J. Phycol. **55,** 1361–1369 (2019)

DOI: 10.1111/jpy.12913

THE MACROMOLECULAR COMPOSITION OF NONCALCIFIED MARINE MACROALGAE[1]

Catherine Fiset[2]

Environmental Science Program, Mount Allison University, 62 York Street, Sackville, New Brunswick E4L 1E2, Canada

Andrew J. Irwin

Department of Mathematics and Computer Science, Mount Allison University, 62 York Street, Sackville, New Brunswick E4L 1E2, Canada

Department of Mathematics and Statistics, Dalhousie University, 6316 Coburg Rd, Halifax, Nova Scotia B3H 4R2, Canada

and Zoe V. Finkel

Environmental Science Program, Mount Allison University, 62 York Street, Sackville, New Brunswick E4L 1E2, Canada

Department of Oceanography, Dalhousie University, 6299 South St, Halifax, Nova Scotia B3H 4R2, Canada

The macromolecular composition of macroalgae influences nutrient flow and food quality in aquatic ecosystems and the value of macroalgae species for human consumption, aquaculture, biofuels, and other applications. We used literature data (125 publications, 1,117 observations) and a hierarchal Bayesian statistical model to estimate the average macromolecular composition, protein, lipid, and carbohydrate of macroalgae as a whole and at the phylum level. Our focus was on marine, noncalcified macroalgae sampled from wild-grown populations in the field. We found that the median macromolecular composition is 9.98% protein, 2.7% lipid, 48.5% carbohydrate, and 31.8% ash as percent dry weight. We compared the median macromolecular content of macroalgae to microalgae and herbaceous plants and test for differences in macromolecular content across macroalgal phyla. Macroalgae were much more enriched in carbohydrate and minerals than the microalgae and lower in protein and lipid than many herbaceous plants. Rhodophyte macroalgae have significantly less lipid and more protein and the Ochrophyte macroalgae have significantly less protein than the average.

Key index words: **ash; carbohydrates; Chlorophyta; lipids; macroalgae; Ochrophyta; protein; Rhodophyta**

***Abbreviations*: DW, dry weight**

Macroalgae are found on coastlines from the Arctic to the Antarctic (Coyer 2007) and cover ~1.4 to 6.8 × 10^6 km^2 (Duarte 2017). Macroalgae can be both abundant and productive (Smith 1981, Duarte and Cebrian 1996, Duarte and Chisnaco 1999). As a consequence, the macromolecular and elemental composition of macroalgae can influence higher trophic levels and biogeochemical cycles in aquatic ecosystems (Dooley 1972, Hawkins and Hartnoll 1983, Lapointe 1986, Duarte 2017). The macromolecular content of macroalgae reflects their own nutritional requirements and physiological capacity to respond to environmental conditions and indicates their nutritional value for grazers. For instance, species with a high protein content tend to have lower carbon to nitrogen ratios and higher nitrogen to phosphorus ratios than species with a lower protein composition and this can influence competition for resources between species (Tilman et al. 1982, Sterner and Hessen 1994, Lynn et al. 2000).

The macromolecular composition of macroalgae is of interest to humans, who use macroalgae for aquaculture feed (Nayar and Bott 2014), food products (Fleurence et al. 1999, Nayar and Bott 2014, FAO 2016), phycocolloid extracts (principally alginate, agar, and carrageenan, which are used as thickening agents; FAO 2016), iodine extraction (FAO 2016, Wells et al. 2017), and possible biomedical and biofuel development (Gosch et al. 2012, Neveux et al. 2014, Deniaud-Bouët et al. 2017). The macromolecular composition of a specific macroalgae species can be used as an indicator of how useful they are to certain applications. For example, the high-protein species *Porphyra tenera* (nori) and *Palmaria palmata* (dulse) are commonly used as food sources due to their nutritive value (Mabeau and Fleurence 1993). High-lipid species are being studied for biofuel development (Gosch et al. 2012), and high-phycocolloid species such as *Kappaphycus*, *Eucheuma*, and *Gracilaria* are used to extract phycocolloids, which are a component of the carbohydrate pool (Ito and Hori 1989). The elemental and macromolecular composition of seaweeds could influence the efficiency of specific seaweeds for coculture with finfish and shrimp aquaculture (Chopin et al. 2001) or for coastal CO_2 sequestration (Chung et al. 2017).

[1]Received 17 September 2018. Accepted 6 August 2019. First Published Online 16 August 2019. Published Online 11 October 2019, Wiley Online Library (wileyonlinelibrary.com).
[2]Author for correspondence: e-mail cfiset@mta.ca.
Editorial Responsibility: C. Amsler (Associate Editor)

Despite the wide interest in the macromolecular composition of macroalgae and observed variance among taxa, the average macromolecular composition of macroalgae and the differences across the major evolutionary lineages are not well quantified. Classic reviews on the macromolecular content of macroalgae (Ito and Hori 1989, Darcy-Vrillon 1993, Mabeau and Fleurence 1993, Fleurence et al. 1999) have provided estimates of the broad ranges of values (see Table 1) but did not provide a measure of central tendency or variance on macromolecular content. This makes it difficult to identify macroalgae phyla or species or measurements with anomalous macromolecular content. The three macroalgal phyla have different characteristic carbohydrates in their cell walls: ulvan in some members of the Chlorophtya; carrageenan and agar in the Rhodophyta; and alginate and sulfated polysaccharides with fucose in the Ochrophyta (Deniaud-Bouët et al. 2017), and different characteristic storage compounds, for example, isofloridoside in Rhodophyta, and mannitol and laminaran in Ochrophyta (Ito and Hori 1989, Wells et al. 2017). A recent meta-analysis quantified statistically significant differences in protein content across macroalgal phyla (Angell et al. 2015, 2016), but lipid and carbohydrate analyses have yet to be determined. In aggregate, protein, lipid, and carbohydrate content accounts for the majority of organic C and N and, therefore, provides a functional basis for understanding macroalgal C to N ratios.

Researchers have been conducting regional surveys on the macromolecular composition of macroalgae to find species with especially high-protein, -lipid, or -carbohydrate content for use in industry, aquaculture, biofuel development, or other applications, so the literature on macroalgae macromolecular content is plentiful (Jayasankar et al. 1990, Heiba et al. 1997, McDermid and Stuercke 2003, Banerjee et al. 2009, Manivannan et al. 2009, Gómez-Ordóñez et al. 2010, Biancarosa et al. 2017). Here, we compiled and analyzed a database of protein, lipid, carbohydrate, and ash (mineral) content in macroalgae to quantify the median macromolecular composition and C:N of macroalgae. We contrasted the median macromolecular composition of macroalgae with microalgae and herbaceous plants and quantify differences in the macromolecular composition across the three macroalgae phyla. Quantifying the macromolecular composition and C:N of macroalgae and the differences across phyla could improve our understanding of the influence macroalgae have on the biogeochemistry of aquatic ecosystems.

METHODS

Macroalgae macromolecular database. We assessed the protein, lipid, and carbohydrate content of macroalgae by analyzing data collected from the literature. Macromolecular data as percent dry weight (% DW) were gathered from the text, tables, and figures of 125 publications. These papers were published between 1931 and 2016 and were identified using Google Scholar Search between August 2015 to July 2016 using the following search terms: seaweed, macroalgae, Chlorophyta, Rhodophyta, Ochrophyta, green seaweeds, red seaweeds, brown seaweeds, macromolecular composition, biochemical composition, chemical composition, protein, fats, lipids, carbohydrates, fibre, nutritional value, nutritional composition, and calorie content. Data were extracted from

TABLE 1. Median macromolecular composition as percent dry weight across all macroalgae phyla in the current study and reviews by Ito and Hori (1989), Mabeau and Fleurence (1993), and Darcy-Vrillon (1993), as well as the median macromolecular composition of microalgae in active growth from Finkel et al. (2016), and the macromolecular composition of some herbs and leaves summarized by Finkel et al. (2016). The top value is the median, the middle values in parentheses represent the 95% credible interval on the median, and the bottom value is the number of experimental observations (*n*). The range and calculated median is given for Ito and Hori (1989), Mabeau and Fleurence (1993), and Darcy-Vrillon (1993) because that is what was provided. The carbohydrate values from Mabeau and Fleurence (1993) represent the fiber fraction of carbohydrates in macroalgae.

Macromolecule	Current study	Ito and Hori (1989)	Mabeau and Fleurence (1993)	Darcy-Vrillon (1993)[a]	Microalgae[b]	Herbs and leaves[b]
Protein						
Median	9.98	20	17.5	19.5	32.2	27.3
95% CI	(9.3, 10.6)	5–35	5–30	3–36	(30.4, 34.0)	
n	827				317	11
Lipid						
Median	2.7	2.0	2	1.8	17.3	5.1
95% CI	(2.5, 3.0)	0.2–3.8	1–3	0.1–3.5	(16.2, 18.2)	
n	406				375	11
Carbohydrate						
Median	48.5	54.5	44	54.5	15.0	56.3
95% CI	(44.1, 52.6)	35–74	33–55	38–71	(13.7, 16.5)	
n	92				308	11
Ash						
Median	31.8	30		21	17.3	
95% CI	(29.7, 33.8)	10–50		6–36	(15.4, 18.7)	
n	491				185	

[a]Data summarized by Darcy-Vrillon (1993) from Sautier (1987) and Sautier (1990).
[b]Data from Finkel et al. (2016).

figures using Image J (https://imagej.nih.gov/ij/). The current taxonomic status of all species documented was determined using AlgaeBase (http://www.algaebase.org/). Note that the nori species *Porphyra tenera* and *Porphyra yezoensis* were reclassified in 2011 into *Pyropia* (Sutherland et al. 2011), but we could not reclassify *Porphyra* sp. in the database due to insufficient data. Along with the macromolecular composition data, the method used to estimate macromolecular content and dry the samples was extracted and recorded. Only macroalgae samples that were collected in the field were included in the database, since laboratory-grown samples are often exposed to conditions that can alter their macromolecular composition (Bird 1984, Shpigel et al. 1999, Viera et al. 2005, Angell et al. 2014).

This macroalgal macromolecular database is available online at figshare (Fiset 2016), and includes references to the articles used to create it. This database is composed of a total of 1,054 observations for protein, 796 for lipids, and 917 for carbohydrates from 364 species gathered from 125 publications. Widely distributed macroalgae species and those of commercial interest, either as food sources or for their chemical properties, are overrepresented in the database. The most commonly occurring species in the database are *Ulva lactuca* ($n = 58$), *Palmaria palmata* ($n = 45$), and *Gracilaria corticata* ($n = 21$). The highly calcified genera *Halimeda*, *Amphiroa*, *Lithothamnion*, and *Calliarthron* were excluded from all analyses of macroalgal macromolecular composition because their calcium content significantly reduces their macromolecular content as % DW (Steneck and Martone 2007). Observations of these species relative to less and noncalcified species using different methods will affect estimates of median macromolecular composition as % DW.

Different biochemical and drying methods can bias estimates of macromolecular content as % DW. A full analysis of the methodological biases present in the database is documented in Fiset et al. (2017). Based on this analysis, we included protein observations based on measurements of nitrogen (converted to protein following Lourenço et al. 2002), amino acids, and colorimetric protein assays. Protein observations were excluded if they had been sun dried or were dried by blotting. Our database is complementary to the protein data compiled by Angell et al. (2016), with about 30% of the data in common. We included lipid observations that used oven and freeze drying and either the Folch et al. (1957) or Bligh and Dyer (1959) extraction methods, but excluded lipid observations generated using the AOAC method or if the methods used were not clearly identified. In addition, observations of lipid from four publications (Heiba 1990, Jayasankar 1993, Kaliaperumal et al. 1994, Gokulakrishnan et al. 2015) were removed from the data set prior to analyses due to consistently anomalously high values. For carbohydrate, observations generated using the By Difference method and freeze-dried or dried at room temperature were included in the analysis. In summary, we analyzed 827, 406, 58, and 491, protein, lipid, carbohydrate, and ash observations, respectively.

Bayesian analyses. To determine the median macromolecular composition of macroalgae, the macromolecular composition as % DW was computed using a hierarchical Bayesian model. We expressed each quantity as a sum of random variables for each species, phylum, and an overall mean as

$$y_i = \mu + P_{p[i]} + S_{s[i]} + e_i$$

where y_i are the observations, μ is the overall mean, P_j and S_j are the estimated means for each phylum and species, respectively, *p[i]* and *s[i]* are the phylum and species associated with observation *i*, respectively, and e_i is the residual error associated with each observation *i*. The estimates μ, P_j, and S_j were described by a normal distribution, and three distinct uninformative hierarchal priors were used for the variances of the species means, phylum means, and the residual error. All analyses were performed using R and Stan (R Core Team 2017, Stan Development Team 2018). The use of the hierarchical model leads to smaller variances than would be obtained using a classical regression, since it partially pools the data across taxonomic levels and allows for sharing of sampling strength across taxa. The overall mean of protein, lipid, and carbohydrate content was calculated from the phylum means weighted by their inverse variances following Finkel et al. (2016). We report the posterior median of the overall mean and the phylum means. We choose to report the median as a measure of central tendency of macromolecular content because it is less sensitive to a skewed distribution than the mean.

We also investigated the average macromolecular ratios (protein:lipid, protein:carbohydrate, lipid:carbohydrate; wt:wt) and C:N ratios (mol:mol) as estimated from the macromolecular composition of macroalgae. Ideally, all ratios would have been computed using the Bayesian hierarchal model described above with paired data in which each observation reported values for two or more macromolecules. This would account for how macromolecular composition varies within individual species. However, with the exception of the protein:lipid ratio ($n = 387$), the majority of our sample sizes for paired data were very limited (protein:carbohydrate, $n = 58$; lipid:carbohydrate, $n = 10$; C:N, $n = 10$). Sample sizes at the phylum level were as low as one observation for certain ratios, and so instead the ratios were calculated using the posterior distributions generated by the model described above. The macromolecular ratios were calculated by dividing the posterior distributions of the macromolecular content. The C:N ratios were calculated using the chemical compositions of the macromolecules as described by Geider and La Roche (2002) and the percent contributions of protein, lipid, and carbohydrate to macroalgae dry weight from their posterior distributions. The grand mean for macroalgae for each ratio was calculated as the mean of the phylum means weighted by their inverse variances. There was a sufficiently large sample size ($n = 387$) to compare the protein:lipid ratio calculated using the posterior distributions with the estimate using paired data. The percent differences ranged from 0.0 to 17% at the phylum level and the grand median in macroalgae (see Table S1 in the Supporting Information).

To test for differences in macromolecular composition across phyla, we constructed 95% credible intervals of the differences between phyla using their posterior distributions. The posterior distributions were generated from 2,000 iterations on four chains using RStan, where only the last 1,000 iterations were used (Stan Development Team 2018). We interpreted phylum-level medians as different if the 95% credible interval of their difference did not include zero.

RESULTS

Median macromolecular composition of macroalgae. The median macromolecular composition of macroalgae as % DW was 9.98% protein, 2.7% lipid, 48.5% carbohydrate, and 31.8% ash (Table 1). For context to previous work and other herbaceous photosynthetic organisms, we compared these results to ranges reported in previous studies on macroalgae, as well as the average macromolecular content of microalgae and selected herbs and leaves (Table 1). Note that the same statistical technique was used to

estimate median macromolecular composition for the microalgae but there are differences in the dominant biochemical methods used to estimate macromolecular composition across these data sets.

Phylum-level differences. Phylum-level differences in macromolecular and ash compositions were observed for macroalgae (Fig. 1). The Ochrophyta are lower in protein than the Chlorophyta and Rhodophyta, as well as the grand median for macroalgae. The Rhodophyta are lower in lipid content than the Chlorophyta and the grand median for macroalgae, and may be lower in lipid content than the Ochrophyta (Fig. 1). No differences in total carbohydrate content or ash were observed between phyla or the grand median.

The macromolecular and C:N ratios of macroalgae also varied according to phylum and reflect the differences observed in the median macromolecular composition (Fig. 2). The Ochrophyta had significantly lower protein:carbohydrate ratios and higher C:N ratios than the other phyla and the average in macroalgae (Fig. 2). No statistical differences were observed in the protein:lipid ratios or the lipid:carbohydrate ratios.

DISCUSSION

Macroalgal macromolecular data have been gathered from sites around the world over many decades to better understand the physiology, ecology, and biotechnological potential of individual species and assemblages from different regions. A series of review papers by Ito and Hori (1989), Darcy-Vrillon (1993), and Mabeau and Fleurence (1993) summarized knowledge, as of the early 1990s, of seaweed macromolecular content and provided estimates for the range of protein, lipid, and carbohydrate content in macroalgae as percent dry weight (% DW). Here, we quantitatively analyze our compilation of hundreds of observations of protein, lipid, carbohydrate, and ash content from more than 100 publications going back to 1931 (Fiset et al. 2017). We use these data to compute the median macromolecular composition of macroalgae from the field, to compare the macromolecular content of macroalgae to microalgae and herbaceous plants, and to test for differences in macromolecular content and C:N across macroalgal phyla. This work complements and extends the recent meta-analysis of protein content in macroalgae conducted by Angell et al. (2016).

The median macromolecular composition of macroalgae collected from the field was 9.98% protein, 2.7% lipid, 48.5% carbohydrate, and 31.8% ash (Table 1; Fig. 1). Despite representing data from a diverse variety of species from the field exposed to different environmental conditions, the variation in macromolecular content, estimated as the width of the 95% credible interval relative to the median, was less than 20%. On average, the four major macromolecules accounted for 93% of total dry weight. Most of the unaccounted for mass was likely residual moisture, nucleic acids, and pigments. Between 5 and 10% of dry weight can be attributed to moisture even after drying (Wong and Cheung 2000, Marinho-Soriano et al. 2007, Khan and Qari 2012, Rohani-Ghadikolaei et al. 2012, Rodrigues et al. 2015). DNA, RNA, and pigments typically account for less than 5% DW in macroalgae (Orduña-Rojas et al. 2002, McDermid and Stuercke 2003, Hong et al. 2007, Rao et al. 2007, Ganesan et al. 2014, Vilg et al. 2015). In summary our new estimates of median macromolecular composition fell within the ranges reported in the classic reviews (Table 1); median carbohydrate was in the bounds of the large range of values previously reported, but median protein was lower, and median lipid was higher than the midpoint of these ranges. The estimates of median

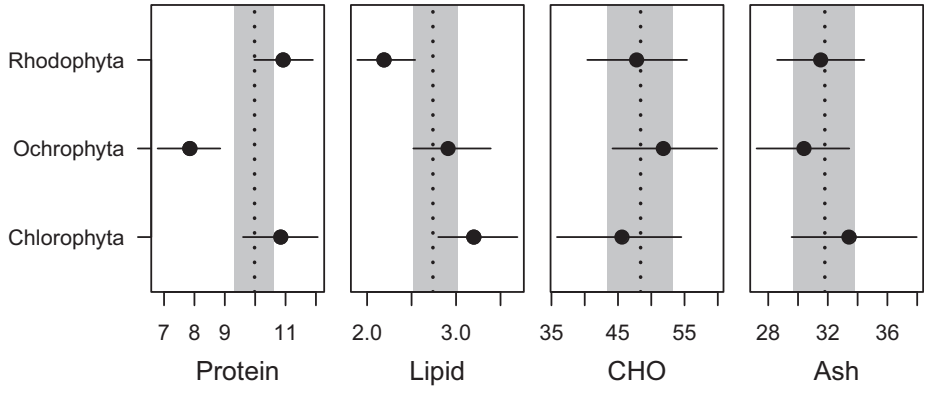

FIG. 1. Median and 95% credible interval of the median macromolecular composition of macroalgae as percent dry weight according to phylum. The gray bars represent the 95% credible interval about the overall distribution of each macromolecule and ash in macroalgae. Sample sizes for protein estimates for the Rhodophyta, Ochrophyta, and Chlorophyta are 366, 214, and 247, respectively. In the same order of phyla, sample sizes for lipid measurements are 182, 87, and 137, and sample sizes for carbohydrate estimates are 20, 21, and 17, and sample sizes for ash measurements are 230, 153, and 108.

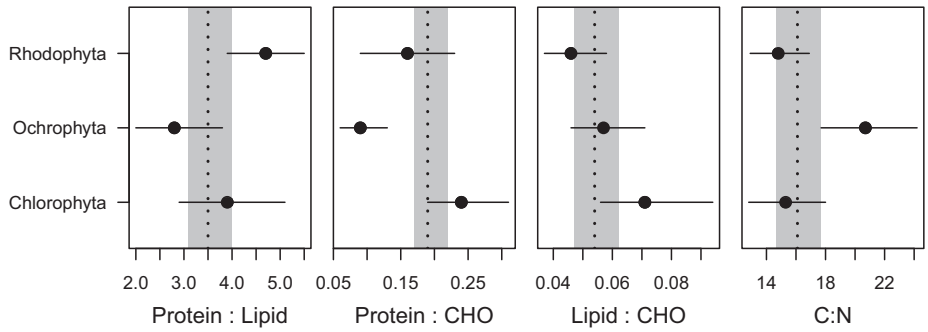

FIG. 2. Median macromolecular and elemental ratios of macroalgae as percent dry weight according to phylum. The C:N ratio refers the molar ratio of carbon to nitrogen. The gray bars represent the 95% credible intervals on the median of the overall distribution of each ratio for macroalgae. The macromolecular and elemental ratios are based on the posterior distributions of the macromolecular data generated from the hierarchical Bayesian analysis reported in Table S1.

protein content computed by Angell et al. (2016) varied with the biochemical method used from 7.78% DW when protein extraction methods are used, 11.6% when total amino acids are used, to 16.6% when using total nitrogen content and a protein conversion factor of 6.25. Our estimate of median protein (9.98% DW), which excludes sun-dried and blotted samples, overlaps the 95% confidence intervals for all methods used to estimate protein by Angell et al. (2016) and is in best agreement with the preferred method of total amino acid content.

The macromolecular composition of macroalgae differs from herbaceous plants and microalgae. Macroalgae are predominantly composed of carbohydrate and minerals (represented by ash content; Table 1). The higher carbohydrate and mineral content of macroalgae provides structural support for upward growth and resistance to a variety of biotic and abiotic stressors (Deniaud-Bouët et al. 2017), including grazing by some predators (Montgomery and Gerking 1980, Pennings and Paul 1992). The mineral content of noncalcified macroalgae considered here (32% DW) is considerably higher than a typical nonmineralized microalgal species (17% DW; Table 1). The highly calcified macroalgae, excluded from our analyses here, can have ash content as high as 89.7% DW (*Halimeda opuntia*, from Renaud and Luong-van 2006), while highly mineralized microalgae, such as the diatoms, are typically 30% DW ash (Finkel et al. 2016). Herbaceous plants and leaves, while similar in carbohydrate content to microalgae and macroalgae, tend to be higher in protein and lipid, whereas actively growing microalgae tend to be much lower in carbohydrate and higher in protein and lipid than macroalgae (Table 1). Most of the carbohydrate fraction in macroalgae is cell wall polysaccharides, largely composed of cellulose, hemicellulose, neutral and sulfated or acid polysaccharides (Holdt and Kraan 2011, Deniaud-Bouët et al. 2017). Unlike terrestrial plants, which often use lignin to solidify their cell walls, macroalgae generally do not contain lignin (but see Martone et al. 2009), suggesting it is not needed for structural support underwater. In addition to cell wall polysaccharides, macroalgal carbohydrates include storage polysaccharides (Holdt and Kraan 2011), which differ in composition and content across species (Ito and Hori 1989). Microalgae tend to store energy in the form of both carbohydrates and lipids, whereas the primary storage form in macroalgae is carbohydrate (Ito and Hori 1989). Algal lipids include glycolipids, which are found in the chloroplasts; and phospholipids, which are an integral component of cell membranes and neutral lipids such as triacylglycerides, which function as carbon and energy storage (Khotimchemko et al. 2002, Suutari et al. 2015). Lipid stores are metabolically more expensive but more energetically dense; as a consequence, lipid storage may be relatively more important in microalgae, where cellular space is limited and lower specific density affects buoyancy regulation (Subramanian et al. 2013).

The higher C:N ratio in the macroalgae relative to the microalgae is due to their higher carbohydrate and lower protein content (Geider and La Roche 2002), while lower lipid values for macroalgae are responsible for their much higher protein to lipid ratio compared to that for microalgae (Table S1). Based on the median macromolecular content of macroalgae, we estimate a mean C:N of 16.1 (mol:mol; Table S1) compared to a mean C:N of 7 for the microalgae (Finkel et al. 2016). Direct carbon and nitrogen measures provide a similar estimate of the mean C:N in macroalgae of ~18.7 (Atkinson and Smith 1983). Most estimates of bulk protein in macroalgae are based on a measure of total nitrogen and a conversion factor (Angell et al. 2016, Fiset et al. 2017), so our macromolecular estimate of C:N is not completely independent of direct elemental analysis.

The Ochrophyta are significantly lower in protein than the Chlorophyta and Rhodophyta (Fig. 1). The Rhodophyta are significantly lower in lipid than the Chlorophyta. These differences in macromolecular content help explain why the Ochrophyta tend to have higher C:N relative to the Rhodophyta and

Chlorophyte (Atkinson and Smith 1983). Angell et al. (2016) also found that the Ochrophyta have consistently lower protein content than the other macroalga phyla regardless of the methods used to harvest or quantify protein content. Differences in nitrogen storage strategies employed by some of the dominant taxa sampled within the major phyla may help explain the lower protein content in the Ochrophyta versus the Rhodophyta and Chlorophyta. Two orders within the Ochrophyta, the Laminariales and Fucales, are recognized for their ability to store large amounts of inorganic nitrogen, and are generally perennial (Chapman and Craigie 1977, Zimmerman and Kremer 1986, Young et al. 2009). When nutrients are scarce, these macroalgae convert their inorganic nitrogen reserves into nitrogen-rich macromolecules such as proteins in order to grow (Chapman and Craigie 1977, Zimmerman and Kremer 1986, Pedersen and Borum 1996, Young et al. 2009). Shorter-lived ephemeral macroalgae tend to take up nitrogen more rapidly from the environment and use it to build proteins and pigments to promote rapid growth (Littler and Littler 1980, Littler et al. 1983). Slow-growing species that store nitrogen have been shown to have slightly lower protein content than fast-growing species like *Ulva* and *Porphyra* (King and Schramm 1976, Tiwari and Troy 2015). Nitrogen storage has also been observed in some macroalgae species within the Rhodophyta, but the literature suggests that nitrogen storage does not seem as prevalent as within the Ochrophyta (Ryther et al. 1981). Since the majority of the Ochrophyta sampled within the current study were kelp or fucoid macroalgae (64%), we hypothesize that their low protein content may account for the lower average protein content of the Ochrophyta.

There were no significant differences in carbohydrate content, ash content, or lipid to carbohydrate ratios across the phyla. The lack of significant taxonomic differences in carbohydrate content was surprising given there are several species, within specific phyla, harvested for their high polysaccharide content. For example, the Ochrophytes: *Saccharina* and *Undaria* and the Rhodophytes: *Gracilaria*, Kappaphycus, and *Gelidium* are widely harvested for their phycocolloids (FAO 2007, Holdt and Kraan 2011) that are used for their gelling properties in food and other industrial products (Dawes et al. 1973, Mouritsen 2013, Tiwari and Troy 2015). In the Rhodophyte *Hypnea musciformis*, carrageenan can make up to 41% of dry weight (Aziza et al. 2008). According to a review by Holdt and Kraan (2011) investigating the bioactive compounds of the 10 most researched and utilized macroalgal species, the three genera with the highest carbohydrate contents were members of the Ochrophyta and Rhodophyta (*Ascophyllum*, up to 64%; *Porphyra*, up to 76%, and *Palmaria*, up to 74% DW). Our inability to detect significant differences in carbohydrate content across phyla may be due to variability associated with pooling data across studies (differences in strains, geographic and environmental conditions, biochemical methods, and laboratory protocols) as well as the variability inherent in the dominant method used to estimate total carbohydrate in macroalgae (Fiset et al. 2017). Species-level differences in bulk macromolecular content are much larger than differences across phyla. While the current study provides a measure of central tendency of the macromolecular composition of macroalgae, it must be noted that macroalgae macromolecular composition is highly plastic and greatly affected by a variety of different environmental variables, including season, nutrient availability, and habitat (Khotimchenko et al. 2002, Holdt and Kraan 2011, Suutari et al. 2015, Angell et al. 2016, Deniaud-Bouët et al. 2017, Wells et al. 2017). For example, seasonal variation in protein content in *Sargassum vulgar* can exceed 2-fold (Marinho-Soriano et al. 2006) and lipid content from brown macrophyte *Dictyota* range between 20.2% and 2.6% DW across studies from different regions (McDermid and Stuercke 2003, McDermid et al. 2007, Chakraborty and Santra 2008, Parthiban et al. 2013). In the current study, observations for each of the macromolecules were pooled regardless of environmental conditions, broadening the variability associated with our median estimates, and reducing our ability to detect finer-scale taxonomic differences. Wider application of a better method to estimate total carbohydrate content in macroalgae may be required to detect global differences in median total carbohydrate content in macroalgae collected from the field from diverse locations (Fiset et al. 2017, Van Wychen and Laurens 2019).

CONCLUSIONS

We present a new quantitative estimate of the median macromolecular content of macroalgae collected from the field. Macroalgae are carbohydrate and mineral rich relative to microalgae and protein and lipid poor relative to both microalgae and herbs and leaves. Our statistical analyses confirm there are significant differences in bulk macromolecular content across the macroalgal phyla:the Ochrophyta are lowest in protein and the Rhodophyta are low in lipid.

ACKNOWLEDGEMENTS

This work was supported by grants from the Gordon and Betty Moore Foundation (#3778), the Simons Collaboration on Computational Biogeochemical Modeling of Marine Ecosystems/CBIOMES Grant #549935 (ZVF), #549937 (AJI) and NSERC and CRC programs.

Angell, A. R., de Nys, R. & Paul, N. A. 2015. The nitrogen, protein and amino acid content of seaweeds [data set]. https://doi.org/10.4225/28/55776d6f45871

Angell, A. R., Mata, L., de Nys, R. & Paul, N. A. 2016. The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. *J. Appl. Phycol.* 28:511–24.

Angell, A. R., Mata, L., Nys, R. & Paul, N. A. 2014. Variation in amino acid content and its relationship to nitrogen content

and growth rate in *Ulva ohnoi* (Chlorophyta). *J. Phycol.* 50:216–26.
Atkinson, M. & Smith, S. 1983. C: N: P ratios of benthic marine plants. *Limnol. Oceanogr.* 28:568–74.
Aziza, M., Givernaud, T., Chikhaoui-khay, M. & Bennasser, L. 2008. Seasonal variation of the growth, chemical composition and carrageenan extracted from *Hypnea musciformis* (Wulfen) Lamouroux harvested along the Atlantic coast of Morocco. *Sci. Res. Essays* 2:509–14.
Banerjee, K., Ghosh, R., Homechaudhuri, S. & Mitra, A. 2009. Biochemical composition of marine macroalgae from Gangetic Delta at the apex of the Bay of Bengal. *Afr. J. Basic Appl. Sci.* 1:96–104.
Biancarosa, I., Espe, M., Bruckner, C. G., Heesch, S., Liland, N., Waagbo, R., Torstensen, B. & Lock, E. J. 2017. Amino acid composition, protein content, and nitrogen conversion factors of 21 seaweed species from Norwegian waters. *J. Appl. Phycol.* 29:1001–9.
Bird, K. T. 1984. Seasonal variation in protein: carbohydrate ratios in a subtropical estuarine alga, *Gracilaria verrucosa,* and the determination of nitrogen limitation status using these ratios. *Bot. Mar.* 27:111–6.
Bligh, E. G. & Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.* 37:911–7.
Chakraborty, S. & Santra, S. 2008. Biochemical composition of eight benthic algae collected from Sunderban. *Ind. J. Mar. Sci.* 37:329.
Chapman, A. & Craigie, J. 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.* 40:197–205.
Chopin, T., Buschmann, A. H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G. P., Zertuche-González, J. A., Yarish, C. & Neefus, C. 2001. Integrating seaweeds into marine aquaculture systems: a key toward sustainability. *J. Phycol.* 37:975–86.
Chung, I. K., Sondak, C. F. & Beardall, J. 2017. The future of seaweed aquaculture in a rapidly changing world. *Eur. J. Phycol.* 52:495–505.
Coyer, J. A. 2007. Algal biogeography. *In* Denny, M. W. & Gaines, S. [Eds.] *Encyclopedia of Tidepools and Rocky Shores.* University of California Press, Berkeley, CA, pp. 24–9.
Darcy-Vrillon, B. 1993. Nutritional aspects of the developing use of marine macroalgae for the human food industry. *Int. J. Food Sci. Nutr. UK* 44:S23–35.
Dawes, C. J., Lawrence, J. M., Cheney, D. P. & Mathieson, A. C. 1973. Ecological studies of Floridian *Eucheuma* (Rhodophyta, Gigartinales). III. Seasonal variation of carrageenan, total carbohydrate, protein, and lipid. *Bull. Mar. Sci.* 24:286–99.
Deniaud-Bouët, E., Hardouin, K., Potin, P., Kloareg, B. & Hervé, C. 2017. A review about brown algal cell walls and fucose-containing sulfated polysaccharides: cell wall context, biomedical properties and key research challenges. *Carbohyd. Polym.* 175:395–408.
Dooley, J. K. 1972. Fishes associated with the pelagic *Sargassum* complex, with a discussion of the *Sargassum* community. *Contrib. Mar. Sci.* 16:1–32.
Duarte, C. M. 2017. Reviews and syntheses: Hidden forests, the role of vegetated coastal habitats in the ocean carbon budget. *Biogeosci.* 14:301.
Duarte, C. M. & Cebrian, J. 1996. The fate of marine autotrophic production. *Limnol. Oceanogr.* 41:1758–66.
Duarte, C. M. & Chisnaco, C. L. 1999. Seagrass biomass and production: a reassessment. *Aquat. Bot.* 65:159–74.
FAO 2007. *The State of Food and Agriculture.* Food and Agriculture Organization of the United Nations, Rome, 1–107.
FAO 2016. *The State of World Fisheries and Aquaculture 2016.* Food and Agriculture Organization of the United Nations, Rome, 1–200.
Finkel, Z. V., Follows, M. J., Liefer, J. D., Brown, C. M., Benner, I. & Irwin, A. J. 2016. Phylogenetic diversity in the macromolecular composition of microalgae. *PLoS ONE* 11: e0155977.
Fiset, C. 2016. Macroalgae macromolecular database. *Figshare.* https://doi.org/10.6084/m9.figshare.4248962.v1
Fiset, C., Liefer, J., Irwin, A. J. & Finkel, Z. V. 2017. Methodological biases in estimates of macroalgal macromolecular composition. *Limnol. Oceanogr. Meth.* 15:618–30.
Fleurence, J., Chenard, E. & Luçcon, M. 1999. Determination of the nutritional value of proteins obtained from *Ulva armoricana. J. Appl. Phycol.* 11:231–9.
Folch, J., Lees, M. & Sloane-Stanley, G. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497–509.
Ganesan, K., Kumar, K. S., Rao, P. S., Tsukui, Y., Bhaskar, N., Hosokawa, M. & Miyashita, K. 2014. Studies on chemical composition of three species of *Enteromorpha. Biomed. Prevent. Nutr.* 4:365–9.
Geider, R. J. & La Roche, J. 2002. Redfield revisited: variability of C:N: P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* 37:1–17.
Gokulakrishnan, S., Raja, K., Sattanathan, G. & Subramanian, J. 2015. Proximate composition of bio potential seaweeds from Mandapam South East coast of India. *Int. Lett. Nat. Sci.* 45:49–55.
Gómez-Ordóñez, E., Jiménez-Escrig, A. & Rupérez, P. 2010. Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. *Food Res. Int.* 43:2289–94.
Gosch, B. J., Magnusson, M., Paul, N. A. & Nys, R. 2012. Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts. *GCB Bioenerg.* 4:919–30.
Hawkins, S. J. & Hartnoll, R. 1983. Grazing of intertidal algae by marine invertebrates. *Oceanogr. Mar. Biol.* 21:195–282.
Heiba, H. 1990. Phytochemical studies on the marine algae of Qatar, Arabian Gulf. *Plant Food Hum. Nutr.* 51:27–34.
Heiba, H. I., Al-Easa, H. S. & Rizk, A. F. M. 1997. Fatty acid composition of twelve algae from the coastal zones of Qatar. *Plant Food Hum. Nutr.* 51:27–34.
Holdt, S. L. & Kraan, S. 2011. Bioactive compounds in seaweed: functional food applications and legislation. *J. Appl. Phycol.* 23:543–97.
Hong, D. D., Hien, H. M. & Son, P. 2007. Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *J. Appl. Phycol.* 19:817–26.
Ito, K. & Hori, K. 1989. Seaweed: chemical composition and potential food uses. *Food Rev. Int.* 5:101–44.
Jayasankar, R. 1993. Seasonal variation in biochemical constituents of *Sargassum wightii* (Grevillie) with reference to yield in alginic acid content. *Seaweed Res. Util.* 16:13–6.
Jayasankar, R., Ramalingam, J. & Kaliaperumal, N. 1990. Biochemical composition of some green algae from the Mandapam coast. *Seaweed Res. Util.* 12:37–40.
Kaliaperumal, N., Chennubhotla, V., Najmuddin, M., Ramalingam, J. & Kalimuthu, S. 1994. Biochemical composition of some common seaweeds from Lakshadweep. *J. Mar. Biol. Assoc. India* 36:316–9.
Khan, F. & Qari, R. 2012. Variation in biomass, biochemical composition and alginic acid contents in *Spatoglossum variabile* and *Stoechospermum marginatum. Int. J. Phycol. Phycochem.* 8:59–68.
Khotimchenko, S., Vaskovsky, V. & Titlyanova, T. 2002. Fatty acids of marine algae from the Pacific coast of North California. *Bot. Mar.* 45:17–22.
King, R. & Schramm, W. 1976. Photosynthetic rates of benthic marine algae in relation to light intensity and seasonal variations. *Mar. Biol.* 37:215–22.
Lapointe, B. E. 1986. Phosphorus-limited photosynthesis and growth of *Sargassum natans* and *Sargassum fluitans* (Phaeophyceae) in the western North Atlantic. *Deep Sea Res. A* 33:391–9.
Littler, M. M. & Littler, D. S. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *Am. Nat.* 116:25–44.
Littler, M. M., Littler, D. S. & Taylor, P. R. 1983. Evolutionary strategies in a tropical barrier reef system: functional-form groups of marine macroalgae. *J. Phycol.* 19:229–37.

Lourenço, S., Barbarino, E., De-Paula, J., Pereira, L. & Marquez, U. 2002. Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. *Phycol. Res.* 50:233–41.

Lynn, S. G., Kilham, S. S., Kreeger, D. A. & Interlandi, S. J. 2000. Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *J. Phycol.* 36:510–22.

Mabeau, S. & Fleurence, J. 1993. Seaweed in food products: biochemical and nutritional aspects. *Trends Food Sci. Tech.* 4:103–7.

Manivannan, K., Thirumaran, G., Devi, G. K., Anantharaman, P. & Balasubramanian, T. 2009. Proximate composition of different group of seaweeds from Vedalai Coastal waters (Gulf of Mannar): Southeast Coast of India. *Middle East J. Sci. Res.* 4:72–7.

Marinho-Soriano, E., Camara, M. R., Cabral, T. & Carneiro, M. A. 2007. Preliminary evaluation of the seaweed *Gracilaria cervicornis* (Rhodophyta) as a partial substitute for the industrial feeds used in shrimp (*Litopenaeus vannamei*) farming. *Aquat. Res.* 38:182–7.

Marinho-Soriano, E., Fonseca, P., Carneiro, M. R. & Moreira, W. 2006. Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresource Technol.* 97:2402–6.

Martone, P. T., Estevez, J. M., Lu, F., Ruel, K., Denny, M. W., Somerville, C. & Ralph, J. 2009. Discovery of lignin in seaweed reveals convergent evolution of cell-wall architecture. *Curr. Biol.* 27:169–75.

McDermid, K. J. & Stuercke, B. 2003. Nutritional composition of edible Hawaiian seaweeds. *J. Appl. Phycol.* 15:513–24.

McDermid, K. J., Stuercke, B. & Balazs, G. H. 2007. Nutritional composition of marine plants in the diet of the green sea turtle (*Chelonia mydas*) in the Hawaiian Islands. *Bull. Mar. Sci.* 81:55–71.

Montgomery, L. W. & Gerking, S. D. 1980. Marine macroalgae as food for fishes: an evaluation of food quality. *Environ. Biol. Fish.* 5:143–53.

Mouritsen, O. G. 2013. *Seaweeds: Edible, Available, Sustainable.* The University of Chicago Press, Chicago, IL, USA, p 304.

Nayar, S. & Bott, K. 2014. Current status of global cultivated seaweed production and markets. *World Aquacult.* 45:32–7.

Neveux, N., Yuen, A., Jazrawi, C., Magnusson, M., Haynes, B., Masters, A., Montoya, A., Paul, N., Maschmeyer, T. & de Nys, R. 2014. Biocrude yield and productivity from the hydrothermal liquefaction of marine and freshwater green macroalgae. *Bioresource Technol.* 155:334–41.

Orduña-Rojas, J., Robledo, D. & Dawes, C. 2002. Studies on the tropical agarophyte *Gracilaria cornea* J. Agardh (Rhodophyta, Gracilariales) from Yucatan, Mexico. I. Seasonal physiological and biochemical responses. *Bot. Mar.* 45:453–8.

Parthiban, C., Saranya, C., Girija, K., Hemalatha, A., Suresh, M. & Anantharaman, P. 2013. Biochemical composition of some selected seaweeds from Tuticorin coast. *Adv. Appl. Sci. Res.* 4:362–6.

Pedersen, M. F. & Borum, J. 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. *Mar. Ecol. Prog. Ser.* 142:261–72.

Pennings, S. C. & Paul, V. J. 1992. Effect of plant toughness, calcification, and chemistry on herbivory by *Dolabella auricularia*. *Ecology* 73:1606–19.

R Core Team. 2017. *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rao, P. S., Mantri, V. A. & Ganesan, K. 2007. Mineral composition of edible seaweed *Porphyra vietnamensis*. *Food Chem.* 102:215–8.

Renaud, S. & Luong-van, J. T. 2006. Seasonal variation in the chemical composition of tropical Australian marine macroalgae. *J. Appl. Phycol.* 18:381.

Rodrigues, D., Freitas, A. C., Pereira, L., Rocha-Santos, T. A., Vasconcelos, M. W., Roriz, M., Rodríguez-Alcalá, L. M., Gomes, A. M. & Duarte, A. C. 2015. Chemical composition of red, brown and green macroalgae from Buarcos bay in Central West Coast of Portugal. *Food Chem.* 183:197–207.

Rohani-Ghadikolaei, K., Abdulalian, E. & Ng, W. K. 2012. Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. *J. Food Sci. Tech.* 49:774–80.

Ryther, J. H., Corwin, N., DeBusk, T. A. & Willimans, L. D. 1981. Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). *Aquaculture* 26:107–15.

Sautier, C. 1987. Les algues en alimentation humaine. *Cah. Nutr. Diét* 22:469–72.

Sautier, C. 1990. Aspects nutritionnels et réglementaires de l'utilization des algues en nutrition humaine. *Revue du Palais de la Découverte* 18:40–6.

Shpigel, M., Ragg, N. L., Lupatsch, I. & Neori, A. 1999. Protein content determines the nutritional value of the seaweed *Ulva lactuca* L for the abalone *Haliotis tuberculata* L. and *H. discus hannai* Ino. *J. Shellfish Res.* 18:227–34.

Smith, S. 1981. Marine macrophytes as a global carbon sink. *Science* 211:838–40.

Stan Development Team. 2018. RStan: the R interface to Stan. R package version 2.17.3. http://mc-stan.org/.

Steneck, R. & Martone, P. 2007. Calcified algae. *In* Denney, M. W. & Gaines, S. D. [Eds.] *Encyclopedia of Tidepools and Rocky Shores.* University of California Press, Berkeley, CA, USA, pp. 21–4.

Sterner, R. W. & Hessen, D. O. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. *Ann. Rev. Ecol. Syst.* 25:1–29.

Subramanian, S., Barry, A. N., Pieris, S. & Sayre, R. T. 2013. Comparative energetics and kinetics of autotrophic lipid and starch metabolism in chlorophytic microalgae implications for biomass and biofuel production. *Biotechnol. Biofuels* 6:150.

Sutherland, J. E., Lindstrom, S. C., Nelson, W. A., Brodie, J., Lynch, M. D., Hwang, M. S., Choi, H. G., Miyata, M., Kikuchi, N. & Oliveira, M. C. 2011. A new look at an ancient order: generic revision of the Bangiales (Rhodophyta). *J. Phycol.* 47:1131–51.

Suutari, M., Leskinen, E., Fagerstedt, K., Kuparinen, J., Kuuppo, P. & Blomster, J. 2015. Macroalgae in biofuel production. *Phycol. Res.* 63:1–18.

Tilman, D., Kilham, S. S. & Kilham, P. 1982. Phytoplankton community ecology: the role of limiting nutrients. *Ann. Rev. Ecol. Syst.* 13:349–72.

Tiwari, B. K. & Troy, D. J. 2015. *Seaweed Sustainability: Food and Non-Food Applications.* Elsevier Inc, London Wall, UK, p 192.

Van Wychen, S. & Laurens, L. M. L. 2019. Total carbohydrate content determination of microalgal biomass by acid hydrolysis followed by spectrophotometry or liquid chromatography. *Meth. Molec. Biol.* https://doi.org/10.1007/7651_2017_106.

Viera, M., Pinchetti, J. G., de Vicose, G. C., Bilbao, A., Suárez, S., Haroun, R. & Izquierdo, M. 2005. Suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculata coccinea* Reeve. *Aquaculture* 248:75–82.

Vilg, J. V., Nylund, G. M., Werner, T., Qvirist, L., Mayers, J. J., Pavia, H., Undeland, I. & Albers, E. 2015. Seasonal and spatial variation in biochemical composition of *Saccharina latissima* during a potential harvesting season for Western Sweden. *Bot. Mar.* 58:435–47.

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E. & Brawley, S. H. 2017. Algae as nutritional and functional food sources: revisiting our understanding. *J. Appl. Phycol.* 29:949–82.

Wong, K. & Cheung, P. C. 2000. Nutritional evaluation of some subtropical red and green seaweeds: Part I—proximate composition, amino acid profiles and some physico-chemical properties. *Food Chem.* 71:475–82.

Young, E. B., Berges, J. A. & Dring, M. J. 2009. Physiological responses of intertidal marine brown algae to nitrogen deprivation and resupply of nitrate and ammonium. *Physiol. Plantarum* 135:400–11.

Zimmerman, R. C. & Kremer, J. N. 1986. *In situ* growth and chemical composition of the giant kelp, *Macrocystis pyrifera*: response to temporal changes in ambient nutrient availability. *Mar. Ecol. Prog. Ser.* 27:277–85.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1. Macromolecular ratios and C:N ratios of the three macroalgae phyla and the grand mean for macroalgae calculated using 1) paired data, and 2) the posterior distributions of the hierarchal Bayesian statistical model (results underlined). The percent difference between the medians of the two methods is also reported and italicized. Microalgae data are from Finkel et al. (2016). CHO is an abbreviation for carbohydrate.