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**UPTAKE KINETICS AND STORAGE CAPACITY OF DISSOLVED INORGANIC
PHOSPHORUS AND CORRESPONDING DISSOLVED INORGANIC NITRATE
UPTAKE IN *SACCHARINA LATISSIMA* AND *LAMINARIA DIGITATA*
(PHAEOPHYCEAE)**

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Abstract

Uptake rates of dissolved inorganic phosphorus (DIP) and dissolved inorganic nitrogen (DIN) under unsaturated (V_S) and saturated conditions (V_M) were studied in young sporophytes of the seaweeds *Saccharina latissima* and *Laminaria digitata* (Phaeophyceae) using a ‘pulse-and-chase’ assay under fully controlled laboratory conditions. In a subsequent second ‘pulse-and-chase’ assay, internal storage capacity (ISC) was calculated based on V_M and the parameter for photosynthetic efficiency F_v/F_m . Sporophytes of *S. latissima* showed a V_S of $0.80 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ and a V_M of $0.30 \pm 0.09 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ for DIP, while V_S for DIN was $11.26 \pm 0.56 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ and V_M was $3.94 \pm 0.67 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$. In *L. digitata*, uptake kinetics for DIP and DIN were substantially lower: V_S for DIP did not exceed $0.38 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ while V_M for DIP was $0.22 \pm 0.01 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$. V_S for DIN was $3.92 \pm 0.08 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ and the V_M for DIN was $1.81 \pm 0.38 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$. Accordingly, *S. latissima*

exhibited a larger ISC for DIP ($27 \mu\text{mol}\cdot\text{cm}^{-2}$) than *L. digitata* ($10 \mu\text{mol}\cdot\text{cm}^{-2}$), and was able to maintain high growth rates for a longer period under limiting DIP conditions. Our standardized data add to the physiological understanding of *S. latissima* and *L. digitata*, thus helping to identify potential locations for their cultivation. This could further contribute to the development and modification of applications in a bio-based economy, for example in evaluating the potential for bioremediation in integrated multi-trophic aquacultures (IMTA) that produce biomass simultaneously for use in the food, feed and energy industries.

Keywords

Laminaria digitata – nitrate uptake – phosphate uptake - *Saccharina latissima* - uptake kinetics

Introduction

Dissolved inorganic phosphorus (DIP) and dissolved inorganic nitrogen (DIN) are essential macronutrients for maintaining the metabolism and growth of seaweeds. P and N are a key components of nucleic acids, phospholipids, adenosine triphosphate (ATP) and are also involved in controlling enzyme reactions and in the regulation of metabolic pathways. After N, P is the second most frequently limiting macronutrient in seaweed growth. Nutrient limitation and shifts in limitation from one element to another can significantly affect the internal composition, physiology and growth of seaweeds (Pederson and Borum 1996, Gevaert et al. 2001). These processes can reflect natural fluctuations, but can also be driven by anthropogenic emissions. For example, agricultural run-off waters contain considerable amounts of inorganic phosphate (PO_4^{3-}) and nitrogenous compounds, like nitrate (NO_3^-) and ammonium (NH_4^+) (Sharpley et al. 1992, Rabalais et al. 2009). Anthropogenic discharge can

also generate nutrient concentration gradients, which are often observed along coastal zones due to the proximity of nutrient sources. This can not only lead to alterations in the type and magnitude of nutrient limitations, but may also cause effects of eutrophication. In the North Sea, measures against eutrophication were first installed in the mid 1980's, when its dramatic effects on marine flora and fauna became evident (Westernhagen and Dethlefsen 1983, Malta and Verschuure 1997, Lyngby et al. 1999). Recently it showed, that the de-eutrophication efforts have led to a large imbalance in the N:P stoichiometry of coastal waters of the North Sea in north-western Europe (Burson et al. 2016). Increasing N:P ratios, which outpace the Redfield ratio of 16:1 were observed (Radach and Pätsch 2007, Grizzetti et al. 2012) and a pronounced P-limitation can be effective in coastal regions of the southern North Sea.

This can have notable effects on the ecosystem communities and growth and functioning of primary producers. It has been reported that N availability mediates the ability of primary producers to access P, as shown for the brown seaweed *Fucus vesiculosus* Linnaeus (Perini and Bracken 2014).

The perennial brown seaweeds (Phaeophyceae) *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders and *Laminaria digitata* (Hudson) J.V. Lamouroux are commonly found on the lower shores of the north Atlantic around the northern North American and European coastlines, including the North Sea. *Saccharina latissima* is also distributed along the shores of the north Pacific. As ecosystem engineers, *S. latissima* and *L. digitata* can affect sedimentation and erosion by reducing water currents (Jones et al. 1994, Bouma et al. 2005) and offer shelter, feedstock and nursery habitats to various fauna, thus enhancing the diversity of their habitat (Jørgensen and Christie 2003). Both seaweeds are rich sources of nutrients and contain large amounts of carbohydrates in the form of structural, storage, and functional polysaccharides, as well as considerable amounts of proteins (Holdt and Kraan, 2011). Aside from the direct use of *S. latissima* and *L. digitata* for culinary and

medicinal purposes, there is great interest in the refinement, extraction and application of carbohydrates and proteins in the energy and animal feed industries, as well as the extraction of important food hydrocolloids, including carrageenan and alginates (McHugh 2003; Troell *et al.* 2006; Holdt and Kraan 2011). However, the content of these compounds varies, depending on nutrient availability, temperature, light and hydrodynamics, alternating in accordance to season and area of cultivation (Murata and Nakazoe 2001, Connan *et al.* 2004).

The vast range of possibilities for using seaweed, especially *S. latissima* and *L. digitata*, has resulted in an enormous surge in interest over the last decades (McLachlan 1985), hence stimulating the efforts towards large-scale cultivation as a supplement to wild harvests (Neori 2008, Bixler and Porse 2011, Holdt and Kraan 2011, Kraan 2013). Although there is much known about the growth requirements of *S. latissima* (Bartsch *et al.* 2008, Reid *et al.* 2013, Marinho *et al.* 2015) and *L. digitata* (Bolton and Lüning 1982, Schaffelke and Lüning 1994, Harrison and Hurd 2001, Gordillo *et al.* 2002, Pederson *et al.* 2010), there is relatively little information available about the DIP uptake kinetics, as well as DIP and DIN management in relation to the internal storage capacity (ISC), the maximal internal duration for growth under external limiting conditions (Pederson *et al.* 2010). This important information, as it allows an estimation of ecological effects on nutrient availability and can contribute to development and modification of cultivation sites. A lot of studies related to uptake kinetics for DIN and DIP in *S. latissima* and *L. digitata* have been conducted under field conditions with weekly to monthly sampling intervals (Bolton and Lüning 1982, Schaffelke and Lüning 1994, Reid *et al.* 2013, Marinho *et al.* 2015) and the majority of studies under laboratory conditions have focused on uptake of nitrogenous compounds, as NO_3^- and NH_4^+ , in *S. latissima* and *L. digitata* (Chapman *et al.* 1978, Conolly and Drew 1985, Harrison *et al.* 1986). Often DIN and DIP uptake is tested independently in short term experiments, usually ranging from minutes

to hours (e.g. Runcie et al. 2003, Martínez and Rico 2004, Luo et al. 2012). Long term responses to DIN and DIP availability remain unknown.

Nutrient uptake by seaweed can be split into three distinct phases, referred to as surge uptake (V_s), metabolic or internally controlled uptake (V_M), and externally controlled uptake (V_e) (Conway et al. 1976, Harrison et al. 1989). V_s refers to the filling of internal nutrient pools, uncoupled from growth (Conway et al. 1976), and has often been described for nutrient-starved seaweeds (e.g. Fujita 1985, Harrison et al. 1989, Dy and Yap 2001). The uptake rates gradually decrease as internal nutrient pools in cytoplasm and vacuoles are filled (Rosenberg et al. 1984, Fujita 1985). When internal nutrient concentrations are constant and relative uptake rates of nutrients remain relatively stable over time, V_M , which is considered equal to the rate of assimilation, is attained (Taylor and Rees 1999, Barr et al. 2004). The previously filled nutrient pools can be utilized at times of low external nutrient availability (Probyn and Chapman 1982, Pederson and Borum 1996). The internal storage capacity (ISC) and temporal duration of the filled nutrient pools under external nutrient depletion conditions has hardly been focused on in seaweeds (Fujita et al. 1985).

Experimental studies under controlled conditions are critical to further understand the role of nutrients and shifts in nutrient ratios, and will strengthen the understanding of nutrient demand and strategies by seaweeds. This is of great ecological and economic importance, as it will open up opportunities to forecast the impacts of nutrient limitation and will shed light on possible competitive advantages of one species versus the other under shifts in limitation from one element to another. It will also facilitate to identify potential locations for seaweed mariculture and provide insight into optimal cultivation practices in regard to nutrient additions.

Material and Methods

In this study, we present the DIP- and DIN-uptake kinetics of young *S. latissima* and *L. digitata* sporophytes exposed to a range of nominal DIP concentrations (0 - 6 $\mu\text{mol}\cdot\text{L}^{-1}$) and non-limiting DIN concentration (50 $\mu\text{mol}\cdot\text{L}^{-1}$) under laboratory conditions, controlling for temperature, light and hydrodynamics in a ‘pulse-and-chase’ assay (i.e. adding a pulse of nutrients and following their removal from the water over time).

In a second ‘pulse-and-chase’ experiment under the same laboratory conditions for light, temperature, and hydrodynamics, sporophytes of both species were exposed to DIP-depleted, DIN-depleted, DIP and DIN-depleted, and DIP- and DIN-enriched seawater. Thereafter, the fluorescence signal F_v/F_m , which is a measure of plant stress/photosynthetic efficiency, was measured over 9 weeks. Based on this data, the DIP- and DIN-uptake kinetics as well as the internal storage capacity of DIP and DIN in *S. latissima* and *L. digitata* were quantified and standardized for surface area (SA).

All experiments and analyses were conducted at the Royal Netherlands Institute for Sea Research (NIOZ) located on Texel, the Netherlands. Cultured sporophytes of *S. latissima* and *L. digitata*, offspring from plants originated and collected from the coastline of Den Helder, the Netherlands, were transferred from incubation tanks at the NIOZ Seaweed Centre (www.nioz.nl/en/expertise/seaweed-research-centre) into 4 separate (2 for each species) transparent 20 L Nalgene™ bottles (Nalge Nunc International Corporation, Rochester, NY, USA), filled with 15L seawater medium, inside a temperature-controlled room ($12.0\pm0.6\text{ }^{\circ}\text{C}$, measured hourly by HOBO temperature loggers (Onset, Bourne, MA, USA)), for an adaptation phase under fully controlled laboratory conditions. Two tubular fluorescent lamps (OSRAM L18 Watt 965, Deluxe cool daylight), attached 50 cm above the flasks and covered by two layers of black mosquito netting, provided a PAR light intensity of $18\pm3\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$

(n=9; light meter ULM- 500, Walz, Germany) inside the glass flasks in a set light/dark period of 16/8 h. The low light concentration was installed to avoid light induced stress on the sporophytes, as previous cultivation of young individuals in light concentrations of 70-80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (after Lüning 1979, Andersen et al. 2011) in a light/dark period of 16/8 h led to frond-bleaching within 2 days (not depicted). A moderate water movement inside the 20 L bottles was provided by aeration, produced by a common air pump (AquaForte V-20, Hailea Group Co., Ltd., China) outside the bottles, which was connected to a PVC hose, connected to a glass pipette (25 ml) inside a bottle.

We used 2 experimental approaches: (1) analysing DIP and DIN uptake kinetics under unsaturated (V_s) and saturated states (V_M) of the two seaweeds, and (2) estimation of their internal storage capacity (ISC) for DIP and DIN, based on V_M and the fluorescence protocol of F_v/F_m , as an indicator for (nutritional) stress. Based on the different 2 experimental approaches to determine DIP and DIN uptake kinetics and ISC for DIP and DIN, the nutrient concentration in the seawater medium inside the 20 L bottles differed during adaptation phase, while other parameters like light, temperature, and hydrodynamics were kept constant.

Experimental approach 1

Sporophytes of both species were maintained in nutrient-depleted seawater ($\text{PO}_4^{3-} = 0.008 \mu\text{mol}\cdot\text{L}^{-1}$, $\text{NH}_4^+ = 0.022 \mu\text{mol}\cdot\text{L}^{-1}$ and $\text{NO}_3^- = 0.003 \mu\text{mol}\cdot\text{L}^{-1}$) for a 15-day adaptation phase in experimental approach 1, which is similar to the experimental set-up used for determining uptake kinetics in *U. lactuca* by Lubsch and Timmermans (2018). Exposing the sporophytes to nutrient-depleted seawater ensured nutrient starvation, as data for their nutritional history was not available. After this starvation phase, 49 randomly picked sporophytes of *S. latissima* and *L. digitata* with a frond size range of 1.5 to 6.5 cm^2 , respectively 5.5 to 29.9 cm^2 (Figure 1) were individually transferred into 200 ml glass jars filled with 100 ml of seawater medium

enriched with a range of dissolved inorganic phosphate levels (DIP: 0.0 $\mu\text{mol}\cdot\text{L}^{-1}$, 0.2 $\mu\text{mol}\cdot\text{L}^{-1}$, 0.4 $\mu\text{mol}\cdot\text{L}^{-1}$, 0.8 $\mu\text{mol}\cdot\text{L}^{-1}$, 1.5 $\mu\text{mol}\cdot\text{L}^{-1}$, 3.0 $\mu\text{mol}\cdot\text{L}^{-1}$ and 6.0 $\mu\text{mol}\cdot\text{L}^{-1}$) and a non-limiting concentration of dissolved inorganic nitrogen (DIN: 50 $\mu\text{mol}\cdot\text{L}^{-1}$). The installed DIP concentrations, as well as DIN concentration, covered the range of observed natural concentrations in coastal areas of the NE Atlantic, respectively neighboring seas like the North Sea, which seasonal extremes (winter concentrations) show an overall average of 2-4 $\mu\text{mol}\cdot\text{L}^{-1}$ for DIP and 60-90 $\mu\text{mol}\cdot\text{L}^{-1}$ for DIN for the years 2006-2014 (OSPAR assessment report 2017; <https://www.oap.ospar.org/en/ospar-assessment/intermediate-assessment-2017/pressures-human-activities/eutrophication/nutrient-concentration>; retrieved in August 2018). The investigation on higher DIP concentrations, as in nominal concentration of 6.0 $\mu\text{mol}\cdot\text{L}^{-1}$ could be of interest for nursery operations of the seaweeds, as well as integrated multi-trophic aquaculture (IMTA) activities with young sporophytes or bioremediation purposes.

The seawater medium was refreshed (“pulsed”) and samples of the day-old medium were taken (“chased”) for dissolved nutrient analysis on a daily basis for 3 weeks, as the removal of DIP and DIN from the seawater medium were referred to uptake rates by the seaweed.

After daily refreshment of the seawater medium, all flasks were randomly distributed to minimize differences in light availability on a rotating table, which provided moderate water movement at a speed of 100 rpm. This constant water movement was maintained for optimal mixing and, hence, availability of nutrients by decreasing the diffusion boundary layers between tissues and the growing medium (e.g. Gonen et al. 1995, Hurd 2000).

Seawater medium

The base for the seawater medium was nutrient-poor seawater from the North Atlantic Ocean (salinity 34.5) with low phosphate (PO_4^{3-} ; 0.008 $\mu\text{mol}\cdot\text{L}^{-1}$), ammonium (NH_4^+ ; 0.022 $\mu\text{mol}\cdot\text{L}^{-1}$)

¹) and nitrate (NO_3^- ; $0.003 \mu\text{mol}\cdot\text{L}^{-1}$) concentrations. The seawater was pasteurized (80°C for 2 h) and salinity was adjusted to 29.5 to reflect the values measured at the NIOZ Seaweed Research Centre and around the island of Texel by mixing with ultrapure water (Milli-Q, Merck KGaA, Massachusetts, USA). Afterwards, potassium-dihydrogen-phosphate (KH_2PO_4) and potassium nitrate (KNO_3) were added as sources for DIP and DIN to create the desired DIP concentrations of 0.0, 0.2, 0.4, 0.8, 1.5, 3.0 and $6.0 \mu\text{mol}\cdot\text{L}^{-1}$ and DIN concentration of $50 \mu\text{mol}\cdot\text{L}^{-1}$. The pH of the medium, after pasteurization and DIN and DIP addition, was 8.1 ± 0.1 ($n=14$) as measured with a pH-Meter (GHM-3511, Greisinger, Germany).

Nutrient analysis

Dissolved inorganic nutrients (DIP and DIN) were measured with colorimetric analysis using a Technicon TRAACS 800 auto-analyzer (Seal Analytical, Germany) in the NIOZ Texel nutrient laboratory. DIP was measured as ortho-phosphate (PO_4^{3-}) at 880 nm after the formation of molybdophosphate complexes (Murphy and Riley 1962). For DIN measurements (nitrate and nitrite), nitrate was first reduced to nitrite through a copperized cadmium coil and color intensity was measured at 550 nm after complexation with sulphanylamide and naphthylethylenediamine (Grasshoff et al. 1983). Ammonium (NH_4^+) was measured at 630 nm after the formation of an indophenol blue complex with phenol and sodium hypochlorite at a pH of 10.5. Citrate was used as a buffer and complexant for calcium and magnesium at this pH (Koroleff 1969 and optimized by Helder and de Vries 1979). The low NH_4^+ -concentration ($0.022 \mu\text{mol}\cdot\text{L}^{-1}$) was not further considered, as no NH_4^+ was added in the experiments. The precision for all the measured channels within the automated nutrient analyzer was better than 0.25 % (personal communication K. Bakker, NIOZ).

DIP and DIN uptake dynamics

DIP and DIN uptake refers to the removal of these nutrients from the medium by *S. latissima* and *L. digitata*. Daily uptake rates (V) were derived from changes in the nutrient concentrations of the seawater medium each day, which were normalized for SA (cm²) and time (day) using the following calculation:

$$V = (T_1 - T_2) \times SA^{-1} \cdot t^{-1},$$

with T₁ as the initial nutrient concentration, T₂ as the nutrient concentration before water exchange after 24 h, SA as surface area (cm²) and t as the incubation time (hours).

Two different uptake rates were classified over time: surge uptake (V_S) after starvation and maintenance uptake with filled nutrient pools (V_M). V_S was calculated from uptake rates under conditions of non-limiting nutrient concentration using the following equation:

$$V_S = (V_2 - V_1) \times (d_2 - d_1)^{-1} = \Delta V \times \Delta d^{-1},$$

where V₁ and V₂ are daily uptake rates on days before a significant decline in uptake rate occurs and no significant variations in nutrient uptake follow. The difference operator between the two days is represented by d₁ and d₂. V_M is calculated as the average uptake rate under non-limiting nutrient concentration after a significant decrease has occurred and subsequent uptake rates show no significant variations.

Surface area analysis

Sporophytes of both species were individually spread flat on a white background, placed next to a ruler for scale, and covered with a transparent Plexiglas sheet to avoid folding of the frond. Photographs (using a Panasonic Lumix DMC-FT5) were taken on a weekly basis, enabling analysis of surface area (SA) by using the open source software ImageJ (ImageJ, U. S. National Institutes of Health, Maryland, USA). Photographs were converted into grayscale (type 8-bit) and transformed into a binary image before SA analysis. The obtained SA

represents one side of the frond. Differences in SA over time were used as indices of growth, with relative growth rates (μ) calculated according to Kain (1987) as follows:

$$\mu = (\ln SA_1 - \ln SA_2) \times t^{-1},$$

where SA_1 represents the initial surface area, and SA_2 represents the final surface area after incubation time t .

Experimental approach 2

In experimental approach 2, young sporophytes of *S. latissima* and *L. digitata* were placed inside 20 L bottles filled with 15 L DIP and DIN enriched seawater medium (DIP: 3 $\mu\text{mol}\cdot\text{L}^{-1}$, DIN: 50 $\mu\text{mol}\cdot\text{L}^{-1}$) for a 21-day adaptation phase under laboratory conditions. The nutrient-enriched seawater was renewed every other day to ensure saturated storage pigments after the adaptation phase. In experimental approach 2, individual sporophytes of *S. latissima* (n=20) and *L. digitata* (n=20) were transferred from the 20 L bottles into 500 ml glass jars filled with 200 ml seawater medium, which were either DIP and DIN enriched (DIP: 3 $\mu\text{mol}\cdot\text{L}^{-1}$, DIN: 50 $\mu\text{mol}\cdot\text{L}^{-1}$, n=5), DIP depleted and DIN enriched (DIP: 0 $\mu\text{mol}\cdot\text{L}^{-1}$, DIN: 50 $\mu\text{mol}\cdot\text{L}^{-1}$, n=5), DIP enriched and DIN depleted (DIP: 3 $\mu\text{mol}\cdot\text{L}^{-1}$, DIN: 0 $\mu\text{mol}\cdot\text{L}^{-1}$, n=5), or DIP and DIN depleted (n=5). The seawater media were refreshed on a daily basis throughout the experiment. Before refreshment of the seawater medium, fluorescence measurements (F_v/F_m) were conducted every other day (see section *Fluorescence measurements*), and after daily refreshment of the seawater medium, all jars were placed on a rotating table (100 rpm) to provide a moderate water movement, while a random distribution of the jars minimized differences in light availability.

Fluorescence measurements

Fluorescence measurements to determine photosynthetic efficiency (F_v/F_m , F_v refers to variable fluorescence and F_m refers to maximum fluorescence) were conducted every other

day over a period of 66 days for *S. latissima* (n=5) and 54 days for *L. digitata* (n=5) in all four treatments of experiment II. Sporophytes were dark-adapted for 20 minutes before photosynthetic efficiency was measured using a pulse-amplitude modulated fluorimeter (JUNIOR-PAM, Walz, Effeltrich, Germany; settings: measuring light intensity=10, pulse width=0.8s, gain=2) attached to a laptop. These measurements were carried out under minimum light conditions (laptop screen as the only light source) in a temperature-controlled room set to 12 °C around the same daytime. Each sporophyte was measured twice at different locations on the frond in an interval of 40 seconds.

Internal storage capacity

The internal storage capacities (ISC) for DIP and DIN were derived from the response of seaweed F_v/F_m when cultured in DIP- and DIN-depleted seawater. All, under the premise that internal storages for DIP and DIN had been filled during adaptation phase and the seaweed was not nutrient starved at the start of the experiment. Either DIN or DIP concentrations that were retrieved as non-limiting in previous experimental set-up I were pulsed for potentially optimal conditions. A control was installed, adding both nutrients, DIP and DIN concentrations. A significant decrease in F_v/F_m under limitation/depletion conditions was postulated to reflect a stress reaction by seaweeds to internal DIP and/or DIN depletion, as parameters, like temperature, light and hydrodynamics were fully controlled. As V_M is considered equal to the rate of assimilation (Taylor and Rees 1999, Barr et al. 2004), the ISC was calculated as follows:

$$ISC = \Delta d \cdot V_M,$$

where Δd represents the duration (days) with initially filled internal nutrient storages under depletion conditions, before a significant decrease in F_v/F_m occurred, and V_M represents the daily maintenance or metabolic uptake rate.

288 *Statistics*

289 Data of both experimental approaches were tested for normality with the Kolmogorov-
290 Smirnov test (KS test) for cumulative probability distribution. A two-sided repeated
291 measures ANOVA was applied to test for significant differences in growth, nutrient uptake
292 rates, and F_v/F_m within and between treatments with different nutrient concentrations.

293

294 **Results**

295 *Experimental approach 1*

296 *Surface area analysis*

297 The increase in SA, as a measure of growth of *S. latissima* and *L. digitata* sporophytes
298 displayed significant differences between DIP treatments over time (respectively ANOVA
299 $F(6, 3)=2.24$, $p=0.042$; $F(6, 5)=9.47$, $p<0.001$). The highest growth rates for *S. latissima* were
300 found in low to intermediate DIP treatments receiving nominal concentrations of $3.0 \mu\text{mol}\cdot\text{L}^{-1}$
301 or less, which were not significantly different in growth from each other (ANOVA $F(5,$
302 $3)=2.28$, $p=0.545$). Mean SA increased by the factor 1.84 ± 0.14 in 23 days ($n=42$, Figure 2),
303 representing a growth rate of $4\% \text{ d}^{-1}$. *S. latissima* cultured in high nominal DIP
304 concentrations of $6.0 \mu\text{mol}\cdot\text{L}^{-1}$ exhibited significantly lower growth compared with
305 sporophytes in other treatments (ANOVA $F(1, 6)=4.04$, $p=0.004$). Mean SA increased by the
306 factor 1.19 ± 0.21 in 9 days, before growth stagnated after 15 days and a negative growth with
307 signs of texture loss and disintegration of the sporophytes was observed on day 23, the final
308 measurement of SA ($n=7$, Figure 2).

309 *Laminaria digitata* showed the highest growth rates when exposed to intermediate nominal
310 DIP concentrations of $1.5 \mu\text{mol}\cdot\text{L}^{-1}$ and $3.0 \mu\text{mol}\cdot\text{L}^{-1}$, and there were no significant

differences in relative increase of SA among these treatments (ANOVA $F(1, 6)=0.46$, $p=0.502$). Mean SA increased by the factor 2.37 ± 0.08 in 35 days ($n=14$, Figure 2), exhibiting a growth rate similar to *S. latissima* in low to intermediate DIP treatments. Sporophytes cultivated under low nominal DIP conditions of $0.8\ \mu\text{mol}\cdot\text{L}^{-1}$ or less, showed a significantly smaller increase in SA (ANOVA $F(3, 6)=3.39$, $p<0.001$), which was comparable to *L. digitata* exposed to high nominal DIP concentration of $6.0\ \mu\text{mol}\cdot\text{L}^{-1}$ (ANOVA $F(1, 6)=11.1$, $p=0.001$). The relative increase in SA of sporophytes in these treatments increased by the factor 1.86 ± 0.05 , respectively 1.81 ± 0.05 , in 35 days ($n=28$, respectively $n=7$, Figure 2), which translates to a growth rate of $2\ \%\ \text{d}^{-1}$.

DIP-uptake dynamics

Sporophytes of *S. latissima* exposed to very low nominal DIP concentration of $0.2\ \mu\text{mol}\cdot\text{L}^{-1}$, $0.4\ \mu\text{mol}\cdot\text{L}^{-1}$ and $0.8\ \mu\text{mol}\cdot\text{L}^{-1}$ depleted all supplied DIP within the daily sampling period of 24 hours throughout the experiment, which indicates non-saturating DIP concentrations to the nutrient starved sporophytes (not depicted). When exposed to nominal DIP concentrations of $1.5\ \mu\text{mol}\cdot\text{L}^{-1}$, all supplied DIP was depleted until day 9, after which uptake significantly decreased (ANOVA $F(1, 6)=6.37$, $p=0.021$) and mean uptake rates levelled off from $0.30\pm0.03\ \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ to $0.22\pm0.01\ \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ until day 22 ($n=7$, Figure 3 A) with no significant variations (ANOVA $F(1, 12)=1.38$, $p=0.220$), indicating saturating DIP conditions. Similarly, but with uptake declining earlier, sporophytes grown in a nominal DIP concentration of $3.0\ \mu\text{mol}\cdot\text{L}^{-1}$ depleted all daily supplied DIP until day 4, followed by a significant decline of mean uptake rates (ANOVA $F(1, 6)=5.91$, $p=0.007$) from $0.80\pm0.03\ \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ to $0.40\pm0.04\ \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ on day 7 ($n=7$, Figure 3 B), after which no significant variations in DIP uptakes rates were found (ANOVA $F(1, 14)=1.29$, $p=0.226$). *S. latissima* exposed to a high nominal DIP concentration of $6.0\ \mu\text{mol}\cdot\text{L}^{-1}$ showed highly significant variations in DIP uptake within treatments (ANOVA $F(1, 6)=7.31$, $p<0.001$) and

over time (ANOVA $F(1, 21)=5.79$, $p<0.001$). Sporophytes depleted all supplied DIP on days 1 and 2 with a mean uptake rate of $1.66\pm0.10 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ ($n=7$), which was followed by a daily decline and final collapse of mean DIP uptake rates to $0.05\pm1.28 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ ($n=7$) on days 21 and 22 (Figure 3 C). At this point, 5 of 7 young sporophytes had lost their texture and started to disintegrate.

Based on DIP uptake rates of *S. latissima* under saturated states in nominal DIP concentrations of $1.5 \mu\text{mol}\cdot\text{L}^{-1}$ and $3.0 \mu\text{mol}\cdot\text{L}^{-1}$, the calculated $V_{\text{M-DIP}}$ was $0.30\pm0.09 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ ($n=14$).

V_{S} for DIP was calculated to be $0.80\pm0.03 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ (average \pm SD, $n=7$), which was based on DIP uptake rates of sporophytes exposed to a nominal concentration of $3.0 \mu\text{mol}\cdot\text{L}^{-1}$ on days 1 to 4. A maximum surge uptake rate was calculated to be $1.66\pm0.10 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ (average \pm SD, $n=7$), based on DIP uptake rates of the young sporophytes exposed to a nominal DIP concentration of $6.0 \mu\text{mol}\cdot\text{L}^{-1}$ on day 1. Sporophytes in this treatment disintegrated within 3 weeks and high uptake rates were referred to as a stress reaction to the unusually high DIP concentrations.

Sporophytes of *L. digitata* cultured in nominal DIP concentrations of $0.2 \mu\text{mol}\cdot\text{L}^{-1}$, $0.4 \mu\text{mol}\cdot\text{L}^{-1}$, $0.8 \mu\text{mol}\cdot\text{L}^{-1}$, $1.5 \mu\text{mol}\cdot\text{L}^{-1}$, and $3.0 \mu\text{mol}\cdot\text{L}^{-1}$ depleted all of the supplied DIP within the 24-hour sampling period throughout the experiment, which indicates non-saturating DIP concentrations (depicted for $3.0 \mu\text{mol}\cdot\text{L}^{-1}$, Figure 4 A). In contrast, mean DIP uptake of *L. digitata* cultured in a high nominal concentration of $6.0 \mu\text{mol}\cdot\text{L}^{-1}$ did not lead to depletion throughout the experiment, thus indicating a saturating concentration. Mean DIP uptake rates varied around $0.37\pm0.03 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ between day 1 and day 10 before a significant decrease occurred (ANOVA $F(1, 11)=8.50$, $p=0.013$). Within day 11 and 21, mean uptake rates stabilized at $0.24\pm0.04 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ ($n=7$, Figure 4 B). The $V_{\text{M-DIP}}$ of *L.*

digitata was calculated to be $0.22 \pm 0.01 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ (n=14), while $V_S\text{-DIP}$ was determined to be approximately twice as high as $V_M\text{-DIP}$ at $0.37 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ (average \pm SD, n=7).

DIN-uptake dynamics

Saccharina latissima showed no significant differences in daily DIN uptake rates among different DIP treatments (ANOVA $F(6, 6)=1.71$, $p=0.116$), but displayed a highly significant difference in uptake over time (ANOVA $F(21, 6)=5.35$, $p<0.000$). No correlation between DIN and DIP uptake ($R=0.415$) was found. The mean DIN uptake oscillated downwards from a high of $11.26 \pm 0.56 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ (n=49) on day 1, which represents the $V_S\text{-DIN}$ of *S. latissima*, to $5.46 \pm 0.77 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ (n=49) by day 14. After these two weeks, the DIN uptake stabilized around $4.07 \pm 0.82 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ without any significant variation (ANOVA $F(7, 6)=1.94$, $p=0.097$) until the end of the experiment on day 22 (Figure 5). A $V_M\text{-DIN}$ of $3.94 \pm 0.67 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ was conclusively calculated, which is approximately three times lower than $V_S\text{-DIN}$.

Laminaria digitata showed no significant differences in DIN uptake rates among different DIP treatments (ANOVA $F(6, 6)=1.21$, $p=0.306$), but exhibited a highly significant difference in DIN uptake over time (ANOVA $F(20, 6)=28.46$, $p<0.001$). Similar to *S. latissima*, no correlation between DIN and DIP uptake ($R=0.229$) was found. DIN uptake rates showed no significant variations within day 1 and 8 (ANOVA $F(7, 6)=0.27$, $p=0.897$) with a mean uptake of $3.72 \pm 0.56 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ (n=49, Figure 6). In correspondence, a $V_S\text{-DIN}$ of $3.92 \pm 0.08 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ (n=49) was calculated. A significant decrease in uptake rates was observed within day 9 and 14 (ANOVA $F(5, 6)=5.44$, $p=0.001$). After this, DIN uptake stabilized without significant variations (ANOVA $F(4, 6)=0.70$, $p=0.590$) at 1.81 ± 0.38

383 $\mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ between day 16 and 21 (Figure 6), which also represents $V_{\text{M-DIN}}$ for *L.*

384 *digitata*.

385 *Experimental approach 2*

386 Based on the results on DIP and DIN uptake kinetics for *S. latissima* and *L. digitata* and in
387 regard to saturating concentrations in experimental approach 1, nominal concentrations of 3.0
388 $\mu\text{mol}\cdot\text{L}^{-1}$ DIP and 50 $\mu\text{mol}\cdot\text{L}^{-1}$ DIN were chosen in experimental approach 2 for the control
389 group (n=5) to ensure non-limiting nutrient availability throughout the experiment without
390 inducing nutritional stress.

391 *Fluorescence measurements*

392 The mean photosynthetic efficiency (F_v/F_m) of *S. latissima* showed significant variations
393 between treatments (ANOVA $F(3, 4)=17.78$, $p<0.001$) and over time (ANOVA $F(33,$
394 $3)=5.09$, $p<0.001$). The control group exposed to treatments with DIP and DIN additions of 3
395 $\mu\text{mol}\cdot\text{L}^{-1}$ and 50 $\mu\text{mol}\cdot\text{L}^{-1}$, respectively, *S. latissima* expressed no significant differences in
396 mean F_v/F_m (ANOVA $F(1, 4)=0.18$, $p=0.686$), but displayed moderate fluctuations around a
397 F_v/F_m of 0.78 ± 0.04 (n=5) over 10 weeks (Figure 7 A). A comparable performance in F_v/F_m
398 was observed in treatments under DIP-depleted conditions, where no significant difference in
399 photosynthetic efficiency was found (ANOVA $F(1, 4)=3.58$, $p=0.095$) and mean F_v/F_m stayed
400 around 0.77 ± 0.04 throughout the experiment (n=5, Figure 7 B). When exposed to DIN-
401 depleted conditions, however, mean F_v/F_m significantly decreased (ANOVA $F(23, 4)=2.04$,
402 $p=0.007$) from 0.78 ± 0.04 to 0.70 ± 0.08 after 6 weeks (n=5, Figure 7 C) with no significant
403 variations thereafter (ANOVA $F(10, 4)=0.17$, $p=0.998$). Similarly, mean F_v/F_m of *S. latissima*
404 sporophytes exposed to total DIP and DIN depletion displayed no significant variations until
405 week 7 (ANOVA $F(22, 4)=1.35$, $p=0.177$), followed by a significant decrease (ANOVA $F(1,$
406 $4)=2.37$, $p=0.002$) from 0.77 ± 0.03 to 0.65 ± 0.16 during week 10 (n=5, Figure 7 D).

The photosynthetic efficiency of *L. digitata* also exhibited significant differences between different treatments (ANOVA $F(3, 4)=11.79$, $p<0.001$) and over time (ANOVA $F(22, 4)=5.26$, $p>0.001$). Sporophytes of the control group exposed to a DIP and DIN concentration of $3\ \mu\text{mol}\cdot\text{L}^{-1}$ and $50\ \mu\text{mol}\cdot\text{L}^{-1}$, respectively, showed no significant variations in F_v/F_m over time (ANOVA $F(22, 4)=0.77$, $p=0.406$) and the mean photosynthetic efficiency was 0.74 ± 0.06 throughout the experiment ($n=5$, Figure 7 E). However, photosynthetic efficiency was more sensitive to DIP and DIN depletion than in *S. latissima*. When exposed to DIP-depleted, DIN-depleted, and both DIP and DIN-depleted seawater medium, the mean F_v/F_m significantly decreased after 6 weeks (ANOVA DIP-depleted: $F(22, 4)=2.41$, $p=0.025$; DIN-depleted: $F(22, 4)=8.51$, $p=0.043$; DIP/DIN-depleted: $F(22, 4)=2.40$, $p<0.001$). Under DIP depletion, the mean photosynthetic efficiency dropped from 0.73 ± 0.05 to 0.50 ± 0.20 during week 8 ($n=5$, Figure 7 F), while mean F_v/F_m under DIN depletion decreased comparably from 0.67 ± 0.10 to 0.53 ± 0.25 during week 8 ($n=5$, Figure 7 G). *L. digitata* sporophytes under DIP and DIN depletion displayed a moderate decrease in mean F_v/F_m from 0.67 ± 0.07 to 0.55 ± 0.15 between week 3 and 6, after which the mean photosynthetic efficiency significantly dropped to 0.34 ± 0.24 during week 8 ($n=5$, Figure 7 H).

Internal storage capacity

Using the fluorescence measurements and duration time, before a significant decrease in F_v/F_m in different treatments occurred and the daily DIP and DIN uptake rates under V_M , we calculated an internal storage capacity (ISC) of $10\ \mu\text{mol}\cdot\text{cm}^{-2}$ ($n=7$) for DIP and $80\ \mu\text{mol}\cdot\text{cm}^{-2}$ ($n=49$) for DIN in *L. digitata*. An ISC-DIN of $160\ \mu\text{mol}\cdot\text{cm}^{-2}$ ($n=49$) was calculated for *S. latissima*, while ISC-DIP could not be calculated from the experimental data collected on this species as no indication of phosphorus nutritional stress was exhibited and no significant decrease in mean F_v/F_m was observed over 66 days (Figure 7 B). However, based on the DIP requirements according to V_M over 66 days and consistent with a DIP:DIN uptake ratio of 1:6

under steady state conditions, we estimated an ISC-DIP of $27 \mu\text{mol}\cdot\text{cm}^{-2}$ (n=14) for *S. latissima*.

Discussion

The growth, productivity and geographical distribution of seaweeds are controlled by environmental factors, such as temperature, irradiance, water movement and nutrient availability. Seasonal fluctuations in nutrient availabilities can also reflect differences in the seasonal growth patterns of seaweeds (Gagne et al. 1982, Zimmerman and Kremer 1986), as for *S. latissima* and *L. digitata* (Conolly and Drew 1985). This study adds to the physiological understanding of dissolved inorganic P and N uptake in *S. latissima* and *L. digitata*, which in turn enables estimation of ecological effects on nutrient availability.

Uptake kinetics are usually expressed as functions of either fresh weight (FW), dry weight (DW) or surface area to volume (SA:Vol), which makes it difficult to compare data. Furthermore, uptake kinetics expressed as a function of dry weight (DW) necessitates destructive sampling through harvesting living biomass. Standardized determination by fresh weight (FW) is even more problematic as small variations in the amount of water attached to the living (and growing) seaweed can lead to large differences in its measured weight, not only between different samples and over time, but also amongst different experimenters. As seaweeds take up nutrients throughout their whole frond, the SA represents a reasonable function to determine uptake kinetics. Standardization of uptake kinetics by SA would allow for intra- and interspecific comparisons over time, for example with observations on the green seaweed *Ulva lactuca* Linneaus by Lubsch and Timmermans (2018). Moreover, phenotypic plasticity of seaweed strongly depends on predominant hydrodynamics of the site (Gerard 1987, Demes et al. 2011), but can also be affected by biotic stress (Molis et al. 2015),

which can make comparison of functions of SA:Vol troublesome. Comparisons between uptake kinetics, such as V_s , V_M , and ISC, of different seaweed species would allow for general insights into seaweed survival and competition in natural environments. It is also an important aspect in scaling up operations to levels of commercial viability, as it enables to estimate the carrying capacity of a cultivation site in regard to nutrient availability and nutrient demand of cultivated species, as well as it allows to adjust duration and quantity of potential nutrient additions according to size (seaweed $\text{SA} \cdot \text{m}^{-2}$) and growth of the operation.

Our results on growth rates for both, *S. latissima* and *L. digitata*, with only a slightly sub-optimal increase of SA observed under non-saturating external DIP conditions within the first 28 days of the first experimental approach, suggests that previously filled internal phosphate storages were utilized during the experiments and were able to compensate for external DIP deficiency (after Probyn and Chapman 1982, Pederson and Borum 1996). This is supported by the reduced, but continuing growth of both species when exposed to DIP-depleted seawater, which clearly indicates that internal phosphate storages had not been depleted after two weeks of starvation during the adaptation phase. The mean growth rates of 4 % d^{-1} under optimal DIP conditions for *S. latissima* and *L. digitata* are within the reported growth rates for both species from the North Sea area (*S. latissima*, Nielsen et al. 2014, Bolderskov et al. 2015; *L. digitata*, Gomez and Lüning 2001). The reduced growth of *L. digitata* exposed to DIP concentrations of $6.0 \mu\text{mol} \cdot \text{L}^{-1}$, which are above the optimal levels, has also been observed for *Ulva lactuca* by Waite and Mitchell (1972) and Steffensen (1976). These studies showed that phosphate concentrations above $4.76 \mu\text{mol} \cdot \text{L}^{-1}$ had negative effects on growth rates for the green seaweed. Similarly, the daily pulsing of high DIP concentrations had a fatal effect on *S. latissima*.

The texture loss and disintegration of juvenile *S. latissima* sporophytes exposed to nominal DIP concentration of $6.0 \mu\text{mol} \cdot \text{L}^{-1}$ within 3 weeks of exposure could have been caused by

epiphytic bacteria. In many cases the level of bacterial populations found on seaweed surfaces depend on the species, thallus section and season (Armstrong et al. 2000, Bengtsson et al. 2010). One cause of a seasonal shift in marine and epiphytic bacterial communities may be a change in external conditions or physical and chemical parameters, such as nutrient stoichiometry and availability. However, studies have demonstrated the ability of certain marine bacteria to degrade various seaweed polymers (Goecke et al. 2010), thus leading to fouling and disintegration of the seaweed. However, *S. latissima* sporophytes only started to disintegrate after 3 weeks of exposure to nominal DIP concentration of $6.0 \mu\text{mol}\cdot\text{L}^{-1}$, and the daily uptake rates within the first week showed the ability of juvenile sporophytes to manage pulses of high DIP concentration for a short time. This ability could also be altered when stress reactions to high external nutrient concentration are initiated (e.g. Fourcroy 1999, Jiang and Yu-Feng 2008), allowing for mobilization and uptake of sufficient DIP to provide temporary relief.

Two different phases of transient responses to nutrient pulses, an initial surge uptake rate (V_S) after starvation and a maintenance or steady state uptake rate (V_M), which is considered equal to the rate of assimilation (Taylor and Rees 1999, Barr et al. 2004) were clearly seen for DIP and DIN-uptake in *S. latissima* and *L. digitata* in our first experimental approach. Depending on total DIP availability, V_S -DIP in *S. latissima* was maintained until the internal storages had been filled and uptake rates gradually decreased to V_M -DIP levels. This is supported by a significant decrease of DIP uptake found in the treatments with saturating nominal DIP concentrations of $1.5 \mu\text{mol}\cdot\text{L}^{-1}$ and $3.0 \mu\text{mol}\cdot\text{L}^{-1}$ on day 9 and day 4, respectively. Similar uptake characteristics were found for *L. digitata* exposed to a nominal DIP concentration of $6.0 \mu\text{mol}\cdot\text{L}^{-1}$; thus, similarly starved *L. digitata* exposed to a nominal DIP concentration twice as high may lead to a shift in uptake rates from V_S to V_M in approximately half the time. This time-shifted phenomenon has also been described for DIP

uptake in the green seaweed *Ulva lactuca* (Lubsch and Timmermans 2018) and corroborates evidence that the filling of internal nutrient pools is uncoupled from growth (Conway et al. 1976, Chapman et al. 1978). The high DIP uptake rates of *S. latissima* exposed to nominal concentrations of $6.0 \mu\text{mol}\cdot\text{L}^{-1}$ within the first 2 days, although referred to as a stress reaction allowing for temporary relief, shows the ability of *S. latissima* to “handle” high DIP concentrations for a short time indicated by a stagnation of growth after 9 days, and in turn the calculated V_s -DIP observed from sporophytes in $3.0 \mu\text{mol}\cdot\text{L}^{-1}$ within 4 days might have been underestimated. It should be mentioned that the daily offered nominal concentration of $6.0 \mu\text{mol}\cdot\text{L}^{-1}$ would correspond to an initial daily DIP availability of approximately $1.6 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ for *S. latissima* and $0.4 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ for *L. digitata*, depending on the SA of the sporophytes. Therefore, the nominal concentration does not represent a fully comparable measure between these species.

DIN uptake rates of *S. latissima* and *L. digitata* under fully saturating DIN conditions followed the same response as DIP uptake rates under saturating DIP conditions. V_M -DIN was attained when internal DIN pools had been filled. In regard to the filling of internal DIN pools, *S. latissima* showed a V_s three times higher than its V_M for DIN, as well as V_s for DIN in *L. digitata*. These values are comparable to the V_s and V_M for DIN, respectively $12.5\pm 5.2 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ and $2.3\pm 0.9 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$, found in the green seaweed *U. lactuca* (Table 1; Lubsch and Timmermans 2018). Nutrient uptake rates can also vary according to the seaweeds age, as uptake of nitrate in first-year plants of *Laminaria groenlandica* Rosenvinge was three times higher than in second- and in third year plants (Harrison et al. 1986).

The oscillatory decrease of DIN uptake in *S. latissima* during V_s suggests that DIN uptake was limited by internal aspects, such as the physical transfer of nutrients to inner tissue and/or enzymatic activity by feedback-controlled processes at the molecular level. Evidence shows that cellular processes are intrinsically rhythmic and follow a circadian metabolic

timekeeping. Although the molecular basis of circadian rhythms in seaweed is poorly understood, many circadian rhythms have been described for microalgae (Wijnen and Young 2006). For example, the green single-cell alga *Chlamydomonas reinhardtii* shows maximal daily uptake of DIN at dawn and maximal nitrate reductase activity around midday (Pajuelo et al. 1995). This rhythmic expression aids in the synchronization of mutually coupled dynamical systems (Progronsky and Nijmeijer 1998). Furthermore, it has been demonstrated that species diversity could be enhanced by different temporal nutrient uptake pattern in micro-algae and even under limitation conditions a coexistence was possible (Ahn et al. 2002).

Survival and growth of perennials also depends on the duration of internal nutrient storages to overcome seasonal minima in nutrient availability. In experimental approach 2, the photosynthetic efficiency (F_v/F_m) was measured as an indication to nutrient stress. F_v/F_m of various seaweeds has often been applied to indicate stress resulting from desiccation (Varela et al. 2006, Schagerl and Möstl 2011, Flores-Molina et al. 2014), photo-period (Magnusson 1997) or light-intensity (Hanelt et al. 1997, Gevaert et al. 2002). The use of F_v/F_m as an indication of nutrient-related stress in the marine sector has been more common in microalgae (Kromkamp and Peene 1999) and corals (Wiedenmann et al. 2012). F_v/F_m values in the range of 0.79 to 0.84 are considered optimal for many plants, while values significantly below are considered to indicate stress (Kitajima and Butler 1975, Maxwell and Johnson 2000). Accordingly, *S. latissima* and *L. digitata* first indicated nutritional stress by a significant decrease in F_v/F_m after 9, respectively 7 weeks of exposure to DIP- and DIN-depleted seawater. This decrease in F_v/F_m can be inferred to indicate depletion of internal storage pools of DIP and/or DIN, as abiotic parameters like light, temperature, and hydrodynamics were kept constant during the pulse-and-chase approach and in relation to the control.

556 The inferred internal storage capacity (ISC) for DIP and DIN in *S. latissima* and *L. digitata*,
557 derived from uptake kinetics (experimental approach 1) and the observed photosynthetic
558 efficiency F_v/F_m (experimental approach 2) are realistic for perennial seaweeds like *S.*
559 *latissima* and *L. digitata*, which are considered K-strategists that seasonally store reserves.
560 The reserve ratio of the ISC for DIN and DIP of 6:1 in *S. latissima* and 8:1 in *L. digitata*,
561 compared to V_M , the rate of assimilation with DIN and DIP uptake ratios of approximately
562 13:1 for *S. latissima* and 8:1 for *L. digitata*, suggests that *S. latissima* is twice as likely to fall
563 under N limitation than P limitation, while in *L. digitata* the storage ratio is equivalent to V_M .
564 A pattern that was reflected by our results on F_v/F_m , which showed a decrease in
565 photosynthetic efficiency to DIN and/or DIP depletion conditions at the same time in *L.*
566 *digitata*, while *S. latissima* exhibited a decrease in F_v/F_m to DIN depletion, but none to DIP
567 depletion, after 6 weeks of exposure. The high demand for DIN (and DIP) in *S. latissima* was
568 also reflected by its high uptake rates under V_s (Table 1), which is comparable to the V_s for
569 DIN and DIP in the opportunistic green seaweed *U. lactuca* (Lubsch and Timmermans 2018),
570 which is considered a promising seaweed for biofiltration purposes (Neori et al. 2003).
571 Unlike *U. lactuca*, which flourishes at relatively high temperatures and light intensities, *S.*
572 *latissima* can be regarded as a winter species, and this could allow for crop rotation in
573 mariculture. Our data provides evidence that *S. latissima* is an effective candidate for
574 bioremediation, for example in close proximity to marine fish farms, potentially able to
575 balance nutrient loads from fish cages, while a relatively fast growth provides valuable
576 biomass at the same time (Reid et al. 2013, Handå et al. 2013, Freitas et al. 2015).

577 Based on our results on DIP and DIN uptake kinetics and calculated ISC, *S. latissima* is
578 predicted to outcompete *L. digitata* in the struggle for nutrients, despite similar spatial and
579 temporal distribution. As mentioned before, multiple environmental factors regulate
580 geographical distribution, and there is no available information about sporophyte recruitment

strategies and the intra- and interspecific competitiveness of gametophytes of *S. latissima* and *L. digitata*. Reed (1990) showed that intra- and interspecific competition was more intense when settlement of gametophytes of *Macrocystis pyrifera* and *Pterygophora californica* were at high densities, but not at low densities. On the other hand, no evidence of competition was found among gametophytes of *Nereocystis luetkeana* (Vadas 1972). Delaying development can also ameliorate the negative effects of intra- and interspecific competition among seaweed gametophytes (Carney and Edwards 2010).

Our standardized data add to the physiological understanding of *S. latissima* and *L. digitata* and can contribute to the development and modification of applications in a bio-based economy, such as in integrated multi-trophic aquacultures (IMTA). Likewise, the obtained physiological data can help to identify potential locations for commercial cultivation and facilitates predicting yields of seaweed biomass in different locations under different environmental conditions using various models (Broch and Slagstad 2012, Van der Molen et al. 2018). These are important applications, as the interest in industrialisation of seaweed culture has increased in Europe throughout the last decades (Holdt and Kraan 2011, Wijesinghe and Jeon 2012).

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Table 1:

	DIP			DIN		
	V_s	V_M	ISC	V_s	V_M	ISC
	$\mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$	$\mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$	$\mu\text{mol}\cdot\text{cm}^{-2}$	$\mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$	$\mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$	$\mu\text{mol}\cdot\text{cm}^{-2}$
<i>S. latissima</i>	0.80±0.03	0.30±0.09	27*	11.26±0.56	3.94±0.67	160*
n	7	14	14	49	49	49
<i>L. digitata</i>	0.38±0.03	0.22±0.01	10*	3.92±0.08	1.81±0.38	80*
n	7	14	7	49	49	49
<i>U. lactuca</i>**	0.66±0.12	0.07±0.04	0.7±0.1	12.5±5.2	2.3±0.9	23±7
n	3	6	9	24	24	24

*approximation based on V_M, DIP:DIN uptake ratio, and photosynthetic efficiency F_v/F_m over time

**data derived from Lubsch and Timmermans (2018)

Figure legends and Table captions:

Table 1. Calculated dissolved inorganic phosphate (DIP) and dissolved inorganic nitrate (DIN) surge uptake rates (V_s), metabolic uptake rates (V_M), and internal storage capacity (ISC) of *Saccharina latissima*, *Laminaria digitata* and *Ulva lactuca*.

Figure 1. Size range of initial surface area (cm²) of *Saccharina latissima* and *Laminaria digitata* sporophytes applied in experiments on nutrient uptake kinetics (experimental approach 1).

Figure 2. Mean growth \pm SD ($\text{cm} \cdot \text{cm}^{-2}$) of young *Saccharina latissima* and *Laminaria digitata* cultivated in different DIP concentration (0 - 6 $\mu\text{mol} \cdot \text{L}^{-1}$, $n=7$) and saturating DIN concentration (50 $\mu\text{mol} \cdot \text{L}^{-1}$) in a 'pulse-and-chase' assay over 5 weeks. Data is depicted according to significant differences in increase of surface area (growth) of the sporophytes in different DIP concentrations.

Figure 3. Mean DIP uptake ($\mu\text{mol} \cdot \text{L}^{-1}$) \pm SD of young *Saccharina latissima* ($n=7$) in saturating nominal DIP concentrations of (A) 1.5 $\mu\text{mol} \cdot \text{L}^{-1}$, (B) 3.0 $\mu\text{mol} \cdot \text{L}^{-1}$ and (C) 6.0 $\mu\text{mol} \cdot \text{L}^{-1}$ and corresponding standardized daily DIP uptake ($\mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$) in a 'pulse-and-chase' assay over 3 weeks.

Figure 4. Mean DIP uptake ($\mu\text{mol} \cdot \text{L}^{-1}$) \pm SD of young *Laminaria digitata* ($n=7$) in (A) unsaturating nominal DIP concentration of 3.0 $\mu\text{mol} \cdot \text{L}^{-1}$ and (B) saturating nominal DIP concentration of 6.0 $\mu\text{mol} \cdot \text{L}^{-1}$ and corresponding standardized daily DIP uptake ($\mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$) in a 'pulse-and-chase' assay over 3 weeks.

Figure 5. Mean DIN uptake ($\mu\text{mol} \cdot \text{L}^{-1}$) \pm SD of young *Saccharina latissima* ($n=49$) cultivated in nominal DIN concentration of 50 $\mu\text{mol} \cdot \text{L}^{-1}$ and corresponding standardized daily DIN uptake ($\mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$) in a 'pulse-and-chase' assay over 3 weeks.

Figure 6. Mean DIN uptake ($\mu\text{mol} \cdot \text{L}^{-1}$) \pm SD of young *Laminaria digitata* ($n=49$) cultivated in nominal DIN concentration of 50 $\mu\text{mol} \cdot \text{L}^{-1}$ and corresponding standardized daily DIN uptake ($\mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$) in a 'pulse-and-chase' assay over 3 weeks.

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862 **Figure 7.** Photosynthetic efficiency F_v/F_m of *Saccharina latissima* (A – D, n=5) and
863 *Laminaria digitata* (E – H, n=5) cultivated in DIP/DIN-enriched (A and E), DIP-depleted (B
864 and F), DIN-depleted (C and G), and DIP/DIN-depleted (D and H) seawater medium in a
865 ‘pulse-and-chase’ assay over 10 weeks, respectively 8 weeks.

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867 Shortened/running title (50/50 characters):

868 DIP and DIN uptake in *S. latissima* and *L. digitata*

869

870 List of abbreviations:

ATP	Adenosine triphosphate
°C	Degree Celsius
DIN	Dissolved inorganic nitrate
DIP	Dissolved inorganic phosphate
DW	Dry weight
d	Day
e.g.	Exempli gratia (‘for example’)
F_v/F_m	F_v refers to variable fluorescence; F_m refers to maximum fluorescence
FW	Fresh weight
h	Hour
IMTA	Integrated multi-trophic aquaculture
ISC	Internal storage capacity
i.e.	Id est (‘in other words’)
L	Liter
ln	Natural logarithm

μ	Relative growth rate
N	Nitrogen
NE	North East
NIOZ	Royal Netherlands Institute for Sea Research
P	Phosphorus
PAM (fluorometry)	Pulse-amplitude-modulation (fluorometry)
SA	Surface area
T	Nutrient concentration
t	Time
Vol	Volume
V	Uptake rate
V_e	Externally controlled uptake
V_M	Maintenance uptake rate
V_S	Surge uptake rate

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882 **Figure 1:**

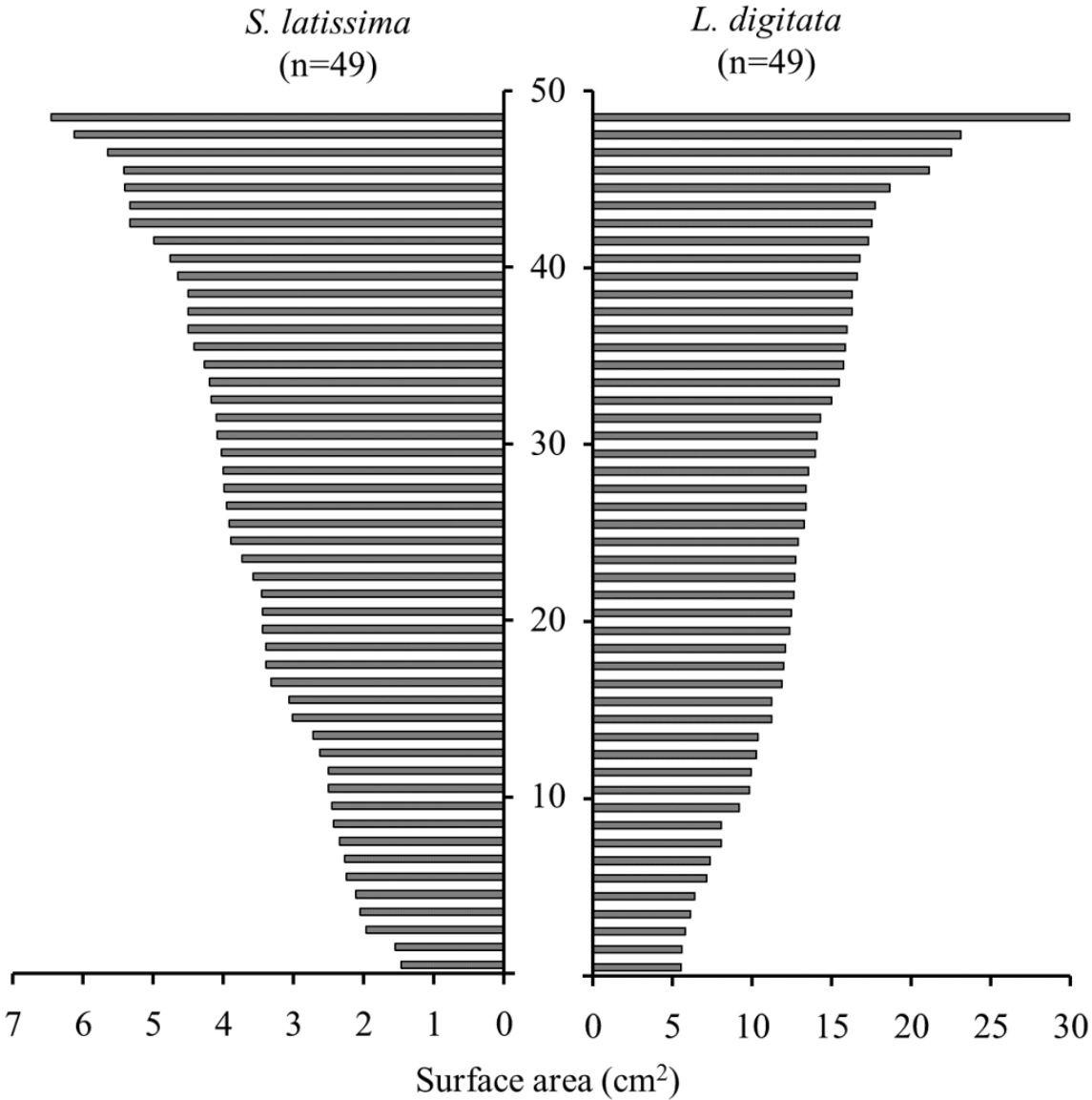
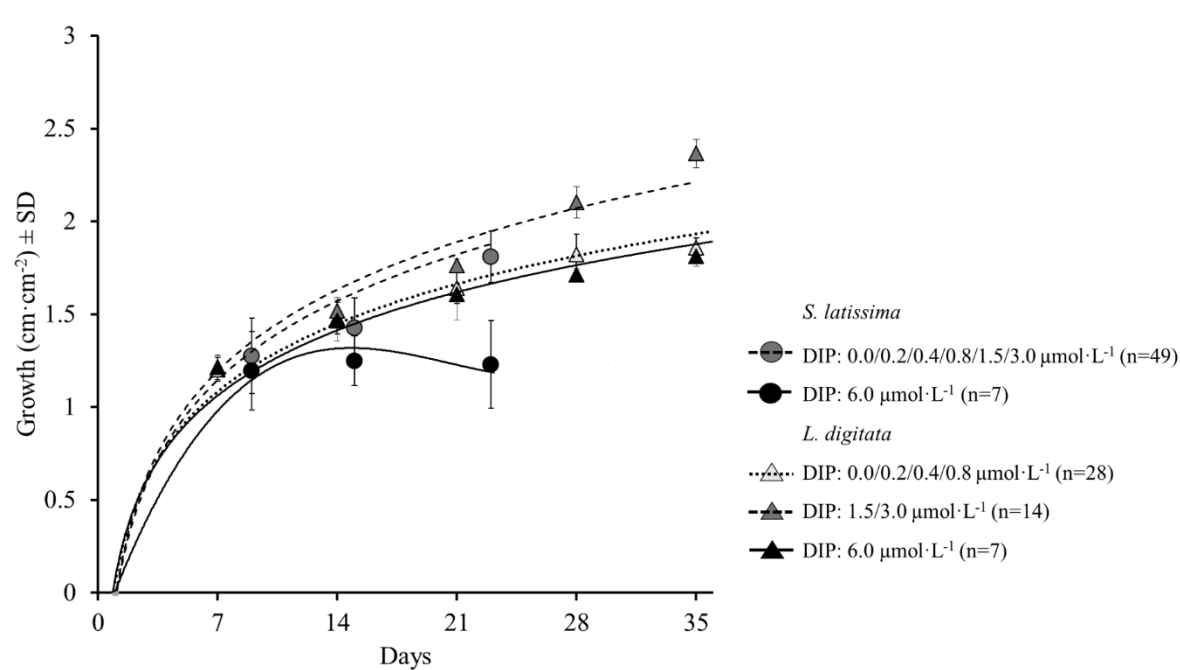
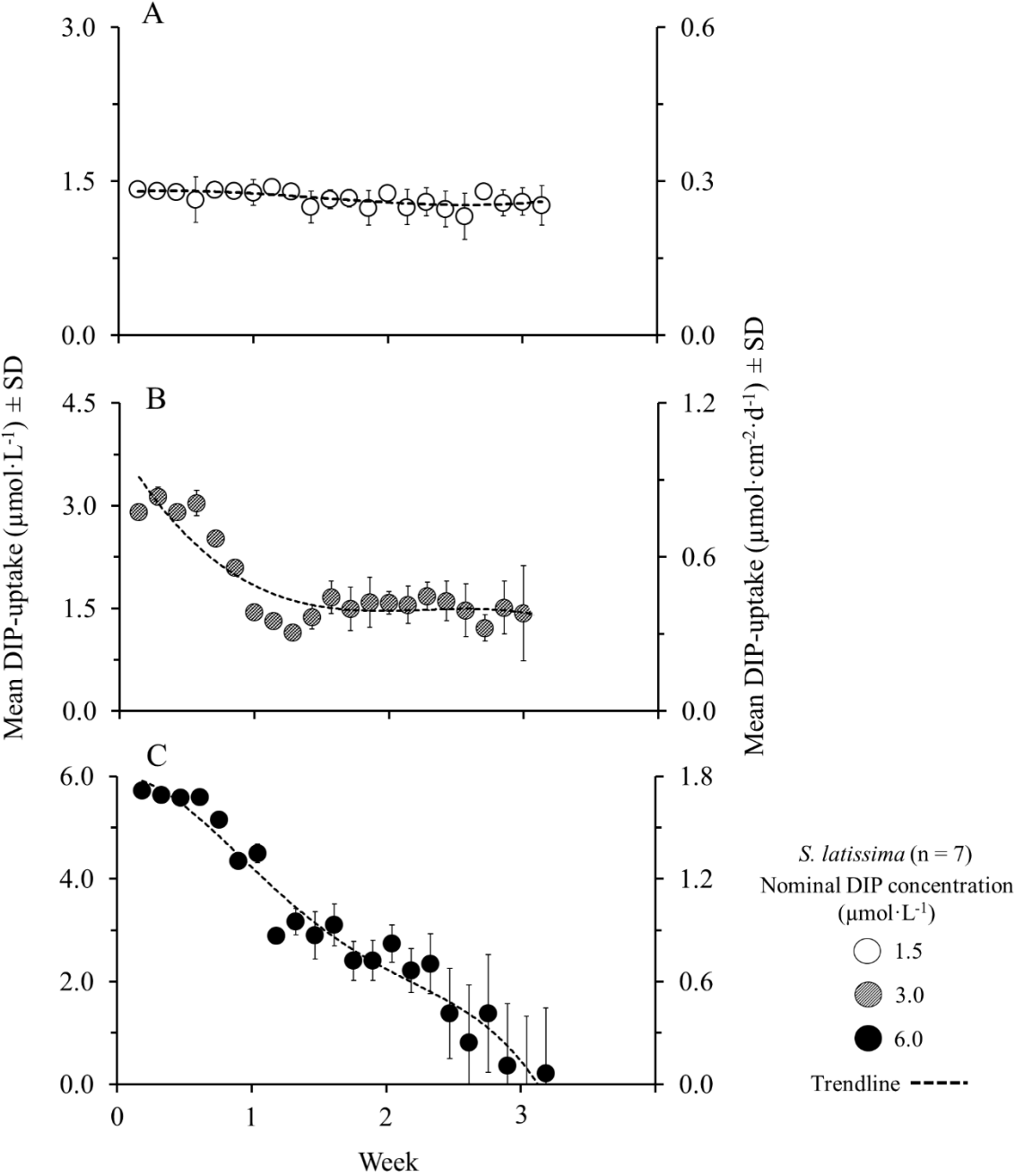


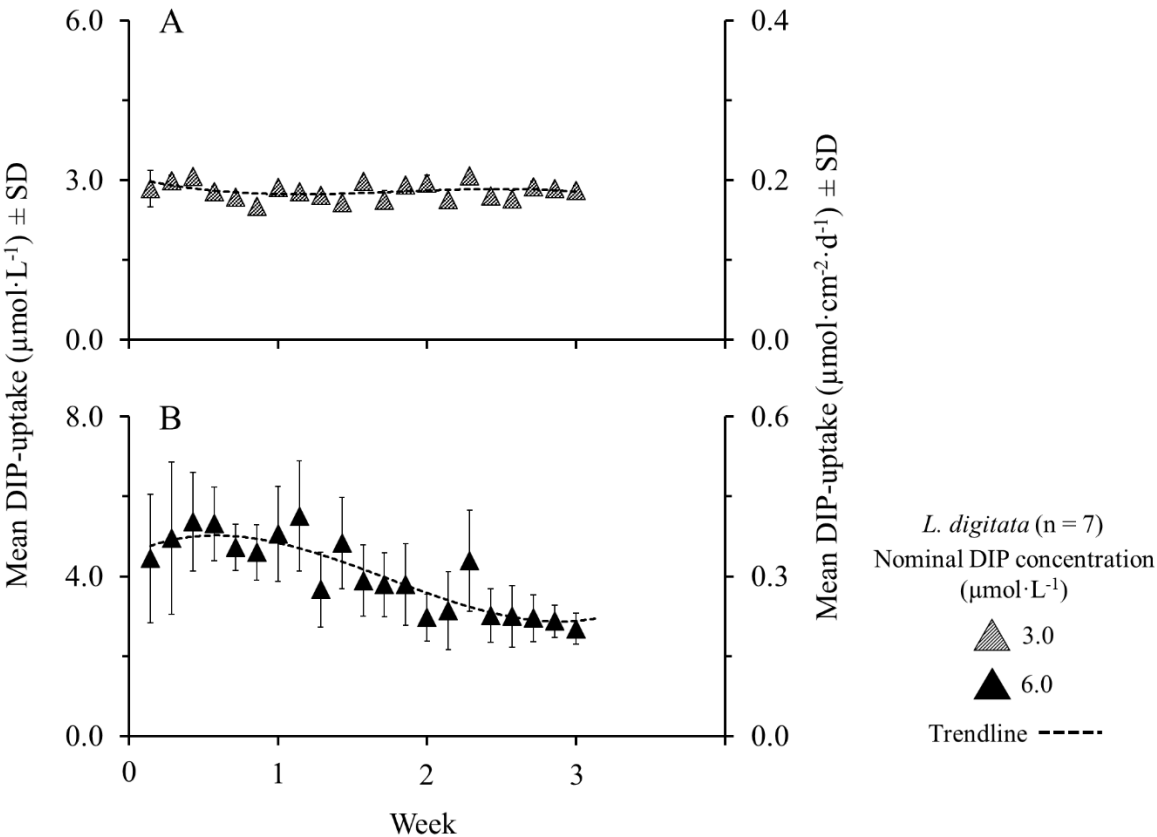
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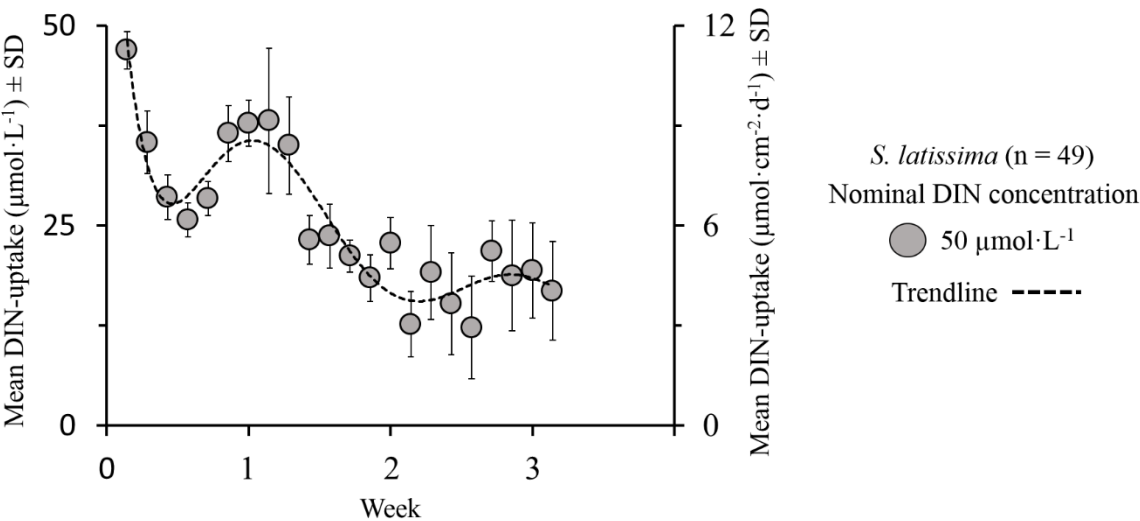
902 **Figure 3:**



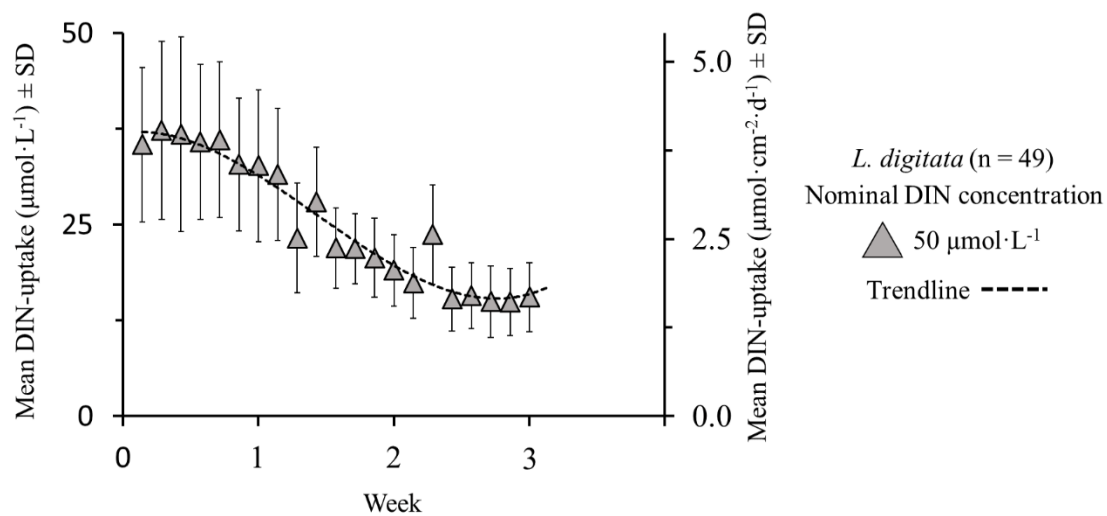
907 **Figure 4:**



909 **Figure 5:**

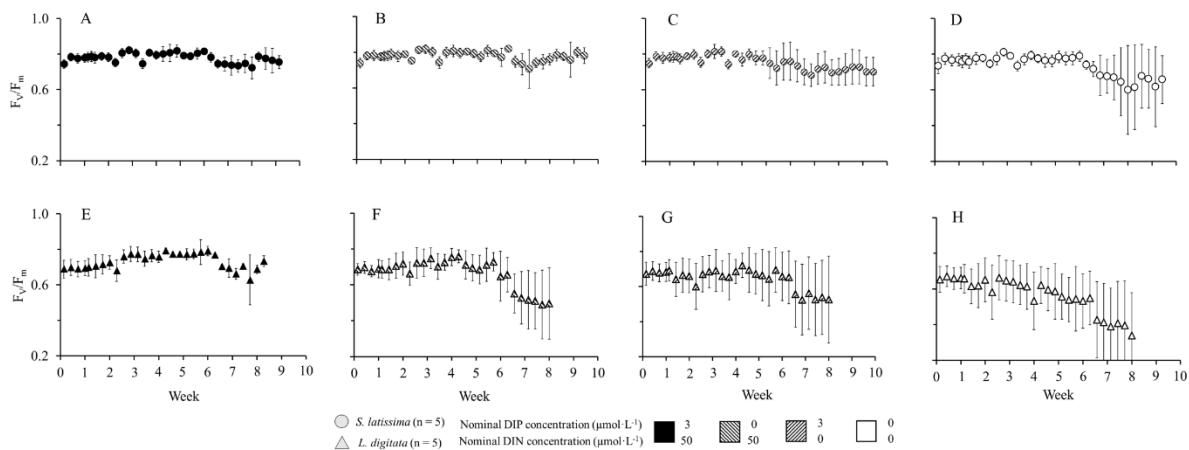


912 **Figure 6:**



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914 **Figure 7:**



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