North Sea seaweeds: DIP and DIN uptake kinetics and management strategies
Lubsch, Alexander

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Chapter 2

Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding N:P dynamics in Ulva lactuca (Chlorophyta)


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2.1 Abstract

Dissolved inorganic phosphorus (DIP) is an essential macronutrient for maintaining metabolism and growth in autotrophs. Little is known about DIP-uptake kinetics and internal P-storage capacity in seaweeds, such as Ulva lactuca (Chlorophyta). U. lactuca is a promising candidate for biofiltration purposes and mass commercial cultivation. We exposed U. lactuca to a wide range of DIP concentrations (1 – 50 µmol·L⁻¹) and a non-limiting concentration of dissolved inorganic nitrogen (DIN) (5000 µmol·L⁻¹) under fully controlled laboratory conditions in a ‘pulse-and-chase’ assay over 10 days. Uptake kinetics were standardized per surface area of U. lactuca fronds. Two phases of responses to DIP-pulses were measured: (1) a surge uptake (Vₛ) of 0.67±0.10
µmol·cm$^{-2}$·d$^{-1}$ and (2) a steady state uptake ($V_M$) of $0.07\pm0.03$ µmol·cm$^{-2}$·d$^{-1}$. Mean internal storage capacity ($ISC_p$) of $0.73\pm0.13$ µmol·cm$^2$ was calculated for DIP. DIP uptake did not affect DIN uptake. Parameters of DIN uptake were also calculated: $V_S$=$12.54\pm5.22$ µmol·cm$^2$·d$^{-1}$, $V_M$=$2.26\pm0.86$ µmol·cm$^2$·d$^{-1}$, and $ISC_N$=$22.90\pm6.99$ µmol·cm$^2$. Combining ISC and $V_M$ values of P and N, nutrient storage capacity of $U. lactuca$ was estimated to be sufficient for approximately 10 days. Both P and N storage capacities were filled within two days when exposed to saturating nutrient concentrations, and uptake rates declined thereafter at 90 % for DIP and at 80 % for DIN. Our results contribute to understanding the ecological aspects of nutrient uptake kinetics in $U. lactuca$ and quantitatively evaluates its potential for bioremediation and/or biomass production for food, feed and energy.

### 2.2 Introduction

Seaweeds are important primary producers. An essential macronutrient for maintaining the metabolism and growth of these autotrophs is dissolved inorganic phosphorus (DIP), along with nitrogen (N). Understanding the demand and management strategy for nutrients by seaweeds is economically and ecologically of central importance as it allows for optimal manipulation in cultivation and biofiltration facilities, as well as it opens opportunities to forecast ecological impacts of nutrient limitation and shifts in limitation from one element to another, which can significantly affect the internal composition, physiology and growth of seaweeds (Pederson & Borum 1996, Gevaert et al. 2001).

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

*Ulva lactuca* (Linnaeus), a seaweed in the division Chlorophyta, is found worldwide and is prolifically abundant where nutrients are readily available (Morand & Merceron 2005). *Ulva lactuca* has been identified as a promising species in water treatment facilities (biofilters) and in integrated multi-trophic aquaculture (IMTA) systems (e.g. Cohen & Neori 1991, Neori et al. 2003). *U. lactuca* is also recognized as a promising species for commercial mass cultivation and subsequent production of food, animal feed and fertilizer (Critchley & Ohno 1998, Sahoo 2000, Thangaraju 2008, Holdt & Kraan 2011). Only a few studies have examined DIP-uptake kinetics and internal DIP-storage capacity in seaweeds in general (e.g. Gordon et al. 1981, Chopin et al. 1997, Gordillo et al. 2002, Pederson et al. 2010) and in *U. lactuca*, in particular (Runcie et al. 2004, Tsagkamilis et al. 2010). The majority of studies related to the efficiency of N and P removal from seawater by *U. lactuca* have been conducted under field conditions (Neori et al. 1991, Neori et al. 2003, Naldi & Viaroli 2002). For example, Tsagkamilis et al. (2010) indicated finding an optimal combination of biomass and water flow rates for satisfactory nutrient uptake by *U. lactuca*, by measuring DIP removal from the effluent in a small-scale water treatment facility. Quantification of DIP uptake kinetics over time, however, and the saturating storage capacity of DIP in *U. lactuca* has not yet been studied. In addition, uptake kinetics are usually expressed as functions of either fresh weight (FW), dry weight (DW) or surface area to volume (SA:Vol), which makes it difficult to compare data accurately without conversion.

In this study, we present the DIP-uptake kinetics of *U. lactuca* exposed to a range of nominal PO$_4^{3-}$ concentrations (1 – 50 µmol·L$^{-1}$). This range of concentrations is equivalent to exposing *U. lactuca* to phosphate concentrations of 0.02 – 0.67 µmol·cm$^{-2}$, which is within the range
of natural concentrations. The experiments were performed under laboratory conditions, controlling for temperature, light and hydrodynamics in a “pulse-and-chase” (i.e. add a pulse of nutrients and follow their removal from the water over time) approach over 10 days. DIP-uptake kinetics and storage capacity were quantified, as well as N:P-uptake dynamics, and all were standardized for SA. In order to make comparisons possible with other standardizations, we calculated factors for conversion to fresh weight (FW) and dry weight (DW).

2.3 Material and methods

All experiments and analyses were conducted at the Royal Netherlands Institute for Sea Research (NIOZ), Texel, The Netherlands. Clean and healthy fronds of *U. lactuca* (after Stegenga and Mol 1983), originally collected from the coastline of the island of Texel in the summer of 2013, were obtained from the NIOZ Seaweed Centre (www.nioz.nl/seaweedcentre) cultivation tanks in September of 2014 and transferred to a temperature-controlled (12.0±0.6 °C) room for a 10-day adaptation phase under fully controlled laboratory conditions in nutrient-depleted seawater (PO$_4^{3-}$ = 0.008 µmol·L$^{-1}$, NH$_4^+$ = 0.022 µmol·L$^{-1}$ and NO$_3^-$ = 0.003 µmol·L$^{-1}$). This ensured that the *U. lactuca* were nutrient starved after 10 days (after Fujita et al. 1985).

Following the adaptation/starvation phase, *U. lactuca* fronds of comparable sizes (76.4±11.5 cm$^2$) were individually transferred into 200 ml glass flasks filled with 100 ml seawater medium and enriched with a range of nominal PO$_4^{3-}$ concentrations (1 – 50 µmol·L$^{-1}$ added) with three replicates for each of the PO$_4^{3-}$ concentrations. The relation between nominal PO$_4^{3-}$ concentration of the seawater medium and comparable SA of *U. lactuca* resulted in a mean DIP availability ranging from 0.02±0.01 to 0.67±0.12 µmol·cm$^{-2}$·d$^{-1}$, resembling a concentration range within the scope of natural phosphate concentration fluxes. The seawater medium was refreshed (“pulsed”) to its intended nominal concentration on a daily basis, and samples for dissolved nutrient analysis were taken (“chased”). Each day, after the seawater medium had been refreshed, all flasks were randomly distributed to minimize differences in light availability on a rotating table.
providing moderate water movement at a speed of 100 rpm. A constant water movement was maintained for optimal mixing and, hence, availability of nutrients by decreasing diffusion boundary layers between tissue and medium (e.g. Gonen et al. 1995, Hurd 2000), assuming that uptake rates become limited by factors such as enzyme activity (Wheeler et al. 1988). Two tubular fluorescent lamps (OSRAM L18 Watt 965, Deluxe cool daylight), attached 50 cm above the flasks, provided a PAR light intensity of 80± 8 µmol·m⁻²·s⁻¹ (light meter ULM- 500, Walz, Germany) inside the glass flasks. A light/dark period of 16/8 h was maintained throughout the experiments.

Seawater medium

As a base for the seawater medium, we used filtered (cellulose acetate filter 0.2 µm, Sartorius, Germany) nutrient-poor seawater from the North Atlantic Ocean (salinity 34.5) with low phosphate (PO₄³⁻; 0.008 µmol·L⁻¹), ammonium (NH₄⁺; 0.022 µmol·L⁻¹) and nitrate (NO₃⁻; 0.003 µmol·L⁻¹) concentrations. After pasteurization of the seawater (80 °C for 2 h), the salinity was adjusted to 29.5, as measured at the NIOZ seaweed centre and around the island of Texel, by mixing with ultrapure water (Milli-Q, Merck KGaA, Massachusetts, USA), followed by adding mono-ammonium-dihydrogen-phosphate ((NH₄)H₂PO₄) and potassium nitrate (KNO₃) as sources for PO₄³⁻, NH₄⁺ and NO₃⁻ until reaching the desired nominal concentrations (treatments) of 1.0, 1.5, 2.5, 4.0, 7.0, 13.0, 25.0 and 50.0 µmol·L⁻¹ of PO₄³⁻ and NH₄⁺. The NO₃⁻ concentration was set to 5000 µmol·L⁻¹ (Table 2-1). The pH of the medium, measured using a pH-Meter (GHM-3511, Greisinger, Germany), was 8.1±0.1 (n=8) after pasteurization and adding nutrients.
Table 2-1. Daily ‘pulsed’ DIP and DIN concentration (µmol·L⁻¹) to Ulva lactuca in a 10-day ‘pulse-and-chase’ experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phosphate</th>
<th>Nitrate</th>
<th>Ammonium</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.0</td>
<td>5000</td>
<td>1.0</td>
</tr>
<tr>
<td>B</td>
<td>1.5</td>
<td>5000</td>
<td>1.5</td>
</tr>
<tr>
<td>C</td>
<td>2.5</td>
<td>5000</td>
<td>2.5</td>
</tr>
<tr>
<td>D</td>
<td>4.0</td>
<td>5000</td>
<td>4.0</td>
</tr>
<tr>
<td>E</td>
<td>7.0</td>
<td>5000</td>
<td>7.0</td>
</tr>
<tr>
<td>F</td>
<td>13.0</td>
<td>5000</td>
<td>13.0</td>
</tr>
<tr>
<td>G</td>
<td>25.0</td>
<td>5000</td>
<td>25.0</td>
</tr>
<tr>
<td>H</td>
<td>50.0</td>
<td>5000</td>
<td>50.0</td>
</tr>
</tbody>
</table>

in µmol·L⁻¹

Nutrient analysis

Nutrients (DIP, DIN=nitrate and ammonium) were measured with colorimetric analysis using a Technicon TRAACS 800 auto-analyzer (Seal Analytical, Germany) in the NIOZ Texel nutrient laboratory. DIP was measured as ortho-phosphate (PO₄³⁻) at 880 nm after the formation of molybdophosphate complexes (Murphy & Riley, 1962). DIN (nitrate and nitrite) was calculated after nitrate reduction to nitrite through a copperized cadmium coil and measured at 550 nm after complexation with sulphanylamide and naphtylethylenediamine (Grasshoff et al. 1983). Ammonium (NH₄⁺) was measured at 630 nm after the formation of an indophenol blue complex with phenol and sodium hypochlorite at pH 10.5. Citrate was used as a buffer and complexant for
calcium and magnesium at this pH (Koroleff 1969 and optimized by Helder & de Vries 1979). Precision for all the measured channels within the automated nutrient analyzer was better than 0.25% (personal communication K. Bakker, NIOZ).

**Nutrient uptake kinetics**

Nutrient uptake is referred to as the removal of dissolved inorganic phosphate (DIP), nitrate and nitrite (DIN), and ammonium from the medium by *U. lactuca*. Daily uptake rates ($V$) were derived from changes in the nutrient concentrations of the seawater medium during each day, normalized for SA ($\text{cm}^2$) and time ($d$), and calculated using the following equation:

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

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

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). The intervals over which $V_s$ and $V_m$ were calculated are indicated in Figure 2-1. $V_s$ was calculated from uptake rates in a non-limiting nutrient concentration using the following equation:

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

where $V_1$ and $V_2$ are 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 $d_2$.

Internal storage capacity (ISC) is the maximum filling capacity of internal nutrient pools, which was calculated using the following equation:

$$ \text{ISC}_{N,P} = \sum (i \epsilon V_s) - n \times V_M, $$

where $i$ represents the daily nutrient uptake from initial exposure and is an element of $V_s$, $n$ accounts for the number of days from initial exposure to when $V_s$ significantly declined and $V_M$ is the daily uptake when nutrient pools are full. A saturation of these pools is indicated by a significant decline in uptake rates (Figure 2-1).
Surface area analysis

*Ulva lactuca* fronds were spread flat on a white background and covered with a transparent Plexiglas sheet to avoid folding and wrinkling of the frond. A ruler was placed next to the Plexiglas for scale comparison. Photographs (Panasonic Lumix DMC-FT5) were taken on days 1, 3, 5, 7 and 10, enabling an analysis of surface area (SA) by using the open source software ImageJ (ImageJ, U. S. National Institutes of Health, Maryland, USA). For analysis of only pigmented areas in a frond and to exclude potential non-pigmented areas and holes using ImageJ, the scanned colored photograph was converted into grayscale (type 8-bit) and further processed into a binary image before ‘particles’ (pixels) of the pigmented SA could be analyzed. The software’s automated threshold displayed the pigmented SA as dark areas within the grayscale. To analyze the SA, including accidentally overlapping tissue (darker), the threshold routine was set to manual mode, which allowed for adjustment of the contrast according to the level of overlapping portions of an individual for a refined analysis. The obtained SA represents one side of the two-cell thick lamina.

**Figure 2-1.** Example graph of nutrient uptake over time (days) illustrated with surge uptake ($V_S$), maintenance uptake ($V_M$), internal storage capacity (ISC), and $d_1$ and $d_2$ as difference operator between days, after a significant decrease in nutrient uptake occurs.
of *U. lactuca*. Differences in SA over time were indicated as growth. Relative growth rates (µ) were calculated according to Kain (1987) using the following equation:

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

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

**Relation of SA to fresh weight (FW) and dry weight (DW)**

In order to make comparisons possible with our uptake kinetics standardized for SA, conversions to fresh weight (FW) and dry weight (DW) were made. Sixty individuals of *U. lactuca* were centrifuged in a top-loading laundry spinner (BOSCH, 2800 U · min⁻¹, 350 W) for 1 minute to dispose of excess water and measured for FW. After this, photographs were taken for SA analysis. Subsequently, to determine DW, the same individuals were quickly rinsed in MilliQ™ to prevent salt residue from forming on the samples after the drying process, and dried for 72 h at 60°C. Both FW and DW were determined using a Mettler Toledo balance (accuracy: 0.01g).

**Statistics**

All data were tested for normality with the Kolmogorov-Smirnoff test (KS test) for cumulative probability distribution. A two-sided ANOVA was performed to test whether growth rates and nutrient uptake rates varied significantly within and between different nutrient concentrations over time.
2.4 Results

*Growth*

The mean initial surface area of *U. lactuca* (n=24) in all experimental treatments was 76.4±11.5 cm$^2$ (SA±SD) and increased to a mean SA of 84.2±14.9 cm$^2$ after 10 days, which represents significant growth (ANOVA, $df=23$, $F=6.20$, $p \leq 0.001$). Mean growth between days 1 and 3 was moderate (4.4 %) and gradually decreased to very low (0.6 %) between days 7 and 10 (Figure 2-2). No significant differences in growth between the different DIP treatments were observed (ANOVA, $df=46$, $F=4.12$, $p=0.087$).

![Figure 2-2](image-url)  
*Figure 2-2.* Mean surface area (SA) ± SD (n=24) of *Ulva lactuca* on day 1, 3, 5, 7, and 10 of all treatments. No significant differences in growth between treatments with different DIP concentrations were found (ANOVA, $df=23$, $F=1.67$, $p=0.113$).

*Relation of Surface Area to FW and to DW*

In order to facilitate conversion of the values determined in our study to other standardizations, for example FW or DW, the SA to FW and to DW relations were determined experimentally for *U. lactuca*. Sixty individuals of *U. lactuca* with SA ranging from 5 to 660 cm$^2$ were analyzed for FW and DW. SA was highly correlated to both FW ($R=0.991$) and DW ($R=0.988$), and showed linearly increasing trends: for FW, $y = 0.013x$; for DW, $y = 0.0026x$ (Figure 2-3).
implies, for example, that an *Ulva* frond of 100 cm² would have a FW of 1.30 g and a DW of 0.26 g. DW was 20% of corresponding FW.

**Figure 2-3.** Relation of fresh weight (FW), dry weight (DW) and surface area (SA) of *Ulva lactuca* (n=60). Trendlines (FW: $y = 0.013x$, $R^2 = 0.978$; DW: $y = 0.0026x$, $R^2 = 0.974$) are illustrated.

*Nutrient uptake kinetics*

*DIP uptake*

When exposed to DIP concentrations of <7 µmol·L⁻¹, *U. lactuca* depleted all the DIP within 24 h, which was faster than the DIP refreshment rate of the medium and indicates non-saturating DIP concentrations (Figure 2-4). When exposed to concentrations >7 µmol·L⁻¹ (13, 25 and 50 µmol·L⁻¹), DIP uptake was initially equal to available DIP but eventually decreased to become lower than DIP availability, indicating saturating concentrations. There was a strong correlation between residual DIP concentration and time of exposure ($R=0.84$). This time lag before a significant reduction in uptake was longer for lower concentrations of DIP availability, occurring on day 5 for 13 µmol·L⁻¹, day 3 for 25 µmol·L⁻¹ and day 2 for 50 µmol·L⁻¹ (Figure 2-4). DIP uptake at concentrations of 13 and 25 µmol·L⁻¹ converged after day 4. For the DIP availability level of 50 µmol·L⁻¹, however, uptake increased again between days 5 and 7 (Figure 2-4) before significantly decreasing between days 7 and 9 (Table 2-2). After day 9, DIP uptake rates at 50 µmol·L⁻¹ were
similar to those that had been reached by the 13 and 25 µmol·L⁻¹ treatments after day 4 (Figure 2-4). The maximum DIP surge uptake rate for *U. lactuca* was calculated to be 0.7±0.1 µmol·cm⁻²·d⁻¹ (average ± SD, n=3) in the 50 µmol·L⁻¹ treatment on day 1. The DIP maintenance uptake rate with filled storage, $V_M$ of DIP, was 0.07±0.03 µmol·cm⁻²·d⁻¹.

**Figure 2-4.** Mean DIP uptake (µmol·L⁻¹) ± SD (n=3) by *Ulva lactuca* in treatments with not-saturating (<7 µmol·L⁻¹) and saturating DIP concentrations (>7 µmol·L⁻¹) and daily supplied (pulsed) DIP.
**DIN uptake**

Similar to DIP uptake, the variations in DIN uptake were strongly correlated with time of exposure (R=0.987) and highly significant over time (ANOVA, \( df=9, F=44.59, p\leq0.001 \)), but not between treatments with varying DIP and \( \text{NH}_4^+ \) concentrations (ANOVA, \( df=23, F=0.57, p=0.944 \)). DIN uptake showed no correlation with DIP uptake (R=0.223) or \( \text{NH}_4^+ \) availability (R= -0.027). Mean DIN surge uptake was 12.5±5.2 \( \mu \text{mol·cm}^{-2}·\text{d}^{-1} \) (Figure 2-5). This surge uptake was followed by a highly significant decrease of DIN uptake on days 2 and 3, after which uptake continued without significant differences between time steps (Table 2-2). Mean initial DIN uptake rates with empty DIN-storage (\( V_S \)) dropped by 80.7 \% within the first 4 days, indicating DIN-storage had been filled and uptake rates only served to maintain metabolism (\( V_M \)). The \( V_M(DIN) \) was calculated to be 2.3±0.9 \( \mu \text{mol·cm}^{-2}·\text{d}^{-1} \).

**Figure 2-5.** Mean DIN uptake (\( \mu \text{mol·L}^{-1} \)) ± SD (n=24) of *Ulva lactuca* in saturating DIN concentration (5000 \( \mu \text{mol·L}^{-1} \)). No significant variances in DIN uptake between DIP treatments (A-H) were found (ANOVA, \( df=23, F=0.57, p=0.944 \)).
Storage capacity

**DIP storage**

Based on DIP uptake dynamics corresponding to the decline of uptake rates over time when exposed to nominal DIP concentration of 13–50 µmol·L⁻¹ (Figure 2-4), we calculated an internal DIP storage capacity of 0.7±0.1 µmol·cm⁻². The significant declines in DIP uptake found on days 5, 3, and 2 when exposed to DIP concentrations of 13, 25 and 50 µmol·L⁻¹, respectively (Table 2-2), indicate a time shift in DIP saturation from accumulation of DIP from the seawater medium on days 4, 2 and 1 (Figure 2-4). This occurred after a mean DIP concentration of 0.7±0.1 µmol·cm⁻² had been removed from the flasks (Figure 2-6).

### Table 2-2. Significances of differences (paired T-test) in DIP and DIN uptake (µmol·cm⁻²·d⁻¹) of Ulva lactuca in treatments with not-saturating (<7 µmol·L⁻¹) and saturating DIP concentrations (>7 µmol·L⁻¹) on consecutive days in a 10-day ‘pulse-and-chase’ experiment.

<table>
<thead>
<tr>
<th>Day</th>
<th>Pulsed DIP conc. (µmol·L⁻¹)</th>
<th>Pulsed DIN conc. (µmol·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.0</td>
<td>13.0</td>
</tr>
<tr>
<td>1 to 2</td>
<td>0.476</td>
<td>0.448</td>
</tr>
<tr>
<td>2 to 3</td>
<td>0.442</td>
<td>0.121</td>
</tr>
<tr>
<td>3 to 4</td>
<td>0.414</td>
<td>0.302</td>
</tr>
<tr>
<td>4 to 5</td>
<td>0.389</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>5 to 6</td>
<td>0.115</td>
<td>0.025</td>
</tr>
<tr>
<td>6 to 7</td>
<td>0.267</td>
<td>0.065</td>
</tr>
<tr>
<td>7 to 8</td>
<td>0.418</td>
<td>0.115</td>
</tr>
<tr>
<td>8 to 9</td>
<td>0.272</td>
<td>0.339</td>
</tr>
<tr>
<td>9 to 10</td>
<td>0.139</td>
<td>0.090</td>
</tr>
</tbody>
</table>

*for DIP n=3; for DIN n=24*
A total mean of 43.3±5.0 μmol·cm⁻² DIN was removed from all flasks by *U. lactuca* within 10 days. 29% of all removed DIN were taken up on day 1 during maximum surge uptake with a mean DIN accumulation of 12.5±1.9 μmol·cm⁻² (Figure 2-7). After no significant variations in daily DIN uptake occurred after day 3 (Table 2-2), we concluded that internal DIN storage had been filled. Accordingly, a DIN storage capacity of 22.9±7.0 μmol·cm⁻² was calculated.
DIP uptake showed no correlation (R=0.223) to DIN uptake, and the initial filling of the internal nutrient pools during $V_S$ indicated an N:P ratio of 20:1. After internal storage cells had been filled and uptake proceeded after reaching $V_M$, the N:P ratio levelled off to 30:1.

2.5 Discussion

*Ulva lactuca* has a maximum thickness of two cell layers; consequently, every cell is in contact with its environment, which makes it an ideal candidate to analyze nutrient uptake kinetics and apply standardized functions of SA for an accurate analysis of nutrient uptake. DIP uptake kinetics and saturating DIP storage capacity, as well as N:P uptake dynamics, were determined under fully controlled laboratory conditions. Growth and nutrient uptake rates in starved *U. lactuca* were not linear over time, and DIP uptake dynamics were clearly different between non-saturating

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**Figure 2-7.** Mean accumulation of daily removed DIN (μmol·cm$^{-2}$) ± SD (n=24) by *Ulva lactuca* in all treatments with DIP concentrations ranging from 1 to 50 μmol·L$^{-1}$.  

\[
\text{Trend: } \quad y = 12.564x^{0.545} \\
R^2 = 0.999 
\]
(<7 µmol·L⁻¹) and saturating (>7 µmol·L⁻¹) DIP concentrations. As growth was not significantly different in treatments with different DIP concentrations, the range of offered nominal DIP concentration (1-50 µmol·L⁻¹) was not the decisive factor for increasing surface area (SA). The increase of total SA is in agreement with reported growth rates for *U. lactuca* (Fortes & Lüning 1980, Fujita 1985). Determination of SA, as a non-destructive method to infer growth, showed a gradual decrease in growth (Figure 2-2).

Two phases of transient responses to nutrient pulses were measured: (1) an initial surge uptake (sensu Conway et al. 1976) after starvation and (2) maintenance (steady state) uptake rates, as measured in continuous cultures (Probyn & Chapman 1982).

In agreement with the total DIP availability in different treatments, VS was maintained until the ISC had been filled and this is supported by the significant decrease of DIP uptake found in all saturating DIP concentrations (Figure 2-4, Table 2-2) This initial filling of internal nutrient pools under VS has often been described for nutrient-starved seaweeds (e.g. Fujita 1985, Harrison et al. 1989, Dy & Yap 2001). Although maximum VS for DIP could not be determined accurately, since all offered DIP was depleted in all the treatments on day 1 (Figure 2-4), an approximation of 0.66±0.12 µmol·cm⁻²·d⁻¹ appears realistic. The VM,DIP for maintenance DIP requirements in *U. lactuca* was calculated as 0.07±0.04 µmol·cm⁻²·d⁻¹, supported by the DIP uptake rates found in *U. lactuca* exposed to nominal concentrations of 7 and 50 µmol·L⁻¹ (Figure 2-6).

*U. lactuca* exposed to 7 µmol·L⁻¹ did not show any significant variations in DIP uptake rates over time (Table 2-2) and removal of DIP from the flasks remained approximately 100 %. The average DIP uptake relative to SA in this treatment was 0.07±0.03 µmol·cm⁻² on day 10, which is equivalent to VM and would approximately account for 100 % of the offered DIP over the 10-day assay (Figure 2-4). *Ulva lactuca* exposed to the DIP concentration of 50 µmol·L⁻¹ showed similar uptake rates on days 3 to 5, after the surge uptake, and on days 9 to 10, after increased DIP uptake had peaked on day 7 (Figure 2-4). This oscillation in the uptake of DIP over a five-day interval in *U. lactuca* exposed to 50 µmol·L⁻¹ DIP could have been caused by various interacting mechanisms,
such as luxury uptake, over-compensation or stress-related responses. In general, luxury uptake describes the ability of plants to store extra nutrients (for seaweeds, e.g. Harrison & Hurd 2001, Naldi & Viaroli 2002) without prior starvation (Eixler et al. 2006). Factors that influence luxury uptake are poorly understood, but external phosphorus concentration is correlated with accumulation and utilization of acid-soluble polyphosphates (ASP) and acid-insoluble polyphosphates (AISP) in microalgae (Powell et al. 2009). Some of these polyphosphates, which are normally involved in metabolic processes, are considered to also form part of the internal short-term phosphorus storage with turnover times of approximately five days (Powell et al. 2009). This 5-day period perfectly matches our finding of re-occurring enhanced DIP uptake rates (Figure 3) when *U. lactuca* was exposed to DIP concentrations of 50 µmol·L⁻¹. Alternatively, over-compensation can be considered as an explanation for oscillating DIP uptake (Cembella et al. 1984). Over-compensation of internally stored phosphorus can occur when phosphorus-starved algae are re-introduced to high concentrations of external DIP (Aitchison & Butt 1973, Chopin et al. 1997). Finally, oscillating uptake can also reflect a stress reaction to high external nutrient concentration (e.g. Fourcroy 1999, Jiang & Yu-Feng 2008), allowing for mobilization and uptake of sufficient DIP to provide temporary relief.

The time-shifting of DIP saturation found in *U. lactuca* exposed to residual concentrations of 13, 25 and 50 µmol·L⁻¹ among days 5, 3 and 2, respectively, clearly suggests that internal DIP storages had been filled before *V_s* turned to *V_M* (Figure 2-4). The calculated ISC for DIP in *U. lactuca* was 0.73±0.13 µmol·cm⁻². This storage can be utilized during times of low external DIP availability (Chapman & Craigie 1977, Pederson & Borum 1996) and considering the *V_M* value (0.07±0.04 µmol·cm⁻²·d⁻¹), a fully filled internal DIP storage system can fuel metabolic processes for 10 days. This corresponds with results from Fujita (1985), which showed inhibited growth of *U. lactuca* after 10 days of exposure to nutrient-depleted seawater.

Similar to DIP uptake, the mean DIN uptake gradually decreased until day 3, and no significant variations in DIN uptake rates were observed afterwards (Table 2-2), indicating a steady
state uptake for DIN. The calculated value of the $V_M$ for DIN in *U. lactuca* (2.3±0.9 µmol·cm$^{-2}$·d$^{-1}$) was approximately 20% of the $V_S$. It should be noted that the presence of ammonium ($\text{NH}_4^+$) can influence the uptake of nitrate in *U. lactuca* (Holdt & Kraan 2011, Ale et al. 2011). In our study, daily DIN uptake was not significantly affected ($R=-0.027$) by the presence of ammonium ($\text{NH}_4^+$). This, in combination with the low $\text{NH}_4^+:\text{DIN}$ ratios and the full removal of $\text{NH}_4^+$ in all treatments throughout the experiment (not depicted), give us full confidence that the presence of ammonium had no significant effects on DIP uptake kinetics.

*Ulva lactuca* showed effects of DIN saturation in all treatments on day 3, represented by a significant decline in DIN uptake rates, which indicated that internal N pools had been filled. Thus, a mean DIN storage capacity of 22.9±7.0 µmol·cm$^{-2}$ was calculated. DIN-ISC was a 10-fold higher than DIN-$V_M$, which is also in agreement with findings of inhibited growth in *U. lactuca* after exposure to nutrient-depleted seawater for 10 days (Fujita 1985).

Uptake rates between starved ($V_S$) to saturated state ($V_M$) differed by a magnitude of 10 for DIP and 5 for DIN. This aspect can reflect the ecological competitiveness for DIN (pulses) in opportunistic seaweed (after Littler & Littler 1980), such as *U. lactuca*. Alternatively, we can conclude that *U. lactuca* was successfully starved of nutrients in the precondition phase of our experiment, independent of its nutritional history. There was no correlation between rates of uptake of DIP and DIN ($R=0.223$), which is contrary to the strong evidence of co-limitation in DIP and DIN in the brown macroalga *Fucus vesiculosus* (Perini & Bracken 2014) and the red macroalgae (Rhodophyta) *Palmaria palmata* (Chapter 6 in this thesis).

Based on $V_M$, an optimal N:P ratio for *U. lactuca* was estimated to be 30:1, consistent with a mean N:P ratio estimated for marine macrophytes (Atkinson & Smith, 1983). Consequently, *U. lactuca* is twice as likely to suffer from N-limitation as P-limitation when considering the Redfield ratio, the relatively consistent stoichiometric atomic ratio of N and P (16:1) found in coastal regions to open ocean. Yet, *U. lactuca* most commonly inhabits coastal zones, which can receive considerable nutrient pulses with high N:P ratios from land-based anthropogenic activities through
rivers (Jickells 1998) or near-shore fish aquaculture (Pearson & Black 2001). Burson et al. (2016) reported an offshore gradient from DIP to DIN limitation in the North Sea during spring, with a nearshore N:P ratio of 375:1 and a 1:1 ratio in the central North Sea. Exactly such a nearshore nutrient stoichiometry can allow *U. lactuca* to thrive, given its low DIP requirements.

A set-up with comparable initial physiological conditions for all organisms is a key element for representative laboratory experiments. *Ulva lactuca* has been reported to be able to grow for 9 days under external nitrogen depletion (Fujita 1985). Accordingly, we assumed that 10 days of nutrient starvation (P and N) would result in *U. lactuca* individuals with similar physiological status with respect to depletion of internal P and N pools, which would lead to representative and comparable responses by all individuals to varying DIP treatments. This assumption is supported by the reproducible DIP and DIN uptake kinetics found in our experiments. Our experimental results moreover confirm the period of time that *U. lactuca* is able to grow under nutrient starvation: using the experimentally determined Vₐ values, ISC depletion is calculated to take exactly 10 days.

In this study we offer correlation factors for SA with FW and DW in *U. lactuca*, which enables conversions between these standardization units and allows for accurate comparison of data to other studies. Moreover, our standardized data adds to the physiological understanding of *U. lactuca*, enables estimation of ecological effects on nutrient availability and can contribute to development and modification of applications in a bio-based economy. In order to predict the efficiency of *U. lactuca* as efficient biofilter, for example in land-based tank systems (e.g. Robertson-Andersson et al. 2008, Copertino et al. 2009) or in situ applied biofilters at inlets of cooling water for power plants, information about uptake kinetics are indispensable and can help to control effluent and productivity for environmentally responsible practices. Despite the quickly filled ISC and the corresponding declines in nutrient uptake rates of approximately 90 % for DIP and 80 % for DIN in saturating concentrations, saturated state uptake rates in *U. lactuca* can significantly contribute to excess nutrient uptake, leading to less eutrophic waters and production of valuable biomass for food, feed and energy.
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