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UPTAKE KINETICS AND STORAGE CAPACITY OF DISSOLVED INORGANIC PHOSPHORUS AND CORRESPONDING N:P DYNAMICS IN *ULVA LACTUCA* (CHLOROPHYTA)

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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 (V_S) of 0.67±0.10 µmol · cm^2 · d^{-1} and (2) a steady state uptake (V_M) of 0.07±0.03 µmol · cm^2 · d^{-1}. Mean internal storage capacity (ISC_P) of 0.73±0.13 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±1.90 µmol · cm^2 · d^{-1}, V_M=2.26±0.86 µmol · cm^2 · d^{-1}, and ISC_N=22.90±6.99 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.

Keywords (5):
Ulva lactuca - uptake kinetics - phosphate uptake - nitrate uptake - storage capacity

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 dissolved inorganic nitrogen (DIN). 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 bioremediation applications (Gao et al.2017). Furthermore, an insight into nutrient management of seaweeds opens opportunities to forecast ecological impacts of nutrient limitation and shifts in limitation from one element to another, all of which can significantly affect the internal composition, physiology and growth of seaweeds (Pederson and 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 and Yap 2001). The uptake rates gradually decrease as internal nutrient pools in cytoplasm and vacuoles are filled (Rosenberg et al. 1984, Fujita 1985). When internal nutrient concentrations are constant and relative uptake rates of nutrients remain relatively stable over time, $V_{M,}$ which is considered equal to the rate of assimilation, is attained (Taylor and Rees 1999, Barr et al. 2004). The previously filled nutrient pools can be utilized at times of low external nutrient availability (Probyn and Chapman 1982, Pederson and Borum 1996).

Ulva lactuca (Linnaeus), a seaweed in the division Chlorophyta, is found worldwide and is prolifically abundant where nutrients are readily available (Morand and Merceron 2005). *U. lactuca* has been identified as a promising species in water treatment facilities (biofilters) and in integrated multi-trophic aquaculture (IMTA) systems (e.g. Cohen and 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 and Ohno 1998, Sahoo 2000, Thangaraju 2008, Holdt and 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, Gao et al. 2017) 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 and 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:V), 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 · L^{-1} · 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).

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 · L^{-1} · 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 (PO_4^{3-}; 0.008 µmol · L^{-1}), ammonium (NH_4^+; 0.022 µmol · L^{-1}) and nitrate (NO_3^-; 0.003 µmol · L^{-1}) concentrations. After pasteurization of the seawater (80 °C for 2h), 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 ($(NH_4)H_2PO_4$) and potassium nitrate (KNO_3) as sources for PO_4^{3-}, NH_4^+ and NO_3^- 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 PO_4^{3-} and NH_4^+. The NO_3^- concentration was set to 5000 µmol · L^{-1} (Table 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.

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 (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 after complexation with sulphanylamide and naphtylethylenediamine (Grasshoff et al. 1983). Ammonium (NH_4^+) was measured at 630 nm after the formation of an indophenol blue complex with phenol and sodium hypochlorite at pH 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). 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 (cm^2) and time (d), and calculated using the following equation:

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

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 (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 Vs and V_M were calculated are indicated in Figure 1. V_S was calculated from uptake rates in a non-limiting nutrient concentration using the following equation:

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

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:

$$\mathbf{ISC_{N,P} = \Sigma(i \epsilon V_S) - n\ V_M},$$

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 1).

Surface area analysis

U. 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 SA and to exclude non-pigmented (dead) areas and holes, 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 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 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:

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

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, 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^{TM} 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.

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, $F_{1,23} = 6.20$, $p \leq 0.001$). 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). No significant differences in growth between the different DIP treatments were observed (ANOVA, $F_{7,23} = 4.12$, $p = 0.087$).

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

223 DW, y = 0.0026x (Figure 3). This implies, for example, that an *Ulva* frond of 100 cm^2 would
224 have a FW of 1.30 g and a DW of 0.26 g. DW was 20% of corresponding FW.
225
226
227 *Nutrient uptake kinetics*
228 DIP uptake
229 The maximum DIP surge uptake rate for *U. lactuca* was calculated to be 0.7±0.1
230 $\mu mol \cdot cm^{-2} \cdot d^{-1}$ (average ± SD, n=3), while the mean DIP maintenance uptake rate with
231 filled storage, V_M of DIP, was 0.07±0.03 $\mu mol \cdot cm^{-2} \cdot d^{-1}$.
232 *U. lactuca* exposed to DIP concentrations <7 $\mu mol \cdot L^{-1}$ depleted all the DIP within 24
233 h, which was faster than the DIP refreshment rate of the medium and indicates non-saturating
234 DIP concentrations (Figure 4). When exposed to 7 $\mu mol \cdot L^{-1}$, *U. lactuca* did not show any
235 significant variations in DIP uptake rates over time (Table 2,) and removal of DIP from the
236 flasks remained approximately 100% (Figure 4). The average DIP uptake relative to SA in
237 this treatment was 0.07±0.03 $\mu mol \cdot cm^{-2}$ on day 10, which is equivalent to V_M and
238 approximately accounts for 100% of the offered DIP over the 10-day assay. When exposed to
239 concentrations >7 $\mu mol \cdot L^{-1}$ (13, 25 and 50 $\mu mol \cdot L^{-1}$), DIP uptake was initially equal to
240 available DIP, but eventually decreased to become lower than DIP availability, indicating
241 saturating concentrations. There was a strong correlation between residual DIP concentration
242 and time of exposure (R = 0.84). This time lag before a significant reduction in uptake was
243 longer for lower concentrations of DIP availability, occurring on day 5 for 13 $\mu mol \cdot L^{-1}$, day
244 3 for 25 $\mu mol \cdot L^{-1}$ and day 2 for 50 $\mu mol \cdot L^{-1}$ (Figure 4). DIP uptake at concentrations of 13
245 and 25 $\mu mol \cdot L^{-1}$ converged after day 4. For the DIP availability level of 50 $\mu mol \cdot L^{-1}$,
246 however, uptake increased again between days 5 and 7 (Figure 4) before significantly
247 decreasing between days 7 and 9 (Table 2). After day 9, DIP uptake rates at 50 $\mu mol \cdot L^{-1}$

248 were similar to those that had been reached by the 13 and 25 µmol · L^{-1} treatments after day 4
249 (Figure 4).
250
251 DIN uptake
252 Similar to DIP uptake, the variations in DIN uptake were strongly correlated with
253 time of exposure (R = 0.987) and highly significant over time (ANOVA, $F_{7,79} = 44.59$, $p \leq$
254 0.001), but not between treatments with varying DIP and NH_4^+ concentrations (ANOVA,
255 $F_{7,23} = 0.57$, $p = 0.944$). DIN uptake showed no correlation with DIP uptake (R = 0.223) or
256 NH_4^+ availability (R = -0.027). Mean DIN surge uptake was 12.5±1.9 µmol · cm^{-2} · d^{-1}
257 (Figure 5). This surge uptake was followed by a highly significant decrease of DIN uptake on
258 days 2 and 3, after which uptake continued without significant differences between time steps
259 (Table 2). Mean initial DIN uptake rates with empty DIN-storage (V_S) dropped by 80.7%
260 within the first 4 days, indicating DIN-storage had been filled and uptake rates only served to
261 maintain metabolism (V_M). The $V_{M\ (DIN)}$ was calculated to be 2.3±0.9 µmol · cm^{-2} · d^{-1}.
262
263 *Storage capacity*
264 DIP storage
265 Based on DIP uptake dynamics corresponding to the decline of uptake rates over time,
266 when exposed to nominal DIP concentration of 13–50 µmol · L^{-1} (Figure 4), we calculated an
267 internal DIP storage capacity of 0.7±0.1 µmol · cm^{-2}. The significant declines in DIP uptake
268 found on days 5, 3, and 2 when exposed to DIP concentrations of 13, 25 and 50 µmol · L^{-1},
269 respectively (Table 2), indicate a time shift in DIP saturation from accumulation of DIP from
270 the seawater medium on days 4, 2 and 1 (Figure 4). This occurred after a mean DIP
271 concentration of 0.7±0.1 µmol · cm^{-2} had been removed from the flasks (Figure 6).
272
273 DIN storage

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 7). After no significant variations in daily DIN uptake occurred after day 3 (Table 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.

N:P dynamics

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.

Discussion

U. 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. Growth and nutrient uptake rates in starved *U. lactuca* were not linear over time, and DIP uptake dynamics were clearly different between non-saturating (<7 µmol · L^{-1}) and saturating (>7 µmol · L^{-1}) DIP concentrations.

Growth

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 and Lüning 1980, Fujita 1985). Determination of SA, as a non-destructive method to infer growth, showed a gradual decrease in growth (Figure 2), which aligns with reported results for *U. lactuca* by other authors (Ale et al. 2011). This decrease in growth may be caused by a shift to a reproductive state, inhibiting vegetative growth in *U. lactuca* (Bruhn et al. 2011).

Nutrient uptake dynamics

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 and Chapman 1982).

DIP uptake

In agreement with the total DIP availability in different treatments, V_S was maintained until the ISC had been filled (Figure 4, Table 2). This initial filling of internal nutrient pools under V_S has often been described for nutrient-starved seaweeds (e.g. Fujita 1985, Harrison et al. 1989, Dy and Yap 2001). Although maximum V_S for DIP could not be determined accurately, since all offered DIP was depleted in all the treatments on day 1 (Figure 4), an approximation of 0.66±0.12 µmol · cm^{-2} · d^{-1} appears realistic. The $V_{M\,(DIP)}$ for maintenance DIP requirements in *U. lactuca* was calculated as 0.07±0.04 µmol · cm^{-2} · d^{-1}. A similar DIP uptake was found by Gao et al. (2017) for a mutant strain of *Ulva rigida*, with an uptake of 5.7±0.04 µmol · g FW^{-1} · d^{-1}, which resembles an uptake of 0.06±0.04 µmol · cm^{-2} · d^{-1}, given our correlation factors (Figure 3).

The oscillation in DIP uptake over a five-day interval, when exposed to DIP concentration of 50 µmol · L^{-1}, 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 and Hurd 2001, and Naldi and 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 $\mu mol \cdot L^{-1}$. 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 and 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 and Yu-Feng 2008), allowing for mobilization and uptake of sufficient DIP to provide temporary relief.

DIP storage capacity

The calculated internal storage capacity (ISC) for DIP in *U. lactuca* was 0.73±0.13 $\mu mol \cdot cm^{-2}$. This storage can be utilized during times of low external DIP availability (Chapman and Craigie 1977, Pederson and Borum 1996) and considering the V_M value (0.07±0.04 $\mu mol \cdot cm^{-2} \cdot d^{-1}$), 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.

DIN uptake

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. DIN uptake was consistent with uptake rates in other published research on *U. lactuca*. Ale et al. (2011) reported nitrate uptake of ~70 µmol · g DW^{-1} · d^{-1} for *U. lactuca*, which is an equivalent to ~3.5 µmol · cm^{-2} · d^{-1}, given our correlation factors (Figure 3). It should be noted that the presence of ammonium (NH_4^+) can influence the uptake of nitrate in *U. lactuca* (Holdt and Kraan 2011, Ale et al. 2011). In our study, daily DIN uptake was not significantly affected (*R*=-0.027) by the presence of ammonium (NH_4^+). This, in combination with the low NH_4^+: DIN ratios and the full removal of 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 in *U. lactuca*.

DIN storage

A mean DIN storage capacity of 22.9±7.0 µmol · cm^{-2} was calculated. Thus the 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).

N:P dynamics

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 and 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 and Bracken 2014) and the red macroalga (Rhodophyta) *Palmaria palmata* (Lubsch and Timmermans, unpublished).

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 and 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 and 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.

Starvation prior to determination of DIP and DIN uptake kinetics

A set-up with comparable initial physiological conditions for all organisms is a key element for representative laboratory experiments. *U. 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 V_M values, ISC depletion is calculated to take exactly 10 days.

399

400 *Applications and Implications*

401 In this study we offer correlation factors for SA with FW and DW in *U. lactuca*,
402 which enables conversions between these standardization units and allows for accurate
403 comparison of data to other studies.

404 Moreover, our standardized data adds to the physiological understanding of *U.*
405 *lactuca*, enables estimation of ecological effects on nutrient availability and can contribute to
406 development and modification of applications in a bio-based economy. In order to predict the
407 efficiency of *U. lactuca* as efficient biofilter, for example in land-based tank systems (e.g.
408 Robertson-Andersson et al. 2008, Copertino et al. 2009) or in *situ* applied biofilters at inlets
409 of cooling water for power plants, information about uptake kinetics are indispensable and
410 can help to control effluent and productivity for environmentally responsible practices.
411 Despite the quickly filled ISC and the corresponding declines in nutrient uptake rates of
412 approximately 90% for DIP and 80% for DIN in saturating concentrations, saturated state
413 uptake rates in *U. lactuca* can significantly contribute to excess nutrient uptake, leading to
414 less eutrophic waters and production of valuable biomass for food, feed and energy.

415

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422

423 **References**

424 Aitchison, P.A. & Butt, V.S. 1973. The relation between the synthesis of inorganic
425 polyphosphate and phosphate uptake by *Chlorella vulgaris*. *J. Exp. Bot.* 24:497-510.

426 Ale, M.T., Mikkelsen, J.D. & Meyer, A.S. 2011. Differential growth response of *Ulva*
427 *lactuca* to ammonium and nitrate assimilation. *J. Appl. Phycol.* 23:345-351.

428 Atkinson, M.J. & Smith, S.V. 1983. C:N:P ratios of benthic marine plants. Limnol.
429 Oceanogr. 28:568-574.

430 Barr, N.G.,Tijssen, R.J., Rees, T.A.V. 2004. The contrasting effects of methionine
431 sulfoximine on uptake and assimilation of ammonium by *Ulva intestinales*. *J. Phycol.*
432 40:697-704.

433 Bruhn, A., Dahl, J., Nielsen, H.B., Nikolaisen, I., Rasmussen, M.B., Markager, S., Olesen, B.,
434 Arias, C. & Jensen, P.D. 2011. Bioenergy potential of *Ulva lactuca*: biomass yield, methane
435 production and combustion. Bioresour. Technol. 102:2595-2604.

436 Burson, A., Stomp, M., Akil, L., Brussaard, C.P.D. & Huisman, J. 2016. Unbalanced
437 reduction of nutrient loads has created an offshore gradient from phosphorus to nitrogen
438 limitation in the North Sea. *Limnol. Oceanogr.* 61:869-888.

439 Cembella, A., Antia, N.J. & Harrison, P.J. 1984. The utilization of inorganic and organic
440 phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary
441 perspective. *CRC Crit. Rev. Microbiol.* 10:317-391.

442 Chopin, T., Lemal, H. & Halcrow, K. 1997. Polyphosphates in the red macroalga *Chondrus*
443 *crispus* (Rhodophyceae). *New Phytologist* 135:587-594.

444 Cohen, I. & Neori, A. 1991. *Ulva lactuca* biofilters for marine fishpond effluents I.
445 Ammonium uptake kinetics and nitrogen content. *Bot. Mar.* 34:475-482.

446 Conway, H.L., Harrison, P.J. & Davis, C.O. 1976. Marine diatoms grown in chemostats
447 under silicate and ammonium limitation. II. Transient response of *Skeletonema costatum* to a
448 single addition of the limiting nutrient. *Mar. Biol.* 35:187-189.

449 Copertino, M.S., Tormena, T. & Seeliger, U. 2009. Biofiltering efficiency, uptake and
450 assimilation rates of *Ulva clathrata* (Roth) Agardh (Chlorophyceae) cultivated in shrimp
451 aquaculture wastewater. *J. Appl. Phycol.* 21:31-45.

452 Critchley, A.T. & Ohno, M. 1998. Seaweed Resources of the World. In: A.T. Critchley and
453 M. Ohno (eds.) JICA.

454 Dy, D.T. & Yap, H.T. 2000. Surge ammonium uptake of the cultured seaweed, *Kappaphycus*
455 *alvarezii* (Doty) Doty (Rhodophyta: Gigartinales). *J. Exp. Mar. Biol. Ecol.* 265:89-100.

456 Eixler, S., Karsten, U. & Selig, U. 2006. Phosphorus storage in *Chlorella vulgaris*
457 (Trebouxiophyceae, Chlorophyta) cells and its dependence on phosphate supply. *Phycologia*
458 45:53-60.

459 Fortes, M.D & Lüning, K. 1980. Growth rates of North Sea macroalgae in relation to
460 temperature, irradiance and photoperiod. *Helgoländer Meeresunters.* 34:15-29.

461 Fourcroy, P. 1999. Iron and oxidative stress in plants. In: M.F. Smallwood, C.M. Calvert,
462 D.J. Bowles (eds.) *Plant Responses to Environmental Stress*. BIOS, Oxford pp. 51-57.

463 Fujita, R.M. 1985. The role of nitrogen status in regulating transient ammonium uptake and
464 nitrogen storage by macroalgae. *J. Exp. Mar. Biol. Ecol.* 99:283-301.

465 Gao, G., Clare, A.S., Rose, C. & Caldwell, G.S. 2017. Reproductive sterility increases the
466 capacity to exploit the green seaweed *Ulva rigida* for commercial applications. Algal Res.
467 24:64-71.

468 Gevaert, F., Davoult, D., Creach, A., Kling, R., Janquin, M.A., Seuront, L. & Lemoine,Y.
469 2001. Carbon and nitrogen content of *Laminaria saccharina* in the eastern English Channel:
470 biometrics and seasonal variations. *J. Mar. Biol. Assoc. U.K.* 81:727-734.

471 Gonen, Y., Kimmel, E. & Friedlander, M. 1995. Diffusion boundary layer transport in
472 *Gracilaria conferta* (Rhodophyta). *J. Phycol.* 31:768-773.

473 Gordon, D.M., Birch, P.B. & McComb, A.J. 1981. Effects of inorganic phosphorus and
474 nitrogen on the growth of an estuarine *Cladophora* culture. *Bot. Mar.* 24:93-106.

475 Gordillo, F.J.L., Dring, M.J. & Saidge, G. 2002. Nitrate and phosphate uptake characteristics
476 of three species of brown algae cultured at low salinity. *Mar Ecol. Prog. Ser.* 234:111-118.

477 Grasshoff, K and H.P. Hansen. 1983. *Methods of seawater analysis*. Verlag Chemie,
478 Weinheim, 419 pp.

479 Harrison, P.J., Parslow, J.S. & Conway, H.L. 1989. Determination of nutrient uptake kinetic
480 parameters: a comparison of methods. *Mar. Ecol. Prog. Ser.* 52:301-312.

481 Harrison, P.J. & Hurd, C.L. 2001. Nutrient physiology of seaweeds: application of concepts
482 to aquaculture. *Cah. Biol. Mar.* 42:71-82.

483 Helder, W & de Vries, R. 1979. An automatic phenol-hypochlorite method for the
484 determination of ammonia in sea- and brackish waters. *Neth. J. Sea Research* 13:154-160.

485 Holdt, S.L. & Kraan, S. 2011. Bioactive compounds in seaweed: functional food applications
486 and legislation. *J. Appl. Phycol.* 23:543-597.

487 Hurd, C.L. 2000. Water motion, marine macroalgal physiology, and production. *J. Phycol.*
488 36:453-472.

Jiang, Y. and Yu-Feng, Y. 2008. Physiological and biochemical response of seaweed *Gracilaria lemaneiformis* to concentration changes of N and P. *J. Exp. Mar. Biol. Ecol.* 367:142-148.

Jickells, T.D. 1998. Nutrient biogeochemistry of the coastal zone. *Science* 228:217-222.

Kain, J.M. 1987. Seasonal growth and photoinhibition in *Plocamium cartilagineum* (Rhodophyta) off the Isle of Man. *Phycologia* 26:88-99.

Koroleff, F. 1969. Determination of total nitrogen in natural waters by means of persulfate oxidation [in Swedish]. In: C.M. Pap (eds.) *Int. Counc. Explor. Sea* (ICES), revised 1970/C:8.

Littler, M.M. & Littler, D.S. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *Am. Nat.* 116:25-44.

Morand, P. & Merceron, M. 2005. Macroalgal population and sustainability. *J. Coast. Res.* 21:1009-1020.

Murphy, J. & Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analyt. Chim. Acta* 27:31-36.

Naldi, M. & Viaroli, P. 2002. Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Po River Delta, Italy). *J. Exp. Mar. Biol. Ecol.* 269:65-83.

Neori, A., Msuya, F.E., Shauli, L., Schuenhoff, A., Kopel, F. & Shpigel, M. 2003. A novel three-stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculture. *J. Appl. Phycol.* 15:543-553.

510 Pearson, T.H. & Black, K.D. 2001. The environmental impacts of marine fish cage culture. *In*
511 K.D. Black (ed.) *Environmental Impacts on Aquaculture*. Sheffield Academic Press,
512 Sheffield, 31 pp.

513 Pederson, M.F. & Borum, J. 1996. Nutrient control of algal growth in estuarine waters.
514 Nutrient limitation and importance of nitrogen requirements and nitrogen storage among
515 phytoplankton and species of macroalgae. *Mar. Ecol. Prog. Ser.* 142:261-272.

516 Pederson, M.F., Borum, J. & Fotel, F.L. 2010. Phosphorus dynamics and limitation of fast-
517 and slow-growing temperate seaweeds in Oslofjord, Norway. *Mar. Ecol. Prog. Ser.* 399:103-
518 115.

519 Perini, V. & Bracken, M.E.S. 2014. Nitrogen availability limits phosphorus uptake in an
520 intertidal macroalga. *Oecologia* 175:667-676.

521 Powell, N., Shilton, A., Chisti, Y. & Pratt, S. 2009. Towards a luxury uptake process via
522 macroalgae – defining the polyphosphate dynamics. *Water Res. 43*:4207-4213.

523 Probyn, T.A. & Chapman, A.R.O. 1982. Nitrogen uptake characteristics of *Chordaria*
524 *flagelliformis* (Phaephyta) in batch mode and continuous mode experiments. *Mar. Biol.*
525 71:129-133.

526 Robertson-Andersson, D.V., Potgieter, M., Hansen, J., Bolton, J.J., Troell, M., Anderson,
527 R.J., Halling, C. & Probyn, T. 2008. Integrated seaweed cultivation on an abalone farm in
528 South Africa. *J. Appl. Phycol.* 20:579-595.

529 Rosenberg, G., Probyn, T.A. & Mann, K.H. 1984. Nutrient uptake and growth kinetics in
530 brown seaweeds: response to continuous and single additions of ammonium. *J. Exp. Mar.*
531 *Biol. Ecol.* 80:125-146.

532 Runcie, J.W., Ritchie, R.J. & Larkum, A.W. 2004. Uptake kinetics and assimilation of
533 phosphorus by *Catenella nipae* and *Ulva lactuca* can be used to indicate ambient phosphate
534 availability. *J. Appl. Phycol.* 16:181-194.

535 Sahoo, D. 2000. *Farming in the Ocean: seaweeds cultivation and utilization*. Aravali Books
536 International, New Delhi, 42 pp.

537 Stegenga, H. & Mol, I. 1983. *Flora van Nederlandse Zeewieren*. 33rd ed. KNNV,
538 Amsterdam, 263 pp.

539 Taylor, M.W., Rees, T.A.V. 1999. Kinetics of ammonium assimilation in two seaweeds,
540 Enteromorpha sp. (Chlorophyceae) and Osmundaria colensoi (Rhodophyceae). *J. Phycol*.
541 35:740-746.

542 Thangaraju, N. 2008. Efficacy of seaweed liquid fertilizers (SLFs) of *Sargassum wightii*
543 Grev. And *Ulva lactuca* on the growth and yield of paddy (Oryza sativa L. var ADT 36)
544 under greenhouse conditions. In *Proceeding of the 11th International Conference on Applied*
545 *Phycology*. Galway-Ireland, 2008.

546 Tsagkamilis, P., Danielidis, D., Dring, M.J. & Katsaros, C. 2010. Removal of phosphate by
547 the green seaweed *Ulva lactuca* in a small-scale sewage treatment plant (Ios Island, Aegean
548 Sea, Greece). *J. Appl. Phycol.* 22:331-339.

549 Wheeler, W.N. 1988. Algal productivity and hydrodynamics – a synthesis. *Prog. Phycol. Res.*
550 6:23-58.

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554 **Figure captions**

555 **Figure 1**. Example graph of nutrient uptake over time (days) illustrated with surge uptake
556 (V_S), maintenance uptake (V_M), internal storage capacity (ISC), and d_1 and d_2 as difference
557 operator between days, after a significant decrease in nutrient uptake occurs.

558 **Figure 2**. Mean surface area (SA) ± SD (n=24) of *Ulva lactuca* on day 1, 3, 5, 7, and 10 of all
559 treatments. No significant differences in growth between treatments with different DIP
560 concentrations were found (ANOVA, $F_{7,23} = 1.67$, $p = 0.113$).

561 **Figure 3**. Relation of freshweight (FW), dryweight (DW) and surface area (SA) of *Ulva*
562 *lactuca* (n = 60). Trendlines (FW: $y = 0.013x$, $R^2 = 0.978$; DW: $y = 0.0026x$, $R^2 = 0.974$) are
563 illustrated.

564 **Figure 4**. Mean DIP uptake (µmol · L^{-1}) ± SD (n = 3) by *Ulva lactuca* in treatments with not-
565 saturating (<7 µmol · L^{-1}) and saturating DIP concentrations (>7 µmol · L^{-1}) and daily offered
566 (pulsed) DIP.

567 **Figure 5**. Mean DIN uptake (µmol · L^{-1}) ± SD (n = 24) of *Ulva lactuca* in saturating DIN
568 concentration (5000 µmol · L^{-1}). No significant variances in DIN uptake between DIP
569 treatments were found (ANOVA, $F_{7,23} = 0.57$ $p = 0.944$).

570 **Figure 6**. Mean accumulation of daily removed DIP (µmol · cm^{-2}) ± SD (n = 3) by *Ulva*
571 *lactuca* in not-saturating (<7 µmol · L^{-1}) and saturating (>7 µmol · L^{-1}) treatments.

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

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576 **Tables**

577 **Table 1**. Daily 'pulsed' DIP and DIN (in µmol · L^{-1}) to *Ulva lactuca* in a 10 day uptake
578 experiment.

Treatment	Phosphate	Nitrate	Ammonium
A	1.0	5000	1.0
B	1.5	5000	1.5
C	2.5	5000	2.5
D	4.0	5000	4.0
E	7.0	5000	7.0
F	13.0	5000	13.0
G	25.0	5000	25.0
H	50.0	5000	50.0

in µmol · L^{-1}

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582 **Table 2**. Significances of differences (paired T-test) in DIP and DIN uptake (µmol · cm^{-2} · d^{-1})
583 of *Ulva lactuca* in treatments with not-saturating (<7 µmol · L^{-1}) and saturating DIP.

Day	**Pulsed DIP conc. (µmol · L^{-1})**				**Pulsed DIN conc. (µmol · L^{-1})**
	7.0	13.0	25.0	50.0	5000
1 to 2	0.476	0.448	0.305	**0.005**	**<0.001**
2 to 3	0.442	0.121	**0.006**	0.317	**0.048**

3 to 4	0.414	0.302	0.061	0.007	0.109
4 to 5	0.389	**0.001**	0.010	0.090	0.083
5 to 6	0.115	0.025	0.075	0.302	0.248
6 to 7	0.267	0.065	0.061	0.146	0.317
7 to 8	0.418	0.115	0.045	0.045	0.272
8 to 9	0.272	0.339	0.161	0.024	0.092
9 to 10	0.139	0.090	0.495	0.424	0.335

for DIP n = 3; for DIN n = 24

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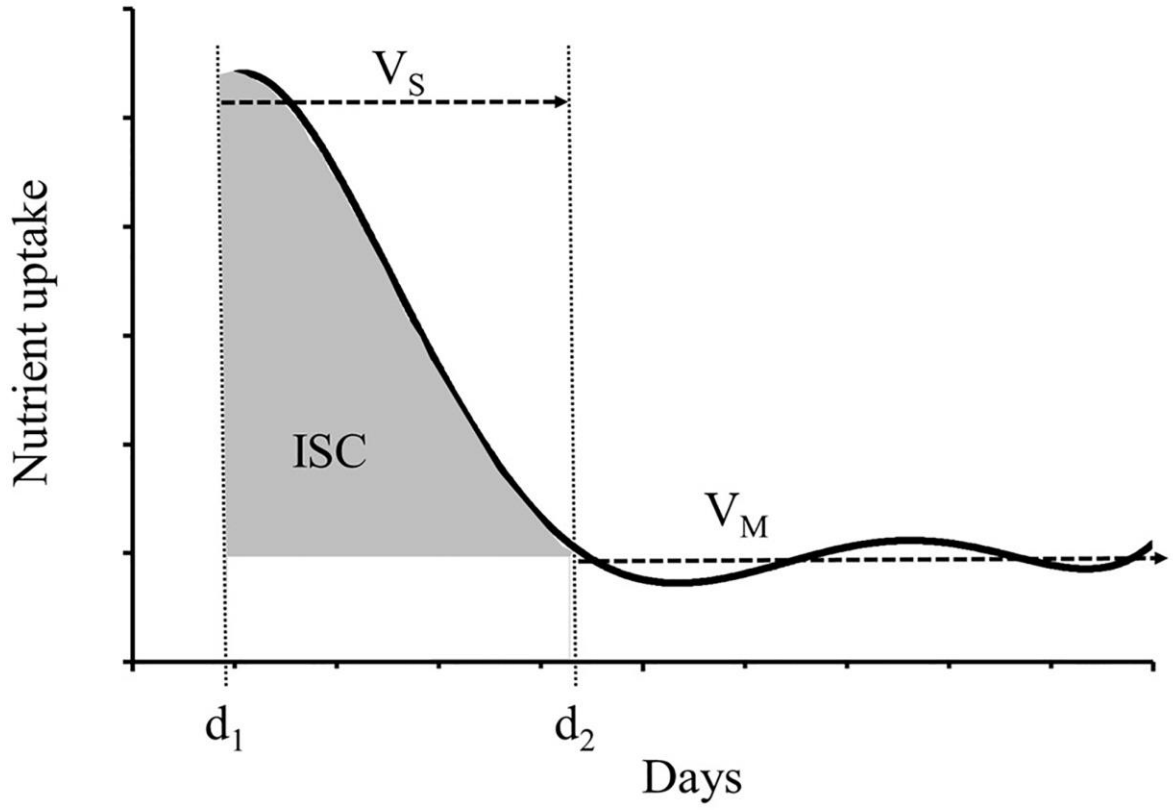
586 **Figures**

587 Figure 1



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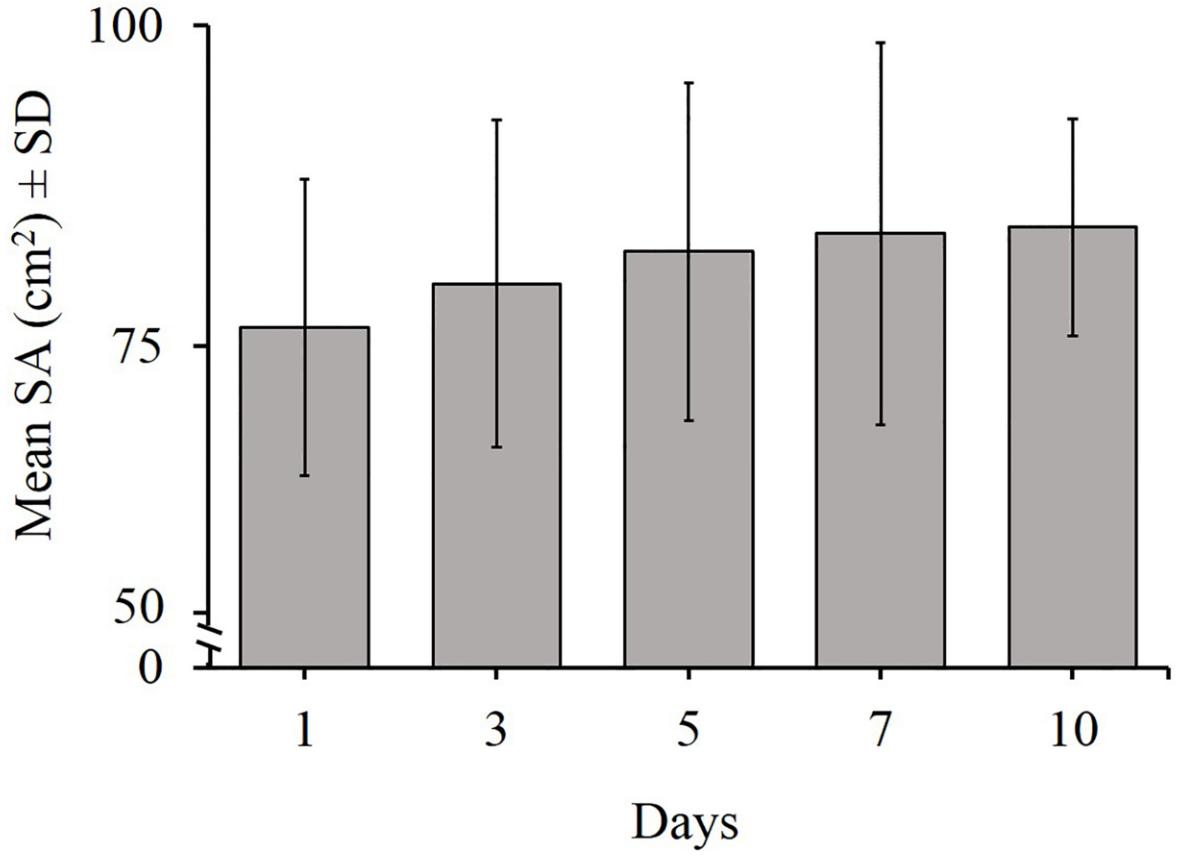
589 Figure 2



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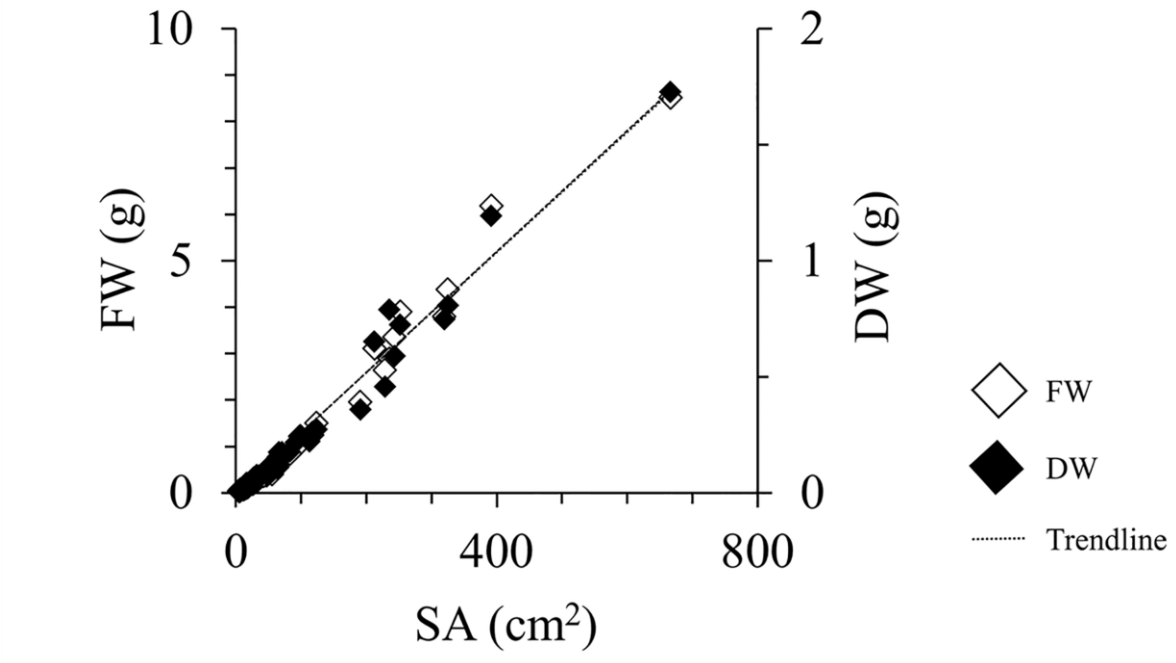

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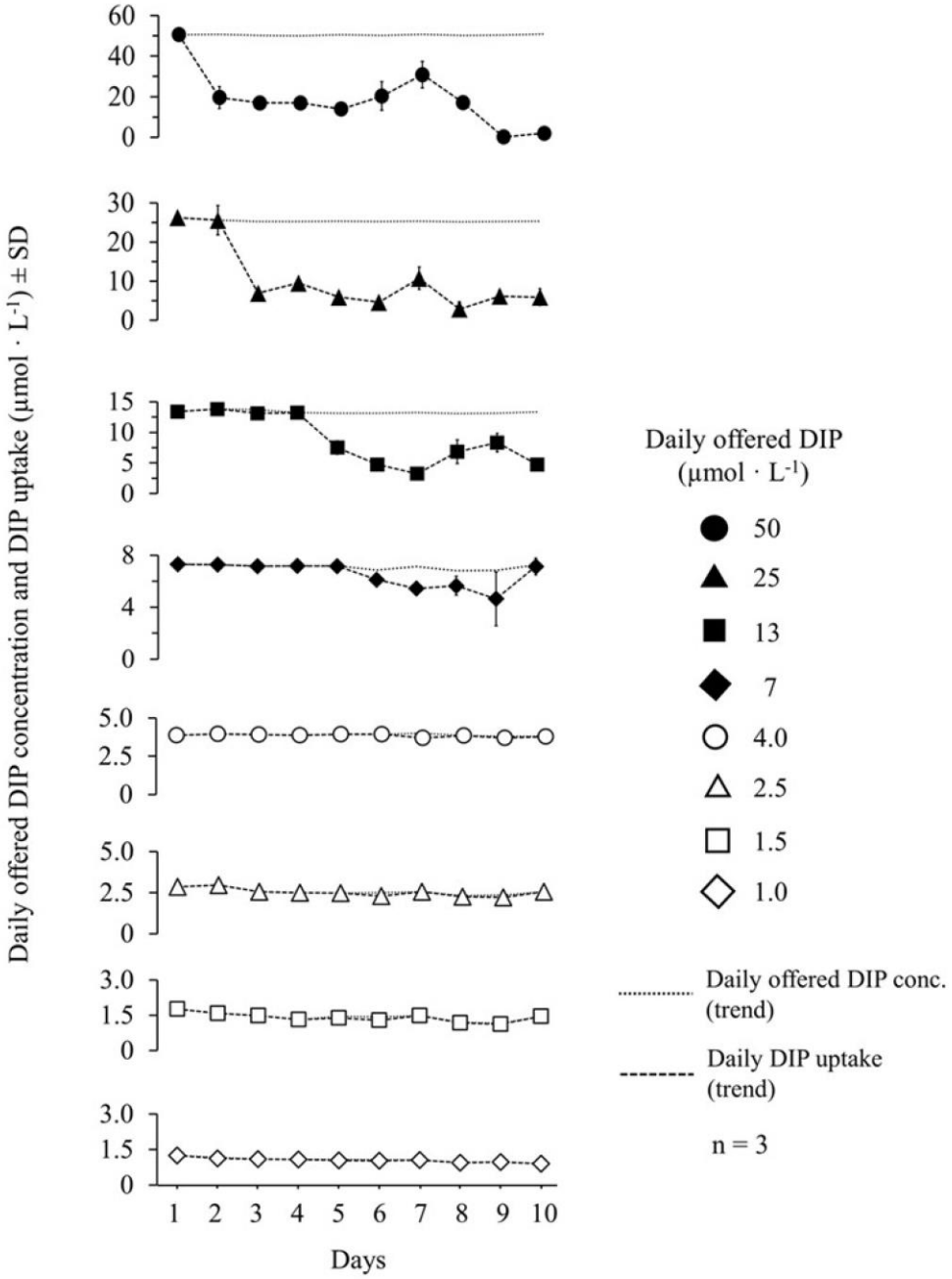
593 Figure 4



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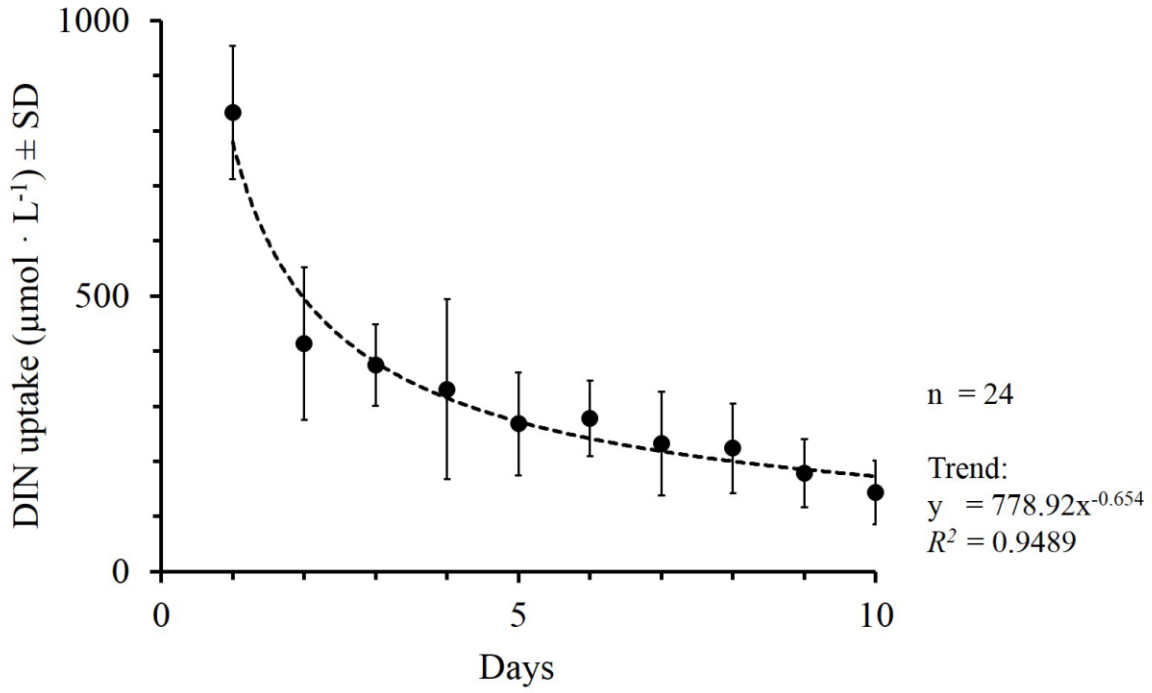
598 Figure 5:



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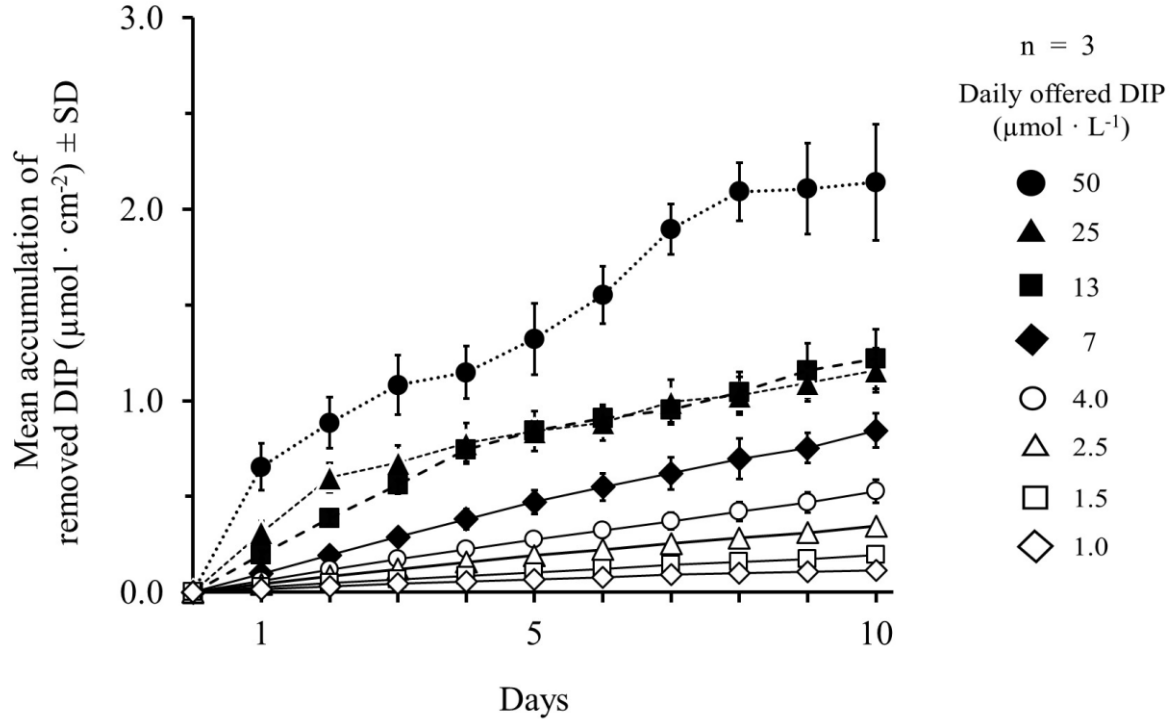
600 Figure 6:



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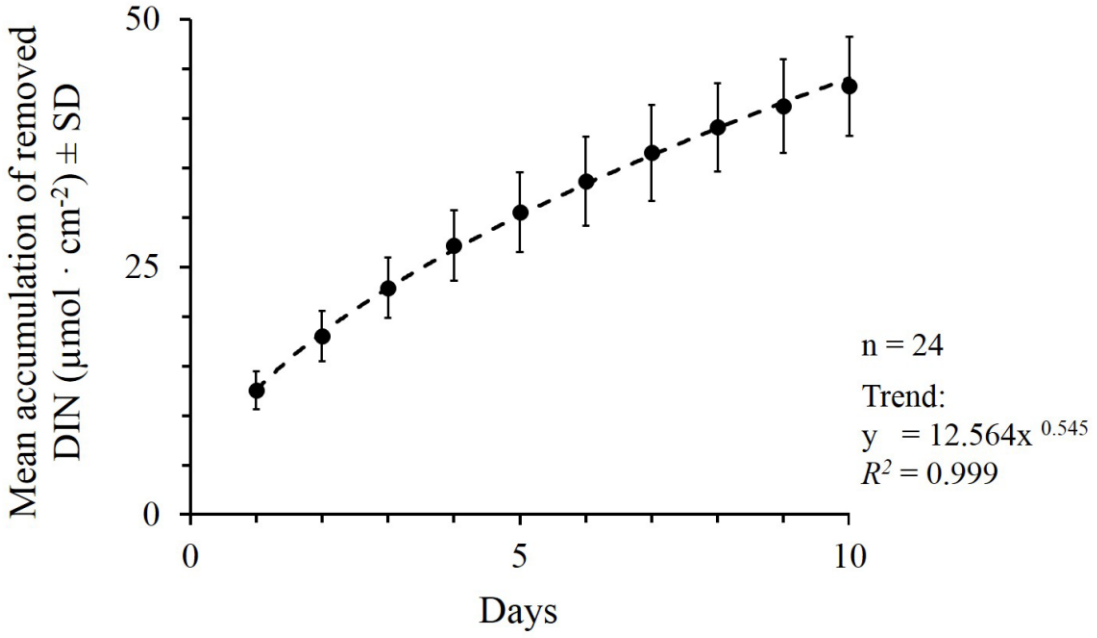
604 Figure 7:



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