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

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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 (Vₛ) after starvation and (2) maintenance uptake with filled nutrient pools (Vₘ). Vₛ 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 Vₘ 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·cm² and DIN of 222 µmol·cm² was determined. A DIP-to-DIN uptake ratio of 1:10 under Vₘ showed a weekly rhythmic uptake pattern, highlighted by a high correlation between DIP and DIN uptake (R = 0.943). Vₛ for DIN did not occur under DIP depletion, but uptake rates increased with increasing DIP availability. Hence, DIN 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; Fₘ/Fₘ, refers to variable fluorescence Fₘ refers to maximum fluorescence; ISC, Internal storage capacity; Kₛ, Half-saturation constant from Michaelis-Menten model; NIOZ, Royal Netherlands Institute for Sea Research; T, Nutrient concentration; Vₛ, Uptake rate; Vₘ, Maintenance uptake rate; Vₛ, 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.
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, alginites 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 efficiency of bioirrigation 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 latissima* 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 μmol · L$^{-1}$) and a DIN concentration of 50 μmol · L$^{-1}$. The seawater medium was exchanged/refresed ("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 (Kühler and Raven 1995). Samples of the seawater medium for dissolved nutrient analyses were taken ("chased") for the initial 20 d of the experiment. After water exchange, all flasks were randomly distributed on a custom
photosynthetic efficiency (K. Bakker, pers. comm.).

Photosynthetic efficiency $F_v/F_m$. Photosynthetic efficiency $F_v/F_m$ was determined on a weekly basis for 5 weeks. All growth conditions, nutrient concentrations (indicated by remaining nutrients in the solution), and biomass were based on the analysis by Lubsch and Timmermans (2018) on Ulva lactuca. Daily uptake rates $V_N$ 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 following the equation:

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

where $T_1$ 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: uptake under 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:

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

where $n_D$ represents the number of days under DIP depletion before $F_v/F_m$ significantly decreased and $V_M$ accounts for the daily DIN uptake under saturating conditions. 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-F5, 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 (Image]. 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 ($\mu$) 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 DIN and DIP uptake dynamics on day without no measurements of SA.
sporophytes were dark adapted for 20 min in glass jars, before \( F_{\text{t}} / F_{\text{m}} \) was determined with a pulse-amplitude-modulated fluorimeter (JUNIOR-PAM, Walz, Effeltrich, Germany; settings: measuring light intensity = 10, pulse width = 0.8 s, 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) 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/Ciocalteu 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 \( F_{\text{t}} / F_{\text{m}} \) 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, \( F_{\text{5,210}} = 1.06, P = 0.391 \)), but a significant difference in growth rates was detected over time (ANOVA, \( F_{\text{1,210}} = 11.30, P < 0.001 \)). The interaction between growth rates and different DIP concentrations showed no significant variations within 5 weeks (ANOVA, \( F_{\text{20,210}} = 0.72, P = 0.832 \)), thus the aged 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 cm² in week 1, which decreased to mean growth of 0.01 ± 0.04 cm² in week 3 and displayed a subsequent increase in week 4 to week 5 with a mean growth of 0.05 ± 0.02 cm² 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 PO₄³⁻). 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 DIP uptake under DIP depletion conditions (ANOVA, \( F_{\text{1,40}} = 10.70, P = 0.002 \)) 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, \( F_{\text{6,190}} = 1.31, P = 0.182 \)). Similarly, no significant variation in DIN uptake rates of sporophytes in nominal DIP concentration of 0.2 µmol L⁻¹ was found (ANOVA, \( F_{\text{6,190}} = 0.04, P = 0.838 \)), 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 DIN availability was observed for sporophytes in treatments of DIN concentrations of 0.4 and 0.8 µmol L⁻¹ with mean uptake rates (\( n = 7 \)) of 11.0 ± 2.1 and 18.9 ± 6.1 µmol cm⁻² d⁻¹ on day 1 (Fig. 2). In nominal DIN 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 DIN concentration of 0.8 µmol L⁻¹ (ANOVA, \( F_{\text{5,20}} = 2.35, P = 0.099 \)). 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 DIN uptake rates (\( n = 7 \)) of 1.42 ± 0.37 and 1.64 ± 0.13 µmol cm⁻² d⁻¹ were observed in nominal DIN concentration of 3.0 and 6.0 µmol L⁻¹. Available DIP was not limiting in these
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, F₁₀,₆ = 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 Vₛ 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, Vₚ of 0.57 ± 0.22 μmol cm⁻² d⁻¹ (n = 14) for DIP and Vₚ 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 Vₚ were a threefold smaller than uptake rates under Vₛ and DIP:DIN uptake ratio under Vₚ was 1:10.

Internal storage capacity. An internal storage capacity (ISC) for DIP of 22.2 μmol cm⁻² was calculated based on Vₚ and the response in Fₚ/Fₚₙ under depletion conditions, including adaptation phase. In correspondence to a DIP:DIN uptake ratio of 1:10 under Vₚ, 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 Fₚ/Fₚₙ. Sporophytes of Palmaria palmata showed no significant variation in photosynthetic efficiency (Fₚ/Fₚₙ) when growing under different DIP concentrations until week 3 (ANOVA, F₆,₁₀₂ = 0.75, P = 0.612). Mean Fₚ/Fₚₙ 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.](https://onlinelibrary.wiley.com/doi/10.1111/jpy.13018)
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 *U. lactuca* (Chlorophyceae), *Saccharina latissima*, *Laminaria digitata* (Phaeophyceae), and *Palmaria palmata* (Rhodophyceae), conducted in ‘pulse-and-chase’ experiments under controlled conditions for light (light:dark: 16:8 h; *U. lactuca*: 80 µmol photons · m$^{-2}$ · s$^{-1}$, *S. latissima* and *L. digitata*: 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.

<table>
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<th>Phaeophyceae</th>
<th>Rhodophyceae</th>
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<td>$V_m$ (µmol · cm$^{-2}$ · d$^{-1}$)</td>
<td>ISC (µmol · cm$^{-2}$)</td>
<td>Daily growth (%)</td>
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<td>Daily growth (%)</td>
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$^*$Data retrieved from Lubsch and Timmermans 2019.
$^*$Data retrieved from Lubsch and Timmermans 2018.
Strong dependency ($R = 0.943$) on DIP availability for nitrate uptake.

Sporophytes were 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, $F_{2,27} = 1.17$, $P = 0.305$). Sporophytes exposed to nonlimiting DIP concentrations of 1.5, 3.0, and 6.0 µmol · L$^{-1}$ showed neither significant variations among sporophytes (ANOVA, $F_{2,40} = 1.18$, $P = 0.315$) 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 ± 0.05, 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.
among treatments (ANOVA, $F_{0.84} = 0.93$, $P = 0.557$), but a significant variation over time (ANOVA, $F_{1.20} = 6.71$, $P = 0.007$). Unlike a steep decrease in $F/F_0$ in sporophytes exposed to limiting DIP concentrations, mean values moderately decreased from $0.60 \pm 0.04$ in week 3 to $0.54 \pm 0.04$ 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, $F_{0.40} = 9.01$, $P < 0.001$), as did the total dissolvable carbohydrate concentration (ANOVA, $F_{0.40} = 6.41$, $P < 0.001$). Mean dissolvable protein concentration in sporophytes was $102 \pm 25 \mu g \cdot mg^{-1} DW$ ($n = 7$) 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, $F_{0.30} = 7.37$, $P < 0.001$). Mean dissolvable protein concentration of sporophytes increased to $202 \pm 47 \mu g \cdot mg^{-1} DW$ ($n = 7$), 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 $186 \pm 33$, $246 \pm 80$, and $206 \pm 28 \mu g \cdot mg^{-1} DW$, 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 $383 \pm 230 \mu g$, $555 \pm 140$, $543 \pm 125$, and $432 \pm 156 \mu g \cdot mg^{-1} DW$, 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, $F_{3.30} = 6.11$, $P < 0.001$). This threshold in mean dissolvable carbohydrate content resulted in concentrations of $221 \pm 36$, $275 \pm 119$, and $294 \pm 60 \mu g \cdot mg^{-1} DW$ 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 $\geq 1.5 \mu mol \cdot L^{-1}$ 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
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 *Halocystis* (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 values for DIP were 11.64–25.40 µmol · L⁻¹, and Kᵣ for nitrate were 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 Vₛ 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 Vₛ and the filling of internal nutrient pools, before Vₘ 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 Vₛ and Vₘ 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 Vₘ 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 Vₛ 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

### 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.

<table>
<thead>
<tr>
<th>DIP concentration (µmol · L⁻¹)</th>
<th>0.0–0.4</th>
<th>0.8</th>
<th>≥1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein/carbohydrate ratio</td>
<td>0.27–0.31</td>
<td>0.47</td>
<td>0.89</td>
</tr>
</tbody>
</table>
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, Gómez 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 VS and VM 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 (Atienza 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 1:1 under V M show the importance of DIP for metabolism and growth in Palmaria palmata, especially compared to the opportunistic seaweed U. 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 VS, did not occur in P. palmata under DIN depletion. Comparably, Martínez and Rico (2004) reported that an enrichment with DIN did not increase growth, if DIN 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 Fₐ/Fₘ 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 Fₐ/Fₘ 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 VS) with filled internal DIP and DIN storages under external depletion conditions. This is comparable to the ISC in the perennial seaweeds Saccharina latissima and Lamna digitata with an ~45 d capacity to storage of DIN. With completely filled internal pools for DIN, 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 Fₐ/Fₘ to nutritional stress. Seaweeds can exhibit a broad range of physiological responses to stress-related conditions, notably an immediate change in the photosynthetic efficiency Fₐ/Fₘ (Parkhill et al. 2001). An Fₐ/Fₘ 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 with limiting DIP concentrations that were in the range of reported values for this species (Morgan et al. 1980, Galland-Irmoulli et al. 1999, Harnedy 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 Fₐ/Fₘ 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, Mutiripah et al. (2014) reported a carbohydrate concentration of 469.8 mg · 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% · 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 (µmol · cm⁻² · 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 µmol · L⁻¹).