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**Global drivers of variation in seaweed
macronutrient composition and nutritional
value of selected seaweed species in
northeastern New Zealand**

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requirements for the degree of

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General Abstract

Seaweeds are taxonomically diverse and have many valuable commercial applications. Globally, the seaweed aquaculture sector is growing exponentially with promising ecological and environmental implications. While the nutritional characteristics of many commercial species are well-recognised, a vast proportion of seaweed taxa remains unexplored. Using a multivariate meta-analytic approach, I examine the reported nutritional composition of 182 seaweed species across 81 published studies using conventional proximate composition analyses to determine the relative amounts of ash, soluble carbohydrate, insoluble fibre, lipid, and protein. I found that although red seaweeds had higher nutritional value compared to brown and green seaweeds, minor differences separated the phyla. Furthermore, I found that procedural effects contributed strongly to variation in the reported values of specific macronutrients. Specifically, I discovered large among-study variation for reported insoluble fibre and soluble carbohydrate content and notable measurement error variation for reported lipid content. Additionally, I found nutritional trade-offs among seaweed species, where some species were higher (lower) in soluble carbohydrate and ash and lower (higher) in insoluble fibre and lipids. My meta-analysis revealed the scope of the impacts of among-study differences and insight into the expected relationships between macronutrients at the species level once procedural and phylum-level effects were accounted for. Unless standardized protocols are adopted, generalisations about the relative nutritional value of different seaweeds or seaweed taxa will be limited. Within northeastern New Zealand, I examined the nutritional composition of eleven coastal seaweeds using proximate composition analyses. On average, *Asparagopsis armata* and *Pterocladia capillacea* were highest in protein, *Corallina officinalis* was highest in ash, and *Xiphophora chondrophylla* was highest in insoluble fibre. Additionally, *Cystophora retroflexa* was highest in lipids, *Carpophyllum maschalocarpum* was highest in soluble carbohydrates, and lastly, *Codium fragile* and *Ulva lactuca* were highest in moisture. Based on their higher protein contents, *A. armata*, and *P. capillacea* may be potentially important for New Zealand's growing commercial seaweed industry. Overall, my thesis examining both large-scale seaweed nutritional composition and underlying variation as well as nutritional properties of northeastern New Zealand seaweeds provides insight into future directions for nutritional research on seaweeds and the implications for the development of seaweed aquaculture in New Zealand.

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Table of Contents

General Abstract	1
Acknowledgements	2
Table of Contents	3
Chapter 1: General Introduction	4
1.1 Rationale for study	4
1.2 Overview of thesis	9
Chapter 2: Investigating drivers of variation in seaweed nutritional composition: A meta-analysis	12
2.1 Abstract	12
2.2 Introduction	13
2.3 Methods	19
2.3.1 Data collection	19
2.3.2 Statistical analysis	20
2.4 Results	22
2.5 Discussion	30
Chapter 3: Nutritional composition of common, coastal seaweeds from Mathesons Bay, Leigh, northeastern New Zealand	37
3.1 Abstract	37
3.2 Introduction	38
3.3 Methods	44
3.3.1 Sample collection	44
3.3.2 Moisture analysis	45
3.3.3 Ash analysis	45
3.3.4 Lipid analysis	46
3.3.5 Insoluble fibre analysis	47
3.3.6 Protein analysis	48
3.3.7 Soluble carbohydrate analysis	49
3.3.8 Statistical analysis	50
3.4 Results	51
3.5 Discussion	54
Chapter 4: General Discussion	62
References (Journal of Applied Phycology style)	68
Appendix 1: List of international studies included in the meta-analysis	118

Chapter 1: General Introduction

1.1 Rationale for study

Seaweeds play key roles in tropical, temperate, and polar coastlines and dominate benthic substrata at higher latitudes (Wiencke et al. 2007; Huovinen and Gómez 2012; Jueterbock et al. 2013; Tano et al. 2016). Seaweeds are multicellular, photoautotrophic organisms (Wernberg et al. 2013; Charrier et al. 2017) with over 18,000 described species within three major phyla: Rhodophyta, Ochrophyta, and Chlorophyta, which are often referred to as the red, brown, and green seaweeds, respectively (Gomez-Zavaglia et al. 2019; Guiry and Guiry 2021). Globally recognised as ‘foundation species’ or ‘ecosystem engineers’, seaweeds are primary producers that provide three-dimensional biogenic habitats which support diverse species assemblages (Dayton 1972, 1985; Jones et al. 1994; Steneck et al. 2002; Schiel and Foster 2006; Smale et al. 2013; Yesson et al. 2015; Charrier et al. 2017; Wernberg et al. 2019). Given their vast morphological variation, ranging from dense turfs or sheets to towering forests, seaweeds create safe havens for marine organisms vulnerable to predators and help extend and diversify niches for different species (Carr 1991; Bertness et al. 1999; Stachowicz 2001; LoDuca et al. 2017). Additionally, seaweeds have a large capacity to assimilate carbon dioxide through photosynthesis and a high uptake rate for inorganic nutrients such as nitrogen and phosphorus (Ritschard 1992; Gao and McKinley 1994; Harrison and Hurd 2001), therefore, they are able to buffer against ocean acidification by effectively reducing carbon dioxide levels (Mongin et al. 2016). Furthermore, seaweeds are integral components of marine ecosystems and can mitigate anthropogenic effects, carbon dioxide emissions, and other impacts of climate change (Hill et al. 2015; Sondak et al. 2017).

A wide range of marine herbivores are heavily reliant on seaweeds (Duffy and Hay 1990; Taylor and Brown 2006; Steneck et al. 2017). Seaweeds provide food, shelter, and refuge for various herbivorous grazers such as crustaceans, gastropods, echinoderms, and fishes (Scheibling 1986; Hay 1991, 1997; Paul et al. 2006; Duarte et al. 2011; Steneck et al. 2017). To tolerate intensive herbivorous grazing, many seaweeds have developed a range of chemical defence strategies to deter particular herbivores (Cronin and Hay 1996a, b; Cetrulo and Hay 2000; Pereira et al. 2000; Bianco et al. 2010), in turn, some herbivores respond by

evolving increased resistance to seaweed defenses (Duffy and Hay 1994; Sotka and Hay 2002). For example, *Ampithoe longimana* selectively grazes and lives on chemically-defended brown seaweeds such as *Dictyopteris* or *Dictyota* spp. that are avoided by herbivores such as large *Diplodus* fishes or sea urchins (Hay et al. 1987; Duffy and Hay 1994). This lowers the susceptibility of *A. longimana* to predation and increases their tolerance for particular chemical metabolites produced by these seaweeds (Hay et al. 1987; Hay et al. 1990; Duffy and Hay 1994; Schnitzler et al. 2001). Moreover, differences in the structural morphology of seaweeds can also influence feeding preferences in marine herbivores, as tougher calcareous seaweeds that form hard crusts are often avoided by herbivores, while fleshy or soft filamentous seaweeds are heavily grazed (Steneck and Watling 1982; Duffy and Hay 1990). Additional to marine herbivores, beach-cast seaweeds and other debris collectively termed 'wrack' support large communities of primary consumers (i.e., amphipods, isopods, and insects), which then become prey for higher trophic predators such as birds or terrestrial mammals (Inglis 1989; Hubbard and Dugan 2003; Dugan et al. 2003; Ince et al. 2007; Fox et al. 2015). Accordingly, beach-cast seaweeds link both marine and terrestrial environments and are of great ecological importance in coastal ecosystems (Mellbrand et al. 2011).

The earliest evidence for human utilisation of seaweeds was approximately 14,000 years ago, where nine seaweed species were recovered from Monte Verde, an archaeological site in southern Chile (Dillehay et al. 2008). The preservation and distribution of the seaweeds suggested their possible usage as both food and medicine. Since 300 BC, particularly in Asian nations such as China and Japan, humans have harvested and consumed seaweeds. Genera such as *Laminaria*, *Undaria*, *Pyropia*, and *Gelidium* were included along with other marine products as tax payment to the Japanese court under the Law of Taihō (AD 701). Additionally, during the Age of Civil Wars or Sengoku period (1467-1507), some seaweeds were also used as army provisions along with other marine life (Nisizawa et al. 1987; Dhargalkar and Pereira 2005). Brown seaweed genera such as *Laminaria*, *Undaria*, *Ecklonia*, and *Sargassum* were stored with other food products during the Edo era (1603-1912) where there were long periods of famine, and various seaweed-based products including confectionaries were locally manufactured across different regions during this period (Nisizawa et al. 1987). During the Tokugawa Era (1600-1800 AD), artificial substrates were constructed by fishermen for fish aquaculture which also allowed for the growth of different seaweed species, which initially facilitated the beginning of seaweed cultivation in

Japan (Buchholz et al. 2012). Since, seaweed cultivation, commercialisation, and research in Japan have drastically increased and a wide variety of seaweed goods have been developed for numerous purposes (Bixler and Porse 2011; Buchholz et al. 2012; Kılınç et al. 2013; Nayar and Bott 2014). At present, popular global commercial seaweed species include *Eucheuma* spp. and *Kappaphycus alvarezii* for carrageenan production, *Gracilaria* spp. for agar production, and *Pyropia* spp., *Saccharina japonica*, *Undaria pinnatifida*, and *Sargassum fusiforme* for food production (Buschmann and Camus 2019). Direct food applications make up the major world market for seaweeds and products are consumed in a variety of ways such as dried, in soup, salads, beverages, condiments, bread, pasta, sushi, and many other products (Holdt and Kraan 2011; Gomez-Zavaglia et al. 2019). Despite widespread cultivation of numerous seaweed species, the global seaweed aquaculture industry is expanding, with rising demand for the addition of new species (Hafting et al. 2015).

Alongside Asian nations, seaweed aquaculture is progressively gaining interest in Western countries (Stévant et al. 2017), with European seaweeds such as *Palmaria palmata*, *Saccharina latissima*, and *Alaria esculenta* being used in a wide variety of food products and seasonings (Mouritsen et al. 2012; Chapman et al. 2015). In Western European countries such as France, Spain, Ireland, and the United Kingdom, both wild and cultivated seaweeds are being used in different food products and sold in fresh or dried form (Stévant et al. 2017). As seaweed aquaculture has evolved, valuable applications for many seaweed species have been discovered, thus, many businesses and researchers anticipate an encouraging future for the growth and success of the global seaweed industry (Buschmann and Camus 2019). Additionally, seaweeds have applications within the nutraceutical, cosmetic, biotechnological, pharmaceutical, and agriculture industries as well as others (Makkar et al. 2016; Buschmann and Camus 2019; Gomez-Zavaglia et al. 2019; Laurens et al. 2020). As previously mentioned, seaweeds contain secondary metabolites that are often used for chemical defense, and thus, research has revealed that metabolites such as phlorotannins show great pharmaceutical potential and have benefits for human health (Rosa et al. 2020). Moreover, specific compounds found in seaweeds such as phycocolloids (agar, carrageenan, and alginate) are highly valuable and are extracted for commercial uses such as thickeners, gelling agents, and stabilizers (Tseng 2001; Rebours et al. 2014). Other seaweed polysaccharides such as fucoïdan, laminarin, and ulvan are known to possess prebiotic, anti-inflammatory, anti-bacterial, and anti-influenza properties (Holdt and Kraan 2011). Moreover, seaweeds hold nutritionally relevant macronutrients such as protein, lipids,

insoluble fibre, and soluble carbohydrates that are becoming increasingly important in both nutrition and aquaculture research (Mabeau and Fleurence 1993; Kumar et al. 2008; Holdt and Kraan 2011).

Given their richness in specific macronutrients, seaweeds are often used as ingredients in formulated aquaculture feeds for various commercially cultured shellfish and finfish species (Ergün et al. 2009; Soler-Vila et al. 2009; Fleurence et al. 2012; Bansemmer et al. 2016). Among all major macronutrients, protein is one of the most important and costly determinants of nutritional value in formulated aquaculture feeds (Tung and Alfaro 2011; Viera et al. 2011), hence, seaweed species such as *Ulva* spp., *Gracilaria* spp., and *Hypnea* sp. are often cultivated in nitrogen-enriched water to increase their protein content (Shpigel et al. 1999; Hernández et al. 2002; Viera et al. 2005; Viera et al. 2011). The co-culture of seaweeds in integrated multitrophic aquaculture (IMTA) systems alongside commercial finfish species can also enable bioremediation, as seaweeds can absorb dissolved organic nitrogen and other excess nutrients released by species such as rainbow trout (*Oncorhynchus mykiss*; Troell et al. 1997), coho salmon (*Oncorhynchus kisutch*; Troell et al. 1997), or Atlantic halibut (*Hippoglossus hippoglossus*; Kim et al. 2013). The role of seaweeds as effective biological filters counteracting environmental effects through intensive nutrient removal increases their value in IMTA systems and efficiency in resource utilization (Neori et al. 2004; Devi and Gowri 2007; Ren et al. 2012; An and Anh 2020).

Research regarding nutritional properties of a large variety of seaweed species is widespread and steadily growing, as they have great potential as food supplements and for the extraction of bioactive substances that have numerous benefits (Kumar et al. 2008; Holdt and Kraan 2011). Worldwide, numerous methods are used to measure the relative proportions of specific macronutrients in plant and animal food materials, namely: moisture, ash, soluble carbohydrate, insoluble fibre, lipid, and protein, which is typically referred to as their 'proximate composition' (Gökođlu and Yerlikaya 2003; Iqbal et al. 2006; Odebunmi et al. 2010; Ubwa et al. 2014; Talreja et al. 2015; Varisco et al. 2020). Moreover, studies investigating the proximate composition of different seaweed species, as well as their amino acid profiles, fatty acid composition, antioxidant activity, and heavy metal content have been published across various countries (Wong and Cheung 2000; McDermid et al. 2007; Marsham et al. 2007; Smith et al. 2010; Tabarsa et al. 2012; Cian et al. 2014; Mwalugha et al. 2015; Ahmad et al. 2016; Jayasinghe et al. 2018). Furthermore, despite the large domain of

research available for nutritional content among seaweeds, global seaweed diversity is vast, hence, knowledge of nutritional characteristics in lesser-known, non-commercial seaweed species is limited, providing opportunities to fill the remaining knowledge gaps in this research field.

Seaweed diversity in New Zealand is extensive and unique, with over 1000 species reported across the ~15,000km coastline (Nelson et al. 2019; O’Callaghan et al. 2019). Before European settlement, many seaweed species such as *Pyropia columbina* (karengo), *Gigartina* spp. (rehia), *Durvillaea antarctica* (rimurapa), and *Ulva* spp. were used as important sources of food and medicine by Māori (Cambie and Ferguson 2003; Hurd et al. 2004; Smith et al. 2010; Fraser et al. 2012; White and White 2020). After the arrival of European immigrants, some *Gigartina* spp. were often used as a thickener in puddings, and many *Pyropia* spp. were readily consumed and sent to New Zealand army troops in the Middle East during the Second World War (Smith et al. 2010). In comparison to other Pacific countries, the New Zealand seaweed industry is currently in its infancy, with only a small range of commercial businesses selling seaweed products for a limited number of species including *Ulva* spp., *Pyropia* spp., *Ecklonia radiata*, *Macrocystis pyrifera*, and the introduced Asian kelp *U. pinnatifida* (White and White 2020). Since the 1940s, the main commercial seaweed-based production in New Zealand has been for agar produced from beach-cast red seaweeds such as *Pterocladia lucida*, *Pterocladia capillacea*, and *Gigartina* spp. collected seasonally from the eastern North Island (White and White 2020). Seaweed cultivation trials in New Zealand started during the late 1980s, and despite small-scale commercial interest to date, recent research projects investigating anti-methanogenic potential of red seaweeds *Asparagopsis armata* and *Bonnemaisonia hamifera*, as well as cultivation of the kelps *E. radiata*, *M. pyrifera*, and *U. pinnatifida* offer promise for a gateway to larger-scale commercial seaweed production in New Zealand (Garbary et al. 2020; Mihaila 2020; Stenton-Dozey et al. 2020; White and White 2020; Nørskov et al. 2021).

While the New Zealand seaweed industry does not currently contribute a substantial seaweed stock to the global market, it has great potential to offer a variety of seaweed taxa of distinctive quality, with valuable applications for nutritional, cosmetic, agricultural, and other commercial industries (White and White 2020). To our knowledge, published studies regarding nutritional composition of New Zealand seaweeds are scarce and are a potential limitation to the development of upscale products for an export market (Stenton-Dozey et al.

2020; White and White 2020). Additionally, a comprehensive knowledge regarding important characteristics of both New Zealand native and introduced seaweeds is crucial for the selection of species for cultivation in future. Identifying seaweed candidates for both potential monoculture and IMTA will assist the growth and progression of the booming aquaculture sector in New Zealand.

1.2 Overview of thesis

This thesis is comprised of four chapters. Chapters two and three are independent publishable units written in manuscript format and Chapter four is a general discussion. I expect to submit chapters two and three for peer-review and publication shortly after receiving feedback from my thesis examiners. These chapters will be submitted as co-authored manuscripts, and accordingly, I use first-person plural (i.e., “we”, “our”) to acknowledge this; regardless, the thesis is my own work and was completed under the guidance of my supervisor. Given that all four chapters presented in this thesis are interrelated, there will be repetition of specific content and methodologies across the thesis. All species names used in this thesis have been standardized to the current species names as indicated in AlgaeBase (Guiry and Guiry 2021).

In Chapter two, we examine the drivers of variability in seaweed proximate composition using a meta-analysis to explore the factors causing variation in nutritional composition among different seaweeds across a global scale. Global interest surrounding the utilization of seaweeds for food consumption and other industrial applications is growing; hence, knowledge regarding the nutritional properties of popular commercial seaweed species is becoming increasingly valuable (Stengel et al. 2011; Fiset et al. 2017). However, while there is wide recognition of factors causing differences in nutritional composition among seaweeds (e.g., environmental conditions), there is a current shortfall of quantitative research synthesizing this information. Accordingly, in this chapter we surveyed the literature for studies assessing nutritional composition in seaweeds, and then analyse the data using a multivariate meta-analytic approach. We expected to find differences in nutritional composition among seaweeds from different phyla and individual species (McDermid and Stuercke 2003; Kılınç et al. 2013; Britton et al. 2021). Regarding individual macronutrients

specifically, we expected that protein and ash content would be greatest in red seaweeds, insoluble fibre and lipid content would be greatest in brown seaweeds, and soluble carbohydrate content would be greatest in green seaweeds (Mabeau and Fleurence 1993; Rupérez and Saura-Calixto 2001; McDermid and Stuercke 2003; Marsham et al. 2007; Chakraborty and Santra 2008; Kasimala et al. 2015). Understanding dominant drivers of variation in seaweed nutritional composition and recognising the magnitude of their effects will provide a greater foundation for future studies regarding the usage of seaweeds in important commercial industries, as well as important recommendations for the field of research.

In Chapter three, we determine the nutritional composition of several coastal seaweed species common to northeastern New Zealand. Seaweeds are a highly valuable food source for marine herbivores, as many species are rich in dietary macromolecules important for growth and development (Steneck et al. 2017). Given their desirable nutritional properties, seaweeds are both a dependable and sustainable source of income for humans and are being increasingly utilized in large commercial industries such as aquaculture as formulated feed ingredients and other uses (Troell et al. 2009; Murty and Banerjee 2011; Bansemer et al. 2016). As demand for sustainability increases, an understanding of seaweed nutritional chemistry is becoming increasingly valuable, and although the nutritional composition for various seaweed taxa has been intensively studied worldwide, knowledge of nutritional attributes in New Zealand seaweeds is somewhat limited. Here, we perform proximate composition analyses to quantify the nutritional composition of eleven common, coastal seaweed species from Mathesons Bay, Leigh, northeastern New Zealand. Identifying sustainable seaweed resources with beneficial nutritional characteristics offers promise for New Zealand's flourishing aquaculture industry.

In Chapter four, I synthesize the results of Chapter two and three. I provide insight into underlying global drivers of variation in seaweed nutritional composition and an early glance at the nutritional profiles of some common northeastern New Zealand seaweeds. I discuss the limitations of my study with reference to methodological biases, data collection, and the frontiers of plausible inferences that can be made from my study. I then explore possible future directions for research and suggest the most appropriate methodologies for determining nutritional content in seaweeds. Ultimately, I address the significance and value

of New Zealand's diverse seaweed flora and the opportunities it presents for the respective aquaculture industry.

Initially, I had planned to determine the suitability of different seaweed species as supplements for commercially farmed black-footed abalone (pāua, *Haliotis iris*) feeds, to gain an understanding of the potential for seaweed aquaculture to supplement abalone farming in New Zealand, as it is commonly practiced in nations such as South Korea (Hwang et al. 2009; Hwang et al. 2013a; Hwang et al. 2014). Additionally, I would have liked to perform depth stratified sampling of seaweeds at multiple locations, to examine the impacts of depth and spatial variation on the nutritional composition of seaweeds from northeastern New Zealand. However, due to the lockdowns and restricted travel during the COVID-19 pandemic, this plan was unable to advance. Nonetheless, I feel I progressed an ambitious research plan and have produced two data chapters of publication quality and look forward to the examiner's suggestions for improvement.

Chapter 2: Investigating drivers of variation in seaweed nutritional composition: A meta-analysis

2.1 Abstract

Seaweeds generally exhibit great differences in their nutritional content, including the composition and richness of specific macronutrients, which are becoming increasingly important as the breadth of usages for seaweed-based products increases. While the nutritional characteristics of many species are well recognised, there are numerous hypothesised drivers of variation in nutritional content and we lack a general consensus on factors determining variation in seaweed nutritional composition. Here, using traditional meta-analytic techniques, we examine global patterns in seaweed nutritional composition and identify factors that contribute strongly to variation in reported macronutrient values for seaweeds. We analysed variation in the five traditional macronutrients considered in proximate analysis (i.e., ash, soluble carbohydrate, insoluble fibre, lipid, and protein) for 182 seaweed species (from three distinct phyla) from 81 internationally published studies. We found relatively small differences in the average proportions of the individual macronutrients among phyla, although overall, the red seaweeds displayed characteristics indicating higher nutritional value. Additionally, we found substantial among-study variation for insoluble fibre and soluble carbohydrate content, and among-species variation for ash, lipid, and protein content, as well as notable sampling error variation for lipid content. Last, we found trade-offs between macronutrients among different species, where ash was strongly negatively correlated with both soluble carbohydrate and protein. Similarly, species higher in soluble carbohydrate and ash content appeared lower in insoluble fibre and lipid content and *vice versa*. Understanding the impacts of laboratory methodologies on the outcome of particular nutritional analyses is crucial in advancing strategies to lessen these procedural artefacts in future. Differences in genetics, morphology, maturity, and life-history traits among seaweed species may underlie among-species variation in ash, lipid, and protein content, and further examination would help uncover important drivers. Overall, although we identify important sources of variation in seaweed biochemistry, the refinement and standardisation of methods for nutritional analyses should be a priority for future research in this field.

2.2 Introduction

Seaweeds are photosynthetic autotrophs that are conspicuous members of many marine ecosystems (Guiry 2012; Wenger et al. 2018). The taxonomic diversity of seaweeds is vast, with recent estimates reporting over 18,000 globally recognised species (Guiry and Guiry 2021). Seaweeds are classified into three major phyla and are often referred to by their characteristic pigmentation: Rhodophyta (red seaweeds), Ochrophyta (brown seaweeds), and Chlorophyta (green seaweeds; Lewis and McCourt 2004; Yoon et al. 2006; Silberfeld et al. 2014). Among the three seaweed phyla, not only are there notable differences in pigmentation, but also reproduction, life history, development, and cell wall structure (Raimundo et al. 2016; Liu et al. 2017). Despite sharing similar features that have evolved in response to the similar environments they occupy, these differences suggest that contemporary seaweeds represent widely divergent evolutionary lineages (Drobnitch et al. 2015; McCoy et al. 2020).

There is a long history of seaweed utilisation by humans (Dhargalkar and Pereira 2005). Despite the degradable nature of seaweeds and poor preservation in archaeological sites, evidence of seaweeds discovered at Monte Verde, a Chilean archaeological site and late Pleistocene settlement, indicated that seaweeds have been utilised for food and medicinal purposes by humans for at least ~14,000 years (Dillehay et al. 2008). Additionally, archaeological remains of *Eisenia* sp. and *Sargassum* sp. have been found amongst shells and fish bones in sites used by inhabitants of the Jomon and Yayoi periods in Japan (Nisizawa et al. 1987). In the 1800's, Australian Aboriginal peoples used *Durvillaea potatorum* (Australian bull kelp) for roofing material in shelters, clothing and footwear, moulds, and food preparation and preservation (Thurstan 2018), and *Betaphycus speciosus* for human consumption (i.e., jelly, blanc-mange) and for construction as an additive in cement (Maiden 1889; Irvine 1957; Guiry and Guiry 2021). In New Zealand, before European settlement, seaweeds such as *Pyropia* spp. were used as food by Māori and *Durvillaea antarctica* was used to hold and preserve muttonbird carcasses (Abbott 1996; Hurd et al. 2004). Based on the discoveries of seaweed remains at archaeological sites and evidence of their early utilisation in food, medicine, and shelter, it is clear that seaweeds were, and continue to be of great importance to indigenous communities.

Given that seaweeds provide natural sources of important dietary nutrients and bioactive compounds, they are cultured and harvested at commercial scale for food and have many applications within the pharmaceutical, cosmetic, agriculture, and aquaculture industries (MacArtain et al. 2007; Holdt and Kraan 2011; Ehrhart et al. 2013). Furthermore, increasing the use of seaweeds in commercial industries is expected to maintain the growth of the seaweed industry in future (Mac Monagail et al. 2017). Over the past 50 years, the global seaweed industry has drastically increased, with annual seaweed production reaching 32.4 million tonnes (mt) in 2018 (Loureiro et al. 2015; FAO 2020). In less than 20 years, global seaweed production has increased three-fold, from 10.6 mt in 2000 to 32.4 mt in 2018 (FAO 2020). Of the total 32.4 mt of wild and cultivated seaweeds harvested in 2018, cultivated seaweeds represented 97.1%, furthermore, global demand for seaweeds has driven the rapid expansion of the seaweed farming industry, alongside encouraging efforts to reduce the over-exploitation of natural seaweed populations. Seaweeds are gaining increasing recognition for their potential to further the development of an environmentally sustainable bioeconomy, through biotechnology and sustainable biofuel production (Mazarrasa et al. 2014; FAO 2020), and recent statistics indicate that the global seaweed industry is worth approximately \$13.3 billion USD (FAO 2020). It is evident that global demand for raw seaweed material has increased and can only be met through intense cultivation practices. Seaweed cultivation is practised in several countries, particularly those in East and Southeast Asia, but opportunities for further expansions are abundantly clear (Mac Monagail et al. 2017; FAO 2020).

Over the last few decades, the number of studies focusing on seaweeds, their biochemical composition, physiological properties, and health benefits, has dramatically increased (Patarra et al. 2011). Seaweeds contain important bioactive compounds and macronutrients, such as protein, lipids, insoluble fibre, and soluble carbohydrates and are also rich in micronutrients such as minerals and vitamins (Ortiz et al. 2006; Yaich et al. 2015). Research has shown that among the many bioactive compounds present in seaweeds, polysaccharides such as agar, alginates, and carrageenan are most important due to their commercial utilisation in the food, pharmaceutical, and cosmetic industries (Tseng 2001; Larsen et al. 2003; Kumar et al. 2008; Cunha and Grenha 2016). Additionally, some seaweed polysaccharides are known to be active against conditions such as cancer and inflammation, given their anticoagulant, antioxidant, antitumor, and antiviral properties (Wijesekara et al. 2011; Rupérez et al. 2013). The various applications of seaweeds as effective nutrient producers are attracting attention and have gained recognition as an extremely valuable food

source on a global scale. Given the wide variety of benefits and uses of raw seaweeds, seaweed-based food additives are being more frequently used in food preparation and nutrition analyses (MacArtain et al. 2007; Mwalugha et al. 2015).

Proximate composition analysis methods are widely used to calculate quantities of particular macronutrients in different food samples (Iqbal et al. 2006; Gul and Safdar 2009). Worldwide, studies have used proximate composition analysis methods to partition and analyse specific macronutrients present in different seaweeds, namely: moisture, ash, soluble carbohydrate, insoluble fibre, lipid, and protein content (Hossain et al. 2003; McDermid and Stuercke 2003; Gómez-Ordóñez et al. 2010; Smith et al. 2010; Gressler et al. 2011; Rohani-Ghadikolaei et al. 2012; Khairy and El-Shafay 2013; Carneiro et al. 2014; Cian et al. 2014; Mwalugha et al. 2015; Aminah and Xiren 2017; Rasyid 2017; Hao et al. 2019). Given their characteristic water-holding capacity, seaweeds usually have a very high moisture content (Gupta et al. 2011; Wan et al. 2019), with some commercially important species ranging between 60% and 94% wet weight (WW; McDermid and Stuercke 2003; Marsham et al. 2007). Ash content widely varies in seaweeds and is generally higher than that of most terrestrial vegetables, commonly ranging between 8% and 40% dry weight (DW; Ito and Hori 1989). Some green seaweed species that grow in multispecific, densely packed assemblages, also known as ‘turfs’, can be structurally tough and high in ash, and species such as *Codium* can exceed 50% ash DW (McDermid et al. 2007; Mwalugha et al. 2015). However, ash contents of heavily calcified red seaweeds such as *Corallina* spp. can exceed 70% DW (Foster and Hodgson 1998; Marsham et al. 2007; You et al. 2014). Typically, most seaweed species have a very low lipid content (< 5% DW; Montgomery and Gerking 1980; Kumari et al. 2010); however, the lipid contents of some brown seaweeds, namely: *Dictyota sandvicensis* and *Dictyota acutiloba*, have been reported as high as 20.2% and 16.1% DW, respectively (McDermid and Stuercke 2003).

Seaweeds are largely comprised of both structural and storage polysaccharides (carbohydrates), and mucopolysaccharides (Murata and Nakazoe 2001). Structural or frame polysaccharides mainly consist of cellulose, constructing the cell wall, whilst storage polysaccharides consist of starch and laminarin. Green seaweeds contain sulfuric polysaccharides, brown seaweeds contain alginic acid, fucoidan, and sargassan, and red seaweeds contain agar-agar and porphyrin (mucopolysaccharides) that take up intercellular space (Murata and Nakazoe 2001). Soluble or digestible polysaccharides are mainly

comprised of agar, alginates, and carrageenan, and commonly range between approximately 55% and 70% DW (Rajapakse and Kim 2011). Soluble carbohydrate content has been shown to range between 4% and 76% DW in a number of seaweed genera, including *Ascophyllum*, *Pyropia*, *Palmaria*, and *Ulva* (Holdt and Kraan 2011; Stiger-Pouvreau et al. 2016), although many green seaweed species reportedly have higher soluble carbohydrate content than red or brown seaweeds (Chakraborty and Santra 2008; Matanjun et al. 2009; Rohani-Ghadikolaei et al. 2012; Kasimala et al. 2017).

Insoluble carbohydrates, also known as ‘insoluble dietary fibre (IDF)’ or ‘crude fibre’ (Jiménez-Escrig and Sánchez-Muniz 2000; Cofrades et al. 2008; Mwalugha et al. 2015) are indigestible to humans and mainly comprised of cell wall compounds such as cellulose, hemicellulose, and lignin (Roehrig 1988; Rupérez and Saura-Calixto 2001). Typically, total dietary fibre (TDF) content in seaweeds ranges between 25-75% DW (Jiménez-Escrig and Sánchez-Muniz 2000) and includes both IDF and soluble dietary fibre (SDF; Wong and Cheung 2000), differing both chemically and physiochemically from that in terrestrial plants and vegetables (Lahaye 1991). For example, IDF generally makes up the lesser proportion of TDF in seaweeds, whereas in terrestrial vegetables, the IDF fraction is often much higher (Lahaye 1991; Prosky et al. 1992). High IDF contents have been reported in brown seaweeds, particularly *Durvillaea antarctica*, *Fucus vesiculosus*, *Laminaria digitata*, with values of approximately 44%, 40%, and 27% DW respectively (Rupérez and Saura-Calixto 2001; Ortiz et al. 2006), however, lower values of IDF (< 20%) have been reported for other brown species such as *Undaria pinnatifida* (Rupérez and Saura-Calixto 2001). Moreover, particular fibrous compounds in seaweeds have positive physiological effects on human health (Jiménez-Escrig and Sánchez-Muniz 2000; Kuda et al. 2005), including increased antioxidant activity, prebiotic efficiency, intestinal regulation, and decreased cholesterol (Wijesekara et al. 2011; Praveen et al. 2019; Peñalver et al. 2020).

Protein content varies significantly among both seaweed species and phyla and can be heavily influenced by geographical location and seasonality (Fleurence 1999). Generally, brown seaweeds have relatively low protein levels commonly ranging between 3% to 15% DW, except for *U. pinnatifida* which can range between 11% to 24% DW (Fleurence et al. 2018). Most red and green seaweeds have higher protein levels, typically ranging from 10% to 30% DW (Mabeau and Fleurence 1993). Nonetheless, there are exceptions for particular species, for example, red seaweeds *Pyropia* spp. and *Palmaria palmata* have reached protein

contents of 43% and 35% DW, respectively (Morgan et al. 1980; Nisizawa et al. 1987; Noda 1993; Marsham et al. 2007; Jung et al. 2016). Protein is considered one of the most crucial and costly components of formulated aquaculture feeds (Tung and Alfaro 2011), and it can also limit growth in marine herbivores, hence, it is regarded as a highly important macronutrient in seaweeds (Horn 1989; Fleming 1995).

The biochemistry of a seaweed can be significantly affected by environmental conditions, seasonality, and maturity (Wong and Cheung 2000; Ortiz et al. 2006; Murakami et al. 2011; Rohani-Ghadikolaei et al. 2012). For example, seaweeds can absorb any available nutrients or compounds (including heavy metals) present in the water column, including those from surface particulate matter (Harrison and Hurd 2001; Schaffelke 2002).

Environmental factors such as water temperature, salinity, sunlight exposure, and nutrient availability can also contribute to significant variation in seaweed macronutrient composition (Kumar et al. 2015a). Studies have revealed that nutrient concentrations of individual seaweed species can peak in different seasons, for example, in Chonburi Province, Thailand, *Sargassum oligocystum* had peak levels of protein, lipid, and insoluble fibre during the monsoon season and when it had reached full maturation (Praiboon et al. 2018). In temperate marine ecosystems, nutrient levels in seaweeds are generally rich during winter and early spring, and slowly decrease as temperatures rise in the warmer seasons of summer and autumn (Mente et al. 2006). On the contrary, in marine ecosystems impacted by aquaculture farms, nutrient levels are usually higher during the warmer seasons due to excessive nitrogen and phosphorus waste released from cage-farmed species such as salmon or trout (Holby and Hall 1991; Hall et al. 1992; Mente et al. 2006).

Given that seaweeds provide natural sources of important dietary nutrients and bioactive compounds, they are being cultivated and harvested at commercial scale (MacArtain et al. 2007; Holdt and Kraan 2011; Ehrhart et al. 2013). Both wild and cultivated seaweeds have exposure to different environmental conditions and grazers, and accordingly, studies have shown that there can be considerable variation in nutritional composition between wild and cultivated populations (Tibbetts et al. 2016). For example, protein and ash content of the brown seaweed *Sargassum hemiphyllum* cultivated in a pond-based aquaculture system differed from that of wild *S. hemiphyllum* collected from Daya Bay, South China Sea (Yu et al. 2013). The protein (6.9%) and ash content (21.5%) of cultured *S. hemiphyllum* being lower than that of wild *S. hemiphyllum* (9.4%) and (26.9%), for protein

and ash, respectively. Similarly, a comparison of two red seaweeds *Gracilaria verrucosa* and *Gracilaria gigas* collected in Indonesia revealed that for both species, ash, lipid, protein, and soluble carbohydrate contents were higher in the wild individuals compared to the cultured individuals (Meinita et al. 2018). On the contrary, some seaweeds cultivated under specific conditions on aquaculture farms such as *Ulva* spp. have been shown to have greater protein contents than their wild counterparts (Shpigel et al. 1999; Neori et al. 2004; Robertson-Andersson et al. 2007). Seaweeds have the potential to absorb waste nutrients such as dissolved nitrogen from effluent produced by aquaculture, which can result in a drastic increase in a seaweed's phosphorus and protein content, thereby affecting its nutritional composition (Harrison and Hurd 2001; Robertson-Andersson et al. 2007; Mortensen 2017).

While there may be many hypotheses for why differences in nutritional composition might occur among seaweeds, we currently lack a quantitative synthesis of knowledge on the major sources of variability in seaweed nutritional composition. Here, we examine the drivers of variability in seaweeds using a formal meta-analysis to dissect the factors causing variation in nutritional composition among different seaweed phyla and species on a global scale. To do so, we perform an extensive search of the available published literature for nutritional composition data, before data curation and preparation for statistical analysis. Factors such as phylum and species were expected to drive changes in seaweed nutritional composition. At the phylum level, we would expect that red seaweeds would have higher protein and ash content, brown seaweeds would have higher lipid and insoluble fibre content, and green seaweeds would have higher soluble carbohydrate content. Overall, our research reveals some dominant drivers of variation in seaweed biochemistry, providing important insights for future research in this field of study.

2.3 Methods

2.3.1 Data collection

To assemble the dataset, an extensive search of the existing literature was undertaken by predominantly using the Google Scholar database. To obtain appropriate studies, the specific terms *seaweed* and *macroalgae* were used in combination with the following key words: *nutritional composition*, *proximate composition*, *proximate analysis*, *biochemical*, *nutritional content*, *nutritional evaluation*, and *nutritional profile*. We also searched the cited literature and reference sections of each study found for additional articles. From these articles, for each seaweed species examined, we extracted the mean percentages of each of the six macronutrients (moisture, ash, soluble carbohydrate, insoluble fibre, lipid, and protein) and their respective standard errors (SE). For published studies where standard deviations (SD) or confidence intervals (CI) were present, we extracted the sample sizes and back-transformed these estimates to SE values.

In order for a study to be included in the analysis, the following criteria had to be met: the titles or abstracts indicated that proximate analysis methods were used to determine the percentages for each of the macronutrients; traditional proximate analysis procedures were used according to the Association of Official Analytical Chemists (AOAC) methods (AOAC 1990) i.e., Kjeldahl (AOAC 988.05), lowry method (Lowry et al. 1951), or elemental analyzer for protein determination (Ebeling 1968; AOAC 968.06), soxhlet method (AOAC 920.39), or chloroform-methanol method (Bligh and Dyer 1959) for lipid determination, freeze-drying, oven drying (AOAC 930.15), or moisture analyzer for moisture determination, incineration method for ash determination (AOAC 942.05), acid-base sequential digestion or crude fibre method (AOAC 978.10) or enzymatic-gravimetric method for insoluble fibre determination, and phenol-sulfuric method (Dubois et al. 1956; Masuko et al. 2005), anthrone method (Yemm and Willis 1954; Hedge and Hofreiter 1962), or nitrogen-free extract (NFE) equation (i.e., $\%NFE = 100\% - [\% \text{ lipid} + \% \text{ protein} + \% \text{ ash} + \% \text{ insoluble fibre}]$) for soluble carbohydrate determination (Lloyd et al. 1978; Bhuiyan et al. 2016); studies had no more than two macronutrients missing; the respective SE values were given or could be calculated for every macronutrient; and the analyses were carried out in duplicate or higher.

Additional data extracted from the studies included: the phylum for each species, the species name, authors and year of study, the location(s) the seaweeds were collected, the year the seaweeds were collected, the season(s) the seaweeds were collected (if the specific month(s) were given, we calculated the solar-calendar season), and whether the seaweeds were collected from the wild or cultivated. Given that many studies had missing data regarding the specific year, months, or season the seaweeds were collected, we chose to exclude these factors in the statistical analysis. Additionally, approximately 60% of the studies included used the 'by difference' or NFE calculation method (Lloyd et al. 1978) to determine soluble carbohydrate content. Given that the macronutrients are constrained to sum to 100% when the NFE method is used, negative correlations between soluble carbohydrate content and the other macronutrients are expected even though that may not be present when analytical methods such as the phenol-sulfuric or anthrone methods are used, hence we excluded values for soluble carbohydrate content calculated using the NFE method from our analysis. Additionally, in this chapter, we chose to exclude moisture content and instead focus on the other five macronutrients. The final database consisted of 81 peer-reviewed studies published between the years 1997-2021 (Appendix 1), with a total of 182 seaweed species analysed across the studies. Once data was collected from studies it was entered into a formatted table in Microsoft Excel (Microsoft 2021) and the dataset was imported into RStudio for statistical analysis (RStudio 2021).

2.3.2 Statistical analysis

To analyse variation in seaweed nutritional composition, we used a linear mixed model fit using the MCMCglmm package for R version 3.6.0 in the RStudio environment (Hadfield 2010; RStudio 2021). We fit a linear mixed model where the fixed effects were phylum and macronutrient (i.e., ash, soluble carbohydrate, insoluble fibre, lipid, protein) as well as phylum by macronutrient interaction, and the random effects were study and species, with random intercepts fitted for each macronutrient at each level of the species and study random effects. Additionally, to control for sampling error in the model, the standard error for each level of each of the five macronutrients was also fitted as a random effect. The response variable was the mean value for each macronutrient for each seaweed species in each study.

For the random effects, a multivariate parameter expanded prior was used and conformed to a scaled non-central F -distribution (Gelman 2006) with a mean vector of zero and a variance equal to a diagonal matrix with the total variance for each macronutrient along the diagonal. For the standard error of each macronutrient, we assumed an inverse-Wishart prior distribution with a value for the shape and scale of 1. For the residual, an inverse-Wishart prior distribution was used with the scale parameter equal to a diagonal matrix with the total variance for each macronutrient along the diagonal and the shape parameter equal to 4.002 as it is commonly used for variance components (Hadfield 2010).

The final MCMC model had a total of 130,000 iterations, a burn-in period of 30,000 iterations, and a thinning interval of 100 iterations, resulting in 1000 posterior samples of the distribution of each term in the model. To examine relationships between the five macronutrients, we calculated the posterior distributions of the correlations for each macronutrient at the level of study and species. If the posterior distributions of the correlations did not overlap zero, we interpreted this as evidence for a significant association between two macronutrients at the level of the particular random effect. The ‘vegan’, ‘corrplot’, and ‘ggplot2’ packages were then used to construct the graphical displays (Dixon 2003; Wickham et al. 2021; Ramasubramanian and Singh 2019).

2.4 Results

Green seaweeds (phylum chlorophyta) were highest in mean ash content ($\bar{x} = 25.706\%$, 95% HPD = 21.874, 29.483; Figure 1A), red seaweeds (phylum rhodophyta) were highest in mean soluble carbohydrate content ($\bar{x} = 35.023\%$, 95% HPD = 25.616, 45.451; Figure 1B) and mean protein content ($\bar{x} = 15.519\%$, 95% HPD = 10.318, 20.203; Figure 1E), and brown seaweeds (phylum ochrophyta) were highest in mean insoluble fibre content ($\bar{x} = 19.979\%$, 95% HPD = 13.003, 26.351; Figure 1C) and mean lipid content ($\bar{x} = 4.596\%$, 95% HPD = 0.115, 9.198; Figure 1D). Brown seaweeds were lowest in mean ash content ($\bar{x} = 23.780\%$, 95% HPD = 20.422, 27.882; Figure 1A), mean soluble carbohydrate content ($\bar{x} = 27.784\%$, 95% HPD = 18.549, 37.896; Figure 1B), and mean protein content ($\bar{x} = 11.945\%$, 95% HPD = 6.604, 16.877; Figure 1E), and red seaweeds were lowest in mean lipid content ($\bar{x} = 2.750\%$, 95% HPD = -1.985, 6.710; Figure 1D), and mean insoluble fibre content ($\bar{x} = 12.809\%$, 95% HPD = 5.643, 18.189; Figure 1C). Given the large overlap in the posterior distributions of the estimates for the mean values of the macronutrients there is no strong evidence for consistent differences in nutritional composition among phyla.

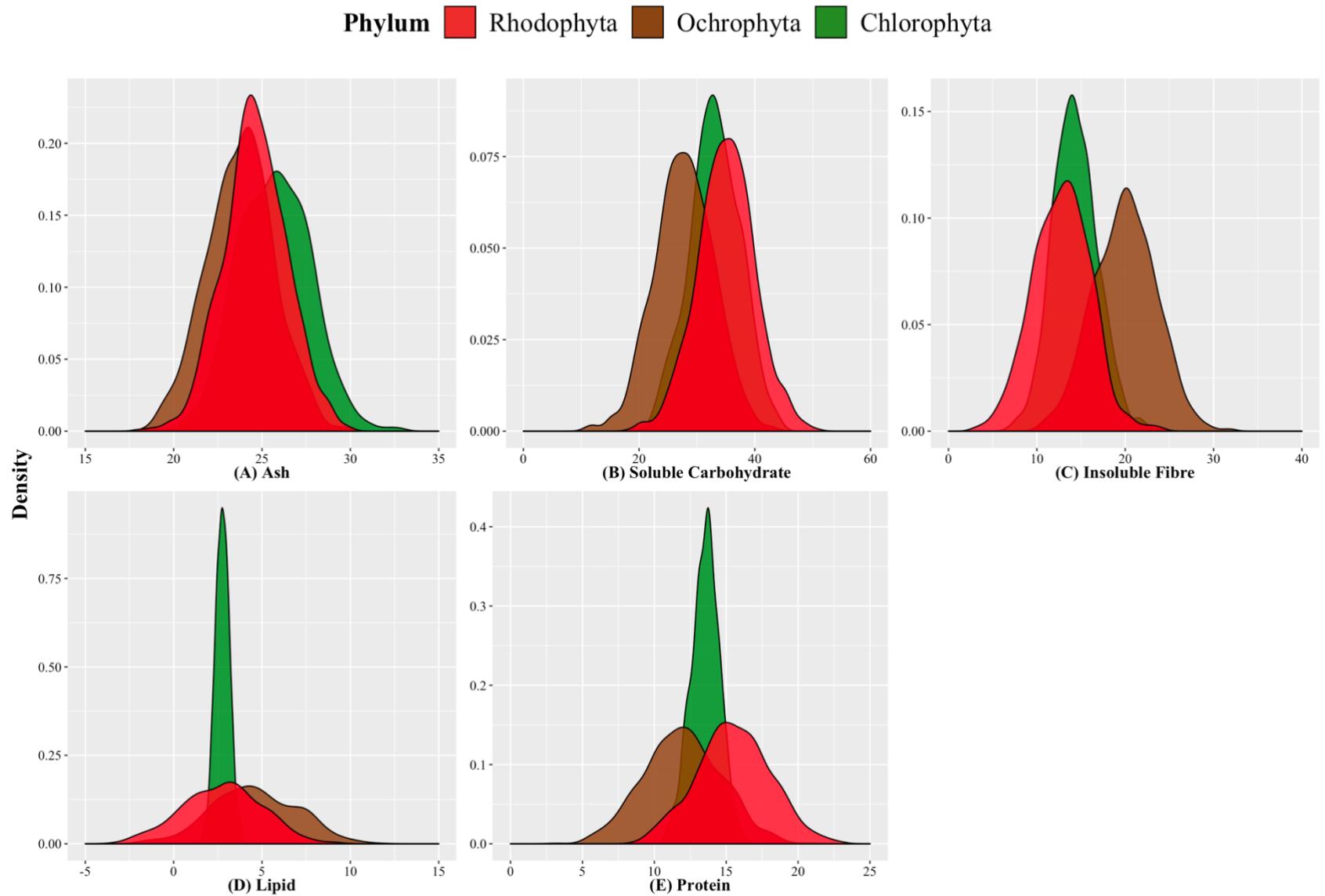


Figure 1. Posterior distributions of the percent contribution of each of the macronutrients (A-E) in each phylum.

Table 1. Variance-covariance matrix for *Study* and *Species* random effects, including both measurement and residual error variances. The posterior mean estimates of the variances for each macronutrient are displayed in bold on the diagonal, posterior mean estimates of the covariances are below the diagonal, and posterior mean estimates of the correlations and 95% highest posterior density (HPD) intervals are above the diagonal.

<u>Random effect</u>	Ash	Soluble Carbohydrate	Insoluble Fibre	Lipid	Protein
<i>Study</i>					
Ash	89.324	-0.457 (-0.758, -0.115)	-0.158 (-0.453, 0.229)	0.036 (-0.245, 0.292)	-0.099 (-0.378, 0.157)
Soluble Carbohydrate	-78.900	341.658	-0.075 (-0.417, 0.307)	-0.317 (-0.626, 0.015)	-0.310 (-0.665, 0.112)
Insoluble Fibre	-24.783	-23.423	273.036	0.200 (-0.219, 0.502)	0.129 (-0.158, 0.460)
Lipid	0.558	-9.158	5.277	2.533	0.141 (-0.135, 0.423)
Protein	-4.819	-29.373	10.796	1.137	25.422
<i>Species</i>					
Ash	89.962	-0.571 (-0.901, -0.153)	0.117 (-0.716, 0.824)	-0.096 (-0.297, 0.087)	-0.592 (-0.754, -0.435)
Soluble Carbohydrate	-23.734	19.948	-0.010 (-0.768, 0.750)	-0.228 (-0.589, 0.126)	0.159 (-0.359, 0.658)
Insoluble Fibre	1.371	0.039	1.434	-0.226 (-0.851, 0.531)	-0.063 (-0.732, 0.663)
Lipid	-1.973	-2.091	-0.720	4.687	0.257 (0.051, 0.443)
Protein	-22.967	2.868	-0.296	2.277	16.860
<i>Measurement error</i>					
	1.206	1.878	4.604	2.118	0.738
<i>Residual error</i>					
	38.117	63.783	18.874	0.622	9.395

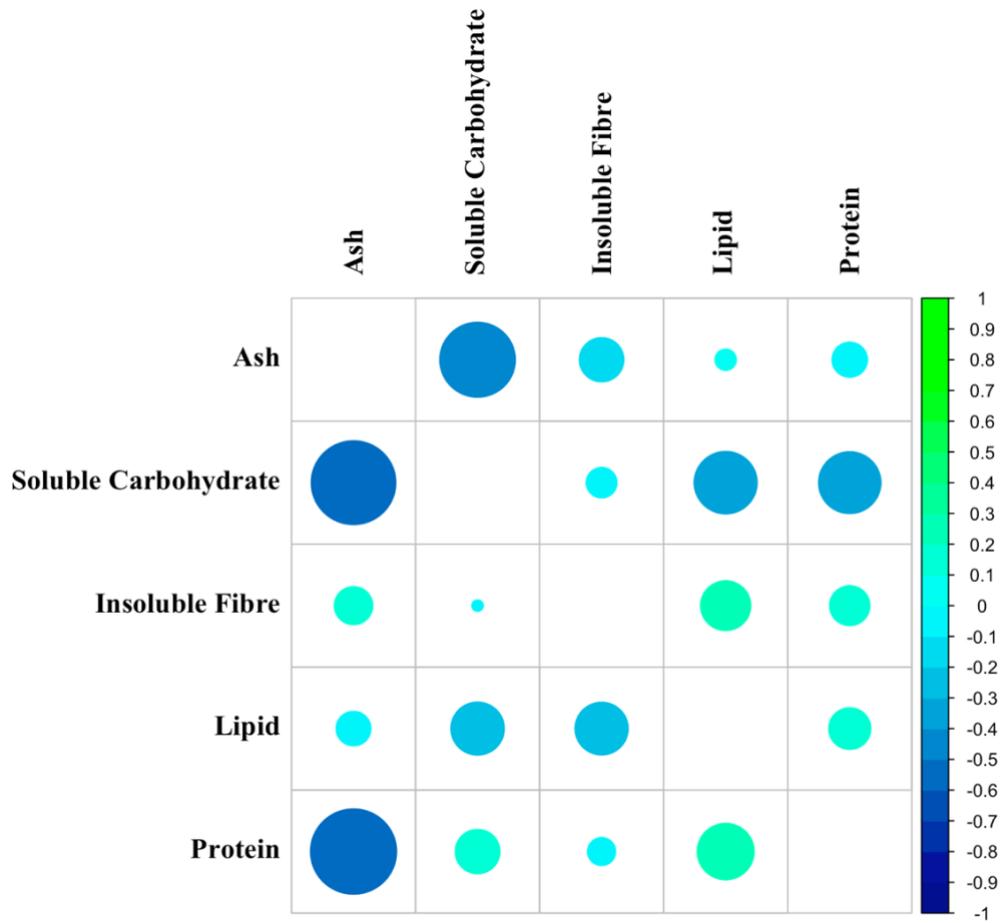


Figure 2. Correlations among the five macronutrients of individual seaweed species (below the diagonal) and seaweeds analysed in different studies (above the diagonal). The coloured bar on the right-hand side indicates the strength and direction of the correlation (see Table 1).

Among-study differences accounted for a very large proportion of the total variation in reported insoluble fibre and soluble carbohydrate values (0.916 and 0.799, respectively, Table 1), whereas for ash, lipid, and protein, among-species differences were associated with the greatest variation (0.412, 0.471, and 0.322, respectively, Table 1). Measurement or sampling error variation contributed most strongly to variation in the reported values for lipid content (0.213, Table 1). At the species level, correlations between the macronutrients were a mixture of positive and negative, with strong negative correlations between ash and protein ($r = -0.592$; Table 1, Figure 2) and ash and soluble carbohydrate ($r = -0.571$; Table 1, Figure 2). Additionally, we found weak positive correlations between ash and insoluble fibre ($r = 0.117$; Table 1, Figure 2), soluble carbohydrate and protein ($r = 0.159$; Table 1, Figure 2), and lipid and protein ($r = 0.257$; Table 1, Figure 2), and weak negative correlations between insoluble fibre and soluble carbohydrate ($r = -0.010$; Table 1, Figure 2), ash and lipid ($r = -0.096$; Table 1, Figure 2), insoluble fibre and protein ($r = -0.063$; Table 1, Figure 2), insoluble

fibre and lipid ($r = -0.226$; Table 1, Figure 2) and soluble carbohydrate and lipid ($r = -0.228$; Table 1, Figure 2).

At the study level, the majority of the correlations between the macronutrients were negative, we found that soluble carbohydrate was moderately negatively correlated with ash ($r = -0.457$; Table 1, Figure 2), lipid ($r = -0.317$; Table 1, Figure 2), and protein ($r = -0.310$; Table 1, Figure 2), and weak positive correlations were shared between ash and lipid ($r = 0.036$; Table 1, Figure 2), insoluble fibre and protein ($r = 0.129$; Table 1, Figure 2), lipid and protein ($r = 0.141$; Table 1, Figure 2) and insoluble fibre and lipid ($r = 0.200$; Table 1, Figure 2). Additionally, weak negative correlations were shared between soluble carbohydrate and insoluble fibre ($r = -0.075$; Table 1, Figure 2), ash and protein ($r = -0.099$; Table 1, Figure 2), and ash and insoluble fibre ($r = -0.158$; Table 1, Figure 2).

After accounting for differences among phyla, species, and measurement error, the phenol-sulfuric method appears to produce larger estimates of mean soluble carbohydrate content than the anthrone method (a mean difference of approximately 11.5%, Figure 3 [top, left]), as well as larger estimates of variation ($\bar{x} = 356.546, 163.410$ for the phenol-sulfuric method and anthrone method, respectively; Figure 3 [top, right]). Examining the coefficient of variation (CV) for the mean and variance of the two methods for estimating soluble carbohydrate content indicated that there was no significant difference in the CV (i.e., the 95% HPD interval for the difference overlaps zero; 95% HPD = $-38.498, 42.827$) and thus, because the phenol-sulfuric method also had a greater posterior mean, differences in variation could be driven by a mean-variance relationship rather than analytical imprecision.

Similar to our findings for soluble carbohydrate content, we found that the study-level estimates of mean insoluble fibre content produced by the acid-base sequential digestion method were higher than that produced by the enzymatic-gravimetric method (a mean difference of approximately 6.4%, Figure 3 [bottom, left]), as well as larger estimates of variation ($\bar{x} = 308.623, 64.944$ for the acid-base sequential digestion method and enzymatic-gravimetric method, respectively; Figure 3 [bottom, right]). Similar to our results for the two most common methods for measuring soluble carbohydrate content above, there was no evidence for a significant difference in the CV between the acid-base sequential digestion method and the enzymatic-gravimetric methods for determining insoluble fibre content as the 95% HPD interval of the CV overlapped zero (95% HPD = $-94.842, 118.514$).

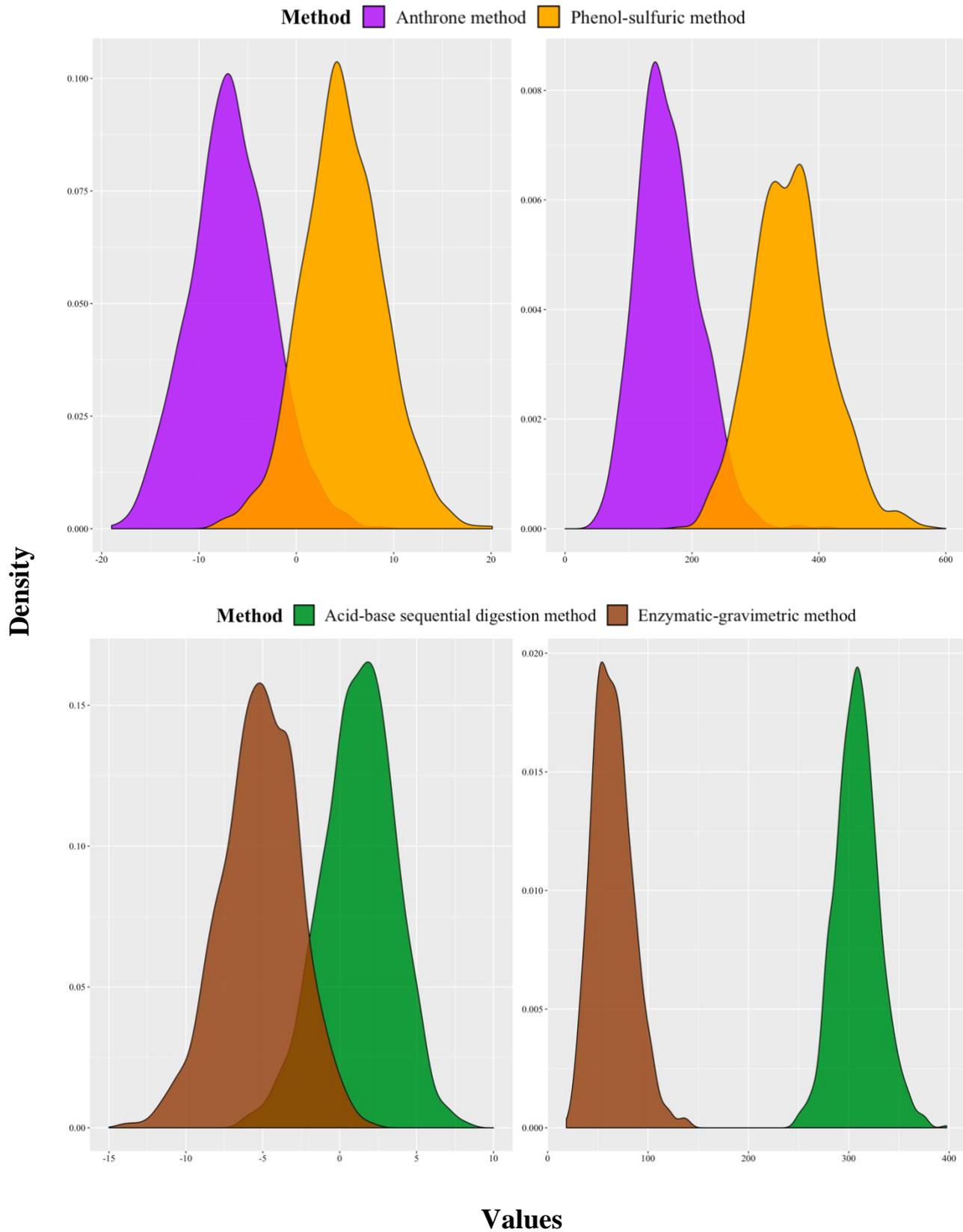


Figure 3. Posterior distributions of the mean (left) and variance (right) for the study-level estimates of reported mean soluble carbohydrate content (top) and mean insoluble fibre content (bottom) obtained using the two most common laboratory methods for each macronutrient.

After accounting for differences in the mean values of the macronutrients in each phylum, among study variation, and measurement error, we discovered that the first principal component of variation in nutritional composition among seaweed species was driven by overall differences in nutritional value. Species such as *Caulerpa cupressoides*, *Carpophyllum plumosum*, *Ulva pertusa* and *Ulva nematoidea* had relatively high values for all macronutrients whereas species such as *Cladophora* spp., *Codium dwarkense*, *Asparagopsis taxiformis*, and *Sargassum obtusifolium* had relatively low values for all macronutrients (Figure 4). Additionally, the second principal component described trade-offs between soluble carbohydrate and insoluble fibre content and between ash and lipid content. Species that appeared relatively high in soluble carbohydrate and ash content but low in insoluble fibre and lipid content included *C. cupressoides*, *S. obtusifolium*, *Cladophora crispula*, and *Acanthophora spicifera*, whereas species that were relatively high in insoluble fibre and lipid content but low in soluble carbohydrate and ash content were *Caulerpa scapelliformis*, *Carpophyllum flexuosum*, *Monostroma oxyspermum*, and *Caulerpa racemosa*.

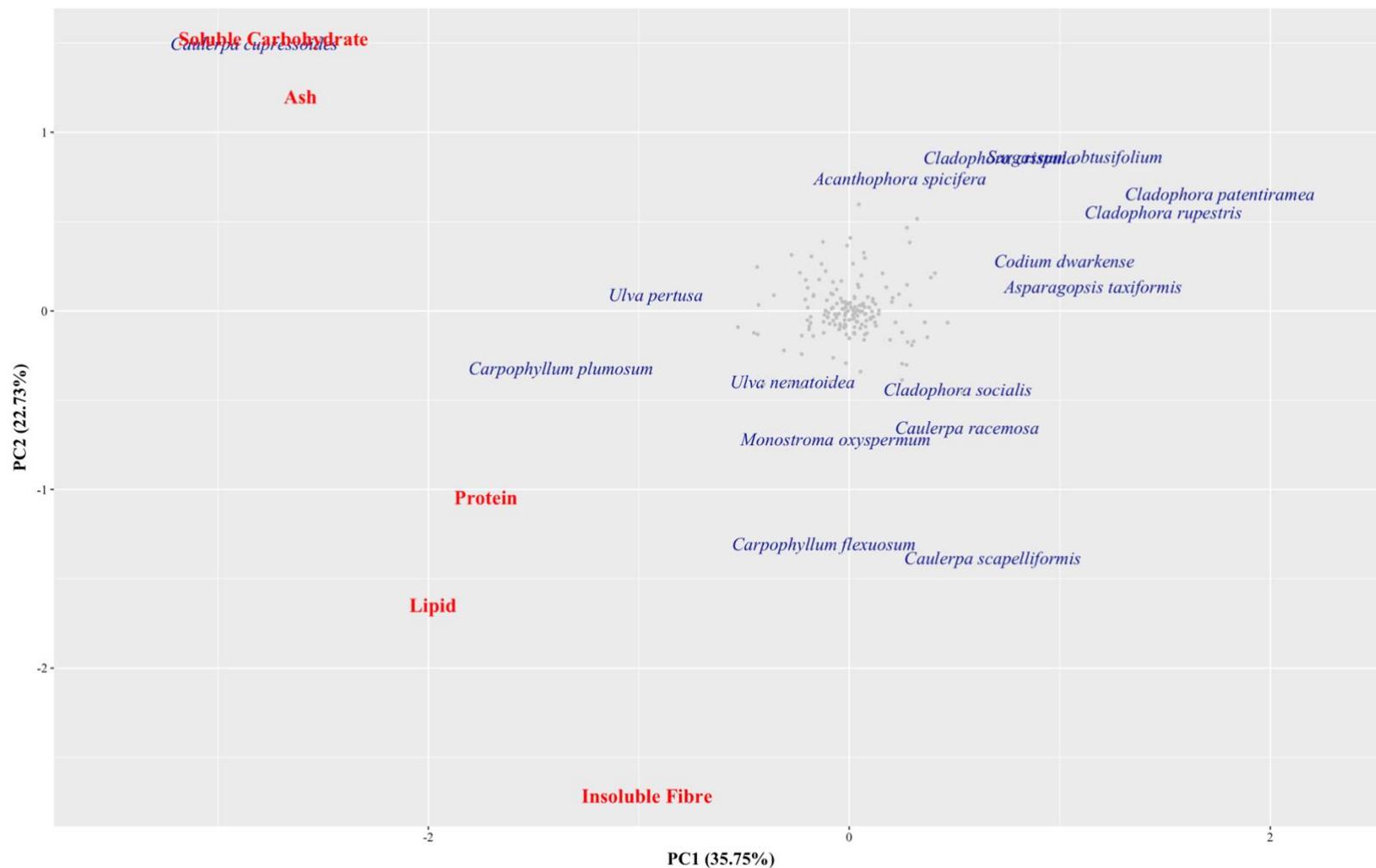


Figure 4. Principal components analysis (PCA) showing variation in nutritional composition among individual seaweed species after accounting for differences in the mean values of the macronutrients in each phylum, among-study variation, and measurement error. The coordinates of the five macronutrients are indicated in red bold text. The species names in the blue italic text indicate the 9% of the species with the most extreme values for the two PC axes and the grey points refer to the other 91% of species for which we omitted the names for clarity of display.

2.5 Discussion

Investigations of the proximate composition of a variety of seaweed taxa from a wide geographical scale have shown large variation in nutritional value among seaweed species and phyla (McDermid et al. 2007; Matanjun et al. 2009; Tabarsa et al. 2012; Chirapart et al. 2014; You et al. 2014; Mwalugha et al. 2015; Ahmad et al. 2016; Muraguri et al. 2016; Dixit et al. 2018). Nonetheless, the nutritional chemistry for a large proportion of seaweed species remains poorly described (Britton et al. 2021), and many species are still yet to be discovered (Nelson et al. 2015; Spalding et al. 2019). Here, we surveyed the existing literature for published studies quantifying the proximate composition of seaweeds and we examined factors causing variation in seaweed nutritional composition. Although red seaweeds are well known for their higher protein contents, and some brown seaweed species for their high lipid contents, after accounting for among-study and among-species variation, differences in nutritional composition among phyla were relatively minor. A notable result was the relatively large contribution of procedural effects (i.e. among-study and measurement error variation) to variation in the published nutritional content values. Last, after accounting for differences in nutrient composition among phyla and procedural effects, we discovered important trade-offs in nutritional contribution among different species, with major repercussions for the nutritional ecology of marine herbivores and for seaweed aquaculture.

At the phylum level, we found that red seaweeds were highest in the reported values for mean soluble carbohydrates and protein, brown seaweeds were highest in mean insoluble fibre and lipids, and green seaweeds were highest in mean ash. Despite higher soluble carbohydrate content in the red seaweeds, and higher ash content in the green seaweeds, the differences in composition were largely expected for the different phyla (McDermid and Stuercke 2003; Holdt and Kraan 2011; Nomura et al. 2013; Fleurence et al. 2018), however, it was interesting that the differences in the macronutrients among phyla were relatively small. Several studies have suggested that red seaweeds are generally higher in protein (Harnedy and FitzGerald 2011; Makkar et al. 2016; Fleurence et al. 2018), brown seaweeds within the order Dictyotales are characteristically higher in lipids (Montgomery and Gerking 1980; McDermid and Stuercke 2003; Gosch et al. 2012), and soluble carbohydrates are typically high in most seaweeds (Holdt and Kraan 2011; Stiger-Pouvreau et al. 2016). In our study, after accounting for among-study and among-species variation, differences among

phyla were much more subtle, with a < 4% difference in mean protein content, < 2% difference in mean lipid content, and a ~7% difference in mean soluble carbohydrate content among phyla. These results suggest that some of the differences we associate with the different phyla are driven by the species of seaweed we tend to analyse in each group rather than a property of the phyla *per se*. For instance, we tend to show particular bias towards red seaweed species with higher protein such as *Pyropia* spp. that are heavily cultivated and commercialised (Hwang and Park 2020), which then biases our perception that red seaweeds are overall higher in protein even though many red species have low protein content (Montgomery and Gerking 1980; McDermid and Stuercke 2003; Pangestuti and Kim 2015). Therefore, previous studies may have indicated that certain phyla might have higher nutritional value than others, however, our analysis suggests that nutritional differences among phyla are relatively small once species choice and methodological biases are accounted for.

The substantial contributions of among-study variation and measurement error were unexpected. The large proportion of the variance for insoluble fibre (91.6%) and soluble carbohydrate (79.9%) attributable to among-study differences, could be due to underlying differences in the laboratory methods used to analyse the macronutrients. Although there was limited evidence to suggest significant differences in both mean and variability in soluble carbohydrate content between the two most common laboratory methods used (i.e., the phenol-sulfuric method and the anthrone method), the phenol-sulfuric method produced larger estimates of soluble carbohydrate content and larger estimates of variation. Similarly, mean and variability in insoluble fibre content also did not significantly differ between the two most common laboratory methods used (i.e., the acid-base sequential digestion method for crude fibre and the enzymatic gravimetric method), however, the acid-base sequential digestion method produced larger estimates of insoluble fibre content as well as larger estimates of variation. Despite the well-known reliability and accuracy of both the phenol-sulfuric method (Dubois et al. 1956; Masuko et al. 2005) and anthrone method (Yemm and Willis 1954) used to analyse soluble carbohydrates, significant variation in soluble carbohydrate content could result from the equipment or specific kit used in a particular study. Additionally, the hydrolysis step in both methods is often influenced by the specific polysaccharides in the sample, the strength of the acid, temperature, and duration (Pakulski and Benner 1992; Woldu and Tsigie 2015), which can cause variation in the results. Moreover, the anthrone method is highly sensitive and susceptible to dust contamination

(Yemm and Willis 1954), which may also be a contributing factor. On the other hand, some methods used to analyse insoluble fibre are considered inaccurate and can underestimate the fraction of insoluble fibre present in seaweeds. For example, despite its popularity and wide usage, the acid-base sequential digestion or crude fibre method fails to recover all indigestible fibre as a large percentage of structural polysaccharides in plant material such as hemicellulose, pentosans, and lignin are removed during the extraction, therefore underestimating the true amount of insoluble fibre present in the sample (Van Soest and McQueen 1973; Knudsen 2001; de-Oliveira et al. 2012). Conversely, some enzymatic-gravimetric methods like the Prosky method (AOAC 985.29; Prosky et al. 1985), are known to overestimate insoluble fibre content as it allows for the inclusion of complex carbohydrates that are not insoluble fibre (McCleary et al. 2012). Evidently, methods for determining insoluble fibre and soluble carbohydrate content are susceptible to bias, and thus can largely influence estimates of these macronutrients. Therefore, there is an urgent need for refinement of the methods currently being used.

Seaweeds generally contain only 1-5% lipids (DW; Kolanjinathan et al. 2014; Makkar et al. 2016), and thus might be more prone to laboratory-related measurement error when compared to other nutrients. In our study, the proportion of the variance for lipid content (21.3%) attributable to measurement error was ~20% greater than that of the other macronutrients. Given that absolute lipid content is very low, it is difficult to achieve high analytical accuracy when using gravimetric methods to quantify such low percentages (i.e. when weighing small samples, the very edge of the capacity of the balance is used). Additionally, factors such as contamination, changes in temperature, sample disruption, or degradation of the sample during the experimental procedure may have strongly influenced the reported values (Randall et al. 1991; Manirakiza et al. 2001). The most common method for lipid determination involves the use of different solvents to extract lipids from the sample (Manirakiza et al. 2001; Fiset et al. 2017), nonetheless, the specific procedures and solvents that provide the largest and most accurate estimations of lipid content have not yet been established (Fiset et al. 2017). The specific extraction method, solvent polarity, sample tissue, and other factors mentioned above, may all influence the amount of lipids acquired (Randall et al. 1991; Manirakiza et al. 2001; Kumari et al. 2011). Additionally, the type of thimble used to hold the sample during the extraction can also affect the amount of lipids recovered, for example, cellulose thimbles are the most commonly used thimbles, however, unlike glass thimbles, they allow the passing of very fine particulate material causing an inaccurate

estimation of lipid content (Manirakiza et al. 2001). Several other factors can contribute to variation in lipid content such as oxidation caused by extended air exposure, phase transitions in a Soxhlet apparatus, or the collection of dust particles on previously used equipment can also affect lipid recovery, and even cause denaturation (Randall et al. 1991; Choe and Min 2006). Overall, our meta-analysis demonstrates that laboratory procedures must be carefully controlled if reported lipid concentration values are to be reliable indicators of biological differences among samples.

Differences in sample preparation methods and laboratory conditions may also contribute to large among-study variation, as drying methods are known to alter and, in some cases, degrade particular macronutrients in seaweeds (Chan et al. 1997; Wong and Cheung 2001; Laurens et al. 2012; Fiset et al. 2017; Nurshahida et al. 2018). If samples are not sufficiently dried, the moisture content will be higher, which causes variation in estimates of the other macronutrients (Fiset et al. 2017). Typically, samples are either oven-dried or freeze-dried before nutrient analysis, however, drying at room temperature, sun-drying, and blotting dry are also used as drying methods (Fiset et al. 2017). In our analysis, most studies used the oven-drying method to determine moisture content, while fewer studies used the freeze-drying or air-drying methods. Consequently, when high temperatures are used during oven-drying, nutrient loss can occur in seaweed samples (Chan et al. 1997), and both oven-drying and freeze-drying methods are known to break down plant polysaccharides (Smith 1973). Storage time and conditions can also cause variation in soluble carbohydrate content, particularly when any moisture is still present in the sample tissues after drying (Smith 1973). Moreover, larger changes in soluble carbohydrate content are more likely to occur in freeze-dried samples, as enzymes can remain functional and cause changes in sample nutrients during storage, depending on the temperature and conditions (Smith 1973).

Our analysis has demonstrated that procedural effects contribute strongly to variation in reported values for nutritional composition in seaweeds, and accordingly, collaborative efforts are needed to ensure the current procedures are updated and advanced, to help minimise avoidable sources of variation and uncertainty in future research. Despite the wide range of methods available, the most commonly used are those that are straightforward, require less effort and use basic equipment, unfortunately, this also makes the methods more susceptible to error (Fiset et al. 2017). There is a need for a standardized set of methods that are widely recognised and easily implemented, which would enable more robust comparisons

between macronutrient concentrations in seaweeds among different studies. Additionally, it is important to acknowledge how differences in factors such as light, temperature, air exposure, and both drying and storage conditions among laboratory environments can influence variation in specific macronutrients (Randall et al. 1991; Regal et al. 2020) and must be accounted for even if standardized methods are employed. Although popular methods in proximate analysis such as the phenol-sulfuric and anthrone methods for soluble carbohydrate determination are considered accurate, the outcome is often heavily dependent on factors surrounding the experiments such as time, temperature, and acid concentration (Pakulski and Benner 1992; Woldu and Tsigie 2015). Furthermore, with large procedural effects stemming from methodological biases in the laboratory it is difficult to both cluster and compare studies to aid the discovery of species with higher nutritional value for their usage in commercial industries (Fiset et al. 2017).

The sizeable proportion of the variance for ash (41.2%), lipid (47.1%), and protein (32.2%) ascribable to among-species differences could have resulted from species-specific variation in structural morphology. For example, heavily calcified seaweeds such as *Corallina* or *Halimeda* spp. are generally much higher in ash, often exceeding 70% DW (Foster and Hodgson 1998; Marsham et al. 2007; Carneiro et al. 2014; You et al. 2014) and lower in protein and soluble carbohydrate content (Kaehler and Kennish 1996; Renaud and Luong-Van 2006). Conversely, species with soft, rubbery blades such as *Ulva reticulata* or *Spatoglossum asperum* appear to have much lower ash contents, sometimes less than 20% (Ratana-arporn and Chirapart 2006; Mwalugha et al. 2015; Hossain et al. 2021). Lipid content and fatty acid concentrations widely vary among seaweed species and are often heavily environmentally and phylogenetically dependent (Kumar et al. 2008; Kumari et al. 2010; Kumari et al. 2014; Gosch et al. 2012). For instance, fatty acid composition can be used as chemotaxonomic markers in seaweeds, as distinguishable patterns in fatty acid profiles have been identified for Dictyotales and Fucales species (Kumari et al. 2010; Galloway et al. 2012). Seaweed species with a greater surface area to volume ratio typically have faster growth rates, higher nutrient requirements, and increased rates of nutrient uptake (Wallentinus 1984; Pedersen and Borum 1996; Taylor et al. 1998). Moreover, faster nitrogen assimilation occurs in smaller seaweeds with a greater surface area to volume ratio than larger, slow-growing seaweeds with thicker thalli (Hein et al. 1995; Pedersen and Borum 1996), therefore, among-species variation in protein content could be dependent on specific morphological characteristics. Other biological factors such as maturity, life-history stage,

and nutrient ecophysiology can also alter the nutritional composition of different seaweeds (Littler and Littler 1980; Ito and Hori 1989; Cronin and Hay 1996a; Harrison and Hurd 2001; Murakami et al. 2011; Elsaied et al. 2015; Praiboon et al. 2018), however, these factors are often influenced by environmental parameters such as salinity, temperature, sunlight exposure, and water motion, which generally vary with sample site and season (Kumar et al. 2015a; Roleda and Hurd 2019; Britton et al. 2021).

Our meta-analytic approach provided important insights into the relationships among the different macronutrients. At the species level, ash was strongly negatively correlated with soluble carbohydrate and protein, indicating that seaweeds with higher ash content such as calcareous species are likely to have lower soluble carbohydrate and protein content, and thus overall lower nutritional value (Montgomery and Gerking 1980). Moreover, after accounting for other known sources of variation, we found that some species had higher values for all macronutrients such as *Caulerpa cupressoides*, *Carpophyllum plumosum*, *Ulva pertusa*, and *Ulva nematoidea*, whereas the species *Cladophora* spp., *Codium dwarkense*, *Asparagopsis taxiformis*, and *Sargassum obtusifolium* had lower nutritional value overall. Furthermore, we discovered notable trade-offs between soluble carbohydrate and insoluble fibre content, and between ash and lipid content. The species *C. cupressoides*, *S. obtusifolium*, *Cladophora crispula*, and *Acanthophora spicifera* had higher soluble carbohydrate and ash contents and lower insoluble fibre and lipid contents, whereas *Caulerpa* spp., *Carpophyllum flexuosum*, and *Monostroma oxyspermum* had higher insoluble fibre and lipid content and lower soluble carbohydrate and ash content. Trade-offs between different macronutrients in seaweeds have been reported in a few studies (Wong and Cheung 2000; Marinho-Soriano et al. 2006; Mwalugha et al. 2015; Kumar et al. 2015a), suggesting that different species may invest more energy into the allocation of particular nutrients, possibly for defence against herbivorous grazers and other threats (Cronin 2001). Furthermore, relationships between different macronutrients among seaweed species could be influenced by cultivation processes and conditions, and further research is required to determine the generality of these trade-offs and the factors influencing the magnitude of the trade-off.

Although nutritional composition data was gathered and analysed from 182 seaweed species across 81 different studies, it is important to note the numerous caveats of these data. Many studies did not provide mean values or their corresponding SD or SE values for all macronutrients, particularly for insoluble fibre and soluble carbohydrate content. Moreover,

approximately 60% of the studies included used the nitrogen-free extract calculation or ‘by difference’ method for soluble carbohydrate determination. Given that this method doesn’t provide an independent analytical measure of soluble carbohydrate content (Fiset et al. 2017), we excluded soluble carbohydrate values for studies using the ‘by difference method’. Consequently, less than half of the published values for soluble carbohydrate content met our quality control criteria and were excluded, influencing the precision of true soluble carbohydrate content estimates among seaweeds in our analysis. Additionally, we excluded studies that had missing values for more than two macronutrients, as they also did not meet our quality control criteria. Many studies also lacked information on the particular laboratory methods used to analyse the macronutrients, more specifically, the type of solvent used for lipid extraction or the nitrogen-to-protein conversion factor used in protein determination, as well as the year, month(s), and seasons the seaweeds were collected, limiting our ability to examine these factors as drivers of variation in seaweed nutritional composition. Despite these caveats, our analysis provides insight into broad-scale patterns in nutritional content among three major seaweed phyla and the determinants of variation in the reported values for macronutrients among different seaweeds.

In summary, our findings reveal both biological and non-biological factors driving variation in nutritional composition among seaweeds across a wide geographical scale. We found considerable among-study variation for both insoluble fibre and soluble carbohydrate content, among-species variation for ash, lipid, and protein content, and notable sampling error variation for lipid content. To an extent, methodological differences among studies can influence nutritional content in seaweeds, although recommendations for managing these impacts are not well known. Our analysis provokes further investigation into the establishment of standardized analytical methods for determining macronutrient composition in seaweeds. Additionally, our findings highlight the nutritional diversity among individual seaweed species and encourage deeper exploration of the potential causes of nutritional differentiation at the species level. Global demand for the commercial utilisation of seaweeds is steadily increasing, as well as knowledge of the nutritional value in commercially important species. Therefore, we must gain a more thorough understanding of seaweed biochemistry by standardising the analytical techniques used to determine their nutritional composition, thus allowing for greater reproducibility for future research in this field.

Chapter 3: Nutritional composition of common, coastal seaweeds from Mathesons Bay, Leigh, northeastern New Zealand

3.1 Abstract

Seaweeds are ecologically important in marine ecosystems, providing biogenic habitat for various marine organisms. Marine herbivores utilise seaweeds for food, as they contain macronutrients necessary for growth. Humans commercially harvest and cultivate seaweeds, and global demand for seaweed-based products is escalating. The utility of seaweeds in commercial industries is often governed by their nutritional value; hence, knowledge of their nutritional composition is becoming increasingly valuable. Despite an advanced understanding of seaweed biochemistry in other countries, there is limited knowledge of nutritional content of New Zealand seaweeds. Here, we analysed the nutritional composition of eleven seaweed species collected from Mathesons Bay, Leigh, northeastern New Zealand using conventional proximate composition analysis methods. We found notable differences in nutritional composition among both phyla and species. At the phylum level, the red seaweeds were highest in protein and insoluble fibre, the brown seaweeds were highest in lipids and soluble carbohydrates, and the green seaweeds were highest in moisture and ash. At the species level, the red seaweeds *Asparagopsis armata* and *Pterocladia capillacea* were highest in protein, while *Corallina officinalis* was highest in ash and much lower in soluble carbohydrates, insoluble fibre, lipids, and protein and thus lower in overall nutritional value. The brown seaweeds *Carpophyllum maschalocarpum* and *Cystophora retroflexa* were highest in soluble carbohydrates, *C. retroflexa* was highest in lipids, and *Xiphophora chondrophylla* had considerably higher insoluble fibre than all other species examined. The green seaweeds *Codium fragile* and *Ulva lactuca* were highest in moisture overall. *Asparagopsis armata* and *P. capillacea* demonstrate potential for aquaculture and thus indicate a need for larger-scale sampling of these species throughout New Zealand. In summary, this study describes differences in nutritional content among coastal northeastern New Zealand seaweeds and provides direction for future analyses.

3.2 Introduction

Seaweeds provide a range of important ecological services in marine ecosystems, and have major roles in coastal habitats (Harley et al. 2012). Several services that seaweeds provide include primary production, protection and shelter for vulnerable organisms, breeding and spawning areas, as well as other services (Terawaki et al. 2001). Additionally, seaweeds are a rich and valuable food source for marine herbivores as they contain various macronutrients that are important for growth in development in many species (Steneck et al. 2017).

Generalist marine herbivores are heavily dependent on seaweed communities and coexist together in the same habitat (Poore et al. 2012; Hawkins et al. 2017; Robin et al. 2017; Navarro-Barranco et al. 2018). Specifically, sea urchins, fish, and molluscs are considered dominant herbivores in both temperate intertidal and subtidal communities (Cebrian and Uriz 2006; Chenelot and Konar 2007; Monteiro et al. 2009; Vergés et al. 2009), grazing directly on kelps and various other seaweed species for nutrition (Montgomery and Gerking 1980; Neighbors and Horn 1991). Marine herbivores can heavily influence the abundance and distribution of seaweeds, as well as benthic community structure across ecosystems (Lubchenco and Gaines 1981; Poore et al. 2012).

Highly mobile herbivores such as fish have much higher energy demands than that of sessile and slow-moving invertebrates and can consume a wide range of seaweeds daily (Hay 1997; Monteiro et al. 2009). Many herbivorous fish species are selective feeders, with some preferring tender and palatable seaweeds, and others regularly consuming structurally rigid or chemically deterrent seaweeds (Horn 1989). For example, adult New Zealand butterfish (*Odax pullus*) are exclusively herbivorous and have shown preferences for brown seaweeds such as *Carpophyllum maschalocarpum*, *Undaria pinnatifida*, *Ecklonia radiata*, and *Macrocystis pyrifera* (Clements and Choat 1993; Baker et al. 2016; Johnson et al. 2017). As juveniles, *O. pullus* have a short carnivorous post-settlement stage and are known to selectively feed on seaweeds with epiphytic red seaweeds which are higher in protein, to help facilitate rapid growth (Clements and Choat 1993). The stichaeid fish species *Cebidichthys violaceus* and *Xiphister mueosus* have a seasonal feeding regime, actively shifting their selection for four annual seaweeds, three red (*Microcladia coulteri*, *Pyropia perforata*, and *Smithora naiadum*) and one green species (*Ulva lobata*), when they are most abundant (Horn et al. 1982). Additionally, the relative selectivity for other perennial red seaweeds increases

in spring and winter seasons when the abundance of the preferred annual seaweeds is low or unavailable. The seasonal change in food preference for *C. violaceus* and *X. mueosus* indicates that these fish could be optimizing growth and a higher nutritional intake through a mixture of different seaweeds (Horn et al. 1982). Furthermore, the grazing behaviour of selective herbivores can influence the abundance, distribution, and community structure of seaweeds, which can heavily impact reef ecosystems (Sala and Boudouresque 1997; Thacker et al. 2001; Russ 2003; Ceccarelli et al. 2006).

In response to herbivory, many seaweeds have evolved secondary metabolites and morphological defence strategies against herbivores, or alternatively, grow in seasons or locations where herbivores are absent (Duffy and Hay 1990; Steneck et al. 2017; Pereira et al. 2017). Alongside nutritional value, chemical deterrents are considered important factors in herbivore feeding preferences, as they can inhibit digestive processes or decrease palatability to different grazers (Targett and Targett 1990; Feeny 1992; Meyer et al. 1994; Hay et al. 1998). Secondary metabolites such as terpenes, aromatic compounds, phlorotannin polyphenolics, bioactive polymers, glycolipids, and amino-acid derived substances are produced by seaweeds (Faulkner 1984, 2001). Many seaweeds store defensive metabolites in areas where they are less exposed to herbivores, for example, Steinberg (1984) found that the kelp *Alaria marginata* stored a greater concentration of phenolic compounds in its reproductive tissues rather than its fronds or blades. Additional to chemical defenses, seaweeds employ morphological and structural defenses and responses, with a diverse range of shapes, sizes, and textures, from intricate filaments, turfs, and tough leathery blades to calcified corallines and solid crusts (Duffy and Hay 1990). Freshly produced segments in *Halimeda* seaweeds are usually nutritionally valuable, however, they also hold a heavy concentration of chemical deterrents. As the segments mature, they become less nutritious and thus less preferable to grazers, therefore, the concentration of defensive chemicals decreases (Hay et al. 1988). Additionally, *Halimeda* seaweeds promote the growth of their newest, most susceptible tissues at night, when predatory herbivorous fish are absent (Steneck et al. 2017). In some brown and green seaweeds, the concentrations of specific nutrients can vary in different areas of the thallus (Westermeyer and Gómez 1996; Cronin and Hay 1996b; Ortiz et al. 2006), for example, the stipes and blades of *Durvillaea antarctica* were found to contain different concentrations of protein and soluble carbohydrates (Duarte et al. 2011). Furthermore, nutritional content in seaweeds is often affected by herbivory, as seaweeds have adapted to grazers by changing the quantities of certain nutritional and

chemical compounds to appear as a less favourable food source, decreasing their vulnerability to grazing.

Studies have shown relationships between seaweed morphology and their vulnerability to herbivores, with many species demonstrating phenotypic responses to different grazing rates and behaviours (Littler and Littler 1980; Steneck and Watling 1982; Lewis et al. 1987). The calcareous, rigid nature of coralline red seaweeds protects them from many herbivores, and therefore are abundant in habitats with intensive grazing (Littler and Littler 1980; Steneck 1983, 1986; Duffy and Hay 1990; Paine and Vadas 1969; Steneck et al. 2017). Furthermore, many seaweeds can identify their herbivores and retaliate by using the most suitable chemical defense to protect themselves (Steneck et al. 2017). Variation in the mouthparts of many herbivores is also correlated with seaweed morphology (Steneck and Watling 1982), for example, modified spatulate chelae and gastric mill of some *Mithrax* crab species enables them to feed on tough, leathery seaweeds (Coen 1987). Additionally, coralline herbivores such as chitons and limpets have evolved flat spiral coiling morphology in their intestine (Sigwart and Schwabe 2017), strong buccal muscles, and enhanced dentition for greater excavation when feeding (Steneck and Watling 1982). Furthermore, it is clear that marine herbivores can shape marine ecosystems dominated by seaweeds, however, seaweeds can respond in many ways and have employed a range of successful defensive strategies for protection.

Many herbivorous species have specific nutritional requirements that vary according to individual characteristics such as energy demand, reproductive status, body composition, growth rate, and age (Raubenheimer et al. 2009; Simpson and Raubenheimer 2012). Accordingly, the nutritional value of seaweeds can play a pivotal role in food choice of herbivorous grazers (Neighbors and Horn 1991), for example, in Barile et al. (2004) sea hares (*Aplysia californica*) were offered the red seaweed *Gracilaria ferox* of varying nutritional value in a number of experimental trial diets and showed strong preference for higher protein and low carbohydrate diets. Furthermore, *A. californica* grew four times faster on higher protein diets than diets with lower protein, indicating that herbivores can optimize their diet through careful food selection. Given their potential as fundamental determinants for nutritional value, protein, lipids, and carbohydrates, are considered essential components in herbivore diets (Neighbors and Horn 1991; Shpigel et al. 1999; Lamare and Wing 2001; Johnson et al. 2017). Accordingly, when limited to lower quality seaweeds, some herbivores

will demonstrate ‘compensatory feeding’ which involves increasing their intake of nutrient-poor seaweeds to both meet and balance their nutritional requirements (Cruz-Rivera and Hay 2001; DeLucia et al. 2012).

Humans are also becoming increasingly reliant on seaweeds (Kumar et al. 2008; Peñalver et al. 2020). In countries such as China, Japan, and Korea, many seaweed species are a major part of the traditional diet, additionally, they are used as delicacies in Western countries and in the food, hydrocolloid, medical, pharmaceutical, aquaculture, and agriculture industries (Kumar et al. 2008; Bixler and Porse 2011; Arioli et al. 2015; Barba 2017; Charrier et al. 2017). The utilization of seaweeds in aquaculture is steadily increasing, as they are being cultivated alongside different aquaculture species for bioremediation and are being used in a wide range of artificial feeds for a variety of commercial shellfish and fish species (Troell et al. 2009; Murty and Banerjee 2011; Bansemer et al. 2016). The commercial importance of seaweeds as ingredients in artificial feeds is determined by their nutritional value and richness in important macronutrients, for example, the partial inclusion of species such as *Pyropia*, *Ulva*, and *Sargassum* in artificial diets for fish species such as red sea bream (Kalla et al. 2008), rainbow trout (Soler-Vila et al. 2009), striped mullet (Wassef et al. 2001), European sea bass (Valente et al. 2006), and olive flounder (Pham et al. 2006) showed significant improvements in growth and development. Macronutrient concentrations in seaweeds can vary greatly, and determining which seaweeds have the potential to improve growth is an important focus of much aquaculture research.

Seaweed nutritional composition largely differs among both individual species and phyla (Chapter two; McDermid and Stuercke 2003; Marsham et al. 2007; Mwalugha et al. 2015; Murugaiyan and Sivakumar 2020), as well as environmental conditions (Dayton 1985; Yates and Peckol 1993; Gerard 1997; Britton et al. 2021). For example, in some species, protein and lipid content is often high in winter and low in summer (Galland-Irmouli et al. 1999; Terasaki et al. 2017), whereas others show high protein content during summer and low lipid content during winter (Khairy and El-Shafay 2013). Other factors such as increased turbidity, phytoplankton abundance, and high nutrient availability in warmer temperatures can affect soluble carbohydrate production in red seaweeds, and accordingly, some species produce higher quantities of soluble carbohydrates when water temperatures cool (Sfriso et al. 2017). Alongside environmental factors, nutrient concentrations in seaweeds can also be affected by the maturity or life-stage of the thallus (Ito and Hori 1989; Muñoz and Fotedar

2011; Chan and Matanjun 2017; Praiboon et al. 2018; Jayasinghe et al. 2018), as higher nutrient uptake in younger, developing sporophytes can elevate their nutrient concentrations at a faster rate than older sporophytes (Mortensen 2017). Nonetheless, there is limited research regarding nutrient physiology of seaweeds at different life-history stages (Roleda and Hurd 2019).

The nutritional chemistry of seaweeds has been widely studied using proximate composition analysis methods (Nguyen et al. 2011; Hwang et al. 2013b; Siddique et al. 2013a; Siddique et al. 2013b; Bhuiyan et al. 2016; Nunraksa et al. 2019). Despite the wide availability of research on seaweed biochemistry, there is still a large proportion of seaweed biodiversity that is yet to be studied (Gressler et al. 2011; Jayasinghe et al. 2018). Despite high seaweed biodiversity in countries such as Australia and New Zealand, their seaweed industries are still in their infancy (Birch et al. 2019; Skrzypczyk et al. 2019; White and White 2020), hence, there is limited research available regarding the nutritional composition of Australasian seaweeds (Hurd et al. 2004; Boulom et al. 2014; McCauley et al. 2015). In New Zealand, studies have focused on calorific content (Lamare and Wing 2001), proximate composition and heavy metal analysis (Brown et al. 1999; Smith et al. 2010), and fatty acid and amino acid profiles (Boulom et al. 2014; Zhou et al. 2015; Diehl et al. 2019). Regarding nutritional content, Lamare and Wing (2001) found high ash content in *Corallina officinalis* and high calorific content in *Cystophora scalaris*, and Smith et al. (2010) found high protein content in *Pyropia* spp. and *Ulva stenophylla*. Nevertheless, research is needed to build a comprehensive understanding of the nutritional potential of New Zealand seaweeds, and unique opportunities that New Zealand's diverse seaweed flora provide.

Here, we analyse the nutritional composition of eleven common, coastal seaweed species from three different phyla using traditional proximate composition analysis methods. The seaweeds were collected from Mathesons Bay, Leigh, New Zealand during the months of September, October, and November, to avoid confounding seasonal effects. Given that seaweed morphology varies widely, we might expect that there will be large differences in the six macronutrients both between phyla and species in each phylum. After reviewing the relevant literature, we expected that protein content would be higher in red seaweeds, lipid and insoluble fibre content would be higher in brown seaweeds, and soluble carbohydrate content would be higher in green seaweeds. Additionally, given its highly calcified structure, we might expect ash content to be highest in red seaweed *Corallina officinalis*, and moisture

content to be high across all species we examine. Our analyses provide insight into the macronutrient profiles of a range of New Zealand seaweeds using proximate composition analysis methods, which may help elucidate important and unique nutritional characteristics of seaweeds for potential commercial aquaculture feed production in New Zealand.

3.3 Methods

3.3.1 Sample collection

Samples of eleven seaweed species were collected from Mathesons Bay, Leigh, Auckland in the North Island of New Zealand (36°18'09"S 174°47'56"E) during the months of September to November 2019 (Table 2). Mathesons Bay is a small beach protected by a rocky islet and further protected from most oceanic swell by Great Barrier Island. The species were chosen due to their abundance at the study site. All samples were collected from live seaweeds attached to rocky substrata either by snorkelling or scuba diving between the depths of approximately 2-10 metres where they were easily accessible. We used a New Zealand seaweed identification guide (Nelson 2013) to identify seaweed species for collection. We collected thalli from five to ten individuals of each species and placed them in two to three large zip-lock bags. The zip-lock bags were filled with seawater to cover entire thalli and kept in a cooler for immediate transferral to the laboratory (Skrzypczyk et al. 2019).

Table 2. Seaweed species collected for this study

Species	Phylum
<i>Asparagopsis armata</i>	Rhodophyta
<i>Corallina officinalis</i>	Rhodophyta
<i>Pterocladia capillacea</i>	Rhodophyta
<i>Carpophyllum maschalocarpum</i>	Ochrophyta
<i>Cystophora retroflexa</i>	Ochrophyta
<i>Ecklonia radiata</i>	Ochrophyta
<i>Sargassum sinclairii</i>	Ochrophyta
<i>Xiphophora chondrophylla</i>	Ochrophyta
<i>Zonaria turneriana</i>	Ochrophyta
<i>Codium fragile</i>	Chlorophyta
<i>Ulva lactuca</i>	Chlorophyta

Within three hours of sample collection, each individual thallus was cleaned and rinsed with seawater to remove excess debris, sand, epiphytes, and any attached organisms (Polat and Ozogul 2009; Rohani-Ghadikolaei et al. 2012; Mwalugha et al. 2015; Ahmad et al.

2016). For each species, thalli were placed into air-tight zip-lock bags labelled with the collection date and species name and stored in a -80°C freezer until further analysis. Given that each zip-lock bag contained thalli from multiple individuals, our analysis did not require correction for repeated measures from individuals and samples were instead considered methodological replicates.

Before chemical analysis and to analyse moisture content on a wet weight (WW) basis, the wet samples were placed into individually labelled 50mL falcon tubes and placed upright on a tray inside a freeze-dryer (LabConco FreeZone 6) without lids for approximately 72 hours at -45°C under 0.100 millibar (mbar) vacuum conditions. Once freeze-dried, the samples were ground into a fine powder using a mortar and pestle, until the particles were able to pass through a 0.5mm sieve (Polat and Ozogul 2009; Benjama and Masniyom 2012; Rohani-Ghadikolaie et al. 2012). The powdered samples were then placed into individually labelled, 50mL flat-bottomed falcon tubes and stored in the -80°C freezer until chemical analysis.

3.3.2 Moisture analysis

The moisture content in each seaweed species was determined according to Association of Official Analytical Chemists (AOAC) 930.15 with slight modifications (AOAC 1990). First, after weighing a fresh, labelled 50mL falcon tube (excluding lid weight), approximately two grams (2.000g) of wet seaweed sample was placed into the tube, and the weight of the tube and sample together was recorded. After freeze-drying the sample, the tube was reweighed, and moisture content was calculated as the difference between the wet and dry samples, accounting for the weight of the tube, and multiplying by 100. All determinations were carried out in triplicate to estimate measurement error in the laboratory.

3.3.3 Ash analysis

The ash content in each seaweed species was determined according to AOAC 942.05 with slight modifications (AOAC 1990). First, a copper crucible was heated for one hour in an oven then cooled in a desiccator for approximately 30 minutes. After labelling the cooled

crucible, the weight was then recorded and approximately 2.000g of the freeze-dried seaweed sample was placed inside the crucible and spread uniformly on the inner base before recording the weight of the crucible and sample together. The sample was then charred over a Bunsen burner inside a fume hood for approximately 20 minutes or until the entire top of the sample was charred to an even black colour. Whilst charring, care was taken to ensure that the sample did not ignite. Once the sample was charred completely, the crucible was placed inside a muffle furnace with the temperature set at 525°C and left for 5-6 hours. After letting the furnace cool down, the crucible containing the remaining ash was then removed from the muffle furnace and placed inside a desiccator for approximately 45 minutes to cool. Once the crucible was cool, it was weighed accurately (0.001g) and ash content was calculated as the difference between the sample weights before and after ashing, accounting for the weight of the crucible, and multiplied by 100. All determinations were carried out in triplicate.

3.3.4 Lipid analysis

The lipid content in each seaweed species was determined using the Soxhlet method, as described by AOAC 920.39 with slight modifications (Soxhlet 1879; AOAC 1990; De Castro and Garcia-Ayuso 1998), using a Büchi B-811 Universal Extraction System with n-hexane as the solvent. Before analysis, the Büchi B-811 Universal Extraction System was set up in a fume hood, to follow the Soxhlet Standard setting, which has three major steps: extraction, rinsing, and drying. Using the operation panel, the settings were adjusted specifically for a six-hour extraction period, a one-hour rinsing period, and a 30-minute drying period. Approximately 5.000g of freeze-dried seaweed sample was accurately weighed into a 25 x 80-millimetre labelled cellulose extraction thimble and placed carefully into an upright position inside the extraction chamber. The condenser was then gently pushed downwards and screwed clockwise onto the chamber until it was closed. The weight of a 250mL glass Büchi beaker was recorded and approximately 170mL of n-hexane was then carefully added to the beaker and gently screwed clockwise into position underneath the extraction chamber, pushing the lever for the heating element upwards and underneath the beaker. Once the apparatus was assembled, the protective shields were pulled downwards and the unit was plugged in and turned on, along with the tap letting cooling water through the condenser. When necessary, n-hexane was carefully added to the beaker through a glass funnel above the

condenser, and a blank determination on the solvent only was carried out at the same time as our focal samples.

Once all three steps were complete, the soxhlet apparatus was turned off and left to cool for approximately 30 minutes. The beaker containing the extracted lipid and any remaining solvent was then carefully unscrewed from the apparatus and left in the fume hood for 48 hours for further drying. Once there was no trace of any solvent in the lipid extract, the weight of the beaker and lipid extract was recorded. The change in weight between the initial and final beaker was the total lipid present in the sample, which was then multiplied by 100. The fat-free sample remaining in the thimble was removed from the apparatus and left to dry in the fume hood for an additional 48 hours. Once dry, the fat-free sample was placed into a clean, labelled, 50mL flat-bottomed falcon tube, and placed into the -80°C freezer, ready for insoluble fibre analysis. All determinations were carried out in triplicate.

3.3.5 Insoluble fibre analysis

The crude fibre content in each seaweed species was carried out using the acid-base digestion method, described by AOAC 978.10 with slight modifications (AOAC 1990). The acid-base digestion method has four main steps: boiling in acid, boiling in base, drying the sample, and incinerating the sample. To prepare 0.128M sulfuric acid (H_2SO_4), approximately 300mL of distilled water was added to a 500mL labelled volumetric flask, followed by 3.60mL of 95% H_2SO_4 , and another 200mL distilled water to make up the final volume of 500mL. The flask was then rotated to mix the contents. To prepare, 0.313M sodium hydroxide (NaOH), 300mL of distilled water was added to a 500mL labelled volumetric flask, followed by 6.250g of pure NaOH pellets. The solution was then swirled a few times until the pellets dissolve completely. Another 200mL distilled water was added to make up the final volume of 500mL. The preparation of reagents was repeated when necessary.

Approximately 200mL of 0.128M H_2SO_4 was then added to a labelled 500mL conical flask, followed by approximately 2.000g of lipid-free seaweed sample, then the solution was swirled to mix the contents. The flask was boiled very gently on a hot plate for exactly 30 minutes, with periodic agitation every five minutes for preventing adherence of the sample to the walls of the flask. The boiled solution was then poured into a glass funnel covered with a

cotton cloth and into a 1000ml waste flask, to drain the acid solution and collect the acid-digested residue on the cloth. The empty flask was then rinsed with hot water, which was poured into the funnel, draining any remaining acid and collecting any leftover residue.

The cloth containing the residue was washed with approximately 200mL of 0.313M NaOH into the cleaned conical flask via a new funnel, ensuring all residue is washed into the new flask. The flask was then swirled to mix the contents and boiled gently for exactly 30 minutes with periodic agitation every five minutes. The solution was then poured into funnel through a new cloth and into the waste flask, draining the NaOH and collecting the base-digested residue on the cloth. The empty flask was then rinsed with hot water, which was poured into the funnel, draining any remaining NaOH and collecting any leftover residue. The residue was then collected from the cloth using a clean and dried 40mL porcelain crucible, leaving no residue remaining on the cloth. The crucible was then placed on the hot plate for 30 minutes to evaporate any excess water, and into a hot air oven at 65°C and left to dry overnight. Next, the crucible was cooled in a desiccator for 30 minutes and weighed. The crucible was then incinerated in a muffle furnace at 550°C for 5 hours, cooled in a desiccator for 30 minutes, and reweighed. The loss in weight between the digested and ashed sample multiplied by 100 is the crude fibre content. All determinations were carried out in triplicate.

3.3.6 Protein analysis

The protein content in each seaweed species was determined using the Kjeldahl method (Kirk 1950; AOAC 1990; Lynch and Barbano 1999), following AOAC 988.05 with slight modifications. There are three major steps in the Kjeldahl method: digestion, distillation, and titration. In the digestion step, approximately 1.000g of freeze-dried seaweed sample was placed onto a piece of filter paper, folded three times, and inserted into a labelled digestion tube. Two Kjeltabs, each containing 3.500g potassium sulfate (K_2SO_4) and 0.0035g selenium (Se), and 25mL 98% sulfuric acid (H_2SO_4), were then added to the digestion tube as catalysts. The 2300 Kjeltac™ Analyzer Unit and Tecator™ Scrubber unit were set up using the 3401 Kj N Animal Feed AOAC setting at a low temperature with gradual increases in heating over time, and finally at 420°C for approximately 60 minutes or until the solution reached a colourless, transparent appearance. For each sample the total digestion time was approximately 120 minutes, and a blank digestion was carried out at the same time (i.e., no

sample, but all reagents). Once the digestion process was complete, the tube was carefully removed from the heating unit and left to cool.

In the distillation step, approximately 50mL 4% boric acid solution was added to a labelled 250mL conical flask. The Kjeltac™ 8100 Distillation Unit was prepared and set up using the Program three setting with a total distillation time of three minutes and 40 seconds. The conical flask was then placed into position underneath the plastic hose in the centre of the unit and once cool, the digestion tube containing the digested sample solution was connected into position with the plastic hose inside the tube. The safety door was closed, and the start button was pressed to begin the distillation process. During the distillation process the boric acid solution changed from reddish pink to a dark green colour, and the digested sample solution was distilled completely, leaving no residue remaining.

Last, in the titration step, the dark green ammonium-borate complex remaining in the conical flask was then titrated with 0.1M hydrochloric acid to a grey-mauve end point, with both the start and finish values recorded. The protein content was calculated by first calculating the percentage of total nitrogen, which involved multiplying the millilitres of HCl used, molarity of HCl used, atomic mass of nitrogen, and 100 together, then dividing by the original sample weight (g) multiplied by 1000. To calculate protein content, the percentage of total nitrogen was multiplied by the nitrogen-to-protein conversion factors recommended by Angell et al. (2016) for each of the three seaweed phyla: 5.10 for red seaweeds, 4.49 for green seaweeds, and 4.56 for brown seaweeds. All determinations for each step were carried out in triplicate.

3.3.7 Soluble carbohydrate analysis

The soluble carbohydrate content or nitrogen-free extract (NFE) in each seaweed species was calculated using the conventional Weende or 'by difference' method (Lloyd et al. 1978; Ranjhan 1993; Talreja et al. 2015). For each seaweed species, NFE was calculated as the difference by subtracting the sum of ash, protein, lipid, and insoluble fibre from 100 (Talreja et al. 2015). The NFE value represents the estimated value of non-structural, soluble carbohydrates in the sample, which includes sugars, some starches, and digestible polysaccharides (Talreja et al. 2015).

3.3.8 Statistical analysis

To examine variability in the six macronutrients across the eleven seaweed species we considered, we used a linear mixed model fit using the MCMCglmm package for R version 3.6.0 in the RStudio environment (Hadfield 2010; RStudio 2021). The five macronutrients moisture, ash, insoluble fibre, lipid, and protein were considered in a single multivariate model. Soluble carbohydrate content was calculated using the ‘by difference’ method, and thus, it is not independent of quantities of the macronutrients used to calculate the difference, and was considered in a separate univariate analysis.

For the multivariate linear mixed model, the response variable represented the values for each of the macronutrients and the fixed effects were macronutrient (i.e., moisture, ash, insoluble fibre, lipid, protein) and species as well as the macronutrient by species interaction. For the residual, an inverse-Wishart prior distribution was used with the scale parameter equal to a diagonal matrix with the total variance for each macronutrient along the diagonal and the shape parameter equal to 4.002 as it is commonly used for variance components (Hadfield 2010). The final MCMC model had a total of 130,000 iterations, a burn-in period of 30,000 iterations, and a thinning interval of 100 iterations, resulting in 1000 posterior samples of the distribution of each term in the model.

For soluble carbohydrate content, the response in the univariate linear mixed model was the values for soluble carbohydrate content and the fixed effect was species. For the residual, the scale parameter was equal to the total variance for soluble carbohydrate content and a shape parameter of 0.002 was used. The final MCMC model had a total of 130000 iterations, a burn-in period of 30,000 iterations, and a thinning interval of 100 iterations, resulting in 1000 posterior samples of the distribution of each term in the model. The ‘emmeans’ package and function was then used to compute the posterior means and corresponding 95% highest posterior density (HPD) intervals for the five macronutrients (Lenth 2021), which were then plotted using the ‘ggplot2’ and ‘ggpubr’ packages (Wickham et al. 2021; Kassambara and Kassambara 2020).

3.4 Results

Moisture content was very high for all seaweeds, ranging from 51.701% to 92.419% (Figure 5A). Green seaweeds (phylum chlorophyta) *C. fragile* and *U. lactuca* were greatest in mean moisture content, with values of 92.419% (95% HPD = 86.891, 98.748) and 85.824% (95% HPD = 80.059, 91.791), respectively. Mean moisture content among the brown seaweeds (phylum ochrophyta) ranged from 63.436% to 83.792%, with the highest values for *E. radiata* and *S. sinclairii* ($\bar{x} > 80\%$). Red seaweeds (phylum rhodophyta) had lower mean moisture content overall ($\bar{x} = 69.129\%$), with the lowest value for *C. officinalis* ($\bar{x} = 51.701\%$, 95% HPD = 46.074, 57.909).

Ash content varied widely, ranging from 19.830% to 82.950% (Figure 5B). *Corallina officinalis* and *C. fragile* were highest in mean ash content, with values of 82.950% (95% HPD = 73.580, 93.282) and 66.905% (95% HPD = 57.384, 77.247), respectively. Overall, green seaweeds had the highest mean ash content ($\bar{x} = 57.477\%$), followed by red seaweeds ($\bar{x} = 49.963\%$), and brown seaweeds were lowest in mean ash content ($\bar{x} = 28.106\%$), with the lowest values for *X. chondrophylla* ($\bar{x} = 19.830\%$, 95% HPD = 11.031, 30.058), *C. maschalocarpum* ($\bar{x} = 23.661\%$, 95% HPD = 12.713, 32.269), and *Z. turneriana* ($\bar{x} = 25.107\%$, 95% HPD = 15.473, 35.240). Soluble carbohydrate content was highly variable among the sampled seaweeds, ranging from 8.145% to 64.477% (Figure 5C). Mean soluble carbohydrate content was highest in brown seaweeds ($\bar{x} = 56.246\%$) with largest values for *C. maschalocarpum* ($\bar{x} = 64.477\%$, 95% HPD = 62.345, 66.617) and *Cystophora retroflexa* ($\bar{x} = 63.682\%$, 95% HPD = 61.519, 65.716). Red and green seaweeds were much lower in mean soluble carbohydrates ($\bar{x} = 30.971\%$, $\bar{x} = 31.673\%$, respectively), with lowest values for *C. officinalis* ($\bar{x} = 8.145\%$, 95% HPD = 6.256, 10.274), and *C. fragile* ($\bar{x} = 23.902\%$, 95% HPD = 21.511, 25.898).

Insoluble fibre content ranged from 2.710% to 22.030% (Figure 5D), with the highest values for *X. chondrophylla* ($\bar{x} = 22.030\%$, 95% HPD = 19.145, 24.808) and *P. capillacea* ($\bar{x} = 11.791\%$, 95% HPD = 9.236, 14.632). Red and brown seaweeds were similar in mean insoluble fibre content ($\bar{x} = 8.336\%$ and $\bar{x} = 8.176\%$, respectively), although across all sampled seaweeds, the brown seaweed *C. retroflexa* was lowest in mean insoluble fibre ($\bar{x} = 2.710\%$, 95% HPD = -0.178, 5.250). Green seaweeds were lower in mean insoluble fibre ($\bar{x} =$

4.972%) compared to the other phyla, with the lowest value for *C. fragile* ($\bar{x} = 2.713\%$, 95% HPD = 0.216, 5.477). Lipids had the smallest contribution to the nutritional composition of the seaweeds we examined, ranging from 0.193% to 2.445% (Figure 5E). Brown seaweeds, on average, had higher mean lipid content ($\bar{x} = 1.489\%$), with the highest values for *C. retroflexa* ($\bar{x} = 2.445\%$, 95% HPD = 1.960, 3.040) and *S. sinclairii* ($\bar{x} = 1.870\%$, 95% HPD = 1.396, 2.477). Red and green seaweeds were lower in mean lipid content ($\bar{x} = 0.754\%$ and $\bar{x} = 0.844\%$, respectively), with the lowest values for *C. officinalis* ($\bar{x} = 0.193\%$, 95% HPD = -0.434, 0.689), *P. capillacea* ($\bar{x} = 0.245\%$, 95% HPD = -0.342, 0.798), and *U. lactuca* ($\bar{x} = 0.464\%$, 95% HPD = -0.072, 0.973).

Protein content ranged from 3.289% to 13.354% and was greatest in the red seaweeds ($\bar{x} = 9.918\%$) (Figure 5F), with the highest values for *A. armata* ($\bar{x} = 13.354\%$, 95% HPD = 11.654, 14.937) and *P. capillacea* ($\bar{x} = 13.111\%$, 95% HPD = 11.533, 14.916). Protein content in *C. officinalis* was lowest across all seaweeds examined ($\bar{x} = 3.289\%$, 95% HPD = 1.449, 5.031). For the brown seaweeds, mean protein content ranged from 4.211% to 7.026%, with the highest values for *E. radiata* ($\bar{x} = 7.026\%$, 95% HPD = 5.486, 8.833) and *X. chondrophylla* ($\bar{x} = 6.961\%$, 95% HPD = 5.174, 8.601), while on average, green seaweeds were lowest in mean protein content ($\bar{x} = 5.032\%$).

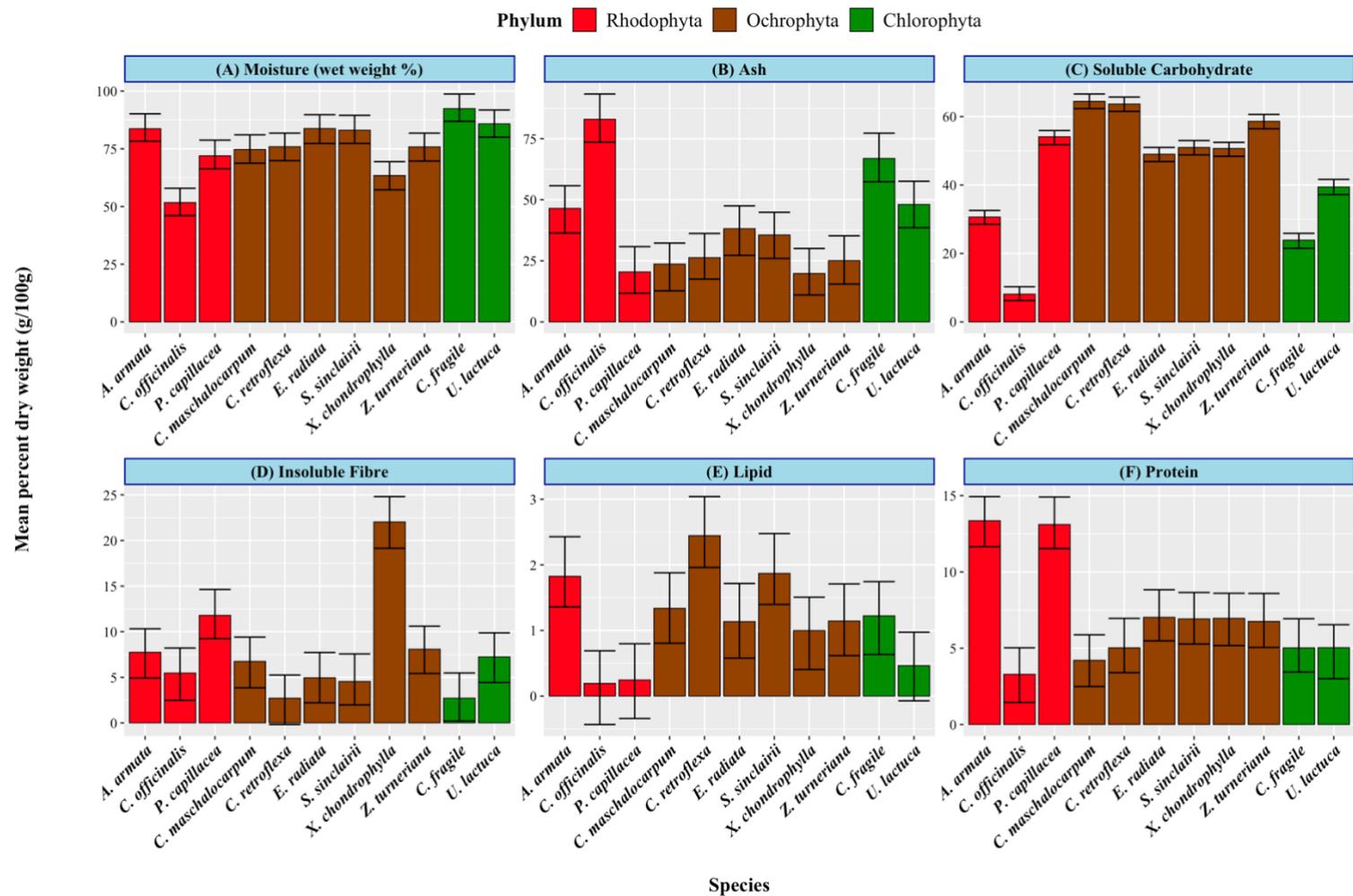


Figure 5. Nutritional composition of eleven common New Zealand seaweed species from three major phyla. Values are represented as posterior means and 95% highest posterior density (HPD) intervals for the six macronutrients considered in proximate analysis. All macronutrients are expressed as a dry weight percentage except moisture content (wet weight %) and analysed in triplicate for each nutritional component for each species. Moisture (A), ash (B), insoluble fibre (D), lipid (E), and protein (F) were considered in a single multivariate model whereas soluble carbohydrate content (C) was calculated using the ‘by difference’ method and was considered in a separate univariate analysis (see methods for details).

3.5 Discussion

Globally, the use of seaweeds in industries such as food production and pharmaceuticals has increased significantly (Kumar et al. 2008; Charrier et al. 2017; Stévant et al. 2017). While the nutritional value of particular seaweeds has been thoroughly studied in many parts of the world, information regarding the nutritional characteristics of New Zealand seaweeds is scarce (Smith et al. 2010; Kindleysides et al. 2012). Here, we analysed the nutritional composition of eleven different seaweed species from three major phyla commonly found across northeastern New Zealand coastlines, using standard proximate composition analyses. Of the species we examined, red seaweeds, on average, were highest in protein and insoluble fibre, brown seaweeds were highest in lipids and soluble carbohydrates, and green seaweeds had greater moisture and ash content. At the species level, the red seaweed *C. officinalis* had the lowest nutritional value overall, whilst *A. armata*, *P. capillacea*, and *C. retroflexa* had attributes (i.e., higher protein, lipid, and soluble carbohydrate content) associated with higher nutritional value. Although additional study is required to thoroughly evaluate the commercial importance and value of the seaweeds examined, some species showed nutritional characteristics that could be potentially useful in New Zealand's small but currently growing seaweed industry (White and White 2020; Stenton-Dozey et al. 2020).

The moisture and ash content of seaweeds is often strongly dependent on the morphological structure of the species and can vary between seasons (Kaehler and Kennish 1996; Renaud and Luong-Van 2006; Polat and Ozogul 2013). For example, *C. fragile* had the greatest mean moisture content ($\bar{x} = 92.419\%$, 95% HPD = 86.891, 98.748) and a high mean ash content ($\bar{x} = 66.905\%$, 95% HPD = 57.384, 77.247). The tubular structure of *C. fragile* may result in a high capacity for retaining moisture (García et al. 2016), with the value reported here comparable to those reported in other *Codium* species and other green seaweeds (> 90% wet weight [WW]; McDermid and Stuercke 2003; Nguyen et al. 2011; Nagappan and Vairappan 2014; García et al. 2016; Ahmad et al. 2016). Additionally, the ash content of *C. fragile* reported here is comparable to previously reported ash contents for *C. fragile* collected from Çanakkale Strait in Turkey during the winter (67.3%) and spring (66.9%) seasons (Irkin and Erdugan 2016). The high ash contents reported for *Codium* spp. could be due to the structural complexity of their interior interwoven filament morphology (Schneider and Searles 1991). The brown seaweeds *E. radiata* and *S. sinclairii* also had high moisture

contents ($\bar{x} > 80\%$ WW), with similar values reported for *Sargassum* spp. in other studies (McDermid and Stuercke 2003; McDermid et al. 2007; Ahmad et al. 2016; Debbarama et al. 2016). The flattened laminar structure of both *E. radiata* and *S. sinclairii* may allow for greater moisture absorption and uptake due to a larger surface area.

Ash content often negatively correlates with other nutrients in seaweeds and hence lowers their overall nutritional value (see Chapter two; Kumar et al. 2015b). For instance, the calcareous coralline seaweed *C. officinalis* was much lower in moisture content ($\bar{x} = 51.701\%$, 95% HPD = 46.074, 57.909) and much higher in ash ($\bar{x} = 82.950\%$, 95% HPD = 73.580, 93.282) relative to the other sampled seaweeds. Similarly, lower moisture contents (< 45% WW) and high ash contents (> 75% dry weight [DW]) have been reported for both *C. officinalis* and *Corallina sessilis* studied in other parts of the world (Lamare and Wing 2001; Marsham et al. 2007; You et al. 2014), and it is likely that the calcareous coralline morphology of *Corallina* spp. underlies both lower moisture and higher ash contents in seaweeds within this genus (Bilan and Usov 2001; Piazzini et al. 2011). *Corallina officinalis* also had the lowest lipid ($\bar{x} = 0.193\%$, 95% HPD = -0.434, 0.689), protein ($\bar{x} = 3.289\%$, 95% HPD = 1.449, 5.031), and soluble carbohydrate content ($\bar{x} = 8.145\%$, 95% HPD = 6.256, 10.274) out of all seaweeds examined, giving it the lowest overall nutritional value. The lower ash contents of brown seaweeds *X. chondrophylla* ($\bar{x} = 19.830\%$, 95% HPD = 11.031, 30.058) and *Z. turneriana* ($\bar{x} = 25.107\%$, 95% HPD = 15.473, 35.240) we recorded were relatively similar to those previously reported for *Xiphophora gladiata* (24.3%) and *Z. turneriana* (33.7%) collected from Doubtful Sound, Fiordland, New Zealand, also sampled during spring (Lamare and Wing 2001). Given that both *X. chondrophylla* and *Z. turneriana* have a rather flattened, fleshy structure (Rice 1989; Phillips 1997; Nelson et al. 2018), it is likely that their ash content would be much lower than that of rigid and more anatomically complex species.

Although seaweeds are usually low in insoluble fibre content, higher values (20-44%) have been reported for particular brown species such as *Fucus* spp., *Gongolaria abies-marina*, and *Zonaria tournefortii* (Rupérez and Saura-Calixto 2001; Paiva et al. 2018; Fonseca et al. 2021). In comparison to the other sampled seaweeds, the brown seaweed *X. chondrophylla* had exceptionally high insoluble fibre ($\bar{x} = 22.030\%$, 95% HPD = 19.145, 24.808). Given the current lack of nutritional research available for *X. chondrophylla*, the

ultimate cause of the greater insoluble fibre content in this species is unknown, although their bushy, clustered morphological structure and structural cell wall constituents could be contributing factors (Rice 1989). Of the species examined, insoluble fibre content was highest among the red seaweeds ($\bar{x} = 8.336\%$) and although the difference between the red and brown seaweeds was slight, this result was somewhat unexpected and possibly due to the high insoluble fibre content found for *P. capillacea* ($\bar{x} = 11.791\%$, 95% HPD = 9.236, 14.632). Among the two green seaweeds examined (*C. fragile* and *U. lactuca*), insoluble fibre content for *C. fragile* ($\bar{x} = 2.713\%$, 95% HPD = 0.216, 5.477) was similar to previously reported values for *C. fragile* (5.10%; Guerra-Rivas et al. 2011), *Codium isthmocladum* (1.01%; Robledo and Freile Pelegrin 1997), and other *Codium* spp. (1.40%; García et al. 2016). Given that low values of both insoluble fibre and soluble carbohydrate content have been reported for *Codium* spp. (Robledo and Freile Pelegrin 1997; McDermid and Stuercke 2003; McDermid et al. 2007; García et al. 2016), the range and abundance of polysaccharides present in Codiaceae seaweeds may be characteristically lower than other seaweeds.

Lipid content in seaweeds generally ranges between 1-5% DW (Montgomery and Gerking 1980; Kumari et al. 2010; Miyashita et al. 2013) and often varies taxonomically, morphologically, phenologically, and environmentally (Nelson et al. 2002; Terasaki et al. 2009; Stengel et al. 2011; Miyashita et al. 2013; García et al. 2016). Brown seaweeds had the greatest lipid content ($\bar{x} = 1.489\%$), with the highest values recorded for *C. retroflexa* ($\bar{x} = 2.445\%$, 95% HPD = 1.960, 3.040) and *S. sinclairii* ($\bar{x} = 1.870\%$, 95% HPD = 1.396, 2.477). Similarly, high lipid contents have been reported for other *Sargassum* spp. (1.56%, 1.45%, 2.02%) in other parts of the world (Li et al. 2010; Rodrigues et al. 2015; Pirian et al. 2020). The relatively low lipid content we recorded for *U. lactuca* ($\bar{x} = 0.464\%$, 95% HPD = -0.072, 0.973) was comparable to 0.60% reported in Jayasinghe et al. (2019) and 0.50% in Marsham et al. (2007). Although the reasons for lower lipid content in *Ulva* spp. are relatively unknown, differences may be caused by environmental effects or specific lipid extraction conditions within the laboratory. For instance, in Kumari et al. (2011) the Cequier-Sánchez method (Cequier-Sánchez et al. 2008) gave a very low lipid yield for *Ulva fasciata* whereas the Folch method (Folch et al. 1957) produced very high lipid yield, therefore, lipid recovery is highly dependent on the solvent used, temperature, and extraction time (Kumari et al. 2011; Santos et al. 2015).

Protein and soluble carbohydrates are important macronutrients in seaweeds, they are often used as ingredients in aquaculture feeds and are integral components of marine herbivore diets (Neighbors and Horn 1991; Bansemer et al. 2016; Johnson et al. 2017). Red seaweeds had the greatest protein content ($\bar{x} = 9.918\%$), with the highest protein values recorded for *A. armata* ($\bar{x} = 13.354\%$, 95% HPD = 11.654, 14.937) and *P. capillacea* ($\bar{x} = 13.111\%$, 95% HPD = 11.533, 14.916), although these values are slightly lower than 17.55% previously reported for *Asparagopsis taxiformis* and 13.40% reported for *P. capillacea* (McDermid et al. 2007; Nunes et al. 2017), which could be due to among-species differences as well as environmental factors such as seasonality or location (Peters et al. 2005; Tabassum et al. 2016a; Fiset et al. 2017). The high protein content and thus high nutritional value for *A. armata* contrasts with our earlier results demonstrating lower overall nutritional value in the congener *A. taxiformis* (see Chapter two, Figure 4 of this thesis). Although this could be indicative of strong among-species differences with the genus, in our meta-analysis we accounted for among-study differences, measurement error, and differences in the mean levels of each macronutrient in each phylum, whereas here we did not partition variation and instead focused on overall nutritional yield in different species among phyla. Soluble carbohydrate content was highest in the two brown seaweeds *C. maschalocarpum* ($\bar{x} = 64.477\%$, 95% HPD = 62.345, 66.617) and *C. retroflexa* ($\bar{x} = 63.682\%$, 95% HPD = 61.519, 65.716) and are comparable to previously reported values of 69% and 66.8% for other *Carpophyllum* spp. (Zhang et al. 2020), and 64.1%, 64.9%, and 67.5% for *Cystophora* spp. (Torbatinejad and Sabine 2001). Nonetheless, the similarity of these values could be due to the concordant usage of the ‘by difference’ method for soluble carbohydrate estimation (see Chapter two of this thesis). Furthermore, the seaweeds we analysed showed unique nutritional diversity, and thus, the higher protein contents of the red seaweeds *A. armata* and *P. capillacea* demonstrate that they could be potentially beneficial candidates for cultivation in future.

Nutritional preferences for different seaweeds have been widely studied in marine molluscs. For example, New Zealand black-foot pāua (*Haliotis iris*), yellow-foot pāua (*Haliotis australis*), and Australian abalone species have shown preferences for red seaweeds over brown seaweeds (Poore 1972; Shepherd 1973; Wells and Keesing 1989; McShane et al. 1994). Although, these preferences could be due to the presence of defensive polyphenolic compounds in many brown seaweeds that deter grazers (Steinberg 1984, 1988, 1989). In

contrast, kelps and brown crustose seaweeds are a highly preferred food source for both *H. iris* and South African abalone *Haliotis midae* (Barkai and Griffiths 1988; Wood and Buxton 1996; Cornwall et al. 2009). Food choice in abalone highly depends on factors such as digestible nitrogen content, chemical defenses, palatability, morphology, accessibility, and abundance (Poore 1972; McShane et al. 1994; Fleming 1995; Wood and Buxton 1996). Given that we found higher protein content in the two red seaweeds *A. armata* and *P. capillacea*, abalone may select seaweeds that will increase their body protein levels and thus increase their growth rate (Fleming 1995). Valuable growth-limiting nutrients such as protein play a key role in abalone feeding preferences and therefore may be indicative of the preference for red seaweeds in some herbivores. Herbivorous fish species reportedly select seaweeds based on both nutritional content and their ability to digest nutrients, however, unlike abalone, they select seaweeds that will maximise their energy from starch components (storage carbohydrates) rather than protein (Zemke-White and Clements 1999). For example, New Zealand black angelfish (*Parma alboscapularis*), marblefish (*Aplodactylus arctidens* and *A. etheridgii*), and parore (*Girella tricuspidata*) reportedly favour green and red seaweeds with higher digestible starch content and lower protein content such as *Ulva* spp., *Caulerpa geminata*, and *Champia novae-zelandiae* (Zemke-White and Clements 1999; Raubenheimer et al. 2005). Although we found low soluble carbohydrate and protein content in the green seaweeds *C. fragile* and *U. lactuca* and higher soluble carbohydrate content in the two brown seaweeds *C. maschalocarpum* and *C. retroflexa*, a direct measure of digestible starch content appears to be a reliable indicator of diet structure in grazing fish (Zemke-White and Clements 1999; Raubenheimer et al. 2005). The ability of marine herbivores to digest and assimilate nutrients from seaweeds plays a key role in their nutritional ecology.

The nutritional composition of the sampled seaweeds we present should be interpreted with the caveat that nutritional composition can be influenced by several environmental factors (Marinho-Soriano et al. 2006; Polat and Ozogul 2013; Khairy and El-Shafay 2013; Britton et al. 2021; Garcia-Vaquero et al. 2021). For instance, the seaweeds were collected in the Austral Spring, when seaweed protein content often peaks (Galland-Irmouli et al. 1999; Osman et al. 2011; Polat and Ozogul 2013; Kumar et al. 2015b; Paiva et al. 2018; Garcia-Vaquero et al. 2021). Similarly, lipid content in some seaweeds has been reported to be higher in winter and spring and lower in summer (Nelson et al. 2002). In some brown seaweeds, soluble carbohydrate content has been reported to accumulate during summer and autumn and is then utilised or consumed as an energy source during winter (Rosell and

Srivastava 1984; Zimmerman and Kremer 1986; Adams et al. 2011; Tabassum et al. 2016a, b). Additionally, higher concentrations of ash have been reported for specific brown seaweeds during winter (Schiener et al. 2015; Landa-Cansigno et al. 2017) and are often influenced by the relative concentrations of inorganic compounds and salts present in the water column (Kumar et al. 2015b). Alongside seasonal variation, small-scale variation in salinity, temperature, depth, wave action, and sunlight exposure could have also affected the nutritional content and nutrient uptake rates in the seaweeds examined here (Floreto and Teshima 1998; Perfeto 1998; Dere et al. 2003; Marinho-Soriano et al. 2006; Hepburn et al. 2007; Nielsen et al. 2016). Given that the sampled seaweeds were collected from a single location during a single season, site-specific variation or seasonal variation between seaweeds was not examined, which would have allowed for more general comparisons in nutritional composition among species and phyla. Seaweeds can exhibit remarkable plasticity in their nutritional content as a response to fluctuating environmental parameters, and further examination of the forces governing this variability is required (Stengel et al. 2011).

Additional to environmental factors, it is important to recognise methodological biases among laboratory procedures used to analyse nutritional composition (see Chapter two of this thesis). Sample preparation methods each have their own biases (Fiset et al. 2017; Nurshahida et al. 2018), for example, insufficient drying of samples before chemical analysis often leads to increased moisture content thereby affecting estimates of macronutrient content (Fiset et al. 2017). Drying methods that use higher temperatures, such as oven-drying, can cause degradative changes and substantial nutrient loss in samples (Chan et al. 1997; Wong and Cheung 2001), therefore, freeze-drying is often recommended as it reduces both sample damage and the occurrence of chemical reactions that could change the nutritional characteristics of the sample (Wong and Cheung 2001). Nonetheless, Smith (1973) discovered that enzymes in freeze-dried sample tissue can remain functional if any moisture is present or the temperature increases during storage. Given that our samples were carefully freeze-dried, although the COVID-19 global pandemic interrupted our laboratory schedule, the samples were stored at -80°C for less than six months and it is unlikely that significant changes in nutritional content occurred during storage. Additionally, research has found that the solvent n-hexane can result in an underestimation of total lipid content as it is weaker in perforating membranes in seaweeds compared to more high-risk solvents such as petroleum ether or chloroform (Kumari et al. 2011; Lu et al. 2015; Yuvarani et al. 2017; Laroche et al. 2019). Due to time constraints, we used the conventional Weende or 'by difference' method

to estimate nitrogen-free extract (NFE) or otherwise known as soluble carbohydrate content. Although the ‘by difference’ method is still widely used for determining soluble carbohydrate content, is not a direct analytical procedure (Merrill and Watt 1955; Cherney 2000; Dennis et al. 2006; Talreja et al. 2015; Fiset et al. 2017). Direct analytical procedures for estimating soluble carbohydrate content such as the phenol-sulfuric or anthrone method are likely to result in lower soluble carbohydrate contents than those produced by the ‘by difference’ method (Fiset et al. 2017). Given its convenience and simplicity, we used the acid-base sequential digestion or crude fibre method to determine insoluble fibre content. Although this method is known to underestimate insoluble fibre content due to the inefficient hydrolysis of structural polysaccharides (Van Soest and McQueen 1973; Knudsen 2001), to minimise error we strictly followed the AOAC recommendations to ensure no deviation or sample loss (AOAC 1990).

While our study provided insight into the nutritional composition of some common New Zealand seaweeds, there are many fruitful future research avenues to consider. First, wider taxonomic diversity and thus the analysis of a larger number of species would have allowed for greater comparisons between phyla. Additionally, sampling seaweeds from different locations across different seasons would enable the assessment of both spatial and temporal variation in the nutritional composition of New Zealand seaweeds. Second, there is currently a lack of research investigating variation in seaweed nutritional composition across different depths, between the juvenile and mature stages, and between different body parts of the seaweeds such as the stipe or lamina. The latter could provide insight into the specific thallus structures of New Zealand seaweeds that hold the heaviest concentrations of macronutrients and how that may correlate with grazing of marine herbivores. Third, regarding laboratory procedures, we would recommend using the phenol-sulfuric method for soluble carbohydrate determination given its ability to detect different classes of carbohydrates and its greater reliability than the outdated ‘by difference method’ (Dubois et al. 1956; Masuko et al. 2005; Fiset et al. 2017). Similarly, we would advise using the modified enzymatic-gravimetric method for determining insoluble fibre content, as it more strongly isolates different fibre fractions in samples and thus provides estimations of insoluble, soluble, and total dietary fibre (McCleary et al. 2012). Due to laboratory safety reasons, we used n-hexane as a lipid extraction solvent; however, a solvent with a higher extraction efficiency such as a chloroform-methanol mixture may provide greater lipid yield (Kumari et al. 2011). Nonetheless, it is important to note that the most appropriate method for

lipid extraction can vary for different seaweed phyla therefore, care must be taken when selecting specific solvents for determining lipid content in particular seaweed species in future (Kumari et al. 2011).

Our research highlights the nutritional diversity and potential value of some common seaweed species found in northeastern New Zealand, and many of our results are comparable to those from similar research both in New Zealand and other parts of the world. Our analysis revealed large differences in specific macronutrients among seaweeds, and we found that protein content in the two red seaweeds *A. armata* and *P. capillacea* may be of potential commercial interest in New Zealand. If additional research was carried out using the same seaweed species collected from multiple locations across different seasons, as well as the measurement of different environmental parameters, we would expect significantly greater differences in nutritional content among species, which would help build a stronger foundation for nutritional research on New Zealand seaweeds. Furthermore, only a few seaweed species with potential for commercial cultivation have been established in New Zealand, therefore, there is a prime opportunity to enhance both the development and innovation of the New Zealand seaweed aquaculture industry.

Chapter 4: General Discussion

In this thesis, I examined drivers of variability in seaweed nutritional composition among different phyla and species at global and local scales. Overall, values for protein and soluble carbohydrate content were generally higher in red seaweeds, values for insoluble fibre and lipid content were higher in brown seaweeds, and values for ash content were higher in green seaweeds. Additionally, I showed that variation in nutritional characteristics of seaweeds can be heavily influenced by biological differences among species, but more significantly, procedural effects arising from methodological differences among studies. In particular, my findings highlighted major biases introduced by methodological choices for both insoluble fibre and soluble carbohydrate determination as well as the effects of measurement error on the reported values for lipid content. Notable variation was apparent among species for ash, lipid, and protein content, along with strong negative correlations between macronutrients likely due to morphological differences, suggesting that calcareous or encrusting seaweeds likely have lower protein and soluble carbohydrate content and thus lower overall nutritional value. Moreover, there were noticeable trade-offs between macronutrients, where some species exhibited higher (lower) soluble carbohydrate and ash content and lower (higher) insoluble fibre and lipid content. I then described the nutritional composition of eleven seaweed species common to northeastern New Zealand, where on average, red seaweeds had greater protein and insoluble fibre content, brown seaweeds had greater lipid and soluble carbohydrate content, and green seaweeds had greater moisture and ash content overall. Furthermore, considerable differences in nutritional content were prevalent across both seaweed phyla and species, which were likely subject to various biological and environmental factors.

My meta-analysis (Chapter two) supported the results of previous nutritional research on seaweeds (Holdt and Kraan 2011; Pereira 2011; Cherry et al. 2019; Shannon and Abu-Ghannam 2019), showing the strong effects of methodological differences between laboratories, methods, equipment, or assays on variation in insoluble fibre, soluble carbohydrate, and lipid content (Randall et al. 1991; Manirakiza et al. 2001; Fiset et al. 2017; Regal et al. 2020). Among the most popular laboratory methods used, the phenol-sulfuric method provided larger estimates of soluble carbohydrate content and larger estimates of variation than the anthrone method, although a significant difference between the two

methods was not found. Similarly, the acid-base sequential digestion method provided larger estimates of insoluble fibre content as well as larger estimates of variation than the enzymatic-gravimetric method. Despite the larger estimates of insoluble fibre produced by the acid-base sequential digestion method, this method often underestimates true insoluble fibre content due to solubilisation of structural tissues (Knudsen 2001) which could be a likely cause of lower insoluble fibre content (< 20%) in most of the seaweed species analysed in Chapter three. Additionally, the variability in the absorbance of different sugars in the phenol-sulfuric assay can be problematic when determining the true sugar content of sensitive samples (Taylor 1995), and both the phenol-sulfuric and anthrone methods include a hydrolysis step that can be subject to characteristic polysaccharides in different seaweeds or other samples (Taylor 1995; Fiset et al. 2017). Furthermore, both soluble carbohydrate and insoluble fibre content in different seaweeds analysed across the studies included in my analysis were likely susceptible to the sensitivity and precision of the different methods used to determine these macronutrients. Therefore, these findings indicate how strongly different laboratory procedures can influence estimates of macronutrient content and recommend that the methods used should be standardized to minimise procedural effects and associated biases.

Variation in reported lipid content attributable to measurement error was prevalent, given that lipids make up a very minimal component of seaweed dry weight (< 5%), lipid content may be especially sensitive to measurement error compared to other macronutrients. For example, sample contaminants, extraction solvents, equipment, sample type, and interference from external factors such as temperature or air exposure could have contributed to variability in lipid content (Randall et al. 1991; Manirakiza et al. 2001; Kumari et al. 2011). Additionally, different drying procedures can cause fluctuation in estimates of lipid content. For instance, given that lipids are prone to oxidation, prolonged drying periods and air exposure can often alter lipid yield (Randall et al. 1991; Choe and Min 2006; Fiset et al. 2017). Elevated temperatures used in oven-drying procedures are known to degrade lipids, and thus, lipid recovery in oven-dried samples is generally lower than that of freeze-dried samples (Randall et al. 1991; Chan et al. 1997; Fiset et al. 2017). While research has found that both lipid and fatty acid composition differ among seaweed species and phyla (Gosch et al. 2012; Santos 2019), the solubility of the lipid components in the extraction solvent used is crucial when determining lipid content and care must be taken when selecting an appropriate solvent for the chosen study species (Kumari et al. 2011). Within the laboratory environment,

many factors govern variability in estimates of lipid content in seaweeds, therefore, methods better suited to specific phyla or species could be employed to improve extraction efficiency in future.

At both global and local scales, on average, red seaweeds were highest in protein content, brown seaweeds were highest in lipid content, and green seaweeds were highest in ash content. Nonetheless, in my meta-analysis, brown seaweeds were highest in insoluble fibre content and red seaweeds were highest in soluble carbohydrate content, whereas in the New Zealand species I examined, red seaweeds were highest in insoluble fibre content and brown seaweeds were highest in soluble carbohydrate content. Further examination is required to determine if differences in both insoluble fibre and soluble carbohydrate between my meta-analysis and the New Zealand species I examined were due to the choice of species I examined in New Zealand. Variation in ash, lipid, and protein content among seaweed species is influenced by biological factors such as, but not limited to, morphology, genetics, life-history traits, and thallus maturity (Littler and Littler 1980; Cronin and Hay 1996b; Harrison and Hurd 2001; Elsaied et al. 2015). Moreover, the strong negative correlation shared between ash and soluble carbohydrate as well as protein implies that morphologically calcareous species such as *Corallina officinalis* are likely to be less nutritionally rich due to the trade-off between ash and other macronutrients, which was also evident in the New Zealand species I examined. Additional trade-offs between soluble carbohydrate and insoluble fibre content and between ash and lipid content present in species such as *Caulerpa* spp., *Sargassum obtusifolium*, *Cladophora crispula*, and *Acanthophora spicifera*, *Carpophyllum flexuosum* and *Monostroma oxyspermum* indicate that different seaweeds may employ individual nutrient allocation strategies, possibly as a defensive response to grazers (Cronin 2001). Although trade-offs between seaweed macronutrients have been reported (Wong and Cheung 2000; Marinho-Soriano et al. 2006; Kumar et al. 2015b), the drivers of these trade-offs are relatively poorly understood.

In Chapter two, I found that *Asparagopsis taxiformis* had low nutritional value compared with the other seaweed species examined, however, in Chapter three I found that *Asparagopsis armata* had high nutritional value. Additionally, species such as *Ulva pertusa* and *Carpophyllum flexuosum* showed characteristics of higher nutritional value in my results for Chapter two but in Chapter three the two New Zealand species *Ulva lactuca* and *Carpophyllum maschalocarpum* had moderate values for nutritional content overall. The

differences between the estimated nutritional value of these species between my two data chapters could have been due to biological differences between species from the same genus or differences in our analytical approach. For instance, because I was interested in dissecting the drivers of variability in nutritional composition in Chapter two, my model accounted for differences in the mean levels of each macronutrient in each phylum, differences among studies, and measurement error as well as among-species differences. In contrast, in Chapter three I specifically focused on identifying seaweed species likely to produce higher yield of the five macronutrients. Accordingly, although some of the high nutritional value associated with species such as *Asparagopsis armata* could be due to phylogenetic effects, the aim of Chapter three was to identify species with high nutritional value, not the drivers of differences in nutritional value. Last, it is important to recognise that seaweed species and their congeners can greatly differ in their nutritional composition, despite their morphological similarity (Stengel et al. 2011).

The influence of environmental factors likely played a part in prompting variability in macronutrients among the seaweeds examined in both Chapters two and three. Given that sampling was carried out during the spring season (Chapter three), specific macronutrients such as protein or lipid content in some species may have responded positively to rising spring temperatures (Nelson et al. 2002; Marinho-Soriano et al. 2006; Madden et al. 2012; Polat and Ozogul 2013; Boulom et al. 2014). Additionally, seasonal temperature changes have been reported to influence both soluble carbohydrate and ash content in brown seaweeds (Adams et al. 2011; Schiener et al. 2015; Kumar et al. 2015a; Tabassum et al. 2016a; Landa-Cansigno et al. 2017). Seasonality is only one of many environmental factors that could have influenced nutritional composition in the seaweeds sampled, as sunlight exposure, temperature, salinity, wave intensity, as well as the availability of nutrients in the water column can contribute to fluctuations in seaweed macronutrient composition (Dawes 1998; Kumar et al. 2015a; Kumar et al. 2015b; Nielsen et al. 2016; Britton et al. 2021). Given that environmental factors are highly variable, it is important to recognise the scope of their influence on seaweed nutritional content. Furthermore, baseline knowledge of both seasonal and spatial variation in seaweed macronutrients is essential to understanding the implications for commercially cultivated species in future.

Although thorough quality control was carried out in Chapters two and three, the data I collected has some limitations. First, given the lack of seasonal, monthly, and yearly data

provided by many studies, I could not assess both seasonal and temporal trends in nutritional content among seaweed proximate composition studies (Chapter two). Additionally, some studies chose not to quantify all six macronutrients and did not specify the laboratory methods used, while others did not provide necessary statistics such as standard deviation or standard error values, or the number of replicates analysed. The wide prevalence of missing data across studies severely limited the sample size available for robust meta-analysis and synthesis. Second, larger sample sizes and a more diverse selection of species across the different seaweed phyla present in New Zealand would have allowed for more general conclusions. If circumstances had allowed, I would have additionally chosen important species such as *Undaria pinnatifida*, *Macrocystis pyrifera*, *Gracilaria chilensis*, *Pyropia* spp., *Durvillaea* spp. (White and White 2020) and a wider range of green species that may include *Caulerpa* spp., other *Ulva* spp., *Codium* spp., and *Cladophora* spp. As previously stated in Chapter three, collecting seaweeds from multiple locations, across different depths, seasons, as well as comparing nutritional content across different aged thalli or different body parts would be beneficial focal areas for future research. Third, given that my time in the laboratory was heavily constrained due to the COVID-19 pandemic, I was limited in the methods I could use to examine insoluble fibre and soluble carbohydrate content. Therefore, in future, I would advise the use of the modified enzymatic-gravimetric method for insoluble fibre content (McCleary et al. 2012) and a modernised phenol-sulfuric assay for soluble carbohydrate content (Masuko et al. 2005). Regardless of these caveats, my study examined major drivers of variation in seaweed nutritional composition, the implications for this variation, recommendations for future work, and a first attempt to catalogue the nutritional composition of some distinctive seaweed species commonly found in northeastern New Zealand.

As the need for climate change mitigation strategies increases, furthering the development of seaweed aquaculture may offer a range of opportunities for reducing global carbon dioxide emissions (Duarte et al. 2017). In particular, research promoting the use of seaweeds as tools for climate change mitigation and adaptation may help relieve the present constraints on commercial seaweed cultivation in New Zealand, and thus a robust knowledge base must be built for species with valuable attributes. Moreover, recognition of the commercial importance of the widely prevalent introduced invasive species *U. pinnatifida* is increasing, as it has the potential to offer a range of high-end products to the international market (White and White 2020). New Zealand's nutrient-rich, temperate coasts along with its

unique seaweed flora make it a prime environment for seaweed aquaculture, and thus, research will provide insight into useful species for commercial utilisation (Hurd et al. 2004). As seaweed research in New Zealand becomes increasingly driven by international market trends, the primary goal for researchers will be to both identify and successfully mass-produce profitable seaweeds (Hurd et al. 2004).

Using similar methods as to what I have implemented here, but focusing on assessing nutritional variation between sites, depths, seasons, bodily structure, as well as young and old thalli will allow for a wider spectrum of comparisons among different seaweed populations in New Zealand. Additionally, future studies should carefully consider the impacts of different quantification methods on the estimates of macronutrients in seaweeds, with a focus on specific effects between phyla and individual species. A proposal for a better specified, standardised suite of proximate composition methods should be constructed for future analyses to enhance the precision and repeatability of methods currently used in nutritional research. Within New Zealand, seaweeds represent a vast and untapped resource that could provide numerous economic and ecological benefits as well as research opportunities. Furthermore, the new and potentially highly active seaweed industry will enable renewable diversification and innovation of the respective aquaculture sector in New Zealand.

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Appendix 1: List of international studies included in the meta-analysis

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