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Seasonal ammonium uptake kinetics of four brown macroalgae: Implications for use in integrated multi-trophic aquaculture

Joanna N. Smart[1] · Matthias Schmid[1,2] · Ellie R. Paine[1] · Damon Britton[1] · Andrew Revill[3] · Catriona L. Hurd[1]

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Abstract

The combined culture of fed species (bivalves, fish) and macroalgae, known as integrated multi-trophic aquaculture (IMTA), has been suggested as a method of mitigating localised nitrogen (N) increase from aquaculture, whilst simultaneously culturing macroalgae for commercial applications. The development of IMTA requires an understanding of the N ecophysiology of candidate macroalga species. We examined seasonal variations in ammonium (NH_4^+) uptake kinetics, carbon to nitrogen (C:N) ratio, pigment content and soluble tissue N of four macroalgae of the phylum Ochrophyta, *Ecklonia radiata, Macrocystis pyrifera, Lessonia corrugata,* and *Phyllospora comosa,* from Tasmania, Australia. This study aimed to determine, (1) if the N physiology of the four macroalgal species was suitable for IMTA applications and (2) whether the species had seasonal variations in N ecophysiology which would influence their suitability for IMTA. *Macrocystis pyrifera*, *L. corrugata,* and *E. radiata* exhibited saturable NH_4^+ uptake kinetics, with a maximum uptake rate (V_{max}) during spring, summer and autumn of 200, 45.8 and 45 µmol gDW^{-1} h^{-1} and half-saturation constants (K_s) of 361.3, 104.2 and 121 µM, respectively. *Phyllospora comosa* exhibited biphasic uptake patterns for three out of four months sampled. There were no noticeable seasonal patterns in pigment content or soluble tissue N for any species. C:N ratios increased from spring (October) to autumn (March) in both *E. radiata* (28.34 – 47.83) and *P. comosa* (24.99 – 51.62), indicating progressive N limitation though summer and into autumn. Results suggest that *M. pyrifera* and *P. comosa* are most suitable for IMTA due to their high NH_4^+ uptake potential.

Keywords ammonium · integrated-multi-tropic aquaculture · macroalgae · nitrogen physiology · Ochrophyta

Introduction

Anthropogenic N inputs into coastal waters have been increasing over the last century (Vitousek 1997; Seitzenger et al. 2002; Seitzinger et al. 2008), in part caused by intensive finfish aquaculture, as fish excrete additional NH_4^+ into the ecosystem (Handy and Poxton 1993; Wild-Allen et al. 2010). Such inputs can have broader scale ecological impacts outside of the farm footprint (Oh et al. 2015). Nutrient loading can cause eutrophication and anoxic sediments leading to broader scale changes in marine community structure such as alteration of benthic fauna and native fish abundance, increased macroalgal growth and epiphyte loading (Black 2001; Read and Fernandes 2003; Soto and Norambuena 2004; Buschmann et al. 2006; Cubitt et al. 2008).

Integrated multi-trophic aquaculture (IMTA) is being tested as a mitigation solution for the effects of increased dissolved inorganic nitrogen (DIN) loading associated with intensive mono-specific aquaculture operations (Wu et al. 2015; Biswas et al. 2020; Knowler et al. 2020; Rugiu et al. 2021). In these systems, fed aquaculture species are farmed in conjunction with extractive species such as macroalgae which take up a portion of the excreted nutrients and can reduce the overall nutrient input into the environment (Chopin et al. 2001; Chopin 2006). Other organisms in the water column also take up DIN surrounding aquaculture facilities including phytoplankton, but IMTA operations utilise species which can then be harvested for commercial gain (Knowler et al. 2020). They can also provide key ecosystem services such as oxygenation and mitigate against coastal

✉ Joanna N. Smart
joanna.smart@utas.edu.au

[1] Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania 7001, Australia

[2] Present Address: Trinity College Dublin, University of Dublin, Dublin, Ireland

[3] CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia

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ocean acidification (Hasselström et al. 2018; Fernández et al. 2018). IMTA principles have been applied to aquaculture operations globally including Korea (Park et al. 2018), China (Wu et al. 2017), Chile (Buschmann et al. 1994, 2008, Vásquez 2008), Europe (Haglund and Pedersén 1993; Sanderson et al. 2008), Israel (Ashkenazi et al. 2019), and North America (Chopin et al. 1999; Carmona et al. 2006). Similarly, there is a strong interest to implement IMTA methods into Australian aquaculture facilities using local macroalga species (Kelly 2020).

Nitrogen is an essential nutrient for macroalgae, found in chlorophylls *a* and *b,* amino acids and cellular enzymes, thus is a key factor limiting macroalgal growth in the marine environment (Hurd et al. 2014). The two main sources of N used by macroalgae are ammonium (NH_4^+) and nitrate (NO_3^-). Ambient concentrations of inorganic N are generally low in seawater, ranging between <5-20 µM depending on location, however concentrations of NH_4^+ can exceed 150 µM immediately surrounding finfish aquaculture facilities (Neori and Shpigel 1999; Carmona et al. 2006). In temperate systems the availability of DIN varies seasonally being generally higher in winter and lower in summer (Hurd et al. 2014). However, spatial variations in inorganic N supply due to anthropogenic inputs can cause localised increases in N concentration regardless of the season (Herbert 1999; Howarth and Marino 2006; Van Alstyne 2018).

One key aspect to assess macroalgal species suitability for IMTA is understanding NH_4^+ uptake kinetics (Roleda and Hurd 2019) because it is the primary waste product of operations incorporating marine finfish, excreted through the gills (Randall and Wright 1987; Wilkie 1997). Also localised increases in NH_4^+ concentrations are observed surrounding finfish cages (Sanderson et al. 2008). NH_4^+ is readily available to macroalgae, where it is taken up through cell membranes via one or more of three mechanisms - passive diffusion, facilitated diffusion and active uptake (Hurd et al. 2014; Roleda and Hurd 2019). Passive transport involves uptake via simple diffusion, whereas facilitated diffusion and active transport utilise proteins to move ions into the cell.

Uptake kinetics can be used to gain an understanding of the mechanisms of inorganic N uptake (Harrison and Druehl 1982; Rosenberg and Ramus 1984; Phillips and Hurd 2004; Roleda and Hurd 2019). Active uptake is indicated by a plot of uptake rate vs. concentration exhibiting saturating kinetics and can be described by the Michaelis-Menten equation (Hurd et al. 2014):

$$V = V_{max}\frac{S}{K_m + S}$$

From this relationship, the parameters maximum uptake rate (V_{max}) and half-saturation constant (K_s) are determined. Desirable V_{max} and K_s values for IMTA are dependent on the objective of the IMTA operation (Chopin et al. 2001). Species with a high V_{max} and K_s values can better take up NH_4^+ at high concentrations, which is desirable for IMTA applications and lower values of K_s indicate a greater ability to procure nutrients at a low concentration. Passive uptake is indicated by a linear relationship between concentration and uptake rate. A combination of linear and saturating components indicates that both active and passive uptake mechanisms are present, known as biphasic or multi-phasic uptake (Roleda and Hurd 2019, See Fig. 6.2 d in Hurd et al. 2014). Biphasic uptake mechanisms also prove desirable for IMTA applications, as the species can take up NH_4^+ at high external NH_4^+ concentrations.

In addition to NH_4^+ uptake kinetics, determining which macroalgal species are most suited to IMTA requires a comprehensive understanding of their underlying N ecophysiology (Neori et al. 2004). Of particular importance are the C:N ratios, photosynthetic pigment content, and soluble tissue nitrogen pools which are used to assess the nitrogen status of the macroalgal tissue (Roleda and Hurd 2019; Rugiu et al. 2021). Comparatively high C:N ratios, low pigment content and low soluble tissue N pools can indicate that macroalgal tissues are depleted in N (Roleda and Hurd 2019; Chopin et al. 1995; Vergara et al. 1993). N depleted species are able to uptake excess DIN in the environment, such as that released from aquaculture operations and are therefore useful in IMTA operations (Pedersen and Borum 1997; Hadley et al. 2018). Soluble tissue N is the amount of N that is stored within the macroalgae cells and provides an indication of nutrient storage capacity, and whether the macroalgae are N depleted at the time of sampling (Roleda and Hurd 2019). Seasonal variation in macroalgal N physiology has been observed for many temperate regions worldwide due to changing light, water temperature, wave motion and nutrient supply (Kain 1989; Lüning 1993). Macroalgae adapt to these changes by seasonally altering pigment content (Flukes et al. 2015), nutrient uptake and storage (Asare and Harlin 1983, Hurd and Dring 1990, Phillips and Hurd 2004), as well as biochemical composition (Wheeler and Björnsater 1992).

Macroalgae of the orders Laminariales and Fucales are key components of temperate reefs worldwide and are candidate species for IMTA in southern Australia. In Tasmania, there are a range of animal aquaculture operations including salmon, mussels, abalone and pacific oysters (DPIPWE 2020), with growing interest to develop IMTA to help mitigate DIN inputs and provide a commercial product. The proportion of DIN up taken my macroalgae in an IMTA setting varies depending on location, stocking densities and hydrodynamics of the area. Modelling studies in Tasmania indicate that *M. pyrifera* can remove up to 11% of DIN input from salmon aquaculture over a nine-month period (Hadley et al. 2018). Four macroalgal species, *Ecklonia radiata* (C. Agardh) J. Agardh, *Macrocystis pyrifera* (Linnaeus) C.

Agardh, *Lessonia corrugata* A.H.S. Lucas, and *Phyllospora comosa* (Labillardière) C. Agardh, have been identified as potential species for IMTA operations in Tasmania due to economic value and potential for high biomass production (Sanderson and Di Bendetto 1988; Kelly 2020). Members of the Laminariales are being trialled in other regions due their comparatively fast growth rate (Barrington et al. 2009), and although the growth rates of the Tasmanian kelps are not well studied, *Macrocystis* and *Ecklonia* are known to have growth rates similar to those of other Laminariales (Miller et al. 2011; Schiel and Foster 2015). Here we determined (1) if the NH_4^+ physiology of the four Tasmanian species studied were suitable for IMTA applications and (2) if the species exhibited seasonal patterns in their NH_4^+ physiology that affect their suitability for IMTA.

Materials and Methods

Sample Collection

Macroalgal samples were collected sub-tidally at 3-5 m depth from two sites in southern Tasmania. Collection occurred four times during Spring – Autumn 2018-2019 at Flowerpot Point, Blackmans Bay (-43° 0'27"S, 147°19'44"E) and the Tessellated Pavements, Eagle Hawk Neck (43° 0'30"S, 147°56'7"E) (Table 1). Mature blades were collected for *M. pyrifera* and *L. corrugata*. Mature, lateral blades were collected for *E. radiata* and *P. comosa*. Five individuals for each species ($n = 5$) were collected at each sampling event. Samples were individually wrapped in damp tissue paper, stored in a dark cool-box and transported back to the laboratory, with 30 min for Flowerpot Point and 60 min for Tessellated Pavements. At the laboratory, samples were immediately wiped with tissue to remove any epibionts and rinsed in filtered, UV sterilised seawater (filtered to 1 µM and UV-sterilised with an Emperor Aquatics Smart HO UV steriliser, 025050-2, 50 W lamp) before being divided into experimental sections (see below). At the time of each collection, replicate 10 mL water samples ($n = 3$) were taken for analysis of ambient NH_4^+ and NO_3^- in seawater. Samples were filtered through a 0.7 µm filter (Whatman GF/F) on-site before being transported back to the laboratory and frozen at -20°C until analysis.

NH_4^+ Uptake Kinetics

To determine the NH_4^+ uptake kinetics of each species, individual blades from each species ($n = 5$) were divided into seven discs using a 3 cm diameter cork borer for *M. pyrifera* (~ 0.5 g), *E. radiata* (~0.5 g) and *L. corrugata* (~ 1.0 g), or seven 5 cm individual apical blade sections for *P. comosa* (~0.25 g). Samples from each individual were placed into separate beakers with filtered seawater and placed on shaker tables set to 100 rpm and a photoperiod of 12:12, which was kept constant across each experiment (at 150 µmol photons m^{-2} s^{-1}) for 24 h to allow wound healing (McDowell et al. 2015).

To determine the maximum uptake rate (V_{max}) and half-saturation coefficient (K_s) of each macroalga species, a multiple flask, constant incubation time, experiment was conducted for (Philips and Hurd 2003) at each sampling event, within 48 h of sample collection. A total of four experiments were conducted for each species. For each species, 37 × 250 mL conical flasks were filled with 200 mL of filtered seawater and enriched with NH_4^+ from a stock solution of NH_4Cl (0.2 M) to give a concentration series of approximately 2, 10, 20, 40, 80, 160 and 240 µM, with five replicates ($n = 5$) for each species. Two additional conical flasks with no macroalgae were used as controls, with one containing filtered seawater and one with filtered seawater enriched to 240 µM.

Before addition of the macroalgae, an initial seawater sample was taken from each flask with a 12 mL syringe filtered through a 0.7 µM filter (Whatman GF/F). Samples were stored in 12 mL polyethylene tubes at -20°C. One piece of alga was then placed into each flask and set on a shaker table at 100 rpm under 150 µmol photons m^{-2} s^{-1}. All flasks were left for two h as no lag or surge phases were detected for any species in the preliminary time-course experiment (data not shown). After two hours, a final water sample was taken, and macroalgae were removed from each flask. Macroalgae were blotted dry, weighed for wet weight (WW) and photographed for surface area. The surface area was calculated using Adobe Photoshop CC 2018 (Adobe Software

Table 1 Location and date of each macroalgal collection event.

Species	Location	Collection Date			
		October 2018 (Spring)	November 2018 (Spring)	January 2019 (Summer)	March 2019 (Autumn)
L. corrugata	Flowerpot Point	4/10/2018	26/11/2018	13/01/2019	08/03/2019
M. pyrifera	Flowerpot Point	4/10/2018	27/11/2018	13/01/2019	08/03/2019
E. radiata	Tessellated Pavements	1/10/2018	29/11/2018	18/01/2019	11/03/2019
P. comosa	Tessellated Pavements	1/10/2018	29/11/2018	18/01/2019	11/03/2019

Inc). Algal pieces were then dried at 60°C for 48 h for determination of dry weight.

Seawater nutrient analysis

NH_4^+ concentrations from the uptake experiments and NO_3^- and NH_4^+ concentrations extracted from tissue (soluble pools) was determined using a QuickChem 8000 Automated Ion Analyser (LaChat Instruments) using the methods outlined in 'Determination of nitrate/nitrite in brackish or seawater by flow injection analysis' (Diamond 2008)

and 'Determination of ammonia in brackish or seawater by flow injection analysis' (Liao 2008).

Calculation of NH_4^+ uptake rates

Uptake rates of NH_4^+ in individual flasks were calculated using the following equation:

$$V = \frac{\left(S_i - S_f\right) \times Vol}{t \times DW}$$

where V = uptake rate (µmol g^{-1} DW h^{-1}), S_i = initial concentration of seawater NH_4^+ (µM), S_f = final concentration after time interval (µM), t = time interval (2 h) and DW = dry weight of macroalgal samples (g) (Harrison and Druehl 1982).

Soluble tissue NO_3^- and NH_4^+ pools

Soluble tissue NO_3^- and NH_4^+ pools were determined in November 2018, January and March 2019 by boiling water extraction (Hurd et al. 1996). One additional tissue sample was taken from each replicate ($n = 5$) of each of the four species prior to the NH_4^+ uptake experiment for analysis of soluble tissue N. These tissue samples were blotted dry and cut into sections of 0.25 g ± 0.01 g. Each piece was placed into a 50 mL boiling tube with 20 mL of deionised water and samples were refrigerated overnight (4°C). Test tubes were then placed in a boiling water bath for 20 min. The samples were left to cool, and the liquid was decanted and filtered through 0.7 µm glass filter paper (Whatman GF/F). The extract was stored at -20°C before analysis using an Automated Ion Analyser described above. This process was repeated three times to ensure all soluble tissue N was extracted. NH_4^+ and NO_3^- contents were calculated using the following equation:

$$N_T = \frac{\left(N_1 + N_2 + N_3\right) \times V}{WW}$$

where N_T is the total concentration of NH_4^+ or NO_3^- extracted, N_1, N_2, and N_3 are the concentrations of NH_4^+ or NO_3^- is the solution after subsequent boiling extractions (µM), V is the volume of water in the boiling tube (L) and WW is the wet weight (g) of the seaweed sample.

Photosynthetic Pigment Content

Photosynthetic pigment content (chlorophyll *a*, chlorophyll *c* and fucoxanthin) was determined using the methods outlined in Seely et al. (1972). Samples of 0.1~ 0.15 g wet weight were taken from the five collected replicates of each species, frozen in liquid nitrogen and stored at -80°C until extraction. Samples were then defrosted and placed into test tubes. Although approximately the same weight (0.1-0.15 g) as other species, the blades of *L. corrugata* were thicker than other species, and so were cut into smaller pieces to facilitate extraction. 4 mL of dimethyl sulfoxide (DMSO) was added to each test tube and samples were left to extract for 10 min. The liquid was then decanted and collected in a test tube. Immediately afterwards 6 mL of 90% acetone v/v was added to the macroalgal tissue and left to extract for 30 min or until tissue was void of pigments, and subsequently decanted into a separate test tube. Test tubes were kept over ice and regularly agitated. Absorbance of the extracts were measured with a S-22 UV/Vis Spectrophotometer (Halo RB-10, Dynamica Scientific Ltd). The absorbance of the DMSO extract was measured at 665, 631, 582, and 480 nm and the acetone extract measured at 664, 631, 581 and 470 nm. Pigment contents were calculated using the equations given by Seely et al. (1972).

C, N and C: N ratio

Tissue carbon, tissue nitrogen and C:N ratio of three replicates per species were determined in October and November 2018 and January and March 2019 using the methods described in Cornwall et al. (2015). An additional 2 cm diameter disk was taken from each blade of the collected species and dried at 60°C for 48 h. A Carlo-Erba NA1500 elemental analyser coupled to a Thermo Scientific Delta V Plus via a Conflo IV was used in analysis with combustion and reduction of samples was achieved at 1020°C and 650°C, respectively. Values were normalised to the Vienna Pee Dee Belemnite (VPDB) scale with a 3-point calibration and both precision and accuracy were ± 0.1 % (1 SD).

Curve fitting and data analysis

The Michaelis-Menten function (i.e., a rectangular hyperbola) was fitted to each replicate of *M. pyrifera, L. corrugata,* and *E. radiata* using SigmaPlot (Systat Software Inc), and V_{max} and K_s obtained for each replicate. The mean values for V_{max} and K_s ± SE were then obtained for each species in each sampling month.

For *P. comosa* the Michaelis-Menten function could only be fitted for data collected in January. In October, December, and March, the pattern of uptake vs. concentration was biphasic with uptake of NH_4^+ appearing to saturate for concentrations <160 µM, however further increased linearly between 160 – 240 µM. Michaelis-Menten curves were therefore fitted to uptake rates at NH_4^+ concentrations <160 µM, and a linear regression was applied to uptake rates at NH_4^+ >160 µM. Negative uptake values were excluded from curve fitting and manuscript figures but are presented in Appendix 1.

The means ± SE were calculated for soluble tissue NH_4^+ and NO_3^-, pigment content, C:N ratio, C and N. Data were analysed using the statistical software R (R core development team 2017). Data were tested for conformity of assumptions of homogeneity of variances and normality of residuals by plotting residuals and fitted values. Transformations to meet these assumptions for V_{max}, K_s, soluble tissue N and pigment determined using the Box-Cox method, and log transformations were applied to the data before analysis. A two-way ANOVA with the factors 'Species', 'Season' and 'Species*Season' was applied where $p < 0.05$. This test compared differences between species, between season and interaction effects and was applied to V_{max}, K_s, soluble tissue N content, pigment content, and C:N ratio. For significant results, a-posteriori multiple comparisons were then conducted using a Tukey's HSD test to elucidate specific differences between species across seasons. Additionally, Pearson's correlation test ($p < 0.05$) was used to determine the relationship between V_{max} and K_s across the sampling months.

Results

Background seawater nutrient concentration

Ambient, *in situ* seawater nutrients did not exceed 3.32 µM, with the highest concentrations recorded at the Tessellated Pavements (Table 2). The range of NO_3^- concentrations across seasons were similar at both Flowerpot Point and the Tessellated Pavements.

Table 2 Range of seawater NH_4^+ and NO_3^- concentrations recorded at each location during the collection events

Location	NH_4^+ concentration (µM)	NO_3^- concentration (µM)
Flowerpot Point	1.16 – 1.55	0.29 – 0.87
Tessellated Pavements	0.56 – 3.32	0.20 – 0.69

NH_4^+ uptake kinetics

Controls showed minimal change in NH_4^+ concentration (0 – 10 %) over the two-hour experimental period and the depletion of NH_4^+ from the seawater with macroalgae was thus attributed to macroalgal uptake. *L. corrugata*, *E. radiata,* and *M. pyrifera* all exhibited saturable uptake (Fig. 1). In contrast, *P. comosa* uptake was biphasic for three of four sampling events (Fig. 2) and V_{max} and K_s values could not be determined.

V_{max} were significantly different between species ($p < 0.05$), and an interaction effect between species and season ($p < 0.05$), indicating that seasonal patterns in V_{max} were not consistent across species (Table 3, Fig 3). An *a-posteriori* Tukey's HSD test revealed no significant differences in *E. radiata* and *L. corrugata* V_{max} over the sampling months ($p > 0.05$). In contrast, *M. pyrifera* V_{max} values varied seasonally and were highest in October 2018 (159 µmoles g^{-1} DW h^{-1} ± 23 SE, Tukey's test, $p < 0.01$) and January 2019 (200 µmoles g^{-1} DW h^{-1} ± 23 SE, Tukey's Test, $p < 0.01$) (Table 3). The highest V_{max} values for *M. pyrifera* were approximately 4.5 times greater than those for *E. radiata* and *L. corrugata,* which ranged between 17 and 45 µmol g^{-1} DW h^{-1}.

For K_s, the two-way ANOVA indicated significant differences in species and season (Table 3). As with V_{max}, an interaction effect of species and season was also present for K_s values, indicating that seasonal effects were not consistent across species. Graphs of mean K_s ± SE are shown in Fig. 3. The K_s of *L. corrugata* did not change significantly between sampling months (Tukey's test, $p > 0.05$), ranging between 104 and 134 µM. In contrast, the K_s of E. *radiata* varied over the sampling months (Tukey's test, $p < 0.05$) and was approximately nine times higher in March 2019 (204.33 µM ± 52.35 SE) than October 2018 (23.50 µM ± 5.05 SE). The K_s of *M. pyrifera* also varied across sampling months, with November K_s values being significantly lower than the other seasons (Tukey's test, $p < 0.05$). Higher K_s values were positively correlated to higher V_{max} values (Pearson correlation, R = 0.808, t = 10.45, df = 58, $p < 0.01$). The high V_{max} values for *M. pyrifera* in October 2018 and January 2019 occurred with the highest observed K_s values (325.85 µM ± 95.83 SE and 361.26 ± 80.26 SE respectively).

Soluble tissue NO_3^- and NH_4^+ pools

Soluble tissue NO_3^- and NH_4^+ content (µmol g^{-1} WW) were variable both across seasons and between species (Table 3). Additionally, an interaction effect of species and season was present with no clear seasonal pattern in soluble tissue N content. For all species, NH_4^+ content was higher than NO_3^- content for all seasons (Fig. 4). As such, variation in total N content reflected changes in tissue NH_4^+. Average NH_4^+

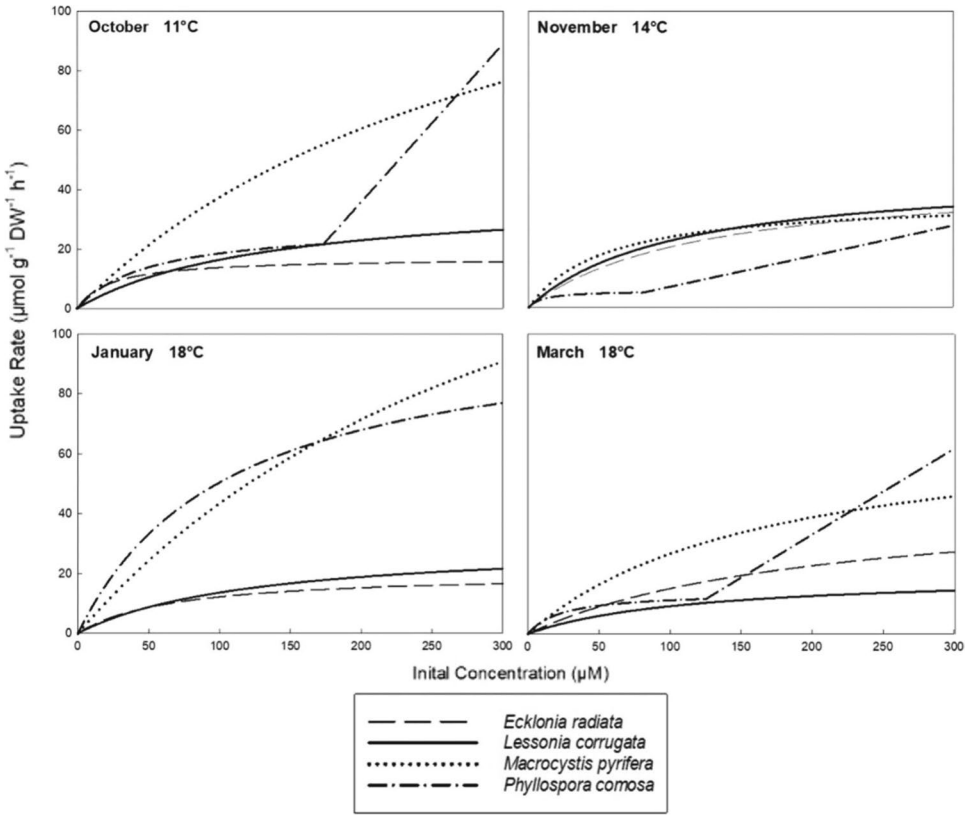

Fig. 1 NH_4^+ uptake rates as a function of substrate concentration for *E. radiata, L. corrugata, M. pyrifera* and *P. comosa* in October and November 2018 and January and March 2019

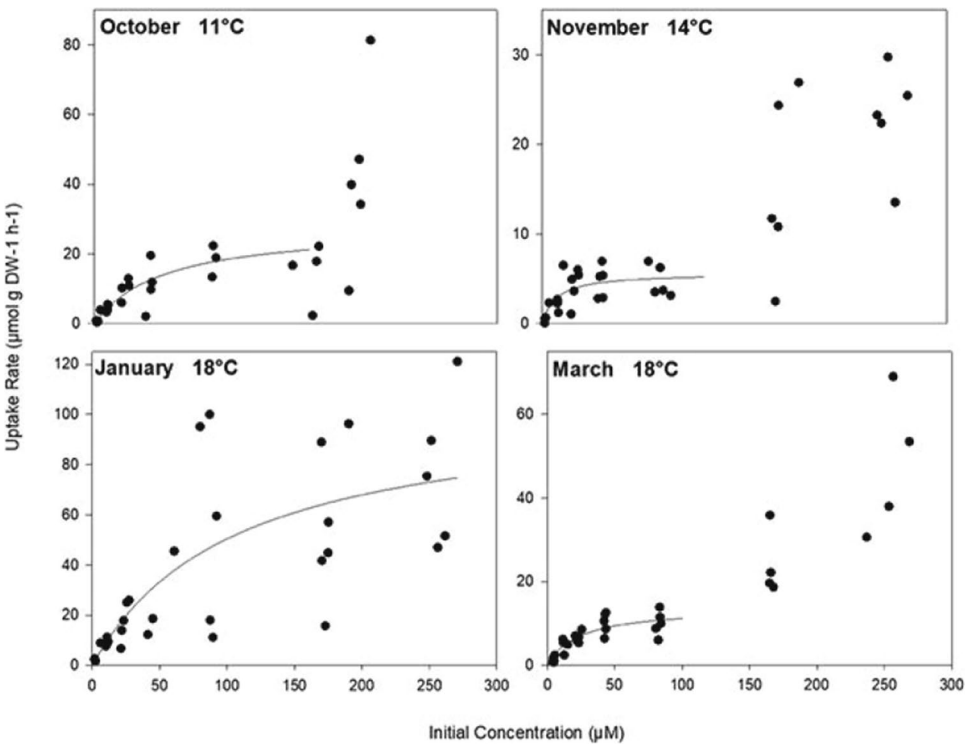

Fig. 2 Rates of NH_4^+ uptake as a function of NH_4^+ concentration for *P. comosa*. Note the y-axis scale range. Each point is a replicate seaweed ($n = 5$)

tissue content as a percentage of total N was 87.66% for *L. corrugata,* 95.49% for *M. pyrifera*, 85.82% for *E. radiata* and 92.87% for *P. comosa*.

For *L. corrugata,* total soluble N content was lowest in summer (January) (0.928 µmol g^{-1} WW ± 0.140 SE, Tukey's test $p < 0.01$) when compared to November and March (~4

Table 3 Summary of two-way ANOVA results for each parameter

Parameter	Degrees of Freedom	Sum of Squares	F Value	*p*-value
Vmax				
Species	2	19.616	35.973	**< 0.001**
Season	3	0.602	0.736	0.536
Species*Season	6	13.5	8.252	**< 0.001**
Ks				
Species	2	11.31	7.928	**< 0.01**
Season	3	5.06	2.363	**< 0.05**
Species*Season	6	17.28	4.038	**< 0.01**
Soluble TissueN				
Species	2	6.153	30.459	**< 0.001**
Season	3	2.563	8.458	**< 0.001**
Species*Season	6	18.72	30.889	**< 0.001**
Pigment Content				
Species	3	9.480	13.026	**< 0.001**
Season	3	4.924	6.767	**< 0.001**
Species*Season	9	5.419	2.482	**< 0.01**
C: N Ratio				
Species	3	1.218	528.9	**< 0.001**
Season	3	1.766	766.8	**< 0.001**
Species*Season	9	1.102	159.5	**< 0.001**
TissueN				
Species	3	0.188	252.4	**< 0.001**
Season	3	0.191	256.0	**< 0.001**
Species*Season	9	0.274	22.4	**< 0.001**
Tissue C				
Species	3	0.416	28.23	**< 0.001**
Season	3	2.281	154.52	**< 0.001**
Species*Season	9	0.632	14.27	**< 0.001**

µmol g^{-1} WW). A similar pattern was seen in *M. pyrifera*, where total soluble N content was approximately three times higher in March (5.435 µmol g^{-1} WW^1 ± 1.090 SE, Tukey's Test $p < 0.01$) than January (0.581 µmol g^{-1} WW ± 0.114 SE). In contrast, total soluble N content for *E. radiata* in January was approximately double the values recorded for November and March (4.519 µmol g^{-1} WW ± 0.693 SE, Tukey's test $p < 0.01$). *P. comosa* soluble tissue N content was highest in November (2.42 µmol g^{-1} WW ± 0.292 SE, Tukey's test, $p < 0.01$).

Photosynthetic pigment content

Pigment content was significantly different between species and between seasons (Table 3). An interaction effect of species and season was also present ($p < 0.05$). Lowest mean pigment contents were recorded for *L. corrugata* samples in January 2019 (0.0591 mg g^{-1} ± 0.0139 SE) and were highest in *P. comosa* in November 2018 (0.326 mg g^{-1} ± 0.138 SE) (Fig. 5). There were no significant differences in pigment content across season for *L. corrugata*, *M. pyrifera* and *P. comosa* (Tukey's test, $p > 0.05$ for all pairwise comparisons). Mean total pigment content of *E. radiata* was approximately three times higher in October (0.309 mg g^{-1} ± 0.0212 SE) than January and March ($p < 0.01$). November pigment content was lower than October (0.149 mg g^{-1} ± 0.0167 SE, $p > 0.01$) and the lowest mean total pigment content for this species was recorded in March (0.0898 mg g^{-1} ± 0.0119 SE).

C, N and C:N ratio

The two-way ANOVA revealed differences in tissue N, tissue C and C:N ratio between seasons and species, with and additional interaction effect (Table 3). For all species,

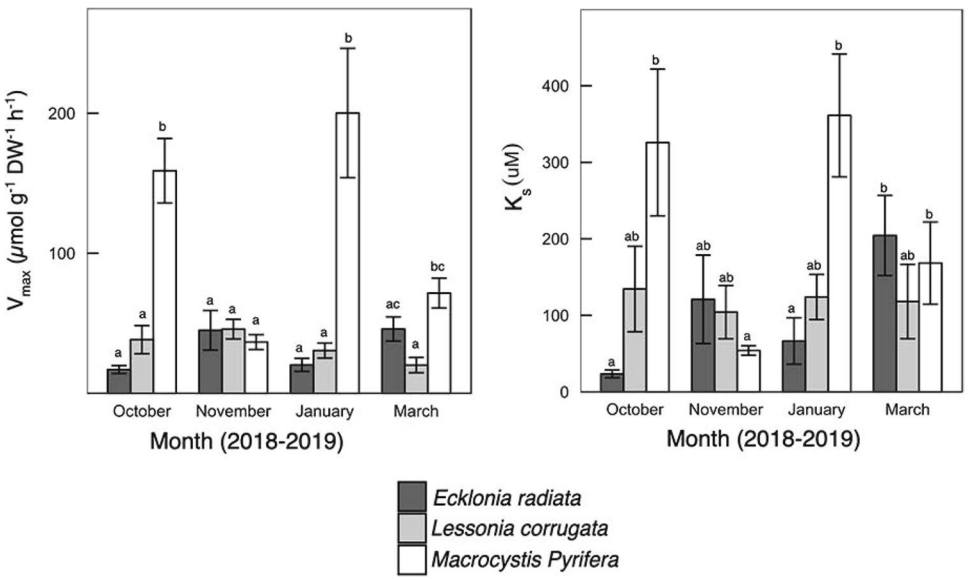

Fig. 3 Maximum uptake rates (V_{max} in µmol g^{-1} DW h^{-1}) and half saturation constants (K_s in µ M) for *E. radiata*, *L. corrugata* and *M. pyrifera* (Mean ± SE, *n = 5). Means with different letters are significantly different (Tukey's test, p < 0.05). Note that V_{max} and K_s values could not be determined for P. comosa due to the biphasic uptake pattern identified in October, November and March*

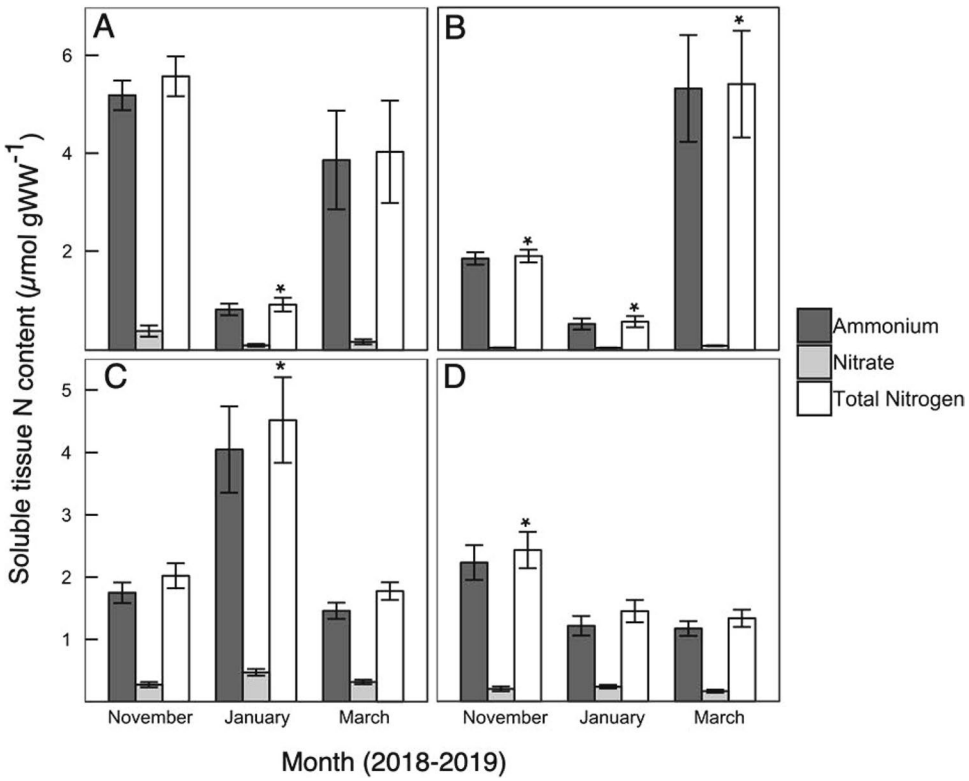

Fig. 4 Soluble tissue NO_3^- and NH_4^+ mean concentrations expressed as µmol g^{-1} WW from (**A**) *L. corrugata*, (**B**) *M. pyrifera*, (**C**) *E. radiata* and (**D**) *P. comosa*. Bars represent mean ± SE, $n = 5$. * denotes total tissue N mean significantly different to other months within a species (Tukey's test, $p < 0.05$)

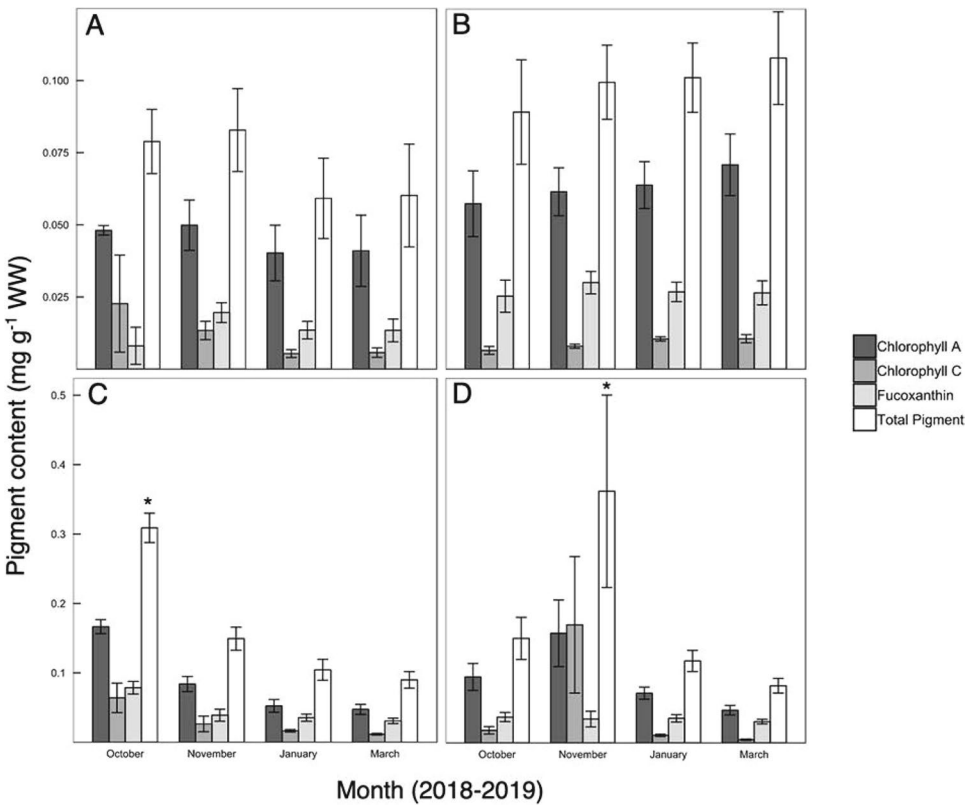

Fig. 5 Pigment content of chlorophyll *a*, chlorophyll *c*, fucoxanthin and total pigment content expressed as mg g^{-1} for (**A**) *L. corrugata*, (**B**) *M. pyrifera*, (**C**) *E. radiata* and (**D**) *P. comosa*. Bars represent mean ± *SE, n = 5. * denotes significant difference in total pigment content from other months within a species (Tukey's test, p < 0.05)*

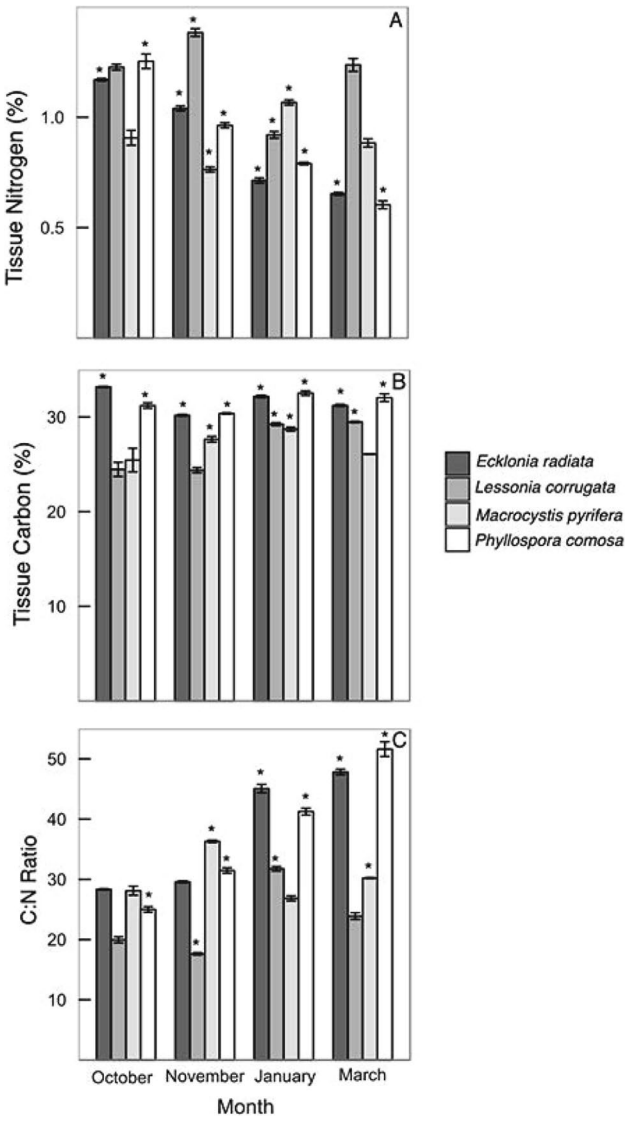

Fig. 6 Tissue C (**A**) and tissue N (**B**) expressed as % of total DW and C:N ratio (based on %) across sampling months. Bars represent mean ± SE, *n*=3. * denotes significant difference (Tukey's test, $p < 0.05$) from other sampling months of the same species

tissue nitrogen did not exceed 1.4%. For *E. radiata* and *P. comosa,* tissue nitrogen decreased by approximately half from October to March ($p < 0.01$) (Fig. 6). Highest N values were recorded in November for *L. corrugata* (1.38% ± 0.017 SE) and January for *M. pyrifera* (1.06% ± 0.012 SE). The highest tissue C values were in *E. radiata* and *P comosa* samples, where C content was always above 30%. For *L. corrugata,* tissue C increased between October 2018 and March 2019 (Tukey's test, $p < 0.01$). For *M. pyrifera*, tissue C was highest in November and January, at 27.6% and 28.72% respectively. The C:N ratio for *E. radiata* increased between October 2018 and March 2019 (Tukey's test, $p < 0.05$ for all pairwise comparisons). The same trend was seen in *P. comosa*. *L. corrugata* and *M. pyrifera* showed no clear seasonal pattern in C: N ratio across the sampling months.

Discussion

For IMTA applications with a primary goal of reducing NH_4^+ output into the environment, macroalgal species with a high potential for DIN uptake (high V_{max}) are required. The maximum V_{max} was recorded for *M. pyrifera* and was ~ 4.5 times greater than for *L. corrugata* and *E. radiata*. The higher nutrient uptake rates seen for *M. pyrifera* are consistent with the fast growth rate of this species (Gerard 1982) and higher NH_4^+ uptake rates have been observed in species with high growth rates (Pedersen and Borum 1997). *M. pyrifera* exhibited the highest K_s value, indicating that this species is better able to take up NH_4^+ at higher concentrations, compared to species with a lower K_s (Roleda and Hurd 2019). Concentrations of NH_4^+ immediately surrounding fin fish aquaculture facilities can exceed 150 µM (Neori and Shpigel 1999; Carmona et al. 2006), compared to background concentrations of DIN of < 20 µM, which as suggested by our results would be rapidly taken up by *M. pyrifera.* Indeed, it has been utilised in IMTA in Chile (Buschmann et al. 2008) and has a high market value compared to other kelp species (Correa et al. 2016; Camus et al. 2019). Of the four species studied here, *M. pyrifera* appears the most suitable for IMTA operations in Tasmania.

M. pyrifera, *L. corrugata* and *E. radiata* all exhibited saturable NH_4^+ uptake kinetics. Saturable NH_4^+ uptake is common in macroalgae, and a summary of species exhibiting saturable NH_4^+ uptake is provided in Table 3. Active uptake mechanisms are particularly important is regions where concentrations are low and can be limiting, as algae must be able to actively pump DIN into their cells against a concentration gradient, for storage and growth. In contrast to the laminarians, the fucalean brown seaweed *P. comosa* exhibited biphasic uptake patterns for all months except summer (January). To our knowledge, this is only the third record of biphasic NH_4^+ uptake in the class Phaeophyceae, and biphasic uptake has been observed only for members of the Fucales: *Fucus distichus* (Thomas et al. 1985) and *Fucus spiralis* (Topinka 1978).

The likely presence of both active and passive N uptake mechanisms, as indicated by bi-phasic uptake as seen in *P. comosa*, is considered an adaptation to areas with large variations in nutrient concentrations (Collos et al. 1997; Lomas and Glibert 1999). Biphasic uptake allows the macroalgae to operate active uptake when nutrient concentrations are low, and passive uptake when nutrient concentrations are high (Buchanan et al. 2000). As such, *P. comosa* may have uses in IMTA operations as its biphasic uptake mechanisms

Table 4 Summary of literature values of V_{max} and K_s values of NH_4^+ for other seaweed species

Macroalgae	Temp (°C)	V_{max} (μmoles g^{-1} DW h^{-1})	K_s	Location	Reference
Ochrophyta:					
Chorda filum	9	23.6	3.44	Sweden, Baltic	Wallentinus 1984
Chordaria flagelliformis	11	61.9	4.35	Nova Scotia, Canada	Probyn and Chapman 1982
Dictyosiphon foeniculaceus	9	54.43	3.6	Sweden, Baltic	Wallentinus 1984
Ecklonia cava	16	29.2	76.8	S. Korea	Kang et al. 2013
Ecklonia radiata	18	Passive uptake	Passive uptake	W. Australia	Paling 1991
*Ecklonia radiata**	18	45	121 ± 57.8	Tasmania	This Study
*Ecklonia radiata**	11	17	23.5 ± 5.05	Tasmania	This Study
Ectocarpus siliculosus	9	39.8	3.46	Sweden, Baltic	Wallentinus 1984
Elachista fucicola	9	133.9	20.93	Sweden, Baltic	Wallentinus 1984
Eudesme virescens	10	38.1	4.78	Sweden, Baltic	Wallentinus 1984
Fucus distichus	15	60	3 – 5	British Columbia	Thomas et al. 1985
Fucus distichus		3.6	13.90	Nova Scotia	Rosenberg and Ramus 1984
Fucus spiralis[c]	15	0.29	5.8	Massachusetts	Topinka 1978
Fucus spiralis[d]	15	0.35	9.6	Massachusetts	Topinka 1978
Fucus spiralis[c]	10	0.26	6.4	Massachusetts	Topinka 1978
Fucus spiralis[d]	5	0.18	6.4	Massachusetts	Topinka 1978
Fucus vesiculosus	15	41	21	Denmark	Pedersen and Borum 1997
Hincksia sordida	15	24.3	12.5	S. Australia	Campbell 1999
Laminaria abyssalis	18	2	4.6	Brazil	Dā Costa Braga and Yoneshigue-Valentin 1996
Laminaria groenlandica	18	Passive uptake	Passive uptake	British Columbia	Harrison et al., 1986a, b
*Lessonia corrugata**	14	45.8	104	Tasmania	This Study
Macrocystis pyrifera	16	23.8	5.3	S. California	Haines and Wheeler 1978
*Macrocystis pyrifera**	14	200	361	Tasmania	This Study
*Macrocystis pyrifera**	14	36.6	54.1	Tasmania	This Study
Macrocystis pyrifera	6 – 9	23.6	50	S. California	Wheeler 1979
*Phyllospora comosa**	18	Biphasic	Biphasic	Tasmania	This Study
Pilayella littoralis	8	35.9	3.57	Sweden, Baltic	Wallentinus 1984
Sargassum baccularia		13	4.8	N. Australia	Schaffelke and Klumpp 1998
Scytosiphon lomentaria	6	69	3.9	Sweden, Baltic	Wallentinus 1984
Scytothamnus australis	15	76	42.8	New Zealand	Phillips 2001b
Undaria pinnatifida	16	10.7	90.9	S. Korea	Kang et al. 2013
Undaria pinnatifida[a]	15	56.7	9.2	S. Australia	Campbell 1999
Undaria pinnatifida[b]	15	32.8	12.4	S. Australia	Campbell 1999
Undaria pinnatifida	10	350	172	Japan	Sato et al. 2016
Undaria pinnatifida	4	Passive uptake	Passive Uptake	New Zealand	Dean and Hurd 2007
Xiphophora chondrophyll	17.5	Passive uptake	Passive uptake	New Zealand	Taylor et al. 1998
Xiphophora gladiata	15	8.7	36.9	New Zealand	Phillips 2001b
Chlorophyta:					
Acrosiphonia centralis	2	115.2	19.07	Baltic	Wallentinus 1984
Caulerpa cupressoides		8.7	48.00	Virgin Islands	Williams and Fisher 1985
Chaetomorpha linum	15	132 ± 29	13 ± 12	Denmark	Pedersen and Borum 1997
Cladophora glomerata	12	356.4	13.2	Sweden, Baltic	Wallentinus 1984
Cladophora serica	15	81	25	Denmark	Pedersen and Borum 1997
Cladophora serica	15	122	13	Denmark	Pedersen and Borum 1997
Cladophora sp.	23	130	20.7	W. Australia	Gordon et al. 1981
Codium decorticatum		13.4	12	N. Carolina	Rosenberg and Paerl 1981
Codium fragile	15	240 ± 61	21 ± 16	Denmark	Pedersen and Borum 1997
Codium fragile	6	13	1.5 ± 0.2	Rhode Island	Hanisak and Harlin 1978

Table 4 (continued)

Macroalgae	Temp (°C)	V_{max} (µmoles g^{-1} DW h^{-1})	K_s	Location	Reference
Codium fragile	24	28	1.4 ± 0.2	Rhoda Island	Hanisak and Harlin 1978
Enteromorpha ahlneriana	13	409.4	16.6	Sweden, Baltic	Wallentinus 1984
Enteromorpha compressa	14	36.8	24	Sweden, Baltic	Kautsky 1982
Enteromorpha prolifera	12 - 14	39 – 188	9.3 – 13.4	Oregon	O'Brien and Wheeler 1987
Enteromorpha sp.	17.5	Passive uptake	Passive uptake	New Zealand	Taylor et al. 1998
Enteromorpha sp.	20	Biphasic	Biphasic	Massachusetts	Fujita 1985
Ulva compressa	16	140.3	268.1	S. Korea	Kang et al. 2013
Ulva lactuca	20	138 ± 78	40.7 ± 8.5	Massachusetts	Fujita 1985
Ulva lactuca	15	190 ± 23	17 ± 6	Denmark	Pedersen 1994
Ulva lactuca		50	5.2	Israel	Cohen and Neori 1991
Ulva sp.	17.5	Passive uptake	Passive uptake	New Zealand	Taylor et al. 1998
Ulva sp.	15	0.9	8.2	S. Australia	Campbell 1999
Ulva rigidia		Passive uptake	Passive uptake	W. Australia	Lavery and McComb 1991
Rhodophyta:					
Agardhiella subulata	20	15.9	3.9	Massachusetts	D'Elia and DeBoer 1978
Apophlaea lyallii	15	11.6	42.08	New Zealand	Phillips 2001b
Arthrocardia sp.	12	2.07		New Zealand	Nguyen et al. 2020
Ceramium rubrum	2	271	29	Denmark	Pedersen and Borum 1997
Ceramium rubrum	15	25.2	3.6	Massachusetts	DeBoer et al. 1983
Ceramium tenuicornea	15	192.1	9	Sweden, Baltic	Wallentinus 1984
Chondrus crispus	15-16	62	35.5	France	Amat and Braud 1990
Crustose coralline sp.	12	0.58		New Zealand	Nguyen et al. 2020
Furcellaria lumbricalis	9	4.88	6.53	Sweden, Baltic	Wallentinus 1984
Gracilaria foliifer	20	23.8	1.6	Massachusetts	D'Elia and DeBoer 1978
Gracilaria gracilis	20	206.7	76.4	South Africa	Smit 2002
Gracilaria incurvata	16	50.6	151.7	S. Korea	Kang et al. 2013
Gracilaria pacifica	16	Passive uptake	Passive uptake	Oregon	Naldi and Wheeler 1999
Gracilaria pacifica	15	30	10	British Columbia	Thomas et al. 1987
Gracilaria tikvahiae	16	Passive uptake	Passive uptake	Florida	Friedlander and Dawes 1985
Gracilaria tikvahiae	20	2.67	24.8	Massachusetts	Fujita 1985
Gracilaria vermiculophylla	20	N/A	N/A	Portugal	Abreu et al. 2009
Gracilariopsis lemaneiformis	27	68	40	N. Carolina	Vergara et al. 1995
Hemineura frondosa[#]	12[e]	0.12	152.93	Tasmania	Paine et al. 2020
Hemineura frondosa[#]	12[f]	0.01	16.17	Tasmania	Paine et al. 2020
Hypnea musciformis	26	115	16.6	Virgin Islands	Haines and Wheeler 1978
Phyllophora truncata	14	9.71	7.03	Sweden, Baltic	Wallentinus 1984
Polysiphonia decipiens	15	4.5	5.7	S. Australia	Campbell 1999
Porphyra sp.	20	Passive uptake	Passive uptake	New Zealand	Taylor et al. 1998
Porphyra yezoensis	16	111.5	248.6	S. Korea	Kang et al. 2013
Pterocladia capillacea	16	65	45	New Zealand	Taylor et al. 1998
Palmaria Palmata	16	12.5 – 19.4	9.28 – 19.81	Spain	Martinez and Rico 2004
Rhodomela confervoides	4	38.1	2318	Sweden, Baltic	Wallentinus 1984
Stictosiphonia arbuscula	15	Passive uptake	Passive uptake	New Zealand	Phillips 2001b

[a]Immature thalli; [b]mature thalli; [c]dark-acclimated; [d]light acclimated; [e]saturating light; [f]limiting light; [*]maximum and minimum values from species from this study; [#]V_{max} measured in µmol cm^{-1} h^{-1}

allows a high NH_4^+ uptake capacity at high external NH_4^+ concentrations.

To compare the values of V_{max} and K_s obtained in our study with those of other macroalgal species, a search of published literature was undertaken and the uptake kinetics of 62 species are reported in Table 4. There were 26 Rhodophyta, 24 Ochrophyta and 16 Chlorophyta, in addition to the four Ochrophyta species studied here. In general, the phyla Chlorophyta and Rhodophyta had higher V_{max} and K_s values than the phylum Ochrophyta, Class Phaeophyceae. This is the first study to examine the NH_4^+ uptake mechanisms of *P. comosa* and *L. corrugata*, and the V_{max} for these species fell within the range of studies on macroalgae of the order Laminariales. For *E. radiata*, Paling (1991) suggested a passive uptake mechanism for NH_4^+ where uptake rate increased proportionally to NH_4^+ concentration, but we found evidence of active uptake as saturating kinetics were observed.

For kelps, a C:N ratio of >15 - 20 is often considered to indicate N limitation (Hurd et al. 2014). For some macroalgae from mid and low latitudes, there are strong seasonal patterns of tissue nitrogen and C:N ratio. For example, C:N ratios of *Ulva olivascens* have been shown to change from 12.9 to 39.4 moving from spring to summer (Altamirano et al. 2000) due to higher levels of nitrogen in the water column in winter months. Such seasonal patterns in soluble tissue N content, N uptake, pigment content and macroalgal growth have been demonstrated by Topinka (1978), Küppers and Weidner (1980), Asare and Harlin (1983), Rosell and Srivastava (1985), Brown et al. (1997), Abreu et al. (2011) and Bearham et al. (2013). In this study, tissue N in *E. radiata* and *P. comosa* declined over summer and C:N ratio increased, indicating progressive N limitation from spring to summer. In contrast, *L. corrugata* and *M. pyrifera* showed no clear seasonal pattern in C:N ratio. The C:N ratio of *M. pyrifera, E. radiata,* and *P. comosa* was >20 for all months, indicating these species are N limited year-round (Hurd et al. 2014) and would therefore be able to uptake NH_4^+ all year in an IMTA scenario. *Lessonia corrugata* was the only species which exhibited a relatively low (<20) C:N ratio year-round. As seawater DIN at the collection locations remained relatively constant over the sampling months, it is possible that *E. radiata* and *P. comosa* had increased growth in the summer months due to higher irradiance and warmer waters, and therefore used any stored N. Additional DIN sources from IMTA operations may prove beneficial for the growth of the species during summer.

Of the species studied, *M. pyrifera* and *P. comosa* appear to be the most suitable for IMTA applications in Tasmanian waters due to their high NH_4^+ uptake potential. *Lessnia corrugata* and *M. pyrifera* did not show any distinct patterns in N ecophysiology that could be attributed to the changing seasons, however *P. comosa* had a depletion in tissue N over the summer, indicating N limitation. Other studies have proposed the introduction of a multi-cultured approach at which different species are grown at different depths. Experiments conducted in Chile have demonstrated that *M. pyrifera* can be best cultivated at 3 m and can be farmed in conjunction with *Gracilaria chiliensis* (Buschmann et al. 2008). This approach may be an option in Tasmania. *Ecklonia radiata* and *L. corrugata* have high commercial value as food products (Sanderson and Di Bendetto 1988) and as sources of extracts (Lorbeer et al. 2015). Farmed in conjunction with a species of high environmental value, such as *M. pyrifera*, a multi-cultured approach may be the best option environmentally and economically.

As the commercial markets for farmed seaweeds grow, the methods used in this study may be used to assess the suitability of other local species including Rhodophyta and Chlorophyta for use in IMTA operations. The work conducted in this study is the first to confirm that *M. pyrifera* in Tasmania has desirable NH_4^+ uptake kinetics for use in IMTA. Additionally in the exploration of NH_4^+ uptake kinetics of *P. comosa*, the works identify a potential species for IMTA that has, to the best of our knowledge, not previously been utilised in aquaculture operations.

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Data Availability Contact corresponding author

Declarations

Conflicts of Interest The authors declare they have no conflicts of interest

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