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

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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 (NH₄⁺) 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 NH₄⁺ uptake kinetics, with a maximum uptake rate (Vₘₐₓ) during spring, summer and autumn of 200, 45.8 and 45 μmol gDW⁻¹ h⁻¹ and half-saturation constants (Kₛ) 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 NH₄⁺ uptake potential.

Keywords ammonium · integrated-multi-trophic 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 NH₄⁺ 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
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 Pedersen 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 macroalgal 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 ($\text{NH}_4^+$) and nitrate ($\text{NO}_3^-$). Ambient concentrations of inorganic $N$ are generally low in seawater, ranging between <5-20 $\mu$M depending on location, however concentrations of $\text{NH}_4^+$ can exceed 150 $\mu$M immediately surrounding finfish aquaculture facilities (Neori and Shpigel 1999; Carmona et al. 2006). In temperate systems the availability of $\text{DIN}$ 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 $\text{NH}_4^+$ 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 $\text{NH}_4^+$ concentrations are observed surrounding finfish cages (Sanderson et al. 2008). $\text{NH}_4^+$ 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 Druhl 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):

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

From this relationship, the parameters maximum uptake rate ($V_{\text{max}}$) and half-saturation constant ($K_m$) are determined. Desirable $V_{\text{max}}$ and $K_m$ values for IMTA are dependent on the objective of the IMTA operation (Chopin et al. 2001). Species with a high $V_{\text{max}}$ and $K_m$ values can better take up $\text{NH}_4^+$ at high concentrations, which is desirable for IMTA applications and lower values of $K_m$ 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 $\text{NH}_4^+$ at high external $\text{NH}_4^+$ concentrations.

In addition to $\text{NH}_4^+$ 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 $\text{DIN}$ 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 Benedetto 1988; Kelly 2020). Members of the Laminariales are being trialed 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 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$_4$Cl (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          |
|---------------|----------------------------|--------------------------|
| *L. corrugata* | Flowerpot Point            | 4/10/2018, 26/11/2018    |
| *M. pyrifera* | Flowerpot Point            | 4/10/2018, 27/11/2018    |
| *E. radiata*  | Tessellated Pavements      | 1/10/2018, 29/11/2018    |
| *P. comosa*   | Tessellated Pavements      | 1/10/2018, 29/11/2018    |
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:

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

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:

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

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$_3^-$ in 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 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.

#### 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 macroagal 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$_4^+$

### 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                 |
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
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 ± 1.090 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, 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 ± 1.090 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).

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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, $p < 0.05$)
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 NH₄⁺ 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 NH₄⁺ uptake rates have been observed in species with high growth rates (Pedersen and Borum 1997). *M. pyrifera* exhibited the highest *Kₛ* value, indicating that this species is better able to take up NH₄⁺ at higher concentrations, compared to species with a lower *Kₛ* (Roleda and Hurd 2019). Concentrations of NH₄⁺ 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 NH₄⁺ uptake kinetics. Saturable NH₄⁺ uptake is common in macroalgae, and a summary of species exhibiting saturable NH₄⁺ uptake is provided in Table 3. Active uptake mechanisms are particularly important in 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 NH₄⁺ 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 $V_{\text{max}}$ and $K_s$ values of $\text{NH}_4^+$ for other seaweed species

| Macroalgae                        | Temp (°C) | $V_{\text{max}}$ (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*                   | 15        | 0.29                                          | 5.8       | Massachusetts     | Topinka 1978                |
| Fucus spiralis*                   | 15        | 0.35                                          | 9.6       | Massachusetts     | Topinka 1978                |
| Fucus spiralis*                   | 10        | 0.26                                          | 6.4       | Massachusetts     | Topinka 1978                |
| Fucus spiralis*                   | 5         | 0.18                                          | 6.4       | Massachusetts     | Topinka 1978                |
| Fucus vesiculosus                 | 15        | 41                                            | 21        | Denmark           | Pedersen and Borum 1997     |
| Hinckia sordida                   | 15        | 24.3                                          | 12.5      | S. Australia      | Campbell 1999              |
| Laminaria abyssalis               | 18        | 2                                             | 4.6       | Brazil            | Dà 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 pyriforma             | 16        | 23.8                                          | 5.3       | S. California     | Haines and Wheeler 1978     |
| Macrocystis pyriforma*            | 14        | 200                                           | 361       | Tasmania          | This Study                  |
| Macrocystis pyriforma*            | 14        | 36.6                                          | 54.1      | Tasmania          | This Study                  |
| Macrocystis pyriforma             | 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        | 69                                            | 4.8       | N. Australia      | Schaffelke and Klumpp 1998  |
| Scytosiphon lomentaria            | 6         | 69                                            | 3.9       | Sweden, Baltic    | Wallentinus 1984            |
| Scyotomumus australis              | 15        | 76                                            | 42.8      | New Zealand       | Phillips 2001b              |
| Undaria pinnatifida               | 16        | 10.7                                          | 90.9      | S. Korea          | Kang et al. 2013            |
| Undaria pinnatifida*              | 15        | 56.7                                          | 9.2       | S. Australia      | Campbell 1999               |
| Undaria pinnatifida*              | 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 decorciceatium             | 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      | Hansik and Harlin 1978      |
### Table 4 (continued)

| Macroalgae                        | Temp (°C) | \(V_{\text{max}}\) (μmoles g\(^{-1}\) DW h\(^{-1}\)) | \(K_s\) | Location              | Reference                           |
|----------------------------------|-----------|------------------------------------------------------|---------|------------------------|-------------------------------------|
| *Codium fragile*                 | 24        | 28                                                   | 1.4 ± 0.2 | Rhode Island           | Hanisak and Harlin 1978            |
| *Enteromorpha ahnneriana*        | 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 | 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 | New Zealand           | Taylor et al. 1998                 |
| *Ulva sp.*                       | 15        | 0.9                                                 | 8.2     | S. Australia          | Campbell 1999                      |
| *Ulva rigida*                    |           | Passive uptake                                     | Passive | 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                   |
| *Gracilariopsis 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 | Oregon                | Naldi and Wheeler 1999             |
| *Gracilaria pacifica*             | 15        | 30                                                 | 10      | British Columbia      | Thomas et al. 1987                 |
| *Gracilaria tikvahiae*            | 16        | Passive uptake                                     | Passive | 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\(^a\) | 0.12                                               | 152.93  | Tasmania              | Paine et al. 2020                  |
| *Hemineura frondosa*              | 12\(^b\) | 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 | 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 – 19.8 | Spain               | Martinez and Rico 2004             |
| *Rhodomela confervoides*          | 4         | 38.1                                               | 2318    | Sweden, Baltic        | Wallentinus 1984                   |
| *Stictosiphonia arbuscula*        | 15        | Passive uptake                                     | Passive | 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; \(^\dagger\)V\(_{\text{max}}\) measured in μmol cm\(^{-1}\) h\(^{-1}\)
allows a high \( \text{NH}_4^+ \) uptake capacity at high external \( \text{NH}_4^+ \) concentrations.

To compare the values of \( V_{\text{max}} \) 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 Rhodomgyta, 24 Ochrophyta and 16 Chlorophyta, in addition to the four Ochrophyta species studied here. In general, the phyla Chlorophyta and Rhodophyta had higher \( V_{\text{max}} \) and \( K_s \) values than the phylum Ochrophyta, Class Phaeophyceae. This is the first study to examine the \( \text{NH}_4^+ \) uptake mechanisms of \( P. \) comosa and \( L. \) corrugata, and the \( V_{\text{max}} \) 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 \( \text{NH}_4^+ \) where uptake rate increased proportionally to \( \text{NH}_4^+ \) 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 \( U. \) 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 \( \text{NH}_4^+ \) all year in an IMTA scenario. Lessnia 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 \( \text{NH}_4^+ \) 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 \( G. \) chilensis (Buschmann et al. 2008). This approach may be an option in Tasmania. \( E. \) 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 Rhodomgyta 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 \( \text{NH}_4^+ \) uptake kinetics for use in IMTA. Additionally in the exploration of \( \text{NH}_4^+ \) 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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