Ulva spp. performance and biomitigation potential under high nutrient concentrations: implications for recirculating IMTA systems

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Abstract
The growth, tissue content and nutrient removal rates of Ulva spp., when exposed to moderate to high nitrogen (0.5–5 mmol L−1) and phosphorus (0.01–0.9 mmol L−1) concentrations, were examined to get a better understanding of recirculating IMTA (Integrated Multi-Trophic Aquaculture) systems with fish and seaweed. It was hypothesized that fish waste effluents might lead to unfavorable nutrient stoichiometry and/or toxic conditions, which might harm seaweeds and, specifically for the present study, reduce Ulva spp. performance. Results demonstrate that: (I) the unfavorable N:P stoichiometry (N:P ≠ Atkinson atomic ratio of 30:1) did not restrict Ulva spp. growth nor tissue content; this indicates that supply of both nutrients exceeded the minimum requirements; (II) a high orthophosphate concentration (0.9 mmol L−1) was toxic to Ulva spp., whereas (III) a high nitrate concentration (5 mmol L−1) did not inhibit phosphorus uptake; (IV) Ulva’s growth was not enhanced when nitrate was exchanged for similarly high ammonium concentrations. However, tissue nitrogen content was 1.4 times higher when exposed to ammonium than nitrate, suggesting that the former N-form was stored faster in the seaweed’s tissue. Therefore, other factors must have limited growth with the high ammonium concentrations. This study also highlights the importance of relatively long acclimatization periods (one week) when maintenance uptake (V_m) is evaluated, as surge uptake (V_s) may result in considerably different and more variable rates. Results of this study contribute to a better understanding of the application of Ulva spp. as extractive component in closed IMTA systems, thus advancing sustainable and circular production techniques.

Keywords Integrated multi-trophic aquaculture · Nitrate · Phosphorus · Ammonium · N:P ratio · Seaweed

Introduction
Green seaweeds belonging to the genus Ulva (Chlorophyta) are well known for their high nutrient uptake capacity and biomass productivity (Bruhn et al. 2011; Kang et al. 2011; Luo et al. 2012; Gao et al. 2018, 2020). Furthermore, they can be cultivated in artificial media and wastewater effluents (Guist Jr and Humm 1976; Cohen and Neori 1991; Luo et al. 2012; Kumari et al. 2013). These characteristics make them an ideal model species for small scale laboratory experiments on eco-physiological processes, like nutrient uptake kinetics (Lubsch and Timmermans 2018), but also for land-based integrated aquaculture systems, for biomitigation of the inorganic waste fraction resulting from fish cultures (Krom et al. 1995; Shpigel and Neori 1996; Neori et al. 2003; Schuenhoff et al. 2003; Msuya et al. 2006). Fish excrete inorganic phosphorus as orthophosphate (PO₄) and inorganic nitrogen in the form of TAN (NH₃-N + NH₄-N), which can convert to the less toxic nitrate (NO₃) by bacterial nitrification. Water renewal is minimized in recirculation aquaculture systems (RAS), and the waste nutrients PO₄ and NO₃ may accumulate in the culture water to high concentrations. Potentially toxic levels of up to 20 mmol L⁻¹ nitrate and 3 mmol L⁻¹ orthophosphate have been reported in various seawater RAS systems (Neori et al. 2007; Tal et al. 2009;
Integrated recirculating fish-seaweed systems can be designed in two ways; either with or without bacterial biofilters. Both designs are characterized by high nutrient concentrations but vary in the form of accumulated nitrogen (TAN or NO₃), with implications to N-removal efficiency (Neori 1996). Nutrient dynamics in integrated aquaculture based on closed systems (i.e., RAS) are different from open-water IMTA systems, where fish wastes are quickly mixed with ambient nutrients (Jansen et al. 2018). Several studies using seaweed as extractive species in integrated aquaculture systems have focussed on biomitigation under low to moderate nutrient conditions (Krom et al. 1995; Al-Hafedh et al. 2012; Kang et al. 2021). These conditions are not always representative for the high nutrient concentrations in RAS effluents.

Molar N:P ratios of inorganic fish waste range between 5 – 35, depending on different factors like fish species and diet type (Wang et al. 2012; Hadley et al. 2014). The Atkinson ratio (N:P 30:1) is considered optimal for seaweeds (Atkinson and Smith 1983), whereas tissue ratios between 16:1 and 24:1 (N:P) have been reported to sustain maximum growth in Ulva spp. (Björnsäter and Wheeler 1990; Tremblay-Gratton et al. 2018). Indeed, suboptimal N:P ratios have reduced the growth rate of seaweed (Björnsäter and Wheeler 1990), indicating that one of the nutrients was limiting. The N:P ratios in fish wastes can be well below the Atkinson ratio, suggesting that phosphorus may accumulate in an integrated RAS system creating suboptimal N:P ratios, and growth of seaweeds might be limited under such conditions. However, at the same time, the high nutrient concentrations in RAS effluents may surpass the minimum values required for maximum growth (0.8 µmol L⁻¹ P and 6.7 µmol L⁻¹ N; Pedersen and Borum 1997, Pedersen et al. 2010). Under this condition, the N:P ratios in the RAS effluent may not limit the uptake of either nutrient and will thus not result in reduced growth of the seaweed.

Apart from potentially limited growth due to unbalanced stoichiometry in fish effluents, high nutrient concentrations may create toxic conditions for seaweeds. As highlighted above, phosphorus may accumulate in integrated RAS systems. Some studies suggest that a high (0.8–3.2 mmol L⁻¹) orthophosphate concentration may reduce seaweed growth (Friedlander and Ben-Amotz 1991; Navarro-Angulo and Robledo 1999). Still, it is unclear whether the cause is the high orthophosphate concentration itself or the low N:P ratios. As far as we know, phosphorus toxicity to Ulva spp. has not been defined. There are, however, indications that exposure of Ulva lactuca to high nitrate concentrations may inhibit phosphorus uptake and, therefore, growth (Lundberg et al. 1989). The opposite has been reported for Fucus vesiculosus (Perini and Bracken 2014), an inconsistency that highlights the limited understanding of the interactions between nitrate and phosphorus uptake kinetics in seaweed.

As nitrate concentrations are high in bacterially-biofiltered RAS effluents, these processes are particularly relevant for such systems, resulting in an even higher phosphorus accumulation in the water and potentially reduced seaweed performance.

Fish excreta consist, among others, of TAN which is transformed into nitrate by the bacterial biofilters in RAS systems (Krom et al. 1995; Neori et al. 2007). Although Ulva spp. can assimilate both nitrogen sources, uptake of nitrate is generally much slower than TAN (Neori 1996; Hadley et al. 2014), which is also reflected in a lower growth (Ale et al. 2011). The different rates of nutrient uptake and growth by Ulva spp. with these two nitrogen forms are related to different uptake and assimilation energy requirements (Shahar et al. 2020). Nitrate, unlike TAN, requires metabolic energy for uptake and assimilation into protein (Taylor et al. 2006). Therefore, seaweed growth in the nitrate-rich RAS IMTA systems might be sub-optimal. An additional consideration is the much lower toxicity of TAN to seaweed than to fish (Harrison and Hurd 2001; Moustafa et al. 2014). Thus, replacing the bacterial biofilter with a seaweed unit may improve water quality for fish (low levels of TAN, consumption of acidity and production of O₂) and at the same time improve seaweed production.

In this study, the growth and nutrient assimilation were measured in Ulva spp., when exposed to relatively high and moderate nitrogen and phosphorus concentrations. Such conditions have been insufficiently addressed in the literature (Lundberg et al. 1989; Demetropoulos and Langdon 2004; Tremblay-Gratton et al. 2018) and would give a better understanding of biomitigation potential and seaweed performance under high nutrient concentrations. The following four hypotheses were examined: (I) Ulva spp. performance is not influenced by N:P stoichiometry in fish waste effluents under high nutrient concentrations, (II) High orthophosphate concentrations typical in RAS effluents are not toxic to Ulva spp., (III) High nitrate concentrations typical in RAS effluents can limit the phosphorus uptake, (IV) High TAN concentrations improve the performance of Ulva spp. compared to comparably high nitrate concentrations.

**Materials & methods**

**Experimental design**

This study consisted of two experiments. The first experiment evaluated maintenance uptake kinetics (Vₘ, i.e., when internal nutrient concentrations remain constant; Lubsch and Timmermans 2018) in Ulva spp. exposed to different nutrient treatments. Separate batches of Ulva spp. were continuously exposed to one of six nutrient treatments (treatments A to F) for two weeks. In treatments A, B, C and D, nitrate
was the N-source, while ammonium was the N-source in treatments E and F. The treatments varied in nutrient concentration between 0.7–5.0 mmol L$^{-1}$ nitrogen (either nitrate or ammonium) and 0.013–0.9 mmol L$^{-1}$ orthophosphate, using dilution steps with a factor of 10, and resulting in stoichiometries that were either high (N:P 60–70) or low (N:P 9–10). For a complete description of the treatments, see Table 1. These treatments reflected nutrient conditions expected from fish effluents in a RAS facility, and tested all four hypotheses. In the second experiment, only hypothesis III was tested for surge uptake ($V_s$, i.e., filling of internal nutrient pools; Lubsch and Timmermans 2018), as the effect of high nitrate concentrations on phosphorus uptake rates might only be visual during surge uptake ($V_s$). This second experiment studied phosphorus uptake for five hours, during which nutrient-starved Ulva spp. was exposed to moderate and high nitrate concentrations (treatment A and B).

### Holding conditions

The experiments were conducted in a temperature- and light-controlled room at the Aquatic Research Facility of the Wageningen University (ARF—Carus, Wageningen, The Netherlands). Ulva spp. was obtained from the greenhouse facility of the Wageningen University and Research Centre (Nergena, Wageningen, The Netherlands), where it has been cultivated since 2012. It was assumed that the culture stock consisted of Ulva lactuca, but recent analysis from the location where initial samples were collected indicates the presence of additional Ulva species (Fort et al. 2020). We, therefore, refer to ‘Ulva spp.’ for the species investigated in this study.

Before the experiments, seaweeds were maintained in a stock tank (1 m$^3$) filled with artificial seawater (Reef Crystals, Aquarium Systems, Inc., Mentor, Ohio, USA), to which once a week plant fertilizer (Pokon® Groene Planten Voeding, Veenendaal, The Netherlands) was added. N:P atomic ratio of this plant fertilizer is 11, which is in line with the low N:P ratio of treatments C, D, and F of the current study (Table 1). Light tubes (T5 TL 24 W – AquaBlue Special—ATI) were placed above the tank (~ 400 μmol photons m$^{-2}$ s$^{-1}$), the temperature was maintained at 17.5 ± 0.5 °C, and aeration was added at the bottom of the tank, to maintain vertical water movement (Msuya and Neori 2008).

### Experiment 1: Maintenance uptake ($V_m$) and growth

Six treatments were formulated varying in nutrient concentrations between 0.7–5.0 mmol L$^{-1}$ nitrogen (either nitrate or ammonium) and 0.013–0.9 mmol L$^{-1}$ orthophosphate, using dilution steps with a factor 10, and resulting in stoichiometries that were either high (N:P 60–70) or low (N:P 9–10) (Table 1). Four experimental systems (header tank plus three associated seaweed cultivation tanks) were available, and treatments were, therefore, divided over two consecutive runs, using new seaweed stocks in each run. The first run included all nitrate-based treatments, while the second run included all ammonium-based treatments. Two identical reference nitrate-based treatments (A1 and A2) were included in both runs to elucidate a potential effect of ‘run’. Treatment concentrations were formulated by daily addition of a mix of artificial seawater (29.7 ± 1.8 %c, Reef Crystals, Aquarium Systems, Inc., Mentor, Ohio, USA), wastewater from a RAS system with sea bass (2L) and stock solutions to header tanks with a total volume of 110 L. The stock solutions were prepared with NaH$_2$PO$_4$ and NaNO$_3$ for the nitrate treatments and NaH$_2$PO$_4$ and NH$_4$Cl for the ammonium treatments. Water samples were collected daily in the header tanks, and were directly stored in the freezer (~ 20 °C) until nutrient analyses.

Seaweed tanks consisted of 15 L white round tanks (0.07 m$^2$ surface area) with an overflow PVC pipe in the center. Each overflow pipe was covered with an additional perforated

### Table 1 Treatment overview including dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentration, and N:P ratio (molar) in the culture media. Planned values refer to the targeted concentrations before the experiment, and realized values are actual daily average concentrations measured in the header tanks. Values are given as mean ± SD.

| Treatment | Run | Planned DIN (mmol L$^{-1}$) | Planned DIP (mmol L$^{-1}$) | Planned N:P | Realized NO$_3$/TAN (mmol L$^{-1}$) | Realized DIN (mmol L$^{-1}$) | Realized DIP (mmol L$^{-1}$) | Realized N:P |
|-----------|-----|-----------------------------|-----------------------------|-------------|---------------------------------|-----------------------------|-----------------------------|-------------|
| A1        | 1   | 5.0 NO$_3$                  | 0.160                       | 30          | 4.9 ± 0.9 NO$_3$                | 5.0 ± 0.9                  | 0.13 ± 0.02                 | 38 ± 3      |
| B         | 1   | 0.5 NO$_3$                  | 0.016                       | 30          | 0.8 ± 0.1 NO$_3$                | 0.8 ± 0.1                  | 0.013 ± 0.003               | 62 ± 10     |
| C         | 1   | 0.5 NO$_3$                  | 0.080                       | 6           | 0.7 ± 0.1 NO$_3$                | 0.7 ± 0.1                  | 0.07 ± 0.01                 | 10 ± 1      |
| D         | 1   | 5.0 NO$_3$                  | 0.800                       | 6           | 5.5 ± 0.4 NO$_3$                | 5.5 ± 0.4                  | 0.86 ± 0.17                 | 7 ± 1       |
| A2$^*$    | 2   | 5.0 NO$_3$                  | 0.160                       | 30          | 4.9 ± 0.6 NO$_3$                | 4.9 ± 0.6                  | 0.14 ± 0.01                 | 34 ± 4      |
| E         | 2   | 0.5 TAN                     | 0.016                       | 30          | 0.6 ± 0.1 TAN                    | 1.0 ± 0.2                  | 0.015 ± 0.003               | 68 ± 13     |
| F         | 2   | 0.5 TAN                     | 0.080                       | 6           | 0.5 ± 0.1 TAN                    | 0.8 ± 0.1                  | 0.09 ± 0.01                 | 9 ± 1       |

$^*$Treatment A2 is identical to A1 and acts as a reference between both runs.
All seaweed samples were analyzed for dry matter, ash, N and P tissue content (see biochemical analyses). Nitrogen and phosphorus removal rates were calculated for week 1 and week 2 separately, using the following equation after Kim et al. (2007):

$$\text{Removal rate (µmol g }^{-1} \text{ DW day }^{-1} ) = \frac{((W_i + TC_f) - (W_i + TC_i))/DW}{T}$$

where $W_i$ is the final biomass in g dry weight, $W_i$ the initial biomass in g dry weight, $TC_f$ the final N or P tissue content (in µmol g }^{-1} \text{ DW}$, $TC_i$ the initial N or P tissue content (in µmol g }^{-1} \text{ DW}$, $DW$ the mean biomass in g dry weight of either week 1 or week 2, and $T$ the number of experimental days.

**Experiment 2: Surge uptake ($V_s$)**

For the measurement of the surge uptake related to hypothesis III, *Ulva* spp. was exposed to either high (5 mmol L }^{-1}) or moderate (0.5 mmol L }^{-1}) nitrate concentrations, both with a fixed orthophosphate concentration (0.026±0.005 mmol L }^{-1}). The solutions were prepared by artificial seawater (29.7±1.8 ‰, Reef Crystals, Aquarium Systems) and NaH$_2$PO$_4$ and NaNO$_3$. Surge uptake measurements were based on the method described by Hurd and Dring (1990). Before the experiment, *Ulva* spp. were nutrient-starved for 3 days. At the start of the experiment, pieces of *Ulva* spp. of comparable weights (1.43±0.03 g fresh weight) were placed in 500 mL glass jars, filled with 400 mL of one of the above-described solutions, in four replicates per treatment. Two control jars were added for each treatment, containing nutrient solution without seaweed. The jars were placed in a water bath shaker, creating a constant water movement for mixing and reducing the diffusion boundary layer between seaweed and the medium. Light tubes (T5 TL 24 W – AquaBlue Special — ATI) were placed above the seaweed tanks, resulting in irradiance of 826±46 µmol photons m }^{-2} s }^{-1} (measured by LI-COR LI193R PAR meter, just underneath the water surface). A slow, intertidal recirculation flow through a UV-light removed potential diffusion boundary layer between seaweed and the medium. Aeration was added via a PVC pipe topped with a perforated cap, preventing seaweed pieces from floating out of the tank. Aeration was added via the bottom of each tank, approximately 5 cm from the center, creating sufficient turbulence to suspend the seaweed pieces and move them through the water column. A slow, internal recirculation flow through a UV-light removed potential microalgal and bacterial contaminants. A double perforated bottom allowed the water to pass the UV light, without damaging the seaweed. Light tubes (T5 TL 24 W – AquaBlue Special — ATI) were placed above the seaweed tanks, resulting in irradiance of 396±46 µmol photons m }^{-2} s }^{-1} (measured by LI-COR LI193R PAR meter, just underneath the water surface). A 12 h light-12 h dark light regime was maintained. Each experimental run consisted of an acclimation week followed by an experimental week. This acclimatization period stabilized the internal nutrient pools (Lubsch and Timmermans 2018). Nutrient removal rates measured in the second week were thus assumed to reflect metabolic controlled uptake rates ($V_m$). At the start of the acclimation period, each seaweed tank was stocked with 60.3±0.4 g, equivalent to 4.02±0.03 g L }^{-1}, fresh *Ulva* spp. material (spin-dried by a lettuce hand-centrifuge). Start samples were collected to determine initial tissue content (n = 3 samples for each run). After the acclimation week, all seaweed material was harvested, spin-dried and weighted to determine fresh weight. Then, 30.5±0.3 g (equivalent to 2.04±0.02 g L }^{-1}) of the harvested *Ulva* spp. material from each tanks was returned to the tank, while the remaining material was collected for analysis. At the end of the second week, all seaweed material was collected, spin-dried and weighted to determine fresh weight. seaweed samples were rinsed with deionized water, spin-dried and thereafter stored in the freezer (-20 °C) until analyses. Specific growth rate (SGR, % day }^{-1}) was calculated based on dry weight (DW) for week 1 and week 2 separately, using the following formula:

$$\text{SGR} = \left(\frac{(\ln(W_f) - \ln(W_i))}{T}\right) \times 100$$

where $W_f$ is the final biomass in g dry weight, $W_i$ the initial biomass in g dry weight, and $T$ the number of experimental days.

The biomass yield was determined for week 2, using the following equation after Revilla-Lovano et al. (2021):

$$\text{Yield (g FW m}^{-2}\text{ day}^{-1}) = \frac{(W_f - W_i)}{T}$$

where $W_f$ is the final biomass (g FW m }^{-2}), $W_i$ is the initial biomass (g FW m }^{-2}), and $T$ the number of experimental days.

Water samples of the growth experiment (maintenance uptake) were analyzed for TAN, nitrate-N and orthophosphate-P using
an auto-analyzer (SANplusSYSTEM, Skalar). Water samples of the surge uptake experiment were analyzed for nitrate–N and orthophosphate-P using a SmartChem 200 Discrete Analyzer. Freeze-dried seaweed samples were ground with a centrifugal grinding mill (Retsch/Brinkmann ZM 100/w 1 mm sieve, Verder NV, The Netherlands). Dry matter and ash were determined according to ISO-6496 (1983) and ISO-5984 (1978), respectively. Phosphorus content in the seaweed was analysed using inductively coupled plasma-mass spectrometry (ICP-OES) according to the standard NEN 15510 (2007). Carbon and nitrogen content of the seaweed were analysed by combusting the samples with an element analyzer (Flash 2000, Therm Fisher) at 1020 °C in the presence of oxygen, converting carbon and nitrogen to CO₂ and NOₓ(NO₂ + NO₃), respectively. Thereafter, NOₓ was reduced to N₂ in a reduction column.

**Statistical analyses**

Statistical analyses were performed in R studio 3.4.0. Before the analysis, residuals of the data were checked for homogeneity of variance and normality using the Shapiro–Wilk and Levene test. A two-way analysis of variance (2-way ANOVA) was used to check for a potential interaction effect between the N:P ratio and N-source (treatments B, C, E, F). Since no interaction effect (p > 0.05) was observed for uptake and growth performance parameters, comparative treatments related to hypotheses I and IV were tested independently. For each hypothesis, differences in uptake and performance of Ulva (i.e., growth, yield, tissue content, Vₘ nutrient removal rates) in the corresponding treatments (Table 1) were tested by one-way analysis of variance (1-way ANOVA). In addition to hypothesis III, a 1-way ANOVA was used to detect potential differences in phosphorus surge uptake rates (Vₛ) by Ulva spp. exposed to either a high or moderate nitrate concentration. All statistics for the Vₘ experiments were based on data collected in the second week only. Paired-Samples T-tests tested the difference in growth and tissue content between week 1 and week 2, for each treatment separately to verify the relevance of acclimation.

**Results**

Realized nutrient concentrations were not always in range with the planned formulated culture media. Especially treatment B and E showed variations and the measured DIN concentrations were almost double the planned ones, resulting in deviations to the N:P ratios (Table 1). Culture media formulated based on NH₄Cl contained not only TAN-nitrogen but also NOₓ-nitrogen, leading to slightly higher DIN concentrations than expected. The origin of these additional NOₓ concentrations remains unknown, but seems to fall within the variation observed for the other treatments and may relate to possible NOₓ contamination in the salt. This was however not analysed. Despite these variations all hypotheses could still be evaluated.

**Hypothesis I: Ulva spp. performance under contrasting N:P ratios**

The two contrasting N:P ratios (9–10 vs 60–70) did not impact growth and yield (Fig. 1) nor tissue content of the seaweed (Fig. 2) (Table 2; 1-way ANOVA; p > 0.05 B vs C; p > 0.05 E vs F). As a result nutrient removal rates were comparable for the high and low N:P treatments (Fig. 3) (Table 2; 1-way ANOVA; p > 0.05 B vs C; p > 0.05 E vs F). Tissue N:P ratios varied between 27–28 for the nitrate-based treatments (B and C) and 39–42 for the ammonium-based treatments (E and F), irrespective of the N:P ratio provided in the culture medium.

**Hypothesis II: Toxicity of high orthophosphate concentrations**

Ulva spp. cultivated under the highest orthophosphate concentration (treatment D; 0.9 mmol L⁻¹ P) showed a different, unhealthy, tissue structure compared to the other treatments; tissue was hard, easy to break and felt brittle, suggesting degradation (visual observation). Most of the material of this treatment was lost during the sampling procedure (spin-drying), and we were therefore unable to derive valid measurements. Treatment D was therefore excluded from the statistical analyses (Table 2; Figs. 1, 2 and 3; no bar shown for treatment D). As described for hypothesis I, no significant differences were observed for the Ulva spp. in the remaining low (treatment B) and medium (treatment C) orthophosphate concentrations.

**Hypothesis III: Inhibiting effects of high nitrate concentration**

Phosphorus removal during maintenance uptake (Vₘ; experiment 1) was approximately 60% higher (Table 2; 1-way ANOVA; p < 0.05) for Ulva spp. cultivated under high nitrate (treatment A) compared to moderate nitrate concentrations (treatment B) (Fig. 3). This difference was not the result of growth, since SGR and yield did not differ between the treatments (Fig. 1; Table 2; 1-way ANOVA; p > 0.05). More likely is that the difference in phosphorus removal was driven by differences in tissue content, as phosphorus content in Ulva spp. of the high nitrate treatment was higher by approximately 25% (Table 2; 1-way ANOVA; p < 0.0001) compared to the moderate nitrate.
treatment (Fig. 2). This P difference coincided with a nearly 25% lower tissue N:P ratio for Ulva spp. in the high nitrate treatment, compared to Ulva spp. cultivated in the moderate nitrate treatment (Fig. 2; Table 2; 1-way ANOVA; \( p = 0.0002 \)). No differences were observed in tissue nitrogen content, tissue C:N ratio and nitrogen removal rates (Figs. 2 & 3; Table 2; 1-way ANOVA; \( p > 0.05 \)). Similarly, surge uptake (\( V_s \); experiment 2) of phosphorus did not vary between the moderate (0.60 ± 0.09 µmol g\(^{-1}\) FW h\(^{-1}\)) and high (0.51 ± 0.04 µmol g\(^{-1}\) FW h\(^{-1}\)) nitrate treatments (Fig. 4; 1-way ANOVA, \( p > 0.05 \)).

**Hypothesis IV: Effect of N-source (nitrate or ammonium)**

The lack of a significant difference in growth between reference treatments A1 and A2 (Table 1; A1 and A2) (1-way ANOVA; \( p = 0.076 \)), validates a comparison between nitrate-based treatments tested in run 1 and ammonium-based treatments tested in run 2. While SGR and tissue phosphorus content were not affected by the type of N-source (Figs. 1 & 2; Table 2; 1-way ANOVA; \( p > 0.05 \) B vs E; \( p > 0.05 \) C vs F), tissue nitrogen content (Fig. 2) was higher for Ulva spp. in the ammonium-based treatments (5.2% DM) compared to the nitrate-based treatments (3.8% DM) (Table 2; 1-way ANOVA; \( p < 0.0001 \) B vs E; \( p < 0.0001 \) C vs F). This was also reflected in a significant higher tissue N:P ratio, but lower tissue C:N ratio for Ulva spp. provided with ammonium-N (Fig. 2). Interestingly, nitrogen removal (in µmol g\(^{-1}\) DM day\(^{-1}\)) did not differ between nitrate and ammonium treatments (Fig. 3; Table 2; 1-way ANOVA; \( p > 0.05 \)). Numerically, the highest nitrogen removal rates were obtained in the ammonium-based treatments (Fig. 3).

**The relevance of acclimatization**

Except for treatment B, a significant increase in tissue nitrogen content was observed between the first and second weeks in all treatments, while tissue phosphorus content remained similar over time (Table S1; Paired-Samples T-tests; \( p > 0.05 \)). However, the increased nitrogen content did not result in a significant difference in tissue N:P ratio between the two weeks. The C:N ratio decreased significantly in the second week only for the Ulva spp. that was cultivated in treatment A1 and treatment F (Table S1; paired-samples T-test; \( p < 0.01 \)). Interestingly, growth seemed to increase over time when nitrogen was provided in the form of ammonium (treatment E & F), while growth decreased in most cases when nitrate was provided (treatment B & C) (Table S1). Nevertheless, a significant time effect for growth was only observed for treatment C (Paired-Samples T-test; \( p < 0.05 \)).

**Discussion**

Our data suggest that Ulva spp. growth is not influenced by (unfavorable) stoichiometry under moderate to high nutrient concentrations, and high nitrate concentrations do not limit phosphorus uptake. This result is promising for closed IMTA systems, where marine fish in RAS are integrated with seaweed. Nevertheless, our data also suggest that high nutrient concentrations (0.9 mmol L\(^{-1}\) orthophosphate) in (simulated) fish waste effluents may, in specific cases, lead...
to reduced Ulva spp. performance. Therefore, the design of IMTA including seaweed as extractive unit should not allow such conditions by matching the uptake capacity of the seaweed unit to the waste production rate (Neori et al. 2001; Neori and Guttman 2017).

**Limiting nutrients**

Under nutrient limiting conditions, the ratio between macro-elements (N:P) regulates growth and nutrient uptake in seaweeds (Björnsäter and Wheeler 1990; Fan et al. 2014; Perini and Bracken 2014), highlighting the importance of studying nutrient interactions, rather than a single nutrient at a time. Under the high nutrient concentrations in the current study, nutrient removal and growth rates were not different for the two contrasting N:P ratios (9–10 vs 60–70; B vs C; E vs F). This observation suggests that neither of the nutrients was limiting at any time. Both nitrogen and phosphorus tissue contents were above the critical tissue values required to sustain maximum growth reported for Ulva spp. (0.20% P of DW and 2.17% N of DW; Pedersen and Borum 1997; Pedersen et al. 2010). This observation must have derived from the non-limiting concentrations that prevailed for both nitrogen and phosphorus in the current study. Steffensen (1976) also reported for U. lactuca that maximum growth could be achieved under a wide variety of N:P ratios in the medium (N:P ratios of 1:48 – 1:2), demonstrating that variations from the Atkinson ratio do not necessarily reduce seaweed growth. When absolute nutrient concentrations are above the saturation threshold, other factors could be limiting seaweed growth. Phosphorus tissue contents measured in the current study were below the highest phosphorus tissue content.
Table 2  Overview of hypotheses and associated treatment comparisons, including statistical results of treatment comparisons. Treatments within a row lacking a common letter differ significantly (p < 0.05) for the parameter(s) indicated in the first column. When treatments are compared separately within a hypothesis (i.e. Hyp I & Hyp IV), the compared treatments share either lower case letters or capital case letters. The ‘na’ (not applicable) denotes missing data due to algal fragmentation in treatment D.

Hypothesis I: *Ulva* spp. performance is not influenced by stoichiometry in fish waste effluents under high nutrient concentrations: Comparison of high and low N:P ratios (B versus C; E versus F)

|            | Treatment B | Treatment C | Treatment E | Treatment F |
|------------|-------------|-------------|-------------|-------------|
| SGR, Yield, Tissue N, Tissue P, Tissue NP, Tissue CN, N removal, P removal | High NO$_3$:P ratio | Low NO$_3$:P ratio | High TAN:P ratio | Low TAN:P ratio |
|            | a           | a           | A           | A           |

Hypothesis II: Orthophosphate concentrations in RAS effluents are not toxic for *Ulva* spp.: Comparison between different orthophosphate concentrations (low = B, moderate = C, high = D)

|            | Treatment B | Treatment C | Treatment D |
|------------|-------------|-------------|-------------|
| SGR, Yield, Tissue N, Tissue P, Tissue NP, Tissue CN, N removal, P removal | low PO$_4$ conc | moderate PO$_4$ conc | high PO$_4$ conc |
|            | a           | a           | na          |

Hypothesis III: High nitrate concentrations in RAS effluents will limit the phosphorus uptake: Comparison between high nitrate (A) and moderate nitrate (B) concentrations

|            | Treatment A | Treatment B |
|------------|-------------|-------------|
| SGR, Yield, Tissue N, Tissue CN, N removal | High NO$_3$ conc | Moderate NO$_3$ conc |
| Tissue P, P removal | a       | a             |
| Tissue NP | b       | a             |

Hypotheses IV: High TAN concentrations will result in better performance of *Ulva* spp. in comparison to comparably high nitrate concentrations: Comparison between nitrate and ammonium conditions (B versus E; C versus F)

|            | Treatment B | Treatment C | Treatment E | Treatment F |
|------------|-------------|-------------|-------------|-------------|
| SGR, Tissue P, N removal, P removal | NO$_3$ (N:P ratio of 62) | NO$_3$ (N:P ratio of 10) | TAN (N:P ratio of 68) | TAN (N:P ratio of 9) |
| Yield | a           | A           | a           | A           |
| Tissue N, Tissue NP | b           | B           | a           | A           |
| Tissue CN | a       | A           | b           | B           |

Fig. 3  Nitrogen (A) and Phosphorus (B) removal rate (μmol g$^{-1}$ dry matter day$^{-1}$) of *Ulva* spp. cultivated in the six experimental treatments. For treatment description, see Table 1, and for a description of the hypotheses tested and the statistical results, see Table 2. Dark grey bars represent nitrate-based treatments, and light grey bars represent ammonium-based treatments. Bars represent mean values (n = 3 tanks treatment$^{-1}$), and error bars represent standard deviations. Due to degradation of the *Ulva* spp. in treatment D, we were unable to derive valid measurements, resulting in no bar shown for treatment D.
values reported in the literature for *Ulva* spp. (0.4 – 1.5% P of DW; Pedersen et al. 2010; Runcie et al. 2004; Lubsch and Timmermans 2018). Tremblay-Gratton et al. (2018) also reported relatively low tissue N and P content under moderate to high nutrient conditions. They suggested that low tissue reserves might have resulted from a deficiency of trace elements in the media. Trace elements are required to stimulate the phosphate transport system of algae (Lobban and Harrison 1994). Both ASW and RAS water, as used in the present experiments, contain trace elements, but their absolute concentrations and potential limiting effects were not analyzed. Therefore, trace elements might have limited phosphorus uptake in our study.

The results of the treatments used to test hypothesis I indicate that nutrients were supplied in concentrations above the minimum requirements for growth. Under these conditions, unfavorable stoichiometry in fish wastes (N:P ≠ Atkinson ratio) did not limit seaweed growth. The possible adverse effect of such high nutrient concentrations on seaweed performance is discussed below.

### Toxicity of high orthophosphate concentrations

This study’s highest orthophosphate concentration (0.9 mmol L⁻¹; treatment D) was sub-optimal for the *Ulva*, which degenerated. Salinity did not vary between treatments and could thus not explain such results. Other studies reported inhibited growth for *Gracilaria cornea* at 824 µmol L⁻¹ P (Navarro-Angulo and Robleda 1999), *G. conferta* at 3.2 mmol L⁻¹ P (Friedlander and Ben-Amotz 1991), *Palmaria mollis* at 83.3 µmol L⁻¹ P (Demetropoulos and Langdon 2004) and *Porphyra columbina* at 120 µmol L⁻¹ P (Frazer and Brown 1995). It remains unclear whether inhibited growth in these studies was caused by high orthophosphate concentrations or low N:P ratios. To the best of our knowledge, it is unknown at what concentration phosphorus becomes toxic for *Ulva* spp. However, Tremblay-Gratton et al. (2018) showed high growth rate of *U. lactuca* at orthophosphate concentrations up to 291 µmol L⁻¹, which is approximately 2.5 times higher than the moderate orthophosphate concentration (0.1 mmol L⁻¹) used in the current study. The exact levels of phosphorus toxicity for *Ulva* spp. therefore need to be further elucidated, but will likely fall within the range of 0.3–0.9 mmol L⁻¹.

### Inhibiting effects of high nitrate concentration

The maintenance (*Vₘ*) uptake of phosphorus was 1.6 times higher under high nitrate concentrations in comparison to moderate nitrate concentrations, and surge uptake (*Vₛ*) was similar for both nitrate concentrations. Although growth did not differ between the treatments, the higher phosphorus removal rate and tissue content in the high nitrate treatment was associated with higher orthophosphate concentrations in the culture media. Despite of the lower orthophosphate concentration supplied in the moderate nitrate treatment, saturating orthophosphate concentrations (0.8 µmol L⁻¹; Pedersen et al. 2010, 7 µmol L⁻¹; Lubsch and Timmermans 2018) were assumed for both treatments.

Unlike in the studies of Lundberg et al. (1989) and Kumari et al. (2013) the present results show no inhibiting effects of high nitrate levels on phosphorus uptake by *Ulva* spp. Similar results were reported by Lubsch and Timmermans (2018) who studied phosphorus uptake kinetics of *U. lactuca* under saturating nitrate concentrations (5 mmol L⁻¹ NO₃). The higher than expected DIN concentration and subsequent high N:P ratio in treatment B are not expected to have influenced the results, as nitrogen concentrations were still contrasting between treatment A and treatment B. It remains unclear why in some studies high nitrate level seem to have an inhibiting effect on phosphorus uptake, while in other studies this is not observed.

### Effect of N-source (nitrate or ammonium) under high nitrogen concentrations

Seaweed provided with ammonium or nitrate as a nitrogen source grew at similar rates. Admittedly, Ale et al. (2011) showed that *U. lactuca* grew about 70% better with ammonium than with nitrate, in batch cultures supplied with 50 µmol L⁻¹ of N. As in these experiments, the culture medium was not replaced or resupplemented, it is likely that these results resemble surge uptake (*Vₛ*) rather than maintenance (*Vₘ*). In that respect they seem to differ from the results in our acclimatization week, which could be regarded as *Vₛ*, and where lower

![Graph showing phosphorus surge uptake (µmol g⁻¹ fresh weight hour⁻¹) for *Ulva* spp. exposed to either high (5 mmol L⁻¹) or moderate (0.5 mmol L⁻¹) nitrate concentration. Bars represent mean values (n = 4 tanks treatment⁻¹) and error bars standard deviations. Treatments did not differ significantly (p < 0.05).](image-url)
growth was observed for the ammonium-based treatments. Both Neori (1996) and Shahar et al. (2020) found better growth of *U. lactuca* with ammonium than with nitrate for non-starved *Ulva*, representing \( \text{V}_m \), and attributed this difference to the different uptake and assimilation pathways that are involved for the two N-sources. Not surprisingly, even though growth rates were comparable between nitrate and ammonium treatments in our study, higher tissue nitrogen contents were achieved in the ammonium based treatments, suggesting an accumulation of nitrogen which is not used for growth. Due to the high tissue nitrogen content, 1.4 times higher nitrogen removal rates are estimated for the ammonium-based treatments.

It is largely unknown what levels of nitrogen are toxic to *Ulva* spp. Waite and Mitchell (1972) suggest that TAN is toxic to *U. lactuca* at concentrations > 65 \( \mu \text{mol L}^{-1} \), whereas other studies (e.g. Fujita 1985; Neori et al. 1991; Ji et al. 2019; Shahar et al. 2020) applied higher concentrations and did not report reduced growth or degenerating seaweed. It seems however unlikely that the high nitrogen concentrations (ammonium nor nitrate) in our study were toxic, since no mortality or debilitation was observed, as seen for the *Ulva* cultured under the high orthophosphate concentration.

**General patterns on the biomitigation potential and seaweed performance in recirculating IMTA systems**

One of the aims of integrated cultures is to remove excess nutrients from the water and improve its quality. Clean water is essential for the health of the cultured organism and, similarly crucial, to the environment that receives the discharged wastewater. In RAS, the receiving environment is the culture itself. Nutrient removal rates observed in the current study were in line with or lower than other studies on different *Ulva* species (summarized in Table 3). This literature describes variable nutrient uptake and removal rates. Besides differences in species, or even strains (Jansen et al. 2022), a potential explanation for the variation might be the method used. Studies either define nutrient removal as a function of biomass increase and nutrient tissue content, while others determine uptake rates based on depletion of nutrients in the medium. Results from both methods may vary, and it was shown that the nutrient depletion method results in a 2 – 4.5 times higher nutrient uptake (Tremblay-Gratton et al. 2018). The nutritional state of the seaweeds may also play a role in the observed variation in literature since nutrient uptake by seaweeds is, among others, a function of their internal nutrient storage (Lobban and Harrison 1994; Hadley et al. 2014). Our study specifically addressed nutrient uptake for maintenance \( (\text{V}_m) \). Unfortunately, some studies do not define rigorously whether their measurements are \( \text{V}_m \) or \( \text{V}_s \) (surge uptake). This difference can considerably change the interpretation of the data because \( \text{V}_s \) uptake rate is much higher than \( \text{V}_m \) (Neori et al. 2003).

Besides the capacity to remove excess nutrients from the system, growth performance and quality (i.e., protein content) also determine extractive species’ success in integrated systems. Growth rates in the current study ranged between 3.6 and 5.0% per day, while biomass yield ranged between 13 and 22 g FW m\(^{-2}\) day\(^{-1}\) under continuous high nutrient concentrations. As for nutrient uptake rates, highly variable growth rates are reported in the literature for *Ulva* species (Table 3), but growth rates and biomass yield measured in the current study were in line with maximum growth rates measured under natural conditions for an *Ulva* strain (SGR up to 6.2% day\(^{-1}\), biomass yield between 20 and 30 g FW m\(^{-2}\) day\(^{-1}\) ) collected from the exact location as the *Ulva* used in the current study (Jansen et al. 2022). Nevertheless, growth rates and biomass yield were low compared to other studies on *Ulva* spp. (Table 3; Revilla-Lovano et al. 2021). Given the high nutrient concentrations that prevailed in the current research prevented the *Ulva*’s growth limitation by the macronutrients), the role of trace elements and other limiting factors should be considered in future studies. Such studies are relevant for integrated RAS, where the main nutrient supply to the extractive species is not controlled but is determined by nutrients that the fed species do not retain.

**Conclusion**

Integrated aquaculture systems, where marine fish in RAS systems are combined with seaweed production are characterised by high nutrient concentrations, which is different to most studies that have previously examined nutrient uptake kinetics of *Ulva* species. Therefore, deliberately exposing *Ulva* spp. to high nutrient concentrations allowed us to answer the hypotheses that were put forward for the present study, as follows:

**Hypothesis (I)—*Ulva* spp. performance is not influenced by stoichiometry in fish waste effluents under high nutrient concentrations:** it was indeed confirmed that nutrient concentrations in the culture medium and tissue content were above the critical threshold for maximum growth, suggesting that other factors than the macronutrients limited seaweed growth. The unfavorable stoichiometry in fish wastes (N:P < < Atkinson ratio) typical for RAS systems does not limit seaweed growth.

**Hypothesis (II)—Orthophosphate concentrations in RAS effluents are not toxic for *Ulva* spp.:** The orthophosphate concentration of 0.9 mmol L\(^{-1}\) was probably toxic since it was associated with the degeneration of the seaweed. The exact details of phosphorus toxicity remain to be further elucidated, but our results suggest that the toxic concentration approaches 0.9 mmol L\(^{-1}\). This hazard requires consideration in the design and operation of integrated RAS systems.
Table 3  A literature overview of *Ulva* performance (tissue content and growth) and nutrient removal rates. DM, dry matter; accl., acclimatization period; exp., experimental period; biomass & tissue content, nutrient removal rate determined based on biomass growth and initial and final tissue content; nutrient depletion, nutrient removal rate determined based on nutrient depletion in the media

| Species          | Tissue content (% DM) | Removal rate (µmol g⁻¹ DM day⁻¹) | Growth (% day⁻¹) | Concentration culture medium (mmol L⁻¹) | Experimental design | Ref                    |
|------------------|-----------------------|-----------------------------------|------------------|----------------------------------------|---------------------|-----------------------|
|                  | N         | P         | DIN   | DIP   | DIN   | DIP   | Removal rate | Performance          |
| *Ulva spp.*     | 3.67–3.82 | 0.30–0.37 | 140–155 | 4–7   | 4.3–5.0 | 0.7–5 NO₃  | 0.01–0.1  | Flow-through, 1 wk accl., 1 wk exp, biomass & tissue content |
|                  | 5.18     | 0.27–0.29 | 191    | 3     | 3.6–4.0 | 0.5–0.6 TAN | 0.01–0.1  | Flow-through, 1 wk accl., 1 wk exp, biomass & tissue content |
| *Ulva sp.*      | 1.30–3.52 |          |        |       | 0.8–6.2 |          |          | Flow-through, 5 months, frequently sampled |
| *Ulva fasciata* | 5.8      | 0.39      | 528    | 260   | 7.5    | 0.7 NO₃  | 0.07–0.08 | Flow-through, 3 wk accl., 4 d exp, nutrient depletion |
|                  | 7.1      | 0.17      | 2736   | 82    | 14.3   | 0.8 TAN  | 0.06–0.07 | Flow-through, 3 wk accl., 4 d exp |
| *Ulva lactuca*  | 0.83     |          | 885    | 27    | 0.6–4.4 | 5 NO₃   | 0.001–0.05 | Daily media replacement, 10 d exp, nutrient depletion |
| *Ulva lactuca*  | 4.41     | 0.26      | 126    | 10    | 1.5–2.8 | 2.9–4.3 NO₃ | 0.195–0.291 | No media exchange, 6 d exp, nutrient depletion; biomass & tissue content |
|                  |          |           | 49–67  | 2     |         |          |          | No media exchange, 6 d exp |
| *Ulva rigida*   | 4.43 (WT) | 2.79 (SM) | 613 (WT) | 22 (WT) | 3.76 (WT) | 0.5 NO₃  | 0.025     | Daily media exchange, 18–27 d exp |
| Wild type (WT)  |          |           | 858 (SM) | 29 (SM) | 13.97 (SM) |          |          | Gao et al. 2017 |
| Sterile mutant (MT) |          |           |         |       |         |          |          |                         |
| *Ulva lactuca*  | 0.14–0.39 |          |        |       | 0.001–0.032 NH₄NO₃ | 0.00005–0.01 | No media exchange, but daily replenishment of N and P based on uptake rates, 12 d exp, nutrient depletion |
|                  |          |           | 100    |       | 0.0006–0.002 NH₄NO₃ |          | Flow-through, 4 h exp, nutrient depletion |
| *Ulva lactuca*  | 52—112   |          |        |       | 0.02   |          |          | No medium exchange, no accl., 18 min exp, nutrient depletion |
|                  |          |           | 600    |       | 0.5 NH₄NO₃ | <0.0005 | No media exchange, 17–18 d exp, nutrient depletion |
|                  |          |           | 1588   |       | 0.5 NH₄NO₃ | 0.05   | No media exchange, 13–15 d exp |
|                  |          |           | 0.35–1.53 |     | 0.04 NH₄NO₃ | 0.001–0.03 | Regular media exchange, 17–18 d exp, nutrient depletion |
| Species       | Tissue content (% DM) | Removal rate (µmol g⁻¹ DM day⁻¹) | Growth (% day⁻¹) | Concentration culture medium (mmol L⁻¹) | Experimental design | Ref                          |
|---------------|-----------------------|-----------------------------------|------------------|----------------------------------------|---------------------|------------------------------|
|               | N P DIN DIP           | DIN DIP                           |                  | DIN DIP                                | Removal rate | Performance                   |
| Ulva rotundata| 2136 69               | 0.03–0.06 TAN                     | Up to 0.005      | No media exchange, 7 h exp., nutrient depletion | Martínez-Aragón et al. 2002; Hernández et al. 2002 |
|               | 1.57–2.62 0.07–0.10 50–90 1.5–5 | 0.02–0.06 TAN                     | Up to 0.005      | Flow-through, 6 d accl. (starved), 1 wk exp., nut. depl |                    |
|               | 1.35–2.32 0.05–0.09 50–150 0–1 | 0.02–0.06 TAN                     | Up to 0.005      | Flow-through, 6 d accl. (non-starved), 1 wk exp., nut. depl |                    |
| Ulva lactuca  | 1728                  | 0.0035–0.085 TAN                  | No media exchange, 4–6 h exp., nutrient deple- | Pedersen and Borum 1997 |
|               | 480                   | 0.0035–0.045 NO₃                  | Up to 0.005      | Flow-through, 3 wk exp                  | Neori et al. 1991  |
| Ulva lactuca  | 7–11                  | 0.010–0.014 TAN                   | No medium exchange, 1-2 h exp., nutrient deple- | Fujita 1985       |
|               | 8–17 15–18            | 0.027–0.048 TAN                   | Up to 0.005      | Medium exchange every other day, 19d exp |                     |
| Ulva lactuca  | 1.15 3312             | 0.04 TAN                          | No medium exchange, 1-2 h exp., nutrient deple- |                     |
|               | 3.59 3312             | 0.2 TAN                           | Up to 0.005      | Flow-through, 3 wk exp                  |                     |
Hypothesis (III)—High nitrate concentrations in RAS effluents limit the phosphorus uptake: The toxic TAN excreted by fish in RAS systems is bacterially transformed into nitrate, which accumulates in the recycled water. In contrast to suggestions in the literature, Ulva V_s and V_m phosphorus uptake were not reduced in the presence of high nitrate concentrations (up to 5 mmol L⁻¹).

Hypothesis (IV)—High ammonium concentrations improve the performance of Ulva spp. compared to comparably high nitrate concentrations: Ulva growth rate was similar with high concentrations of both N-forms, but the nitrogen content in tissue increased significantly in the ammonium-based treatments. Thus, at the tested high concentrations, the advantage of ammonium nutrition was not in growth but much higher rates of uptake and the accumulation of tissue nitrogen.

Growth and nitrogen removal rates did not differ between high and moderate nitrate concentrations, nor between high versus low N:P ratios, suggesting that maximum growth and nitrogen removal rates were achieved under all these conditions. These results contribute to a better understanding of the application of Ulva spp. as an extractive component in closed IMTA systems, where a continuous state of moderate to high nutrient concentrations prevails.

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Data Availability The datasets belonging to the current study are available from the corresponding author upon reasonable request.

Declarations

Conflicts of interest/Competing interests The authors have no conflict of interest or competing interests to declare relevant to the content of this manuscript.

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References

Ale MT, Mikkelsen JD, Meyer AS (2011) Differential growth response of Ulva lactuca to ammonium and nitrate assimilation. J Appl Phycol 23:345–351
Al-Hafedh YS, Alam A, Buschmann AH, Fitzsimmons KM (2012) Experiments on an integrated aquaculture system (seaweeds and marine fish) on the Red Sea coast of Saudi Arabia: efficiency comparison of two local seaweed species for nutrient biofiltration and production. Rev Aquacult 4:21–31
Atkinson M, Smith S (1983) C:N: P ratios of benthic marine plants. Limnol Oceanogr 28:568–574
Björnsäter BR, Wheeler PA (1990) Effect of nitrogen and phosphorus supply on growth and tissue composition of Ulva fenestrata and Enteromorpha intestinalis (Ulvales, Chlorophyta). J Appl Phycol 26:603–611
Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Rasmussen MB, Markager S, Olesen B, Arias C, Jensen PD (2011) Bioenergy potential of Ulva lactuca: biomass yield, methane production and combustion. Bioresour Technol 102:2595–2604
Cohen I, Neori A (1991) Ulva lactuca biofilters for marine fishpond effluents. I. Ammonia uptake kinetics and nitrogen content. Bot Mar 34:475–482
Demetropoulos CL, Langdon CJ (2004) Enhanced production of Pacific dulse (Palmaria prolifica) for co-culture with abalone in a land-based system: nitrogen, phosphorus, and trace metal nutrition. Aquaculture 235:433–455
Fan X, Xu D, Wang Y, Zhang X, Cao S, Mou S, Ye N (2014) The effect of nutrient concentrations, nutrient ratios and temperature on photosynthesis and nutrient uptake by Ulva prolifera: implications for the explosion in green tides. J Appl Phycol 26:537–544
Frazer AW, Brown MT (1995) Growth of the conchocelis phase of Porphyra columbina (Bangiales, Rhodophyta) at different temperatures and levels of light, nitrogen and phosphorus. Phycol Res 43:249–253
Friedlander M, Ben-Amotz A (1991) The effect of outdoor culture conditions on growth and epiphytes of Gracilaria conferta. Aquat Bot 39:315–333
Fort A, McHale M, Cascella K, Potin P, Perrineau MM, Kerrison P, da Costa E, Calado R, Domingues M, Costa Azvedo I, Sousa-Pinto I, Gachon C, van der Werf A, de Visser W, Beniers J, Jansen H, Guiry M, Sulpcie R (2020) Exhaustive reanalysis of barcode sequences from public repositories highlights ongoing misidentifications and impacts taxa diversity and distribution: a case study of the Sea Lettuce. Mol Ecol Resour 22:86–101
Fujita RM (1985) The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. J Exp Mar Biol Ecol 92:283–301
Gao G, Clare AS, Rose C, Caldwell GS (2017) Reproductive sterility increases the capacity to exploit the green seaweed Ulva rigida for commercial applications. Algal Res 24:64–71
Gao G, Clare AS, Rose C, Caldwell GS (2018) Ulva rigida in the future ocean: potential for carbon capture, bioremediation and biomethane production. GCB Bioenergy 10:39–51
Gao G, Burgess JC, Wu M, Wang S, Gao K (2020) Using macroalgae as biofuel: current opportunities and challenges. Bot Mar 63:355–370
Guist Jr GG, Humm H (1976) Effects of sewage effluent on growth of Ulva lactuca. Florida Scientist 39:267–271
Neori A, Guttmann L (2017) Thoughts on algae cultivation toward an expansion of aquaculture to the scale of agriculture. In: Proceedings of 7th International Conference on Innovation in Chemical, Agricultural, Biological and Environmental Sciences (ICABES-2017), pp 53–58

Hadley S, Wild-Allen K, Johnson C, Macleod C (2014) Modeling macroalgae growth and nutrient dynamics for integrated multitrophic aquaculture. J Appl Physcol 27:901–916

Harrison PJ, Hurd CL (2001) Nutrient physiology of seaweeds: application of concepts to aquaculture. Cah Biol Mar 42:71–82

Hernández I, Martínez-Aragón JF, Tovar A, Pérez-Llorens JL, Vergara JJ (2002) Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (Dicentraurus labrax) waste waters. I. Phosphate J Appl Physcol 14:365–374

Msuya FE, Kyewalyanga MS, Salum D (2006) The performance of the seaweed Ulva reticulata as a biofilter in a low-tech, low-cost, gravity generated water flow regime in Zanzibar, Tanzania. Aquaculture 254:284–292

Msuya FE, Neori A (2008) Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed Ulva lactuca cultured in seawater tanks. J Appl Physcol 20:1021–1031

Moustafa YT, Bougaran G, Callier M, Blancheton JP (2014) Bio-physiological response of biofilter algal candidate Ulva sp. to different nitrogen forms and fluxes. Int J Physiol Biochem Biochem 6:71–79

Navarro-Angulo L, Robledo D (1999) Effects of nitrogen source, N: P ratio and N-pulse concentration and frequency on the growth of Gracilaria cornea (Gracilariales, Rhodophyta) in culture. Hydrobiologia 398:315–320

NEN-EN 15510 (2007) Animal feeding stuffs—determination of crude Ash (ISO 5984)

ISO (International Organization for Standardization) (1978) Animal Feeding Stuffs—Determination of Crude Ash (ISO 5984)

ISO (International Organization for Standardization) (1983) Animal Feeding Stuffs—Determination of Dry Matter content (ISO 6496)

Jansen HM, Broch OJ, Bannister R, Cranford P, Handa A, Husa V, Jiang Z, Stroehmeier T, Strand Ø (2018) Spatio-temporal dynamics in the dissolved nutrient waste plume from Norwegian salmon cage aquaculture. Aquacult Environ Interact 10:385–399

Jansen HM, Bernard MS, Nederlof MAJ, van der Meer IM, van der Werf A (2022) Seasonal variation in productivity, chemical composition and nutrient uptake of Ulva spp. (Chlorophyta). strains. J Appl Physcol. https://doi.org/10.1007/s10811-022-02708-z

Ji Z, Zou D, Gong J, Liu C, Ye C, Chen Y (2019) The different responses of growth and photosynthesis to N: P enrichments between Gracilariosis lemaniformis and its epiphytic alga Ulva lactuca grown at elevated atmospheric CO₂. Mar Pollut Bull 144:173–180

Kang YH, Park SR, Chung IK (2011) Biofiltration efficiency and biochemical composition of three seaweed species cultivated in a fish-seaweed integrated culture. Algae 26:97–108

Kang YH, Kim S, Choi SK, Lee HJ, Chung IK, Park SR (2021) A comparison of the bioremediation potential of five seaweed species in an integrated fish-seaweed aquaculture system: implication for a multi-species seaweed culture. Rev Aquacult 13:353–364

Kim JK, Kraemer GP, Neefus CD, Chung IK, Yarish C (2007) Effects of temperature and ammonium on growth, pigment production and nitrogen uptake by four species of Porphyra (Bangiales, Rhodophyta) native to the New England coast. J Appl Physcol 19:431–440

Krom M, Ellner S, Van Rijin J, Neori A (1995) Nitrogen and phosphorus cycling and transformations in a prototype ‘non-polluting’ integrated mariculture system, Eilat, Israel. Mar Ecol Prog Ser 118:25–36

Kumari P, Kumar M, Reddy CRK, Jha B (2013) Nitrate and phosphate regimes induced lipidomic biochemical changes in the intertidal macroalga Ulva lactuca (Ulvoophyceae, Chlorophyta). Plant Cell Physiol 55:52–63

Lobban CS, Harrison PJ (1994) Seaweed ecology and physiology. Cambridge University Press, Cambridge

Lubsch A, Timmermans K (2018) Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding N: P dynamics in Ulva lactuca (Chlorophyta). J Physcol 54:215–223

Lundberg P, Weich RG, Jensen P, Vogel HJ (1989) Phosphorus-31 and nitrogen-14 NMR studies of the uptake of phosphorus and nitrogen compounds in the marine macroalga Ulva lactuca. Plant Physiol 89:1380–1387

Luo MB, Liu F, Xu ZL (2012) Growth and nutrient uptake capacity of two co-occurring species, Ulva prolifera and Ulva linza. Aquat Bot 100:18–24

Martínez-Aragón JF, Hernández I, Pérez-Llorens JL, Vázquez R, Vergara JJ (2002) Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (Dicentraurus labrax) waste waters. I. Phosphate J Appl Physcol 14:365–374

Msuya FE, Kyewalyanga MS, Salum D (2006) The performance of the seaweed Ulva reticulata as a biofilter in a low-tech, low-cost, gravity generated water flow regime in Zanzibar, Tanzania. Aquaculture 254:284–292

Msuya FE, Neori A (2008) Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed Ulva lactuca cultured in seawater tanks. J Appl Physcol 20:1021–1031

Moustafa YT, Bougaran G, Callier M, Blancheton JP (2014) Bio-physiological response of biofilter algal candidate Ulva sp. to different nitrogen forms and fluxes. Int J Physiol Biochem Biochem 6:71–79

Navarro-Angulo L, Robledo D (1999) Effects of nitrogen source, N: P ratio and N-pulse concentration and frequency on the growth of Gracilaria cornea (Gracilariales, Rhodophyta) in culture. Hydrobiologia 398:315–320

Neori A, Cohen I, Gordin H (1991) Ulva lactuca biofilters for marine fishpond effluents. II. Growth rate, yield and C: N ratio. Bot Mar 34:483–489

Neori A (1996) The type of N-supply (ammonia or nitrate) determines the performance of seaweed biofilters integrated with intensive fish culture. Bomidgeh 48:19–27

Neori A, Shipigel M, Scharfstein B (2001) Land-based low-pollution integrated mariculture of fish, seaweed and herbivores: principles of development, design, operation and economics. Eur Aquacult Soc Special Publ 29:190–191

Neori A, Msuya F, Shauli L, Schuennhoff A, Kopel F, Shipigel M (2003) A novel three-stage seaweed (Ulva lactuca) biofilter design for integrated mariculture. J Appl Physcol 15:543–553

Neori A, Krom MD, van Rijin J (2007) Biogeochemical processes in intensive zero-effluent marine fish culture with recirculating aerobic and anaerobic biofilters. J Exp Mar Biol Ecol 349:235–247

Pedersen MF, Borum J (1997) Nutrient control of estuarine macroalgal: growth strategy and the balance between nitrogen requirements and uptake. Mar Ecol Prog Ser 161:155–163

Pedersen MF, Borum J, Fotel FL (2010) Phosphorus dynamics and limitation of fast- and slow-growing temperate seaweeds in Oslofjord, Norway. Mar Ecol Prog Ser 399:103–115

Perini V, Bracken ME (2014) Nitrogen availability limits phosphorus uptake in an intertidal macroalga. Oecologia 175:667–676

Revilla-Lovano S, Sandoval-Gil JM, Zertuche-Gonzalez JA, Camacho-Ibar VF, Muniz-Salazar R, Avila-Esteller J, Rangel-Mendoza LK, Moustafa YT, Arrieta A, Peruji V, Bracken ME (2014) Physiological responses and productivity of the seaweed Ulva ohnoi (Chlorophyta) under changing cultivation conditions in pilot large land-based ponds. Algal Res 56:102316

Runcie JW, Ritchie RJ, Larkum AW (2004) Uptake kinetics and assimilation of phosphorus by Catenella nipae and Ulva lactuca can be used to indicate ambient phosphate availability. J Appl Physcol 16:181–194
Schuenhoff A, Shpigel M, Lupatsch I, Ashkenazi A, Msuya FE, Neori A (2003) A semi-recirculating, integrated system for the culture of fish and seaweed. Aquaculture 221:167–181
Shahar B, Shpigel M, Barkana R, Masasa M, Neori A, Chernova H, Salomona E, Kiflawi M, Guttman L (2020) Changes in metabolism, growth and nutrient uptake of Ulva fasciata (Chlorophyta) in response to nitrogen source. Algal Res 46
Shpigel M, Neori A (1996) The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: I. Proportions of size and projected revenues. Aquacult Eng 15:313–326
Steffensen DA (1976) The effect of nutrient enrichment and temperature on the growth in culture of Ulva lactuca L. Aquat Bot 2:337–351
Tal Y, Schreier HJ, Sowers KR, Stubblefield JD, Place AR, Zohar Y (2009) Environmentally sustainable land-based marine aquaculture. Aquaculture 286:28–35
Taylor MW, Barr NG, Grant CM, Rees TAV (2006) Changes in amino acid composition of Ulva intestinalis (Chlorophyceae) following addition of ammonium or nitrate. Phycologia 45:270–276
Tremblay-Gratton A, Boussin J-C, Tamigneaux É, Vandenberg GW, Le François NR (2018) Bioremediation efficiency of Palmaria palmata and Ulva lactuca for use in a fully recirculated cold-seawater naturalistic exhibit: effect of high NO₃ and PO₄ concentrations and temperature on growth and nutrient uptake. J Appl Phycol 30:1295–1304
Waite T, Mitchell R (1972) The effect of nutrient fertilization on the benthic alga Ulva lactuca. Bot Mar 15:151–156
Wang X, Olsen LM, Reitan KI, Olsen Y (2012) Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquacult Environ Interact 2:267–283
Van Bussel CG, Mahlmann L, Kroeckel S, Schroeder JP, Schulz C (2013) The effect of high ortho-phosphate water levels on growth, feed intake, nutrient utilization and health status of juvenile turbot (Psetta maxima) reared in intensive recirculating aquaculture systems (RAS). Aquacult Eng 57:63–70
Yogev U, Sowers KR, Mozes N, Gross A (2017) Nitrogen and carbon balance in a novel near-zero water exchange saline recirculating aquaculture system. Aquaculture 467:118–126

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