



# Gelatinous and soft-bodied zooplankton in the Northeast Pacific Ocean: organic, elemental, and energy contents

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**ABSTRACT:** Gelatinous and soft-bodied zooplankton (GZ) have long been considered to have low energetic value ('trophic dead end hypothesis') and be insufficient to sustain higher trophic levels. However, the nutritional composition and energy content of GZ are often poorly known for entire groups, ignoring species-, size-, and stage-specific differences. In this study, organic matter and elemental composition (carbon and nitrogen) were measured for >1000 specimens from 34 GZ species collected from neritic and oceanic waters of the Northeast Pacific between 2014 and 2020. Species included 3 gastropods, 16 hydrozoans, 2 nude ctenophores, 6 scyphozoans, 3 tentaculate ctenophores, and 4 thaliaceans. Organic content and elemental composition were used to estimate energy content using published conversion factors and differed between and within taxonomic classes. Size-dependent variability was shown for several species. Differences in organic content and elemental composition by development stage were observed in a salp and scyphomedusa species, highlighting the need to consider life cycle stages separately. The relative energy values of GZ were generally low and highly variable, although some taxa were comparable to crustaceans. The findings of the present study emphasise the need for a more detailed consideration of GZ in marine food web models and time series analyses, to take into account their inter- and intraspecific variability.

**KEY WORDS:** Subarctic Pacific · Jellyfish · Elemental composition · Salish Sea · Ctenophore · Energy content · Salp · Doliolid · Pteropod · Heteropod

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## 1. INTRODUCTION

Historically, gelatinous and soft-bodied zooplankton (GZ; medusae, siphonophores, ctenophores, pelagic tunicates, pelagic gastropods) have been assumed to represent a 'trophic dead end', i.e. the channelling of energy towards functional groups that contribute little to the production of higher trophic levels (Hans-

son & Norrman 1995, Verity & Smetacek 1996, Parsons & Lalli 2002, Sommer et al. 2002). However, feeding on GZ can provide several advantages for predators, such as fast digestibility, low capture and handling costs, easy access during bloom times, and high prey-replenishment rates. In addition, selective feeding on energy-rich tissues and organs of GZ can yield greater benefits than would be estimated from

whole organisms (Purcell 1991, Hays et al. 2018). GZ have been identified in stomachs of predators by traditional gut content analyses (e.g. Pawelec et al. 2016, M. J. Miller et al. 2019). However, due to the lack of hard structures and high digestibility as well as rapid dissolution in conventional preservatives, GZ were likely underrepresented in dietary studies (Hays et al. 2018). Traditional and new, state of the art approaches, e.g. stable isotope analysis (Cardona et al. 2012, Chi et al. 2021), animal-borne camera recordings (Thiebot et al. 2017), remotely operated vehicle observations (Robison 2004, Choy et al. 2017), and DNA metabarcoding of stomach contents and faecal pellets (Jarman et al. 2013, McInnes et al. 2016), have revealed that GZ are frequently consumed by a diverse set of marine vertebrate and invertebrate predators, including other GZ (Harrison 1984, Henschke et al. 2013, Choy et al. 2017, Thiebot et al. 2017, Brodeur et al. 2021).

GZ are ubiquitous components of marine food webs, which warrants greater attention to their ecological roles, particularly with respect to how they may shape the response of ecosystems in the face of environmental change (Hays et al. 2018, Schaafsma et al. 2018). GZ are infrequently included in food web models or included only as a single functional group, which ignores their diversity (Pauly et al. 2009, Lamb et al. 2019). In widely used Ecopath models, wet weights (WW) are the standard biomass metric. However, this metric is inappropriate for GZ due to their high water content (often >95% of WW) that likely results in an overestimation of the GZ contribution to the food web dynamics. This is one reason that energy units may be a more appropriate metric in such models (Pauly et al. 2009).

The nutritional value of GZ depends on their energy content and chemical composition. A number of studies have attempted to determine the energetic value of salps (e.g. Madin et al. 1981, Dubischar et al. 2006, 2012), scyphozoan jellyfish (Doyle et al. 2007, Khong et al. 2016, Schaafsma et al. 2018), and a broader spectrum of GZ (Ikeda 1974, Percy & Fife 1981, Larson 1986, Clarke et al. 1992, Donnelly et al. 1994) using proximate-composition and bomb-calorimetry approaches. The biochemical composition of GZ—major elements such as carbon (C) and nitrogen (N)—are important data for studying biochemical cycles and can be directly linked to energy content. Henschke et al. (2013) showed that the C:N ratio (in multicellular animals, an indicator of food quality due to high lipid content) of the salp *Thetys vagina* is comparable with the ratio in phytoplankton (in plants, a high C:N ratio typically indicates a low

nutritional value, e.g. Mei et al. 2005). However, knowledge of energetic values and C:N ratios of GZ taxa is still limited to a few species from selected ecosystems or commercial perspectives of edible jellyfish (e.g. Khong et al. 2016). There is a need to extend data on energy values and chemical compositions of GZ species (especially for smaller, cryptic species), to better represent GZ functional groups in food web models (Pauly et al. 2009, Schaafsma et al. 2018, Lamb et al. 2019). In addition to species-specific differences, it is likely that the energetic value of GZ is variable among seasons, regions, temperatures, food supply, sexes, sizes, and development stages (e.g. Schaafsma et al. 2018). Fatty acid concentrations of the common scyphomedusa *Aurelia aurita* have been shown to change seasonally, indicating variable energy values (Stenvers et al. 2020), but no seasonal differences in biochemical composition were detected for *Ihlea racovitzai* and *Salpa thompsoni* sampled in the Lazarev Sea (Dubischar et al. 2012).

The GZ fauna of the coastal and offshore waters of the Northeast Pacific from Alaska to California is among the best-studied worldwide (reviewed by Uye & Brodeur 2017). The recent introduction of subtropical and tropical species to the study region during the 2013–2015 marine heatwave (Brodeur et al. 2018, R. R. Miller et al. 2019), and associated occurrence of large-scale unprecedented pelagic tunicate blooms, have raised questions about the potential response of other food web components to a 'gelatinous future' (Richardson et al. 2009, Li et al. 2013, Brodeur et al. 2018, Galbraith & Young 2018). This extends beyond the expectation that facultative 'jellyvorous' (Cardona et al. 2012) or 'gelativorous' (Brodeur et al. 2019) predators could take advantage of new prey, and calls for focussed studies to better resolve GZ functional groups in food web models, to more realistically capture ecosystem responses and predict future change.

The aim of the present study was to measure species-specific elemental composition and energy content for Northeast Pacific GZ, including hydro- and scyphomedusae, pelagic gastropods, ctenophores, salps, and doliolids. To improve the estimates of GZ nutritional value, we also aimed to resolve size- and development stage-dependent differences within species. Further, weight-specific energy values and C:N ratios were compared with published values of the same or sister species from other regions and other non-GZ components of the Northeast Pacific pelagic food web. Ultimately, it was our intention to provide energy-based food web models with better-resolved energetics for GZ.

## 2. MATERIALS AND METHODS

### 2.1. Study area and collection of specimens

GZ were collected in coastal and offshore areas of British Columbia (BC) and the Gulf of Alaska. From 2014 to 2020, 11 cruises and 5 land-based collections were carried out between February and September in the following areas: southern Gulf of Alaska, Queen Charlotte Strait, Queen Charlotte Sound, west coast of Vancouver Island, Strait of Georgia, and Juan de Fuca Strait (46.36°–54.57° N, 123.07°–147.50° W; Fig. 1; Table S1 in Supplement 1 at [www.int-res.com/articles/suppl/m665p019\\_supp1.pdf](http://www.int-res.com/articles/suppl/m665p019_supp1.pdf)). Additionally, 2 visits were made to a local public aquarium to collect samples of different life cycle stages. More than 1000 specimens were collected from 202 stations, comprising 34 species: 3 gastropods, 16 hydrozoans, 6 scyphozoans, 2 nude ctenophores, 3 tentaculate ctenophores, and 4 thaliaceans. Midwater trawls, and Bongo, Juday, Multinet, neuston, and dip nets were used for onboard specimen collection between the surface and 1200 m depth (Table S1). Shore-based collections were performed in marinas and from beaches using dip nets. Siphonophores and larvaceans were not included in the present study, because the net types created difficulties in getting intact specimens.

Samples collected on ships or land-based excursions were sorted for GZ immediately after coming onboard or to shore, prior to preservation. GZ were identified to genus and, where possible, to species

level, based on taxonomic criteria from Arai & Brinckmann-Voss (1980), Wrobel & Mills (1998), Boltovskoy (1999), and Bouillon et al. (2006). GZ specimens were counted, separated by species, and measured for size: umbrella diameter for medusae; total length for ctenophores, salps, doliolids, and pelagic gastropods. Measurements were taken of maximum extent to the nearest millimetre, and individuals were immediately frozen. Small groups of selected species were stored in Petri dishes or single-use plastic bags at –20 or –80°C for further analysis. WW of larger scypho- and hydromedusae was measured within minutes of being removed from the net. WW of other taxa was not recorded. For some salp individuals, size was measured using a ruler to the nearest millimetre immediately after thawing in the laboratory. No great difference in size was expected between fresh and thawed specimens. Salps, especially the blastozooids, possess long projections that vary in size intraspecifically. Using the total length, instead of the more conservative oral-atrial length, may have introduced some bias to these size measurements.

The early life cycle stages of the scyphozoan *Aurelia labiata* were obtained from the Shaw Centre for the Salish Sea in Sidney, BC in January and June 2020. This aquarium facility has maintained a culture of *A. labiata* for about 10 yr, periodically supplemented with specimens from the field. Medusae were kept in large plankton kreisels year-round at about 10°C and a salinity of 30 and were regularly fed with brine shrimps (*Artemia* sp.). All life cycle stages were maintained at the aquarium, and ephyrae, kept in a

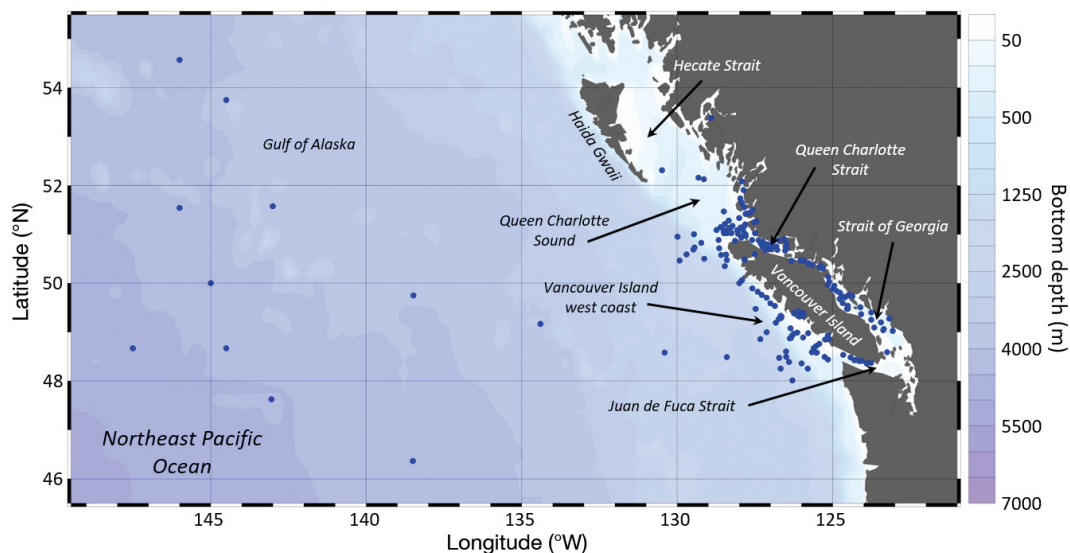


Fig. 1. Stations sampled (blue dots) in the Northeast Pacific between 2014 and 2020. Bathymetry obtained from ODV version 5.2 (Schlitzer 2020)

separate aerated aquarium, were sampled by gently spooning them out of the tank and freezing them in small groups at  $-20^{\circ}\text{C}$ . Ephyra diameters were measured with a ruler to the nearest millimetre. Planulae were taken directly from the breeding pouches of adult females (umbrella diameters: 108, 120, 152, and 160 mm) using 1-way pipettes and were transferred to plastic bags. A few millilitres of mucus-planulae mix were frozen at  $-20^{\circ}\text{C}$  and stored for further analysis.

## 2.2. Dry weight, ash-free dry weight, and C and N content determination

Specimens of all taxa were divided into 2 groups of equal size and similar size-frequency distribution: 1 group was used for elemental composition analysis, while the other group was used for organic content determination (Fig. 2). Both analyses were performed on freeze-dried GZ (individuals, or groups that were clumped before analysis). Frozen specimens were dried in an Edwards Modulyo Freeze Dryer at  $-45^{\circ}\text{C}$  for 68–434 h until a constant weight was reached. No parasites were detected on GZ collected for this analysis. Juvenile stages of *Aurelia labiata* (planulae, ephyrae) were not included in this calculation. Dry weight (DW) of each specimen (individuals or groups depending on the species) was determined using an analytical balance (Mettler Toledo; 10  $\mu\text{g}$  precision) before homogenisation with pestle and mortar. Approximately half of the samples (not homogenised) were combusted in a muffle furnace (SYBRON Thermolyne) at  $500^{\circ}\text{C}$  for 24 h, and ash-free dry weight (AFDW) was determined as the difference between DW and ash weight (AW). Blanks were run on empty sampling dishes throughout the analysis. The DW measurement contained residual water (i.e. water bound in collagen and not evaporated during freeze-drying) that leads to an overestimation of the AFDW. Therefore, a correction factor was applied, assuming that 11.7% of the DW was residual water (Larson 1986). This correction factor (i.e. corrected AFDW as a percentage of DW, AFDW % DW) was applied to

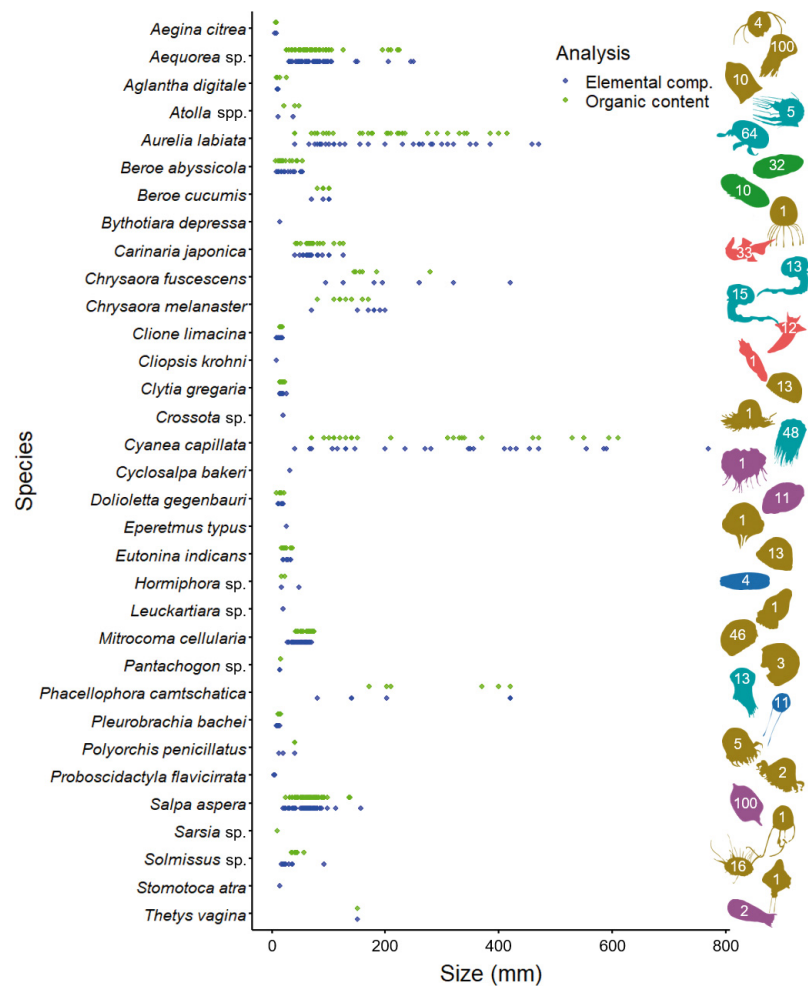


Fig. 2. Size spectrum overview of samples used in organic content and elemental composition determination (balanced design). Colours of silhouettes refer to class (dark yellow: Hydrozoa; light blue: Scyphozoa; green: Nuda; red: Gastropoda; purple: Thaliacea; dark blue: Tentaculata), and numbers indicate the number of analytical samples used in both analyses (total  $N = 593$ , only adults included). Note: 1 analytical sample can contain several specimens (see Tables 1 & 3). *Atolla* spp.: *A. vanhoeffeni* and *A. wyvillei*. *Bolinopsis infundibulum* ( $N = 9$ ) data not shown because of difficulties in size determination

DWs of all Hydrozoa, Scyphozoa, Nuda, Tentaculata, and Thaliacea species, ignoring potential species-specific differences. The DW of gastropods was not corrected for residual water since they are expected to contain considerably less collagen compared to other gelatinous species (Percy & Fife 1981, Kiørboe 2013). Removal of guts and gut contents under ship conditions, especially for smaller specimens, was not feasible and thus was not performed, to ensure consistency among samples. Contents of gastrovascular systems and guts may have influenced the total elemental composition and organic content data. The decision to accept this source of error was in line with earlier studies (Hays et al. 2018).

Homogenised samples for elemental composition analysis were collected in tin caps (8 × 5 mm) and weighed using a precision balance (Mettler Toledo; 1 µg precision). Empty tin caps were used as blanks (every 20 samples), while acetanilide was used as a standard (every 9 samples). Total C and N contents were determined in a CNS vario MICRO cube (Elementar Americas) and expressed per unit DW. Where there was sufficient material, samples were run in duplicate or triplicate. The minimum material used in elemental composition analysis was 1 mg. It should be noted that due to the DW containing salts, which does not affect AFDW and C:N estimates, elemental content expressed as a percentage of DW should be treated as an underestimate and considered conservative.

### 2.3. Energy content determination

Previous studies have determined energy content either through bomb calorimetry (e.g. Percy & Fife 1981, Schaafsma et al. 2018), or an estimation based on elemental and proximate composition (e.g. Clarke et al. 1992, Gorbatenko et al. 2009), or both (e.g. Doyle et al. 2007; see also our Table S2 in Supplement 2 at [www.int-res.com/articles/suppl/m665p019\\_supp2.xlsx](http://www.int-res.com/articles/suppl/m665p019_supp2.xlsx)). Initially, the intent of the present study was to perform bomb-calorimetric measurements on samples. However, the reproducibility of measurements was poor and the results obtained were orders of magnitude lower than those reported in the literature. This challenge was encountered by 2 independent laboratories: Center for Aquaculture & Environmental Research, Fisheries and Oceans Canada (Canada), and Royal Netherlands Institute for Sea Research (Netherlands). Therefore, we decided to use published conversion factors for diverse sets of species to estimate the energy content of GZ from C (Platt et al. 1969), or AFDW content of DW (our Table S3 in Supplement 1; Thayer et al. 1973, Båmstedt 1981).

### 2.4. Statistical analyses

Generalised linear models (GLMs) were used to explore the effect of size (diameter or length) on weight (WW, DW, and AFDW), elemental content (C, N), AFDW % DW, or C:N ratio (Gamma error structure, inverse-link, significant  $\alpha$  level of 0.05) in the R package 'lme4' (Bates et al. 2015). One-way ANOVA followed by Tukey-HSD post hoc tests for pairwise

comparisons were used to investigate differences in AFDW % DW, C % DW, N % DW, C:N ratio, and energy content derived from 3 different conversions between taxonomic classes or development stages of *Salpa* and *Aurelia*, respectively. Organic, elemental, and energy content as well as C:N data showed heteroscedasticity and the residuals were non-normally distributed (Shapiro-Wilk normality test). To meet the assumptions of the ANOVA (i.e. normality of data, homoscedasticity of variances, independence of data), data were  $\log_{10}$ -transformed. All statistical analyses were performed in R (R Core Team 2020) version 3.6.0.

## 3. RESULTS

### 3.1. Organic content

The organic content (i.e. AFDW % DW) varied considerably among the 26 species from 6 classes that were examined (Table 1, Fig. 3). WW-size relationships of the 6 most abundant scypho- and hydromedusa species are given in Table S4 in Supplement 1. Significant differences in organic contents were seen among taxonomic classes (Table S5 in Supplement 1) and between species within classes, for instance between *Mitrocoma cellularia* and *Aglantha digitale* ( $F_{1,25} = 49.98$ ,  $p < 0.001$ ). Of the 2 multi-species genera examined in this study, significant differences were seen within *Beroe* spp. ( $F_{1,19} = 81.54$ ,  $p < 0.001$ ), but not for *Chrysaora* spp. ( $F_{1,12} = 0.004$ ,  $p = 0.95$ ). Among all species, *Thetys vagina* had the highest organic content (75.3%), whereas *Bolinopsis infundibulum* had the lowest ( $13.1 \pm 0.9\%$ , mean  $\pm$  SD). When AFDW and DW were scaled by the size of the specimen (Table 2, Fig. 4), salps and hydro- and scyphomedusae showed decreasing organic contents with increasing size, but a neutral and even positive trend could be seen for the pelagic gastropod *Carinaria japonica* and the nude ctenophore *Beroe abyssicola*, respectively (Fig. 4). Although size-dependent trends were only significant for *Aurelia labiata*, *Cyanea capillata*, and *M. cellularia* ( $p \leq 0.02$ ), a size effect was apparent in most species. The organic content of a species was also stage-specific (e.g. *Salpa aspera* and *A. labiata*, Table S6 in Supplement 1, Fig. 5). In the case of *S. aspera*, blastozooids (aggregate stage) had a significantly higher organic content compared to oozooids (solitary stage) ( $F_{1,47} = 7.30$ ,  $p < 0.01$ ). In contrast, the organic content of medusae was significantly lower than in planula larvae ( $F_{1,37} = 28.14$ ,  $p < 0.001$ ) and in ephyrae ( $F_{1,35} =$

Table 1. Ash-free dry weight (AFDW) as a percentage of dry weight (DW) of 26 gelatinous and soft-bodied zooplankton species ( $N=291$ ) from 6 classes.  $N$ : number of analytical samples;  $n$ : number of specimens per analytical sample; *Atolla* spp.: *A. vanhoeffeni* and *A. wyvillei*. Corrected AFDW % DW assumed residual water is 11.7% of DW of gelatinous species (Larson 1986). Values for gastropods were not corrected. Data are mean  $\pm$  SD

Class Species	$N$	$n$	AFDW % DW	Corrected AFDW % DW
<b>Gastropoda</b>	21	1–9	54.0 $\pm$ 11.1	
<i>Carinaria japonica</i>	16	1	50.5 $\pm$ 9.5	
<i>Clione limacina</i>	5	2–9	65.0 $\pm$ 9.0	
<b>Hydrozoa</b>	104	1–15	26.7 $\pm$ 7.3	17.0 $\pm$ 8.2
<i>Aegina citrea</i>	2	1	37.1 $\pm$ 3.5	28.8 $\pm$ 4.0
<i>Aequorea</i> sp.	50	1–3	25.0 $\pm$ 5.1	15.1 $\pm$ 5.8
<i>Aglantha digitale</i>	5	1–15	37.3 $\pm$ 3.1	29.0 $\pm$ 3.5
<i>Clytia gregaria</i>	6	1–4	25.7 $\pm$ 3.8	15.9 $\pm$ 4.3
<i>Eutonina indicans</i>	7	2–5	26.8 $\pm$ 3.6	17.1 $\pm$ 4.0
<i>Mitrocoma cellularia</i>	22	1–4	24.5 $\pm$ 3.0	14.5 $\pm$ 3.4
<i>Pantachogon</i> sp.	1	1	58.3	52.8
<i>Polyorchis penicillatus</i>	2	1	54.3 $\pm$ 3.1	48.3 $\pm$ 3.5
<i>Sarsia</i> sp.	1	1	50.0	43.4
<i>Solmissus</i> sp.	8	1–3	23.8 $\pm$ 2.9	13.7 $\pm$ 3.2
<b>Nuda</b>	21	1	36.2 $\pm$ 7.9	27.7 $\pm$ 8.9
<i>Beroe abyssicola</i>	16	1	39.9 $\pm$ 4.5	31.9 $\pm$ 5.1
<i>Beroe cucumis</i>	5	1	24.3 $\pm$ 1.5	14.3 $\pm$ 1.7
<b>Scyphozoa</b>	79	1	30.4 $\pm$ 6.5	21.1 $\pm$ 7.3
<i>Atolla</i> spp.	3	1	33.8 $\pm$ 1.9	25.1 $\pm$ 2.2
<i>Aurelia labiata</i>	32	1	27.7 $\pm$ 5.3	18.1 $\pm$ 5.9
<i>Chrysaora fuscescens</i>	6	1	30.6 $\pm$ 1.3	21.4 $\pm$ 1.5
<i>Chrysaora melanaster</i>	8	1	31.0 $\pm$ 5.5	21.8 $\pm$ 6.2
<i>Cyanea capillata</i>	24	1	32.6 $\pm$ 7.6	23.6 $\pm$ 8.6
<i>Phacellophora camtschatica</i>	6	1	32.7 $\pm$ 9.7	23.8 $\pm$ 11.0
<b>Tentaculata</b>	11	1–18	25.0 $\pm$ 2.8	15.1 $\pm$ 3.2
<i>Bolinopsis infundibulum</i>	4	1	23.2 $\pm$ 0.8	13.1 $\pm$ 0.9
<i>Hormiphora</i> sp.	2	1	27.5 $\pm$ 6.7	17.9 $\pm$ 7.6
<i>Pleurobrachia bachei</i>	5	2–18	25.4 $\pm$ 1.3	15.5 $\pm$ 1.5
<b>Thaliacea</b>	55	1–30	39.7 $\pm$ 10.5	31.7 $\pm$ 11.9
<i>Doliolletta gegenbauri</i>	5	1–30	47.6 $\pm$ 15.6	40.7 $\pm$ 17.6
<i>Salpa aspera</i>	49	1–3	38.1 $\pm$ 8.0	29.9 $\pm$ 9.1
<i>Thetys vagina</i>	1	1	78.2	75.3

7.47,  $p < 0.01$ ). However, the organic content did not differ significantly between planulae and ephyrae ( $F_{1,10} = 1.51$ ,  $p = 0.25$ ) (Table S6). Trends depicted in Figs. 4 & 5 clearly show that differences not only occurred interspecifically (Table 1), but also intraspecifically. A comparison between our organic content results and those from the literature is presented in Fig. 6.

### 3.2. Elemental composition

C and N contents were determined for 33 species from 6 classes (Table 3, Fig. 3). C and N content, expressed as percent of DW, encompassed a wide

range (Table 3), from  $30.2 \pm 4.1$  C % DW and  $6.8 \pm 0.7$  N % DW in *Clione limacina* to  $1.0 \pm 0.6$  C % DW and  $0.3 \pm 0.2$  N % DW in *Solmissus* sp. The C and N content of GZ were positively correlated (Fig. 7), although the class Thaliacea had a lower N content at a comparably high C content. The latter was also reflected in the relatively high C:N ratio of this group (Table 3). The C and N contents were dependent on the size of the specimen (Table 4). Among all species, *T. vagina* and *Cyclosalpa bakeri* had the highest C:N ratio (6.8 and 6.9, respectively), whereas *Crossota* sp. had the lowest (3.1). Significant differences among classes (e.g. the C:N ratio of Thaliacea was significantly higher than in all other classes) and between species within classes occurred (Table S7 in Supplement 1). The C:N ratio of GZ varied considerably among the various taxa (Table 3), as well as within species with respect to size (Fig. 8), although no clear trend among classes was apparent in the latter. *Salpa aspera* was the only taxon to show a significant size-dependent trend (Fig. 8G;  $p = 0.03$ ). Stage specificity for elemental composition and C:N ratio was noted for *S. aspera* and *A. labiata* (Table S6, Fig. 5). *S. aspera* blastozooids (aggregate stage) had significantly higher C and N contents than oozoids (solitary stage) ( $p < 0.001$ ), while the C:N ratio did not differ significantly between stages

( $F_{1,47} = 0.12$ ,  $p = 0.73$ ). The pattern was more complex in *A. labiata*. The C % DW of ephyrae was significantly lower than in planula larvae ( $F_{1,10} = 9.19$ ,  $p = 0.01$ ) and in medusae ( $F_{1,35} = 62.42$ ,  $p < 0.001$ ), but did not differ between planulae and medusae ( $F_{1,37} = 0.32$ ,  $p = 0.57$ ; Table S6). Similar differences in N % DW were found among stages. The C:N ratio of planula larvae was significantly higher than in medusae ( $F_{1,37} = 319.3$ ,  $p < 0.001$ ), but was similar to that of ephyrae ( $F_{1,10} = 3.85$ ,  $p = 0.08$ ). It should be noted that data for planulae should be viewed with caution, as maternal material may have been included during sample collection. Trends depicted in Figs. 5 & 8 showed that differences occurred both inter- and intraspecifically. A comparison between

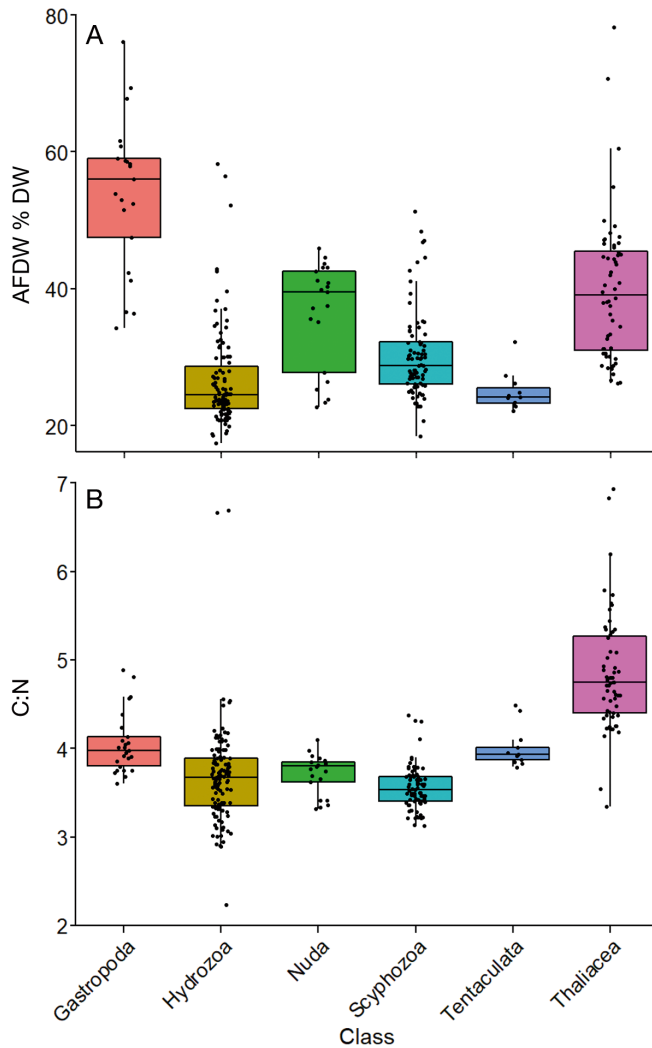


Fig. 3. (A) Organic content (ash-free dry weight as a percentage of dry weight, AFDW % DW; not corrected for residual water) and (B) C:N ratio (by mass) of Gastropoda (red;  $N = 21$  and  $25$ ), Hydrozoa (dark yellow;  $N = 103$  and  $114$ ), Nuda (green;  $N = 21$  and  $21$ ), Scyphozoa (light blue;  $N = 79$  and  $79$ ), Tentaculata (dark blue;  $N = 11$  and  $13$ ), and Thaliacea (purple;  $N = 56$  and  $59$ ). For numbers of respective specimens, see Tables 1 & 3 and Fig. 2. Line: median; box: interquartile range (IQR); whiskers: max./min. value  $\leq 1.5 \times$  IQR above/below box; dots: outliers

our C:N results and those from the literature is presented in Fig. 6.

### 3.3. Energy content

The measured organic content and elemental composition data were used to estimate weight-specific energy content. In the absence of GZ-specific conversion equations, we applied 3 conversions developed from general zooplankton assemblages (Table S3) and compared their results. The estimated values from the 3 equations, 2 of them using AFDW % DW data and 1 using C % DW data, aligned well (Fig. 9), supporting the use of these conversions. In all taxonomic classes, median energy content values derived from Båmstedt (1981) were highest, but were not always significantly different from energy content derived from the other 2 conversions. The conversion equation from Platt et al. (1969) used C % DW in contrast to the other 2 methods and can be considered conservative due to salt-related DW overestimation. However, the resulting energy values were in good agreement among approaches, which supported using conversions in this case.

Sampling conditions (i.e. salinity range, sampled depth, temperate-boreal species assemblage) used by Båmstedt (1981) were similar to our study and his mixed macrozooplankton included *Aglantha digitale* and *Pleurobrachia pileus*. Therefore, only the energy conversion equation based on AFDW % DW given by Båmstedt (1981) was used for further analyses. Species energy content ranged from  $20.9 \text{ kJ g DW}^{-1}$  in *T. vagina* to  $3.2 \pm 0.2 \text{ kJ g DW}^{-1}$  in *Bolinopsis infundibulum* (Table 5). Pairwise comparisons between classes revealed that gastropods had significantly higher energy content than all other taxonomic classes ( $p < 0.001$ ). Thaliacea generally had higher energy contents than other groups

Table 2. Corrected dry and ash-free dry weights (DW, AFDW) of 4 gelatinous and soft-bodied zooplankton species as a function of umbrella diameter ( $d$ , for hydromedusae) or length ( $L$ , for ctenophores and salps) following power functions  $DW = a \times d^b$  or  $a \times L^b$  and  $AFDW = c \times d^e$  or  $c \times L^e$ .  $N$ : number of specimens. Only species with a minimum of 16 specimens were included in generalised linear models. Many *Aequorea* sp. and *Mitrocoma cellularia* specimens were only sub-sampled and no total DW and AFDW are available. All:  $p < 0.01$

Species	Corrected DW					Corrected AFDW				
	$N$	Size range (mm)	$a$	$b$	$R^2$	$N$	Size range (mm)	$c$	$e$	$R^2$
<i>Aequorea</i> sp.	59	25–105	0.0019	2.91	0.73	42	25–105	0.0038	2.30	0.66
<i>Beroe abyssicola</i>	32	6–53	0.1873	1.78	0.84	16	6–53	0.0496	1.88	0.90
<i>Mitrocoma cellularia</i>	40	27–74	0.0001	3.92	0.84	21	42–74	0.0061	2.71	0.76
<i>Salpa aspera</i>	81	20–156	0.0004	2.91	0.90	46	24–137	0.0001	2.85	0.82

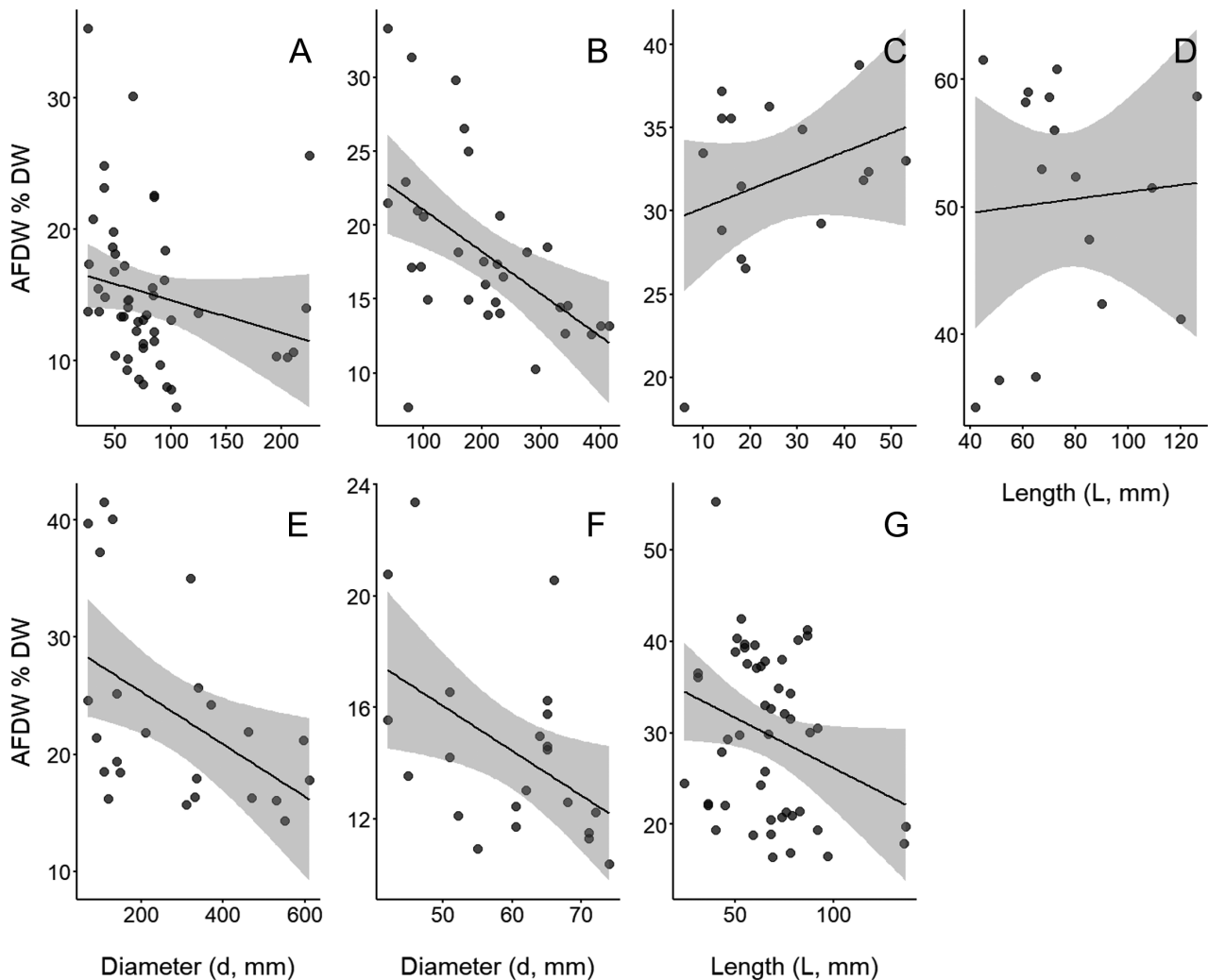


Fig. 4. Relationship of corrected ash-free dry weight as a percentage of dry weight (AFDW % DW) with size (diameter, d, for hydro- and scyphomedusae; length, L, for ctenophores, gastropods, and salps) for (A) *Aequorea* sp., (B) *Aurelia labiata*, (C) *Beroe abyssicola*, (D) *Carinaria japonica*, (E) *Cyanea capillata*, (F) *Mitrocoma cellularia*, and (G) *Salpa aspera*. Linear regression line shown with 95% confidence intervals (grey shading). Significant slopes ( $p < 0.05$ ) detected for *A. labiata*, *C. capillata*, and *M. cellularia*

except for Gastropoda and differed significantly from all classes except Nuda ( $p = 0.52$ ). The energy content of Hydrozoa, Scyphozoa, and Tentaculata was generally  $< 5 \text{ kJ g DW}^{-1}$ .

A comparison between our energy content results and those from the literature is presented in Fig. 6. As shown in Fig. 10 & Fig. S1 (in Supplement 1), crustaceans had the highest weight-specific (i.e. relative) energy content, and only a few pelagic gastropod and tunicate specimens approached these values. However, due to their large size, the total available energy from prey organisms was higher in GZ, especially from the classes Hydrozoa, Nuda, Scyphozoa, and Thaliacea.

## 4. DISCUSSION

### 4.1. Differences in organic content and elemental composition among species and classes

The results from our study confirmed that the organic content (i.e. AFDW % DW) of GZ was low compared to crustacean zooplankton from the Northeast Pacific, consistent with expectations of their low nutritional and energetic value (Hansson & Norrman 1995, Arai 2005). However, there was significant variability in organic content among species and development stages, reflecting the taxonomic diversity of GZ in this region.



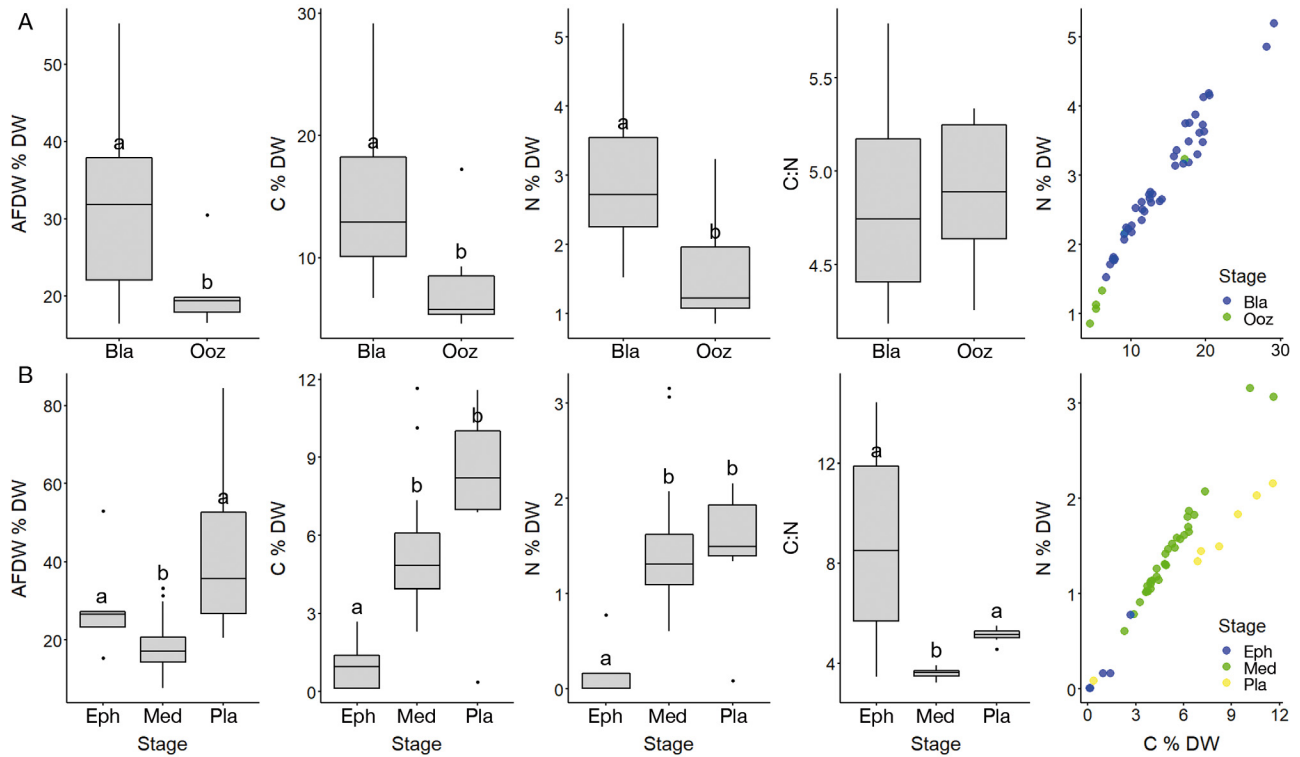


Fig. 5. Stage specificity of corrected ash-free dry weight as a percentage of dry weight (AFDW % DW), carbon and nitrogen content as a percentage of dry weight (C % DW, N % DW), and C:N ratio (by mass) in (A) *Salpa aspera* (Bla: blastozoid; Ooz: oozoid) and (B) *Aurelia labiata* (Eph: ephyra; Med: medusa; Pla: planula). Letters indicate significantly different stages ( $p < 0.05$ ). Box plot parameters as in Fig. 3

Variability in organic matter content among species appeared to be influenced, at least in part, by GZ life history traits. For instance, *Carinaria japonica* ( $50.5 \pm 9.5\%$ ) had significantly lower organic content than *Clione limacina* ( $65.0 \pm 9.0\%$ ). Both pelagic gastropod species are holoplanktonic and have partly reduced or absent shells. The within-class difference is likely a result of their preferred prey: *C. japonica* mostly feeds on salps and doliolids (comparably low organic content), whereas *C. limacina* almost exclusively consumes *Limacina helicina* (Wrobel & Mills 1998). Similarly, the narcomedusa *Solmissus* sp. that feeds exclusively on other GZ would be expected to have lower organic content than other hydromedusae that feed on 'richer' crustacean zoo- and ichthyoplankton. In the class Hydrozoa, the interspecific organic content variability was especially pronounced (Table 1), and *Solmissus* sp. indeed had the lowest organic content of all GZ measured ( $13.7 \pm 3.2\%$ ), though not always significantly.

When comparing organic content among GZ species and with crustaceans, the problem of residual water bound to collagen in gelatinous mesoglea and not reduced during drying needs to be taken into

consideration (Larson 1986). The residual water percentage may also be a function of size (Hirst & Lucas 1998), thus the single conversion factor used here may be an oversimplification. While this is not of relevance for other zooplankton taxa, Kogovšek et al. (2014) and MacKenzie et al. (2017) recommended standardised sample preparation when working with GZ. In the present study, all samples were prepared for analyses in the same fashion. Our data on organic content generally agreed well with values previously reported in the literature. The slightly lower values in our data may have occurred due to the residual water standardisation applied to all taxa other than Gastropoda, which was not done in most earlier studies.

Wright & Purcell (1997) and Hirst & Lucas (1998) noticed that ambient salinity has a considerable effect on DW and AW of jellyfish. Jellyfish are osmoconformers and thus have a high and variable body salt content, which can lead to an overestimation of the AFDW (i.e. organic content). Salinity was not measured systematically during all sampling events in this study, but can be roughly estimated to be between 27 and 35. Thus, the effect of salinity on DW and AW measurements was expected to be small in

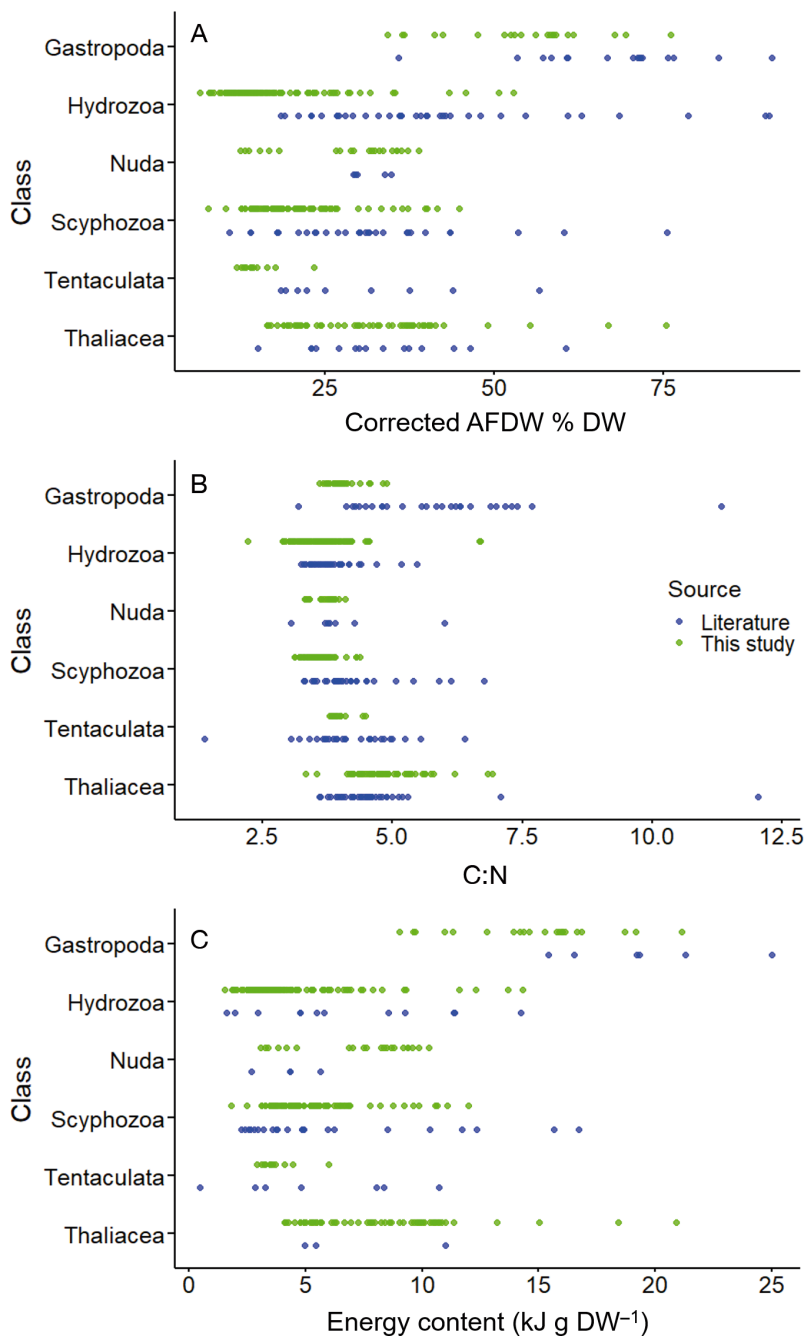


Fig. 6. Comparison of (A) organic content (corrected ash-free dry weight as a percentage of dry weight, AFDW % DW), (B) C:N ratio, and (C) energy content (conversion from Båmstedt 1981) by taxonomic class between the present study and previously published values. For details, see Table S2 (in Supplement 2) and Fig. 3

comparison to the salinity range of 10–33 in Hirst & Lucas (1998). While the effect of ambient salinity on weight changes of scyphozoan jellyfish has been explored sporadically, almost no effect studies exist for other GZ classes.

Ikeda (2014a,b) aimed at compiling a global data set of elemental composition and organic content values of GZ, but many studies available at that time were not included and more studies have been published since then. When compared to the literature, our C:N ratio data mirror the patterns seen in earlier studies, even though some were molar and not mass-specific ratios. The current global GZ data are understandably still incomplete, with large regions still underrepresented (e.g. Arctic, Indian, South Atlantic, central Pacific Oceans; Table S2). Thus, we encourage unpublished data to be made available, to build on the findings of Ikeda (2014a,b) for hydromedusae, scyphomedusae, and pelagic gastropods, including doliolids, pyrosomes, larvae, and cubomedusae (Table S2).

For GZ collected in Saanich Inlet, BC, Larson (1986) observed that the C % DW was generally below 15% (content of gonads was considerably higher), substantially lower than values reported in our study (up to 35%, Fig. 7). However, Larson (1986) did not include pelagic gastropods, tunicates, and ctenophores, which were largely responsible for C % DW values >15% in our study. In addition to variability among taxa, we also observed intra-specific variability, as previously discussed by Hirst & Lucas (1998). Inter-region comparisons of GZ population (and life cycle stage) organic content and elemental composition, as presented in Ikeda (2014a), are still hampered by the low number of analytical studies of the same species experiencing different environmental conditions. One of the best-studied species in this respect is *Aglantha digitale*, a small trachymedusa found in cold-temperate to arctic environments that shows remarkable plasticity (Table S2).

While the C:N ratio of *A. digitale* had a rather narrow range in this study (3.4–3.9), the organic content varied between 39.1 and 60.8% (mean  $\pm$  SD: 29.0  $\pm$  3.5%). This variability may be explained by different ambient temperatures, development/reproductive

Table 3. Elemental percentages of dry weight (DW) of 33 gelatinous and soft-bodied zooplankton species ( $N = 311$ ) from 6 classes.  $N$ : number of analytical samples;  $n$ : number of specimens per analytical sample. C:N ratio is by mass. Data are mean  $\pm$  SD

Class Species	$N$	$n$	C % DW	N % DW	C:N ratio
<b>Gastropoda</b>	25	1	23.7 $\pm$ 6.7	5.8 $\pm$ 1.3	4.1 $\pm$ 0.3
<i>Carinaria japonica</i>	17	1	21.0 $\pm$ 6.0	5.4 $\pm$ 1.4	3.9 $\pm$ 0.2
<i>Cliome limacina</i>	7	1	30.2 $\pm$ 4.1	6.8 $\pm$ 0.7	4.5 $\pm$ 0.3
<i>Cliopsis krohnii</i>	1	1	23.4	5.7	4.1
<b>Hydrozoa</b>	114	1–12	6.9 $\pm$ 6.7	1.9 $\pm$ 1.8	3.7 $\pm$ 0.6
<i>Aegina citrea</i>	2	1	24.4 $\pm$ 4.3	5.9 $\pm$ 0.8	4.1 $\pm$ 0.2
<i>Aequorea</i> sp.	50	1–3	5.9 $\pm$ 5.7	1.7 $\pm$ 1.6	3.5 $\pm$ 0.4
<i>Aglantha digitale</i>	5	2–9	14.6 $\pm$ 5.3	4.2 $\pm$ 1.3	3.5 $\pm$ 0.2
<i>Bythotiara depressa</i>	1	1	6.4	1.0	6.7
<i>Clytia gregaria</i>	7	1–10	4.6 $\pm$ 1.4	1.2 $\pm$ 0.4	3.9 $\pm$ 0.2
<i>Crossota</i> sp.	1	1	16.2	5.2	3.1
<i>Eperetmus typus</i>	1	1	7.5	1.9	4.0
<i>Eutonina indicans</i>	6	1–12	4.5 $\pm$ 0.9	1.3 $\pm$ 0.3	3.5 $\pm$ 0.6
<i>Leuckartiara</i> sp.	1	1	10.1	2.6	3.8
<i>Mitrocoma cellularia</i>	24	1–3	6.5 $\pm$ 7.6	1.7 $\pm$ 2.0	3.8 $\pm$ 0.3
<i>Pantachogon</i> sp.	2	1–2	8.7 $\pm$ 0.7	1.6 $\pm$ 0.6	5.6 $\pm$ 1.5
<i>Polyorchis penicillatus</i>	3	1	16.8 $\pm$ 9.4	4.4 $\pm$ 2.2	3.7 $\pm$ 0.3
<i>Proboscidactyla flavicirrata</i>	2	2–6	15.2 $\pm$ 4.8	3.5 $\pm$ 0.9	4.4 $\pm$ 0.3
<i>Stomotoca atra</i>	1	3	12.7	2.9	4.2
<i>Solmissus</i> sp.	8	1–2	1.0 $\pm$ 0.6	0.3 $\pm$ 0.2	3.3 $\pm$ 0.2
<b>Nuda</b>	21	1	11.4 $\pm$ 4.8	3.0 $\pm$ 1.2	3.7 $\pm$ 0.2
<i>Beroe abyssicola</i>	16	1	13.8 $\pm$ 2.1	3.6 $\pm$ 0.5	3.8 $\pm$ 0.1
<i>Beroe cucumis</i>	5	1	3.7 $\pm$ 1.3	1.1 $\pm$ 0.4	3.4 $\pm$ 0.0
<b>Scyphozoa</b>	79	1–2	6.6 $\pm$ 2.8	1.9 $\pm$ 0.8	3.6 $\pm$ 0.2
<i>Atolla vanhoeffeni</i>	2	1–2	7.9 $\pm$ 1.5	2.2 $\pm$ 0.3	3.6 $\pm$ 0.2
<i>Aurelia labiata</i>	32	1	5.2 $\pm$ 1.9	1.4 $\pm$ 0.6	3.6 $\pm$ 0.2
<i>Chrysaora fuscescens</i>	7	1	8.3 $\pm$ 1.1	2.3 $\pm$ 0.3	3.6 $\pm$ 0.4
<i>Chrysaora melanaster</i>	7	1	7.8 $\pm$ 2.7	2.1 $\pm$ 0.9	3.8 $\pm$ 0.4
<i>Cyanea capillata</i>	24	1	7.2 $\pm$ 3.4	2.1 $\pm$ 1.0	3.5 $\pm$ 0.2
<i>Phacellophora camtschatica</i>	7	1	7.4 $\pm$ 3.5	2.3 $\pm$ 1.2	3.3 $\pm$ 0.1
<b>Tentaculata</b>	13	1–7	3.6 $\pm$ 2.1	0.9 $\pm$ 0.5	4.0 $\pm$ 0.2
<i>Bolinopsis infundibulum</i>	5	1	1.6 $\pm$ 0.4	0.4 $\pm$ 0.1	3.9 $\pm$ 0.1
<i>Hormiphora</i> sp.	2	1	5.6 $\pm$ 2.8	1.4 $\pm$ 0.7	3.9 $\pm$ 0.0
<i>Pleurobrachia bachei</i>	6	1–7	4.6 $\pm$ 1.5	1.1 $\pm$ 0.3	4.1 $\pm$ 0.3
<b>Thaliacea</b>	59	1–13	14.5 $\pm$ 7.0	3.0 $\pm$ 1.4	4.8 $\pm$ 0.7
<i>Cyclosalpa bakeri</i>	1	6	5.4	0.8	6.9
<i>Doliioletta gegenbauri</i>	6	1–13	20.9 $\pm$ 12.9	4.8 $\pm$ 2.6	4.1 $\pm$ 0.6
<i>Salpa aspera</i>	51	1–3	13.7 $\pm$ 5.6	2.8 $\pm$ 0.9	4.9 $\pm$ 0.5
<i>Thetys vagina</i>	1	1	26.2	3.8	6.8

stage, and/or depth at collection time (Ikeda 2014a), e.g. size and stage composition are known to vary with depth (0–1000 m, Mańko et al. 2020). Importantly, these findings demonstrate that the ‘reductionist approach’ of using generic values for species can lead to a significant over- or underestimation of elemental or energy content of GZ. The few species that have been studied extensively, such as *C. limacina*, *Pleurobrachia pileus*, and *Salpa thompsoni*, would allow for further research into inter-population variability. Few data exist for the vast majority of GZ species, leaving space for speculation about abi-

otic drivers of species biochemistry. However, it should be noted that GZ are increasingly being recognised as major contributors of vertical nutrient fluxes and are important in structuring marine bacteria assemblages (Pitt et al. 2009, Henschke et al. 2016). An in-depth understanding of their elemental and proximate composition should be continued to provide a more realistic parameterisation of GZ, including biochemical flux and cycling studies.

#### 4.2. Size and stage specificity of organic content and elemental composition

In the present study, we found differences in the C:N ratio among life cycle stages of *Aurelia labiata*, but not between blastozoids and oozoids of *Salpa aspera*. This may have been due to the small sample size, as stage specificity of the C:N ratio has previously been found for the lobate ctenophore *Mnemiopsis leidyi* (cydippid larvae versus lobate adults; Javidpour et al. 2020). However, blastozoids and oozoids differed significantly in organic and elemental content, as shown previously for *S. thompsoni* (Dubischar et al. 2006, 2012). In other investigations, Heron et al. (1988) and Iguchi & Ikeda (2004) did not find stage-specific differences in elemental contents, in either *Thalia democratica* or *S. thompsoni*. On the contrary, Stenvers et al. (2020) showed differences in total fatty acid concentration and composition between 3 planktonic scyphozoan jellyfish life cycle stages, indicating differing nutritional values. Thus, analysing groups that are truly comparable in terms of stage and size composition is critical to avoid erroneous conclusions.

Stage-dependent differences were detected for organic and elemental content and C:N ratio in ephyrae, medusae, and planulae of *A. labiata*. Planulae showed significantly higher organic and elemental content when compared to the adults, in line with their non-feeding lecithotrophic trait (Suzuki et al. 2019). Ephyrae had high organic content, but low C and N content, underscoring their poorer nutritional

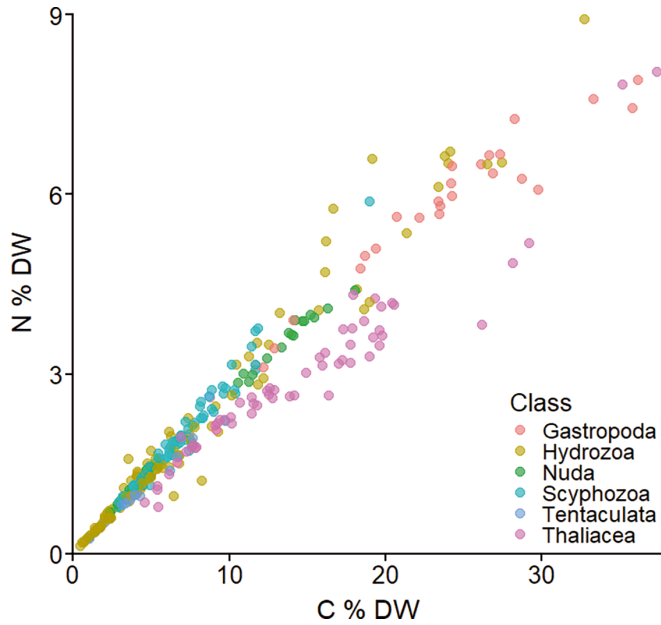


Fig. 7. Relationship between C and N expressed as percent of dry weight ( $N = 311$ ). Only adult specimens were included. For numbers of respective specimens, see Table 3 and Fig. 3

condition. When compared to published values, the organic and elemental content of planulae and ephyrae from our study were low (Table S6). This dif-

ference may have been due to a considerably smaller size of ephyrae in our study (ephyrae in our study were 2–6 mm, but 4–12 mm in other studies) or species specificity. Apparently, the organic content of anthozoan planulae (Richmond 1987, Ben-David-Zaslow & Benayahu 2000) is considerably higher than those of scyphozoans, but this requires further examination.

Organic and elemental content vary with individual size, as shown for 7 frequently encountered GZ species (Tables 2 & 4, Figs. 4 & 8). With increasing body size, the ratio between cellular tissue and acellular mesoglea decreases disproportionately in gelatinous species, so that smaller ctenophores, medusae, and tunicates have higher weight-specific contents than larger conspecifics, as shown by Iguchi & Ikeda (2004) for *S. thompsoni*. In *Aequorea* sp., *Beroe abyssicola*, *Carinaria japonica*, and *Salpa aspera*, the organic content-size correlation was not significant, potentially due to either small sample sizes, other morphological changes, or independence of organic content from size. In contrast, Iguchi et al. (2017) reported increasing C and N % DW values with increasing umbrella diameter in the large rhizostome jellyfish *Nemopilema nomurai*. In our study, the C:N ratio did not change significantly with size for any of the 7 species, except for *S. aspera*. Riascos et al. (2015) also did not find a size-dependent

Table 4. Carbon and nitrogen content (as percentage of dry weight: C % DW, N % DW) of various gelatinous and soft-bodied zooplankton species as a function of umbrella diameter (d, for hydro- and scyphomedusae) or length (L, for ctenophores, gastropods, and salps) following linear functions  $C \% DW = a \times d + b$  or  $a \times L + b$  and  $N \% DW = c \times d + e$  or  $c \times L + e$ . N: number of specimens. Only species with a minimum of 16 specimens were included in generalised linear models. **Bold**: significant ( $p < 0.05$ ) slopes

Species	N	Size range (mm)	a	b	R <sup>2</sup>	t	p
<i>Aequorea</i> sp.	49	30–250	-0.004	5.686	0.01	0.31	0.757
<i>Aurelia labiata</i>	32	40–470	-0.007	6.647	0.21	3.05	<b>0.005</b>
<i>Beroe abyssicola</i>	16	8–53	-0.074	15.773	0.31	2.60	<b>0.021</b>
<i>Carinaria japonica</i>	17	40–125	-0.135	30.822	0.24	2.10	0.053
<i>Cyanea capillata</i>	24	40–770	-0.002	8.007	0.02	0.65	0.523
<i>Mitrocoma cellularia</i>	24	27–70	-0.324	22.320	0.31	3.21	<b>0.004</b>
<i>Salpa aspera</i>	51	20–156	-0.111	20.680	0.22	3.71	<b>0.001</b>
Species	N	Size range (mm)	c	e	R <sup>2</sup>	t	p
<i>Aequorea</i> sp.	49	30–250	-0.001	1.656	0.01	0.35	0.726
<i>Aurelia labiata</i>	32	40–470	-0.001	1.863	0.21	3.03	<b>0.005</b>
<i>Beroe abyssicola</i>	16	8–53	-0.019	4.100	0.29	2.92	<b>0.011</b>
<i>Carinaria japonica</i>	17	40–125	-0.034	7.836	0.12	2.38	<b>0.031</b>
<i>Cyanea capillata</i>	24	40–770	-0.001	2.233	0.01	0.43	0.671
<i>Mitrocoma cellularia</i>	24	27–70	-0.088	6.012	0.32	3.53	<b>0.002</b>
<i>Salpa aspera</i>	51	20–156	-0.018	3.907	0.36	3.34	<b>0.002</b>

change in C:N ratio in *Chrysaora plocamiamia* medusae. As the elemental composition of salps depends on the gut content fullness and composition, it cannot be ruled out that specimens collected between February and October had food ingested that contributed differently to the total C:N. Dubischar et al. (2012) and Koppelman et al. (2013), however, found for several salp and pteropod species from the Southern Ocean and Benguela Upwelling System, respectively, that the C:N ratio was not influenced by the sampling season. Until better time-resolved sampling for *S. aspera* in the Northeast Pacific has been conducted, the reason for the size-dependent change in the C:N ratio will remain unanswered. Ikeda (2014a,b) reviewed the existing literature on the biochemistry of pelagic cnidarians, ctenophores, and gastropods and argued that the decrease in C and N with specimen size occurs simultaneously with an increase in ash (i.e. reduction in AFDW % DW)

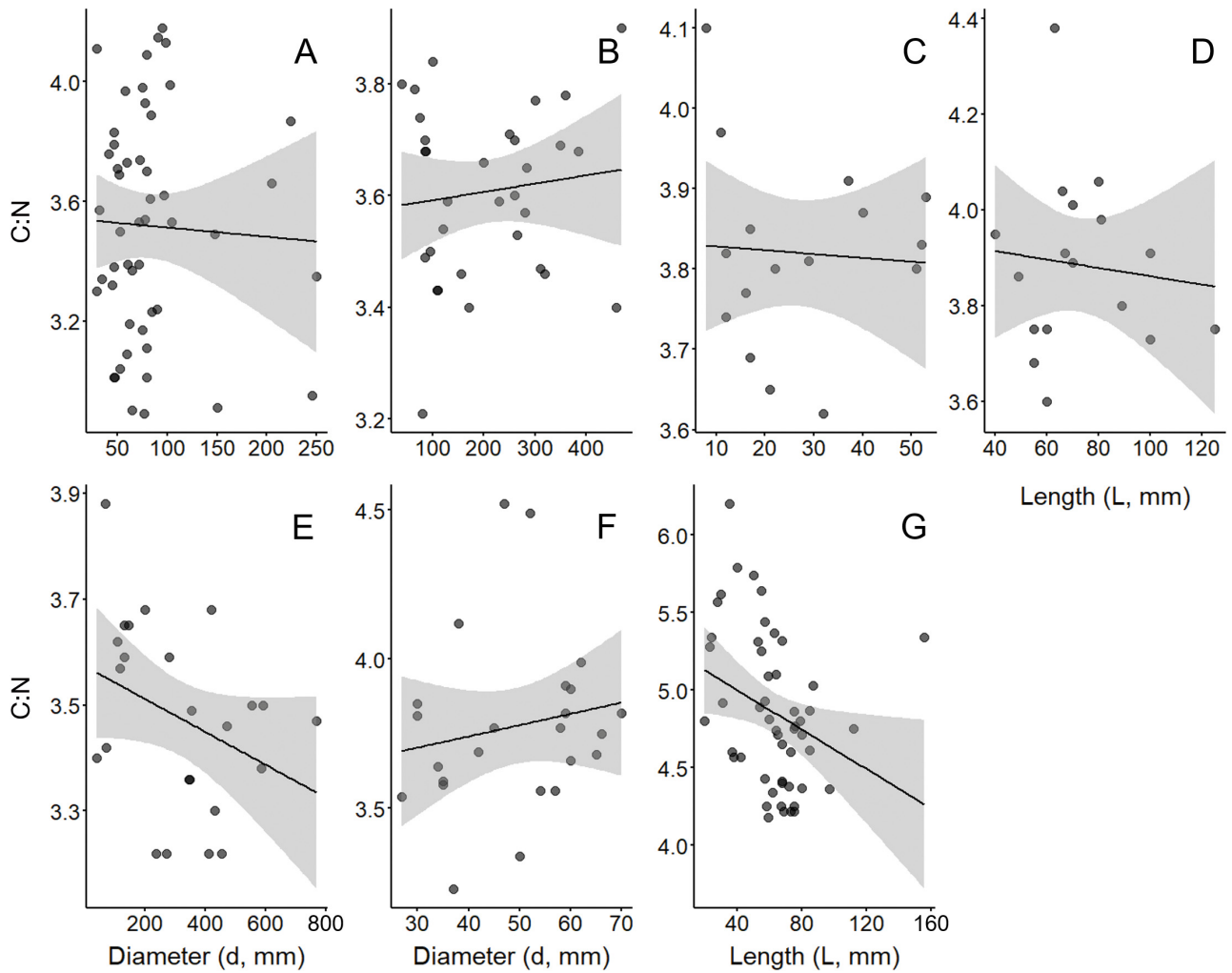


Fig. 8. Relationship of C:N ratio (by mass) with size (diameter,  $d$ , for hydro- and scyphomedusae; length,  $L$ , for ctenophores, gastropods, and salps) for (A) *Aequorea* sp., (B) *Aurelia labiata*, (C) *Beroe abyssicola*, (D) *Carinaria japonica*, (E) *Cyanea capillata*, (F) *Mitrocoma cellularia*, and (G) *Salpa aspera*. Linear regression line shown with 95% confidence intervals (grey shading). Significant slope ( $p < 0.05$ ) detected for *S. aspera*

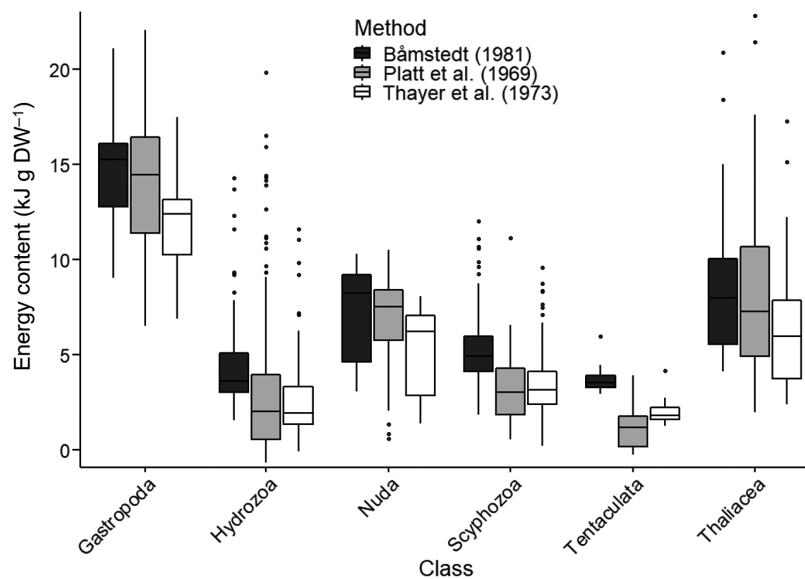


Fig. 9. Energy contents of gelatinous and soft-bodied zooplankton species ( $N = 34$ ) from 6 classes. For conversion factors, see Table S3 in Supplement 1. Box plot parameters as in Fig. 3

Table 5. Energy content (E) of 26 gelatinous and soft-bodied zooplankton species ( $N = 291$ ) from 6 classes, calculated using equation from Båmstedt (1981).  $N$ : number of analytical samples; DW: dry weight; *Atolla* spp.: *A. vanhoeffeni* and *A. wyvillei*. Data are mean  $\pm$  SD

Class Species	$N$	E (kJ g DW <sup>-1</sup> )
<b>Gastropoda</b>	21	14.7 $\pm$ 3.2
<i>Carinaria japonica</i>	16	13.7 $\pm$ 2.7
<i>Clione limacina</i>	5	17.9 $\pm$ 2.6
<b>Hydrozoa</b>	104	4.4 $\pm$ 2.4
<i>Aegina citrea</i>	2	7.5 $\pm$ 1.1
<i>Aequorea</i> sp.	50	3.8 $\pm$ 1.6
<i>Aglantha digitale</i>	5	7.6 $\pm$ 1.0
<i>Clytia gregaria</i>	6	6.0 $\pm$ 1.1
<i>Eutonina indicans</i>	7	4.3 $\pm$ 1.1
<i>Mitrocoma cellularia</i>	22	3.6 $\pm$ 0.9
<i>Pantachogon</i> sp.	1	14.3
<i>Polyorchis penicillatus</i>	2	13.0 $\pm$ 1.0
<i>Sarsia</i> sp.	1	11.6
<i>Solmissus</i> sp.	8	3.3 $\pm$ 0.9
<b>Nuda</b>	21	7.2 $\pm$ 2.4
<i>Beroe abyssicola</i>	16	8.4 $\pm$ 1.4
<i>Beroe cucumis</i>	5	3.6 $\pm$ 0.5
<b>Scyphozoa</b>	79	5.4 $\pm$ 2.0
<i>Atolla</i> spp.	3	6.5 $\pm$ 0.6
<i>Aurelia labiata</i>	32	4.6 $\pm$ 1.6
<i>Chrysaora fuscescens</i>	6	5.5 $\pm$ 0.4
<i>Chrysaora melanaster</i>	8	5.6 $\pm$ 1.7
<i>Cyanea capillata</i>	24	6.1 $\pm$ 2.4
<i>Phacellophora camtschatica</i>	6	6.2 $\pm$ 3.0
<b>Tentaculata</b>	11	3.8 $\pm$ 0.9
<i>Bolinopsis infundibulum</i>	4	3.2 $\pm$ 0.2
<i>Hormiphora</i> sp.	2	4.5 $\pm$ 2.0
<i>Pleurobrachia bachei</i>	5	3.9 $\pm$ 0.4
<b>Thaliacea</b>	55	8.3 $\pm$ 3.4
<i>Dolioletta gegenbauri</i>	5	10.9 $\pm$ 5.0
<i>Salpa aspera</i>	49	7.8 $\pm$ 2.5
<i>Thetys vagina</i>	1	20.9

since the sum of these 3 components is independent of body mass (= body size).

The uncertainty related to the size- and stage-specific organic content and elemental composition warrants a precautionary approach when using whole species data in comparisons. Intraspecific variability may be introduced by numerous factors (e.g. sex, reproductive state, season, or feeding condition), but a lack of data puts these factors beyond the scope of this discussion.

### 4.3. Energetic gain for predators

Significant differences in energy content among species were observed in the present study. Differ-

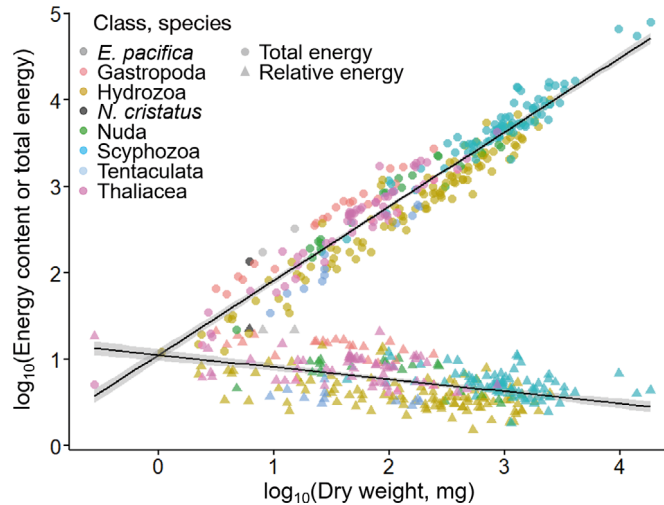


Fig. 10. Relative individual (kJ g DW<sup>-1</sup>, conversion from Båmstedt 1981) and total energy (i.e. product of relative energy contents and individual dry weight, kJ specimen<sup>-1</sup>) contents as a function of dry weight (mg), with linear regression models and 95% confidence intervals (grey shading) indicating trends.  $\log_{10}(\text{relative energy content}) = -0.14 \times \log_{10}(\text{DW}) + 1.05$  ( $R^2 = 0.24$ ,  $p < 0.001$ ),  $\log_{10}(\text{total energy content}) = 0.86 \times \log_{10}(\text{DW}) + 1.05$  ( $R^2 = 0.92$ ,  $p < 0.001$ ). For species-specific relative energy contents, see Table 5. Energy data for *Euphausia pacifica* and *Neocalanus cristatus* originate from Table S8 in Supplement 1

ences in AFDW % DW among species and taxonomic classes can result in variation of their energy content (Tables 1 & 5). Ignoring species-specific differences, e.g. through the application of generic or region-specific conversion factors, has the potential to introduce significant bias and erroneous results. For example, the use of values from *Aequorea* sp., *Aurelia labiata*, and other conspicuous species in food web models may undermine the overall trophic importance of GZ and their contribution to predator energy budgets. When compared to the literature, our energy content data reflected the taxonomic patterns documented in earlier studies, regardless of whether they were derived from conversions, proximate and elemental composition, or bomb calorimetry.

GZ water content can further complicate the situation because grouping several specimens in 1 analytical sample to obtain a clearer signal would remove the intraspecific variability. Arai et al. (1989) experienced a problem during bomb calorimetry because GZ generated a small, often undetectable, temperature change, and when samples were spiked with benzoic acid, its signal masked the original sample signal. We found that smaller species such as *Aegina citrea*, *Aglantha digitale*, and *Polyorchis penicillatus* had higher energy contents per unit bio-

mass and also higher organic contents compared to larger hydromedusa such as *Aequorea* sp. (Table 5). This may be explained by a lower ratio between tissue and mesoglea in *Aequorea* sp. and warrants closer considerations when selecting GZ energy data. Foxton (1966) and Heron et al. (1988) speculated that smaller GZ specimens (newly released salp embryos and young blastozooids, respectively) are consumed preferentially by predators due to their biochemical and higher energetic properties.

Many GZ species do not contain the same amount of weight-specific energy as co-occurring crustacean zooplankton organisms (e.g. *Euphausia pacifica* and *Neocalanus cristatus*, Table S8 in Supplement 1), often just 15–65 % of crustacean energy contents. However, some species of the classes Gastropoda and Tunicata come close, and considering their larger size and reduced mobility, may even be targeted prey (Hays et al. 2018).

Two co-occurring gelativorous pelagic predators with different prey size spectra were chosen to illustrate their impacts on fish predators (Fig. S1). Pacific herring consumes prey items  $\leq 20$  mm (Haegele 1997), and spiny dogfish typically consume prey between 21 and 300 mm in size (Scharf et al. 2000). Both species are known to consume GZ during specific periods of the year to some degree, intensified in years with high GZ abundances. The 2 examples show that even though most GZ have lower relative energy contents, the total predator energy gain may be comparable or even exceed that from crustacean prey. The overwhelming published evidence reflecting GZ consumption by predators, and GZ diversity in life cycles, feeding, and dispersal, highlights a diverse functional group that warrants an appropriate representation in food web models (Henschke et al. 2016, Hays et al. 2018, Thiebot & McInnes 2020). It should be clear that species- and stage-specific energy contents as well as their spatial and temporal variability are information required to reflect the complexity and importance of the GZ functional group.

#### 4.4. Summary

Large inter- and intraspecific differences in organic content and elemental composition were found for 34 GZ taxa from the Northeast Pacific. Differences in composition occurred within species, with values varying by size and development stage. Pelagic gastropods showed higher organic content than other classes. Organic content was not significantly different between Thaliacea and Nuda, whereas all other

classes differed significantly from each other. Thaliacea showed the highest C:N ratio among all tested classes. The C:N ratios of Gastropoda and Hydrozoa as well as Scyphozoa and Thaliacea were significantly different. This highlights the inappropriateness of representing GZ organic, elemental, and energy content data as a single mean for this functional group. In several species, the organic content and the C:N ratio decreased with increasing body size. These results have important implications for the outdated concept of GZ being a ‘trophic dead end’ and should be incorporated in the thinking of fisheries scientists, ecosystem modellers, and ecosystem service managers.

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*Data availability.* Raw data have been deposited in PANGAEA (<https://doi.pangaea.de/10.1594/PANGAEA.929888>).

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