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Total lipid and fatty acid composition of mesozooplankton functional group members in the NE Pacific over a range of productivity regimes

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ABSTRACT: Fish, whales, and seabirds along the productive west coast of Vancouver Island (WCVI), NE Pacific, rely on copepod prey that are assumed to be rich in lipid. However, the total lipid (TL) and fatty acid content of most copepod species and other mesozooplankton in this region have not been measured. To assess the diets and quality of zooplankton prey off the WCVI, we investigated differences in lipid content and composition of mesozooplankton according to their taxonomic versus functional group identities. Copepods, chaetognaths, euphausiids, and amphipods, belonging to 3 different functional groups, were sampled on the WCVI during pre-, mid-, and post-bloom conditions. Compared to taxonomic classifications, the functional group approach was superior in its ability to discern statistical differences in TL among zooplankton and between seasons. Furthermore, multivariate analyses using zooplankton fatty acid composition from all oceanographic regimes produced 3 to 4 statistically different clusters of species that corroborated functional group designations. However, some trophic flexibility was observed in several copepod species, particularly *Calanus marshallae*, that may relate to food scarcity or the presence of potentially noxious prey. The omnivorous-herbivorous functional group frequently contained higher amounts of TL and masses of essential fatty acids than the carnivorous group. Euphausiids and *C. marshallae* contributed the most lipid (mg m^{-3}) to the pelagic 'lipid pool', due to very high lipid content and high abundance, respectively. However, on the continental slope and shelf break, chaetognaths and *Neocalanus* spp. copepods contributed substantially to the lipid pool.

KEY WORDS: Zooplankton · Fatty acids · Total lipid · NE Pacific Ocean · Functional groups

1. INTRODUCTION

Zooplankton are integral members of marine ecosystems: they repackage carbon at the base of the food web by feeding on photosynthetic, mixotrophic, and heterotrophic protists (Ohman & Runge 1994, Liu et al. 2005), as well as marine snow, detritus (Park et al. 2011), and other zooplankton (Auel 1999). In turn, zooplankton serve as primary food items for fish (Brodeur 1989, Bollens et al. 2010, Hertz et al. 2015), whales (Dunham & Duffus 2001, Miller et al. 2019), seabirds (Bertram et al. 2009), and cephalopods

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(Uchikawa et al. 2004). Because zooplankton are involved in multiple trophic pathways in marine food webs, determining their diets over a range of oceanographic conditions is a primary research goal. In addition, understanding variation in the nutritional properties of zooplankton themselves (e.g. lipid mass) is crucial to understanding how their availability and energy content impact higher trophic levels.

One method of estimating the diet and energy content of wild populations of marine zooplankton is to sample the species of interest in the field and extract their lipids in the laboratory. Fatty acid biomarkers can be used to identify zooplankton prey items because they vary with the taxonomic composition of the prey and are incorporated relatively unchanged into consumer tissues (Lee et al. 1971). The fatty acid biomarker approach is not prone to the same biases as gut content analysis, where soft-bodied prey may not be preserved (Stoecker & Pierson 2019), and it does not rely on the consumers possessing full guts. Fatty acids have been successfully used as proxies for the dietary preferences of zooplankton from several marine systems, including pelagic environments in the Arctic (Scott et al. 2000, Auel et al. 2002, Stevens et al. 2004b, Connelly et al. 2014, Grigor et al. 2015), Antarctic (Graeve et al. 1994b, Kruse et al. 2010), tropics (Cass et al. 2014, Metillo & Aspiras-Eya 2014, Moura et al. 2016), and the temperate ocean (Kattner & Krause 1989, Wilson et al. 2010, Yamada et al. 2016). However, very little is known about the lipid composition of zooplankton in some regions of the North Pacific, including the west coast of Vancouver Island (WCVI), Canada.

The WCVI is located at the northern end of the California Current System, one of 4 major eastern boundary upwelling ecosystems (Chavez & Messié 2009). These ecosystems are characterized by high biological productivity (Checkley & Barth 2009), including prolific commercial fisheries (Pauly & Christensen 1995), and are used by animals as 'stepping stones' for foraging during annual migrations (cf. McKnight et al. 2013). Zooplankton biomass and diversity have been monitored in the WCVI region for 30+ yr in an attempt to understand the effects of climatic forcing on ecosystem function (McFarlane et al. 1997, Mackas et al. 2007, Mackas & Beaugrand 2010). The WCVI ecosystem supports large stocks of resident and migratory fish species including Pacific herring, Pacific hake, Chinook salmon, and lingcod (Ware & McFarlane 1995), most of which have strong connections to lipid-rich zooplankton (Emmett & Krutzikowsky 2008, Coyle et al. 2011, Dale et al. 2017).

Off the WCVI, lipids have been analyzed in only 1 zooplankton species, the copepod *Calanus marshallae* (Bevan 2015). More extensive lipid work has been conducted in the nearby Strait of Georgia, where 3 copepod trophic levels were identified using a combination of fatty acids and stable isotopes (El-Sabaawi et al. 2009). The carnivorous copepod *Paraeuchaeta elongata* (formerly *Euchaeta elongata*) was at the apex, herbivorous *Eucalanus bungii* was at the base, and *Neocalanus plumchrus* was in the intermediate, omnivorous position. The fatty acid composition of non-copepod zooplankton taxa has also been determined in the Strait of Georgia, but individual species were not always resolved (Costalago et al. 2020). In particular, the fatty acid composition of chaetognath species from this part of the NE Pacific has not been determined. Chaetognaths are constant, persistent community residents that make important contributions to total zooplankton abundance in marine systems (Brodeur & Terazaki 1999, Mackas & Galbraith 2002, Søreide et al. 2003). While the lipid studies cited above have improved our understanding of trophic linkages among copepods in the coastal NE Pacific, none have quantified the total lipid mass of individual zooplankton species, nor the masses of fatty acids essential to the growth and survival of fish (i.e. EPA [eicosapentaenoic acid, 20:5n-3]; DHA [docosahexaenoic acid, 22:6n-3]) (Copeman et al. 2002). These lipid masses, and their variation in space and time, are needed to understand the contribution of individual species and taxa to the 'marine lipidscape' (cf. Record et al. 2018).

Analyzing data using a species-specific approach, with lipids or otherwise, yields a fine trophic resolution that may be crucial to unraveling the ecology of key zooplankton species (Jakubas et al. 2007, Bertram et al. 2017, Schmid et al. 2018). However, given the diversity in size, trophic niche, and reproductive strategies of marine pelagic zooplankton, as well as the large number of global species (cf. Hébert et al. 2016a: copepods), integrating species-specific information into functional groups may confer advantages and simplify the interpretation of data (Violle et al. 2007, Litchman et al. 2013). The functional traits used to place organisms into functional groups (e.g. morphological, physiological, and reproductive characteristics) represent critical ecological functions that define their ecological niche (Litchman et al. 2013). Functional traits influence organismal fitness and affect key ecosystem processes such as productivity and energy transfer (Hébert et al. 2016b). Dietary preference (e.g. herbivore, carnivore, omnivore) has been important in defining zooplankton functional groups. Demonstrated relationships between zooplankton functional groups and *in situ*

environmental variables may prove useful in forecasting the impacts of climate change on local zooplankton populations (Benedetti et al. 2018, Durán-Campos et al. 2019, Venello et al. 2021). While the functional group approach shows much promise in the interpretation of multi-species datasets, it needs to be validated using independent methods, which was a primary goal of this study.

In this study, we compared the total lipid content, fatty acid composition, and masses of EPA and DHA in 25 species of mesozooplankton from 4 taxa: copepods, chaetognaths, euphausiids, and hyperiid amphipods. We compared lipid levels using 3 different organizational methods: broad taxonomic groups (e.g. phylum, subclass, order), functional groups, and individual species. Sampling was conducted on the continental shelves and slopes off the WCVI over a range of productivity regimes, including pre-bloom, mid-bloom, and post-bloom conditions during 2018 and 2019. To our knowledge, no studies to date have used lipid profiles to validate zooplankton functional group designations.

2. MATERIALS AND METHODS

2.1. Sampling location

All samples were collected during the long-standing La Perouse (WCVI) and Line P monitoring surveys conducted by the Department of Fisheries and Oceans Canada (DFO). The samples presented in the present study were taken during 3 La Perouse cruises (7 to 18 May 2018, 1 to 11 September 2018, 21 May to 2 June 2019) and 1 Line P cruise (2 to 18 June 2019). Because the May 2019 and June 2019 cruises ran back-to-back, we have grouped these as 'May–June 2019', and we included only Line P stations that were physically located within the WCVI monitoring area. During each cruise, we focused on 8 to 12 stations, including those classified as northern Vancouver Island ('North') and southern Vancouver Island ('South'). We designated stations sampled on the shelf as 'IS' (inshore), those on the shelf break and continental slope as 'SBS', and those further offshore along Line P as 'Oceanic' (Table 1, Fig. 1).

2.2. Oceanographic data collection and phytoplankton sampling

At most stations, a CTD profile was obtained during deployment of a sampling rosette for water collection. Water samples from 5 depths (WCVI) or 11 to 23 depths (Line P) were filtered onto 25 mm GF/F filters and extracted chl *a* was measured (Holm-Hansen et al. 1965). Please note that we consider only data from 0 to 50 m in our characterization of bloom state/phytoplankton biomass. Samples for phytoplankton identification were taken from Niskin bottles at a depth of 5 m at Stns N1/N5, N11/N13, S3/S5, S11/S17 (Fig. 1). A 250 ml subsample of water from each of these stations was preserved in an amber glass bottle with 1 ml Lugol's acid iodine (Sournia 1978). In the laboratory, a 25 ml aliquot was settled for 24 h in an Utermohl chamber and protist cells identified and enumerated using a Zeiss Axio Vert A.1 inverted phase contrast microscope.

2.3. Zooplankton sampling

Zooplankton for lipid analysis were collected by Bongo nets equipped with 236 µm mesh (Table 1) using the standardized sampling methodology developed by DFO (Mackas 1992, Mackas & Galbraith 2002). Briefly, Bongo nets were towed vertically between approximately 250 m and the surface, or from approximately 5–10 m off the sea floor to the surface at stations that had bottom soundings <250 m. Once onboard, the contents of one of the Bongo cod ends was immediately frozen for biomass analysis. Individuals for lipid analysis were picked from the second cod end, recorded in the log, and the remainder preserved in buffered formalin for zooplankton counts (i.e. abundance). Therefore, zooplankton sampling generally consisted of 1 surface layer Bongo tow per station; however, at some stations deep Bongos and deep MultiNet tows (mesh size 250 µm; 0.5 × 0.5 m opening) were done. From this point forward, we do not distinguish between zooplankton collected in shallow or deep tows, because the lipid content and fatty acid composition of zooplankton was unrelated to sampling depth (data not shown), and we include zooplankton compositional data from the upper water column only (≤300 m). Large, dominant mesozooplankton, i.e. calanoid copepods, chaetognaths, euphausiids and hyperiid amphipods, were picked from net catches and identified to species using a dissecting scope. They were then rinsed with deionized water and placed in pre-weighed, lipid-clean glass vials and frozen at −80°C. Samples consisted of between 1 and 16 individuals per species, depending on abundance and size (see Table 1 for sample sizes). In terms of the copepods, the developmental stage sampled was either copepodite stage V or mature females/males.

Table 1. Descriptions of samples taken in 2018 and 2019 for zooplankton lipid analysis off the west coast of Vancouver Island, NE Pacific, during pre-bloom conditions (May 2018, n = 47), mid-bloom conditions (May–June 2019, n = 41), and post-bloom conditions (September 2018, n = 54). In some cases, more than 1 sample per species was collected at a given station. Gelatinous chaetognath species codes are underlined in the table. SBS: shelf break/slope; IS: inshore. Monitoring station names are those used by Fisheries and Oceans Canada (DFO) during monitoring cruises off Vancouver Island and along Line P. Station bottom depth is indicated in brackets after sampled depth stratum (m)

Stn	Monitoring station	Region	Sampled stratum	Zooplankton species[a]
Pre-bloom				
N2	CS02	North_SBS	0–249 m (1800)	Cm, Er, Nc, Np, Ps, Tp
N10	LBP8	North_SBS	0–249 m (2070)	Cm, Eb, Nc, Np, Eh, Ps, Pa
N7	CS09	North_IS	0–188 m (195)	Ts, Ps
N13	LBP3	North_IS	0–160 m (172)	Cm, Pe, Pse
S1	LG09	South_SBS	0–1200 m (2056)	Eg, Er, Nc, Np, Eh, Ps, Ep, Ts, Tp
S11	LC09	South_SBS	0–246 m (611)	Ec, Mp, Nc, Np, Eh, Pse
S5	LG02	South_IS	0–90 m (105)	Cm, Eb, Pse, Ep, Tp
S16	LC04	South_IS	0–146 m (167)	Cm, Eb, Np, Pse, Ep, Ts, Hm
Mid-bloom				
N2	CS02	North_SBS	0–250 m (1800)	Cm, Nc, Eh, Ps
N7	CS09	North_IS	0–180 m (195)	Cm, Eh
N13	LBP3	North_IS	0–154 m (172)	Cm, Peu
S1	LG09	South_SBS	0–250 m (2056)	Cm, Nc, Pe, Eh, Peu, Ps
S7	LD11	South_SBS	0–250 m (1560)	Ps
S11	LC09	South_SBS	0–250 m (611)	Cm, Nc, Np, Pe, Eh, Peu
P4	P4	South_SBS	0–250 m (1300)	Nc, Pe, Eh, Peu
S5	LG02	South_IS	0–86 m (105)	Cm, Peu
S15	LC05	South_IS	0–55 m (65)	Cm
P2	P2	South_IS	0–110 m (114)	Cm, Peu, Pse
P8	P8	Oceanic	0–1200 m (2440)	Nc, Np, Eh, Ps
P12	P12	Oceanic	0–250 m (3300)	Nc, Np, Eh, Ps
Post-bloom				
N10	LBP8	North_SBS	0–250 m (2070)	Tp
N10	LBP8	North_SBS	0–2000 m (2070)	Nc
N10	LBP8	North_SBS	1500–2000 m (2070)	Nc, Np, Ec, Pe, Eh, Tp
N3	CS03	North_IS	0–225 m (240)	Cm, Tis
N7	CS09	North_IS	0–190 m (195)	Cm, Np, Pse, Ep, Ti, Tl, Ts, Tp
S1	LG09	South_SBS	500–1500 m (2056)	Nc, Np
S9	LC12	South_SBS	0–1200 m (2510)	Ec, Nc, Nf, Np, Pe, Pp, Sc, Eh, Pse, Tp
S11	LC09	South_SBS	0–250 m (611)	Cm, Eb, Ec, Er, Nc, Eh, Ps, Ep, Hm, Tp
S5	LG02	South_IS	0–90 m (105)	Np, Pse, Tp
S16	LC04	South_IS	0–160 m (167)	Cm, Cp, Pse, Ep, Tp

[a]ind.: number of zooplankton individuals per lipid sample. Copepods: Cm: *Calanus marshallae* (2–16 ind.), Cp: *C. pacificus* (10 ind.), Eg: *Euaugaptilus graciloides* (2 ind.), Eb: *Eucalanus bungii* (1–8 ind.), Ec: *Eucalanus californicus* (3–7 ind.), Er: *Euchirella rostrata* (1–7 ind.), Mp: *Metridia pacifica* (8 ind.), Nc: *Neocalanus cristatus* (3–13 ind.), Nf: *N. flemingeri* (2 ind.), Np: *N. plumchrus* (2–15 ind.), Pe: *Paraeuchaeta elongata* (3–9 ind.), Pp: *Pseudhaloptilus pacificus* (1 ind.), Sc: Scaphocalanidae (no species ID; 1 ind.); Chaetognaths: Eh: *Eukrohnia hamata* (2–12 ind.), Pse: *Parasagitta elegans* (1–14 ind.), Peu: *Parasagitta euneritica* (5–12 ind.), Ps: *Pseudosagitta scrippsae* (1–7 ind.); Euphausiids: Ep: *Euphausia pacifica* (1–5 ind.), Ti: *Thysanoessa inermis* (1 ind.), Tis: *T. inspinasa* (1 ind.), Tl: *T. longipes* (2 ind.), Ts: *T. spinifera* (1–5 ind.); Amphipods: Hm: *Hyperoche medusarum* (1–3 ind.), Pa: *Primno abyssalis* (1 ind.), Tp: *Themisto pacifica* (1–10 ind.)

2.4. Zooplankton functional groups

The zooplankton species sampled in this study belonged to 3 of the 8 functional groups developed by Venello et al. (2021) in the NE Pacific Ocean. A high proportion of our sampled zooplankton species were classified as 'Omnivore-Herbivores' (OH), and this functional group included the copepods *Calanus marshallae*, *C. pacificus*, *Eucalanus bungii*, *E. californicus*, *Metridia pacifica*, *Neocalanus cristatus*, *N. plumchrus*, and *N. flemingeri*, and the euphausiids *Euphausia pacifica*, *Thysanoessa spinifera*, *T. inermis*, and *T. inspinasa*. The euphausiid *T. longipes* was not included in the study by Venello et al. (2021), but we placed it in the OH group with other members of the *Thysanoessa* genus. We sampled an unidentified scaphocalanid copepod that belonged to the functional group 'Detrivores' (D); we also placed the

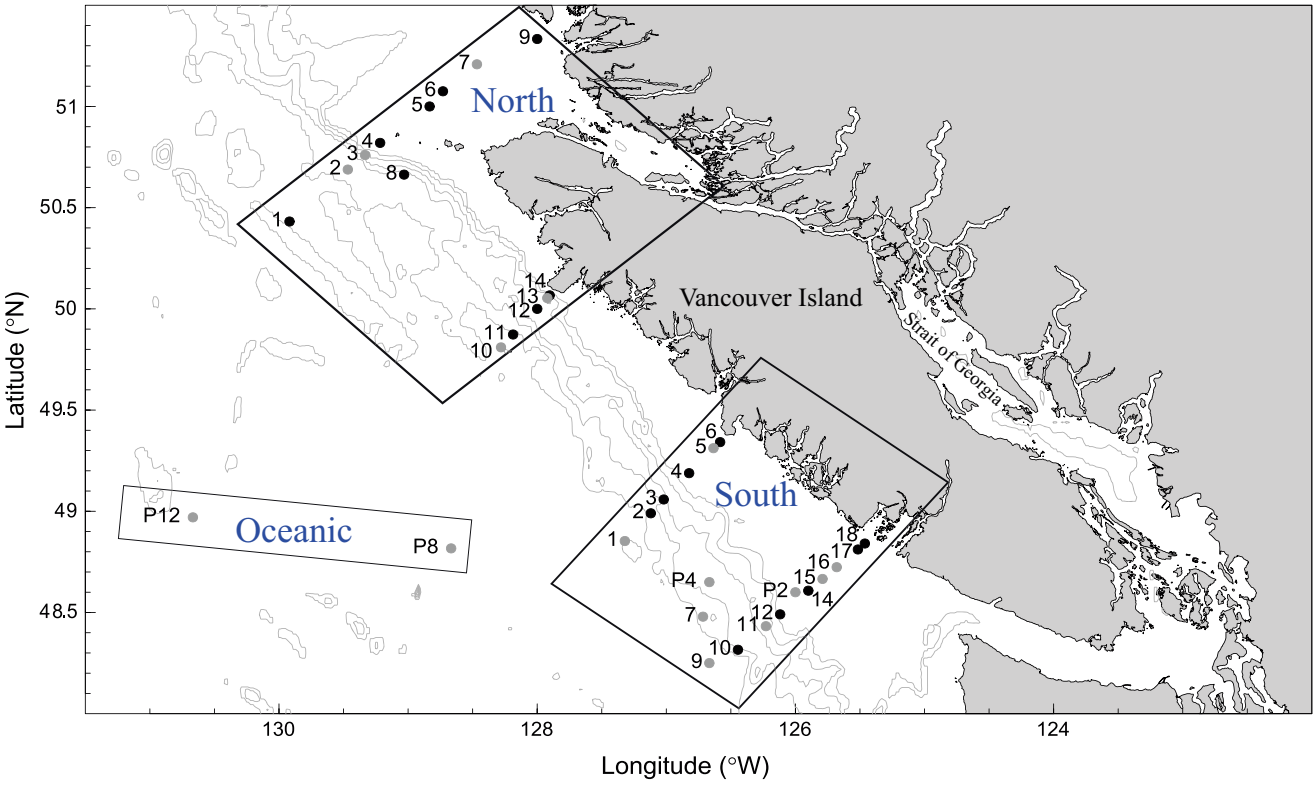

Fig. 1. Location of stations sampled off the west coast of Vancouver Island, NE Pacific, during May 2018, September 2018, and May–June 2019. North, south, and oceanic regions, identified by boxes, were defined by Fisheries and Oceans Canada during routine monitoring of this region over 30+ yr. (•) Stations where zooplankton lipid samples were taken; (•) stations where we took samples for zooplankton taxonomy and/or protist taxonomy. In some cases, taxonomic samples were taken at the same stations as the lipid samples

copepod *Euchirella rostrata* in D since it was classified as such by Benedetti et al. (2016). Although we had only 4 samples from the D group, we included them out of ecological interest; however, few conclusions can be drawn from these data. The remainder of our sampled species fell into the 'Egg-Brooding Carnivores' (EBC) functional group developed by Venello et al. (2021), or we classified them as EBC based on literature descriptions of their carnivorous feeding mode and/or egg-brooding behavior (von Westernhagen 1976, Kinoshita 1982, Yamada et al. 2002, Ikeda et al. 2006, Kosobokova et al. 2007, Grigor 2017). Therefore, in this study, the EBC included 3 different taxonomic groups: copepods (*Paraeuchaeta elongata*, *Euaugaptilus graciloides*, *Pseudhaloptilus pacificus*), chaetognaths (*Eukrohnia hamata*, *Parasagitta elegans*, *P. euneritica*, *Pseudosagitta scrippsae*), and hyperiid amphipods (*Themisto pacifica, Hyperoche medusarum*, *Primno abyssalis*). It should be noted that of the chaetognaths included in the EBC group, only *E. hamata* brood their eggs. However, 'reproductive mode' was 1 of 4 functional traits used to statistically differentiate the EBC from other functional groups (Venello et al. 2021).

2.5. Lipid analysis

2.5.1. Lipid extraction and determination of total lipid

Prior to extraction, each sample was removed from −80°C storage, the cap removed, and wet mass (WM) determined. Samples were then freeze-dried for 24 h and re-weighed on a Mettler analytical single-pan H5 balance (±0.1 mg). Teflon-lined caps were replaced under nitrogen gas and sealed with Teflon tape. Samples were stored at −80°C until lipid extraction. Zooplankton samples were extracted following Parrish (1999), as modified from Folch et al. (1957). Extracts were weighed to determine total lipid (TL). The extract was then re-suspended in approximately 0.5 ml of chloroform, capped under nitrogen gas, and stored at −20°C until the fatty acid methyl ester (FAME) derivatives were made. For each set of oceanographic conditions, we computed an average,

region-specific TL value for each species (mg per individual [ind.$^{-1}$]). We then multiplied the TL by the abundances (ind. m^{-3}) for the same species and regions, using data from the upper water column (surface to ≤300 m). What emerged were species-specific estimates of contributions to the lipid pool available to higher order consumers, per unit volume (mg m^{-3}; see Fig. 8).

2.5.2. Fatty acid synthesis, analysis, and biomarkers

Before FAME synthesis of the lipid extract, a known amount of 23:0 (based on zooplankton TL) was added as an internal standard. The extract was then evaporated to dryness under nitrogen gas. In 2018, BF_3-CH_3OH was used to make the FAME (cf. Stevens et al. 2004a), while in 2019, we used the H_2SO_4 method of Toyes-Vargas et al. (2020). In 2018, the zooplankton FAME were analyzed by gas chromatography (GC) at the Centre for Aquaculture and Environmental Research (DFO) as per Costalago et al. (2020). Through comparison of the retention times to a commercial standard, the fatty acids were identified following Ackman (1986). As an inter-laboratory comparison, 2 lipid-rich derivatives were halved and sent to the Aquatic Research Cluster (ARC) at the Memorial University of Newfoundland (MUN) to undergo GC analysis on a more sensitive column (refer to Toyes-Vargas et al. 2020 for details). In 2019, all FAME derivatives were analyzed at MUN.

Certain fatty acids in pelagic zooplankton tissues can be attributed to specific prey items, such as diatoms, dinoflagellates, bacteria, and other zooplankton (Lee et al. 1971, Dalsgaard et al. 2003). Diatoms are characterized by high relative amounts of the fatty acids 16:1n-7, 16:4n-1, 20:5n-3 (EPA), and other 16- and 20-carbon polyunsaturated fatty acids (PUFA; Viso & Marty 1993). Flagellates contain high amounts of 22:6n-3 (DHA) and 18:4n-3, while bacterial biomarkers are 18:1n-7 and odd-numbered and/or branched chain fatty acids (OBFA; Viso & Marty 1993, Stevens et al. 2004b, Wilson et al. 2010). The fatty acid 18:1n-9 is used to indicate carnivory (Graeve et al. 1994b, Richoux 2011), as is the DHA to EPA ratio (i.e. 22:6n-3 to 20:5n-3) under certain conditions (El-Sabaawi et al. 2009, Richoux 2011, Parrish et al. 2015).

2.6. Statistical analysis

All statistical analyses were performed in R, version 4.0.0 (R Core Team 2020). To test for differences in masses of TL and the essential fatty acids EPA and DHA in zooplankton tissues, as well as proportions of fatty acid biomarkers, we used a Kruskal-Wallis test in conjunction with a post-hoc Dunn's test (Bonferroni correction), or a Wilcoxon Rank Sum Test in the case of <3 means. Seasonal patterns in raw percentage biomarker data are shown (see Fig. 7), but the statistics were performed on arcsine-transformed values. For all univariate tests, all data were included (i.e. all species/taxa/functional groups, see Figs. 4, 5 & 7) but for the multivariate analysis (see Fig. 6), only data from copepods and chaetognaths were used. In 2018, 4 zooplankton taxa were sampled (i.e. copepods, chaetognaths, amphipods, euphausiids), while in 2019, samples of only 2 taxa were taken (i.e. copepods and chaetognaths). To maximize comparability between the 3 individual non-metric, multi-dimensional scaling (NMDS) ordinations, we included only copepods and chaetognaths in each.

NMDS was conducted using the function 'metaMDS' from the vegan package (Oksanen et al. 2019), calculated with the Bray-Curtis distance. A square-root transformation was applied to all fatty acid data used in the NMDS. NMDS ranks the samples within a similarity matrix, and then plots these ranks in 2-dimensional space, with greater proximity indicating greater similarity (Clarke 1993). The stress coefficient is calculated based on the amount of disagreement in configuration between the 2 axes represented, with a stress value <0.2 generally providing an acceptable solution (Clarke 1993). Fatty acid compositional data from May 2018, September 2018, and May–June 2019 were analyzed in separate NMDS ordinations because temperature, phytoplankton composition, and chl *a* concentrations indicated that different productivity regimes were operating during each of the 3 cruises (presented in Section 3).

We used hierarchical cluster analysis (function 'hclust') to visualize the sample clusters present in the NMDS ordinations. Using these cluster designations, ellipses were added to the NMDS plots at the 95% confidence level (function 'stat_ellipse'). A permutational multivariate analysis of variance (PERMANOVA) was run to determine whether the zooplankton fatty acid compositions were statistically significant in terms of cluster designation, species, taxon, functional group, and WCVI region (function 'adonis'). Finally, we conducted a similarity percentages (SIMPER; Clarke 1993) analysis (function 'simper') to identify the individual fatty acids that accounted for the most dissimilarity between clusters. Please note that during May 2018 and May–June 2019, an 'outgroup' was present in the cluster den-

drograms. We chose to leave these samples in the analysis, but they were too small to be captured by an ellipse at the 95% confidence level. We do not discuss the fatty acid properties of these outgroups.

3. RESULTS

3.1. Oceanographic regimes and prey availability at the time of sampling

Several oceanographic properties revealed that each of the cruises was characterized by a different productivity regime. Ocean chlorophyll concentrations from the Giovanni website (https://giovanni.gsfc.nasa.gov/giovanni/), derived from the MODIS-AQUA satellite/instrument system, indicated that the spring bloom had not yet started when we sampled in May 2018. Maximum extracted chl *a* concentrations between 0 and 50 m were also low, ranging from 0.72 to 7.88 mg m^{-3} throughout the May 2018 cruise, with only 3 inshore stations experiencing moderate levels (e.g. >5 mg m^{-3}; Stns N5, S17, S18; Table 2). Moderate to high proportions of mixotrophic and heterotrophic cells were observed during May 2018, although at Stn N5, diatoms were the dominant protist group (Fig. 2). The diatoms present at Stn N5 in May 2018 were the centric species *Chaetoceros socialis*. Maximum temperatures in the upper 50 m ranged from 9.86 to 12.15°C during May 2018. Based on these oceanographic parameters, we classified the May 2018 cruise as 'pre-bloom'. We consider the May–June 2019 cruises as 'mid-bloom' because maximum chl *a* concentrations between 0 and 50 m were moderate (e.g. >5 mg m^{-3}) to high (e.g. >10 mg m^{-3}) at several stations, particularly those inshore (i.e. Stns N5, S6, P2, S16) (Table 2), and diatoms were proportionally more abundant than they were under pre-bloom conditions (Fig. 2). As in May 2018, during May–June 2019, Stn N5 was dominated by the centric diatom *C. socialis*, while at most other stations the dominant diatom was the pennate

Table 2. Maximum temperature and chl *a* concentrations in the upper 50 m at northern, southern, and oceanic sampling stations under pre-bloom (May 2018), mid-bloom (May–June 2019), and post-bloom (September 2018) conditions. –: stations along Line P (P2, P4, P8, P12) were sampled under mid-bloom conditions only. Station names are those used by Fisheries and Oceans Canada (DFO) during monitoring cruises off Vancouver Island and along Line P; SBS: slope/shelf break; IS: inshore

Stn	Monitoring station	Region	Maximum temperature (°C)			Maximum chl *a* (mg m^{-3})		
			Pre-bloom	Mid-bloom	Post-bloom	Pre-bloom	Mid-bloom	Post-bloom
North								
N1	CS00	North_SBS	10.97	11.90	15.77	0.89	3.45	1.52
N2[a]	CS02	North_SBS	10.79	11.80	15.30	1.67	1.83	2.02
N11	LBP7	North_SBS	10.94	11.90	15.93	1.40	2.59	1.53
N12	LBP5	North_SBS	9.86	9.89	11.68	1.42	5.48	11.73
N4	CS04	North_IS	10.48	11.10	12.61	3.55	2.45	4.05
N5	CS06	North_IS	10.88	10.41	10.60	6.47	16.02	25.14
N7[a]	CS09	North_IS	11.57	10.70	13.27	1.59	0.99	3.86
N13[a]	LBP3	North_IS	12.20	9.41	11.78	1.67	1.05	22.68
South								
S3	LG06	South_SBS	11.25	10.83	13.31	0.72	4.20	6.33
S10	LC11	South_SBS	11.23	11.62	13.90	0.87	1.30	4.94
S11[a]	LC09	South_SBS	11.33	11.51	12.92	0.86	2.66	4.17
P4[a]	P4	South_SBS	–	11.64	–	–	6.03	–
S4	LG04	South_IS	11.76	11.19	10.80	2.03	3.51	6.06
S5[a]	LG02	South_IS	10.65	10.96	12.28	1.09	7.89	17.51
S6	LG01	South_IS	11.20	11.41	11.48	0.79	18.68	10.99
S12	LC08	South_IS	11.27	11.21	12.80	1.16	3.41	3.06
S14	LC06	South_IS	11.31	12.55	12.51	2.03	6.07	3.88
S16[a]	LC04	South_IS	11.70	13.65	12.37	2.69	26.73	4.60
S17	LC02	South_IS	12.15	13.36	13.49	5.41	5.97	13.72
S18	LC01	South_IS	10.87	13.75	12.76	7.88	5.93	6.73
P2[a]	P2	South_IS	–	12.80	–	–	13.41	–
Oceanic								
P8[a]	P8	Oceanic	–	12.29	–	–	0.51	–
P12[a]	P12	Oceanic	–	11.80	–	–	0.28	–

[a]Stations where lipid samples were taken

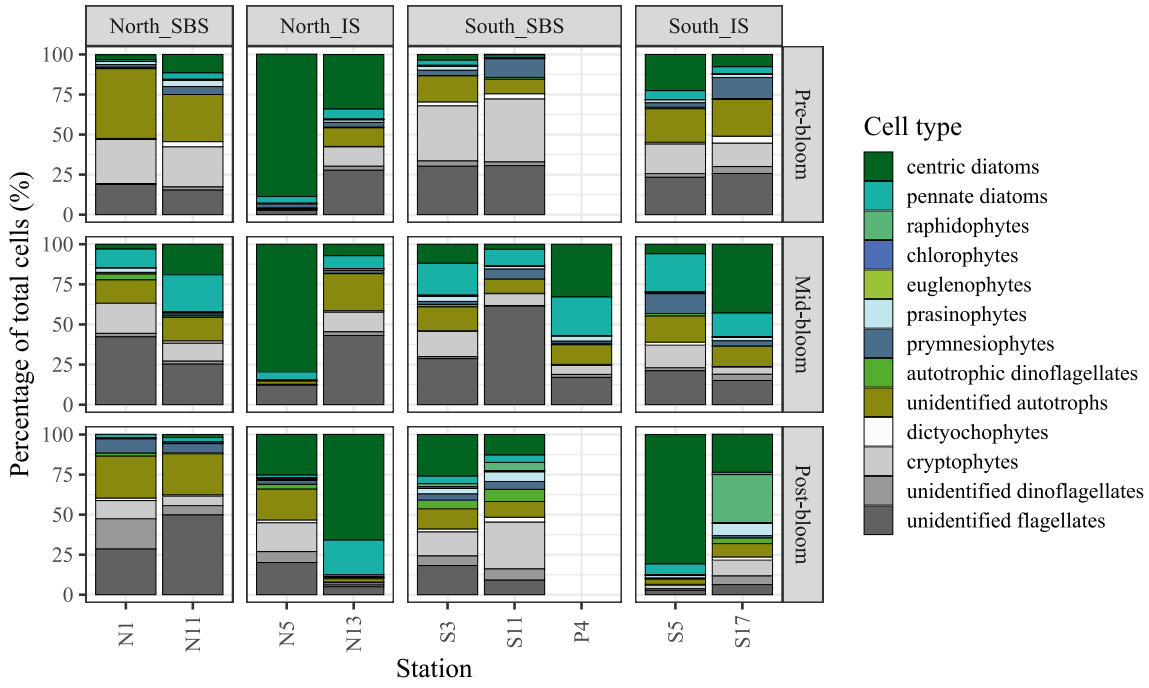

Fig. 2. Taxonomic composition of protist cells from 5 m depth off the west coast of Vancouver Island, collected during pre-bloom, mid-bloom, and post-bloom conditions in 2018 and 2019. Grey shading indicates groups that are predominantly mixotrophic or heterotrophic. SBS: shelf break/slope stations; IS: inshore stations

species *Pseudo-nitzschia* cf. *fraudulenta*. Ocean chlorophyll data from the Giovanni website indicated that during 2019 the spring bloom began in late April to early May, with a lull in phytoplankton biomass in late May, and a return to higher, localized phytoplankton levels in early June. Maximum temperatures in the upper 50 m during May-June 2019 ranged from 9.41 to 13.75°C (Table 2). During September 2018, which we consider to be 'post-bloom', maximum temperatures from 0 to 50 m were markedly higher than they were under pre- and mid-bloom conditions, ranging from 10.60 to 15.93°C (Table 2), and the water column was stratified (data not shown). However, there was persistent productivity during September 2018. Extracted chl *a* concentrations ranged from 1.52 to 25.14 mg m^{-3} (Table 2), with significant levels (e.g. >10 mg m^{-3}) found at several inshore and shelf break/slope stations (i.e. Stns N5, N12, N13, S5, S6, S17), and at many of these productive stations, centric diatoms were dominant (Fig. 2). The centric diatoms present under post-bloom conditions were *C. socialis*, *Chaetoceros* spp., and *Thalassiosira* spp. Although our 3 productivity regimes (i.e. pre-, mid-, post-bloom) did not occur in the same calendar year, 2018 and 2019 were very similar oceanographically (Boldt et al. 2019, 2020). Both years were still strongly affected by the marine heatwaves of 2014 to 2016, such that sea-surface temperatures remained relatively high in the region. In addition, phytoplankton and zooplankton biomass and community composition were similar in 2018 and 2019.

3.2. Abundances of mesozooplankton species collected for lipid analysis

In this section, we consider only the abundances of zooplankton species that were analyzed for lipids. Furthermore, species for which we had only 1 lipid sample are not shown (i.e. *Euaugaptilus graciloides*, *Metridia pacifica*, *Neocalanus flemingeri*, *Pseudhaloptilus pacificus*, unidentified scaphocalanid, *Thysanoessa inermis*, *T. inspinasa*, *T. longipes*, *Primno abyssalis*). Abundances of our target species were generally an order of magnitude higher under mid-bloom conditions, as compared to data from the pre- and post-bloom cruises, and abundances were usually higher at IS stations (Fig. 3). Considering the copepod species, at SBS stations under pre-bloom (North_SBS and South_SBS) and mid-bloom conditions (South_SBS only), *Neocalanus* spp. were the dominant species (Fig. 3). However, at most other stations and conditions, *Calanus marshallae* was the most abundant copepod. Euphausiids contributed significantly to the overall abundance of our target

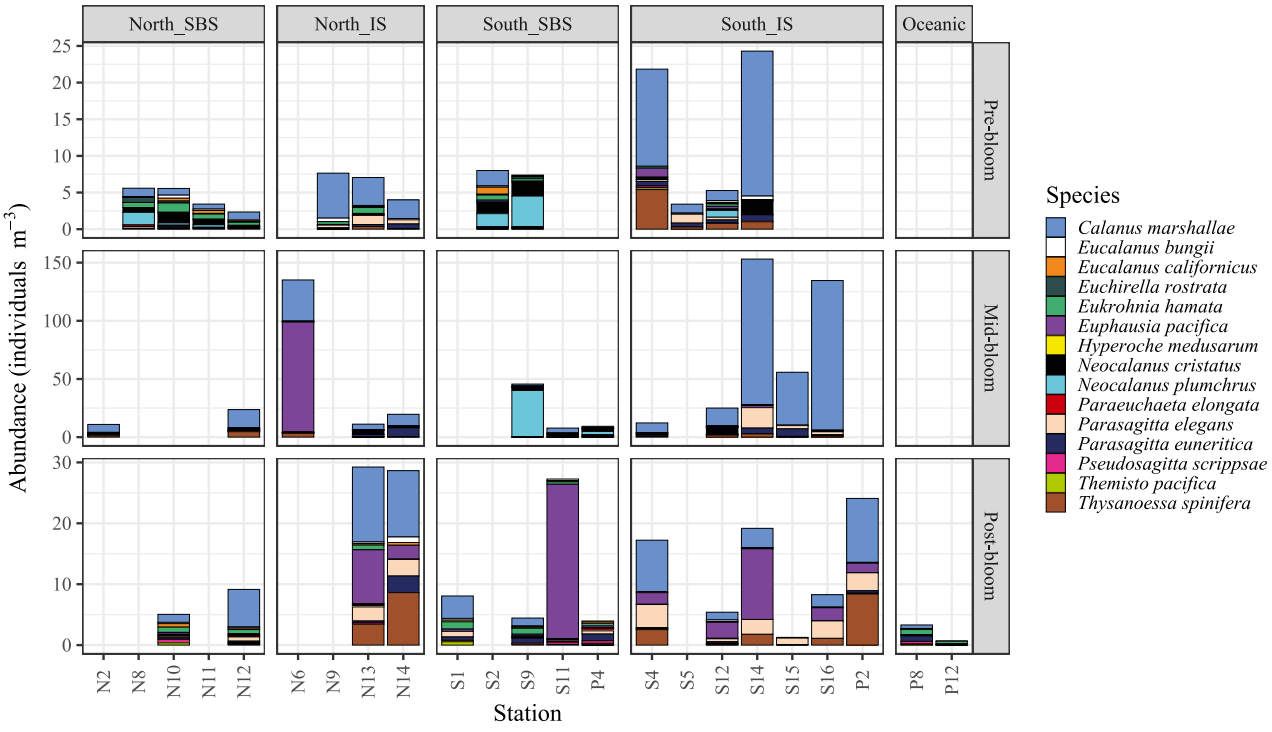

Fig. 3. Abundances of zooplankton species that were collected for lipid analysis during pre-bloom, mid-bloom, and post-bloom conditions in 2018 and 2019. SBS: shelf break/slope stations; IS: inshore stations

zooplankton during post-bloom conditions only, with the exception of Stns S4 (pre-bloom *Thysanoessa spinifera*) and N6 (mid-bloom *Euphausia pacifica*). At several post-bloom stations, in particular IS stations, the chaetognath *Parasagitta elegans* was relatively abundant. *Eukrohnia hamata* was present in low numbers during pre- and post-bloom conditions, but was virtually absent during the mid-bloom cruise. *Parasagitta euneritica* was present in small concentrations at several stations across all 3 sets of oceanographic conditions. Several species that were sampled for lipids were present in very low numbers throughout our study; this included both hyperiid amphipod species (*Themisto pacifica*, *Hyperoche medusarum*), several copepods (*Euchirella rostrata*, *Paraeuchaeta elongata*, *Eucalanus bungii*, *Eucalanus californicus*), and the chaetognath *Pseudosagitta scrippsae*.

3.3. Total lipid content and masses of essential fatty acids in zooplankton

3.3.1. Broad zooplankton taxa

During pre-bloom conditions, the mean TL content of zooplankton was highest in copepods (41.8 ± 48.0 mg g^{-1} WM) and amphipods (35.4 ± 44.8 mg g^{-1} WM), and lower in euphausiids (20.5 ± 15.5 mg g^{-1} WM) and chaetognaths (17.2 ± 17.4 mg g^{-1} WM), yet these differences in TL between taxa were not statistically significant (Fig. 4). Mean TL levels in copepods collected under mid-bloom conditions (59.4 ± 58.7 mg lipid g WM^{-1}) were statistically higher than those in the chaetognaths (32.2 ± 37.5 mg g^{-1} WM) ($p = 0.001$; Wilcoxon test) (Fig. 4). Post-bloom mean TL levels were significantly higher in copepods (79.1 ± 67.7 mg g^{-1} WM) and chaetognaths (50.4 ± 47.0 mg g^{-1} WM), as compared to TL in those 2 taxa during pre-bloom conditions (copepods: $p = 0.003$; Kruskal-Wallis test + Dunn's test [Bonferroni]; chaetognaths: $p = 0.024$; Kruskal-Wallis test + Dunn's test [Bonferroni]). Under post-bloom conditions, mean TL levels in euphausiids (68.4 ± 43.6 mg g^{-1} WM) and amphipods (42.7 ± 19.3 mg g^{-1} WM) were also higher than they were before the spring bloom, but these differences were not significant.

Mean masses of the essential fatty acid EPA (i.e. 20:5n-3) in zooplankton taxa during pre-bloom conditions were lowest in chaetognaths (0.6 ± 0.9 µg mg^{-1} WM), as compared to copepods (2.0 ± 2.8 µg mg^{-1} WM), euphausiids (1.5 ± 0.8 µg mg^{-1} WM), and amphipods (1.7 ± 3.2 µg mg^{-1} WM) (Fig. 4). Similarly, under mid-bloom conditions, the chaetognaths (0.9 ± 0.9 µg mg^{-1} WM) contained less EPA than the cope-

pods (1.3 ± 0.9 µg mg^{-1} WM). During the post-bloom cruise, mean masses of EPA were very similar across taxa (copepods: 1.7 ± 2.5 µg mg^{-1} WM; chaetognaths: 1.8 ± 1.3 µg mg^{-1} WM; euphausiids: 1.8 ± 1.5 µg mg^{-1} WM; amphipods: 1.2 ± 1.3 µg mg^{-1} WM). No statistically significant differences were found in masses of EPA between taxa or between cruises. Mean amounts of DHA (i.e. 22:6n-3) in zooplankton tissues were lower than those of EPA, and ranged from 0.5 to 1.4 µg mg^{-1} WM across all taxa and cruises (Fig. 4). Under mid-bloom conditions, copepods (1.2 ± 1.0 µg mg^{-1} WM) contained significantly more DHA then the chaetognaths (0.5 ± 0.5 µg mg^{-1} WM) (p = 0.019; Wilcoxon test), but this was the only significant difference found in DHA mass between taxa and bloom states.

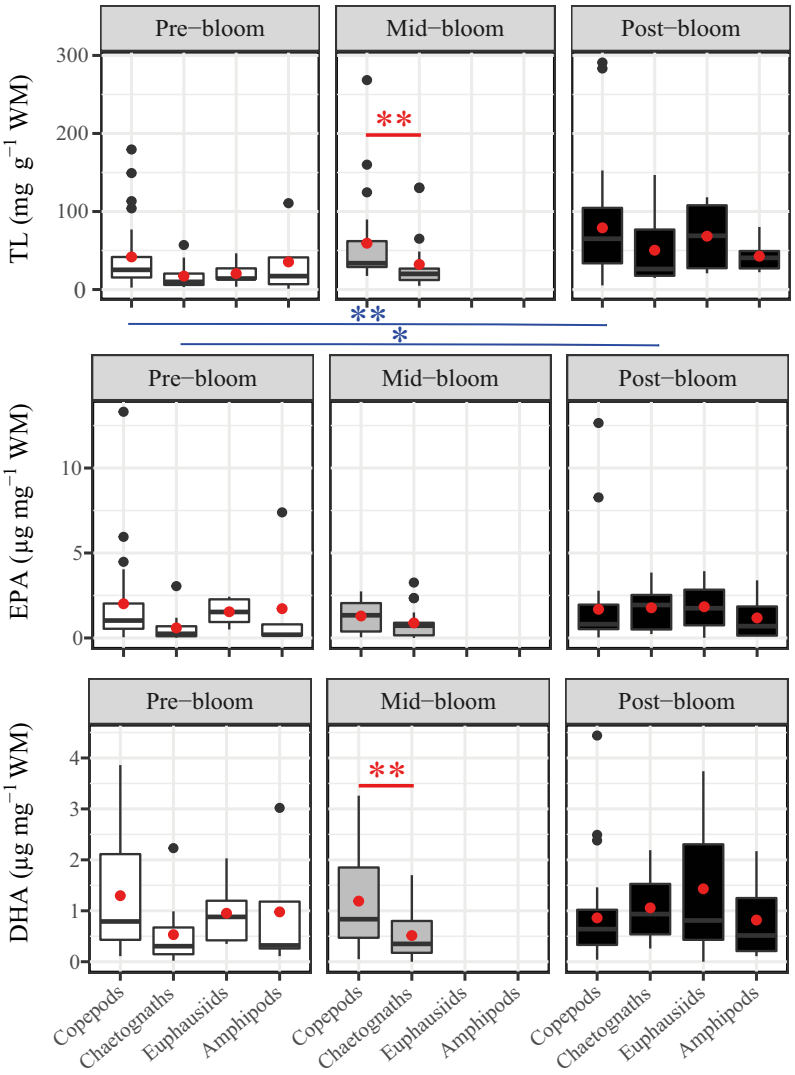

Fig. 4. Masses of total lipid (TL), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) per mass of wet zooplankton tissue (WM) in broad taxonomic groups off the west coast of Vancouver Island. (•) Variable means; (*) statistical significance within oceanographic regime/cruise (i.e. pre-bloom, mid-bloom, post-bloom); (*) statistical significance between cruises for a given taxon. *p < 0.05, **p < 0.01. Boxes represent the first and third quartiles, and the black line bisecting the boxes is the median. Whiskers represent extreme values that are no greater than 1.5 times the interquartile range. Single black points are outliers

3.3.2. Zooplankton functional groups

In contrast to the previous analysis, where we tested for differences in masses of TL, EPA (i.e. 20:5n-3), and DHA (i.e. 22:6n-3) between broad taxonomic groups, many more statistical differences were discerned when we compared lipids across functional groups of zooplankton (Fig. 5). For this reason, we focus primarily on differences in lipids between functional groups and species for the remainder of this paper. During mid-bloom conditions, the OH functional group contained significantly higher amounts of TL than the EBC (p = 0.006; Wilcoxon test), whereas under pre- and post-bloom conditions, no differences in TL were found between functional groups (Fig. 5). Under pre-bloom conditions, the TL of the OH was significantly lower than it was during both mid- (p = 0.006; Kruskal-Wallis test + Dunn's test [Bonferroni]) and post-bloom conditions (p < 0.001; Kruskal-Wallis test + Dunn's test [Bonferroni]). In addition, the EBC functional group contained significantly higher amounts of TL during post-bloom conditions, relative to pre-bloom samples (p = 0.008; Kruskal-Wallis test + Dunn's test [Bonferroni]). In terms of EPA, under pre-bloom conditions, the OH contained significantly higher masses than the EBC (p = 0.002; Kruskal-Wallis test + Dunn's test [Bonferroni]). The EBC contained more EPA in post-bloom conditions than they did during pre-bloom conditions (p = 0.03; Kruskal-Wallis test + Dunn's test [Bonferroni]). Few differences in masses of DHA could be discerned between functional group and oceanographic regimes, but the OH contained more DHA than the EBC during pre-bloom conditions (p = 0.01; Kruskal-Wallis test + Dunn's test [Bonferroni]).

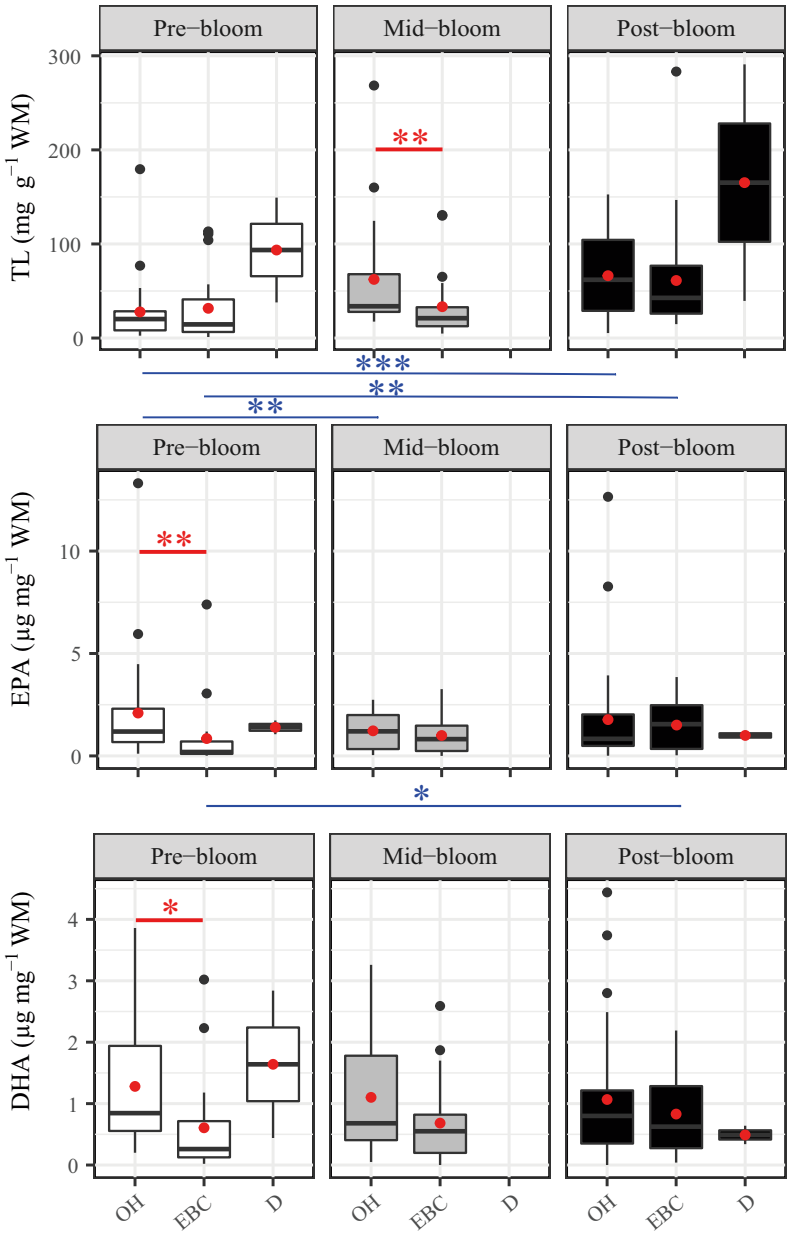

Fig. 5. Masses of total lipid (TL), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) per mass of wet zooplankton tissue (WM) in different functional groups (OH: Omnivore-Herbivores; EBC: Egg-Brooding Carnivores; D: Detritivores; per Venello et al. 2021) off the west coast of Vancouver Island. (•) Variable means; (*) statistical significance within oceanographic regimes (i.e. pre-bloom, mid-bloom, post-bloom); (*) statistical significance between cruises for a given taxon. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Boxes represent the first and third quartiles, and the black line bisecting the boxes is the median. Whiskers represent extreme values that are no greater than 1.5 times the interquartile range. Single black points are outliers

3.3.3. Zooplankton species

Presenting TL data on a mass basis (e.g. mg g^{-1} WM) facilitates comparisons across different zooplankton groups. However, since most of the scientific literature reports lipid mass per individual zooplankter, we will focus on these units for our species comparisons. We make no distinction here between the different cruises, because only 1 species contained significantly different amounts of TL as a function of oceanographic conditions. The copepod *Neocalanus cristatus* had a significantly higher mean TL content during post-bloom conditions as compared to both mid-bloom ($p = 0.02$; Kruskal-Wallis test + Dunn's test [Bonferroni]) and pre-bloom ($p = 0.001$; Kruskal-Wallis test + Dunn's test [Bonferroni]) conditions (Table 3). Most copepod species contained a mean TL of between approximately 0.3 and 1.0 mg ind.$^{-1}$, but some had markedly higher or lower amounts. *Euaugaptilus graciloides* and the unidentified scaphocalanid contained 27.35 and 4.80 mg ind.$^{-1}$, respectively, and mean TL in the copepods *Eucalanus bungii*, *E. californicus*, *Metridia pacifica*, and *Calanus pacificus* was usually <0.3 mg ind.$^{-1}$. The chaetognaths *Eukrohnia hamata*, *Parasagitta elegans*, and *P. euneritica* were characterized by similar amounts of TL (<1 mg ind.$^{-1}$), while *Pseudosagitta scrippsae* contained 1.3 to 7.0 mg ind.$^{-1}$. The euphausiid species in our dataset generally contained high amounts of TL, particularly *Thysanoessa spinifera* (mean TL up to 8.6 mg ind.$^{-1}$). Mean TL of the 3 hyperiid amphipods ranged from 0.2 to 2.8 mg ind.$^{-1}$.

3.4. Fatty acid composition of zooplankton species — multivariate ordinations

An NMDS ordination using the pre-bloom zooplankton fatty acid compositional data had a stress value of 0.162, and 21 fatty acids/fatty acid sums contributed significantly to the relative positions of the samples in multivariate space (Fig. 6). Three clusters of zooplankton samples were revealed by hierarchical cluster analysis, and using these cluster designations,

Table 3. Total lipid content in mesozooplankton collected off the west coast of Vancouver Island during pre-bloom (May 2018), mid-bloom (May–June 2019), and post-bloom (September 2018) conditions. Total lipid values are expressed as means ± 1 SD where the number in brackets represents the number of samples included in each mean. –: no samples collected

Species	Total lipid (mg g^{-1} wet mass)			Total lipid (mg $ind.^{-1}$)		
	Pre-bloom	Mid-bloom	Post-bloom	Pre-bloom	Mid-bloom	Post-bloom
Copepods						
Calanus marshallae	31.53 ± 32.31 (5)	98.64 ± 77.08 (9)	58.88 ± 62.69 (4)	0.33 ± 0.47 (5)	0.98 ± 0.82 (9)	0.31 ± 0.20 (4)
Calanus pacificus	–	–	13.65 (1)	–	–	0.11 (1)
Euaugaptilus graciloides	113.30 (1)	–	–	27.35 (1)	–	–
Eucalanus bungii	24.96 ± 3.77 (3)	–	8.29 (1)	0.34 ± 0.14 (3)	–	0.06 (1)
Eucalanus californicus	2.91 (1)	–	56.07 ± 47.64 (4)	0.03 (1)	–	0.35 ± 0.26 (4)
Euchirella rostrata	93.56 ± 78.76 (2)	–	39.47 (1)	0.66 ± 0.49 (2)	–	0.60 (1)
Metridia pacifica	18.45 (1)	–	–	0.06 (1)	–	–
Neocalanus cristatus	17.40 ± 11.34 (5)	30.44 ± 8.46 (7)	91.90 ± 24.41 (7)	0.47 ± 0.38 (5)	0.92 ± 0.22 (7)	1.51 ± 0.34 (7)
Neocalanus plumchrus	51.62 ± 72.10 (5)	27.81 ± 5.96 (3)	68.94 ± 38.28 (7)	0.29 ± 0.26 (5)	0.53 ± 0.32 (3)	0.33 ± 0.20 (7)
Neocalanus flemingeri	–	–	34.48 (1)	–	–	0.40 (1)
Paraeuchaeta elongata	104.05 (1)	40.68 ± 15.48 (3)	145.74 ± 119.34 (3)	1.08 (1)	0.90 ± 0.30 (3)	1.60 ± 0.19 (3)
Pseudhaloptilus pacificus	–	–	41.44 (1)	–	–	1.50 (1)
Scaphocalanidae	–	–	290.91 (1)	–	–	4.80 (1)
Chaetognaths						
Eukrohnia hamata	19.57 ± 12.65 (3)	33.95 ± 42.64 (7)	56.85 ± 35.90 (3)	0.29 ± 0.07 (3)	0.71 ± 0.76 (7)	0.49 ± 0.16 (3)
Parasagitta elegans	13.01 ± 18.64 (4)	10.76 (1)	54.45 ± 61.68 (4)	0.13 ± 0.15 (4)	0.19 (1)	0.36 ± 0.12 (4)
Parasagitta euneritica	–	43.94 ± 44.21 (6)	–	–	0.69 ± 0.81 (6)	–
Pseudosagitta scrippsae	19.06 ± 21.60 (5)	19.86 ± 25.56 (5)	14.78 (1)	1.73 ± 0.49 (5)	7.04 ± 9.33 (5)	1.30 (1)
Euphausiids						
Euphausia pacifica	10.43 ± 5.87 (3)	–	41.21 ± 23.98 (3)	0.58 ± 0.72 (3)	–	1.89 ± 1.07 (3)
Thysanoessa inermis	–	–	118.24 (1)	–	–	3.50 (1)
Thysanoessa inspinasa	–	–	20.83 (1)	–	–	0.70 (1)
Thysanoessa longipes	–	–	98.23 (1)	–	–	2.50 (1)
Thysanoessa spinifera	30.55 ± 16.29 (3)	–	117.65 (1)	2.33 ± 1.29 (3)	–	8.60 (1)
Amphipods						
Hyperoche medusarum	17.19 (1)	–	27.46 (1)	0.20 (1)	–	1.70 (1)
Primno abyssalis	110.67 (1)	–	–	2.80 (1)	–	–
Themisto pacifica	16.37 ± 21.69 (3)	–	44.86 ± 19.79 (7)	0.42 ± 0.28 (3)	–	0.41 ± 0.11 (7)

we fit 3 ellipses to the ordination at the 95% confidence level. A SIMPER analysis identified 11 fatty acids responsible for driving the separation between the 3 clusters (Table S1 in the Supplement at www.int-res.com/articles/suppl/m687p043_supp.pdf), most of which had been deemed significant by the NMDS analysis.

Pre-bloom Cluster 1 was characterized by very high levels of the carnivory marker 18:1n-9 and ∑MUFA (monounsaturated fatty acids), and low proportions of ∑PUFA, including 20:5n-3 and 22:6n-3 (Fig. 6; Table S1). Five of the 6 zooplankton samples contained in this cluster were copepods (*Euchirella rostrata*, *Euaugaptilus graciloides, Metridia pacifica*, *Paraeuchaeta elongata*), with 1 chaetognath sample (*Eukrohnia hamata*), and they belonged to 3 different functional groups (see Section 2.4): OH, EBC, and D. Cluster 2 contained a mixture of OH copepod species (n = 5 samples; *Neocalanus cristatus*, *N. plumchrus*, *Calanus marshallae*, *Eucalanus bungii*, *E. californicus*) and 3 chaetognath species (n = 9 samples; *Eukrohnia hamata*, *Pseudosagitta scrippsae*, *Parasagitta elegans*) classified as EBC. The zooplankton in Cluster 2 were typified by a high ∑PUFA content, mainly in the form of dinoflagellate markers (i.e. 22:6n-3, ∑18C+22C PUFA), and moderate 18:1n-9 levels. The bacterial fatty acid 18:1n-7 was also enriched in the zooplankton from Cluster 2. In contrast to the previous 2 pre-bloom clusters, Cluster 3 was composed solely of copepods from the OH functional group; specifically, *N. cristatus*, *N. plumchrus*, *C. marshallae*, and *E. bungii* (n = 13 samples). The zooplankton in Cluster 3 had relatively high amounts of 14:0, ∑SFA (saturated fatty acids), and the diatom markers 20:5n-3 and ∑16C+20C PUFA, and low proportions of 18:1n-9. In addition, Cluster 3 zooplankton contained notable levels of the bacterial fatty acids ∑OBFA, 15:0, and i-15:0.

PERMANOVA tests showed that the fatty acid compositions of zooplankton in the 3 pre-bloom clusters/ellipses were significantly different ($p = 0.006$), as were differences between taxa ($p = 0.005$). Additionally, statistically significant differences were found

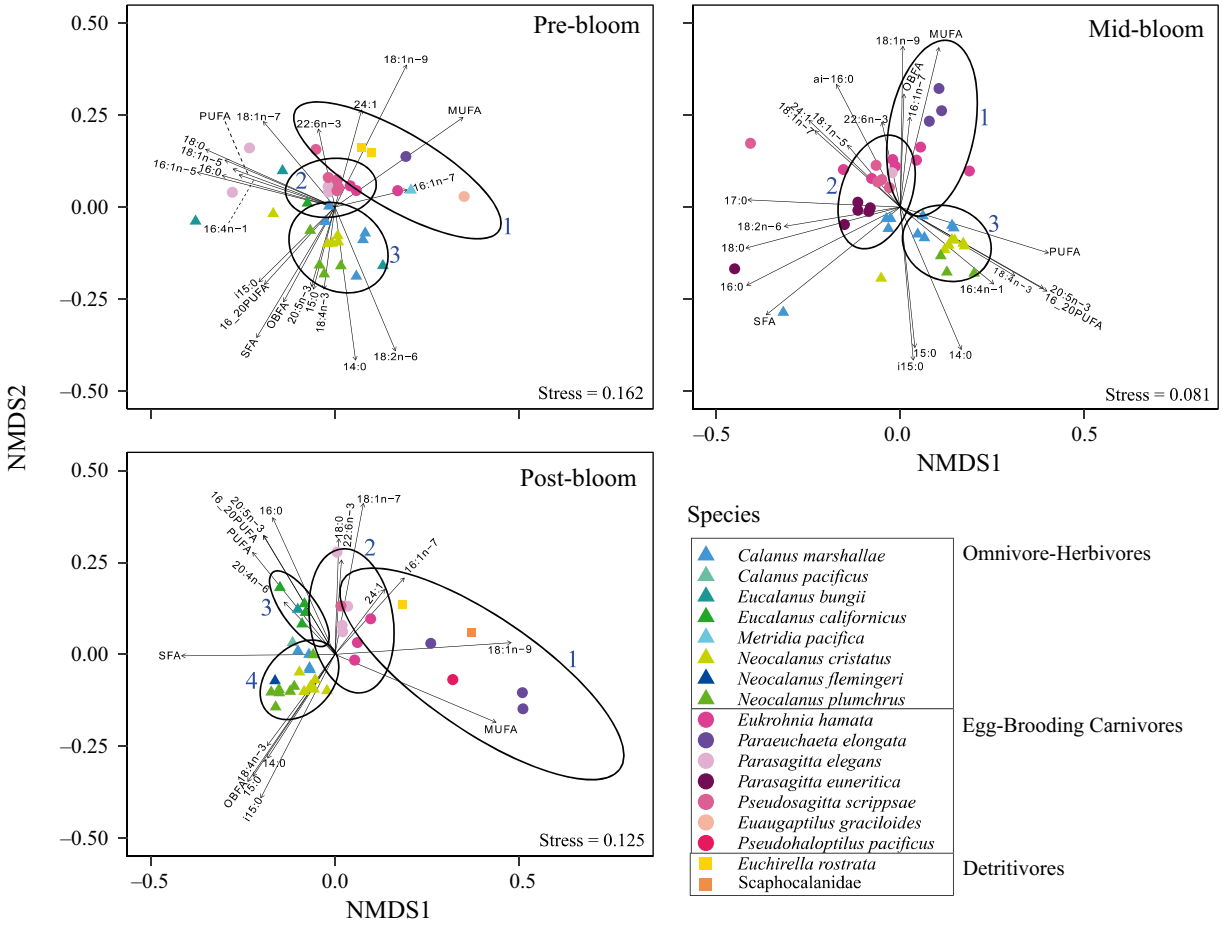

Fig. 6. NMDS ordinations of fatty acid compositional data from copepods and chaetognaths collected during pre-bloom, mid-bloom, and post-bloom conditions during 2018 and 2019 off the west coast of Vancouver Island. The plotted vectors are fatty acids deemed significant during the ordination analysis (SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; OBFA: odd and/or branched fatty acids). Ellipses were added at the 95% confidence level. Cluster designations are shown in blue

between the fatty acid compositions of the OH and EBC functional groups ($p = 0.014$). The fatty acid composition of the D functional group was not significantly different from the OH nor the EBC groups. No differences in the fatty acid composition of zooplankton could be discerned between sampling regions (i.e. North versus South, inshore versus onshore).

The NMDS ordination using the mid-bloom zooplankton fatty acid data yielded a more favorable stress value of 0.081, and 3 ellipses based on cluster membership were fit to the data at the 95% confidence level (Fig. 6). There were 22 significant fatty acids that contributed to the relative positions of the samples, and the SIMPER analysis identified 11 fatty acids that were important in distinguishing between clusters (Table S2). Mid-bloom Cluster 1 consisted of 3 samples of *Eukrohnia hamata*, and 3 samples of *Paraeuchaeta elongata*, with both of these species classified as EBC. The samples in Cluster 1 contained high relative amounts of the carnivory marker 18:1n-9, ∑MUFA, and the bacterial fatty acid group ∑OBFA, and were comparatively low in diatom biomarkers (e.g. 20:5n-3, ∑16C+20C PUFA). Cluster 2 contained all EBC group members (n = 14 samples; *Parasagitta euneritica*, *P. elegans*, *Pseudosagitta scrippsae*, *E. hamata*), apart from 3 samples of *C. marshallae*, that belong to the OH functional group. Zooplankton in Cluster 2 were characterized by moderate amounts of 18:1n-9, high levels of 16:0, ∑SFA, the dinoflagellate markers 22:6n-3 and ∑18C+22C PUFA, and the bacterial markers i16:0, 17:0, and 18:1n-7. Cluster 3 was composed of all the *Neocalanus* spp. samples (n = 10) and 5 *C. marshallae* samples; all belong to the OH group. The copepods in this cluster had high levels of the diatom markers 20:5n-3, 16:4n-1, and ∑16C+20C PUFA, as well as 14:0 and the bacte-

rial fatty acids i-15:0 and 15:0. The zooplankton in the 3 clusters had significantly different fatty acid compositions (p = 0.006; PERMANOVA). In addition, the fatty acid compositions of the 2 taxa were significantly different from each other (p = 0.001; PERMANOVA), as were the 2 functional groups (p = 0.001; PERMANOVA). No significant difference was found in the fatty acid composition of zooplankton as a function of sampling region.

The stress value for the NMDS ordination using post-bloom samples was 0.125. Hierarchical cluster analysis revealed 4 clusters of zooplankton, all with statistically distinct fatty acid compositions (p = 0.006; PERMANOVA), and 4 ellipses were fit to the data (Fig. 6). There were 18 fatty acids that were determined to be significant during the ordination analysis (Fig. 6), and SIMPER identified 12 key fatty acids (Table S3). Post-bloom Cluster 1 was composed of 5 copepod samples: *Paraeuchaeta elongata* (n = 3), *Pseudhaloptilus pacificus* (n = 1), and an unidentified scaphocalanid (n = 1). *P. elongata* and *P. pacificus* are EBC, while the scaphocalanid belongs to the D functional group. The copepods in Cluster 1 were characterized by high relative amounts of the carnivory marker 18:1n-9, the diatom marker 16:1n-7, and ∑MUFA, and low levels of 20:5n-3, 22:6n-3, and ∑PUFA. Cluster 2 contained all of the chaetognaths from the post-bloom subset of data (n = 9 samples), and was typified by the following fatty acids: 18:0, 22:6n-3, ∑18C+22C PUFA, ∑PUFA, 18:1n-7, and moderate levels of 18:1n-9. Cluster 3 was monogeneric, containing 1 sample of *Eucalanus bungii* and 4 samples of *E. californicus*, all from the OH group. The dominant fatty acids in the Cluster 3 zooplankton group were the diatom markers 16:1n-7, 20:5n-3, and ∑16C+20C PUFA, as well as ∑SFA and 16:0. Post-bloom Cluster 4 was a large group containing *Neocalanus cristatus* (n = 7), *N. plumchrus* (n = 7), *N. flemingeri* (n = 1), *C. marshallae* (n = 4), and *C. pacificus* (n = 1), all belonging the OH functional group. The fatty acid composition of the zooplankton in Cluster 4 was not vastly different from that of Cluster 3, but generally contained more 14:0, ∑18C+22C PUFA, 18:4n-3, and the bacterial fatty acids i-15:0, 15:0, and ∑OBFA. PERMANOVA tests showed that the OH was statistically different from both EBC (p = 0.003) and D (p = 0.009) in terms of its fatty acid composition, but the EBC did not differ from D. As with the 2 previous ordinations, the 2 taxa (i.e. copepods versus chaetognaths) had significantly different fatty acid compositions (p = 0.005; PERMANOVA), but sampling region did not affect the fatty acid composition of the zooplankton.

3.5. Seasonal differences in trophic biomarkers

While the NMDS clusters indicated differences between the fatty acid compositions of individual species and functional groups, we were also interested in the seasonal aspect of the overall degree of omnivory versus carnivory. There were only a few species for which we had adequate coverage across all 3 cruises (i.e. *C. marshallae*, *E. hamata*, *N. cristatus*, *N. plumchrus*), so we performed this analysis for functional groups only (Kruskal-Wallis test + Dunn's test [Bonferroni]). Overall, the OH group had more significant differences as a function of season than the EBC, where the fatty acid composition appears to have been more fixed (Fig. 7). During mid-bloom conditions, the OH had significantly higher 20:5n-3 and 16:4n-1 than they did under pre- (20:5n-3: p = 0.004; 16:4n-1: p < 0.001) and post-bloom conditions (20:5n-3: p < 0.001; 16:4n-1: p < 0.001). Total PUFA in the OH functional group was significantly lower during pre-bloom conditions than it was under mid-bloom conditions (p = 0.041), but higher than post-bloom levels (p < 0.001). Levels of the carnivory markers 18:1n-9 and the DHA/EPA ratio in the OH were significantly lower in mid-bloom samples as compared to both pre- (18:1n-9: p = 0.001; DHA/EPA: p = 0.014) and post-bloom samples (18:1n-9: p = 0.039; DHA/EPA: p = 0.002). Similarly, proportions of the bacterial marker 18:1n-7 were significantly lower in mid-bloom samples as compared to both pre- (p < 0.001) and post-bloom samples (p = 0.025). The EBC group also contained lower percentages of the bacterial fatty acid 18:1n-7 during the mid-bloom cruise than they did during both pre- (p < 0.001) and post-bloom conditions (p < 0.001). The diatom marker 16:4n-1 was significantly elevated in the EBC during mid-bloom conditions, as compared to the post-bloom cruise (p < 0.001).

3.6. Total lipid available for higher trophic levels

Because the euphausiids *Euphausia pacifica* and *T. spinifera* contained a significant amount of lipid (up to 1.9 and 8.6 mg ind.$^{-1}$, respectively), they represented a large proportion of the TL at stations where they were abundant (Fig. 8A). This was particularly true of northern and southern IS stations during the post-bloom cruise, where they essentially masked the lipid contributions of the other mesozooplankton species. Please note that euphausiids were not sampled during mid-bloom conditions so we had no TL

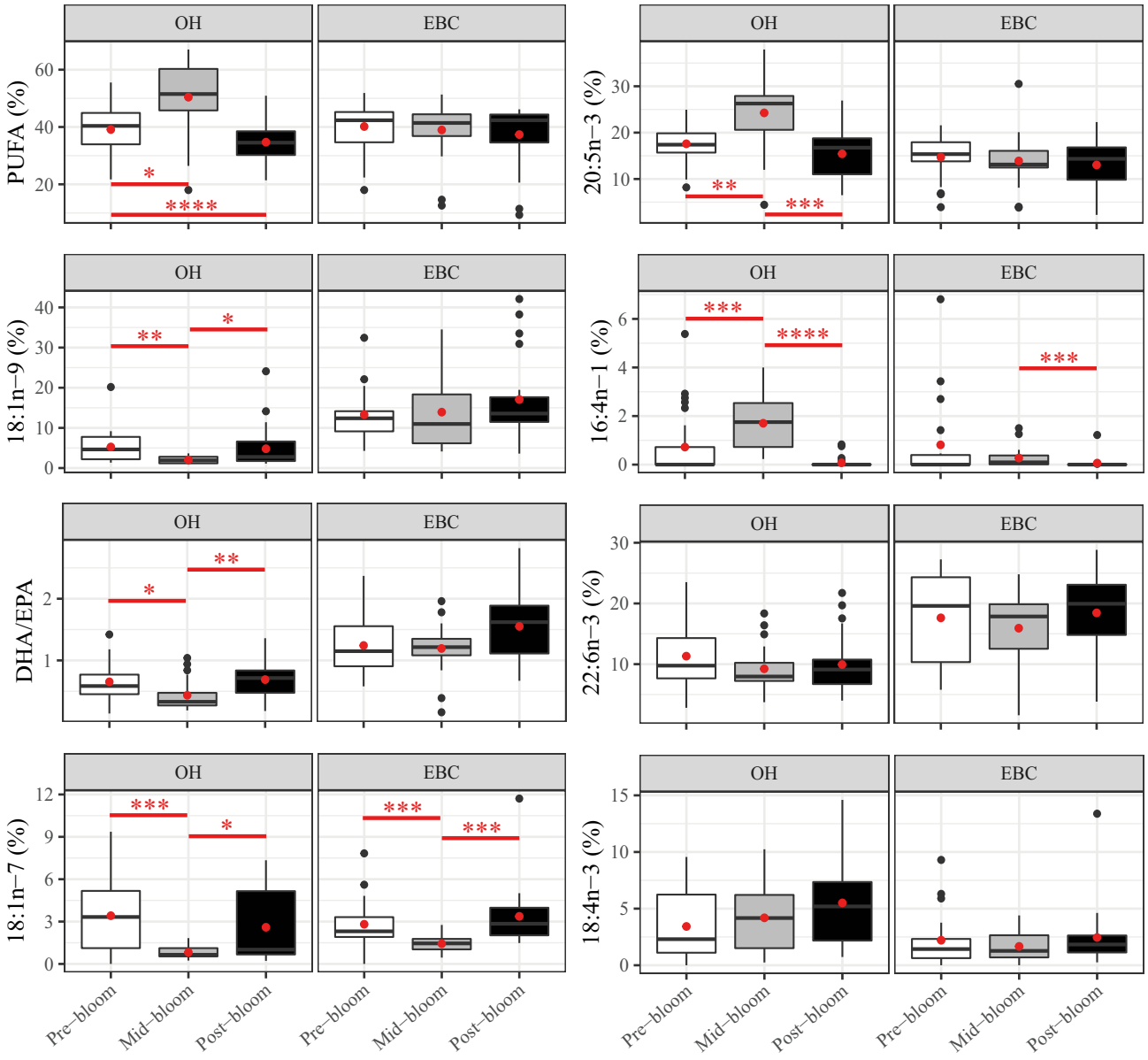

Fig. 7. Relative amounts (%) of fatty acid biomarkers and condition indices in the Omnivore-Herbivore (OH) and Egg-Brooding Carnivore (EBC) functional groups between the 3 sets of oceanographic conditions (i.e. pre-bloom, mid-bloom, post-bloom). The fatty acid 18:1n-9 and the DHA/EPA ratio indicate carnivory, 18:1n-7 is a marker for bacteria, 20:5n-3 and 16:4n-1 are used as diatom biomarkers, 22:6n-3 and 18:4n-3 indicate dinoflagellates, and ∑PUFA (polyunsaturated fatty acids) is an indication of condition. (•) Variable means. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.00001$. Boxes represent the first and third quartiles, and the black line bisecting the boxes is the median. Whiskers represent extreme values that are no greater than 1.5 times the interquartile range. Single black points are outliers

measurements with which to calculate lipid contributions. At some stations sampled during post-bloom conditions, *T. spinifera* contributed up to 74 mg m^{-3} (Stn N14) and *E. pacifica* up to 48 mg m^{-3} (Stn S11). When euphausiids were excluded from the lipid pool calculations, contributions from other mesozooplankton were visible (Fig. 8B). Under pre-bloom and post-bloom conditions, the lipid from all of our target zooplankton combined was <10 mg m^{-3}, whereas during the mid-bloom cruise *C. marshallae* contributed substantial amounts of lipid that reached 126 mg m^{-3} (Stn S16). During pre-bloom and mid-bloom bloom cruises it was mostly the large copepods (i.e. *C. marshallae*, *Neocalanus* spp.) that contributed the most lipid, but at a number of pre-bloom stations the chaetognaths *Eukrohnia hamata* and *Pseudosagitta scrippsae* contributed small amounts of lipid (<1 mg m^{-3}). Additionally, the chaetognath

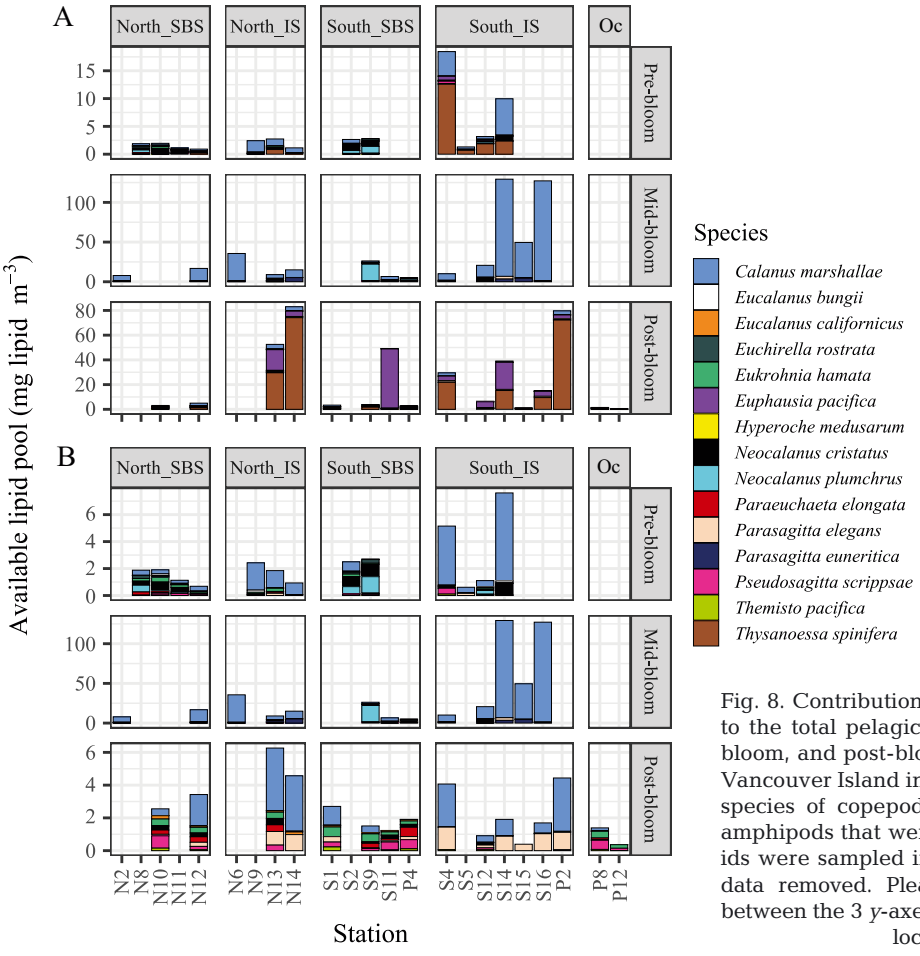

Fig. 8. Contributions of individual zooplankton species to the total pelagic lipid pool during pre-bloom, mid-bloom, and post-bloom conditions off the west coast of Vancouver Island in 2018 and 2019 (A) for all dominant species of copepods, chaetognaths, euphausiids, and amphipods that were analyzed for lipids (no euphausiids were sampled in 2019) and (B) with all euphausiid data removed. Please note the differences in scales between the 3 *y*-axes. SBS: shelf break/slope; IS: inshore locations; Oc: oceanic

Parasagitta euneritica made small contributions to the lipid pool during the mid-bloom cruise, reaching 3 to 5 mg m^{-3} (Stns N14, S14, S15). Under post-bloom conditions, lipid contributions from carnivorous zooplankton were more obvious, particularly among the chaetognaths *Parasagitta elegans* (up to 1.4 mg m^{-3}), *P. scrippsae* (up to 0.8 mg m^{-3}), and *E. hamata* (up to 0.6 mg m^{-3}), the copepod *Paraeuchaeta elongata* (up to 0.6 mg m^{-3}), and the hyperiid amphipod *Themisto pacifica* (up to 0.2 mg m^{-3}).

4. DISCUSSION

4.1. Diets and trophic role of WCVI zooplankton: fatty acid composition corroborates functional group designation

The structure of the mesozooplankton food web off the WCVI was similar to that observed in the nearby Strait of Georgia, with the carnivorous copepod *Paraeuchaeta elongata* at the apex of the food web, followed by the 4 chaetognath species *Eukrohnia hamata*, *Parasagitta elegans*, *Pseudosagitta scrippsae* and *Parasagitta euneritica*, and at the base, the omnivorous (e.g. *Calanus marshallae*, *Neocalanus* spp.) and herbivorous copepods (e.g. *Eucalanus* spp.) (El-Sabaawi et al. 2009, Costalago et al. 2020). These relative trophic positions in our dataset were primarily based on differences in proportions of the carnivory biomarker 18:1n-9 versus those fatty acids associated with phytoplankton and bacteria. The very high proportions of 18:1n-9 found in *P. elongata* off the WCVI were among the highest observed in this study, and similar levels have been found in *Paraeuchaeta*/*Euchaeta* spp. in other marine ecosystems (Auel 1999, Lee et al. 2006, Wilson et al. 2010). The lipid composition of *P. elongata* suggests strict carnivory, and this is supported by observations of copepod remains in fecal pellets (Fleddum et al. 2001) and guts (Øresland 1991) of *Paraeuchaeta*/*Euchaeta* spp.

The 4 chaetognath species appeared to feed carnivorously throughout the WCVI, and across all 3 productivity regimes, and our data do not support

significant herbivory or omnivory (cf. Grigor et al. 2020). The chaetognath species generally grouped together, or with other members of the EBC and D functional groups. Chaetognaths are ambush predators whose primary source of prey is copepods (Feigenbaum & Maris 1984, Terazaki 2004), although the remains of diverse types of food have been found in their guts (e.g. fish larvae, other chaetognaths, amphipods, protists; Feigenbaum & Maris 1984). There were small differences in the fatty acid composition between the 4 species of chaetognaths from the WCVI, such that *E. hamata* usually had higher proportions of 18:1n-9 than the others, and was more likely to contain the copepod biomarkers 20:1n-9 and 22:1n-11. These 20- and 22-carbon MUFA are produced de novo by many species of calanoid copepods (Sargent & Falk-Petersen 1988). The opposite result was found in the Arctic, where *E. hamata* regularly ingested diatoms and detritus, while *P. elegans* was less connected to phytoplankton and microbe-rich prey (Grigor et al. 2020). Accurately determining dietary differences among chaetognaths requires depth-stratified sampling to account for the vertical distributions of individual species and prey fields (cf. Grigor et al. 2020), which sampling constraints did not allow in the present study.

The fatty acid compositions of the copepods *C. marshallae*, *N. cristatus*, and *N. plumchrus* were broadly similar and were associated with 20:5n-3, 18:4n-3, and several OBFA, but never with 22:6n-3. This fatty acid composition suggested a diet based on diatoms and flagellated protists, and perhaps bacterivorous ciliates. Grazing experiments with *Neocalanus* spp. in the NE Pacific have shown that these copepods can act as secondary consumers, feeding not on the phytoplankton themselves, but on heterotrophic ciliates and flagellates (Gifford 1993, Liu et al. 2005). *N. plumchrus* and *C. marshallae* in the Strait of Georgia also feed omnivorously, and have similar fatty acid compositions to what we found off the WCVI (El-Sabaawi et al. 2009). In the multivariate ordinations, *Eucalanus bungii* and *Eucalanus californicus* grouped with other members of the OH functional group or the chaetognaths under pre-bloom conditions, but formed a separate and distinct cluster during post-bloom conditions. Samples of *E. bungii* and *E. californicus* from the post-bloom cruise were associated with diatom fatty acids, supporting their herbivorous diet (El-Sabaawi et al. 2009). Furthermore, DHA/EPA ratios in *E. bungii* and *E. californicus*, which can indicate carnivory (El-Sabaawi et al. 2009, Richoux 2011, Parrish et al. 2015), were among the lowest of our dataset.

Generally speaking, the fatty acid composition of zooplankton from the WCVI corroborated the functional group designations for NE Pacific species (Pomerleau et al. 2015, Venello et al. 2021). In the post-bloom ordination, clusters were mostly composed of single functional groups: 1 cluster of EBC and D copepods, 1 cluster of chaetognaths (EBC), 1 cluster solely composed of *Eucalanus* spp. (OH), and 1 cluster of *N. cristatus*, *N. plumchrus*, and *C. marshallae* (OH). Our fatty acid data therefore validated the assumption that the diets of the OH and EBC were different, and we were also able to detect some within-group differences in lipid composition. When zooplankton switch between 2 dominant prey types, it can take 4 to 6 wk for the initial fatty acid prey signal to be replaced by the second one (Graeve et al. 1994a, Stevens et al. 2004a). Thus the fatty acid composition of the post-bloom samples was presumably the result of zooplankton feeding during an extended period of productivity on the WCVI. Because prey were plentiful, it is possible that all species were well-fed and consuming their preferred prey; this may explain the purity of the post-bloom NMDS clusters, in terms of functional group.

Food quantity and quality, which were both reduced during pre-bloom conditions in this study, can lead to increased competition between consumers in a range of habitats (reviewed by Campanyà-Llovet et al. 2017). Increased competition could potentially result in mixed functional group clusters; for example, those observed in the pre-bloom fatty acid data. Pre-bloom NMDS clusters were frequently a mixture of more than 1 functional group, such that OH copepods were found in 3 different clusters. More than 50% of the time, the OH member appearing in more than 1 cluster was *C. marshallae*, and this was also the case under mid-bloom conditions. This species has a diet that overlaps significantly with *N. cristatus* and *N. plumchrus*, but it appears to also feed on prey not commonly consumed by members of OH group. In the Arctic, some lipid-based estimates of omnivory in *Calanus glacialis* were closer to the omnivore *Metridia longa* than they were to *Calanus hyperboreus* (Stevens et al. 2004c). Until the 1970s, no distinction was made between *C. glacialis* and *C. marshallae* because they are very similar morphologically and parts of their ranges overlap (Frost 1974); it is probable that they fulfil similar ecological roles, including omnivorous feeding. It is unclear why the mid-bloom ordination did not yield pure functional group clusters, since diatoms were abundant and satellite data indicated that coastal waters off the WCVI had been productive for weeks. However, the

dominant diatom present in May and June 2019 was the toxigenic genus *Pseudo-nitzschia*, which may be actively avoided by mesozooplankton in the NE Pacific (Olson et al. 2006).

4.2. Zooplankton lipids show seasonal variation related to food availability

Expressing TL in terms of functional group, instead of taxa, was more sensitive to the detection of seasonal differences in lipid composition. The seasonal pattern we observed in TL, where masses of zooplankton lipid were elevated in the fall, is well-known for temperate and polar copepods. Several copepods from these marine systems sequester large amounts of lipid that they use as metabolic fuel while undergoing diapause in deep water during autumn and winter (Lee et al. 2006, Baumgartner & Tarrant 2017, Schmid et al. 2018). Although both the OH and EBC had higher TL levels in post-bloom samples, as compared to pre-bloom, the only individual species that also showed this significant difference was *N. cristatus*. This species, in addition to *N. plumchrus*, forms thick aggregations in deep waters of nearby Barkley Canyon during winter (De Leo et al. 2018). There were very few differences observed in masses of the essential fatty acids EPA and DHA between seasons. Because DHA is important for proper neural development (Bell et al. 2003), it is highly conserved in consumer tissues (Graeve et al. 2005, Connelly et al. 2014).

Our fatty acid data showed differences in the relative degree of carnivory, and connections to the microbial food web, between seasons. The OH group appeared more responsive to changes in prey availability than the EBC, since condition indices (∑PUFA) and diatom biomarkers (EPA, 16:4n-1) were significantly elevated in mid-bloom samples. Furthermore, carnivory (18:1n-9) and bacterial (18:1n-7) biomarkers in the OH were significantly lower during mid-bloom conditions. Copepods switch from phytoplankton to ciliates when the microbial food web dominates after the spring bloom (Ohman & Runge 1994, Atkinson 1996, Liu et al. 2005). Non-photosynthetic food was more abundant at 5 m depths (grey shading in Fig. 2) during both pre-bloom and post-bloom conditions than it was during the spring bloom, where diatoms were proportionally more abundant. DHA/EPA ratios, used here as a carnivory index (cf. El-Sabaawi et al. 2009), were also lowest in mid-bloom OH samples. Because DHA is sequestered by zooplankton, carnivorous species will have proportionally higher amounts than those feeding herbivorously (Connelly et al. 2014).

4.3. Condition and abundance of WCVI zooplankton: food for higher trophic levels

Our study is the first to measure TL in a broad suite of zooplankton from off the WCVI. TL in our samples of *N. cristatus* and *N. plumchrus* corresponded well to levels found in these same species from other areas of the north Pacific Ocean (Saito & Kotani 2000, Lee et al. 2006, Yamada et al. 2016), and *N. cristatus* generally contained 50% more lipid than *N. plumchrus* (this study). *N. tonsus*, an important contributor to zooplankton biomass in the Southern Ocean (Bradford-Grieve et al. 2001), contains less lipid than either of the aforementioned species (Lee et al. 2006). Although there do not appear to be any TL measurements for *C. marshallae* in the literature, levels in this species were similar to other *Calanus* spp., including *C. pacificus*, *C. australis*, and mid-season (i.e. well before diapause) *C. glacialis*, and TL in *C. marshallae* was generally higher than that of *C. finmarchicus*, *C. helgolandicus*, and *C. carninatus* (Flint et al. 1991, Stevens et al. 2004b, Lee et al. 2006). While the majority of large-bodied copepods store <1 mg lipid (per individual) throughout their life cycles, late-stage copepodites of *C. hyperboreus*, *C. glacialis*, and *N. cristatus* usually amass >1 mg in preparation for diapause (Stevens et al. 2004b, Lee et al. 2006, Connelly et al. 2012, Yamada et al. 2016). *P. elongata*, which does not diapause, also contained >1 mg ind.$^{-1}$ off the WCVI (this study), which is similar to levels in *Paraeuchaeta* spp. and *Euchaeta* spp. collected in the north Pacific, Arctic, and Antarctic (Lee et al. 2006). These carnivorous copepods likely amass large quantities of lipids due to feeding on lipid-rich copepods (Hagen et al. 1995). Off the WCVI, the copepods *Eucalanus bungii* and *E. californicus* contained less lipid than most of the other large copepods in our study, but were comparable to *E. bungii* collected in the western subarctic Pacific Ocean (Saito & Kotani 2000, Yamada et al. 2016), and were not as lipid-poor as *E. inermis* and *E. elongatus* f. *hyalinus* (Flint et al. 1991).

Most of the lipid studies on chaetognaths (Connelly et al. 2012, Grigor et al. 2020) and amphipods (Lee 1975, Auel et al. 2002) have been conducted in the Arctic and Antarctic, where organisms generally contain elevated amounts of lipid (Lee et al. 2006). TL in the 4 chaetognath species off the WCVI, in addition to the hyperiid amphipod *Themisto pacifica*, were therefore on the low end of the lipid content

measured in these groups at the poles. Most of the lipid levels in Antarctic and Arctic euphausiids were higher than what we found in *Euphausia pacifica* and *Thysanoessa* spp. off the WCVI (Lee et al. 2006, Connelly et al. 2012). In particular, the Antarctic euphausiids *Euphausia superba* and *Euphausia crystallophorus* contain massive amounts of lipid that are an order of magnitude higher than the levels measured off the WCVI (Lee et al. 2006). However, TL measurements in *E. pacifica* and *Thysanoessa* spp. from the WCVI were not appreciably different from Antarctic *Thysanoessa macrura* or *Euphausia* spp. from the north and south Atlantic and Pacific Oceans (Lee et al. 2006).

Under post-bloom conditions and warm water events, gelatinous zooplankton may become important dietary items for some vertebrate consumers. Several species of commercially important fish in the NE Pacific regularly prey on jellyfish (i.e. cnidarians and ctenophores) and urochordates (i.e. pelagic tunicates), and the contribution these gelatinous organisms made to fish diet was highest in spring and summer months (Brodeur et al. 2021). In our study, the contribution of gelatinous chaetognaths to the available lipids became important under post-bloom conditions; such conditions may mimic longer-term scenarios relating to increasing ocean temperatures. Carnivorous functional groups of zooplankton have been associated with oligotrophic conditions in the Mediterranean Sea (Benedetti et al. 2018). As we have seen in this study, chaetognaths have very favorable DHA/EPA ratios, are not lipid-poor, and are a common food item of some predators (Uchikawa et al. 2004).

5. CONCLUSIONS

The zooplankton functional group framework developed with NE Pacific species, which takes into account morphological, physiological, reproductive, and behavioral traits (Pomerleau et al. 2015, Venello et al. 2021), aligned closely with our NMDS ordinations of zooplankton fatty acid composition. Although good separation between functional groups was obtained using samples collected during pre-, mid-, and post-bloom oceanographic conditions, the best statistical resolution resulted from analysis of the post-bloom dataset. During the latter, statistical clusters were generally composed of a single functional group. The purity of the hierarchical clusters among the post-bloom zooplankton samples may reflect the prolonged availability of high-quality prey (i.e. centric diatoms + high extracted chl *a* concentrations) that preceded sampling. Differences in the fatty acid composition between functional groups were not as well-defined when prey was scarce (pre-bloom) or potentially noxious (mid-bloom). The lipid pool available to predators on the WCVI was primarily related to the abundance and distribution of individual zooplankton species, and the amount of stored lipid. When euphausiids are even moderately abundant, as is common at inshore locations, their large lipid mass masked the contributions made by all other mesozooplankton. *Calanus marshallae* generally supplied most of the copepod lipid off the WCVI because it frequently dominated zooplankton abundance. However, at offshore stations under post-bloom conditions, carnivorous zooplankton supplied moderate amounts of lipid to the pelagic lipid pool. Post-bloom conditions, such as those investigated during this study, may become increasingly common since marine heatwaves continue to occur (Boldt et al. 2020). Warm, oligotrophic oceans are characterized by gelatinous and carnivorous functional zooplankton groups (Benedetti et al. 2018) that may contain less lipid than omnivorous and herbivorous crustaceans. In future, total lipid must be measured in these groups (e.g. thaliacians, ctenophores, and hydrozoans) to quantify their potential importance as prey for higher trophic levels. Gelatinous zooplankton have long been considered trophic 'dead ends', yet a large number of fish do ingest jellyfish prey under some conditions (Brodeur et al. 2021).

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