Initial short-term nitrate uptake in juvenile, cultivated *Saccharina latissima* (Phaeophyceae) of variable nutritional state

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ABSTRACT

To reach the goal of large-scale seaweed cultivation in Norway, new knowledge concerning commercially important species like the kelp *Saccharina latissima* is essential. This includes fundamental understanding of physiological mechanisms like nutrient uptake kinetics to better understand its ecological niche and nutritional requirements. The initial short-term nitrate (NO_3^-) uptake kinetics in cultivated *S. latissima* juvenile sporophytes were evaluated under nitrogen-saturated and nitrogen-limited conditions. The uptake was measured for concentrations in a gradient from 2 to 18 μM NO_3^- which is representative of Norwegian coastal waters in the main growth season of winter and early spring. Preconditioning treatments led to internal nitrogen pools (total tissue nitrogen and intracellular nitrate) that were significantly lower for the N-limited than for the N-saturated sporophytes prior to the experiment. Nitrate uptake rates, related to biomass (V) and intracellular nitrogen content (U), were linearly related to the substrate concentrations for both N-limited and N-saturated sporophytes, indicating that *S. latissima* requires high ambient nitrate concentrations to maintain rapid growth. The sporophytes with deficient internal nitrogen pools exhibited higher uptake rates of NO_3^- than sporophytes with higher internal pools of nitrogen. Mathematical modelling was used to investigate the temporal development of total tissue nitrogen (Q_N) based on the nitrogen-specific uptake rates (U) and revealed a near linear response of U to changes in Q_N. The model also found that a maximum estimated value for Q_N was only approached after more than 20 days at external NO_3^- concentrations of 8 μM. These results expand the physiological understanding of cultivated *S. latissima* and are important for a sustainable upscaling of seaweed farm production.

1. Introduction

Saccharina latissima (Linnaeus) Lane, Mayes, Druehl, and Saunders 2006 is a cold water kelp species that grows rapidly in late winter and early spring when dissolved inorganic nutrients like nitrate (DIN) are available in excess and other environmental factors such as temperature, light conditions and salinity are favorable (Kerrison et al., 2015). Cultivation of *S. latissima* in Norway normally involves deployments during the autumn and winter (Stévant et al., 2017) and harvesting in the period from May-August, depending on the latitude and degree of epifouling on the biomass (Forbord et al., 2020). The interest in seaweed cultivation has increased in Norway over the last decade, but the production of *S. latissima* did not reach more than 174 metric tons in 2018 (Directorate of Fisheries, 2020), compared to the total world production of almost 30 million metric tons of seaweed (FAO, 2018). To upscale and industrialize seaweed production, more knowledge on how to utilize and optimize farm locations in terms of key resources such as light and nutrient availability is essential. This would enable farmers to identify suitable cultivation depths and seasons and, ideally, predict potential biomass production.

Nitrogen is considered to be the potential limiting nutrient for macroalgal growth, both globally and in Norwegian coastal waters, especially during summer months after phytoplankton blooms have depleted the concentration of DIN to almost zero (Hanisak, 1983; Ibrahim et al., 2014). The major sources of inorganic nitrogen available for macroalgae are nitrate (NO_3^-), ammonium (NH_4^+), and urea (Hurd et al., 2014). North-East Atlantic deep water contains around 10 μmol L^{-1} (μM) of NO_3^-, representing the highest concentration of nitrate that the algae are exposed to in its natural environment (Forbord et al., 2012). The concentrations of NH_4^+ are normally lower, but variable and dependent on regeneration processes in the water column (Hurd et al., 2014). NO_3^- can be stored inside the seaweed cells at concentrations exceeding

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ambient concentrations and is therefore assumed to require active uptake (Harrison and Druehl, 1982; Harrison and Hurd, 2001); however, this has only been confirmed for a few species (Roleda and Hurd, 2019). With an active mechanism of uptake, this will normally exhibit saturation kinetics, i.e. the rate of uptake can be described by a rectangular hyperbola like the Michaelis-Menten equation (Chapman et al., 1978; Harrison and Druehl, 1982; Wallentinus, 1984). In other cases, saturation does not occur at high nutrient concentrations, and a linear uptake may be experienced. Unsaturated uptake is common for NH_4^+ (Phillips and Hurd, 2004; Abreu et al., 2011; Martínez et al., 2012), but it has also been described for NO_3^- (Harrison et al., 1986; Ahn et al., 1998; Sánchez-Barredo et al., 2011; Martínez et al., 2012).

Nutrient uptake rates in seaweed are affected by, and vary considerably with, chemical (concentration of nutrients in their ionic or molecular form), physical (light, salinity, temperature, desiccation and water motion) and biological factors (Thomas et al., 1987; Harrison and Hurd, 2001). The latter includes the ratio of surface-area:volume (SA:V), type of tissue, age, nutritional state and variability between species (Wallentinus, 1984; Hurd et al., 2014). The nitrate taken up can be stored in intracellular pools (I-DIN) in the vacuoles (Hurd et al., 2014) or reduced to nitrite (NO_2^-). Incorporation in vacuoles may occur when the uptake rate of NO_3- is greater than the conversion rate to NO_2^-. Nitrite is further reduced to NH_4^+ and incorporated into biomolecules such as amino acids (Harrison and Hurd, 2001). Dependent on the size of this I-DIN pool, it has been suggested that the algae may be more capable of growing in areas where the nutrient availability varies over the seasons (Hanisak, 1979). Other potential nitrogen reserve pools in macroalgae include proteins, nucleic acids, free amino acids and pigments (Lapointe and Duke, 1984; Jones et al., 1996; Young et al., 2007; Naldi and Wheeler, 1999), where proteins account for the largest N-source (Pueschel and Korb, 2001). High contents of these components may be a result of nutrient saturation, whereas low levels could suggest nutrient sub-saturation.

The short-term nitrate uptake reflects the instantaneous uptake following a perturbation in nutrient concentration. The uptake rate will be constant for a short time after perturbation, before intracellular feedback from the increasing nutrient content in the algal tissues and before ambient concentration has been substantially reduced. Uptake rates measured over a long period of time will become proportional to and vary with the growth rate. Short-term uptake following a perturbation in ambient concentration is not quantitatively associated with the growth rate, but the uptake rate depends on the initial nutritional state of the algae.

The aim of this study was to determine the initial short-term nitrate uptake rates and the total nitrogen and internal nitrate storage capacity for cultivated, juvenile N-saturated and N-limited *S. latissima* exposed to nitrate concentrations that are representative of Norwegian coastal waters. These results, under controlled laboratory conditions with ecologically relevant nutrient concentrations, provide new and valuable knowledge that expands the physiological understanding of cultivated *S. latissima* with different nutritional histories.

2. Materials and methods

2.1. Experimental design

Saccharina latissima sporophytes used in this experiment were prepared according to methods described by Forbord et al. (2018) and cultivated at sea from September 2016 to March 2017 at the seaweed farm Taraskjæret in Central Norway (63°42′N 08°52′E). The ambient nitrate concentration at this site in mid-March is typically around 6 µmol L^{-1} at a depth of 0−8 m (unpublished data). Visible epiphyte-free sporophytes with a length of 7-13 cm were chosen for the experiment. Half of the sporophytes were placed in a tank for nitrogen saturation for 8 days with running flow-through deep water from a depth of 90 m with a nitrate concentration of ~10 µmol L^{-1}. The other half of the

sporophytes were placed in artificial seawater (Grasshoff et al., 2009) enriched with modified f/2 medium without nitrogen and silicate for nitrogen depletion for 8 days prior to the experiment. The medium was replaced once in that period. After this, the sporophytes started to bleach and decompose, suggesting severe nitrogen starvation.

The experiment had a duration of 300 min for each group of pre-treated sporophytes and was conducted with a combination of the perturbation- and multiple flask techniques (Harrison et al., 1989) to examine the uptake of NO_3^- as the only available nitrogen source for saturated and depleted *S. latissima* sporophytes added to a concentration gradient (Table 1). The experiment was performed over two following days with the N-saturated experiment run the first day and the N-limited experiment the day after. The laboratory conditions were kept constant over the two days holding a temperature of 10 °C and a light intensity of 40 µmol m^{-2} s^{-1} above the experimental flasks to ensure sufficient energy supply and photosynthetic activity, and the same equipment was used. 250 mL flasks were filled up with artificial seawater without nitrogen and silica and added nitrogen stock solution to create the target concentration. Water motion to reduce boundary layer effects that could reduce uptake rates was achieved by placing the flasks on orbital shakers. Five specimens of whole, untrimmed *S. latissima* sporophytes of approximately similar sizes were haphazardly collected from the appropriate preconditioning tank and added to each replicate flask. The flasks were placed on the orbital shakers at 100 rpm. Deviations in the initial measured concentrations compared to the planned ones (0.25, 0.5, 1, 2, 4, 8 and 16 µM) were found for all treatments due to the fact that the artificial seawater had a background N-content of ~1 µM or more (Table 1). In addition to seven concentrations, with six replicates each (n = 6), two control samples without *S. latissima* biomass were included in both experiments (saturated and depleted) corresponding to low (2.0 and 1.7 µM) and high (16.7 and 18.5 µM) concentrations to monitor potential changes in nitrogen during the experiments due to microbiological activity.

Upon sampling, 2 mL water samples were collected from each flask after 5, 10, 20, 30, 50 and 90, 180 and 300 min of incubation. Samples from the controls were taken at the first and last sampling points. All samples were transferred to pre-marked 15 mL plastic tubes and frozen until analysis. After the experiment, the sporophytes from all experimental flasks were gently dried with paper towels and the total wet biomass per flask was weighed. Two sporophytes from each replicate were weighed individually, dried at 80 °C for 24 h, and weighed again to estimate the total dry weight biomass for each flask.

2.2. Chemical analysis of nitrate

Prior to analysis, the frozen water samples were thawed at room temperature and filtered using a 0.45 µm syringe filter to remove algal debris. Water samples were analyzed colorimetrically at 550 nm after the reduction of NO_3^- to NO_2^- through a copperized cadmium coil in a Flow Solution IV System, O. I. Analytical Auto Analyzer following Norwegian Standard 4745 (NSF, 1975).

2.3. Calculation of uptake rates

Substrate concentrations of NO_3^- were measured at each sampling point to follow the change in concentration over time, reflecting the disappearance of the nutrient from the medium. The substrate concentrations were related to biomass (grams dry weight; g DW) in each flask

Table 1
Seven different incubation concentrations of nitrate (µM) used for the N-saturated and N-limited experiments.

Nutritional history	Initial concentration (µM)						
N-saturated	2.0	2.5	3.2	3.6	5.2	7.6	16.7
N-limited	1.7	2.2	2.4	3.8	5.3	10.2	18.5

and subsequently used to calculate uptake rates. The uptake rates were determined in each replicate flask according to Eq. 1:

$$V = \frac{(S_i - S_f) \text{ x vol}}{t \text{ x DW}} \text{ x } 24 \qquad (1)$$

where V is the specific uptake rate ($\mu g \text{ g}^{-1}$ DW day^{-1}), S_i is the initial substrate concentration ($\mu g \text{ L}^{-1}$), S_f is the final substrate concentration ($\mu g \text{ L}^{-1}$), vol is the volume (L), t is the time between sampling (h) and DW is the dry weight of the total biomass in the flask (g). This calculation was done for the six replicate flasks and a mean value ± standard error (SE) expressed the final results. The uptake rate remained constant over time in the period from 5−90 min of incubation before levelling off; therefore, the measurements made beyond 90 min were not included in the calculation of uptake rates.

The initial uptake of NO_3^- per unit of cellular N was calculated for each replicate flask according to Eq. 2:

$$U = \frac{V}{Q_N} \text{ x } 24 \qquad (2)$$

where U is the specific initial cellular N based uptake rate (day^{-1}), V is the DW specific uptake rate calculated from Eq. 1 ($\mu g \text{ g}^{-1}$ DW h^{-1}), and Q_N is the total tissue nitrogen ($\mu g \text{ N g}^{-1}$ DW) measured from the start of the experiment.

2.4. Total intracellular nitrogen analysis (Q_N)

Total intracellular tissue nitrogen (Q_N) contents were analyzed for the whole thalli for N-saturated and N-limited sporophytes. Three sporophytes taken from each conditioning tank immediately before the uptake experiment were frozen at −20 °C and later stored at −80 °C until freeze-dried (Hetosicc CD 13−2) at −40 °C for 48 h. The dried kelp was homogenized into a fine powder. Samples of 0.4−1 mg freeze-dried kelp were transferred to tin capsules and analyzed for N in quadruplicate with a Carlo Erba element analyzer (model 1106).

2.5. Intracellular nitrate content (I-DIN)

After 300 min incubation, three sporophytes from three of the replicate flasks from each concentration were haphazardly collected and frozen at −20 °C. For the analysis of intracellular nitrate content (I-DIN), 0.06 g semi-frozen *S. latissima* material from each sample was transferred to a test tube with a cork and filled with 6 mL of distilled water. The samples were boiled for 30 min, cooled and filtered through a 0.45-μm polysulfone syringe filter to remove algal debris. The tubes were kept frozen at −20 °C, then defrosted and shaken prior to analysis. The extracts were used to determine the concentration of I-DIN using an Auto Analyzer (Flow Solution IV System, O.I Analytical) after Norwegian Standard 4745 (NSF, 1975).

2.6. Data treatment and statistical analysis

Large outlying substrate concentrations were determined as extreme outliers (outside of the ranges 3rd quartile + 3*interquartile range and 1st quartile – 3*interquartile range) and were removed from the data set as they were likely to represent contamination in the flasks. This applies for one replicate at two concentrations obtained for N-saturated sporophytes and one replicate at three concentrations obtained for N-limited sporophytes (n = 5). Data from the 180- and 300-min time intervals were excluded because the uptake was only constant over time up to 90 min.

Simple linear regression analyses were performed to reveal correlations between the following measurements for both N-saturated and N-limited sporophytes; reduction in nitrate concentrations in the medium *versus* time, uptake rates (V and U) *versus* substrate concentrations and internal nitrate (I-DIN) concentration *versus* substrate concentration.

The Pearson correlation coefficient (r) was calculated to measure the linear correlation between the different variables. The mean I-DIN and Q_N values for N-saturated and N-limited treatments were compared using an independent-samples *t*-test after confirming the assumption of normality (Shapiro-Wilk's test) and homogeneity of variance (Levene's test). Data are presented as mean ± standard error (SE) unless otherwise stated. The significance level was set to p = 0.05. Statistical analysis was performed using IBM SPSS Statistical software (Version 25) and plots were made using Systat SigmaPlot software (version 14) and MATLAB (Release 2017).

2.7. Mathematical modelling of Q_N dynamics

A simple ordinary differential equation for the dynamics of total tissue nitrogen (Q_N) was formulated based on the results from the uptake experiment described in Sections 2.1-2.4. A Monte Carlo type simulation was performed in order to analyze the variability in how the N-affinity may change with Q_N for Q_N values outside of the experimental range, i. e., the functional response of the N-specific uptake rates (U) to the values of Q_N. Using the model, the implications of the uptake results on the temporal changes in Q_N were assessed for different external concentrations of NO_3^-.

3. Results

3.1. Initial uptake rates of nitrate

The uptake of nitrate in *S. latissima* was measured as the reduction in nitrate concentration over time in the water samples with different initial substrate concentrations added. The linear reduction of nitrate found in the 90 min experimental period revealed a constant uptake rate for each concentration, both for the N-saturated (Fig. 1a) and N-limited (Fig. 1b) sporophytes. The uptake rate levelled off after 90 min (data not shown) and nitrate was not depleted during the experimental period (up to 300 min). Linear regression showed a significant (p < 0.05) relationship between concentration and time for all initial concentrations tested, except for 2.5 μM for the N-saturated sporophytes (Table 2).

3.2. Nitrate uptake rates as a function of substrate concentrations

Uptake rates of NO_3^- (V) normalized to dry weight ranged from 64.8 ± 18.5−1414 ± 170 $\mu g \text{ N g}^{-1}$ DW day^{-1} for N-saturated sporophytes (Fig. 2), increasing with increasing nitrate concentration to which a strong positive (r = 0.91) and significant correlation was apparent (R^2 = 0.83, p < 0.001). The V-values for N-limited sporophytes ranged from 179 ± 14.2–2407 ± 111 $\mu g \text{ N g}^{-1}$ DW day^{-1} (Fig. 2); the values increased with increasing nitrate concentration, revealing a strong positive relationship (r = 0.98, R^2 = 0.95, p < 0.001). For both experiments, there was a positive and linear relationship between uptake rate and nitrate concentration throughout. The slopes of the two regression lines (0.26 ± 0.03 for N-saturated and 0.41 ± 0.03 for N-limited sporophytes), describing the substrate specific affinity, were significantly different (p < 0.001).

Mean nitrogen-specific uptake rates (U, day^{-1}) as a function of nitrate concentrations for N-saturated and N-limited sporophytes are shown in Fig. 3. U expresses nitrogen uptake rates normalized to tissue nitrogen (Q_N) of the algae instead of biomass (DW). Nitrogen-specific uptake rates (U) for N-saturated sporophytes ranged from 0.002 ± 0.001 to 0.048 ± 0.006 day^{-1}, increasing with increasing nitrate concentrations to which a strong positive (r = 0.87) and significant correlation was apparent (R^2 = 0.75, p < 0.001). The values of U for N-limited sporophytes ranged from 0.009 ± 0.001 to 0.122 ± 0.006 day^{-1}, increasing with increasing nitrate concentration, revealing a strong positive relationship (r = 0.98, R^2 = 0.95, p < 0.001). The slopes of the two regression lines (0.003 ± 0.001 for N-saturated and 0.007 ± 0.001 for N-limited sporophytes) were significantly different (p < 0.001).
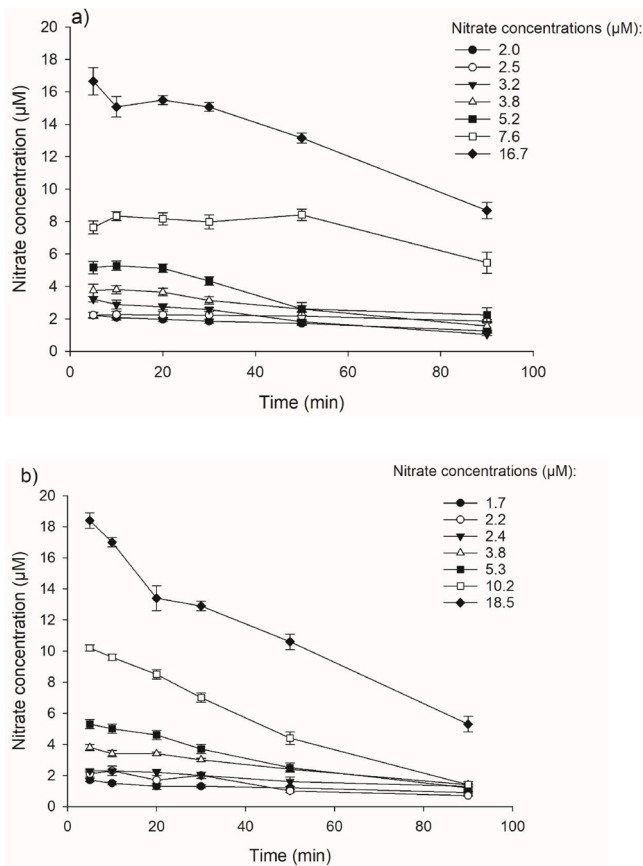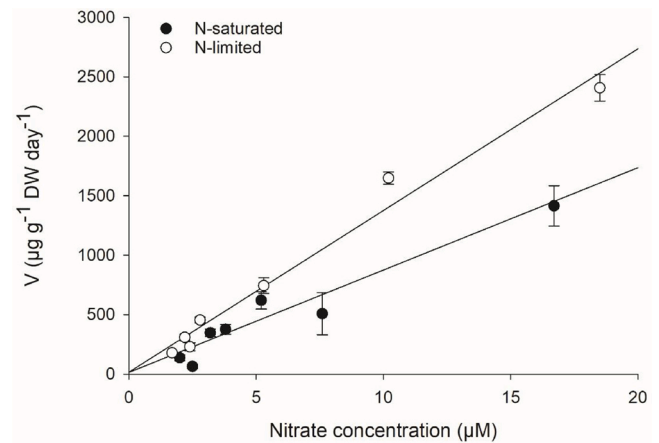
Fig. 2. Mean uptake rates of NO_3^- (V, µg g^{-1} DW day^{-1}) for N-saturated and N-limited sporophytes as a function of initial ambient NO_3^- concentration. The value for N-saturated sporophytes perturbed with 7.6 µM nitrate deviated somewhat from the other values and was not included in the line fitting by linear regression. Mean ± SE, n = 5-6.

Fig. 1. Nitrate (µM) removal from the medium over a period of 90 min for seven different initial concentrations for N-saturated (a) and N-limited (b) sporophytes. Legends show initial nitrate concentrations. Mean ± SE, n = 5-6.

Table 2
Linear regression coefficients (slope, R^2 and p-values) of nitrate concentration *versus* time of uptake for the different initial concentrations (µM) for N-saturated and N-limited sporophytes of *S. latissima*.

N-saturated sporophytes				N-limited sporophytes			
Initial conc.	Slope	R^2	p	Initial conc.	Slope	R^2	p
2.0	−48.6	0.52	<0.001	1.7	−65.5	0.56	<0.001
2.5	−16.6	0.08	**0.105**	2.2	−29.9	0.58	<0.001
3.2	−31.8	0.80	<0.001	2.4	−38.5	0.52	<0.001
3.8	−23.2	0.64	<0.001	3.8	−30.3	0.83	<0.001
5.2	−15.8	0.64	<0.001	5.3	−16.1	0.80	<0.001
7.6	−11.4	0.31	<0.001	10.2	−9.00	0.94	<0.001
16.7	−9.04	0.79	<0.001	18.5	−6.12	0.89	<0.001

In the control samples run without seaweed, only a small increase (< 2.5 %) in NO_3^- concentration was measured in the culture media during the experimental period for the highest initial concentration. The measured nitrate reduction in the seawater was therefore attributed to algal uptake.

3.3. Total intracellular nitrogen content (Q_N)

The N-saturated sporophytes had a total tissue N-content (Q_N) of 29.5 ± 0.5 mg g^{-1} DW before the start of the experiment, which was significantly higher (p < 0.001) than the Q_N value found for N-limited sporophytes of 19.7 ± 0.6 mg g^{-1} DW.
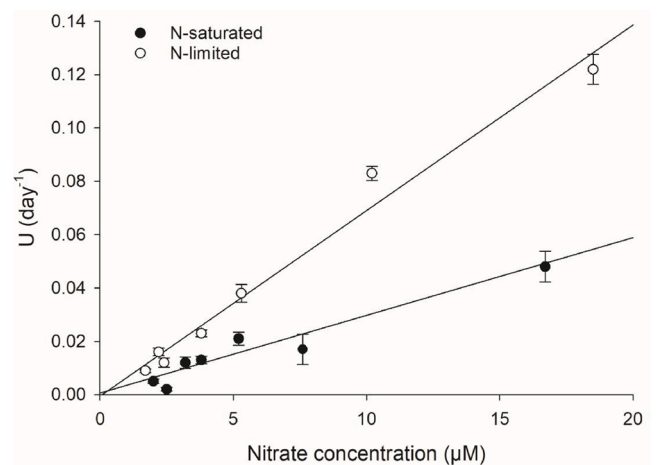
Fig. 3. Mean nitrogen-specific uptake rates (U, day^{-1}) for N-saturated and N-limited sporophytes as a function of initial nitrate concentration. The value for N-saturated sporophytes perturbed with 7.6 µM nitrate deviated somewhat from the other values and were not included in the line fitting. Mean ± SE, n = 5-6.

3.4. Mathematical modelling and simulated Q_N dynamics

In order to discuss the temporal development of Q_N, it is necessary to know or assume something about the N-uptake rates as well as for values of Q_N between and outside the experimental values for the N-saturated and N-limited sporophytes described above. Here, we first show that under certain assumptions the choice of response of U to Q_N is relatively low, and then use this to study the temporal development of Q_N under some relevant external concentrations of NO_3^- like those in the current experiment (Fig. 3).

The main assumption is that the N-specific uptake rate *U* (Eq. 2) is a linear function of the external concentration of NO_3^- (denoted by [NO$_3$]). Thus, it was assumed that

$$U = U(Q_N, [NO3]) = f(Q_N)[NO3] \tag{3}$$

where *f* is *some* response function of the N-specific uptake rate to Q_N. For each Q_N, $f(Q_N)$ represents the N-affinity of the sporophytes, the slope of the U *versus* [NO$_3$] line (c.f., Fig. 3). It can be assumed that the function *f* decreases with increasing Q_N. The function was assumed to have the

form:

$$f(Q_N) = a - bQ_N^\alpha \tag{4}$$

for exponents α ranging from 0 to 2. Values of $\alpha < 1$ will give concave response curves, as found for microalgae (Olsen, 1989), while $\alpha > 1$ implies convex curves. Choosing $\alpha = 1$ gives a linear response of the uptake rates to changes in Q_N.

A Monte Carlo type simulation was performed to analyze the variability and uncertainty in the choice of f. For each simulation, the value of α was selected pseudo-randomly from a uniform distribution (α in [0, 2]), while the values of $f(19)$ and $f(29)$ were selected pseudo-randomly from normal distributions with a mean and standard deviation (SD), as shown in Fig. 3. For each choice of α, the parameters a and b were determined based on the selected $f(19)$ and $f(29)$ (known values, the slopes of the lines in Fig. 3).

On average, the simulations reveal a near linear response of the N-specific uptake rate (U) to changes in Q_N (Fig. 4, black curve). The SDs for Q_N values between 19 and 29 were smaller than the SDs for the slopes of the curves in Fig. 3 (cf. Fig. 4, red bars and dots). Outside the interval [19, 29] for Q_N, the SD increased, reflecting the increased ranges in the functional responses (Eq. 4) outside of this interval.

Fig. 4 also reveals that the simulated theoretical average maximum Q_N of the algae was 36 mg N g^{-1} DW (the intersection of the black line with the horizontal axis) with a range from 32 to 48 mg N g^{-1} DW (the intersections of the boundary of the grey region with the horizontal Q_N axis).

How Q_N changes with time, can be expressed through an appropriate differential equation. The relationship between dry weight (DW g), total tissue N (Q_N, mg N g^{-1} DW) and absolute nitrogen content (N_{abs} g N) (see Section 2.3 above) is

$$Q_N = N_{abs}/DW$$

This means that:

$$\frac{1}{Q_N}\frac{dQ_N}{dt} = \frac{1}{N_{abs}}\frac{dN_{abs}}{dt} - \frac{1}{DW}\frac{dDW}{dt} = U - \mu \tag{5}$$

where U is the nitrogen-specific uptake rate (Eqs. 2 and 3) and μ is the biomass specific growth rate. Q_N must therefore satisfy the following differential equation:
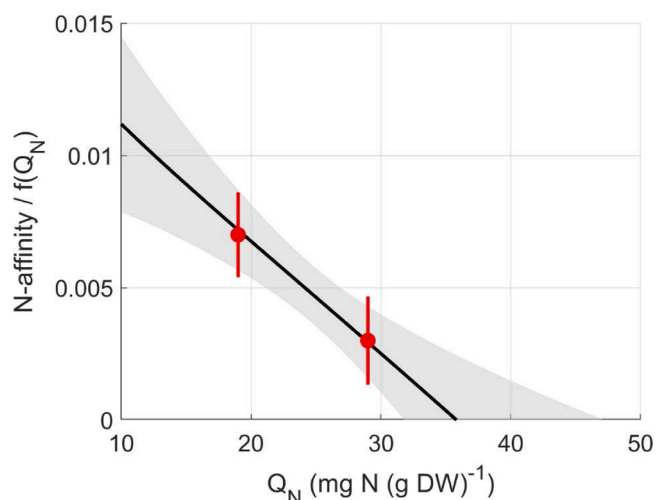
$$\frac{dQ_N}{dt} = Q_N(f(Q_N)[NO3] - \mu) \tag{6}$$

All solutions to Eq. 6 have a sigmoid shape, and for $\alpha = 1$ in Eq. 4 and $\mu = 0$ in Eq. 6 we get the standard logistic equation when [NO$_3$] is kept fixed (Murray, 2002). Eq. 6 was solved, corresponding to each choice of f by the Monte Carlo simulation, by a standard forward Euler method with short time step.

The temporal dynamics of Q_N by Eq. 6 were simulated for [NO$_3^-$] = 1, 4, and 8 μmol L^{-1}. The results illustrated how Q_N over a longer time period varied with external concentrations and time (Fig. 5). The uptake and following influence on Q_N were slow, a maximum value (theoretical/model) was only approached after > 20 days at external concentrations of [NO$_3^-$] = 8 μmol L^{-1}.

3.5. Intracellular nitrate content (I-DIN)

The N-saturated sporophytes showed an I-DIN content of 0.46 $\pm$ 0.06 mg g^{-1} DW prior to incubation (0 μM, Fig. 6), whereas the N-limited sporophytes showed an I-DIN content of 0.020 $\pm$ 0.002 mg g^{-1} DW, which was significantly lower than that of the saturated sporophytes (p = 0.002). The highest I-DIN values were found for the N-saturated sporophytes for all initial nitrate concentrations throughout. Values for the N-saturated sporophytes ranged from 0.25 $\pm$ 0.05 to 0.60 $\pm$ 0.08 mg g^{-1} DW (mean 0.44 $\pm$ 0.04 mg I-DIN g^{-1} DW), while the values for N-limited sporophytes ranged from 0.02 $\pm$ 0.00 and 0.12 $\pm$ 0.01 mg g^{-1} DW (mean 0.06 $\pm$ 0.01 mg I-DIN g^{-1} DW). Linear regression did not reveal a significant relationship between I-DIN content and nitrate concentration for either N-saturated (r = 0.196, R^2 = 0.038, p = 0.359) or N-limited sporophytes (r = 0.034, R^2 = 0.001, p = 0.880).

The quantitative nitrogen amount accumulated in I-DIN pools was low compared to total Q_N of the algae. Percentage I-DIN of total intracellular N (Q_N) was 1.6 % for N-saturated and 0.02 % for N-limited sporophytes. I-DIN is accordingly not an important nitrogen storage that can support algal growth for a long time. However, it does reflect the nutritional nitrogen state of the algae.

4. Discussion

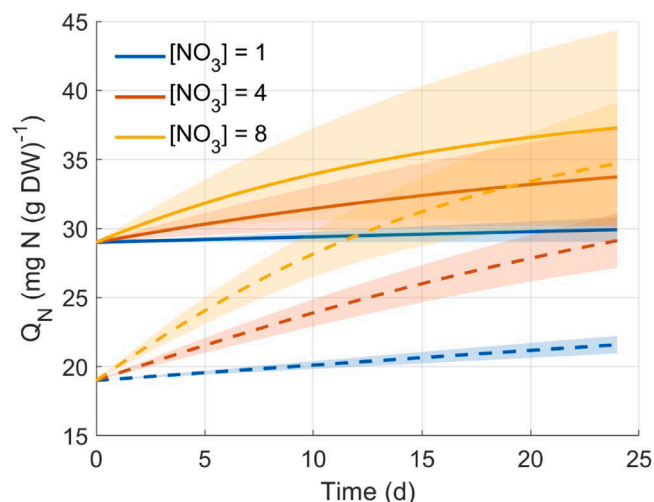We found that sporophytes of *S. latissima* with deficient internal

Fig. 4. Plot of the theoretical affinity to NO$_3^-$ (y-axis) as a function f of Q_N (x-axis). The *slopes* of the lines in Fig. 3 for $Q_N = 19$ and $Q_N = 29$ are represented by the red dots, with corresponding standard deviations (vertical red lines). The black curve and the grey region represent the results of a Monte Carlo-type simulation (n = 3000 runs) of f (in Eq. 3) as a function of Q_N. The black curve indicates the mean values of f for each value of Q_N and corresponding SD (grey region).

Fig. 5. Simulated temporal development of Q_N from Eq. 6 for two initial values of Q_N (Q_N (0) = 19 (dashed lines) and Q_N (0) = 29 (solid lines)) and three different external concentrations of NO$_3^-$ (blue: NO$_3^-$ = 1; red: NO$_3^-$ = 4; yellow: NO$_3^-$ = 8). The curves are mean values of n = 3000 simulations, while the shaded regions represent the SDs. The growth rate was assumed to be $\mu = 0$.
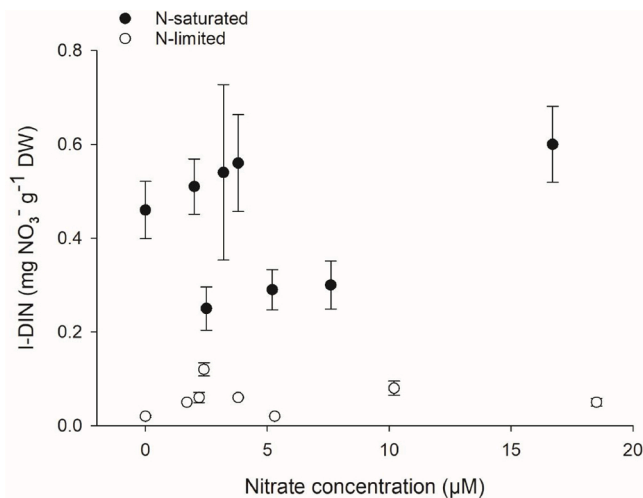
Fig. 6. Mean intracellular nitrate concentration (I-DIN, mg g^{-1} DW) for the different initial incubation concentrations (µM) after 300 min incubation of N-saturated and N-limited sporophytes. Mean ± SE, n = 3.

nitrogen pools (Q_N and I-DIN) exhibited higher uptake rates of NO_3^- than sporophytes with higher internal nitrogen pools. For both nutrient-deficient and -saturated sporophytes, the uptake rates of nitrate were linearly related to the substrate concentration. No clear saturation level was found for nitrate concentrations exceeding double the maximum deep-water concentration potentially experienced in natural North-East Atlantic water. A mathematical model was used to investigate the temporal development of Q_N for N-saturated and N-limited sporophytes based on the nitrogen-specific uptake rates (U), and revealed a near linear response of U to changes in Q_N. The model also found that a maximum estimated value for Q_N was only approached after more than 20 days at external NO_3^- concentrations of 8 µM.

4.1. Uptake kinetics of nitrate

The uptake of NO_3^- has generally been found to exhibit rate-saturation with increasing concentrations, suggesting an active transport mechanism (Chapman et al., 1978; Phillips and Hurd, 2004). This is not supported by the findings in our study, as the uptake of NO3- showed an unsaturated response with a linear increase for both N-saturated and N-limited sporophytes, even for nitrate concentrations higher than the maximum concentrations found in North-East Atlantic deep-water (Voss et al., 2013; Ibrahim et al., 2014). Saturation will, however, most likely be met for higher nitrate concentrations and for a longer uptake period, and an indication of an upcoming saturation could be seen for the N-depleted sporophytes at the highest N-concentration

It appears that several kelp species do not exhibit saturation kinetics for nitrate uptake for nutrient concentrations well beyond the highest natural concentrations (Harrison et al., 1986). This has been found for *Laminaria groenlandica* (Harrison et al., 1986), *Macrosystis pyrifera* (Kopczak, 1994) and *Eisenia arborea* (Sánchez-Barredo et al., 2011). It has been suggested that this characteristic kinetic response is mainly dependent on the nutritional state and/or life-history of the individuals rather than the species (Thomas et al., 1985). Moreover, for intertidal seaweeds from New Zealand, both saturated and non-saturated uptake patterns were found for different individuals of the same population (Phillips and Hurd, 2004), supporting this idea. While other studies have run experiments with NO_3^- concentrations from 30 to 450 µM for various species (Harrison et al., 1986; Martínez and Rico, 2004; Phillips and Hurd, 2004; Abreu et al., 2011; Li et al., 2019), the maximum NO3-concentrations of 16–18 µM in our experiment were much lower, but still above 2–8 µM, which is the ecologically relevant range of concentrations along the Norwegian coast during the winter and early spring

when cultivated *S. latissima* has its highest growth rates (Broch et al., 2019; Forbord et al., 2020).

The affinity and uptake capabilities were lower for N-saturated than N-limited sporophytes, in agreement with several previous studies (Druehl et al., 1989; McGlathery et al., 1996; Ahn et al., 1998). It has also been found that nitrate uptake in macroalgae generally proceeds at considerably lower rates than the uptake of ammonium (NH_4^+) for both N-saturated and N-limited sporophytes (Pedersen and Borum, 1997; Corey et al., 2013; Liu et al., 2016). A recent experiment showed that when offered both NH_4^+ and NO_3^- simultaneously, the sporophytes selectively took up NH_4^+ at a higher rate until the concentration became low (~3µM). The uptake of NO3- then proceeded at a lower rate than when just offered alone (Etter et al., unpublished data).

It is important to consider type of tissue, age, nutrient pre-conditioning, biomass-to-volume of medium during incubation, incubation time and normalization of uptake rates when comparing published results from different experiments.

4.2. Nitrogen-specific uptake rates and simulated Q_N dynamics

The value of the nitrogen-specific uptake rate (U, day^{-1}) during steady-state of growth is equal to the carbon specific growth rate (µ) and can reflect the capacity of *S. latissima* to sustain growth at given ambient concentrations of nitrate even though uptake and growth are not directly coupled. Thus, under steady state conditions, when the processes of uptake and growth are in balance and gives a constant Q_N, an estimated U value can potentially correspond to the steady state growth rate (µ, day^{-1}) that is achievable by the algae at that concentration. The sporophytes in the current experiment were acclimated to nutrient deficient and nutrient sufficient conditions, aiming to achieve growth at steady state conditions. Fig. 3 shows the potential maximum relative growth rates (RGR, day^{-1}), equal to U during steady state, which can be achieved for given nitrate concentrations and nutritional states, reflected by Q_N. The current U values correspond well with the RGR (day^{-1}) of cultivated *S. latissima* based on an increase in length in the period from May-June in Central Norway, where the RGR fluctuated between 0.02 and 0.05 day^{-1} (Forbord et al., 2019). The ambient NO3-concentration is found to vary between 0.4–6.6 µM in that region in that particular period (Forbord et al., unpublished data).

It appears that *S. latissima* requires high ambient NO_3^- concentrations for maintaining rapid growth and are not able to compete for available nutrients with the more efficient phytoplankton during late spring and summer. This means that *S. latissima* have to take up most of the NO_3^- needed early in the season when ambient concentrations are high, and that the period after the spring bloom represents a negative shift in nutrient uptake that involves sporophyte growth mainly based on internal inorganic- and organic nitrogen components. The coupling between extracellular nitrate concentration, initial NO_3^- uptake kinetics, intracellular NO_3^- concentration and specific growth rate is as far as we understand new fundamental knowledge which makes us better understand the ecological niche and the nutritional requirements of *S. latissima*. Such quantitative knowledge is paramount for understanding both the nutrient- and environmental conditions required for efficient cultivation of *S. latissima* as it will have an essential importance for future large-scale production. This also underscore the importance of careful site selection and for finding the best suited cultivation and harvesting periods for optimal nutrient utilization.

Our experimental results did not reveal whether the value of U supporting the maximum growth rate (U_{max}) was linearly or non-linearly related to the nutritional state of the algae, expressed in terms of Q_N. Under the assumptions made, U can be assumed to be a linear function of both Q_N and the ambient nitrate concentration in the [19, 29] interval (Fig. 3). It is important to note that the relations between Q_N and uptake capabilities are most uncertain for minimal and maximal values of Q_N.

4.3. Total intracellular nitrogen content (Q_N)

The N-limited sporophytes had significantly lower Q_N (19.7 mg N g^{-1} DW) than the N-saturated ones (29.5 mg N g^{-1} DW), as a result of the preconditioning treatments, and in agreement with Gerard (1997). Bleaching of tissue, as observed for our N-limited sporophytes after 8 days of starvation, suggested severe N-limitation, and was most likely due to the loss of pigment-protein complexes (Chapman et al., 1978; Hanisak, 1990). The affinity is unlikely to reach zero, reflecting zero uptake, meaning that the algae will exhibit a minimum affinity for Q_N below 36 mg N g^{-1} DW. In the same way, under steady state conditions where U = μ, it is expected that algae can maintain a maximum growth rate at a similar Q_N value (c.f., Q_M for microalgae; Droop, 1968). This means that our N-saturated sporophytes were close to being nitrogen-sufficient, with capabilities of growing close to their maximum growth rate. A Q_N content of 35 mg N g^{-1} DW has been previously reported for N-saturated, juvenile *S. latissima* (Gerard, 1997), which also agrees well with the results from our model.

The content of Q_N has been found to increase with increasing ambient nitrogen concentrations (Young et al., 2007; Martínez et al., 2012), and has been suggested to act as a reliable indicator of the physiological nutritional state of the seaweeds (Manns et al., 2017; Forbord et al., 2020). The feasibility of Q_N as an indicator of nutritional state for *S. latissima* has been previously shown over longer cultivation periods along the Norwegian coast where Q_N was highest early in the season and at greater depths where nitrate was not yet depleted by phytoplankton blooms (Forbord et al., 2020). The decline in Q_N content in macroalgae during the spring and summer are a consequence of metabolic demands exceeding nutrient uptake during growth.

During the course of the uptake experiment, Q_N would potentially increase only by 0.7 % after 90 min of incubation given the highest uptake rates (V) for N-limited sporophytes, and even less for the N-saturated ones (0.3 %). This shows that altering the value of Q_N is a slow process and that the initial steady state situation is not greatly affected during the experiment. Moreover, a maximum value for Q_N estimated from the mathematical model was only approached after more than 20 days at external NO_3^- concentrations of 8 μM. It therefore follows that the reduced nitrate uptake beyond 90 min incubation was mainly an effect of a reduced ambient nitrate concentration and not an effect of increased feedback on uptake following increased Q_N, reflecting nutritional state. Our results are in line with findings showing no significant changes in total Q_N content one week after nitrate resupply in two *Fucus* species that were N-deprived for 15 weeks (Young et al., 2009).

4.4. Intracellular nitrate content (I-DIN)

Our results revealed that young, cultivated sporophytes of *S. latissima* did not hold large reserves of intracellular nitrate (I-DIN) at the conditions of our experiment. We also found that neither of the sporophyte-groups exhibited a significant increase in the content of I-DIN, even after 300 min exposure to more than 16 μM of ambient nitrate.

Potentially, if all nitrate taken up during the experiment at the highest concentration was accumulated directly in the I-DIN pools, the N-saturated sporophytes would increase their I-DIN content by 17 %, which lies within the range of the I-DIN concentrations measured after the experiment. This suggests that the N-saturated sporophytes could store most of the surplus nitrate accumulated in the algae without any need for reduction and incorporation in biomolecules. On the other hand, if the N-limited sporophytes stored all of the nitrate taken up during the experiment at the highest concentration, their I-DIN pool would increase by over 700 %, which was not the case. As a result, we can infer that the intracellular equilibration process between N-compartments is a slow process and that the sporophytes must have incorporated nitrate taken up after perturbation into small molecular N-components, amino acids and pigments rather than depositing nitrate in the vacuoles.

The highest measured I-DIN value of 0.6 mg NO_3^- g^{-1} DW for N-saturated sporophytes was comparable to the higher range of values found for nitrogen-sufficient sporophytes of *S. latissima* (Jevne et al., unpublished data), measuring the I-DIN content in adult, first-year sporophytes that had been supplied with nutrient rich deep-water and maintained in tanks at relatively low light intensities. Similarly, cultivated *S. latissima* at sea followed a seasonal I-DIN pattern with the highest content in spring at a depth of 8−9 m (0.7 mg NO_3^- g^{-1} DW) compared to barely detectable contents towards the summer when ambient nitrate was depleted (Forbord et al., 2020). As for Q_N, I-DIN can be used to express the nutritional state of the alga because studies have revealed that there is a close and significant relationship between I-DIN and both growth rate and ambient nitrate concentrations (Wheeler and Weidner, 1983; Young et al., 2007; Jevne et al., unpublished data). I-DIN concentration is also easily measurable.

The current study did not show a positive linear relationship between I-DIN content and increasing substrate concentration for either of the two pre-treatments during 300 min of perturbation, confirming the slow storage process. This is supported by Naldi and Wheeler (1999), who found a significant increase in I-DIN only after 8−9 days of nitrogen enrichment in *Ulva fenestrata* and *Gracilaria pacifica*, resulting in an increase from < 1%–7% I-DIN of total Q_N for the former species and from < 1% up to 2% of Q_N for the latter species. I-DIN contributed to only 1.6 % of Q_N for N-saturated and 0.02 % of Q_N for the N-limited sporophytes in the current experiment.

New and existing growth models for *S. latissima* and other commercially important seaweeds (Broch and Slagstad, 2012; Hadley et al., 2015; van der Molen et al., 2018) will always require updates and further development. Information like the seaweed's nutrient uptake capabilities of ecologically relevant concentrations can support users in finding suitable cultivation sites, validate their production expectations and in the longer term predict the chemical composition, e.g., the amount of harvestable proteins. Nutrient uptake rates can also be used when calculating the seaweeds capability and efficiency for bioremediation, especially in areas with heavy nitrogen run-off from land. Experimental studies under controlled conditions are crucial to further explore the importance of nitrogen uptake and will strengthen the understanding of macroalgae's nutrient demand (Lubsch and Timmermans, 2019).

5. Conclusions

Seaweed-based ecosystems are potentially very productive. However, this productivity must be sustained through the acquisition and utilization of nutrients, particularly nitrogen. *S. latissima* is abundant along the Norwegian coast and has its highest growth and nutrient uptake rates during periods with high ambient nitrate concentration, typically late autumn to early spring, dependent on latitude. The nitrate concentrations of 2–8 μM used in the current experiment were ecologically relevant for Norwegian coastal waters, but saturation kinetics were not found for these concentrations or for concentrations twice as high as the maximum values found in the natural environment of *S. latissima*. This indicates that *S. latissima* cannot compete efficiently for nitrate in the late spring and summer, where nutrient starvation is experienced and an increase in biomass in these periods is based on intracellular nitrogen components (U < μ_m), cf. Eq. 5. This pool of intracellular nutrients mainly consists of nitrogen-containing compounds like proteins and pigments, and stored inorganic nitrate to a smaller extent, which most likely needs a long time of increased uptake at high ambient nitrate concentrations to reach high levels. The conservative growth strategy and low nutrient uptake of *S. latissima* is ecologically advantageous in physically stable environments with restricted but predictable nutrient resource availability, like in North-Atlantic coastal waters during winter. Knowledge of nitrate uptake kinetics and utilization capabilities in *S. latissima* are important aspects for a sustainable scaling up of seaweed farm production. This enables the farmers to identify potential

locations for their cultivation, to estimate the carrying capacity of the cultivation site, and could predict the potential biomass production based on the nutritional history of the seaweed.

CRediT authorship contribution statement

Declaration of Competing Interest

None.

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References

Abreu, M.H., Pereira, R., Buschmann, A., Sousa-Pinto, I., Yarish, C., 2011. Nitrogen uptake responses of *Gracilaria vermiculophylla* (Ohmi) Papenfuss under combined and single addition of nitrate and ammonium. J. Exp. Mar. Biol. Ecol. 407 (2), 190–199.

Ahn, O., Petrell, R.J., Harrison, P.J., 1998. Ammonium and nitrate uptake by *Laminaria saccharina* and *Nereocystis luetkeana* originating from a salmon sea cage farm. J. Appl. Phycol. 10 (4), 333–340.

Broch, O.J., Slagstad, D., 2012. Modelling seasonal growth and composition of the kelp *Saccharina latissima*. J. Appl. Phycol. 24 (4), 759–776.

Broch, O.J., Alver, M.O., Bekkby, T., Gundersen, H., Forbord, S., Handå, A., Skjermo, J., Hancke, K., 2019. The kelp cultivation potential in coastal and offshore regions of Norway. Front. Mar. Sci. 5 (529).

Chapman, A., Markham, J., Lüning, K., 1978. Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. J. Phycol. 14 (2), 195–198.

Corey, P., Kim, J.K., Duston, J., Garbary, D.J., Prithiviraj, B., 2013. Bioremediation potential of *Palmaria palmata* and *Chondrus crispus* (Basin Head): effect of nitrate and ammonium ratio as nitrogen source on nutrient removal. J. Appl. Phycol. 25 (5), 1349–1358.

Directorate of Fisheries, 2020. Akvakulturstatistikk (tidsserier)-Alger (*in Norwegian*). Accessed 30.01 2020. http://www.fiskeridir.no/Akvakultur/Statistikk-akvaku ltur/Akvakulturstatistikk-tidsserier/Alger.

Droop, M.R., 1968. Vitamin B 12 and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. J. Mar. Biol. Assoc. U.K. 48 (3), 689–733.

Druehl, L., Harrison, P., Lloyd, K., Thompson, P., 1989. Phenotypic variation in N uptake by *Laminaria groenlandica* Rosenvinge (Laminariales, Phaeophyta). J. Exp. Mar. Biol. Ecol. 127 (2), 155–164.

FAO, 2018. The Global Status of Seaweed Production, Trade and Utilization, Vol 124. Globefish Research Programme, Rome.

Forbord, S., Skjermo, J., Arff, J., Handå, A., Reitan, K., Bjerregaard, R., Lüning, K., 2012. Development of *Saccharina latissima* (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. J. Appl. Phycol. 24 (3), 393–399.

Forbord, S., Steinhovden, K.B., Rød, K.K., Handå, A., Skjermo, J., 2018. Cultivation protocol for *Saccharina latissima*. In: Charrier, B., T W., Reddy, C. (Eds.), Protocols for Macroalgae Research, 1st edn. CRC Press, Taylor Francis Group, Boca Raton, FL, London, New York, NY, pp. 37–59.

Forbord, S., Steinhovden, K.B., Solvang, T., Handå, A., Skjermo, J., 2019. Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case-study. J. Appl. Phycol. https://doi.org/10.1007/s10811-020-02042-2.

Forbord, S., Matsson, S., Brodahl, G.E., Bluhm, B.A., Broch, O.J., Handå, A., Metaxas, A., Skjermo, J., Steinhovden, K.B., Olsen, Y., 2020. Latitudinal, seasonal and depth-dependent variation in growth, chemical composition and biofouling of cultivated *Saccharina latissima* (Phaeophyceae) along the Norwegian coast. J. Appl. Phycol. https://doi.org/10.1007/s10811-020-02038-y.

Gerard, V.A., 1997. The role of nitrogen nutrition in high-temperature tolerance of the kelp, *Laminaria saccharina* (Chromophyta). J. Phycol. 33 (5), 800–810.

Grasshoff, K., Kremling, K., Ehrhardt, M., 2009. Methods of Seawater Analysis, third edn. John Wiley & Sons, Weinheim.

Hadley, S., Wild-Allen, K., Johnson, C., Macleod, C., 2015. Modeling macroalgae growth and nutrient dynamics for integrated multi-trophic aquaculture. J. Appl. Phycol. 27 (2), 901–916.

Hanisak, M., 1979. Nitrogen limitation of *Codium fragile* ssp. tomentosoides as determined by tissue analysis. Mar. Biol. 50 (4), 333–337.

Hanisak, M., 1983. The nitrogen relationships of marine macroalgae. In: Carpenter, E.J., Capone, D.G. (Eds.), Nitrogen in the Marine Environment. Academic Press, New York, pp. 699–730.

Hanisak, M.D., 1990. The use of *Gracilaria tikvahiae* (Gracilariales, Rhodophyta) as a model system to understand the nitrogen nutrition of cultured seaweeds. Hydrobiologia 204 (1), 79–87.

Harrison, P., Druehl, L., 1982. Nutrient uptake and growth in the Laminariales and other macrophytes: a consideration of methods. In: Srivastava, L.M. (Ed.), Synthetic and Degradative Process in Marine Macrophytes. Walter de Gruyter Academic Press, pp. 99–120.

Harrison, P.J., Hurd, C.L., 2001. Nutrient physiology of seaweeds: application of concepts to aquaculture. Cah. Biol. Mar. 42 (1-2), 71–82.

Harrison, P., Druehl, L., Lloyd, K., Thompson, P., 1986. Nitrogen uptake kinetics in three year-classes of *Laminaria groenlandica* (Laminariales: phaeophyta). Mar. Biol. 93 (1), 29–35.

Harrison, P., Parslow, J., Conway, H., 1989. Determination of nutrient uptake kinetic parameters: a comparison of methods. Mar. Ecol. Prog. Ser. 301–312.

Hurd, C.L., Harrison, P.J., Bischof, K., Lobban, C.S., 2014. Seaweed Ecology and Physiology, 2 edn. Cambridge University Press, Cambridge.

Ibrahim, A., Olsen, A., Lauvset, S., Rey, F., 2014. Seasonal variations of the surface nutrients and hydrography in the Norwegian Sea. Int. J. Environ. Sci. De. 5 (5), 496–505.

Jones, A.B., Dennison, W.C., Stewart, G.R., 1996. Macroalgal responses to nitrogen source and availability: amino acid metabolic profiling as a bioindicator using *Gracilaria edulis* (Rhodophyta). J. Phycol. 32 (5), 757–766.

Kerrison, P.D., Stanley, M.S., Edwards, M.D., Black, K.D., Hughes, A.D., 2015. The cultivation of European kelp for bioenergy: site and species selection. Biomass Bioenergy 80, 229–242.

Kopczak, C.D., 1994. Variability of nitrate uptake capacity in *Macrocystis pyrifera* (Laminariales, Phaeophyta) with nitrate and light availability. J. Phycol. 30 (4), 573–580.

Lapointe, B.E., Duke, C.S., 1984. Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. J. Phycol. 20 (4), 488–495.

Li, H., Zhang, Y., Chen, J., Zheng, X., Liu, F., Jiao, N., 2019. Nitrogen uptake and assimilation preferences of the main green tide alga *Ulva prolifera* in the Yellow Sea, China. J. Appl. Phycol. 31 (1), 625–635.

Liu, H., Wang, F., Wang, Q., Dong, S., Tian, X., 2016. A comparative study of the nutrient uptake and growth capacities of seaweeds *Caulerpa lentillifera* and *Gracilaria lichenoides*. J. Appl. Phycol. 28 (5), 3083–3089.

Lubsch, A., Timmermans, K.R., 2019. Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding dissolved inorganic nitrate uptake in *Saccharina latissima* and *Laminaria digitata* (Phaeophyceae). J. Phycol. 55, 637–650.

Manns, D., Nielsen, M.M., Bruhn, A., Saake, B., Meyer, A.S., 2017. Compositional variations of brown seaweeds *Laminaria digitata* and *Saccharina latissima* in Danish waters. J. Appl. Phycol. 1–14.

Martínez, B., Rico, J.M., 2004. Inorganic nitrogen and phosphorus uptake kinetics in *Palmaria palmata* (Rhodophyta). J. Phycol. 40 (4), 642–650.

Martínez, B., Pato, L.S., Rico, J.M., 2012. Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies. Aquat. Bot. 96 (1), 14–22.

McGlathery, K.J., Pedersen, M.F., Borum, J., 1996. Changes in intracellular nitrogen pools and feedback controls on nitrogen uptake in *Chaetomorpha linum* (Chlorophyta). J. Phycol. 32 (3), 393–401.

Murray, J.D., 2002. Mathematical Biology: I. An Introduction, 3 edn. Springer, New York.

Naldi, M., Wheeler, P.A., 1999. Changes in nitrogen pools in *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) under nitrate and ammonium enrichment. J. Phycol. 35 (1), 70–77.

NSF, 1975. NS 4746 Water Analysis. Determination of the Sum of Nitrite-nitrogen and Nitrate-nitrogen (*in Norwegian*). Norges Standardiseringsforbund, Oslo, Norway.

Olsen, Y., 1989. Evaluation of competitive ability of *Staurastrum luetkemuellerii* (Chlorophyceae) and *Microcystis aeruginosa* (Cyanophyceae) under P limitation. J. Phycol. 25 (3), 486–499.

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

Phillips, J.C., Hurd, C.L., 2004. Kinetics of nitrate, ammonium, and urea uptake by four intertidal seaweeds from New Zealand. J. Phycol. 40 (3), 534–545.

Pueschel, C.M., Korb, R.E., 2001. Storage of nitrogen in the form of protein bodies in the kelp *Laminaria solidungula*. Mar. Ecol. Prog. Ser. 218, 107–114.

Roleda, M.Y., Hurd, C.L., 2019. Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. Phycologia 58 (5), 552–562.

Sánchez-Barredo, M., Ladah, L.B., Zertuche-González, J.A., 2011. Nitrate uptake and duration of internal nitrogen reserves in the kelp *Eisenia arborea*. Bot. Mar. 54 (5), 441–446.

Stévant, P., Rebours, C., Chapman, A., 2017. Seaweed aquaculture in Norway: recent industrial developments and future perspectives. Aquac. Int. 25 (4), 1373–1390.

Thomas, T., Harrison, P., Taylor, E., 1985. Nitrogen uptake and growth of the germlings and mature thalli of *Fucus distichus*. Mar. Biol. 84 (3), 267–274.

Thomas, T., Harrison, P., Turpin, D., 1987. Adaptations of *Gracilaria pacifica* (Rhodophyta) to nitrogen procurement at different intertidal locations. Mar. Biol. 93 (4), 569–580.

van der Molen, J., Ruardij, P., Mooney, K., Kerrison, P., O`Connor, N.E., Gorman, E., Timmermans, K., Wright, S., Kelly, M., Hughes, A.D., 2018. Modelling potential production of macroalgae farms in UK and Dutch coastal waters. Biogeosciences 15 (4), 1123–1147.

Voss, M., Bange, H.W., Dippner, J.W., Middelburg, J.J., Montoya, J.P., Ward, B., 2013. The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change. Phil. Trans. R. Soc. B 368, 20130121.

Wallentinus, I., 1984. Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Mar. Biol. 80 (2), 215–225.

Wheeler, W., Weidner, M., 1983. Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. J. Phycol. 19 (1), 92–96.

Young, E.B., Dring, M.J., Savidge, G., Birkett, D.A., Berges, J.A., 2007. Seasonal variations in nitrate reductase activity and internal N pools in intertidal brown algae are correlated with ambient nitrate concentrations. Plant Cell Environ. 30 (6), 764–774.

Young, E.B., Berges, J.A., Dring, M., 2009. Physiological responses of intertidal marine brown algae to nitrogen deprivation and resupply of nitrate and ammonium. Physiol. Plant. 135 (4), 400–411.