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RESEARCH

Fatty acid profiles and stable isotope composition of Antarctic macroalgae: a baseline for a combined biomarker approach in food web studies

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Abstract

The Western Antarctic Peninsula supports a diverse assemblage of > 100 described macroalgal species that contribute to the base of coastal food webs, but their contribution to local nearshore food webs is still uncertain across larger spatial scales. The analysis of biomarkers, specifically fatty acids and stable isotopes, offers a tool to clarify the trophic role of Antarctic macroalgae. The aim of this study was to describe the fatty acid profiles and stable isotope values of 31 algal species from three divisions (Chlorophyta—1, Ochrophyta—8, Rhodophyta—22) collected at the same sites for both biomarkers. Of these, 13 species had no previously published fatty acid profiles. Most species were rich in polyunsaturated fatty acids (PUFAs), suggesting they are highly nutritious and could be a resource of essential fatty acids for consumers. This was specifically noticeable in the overall low PUFA ratio ($\sum\omega6/\sum\omega3$ ratio), with low ratios being an indicator of high nutritional quality for consumers. Fatty acid profiles of macroalgae grouped strongly by phylogeny (at the levels of division, order, and family), while stable isotope groupings were more driven by the physiological properties of the species. Specifically, some closely related red algal species exhibited very different stable isotope values based on their carbon concentrating mechanisms, with highly ^{13}C-depleted values in several Rhodophyta species. The fact that the two biomarker approaches created different groupings of Antarctic macroalgae collected at the same locations emphasizes that their combined application can be a powerful tool in Antarctic coastal food web studies.

Keywords Fatty acids · Stable isotopes · Chemotaxonomy · Food web · Nearshore ecology · Western Antarctic Peninsula

Introduction

Along the coastal Western Antarctic Peninsula (WAP) dense nearshore macroalgal forests provide habitat and carbon to a diverse assemblage of marine organisms (Wiencke et al. 2014; Valdivia et al. 2015; Oliveira et al. 2020). Nearshore marine habitats of the WAP (0–50 m depth) contain the greatest macroalgal biomass found on the continent, with approximately 110 species of an overall diversity of 151 described macroalgal species in Antarctica (Mystikou et al.

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2014; Pellizzari et al. 2017; Oliveira et al. 2020). These macroalgal species make up the majority of benthic primary production along the WAP (Wiencke et al. 2014; Barnes 2017). It is estimated that much of the total macroalgal biomass production is channeled annually into the coastal system (Quartino and Boraso de Zaixso 2008) or transported to adjacent, deeper habitats as a spatial subsidy (Fischer and Wiencke 1992). Multiple studies along the WAP have identified the overall importance of macroalgae in supporting the coastal food web (e.g., Iken et al. 1997, 2023; Dunton 2001; Huang et al. 2006; Aumack et al. 2017; Zenteno et al. 2019; Cardona et al. 2021); however, the contributions of individual macroalgal species to the Antarctic food web on larger scales are less well understood.

The role that macroalgae play in the WAP coastal food web is complex, and is controlled by various factors, including physiological differences in macroalgae marked by the presence of chemical defense compounds (Amsler et al. 2005; 2009), and environmental factors such as sea ice cover (Quartino et al. 2013; Amsler et al. 2023), temperature (Becker et al. 2010; Cordone et al. 2018), and light (Deregibus et al. 2016). Amphipods and gastropods are among the most abundant mesograzers in the Antarctic subtidal and mostly consume small epiphytes growing on the larger, often chemically defended macroalgae (Iken 1999; Aumack et al. 2017). These small mesograzers are important prey for other predators, and they may serve as a link between benthic primary production and higher trophic levels (e.g., Dauby et al. 2003; Zamzow et al. 2011; Casaux and Barrera-Oro 2013). However, some consumers prefer chemically defended species to reduce competition for food resources or capitalize on the chemicals for their own defenses (Amsler et al. 2013; Heiser et al. 2022). In addition, chemical defenses of Antarctic macroalgae can dissipate once algae senesce or die, increasing the palatability to consumers once the algal material enters the detrital pathway (Reichardt and Dieckmann 1985; Amsler et al. 2012; Schram et al. 2019).

Antarctic macroalgae also differ in other attributes important to consumers, such as morphological or biochemical characteristics. For example, thallus toughness mediates palatability for some consumers (Amsler et al. 2005). Also, while nutritional quality of Antarctic macroalgae is typically high (Weykam et al. 1996; Amsler et al. 2005), there are differences in biochemical content that increase the nutritional quality of some algal species over others (Peters et al. 2005). One of these nutritional sources that is especially important to consumers are fatty acids (FAs). The FAs of both Arctic and Antarctic seaweeds are known to differ among taxonomic groups (e.g., Graeve et al. 2002; Aumack et al. 2017; Berneira et al. 2020). The strong differentiation of FA composition by phylogenetic relationships of the groups aligns with well-established FA patterns in macroalgae elsewhere, with closely related taxa having high similarity in FA profiles (Galloway et al. 2012; Kelly and Scheibling 2012). FAs are vital to macroalgal metabolic functions, such as maintaining membrane fluidity (Santos et al. 2017), in addition to contributing critical nutrients to the metabolic processes of most consumers (Dalsgaard et al. 2003; Budge et al. 2006).

Some of the more comprehensive studies on FAs in Antarctic macroalgae established that they contain significant amounts of polyunsaturated fatty acids (PUFAs) (Graeve et al. 2002; Santos et al. 2017; Schram et al. 2019; Teixeira et al. 2019; Berneira et al. 2020), especially intermediate- and long-chained (C18 and C20, respectively) PUFAs, which have been identified as key nutritional components in aquatic food webs (Ruess and Müller-Navarra 2019). PUFAs, especially the long-chained PUFAs Arachidonic acid, Eicosapentaenoic acid, and Docosahexaenoic acid, are important for growth, production, and fecundity in marine consumers (e.g., Parrish 2009; Pereira et al. 2012) and enhance the energy transfer efficiency among trophic levels in marine systems (Müller-Navarra et al. 2000; Troch et al. 2012; Eddy et al. 2021). In Antarctic coastal food webs, PUFAs and other FAs from macroalgae are effectively assimilated by consumers (Aumack et al. 2017; Schram et al. 2019), likely contributing to their nutrition and performance.

In addition to providing important nutrition value, FAs can also be used in food web studies to trace the consumption of various macroalgal species by consumers (Kelly and Scheibling 2012). This applies especially to essential fatty acids (EFAs), which are organic molecules required for biological processes that are only synthesized by primary producers and must be assimilated into consumer tissues through consumption (Arts et al. 2001; Galloway and Winder 2015). These EFAs, along with other FAs, can be used as biomarkers to trace the flow of trophic resources through a food web and clarify relationships between specific producers and consumers (Dalsgaard et al. 2003; Budge et al. 2006). This is enhanced by the fact that FA profiles of macroalgal species seem mostly conserved across large geographical scales, making them conservative markers for a source species (Khotimchenko et al. 2002). EFAs, in particular, can provide important information on what resources a consumer has been assimilating, generating quantitative estimates of the consumer's diet (Iverson et al. 2004; Galloway et al. 2014; Bromaghin 2017; Guerrero and Rogers 2020).

At the same time, a combination of different biomarkers, such as FAs with stable isotope (SI) information, can be especially powerful in distinguishing different primary producer sources in benthic food webs (Hanson et al. 2010; Kelly and Scheibling 2012; Dethier et al. 2013; Aumack et al. 2017). The combined biomarker approach is widely applicable across many marine ecosystems, trophic levels, and a variety of scientific questions (see Nielsen et al. 2018). SIs are typically used to provide information about

food resources ($^{13}C/^{12}C$; $\delta^{13}C$) and trophic position ($^{15}N/^{14}N$; $\delta^{15}N$) in food web analyses, where the $\delta^{13}C$ values of different primary producer sources are preserved with minimal fractionation (~ 1 ‰) between consumer trophic levels (DeNiro and Epstein 1978; Caut et al. 2009), and can, thus, be traced through the food web (Boecklen et al. 2011). This applies, however, mostly to differences among larger groupings of primary producer sources in coastal systems, such as phytoplankton and macroalgal production or terrestrial organic matter (France 1995; Peterson 1999; Raven et al. 2002). However, the ability of SIs to distinguish high-resolution (e.g., species-level) taxonomy is limited. Using SIs to distinguish trophic levels in food webs is particularly valuable as FAs typically cannot determine trophic level (Kelly and Scheibling 2012). Data collected during SI analyses also allow for the calculation of the C:N ratio, which is a useful measure of the nutritional value of macroalgae as a food source (e.g., Weykam et al. 1996; Peters et al. 2005), and can provide additional information about trophic resource use.

Here, we provide FA profiles of 31 species of Antarctic macroalgae across the three major macroalgal divisions (Chlorophyta, Ochrophyta, Rhodophyta), significantly expanding the published profiles of 18 species (Graeve et al. 2002; Aumack et al. 2017; Santos et al. 2017; Schram et al. 2019; Teixeira et al. 2019; Berneira et al. 2020). In addition, we provide the carbon and nitrogen SI values and C:N ratios for most of the same species from the same locations. Our expanded biomarker baselines, especially for FA profiles, are obtained from field-collected specimens, which may differ from profiles of some specimens previously analyzed from long-term lab cultures (Graeve et al. 2002). We also assess the ability of the two biomarker types, individually and combined, to distinguish macroalgal species and the value of a multiple biomarker (FAs and SIs) approach in Antarctic coastal food web studies. Specifically, we used the various biomarker data sets to test how well they could differentiate some taxonomically close and morphologically similar macroalgal species, but that may contribute differently to coastal food webs.

Methods

Site selection

Macroalgae were collected in 2019 at 15 sites spanning the WAP from the Joubin Islands (−64.0°) in the north to Millerand Island (–68.0°) in the south (Fig. 1, Online Resources Table 1). Sites were accessed using small boats from the Antarctic Research and Support Vessel *Laurence M. Gould*. Collections were made opportunistically as part of a larger project assessing the macroalgal distribution and their role in the coastal food web along a gradient of sea ice cover (Amsler et al. 2023). Collections for the present study were aimed to capture the diversity of macroalgal species across all sites but were not a comprehensive species collection at all sites.

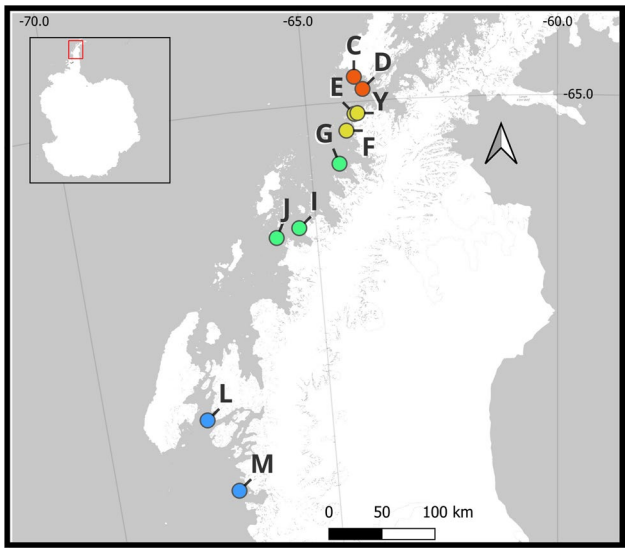


Fig. 1 Map of study locations along the Western Antarctic Peninsula. Warmer colors indicate higher latitude. Land masses are in white and ocean is in grey. Study locations are indicated by letters according to Online Resource Table 1

Field collections

Algal samples were collected between April 28 and May 18 of 2019 using SCUBA, between 5 and 40 m depth. Algal samples were returned to the lab within 1 h of collection where they were sorted and identified. Team phycologists verified the identities of the samples from morphological features, as well as later confirmation from pressed voucher specimens, described in Amsler et al. (2023). A maximum of seven replicates and a minimum of one algal tissue sample per species were taken (> 20 mg per sample when possible) for FA analysis and frozen at −80 °C in microcentrifuge tubes. In most cases, up to three replicates per species were collected for bulk SI analysis at the same, or a subset of the collection sites for FA analysis (Online Resources Table 1, 2). More specifically, these SI samples were mostly from the same thalli as those for FA analysis but on occasion, specimens were too small and different thalli were sampled for the two biomarker approaches at the same sites. Algal tissues for SI analysis were dried at 60 °C until constant weight (at least 24 h).

Table 1 Macroalgal species collected during the 2019 Western Antarctic Peninsula Project cruise for FA analysis

Phylum	Order	Family	Species	Sites collected	References
Chlorophyta	Bryopsidales	Bryopsidaceae	*Lambia antarctica*	B	Graeve et al. (2002)
Ochrophyta	Ascoseirales	Ascoseiraceae	*Ascoseira mirabilis*	Y	Teixeira et al. (2019)
	Desmarestiales	Desmarestiaceae	*Desmarestia anceps*	A,B,G	Aumack et al. (2017), Teixeira et al. (2019, Schram et al. (2019)
			Desmarestia antarctica	A, C, J	Graeve et al. 2002)
			Desmarestia menziesii	A, E, G	Aumack et al. (2017)
			Himantothallus grandifolius	C, D, E	Schram et al. (2019)
	Ectocarpales	Adenocystaceae	*Adenocystis utricularis*	F	Teixeira et al. (2019, Berneira et al. (2020)
	Fucales	Seirococcaceae	*Cystosphaera jacquinotii*	A, B	
	Syringodermatales	Syringodermataceae	*Microzonia australe*	B	
Rhodophyta	Balliales	Balliaceae	*Ballia callitricha*	A	
	Bangiales	Bangiaceae	*Porphyra plocamiestris*	F	
	Bonnemaisoniales	Bonnemaisoniaceae	*Delisea pulchra*	F	Schmid et al. (2018)
	Ceramiales	Callithamniaceae	*Georgiella confluens*	A	Graeve et al. (2002)
		Delesseriaceae	*Myriogramme smithii*	A, B	Graeve et al. (2002)
			Myriogramme manginii	B, C, E	Berneira et al. (2020)
			Pantoneura plocamioides	A	Graeve et al. (2002)
			Paraglossum salicifolium	A	
		Rhodomelaceae	*Picconiella plumosa*	C	
	Gigartinales	Cystocloniaceae	*Meridionella antarctica*	A	
		Gigartinaceae	*Sarcopeltis antarctica*	A,B	Graeve et al. (2002), Aumack et al. (2017), Berneira et al. (2020)[a]
			Iridaea cordata	B:E, I	Santos et al. (2017)
		Kallymeniaceae	*Austropugetia crassa*	B	
			Callophyllis atrosanguinea	I, M	
		Phyllophoraceae	*Gymnogongrus antarcticus*	A, B	
			Phyllophora antarctica	B, E, G	
	Gracilariales	Gracilariaceae	*Curdiea racovitzae*	A	Berneira et al. (2020)
	Halymeniales	Halymeniaceae	*Pachymenia orbicularis*	B	
	Palmariales	Palmariaceae	*Palmaria decipiens*	A	Graeve et al. (2002), Santos et al. (2017), Schram et al. (2019)
	Plocamiales	Plocamiaceae	*Plocamium* sp.	B, E, J	Santos et al. (2017), Aumack et al. (2017)[b]
		Sarcodiaceae	*Trematocarpus antarcticus*	A, C, F	
	Rhodymeniales	Fryeellaceae	*Hymenocladiopsis* sp.	B, D, G	Graeve et al. (2002)[c]

Species highlighted in gray do not have a published FA profile

[a] As *Gigartina skottsbergii*

[b] *Plocamium cartilagineum*

[c] *Hymenocladiopsis prolifera (crustigena)*

Fatty acid analysis

Collections of each macroalgal species were freeze-dried in a lyophilizer for 48 h (or until completely dry) within 8 months of collection. Homogenized tissue was then processed for FA extraction at the Oregon Institute of Marine Biology lab following Taipale et al. (2016).

Extraction. Approximately 10–20 mg of dried macroalgal tissue was homogenized from each sample with a mortar and pestle, suspended in 2 mL of chloroform in a 10 mL graduated centrifuge tube under N2, and held at -20 °C overnight. Tissue samples were sufficiently large for each replicate so that samples did not need to be pooled. After 24 h extraction, 70 µL C19 standard (GLC Reference Standard 566 C, Nu-Check-Prep, Elysian, MN), 1 mL methanol, and 0.75 mL 0.9% NaCl water solution were added to each tube containing the tissues. Between each step throughout the extraction and transesterification

Table 2 PERMANOVA table for results across all biomarker and taxonomic analyses

Biomarkers	df	SS	*F*	*p*(perm)
FAs only				
Division				
Division	2	4.235	54.847	0.001
Residual	103	3.977		
Total	105	8.212		
Order				
Order	15	5.939	15.673	0.001
Residual	90	2.274		
Total	105	8.212		
Family				
Family	21	6.834	19.832	0.001
Residual	84	1.378		
Total	105	8.212		
Species				
Species	30	7.419	23.383	0.001
Residual	75	0.793		
Total	105	8.212		
FA reduced				
Division				
Division	2	4.184	64.527	0.001
Residual	103	3.339		
Total	105	7.523		
Order				
Order	15	5.626	17.792	0.001
Residual	90	1.897		
Total	105	7.523		
Family				
Family	21	6.451	24.063	0.001
Residual	84	1.072		
Total	105	7.523		
Species				
Species	30	6.905	27.922	0.001
Residual	75	0.618		
Total	105	7.523		
SIs only				
Division				
Division	2	0.262	10.794	0.001
Residual	89	1.080		
Total	91	1.342		
Order				
Order	11	0.624	6.312	0.001
Residual	80	0.718		
Total	91	1.342		
Family				
Family	17	1.016	13.584	0.001
Residual	74	0.326		
Total	91	1.342		
Species				
Species	24	1.136	15.410	0.001
Residual	67	0.206		
Total	91	1.342		
Reduced FA + SI				
Division				
Division	2	0.297	13.251	0.001
Residual	73	0.818		
Total	75	1.115		
Order				
Order	11	0.594	6.661	0.001
Residual	64	0.520		
Total	75	1.115		
Family				
Family	17	0.888	13.334	0.001
Residual	58	0.227		
Total	75	1.115		
Species				
Species	24	1.001	18.599	0.001
Residual	51	0.114		
Total	75	1.115		
All FAs + SIs				
Division				
Division	2	0.301	13.293	0.001
Residual	73	0.827		
Total	75	1.128		
Order				
Order	11	0.603	6.673	0.001
Residual	64	0.526		
Total	75	1.128		
Family				
Family	17	0.897	13.236	0.001
Residual	58	0.231		
Total	75	1.128		
Species				
Species	24	1.012	18.443	0.001
Residual	51	0.117		
Total	75	1.128		

process (see below), sample tubes were topped off with N2 to avoid FA oxidation. Samples were then sonicated in an ice water bath for 10 min. Each tube was vortexed for 10 s and centrifuged for 5 min at 3000 rpm at 4 °C. The separated chloroform solution was transferred to an 8 mL scintillation vial, and the remaining material including algal tissues in the centrifuge tube was topped off with 1 mL chloroform and re-run through the vortexing, centrifuging, and chloroform removal process. All chloroform fractions from one sample were combined in the same scintillation vial. The chloroform solution was then evaporated under N2 to approximately 1.5 mL, and 1 mL of this remaining

extract was moved to a 10 mL graduated centrifuge tube for FA transesterification. This 1 mL chloroform subsample of each extract in the centrifuge tubes was evaporated to dryness under N2 and 1 mL toluene was added to each sample, and the remaining sample volume was dried and total lipid content gravimetrically determined (data not reported here).

Transesterification. To transesterify each sample, 2 mL of 1% H2SO4 in methanol was added to each toluene extract and vortexed for 10 s. Centrifuge tubes were held at 90 °C for 90 min in a water bath. After the incubation period, 1.5 mL 2% KHCO3 and 2 mL hexane were added to each tube and vortexed for 10 s. Tubes were then centrifuged for 2 min at 1500 rpm at 4 °C. The resulting fatty acid methyl esters (FAME) were transferred from the top phase into a new tube for evaporation under N2 in a ~ 30 °C water bath. An additional 2 mL hexane was added to the remaining lower phase in the centrifuge tube, vortexed, and centrifuged again as above. The FAME was then combined into each respective sample's tube in the water bath. Samples were evaporated to dryness, then 1.5 mL hexane was added to each, and the resulting FAME was transferred to a GC vial and held at −20 °C.

Mass spectrometry. The FA content of each sample was quantified in a gas chromatograph equipped with a mass spectrometer (GC–MS, Model QP2020, Shimadzu), with a DB-23 column 29.6 m long, 0.15 µm thick, and 0.25 mm diameter, using helium as a carrier gas and a single quadrupole mass analyzer. For each sample, 1 µL of FAME was run through the following heating protocol: 50 °C for 1 min, increased by 20 °C min^{-1} to 240 °C, and held for 10 min. Retention time and major ions were used to identify specific FAs in the sample. A calibration curve using four serial dilutions (15 ng mL^{-1}, 50 ng mL^{-1}, 100 ng mL^{-1}, 250 ng mL^{-1}) of known FAs (GLC Reference Standard 566 C, Nu-Check-Prep, Elysian, MN; Pearson correlation coefficient > 0.99) and the major ions for each identified sample peak were used to quantify FA concentrations. Total concentrations of FAs in each sample were extracted using GCMS Postrun Analysis software and converted to proportions (v4.41, Shimadzu Corporation, Kyoto, Japan). FAs quantified were those included in the GLC Reference Standard 566 C, Nu-Check-Prep, Elysian, MN (Online Resource Table 3) to ensure confident identification. The PUFA 18:4ω3, which is a diagnostic biomarker for brown algae, and several 16C PUFAs associated with green and red algae, while not part of the standard mix, were also included in our analyses as they could be identified with confidence in chromatogram outputs. FAs are presented here as percentages and according to the omega nomenclature including the number of carbons in the FA, the number of double-bonds, and the number of carbons from the methyl end to the first carbon in the double bond closest to the methyl end.

Table 3 SIMPER analysis of contribution of FAs to driving differences across all samples, through 80% of cumulative variation including the average contribution, standard deviation, and additive cumulative contribution

FA	Mean	SD	Cumulative sum
20:5ω3	0.080	0.060	0.220
20:4ω6	0.072	0.059	0.418
16:0	0.049	0.035	0.552
18:4ω3	0.026	0.029	0.622
18:3ω3	0.025	0.034	0.692
18:1ω9	0.023	0.027	0.755
18:1ω7	0.015	0.012	0.830

Stable isotope analysis

Dried macroalgal samples were homogenized to a powder and the carbon and nitrogen SI composition determined at the Alaska Stable Isotope Facility (ASIF) at the University of Alaska Fairbanks. Analyses were conducted on a continuous-flow isotope ratio mass spectrometer (CFIRMS) using a Thermo Scientific Flash 2000 elemental analyzer and Thermo Scientific Conflo IV interfaced with a Thermo Scientific DeltaV^{Plus} Mass Spectrometer. Approximately 0.8–1.2 mg macroalgal material were weighed into tin capsules for analysis. Results are expressed as conventional δ notation in parts per thousand (‰) according to the following equation: $\delta X\ (‰) = ([R\text{sample}/R\text{standard}] - 1) \cdot 1000$, where X is ^{13}C or ^{15}N of the sample and R is the corresponding ^{13}C:^{12}C or ^{15}N:^{14}N ratio. Pee Dee Belemnite and atmospheric N2 served as standards for carbon and nitrogen, respectively. Instrument error at ASIF was < 0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. The molar ratio of carbon to nitrogen (C:N ratio) was also calculated from the outputs of the SI analysis.

Statistical analysis

Descriptive patterns of mean FA compositional and SI data for each species were visualized in R (version 4.3.1) using the tidyverse and viridis packages (Wickham et al. 2019; Garnier et al. 2021; R Core Team 2022). Additionally, a contribution of variables to similarity (SIMPER) analysis was used to identify FAs that had the strongest effect (up to 80% cumulative) on sample differences, which were then used in reduced-biomarker principal components analysis (PCA) and permutational analysis of variance (PERMANOVA) analyses using the vegan package (Oksanen et al. 2018). The effect of site on observed FA profiles within species was also tested with PERMANOVA; site had no effect and was subsequently excluded. All PERMANOVA were run on individual samples grouped by the different phylogenetic groupings. Both sets of biomarkers were visualized using cluster

analysis and non-metric multidimensional scaling (nMDS). To determine how FA and SI biomarkers drive differences among individual samples and taxonomic groupings, a combination of PCA analysis, cluster analysis, and PERMANOVA were run using the vegan and factoextra packages (Oksanen et al. 2018; Kassambara and Mundt 2020) for: all samples with FA data; all samples with SI data; samples for which both types of data were available; select sample pairs of species that are morphologically and taxonomically similar. Differences in C:N ratios (log transformed to meet assumptions of normality) among taxonomic divisions were tested with one-way ANOVA and a post hoc Tukey test.

Results

We quantified 44 FAs across all samples, 13 of which constituted at least 3.4% (global mean) of the mean proportion of FA content across species (Fig. 2, Online Resource Table 2). These 13 'common' FAs included saturated and unsaturated FAs, including EFAs such as 18:3ω3 (Alpha-linolenic acid), 18:4ω3 (Stearidonic acid), 20:4ω6 (Arachidonic acid), 20:5ω3 (Eicosapentaenoic acid), and 22:5ω3 (Docosapentaenoic acid). Among the other most common FAs was the saturated FA (SAFA) 16:0 (Palmitic acid). Macroalgal samples contained between 17.1 and 43.5% SAFAs and 44.9–72.5% PUFAs. *Lambia antarctica*, the only green algal representative in our collection, was particularly rich in 18:3ω3 and 16:3ω3 compared to other taxa (Fig. 2). Within this reduced set of most common FAs, most brown algal species contained high proportions of 18:1ω7 (Vaccenic acid), 18:1ω9 (Oleic acid), and the EFA 18:2ω6 (Linoleic acid) compared to red algae, which had overall higher proportions of 20:5ω3, an especially nutritionally important FA for heterotrophs. In contrast, the PUFA 22:6ω3 (Docosahexaenoic acid), another important nutritional FA for consumers, was not overly abundant in most macroalgae, except the Rhodophyta *Ballia callitricha* (close to 3%). The PUFA ratio ($\sum\omega6/\sum\omega3$ ratio), an indicator of macroalgal nutritional quality, was below 5 in all species, which indicated high nutritional value. The ratio was specifically low in all Ochrophyta ($\sum\omega6/\sum\omega3 < 1$) and only slightly higher in the Rhodophyta *Paraglossum salicifolium* ($\sum\omega6/\sum\omega3 = 4.1$), *Trematocarpus antarcticus* ($\sum\omega6/\sum\omega3 = 3.4$), *Austropugetia crassa* ($\sum\omega6/\sum\omega3 = 2.6$), and *Callophyllis atrosanguinea*, *Gymnogongrus antarcticus* and *Phyllophora antarctica* ($\sum\omega6/\sum\omega3 = 1.4$, 1.4 and 1.8, respectively) (Online Resource Table 2).

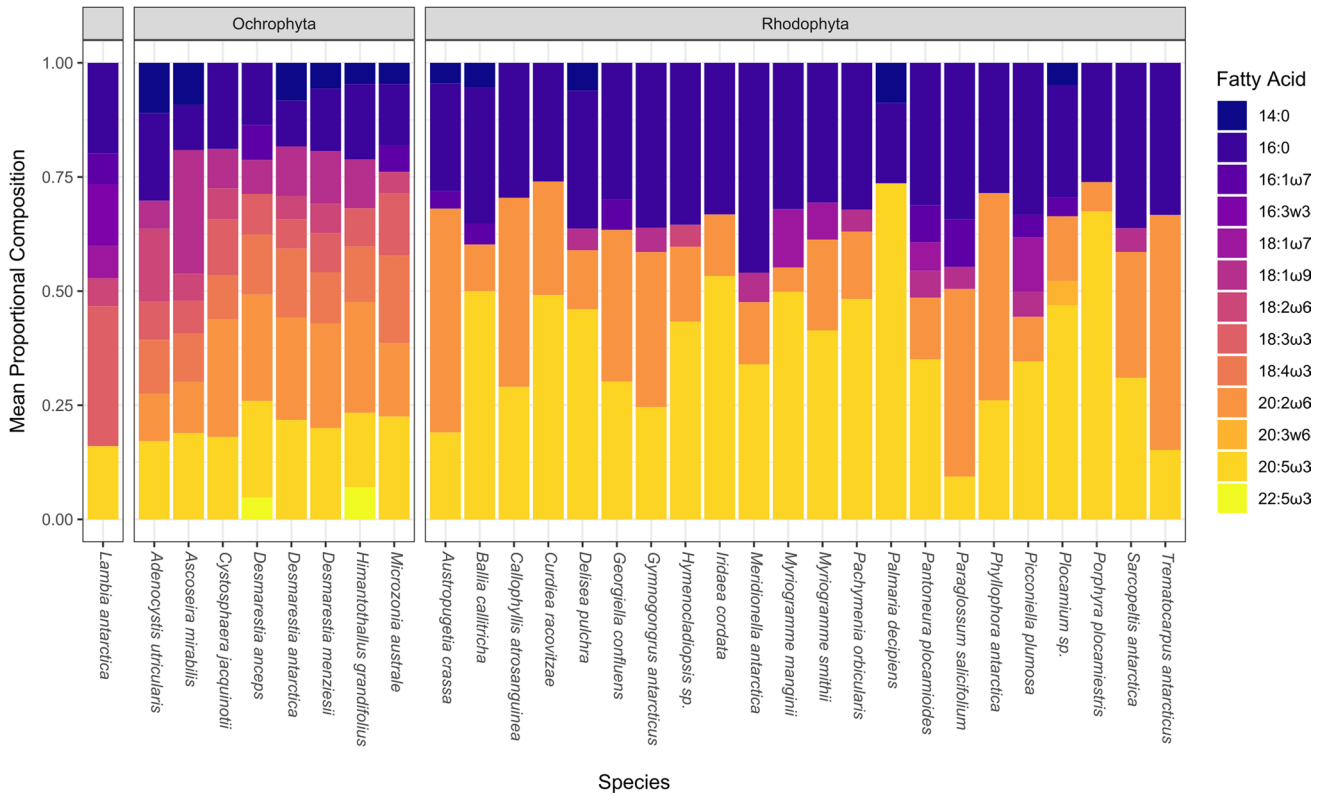


Fig. 2 Mean proportional composition of individual FAs that contributed at least 3.4% of total FA content to each macroalgal species. Species are organized in groups by division, with the first column being Chlorophyta, followed by Ochrophyta, and lastly Rhodophyta. FAs are listed in the legend according to the omega nomenclature

Cluster analysis identified four primary groupings of all algal species based on the full suite of 44 FAs quantified (Fig. 3). These groupings matched division-level differences, with the Chlorophyta and Ochrophyta separating out into individual groupings, while the Rhodophyta were further subdivided into two primary groupings. The phylogenetic resolution of these division-level cluster groupings was confirmed by nMDS and extended to the levels of order and even family (Fig. 4; see Table 1 for taxonomic information). Additionally, the full suite of FAs detected supported this division-level separation of the macroalgal species in PCA analysis, with PC1 and PC2 explaining 16.1% and 9.6% of the variation, respectively (Fig. 5). Ochrophyta grouped distinctly and tightly along PC1 and PC2, while Rhodophyta were separated along PC1 from the Ochrophyta but also had a larger spread. Species-level differences in full FA profiles were also detected (PERMANOVA, Table 2), though there was much overlap among species (Fig. 5). There was no effect of site on FA profiles within species (PERMANOVA: $df = 10$, $ss = 0.1270$, $F = 0.01546$, $p(\text{perm}) = 0.191$). A subset of FAs driving differences among samples was determined with SIMPER analysis and those that contributed > 80% to the cumulative SIMPER sum were used in additional PCA analyses to confirm that major division-level groupings were still apparent (Fig. 6, Tables 2, 3). This reduced PCA included primarily FAs that were also part of the suite of common FAs that comprised more than 3.4% of the total proportional contribution (see Fig. 2). This reduced PCA explained 44.4% (PC1) and 23.8% (PC2) of variation among samples. The pattern observed with the FA subset was similar to that seen when the full suite of FAs was considered; however, the phylogenetic groupings were even more distinct. Overall, division-level differences were supported more strongly than other taxonomic levels between both the full and reduced FA analyses, and species-level effects were more clearly pronounced than by order or family (Table 2).

Stable isotope values were variable across the Rhodophyta and Ochrophyta, with $\delta^{13}C$ ranging from −37.9‰ to −11.4‰ (mean −30.1‰ ± 6.2‰, $n = 67$, Rhodophyta) and −33.4‰ to −22.8‰ (mean −27.2‰ ± 2.9‰, $n = 22$, Ochrophyta) (Online Resource Table 2). SI values within species were not influenced by site (Iken et al. 2023). The $\delta^{13}C$ range among the Rhodophyta was particularly large and included two broad groupings, one with values highly depleted in ^{13}C and another grouping with $\delta^{13}C$ values more similar to those of many Ochrophyta (Fig. 7a, b). The green

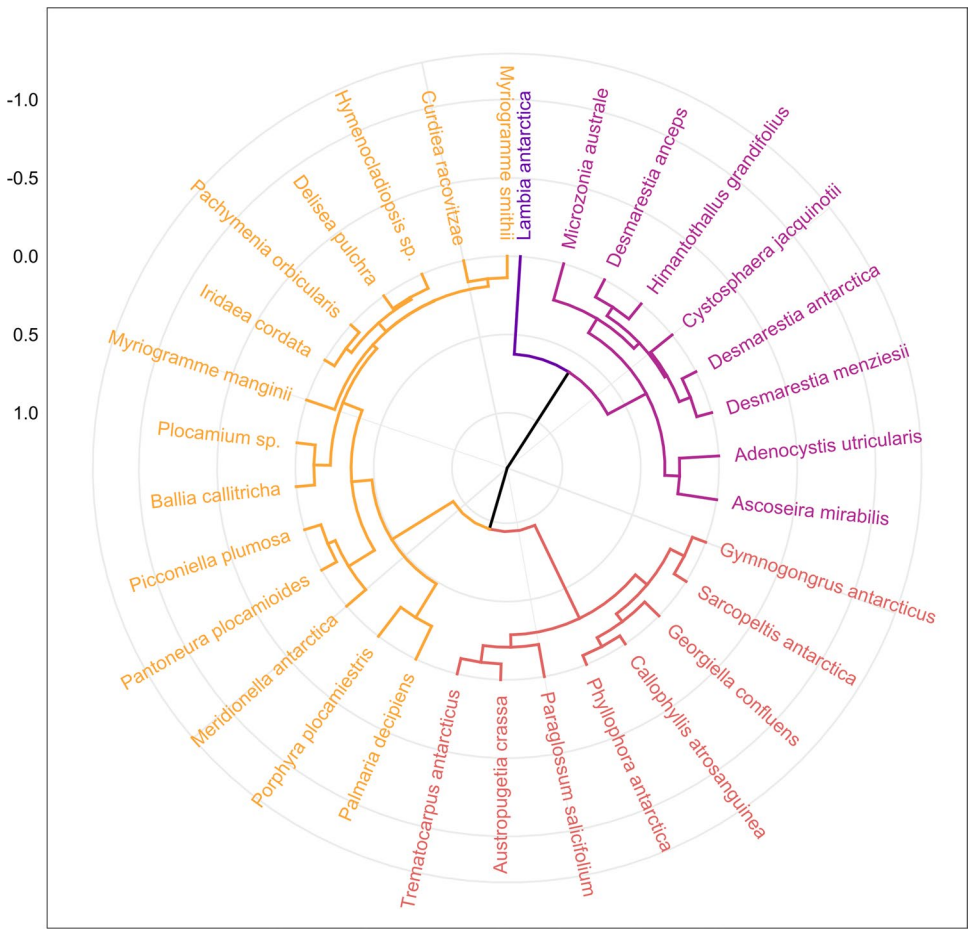


Fig. 3 Cluster diagram of macroalgal species' average fatty acid profiles based on 44 FAs analyzed in this study. Colors represent major groupings of algal species by FA profiles. Algal species group phylogenetically by division, with Ochrophyta as one group (pink), followed by a single species group for Chlorophyta (purple), and two Rhodophyta groups (yellow and orange)

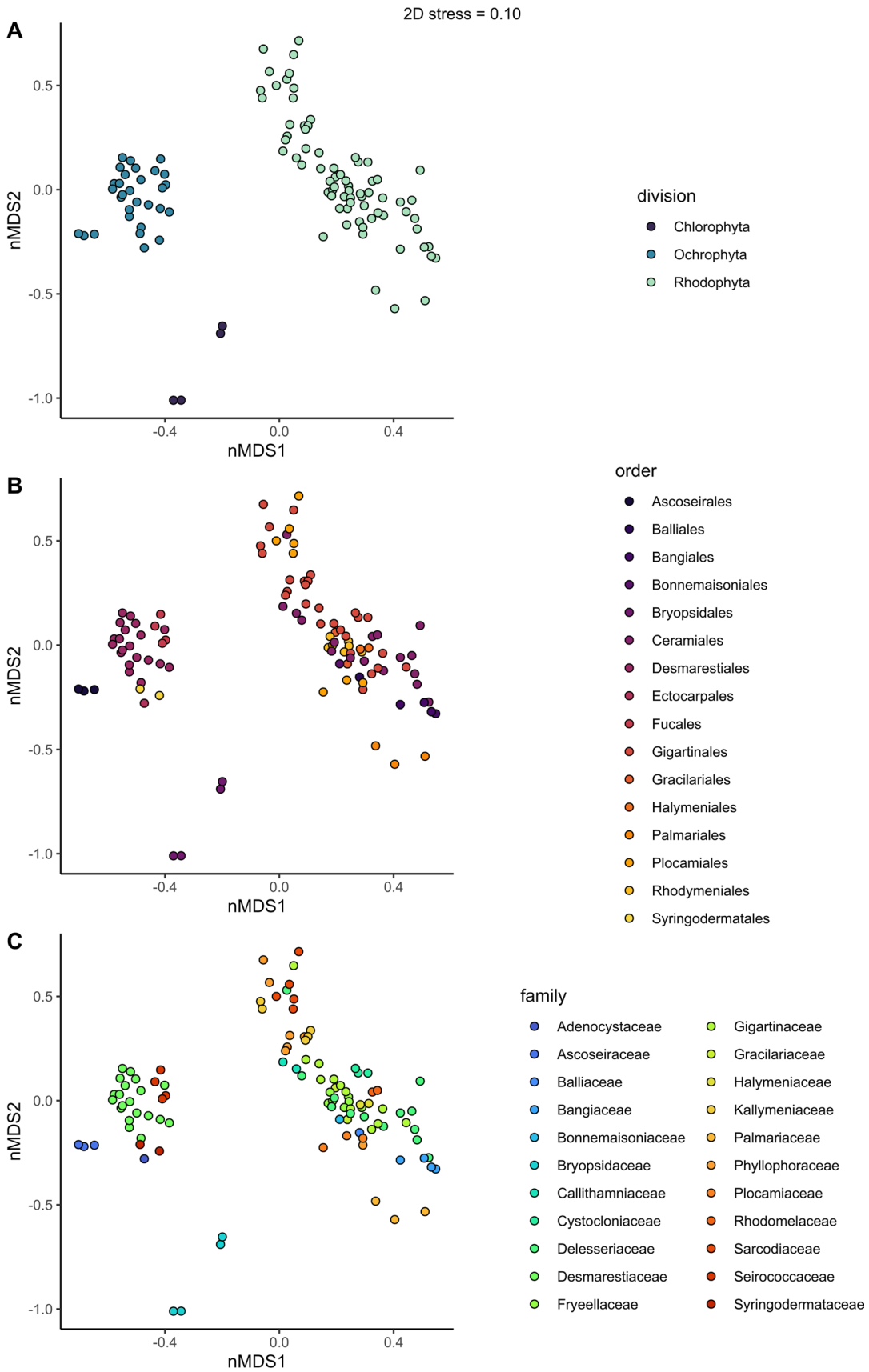


Fig. 4 nMDS of all macroalgal FA profiles coded by division (**a**), order (**b**), and family (**c**)

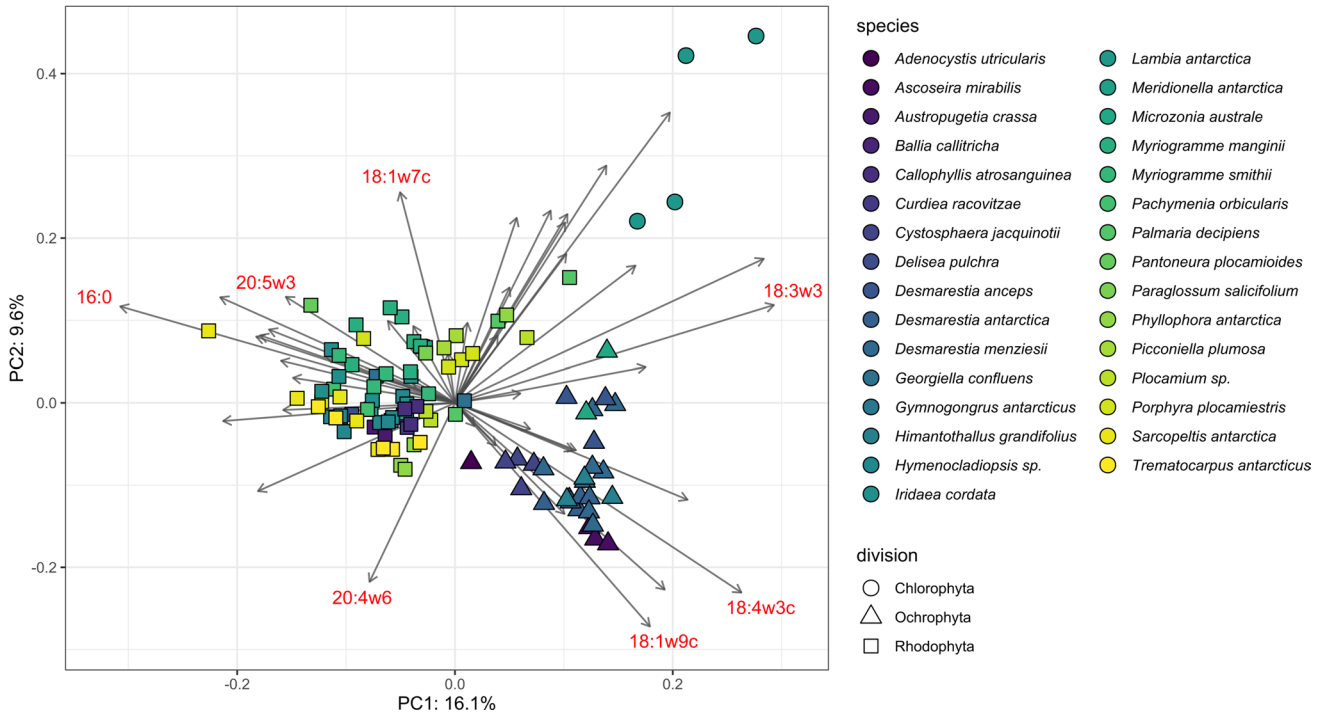


Fig. 5 Principal component analysis (PCA) of all macroalgal samples based on the full suite of 44 FAs. Samples are color coded by species and shapes denote phylum. All individual replicates of the algal species are represented. Vectors represent individual FAs, with the length and direction indicating the amount of variability and the direction associated with each FA according to the principal component axes. Only those FAs identified as primary contributors to axes in a Similarity Percentage analysis (SIMPER, see Fig. 6) are labeled here for clarity. Variance explained by each axis is given as percent along each axis

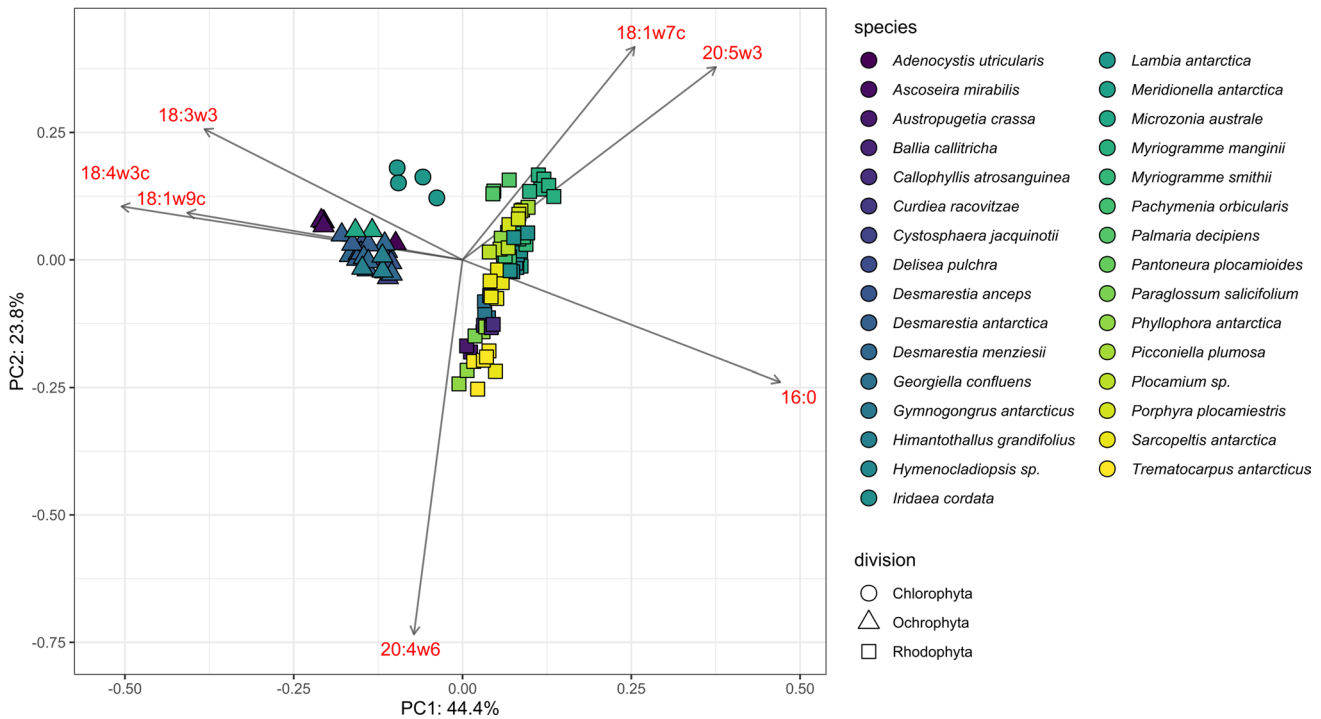


Fig. 6 Principal component analysis (PCA) of all macroalgal samples based on a reduced set of seven FAs as determined by a similarity percentage (SIMPER) analysis. See Fig. 5 for details on the PCA arrangement

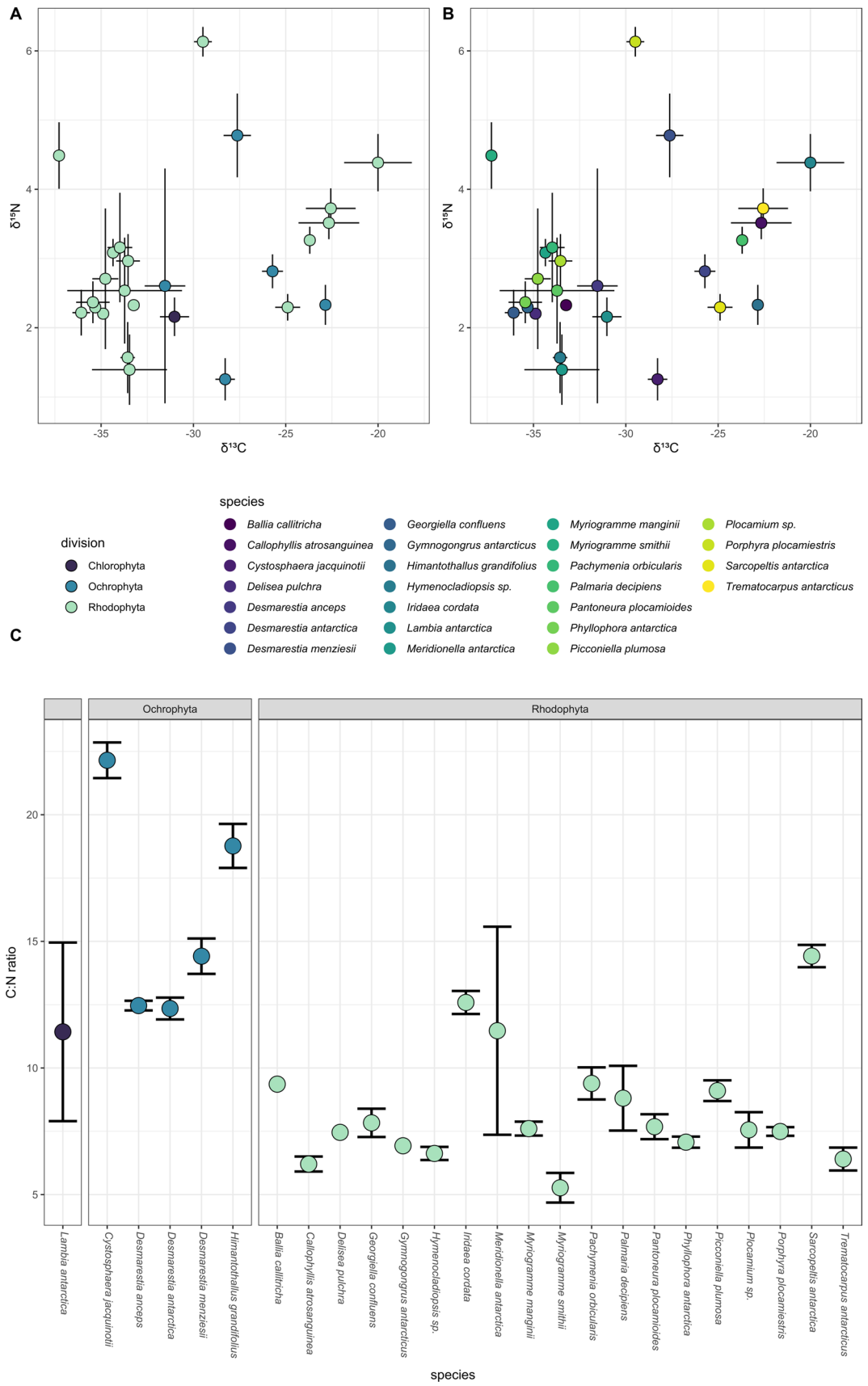
alga *L. antarctica* and one Ochrophyta (*Desmarestia anceps*) aligned closely with the ^{13}C-depleted Rhodophyta group. δ^{15}N ranged from 1.4 to 6.1‰ (mean 3.0‰ ± 1.3‰, $n = 67$, Rhodophyta), and 1.3‰ to 4.8‰ (mean 3.1‰ ± 1.9‰, $n = 22$, Ochrophyta; Fig. 7a, b, Online Resource Table 2). The single Chlorophyta had a mean δ^{13}C of -31.0‰ ± 1.4‰ and a mean δ^{15}N of 2.2‰ ± 0.5‰ ($n = 3$). Four macroalgal species were distinct with mean δ^{15}N values > 4‰; these were the Ochrophyta *Desmarestia menziesii*, and the Rhodophyta *Iridaea cordata*, *Myriogramme smithii*, and *Porphyra plocamiestris*. The C:N ratios across all macroalgal divisions were variable but overall lower in the Rhodophyta than the other divisions with means ranging from 5.3 to 14.4 (mean 8.8 ± 3.4, $n = 67$, Rhodophyta), 12.3 to 22.2 (mean 15.7 ± 4.0, $n = 22$, Ochrophyta), and 11.4 (Chlorophyta) (Fig. 7c). Differences in C:N ratio were significantly different between Rhodophyta and Ochrophyta, but not between Chlorophyta and the other divisions (ANOVA: df = 2, ms = 392.5, $F = 30.49$, $p < 0.0001$; Tukey-test: p-adj. < 0.0001). Cluster analysis of stable isotope values (δ^{13}C, δ^{15}N) and the C:N ratio resulted in four groupings of algae, although these did not resolve distinctly along the phylogenetic divisions (Online Resource Fig. 1, see Table 1 for taxonomic information). Although taxonomic groupings were not readily apparent in nMDS and clusters, PERMANOVA revealed significant division, order, and family groupings (Online Resource Fig. 2, Table 2). A PCA of macroalgal samples based only on carbon and nitrogen SI values and C:N ratios explained 48.7% (PC1) and 36.4% (PC2) of variation among samples (Fig. 8). This PCA also showed clear division-level groupings although the separation of Rhodophyta and Ochrophyta along PC1 was not as large as it was in the ordination of macroalgal species based on FA analysis (see Fig. 5). Analyses across all samples provided support for groupings at all taxonomic levels; however, species-level effects were strongest (Table 2).

A combined PCA based on either total or reduced FA with carbon and nitrogen SI and C:N ratio data from samples that were analyzed for both types of biomarkers (Fig. 9, Online Resource Fig. 3) produced very similar patterns as seen in the PCA based solely on total or reduced FAs (Figs. 5, 6), with clear delineation of the macroalgal divisions. The combination of both biomarkers with the full suite of FAs slightly increased the explanatory power for PC1 (16.6%) and PC2 (9.8%), and created a larger separation between Ochrophyta and Rhodophyta (Fig. 9) compared to the FA-only PCA (Fig. 5). These groupings were maintained, though not as tightly, when comparing the reduced FAs combined with SIs (Online Resource Fig. 3) with the reduced-FA only PCA (Fig. 6). It showed a stronger effect at the species-level than at the divisional level, which was in contrast to the reduced FA-only groupings that had stronger division than species groupings (Table 2).

The morphologically similar Ochrophyta species *D. menziesii* and *D. anceps,* and the Rhodophyta *P. antarctica* and *C. atrosanguinea* were tested for differences based on FA profiles and carbon and nitrogen SI composition. *D. menziesii* and *D. anceps* were significantly different from one another with regards to both their FA profiles (PERMANOVA: df = 1, ss = 0.037, $F = 3.166$, p(perm) = 0.034), and SI values (PERMANOVA: df = 1, ss = 0.015, $F = 5.730$, p(perm) = 0.015). However, *P. antarctica* and *C. atrosanguinea* were not significantly different from one another with regards to their FA profiles (PERMANOVA: df = 1, ss = 0.011, $F = 0.380$, p(perm) = 0.764), but were different in SI values (PERMANOVA: df = 1, ss = 0.068, $F = 38.609$, p(perm) = 0.018).

Discussion

In this study, we quantified the FA profiles and SI values of 31 Antarctic algal species to assess their biochemical and biogeochemical composition and the possible value of using these metrics as biomarkers to differentiate macroalgal species in food web applications. Our work extended the number of published Antarctic macroalgal FA profiles by 13 species not covered in previous studies (Graeve et al. 2002; Aumack et al. 2017; Santos et al. 2017; Schmid et al. 2018; Schram et al. 2019; Berneira et al. 2020). This not only expands the knowledge of biomarker characteristics to a much broader range of macroalgal species, including new orders, but it also presents a comprehensive view of both FA and SI data for the same species collected from the same sites and across a large spatial scale. The use of multiple biomarker tools in food web studies is becoming more prominent (e.g., Nielsen et al. 2018) and could be especially important for understanding the importance of macroalgal contributions to coastal food webs along the WAP where rapid warming results in decreases of sea ice cover and the potential expansion of macroalgal habitat (Amsler et al. 2023). As has been established for macroalgae elsewhere (Galloway et al. 2012), the FA profiles of WAP macroalgal species differed phylogenetically by the major algal divisions of Chlorophyta, Ochrophyta, and Rhodophyta, and the separation was robust also on the order and even the family levels. The grouping by division was preserved when only a small subset of FAs was applied, including several PUFAs, most of them EFAs. SI information was less specific to the taxonomic affiliation of the species, although divisional-level separation was still noticeable. We show that the combination of the two biomarker approaches could be especially valuable for differentiating morphologically similar or taxonomically close macroalgal species groups in food web studies.


A
B
δ15N
δ13C
division
Chlorophyta
Ochrophyta
Rhodophyta
species
Ballia callitricha
Callophyllis atrosanguinea
Cystosphaera jacquinotii
Delisea pulchra
Desmarestia anceps
Desmarestia antarctica
Desmarestia menziesii
Georgiella confluens
Gymnogongrus antarcticus
Himantothallus grandifolius
Hymenocladiopsis sp.
Iridaea cordata
Lambia antarctica
Meridionella antarctica
Myriogramme manginii
Myriogramme smithii
Pachymenia orbicularis
Palmaria decipiens
Pantoneura plocamioides
Phyllophora antarctica
Picconiella plumosa
Plocamium sp.
Porphyra plocamiestris
Sarcopeltis antarctica
Trematocarpus antarcticus
C
Ochrophyta
Rhodophyta
C:N ratio
species

◂**Fig. 7** Biplot of mean values for $\delta^{15}N$ and $\delta^{13}C$ with standard error bars coded by division (**a**) and species (**b**). Mean C:N ratio (**c**) is also shown with mean and standard error and each species (column) is coded by division as in panel **a**

Macroalgal species from the WAP varied in their proportional FA compositions, but all contained high proportions of intermediate- and long-chained PUFAs, often comprising more than 50% of the total FAs. The low $\sum\omega6/\sum\omega3$ ratio (≤ 5 in all species and mostly < 1) indicates that Antarctic macroalgae are rich in EFAs and particularly rich in ω3 PUFAs, which are considered most important for consumers (Pereira et al. 2012). While this nutritional indicator tends to be generally high (i.e., low ratio values) in macroalgae, the values reported here and by others (Santos et al. 2017) reflect Antarctic macroalgae to have especially high nutritional quality, with respect to lipids, compared to macroalgae elsewhere (Pereira et al. 2012). We do note that lipids are only one factor contributing to the nutritional value of these macroalgae to consumers; the fact that many WAP macroalgae are highly chemically defended (Amsler et al. 2005) also mediates the direct value of these resources to heterotrophs.

The Rhodophyta in our study were largely composed of the SAFA 16:0 (mean 27.8% ± 5.5%, n = 72) and the PUFA 20:5ω3 (mean 35.2% ± 14.0%, $n = 72$), which is generally comparable to means previously published for Antarctic red algae (30.9% and 28.8%, respectively; see Table 4; averaged from Graeve et al. 2002; Aumack et al. 2017; Santos et al. 2017; Schmid et al. 2018; Berneira et al. 2020). However, 20:4ω6 was more prominent in our samples (mean 20.0% ± 15.2%, $n = 72$) compared to previously published Antarctic Rhodophyta (9.0%, references as above, Table 4), although 20:4ω6 has been identified as a major FA in Rhodophyta elsewhere (e.g., Dalsgaard et al. 2003; Kumari et al. 2013; Sohrabipour 2019). The monounsaturated FA 16:1ω7 was reported as one of the top five contributing FAs for Antarctic Rhodophyta in the literature (mean 6.5%, Table 4) but was negligible when considering the same set of species in our study (Table 4). However, when only considering new Rhodophyta species not previously investigated for FAs, 16:1ω7 was detectable (mean 2.6% ± 2.7%, $n = 30$). Some differences to previous Antarctic macroalgal records could be based on the fact that FAs are reported in percentages instead of absolute concentrations, making these mean values dependent on the total number of FAs detected. In our study, we targeted 44 FAs, while other studies analyzed between 11 and 35 FAs (Graeve et al. 2002; Aumack et al. 2017; Santos et al. 2017; Schmid et al. 2018; Berneira et al. 2020), although the identity of the major FA contributors should still be similar, with just differences in the exact percentages. Sixteen PUFAs previously noted in two red algal species (*G. confluens* and *P. plocamioides*, Graeve et al. 2002) were not prominent in our analysis for these species.

The identity as well as the relative proportion of the main FA contributors in Ochrophyta in our study were extremely similar to those reported in the literature (Table 4). The Ochrophyta in our study as well as those reported in the literature (Graeve et al. 2002; Aumack et al. 2017; Berneira et al. 2020) contained high proportions of the same set of FAs: PUFAs 20:4ω6 (20.0% ± 5.2%, $n = 30$, in our study vs. 16.0% in the literature) and 20:5ω3 (18.2% ± 2.7%, $n = 30$, vs. 15.7%), the SAFA 16:0 (12.4% ± 3.2%, $n = 30$, vs. 15.2%) as well as the PUFAs 18:4ω3 (11.6% ± 3.5%, $n = 30$, vs. 12.0%) and 18:1ω9 (10.6% ± 6.1%, $n = 30$, vs. 10.5%). In part, this consistency may be based on the large overlap in species between the literature and our study, including six out of the eight total Ochrophyta we investigated (see Table 1). However, we added not only two new species (*Cystosphaera jacquinotii* and *Microzonia australe*) in our investigation but these species belong to different orders (Fucales and Syringodermatales, respectively) that have not been included in any previous analyses. Despite minor differences in the rank order of the main contributing FAs, depending on which species set is included (Table 4), the very high consistency we found in FA composition even after adding representatives of two new orders confirms the conservative FA makeup of Ochrophyta. The conservative nature of FA profiles does not only apply to the Antarctic Ochrophyta but is typical of the main FA composition of Ochrophyta in general from a variety of regions and climate regimes (e.g., Khotimchenko 1998; Khotimchenko et al. 2002; Dalsgaard et al. 2003; Pereira et al. 2012; Sohrabipour 2019 and references therein).

Chlorophyta is the least diverse macroalgal division in the Antarctic, with many species only occurring at lower latitudes along the WAP (Oliveira et al. 2020). Our study contained one green algal species, *L. antarctica*, which was only previously investigated for its FA composition by Graeve et al. (2002). FA composition for *L. antarctica* matched well with some of the main FA contributors in this past study, such as the SAFA 16:0 (19.8% in Graeve et al. 2002 and 20.3% ± 1.3%, $n = 4$, in this study). Our *L. antarctica* profiles also matched reasonably well for 18:3ω3 (23.7% in Graeve et al. 2002 and 25.9% ± 7.0%, $n = 4$, in this study), but it differed considerably for 18:2ω6, which contributed nearly a quarter (22.3%) of FAs in Graeve et al. (2002), but only 5.1% ± 0.08% ($n = 4$) in our analysis. Conversely, 20:5ω3 was the third-largest contributing FA in *L. antarctica* in our study while this FA was only represented with 5.0% in Graeve et al. (2002). This green alga was notably high in the proportions of 16 PUFAs, especially 16:3ω3, which has not been quantified in the previous study on this species (Graeve et al. 2002). *L. antarctica* is a siphonous, unicellular species, so it is possible that cell content is lost to various degrees when damaged during collection, and the thallus also disintegrates rapidly. This potential damage may

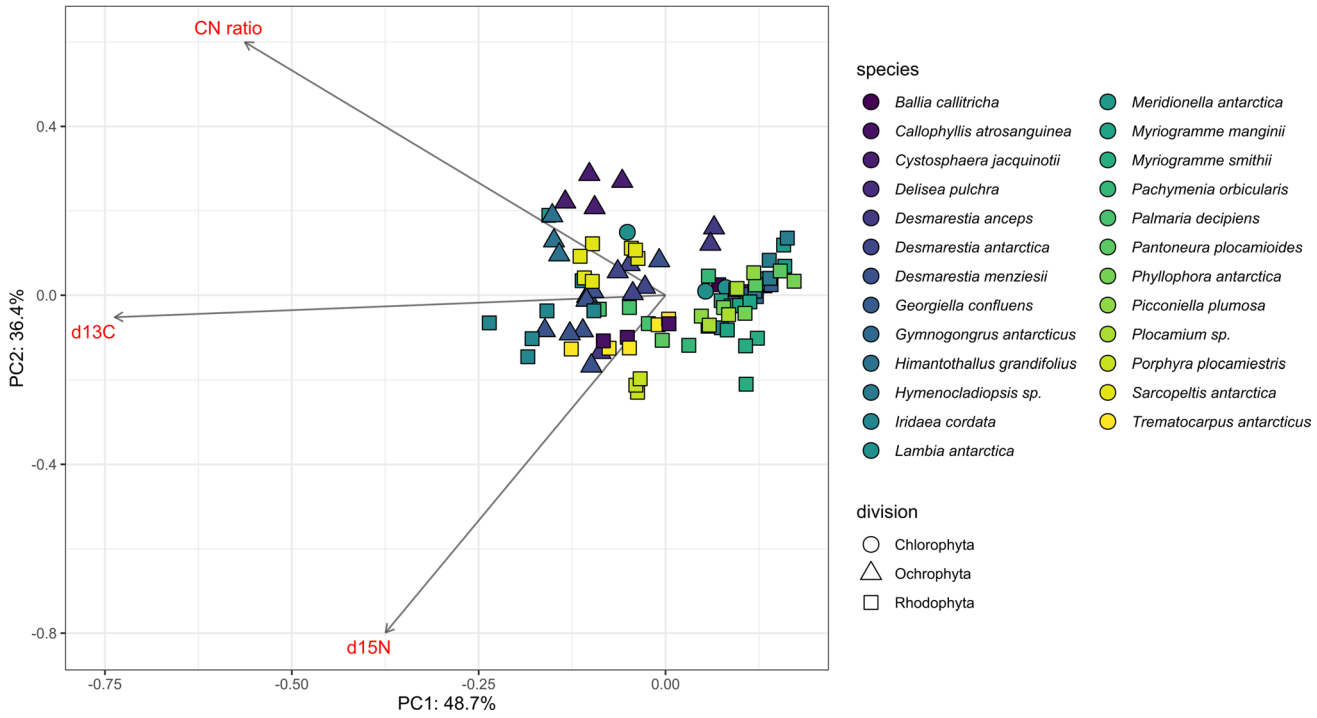


Fig. 8 Principal Component Analysis (PCA) of all macroalgal samples based on their carbon and nitrogen stable isotope values as well as their C:N ratio. See Fig. 5 for details on the PCA arrangement

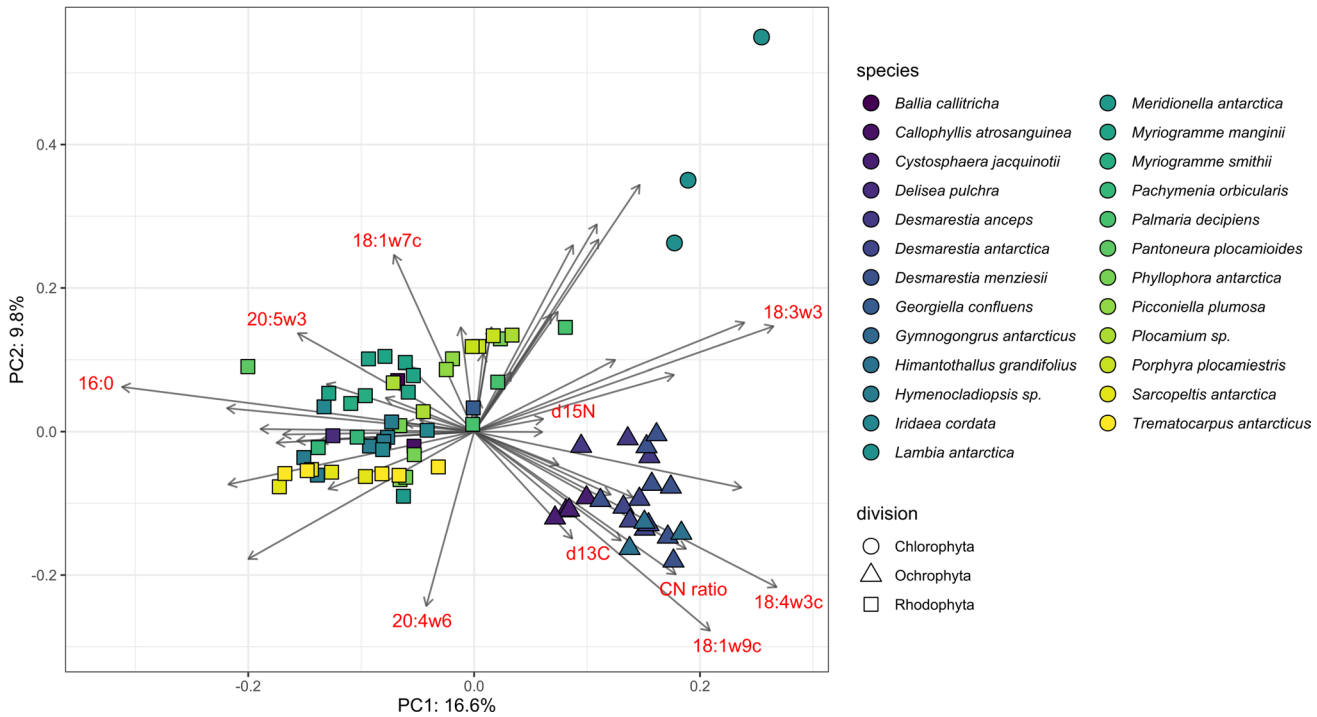


Fig. 9 Principal Component Analysis (PCA) of all macroalgal samples based on the full suite of 44 fatty acids as well as the carbon and nitrogen stable isotope values and the C:N ratio. See Fig. 5 for details on the PCA arrangement

Table 4 Most abundant pooled FAs found in samples ranked by taxonomic division and source of values (published = published literature values, literature species = species in our dataset with published counterparts, new species = species in our dataset with no published profiles, both = overall mean of all species within our dataset)

Rank	Ochrophyta				Rhodophyta			
	This study				This study			
	Published[a]	Literature species	New species	Both	Published[b]	Literature species	New species	Both
1	20:4ω6 (16.0%)	20:4ω6 (19.0%)	20:4ω6 (20.8%)	20:4ω6 (19.5%)	16:0 (30.9%)	20:5ω3 (40.0%)	20:5ω3 (28.5%)	20:5ω3 (35.2%)
2	20:5ω3 (15.7%)	20:5ω3 (18.9%)	20:5ω3 (16.6%)	20:5ω3 (18.2%)	20:5ω3 (28.8%)	16:0 (27.4%)	20:4ω6 (28.2%)	16:0 (27.8%)
3	16:0 (15.2%)	18:1ω9 (11.9%)	16:0 (15.2%)	16:0 (12.4%)	20:4ω6 (9.0%)	20:4ω6 (14.0%)	16:0 (28.2%)	20:4ω6 (19.9%)
4	18:4ω3 (12.0%)	18:4ω3 (11.8%)	18:4ω3 (11.2%)	18:4ω3 (11.6%)	18:1ω9 (6.6%)	18:1ω7 (3.4%)	18:1ω9 (2.7%)	18:1ω9 (2.7%)
5	18:1ω9 (10.5%)	16:0 (11.2%)	18:3ω3 (10.1%)	18:1ω9 (10.6%)	16:1ω7 (6.5%)	18:1ω9 (2.7%)	16:1ω7 (2.6%)	18:1ω7 (2.6%)

Only FAs included in our analyses were extracted from the literature

[a]Values from Graeve et al. (2002) , Aumack et al. (2017), and Berneira et al. (2020)

[b]Values from Graeve et al. (2002), Aumack et al. (2017), Santos et al. (2017), Schmid et al. (2018), and Berneira et al. (2020)

contribute to some of the differences in FA profiles we saw compared to the previously published record.

Marine macroalgae are a polyphyletic group, where the major divisions differ in many of their cellular and molecular properties (Chapman et al. 2012; Belghit et al. 2017). Taxonomic relationships help explain the consistency of differences in major FA profiles among the three main divisions, which has been reported numerous times for macroalgae worldwide (e.g., Dunstan et al. 2005; Hanson et al. 2010; Galloway et al. 2012; Pereira et al. 2012; Kumari et al. 2013). This strong divisional grouping has also been observed previously for Antarctic macroalgae (Graeve et al. 2002) and our addition of 13 newly profiled species expanded this finding. Some previous studies have documented similar separations at the order and even the family level for non-Antarctic species (e.g., Galloway et al. 2012; Kumari et al. 2013). Here, we documented, for the first time, that Antarctic macroalgal FA profiles are similarly conservative at the order and family levels.

A subset of seven FAs described patterns of phylogenetic differentiation equally well if not more distinctly than the full suite of 44 FAs. The FAs that were driving differences among macroalgal divisions were primarily EFAs, including the strict EFA 18:3ω3, as well as what can be characterized as physiologically EFAs (20:5ω3, 20:4ω6, 18:4ω3, 18:1ω9, and 20:3ω3) since they are required by heterotrophic consumers but can potentially be biosynthesized from the strict EFA precursors (Das 2006; Pereira et al. 2012). Only one SAFA (16:0) contributed strongly to these patterns. A similar observation was made for Northeast Pacific macroalgae, where robust phylogenetic separation was also observed when only applying the broader group of EFAs (Galloway et al. 2012). EFAs are typically used as key markers in food web analysis as they cannot be synthesized in most higher consumers (Budge et al. 2006; Brett et al. 2016), although some organisms are able to synthesize some EFAs from precursors, as mentioned above. EFAs also are routinely recorded in the published literature of macroalgal FAs, allowing the published profiles to be 'mined' for applications in trophic analyses. A caveat of this approach may be that, despite the overwhelming consistency reported in the literature for characteristic FA profiles of macroalgal divisions, there are reports that macroalgal FAs can vary seasonally in content and relative proportions, which may make taxon-specific profiles more difficult to resolve (Hernández-Carmona et al. 2009; Dethier et al. 2013; Barbosa et al. 2020). However, macroalgae living in narrow temperature windows may be limited in their ability to modulate their FA profiles (Barkina et al. 2019), suggesting that Antarctic macroalgal FA profiles, especially EFAs, are likely reasonably consistent over time. Future work could investigate seasonal changes in FA composition at least in some target species. Temperature exposure in short-term mesocosm studies on one species of Antarctic Rhodophyta (*P. decipiens*) indicates that FA composition (especially, SAFA/PUFA ratios) changes with higher temperatures in intertidal specimens, assumed to be an adaptation in membrane fluidity in response to stressful conditions (Becker et al. 2010). Longer-term studies spanning the entire season would be useful to better determine and understand FA composition changes across environmental gradients.

When solely considering SIs and C:N ratios, macroalgal species resolved into three major groupings that did not fully align with phylogenetic groups or match those obtained based on FA profiles. Instead, groupings seemed to be driven more by the physiological properties of the species. Specifically, $\delta^{13}C$ values are strongly influenced by the carbon uptake mechanisms of the macroalgal species, resulting in a separation of macroalgal species into two groups based on their $\delta^{13}C$ values. Despite generally employing C3

photosynthesis, some marine florideophyte Rhodophyta lack pyrenoids and carbon-concentrating mechanisms (CCMs), rendering them highly depleted in ^{13}C, typically resulting in $\delta^{13}C$ values of -30‰ or lower (Raven et al. 2002, 2005, 2012). Some Antarctic red algae, however, can have low $\delta^{13}C$ values despite being able to use bicarbonate and having CCMs (Beardall and Roberts 1999). The largest macroalgal grouping in our analysis were species with such low $\delta^{13}C$ values, suggesting that underlying physiological differences in photosynthetic processes drive this separation. While the occurrence of macroalgae with low $\delta^{13}C$ values is typically rare, it was a fairly widespread phenomenon in our dataset. This is confirmed by similar SI records in previous studies of several of the same Antarctic macroalgal species as examined in this study (e.g., Fischer and Wiencke 1992; Dunton 2001). Interestingly, one Ochrophyta (*D. anceps*) and the sole Chlorophyta in our study (*L. antarctica*) also grouped with this low $\delta^{13}C$ value group. However, we are not aware of CCMs in marine brown or green algae, suggesting there may be other physiological processes at play. Accordingly, the $\delta^{13}C$ values also were the strongest driver separating algal species with our PCA based on SI data and C:N ratios. It is important to note in this context that the carbon and nitrogen SI values of the most abundant macroalgal species did not change across the same sampling range (spanning > 3° of latitude) (Iken et al. 2023). This indicates that inorganic carbon substrates or the fractionation during photosynthesis, both of which are important drivers of carbon isotope values in macroalgae (Hayes 2001; Chikaraishi 2014; Velázquez-Ochoa et al. 2022), did not vary, at least during the fall timing of our sampling.

Some of the macroalgal species formed a loose grouping based on high $\delta^{15}N$ values. Of particular note is *P. plocamiestris*, which had high mean $\delta^{15}N$ values > 6‰. The $\delta^{15}N$ values of macroalgae can be driven by environmental conditions, such as inorganic nitrogen availability in the surrounding waters (Viana and Bode 2013; Lemesle et al. 2016) or selective release of nitrogen during photorespiration (Kim et al. 2013). The former is unlikely to be a driving factor in Antarctic waters as anthropogenic influences of eutrophication are not present on larger spatial scales, and point sources such as guano from penguin or other seabird colonies that could be implicated in the isotope values of marine organisms (Rossi et al. 2019) were not present in our sampling locations. The high $\delta^{15}N$ values of > 6‰ also are indicative of the higher fractionation in macroalgae with ample inorganic nitrogen concentrations (Swart et al. 2014), although this could be an effect of the time of sampling in the late fall when nitrate levels are replenished after summer drawdown with high (phytoplankton) production (Henley et al. 2017). High tissue nitrogen SI values have been associated with protein breakdown during photorespiration in shallow-water macroalgae, especially during emersion in intertidal algae (Kim et al. 2013). While we did not collect *P. plocamiestris* intertidally, it is a very shallow-water species that could experience periodic emergence, which could drive its very high $\delta^{15}N$ value.

We also included the C:N ratio of macroalgae in the ordination based on SI tracers as an added dimension, a not uncommon approach when applying dietary mixing models including primary producer sources (e.g., Brett et al. 2016; Wen et al. 2016; Schloemer et al. 2023), although it should be noted that the C:N ratio itself is not a dietary tracer. But briefly, C:N ratios were generally low, reflecting high tissue nitrogen concentrations driven by the nitrogen-replete waters of coastal Antarctica (Grotti et al. 2001). C:N ratios in our study were lower in Rhodophyta than Ochrophyta, confirming earlier findings by Weykam et al. (1996), although differences in thallus structure have also been implicated in driving C:N ratios in Antarctic macroalgae and likely contributed to the variability we observed in the C:N ratios in the Rhodophyta. The higher C:N ratios in the large Ochrophyta are indicative of high levels of structural cell components (cell walls, phlorotannins, e.g., Iken et al. 2007). Thus, C:N ratios added to the divisional separation of macroalgae when combined in multivariate ordinations with the stable carbon and nitrogen isotope markers.

The underlying processes driving macroalgal FA composition versus SI values among macroalgal species are quite different, with FAs primarily driven by metabolic pathways rooted in phylogenetic identity, while SI values are usually more influenced by environmental factors such as light, temperature and the ability to sequester inorganic carbon (Wiencke and Fischer 1990; Fischer and Wiencke 1992; Dalsgaard et al. 2003; Guest et al. 2010; Mackey et al. 2015). In this context, it is of note that we did not find any spatial trends either in FA profiles (see results, and Galloway et al. in revision) or SI values (Iken et al. 2023) of the macroalgal taxa across an environmental gradient spanning > 3° latitude and a range of sea ice cover along the WAP. At least for the specific time of sampling (fall), this indicates that the data provided here for both biomarkers are representative of the taxa in general. It remains to be shown if similar consistency exists across seasons, at other seasonal times, and/or across even larger latitudinal ranges. Based on our current results, we propose the combination of the two biomarkers to add to the ability to separate macroalgal groupings, ultimately for the purpose of tracing food web connections. This has been increasingly suggested or applied in several other systems across the world (e.g., Hanson et al. 2010; Kelly and Scheibling 2012; Dethier et al. 2013), where the combination of multiple biomarkers aided in finer trophic resolution of benthic primary producers. We found that the combination of FA profiles with SI markers and C:N ratios only slightly improved the separation of macroalgal species or groupings in multivariate space. This marginal

improvement may be explained by the much larger number of FA compared to isotope markers. It is possible that the more nuanced SI separation of macroalgae based on physiological processes is overwhelmed by the strong phylogenetic origins of FA profiles that also underlie some of the SI patterns. An important next step in this research would be to employ compound-specific SI analyses, especially of FAs, which may be able to produce a finer isotopic separation of macroalgal species (Twining et al. 2020). Trophic level baselines could also be refined in food web studies through the use of amino-acid specific isotope values (Whiteman et al. 2019). Compound-specific work would generally increase the value of the combined use of two biomarker approaches for Antarctic macroalgae in food web studies.

The small improvement in multivariate grouping does not mean that a combination of FA and SI biomarkers would not be useful in Antarctic coastal food web studies, and we propose the benefit of a combined approach for several reasons. First, SIs are commonly used to analyze trophic connections (Peterson and Fry 1987; Layman et al. 2007), and while there is still uncertainty about fractionation and trophic enrichment (Post 2002), our understanding of SIs in food webs is greater than that of FAs. There is still a need for controlled experimental work to better understand FA uptake and trophic transfer (Galloway and Budge 2020). An example of the usefulness of a combined approach has been shown for Antarctic amphipods feeding on macro- and microalgae, where general food sources identified by SI data were confirmed and refined by FA analysis (Aumack et al. 2017). Also, the different underlying processes driving FAs and SIs in macroalgae can be particularly useful when aiming to differentiate the contribution of phylogenetically close macroalgal species to a consumer. Based on FAs, those species would likely be difficult to trace but if they differed profoundly in their SI composition, the combination of the two biomarkers could prove very useful. For example, the two red algal species, *P. antarctica* and *C. atrosanguinea,* were almost identical in their FA profiles, and they also are morphologically difficult to distinguish (Wiencke and Clayton 2002). Isotopically, however, these two species were vastly different, with *P. antarctica* having very low $\delta^{13}C$ values of around -35‰ ± 2‰ ($n = 5$) compared to -23‰ ± 3‰ ($n = 3$) in *C. atrosanguinea* in our study, similar to values reported previously (e.g., Norkko et al. 2004; Marconi et al. 2011 for genus-level value for *Callophyllis*). Therefore, using multiple lines of evidence in tracing macroalgal species in the coastal food web could prove especially useful. As FA analyses can be labor and time intensive, using a targeted FA approach as suggested in our work could allow a broader application of Antarctic macroalgal FAs in food web studies in combination with SI data. Therefore, the comprehensive dataset presented here across a large number of macroalgae for both FAs and SIs for specimens collected at the same locations and at a specific time of year provides an important first baseline for the future application of these tracers in Antarctic food web studies.

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Author contributions This study was conceived by CDA, KI, JBM, AGK, and AWEG. Field collections were made by CDA, MOA, KI, SH, ATL, JBS, AWEG, and RW. Fatty acid biomarker extraction, lab work, and chromatogram analyses were done by RW and JBS, and stable isotope data were prepared by KI. Statistical analyses were done by RW. RW wrote the manuscript with editorial input from all authors.

Data availability Fatty acid and analysis scripts and data associated with this manuscript are available through Zenodo at https://doi.org/10.5281/zenodo.10524920. Stable isotope data associated with this manuscript are stored at the USAP-DC under https://doi.org/10.15784/601653, linked to project https://www.usap-dc.org/view/project/p0010104.

Declarations

Competing interest The authors declare no competing interests.

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