letters indicate data source: "a" NOVANA, n = 10–27, "b" measured at time of algae collection, n = 1, "c" provided by Pure Algae Denmark. Distance to monitoring station of Pure Algae Denmark is not relevant, as this is a land-based cultivation facility with manipulation of abiotic conditions.

Collection site	Distance to monitoring station (km)	Surface water salinity	Surface water temperature (°C)	Total water dissolved inorganic nitrogen (μM)	Total water phosphorus (μM)
Fornæs	36.6	19.8 ± 0.5[a]	15.9 ± 0.3[a]	0.84 ± 0.3 (0.29–5.2)[a]	0.12 ± 0.02 (0.03–0.29)[a]
Mariager Fjord	Not relevant	18[b]	16.5[b]	14.0[b]	2.0[b]
Nakkebølle Fjord	4.5	16.1 ± 0.3[a]	16.5 ± 0.3[a]	0.45 ± 0.5 (0.29–1.16)[a]	0.11 ± 0.02 (0.03–0.32)[a]
Nykøbing Mors	22	27.8 ± 0.2[a]	15.7 ± 0.4[a]	1.05 ± 0.2 (0.32–4.57)[a]	1.06 ± 0.09 (0.23–1.78)[a]
Seden Beach	6.2	20.4 ± 0.4[a]	15.1 ± 0.4[a]	4.55 ± 1.2 (0.35–29.34)[a]	0.80 ± 0.08 (0.03–1.91)[a]
Skive Fjord	7.0	24.7 ± 0.6[a]	15.7 ± 0.5[a]	4.02 ± 1.0 (0.3–17.49)[a]	2.23 ± 0.3 (0.11–5.49)[a]
Pure Algae Denmark	Not relevant	24[c]	~17[c]	223[c]	112[b]
Virksund	1.2	27[b]	15.7[b]	5.70 ± 3.3 (0.29–55.5)[a]	0.51 ± 0.2 (0.03–3.55)[a]

measuring the fresh weight. Biomass corresponding to the initial stocking density was then transferred back to the tanks. The fresh weight specific growth rate (SGR, % FW d^{-1}) was calculated weekly as:

$$SGR = 100 \cdot (ln(FW_t/FW_0)/t)$$

where FW_0 was the initial fresh weight and FW_t was the fresh weight at harvest after t days.

2.5. Experiment 2 - temperature responses

Two of the nine strains from Experiment 1 (UO and UC3) were selected for the temperature response experiments based on their high growth rates and tissue nitrogen content and kept in plexiglass tanks under same constant conditions as in Experiment 1 (Section 2.4) for four months. Biomass and thallus size regulation as well as growth medium exchange was performed weekly to keep the initial stocking density at 1.5–2 g FW L^{-1}. Prior to the temperature response experiments, the algae were gradually acclimated to the experimental temperatures (10, 16, 22, and 28 °C) over three weeks, adjusting the temperature by two degrees every 3–4 days, until the target temperature was reached. The algae were then exposed to this target temperature for at least seven days before the experiment began.

2.5.1. Biomass yield

Following temperature acclimation, the two selected algae strains were cultured in a five-replicate setup (biological replicates) with light and salinity conditions as described in Experiment 1 (Section 2.4). The growth medium volume was 3 L, and the initial stocking density was 1.5 g FW L^{-1}. Based on an environmental assessment report regarding a Danish land-based recirculating *S. salar* aquaculture [31] it was decided to increase nutrient enrichment to 161.9% f/2 stock [34] which was added twice a week, resulting in a total weekly addition of 2860 μmol NO_3^--N and 136 μmol PO_4^-P per L culture. For each temperature treatment a single tank without algae biomass was set up to serve as a control when sampling for water nutrient concentrations (Section 2.5.2). The control tanks were treated in the same manner as tanks containing algae biomass. The experiment lasted for three weeks with biomass harvest and renewal of cultivation medium every week. Upon harvest, biomass corresponding to the initial stocking density was transferred back to the tanks while the remaining biomass was used for analyses of biomass composition (Section 2.6). Biomass yield (BY, g DW m^{-2} day^{-1}) was calculated as:

$$BY = \frac{(FW_t \cdot DM_t - FW_0 \cdot DM_0)}{A} \Big/ t$$

where A was the surface area of the cultivation tanks, t the duration of the experiment, FW the fresh weight and DM the tissue dry matter content (% of FW) (Section 2.6).

2.5.2. Dissolved inorganic nitrogen in the growth medium

The concentration of dissolved inorganic nitrogen (DIN, g N L^{-1}) in the growth medium was analysed during the second week of the experiment. Water samples from both control tanks and tanks containing algae were taken on day 0, 4, and 7 of the experimental week. The DIN concentration (g N L^{-1}) was analysed using a five channel SKALAR SAN Plus segmented flow autoanalyzer (Breda, The Netherlands). All methods were adopted by Grasshoff et al. [36]. The measured DIN concentrations were used to calculate the following variables:

The "total DIN concentration added" (DIN_{tot}, g N L^{-1}) was calculated as the sum of DIN measured for each of the empty control tanks on day 0 and 4 after nutrient addition. No replicates were available for control tanks and hence the "total DIN added" were grouped across temperature treatments to accommodate for potential pipetting errors or evaporation effects (n = 4).

The "final DIN concentration" (g N L^{-1}) was measured in all tanks on day 7 of the experimental week. The DIN concentration measured in the empty control tanks was compared to the "total DIN concentration added" to examine if nitrogen was removed in the empty tanks (Section 2.8.3) (n = 4). The final DIN concentrations measured in tanks containing algae (DIN_{algae}) on day 7 were used to compare temperature and strain effects (Section 2.8.3) (n = 3–5).

The "removed DIN concentration" (g N L^{-1} $Week^{-1}$) was calculated as the difference between DIN_{tot} and DIN_{algae} and compared to the nitrogen which was assimilated in the algae biomass ("volume specific nitrogen uptake potential", Section 2.6.2) (n = 3–5).

2.6. Composition of the algae biomass

2.6.1. Dry matter content

The harvested algae biomass was oven dried at 60 °C until constant weight. The dried algae biomass was weighed, and the dry matter content (% of FW) was estimated as the ratio between dry weight (DW) and fresh weight.

2.6.2. Tissue carbon and nitrogen content

To determine tissue carbon and nitrogen (% of DM), the dry biomass samples were milled, homogenized and analysed using a CN analyser (Vario EL Cube, Elementar, Langenselbold, Germany).

To compare DIN reduction in the growth media with algae tissue nitrogen, the biomass yield was standardised to tissue nitrogen content as "volume specific nitrogen uptake potential" (g N L^{-1} $Week^{-1}$) calculated as:

$$\text{"Volume specific nitrogen uptake potential"} = DW_t \cdot N_t - DW_0 \cdot N_0$$

where DW was the dry weight of the algae biomass per litre growth medium (g L^{-1}) and N was the tissue nitrogen content (% of DW) at the beginning (0) and end (t) of the experimental week.

2.6.3. Pigment content

The tissue content of total chlorophyll (Chl. a + b, mg g^{-1} DW) and total carotenoids (xanthophylls and carotenes, mg g^{-1} DW) were determined spectrophotometrically after lyophilization and 24 h dark extraction in 96% ethanol according to Lichtenthaler [37].

2.7. Photosynthetic parameters

The photosynthetic light response of the *Ulvaceae* strains was measured with a Clark-type electrode connected to an oxygen monitor (Chlorolab, 3 System, Hansatech Instruments, Norfolk, UK). Fresh algae tissue was cut to 3 cm^2 pieces, corresponding to approx. 0.2 g FW and placed in darkness for at least 30 min prior to measurements. The tissue was incubated in the photosynthetic chamber in 10 mL growth medium and the same temperature as in the experiments (15 °C for Experiment 1 and 10, 16, 22, or 28 °C for Experiment 2). The algae were fixated in the chamber to prevent self-shading. Irradiance was provided from a LED light source (LH36/2R, Hansatech Instruments, Norfolk, UK) at nine different photon flux densities and was progressively increased from 0 to 700 μmol m^{-2} s^{-1}. The maximum photosynthetic rate (P_{max}, μmol O_2 g^{-1}DW h^{-1}), dark respiration rate (R_d, μmol O_2 g^{-1}DW h^{-1}), and light saturation point (I_k, μmol photons m^{-2} s^{-1}) were estimated by fitting the hyperbolic tangential model of Jassby and Platt [38] modified to include dark respiration in SigmaPlot 14 (Systat Software Inc., San Jose, USA):

$$P = P_{max} \cdot tanh\left(I_k \cdot \frac{I_d}{P_{max}}\right) + R_d$$

where I_d is irradiance (μmol photons m^{-2} s^{-1}) and P is the net photosynthetic rate at I_d.

2.8. Data analysis

2.8.1. General data treatment

All data were tested for homogeneity of variance using Levene's test or Mauchly's test of sphericity, and for normality using Shapiro-Wilk's test. When assumptions were met, the data were analysed with ANOVA, considering $p < 0.05$ as significant and $p = 0.05$–0.1 as a "trend". Tukey's post-hoc tests were conducted for significant ANOVA results ($p < 0.05$). If the assumption of normality was not met, a non-parametric test was applied (PERMANOVA or unpaired Wilcoxon signed-sum exact test) [39] without pairwise comparison of means. All data showed variance homogeneity though "biomass yield" and "volume specific nitrogen uptake potential" were not normally distributed during Experiment 2. Results were presented as means with standard deviations, or boxplots using R-console and R-studio (packages: *ggplot2*, *multcompview*, *vegan)* [40–42].

2.8.2. Experiment 1

Algae strains that sporulated during the experiment were removed from statistical analysis due to incomparability with the non-sporulating strains. The SGR was analysed for effects of strain and harvest number and their interaction with a two-way ANOVA with replicate ID as a repeated measure. Photosynthetic parameters and tissue biochemical composition were sampled only in the final experimental week, hence only the effect of strain and not the effect of harvest number was analysed using a one-way ANOVA on these parameters.

2.8.3. Experiment 2

The first-week data were removed, because biomass yield, dry matter content and tissue nitrogen concentration suggested incomplete algal acclimation. *Ulvaria obscura* degraded during the acclimation at 28 °C, which resulted in no biomass for the experiment and, consequently, no data for this treatment. Biomass yields were analysed for effects of strain, temperature and harvest number in a 3-way PERMANOVA using replicate ID to restrict permutations to account for the repeated measures (9999 permutations).

Photosynthetic parameters and tissue biochemical parameters were only sampled at the final week of the experiment and therefore only the effect of strain, temperature and their interaction were statistically tested with a two-way ANOVA.

A one-way ANOVA compared the DIN concentration at the beginning and the end of the experimental week in the control tanks without algae to assess if nitrogen was removed significantly when algae were not present. A two-way ANOVA analysed the effects of strain and temperature on the DIN concentrations at the end of the experimental week in the tanks containing algal biomass ($n = 3$–5).

Differences between the removed DIN from the medium and the nitrogen which was taken up in the algal tissue (volume specific nitrogen uptake potential, Section 2.6) were compared with an unpaired Wilcoxon signed-sum exact test across all temperatures and strains.

Optimum temperatures for all parameters for UC3 were assessed, excluding UO due to degradation at 28 °C. Non-linear regression with a Gaussian equation was used to estimate the optimum temperature:

$$a^{*}e^{-\left(0.5^{*}\left(\frac{\text{Temperature}-b}{c}\right)^{2}\right)}$$

where a was the peak value, b the optimal temperature (critical point), and c the regression growth rate. The optimum temperatures were presented with 95% confidence intervals, if significant by X^2 probability test ($p < 0.05$). The analysis was done in JMP Pro 16 (SAS Institute Inc.)

3. Results

3.1. Species identification

Species identification was successful for eight out of the nine collected strains with the algae strain "U" remaining unidentified. A total of six species were identified (Table 1) which included five *Ulva* species (*U. compressa*, *U. australis*, *U. lacinulata*, *U. fenestrata* and *U. intestinalis*) and one *Ulvaria* species (*Ulvaria obscura*). Notably, three strains of *Ulva compressa/Ulva mutabilis* (UC1, UC2 and UC3) were identified from three different locations (Seden Beach, Skive Fjord and Pure Algae Denmark). *U. compressa* was considered conspecific to *U. mutabilis* according to Steinhagen et al. [32] and will hereafter only be mentioned as *U. compressa*. A more detailed description of the phylogenetic groupings is available in Tran et al. (in prep.).

3.2. Experiment 1 - strain selection

3.2.1. Specific growth rates

The SGR varied from −20 to 22% FW d^{-1} (Fig. 2) across all strains and weekly harvests. Five out of the nine algae strains (U, UA, UF, UI, and UL) sporulated spontaneously during the experiment which led to variable and sometimes negative growth rates between harvest weeks (Fig. 2). We differentiated between sporulation and degradation events by the observation of spores or gametes and formation of algae clusters [43]. As the focus of this study was the direct applicability of *Ulvaceae* strains in land-based cultures, with the need for robust cultures, only strains without spontaneous sporulation were further analysed.

The SGR of the four remaining strains (UC1, UC2, UC3 and UO) increased significantly over the three-week experiment ($p < 0.001$, Table 3). The SGR was significantly affected by strain ($p < 0.001$) with the *U. compressa* strain UC2 having a significantly higher growth rate (17.2 ± 4% FW d^{-1}) than UC1, UC3, and UO (range 11.0–12.1% FW d^{-1}).

3.2.2. Tissue dry matter, nitrogen, and carbon content

The dry matter content did not vary between the four non-sporulating strains ($p = 0.17$) and ranged between 10 ± 2% and 13 ± 2% of FW (Tables 4, 5).

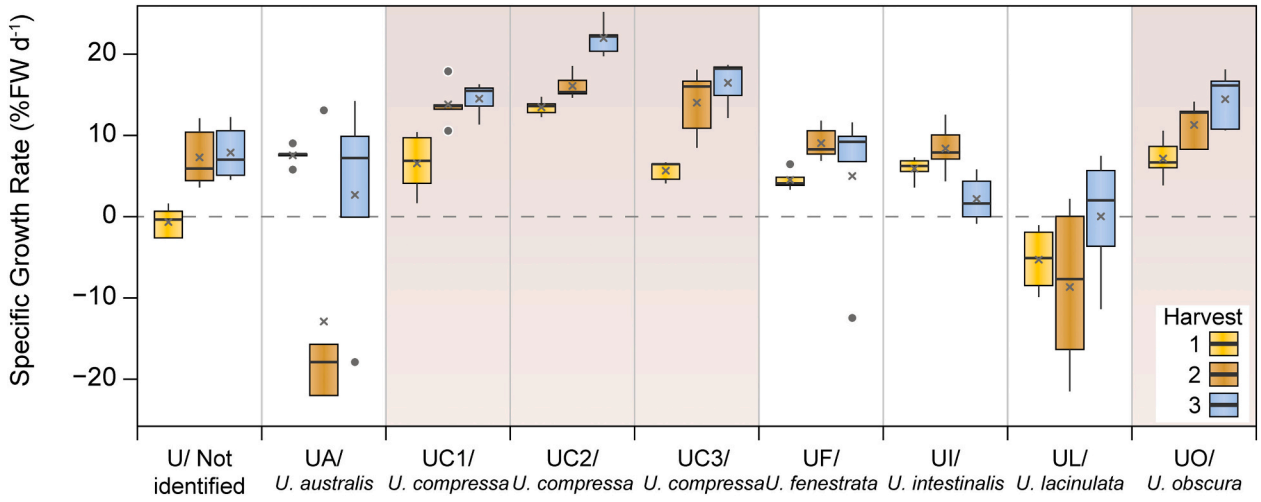

Fig. 2. Specific growth rates (% FW d^{-1}) of all *Ulvaceae* strains at three weekly harvests (Harvest 1, 2 and 3; n = 4–5). Grey-toned background indicates non-sporulating strains. Boxplots show the 25% and 75% quartiles, with black dots indicating individual measurements, crosses represent means, and horizontal lines represent medians. Species names are listed below strain IDs. For collection sites see Table 1.

Table 3
Results of two-way ANOVA with repeated measures analysing the effect of strain and harvest on fresh weight specific growth rate (SGR) for the algae strains that did not sporulate (UC1, UC2, UC3, and UO) during Experiment 1 (n = 5). Degrees of freedom (Df), F-values (F) and p-values (P) are presented in the table. Asterisks indicate significant effects on response variables.

Variable	Effect	Df	F	P
SGR	Between subjects			
	Strain	3	12.64	<0.001 *
	Residuals	16		
	Within subjects			
	Harvest	2	64.31	<0.001 *
	Strain * Harvest	6	1.96	0.101
	Residuals	32		

Ulvaria obscura showed a significantly higher tissue nitrogen content (5.3 ± 1.4% of DW) than two of the *U. compressa* strains (UC2 and UC3) (2.3–3.6% of DW) (p = 0.002). Additionally, *Ulvaria obscura* also had a significantly higher (p = 0.01) tissue carbon content (39.7 ± 0.8% of DW) than the two *U. compressa* strains UC1 and UC2 (32.2 ± 2.5–32.4 ± 5.6% DW).

3.2.3. Pigment content

Ulvaria obscura had a significantly higher (p = 0.002) total chlorophyll content (14.4 ± 2.1 mg g^{-1} DW) than UC1 and UC2 (Tables 4, 5), and a significantly higher (p < 0.001) total carotenoid content (3 ± 0.3 mg g^{-1} DW) compared to all three *U. compressa* strains (UC1, UC2 and UC3).

3.2.4. Photosynthetic parameters

The photosynthetic parameters (P_{max}, R_d and I_k) did not differ between the four non-sporulating strains (UC1, UC2, UC3, and UO) (ANOVAs, p = 0.19, 0.25, and 0.27, respectively) (Tables 4, 5). P_{max} and R_d ranged for all strains between 529.4 and 841.3 μmol O_2 g^{-1} DW h^{-1} and 42.3–91.9 μmol O_2 g^{-1} DW h^{-1} respectively. I_k ranged between 98.7 and 130.3 μmol photons m^{-2} s^{-1}.

3.3. Experiment 2 - temperature responses

3.3.1. Biomass yield

The two selected strains, *U. compressa* (UC3) and *U. obscura* (UO), exhibited biomass yields which varied significantly in response to strain (p = 0.03) and temperature (p = 0.03) as well as harvest (p = 0.025) but with significant interactions between strain and temperature (p = 0.03) and between temperature and harvest (p = 0.023) (Fig. 3, Table 6). Hence, the biomass yield of UC3 increased with temperature and particularly so at 28 °C during the third week of experiments. In contrast, biomass yields of UO decreased with temperature and it was unable to grow at 28 °C. Biomass yields were similar for the two strains at 10 and 16 °C but were higher for UC3 at 22 °C (23.1–24.2 g DW m^{-2} day^{-1}) than for UO (15.5–16.6 g DW m^{-2} day^{-1}). There was little variation in biomass yield between harvests with the exception of UC3 cultivated at 28 °C where biomass yields increased over time.

3.3.2. Dissolved inorganic nitrogen and nitrogen removal in the growth media

The DIN concentration decreased markedly during the experimental

Table 4
Dry matter content (% of FW), nitrogen and carbon (% of DW), total chlorophyll and total carotenoid content (mg g^{-1} DW), maximum photosynthetic rate (P_{max}, μmol O_2 g^{-1} DW h^{-1}), dark respiration rate (R_d, μmol O_2 g^{-1} DW h^{-1}) and light saturation point (I_k, μmol photons m^{-2} s^{-1}) for the three *Ulva compressa* strains and the single *Ulvaria obscura* strain that did not sporulate during Experiment 1. Mean values (± SD), n = 3–5. Superscript letters present significant differences in means (Tukey post hoc test).

Species/strain ID	Dry matter content	Nitrogen content	Carbon content	Total chlorophyll content	Total carotenoid content	P_{max}	R_d	I_k
U. compressa/ UC1	10 ± 2	3.6 ± 1.2 ^{ab}	32.4 ± 5.6 ^{b}	9.3 ± 1.9 ^{b}	1.2 ± 0.2 ^{b}	841.3 ± 105.5	91.9 ± 31.9	130.3 ± 14.0
U. compressa/ UC2	11 ± 1	2.3 ± 0.2 ^{b}	32.2 ± 2.5 ^{b}	7.7 ± 0.7 ^{b}	1.1 ± 0.1 ^{b}	662.1 ± 192.5	72.1 ± 40.9	105.4 ± 28.3
U. compressa/ UC3	13 ± 2	3.2 ± 0.7 ^{b}	35.0 ± 3.2 ^{ab}	11.5 ± 2.9 ^{b}	1.6 ± 0.5 ^{b}	784.3 ± 222.5	53.5 ± 17.3	98.9 ± 19.4
U. obscura/UO	11 ± 3	5.3 ± 1.4 ^{a}	39.7 ± 0.8 ^{a}	14.4 ± 2.1 ^{a}	3 ± 0.3 ^{a}	529.4 ± 129.6	42.3 ± 31.1	98.7 ± 20.1

Table 5
Results of one-way ANOVA testing the effect of strain on dry matter content, tissue nitrogen, carbon content, total chlorophyll content, total carotenoid, maximum photosynthetic rate (P_{max}), dark respiration rate (R_d), and light saturation point (I_k). Only biomass from Experiment 1, harvest 3, and algae strains UC1, UC2, UO and UC3 was analysed. Degrees of freedom (Df), F-values (F), p-values (P) are presented in the table. Asterisks indicate a significant effect on response variables.

Variable	Effect	Df	F	P
Dry matter content	Strain	3	1.89	0.17
	Residuals	15		
Nitrogen content	Strain	3	7.76	0.002 *
Carbon content	Strain	3	5.0	0.01 *
	Residuals	16		
Total chlorophyll	Strain	3	8.30	0.002 *
Total carotenoid	Strain	3	32.3	<0.001 *
	Residuals	13		
P_{max}	Strain	3	1.88	0.19
R_d	Strain	3	1.56	0.25
I_k	Strain	3	1.47	0.27
	Residuals	12		

week in tanks containing algae (Fig. 4A). For control tanks with no algal biomass, there was no significant difference between the total added DIN concentration (0.042 ± 0.004 g N L^{-1}) and the final DIN concentration after 7 days (0.05 ± 0.01 g N L^{-1}) ($F_1 = 4.28$, $p = 0.08$) (Fig. 4A). In tanks with algal biomass, the DIN concentration ranged between 0.002 ± 0.001 and 0.015 ± 0.005 g N L^{-1}, showing overall significant effects of both strain ($p = 0.03$) and temperature ($p < 0.001$) but no significant effect of the factor interaction ($p = 0.14$) (Table 7). However, in tanks with UO, the DIN concentrations did not differ across temperatures, whereas the DIN content in tanks with UC3 varied by temperature, with the highest DIN concentration observed at 28 °C (0.015 ± 0.005 g N L^{-1}). The DIN pool in the medium was dominated by nitrate (range 6–4600 μM), while concentrations of ammonium (range 5.1–46.8 μM) and nitrite (range 0.1–7.8 μM) remained low during the experiment.

The DIN removed from the growth medium was significantly higher (0.034 ± 0.005 g N L^{-1} $Week^{-1}$) than the volume specific nitrogen uptake potential (0.029 ± 0.006 g N L^{-1} $Week^{-1}$) when grouping data from all temperature treatments ($W_1 = 732$, $p = 0.003$) (Fig. 4B). Hence, 87% of the total removed DIN from the tanks was found in the algae biomass, while 13% remained unaccounted for during the experiment. Additionally, the volume-specific nitrogen uptake potential accounted for 69% of the total added DIN (64% for *U. obscura* and 74% for *U. compressa*), while 12% of the total added DIN was unaccounted for.

3.3.3. Tissue dry matter, nitrogen and carbon content

Tissue dry matter content was significantly affected by strain ($p = 0.038$), temperature ($p < 0.001$) and their interaction ($p < 0.001$, Table 7). For *U. obscura,* the dry matter content was higher at 16 and 22 °C (23 ± 2% and 21 ± 1% of FW respectively) as compared to 10 °C, while *U. compressa* showed the highest DM content at 22 °C (22 ± 1% of FW) compared to that of 10, 16, and 28 °C (19 ± 1% FW) (Table 8).

Tissue nitrogen varied between 3.2 and 5.4% of DW and was significantly affected by strain ($p < 0.001$) and temperature ($p < 0.001$) with highest tissue nitrogen concentrations in UO cultivated at 10 °C and lowest concentrations found in UC3 cultivated at 22 °C and 28 °C (Tables 7, 8). The carbon content was unaffected by strains ($p = 0.05$) and temperature ($p = 0.44$) and varied from 29.3 to 32.6% of DW across the two strains and temperatures (Table 8).

3.3.4. Pigment content

The total chlorophyll content significantly ($p < 0.001$) increased in biomass cultivated at temperatures between 10 and 22 °C for UO and UC3 (10.6–15.4 and 6.3–15.3 mg g^{-1} DW, respectively) but decreased at

Table 6
Results of 3-way PERMANOVA with restricted permutations testing the effect of strain, temperature, and harvest on biomass yield (n = 4–5) during Experiment 2. Degrees of freedom (Df), Pseudo F (F) and p-values (P) are presented in the table. Asterisks represent significant effects on response variables.

Variable	Effect	Df	F	P
Biomass yield	Strain	1	63.20	0.03 *
	Temperature	3	15.47	0.03 *
	Harvest	1	10.03	0.025 *
	Strain * Temperature	2	14.00	0.03 *
	Strain * Harvest	1	3.69	0.089
	Temperature * Harvest	3	16.96	0.023 *
	Strain * Temperature * Harvest	2	0.32	0.487
	Residuals	56		

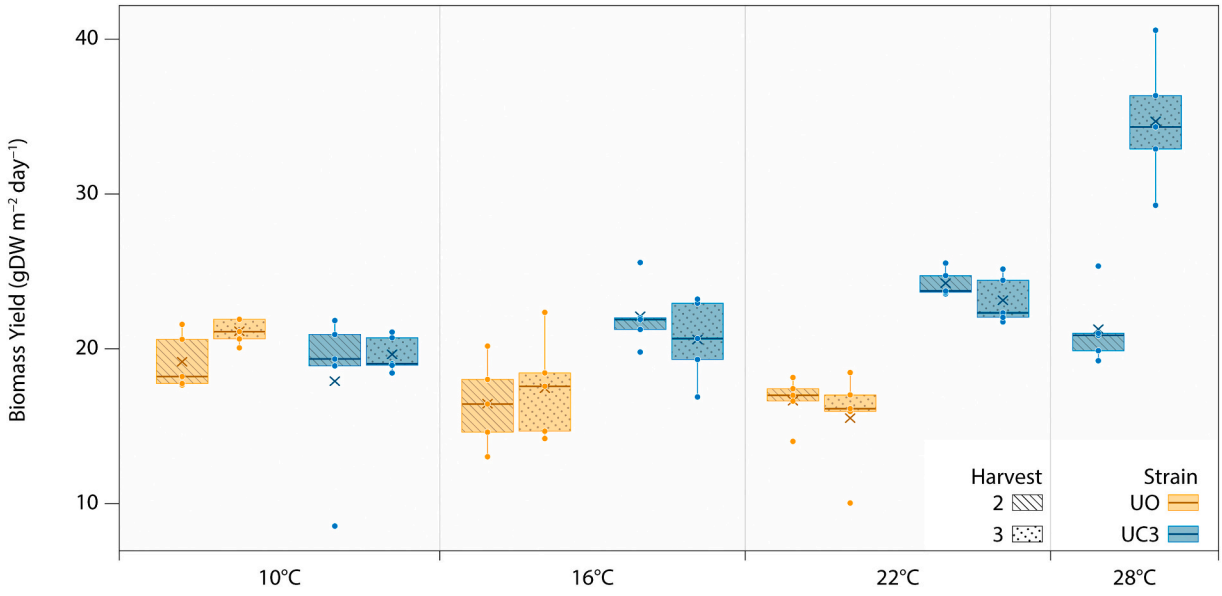

Fig. 3. Biomass yields (g DW m^{-2} day^{-1}) of UO (*U. obscura*) and UC3 (*U. compressa*) for harvest 2 and 3 during Experiment 2 (n = 5). Boxplots show 25% and 75% quartiles, crosses indicate means, and horizontal lines represent median values. Individual measurements are shown as dots. UO degraded at 28 °C and was excluded from further analysis.

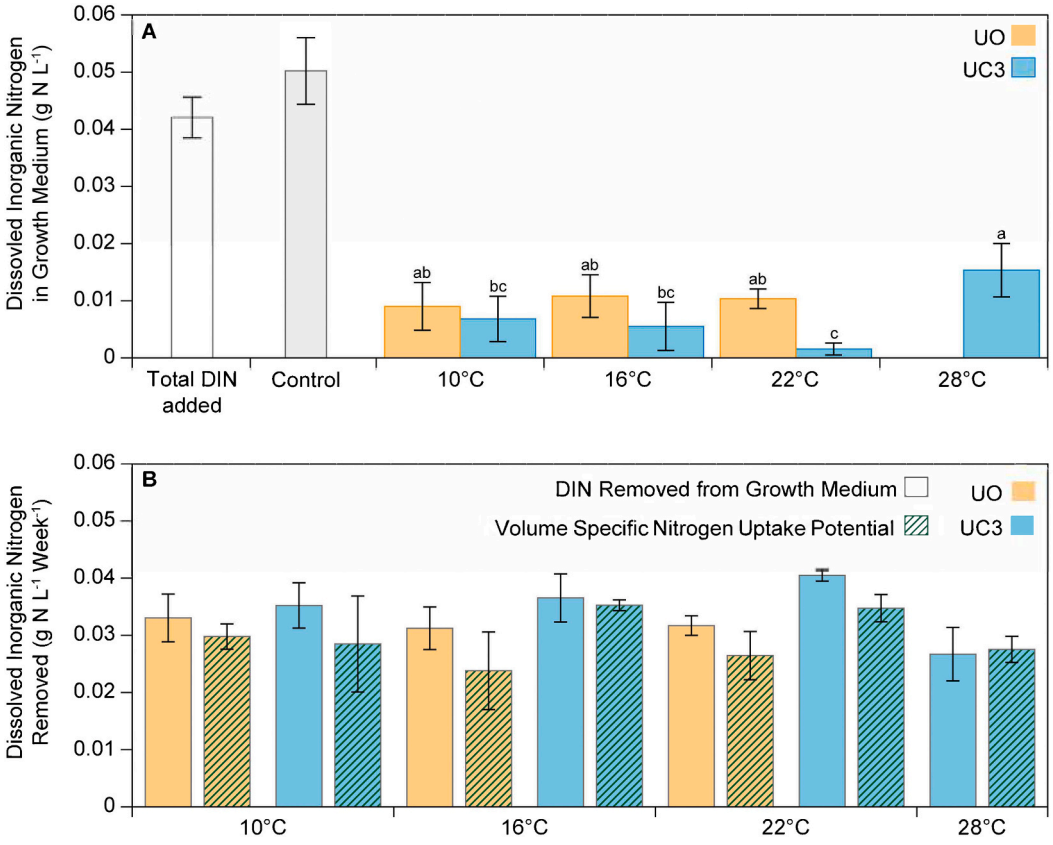

Fig. 4. (A) Dissolved inorganic nitrogen (DIN) concentration (± SD) (g N L^{-1}) added to the culture medium (n = 4) and after seven days in tanks without algae (control) (n = 4) and with algal biomass (n = 3–5) and (B) the removed DIN from the medium compared to the nitrogen uptake potential of the algae (g N L-1 Week) (± SD). Tanks with algae were grouped by strain and temperature (n = 3–5) while "control" tanks and "total added DIN" were not grouped by temperature (n = 4) (Section 2.5.2). Superscript letters indicate significant differences. *U. obscura* (UO) degraded at 28 °C and was excluded from analysis.

the higher temperatures for UC3 (Tables 7, 8).

There was no overall effect of temperature on total carotenoid content ($p = 0.23$), but significant differences between strains ($p = 0.006$) and interaction between strains and temperature ($p = 0.028$) indicated that UO had similar carotenoid contents at all temperatures (1.67–1.85 mg g^{-1} DW), whereas it for UC3 increased from 0.54 mg g^{-1} DW at 10 °C, to 1.6 ± 0.3 mg g^{-1} DW at 22 °C (Tables 7, 8).

3.3.5. Photosynthetic parameters

The light saturated photosynthetic rates ranged between 1038.9 and 1966.7 μmol O_2 g^{-1} DW h^{-1} across temperatures, with the two strains showing similar rates ($p = 0.15$) but with a significant temperature effect ($p < 0.001$), peaking around 22 °C (Fig. 5A).

The dark respiration rate, R_d, also varied with temperature ($p = 0.01$) from 47.2 to 163.3 μmol O_2 g^{-1} DW h^{-1} and tended to be highest at 16 °C for UO and at 28 °C for UC3 (Fig. 5B).

The light saturation points were similar across temperature treatments and strains (96–116.8 μmol photons m^{-2} s^{-1}) (Fig. 5C).

3.3.6. Optimum temperature range for U. compressa yield, photosynthesis and tissue content

Biomass yield, tissue carbon content, and dark respiration rate of *U. compressa* did not conform to a bell-shaped regression model ($p > 0.8$). The remaining parameters grouped into three groups which either differed in 95% confidence intervals (CI) or the regression fit R^2 values (Fig. 6) ($p < 0.001$ for all parameters). Firstly, several parameters (P_{max}, chlorophyll content, carotenoid content, and volume specific nitrogen uptake potential) had an optimum temperature around 19–20 °C and showed overlapping CI. The optimum temperature was for, both chlorophyll and carotenoid content, around 21.1 °C and had similar 95% CI from 20 to 23 °C ($R^2 = 0.76$ and 0.82 for carotenoids and chlorophyll respectively). P_{max} was estimated to have an optimum temperature at 20.5 °C with an CI of 18.7–22.2 °C ($R^2 = 0.63$). The volume specific nitrogen uptake potential was estimated to have an optimum temperature at 18.7 °C with a CI between 16.8 and 20.6 °C ($R^2 = 0.39$). Furthermore, the dry matter content and I_k showed low regression fits (0.12 and 0.1 respectively) which made it harder to estimate the exact temperature optimum for these parameters. The non-linear regression estimated that the dry matter content and I_k had an optimum temperature at 20.7 °C (CI = 16–25.5 °C) and 22.1 °C (CI = 10.1–34.0 °C) respectively. Lastly, the tissue nitrogen content was estimated to have an optimum temperature around 14.3 °C (CI = 13.0–15.7 °C) which was lower than the other parameters ($R^2 = 0.89$). Hence, most investigated parameters for *U. compressa* had an optimum temperature range between 16 and 22 °C except for tissue nitrogen content which had a lower optimum temperature range (Fig. 6).

4. Discussion

The integration of *Ulvaceae* cultivation in land-based aquaculture systems offers an opportunity to recycle nutrients and support a circular, blue bioeconomy. However, selecting the appropriate *Ulvaceae* strains and determining the optimal culture conditions is required to optimise seaweed cultivation. In this study, we introduced four *Ulvaceae* strains as candidates for free-floating culture cultivation and documented a potential for nitrogen bioremediation for two of these strains.

This study highlighted the varying responses of *Ulvaceae* strains in high nutrient cultivation systems. The growth rates in this study (15–22% FW d^{-1}) were comparable to that of other *U. compressa, U. obscura,* and *U. fenestrata* strains (10% d^{-1}, 10% d^{-1}, and 15% d^{-1},

Table 7
Results of two-way ANOVA testing the effect of strain and temperature on maximum photosynthetic rate (P_{max}), dark respiration rate (R_d) light saturation point (I_k), total chlorophyll content and total carotenoid content. DIN Concentrations in the growth media was analysed in the second week of Experiment 2 while the remaining parameters were analysed in the third week (n = 3–5). Degrees of freedom (Df), F-values (F), and p-values (P) are presented in the table. Asterisks represent significant effects on response variables.

Variable	Effect	Df	F	P
DIN Concentration in Growth Media	Strain	1	5.01	0.033 *
	Temperature	3	11.86	<0.001 *
	Strain * Temperature	2	2.12	0.14
	Residuals	28		
Dry matter content	Strain	1	4.73	0.038 *
	Temperature	3	13.78	<0.001 *
	Strain * Temperature	2	24.92	<0.001 *
	Residuals	28		
Tissue nitrogen content	Strain	1	21.57	<0.001 *
	Temperature	3	16.4	<0.001 *
	Strain * Temperature	2	0.29	0.75
Tissue carbon content	Strain	1	4.12	0.053
	Temperature	3	0.93	0.44
	Strain * Temperature	2	0.51	0.61
	Residuals	26		
Total chlorophyll content	Strain	1	7.33	0.017 *
	Temperature	3	17.63	<0.001 *
	Strain * Temperature	2	2.66	0.1
Total carotenoids content	Strain	1	10.36	0.006 *
	Temperature	3	1.61	0.23
	Strain * Temperature	2	4.64	0.028 *
P_{max}	Strain	1	2.33	0.15
	Temperature	3	10.1	<0.001 *
	Strain * Temperature	2	0.12	0.89
R_d	Strain	1	4.53	0.052
	Temperature	3	5.04	0.014 *
	Strain * Temperature	2	1.52	0.25
I_k	Strain	1	0.30	0.59
	Temperature	3	0.79	0.51
	Strain * Temperature	2	0.28	0.76
	Residuals	14		

respectively) when cultivated in similar systems [21,44,45]. However, the reproductive responses of *Ulva* still pose challenges in establishing stable free-floating cultures without formation of spores and cluster cultures ([21]; this study). Although high biomass yields can be obtained with cluster cultures as found in Lawton et al. [21], the use of cluster cultures would require additional testing to verify that the algae do not sporulate rhythmically as documented in *Ulva lactuca* and *Ulva fasciata* strains [46,47]. In the context of our results, the relatively short acclimation period could have caused the sporulation observed in this study, as high amplitude nitrogen pulses have been used to produce spores for open-water *Ulva* cultivation [48,49]. However, in land-based cultivation it may be more efficient to cultivate *Ulvaceae* strains that can tolerate high and varying nutrient loads without sporulation. Hence, the use of non-sporulating *Ulvaceae* strains could reduce the need to dilute the waste-water medium as done in Nielsen et al. [29] and Stedt et al. [44], where concentrated ammonium sources were diluted to concentrations of 20–100 μM to prevent negative impacts on growth.

We found that species collected from green tide environments with high nitrogen concentrations were more likely to exhibit high growth rates in free-floating cultures, which support the findings of Fort et al. [20], where 28 European green tide *Ulva* strains of different species, grew 1.25-fold faster than 100 "non-green tide" *Ulva* strains. Additionally, three out of the four non-sporulating strains found in this study were identified as *U. compressa*. The SGRs of the two different *U. compressa* strains UC1 and UC3 were comparable even though UC3 had been cultivated in a land-based cultivation system for at least one month prior to experimentation and could be considered to have had a longer acclimation to the high nutrient environments than UC1. The *U. obscura* strain used in our experiments also tolerated our high nutrient concentrations, which was evident from the stable SGR during experimentation, although, this result might be influenced by the exposure to higher nutrient concentrations during the initial acclimation than the other *Ulvaceae* strains. However, these findings indicate that European *Ulvaceae* strains from green tide habitats and especially *U. compressa* strains are adapted for the high nutrient conditions present in IMRAS cultivation and could guide and improve future strain selection procedures without relying on mutagenic technologies as seen in Gao et al. [50]. An alternative method of *Ulvaceae* species selection can be conducted by common garden experiments, where a series of algae strains are screened when exposed to a single environmental condition [22]. Here it could be possible to screen several green-tide *Ulvaceae* strains for a tolerance to high nutrient environments and potentially identify the strains that outperform others [22]. Having identified strains that tolerate and perform well in high nutrient conditions, selection efforts can subsequently focus on optimising cultivation conditions regarding i.e. culture temperatures.

This study did not identify a clear optimum temperature for biomass production of the two high nutrient tolerant algae species. For *U. obscura*, the decreasing biomass yields at temperatures above 10 °C suggested that its temperature optimum was within a lower range than the temperatures applied in this study. This indicates that *U. obscura* may be suitable for cold-water IMRAS aquacultures such as in combination with *S. salar* production but not for warmer aquaculture conditions. In contrast, the biomass yields of the *U. compressa* strain remained stable across all temperatures, which suggest a broad temperature tolerance for this strain. However, temperatures between 18 and 22 °C could be considered optimal for cultivation of *U. compressa* as optimum temperatures for P_{max} and volume specific nitrogen uptake potential were observed within this range. From a cultivation perspective this suggests that *U. compressa* is culturable in a variety of culture conditions,

Table 8
Biochemical parameters for UO (*U. obscura*) and UC3 (*U. compressa*) during Experiment 2. Presented are: dry matter content (% of FW), tissue nitrogen and carbon content (% of DW), and total chlorophyll and carotenoid content (mg g^{-1} DW). Only biomass from harvest 3 was analysed. Superscript letters indicate significant difference of means according to the Tukey post hoc test. Mean values ± SD, n = 3–5.

Species/strain ID	Temperature	Dry matter content	Nitrogen content	Carbon content	Total chlorophyll content	Total carotenoid content
U. obscura/UO	10 °C	18 ± 1 ^{c}	5.4 ± 0.5 ^{a}	32.5 ± 2.3	10.64 ± 0.89 ^{bc}	1.85 ± 0.78 ^{a}
	16 °C	23 ± 2 ^{a}	5.2 ± 0.6 ^{ab}	32.6 ± 3.7	13.33 ± 0.9 ^{ab}	1.67 ± 0.23 ^{a}
	22 °C	21 ± 1 ^{ab}	4.9 ± 0.4 ^{ab}	32.1 ± 2.9	15.4 ± 2.19 ^{a}	1.69 ± 0.31 ^{a}
	28 °C	–	–	–	–	–
U. compressa/UC3	10 °C	19 ± 1 ^{bc}	4.9 ± 0.1 ^{ab}	32.3 ± 0.6	6.32 ± 1.09 ^{c}	0.54 ± 0.12 ^{b}
	16 °C	19 ± 1 ^{c}	5.0 ± 0.2 ^{ab}	30.4 ± 1.2	12.25 ± 2.09 ^{ab}	1.47 ± 0.38 ^{ab}
	22 °C	22 ± 1 ^{a}	4.5 ± 0.3 ^{b}	30.5 ± 1.9	15.33 ± 2.65 ^{a}	1.6 ± 0.3 ^{a}
	28 °C	19 ± 0.3 ^{bc}	3.2 ± 0.3 ^{c}	29.3 ± 0.9	10.65 ± 0.63 ^{bc}	1.18 ± 0.16 ^{ab}

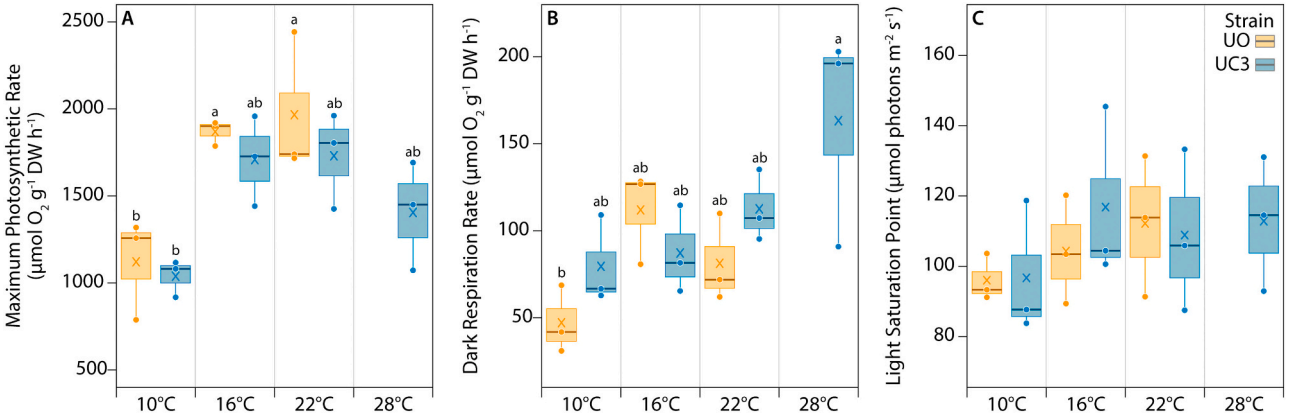

Fig. 5. (A) Maximum photosynthetic rate (µmol O_2 g^{-1} DW h^{-1}), (B) dark respiration rate (µmol O_2 g^{-1} DW h^{-1}), and (C) light saturation point (µmol photons m^{-2} s^{-1}) for the strains UO (*Ulvaria obscura*) and UC3 (*Ulva compressa*) cultured at four temperatures. Only biomass from harvest 3 was analysed. Boxplots show 25% and 75% quartiles, crosses indicate means, horizontal lines represent median values, and individual measurements are represented as dots, n = 3. Superscript letters indicate significant differences. Note that strain UO degraded at 28 °C and was excluded from analysis.

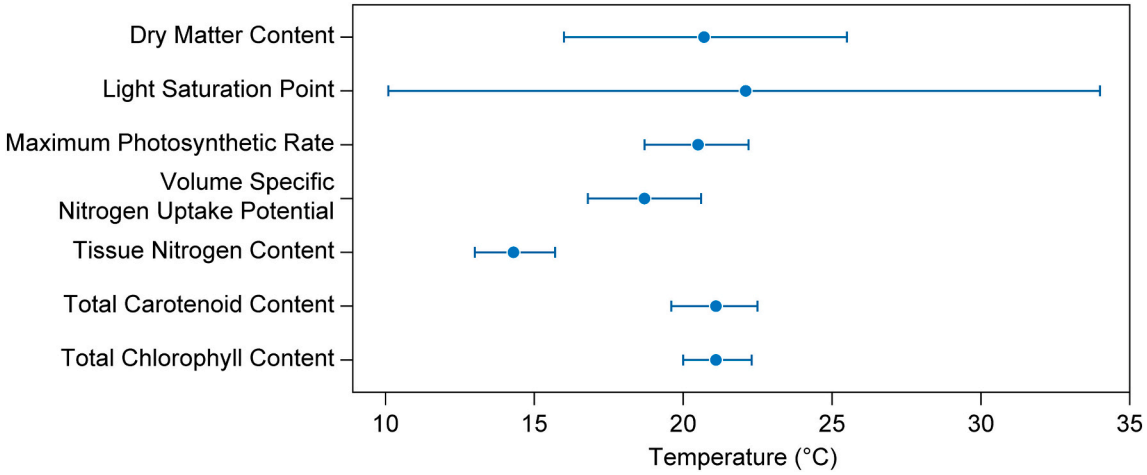

Fig. 6. Optimum temperatures (°C) and 95% confidence intervals for photosynthetic and tissue biochemical parameters following a non-linear bell-shaped regression (Gaussian model) during Experiment 2. Only data from strain UC3 (*U. compressa*) with significant critical values (Probability $X^2 < 0.05$) are presented here. The analysed data was from harvest 3 except for "Volume Specific Nitrogen Uptake Potential", which was from harvest 2.

but that the efficacy of i.e. bioremediation will change with cultivation temperature. Similar consistent growth rates (10–20% d^{-1}) of *U. compressa* have previously been described when grown over a broad temperature range (15–27 °C) and support the result of this study [51]. Additionally, temperate *Ulva* and *Ulvaria* species often reach optimal growth around 19–22 °C which coincided with the optimum temperature ranges for photosynthetic parameters, pigment content, and nitrogen uptake found in this study [25,52–55,68]. However, some of these earlier studies were conducted in experimental settings with lower nitrogen concentrations as *Ulvaceae* cultivation was not the primary objective of these studies and may therefore not reflect the *Ulvaceae* algae responses in high nutrient conditions. Hence, our study together with the studies of Xiao et al. [23] and Toth et al. [27] provide insights in how *Ulvaceae* algae respond to abiotic factors when grown in environments with excess nutrients. However, lower temperature optimum of *U. compressa* has been documented by Taylor et al. [56] who found that the growth of *U. compressa* decreased from 8% d^{-1} at 10 °C to 2% d^{-1} at 30 °C. Taylor et al. [56] presents an example of intraspecies variation which is also documented in a variety of European *Ulva* species [19]. Furthermore, Xiao et al. [23] argue that the growth of *Ulva prolifera* is a result of interactions between water salinity and temperature, showing that salinity (14–32) affected growth rates more at temperature extremes (5 and 32 °C) compared to medium temperatures (20 °C) which adds further complexity to the study of *Ulvaceae* algae. The complexity of *Ulvaceae* growth dynamics and intraspecies variations make it challenging to apply experimental findings directly to large-scale commercial cultivation and highlight the need for strain-specific studies to optimise cultivation conditions.

Ulvaceae cultivation must offer aquaculture companies a beneficial opportunity to implement IMRAS and lower their environmental footprint while producing marketable products [5,6,57,58]. In this study, 63% and 74% of the added dissolved inorganic nitrogen was removed by *U. obscura* and *U. compressa,* respectively. For *U. compressa*, the nitrogen uptake was estimated to be highest between 16.8 and 20.6 °C, where chlorophyll content and photosynthetic activity were at a maximum. However, while the nitrogen uptake found in this study indicates that *Ulvaceae* cultivation in aquaculture systems could substantially reduce IMRAS DIN emissions to the aquatic environment, there are limitations when extrapolating the results of laboratory studies to large scale IMRAS systems:

Laboratory studies often use small batch cultures of just a few litres, which makes it difficult to compare these results to commercial production units. Firstly, the nutrient dynamics in batch cultures do not resemble IMRAS facilities with constant water flow as the nutrient concentrations will inherently decrease over time if nutrients are not renewed equivalent to the algae nutrient uptake. This is acknowledged

in studies such as Zollmann et al. [59], where nitrogen depletion occurred in batch cultures after a few days. Zollmann et al. [59] further highlighted that nitrogen uptake by *Ulva* increased when using continuous nutrient regimes, which suggest that nitrogen uptake in IMRAS will also be higher than in batch systems. In this study, the DIN concentration was low at the end of the experimental week which suggests a potential depletion of DIN in the growth medium. However, the tissue nitrogen content was 3.2–5.4% of DW, which is indicative of sufficient nitrogen availability to sustain maximum growth rates of *Ulva* sp. (>2.2% of DW) [29,35,44,60]. Additionally, 12% of the total added DIN was unaccounted for in the algae biomass and was potentially assimilated by microbes or lost as dissolved or particulate organic matter due to the mechanical erosion by the aeration bubbles [61]. Hence, we potentially underestimate the nitrogen uptake potential of the *Ulvaceae* algae in our study.

Another limitation for laboratory studies is the use of small pilot tanks and the complexities of upscaling. Continued experimentation into the effects of upscaling is needed before commercial adaptations in IMRAS are possible as the physio-chemical parameters of water mixing, light attenuation and gas exchange will likely change when upscaling the cultivation tanks. Upscaling has been investigated in microalgae raceways cultivation, where gas-exchange and water mixing are identified as main complications during upscaling and make the availability of nutrient, CO_2, and light less stable for the produced microalgae [62,63]. Despite several limitations in interpreting laboratory results to industry, it might still be relevant to extrapolate the results of this study to a full scale IMRAS system. This could provide perspectives in how land-based aquacultures might integrate *Ulvaceae* cultivation to reduce nitrogen emissions:

A Danish land-based fish producer emits 26.2 metric tonnes of inorganic dissolved nitrogen annually while producing approximately 2750 tonnes of *S. salar* [31]. Based on the volume specific nitrogen uptake potential found in this study (0.026–0.031 g N L^{-1} $Week^{-1}$, corresponding to 63–74% of total added DIN), 17–19 tonnes of this inorganic nitrogen could be captured and removed annually by *Ulvaceae* cultivation, corresponding to approximately 360 tonnes of dried algae product (assuming a 5% of DW tissue nitrogen content). Optimising stocking densities could further optimise nitrogen extraction. This will require system specific optimisation of stocking density, as demonstrated in an outdoor *Ulva* cultivation system, where biomass yields peaked with stocking densities beyond what was used in this study [28]. Although the implementation of *Ulvaceae* cultivation will likely not be without costs [64], land-based aquaculture companies may benefit from taking a more precautious approach to nutrient emissions, as increased focus on eutrophication and biodiversity in regional legislation and global resolutions is expected to lead to demands for reduced nutrient emissions [65–67].

CRediT authorship contribution statement

Kristoffer Larsen-Ledet: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Teis Boderskov:** Writing – original draft, Visualization, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Birgit Olesen:** Writing – original draft, Formal analysis, Conceptualization. **Martin Mørk Larsen:** Writing – original draft, Investigation, Formal analysis, Data curation. **Nina Simonsen:** Writing – review & editing, Investigation, Formal analysis. **Esben Rimi Christiansen:** Writing – review & editing, Conceptualization. **Lasse Hornbek Nielsen:** Writing – review & editing, Conceptualization. **Lan-Anh T. Tran:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Sofie D'Hondt:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Olivier De Clerck:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Annette Bruhn:** Writing – original draft, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Microsoft Copilot 1.0 to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Annette Bruhn reports financial support was provided by Innovation Fund Denmark. Olivier De Clerck reports financial support was provided by Research Foundation Flanders. Annette Bruhn reports financial support was provided by Horizon 2020 European Innovation Council Fast Track to Innovation. Olivier De Clerck reports financial support was provided by European Marine Biological Resource Centre Belgium. Esben Rimi Christiansen reports a relationship with Pure Algae Denmark that includes: employment. Lasse Hornbek Nielsen reports a relationship with Pure Algae Denmark that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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