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An Evaluation of Surge Uptake Capability in *Macrocystis pyrifera* in Response to Pulses of Three Different Forms of Nitrogen

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by

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ABSTRACT

An Evaluation of Surge Uptake Capability in *Macrocystis pyrifera* in Response to Pulses of Three Different Forms of Nitrogen

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This thesis examined the capacity of the foundation species *Macrocystis pyrifera*, giant kelp, to exhibit surge uptake as a mechanism to enhance nitrogen assimilation during seasons when nitrate is depleted. The ability of *M. pyrifera* to rapidly take in pulses of ^{15}N-labeled nitrate, ammonium, and urea, was assessed in the spring and summer using whole blade incubation experiments coupled with mass spectrometry analysis. The incubation experiments showed evidence of surge uptake only for ammonium, and only during the summer. However, given the ephemeral nature of potential ammonium pulses, the ecological importance of this physiological adaptation is questionable. Rather it seems more likely that a combination of uptake of ammonium and urea at ambient concentrations combined with uptake of nitrate during longer duration pulses of high concentrations (e.g., 30 – 60 minutes during internal waves), sustains giant kelp during seasons when ambient concentrations of nitrate are low.

INTRODUCTION

In many marine environments, nutrient availability is an important, and often limiting, factor controlling the species composition and performance of macrophytes (Borum 1996, Pedersen and Borum 1997, Taylor et al. 1999). In temperate coastal marine systems, primary production of macrophytes is generally limited by nitrogen especially in summer months (Ryther and Dunstan 1971, Topinka and Robbins 1976, Chapman and Craigie 1977, Thomas and Harrison 1987). In the southern California coastal system, this strong seasonality is due to seasonal patterns of coastal upwelling, the dominant controller of nutrient delivery. Starting in early summer, upwelling begins to weaken, giving way to stratification and subsequent near-oligotrophic conditions that extend into autumn (Brzezinski and Washburn 2011). With the low capacity for nutrient storage common within temperate macrophytes and in the ecosystems that they occupy (Brzezinski et al. 2013), these organisms rely on persistent, if not continuous, sources of nitrogen to sustain continuous growth. Nitrogen supply is most often facilitated by external processes that variably replenish the nutrient stock by means of currents, tides, wind, and runoff (Gerard 1982a, Brzezinski et al. 2013, Washburn and McPhee-Shaw 2013).

Macrocystis pyrifera, a perennial brown macroalga commonly known as giant kelp, grows mostly on shallow coastal reefs in the eastern Pacific and Southern oceans (Graham et al. 2007), and serves as a foundation species in these southern California coastal systems (Miller et al. 2018, Castorani et al. 2018, Lamy et al. 2020). The development of near-oligotrophic conditions during summer and autumn causes kelp forest systems to be potentially limited by nitrogen availability (Haines and Wheeler 1978, Wheeler et al. 1981, Zimmerman and Kremer 1984).

The main form of nitrogen available to *M. pyrifera*, nitrate (NO_3), displays high seasonality in its concentration (Gerard 1982a, Fram et al. 2008, Stewart et al. 2009, Brzezinski et al. 2013) due to variation in coastal upwelling. During stratified months (typically July through November), the standing concentration of nitrate reaches lows of less than 1 μmol L^{-1} (Brzezinski et al. 2013), and the nitrate supply becomes dominated by smaller, shorter lived, pulsed delivery of high nitrate water (McPhee-Shaw et al. 2007). With a minimum nitrogen requirement of 1-2 μmol L^{-1} (Gerard 1982b), the expected observation would be very limited to no growth of *M. pyrifera* populations in summer. However, the Santa Barbara Coastal LTER has routinely observed continued growth of *M. pyrifera* throughout the year (Rassweiler et al. 2018) suggesting the possible importance of other N sources during stratified periods, and motivating a reassessment of the importance of nutrient pulses in sustaining kelp.

A major pulse source of nitrate comes in the form of internal waves. These shoaling subsurface waves propagate through kelp forests in time scales on the order of an hour and can transport cold, nitrate-rich waters into kelp forests (Garrett and Munk 1979, Fram et al. 2008, Richards et al. 2013). These diurnal internal oscillations are considered to have a substantial influence on the nitrate supply during stratified summers (Zimmerman and Kremer 1984, McPhee-Shaw et al. 2007, Brzezinski et al. 2013, Reid et al. 2019). Nevertheless, Fram et al. (2008) concluded, based on modeled estimated of kelp nitrogen demand, that internal waves were not frequent enough off Santa Barbara to fulfill demand (fulfills ~27%) and there still exists a relatively large unexplained N source (Fram et al. 2008).

Another potential explanation for sustained growth of *M. pyrifera* despite apparently inadequate nitrate is reliance on regenerated nitrogen (Wheeler et al. 1981, Zimmerman and Kremer 1984, Brzezinski et al. 2013). Most commonly in the form of ammonium (NH_4),

regenerated nitrogen represents a continuous, mostly constant, flux of nitrogen in coastal waters of southern California throughout the year (Eppley et al. 1979, Herbert 1999). Although this could increase the nitrogen stock available to *M. pyrifera*, not enough is known about ammonium concentrations or the capacity of *M. pyrifera* to utilize ammonium to assess its role in sustaining kelp growth. As opposed to the large diurnal pulses of nitrate, ammonium pulses, commonly delivered through excretion by consumers within the system (Bray et al. 1988, Raikar and Wafar 2006, Peters et al. 2019), are thought to propagate through the water sporadically in small patches (Raikar and Wafar 2006). Due to the size of these patches, exposure to them occurs at spatial scales of individual blades or less and timescales of minutes, but at potentially high concentrations of nitrogen (Dy and Yap 2001, Raikar and Wafar 2006). Excretion of ammonium from resident fish populations has been shown to enhance the nitrogen uptake in juvenile *M. pyrifera* (Bray et al. 1986) and has been observed to increase primary production during low nitrate conditions in coral reefs (Holbrook et al. 2008, Burkepile et al. 2013, Shantz et al. 2015).

Urea (CH_4N_2O) may also play an important part in the nitrogen flux as it is a widely available source of nitrogen off the southern California coast (Smith et al. 2018). Most studies up to now have focused on the role of nitrate and ammonium as major nitrogen sources to kelp, but recent data has shown that *M. pyrifera* is capable of directly taking up urea (Smith et al. 2018). This form of DON is also a major component of excretion by common marine consumers (Corner and Newell 1967, Remsen 1971, Regnault 1987). Urea has also been shown to contribute to the nitrogen pool via sources such as runoff due to its use as fertilizer (Glibert et al. 2006). Although studies have been done on the effects of urea on phytoplankton

communities (Mulholland and Lomas 2008), our understanding of the role of urea in supporting macroalgal growth is lacking (Smith et al. 2018).

The ability to quickly increase the rate of uptake of a nutrient delivered in short, but potentially concentrated, pulses could be a very beneficial and potentially competitively advantageous adaptation. Surge uptake, also referred to as enhanced or transient uptake (Rosenberg et al. 1984, Thomas and Harrison 1987, Dy and Yap 2001) is a form of non-linear uptake that would facilitate use of nutrient pulses. By being pre-adapted for rapid uptake upon initial contact with a concentrated pulse the organism can make the most of the available nutrient while it is present. The threshold of what is considered surge uptake can be very dependent on the organism that is being examined. For phytoplankton communities, an increased rate during the first 2 hours has been considered to be evidence of surge uptake (Glibert and Goldman 1981). By contrast, surge uptake by macroalgae has been defined as a significantly increased rate of uptake within the first 15 to 30 minutes of exposure to a nutrient pulse (Thomas and Harrison 1987). Although surge uptake is most common in ephemeral macroalgae, it has also been observed in perennial macroalgae (Thomas and Harrison 1987).

Evidence towards the adaptation of surge uptake under low-nutrient conditions is seen in studies that have also pointed to previously nitrogen-starved cells being best at readily taking in pulses. Phytoplankton studies have shown that nitrogen-limited cells are able to take up nitrogen at rates in excess of their growth rates (Glibert and Goldman 1981) allowing increases in cell N. Thomas and Harrison (1987) examined surge uptake of two forms of nitrogen (nitrate and ammonium) in five species of macroalgae. Surge uptake of ammonium was observed in three ephemeral species, but nitrate uptake rates did not display a surge effect (Thomas and Harrison 1987). A study by Dy and Yap (2001) was conducted on a common commercial

species, *Kappaphycus alvarezii*, which is commonly grown in nitrogen-depleted environments. Using an incubation technique to determine rate of uptake over a short time scale, they found that *K. alvarezii* experienced patterns of ammonium uptake indicative of surge uptake.

These studies show that surge uptake is possible in macrophytes and potentially beneficial when nitrogen is delivered in concentrated pulses. The potential for surge uptake of nitrate by *M. pyrifera* during short (minutes to hours) pulses via internal waves during stratified conditions could potentially have a large impact on sustaining its growth (Zimmerman and Kremer 1984). By contrast ammonium and urea pulses show little seasonality in their prevalence, but they are likely to be highly patchy in time and space. Surge uptake of these forms of nitrogen would likely occur year-round though their importance would presumably be low in the presence of high nitrate concentrations associated with upwelling and other transport processes (Washburn and McPhee-Shaw 2013).

Once nitrogen sources become highly variable, the forms and extent to which these short-lived pulses can be utilized becomes a larger question. Even if uptake is greater during these shorter time scales, if the rate, and the total amount of nitrogen taken in, is not high enough to sustain growth, then this process would have little ecological relevance.

If evidence of this is found, it could point towards *M. pyrifera*, possibly compensating for low nitrate concentrations by rapidly taking in pulses of nitrogen as they become available. This thesis serves to examine the physiological capacity of *M. pyrifera* for surge uptake of these three nitrogen forms as well as the ecological relevance of surge uptake based on the kelp's uptake response and differences in the delivery patterns of pulses of each nitrogen form in nature.

METHODS

Surge uptake was evaluated by conducting incubation experiments with three common forms of nitrogen: nitrate, ammonium, and urea. Surge uptake has most often been observed in organisms that were nitrogen starved (D'Elia and DeBoer 1978), so experiments were conducted five times in nitrate-rich spring and five times during nitrate-depleted late summer in 2019.

a. Sample collection and preparation. Actively growing *M. pyrifera* blades were collected from natural populations near Santa Barbara, California. Single blades were collected 2 meters from the growing end of a frond on 30 different haphazardly chosen plants. Blades were collected 1-2 days prior to each experiment and kept in a holding tank, illuminated under artificial LED lamps on a 12:12 hour day:night cycle, with constantly flowing sand-filtered seawater. All blades on a given day were from the same kelp forest.

b. Incubation experiment. Nitrogen uptake by *M. pyrifera* was measured by exposing entire blades to a labelled form (^{15}N) of the desired nitrogen source and allowing them to incubate for pre-determined amounts of time. For each trial, single blades were placed in 1 L trays filled with nitrogen-stripped sea water. Sea water used in all experiments was stripped through inoculation of phytoplankton enriched seawater. Once sea water was assumed depleted (approximately 4 weeks), the water was filtered and stored at ambient temperature. Each tray was fitted with an air stone driven by an aquarium pump to maintain constant water movement. The length of exposure to the pulse was varied to determine how the rate of uptake changed as a function of the duration of the simulated pulse. Based on the definition of surge uptake by Thomas and Harrison (1987) the first 45 minutes of exposure were examined. Four exposure times were tested: 1 minute, 5 minutes, 15 minutes, and 45 minutes. To minimize uptake rate

differences caused by changes in available nitrogen, 10 uM pulses of the desired nitrogen isotope tracers were introduced at the start of the experiment, before blade addition, to simulate a naturally occurring pulse such as an internal wave (Brzezinski et al. 2013) or ammonium excretion from fish (Bray et al. 1986). Each exposure time was replicated across three blades, totaling 12 blades per experiment day. All experiments were done under conditions of light saturating irradiance, >169 umol photons m^{-2} s^{-1}, to eliminate light as a limiting factor (Colombo-Pallotta et al. 2006).

At the end of each trial, blades were processed and cleaned of any remaining tracer or epiphytes by dipping each blade in 10% HCl and then deionized water. Each blade was weighed damp immediately after the trial and then dried overnight at 60°C to obtain dry weight. Control blades, not run through any incubation, were also processed in order to measure the baseline nitrogen isotope composition of the blades and to assess initial blade condition during each season. Dried tissue subsamples were analyzed on an EA-IRMS Mass Spectrometer to determine the $\delta^{15}N$ and the percent weight nitrogen (%N) of each blade, which was used to calculate uptake rates for each incubation treatment.

c. Establishing evidence of surge. Surge uptake was evaluated by calculating differences in specific uptake rate (V) using a modified version of an equation by Legendre and Gosselin (1996):

$$V = \frac{(n_t - n_{t0})}{(n_s - n_{s0}) \times t} \qquad (1)$$

where n_t was the atom % ^{15}N in the blade after incubation; n_{t0} was the average concentration of isotope in the control blades; n_S was the atom % ^{15}N of the nutrient in the seawater at the start of the incubation; n_{S0} was the atom % ^{15}N in the nitrate pool before isotope tracer was added; and t was the length of the incubation in minutes. Final units of V were h^{-1}.

The discrete pulse duration V's calculated in eq (1) were used to assess how the physiological uptake changed over time in response to exposure to a high-concentration N pulse.

A caveat of this calculation was that although it provided insight into the overall effect of a single pulse during its lifespan, the dynamics of the uptake patterns were dampened due to the fact that they were averaged over the total pulse duration and thus unable to reveal instantaneous rates of uptake rate at any given point in time. To provide a sense of the temporal evolution of uptake over 45 minutes, average values of V for intervals between the focused time points were calculated by integrating V from discrete points over previous intervals into the total time as follows:

$$V_I = \frac{(V_f \times t_f) - (V_i \times t_i)}{t_{f-i}} \qquad (2)$$

Here V from the start of the interval was multiplied by its pulse duration and then subtracted from V at the end of the interval multiplied by its pulse duration. The resulting difference was divided over the time duration of the interval to obtain an average V for the whole interval. This resulted in average estimates of V for 0-1 minute, 1-5 minutes, 5-15 minutes, and 15-45 minutes.

d. Assessing the ecological impact of uptake rates. The ecological implication of the uptake response to pulsed nutrient supplies was assessed by examining the biomass-normalized uptake rate (ρ) using Equation (3):

$$\rho = V \times \frac{m_N}{m_{dw} \times 60} \qquad (3)$$

m_N was the mass of isotope in the subsample taken for isotope analysis (ug); m_{dw} was the dried weight subsample (g). (Legendre and Gosselin 1997). This created units of ug N $(g\ dw)^{-1}$ min^{-1}.

ρ was also compared to that expected based on rates of net primary production (NPP) by *M. pyrifera* obtained from long-term data collected by the SBC-LTER (Rassweiler et al. 2018). Values of ρ in control blades collected along with spring and summer experimental trial blades surveys were used to determine the frequency and intensity of pulses necessary to meet the demand measured by Rassweiler et al. (2018) to evaluate the likelihood that the uptake of pulses, and surge uptake in particular, make a significant contribution to nitrogen demand.

e. Test of Assumptions. To adequately replicate the conditions needed for surge uptake as well as the effects of a natural pulse, the concentration of the test substrate needed to remain at saturating concentrations during the entire experiment. For all calculations, the nitrogen pool was assumed to be constant over the course of the whole exposure time to allow the blades to operate at a high exposure over the entire experiment. To check the degree to which this assumption was met, the theoretical ratio of $V:V_{max}$ was calculated using a modified kinetics equation (4) to see how uptake may have changed by the end of the exposure time.

$$\frac{V}{V_{max}} = \frac{S}{K_s+S} \qquad (4)$$

Here K_s represented the half saturation constant for the form of nitrogen the blade was exposed to, obtained from Brzezinski et al. (2013) for nitrate, Haines and Wheeler (1978) for ammonium, and Smith et al. (unpublished) for urea. S was the concentration of isotope remaining in the pool after incubation, calculated as follows:

$$S = n_{t0} - (\rho * t * m_{dw}) \quad (5)$$

f. Examination of nitrogen starvation as a driver of surge uptake. The nitrogen content of kelp blades in spring and summer was compared to test whether surge uptake varied with potential nitrogen starvation during the summer. This was accomplished by measuring the percent N of ”control” blades that were collected with experimental blades in spring and summer and

immediately processed without exposure to experimental nitrogen incubations. Because it was not possible to analyze the % N of a blade prior to incubation without damaging the blade, the % N measured at the end of the incubation was used as an indicator of a blade's initial nitrogen status.

g. Statistical analysis. The mean percent N of control blades was compared between seasons using a t-test. The effects of season, pulse duration (or pulse interval) on specific uptake rate (V) were analyzed separately for each nitrogen form using a type III fixed factor ANOVA. The effects of season, pulse duration and nitrogen status (% N) on biomass normalized uptake rate (ρ) were analyzed separately for each nitrogen form using ANCOVA in which season and pulse duration were considered fixed factors and nitrogen status a covariate. If pulse duration (or pulse time interval) was found to be significant, then a post-hoc Tukey Test was done to determine which pulse durations or time intervals differed from each other.

Correlation analysis that compared the relationship between the % N of experimental blades at the end of the incubation and the duration of the incubation was used to determine whether the % N at the end of an experiment was useful indicator of the nitrogen status of the blade prior to the addition of pulsed nitrogen. The assumption here was that if % N was unrelated to the duration of pulsed nitrogen, then uptake by the blade during the experiment did not have an appreciable effect on its % N.

Surge uptake was defined as an increased rate within the first 30 minutes, so there was heightened interest in determining significant differences in uptake rates for short pulses within the first 15 minutes of incubation compared to the 45-minute pulse durations. Significantly higher uptake during any interval < 15 min was taken as evidence of surge uptake.

RESULTS

Kinetic Parameters of Experimental Conditions

Using the kinetic parameter estimations and the depletion of the nitrogen sources in the experiments it was estimated that uptake rates were >75% of V_{max} during the entire exposure duration for all 3 nitrogen forms, validating my assumption of saturating uptake across experiments.

Determination of Surge Uptake

The average uptake rates between exposure intervals for both nitrate and urea (equation 2) did not vary significantly with season (Fig.1; nitrate: $F_{1,32} = 1.03$, $p = .319$ and urea: $F_{1,32} = .669$, $p = .420$) or time (nitrate: $F_{3,32} = .476$, $p = .701$ and urea: $F_{3,32} = .042$, $p = .988$) . The specific uptake of ammonium displayed a more dynamic pattern with higher uptake in summer compared to spring that was most pronounced at short exposure durations ($F_{1,32} = 5.22$, $p = .029$). As seen in Figure 1, elevated rates of ammonium uptake were maintained for the first 5 minutes, but after 15 minutes there was a significant decrease in uptake ($F_{3,32} = 1.45$, $p = .246$), indicating a brief period of surge uptake. This pattern was most pronounced in summer, leading to a marginally significant interaction between season and pulse duration ($F_{3,32} = 2.78$, $p = .057$). The average %N for control blades in spring (1.65 ± .17 %N/wt.) and summer (1.51 ± .24 %N/wt.) were not statistically different (t-test = 1.10, df = 46.66, p-value = .278) suggesting % N did not account for the higher rates of ammonium uptake observed in summer.

Like the pattern for specific rates estimated for discrete time intervals, specific rates of average cumulative nitrate uptake (Fig. 2) were not significantly different in summer compared to spring ($F_{1,32} = 1.15$, $p = .292$) and did not differ significantly among exposure durations ($F_{3,32} = .403$, $p = .752$). The significantly higher rates of specific uptake of

ammonium in summer observed for discrete time intervals was also found for cumulative uptake rates averaged over time ($F_{1,32} = 5.72$, $p = .023$). Cumulative pulse duration showed no significant effect ($F_{3,32} = 1.07$, $p = .375$), and there was no significant interaction detected ($F_{3,32} = 1.91$, $p = .148$). No significant effects of either season ($F_{1,32} = .926$, $p = .343$) or cumulative pulse duration ($F_{3,32} = .032$, $p = .992$) on specific uptake rates of urea were observed.

Evaluation of Ecological Significance

Results of correlation analysis showed no significant relationship between tissue nitrogen at the end of an experiment and the duration of a pulse for all three forms of nitrogen (nitrate: $R^2 = .0001$, df = 38, $p = .943$; ammonium: $R^2 = .009$, df = 38, $p = .560$; urea: $R^2 = .053$, df = 38, $p = .153$) indicating that nitrogen uptake during the experiment did not lead to appreciable changes in the % N of a blade, and that the %N or a blade following the pulsed addition of nitrogen was a good indication of its nitrogen status at the beginning of the experiment.

Like rates of specific uptake, the biomass-normalized rate, ρ, of nitrate uptake also showed a significant difference between spring and summer ($F_{1,31} = 5.74$, $p = .023$) with higher rates during summer, but no effect of cumulative pulse duration ($F_{3,31} = .481$, $p = .698$) and no seasonal interaction ($F_{3,31} = .525$, $p = .668$). For ammonium, ρ was significantly higher in summer compared to spring ($F_{1,32} = 10.7$, $p = .003$) and differed significantly among cumulative pulse durations ($F_{3,31} = 4.15$, $p = .014$), as ρ tended to decline with increasing pulse durations especially during summer (Figure 3; $F_{3,31} = 2.40$, $p = .087$ for season*exposure duration interaction), leading to a marginally significant interaction effect ($F_{3,31} = 2.40$, $p = .087$). For urea pulses, there were also no significant differences due to

season ($F_{1,31} = 2.41$, $p = .131$) or duration of pulse ($F_{3,31} = .663$, $p = .581$) individually, but there appeared to be a possible interaction between the two ($F_{3,31} = 2.29$, $p = .098$).

The relationship between %N and ρ showed no significant relationship for ammonium pulses ($F_{1,31} = 3.03$, $p = .092$). However, there was a significant negative relationship for nitrate ($F_{1,31} = 8.82$, $p = .006$) and urea ($F_{1,31} = 9.74$, $p = .004$).

DISCUSSION

In these experiments, *M. pyrifera* exhibited surge uptake of ammonium, which was most prominent in summer. By contrast, surge uptake of nitrate and urea was not evident in either spring or summer. In the case of nitrate both V and ρ tended to be higher during the 1-minute exposure, indicating that *M. pyrifera* may exhibit some capacity for surge uptake for a very short duration. Considering a major source of nitrate pulses is propagated by internal waves that persist for up to an hour, an increased uptake capacity that lasted only a minute would not significantly impact *M. pyrifera* N content or growth during oligotrophic summer and autumn periods. There was, however; a significant difference in nitrate uptake between the two seasons, with higher uptake capacity during summer. This could be indicative of a separate mechanism in place to allow for generally higher uptake during nitrogen depleted environments, but not via a surge mechanism.

For ammonium exposure, the summer data presented the clearest evidence of surge uptake. Ammonium pulses had a noticeable spike in ρ for the first 5 minutes during the summer, providing evidence of surge uptake of ammonium being a potential strategy of N acquisition. Since the surge pattern only appeared during the summer experiment, this potentially reflects an adaptation affected by seasonal change. However, prior nitrogen starvation as a driver was ruled out based on the %N of blades. First, the examination of control blades showed no

significant difference between the mean % N of blades in spring and summer, suggesting that even with the changes in available nitrogen, blades were in similar states and were apparently not more starved in one season over the other. It should be noted, however, that although it was not observed in this experiment, seasonal differences in the N content of *M. pyrifera* is a common occurrence in the Santa Barbara Channel (Reed et al. 1996, Brzezinski et al. 2013) that would afford greater advantage to a surge uptake capacity in summer.

The lack of relationship between %N and ρ for ammonium differed from that of nitrate and urea, which both seemed to be influenced by the kelp's tissue nitrogen status. It should be noted, however, that this may be a difference that is specific to *M. pyrifera*, since in other macroalgae, *Neoagardhiella baileyi* and *Gracilaria foliifera,* N-limitation doubled short term (5 minutes) ammonium uptake rates compared to more N replete specimens (D'Elia and DeBoer 1978). *Chordaria flagelliformis and Fucus distichus* also had highest rates of non-linear uptake during the initial 30 minutes of exposure to ammonium in individuals with the lowest thalli N content (Rosenberg et al. 1984).

To evaluate the ecological significance of how *M. pyrifera* responds to pulses of different forms of nitrogen, the prevalence and duration of these pulses in nature must be considered. Using the net primary production of nitrogen and average dry weight per area taken from a 15-year record of net primary production of *M. pyrifera* at three kelp forests near Santa Barbara (Rassweiler et al. 2018), the average daily nitrogen uptake of *M. pyrifera* was calculated to be 315.89 ug N (g dry wgt)$^{-1}$ d^{-1} in the spring and 268.87 ug N (g dry wgt)$^{-1}$ d^{-1} in the summer. From Fram et al. (2008), nitrate pulses were shown to propagate most commonly in southern California coastal systems, in the form of twice-daily internal waves with durations of a few hours (Zimmerman and Kremer 1984, Brzezinski et al. 2013)before being completely

dissipated in the kelp forest. Using values of ρ determined for 45 minute exposure duration of a 10 uM pulse in this experiment to represent the response of *M. pyrifera* to internal waves, it was determined that approximately 7 pulses d^{-1} would be required in the spring and 6 pulses d^{-1} in the summer to meet the total estimated N demand. Considering the internal waves in this system occur approximately twice per day (Fram et al. 2008), the uptake of nitrate from internal waves could meet about a third of *M. pyrifera*'s nitrogen demand during the summer. These results align with the findings from Fram et al. (2008) which concluded that internal waves make up 27% of the nitrogen demand of *M. pyrifera* during the summer in the Santa Barbra Channel.

Ammonium and urea pulses on the other hand are harder to quantify. Due to their presumed sporadic and ephemeral nature (Raikar and Wafar 2006), the shorter pulse durations may be most representative of naturally occurring surges. Raikar and Wafar (2006) described such pulses as being extremely short-lived, and in the case of excretion they could dissipate within minutes, leading to their having an effect only on their immediate area. Therefore, for this comparison both 1- and 5-minute pulse durations were used to evaluate the ecological potential of ammonium and urea pulses. During spring, 122 1-minute pulses of 10 µM ammonium, or 34 5-minute pulses, would be needed per day to meet *M. pyrifera*'s total nitrogen demand, while in the summer, 71 1-minute pulses or 18 5-minute pulses would be required each day. This calculation assumes a pulse size that encompasses the entire plant. Scaling down to the mm or cm scale associated with an ammonium excretion event by an individual consumer would increase these estimates by orders of magnitude, diminishing the ecological importance of surge uptake. Similarly, for a 10 µM urea pulse to meet total N demand requires 464 1-minute or 99 5-minute pulses in the spring, and 435 1-minute or 96 5-

minute pulses in the summer. Like for ammonium these estimates must be scaled to the size of a concentrated patch of urea, which again would diminish the role of pulsed supply and need for surge uptake. Despite the result of these patches not being relevant, it should still be noted that both ammonium and urea exist at a lower concentration as a constant recycled form of N (Eppley et al. 1979, Herbert 1999, Smith et al. 2018). Thereby, their role in meeting the N demand most likely comes from the constant uptake of this background concentration.

There was a large difference in the maximum ρ (from any pulse duration during either seasons) for each form of N, more than doubling between urea (.92 ug N (g dry wgt)$^{-1}$ min^{-1} for 15 minute pulse in the summer) to nitrate (2.28 ug N (g dry wgt)$^{-1}$ min^{-1} for 1 minute pulse in the summer) with another doubling for ammonium uptake to ammonium (5.00 ug N (g dry wgt)$^{-1}$ min^{-1} 1-minute pulse in the summer). This provided evidence for a potential hierarchy of uptake and utilization among the different nitrogen forms.

Summary - The findings presented here assessed the physiological capacity of *M. pyrifera* for surge uptake and its ecological relevance for meeting the nitrogen demand of the alga. This research showed that in situations when nitrate concentrations are below the minimum nitrogen requirement, such as in the summer, *M. pyrifera* is able to increase its uptake of nitrate and ammonium, exhibiting a surge response to short (< 30 min) pulses of ammonium, although this may not be a result of the low N conditions directly. If the patterns observed here are indicative of summer patterns of uptake for *M. pyrifera* populations, surge uptake of ammonium or direct exploitation of patches of elevated ammonium or urea released by consumers appears to hold little potential for meeting measured N demand. The lack of surge uptake in nitrate on minute timescales would not interfere with the ability of kelp to exploit

elevated nitrate in internal waves lasting a few hours; yet nitrate uptake, even with internal waves, appears capable of only supplying a third of the kelp N demand (Fram et al. 2008). The results presented here suggest that if this deficit is met by regenerated nutrients like ammonium and urea it is unlikely through surge uptake. Rather low persistent uptake of the 'background' ammonium and urea (0.2-1.5 uM in this environment) is a more likely mechanism employed by *M. pyrifera* to meet this deficit.

This thesis served to examine the uptake rate of different forms of nitrogen independently both physiologically and ecologically. It did not explore the potential impacts of interactions between the multiple nitrogen forms at once and their subsequent effect on the uptake rate. Ross et al. (2018) examined the effect of multiple nitrogen sources, both independently and together, on the uptake rate and daily growth rate of *Cladophora parriaudii* and found that in the presence of urea, the uptake of other co-existing nitrogen forms was enhanced and daily growth rate was increased (Ross et al. 2018). As mentioned above, the suppression of nitrate uptake in the presence of ammonium has also been a point of debate (D'Elia and DeBoer 1978, Haines and Wheeler 1978, Thomas and Harrison 1987), therefore examining these interactions could give greater context to in-situ nitrogen uptake.

The results presented here provide new insight into the question of how *M. pyrifera* maintains its continuous growth in nitrate depleted conditions. With longer, warmer summers being a consequence of an evolving climate, the need for greater knowledge on how this important foundation species will react and/or adapt to its changing conditions will be increasingly important moving forward. Much still remains unknown about the complexities of nitrogen uptake by *M. pyrifera,* and the results presented here provide useful guidance for future research on this topic.

FIGURES

Fig. 1. **Specific uptake rates over time intervals for each nitrogen form**. Extrapolated V between experimental pulse exposure duration. Rates shown for both spring (black) and summer (white). Error bars represent standard error. Lines above bars, represent groupings of similarity for spring (blue) and summer (orange) time intervals.

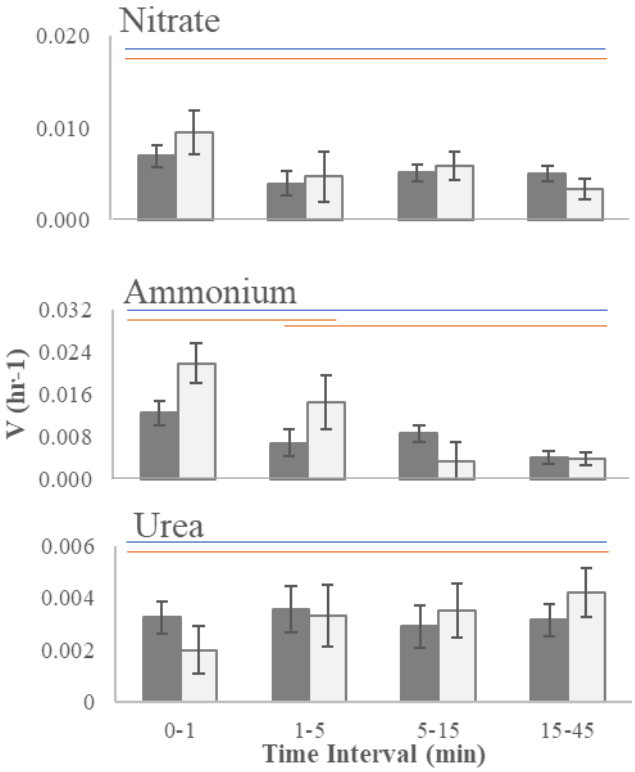


Fig. 2. **Seasonal uptake rates of nitrogen pulses over duration of pulse**. Specific uptake rates of each nitrogen isotope over pulse exposure duration (min) during spring (black) and summer (white). Error bars represent standard error. Lines above bars, represent groupings of similarity for spring (blue) and summer (orange) exposure times.

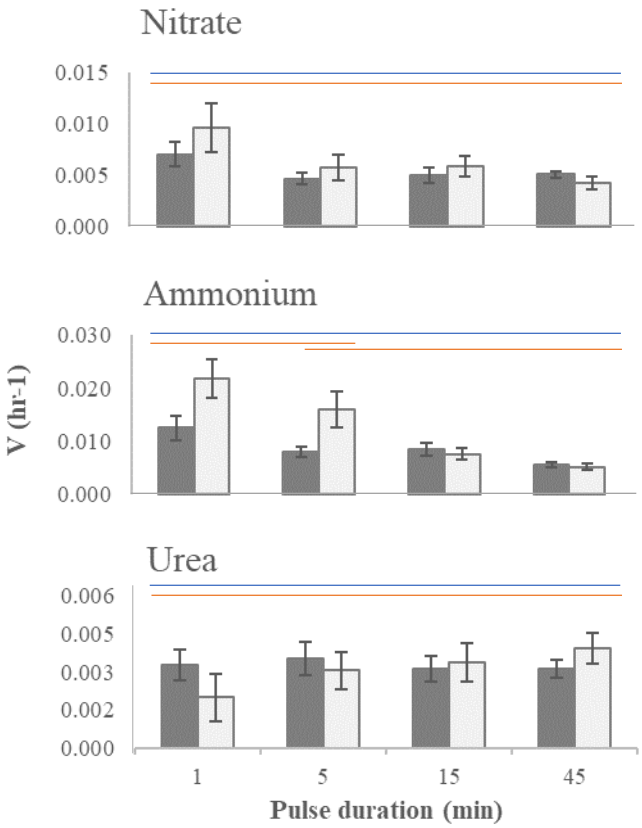
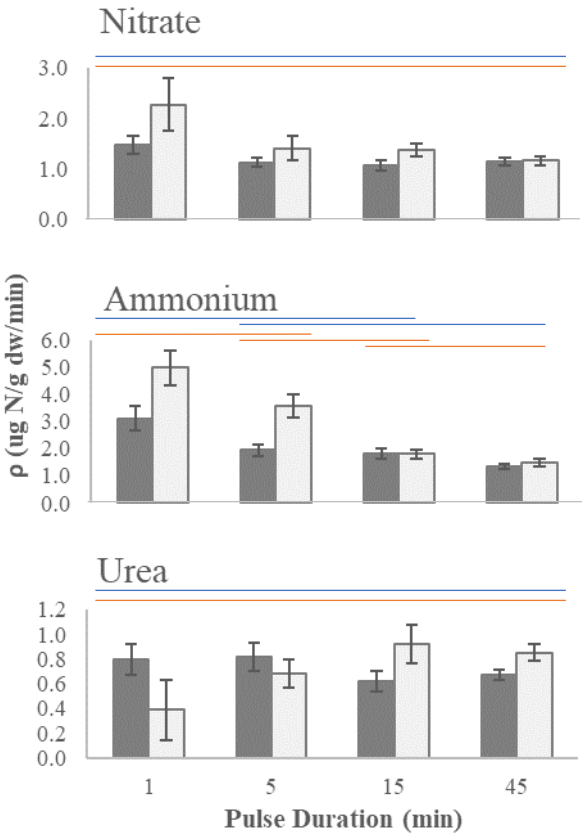


Fig. 3. **Seasonal net transport rates of nitrogen pulses over pulse duration**. Net transport rates of each nitrogen isotope over pulse exposure duration (min) during spring (black) and summer (white). Error bars represent standard error. Lines above bars, represent groupings of similarity for spring (blue) and summer (orange) time points.



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