



NOTE

# Calcified coralline algae have similar caloric value to uncalcified algae

Isaak Haberman<sup>1,2,\*</sup>, Patrick T. Martone<sup>1</sup>

<sup>1</sup>Department of Botany and Biodiversity Research Centre, University of British Columbia, 6270 University Blvd, Vancouver, BC V6T 1Z4, Canada

<sup>2</sup>Present address: Department of Marine Science, California State University, Monterey Bay, 6200 6th Ave, Seaside, CA 93955, USA

**ABSTRACT:** A long-held paradigm in marine herbivore ecology is that calcified coralline algae are less nutritious than their uncalcified, fleshy counterparts, partially explaining why herbivores prefer to consume uncalcified algae. The basis for this assumption is that calcium carbonate ( $\text{CaCO}_3$ ) comprises a large portion of coralline thalli and is a dense, non-nutritious compound that lowers the relative caloric value. Caloric analyses generally make comparisons using dry weight, which is not a biologically relevant metric to assess algal caloric value: algae are not consumed dry and are consumed by volume rather than by weight. We determined the caloric value of 5 fleshy, uncalcified algal species and 5 calcified coralline algal species on a per-dry-weight and per-volume basis. Results clearly indicated that when compared on a per-dry-weight basis, uncalcified thalli were more calorie rich than coralline thalli. However, on a per-volume basis, coralline and uncalcified thalli were more similar, and the caloric value of some corallines exceeded that of some kelps. Our data show that calcified and uncalcified thalli have similar proportions of organic tissue, with a large portion of uncalcified thalli composed of water and a similar portion of calcified thalli composed of water and  $\text{CaCO}_3$ . We argue that a volumetric assessment of the caloric values of macroalgae is more relevant and accurate than a dry-weight metric. The widespread observation that herbivores prefer uncalcified over coralline algae cannot be explained by differences in caloric content, indicating that another mechanism must account for many marine herbivores' avoidance of calcified coralline algae.

**KEY WORDS:** Caloric value · Coralline algae · Kelp · Volumetric · Herbivore ecology

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## 1. INTRODUCTION

Coralline algae are a distinctive group of red macroalgae that calcify, depositing calcium carbonate ( $\text{CaCO}_3$ ) into their cell walls as they grow (Johansen 1981, Steneck 1986). In marine ecosystems, when grazer abundance is high, coralline red algae often dominate macrophyte communities (Steneck 1986, Johnson & Paine 2016). Past work has demonstrated

that some grazers strongly prefer kelps and other uncalcified, fleshy macroalgae over corallines (Paine & Vadas 1969b, Dayton 1975, Vadas 1977, Johnson & Paine 2016). For example, when urchin populations expand, kelp populations are decimated, leaving 'urchin barrens' composed primarily of crustose and articulated coralline algae (Filbee-Dexter & Scheibling 2014, Hind et al. 2019). Such observations have led to the conclusion that coralline algae are resistant

\*Corresponding author: [ihaberman@csumb.edu](mailto:ihaberman@csumb.edu)

to herbivores, perhaps because of their calcified thalli (Littler et al. 1983, Paul & Hay 1986, Steneck 1986). Yet despite varied levels of protection attributed to thallus morphology (Padilla 1984, Steneck 1986, Martone et al. 2021), a generalized mechanism of herbivore resistance provided by calcification is unclear.

One hypothesis states that calcified coralline thalli are physically more difficult to eat than uncalcified fleshy algae. However, to the contrary, Padilla (1985) showed that it is easier for patellogastropod radula to rasp/scrape coralline thalli than some uncalcified algae thalli—just as it can be easier to write with chalk on a chalkboard than on stretchy fabric. Moreover, removing  $\text{CaCO}_3$  from coralline thalli does not necessarily increase grazing rates on those thalli (Martone et al. 2021), suggesting that calcification alone cannot necessarily explain reduced rates of herbivory.

An alternative hypothesis is that coralline algae are less nutritious than uncalcified macroalgae. Indeed, Paine & Vadas (1969a) determined caloric value (kcal) and ash-free dry weight (AFDW; a measure of organic carbon content) for 70 macroalgal species and concluded that coralline algal species were calorically less rich than uncalcified species, likely due to their  $\text{CaCO}_3$  content. However, despite various calculations based on wet weight, the conclusions presented by Paine & Vadas (1969a) were based on energetic values calculated per dry mass (i.e. kcal per dry gram of algae), which is not necessarily a biologically relevant comparison. To the contrary, Maneveldt & Keats (2008) showed that calcified algal thalli may contain similar amounts of organic content on a per-volume basis. Herbivore grazing is not limited by the dry weight of food but rather by the volume of food that is removed with each bite or feeding stroke. Moreover, non-nutritious components, such as water and  $\text{CaCO}_3$ , are often removed from per-weight measurements of caloric value even though these components are still ingested. Caloric data are especially biased towards uncalcified algal species since the most common metric, dry weight, excludes water weight but includes  $\text{CaCO}_3$ , despite both being calorically deficient. Reporting such manipulated data potentially skews our understanding of edible algal species. In addition, on a per-weight basis, lower-density food (such as uncalcified macroalgal species) may appear to have higher caloric value simply because more tissue must be analyzed for each measurement of comparable mass. Thus, we would argue that comparing caloric content of macroalgae on a per-volume basis

is more biologically relevant than comparisons on a per-weight basis.

The present study compares the caloric value of several species of calcified coralline macroalgae and uncalcified, fleshy macroalgae on a per-volume basis. We account for water and  $\text{CaCO}_3$  content but ultimately present the energetic value per volume (e.g. per 'biteful' or feeding stroke) of algae. Using this new framework, we reconsider the long-standing hypothesis that coralline algae are less nutritious than their uncalcified counterparts.

## 2. MATERIALS AND METHODS

*Fucus distichus* Linnaeus was collected from Stanley Park (49.300° N, 123.118° W) in Vancouver, BC, Canada, on 9 February 2020. *Hedophyllum sessile* (C. Agardh) Setchell, *Alaria marginata* Postels & Ruprecht, *Bossiella frondifera* (Manza) P.W. Gabrielson, K.A. Miller, Martone & K.R. Hind, and *Corallina vancouveriensis* Yendo were collected from Brady's Beach (48.827° N, 125.154° W) near Bamfield, BC, on 22 February 2020. *Corallina chilensis* Decaisne, as well as additional samples of *H. sessile* and *C. vancouveriensis* were collected from the same location on 12 June 2021. *Macrocystis pyrifera* (Linnaeus) C. Agardh was collected from Eagle Bay (48.835° N, 125.145° W) near Bamfield, BC, on 14 March 2020 and 12 June 2021. *Calliarthron tuberculosum* (Postels & Ruprecht) E. Y. Dawson and *Chamberlainium tumidum* (Foslie) Caragnana, Foetisch, Maneveldt & Payri were collected from Calvert Island (51.572° N, 128.119° W) on 15 March 2020. Each sample was independent, taken from a different thallus. Kelp samples were taken from thallus blades and were no larger than 8 × 8 cm. Coralline samples were taken from whole thalli and included 1–2 fronds. *F. distichus* samples consisted of 1–2 fronds with branches and blades. Sample sizes were as follows: *A. marginata* (n = 7), *B. frondifera* (n = 3), *C. tuberculosum* (n = 6), *C. tumidum* (n = 6), *C. chilensis* (n = 6), *C. vancouveriensis* (n = 8), *F. distichus* (n = 6), *H. sessile* (n = 6), *M. pyrifera* (n = 6), and *N. luetkeana* (n = 7). Samples were stored in a water table with recirculating seawater and adequate lighting (10–15 µE) until volumes and wet weights were measured, which occurred within 6 to 30 d after collection for all species except *C. tumidum*, for which weighing occurred 120 d after collection. Prior to volume and weight measurements, all thalli were cleaned to remove any epiphytes or fouling organisms. The volume of each

sample was assessed by measuring water displacement in graduated cylinders. Coralline samples were analyzed using a 10 or 5 ml graduated cylinder with marks every 0.1 ml, and kelp and *F. distichus* samples were measured using a 25 ml graduated cylinder with marks every 0.2 ml. A metal spatula was used to remove any air bubbles prior to volume measurement. Water on the surface of each sample was removed by blotting with a paper towel prior to weighing. Wet weights of *B. frondifera* and *C. tuberosum* were not measured. After wet weight was collected, samples were wrapped in wax paper and aluminum foil packets, dried in an oven at 62°C for 16 h, and re-weighed. Samples were then transported to the Department of Fisheries and Oceans Canada laboratory in West Vancouver, BC, and freeze-dried for an additional 16 h in a 1994 Labconco FreeZone freeze dryer at -50°C and re-weighed. The caloric content of tissue samples was then analyzed using a bomb calorimeter (IKA C5000 Calorimeter). In order to encourage combustion, coralline samples were combined with 1 g of benzoic acid standard with a known caloric value which was subtracted from the calorimetry output. To account for the endothermic processes that take place upon the combustion of CaCO<sub>3</sub> in coralline algae samples, an addition of 0.14 kcal g<sup>-1</sup> of CaCO<sub>3</sub> was made as per Paine (1971). The weight of CaCO<sub>3</sub> present in each sample was calculated using the dry weight CaCO<sub>3</sub> content from Martone et al. (2021) for *C. chilensis* (82% CaCO<sub>3</sub>), and from Guenther (2011) and Guenther & Martone (2014) for *C. vancouveriensis* (64% CaCO<sub>3</sub>) and *C. tuberosum* (84% CaCO<sub>3</sub>). CaCO<sub>3</sub> content for *C. tumidum* (89.31% CaCO<sub>3</sub>) and *B. frondifera* (85.56% CaCO<sub>3</sub>) were provided by E. Porcher (unpubl. data) and G. Colwell (unpubl. data), respectively, using methods from Martone et al. (2021).

The proportion of each thallus weight that was organic tissue, water, and CaCO<sub>3</sub> was derived for species with wet and dry weights. The proportion of each thallus that was water was calculated using the following:

$$\text{proportion of water} = (\text{wet weight} - \text{dry weight}) / \text{wet weight}$$

For uncalcified species, the proportion of the thallus that was organic tissue was 1 - water proportion. The same was done with the calcified species; however, to differentiate between organic tissue and CaCO<sub>3</sub>, wet weight CaCO<sub>3</sub> content (calculated using water and dry weight CaCO<sub>3</sub> proportions) for *C.*

*chilensis*, *C. tuberosum*, *C. vancouveriensis*, and *C. tumidum* were additionally subtracted to identify the portion of each thallus that was composed of organic tissue. Organic tissue is therefore defined as any portion of the thallus that is not composed of water (uncalcified species) or water and CaCO<sub>3</sub> (calcified species). Reported water, CaCO<sub>3</sub>, and organic tissue proportions represent the average for each species across all samples (see Fig. 2, Table 1) except *C. tuberosum*, where water proportion was taken from Guenther (2011) and Guenther & Martone (2014). Reported wet weight CaCO<sub>3</sub> proportions were calculated using dry weight CaCO<sub>3</sub> proportions from Guenther (2011), Guenther & Martone (2014), Martone et al. (2021), and E. Porcher (unpubl. data) and G. Colwell (unpubl. data). Statistical analyses were not performed on proportional results.

A 1-way ANOVA was used to compare the caloric content of different species based on weight. A Tukey's post hoc test was used to assess differences in kcal g<sup>-1</sup> among species. The volumetric data did not have equal variances among species (Levene's test,  $F_{9,51} = 2.74$ ,  $p = 0.011$ ), nor were the data normally distributed (Shapiro-Wilk  $W = 0.95$ ,  $p = 0.021$ ), so a Kruskal-Wallis ANOVA followed by a Dunn's multiple comparison post hoc test was used to account for heteroscedasticity and non-normality. To compare the caloric value of uncalcified species to calcified species in terms of weight, Welch's 2-sample *t*-test was used since data exhibited heteroscedasticity (Levene's test,  $F_{1,59} = 14.86$ ,  $p < 0.001$ ). To complete the same analysis in terms of volume, a Student's *t*-test was used since all assumptions were met. All assumptions of linear regression were checked prior to statistical analysis, and an alpha of 0.05 for significance was applied for all statistical analyses. Reported *p*-values between comparisons of caloric values between species were adjusted using the Benjamini-Hochberg adjustment method to reduce the likelihood of false rejections of the null hypothesis due to multiple comparisons. All analyses were done in R (v.4.2.1).

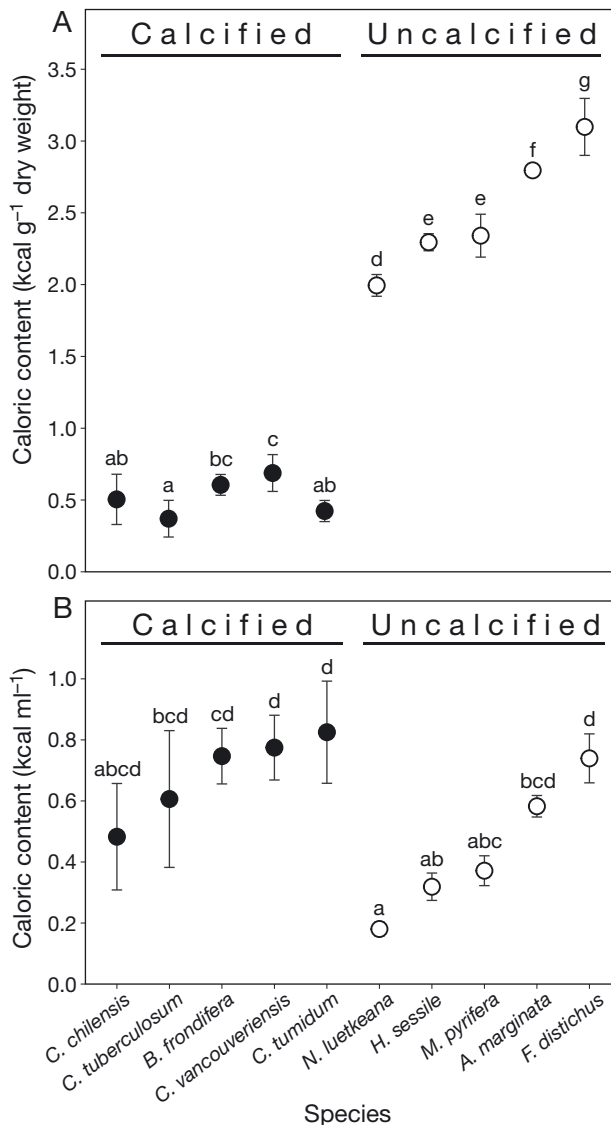
### 3. RESULTS

Per unit dry weight, uncalcified species ranged in caloric value (mean ± SE) from 1.995 ± 0.039 kcal g<sup>-1</sup> (*Nereocystis luetkeana*) to 3.098 ± 0.101 kcal g<sup>-1</sup> (*Fucus distichus*), and calcified coralline species ranged from 0.370 ± 0.065 kcal g<sup>-1</sup> (*Calliarthron tuberosum*) to 0.688 ± 0.065 kcal g<sup>-1</sup> (*Corallina vancouveriensis*) (Table 1, Fig. 1A). There were sig-

Table 1. Caloric values ( $\pm$ SE) and organic and inorganic proportions per wet weight of each species. Organic tissue is defined as any portion of the thallus that is not composed of water (uncalcified species) or water and  $\text{CaCO}_3$  (calcified species)

Species	kcal $\text{g}^{-1}$	kcal $\text{ml}^{-1}$	% organic tissue	% water	% $\text{CaCO}_3$	Calcified?
<i>Bossiella frondifera</i>	0.605 $\pm$ 0.037	0.746 $\pm$ 0.046	–	–	–	Yes
<i>Calliarthron tuberculosum</i>	0.370 $\pm$ 0.065	0.606 $\pm$ 0.114	11.8	26.0 <sup>a</sup>	62.2 <sup>b</sup>	Yes
<i>Chamberlainium tumidum</i>	0.423 $\pm$ 0.038	0.825 $\pm$ 0.085	9.2	14.2	76.6 <sup>c</sup>	Yes
<i>Corallina chilensis</i>	0.504 $\pm$ 0.089	0.483 $\pm$ 0.089	11.9	33.8	54.3 <sup>d</sup>	Yes
<i>Corallina vancouveriensis</i>	0.688 $\pm$ 0.065	0.775 $\pm$ 0.054	25.3	29.8	44.9 <sup>b</sup>	Yes
<i>Alaria marginata</i>	2.796 $\pm$ 0.017	0.583 $\pm$ 0.018	14.3	85.7	0	No
<i>Fucus distichus</i>	3.098 $\pm$ 0.101	0.739 $\pm$ 0.041	17.9	82.2	0	No
<i>Hedophyllum sessile</i>	2.295 $\pm$ 0.030	0.319 $\pm$ 0.023	13.5	86.5	0	No
<i>Macrocystis pyrifera</i>	2.341 $\pm$ 0.076	0.371 $\pm$ 0.025	14.3	85.7	0	No
<i>Nereocystis luetkeana</i>	1.995 $\pm$ 0.039	0.180 $\pm$ 0.011	8.8	91.2	0	No

<sup>a</sup>From Guenther (2011) and Guenther & Martone (2014); <sup>b</sup>Calculated using dry weight  $\text{CaCO}_3$  content from Guenther (2011) and Guenther & Martone (2014); <sup>c</sup>Calculated using dry weight  $\text{CaCO}_3$  content from E. Porcher (unpubl. data); <sup>d</sup>Calculated using dry weight  $\text{CaCO}_3$  content from Martone et al. (2021)



nificant differences in caloric value per unit dry weight within both calcified and uncalcified species (ANOVA,  $F_{9,51} = 296.4$ ,  $p < 0.001$ ; Appendix Table A1). *C. vancouveriensis* had a higher caloric value than *C. tuberculosum* (Tukey's post hoc,  $p < 0.001$ ), *Chamberlainium tumidum* ( $p = 0.003$ ), and *Corallina chilensis* ( $p = 0.041$ ), but similar caloric values to *Bossiella frondifera* ( $p = 0.458$ ). *F. distichus* had a higher caloric value than *Alaria marginata* ( $p = 0.001$ ), which had a higher caloric value than *Hedophyllum sessile* ( $p < 0.001$ ) and *Macrocystis pyrifera* ( $p < 0.001$ ). *H. sessile* and *M. pyrifera* had similar caloric values ( $p = 0.614$ ), and both species exhibited larger caloric values than *N. luetkeana* ( $p = 0.001$  and  $p < 0.001$ , respectively) (Fig. 1A). Per dry weight, all uncalcified species had significantly higher caloric value than all calcified species when grouped (Welch's *t*-test,  $t_{44.9} = -23.7$ ,  $p < 0.001$ ).

When measured in terms of volume, calcified species exhibited significantly higher caloric values than uncalcified species (Student's *t*-test,  $t_{59} = 4.45$ ,  $p < 0.001$ ). Per volume, the caloric value of uncalcified species ranged from  $0.180 \pm 0.011$  kcal  $\text{ml}^{-1}$  (*N. luetkeana*) to  $0.739 \pm 0.041$  kcal  $\text{ml}^{-1}$  (*F. distichus*), and calcified coralline species ranged from  $0.483 \pm 0.089$  kcal  $\text{ml}^{-1}$  (*Corallina chilensis*) to  $0.825 \pm 0.085$  kcal  $\text{ml}^{-1}$  (*C. tumidum*) (Table 1, Fig. 1B). Significant differences in caloric value between species

Fig. 1. Mean caloric content in (A) kcal  $\text{g}^{-1}$  dry weight and (B) kcal  $\text{ml}^{-1}$  for 10 species of macroalgae, including 5 calcified species (white circles) and 5 uncalcified species (black circles). Different letters indicate  $p < 0.05$  for a Tukey's (A) or Dunn's (B) post hoc ANOVA. Error bars: 95% confidence intervals from the mean. See Table 1 for full species names

remained when measured in terms of volume (Kruskal-Wallis,  $\chi^2_9 = 42.1$ ,  $p < 0.001$ ). Differences between *F. distichus* and *N. luetkeana*, *H. sessile*, and *M. pyrifera* were still significant when analyzed per volume (Dunn's post hoc,  $p = 0.001$ ,  $p = 0.015$ , and  $p = 0.028$ , respectively), but differences between *F. distichus* and *A. marginata* diminished ( $p = 0.197$ ). Moreover, per volume, the caloric value of *C. vancouveriensis* and *C. tumidum* were more than double the caloric value of *N. luetkeana* ( $p < 0.001$  for both), significantly larger than *H. sessile* ( $p = 0.009$  and  $p = 0.011$ , respectively) and *M. pyrifera* ( $p = 0.015$  and  $p = 0.019$ , respectively), and similar to *F. distichus* ( $p = 0.981$  and  $p = 0.980$ , respectively) and *A. marginata* ( $p = 0.154$  and  $p = 0.768$ , respectively). *B. frondifera* had a significantly higher caloric value than *H. sessile* ( $p = 0.036$ ) and *N. luetkeana* ( $p = 0.006$ ). *C. tuberculosisum* showed a significantly greater caloric value than *N. luetkeana* ( $p = 0.014$ ). Caloric values for *C. chilensis* were not significantly different from any of the uncalcified species in terms of volume. No uncalcified species exhibited significantly greater caloric values than any of the calcified species. Despite differing proportions of weight attributed to water and  $\text{CaCO}_3$ , uncalcified and calcified species had similar proportions of

weight attributed to organic content (Table 1, Fig. 2). The organic content of *C. vancouveriensis* was more than double that of *N. luetkeana*.

#### 4. DISCUSSION

Conclusions about the interspecific differences in caloric content depend greatly on whether caloric value is calculated in terms of dry weight or volume. Per dry weight, uncalcified species have a much higher caloric value than calcified species, findings which are supported by Paine & Vadas (1969a). Yet this broad distinction disappears when comparisons are made per volume (i.e. the more ecologically meaningful, 'biteful'), with any remaining differences likely unrelated to calcification.

Sample density and tissue composition clearly affect caloric value depending on the metric used. Despite exhibiting similar proportions of organic tissue, calcified and uncalcified algal species had different proportions of inorganic material: calcified coralline species are largely composed of  $\text{CaCO}_3$  (density:  $2.71 \text{ g cm}^{-3}$ ; Weast 1986), whereas uncalcified species are mainly composed of water (density:  $1.02 \text{ g cm}^{-3}$ ; Vogel 1988). These differences in tissue

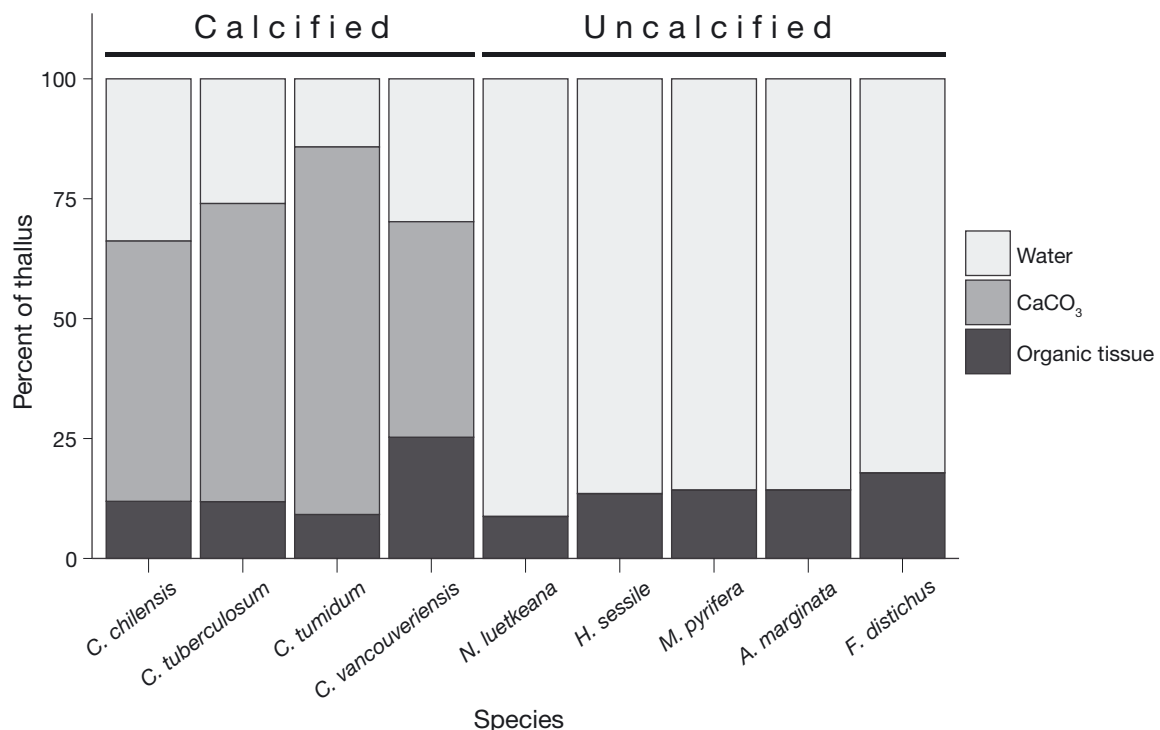


Fig. 2. Proportional representations of water, calcium carbonate ( $\text{CaCO}_3$ ), and organic tissue per wet weight for 9 algal species, including 4 calcified species and 5 uncalcified species. Organic tissue is defined as any portion of the thallus that is not composed of water (uncalcified species) or water and  $\text{CaCO}_3$  (calcified species). See Table 1 for full species names

composition and density likely have a large effect on weight-based comparisons, deflating values for calcified species. These issues may be compounded when using sample dry weight or AFDW since components like water and  $\text{CaCO}_3$  occur in different proportions across different species of macroalgae and, therefore, the effects of removing them from analyses will be non-uniform. Dry weight excludes water but retains  $\text{CaCO}_3$ , deflating relative caloric values of calcified species. AFDW excludes both water and  $\text{CaCO}_3$ , which may seem reasonable, but this metric excludes 2 components that herbivores still ingest, raising questions about its biological relevance. We assert that comparing caloric values of macroalgae must be done on a volumetric basis where all tissue ingested is included and differences in tissue density are mitigated. Applying this method, we found that the caloric value of macroalgae per volume is unrelated to calcification, and herbivores ingest a similar number of calories in any given bite or feeding stroke regardless of whether algae are calcified or not. This likely reflects the similar proportions of organic tissue comprising both calcified and uncalcified macroalgal species, avoids the disparity that occurs when only considering dry weight, and supports previously published work on coralline nutritional value (Maneveldt & Keats 2008).

Our findings suggest that another mechanism besides caloric value must be responsible for the avoidance of calcified species by marine herbivores. Alternative hypotheses may relate to the cost of ingesting  $\text{CaCO}_3$  and perhaps the additional time required to clear  $\text{CaCO}_3$  from the gut. Hay et al. (1994) found that  $\text{CaCO}_3$  interacts with secondary metabolites in calcified green algae, increasing their effectiveness in grazing resistance. However, past researchers have searched for anti-herbivory compounds in coralline algae but failed to find any candidates (M. Borowitzka pers. comm.), aside from those posited to deter epiphytic growth (Suzuki et al. 1998). Given the growing recognition of the taxonomic and ecological diversity of coralline algae, additional studies evaluating basic assumptions of coralline herbivory are warranted.

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### Appendix.

Table A1. ANOVA results for the effect of algal species on kcal g<sup>-1</sup>

Source of variation	SS	df	MS	F
Species	71 817 502	9	7 979 722	328.19
Residuals	1 240 029	51	24 314	
Total	73 057 531	60		

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