Nutritional quality of kelp as a key driver of commercial abalone productivity.

Final contracted report for the Abalone Industry Reinvestment Fund
(AIRF Project 2020/47)

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Executive summary

The primary aim of this project was to identify whether there were distinct regional patterns in nutritional quality of understory seaweeds that correlate with *Haliotis rubra* productivity. The project achieved this aim and identified that the southern and south-eastern regions are characterised by a higher biomass of palatable red seaweeds and a higher availability of protein and essential fatty acids. Moreover, the project identified seasonal patterns in the proportion of essential fatty acids available per gram of seaweed, with reductions in availability during warmer seasons. This seasonal decline in food quality may contribute to the decline in *H. rubra* condition observed during summer. The findings presented here suggest that the seaweed communities in the southern and south-eastern regions likely support higher rates of productivity in *H. rubra*. This may in turn allow reefs in these regions to support greater harvest levels than their northern counterparts.

The secondary aim of this study was to identify how temperature and inorganic nutrient concentrations influenced the performance and nutritional quality of key seaweeds in *H. rubra* habitat. The project identified that protein and essential fatty acid contents of seaweeds are likely to decline under elevated temperatures. Furthermore, the project highlighted the susceptibility of two key seaweed species in *H. rubra* habitat to elevated temperatures (*Phyllospora comosa* and *Lessonia corrugata*). These findings suggest that under ocean warming and marine heatwave events, the quality of *H. rubra*'s food source is likely to decline, and we may see reductions in biomass of these key habitat-forming species. Together these changes may exacerbate the direct effects of elevated temperatures on *H. rubra*.

These data contribute to the ongoing management of *H. rubra* populations by providing critical knowledge on how the nutritional quality of *H. rubra*'s food source varies on regional and seasonal scales. Moreover, the data provide a preliminary forecast of how food quality available to *H. rubra* may change under elevated temperatures. Combined these findings can assist management in making informed decisions around regional scale catch limits by filling a valuable knowledge gap that is required to determine the carrying capacity of *H. rubra* on Tasmanian reefs in a changing ocean.

Contents

Background

A latitudinal gradient of commercial Blacklip abalone (*Haliotis rubra*) fishery productivity occurs along the East coast of Tasmania, with the highest productivity in the south despite temperatures being well below published optimum growth temperatures throughout the year (Gilroy and Edwards 1998). Reasons for this apparent paradox are unclear but several explanatory factors include wave regime, water temperature, food availability and food quality. A preliminary study by Britton et al. (2021) suggests that seaweed communities in southern Tasmania are characterised by a higher proportion of red algae than their northern counterparts, resulting in a greater availability of nutritional compounds such as protein and essential fatty acids. The current project furthered the work of Britton et al. (2021) by undertaking an extensive seasonal survey of the nutritional quality of seaweed communities at multiple sites in three regions (north-east, south-east and south). The project further investigated the role of climate change drivers (marine heatwaves, ocean warming and background nutrient levels) in influencing the nutritional quality of key seaweed species to provide indications of changes expected in a future ocean.

Seaweeds are key to the productivity of abalone populations; they create essential habitat and are the primary component of abalone diets, thus providing energy and essential nutrients to sustain growth and reproduction. Abalone prefer the structure provided by large brown seaweeds as the primary habitat and utilise understory seaweeds as secondary habitat and a food source (Hurd et al., 2014; Shepherd and Steinberg, 1992; Steneck et al., 2002). Seaweeds are rich sources of nutritionally important compounds such as protein and polyunsaturated fatty acids (PUFA, Schmid et al. 2018). Seaweeds containing high levels of protein are known to promote growth in abalone and are the primary macronutrient used in building tissue (Bollon et al. 2023; Fleming 1995; Fleming, VanBarneveld, & Hone 1996; McShane et al., 1994). Abalone are unable to synthesise several specific PUFA known as essential fatty acids and must obtain from them from their diet (Mai et al., 1996; McShane et al., 1994; Nelson et al., 2002). High levels of these compounds are therefore essential for abalone to maintain growth and reproduction and may help mitigate the negative effects of stress associated with elevated temperatures (Bullon et al 2023).

Seaweeds comprise of three groups based on their phylum: Rhodopyhta (reds), Chlorophyta (greens) and Orchrophyta (browns, Hurd et al. 2014). The three groups differ in their nutritional profiles, with the reds in eastern Tasmania generally being richer sources of protein and certain essential fatty acids than the browns or greens (Britton et al 2021; Schmid et al. 2018). As such changes in the relative abundance of these groups can have flow on effects to the types of nutrients available to abalone. This is particularly important as there appears to be a higher abundance of red seaweeds in the south relative to the north-east (Britton et al 2021). However, it is unclear whether the higher abundance of reds leads to an overall increase in the amount of essential nutritional compounds available to abalone. Moreover, there is still some debate regarding the dietary preference of *H. rubra* for the different seaweed groups. Historically it has been unclear whether brown or red seaweeds form the major component of *H.rubra* diets (Foale and Day 1992; Guest et al., 2008; Shepherd 1975). However, recent advancements in statistical models provide an opportunity to use fatty acid profiles of both abalone and seaweed to determine the relative diet proportions of each group. These models work on the principle of "you are what you eat," with the unique fatty acid signature of each seaweed group being reflected in the tissue of the abalone consuming the seaweed. The broad geographic and temporal coverage of the samples collected during this project provide an ideal dataset to identify the relative proportions of each food source.

Reefs in eastern Tasmania have experienced substantial changes in oceanography in recent decades, driven primarily by global climate change (Hobday and Pecl 2014; Oke et al 2019; Ridgway 2007). Eastern Tasmania is an ocean warming hotspot, with the southward incursion of the warm nutrient poor east Australian current causing significant changes in local ecology, most notably the > 95 % declines in the giant kelp *Macrocystis pyrifera* (Butler et al. 2020). The rapid warming has likely caused or may yet cause substantial declines in other seaweed species key to the life cycle of the *H. rubra* (Britton et al. 2021; Martínez et al. 2018), yet these remain poorly understood or documented due to the lack of historical data. Moreover, the increase in temperature and declines in nutrient availability are likely to have flow on effects to the quality of the seaweeds as a food source, by reducing abundance and direct impacts on the availability of nutritional compounds in a given seaweed. For example, elevated temperature causes declines in the amount of nutritionally important PUFA (Becker, Graeve, & Bischof, 2010; Britton et al. 2020; Gosch, Lawton, Paul, de Nys, & Magnusson, 2015), while low seawater inorganic nitrogen concentrations can lead to reductions in overall protein content. As such, this project investigated the role of temperature and nutrient concentrations in influencing performance and nutritional quality of key seaweeds using a mixture of natural analogues and targeted laboratory experiments.

Yield of *H. rubra* is greatest along the southern Tasmanian coastline and appear to be correlated with the preliminary data suggesting higher nutritional quality in these regions. As such the primary aim of the project was to identify whether there were distinct regional patterns in nutritional quality that correlated with abalone productivity. We further aimed to investigate how temperature and inorganic nutrient concentrations altered the nutritional quality and performance of key seaweeds species in *H. rubra* habitat to provide context of how food quality and habitat may change in the future.

Methods

Seasonal survey collections

Sampling design

The sampling design for a seasonal survey of biomass and nutritional quality of seaweed assemblages was a hierarchical design with two sites within each of three regions: North-east (The Gardens, -41.166560, 148.294006, Seymour, -41.741230, 148.303856), South-east (The Sisters, -42.971205, 147.988084, The Thumbs, -43.101587, 147.972224) and South (Black Reef, -43.538647, 146.971623, Mouldy Hole, -43.594561, 146.921886). See Figure 1 for location of sites. Each site was sampled once per season (Winter 2021, Spring 2021, Summer 2022 and Autumn 2022) for both biomass and nutritional quality (see – *Heatwave sampling* for additional samplings at Black Reef).

Understory biomass and density of canopy formers

Understory seaweeds were defined as > 2 cm and < 40 cm high as per Britton et al. (2021). Biomass of understory seaweeds were estimated by divers on SCUBA once per site each season. Eight 1 m^2 quadrats were placed haphazardly within a 50 m transect along the 5-7 m depth contour. In each quadrat divers collected all understory seaweeds by hand into mesh bags. The density and length of canopy forming brown seaweeds was also recorded in each quadrat. Biomass samples were frozen upon return to the laboratory and subsequently thawed, sorted to species level or genus level if species identification was not possible. They were then dried at 60 °C and weighed to determine biomass. Biomass is presented as g/unit area.

Collection of samples for nutritional quality

Samples were collected for determination of nutritional quality alongside samples for biomass estimates. At each site and season four replicate individuals of the visually abundant species (regardless of Phylum) were haphazardly collected along the transect (typically 6-10 species per site and season combination, n = 4 for each species). Upon return to the surface, samples were kept on ice in an insulated container until return to laboratory (typically < 12 hours). Once back in the laboratory samples were frozen at -20 °C and then freeze-dried as soon as possible (typically < 2 weeks). All samples were kept at -20 °C until analysis (see – *Laboratory analysis of nutritional quality*). Samples of abalone muscle were also collected at the same time as seaweeds. These samples were treated the same as the seaweed samples and used in estimating the contribution of each seaweed group to the abalone diets (see – *Dietary analysis*). All freeze-dried samples for analysis were ground with a mortar and pestle into a fine powder except for abalone tissue which was ground to a fine powder with a spice grinder.

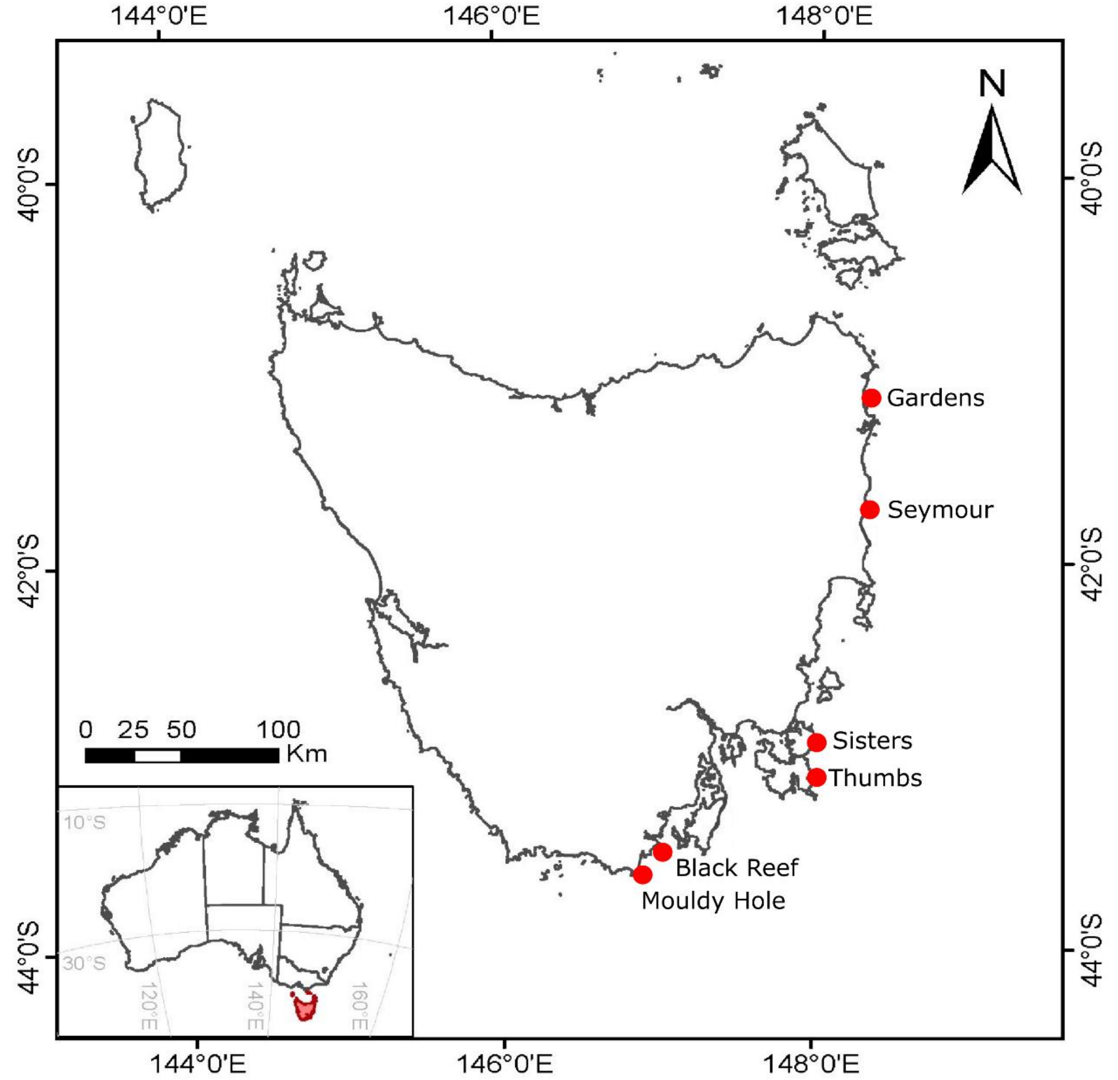

Figure 1: locations of sites sampled once per season for biomass and nutritional quality estimates. North-east: Gardens and Seymour, South-east: Sisters and Thumbs, South: Black Reef and Mouldy Hole.

Heatwave sampling

In January and February 2022, a marine heatwave event occurred at our Black Reef site (Table 1). Our scheduled summer sampling coincidently occurred during the heatwave event in late January. To investigate whether there were lasting effects of the heatwave, at Black Reef we undertook a second (post-heatwave 1) and third (post-heatwave 2) sampling of five key seaweed species (*Ecklonia radiata*, *Phyllospora comosa, Zonaria spp., Cystophora platylobium* and *Plocamium dilatatum*) for nutritional quality in late February and late March 2022 for each species (n = 4). Samples were treated the same as all other biochemical composition samples (see – *Laboratory analysis of nutritional quality*)

Table 1: Duration, start and end dates, date of peak intensity and maximum intensity of the marine heatwave that occurred at Black Reef site in January and February 2022.

Duration (days)	Start date	End date	Date of peak	Max intensity (°C)
17	16/01/2022	1/02/2022	24/01/2022	2.35

Eastern and western comparison

To test for differences in nutritional quality between seaweeds on the east and north-west coast, samples of the canopy forming species *Ecklonia radiata*, *Phyllospora comosa* and *Durvillaea spp.* were collected during December 2022. Samples (n = 4) from two sites on each of the eastern (Eddystone Point and Waubs Bay) and north-western coasts (Couta rocks and Trial harbour) of Tasmania. The eastern sites were selected based on similar latitude to the western sites. Samples were treated the same as all other biochemical samples (see – *Laboratory analysis of nutritional quality*).

Laboratory analysis of nutritional quality

% N and protein content

Approximately 1.5 – 2.0 mg of sample was weighed into tin capsules and total nitrogen, was determined using a flash combustion isotope ratio mass spectrometer at the Central Science Laboratory, University of Tasmania. Total protein content was determined using the seaweed specific nitrogen to protein conversion factor of five (Angell et al. 2016).

Fatty acid composition

Fatty acids were extracted and analysed by direct-transmethylation. Freeze-dried biomass (approximately 40 mg) was transferred into borosilicate glass test tubes fitted with a PTFElined screw cap and weighed. Fatty acid methyl esters (FAME) were extracted using the methods outlined in Stengel et al. (2015) with known amounts of C19 FAME and C23 used as surrogate and internal standards respectively. FAME were analysed using GC-MS at the Central Science Laboratory, University of Tasmania.

Data analysis – seasonal survey

Nutritional quality per gram of seaweed tissue and unit area

We estimated the average nutritional quality per gram of seaweed tissue, pooled across reds and browns for each site and season combination by calculating a weighted average based on the proportion a species contributed to the overall biomass within a quadrat (Equation 1).

Equation 1

$$N_{weighted} = \sum N_{species\ A} \times P_A + N_{species\ B} \times P_B \ldots\ldots N_{species\ Z} \times P_Z$$

Where the weighted average of a nutritional compound (N_{weighted}) is the sum of the nutritional value of each species ($N_{\text{species A,B…Z}}$) multiplied by the proportion each species contributes to the overall biomass ($P_{\text{A,B…Z}}$).

To estimate the nutritional quality per m^2 for each site and season combination, the biomass of each species within a quadrat was multiplied by the amount of each nutritional compound per gram of seaweed tissue for that species. Where we did not have samples for nutritional quality of a species but had measured them in a quadrat for biomass (typically < 5 – 10 % of total biomass) we used the following rules: 1) A species biomass was multiplied by the nutritional value of the same genus if another was available from that site and season combination or 2) in all other cases the species biomass was multiplied by the average nutritional quality value for each group (brown, red or green) at each site and season combination. These rules were followed as species from the same group are known to have similar nutritional quality (Galloway et al. 2012) and ensured we could estimate nutritional quality per unit area.

Individual nutritional compounds and biomass analysis

Differences in biomass, and the key nutritional compounds: protein, PUFA and the essential fatty acids: linoleic acid (LIN), arachidonic acid (ARA) and eicosapentaenoic acid (EPA) were compared across region and season for both the amount per unit area and amount per gram of seaweed tissue. Each compound was analysed with a two-way nested ANOVA with the fixed factors: “Season” (4 levels: Summer, Autumn, Winter and Spring), “Region” (3 levels: North-east, South-east and South), the Region × Season interaction and the random nested factor “Site” (2 levels per region). When significant effects of Region or Season were detected in the overall ANOVA, Tukey’s Honestly Significant Different (THSD) post-hoc tests were conducted to identify differences between groups within each factor. When a significant interaction was detected in the overall ANOVA separate generalised linear mixed models (GLMM) were conducted for to test for differences between Seasons within each level of Region. In the GLMMs, Site was included as a random factor. When a significant effect of Season was found in these models THSD post-hoc tests were conducted to identify differences between seasons.

Eastern and western coast comparison and heatwave sampling

For the eastern and western coast comparison, differences in the content of PUFA, saturated fatty acids (SFA) and the essential fatty acids LIN, ARA and EPA in the three seaweed species were compared across the eastern and western coasts using a GLMM with the fixed factor: Region (2 levels: east and west) and the random factor Site (2 levels in each Region). To examine correlations of nutritional quality with latitude, a linear regression of latitude and nutritional compound was undertaken for pooled samples from both coasts when no differences between coasts were detected in the GLMM. For the heatwave samples, differences in total fatty acids (TFA), total protein, PUFA and the essential fatty acids LIN, ARA and EPA were compared visually to identify trends in responses to the heatwave relative to the Spring and Autumn samples. Statistical tests were not conducted on this data as we had no baseline immediately prior to the heatwave.

Dietary analysis

To examine the relative proportion of each of the major seaweed groups contribution to the diet of *H. rubra* the fatty acid profiles of both abalone and seaweed were compared using the methods outlined in Stock et al. (2018). Briefly, the analysis only considered the fatty acids that contributed to ≥ 1 % of TFA of either abalone or any of the seaweed groups. Green seaweeds were only present at two sites in one season and were subsequently excluded from all dietary analyses. In some seasons at particular sites, we were unable to collect sufficient samples of both red and brown seaweeds due to low abundance of red seaweeds at some sites. As such we analysed sites separately and only considered seasons within each site that had both red and brown seaweeds present. Each dietary analysis model was conducted in the R package *MixSIAR* and included the random factor Season (1 – 4 levels depending on site).

Laboratory experiments

Thermal baseline of *Phyllospora comosa*

To examine the influence of thermal baselines in mediating the response of seaweeds to elevated temperatures, thermal performance curves (TPCs) were constructed for net photosynthetic rates of juveniles of the key habitat forming brown seaweed *Phyllospora comosa*. TPCs were constructed for individuals from 4 regions with 2 sites within each region. Six individuals were collected from each site and transported to the laboratory at IMAS in late December 2022 and early January 2023. For all individuals, net photosynthetic rates were measured at seven temperatures (6 – 30 °C) using the methods of Britton et al. (2020). These rates were used to construct thermal performance curves and identify thermal optimum of kelp at each site. TPCs were conducted using the methods outlined in Padfield and O’Sullivan, (2021). Samples of seaweed tissue were collected following incubations to measure % N and fatty acid profiles.

Thermal performance curves – *Lessonia corrugata*

Thermal performance and the influence of temperature on nutritional quality of the kelp *Lessonia corrugata* was investigated by measuring growth, net photosynthesis and nutritional quality (protein content, fatty acid profiles) over temperatures ranging from 4 – 22 °C in a three-week laboratory experiment. Juveniles of *L. corrugata* for the experiment were collected from Coal Point, Bruny Island (-43.3353, 147.3247) in May 2022. Responses were used to construct TPCs for growth, and net photosynthesis and identify thermal optima as per the methods in Padfield and O’Sullivan (2021). For nutritional quality, regressions were undertaken to examine the influence of temperature on nutritional quality.

Interaction of warming and nitrate availability – *Lessonia corrugata*

A three-week laboratory experiment was undertaken to investigate the interactive effects of seawater nutrient concentrations and warming on kelp performance and nutritional quality. Individuals of *L. corrugata* collected from Coal Point, Bruny Island (-43.3353, 147.3247) in July 2022 were cultured in temperatures at and above the optimum (16 – 23 °C) with low (2.5 µmol/L per day) and high (50 µmol/L per day) nitrate availability (n = 10 for each nitrate level and temperature combination). Growth, net photosynthesis and total protein content was measured following the completion of the experiment. Data were analysed using a two-way ANOVAs with the fixed factors: “Temperature” (4 levels: 16, 18.5, 21, 23.5) and “Nitrate” (2 levels: high and low) and the Temperature × Nitrate interaction. When a significant interaction was detected, a separate one-way ANOVA was conducted to determine differences between Nitrate groups for each level of Temperature. When main effects were found to be significant, THSD post-hoc tests were used to identify which groups differed within a factor.

Results

Seasonal survey

Overstory canopy

The main overstory species at all six sites was *Phyllospora comosa*, which was present in similar densities at all sites year round (2-6 individuals per m, Figure 2). *Ecklonia radiata* was also abundant but was more varied across sites (0.2 – 5 individuals per m, Figure 2) with the lowest abundances in the two southern sites. The two southern sites also had low abundances of the species *Durvillaea spp.* and *Cystophora platylobium*, which were not present at the other sites. Mean length of *P. comosa* and *E. radiata* is shown in Figure A1.

Biomass and nutritional quality availability per unit area

There was significantly higher biomass of understory seaweeds in the southern and south-eastern regions relative to the northern region (Figure 3,Table 2) This elevated biomass was driven by the greater abundance of red seaweeds at the south-eastern and southern sites. No seasonal differences in biomass were detected in any regions. Similar to biomass, the amount of protein, TFA and PUFA per metre were significantly higher in the south and south-east relative to the north (Figure 3, Table 2). No seasonal trends in the availability of these compounds per metre were detected. There were no differences in the amount of the essential fatty acid LIN across regions or seasons (Figure 3,Table 2). In contrast the amount of the essential fatty ARA was higher in the southern and south-eastern regions relative to the northern region (Figure 3,Table 2). The essential fatty acid EPA displayed seasonal changes that were different in each region (Figure 4,Table 2). In the northern region, EPA was highest in spring and similar in all other seasons, while in the south east and southern regions EPA was highest in winter and autumn respectively (Figure 4,Table 2). There was also a trend of higher EPA content in general in the southern site (Figure 4).

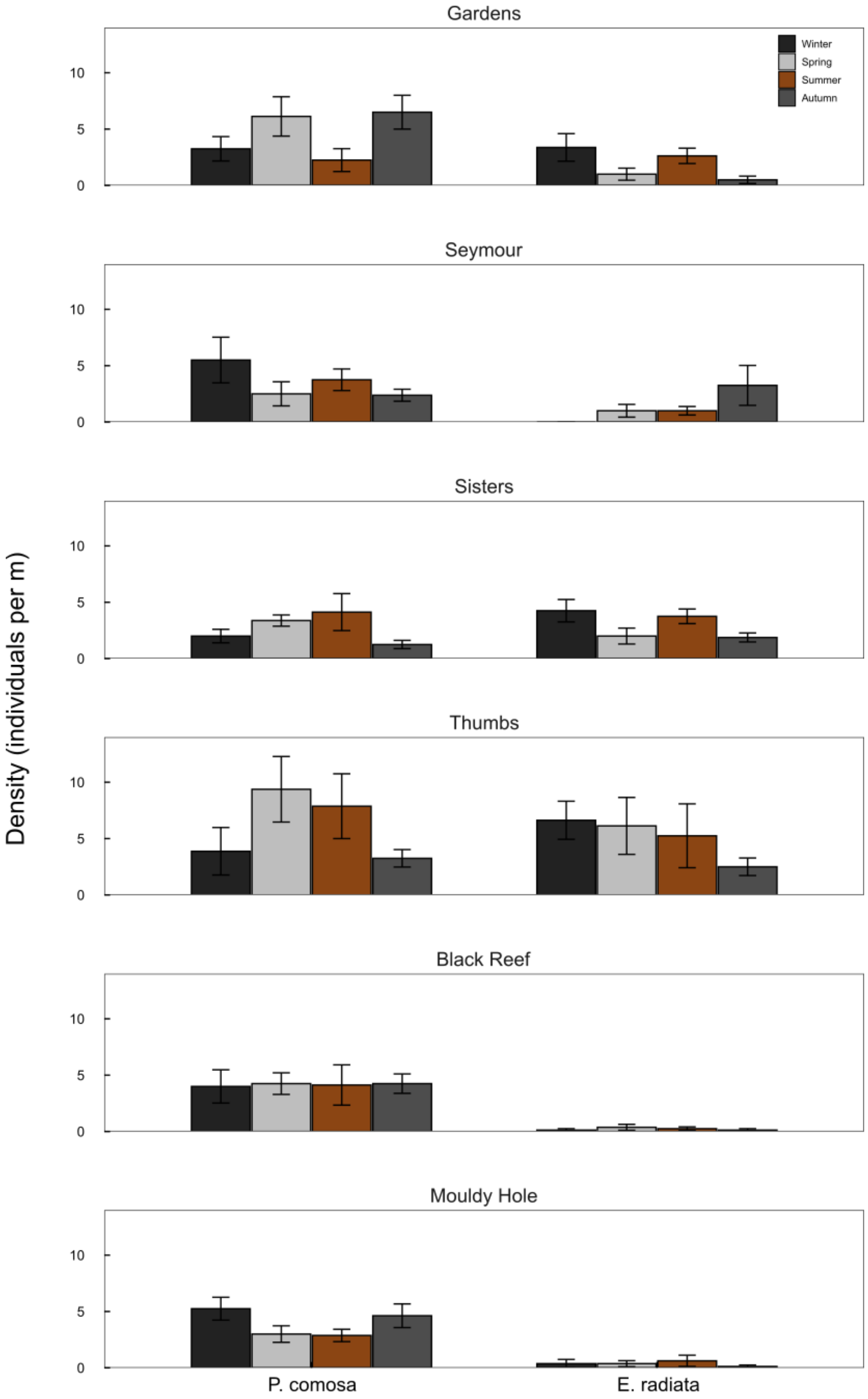

Figure 2: Density of dominant the canopy species *Phyllospora comosa* and *Ecklonia radiata* at each site across all seasons: black bars = Winter, light grey bars = Spring, brown bars = Summer, dark grey bars = Autumn.

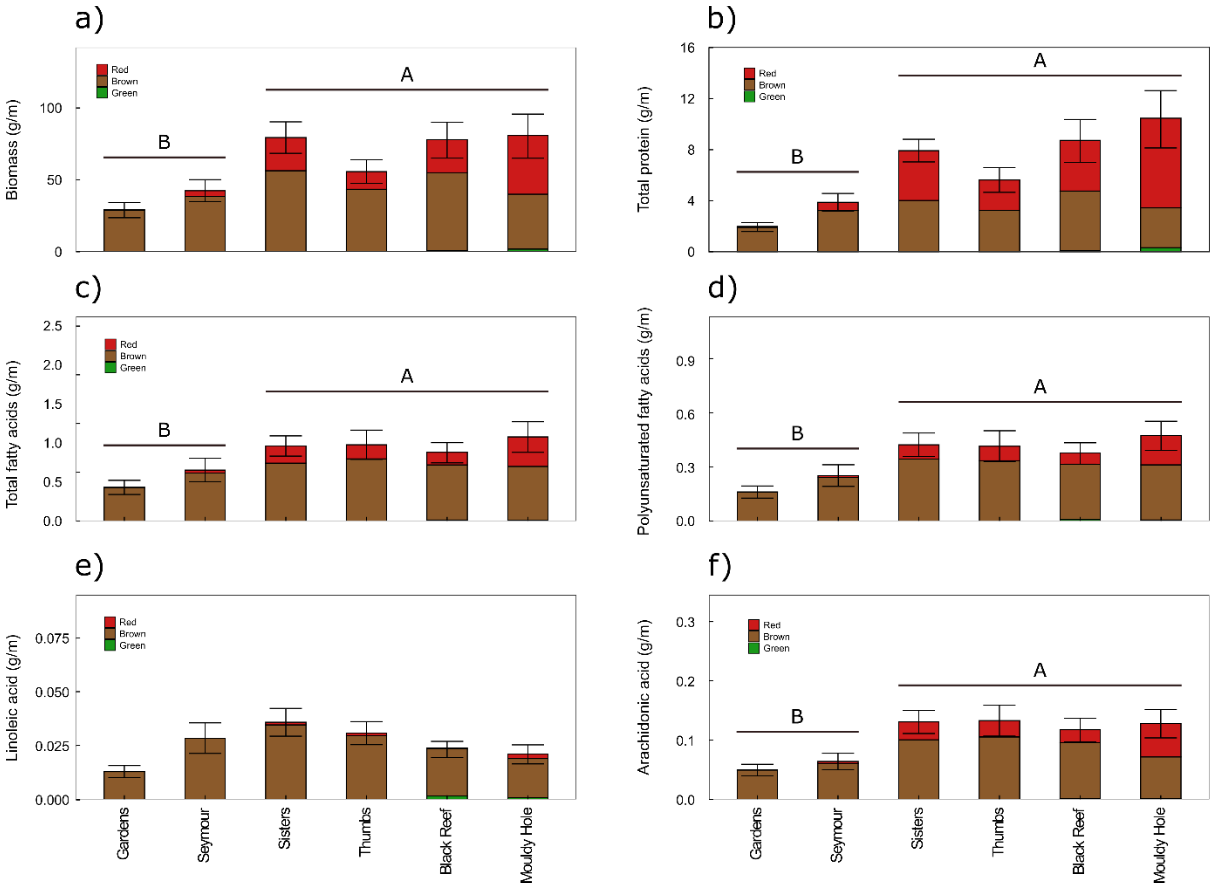

Figure 3: Total grams per metre for a) biomass, b) total protein content, c) total fatty acids, d) polyunsaturated fatty acids and the essential fatty acids e) linoleic acid and f) arachidonic acid at all sites. The proportion each seaweed group (Red, Brown and Green) contribute to the each site are displayed as colours. Data are displayed as pooled means ± standard error across seasons as no effect of season or the interaction was detected in the overall ANOVA. Letters display groups that are not significantly different from each other using Tukey’s Honestly Significant Different post-hoc tests when the overall ANOVA was significant.

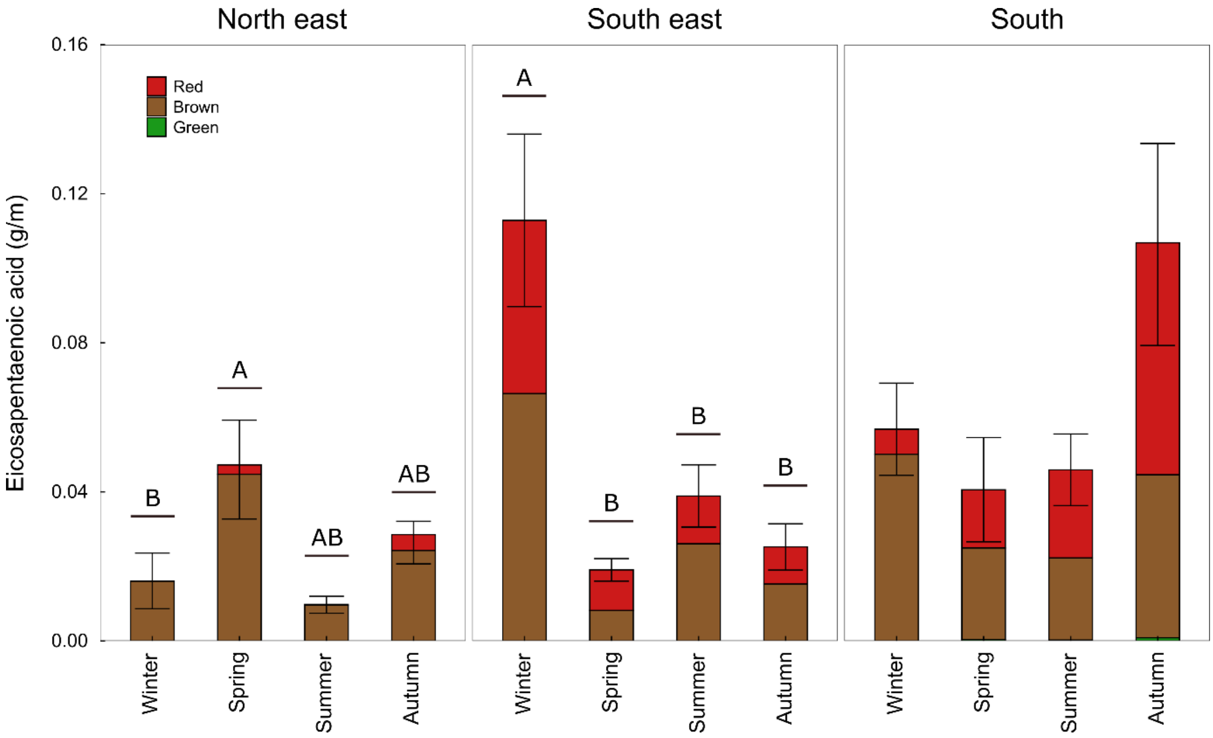

Figure 4: Total amount of the essential fatty acid eicosapentaenoic acid in all seasons for the three regions (sites pooled within region). The proportion each seaweed group (Red, Brown and Green) contribute to each site are displayed as colours. Data are displayed as means ± standard error for each season and region combination as a significant interaction was detected in the overall ANOVA. Letters display groups that are not significantly different from each other using Tukey's Honestly Significant Different post-hoc tests, no letters indicate no differences in means.

Table 2: ANOVA table for amount of biomass and nutritional quality compounds per m^2. Factors where significant differences occur have P-values in bold. THSD post-hoc shows differences between groups within a factor when the overall ANOVA was significantly different. For the significant interaction (EPA), differences between seasons are shown within each region. S = South, SE = South-east, NE = North-east, Sp = Spring, Su = Summer, Wi = Winter and Au = Autumn.

Variable	Factor	F -value	P- value	THSD post-hoc
Biomass	Region	5.88	**0.0166**	South, south-east > North-east
	Season	1.11	0.3842	
	Region × Season	1.17	0.3854	
	Site	3.02	**0.0007**	
Protein	Region	7.86	**0.0066**	South, south-east > North-east
	Season	1.21	0.3489	
	Region × Season	1.35	0.3089	
	Site	3.29	**0.0003**	
TFA	Region	6.04	**0.0153**	South, south-east > North-east
	Season	0.35	0.7931	
	Region × Season	2.39	0.0934	
	Site	2.48	**0.0051**	
PUFA	Region	7.14	**0.0091**	South, south-east > North-east
	Season	1.04	0.4081	
	Region × Season	2.59	0.0754	
	Site	2.71	**0.0023**	
LIN	Region	3.63	0.0585	
	Season	0.21	0.8898	
	Region × Season	1.85	0.1710	
	Site	2.66	**0.0028**	
ARA	Region	9.02	**0.0041**	South, south-east > North-east
	Season	0.36	0.7830	
	Region × Season	2.61	0.0745	
	Site	2.07	**0.0216**	
EPA	Region	8.90	**0.0043**	
	Season	0.88	0.4771	
	Region × Season	3.41	**0.0336**	S: n.d, SE: Sp > Wi, Su, Au, NE: Sp > Wi
	Site	2.18	**0.0145**	

Average nutritional content per g seaweed

The amount of protein per gram of seaweed was significantly higher in the southern and south-eastern regions relative to the north and this was consistent across seasons (Figure 5, Table 3). In contrast the amount of TFA, PUFA, ARA and EPA per gram of seaweed was similar across regions but differed significantly between seasons (Figure 6, Table 3). TFA was significantly higher in winter than all seasons, while spring was significantly higher than summer and autumn (Figure 6,Table 3). PUFA, ARA and EPA content showed a similar response with highest values recorded in winter with a seasonal trend of a decline in spring and summer and a slight increase in autumn (Figure 6,Table 3). LIN content displayed different patterns across seasons within regions (Figure 7,Table 3). Subsequent analysis within each region showed that in the north-east, LIN content was significantly higher than all other seasons in spring, whereas the opposite pattern was observed in the south-east. In the southern region LIN content was significantly higher in winter than all other seasons (Figure 6,Table 3).

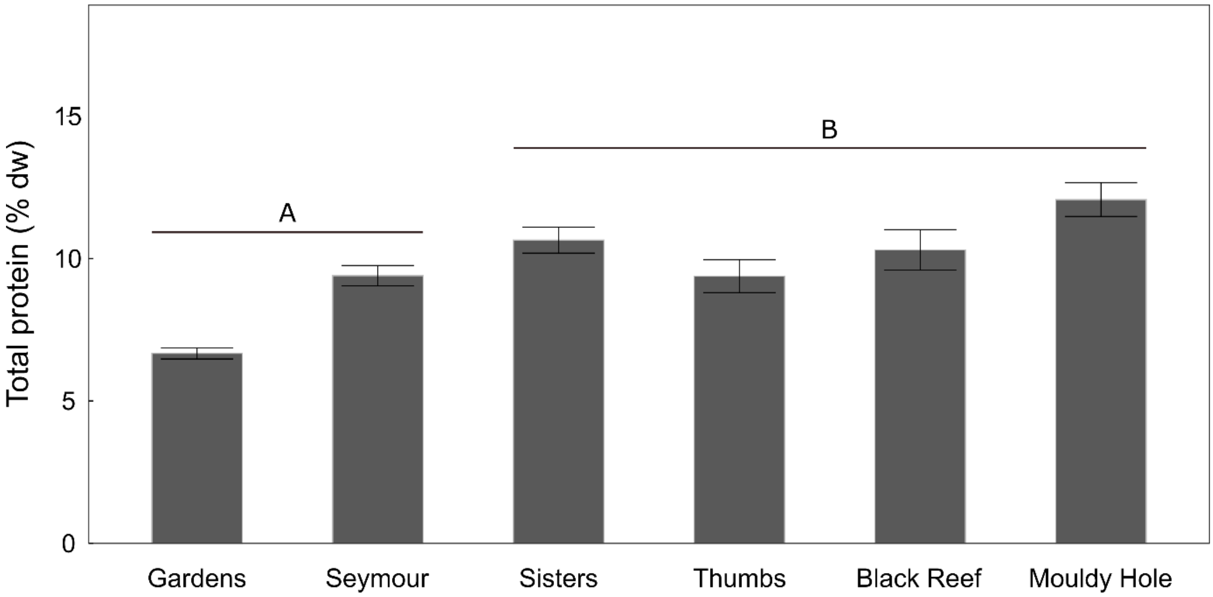

Figure 5: Total protein content per g of seaweed (dry weight) at each site pooled across seasons. Data are displayed as pooled means ± standard error across seasons as no effect of season or interaction was detected in the overall ANOVA Letters display groups that are not significantly different from each other using Tukey's Honestly Significant Different (THSD) post-hoc tests when the overall ANOVA was significant.

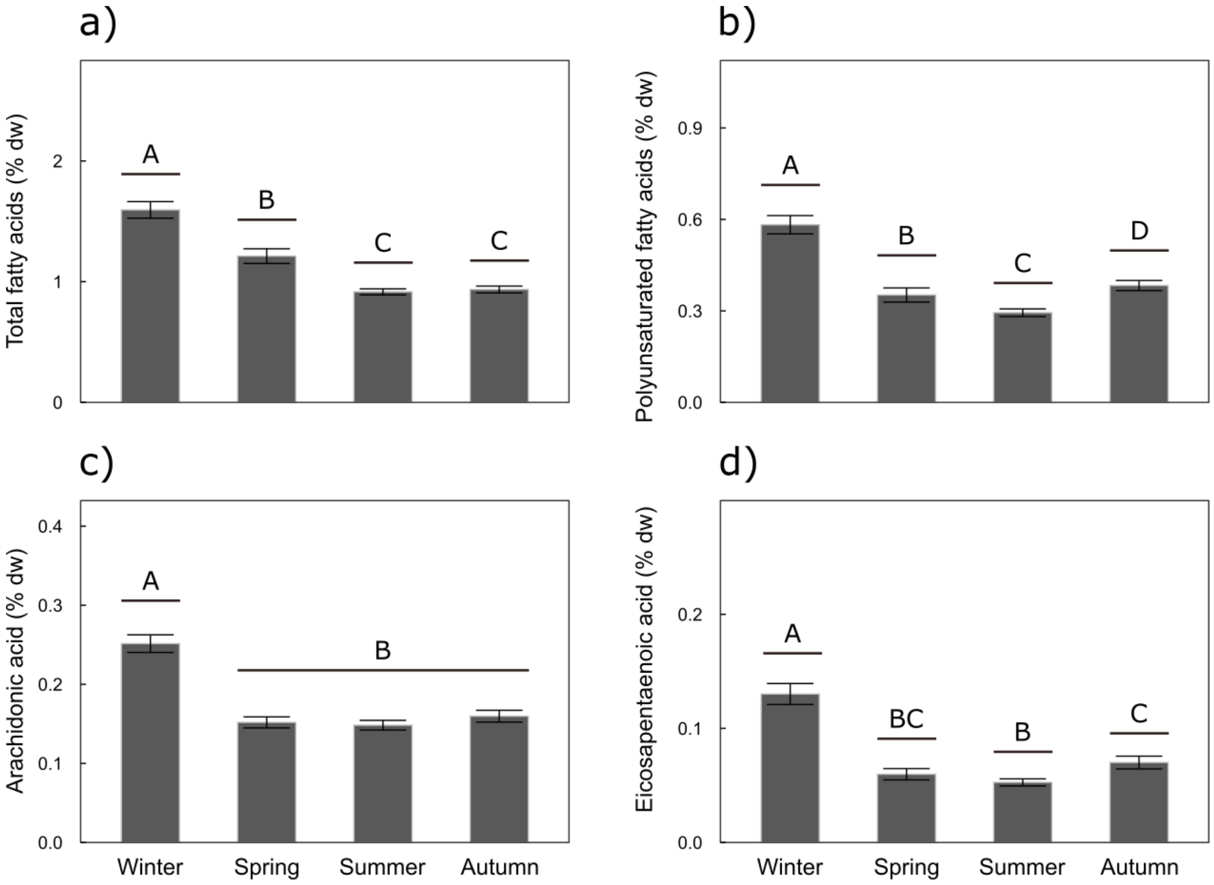

Figure 6: a) total fatty acids, b) polyunsaturated fatty acids and the essential fatty acids c) arachidonic and d) eicosapentaenoic acid per g of seaweed (dry weight) in each season. Data are displayed as pooled means ± standard error as no effect of region was detected in the overall ANOVA. Letters display groups that are not significantly different from each other using Tukey's Honestly Significant Different post-hoc tests when the overall ANOVA was significant.

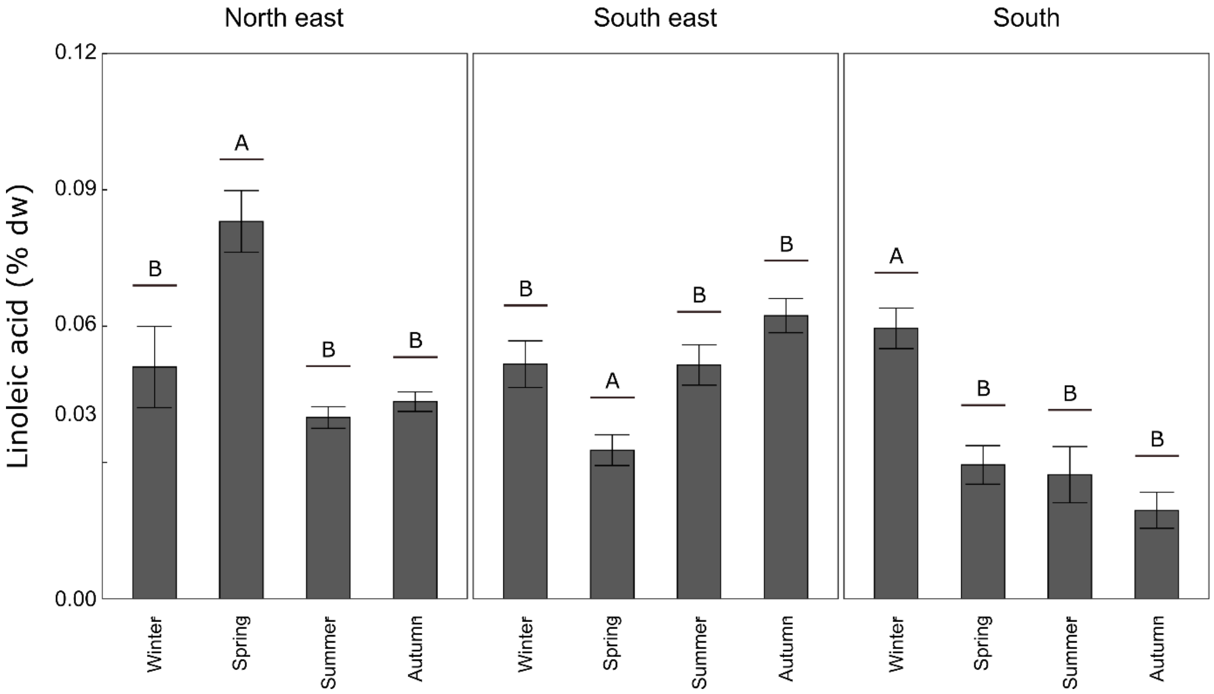

Figure 7: Total amount of the essential fatty acid linoleic acid per g of seaweed (dry weight) in all seasons for the three regions. Data are displayed as means ± standard error for each season and region combination as a significant interaction was detected in the overall ANOVA. Letters display groups that are not significantly different from each other using Tukey's Honestly Significant Different post-hoc tests, no letters indicate no differences in means.

Table 3: ANOVA table for amount of biomass and nutritional quality compounds per g of seaweed tissue pooled across reds and browns. Factors where significant differences occur have P-values in bold. THSD post-hoc shows differences between groups within a factor when the overall ANOVA was significantly different. For the significant interaction (LIN), differences between seasons are shown within each region. S = South, SE = South-east, NE = North-east, Sp = Spring, Su = Summer, Wi = Winter and Au = Autumn.

Variable	Factor	F -value	P- value	THSD post-hoc
Protein	Region	4.51	**0.0345**	South, south-east > North-east
	Season	1.68	0.2236	
	Region × Season	1.98	0.1478	
	Site	5.67	**<0.0001**	
TFA	Region	0.72	0.5073	
	Season	8.04	**0.0033**	Wi > Sp > Su, Au
	Region × Season	1.89	0.1636	
	Site	15.69	**<0.0001**	
PUFA	Region	0.05	0.9541	
	Season	6.82	**0.0062**	Wi > Au > Sp > Su
	Region × Season	2.74	0.0648	
	Site	12.76	**<0.0001**	
LIN	Region	5.19	**0.0238**	
	Season	1.39	0.2923	
	Region × Season	3.33	**0.0363**	S: Wi > Sp, Su, Au SE: Sp < Wi, Su, Au NE: Sp > Wi, Su, Au
	Site	6.50	**<0.0001**	
ARA	Region	1.00	0.3974	
	Season	8.44	**0.0028**	Wi > Sp, Su, Au
	Region × Season	2.88	0.0563	
	Site	14.74	**<0.0001**	
EPA	Region	0.55	0.5908	
	Season	4.90	**0.0190**	Wi > Sp, Su, Au, Au > Su
	Region × Season	1.15	0.3930	
	Site	12.33	**<0.0001**	

Heatwave results

There were substantial differences in the nutritional quality of the seaweed species sampled during the heatwave (the summer sampling is referred to as the heatwave as this coincided with the heatwave peak) and following the heatwave at Black Reef. These trends were inconsistent across species (Figure 8, 9). The two primary habitat forming species *P. comosa* and *E. radiata* displayed a trend of a reduction in TFA and total protein content during the heatwave relative to the spring sampling and increases in PUFA, ARA and EPA content (Figure 8, 9). LIN content was relatively unchanged across samplings. There was a reduction in TFA following the heatwave sampling and an increase in PUFA content during the heatwave and post heatwave relative to spring in *C. platylobium,* while protein content was relatively unaffected (Figure 8). Essential fatty acid content in *C. platylobium* showed varied responses: LIN decreased during and post-heatwave, while EPA and ARA increased. The red understory species *P. dilatatum* displayed a trend of a decline in TFA, PUFA, EPA and total protein during the heatwave but levels of these compounds generally returned to that of spring in subsequent samplings. (Figure 8, 9) The essential fatty acid LIN was only present in trace amounts and ARA remained stable during the heatwave but increased in the post-heatwave sampling in this species (Figure 9). The brown seaweed *Zonaria spp.* displayed a trend of an increase in PUFA, and EPA during and post heatwave but no clear trends were evident in the other fatty acids metrics (Figure 8, 9). There was a trend of a decrease in protein content relative to spring in the heatwave and all other samplings (Figure 8).

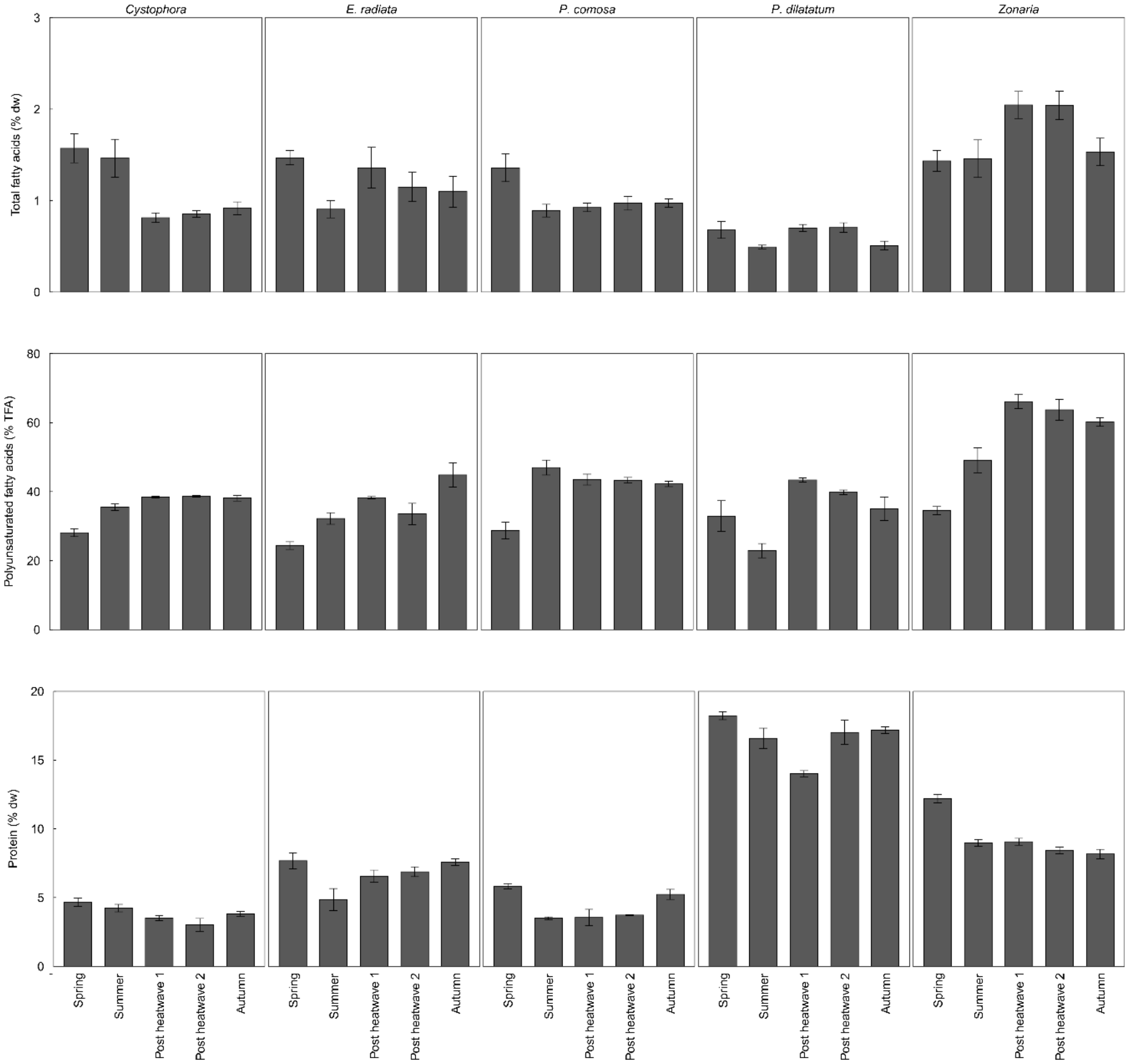

Figure 8: Total fatty acids (% dw, top panel), polyunsaturated fatty acid content (% of TFA, middle panel), and protein content (% dw, bottom panel) for 5 species (*C. platylobium, E. radiata, P. comosa, P. dialtatum* and *Zonaria spp*) before (Spring), during (Summer), and after (1 and 2 months post the heatwave event and Autumn) that occurred at the Black Reef site in January 2022. Data are displayed as means ± standard error.

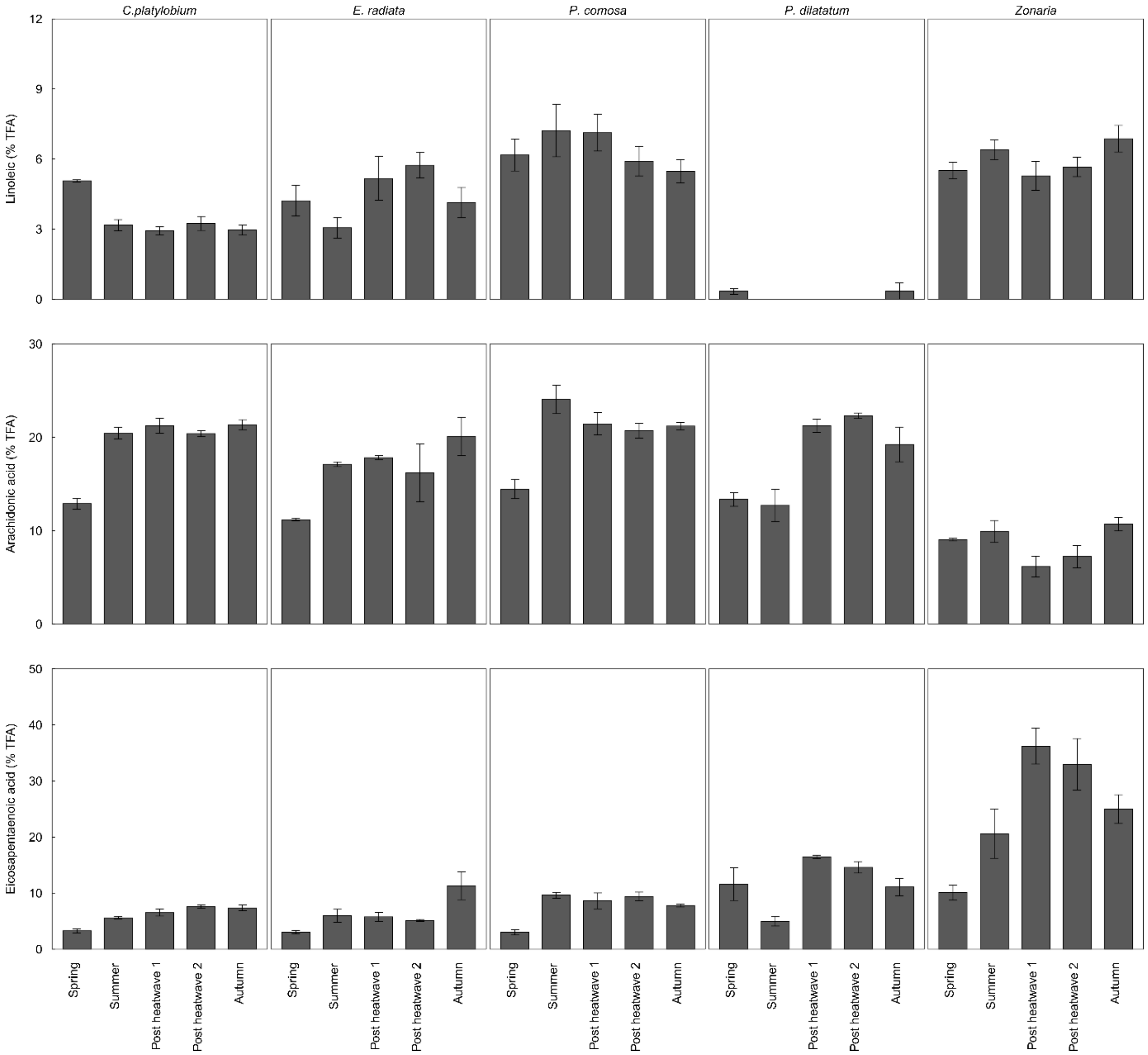

Figure 9: Essential fatty acids (% of TFA): linoleic acid (top panel), arachidonic acid (middle panel) and eicosapentaenoic acid (bottom panel) for 5 species (*C. platylobium, E. radiata, P. comosa, P. dialtatum* and *Zonaria spp*) before (Spring), during (Summer), and after (1 and 2 months post the heatwave event and Autumn) that occurred at the Black Reef site in January 2022. Data are displayed as means ± standard error.

East and west comparison

PUFA content displayed a similar trend in all three species with the highest proportion of PUFA in the southern sites (Figure 10). SFA displayed the opposite trend to PUFA for all species with higher proportions of SFA in the north relative to south (Figure 10). There was no effect of coast on PUFA or SFA content in any species (Table 4). Protein content and the essential fatty acids LIN, ARA did not display clear patterns with latitude for any species and there were no significant differences between coasts (Figure 10, Table 4). In contrast, EPA displayed similar patterns to PUFA content with higher proportion in the southern sites which was correlated with latitude (Figure 10). No effect of coast was detected for EPA (Table 4).

Table 4: ANOVA table comparing the amount of PUFA, SFA, LIN, ARA, EPA (% TFA) and protein (% dw) between the west and east coasts sites in December 2022. Where significant differences were detected, P-values are displayed in bold.

Variable	Species	F-value	P-value
PUFA	*E. radiata*	0.10	0.78
	P. comosa	0.52	0.55
	Durvillaea	0.12	0.76
SFA	*E. radiata*	0.04	0.86
	P. comosa	0.41	0.59
	Durvillaea	0.37	0.61
Protein	*E. radiata*	0.35	0.61
	P. comosa	1.60	0.33
	Durvillaea	2.02	0.29
LIN	*E. radiata*	0.14	0.74
	P. comosa	0.36	0.61
	Durvillaea	0.01	0.94
ARA	*E. radiata*	0.32	0.63
	P. comosa	0.17	0.72
	Durvillaea	0.06	0.83
EPA	*E. radiata*	0.30	0.64
	P. comosa	0.03	0.87
	Durvillaea	1.04	0.42

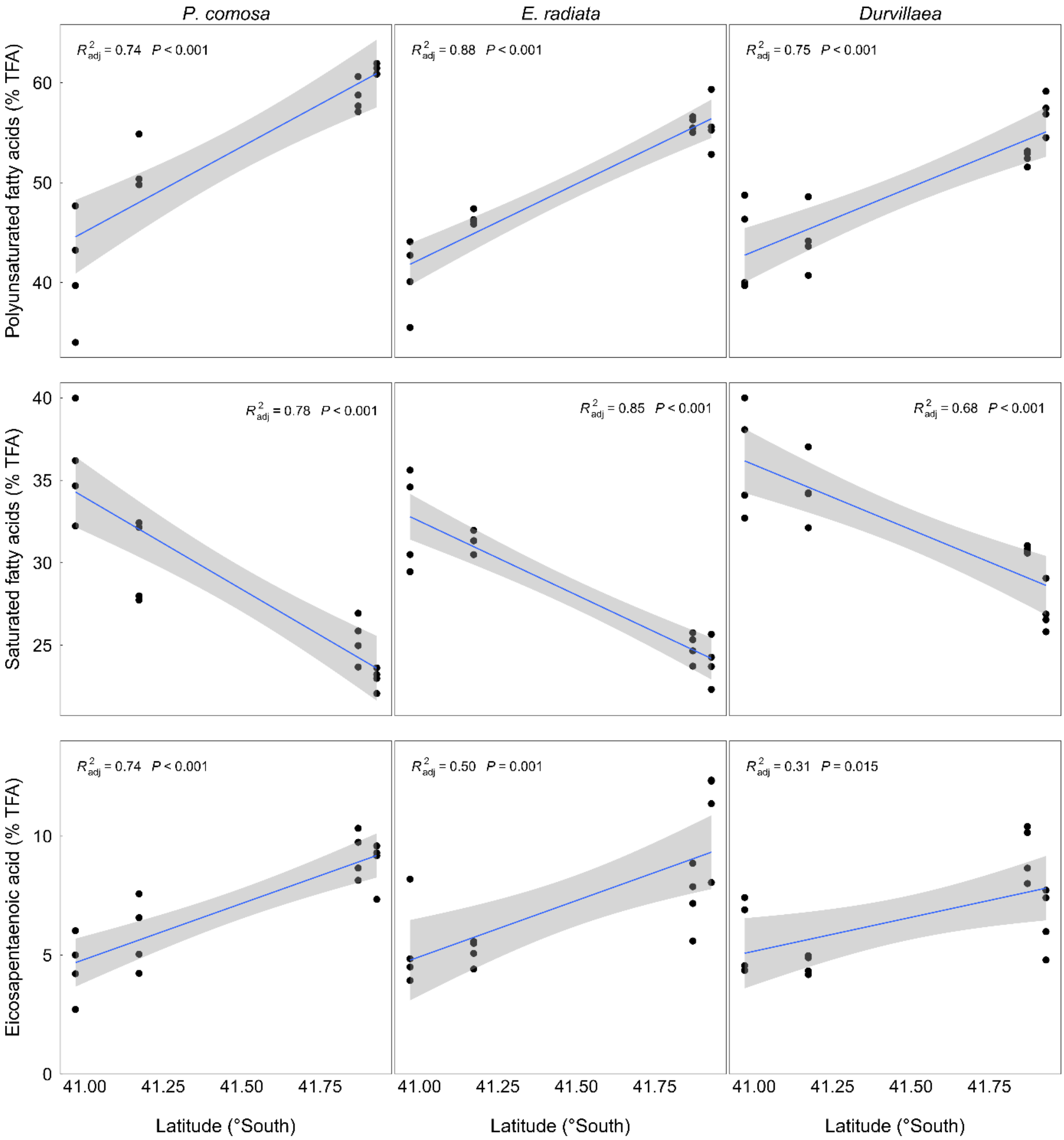

Figure 10: Correlations of polyunsaturated fatty acids (top panel), saturated fatty acids (middle panel) and eicosapentaenoic acid (bottom panel) with latitude on the east and west coasts for the species: *E. radiata* (left), *P. comosa* (middle), and *Durvillaea spp.* (right). Correlations are on pooled samples from both coasts as no differences between coasts were detected.

Dietary analysis

The dietary analysis of the proportion of red or brown seaweeds eaten by abalone suggested a trend at all sites in which the mean proportion of reds consumed was larger than that of the browns (Table 5). However, there was significant overlap in the standard deviations, indicating that any statistical differences between the proportions were unlikely to be significant (Table 5). There were no clear latitudinal trends in the proportion of each group eaten at each site (Table 5).

Table 5: Proportion of each seaweed group eaten at each site based on the dietary analysis models comparing fatty acid profiles of *H. rubra* and fatty acids that contributed to ≥ 1 % of total fatty acids of either abalone or either of the seaweed groups. Data are shown as means (Proportion) and standard deviation.

Site	Region	Group	Proportion	Standard deviation
Gardens	North-east	Brown	0.45	0.25
		Red	0.55	0.25
Seymour	North-east	Brown	0.34	0.26
		Red	0.66	0.26
Sisters	South-east	Brown	0.29	0.27
		Red	0.71	0.27
Thumbs	South-east	Brown	0.39	0.24
		Red	0.61	0.24
Black Reef	South	Brown	0.35	0.23
		Red	0.65	0.23
Mouldy Hole	South	Brown	0.29	0.27
		Red	0.71	0.27

Laboratory experiments

Net photosynthesis of kelp from different thermal baselines

The thermal optima for net photosynthesis of *P. comosa* differed between regions (Figure 11). The thermal optima in the northern and eastern sites were ~ 18-20 °C. In contrast, the thermal optimum was higher in the south-eastern sites (~ 21-23 °C) and higher again in the southern sites (~ 23-24 °C). There were minimal differences in the % N content or the proportion of PUFA and SFA between the individuals from each region (Table 6).

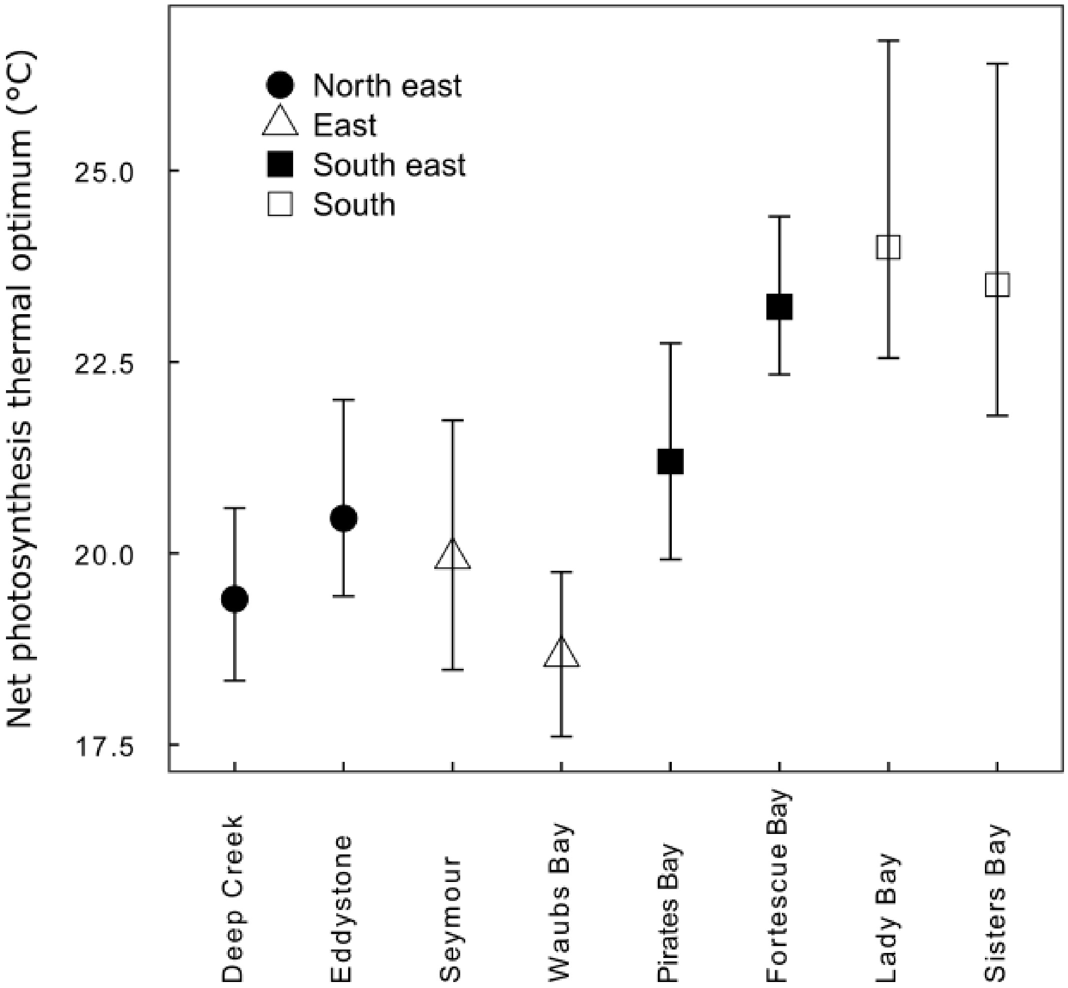

Figure 11: Thermal optimum for photosynthesis of *Phyllospora comosa* from 8 sites in north-eastern Tasmania (Deep creek and Eddystone), eastern Tasmania (Seymour and Waubs Bay), south-eastern Tasmania (Pirates Bay and Fortescue Bay), and southern Tasmania (Lady Bay and Sisters Bay). Data are displayed as means and 95 % confidence intervals. Shapes display region individuals were collected from (filled circles = North-east, open triangles = East, filled squares = South-east, and open squares = South.

Table 6: Means and standard deviation of nitrogen content (% dw), PUFA (% TFA) and SFA (% TFA) for *P. comosa* individuals collected from the four regions with differing thermal baselines. SD refers to standard deviation.

Region	Site	% N (% dw)	SD	PUFA (% TFA)	SD	SFA (% TFA)	SD
North east	Deep Creek	1.09	0.08	60.02	1.16	25.47	0.84
North east	Eddystone	1.39	0.14	60.71	1.32	24.98	0.77
East	Seymour	1.23	0.20	60.30	1.40	25.83	1.46
East	Waubs Bay	1.45	0.12	62.13	1.64	23.94	1.24
South east	Fortescue Bay	1.49	0.08	58.57	1.78	26.35	1.28
South east	Pirates Bay	1.21	0.15	55.82	2.19	28.33	1.21
South	Lady Bay	1.07	0.22	60.46	0.84	25.10	0.53
South	Sisters Bay	1.18	0.12	60.18	1.59	25.10	1.30

Thermal performance of the kelp *Lessonia corrugata*

The thermal optima of *Lessonia corrugata* was identified to be ~ 16 °C with an upper thermal limit of ~ 22-23 °C (Figure 12, Figure *13*). The upper thermal limit did not change when *L. corrugata* was cultured under elevated nitrate concentrations, however the growth rate at the optimum was slightly higher under elevated nitrate (~ 20 %, Figure 13). Protein, PUFA, EPA and LIN content all displayed a trend of a decrease with increasing temperatures (Figure 14, Figure *15*).

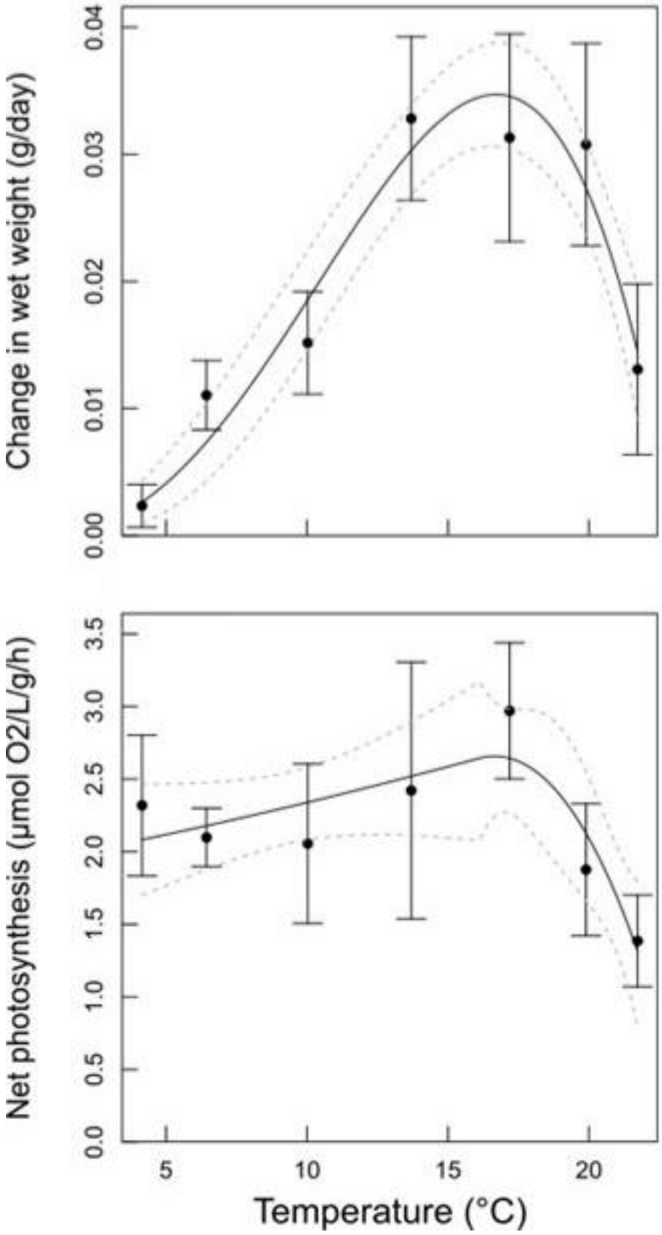

Figure 12: Thermal performance curves for the kelp *Lessonia corrugata* grown in 3-week culture experiments. Top panel = Change in wet weight (g/day), bottom panel = Net photosynthesis (µmol O_2/L/g/h). Data are displayed as means ± standard error. Lines show model predictions with standard of model predictions as dashed lines.

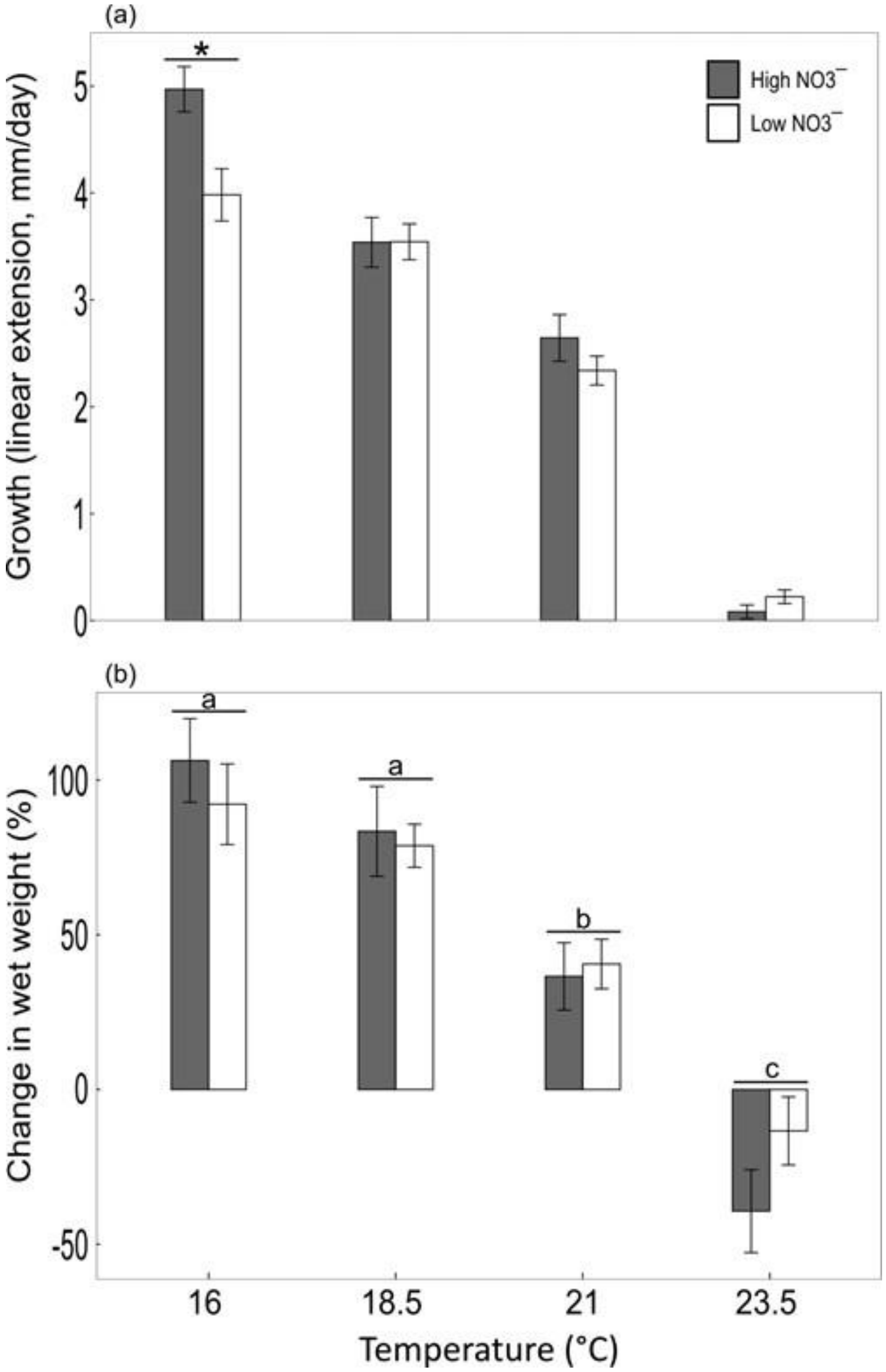

Figure 13: Growth (mm/day, top panel) and change in wet weight (%, bottom panel) of *L. corrugata* grown under low (5 µmol/day) and high (50 µmol/day) nitrate concentrations at 16, 18.5, 21 and 23.5 °C for weeks, data are presented as means ± standard error (n = 10).

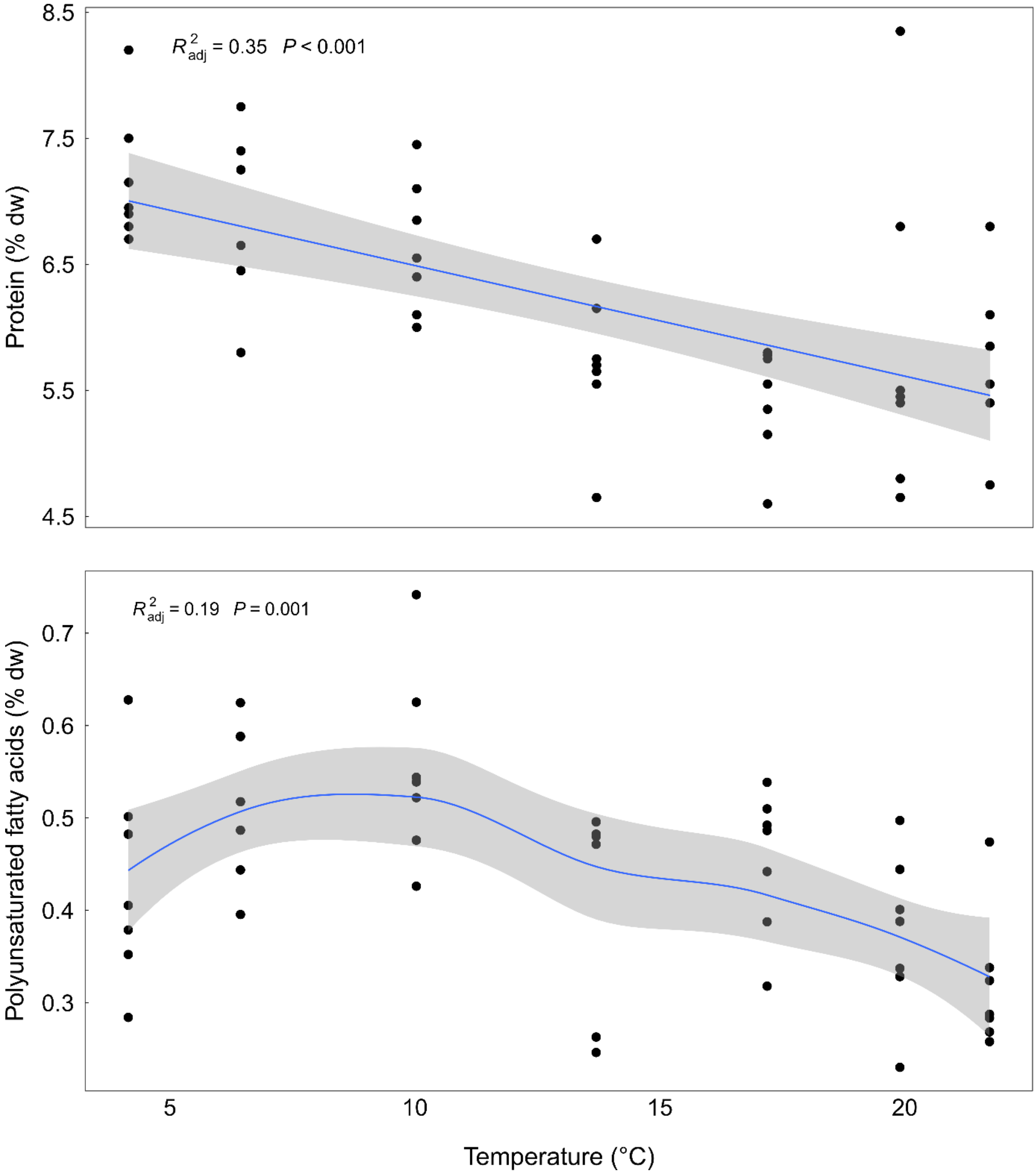

Figure 14: Correlation of protein (top panel) and PUFA content (bottom panel) and temperature for the kelp *Lessonia corrugata* grown in the laboratory for 3 weeks under seven temperature conditions.

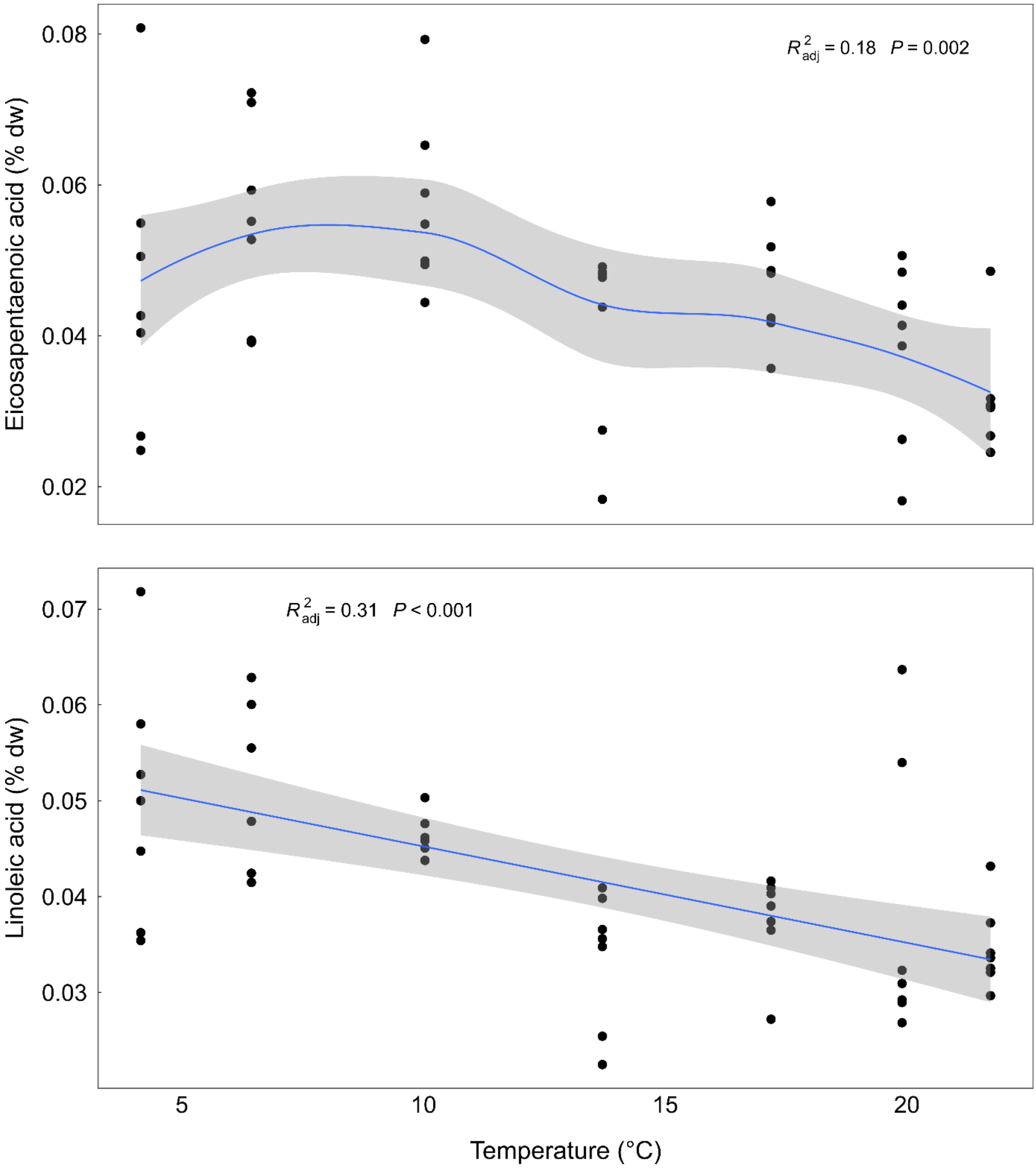

Figure 15: Correlation of EPA (top panel) and LIN content (bottom panel) and temperature for the kelp *Lessonia corrugata* grown in the laboratory for 3 weeks under seven temperature conditions.

Discussion and implications for management

Seasonal and regional patterns in nutritional quality of seaweeds

Understory biomass

Biomass of understory seaweeds increased with increasing latitude and was primarily driven by a greater abundance of red seaweeds in the southern sites. This result is consistent with the findings of Britton et al (2021) and provides further evidence of a latitudinal gradient in seaweed biomass. The higher biomass of red seaweeds in the southern regions is a key finding in the context of abalone nutrition as red seaweeds are generally more palatable to *H. rubra* than leathery brown seaweeds such as kelps and fucoids (McShane et al 1994; Steneck and Watling, 1982; Roussel et al 2020). The greater palatability of red seaweeds is likely due to the structure of the abalone radula used in feeding, which due to lack of hardened teeth is limited in its capability to penetrate tough leathery seaweed tissue (Steneck and Watling, 1982). Physical toughness has been proposed as one of the main feeding deterrents of seaweed to *H. rubra* (and other haliotids e.g. Roussel et al 2020), with ingestion rates strongly correlated with resistance of the seaweed thalli to penetration (McShane et al 1994; Shepherd and Steinberg 1992). Furthermore, red seaweeds have been proposed as the preferred food source when abundant (Shepherd 1975) and some haliotids are known to prefer seaweeds with high protein (Roussel et al 2020). This preference for high protein red seaweeds was reflected in our dietary analysis with a greater proportion of reds eaten at all sites. However, there was substantial variability in these proportions and this finding contrasts with other studies on *H. rubra* diets (Guest et al. 2008; Holland et al. 2021). It is important to note that local availability is a driver of food preference and study sites in Victoria where Holland et al (2021) was conducted are likely to have a lower abundance of red algae. Moreover, Holland et al (2021) relied on extracts from stomach contents, which are known to overestimate the contribution of brown seaweeds to abalone diet (Foale & Day, 1992). Nevertheless, regardless of the exact proportion consumed, it appears that at least a substantial component of *H. rubra* diet consists of red seaweeds. Given this, the higher proportion of palatable red species in the southern region is likely to contribute to the elevated productivity of the *H. rubra* fishery in this region.

Availability of nutritional compounds per unit area

The seasonal survey indicated that the availability of nutritional compounds on a per unit area basis was substantially higher in the southern and south-eastern regions. Protein availability was substantially higher in the south and south-east, which likely explains, at least in part, the higher productivity of *H. rubra* in these regions (Mundy and McAllister, 2022). Protein is the main macronutrient used to build tissue in abalone (Bollon et al. 2023; Fleming, VanBarneveld, & Hone 1996), and aquaculture feed trials suggest that the protein content of seaweeds are typically lower than optimum dietary levels (Bansemer et al. 2016; Bautista-Teruel and Millamena 1999; Dunstan 2010). As such, it is likely that the availability of protein is a limiting constraint on wild abalone growth. This is certainly supported by the lower growth rates of abalone when fed natural versus protein enriched diets (Bautista-Teruel and Millamena 1999), and may indicate that reduced rates of growth in abalone from the northern regions is partly driven by lower protein availability. The southern and south-eastern regions also had greater availability of TFA, PUFA and the essential fatty acids EPA and ARA. The greater availability of EPA and ARA is particularly important as abalone are unable to synthesise essential fatty acids and must obtain these from their food source. Because of this, high levels of EPA and ARA are correlated with high specific growth rates in the numerous abalone species *Haliotis tuberculata, Haliotis discus hannai, Haliotis asinina* and *Haliotis fulgens* (Bautista-Teruel et al. 2011; Durazo-Beltrán et al. 2004; Mai et al. 1996; Nelson et al. 2002). While there have been no studies examining the direct effect of diets containing high levels of EPA/ARA on growth of *H. rubra*, they are two of the most abundant fatty acids found in *H. rubra* tissue (Dunstan et al. 1996; Grubert, Dunstan and Ritar, 2004). This suggests they are key nutritional requirements, which is certainly the case in other abalone species (Grubert, Dunstan and Ritar, 2004). Identifying the physiological benefits for *H. rubra* consuming diets high in these essential fatty acids will be key to directly linking food quality to enhanced productivity of abalone in the southern and south-eastern regions.

Availability of nutritional compounds per gram of seaweed

The availability of nutritional compounds available per gram of seaweed tissue is an important metric to consider as for a given amount of biomass, a richer source of a compound will require less energy to consume. Protein content per gram of seaweed was higher in the southern and south-eastern regions relative to the north, and this was consistent across seasons. This provides further evidence of protein content being a key driver of abalone productivity as not only is the total amount of protein available per metre higher in the south, the energy required to extract this protein is also less. In contrast to protein, there were no regional differences in the amount of essential fatty acids per gram of seaweed. However, there were distinct seasonal patterns that were consistent across sites. The typical pattern observed for TFA, PUFA, ARA and EPA was a peak in winter, a decline through spring and summer followed by a slight increase in autumn. This pattern is likely driven by seasonal patterns in temperature, with seaweeds known to alter fatty acid composition in response to elevated temperature by reducing the proportion of PUFA (Britton et al. 2020; Schmid et al. 2020). However, PUFA and TFA are known to increase under both elevated nutrients and lower light levels (Floreto et al 1998; Khotimchenko and Yakovleva 2005; Kumari et al. 2013), which are typical conditions observed in winter. As such, it is a likely a complex interplay of all three environmental factors drives the patterns observed here. However, regardless of the drivers, the seasonal decline in TFA, PUFA and essential fatty acids could have important implications for abalone nutrition and partially explain the seasonal decline in condition observed in summer months, as the combined stress of elevated temperatures coincides with a decline in food quality. Furthermore, abalone food intake requirements can increase with elevated temperature (Frederick et al. 2022) or can alter the relative energy allocation between growth and reproduction (Lopez and Tyler, 2006), potentially exacerbating the impact of declines in food quality in warmer seasons.

Temperature impacts on seaweed performance

We conducted three laboratory experiments investigating the thermal performance of kelp. Two examined the endemic kelp *Lessonia corrugata* that is abundant in Storm Bay; one identifying thermal optima and another the interactive effects of temperature and nutrient availability. The third experiment examined the thermal performance of crayweed (*P. comosa*) from different thermal baselines. *L. corrugata* displayed a thermal optimum of ~ 16 °C for growth and photosynthesis, with a decline in performance at temperatures above this and a maximum thermal limit of ~22 °C. Moreover, this pattern was not influenced by the availability of nutrients. We saw no increase in the thermal tolerance at temperatures above the optimum when cultured under high nutrient conditions, contrasting with that of other kelp species in Tasmania (Fernandez et al 2020). The upper thermal limit of ~ 22 °C for *L. corrugata* is a particular concern as this temperature is very close to the summer peak in the north of Tasmania. Furthermore, these temperatures will become more frequently reached in southern regions under climate change, potentially leading to contraction of this species further south, or widespread declines of this species. Whilst we did not test the thermal performance of other species (excluding *P. comosa* – see below), it is likely that many species in Tasmania will be equally challenged. Tasmania has one of the most diverse seaweed assemblages in the world with high rates of endemism (Hurd et al. 2023), yet little is known about the thermal tolerances of most species, particularly the reds. Given that red seaweeds appear to be critically important for abalone in this region, further research identifying susceptibility of these species is required to prioritise management of these systems.

The experiment examining the thermal performance of *P. comosa* from different thermal baselines found highly unexpected results. We had predicted that *P. comosa* in the northern sites would have higher thermal optima than their southern counterparts, however the opposite pattern was observed. Reasons for this are unclear but maybe be related to higher nutrient levels in the southern sites. However, in the samples collected for the TPCs, there was no evidence of elevated nitrogen content in southern samples. The southern sites are certainly less influenced by the warm, nutrient poor east Australian current than those of the north-east and east (Richardson et al 2019; Ridgway et al. 2007). As such, it may be that *P. comosa* from the northern sites were limited by nutrients other than nitrogen, or differences are due to genotypic or phenotypic plasticity. Regardless of the mechanism, these results provide concern for *P. comosa* in the northern regions as they indicate that the main habitat forming seaweed in abalone habitat is not only exposed to warmer temperatures, but may be less tolerant of them, potentially impacting the availability of abalone habitat in the future. These results are consistent with recent modelling work on *P. comosa* that suggest it is a species at risk of decline due to climate change (Holland et al. 2021; Martínez et al. 2018). Further research is required to assess whether the latitudinal patterns of thermal tolerance observed here are widespread amongst *P. comosa* populations and identify the mechanisms driving this pattern.

Temperature impacts on seaweed nutritional quality

Two components of this project allowed us to examine how temperature directly influences nutritional quality: 1. The laboratory experiment constructing thermal performance curves for *L. corrugata*, and 2. The marine heatwave event in which we sampled during, one and two months post the heatwave event at Black Reef. The laboratory experiment on *L. corrugata* was conducted over a temperature range similar to that seen in the field over the course of a year (with slightly lower minimum temperatures), and there was a trend of a decline in protein content as temperatures increased. Likewise, an inverse relationship was found between amount of the essential fatty acids LIN, EPA and overall PUFA content with temperature. The decline in protein content under elevated temperature has also been observed in *P. comosa* and the red understory seaweed *Callophyllis lambertii* (Britton et al. 2019, 2020), and similar changes in fatty acid composition have been demonstrated in Tasmania (Britton et al. 2020) and elsewhere (Becker, Graeve, & Bischof, 2010; Gosch, Lawton, Paul, de Nys, & Magnusson, 2015). These responses, in conjunction with the seasonal survey showing a reduction in the availability of essential fatty acids during the warmer seasons, indicate that this trend is widespread. As such it is likely that under ocean warming and during marine heatwaves there will be reductions in the nutritional quality of food available to abalone.

The sampling undertaken post-heatwaves was opportunistic and attempted to identify any major persistent changes in the nutritional quality of the species sampled following cessation of the heatwave. Unfortunately, as there were no baseline samples collected immediately prior to the onset of the heatwave, attributing changes to the heatwave per se is difficult, particularly as species displayed varied responses. There was some indication that the marine heatwave caused declines in the nutritional quality of some seaweed species. For example, protein content declined in all species excluding *C. platylobium*, while TFA declined in all species except *Zonaria spp.* However, other compounds such as EPA and ARA increased in some species making inferences difficult. The nearest baseline sample was taken in spring (~ 3 months earlier), and as such, attributing changes in nutritional quality to the heatwave as opposed to normal seasonal variability is not possible. Nevertheless, the opportunistic sampling undertaken here and the variability in responses highlights the complexity of understanding the impact of marine heatwaves on food quality, over and above the normal summer patterns. A key consideration for the future is to undertake summer monitoring of seaweed nutritional quality at more frequent time intervals (weeks) over multiple years to separate the impact of heatwaves from interannual and seasonal variability. Doing so will improve our understanding of how marine heatwaves directly impact abalone, through changes to their food quality. Expanding the range of species subjected to thermal performance analyses will assist with a broader understanding of declines in seaweed nutrition during the summer period, and during marine heat wave events.

Eastern and western comparison

There were no differences in the nutritional quality of seaweeds collected from the north-western and eastern coasts. We had predicted that due to the increased influence of the east Australian current that the food quality of the seaweeds in the east would be lower than their western counterparts. Reasons for this are unclear but it may be that the influence of the east Australian current during the sampling period (December) was not yet large enough to have a major influence. Alternatively, temperature may be the key driver of these responses. All species from both coasts displayed near identical responses in which PUFA and EPA increased from north to south along the temperature gradient. This response mirrored that of the laboratory experiment on *L. corrugata* and other seaweeds described above (see – *Temperature impacts on seaweed nutritional quality*). As such, this finding provides further evidence that under ocean warming and marine heatwaves we can expect to see declines in seaweed nutritional quality, potentially impacting productivity of abalone populations that utilise them as a food source.

Summary and implications for management

The data presented here provide evidence that food quality for abalone in temperate rocky reefs in southern and south-eastern Tasmanian are higher than those in the north-east. This higher food quality is driven by a higher biomass of palatable red seaweeds in the southern and south-eastern regions and a greater availability of protein and essential fatty acids. As such, the seaweed communities in these regions likely support higher rates of productivity in *H. rubra*, which may allow these reefs to support greater harvest levels. These data contribute to the ongoing management of *H. rubra* populations by providing key knowledge on the factors driving productivity of *H. rubra* and how these vary on regional and seasonal scales. Furthermore, the data provide evidence that nutritional quality and performance of seaweeds in Tasmania will decrease under ocean warming and marine heatwave events, leading to declines in food quality. This will likely exacerbate the direct effects of elevated temperatures on *H. rubra*. Future research should investigate how the nutritional quality of seaweed assemblages are altered by marine heatwaves in the summer months, through targeted field sampling. This research, if conducted over multiple years, will identify direct links between marine heatwave events and seaweed nutritional quality, providing invaluable information to inform management of the risks associated with climate change.

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Appendices

Length of overstory species

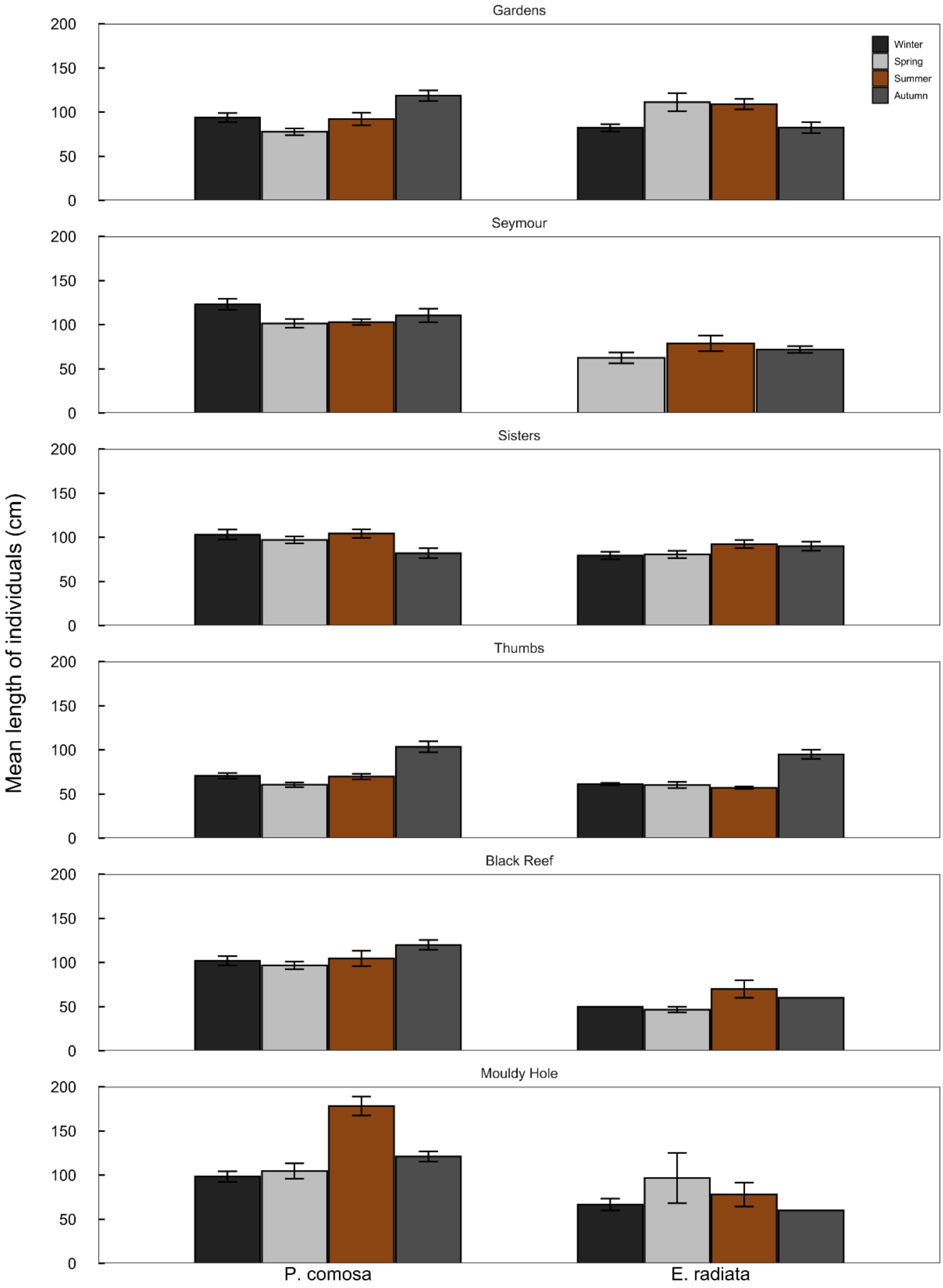

Figure A1*:* Mean length of the dominant the canopy species *Phyllospora comosa* and *Ecklonia radiata* at each site across all seasons: black bars = Winter, light grey bars = Spring, brown bars = Summer, dark grey bars = Autumn.

Regional and seasonal overview – data analysis

To visualise overall region and seasonal differences in fatty acid and protein availability per m^2 and fatty acid and protein availability per g of seaweed in multivariate space, a Canonical analysis of Principle components (CAP) was undertaken for the factors Region and Season. To test for overall differences between Regions and Seasons a Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted with the fixed factors: “Season” (4 levels: Summer, Autumn, Winter and Spring), “Region” (3 levels: North east, South east and South), the Site × Season interaction and the random nested factor “Site” (2 levels per region). All resemblance matrices were based on the Euclidean distance measure and were either square (availability per metre) or 4th (availability per g) root transformed. Results can be viewed in Appendices.

Regional and seasonal overview – results

There were substantial differences in both the amount of fatty acids and protein available per m^2 regions (Table A1, Figure A2) and between seasons (Table A1, Figure A3). Pair-wise comparisons for region indicated that both the southern and south-eastern and southern region were differed significantly from the north-east region ($p < 0,05$, Table A1). Pair-wise comparisons for seasons indicated that spring differed from all other seasons ($p < 0,05$, Table A1). All regions tended to differ from each other an equal amount. In contrast, winter was substantially different from all other seasons that only differed from each other slightly. There were no significant differences in the amount of fatty acids and protein available per g of seaweed tissue between regions (Table A2, Figure A4). In contrast, there were significant differences between seasons (Table A2, Figure A5), with spring being significantly different from all other seasons in the pair-wise comparisons ($p < 0.05$, Table A2).

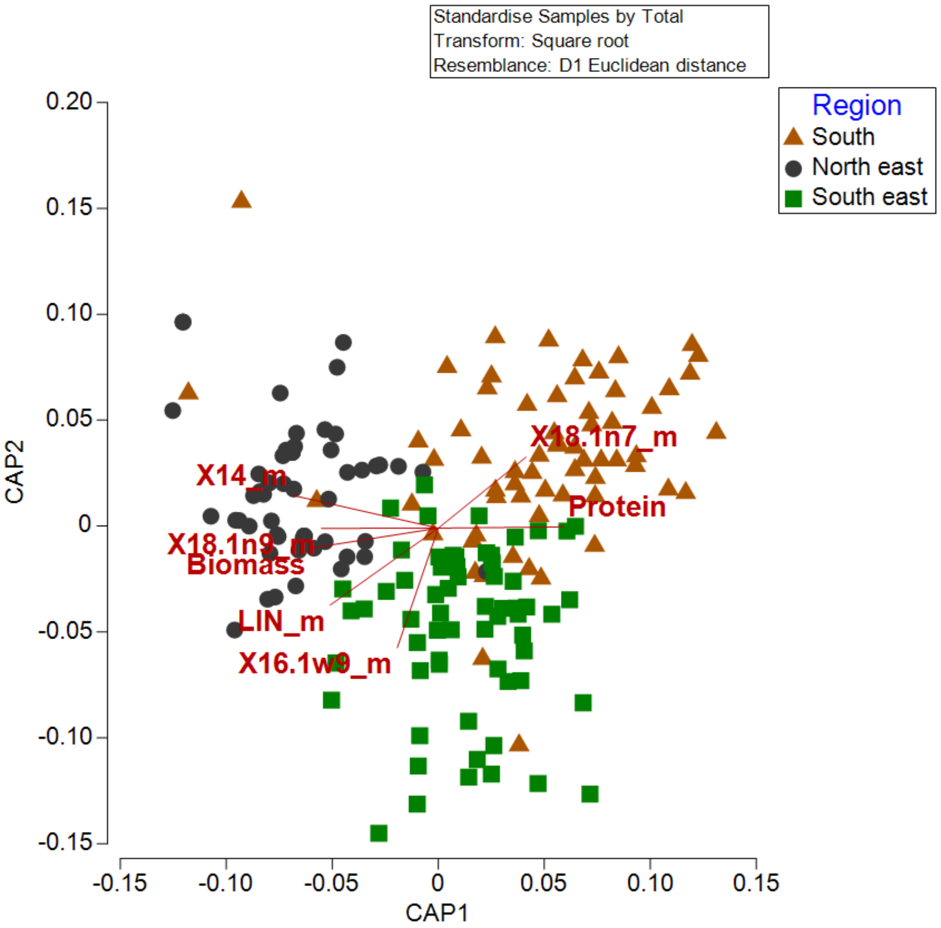

Figure A2: Canonical Analysis of Principal Components (CAP) showing separation of regions based on total biomass, protein, total fatty acids and abundant individual fatty acids (> 1% of total fatty acids). All variables are in total grams per metre. Overlays show key drivers of differences between regions.

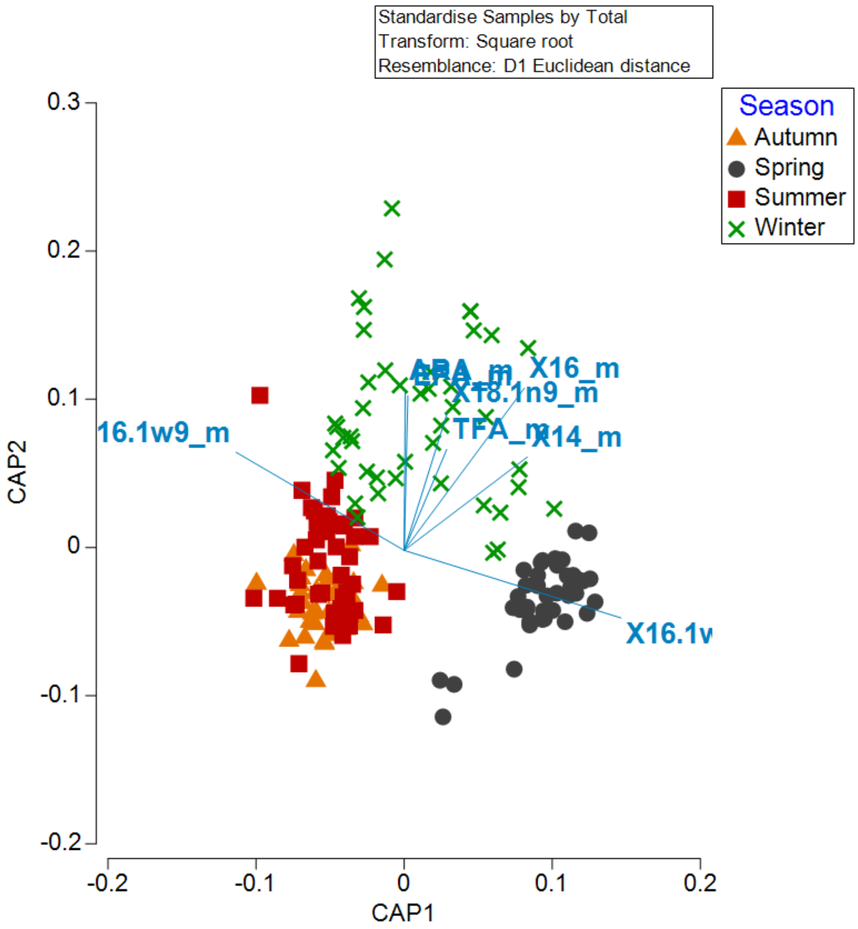

Figure A3: Canonical Analysis of Principal Components (CAP) showing separation of seasons based on total biomass, protein, total fatty acids and abundant individual fatty acids (> 1% of total fatty acids). All variables are in total grams per metre. Overlays show key drivers of differences between seasons.

Table A1: Permutational MANOVA table for the amount of fatty acids and protein available per m^2 within the three Regions (North east, south east and south) and Seasons (Winter, Spring, Summer and Autumn), the Region × Season interaction and the random nested factor Site. Significant differences have p-values are displayed in bold. Pair-wise tests show groups that are significantly different from each other and are designated by a not equal symbol.

Factor	df	F-value	P-Value	Pair-wise tests
Region	2	3.38	**0.0154**	South, South-east ≠ North-east
Season	3	3.06	**0.0137**	Autumn, Winter, Summer ≠ Spring
Region x Season	6	1.81	0.0769	
Site	12	7.06	**0.0001**	
Residuals	164			

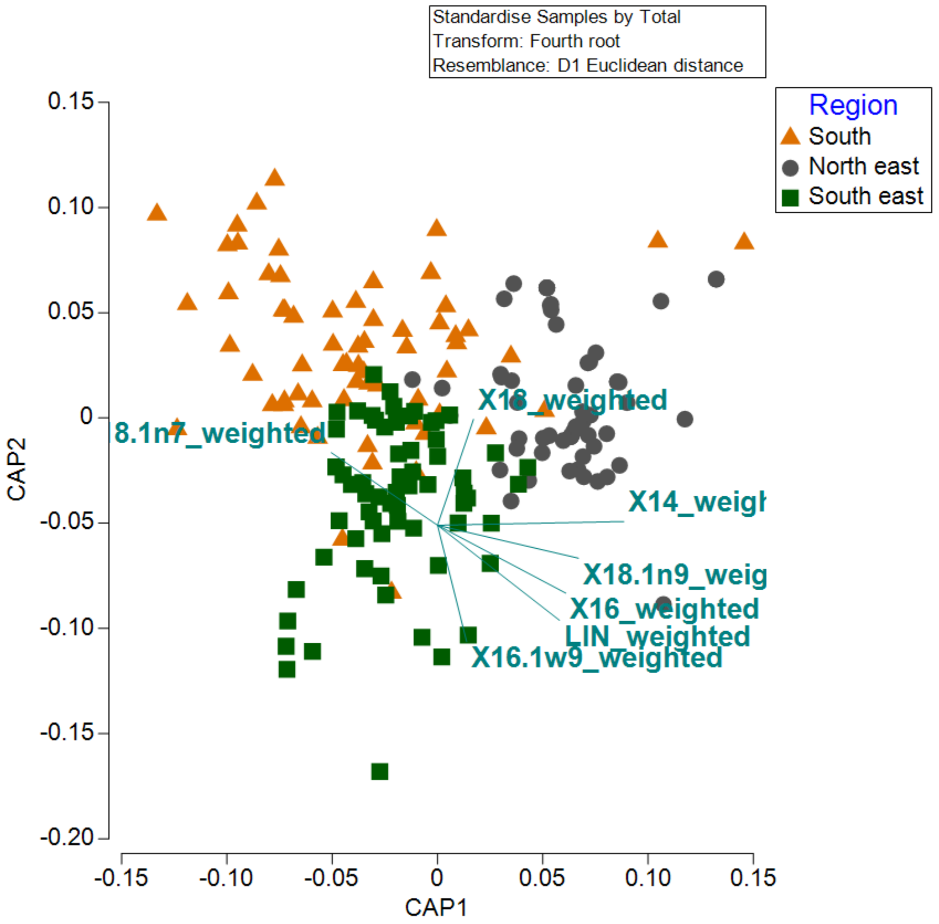

Figure A4: Canonical Analysis of Principal Components (CAP) showing separation of regions based on total protein, total fatty acids and abundant individual fatty acids (> 1% of total fatty acids). All variables are in average grams per gram of seaweed tissue. Overlays show key drivers of differences between regions.

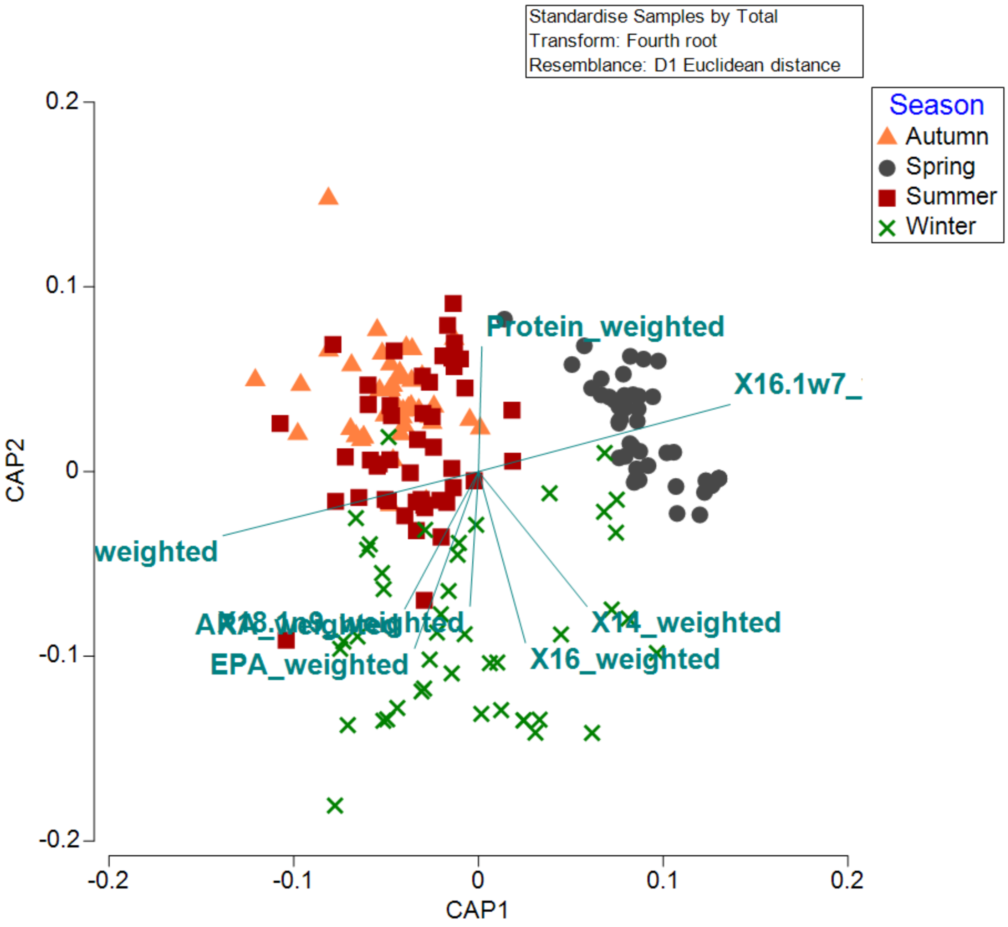

Figure A5: Canonical Analysis of Principal Components (CAP) showing separation of seasons based on total protein, total fatty acids and abundant individual fatty acids (> 1% of total fatty acids). All variables are in average grams per gram of seaweed tissue. Overlays show key drivers of differences between seasons.

Table A2: Permutational MANOVA table for the amount of fatty acids and protein available per g of seaweed tissue within the three Regions (North east, south east and south) and Seasons (Winter, Spring, Summer and Autumn), the Region × Season interaction and the random nested factor Site. Significant differences have p-values are displayed in bold. Pair-wise tests show groups that are significantly different from each other and are designated by a not equal symbol.

Factor	df	F-value	P-Value	Pair-wise tests
Region	2	1.92	0.0599	
Season	3	4.05	**0.0002**	Autumn, Winter, Summer ≠ Spring
Region x Season	6	1.59	0.0642	
Site	12	13.61	**0.0001**	
Residuals	164			