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PHOSPHATE AND NITRATE UPTAKE DYNAMICS IN *PALMARIA PALMATA* (RHODOPHYCEAE): ECOLOGICAL AND PHYSIOLOGICAL ASPECTS OF NUTRIENT AVAILABILITY[1]

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Uptake dynamics of dissolved inorganic phosphate (DIP) and dissolved inorganic nitrate (DIN) in young *Palmaria palmata* ($n = 49$), cultivated in a range of DIP concentrations (0.0–6.0 µmol · L^{-1}) and nonlimiting DIN concentration (50 µmol · L^{-1}) under fully controlled laboratory conditions, were quantified in a 'pulse-and-chase' approach over 5 weeks. Two different uptake rates were specified: (1) surge uptake (V_S) after starvation and (2) maintenance uptake with filled nutrient pools (V_M). V_S for DIP of 1.57 ± 0.29 µmol · cm^{-2} · d^{-1} and DIN of 15.6 ± 4.3 µmol · cm^{-2} · d^{-1}, as well as V_M for DIP of 0.57 ± 0.22 µmol · cm^{-2} · d^{-1} and DIN of 5.6 ± 2.1 µmol · cm^{-2} · d^{-1} were calculated. In addition, an absolute size of the internal storage capacity (ISC) for DIP of 22 µmol · cm^2 and DIN of 222 µmol · cm^2 was determined. A DIP-to-DIN uptake ratio of 1:10 under V_M showed a weekly rhythmic uptake pattern, highlighted by a high correlation between DIP and DIN uptake ($R = 0.943$). V_S for DIN did not occur under DIP depletion, but uptake rates increased with increasing DIP availability. Hence, DIP availability limited access to DIN, which was also reflected by total dissolvable protein concentrations in sporophytes, which ranged from 10.2 ± 2.5% to 24.6 ± 8.0% dry weight depending on DIP availability. Similarly, total dissolvable carbohydrate concentration ranged from 22.1 ± 3.6% to 54.3 ± 12.3% dry weight. The data presented in this study open further insight into ecological and physiological aspects of nutrient availability in *P. palmata* and allow for an optimization in cultivation.

Key index words: **carbohydrate content; circadian rhythm; coupled uptake; nitrate uptake; *Palmaria palmata*; phosphate uptake; protein content; rhythmic uptake; seaweed; uptake dynamics**

***Abbreviations*: BSA, Bovine serum albumin; F_v/F_m, F_v refers to variable fluorescence F_m refers to maximum fluorescence; ISC, Internal storage capacity; K_S, Half-saturation constant from Michaelis-Menten model; NIOZ, Royal Netherlands Institute for Sea Research; T, Nutrient concentration; V_D, Uptake rate; V_M, Maintenance uptake rate; V_S, Surge uptake rate**

Dissolved inorganic phosphorus (DIP) and dissolved inorganic nitrogen (DIN) are two of the most important macronutrients in the metabolism and growth of seaweeds. A nutrient limitation can significantly affect growth, physiology, reproduction, and internal composition of seaweeds and thus can affect the nutritional value, as well as render their spatial and temporal distribution (Lobban and Harrison 1994, Pederson and Borum 1996). Thus, resource availability is a key element for survival of species in any given environment and hence drives the outcome of biological interactions, shaping community composition and structure (Chapin et al., 2000). It has been demonstrated that species diversity in microalgae was enhanced by temporal stratification of nutrient uptake, resulting in oscillating or rhythmic pattern, and even under limitation conditions a coexistence was possible with this strategy (Ahn et al. 2002). The general nutrient uptake mechanisms in seaweeds are basically known (e.g., Lüning 1992, Lobban and Harrison 1994, Harrison and Hurd 2001). However, there is a paucity of information on nutrient uptake pattern in seaweeds, particularly on phosphate uptake and its relationship to nitrogen utilization. New approaches are needed to fully understand nutrient uptake dynamics in seaweeds in order to gain knowledge on effects of nutrient limitation and shifts in limitation

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from one element to another. This can also contribute to economical endeavors, as it allows to identify potential locations for mariculture and enables optimization in cultivation. The demand of seaweed products, for example, alginates or carrageenan, have been increasing globally during the last decades (Bixler and Porse 2011, Porse and Rudolph 2017). Edible seaweeds are on the verve to enter the market for human food in the western hemisphere, as they are marketed as super-food with high values of various minerals, vitamins, carbohydrates, proteins, and a low fat content (e.g., McHugh 2003, Troell et al. 2006, Holdt and Kraan 2011).

The red alga *Palmaria palmata* is a temperate seawater species, which can be found in the intertidal zone along the North Atlantic Ocean. Due to its nutritional value with protein levels higher than soybeans (Morgan et al. 1980, Arasaki and Arasaki 1983, Galland-Irmoulli et al. 1999) and with its distinctive umami flavor (when dried, roasted, or fried), *P. palmata* is considered a novel and tasty marine vegetable. With an increasing interest in novel and functional foods and the successful commercialization of *P. palmata* for feed in aquaculture, for example, in abalone farms (Evans and Langdon 2000, Rosen et al. 2000), the natural resources of *P. palmata* have become short in supply. As a result, studies on the development of cultivation methods have been performed thoroughly to understand the life cycle and hence control reproduction (Van der Meer and Todd 1980, Wikfors and Ohno 2001, Grote 2019). A few studies have aimed at the yield and effectiveness of bioremediation by *P. palmata* in pilot-scale offshore cultivation, for example, in the vicinity of fish farms (Sanderson et al. 2012), as well as in land-based tank systems (Gall et al. 2004, Pang and Lüning 2004, Corey et al. 2014, Grote 2016, Tremblay-Gratton et al. 2018). A majority of these studies have focused on yield and the efficiency to remove nitrogenous compounds like ammonium (NH_4^+) and nitrate (NO_3^-) from the water column. Less attention has been paid to phosphate uptake and the potential of colimitation between nutrients with one limiting nutrient hindering uptake of a second nutrient (Harpole et al. 2011), which has been observed in many microalgae (e.g., Rhee 1974, D'Elia and DeBoer 1978, Haines and Wheeler 1978). Martínez and Rico (2004) demonstrated a biphasic nutrient uptake for DIP and DIN in *P. palmata* by incubating sporophytes in various DIN and DIP concentrations and following the uptake rates over ~6 h. A biphasic nutrient uptake has often been described for nutrient-starved seaweeds (e.g., Fujita 1985, Dy and Yap 2001), including a surge uptake (V_S), which refers to the filling of internal nutrient pools, uncoupled from growth, and an internally or metabolic uptake (V_M), which is considered equal to the rate of assimilation (Taylor and Rees 1999, Barr et al. 2004). Little focus has been rewarded given to the absolute size and, thus (in combination with daily requirements), the time internal nutrient pools or internal storage capacity (ISC) would be sufficient to overcome seasonal minima in nutrient availability without significant forfeit to growth (Fujita 1985, Pederson and Borum 1996, 1997, Pedersen et al. 2010). Perennial seaweeds, like *P. palmata*, rely on stored N and P, which are gained during autumn and winter, when nutrient availability is high, and benefit from this internal storage during spring and summer with increased day length, temperatures, and typically low nutrient availability (e.g., Martínez and Rico 2002). In *P. palmata*, protein has been described as the major N storage pools, which constitutes a large fraction of the seaweed's dry weight (DW; Morgan et al. 1980).

This study adds to eco-physiological research of *Palmaria palmata* under fully controlled laboratory conditions and contributes to ecological aspects of nutrient uptake dynamics and nutrient management strategy for DIP and DIN. Uptake dynamics and ISC were quantified and standardized for surface area (SA), comparable to experiments on the green seaweed *Ulva lactuca* and the brown seaweeds *Saccharina latissimi* and *Laminaria digitata* by Lubsch and Timmermans (2018, 2019). In addition, total dissolvable protein concentration and total dissolvable carbohydrate concentration in the fronds were determined after 5 weeks exposure to limiting and nonlimiting nutrient concentrations. More information on the eco-physiology of seaweeds, presented in a comparable and comprehensive fashion, would strengthen the ecological understanding of eco-system dynamics and could initiate and expand bio-based activities in a responsible manner.

MATERIAL AND METHODS

Experimental set-up. All experiments and analysis were conducted at the Royal Netherlands Institute for Sea Research (NIOZ), Texel, the Netherlands. Young sporophytes of *Palmaria palmata*, which parental plants had originated from the Irish coastline, were cultivated at the NIOZ Seaweed Research Centre (https://www.nioz.nl/en/expertise/seaweed-research-centre) and brought to a temperature-controlled room (set 12°C) for a 10-d adaptation phase under laboratory conditions. During this adaptation phase, the sporophytes received DIP- and DIN-depleted seawater medium to ensure nutrient starvation (after Fujita 1985). After adaptation, 49 randomly collected sporophytes with a mean surface area (SA) of 1.9 ± 0.7 cm^2 were individually transferred into 200-mL glass jars filled with 100-ml seawater medium, enriched with a range of DIP concentrations (0.0 – 0.2 – 0.4 – 0.8 – 1.5 – 3.0 – 6.0 $\mu mol \cdot L^{-1}$) and a DIN concentration of 50 $\mu mol \cdot L^{-1}$. The seawater medium was exchanged/refreshed ("pulsed") on a daily basis throughout the experimental time, which not only provided a constant daily pulse of nutrients but also would mitigate effects of elevated or low CO_2 levels on growth rates (Kübler and Raven 1995). Samples of the seawater medium for dissolved nutrient analysis were taken ("chased") for the initial 20 d of the experiment. After water exchange, all flasks were randomly distributed on a custom

platform (100 × 60 × 1 cm) on a rotating table, slowly brought to a speed of 100 rpm to provide a moderate water movement. This constant water movement was maintained for optimal mixing and, hence, availability of nutrients by decreasing the diffusion boundary layers between tissue and growing medium (e.g., Gonen et al. 1995, Hurd 2000). Two tubular fluorescence lamps (OSRAM L18 Watt 965, Deluxe cool daylight) attached 50 cm above the flasks provided a light intensity of 60 ± 8 μmol photons · m^{-2} · s^{-1} to reach light saturation for the sporophytes (Kübler and Raven 1995) and a light:dark period of 16:8 h was arranged (Pang and Lüning 2004). Growth rates as a measure of surface area (SA) and the photosynthetic efficiency F_v/F_m were followed on a weekly basis for an additional 2 weeks. A standardization of growth, respectively, uptake kinetics, to SA was chosen, as it enables an intra- and interspecific comparison of the seaweeds over time, while uptake kinetics expressed as a function of dry weight (DW) define the end of the living biomass. The nondestructive method of standardized determination of fresh weight (FW) was not regarded as a useful parameter for small amount of biomass, as little variations in the amount of water attached to the living (and growing) seaweed can lead to huge differences in its weight, not only between different samples and over time but also among different experimentators.

Total dissolvable protein concentration as well as the total dissolvable carbohydrate concentration of all sporophytes (n = 49) were determined after a total experimental time of 5 weeks.

Seawater medium. Natural, filtered (0.2 μm) North Atlantic seawater with low phosphate (PO_4^{3-}: 0.011 μmol · L^{-1}), ammonium (NH_4^+: 0.032 μmol · L^{-1}), and nitrate (NO_3^-: 0.004 μmol · L^{-1}) concentrations and a salinity of 34.5 was used as a base for the seawater medium. After pasteurization of the seawater (80°C for 2 h), the salinity was adjusted to 29.5 by mixing ultrapure water (Milli-Q, Merck KGaA, Bedford, MA, USA) to bring the salinity to levels of the cultivation tanks. Afterward potassium–dihydrogen–phosphate (KH_2PO_4) and potassium nitrate (KNO_3) were added as sources for DIP and DIN to create DIP concentrations of 0.2 – 0.4 – 0.8 – 1.5 – 3.0 – 6.0 μmol · L^{-1} and a DIN concentration of 50 μmol · L^{-1}. The mean pH of all seven seawater medium stocks with varying DIP concentration was 8.1 ± 0.1 (n = 14), measured with a pH-Meter (GHM-3511, Greisinger, Germany).

Seawater analysis. Dissolved inorganic nutrients (DIP and DIN) were measured with a colorimetric analysis using a Technicon TRAACS 800 auto-analyzer (Seal Analytical, Norderstedt, Germany) in the NIOZ Texel nutrient laboratory. DIP was measured as orthophosphate (PO_4^{3-}) at 880 nm after the formation of molybdophosphate complexes (Murphy and Riley 1962). DIN (nitrate and nitrite) was calculated, after nitrate reduction to nitrite through a copperized cadmium coil and measured at 550 nm, posterior to a complexation with sulphanylamide and naphtylethylenediamine (Grasshoff and Hansen 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 μmol · L^{-1}) were not further considered, as no NH_4^+ was added for the experiments. Nominal nitrite concentration was measured in all cases <0.05 μmol · L^{-1} and hence played only a subordinate role in the (nitrate dominated) DIN concentration. Precision for all measured channels within the automated nutrient analyzer was better than 0.25% (K. Bakker, pers. comm.).

Surface area (SA) analysis. The sporophytes were individually spread flat on a white plastic board next to a ruler, used for scale comparison, and covered with a transparent Plexiglas sheet to avoid corrugations. Photographs (Panasonic Lumix DMC-FT5, Oaza Kadoma, Osaka, Japan) of the samples were taken from a 90° angle, enabling an analysis of surface area (SA) by using the open-source software ImageJ (ImageJ, US National Institutes of Health, Bethesda, MD, USA). The images of *Palmaria palmata* were converted into grayscale (type 8-bit) and transformed into a binary image before the SA was analyzed. The obtained SA represents one side of the frond.

Growth. Differences in SA over time were interpreted as 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. The results were used to calculate DIP and DIN uptake dynamics on d with no measurements of SA.

DIP and DIN uptake dynamics. Uptake is referred to the removal of dissolved inorganic phosphate (DIP), dissolved inorganic nitrate (DIN) by *Palmaria palmata* sporophytes. Determination of daily uptake rates was comparable to the analysis by Lubsch and Timmermans (2018) on *Ulva lactuca*. Daily uptake rates (V_D) were derived from changes in the nutrient concentrations of the seawater medium during each day, normalized for SA (cm^2) and time (d), and calculated using the following equation:

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

with T_1 as the initial nutrient concentration, T_2 as the nutrient concentration before water exchange after 24 h, SA as surface area (cm^2), and t as the incubation time.

Two different uptake rates over time were categorized: surge uptake (V_S, S for surge) after starvation, and maintenance uptake with filled nutrient pools (V_M, M for maintenance). V_S was calculated from uptake rates in nonlimiting nutrient concentrations (indicated by remaining nutrients after sampling interval), using the following equation:

$$V_S = (V_{D2} - V_{D1}) \times (d_2 - d_1)^{-1} = \Delta V \times \Delta d^{-1},$$

with V_{D1} and V_{D2} as daily uptake rates on days before a significant decline in uptake rates occurs and no significant variations in nutrient uptake follow. The difference operator between the two days is represented by d_1 and d_2.

The internal storage capacity (ISC) is the maximum filling capacity of internal nutrient pools for DIN and DIP and was calculated based on V_M and the response of the photosynthetic efficiency F_v/F_m under DIN- and DIP-limitation, respectively, depletion. The calculated ISC also accounts for the 10-d adaptation phase under depletion conditions, and was determined as follows:

$$ISC_{DIP} = n_M \times V_M,$$

where n_M represents the number of days under DIP depletion before F_v/F_m significantly decreased and V_M accounts for the daily DIP uptake under saturating conditions.

Photosynthetic efficiency F_v/F_m. Photosynthetic efficiency F_v/F_m was determined on a weekly basis for 5 weeks. All

sporophytes were dark adapted for 20 min in glass jars, before F_v/F_m was determined with a pulse-amplitude–modulated fluorimeter (JUNIOR-PAM, Walz, Effeltrich, Germany; settings: measuring light intensity = 10, pulse width = 0.8s, gain = 2) by measuring each sporophyte twice on different locations of the frond with an interval of 40 s between the two measurements. The measurements were done under minimum light conditions (laptop screen as the only light source) in a temperature-controlled room (set to 12°C) at approximately the same daytime.

Total dissolvable protein and carbohydrate analysis. All sporophytes were individually rinsed in fresh (MilliQ™) water to remove saltwater residue, immediately frozen (−40°C), freeze-dried (24 h), and homogenized for the determination of total dissolvable protein concentration (after Lowry et al. 1951), as well as total dissolvable carbohydrate concentration (Anthrone method after Trevelyan et al. 1952). A suspension of a homogenized *Palmaria palmata* sample (10 mg) and deionized, ultrapure water (10 mL) was made, and the Lowry reagents, respectively, the Anthrone reagents, were added. The solution with Lowry reagents was incubated at room temperature for 10 min, before the Folin/Cioccalteus reagent was added and the solution incubated for 30 min at room temperature. The solution with Anthrone reagents was placed in a heating chamber for 6 min at 95°C. Both solutions developed a blue color, which was analyzed with a photometer (SpectraMax M2, Molecular Devices, LLC, San Jose, CA, USA) at a wavelength of 660 nm, respectively, 620 nm. The total dissolvable protein concentration was calculated using a calibration curve based on a bovine serum albumin (BSA) stock solution with known protein concentration. Comparably, the total dissolvable carbohydrate concentration was determined by a glucose stock solution with known concentration.

Statistics. All data were tested for normality with the Kolmogorov–Smirnoff test (KS test) for cumulative probability distribution. A two-sided ANOVA with repetition was performed to test whether growth rates, nutrient uptake rates, total dissolvable protein, carbohydrate content, and F_v/F_m varied significantly within and between different nutrient concentrations over time.

RESULTS

Growth. The increase in surface area (SA), referred to as growth, of *Palmaria palmata* showed no significant variations among treatments with different DIP concentrations (ANOVA, $F_{6,210} = 1.06$, $P = 0.391$), but a significant difference in growth rates was detected over time (ANOVA, $F_{4,210} = 11.30$, $P < 0.001$). The interaction between growth rates and different DIP concentrations showed no significant variations within 5 weeks (ANOVA, $F_{24,210} = 0.72$, $P = 0.832$), thus the averaged growth of all sporophytes in different DIP concentrations was depicted (Fig. 1). The SA of all young sporophytes ($n = 49$) showed a mean increase of 0.12 ± 0.04 cm^2 in week 1, which decreased to mean growth of 0.01 ± 0.04 cm^2 in week 3 and displayed a subsequent increase in week 4 to week 5 with a mean growth of 0.05 ± 0.02 cm^2 per week (Fig. 1). The total increase in SA over 5 weeks resulted in a daily growth rate of 1%.

DIP and DIN uptake dynamics. Palmaria palmata sporophytes exposed to nominal DIP concentrations of 0.2, 0.4, and 0.8 μmol · L^{-1} depleted all offered DIP within the daily sampling of 24 h and throughout the experimental time (here referred to as limiting concentrations of PO_4^{3-}). The daily supplied DIN concentration of 50 μmol · L^{-1} was nonlimiting in all treatments and over experimental time. In treatments with DIP additions, DIN uptake was significantly higher than DIN uptake under DIP depletion conditions (ANOVA, $F_{1,40} = 10.70$, $P = 0.002$) and mean uptake rates increased in accordance with DIP availability (Fig. S1 in the Supporting Information). A strong positive correlation between DIP and DIN uptake was found (R = 0.943). When exposed to DIP-depleted seawater medium, sporophytes showed a mean DIN uptake rate of 5.8 ± 1.0 μmol · cm^{-2} · d^{-1} (Fig. 2) without significant variations over 20 d (ANOVA, $F_{6,19} = 1.31$, $P = 0.182$). Similarly, no significant variation in DIN uptake rates of sporophytes in nominal DIP concentration of 0.2 μmol · L^{-1} was found (ANOVA, $F_{6,19} = 0.04$, $P = 0.838$), but a mean DIN uptake rate of 7.6 ± 2.0 μmol · cm^{-2} · d^{-1} over 20 d was moderately higher than uptake rates under DIP depletion. An analogous increase in DIN uptake rates in accordance to DIP availability was observed for sporophytes in treatments of DIP concentrations of 0.4 and 0.8 μmol · L^{-1} with mean uptake rates ($n = 7$) of 11.0 ± 2.1 and 16.9 ± 6.1 μmol · cm^{-2} · d^{-1} on day 1 (Fig. 2). In nominal DIP concentrations of 1.5, 3.0, and 6.0 μmol · L^{-1}, initial DIN uptake rates showed no significant variations to initial uptake rates in nominal DIP concentration of 0.8 μmol · L^{-1} (ANOVA, $F_{3,20} = 2.33$, $P = 0.099$). Mean DIN uptake rates ($n = 7$) in these treatments were 16.0 ± 4.3, 11.8 ± 2.7, and 11.3 ± 1.0 μmol · cm^{-2} · d^{-1} on day 1, respectively, 15.6 ± 4.3, 17.9 ± 3.8, and 15.6 ± 2.3 μmol · cm^{-2} · d^{-1} on day 2 (Fig. 2). At the same time, maxima in mean DIP uptake rates ($n = 7$) of 1.42 ± 0.37 and 1.64 ± 0.13 μmol · cm^{-2} · d^{-1} were observed in nominal DIP concentration of 3.0 and 6.0 μmol · L^{-1}. Available DIP was not limiting in these

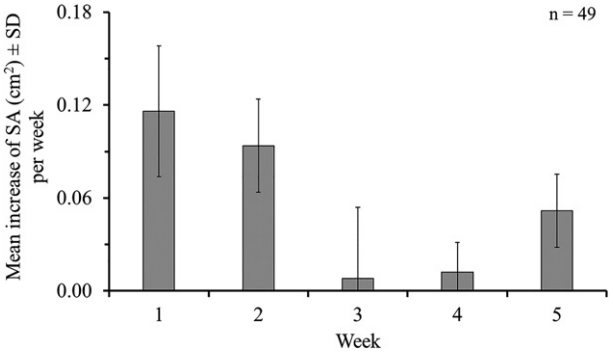

FIG. 1. Mean increase in surface area (cm^2) ± SD per week of young *Palmaria palmata* sporophytes ($n = 49$) cultivated in a range of dissolved inorganic phosphate (DIP) concentration (0–6 μmol · L^{-1}) and saturating dissolved inorganic nitrate (DIN) concentration (50 μmol · L^{-1}) in a 'pulse-and-chase' assay over 5 weeks.

treatments, unlike DIP in nominal concentration of 1.5 μmol · L^{-1}. Sporophytes exposed to 1.5 μmol · L^{-1} had depleted all daily supplied DIP until day 3, before a significant decrease in DIP removal from the seawater medium occurred on day 4 (ANOVA, $F_{3,6} = 13.05$, $P < 0.001$) with a mean uptake rate of 0.29 ± 0.05 μmol · cm^{-2} · d^{-1} (Fig. 2). DIP depletion was also recorded on days 9 and 14 in this treatment. Based on uptake rates of *P. palmata* in nominal DIP concentration of 3.0 and 6.0 μmol · L^{-1}, a mean V_S of 1.57 ± 0.29 μmol · cm^{-2} · d^{-1} for DIP ($n = 14$) was calculated for sporophytes in both treatments (Table 1).

After elevated uptake rates on day 1 and day 2, sporophytes in treatments with DIP availability >0.8 μmol · L^{-1} showed a rhythmic DIP and DIN uptake pattern with recurring maxima in uptake rates within the magnitude of initially elevated uptake on days 9 and 14, and minima with very low or hardly any detectable DIP and DIN uptake on days 12 and 18 (Fig. 2). For example, mean DIP uptake rates as low as 0.08 ± 0.10 and 0.10 ± 0.10 μmol · cm^{-2} · d^{-1} were measured in treatments with nominal DIP concentration of 1.5 μmol · L^{-1} during minima on day 12, respectively, day 18 (Table 1). At the same time, low DIN uptake rates of 4.0 ± 1.0 and 2.2 ± 0.6 μmol · cm^{-2} · d^{-1} were observed in this treatment (Fig. 2). The rhythmic recurrence of minima and maxima in DIP and DIN uptake rates resulted in mean uptake rates of 0.31 ± 0.15 μmol · cm^{-2} · d^{-1} for DIP and 5.4 ± 2.2 μmol · cm^{-2} · d^{-1} for DIN during the last rhythmic interval recorded in week 3 (Table 1). Similarly, the rhythmic uptake pattern of sporophytes in nominal DIP concentration of 3.0 and 6.0 μmol·L^{-1} showed mean uptake rates of 0.59 ± 0.23 μmol · cm^{-2} · d^{-1} for DIP and 5.6 ± 3.1 μmol · cm^{-2} · d^{-1} for DIN, respectively, 0.56 ± 0.23 μmol · cm^{-2} · d^{-1} for DIP and 7.8 ± 4.3 μmol · cm^{-2} · d^{-1} for DIN, during in the rhythmic interval in week 3. Based on the data, V_M of 0.57 ± 0.22 μmol · cm^{-2} · d^{-1} ($n = 14$) for DIP and V_M of 5.6 ± 2.1 μmol · cm^{-2} · d^{-1} ($n = 28$) for DIN were calculated (Table 1). Uptake rates for DIP and DIN under V_M were a threefold smaller than uptake rates under V_S and DIP:DIN uptake ratio under V_M was 1:10.

Internal storage capacity. An internal storage capacity (ISC) for DIP of 22.2 μmol · cm^{-2} was calculated based on V_M and the response in F_v/F_m under depletion conditions, including adaptation phase. In correspondence to a DIP:DIN uptake ratio of 1:10 under V_M, an ISC for DIN of 222 μmol · cm^{-2} was deduced. The internal storages for both, DIP and DIN, are equivalent to 40 days to maintain growth under depletion conditions.

Photosynthetic efficiency F_v/F_m. Sporophytes of *Palmaria palmata* showed no significant variation in photosynthetic efficiency (F_v/F_m) when growing under different DIP concentrations until week 3 (ANOVA, $F_{6,42} = 0.75$, $P = 0.612$). Mean F_v/F_m of all

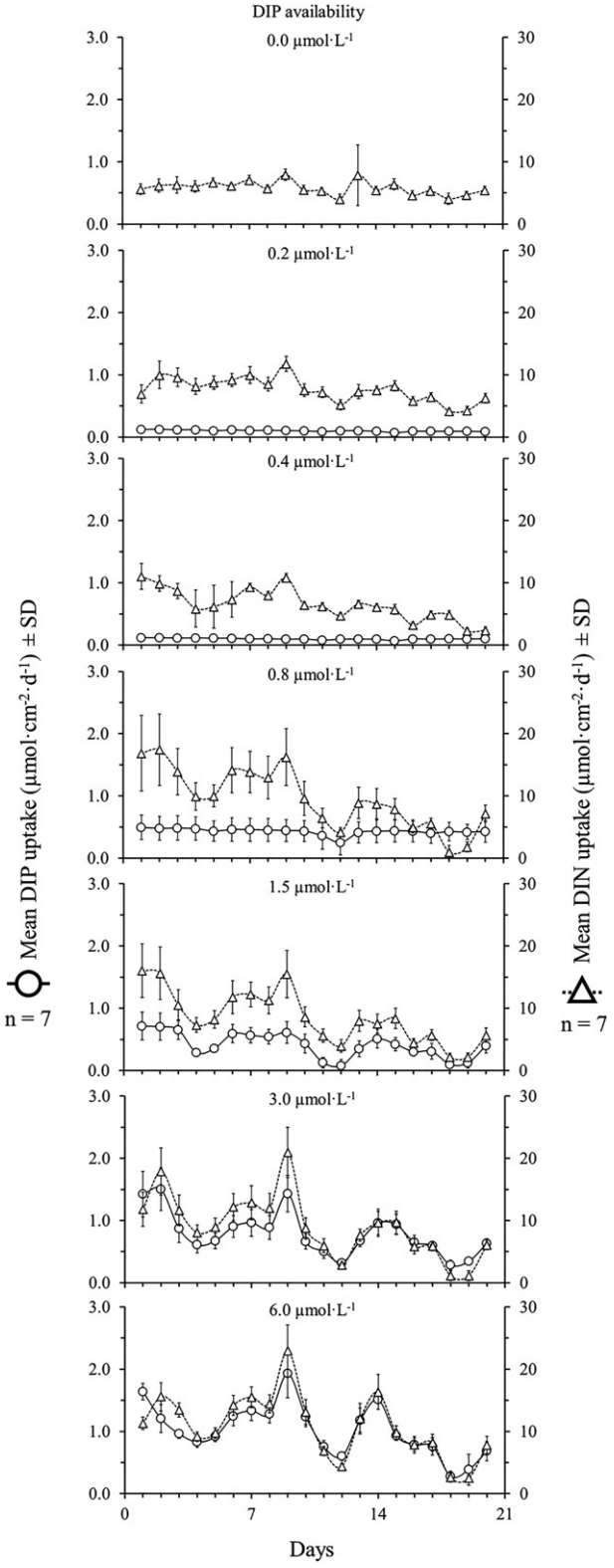

FIG. 2. Mean uptake rates (μmol · cm^{-2} · d^{-1}) ± SD of dissolved inorganic phosphate (DIP) and dissolved inorganic nitrate (DIN) of young *Palmaria palmata* sporophytes ($n = 7$) exposed to a range of available DIP concentration (0.0 – 0.2 – 0.4 – 0.8 – 1.5 – 3.0 – 6.0 μmol · L^{-1}) and saturating DIN concentration (50 μmol · L^{-1}) in a 'pulse-and-chase' assay over 20 d.

TABLE 1. Results on uptake dynamics (surge uptake V_S, maintenance uptake V_M, uptake ratios, internal storage capacity ISC, and growth) for dissolved inorganic nitrate (DIN) and dissolved inorganic phosphate (DIP) in *Ulva lactuca*[1] (Chlorophyceae), *Saccharina latissima*[2], *Laminaria digitata*[2] (Phaeophyceae), and *Palmaria palmata* (Rhodophyceae), conducted in 'pulse-and-chase' experiments under controlled conditions for light (light:dark: 16:8 h; *U. lactuca*[1]: 80 µmol photons · m^{-2} · s^{-1}, *S. latissima*[2] and *L. digitata*[2]: 18 µmol photons · m^{-2} · s^{-1}, *P. palmata*: 60 µmol photons · m^{-2} · s^{-1}), temperature (12 ± 1°C), and hydrodynamics over several weeks.

Class		Chlorophyceae	Phaeophyceae		Rhodophyceae
Species		*U. lactuca*[1]	*S. latissima*[2]	*L. digitata*[2]	*P. palmata*
V_S (µmol · cm^{-2} · d^{-1})	DIN	12.5 ± 5.2	11.3 ± 0.6	3.9 ± 0.1	15.6 ± 4.3*
	DIP	0.66 ± 0.12	0.80 ± 0.03	0.38 ± 0.03	1.57 ± 0.29
V_M (µmol · cm^{-2} · d^{-1})	DIN	2.3 ± 0.9	3.9 ± 0.7	1.8 ± 0.4	5.6 ± 2.1
	DIP	0.07 ± 0.04	0.30 ± 0.09	0.22 ± 0.01	0.57 ± 0.22
	N:P ratio	32:1	13:1	8:1	10:1
ISC (µmol · cm^{-2})	DIN	23 ± 7	49	80	222
	DIP	0.7 ± 0.1	14	>10	22
Daily growth (%)		4	3	2	1

[1]Data retrieved from Lubsch and Timmermans 2018.
[2]Data retrieved from Lubsch and Timmermans 2019.
*Strong dependency (R = 0.943) on DIP availability for nitrate uptake.

sporophytes was 0.61 ± 0.04 (n = 49; Fig. 3). After week 3, F_v/F_m of sporophytes exposed to limiting DIP concentrations of 0.0, 0.2, 0.4, and 0.8 µmol · L^{-1} significantly decreased (ANOVA, $F_{2,27}$ = 3.87, P = 0.027) to a mean value of 0.49 ± 0.06 (n = 28) with no significant variations among sporophytes (ANOVA, $F_{27,54}$ = 1.17, P = 0.305). Sporophytes exposed to nonlimiting DIP concentrations of 1.5, 3.0, and 6.0 µmol · L^{-1} showed neither significant variations among sporophytes (ANOVA, $F_{20,40}$ = 1.18, P = 0.315) nor a significant decrease within the first 3 weeks of the experiment (ANOVA, $F_{2,20}$ = 0.52, P = 0.595). Photosynthetic efficiency of sporophytes exposed to limiting DIP concentration of 0.0, 0.2, and 0.4 µmol · L^{-1} continued to significantly decrease between weeks 4 and 5 (ANOVA, $F_{1,20}$ = 18.97, P < 0.001) to a mean value of 0.44 ± 0.06 (n = 21). Although no significant variation in F_v/F_m among sporophytes in limiting DIP treatments was found until week 4 (ANOVA, $F_{20,80}$ = 1.17, P = 0.299), photosynthetic efficiency leveled off in accordance with available DIP concentrations. Sporophytes exposed to a concentration of 0.0 µmol · L^{-1} DIP showed the steepest and deepest drop in F_v/F_m with a mean of 0.46 ± 0.09 (n = 7) in week 4 and 0.40 ± 0.08 in week 5, compared to values exhibited by sporophytes in DIP concentration of 0.2 and 0.4 µmol · L^{-1}, which decreased to 0.51 ± 0.04, respectively, 0.49 ± 0.07 in week 4 and reached values of 0.41 ± 0.03, respectively, 0.43 ± 0.03 in week 5 (Fig. 3). In contrast, F_v/F_m of sporophytes in DIP concentration of 0.8 µmol · L^{-1} showed no significant variation between weeks 4 and 5 (ANOVA, $F_{1,6}$ = 4.21, P = 0.086) and a mean value of 0.51 ± 0.04 persisted (Fig. 3).

Similar to *Palmaria palmata* exposed to limiting DIP concentrations, F_v/F_m of sporophytes in nonlimiting DIP concentrations of 1.5, 3.0, and 6.0 µmol · L^{-1} showed no significant variation

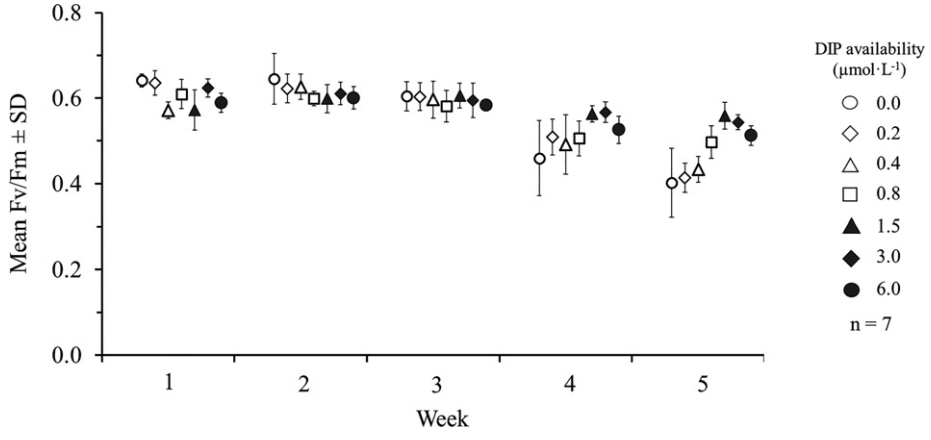

FIG. 3. Mean photosynthetic efficiency F_v/F_m ± SD of young *Palmaria palmata* sporophytes (n = 7) cultivated in a range of available dissolved inorganic phosphate (DIP) concentrations (0.0 – 0.2 – 0.4 – 0.8 – 1.5 – 3.0 – and 6.0 µmol · L^{-1}) and a dissolved inorganic nitrate (DIN) concentration of 50 µmol · L^{-1} in a 'pulse-and-chase' assay over 5 weeks

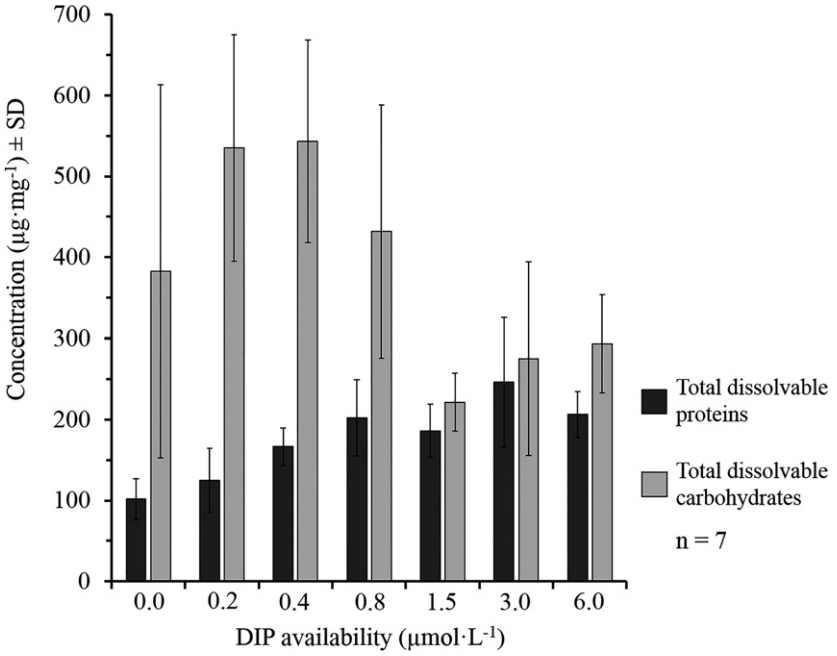

FIG. 4. Mean total dissolvable protein and carbohydrate concentration ($\mu g \cdot mg$ of dry $weight^{-1}$) ± SD of young *Palmaria palmata* sporophytes ($n = 7$), after cultivation in a range of dissolved inorganic phosphate (DIP) concentrations (0.0 – 0.2 – 0.4 – 0.8 – 1.5 – 3.0 –6.0 $\mu mol \cdot L^{-1}$) and a dissolved inorganic nitrate (DIN) concentration of 50 $\mu mol \cdot L^{-1}$ in a 'pulse-and-chase' assay for 5 weeks.

among treatments (ANOVA, $F_{20,84} = 0.93$, $P = 0.557$), but a significant variation over time (ANOVA, $F_{4,20} = 6.71$, $P = 0.007$). Unlike a steep decrease in F_v/F_m in sporophytes exposed to limiting DIP concentrations, mean values moderately decreased from 0.60 ± 0.04 in week 3 to 0.54 ± 0.04 in week 5 (Fig. 3).

Total dissolvable protein and carbohydrate concentrations. The total dissolvable protein concentration in *Palmaria palmata* showed significant variations among treatments with different nominal DIP concentrations (ANOVA, $F_{6,40} = 9.01$, $P < 0.001$), as did the total dissolvable carbohydrate concentration (ANOVA, $F_{6,40} = 6.41$, $P < 0.001$). Mean dissolvable protein concentration in sporophytes was 102 ± 25 $\mu g \cdot mg^{-1}$ DW ($n = 7$) after DIP depletion conditions for 6.5 weeks (adaptation and experimental time; Fig. 4) and showed significant variations to mean protein concentration of sporophytes with DIP availability (ANOVA, $F_{6,30} = 7.37$, $P < 0.001$). Mean dissolvable protein concentration of sporophytes increased to 202 ± 47 $\mu g \cdot mg^{-1}$ DW ($n = 7$), as DIP availability increased to a nominal DIP concentration of 0.8 $\mu mol \cdot L^{-1}$ (Fig. 4). No significant variation in dissolvable protein concentration in sporophytes exposed to nominal DIP concentration of 0.8 $\mu mol \cdot L^{-1}$ and higher were found (ANOVA, $F_{3,22} = 1.62$, $P = 0.214$) and mean dissolvable protein concentrations in sporophytes exposed to nominal DIP concentrations of 1.5, 3.0, and 6.0 $\mu mol \cdot L^{-1}$ ($n = 7$) was 186 ± 33, 246 ± 80, and 206 ± 28 $\mu g \cdot mg^{-1}$ DW, respectively (Fig. 4).

The total dissolvable carbohydrate concentration in *Palmaria palmata* showed no significant variation, when exposed to limiting DIP concentrations of 0.0, 0.2, 0.4, and 0.8 $\mu mol \cdot L^{-1}$ (ANOVA, $F_{3,24} = 1.81$, $P = 0.171$) and mean dissolvable concentrations were 383 ± 230 µg, 535 ± 140, 543 ± 125, and 432 ± 156 $\mu g \cdot mg^{-1}$ DW, respectively (Fig. 4). Similarly, no significant variation in dissolvable carbohydrate concentrations among treatments with nonlimiting DIP concentrations was found (ANOVA, $F_{2,16} = 1.53$, $P = 0.247$), but concentrations of dissolvable carbohydrates were significantly lower than concentrations in sporophytes exposed to limiting DIP concentrations (ANOVA, $F_{4,30} = 6.11$, $P < 0.001$). This threshold in mean dissolvable carbohydrate content resulted in concentrations of 221 ± 36, 275 ± 119, and 294 ± 60 $\mu g \cdot mg^{-1}$ DW were measured after 5 weeks exposure to nonlimiting DIP concentrations of 1.5, 3.0, and 6.0 $\mu mol \cdot L^{-1}$, respectively (Fig. 4).

In DIP depletion and limitation conditions (0.0–0.4 $\mu mol \cdot L^{-1}$), the protein:carbohydrate ratio in the sporophytes ranged from 0.27 to 0.31, the critical protein:carbohydrate ratio. A ratio of 0.47 was exhibited, when daily DIP pulses of 0.8 $\mu mol \cdot L^{-1}$ were supplied. Sporophytes exposed to DIP concentrations ≥1.5 $\mu mol \cdot L^{-1}$ showed a protein:carbohydrate ratio as high as 0.89 (Table 2).

DISCUSSION

Seaweeds acquire their resources from the surrounding seawater by uptake across their entire SA, and growth rates in nature are often constrained by rates of uptake and assimilation of nutrients per cm^2 surface area (Rees 2007). The determination of

TABLE 2. Ratio of total dissolvable protein and total dissolvable carbohydrate concentration in *Palmaria palmata* ($n = 7$) cultivated in nonlimiting dissolved inorganic nitrate (DIN) concentration (50 μmol · L^{-1}) and a range of dissolved inorganic phosphate (DIP) concentrations (0.0 – 0.2 – 0.4 – 0.8 – 1.5 – 3.0 – 6.0 μmol · L^{-1}) in a 'pulse-and-chase' experiment (daily refreshment of seawater medium; light:dark: 16:8 h, light intensity: 60 μmol photons · m^{-2} · s^{-1}) over 5 weeks.

DIP concentration (μmol · L^{-1})	0.0–0.4	0.8	≥1.5
Protein/carbohydrate ratio	0.27–0.31	0.47	0.89

the SA as a nondestructive method to infer to growth showed no significant variations in treatments with different nominal DIP concentrations, thus, the nutrient supply was not decisive for growth. This was supported by the ongoing growth and high photosynthetic efficiency of *Palmaria palmata* sporophytes under DIP depletion, which clearly demonstrated that internal storage pools were not completely depleted, during the 10-d adaptation phase. The latter was during the experiments confirmed, after calculation of maintenance uptake and ISC for *P. palmata*. Moreover, the determination of SA revealed a rhythmic growth pattern in *P. palmata*, conceivably in a monthly growth cycle. Circadian rhythms in growth have been often documented for plants, including seaweeds, and are attributed to survival and competitive advantages, although the contribution to plant fitness remains unknown (e.g., Michael et al. 2003, Dodd et al. 2005). Reproduction and growth cycles in monthly, respectively, moon-related periods, have been reported for several brown seaweed genera, including *Fucus*, *Dictyota*, and *Sargassum* (Schad 2001). Similar lunar or semilunar periodicities were also found in the green seaweeds *Ulva*, *Enteromorpha*, *Halimeda*, and *Halicystis* (Schad 2001). Obviously most seaweeds live in (inter-)tidal zones of coastal habitats and it is not surprising that physiological pattern have adapted to their environment in a more or less strict, genetically fixed amount (Schad 2001).

Biphasic responses to nutrient pulses are well known for seaweeds (Hurd and Dring 1990, Lotze and Schramm 2000, Lubsch and Timmermans 2018, 2019) and also have been reported for *Palmaria palmata* in short-time experiments (Martínez and Rico 2004). The responses to DIP and DIN pulses in *P. palmata* reported by Martínez and Rico (2004) showed a biphasic uptake with a V_{max} for DIP of 6.29–10.21 μmol · h^{-1} · g DW^{-1} and V_{max} for DIN of 16.96–24.67 μmol · h^{-1} · g DW^{-1} (V_{max} refers to the maximal uptake rate from the Michalis-Menten model, which is equivalent to V_S in this study), followed by a regression in uptake rates. This regression was described by the half-saturation concentration K_s from the Michaelis-Menten model and values for DIP were 11.64–25.40 μmol · L^{-1}, and K_s for nitrate was reported to be between 15.28 and 30.53 μmol · L^{-1} (Martínez and Rico 2004). A comparison of uptake kinetics to results of this study is troublesome, as no conversion factor for DW to SA for *P. palmata* was available. Uptake kinetics expressed as a function of dry weight (DW) necessitates destructive sampling through harvesting living biomass and, as seaweeds take up nutrients throughout their whole frond, the SA represents a more appropriate function to determine uptake dynamics in this study, comparable to publications on uptake dynamics and management strategies in *U. lactuca* (Lubsch and Timmermans 2018), *S. latissima*, and *L. digitata* (Lubsch and Timmermans 2019; Table 1). Rees (2007) reviewed available data (i.e., maximum uptake rates of nitrate and growth rates in several marine microalgae and macroalgae) which provided values or enabled to calculate for these parameters in relationship to their SA. The reported maximum uptake rates per SA and hour in macroalgae by Rees (2007) ranged from 40.3 nmol · cm^{-2} · h^{-1} for *Ulva intestinalis* (after Taylor et al. 1998) to 342.0 nmol · cm^{-2} · h^{-1} for *Fucus spiralis* (after Topinka 1978 and Nielsen and Sand-Jensen 1990), which meet the result on V_S in *P. palmata* (325 nmol · cm^{-2} · h^{-1}) in this study. Nevertheless, it has to be clarified that results on uptake dynamics in this study refer to the whole plant, while only one side of the sporophyte's SA is presented (see Material and Methods), which is an important factor, particularly for the implementation of data in 3-D models.

Clearly, elevated DIP and DIN uptake rates in saturating DIP treatments at the beginning of the assay can be attributed to V_S and the filling of internal nutrient pools, before V_M is attained, which is considered equal to the rate of assimilation (Taylor and Rees 1999, Barr et al. 2004). The DIP and DIN uptake rates quantified during V_S and V_M showed a ratio of 3:1, which is consistent with observations on uptake kinetics in *Palmaria palmata* by Martínez and Rico (2004). Uptake rates under V_M for both nutrients, DIP and DIN, were notably rhythmic in approximately weekly intervals, in contrast to an almost linear DIP and DIN uptake pattern during V_M in the brown seaweeds *Saccharina latissima* and *Laminaria Devaleraea* (Lubsch and Timmermans 2019) and the green seaweed *Ulva lactuca* (Lubsch and Timmermans 2018). Corresponding results to nutrient pulses were also found for growth in Devaleraea *mollis* in land-based co-culture systems with abalone (Demetropoulos and Langdon 2004): the supply of nutrients every 5–7 d resulted in no significant difference in *D. mollis* growth compared with daily nutrient supply, and was more effective in controlling epiphyte growth, as were daily nutrient additions during the dark cycle, compared with the light cycle.

The synchronized rhythmic uptake pattern of *Palmaria palmata* in different treatments provides evidence for physiological cell synchronization. Synchronization of nutrient uptake, growth, and

reproduction has often been applied to harmonize the response of seaweed cultures and can be achieved, for example, by the regulation of abiotic factors, for example, photoperiod, temperature, and nutrient supply over an extended period of time (e.g., Lüning 1993, Gomez and Lüning 2001, Bogaert et al. 2016). A weekly oscillating or rhythmic uptake pattern as observed during our experiments with *P. palmata* can be referred/linked to a physiological response to intra- and interspecific competition. Arino et al. (2003) demonstrated that a competitor-mediated coexistence, respectively, a competitive exclusion strategy, can result in oscillatory coexistence of more than one species with regards to nutrient uptake and growth (yield) of microorganisms in mathematical simulations. The rhythmic uptake pattern under V_M and high nutrient uptake rates under both V_S and V_M suggest a competitive exclusion strategy by *P. palmata*, which can often be found as large epiphytic populations, for example, on *Laminaria* stipes (Whittick 1983). It is conceivable that *Laminaria* stipes resemble a beneficial substratum to settle on, as big *Laminaria* fronds can provide shade and reduce effects of harmful light intensities to *P. palmata*, such as ultraviolet-induced genotoxicity (Atienzar et al. 2000). *Laminaria* stipes can mitigate the impact by hydrodynamic forces on *P. palmata*, and by this avoid damage or dislodgement by drag, the primary wave-induced force to intertidal seaweeds (Denny and Gaylord 2002). High uptake rates, especially during V_S, in *P. palmata* suggest a competitive advantage for nutrients, compared to nutrient uptake rates in *L. digitata*, which showed a V_S and V_M for DIP of 0.38 ± 0.03 and 0.22 ± 0.01 μmol · cm^{-2} · d^{-1}, respectively, for DIN 3.9 ± 0.1 and 1.8 ± 0.4 μmol · cm^{-2} · d^{-1} in comparable conditions of light, temperature, and hydrodynamics (Table 1; Lubsch and Timmermans 2019). The rhythmic uptake strategy during V_M by *P. palmata* bypasses the strategy of linear uptake under V_M in *L. digitata* (Lubsch and Timmermans 2019), and by that ensures a coexistence with regard to nutrient resources.

The strong correlation of DIP to DIN uptake, as well as the uptake ratio of 1:10 under V_M show the importance of DIP for metabolism and growth in *Palmaria palmata*, especially compared to the opportunistic seaweed *Ulva lactuca*, which showed an uncorrelated DIP and DIN uptake with a ratio of 1:32 under V_M (Lubsch and Timmermans 2018). Moreover, an elevated DIN uptake, as in V_S, did not occur in *P. palmata* under DIP depletion. Comparably, Martínez and Rico (2004) reported that an enrichment with DIN did not increase growth, if DIP was not added to the cultivation medium and a proper enrichment with both, N and P, was the only way to enhance growth rate in *P. palmata*.

The ISC calculated for DIP is an approximation, as internal nutrient pools had not been depleted during the adaptation/starvation phase, indicated by an ongoing growth, as well as high values of F_v/F_m under DIP depletion into 3 weeks, respectively, 4 weeks of the assay. Nevertheless, a rational estimation of ISC for DIP was possible based on the decrease in F_v/F_m of sporophytes that leveled off in accordance with the dosage of limiting DIP availability under completely controlled conditions for light, temperature, and hydrodynamics over the 5 weeks experimental period. It can be estimated that it will take some 40 d for *Palmaria palmata* to maintain its assimilation rate (equivalent to V_M) with filled internal DIP and DIN storages under external depletion conditions. This is comparable to the ISC in the perennial seaweeds *Saccharina latissima* and *Laminaria digitata* with an ~45 d capacity to storage of DIN. With completely filled internal pools for DIP, it can be estimated that *S. latissima* can maintain its assimilation rate under DIP-depleted conditions for ~90 d (Lubsch and Timmermans 2019). In contrast, the opportunistic seaweed *U. lactuca* exhibited an ISC that would last 10 d for DIP and DIN (Fujita 1985, Lubsch and Timmermans 2018). It should be realized that the estimation of ISC is largely based on the response of photosynthetic efficiency F_v/F_m to nutritional stress. Seaweeds can exhibit a broad range of physiological responses to stress-related conditions, notably an immediate change in the photosynthetic efficiency F_v/F_m (Parkhill et al. 2001). An F_v/F_m value between 0.79 and 0.84 is considered the optimal value for many plants, while values significantly below that range are considered to stress (Maxwell and Johnson 2000). *Palmaria palmata* showed values significantly below the considered optimum of 0.79 to 0.84, when exposed to nonlimiting DIP and DIN treatments, but were in agreement within the optimum range of F_v/F_m generally measured in red algae (Bose et al. 1988, Hanelt et al. 1993, Dring et al. 1996), including *P. palmata* (Liu and Pang 2010). Accordingly, *P. palmata* first indicated nutritional stress by a significant decrease in F_v/F_m, in limiting DIP concentrations.

The total dissolvable protein and carbohydrate concentrations in *Palmaria palmata* were in the range of reported values for this species (Morgan et al. 1980, Galland-Irmoulli et al. 1999, Harnedy and FitzGerald 2013). The dissolvable protein concentrations, ranging from 10 to 25% DW in sporophytes exposed to different limiting and nonlimiting DIP treatments in our experiments, perfectly aligned with observed seasonal variations in protein concentrations in natural populations of *P. palmata*: the lowest protein concentrations of ~8% DW were measured during summer months, when nutrient concentration of the seawater was lowest, and protein concentrations of ~30% DW were found during winter and early spring, when nutrient concentrations of the seawater showed an annual high (Martínez and Rico 2002, Rødde et al. 2004). Galland-Irmoulli et al. (1999) reported a protein content as high as

21.9 ± 3.5% DW in natural populations of *P. palmata*. The significant differences in dissolvable protein concentration in our experiments with *P. palmata* under different treatments of limiting DIP concentrations can not only reflect seasonal variations but also show the strong dependency on available DIP in order to take up nitrate, as nitrogen represents a key element in the protein production, respectively, amino-acid synthesis. For *P. palmata*, it has been shown that some forms of N increase growth rate, whereas other forms increase tissue N, and therefore protein content (Morgan and Simpson 1981, Grote 2016). Nitrate was found superior to ammonium as a source of nitrogen for growth and *P. palmata* supplied with ammonium accumulated more tissue nitrogen than plants supplied with nitrate within the same time span (Morgan and Simpson 1981, Demetropoulos and Langdon 2004). Similar to N and P, carbon can be stored as reserves in the form of carbohydrates and can be utilized to profit during times of high external DIP and DIN availability. In addition to nutrient availability, light (irradiance) has been identified as a main factor affecting nutrient reserves in *P. palmata*. Sun-acclimated *P. palmata* in northern Spain showed lower N and P and higher C content than shade-acclimated individuals, irrespective of transient high nutrient concentrations due to upwelling (Martínez and Rico 2008). The storage of C from high light exposure was shown to be the driving factor for metabolic adjustments at the end of summer. Environmental parameters vary according to season and the ecological conditions can stimulate or inhibit the biosynthesis of chemical composition in seaweed (Lobban and Harrison 1994).

In this study, the storage of C during times of low external DIN and DIP availability was clearly shown by high concentrations of dissolvable carbohydrates and low-to-moderate concentrations of dissolvable proteins in sporophytes exposed to limiting DIP concentrations, and vice versa in nonlimiting DIP conditions (Table 2). Total dissolvable carbohydrate concentrations ranged from 20% to 55% DW, and concentrations were consistent with reported values for this species. For example, Mutripah et al. (2014) reported a carbohydrate concentration of 469.8 $mg \cdot g^{-1}$ DW in *Palmaria palmata*, the highest carbohydrate content of 20 seaweed species evaluated.

Our results showed that increased DIP availability led to increased nitrate uptake in *Palmaria palmata*, which in turn increased the protein:carbohydrate ratio (Table 2). Similar protein: carbohydrate ratios were found, for example, in seasonal patterns in natural populations of the red alga *Gracilaria verrucosa*, with the highest ratio in the winter months associated with high inorganic nitrogen concentration of the seawater, low water turbidity, and low temperatures (Bird 1984). A critical protein:carbohydrate ratio for the subtropical *G. verrucosa* was documented at 0.38. It was suggested that the thallus constituents of protein and carbohydrates could be used to evaluate the nutrient deficiency status of *G. verrucosa* (Bird 1984).

The results on DIP and DIN uptake dynamics, as well as on dissolvable protein and carbohydrate concentrations under limiting and nonlimiting DIP conditions that are presented in this study match existing information on *Palmaria palmata* and add to the eco-physiological understanding, help to interpret nutrient management strategies, and open further insight into ecological aspects of nutrient availability. Moreover, our data allow to contribute to a viable seaweed mariculture, as well as a modern land-based cultivation in an economical and environmentally responsible manner. For example, our data on uptake dynamics and growth rates support *P. palmata* to be a potent species for bioremediation purposes in layered multispecies cultures, especially regarding the N:P stoichiometry, while a considerable amount of valuable proteins and carbohydrates is produced at the same time. For example, to improve efficiency in bioremediation and enhance yield, the slow growth rates and oscillating uptake strategy by *P. palmata* can be complemented by *S. latissima*, which showed a mean growth of $4\% \cdot d^{-1}$ and similarly high, but uncorrelated and linear uptake rates of DIP and DIN in a ratio of 1:13 under V_M in comparable conditions (Table 1; Lubsch and Timmermans 2019). Such a multispecies culture can be useful, especially in close proximity to fish farms, which commonly generate large amounts of effluents in fluctuating quantities, including nitrogenous compounds and phosphates. Limitations or shifts in limitation from one element to another can be accounted for by nutrient additions in appropriate frequency and ratio, as well as by crop rotation of different species in accordance to DIP and DIN uptake ratios (Table 1). Moreover, bioremediation efforts can be encountered in an environmentally sustainable manner, as it has recently been shown, that de-eutrophication efforts in north-western Europe in the 1980s have led to a large imbalance in the N:P stoichiometry of coastal waters of the North Sea (Burson et al. 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Mean uptake rates ($\mu mol \cdot cm^{-2} \cdot d^{-1}$) ± SD of dissolved inorganic nitrate (DIN) of young *Palmaria palmata* sporophytes ($n = 7$) in accordance to dissolved inorganic phosphate (DIP) availability (0.0 – 0.2 – 0.4 – 0.8 – 1.5–3.0 – 6.0 $\mu mol \cdot L^{-1}$).