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# Supply- and demand-driven phosphate uptake and tissue phosphorus in temperate seaweeds

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ABSTRACT: High *in situ* rates of phosphate uptake should coincide with high tissue phosphorus content and/or high growth rate and be either supply-driven (largely controlled by the phosphate concentration in the surrounding seawater) or demand-driven (largely dictated by the maximum uptake rate, $V_{max}$, and under the control of the organism). To test this hypothesis, 6 common New Zealand seaweed species (*Cystophora torulosa*, *Melanthalia abscissa*, *Pterocladia lucida*, *Ulva intestinalis*, *Xiphophora chondrophylla* and *Zonaria turneriana*) were used. We calculated *in situ* rates of phosphate uptake from the kinetic constants of uptake, monthly rates of uptake at a fixed phosphate concentration and seawater phosphate concentration, and compared these rates with monthly tissue phosphorus content. There were no significant differences in the half-saturation constant ($K_m$) values for phosphate uptake by the 6 species. $V_{max}$ and affinity ($V_{max}/K_m$) were largely a function of the seaweed surface area:volume quotient. In the 5 species where there was a peak in tissue phosphorus levels, it occurred in July or September/October. Peaks in tissue phosphorus in *M. abscissa*, *P. lucida*, *U. intestinalis* and *Z. turneriana* coincided with, or occurred soon after, peaks in calculated *in situ* rates of phosphate uptake. Maximum rates of *in situ* phosphate uptake were demand-driven in all subtidal species and supply-driven in the only intertidal alga *U. intestinalis*.

KEY WORDS: Phosphate uptake · Phosphorus · Seaweeds · Uptake kinetics

## INTRODUCTION

Seaweeds require phosphorus for growth, as it is a major constituent of RNA and, consequently, is involved in protein synthesis (Sterner & Elser 2002). It is also a constituent of phospholipid, sugar phosphates and nucleotides such as ATP. For marine algae, irrespective of the source of phosphate (dissolved in seawater and/or the product of extracellular alkaline phosphatase activity), it has to be taken up across the cell membrane. The amount of phosphorus present in the tissues of a seaweed can change depending on the rates of input (rate of net uptake) and output (growth, reproduction and other loss of tissue). The balance between these 2 is the cellular phosphorus content of intact tissue. If the input exceeds the output, the tissue phosphorus content will tend to increase. The rate of phosphate uptake may depend on supply, as determined by the concentration of phosphate in the surrounding seawater, and demand, which is determined by the kinetic constants of phosphate uptake, particularly the maximum rate of uptake (see below). The former is out of the control of the alga; the latter is largely under the control of the alga, though the supply will continue to have an impact on the actual *in situ* rate of uptake. Currently we know remarkably little about the relationship between phosphate uptake and tissue phosphorus (e.g. Runcie et al. 2004, but see Pedersen et al. 2010), in particular what determines the *in situ* rate of uptake.

Though less common than nitrogen limitation, phosphorus limitation of growth in temperate marine macroalgae does occur (Birch et al. 1981, Pedersen et

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al. 2010), but with increased nitrogen loading into coastal water, the incidence of phosphorus limitation of algal growth is likely to increase in the future (Turner et al. 2003). Consequently, an improved understanding of phosphorus metabolism is essential to mitigate this adverse effect of increased nitrogen loading.

The Michaelis-Menten model is commonly used to describe nutrient uptake by seaweeds, including phosphate (Rees 2003). $V$ is the uptake rate, which increases with increasing substrate concentration up to a maximum at infinite substrate concentration where the rate is $V_{max}$. The half-saturation constant ($K_m$) is the concentration where the reaction rate is half the maximum value ($V_{max}$). The affinity ($V_{max}/K_m$) quantifies the ability to take up a nutrient at low concentrations (Healey 1980). Relatively little is known about the kinetics of phosphate uptake in seaweeds, whereas the kinetics of inorganic nitrogen uptake has been more widely studied (Rees 2003). Even less is known about other aspects of phosphate uptake, but there are a few studies that have investigated phosphate uptake in relation to season, shoreline position and desiccation (Hurd & Dring 1990, 1991, Kim et al. 2008). However, none of these studies have related rates of uptake to tissue phosphorus content.

Here we describe seasonal changes in rates of phosphate uptake and tissue phosphorus content in 6 common New Zealand seaweeds. We hypothesized that there would be a supply and demand relationship with the calculated *in situ* rate of phosphate uptake and that whichever was greater would coincide with the maximum tissue phosphorus content and/or maximum output.

## MATERIALS AND METHODS

To examine phosphate uptake 6 seaweed species were used. Green seaweeds were represented by *Ulva intestinalis*; red seaweeds by *Melanthalia abscissa* and *Pterocladia lucida*; and brown seaweeds by *Cystophora torulosa*, *Xiphophora chondrophylla* and *Zonaria turneriana*. All taxa are perennial—except for *U. intestinalis* which is semi-perennial—and common in north-eastern New Zealand. Samples were collected monthly (April to December) at 1 to 3 m depth from Waterfall Reef at Goat Island (36° 16′ 07.43″ S, 174° 47′ 58.80″ E) in temperate, north-eastern New Zealand, except for *U. intestinalis*, which was collected from intertidal rock pools adjacent to Waterfall Reef. These rock pools are regularly flushed by fresh seawater at high tide.

### Seawater nutrients

Three replicate seawater samples were collected monthly (at the same time as seaweed collection) from 5 m depth at Waterfall Reef for nutrient analysis. Seawater samples were stored frozen (–18°C) and unfiltered until analysis. Independent experiments verified that this procedure had no effect on the results obtained and that there was no significant difference in nutrient concentrations between filtered and unfiltered seawater samples (Barr & Rees 2003). A problem that we have encountered (specifically with nitrite) is that filtering introduces contamination (B. C. Dobson unpubl.) and contamination through the use of filters has been observed by others (Knefelkamp et al. 2007). Ammonium (Koroleff 1983a), nitrite and nitrate (Parsons et al. 1984), and phosphate (Koroleff 1983b) were determined as previously described. The detection limits for ammonium, nitrite, nitrate and phosphate were 0.05, 0.01, 0.05 and 0.01 µM, respectively.

### Phosphate uptake

Seaweeds for uptake experiments were collected within 48 h prior to use and kept in a large (1.3 $m^3$) outdoor holding tank with regular seawater (from the same location as where the seaweeds were collected) flow, turbulence and mixing (input water was via a dumping system of about 10 l every 2 min) (Barr et al. 2008). Trials comparing algae that were maintained in this system for 48 h and freshly collected seaweed showed no significant difference in phosphate uptake rate (authors' unpubl. data). Uptake of phosphate was measured as its disappearance from seawater. Experiments were done with entire algae which had been cleaned of all visible epibiota and sand. Temperature was held constant at 17.5°C and photon flux density (photosynthetically active radiation) at 160 µmol $m^{-2}$ $s^{-1}$. Phosphate uptake experiments were done with 200 ml seawater in 250 ml Perspex chambers for the small seaweeds (*M. abscissa*, 15 g fresh weight, *U. intestinalis*, 1 g fresh weight, and *Z. turneriana*, 10 g fresh weight) and with 1 l seawater in 3 l Perspex chambers for the larger seaweeds (*C. torulosa*, *P. lucida* and *X. chondrophylla*, each 65 g fresh weight). Mixing in the chambers was achieved either manually (3 l chambers) or with constant mixing via a magnetic stirrer (250 ml chambers). There was no significant difference between these 2 mixing methods on rates of phosphate uptake (authors' unpubl. data).

Samples (5 ml) were taken from each container after the addition of the appropriate amount of $K_2HPO_4$ and thorough mixing, but prior to the addition of the seaweed, and 0.5, 1, 2, 4 and 6 h thereafter. Phosphate concentration in each sample was measured using a malachite green reagent (Geladopoulos et al. 1991). Absorbances were converted to concentration using a standard curve obtained with known phosphate concentrations. The rate of uptake for each alga was then calculated by taking the slope at time zero of an exponential rise curve fitted to a plot of cumulative phosphate in the seaweed versus time (see Taylor & Rees 1998). Dry weights of seaweeds used in the experiments were determined by drying all individuals at 80°C to constant weight following uptake experiments in order to express results per unit dry weight.

The effect of seawater phosphate concentration on the rate of phosphate uptake was investigated in the 6 species. Rates of uptake were determined as described above at 6 phosphate concentrations (1, 2, 5, 10, 15 and 20 µM), with experiments repeated 3 times for each species in April/May in 2008. Two constants were used to describe uptake rates: $V_{max}$ (maximum rate of uptake, µmol $g^{-1}$ dry wt $h^{-1}$) and $K_m$ (concentration of phosphate that gave half the maximum rate of uptake, µM).

### Temporal changes in rates of phosphate uptake

Monthly determinations of rates of phosphate uptake were done in triplicate between April 2008 and December 2008 for the 6 species as described above, at an external phosphate concentration of 5 µM. For each species, *in situ* phosphate uptake rates at ambient seawater concentrations were calculated using $V_{max}$ and $K_m$ values and monthly uptake rates at 5 µM phosphate concentration. This method was intended to provide an estimate of, rather than a precise value for, *in situ* rates of phosphate uptake (see 'Discussion'). *In situ* rates of uptake were calculated using the formulae expressed below, where $V_5$ is the rate at 5 µM phosphate (in original kinetic experiments), $V_{Natural}$ is the rate at the natural concentration (based on kinetic experiments), [P] is the measured ambient seawater phosphate concentration, $X$ is $V_{Natural}$ as a proportion of $V_5$, and $R_5$ is the measured monthly rate of phosphate uptake at 5 µM. Finally, $V_{Ambient}$ is the calculated rate of phosphate uptake at ambient seawater phosphate concentrations.

$$V_5 = \frac{5 \times V_{max}}{5 + K_m} \quad (1)$$

$$V_{Natural} = \frac{[P] \times V_{max}}{[P] + K_m} \quad (2)$$

$$X = \frac{V_{Natural}}{V_5} \quad (3)$$

$$V_{Ambient} = R_5 \times X \quad (4)$$

### Tissue phosphorus

Tissue phosphorus content was determined according to the method of Solórzano & Sharp (1980), as modified by Zhou et al. (2003). Tissue was dried at 80°C for at least 24 h and then ground into a fine powder using a mortar and pestle. Samples were either processed immediately or stored dry at –80°C. For a given sample, approximately 50 mg of dried tissue (with precise weight recorded) was placed in a borosilicate glass scintillation vial with 1 ml auxiliary (0.1 M $MgCl_2.6H_2O$) and dried at 60°C. Samples were then ashed in a muffle furnace for 3 h at 500°C. Once cool, 10 ml 0.2 M HCl was added to each sample followed by heating at 80°C in a dry bath for 30 min. Following heating, 1 ml samples from each glass scintillation vial were taken and added to 9 ml distilled water in polypropylene scintillation vials. From these diluted samples, 5 ml were taken for phosphate determination using a malachite green reagent (Geladopoulos et al. 1991). Values are expressed as % dry weight.

### Data analysis

Data from the kinetics experiments were used to generate a rectangular hyperbolic regression analysis giving the model parameters $V_{max}$ and $K_m$. Differences in $K_m$ were investigated with 1-way ANOVA on natural log-transformed data, to determine whether any differences existed between the species. To gain a maximum estimate of error associated with the measurements of *in situ* rates of phosphate uptake, the highest rate of uptake at 5 µM for each species was matched with the highest seawater phosphate concentration, and the lowest rate of uptake at 5 µM for each species was matched with the lowest seawater phosphate concentration in calculating the *in situ* rates. For time series data we used a third-order polynomial regression. All analyses were done using Sigmaplot 11.0.

## RESULTS

Seawater concentrations of phosphate and total inorganic nitrogen (ammonium, nitrite and nitrate) at Waterfall Reef varied seasonally, with a peak in nutrients in winter (July) (Fig. 1). There was a 3.4-fold (0.20 to 0.68 µM) range of phosphate concentration and a 6.6-fold (0.92 to 6.11 µM) range in total inorganic nitrogen concentration over the sampling period.

Typical time courses as cumulative uptake against time for all species are shown in Fig. 2. Though half-saturation constant ($K_m$) values for phosphate uptake were greater for brown than green and red seaweeds (Fig. 3, Table 1), the differences were not significant ($p = 0.061$). $V_{max}$ and affinity were largely a function of the seaweed surface area:volume quotient (SA:V) with *Ulva intestinalis* (high SA:V) having the highest values for both parameters relative to other species.

Rates of phosphate uptake at an external concentration of 5 µM phosphate reached a peak in spring (September/October) for 4 species (*Melanthalia abscissa*, *Pterocladia lucida*, *U. intestinalis* and *Zonaria turneriana*) (Fig. 4). However, stronger patterns emerged with comparisons of calculated *in situ* rates of phosphate uptake (Fig. 5). Three species (*M. abscissa*, *P. lucida* and *Z. turneriana*) continued to exhibit peaks in uptake rates in September and October despite accounting for changes in seawater phosphate concentration, but the peak in uptake in *U. intestinalis* shifted to July (Fig. 5).

Maximum tissue phosphorus levels occurred in winter (July) or spring (September/October) (Fig. 6). Only *U. intestinalis* had a peak in tissue phosphorus in July, all the others, except *Cystophora torulosa* for which there was no discernible peak, had maxima in spring. Peaks in tissue phosphorus in *M. abscissa*, *P. lucida* and *Z. turneriana* coincided with peaks in rates of uptake in the presence of 5 µM phosphate. However, there was a better correspondence between peaks of *in situ* rates of phosphate uptake and peaks of tissue phosphorus. Tissue phosphorus peaks for *M. abscissa*, *P. lucida*, *U. intestinalis* and *Z. turneriana* coincided with, or occurred soon after, peaks of *in situ* rates of phosphate uptake.

## DISCUSSION

The values for both seawater inorganic phosphate concentration and the kinetic constants for seaweed phosphate uptake were similar to those obtained for seaweeds in Norway (Pedersen et al. 2010). Though it may consequently be tempting to suggest some degree of phosphate limitation for New Zealand seaweeds, as reported for Norwegian seaweeds by Pedersen et al. (2010), the maximum seawater total inorganic nitrogen concentration in Norway is over 3 times greater than that observed by us. However, this does serve to emphasise the potential importance of increased nitrogen inputs into coastal waters in forcing the primary producers towards phosphorus limitation.

Some brown seaweeds have very low $K_m$ values for phosphate uptake: *Laminaria japonica* (Ozaki et al. 2001) and *Sargassum baccularia* (Schaffelke & Klumpp 1998) have $K_m$ values of 0.14 and 0.26 µM, respectively. However, red and green seaweeds in general have lower $K_m$ values for phosphate uptake (4 µM or less) than brown seaweeds (Rees 2003). Affinity values can be used to deduce how well seaweeds can take up phosphate at very low concentra-

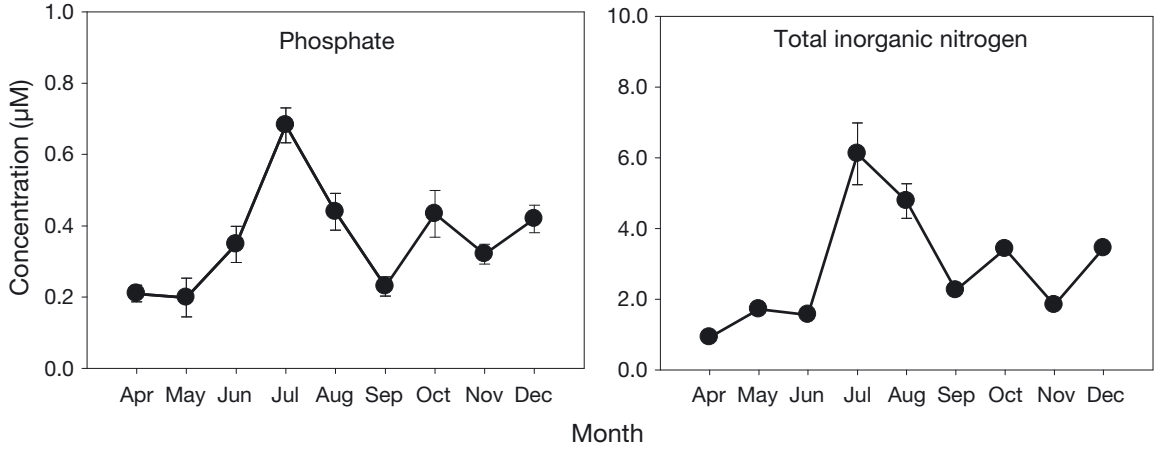


Fig. 1. Total inorganic nitrogen (ammonium, nitrate and nitrite) and phosphate concentrations in seawater at Waterfall Reef in 2008. Data are mean values ± SE

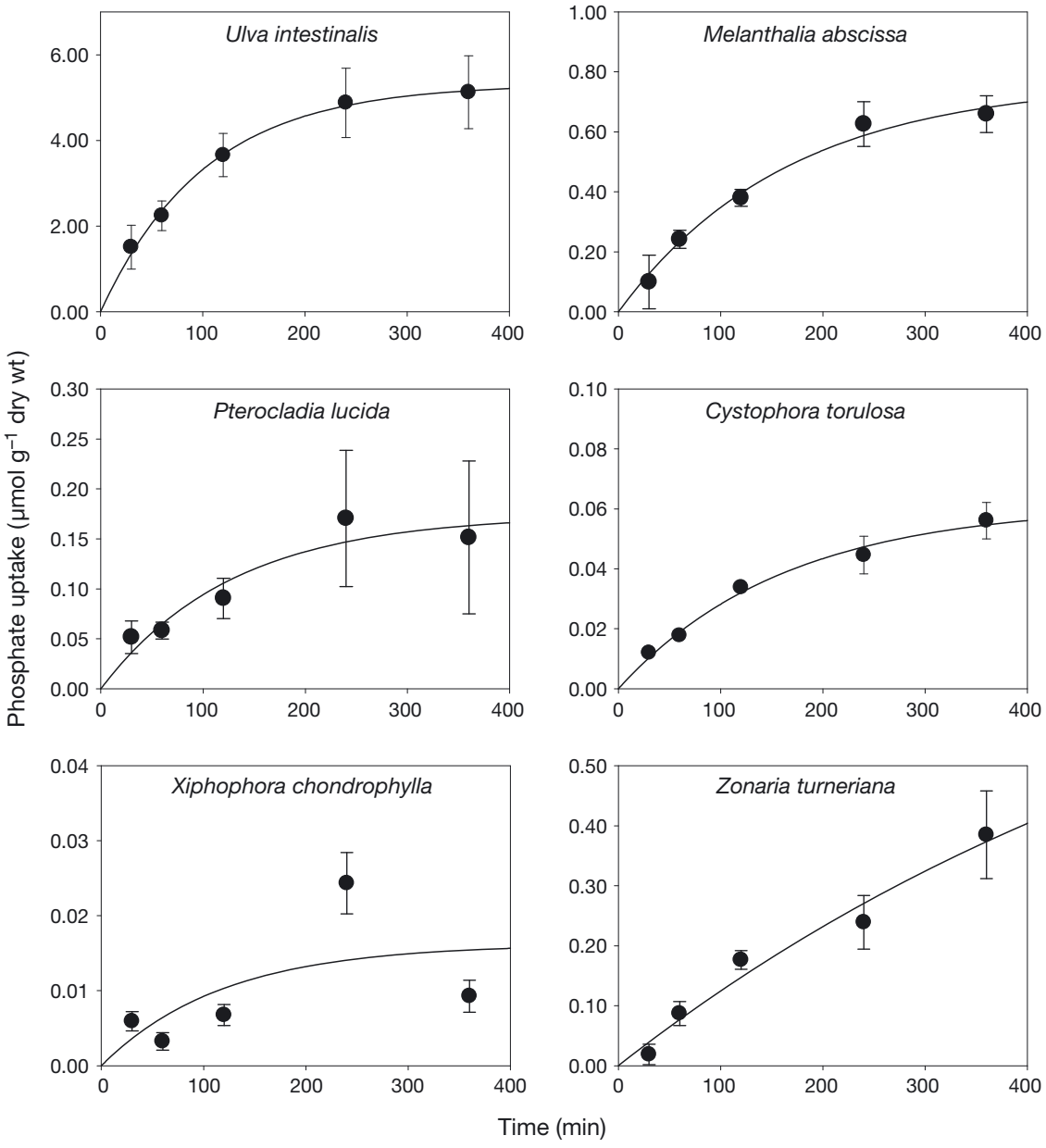


Fig. 2. Time courses of phosphate uptake by 6 species (*Ulva intestinalis*, *Melanthalia abscissa*, *Pterocladia lucida*, *Cystophora torulosa*, *Xiphophora chondrophylla* and *Zonaria turneriana*) of New Zealand seaweeds in July. The rate of uptake for each alga was calculated by taking the slope at time zero of the exponential rise curve fitted to each plot of cumulative phosphate in the seaweed versus time. Note: *y*-axis scale differs among plots. Data are mean values ± SE

tions (Rees 2003), and therefore are indicative of competitive ability. Wallentinus (1984) investigated species with different morphologies and life histories from the Baltic Sea and found that life history traits and morphology are important factors in determining nutrient uptake rates and affinities. Low uptake rates and affinity values occur in long-lived species with low SA:V, whereas high uptake rates and high affinity values are characteristic of opportunistic species with short life spans and high SA:V (Wallentinus 1984). Our data are consistent with her interpretation.

The measurement of phosphate uptake rates of seaweeds at natural concentrations of phosphate *in situ* is, unfortunately, very difficult to achieve. A reasonable and potentially informative estimate of the *in situ* rate of phosphate uptake, however, can be derived from the kinetic constants of uptake and seawater phosphate concentration. The procedure presented in this study makes 3 assumptions: (1) a con-

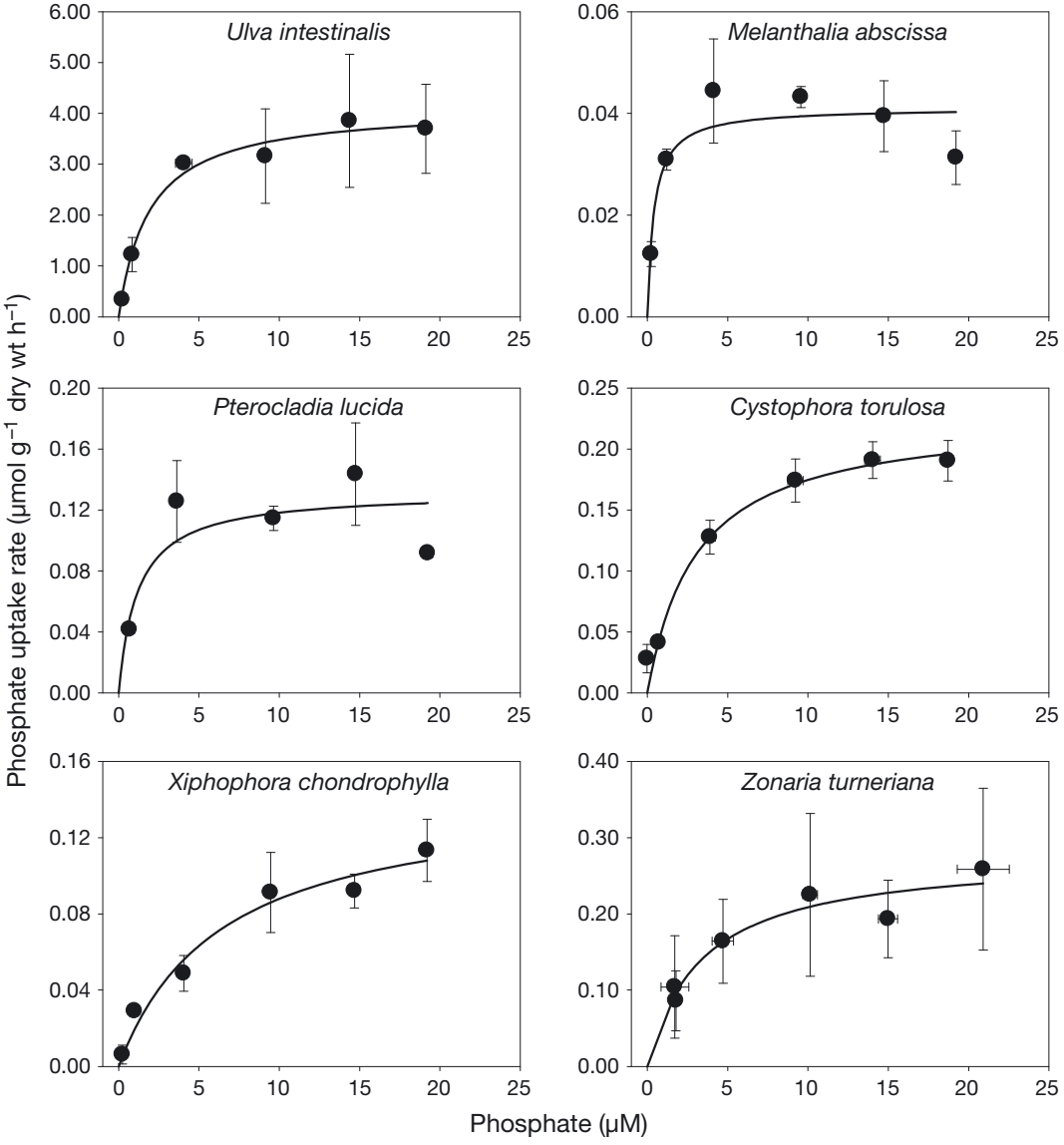


Fig. 3. Effect of external phosphate concentration on rates of phosphate uptake for 6 species (*Ulva intestinalis*, *Melanthalia abscissa*, *Pterocladia lucida*, *Cystophora torulosa*, *Xiphophora chondrophylla* and *Zonaria turneriana*) of New Zealand seaweeds. The fitted curves are rectangular hyperbolae. Note: *y*-axis scale differs among plots. Data are mean values ± SE

stant $K_m$, (2) $V_{max}$ values are proportional to rates of uptake measured at an external concentration of 5 µM phosphate and (3) temperature has little effect on the rate of uptake. The available evidence (Hurd & Dring 1990) suggests that any seasonal changes in $K_m$ values are likely to be minor. Possibilities involving changes in $K_m$ would be (1) synthesis of a low-affinity transport system or (2) a constant affinity, with an increase in $V_{max}$ being accompanied by a proportional increase in $K_m$. There is evidence for low-affinity phosphate transporters in plants, but it is difficult to envisage a situation where one would be required or effective in a marine alga (but see Gordillo et al. 2002). Terrestrial plants experience similar soil concentrations of phosphate and have similar $K_m$ values for uptake (Dunlop et al. 1997) as seaweeds. The effect of any low-affinity transporter at these concentrations is negligible (Dunlop et al. 1997). Increases in $V_{max}$ for phosphate uptake in plants are accompanied by no change in $K_m$ (Dunlop et al. 1997) or a decrease (Lee 1982, Cogliatti & Clarkson 1983, Rubio et al. 1997, Xu et al. 2007); in both instances there is an increase in affinity. In one instance (Jungk et al. 1990), increases in $V_{max}$ are accompanied by increases in $K_m$, but the latter are minor compared to the former, i.e. affinity increases.

Table 1. Maximum uptake rate ($V_{max}$), half-saturation constant ($K_m$), affinity ($V_{max}/K_m$) and surface area:volume (SA:V) quotient values for phosphate uptake in 6 species of New Zealand seaweeds arranged in ascending order of $K_m$ values. SA:V values are from Taylor et al. (1999). Values of $K_m$, $V_{max}$ and affinity are means ± SE for 3 separate determinations

| | Species | $K_m$ (µM) | $V_{max}$ (µmol $g^{-1}$ dry wt $h^{-1}$) | Affinity (l $g^{-1}$ dry wt $h^{-1}$) | SA:V ($cm^{-1}$) |
|---|---|---|---|---|---|
| Rhodophyta | *Melanthalia abscissa* | 0.65 ± 0.25 | 0.04 ± 0.01 | 0.09 ± 0.01 | 30 |
| | *Pterocladia lucida* | 1.64 ± 0.84 | 0.14 ± 0.02 | 0.23 ± 0.17 | – |
| Chlorophyta | *Ulva intestinalis* | 1.97 ± 0.35 | 4.25 ± 1.17 | 2.08 ± 0.22 | 315 |
| Phaeophyceae | *Cystophora torulosa* | 4.09 ± 1.03 | 0.24 ± 0.03 | 0.07 ± 0.01 | 18 |
| | *Zonaria turneriana* | 8.11 ± 6.25 | 0.31 ± 0.07 | 0.12 ± 0.06 | 97 |
| | *Xiphophora chondrophylla* | 9.90 ± 4.04 | 0.17 ± 0.03 | 0.03 ± 0.01 | 21 |

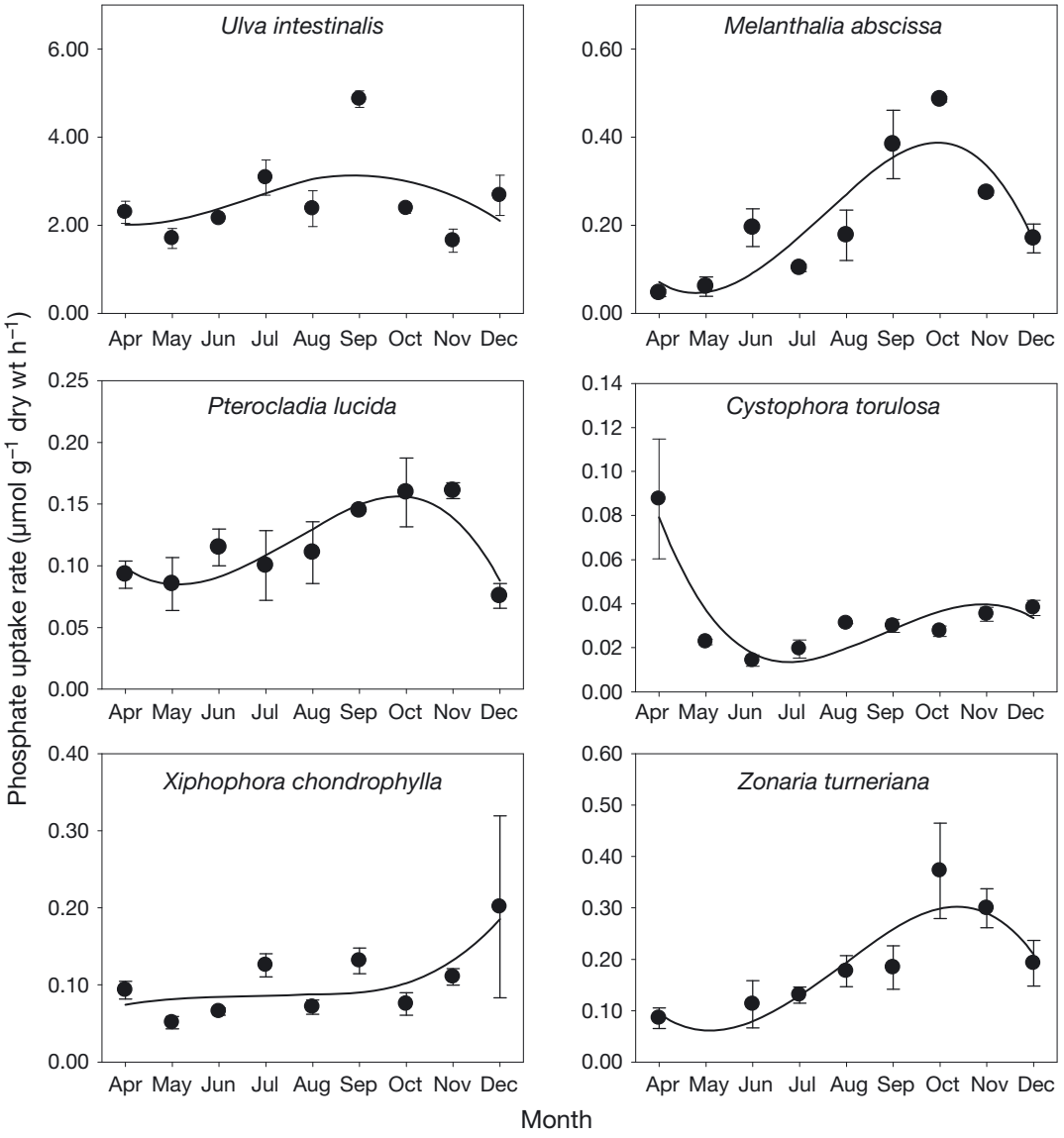


Fig. 4. Temporal changes in rates of phosphate uptake at an external phosphate concentration of 5 µM by 6 species (*Ulva intestinalis*, *Melanthalia abscissa*, *Pterocladia lucida*, *Cystophora torulosa*, *Xiphophora chondrophylla* and *Zonaria turneriana*) of New Zealand seaweeds. The fitted curves are third-order polynomials. Note: *y*-axis scale differs among plots. Data are mean values ± SE

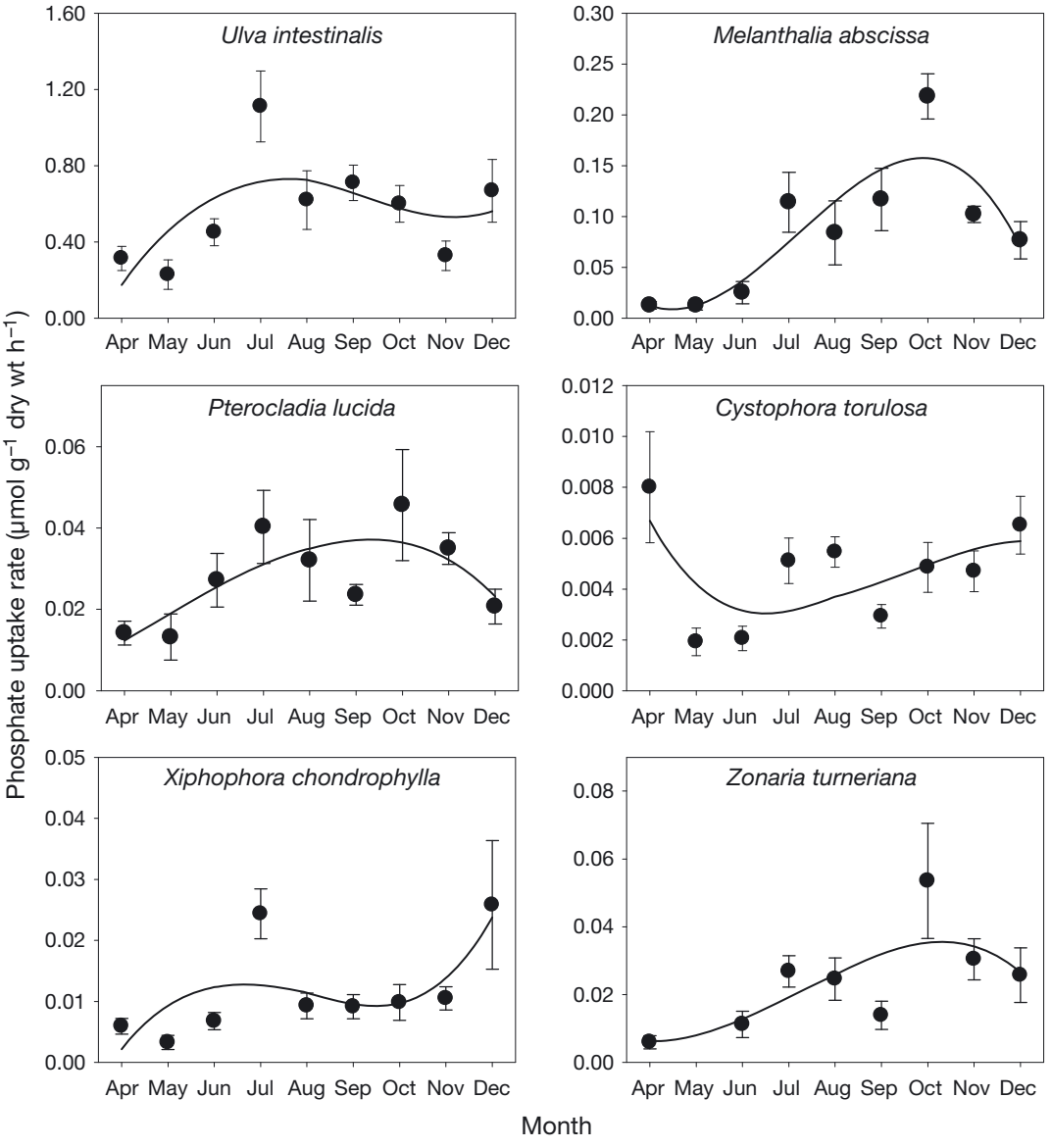


Fig. 5. Temporal changes in calculated *in situ* rates of phosphate uptake for 6 species (*Ulva intestinalis*, *Melanthalia abscissa*, *Pterocladia lucida*, *Cystophora torulosa*, *Xiphophora chondrophylla* and *Zonaria turneriana*) of New Zealand seaweeds at ambient seawater concentrations. The fitted curves are third-order polynomials. Note: *y*-axis scale differs among plots. Data are mean values ± SE

The affinity for phosphate uptake in *Fucus vesiculosus* from a nitrogen-limited ecosystem increases in response to nitrogen enrichment (Perini & Bracken 2014). These data would suggest that our calculations of *in situ* uptake rates are conservative. Maintaining a constant affinity by increasing $K_m$ proportionately as $V_{max}$ increases would provide little competitive advantage to an alga, as the increase in $K_m$ effectively negates any increase in $V_{max}$. If a constant affinity was maintained, the *in situ* rate of uptake would be entirely supply-driven and track changes in seawater phosphate concentration. If the $K_m$ is constant, then the rate at an external concentration of 5 µM phosphate has to be proportional to $V_{max}$. Temperature has little effect on the phosphate uptake rate of *Porphyra* spp. (Pedersen et al. 2004), and the range of seawater temperature measured during our study (April to December) was relatively small (6.3°C) compared with other temperate locations. If we apply the highest $Q_{10}$ value (6.6) of Raven & Geider (1988), the peak in the *in situ* rate of phosphate uptake for all 6 species remain in exactly the same month as reported here. The pattern obtained using this approach was different from that obtained by

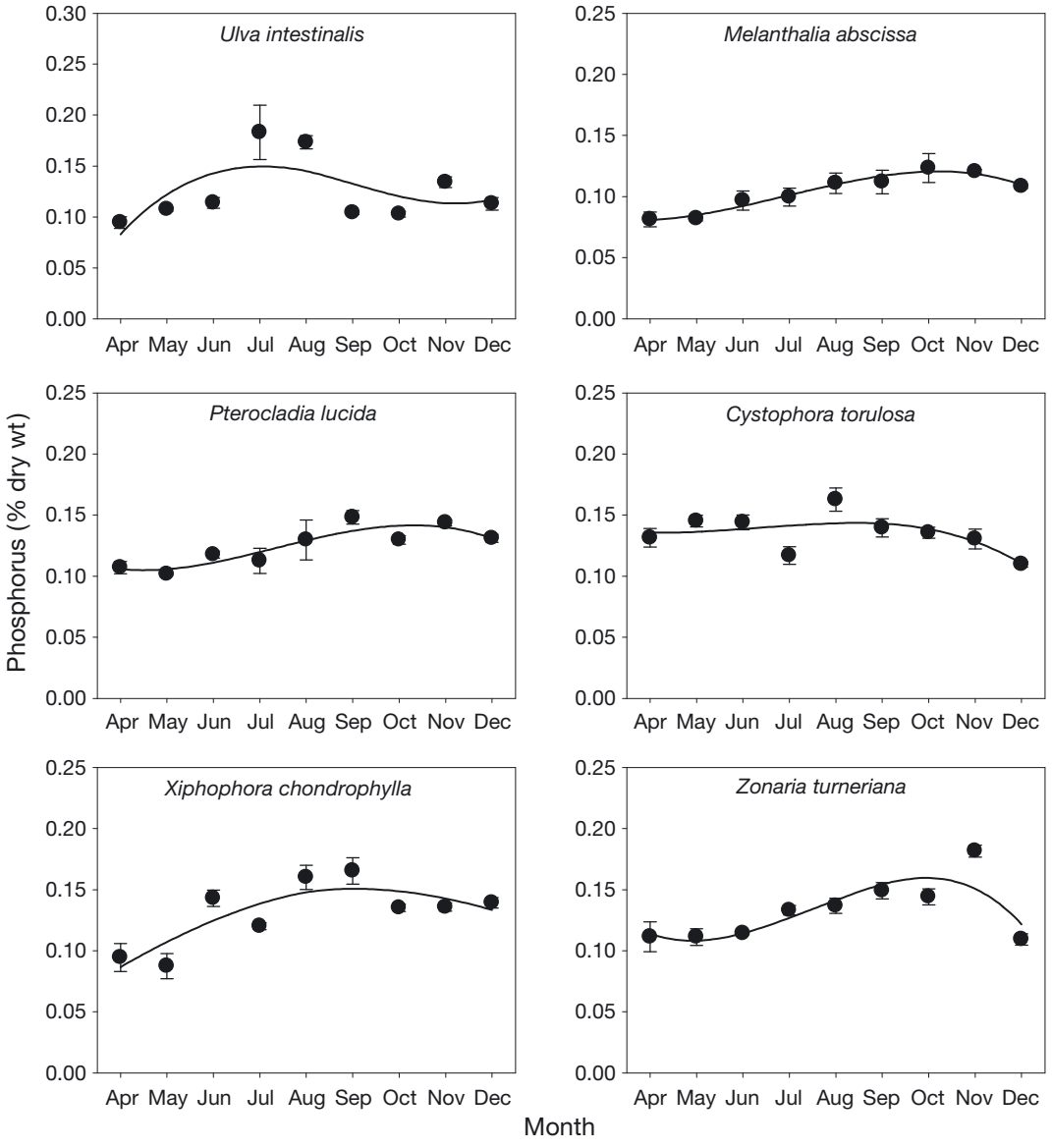


Fig. 6. Monthly mean tissue phosphorus content in 6 species (*Ulva intestinalis*, *Melanthalia abscissa*, *Pterocladia lucida*, *Cystophora torulosa*, *Xiphophora chondrophylla* and *Zonaria turneriana*) of New Zealand seaweeds. The fitted curves are third-order polynomials. Data are mean values ± SE

simply measuring rates at a constant phosphate concentration. Winter peaks for *in situ* rates of phosphate uptake were largely due to supply (higher seawater phosphate concentrations) and spring peaks due to increased demand or intrinsic rates of uptake. In addition, for most seaweeds examined, peak tissue phosphorus levels coincided with, or occurred soon after, the peaks in *in situ* rates of phosphate uptake.

The concentration of phosphate and other nutrients can vary over very short time intervals in estuaries (Litaker et al. 1993, Glibert et al. 2008). Though there is no information for coastal waters, it is possible that similar changes occur. However, though calculated *in situ* uptake rates would change with rates increasing with increasing phosphate concentrations, this would not alter whether uptake is demand-driven as this is dictated by the alga.

There are a number of factors that can influence seasonal tissue phosphorus levels, which can be classified broadly as rates of input (dictated by the effect of supply and demand on phosphate acquisition) and output (growth, reproduction and tissue loss). The balance between input and output is the tissue phosphorus content. Two major determinants of the rate of input are the maximum rate of phosphate uptake and the concentration of phosphate in the surround-

ing seawater, though other factors such as water motion (Barr et al. 2008) will also affect rates of acquisition. The major determinant of demand is likely to be growth rate, but it will include any other process that increases the demand for phosphorus. One example of the latter is reproduction, which can involve an increased demand for phosphorus to create reproductive tissue and, through gamete and spore release, cause loss of phosphorus from the tissue. The kelps *Undaria pinnatifida* and *Alaria crassifolia* require increased levels of tissue phosphorus for sporophyll growth and zoospore formation (Kumura et al. 2006), and *Laminaria* species also require critical levels of tissue nitrogen and phosphorus for growth of reproductive sori (Nimura et al. 2002).

In general, tissue phosphorus was greatest in winter or spring. The growth rate of these seaweeds in winter is likely to be constrained by temperature and/or photon flux density (Chopin et al. 1990). Consequently, the input of phosphorus in winter is largely due to increased supply of phosphate and exceeds output (as growth), causing tissue phosphorus levels to increase. Seaweeds would respond to increased temperature and photon flux density in spring by increasing growth rates. This requires phosphorus, and the increased input is largely under the control of the alga through increased demand-driven uptake of phosphate. This results in increased tissue phosphorus that is subsequently 'diluted' by the creation of new tissue.

The importance of phosphate demand to 'input' is easier to interpret if (1) phosphorus is not limiting growth, (2) there is no luxury uptake and (3) there is no change in tissue phosphorus content (e.g. Viaroli et al. 1996). The available evidence (Carter et al. 2005) suggests that nitrogen is the nutrient most likely to be limiting growth of algae in New Zealand coastal waters, mainly in summer. At other times of the year, particularly winter, it is likely that light and/or temperature limit growth. If we assume that the seaweeds in this study were not limited by phosphorus availability, this raises the issue of whether luxury consumption (Sterner & Elser 2002), defined as input in excess of requirements for growth resulting in polyphosphate storage (Raven 1980), was occurring. It is unlikely that luxury consumption played an important role in this study for 2 reasons. The range of tissue phosphorus values was small, varying from 1.46-fold in *Pterocladia lucida* to 1.96-fold in *Ulva intestinalis*, and the maximum values for tissue phosphorus (0.123 to 0.183%) were low compared with other temperate seaweeds (e.g. Wheeler & Björnsäter 1992), and comparable to some seaweeds from coral reefs (e.g. Schaffelke & Klumpp 1998, Lapointe et al. 2005, Tsai et al. 2005). These ranges of tissue phosphorus values are smaller than the ranges in seawater phosphate concentrations (3.43-fold) and phosphate uptake rates at 5 µM, which varied from 2.88-fold in *P. lucida* to 10.94-fold in *Melanthalia abscissa*. The only other seasonal values for tissue phosphorus in a New Zealand alga are for *Macrocystis pyrifera* from Otago Harbour in the South Island. The range (2-fold) was similar to the values reported here, but the minimum value was 0.23% in September (spring) and the maximum 0.46% in July (winter) (Walsh & Hunter 1992), which is greater than any of the values we obtained for North Island seaweeds, possibly because of the urban nature of Otago Harbour. Of interest is that though the winter value coincided with numerous polyphosphate bodies in *Macrocystis pyrifera* cells, there were no polyphosphate bodies in the cells of the alga in April, when there was lower (0.25%) tissue phosphorus (Walsh & Hunter 1992). As this value exceeds our highest observed value for tissue phosphorus, it suggests that the seaweeds in this study had little or no luxury consumption that led to the formation of polyphosphate.

Maximum rates of phosphate uptake coincide with nitrogen enrichment in intertidal *F. vesiculosus* from nitrogen-deficient coastal waters in Maine (Perini & Bracken 2014). The available evidence suggests that the temperate coastal waters surrounding New Zealand are also nitrogen deficient (Carter et al. 2005). Peaks in *F. vesiculosus* tissue phosphorus content coincided with maximum seawater nitrate concentrations and algal tissue nitrogen content (Perini & Bracken 2014). In contrast, maximum *in situ* rates of phosphate uptake and, where they occurred, maximum tissue phosphorus levels in our subtidal seaweeds occurred in September and October, and did not coincide with maximum seawater concentrations of nitrate (July) or phosphate (July). Of interest, however, is that the pattern in the only intertidal alga we studied, *U. intestinalis*, appears to be very similar to that for the intertidal alga *F. vesiculosus* (Perini & Bracken 2014). Whether our and Perini & Bracken's observations are a specific feature of intertidal algae remains to be determined.

We would suggest that tissue nitrogen in subtidal algae is stored in winter and as light and temperature increase, this nitrogen is used in spring growth. Our unpublished data show peaks in tissue nitrogen in June for 2 of the species (*U. intestinalis* and *Xiphophora chondrophylla*) used here. It should be noted that this nitrogen may be stored as a variety of different compounds from chlorophyll-protein complexes

in green algae (Barr & Rees 2003), L-citrullinyl-L-arginine and/or gigartinine in red algae (Laycock & Craigie 1977) to specific storage proteins in brown algae (Pueschel & Korb 2001), but that all of these need to be metabolised to provide the amino acids required for the synthesis of proteins associated with growth. This protein synthesis generates an increase in demand-driven uptake of phosphate, which is required mainly for the synthesis of RNA, in particular ribosomal RNA (Sterner & Elser 2002).

The central aim was to determine the relationship between supply and demand on rates of *in situ* phosphate uptake. Increased growth rate requires more phosphorus (for RNA) for protein synthesis and this should coincide with the period of greatest demand. Alternatively, if seaweeds are driven by phosphate supply, tissue phosphorus would track phosphate concentration in the surrounding water. For all subtidal species, maximum *in situ* rates of phosphate uptake were demand-driven, but in the only intertidal alga, *U. intestinalis*, uptake was supply-driven.

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