Taxonomic, Temporal, and Spatial Variations in Zooplankton Fatty Acid Composition in Puget Sound, WA, USA

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Abstract

Fatty acid (FA) content and composition of zooplankton in Puget Sound, Washington (USA) was studied to investigate the nutritional quality of diverse zooplankton prey for juvenile salmon (*Oncorhynchus* spp.) in terms of their essential fatty acid (EFA) content. The study focus was on eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA) as these are key FA needed to maintain growth and development of juvenile fish. The different zooplankton taxa varied in their FA composition. Much of the variation in FA composition was driven by 18:1 ω 9 (a biomarker of carnivory), ARA, DHA, and FA characteristic of diatoms, which are linked to zooplankton diet sources. Gammarid and hyperiid amphipods contained the highest amount of EFA, particularly the gammarid amphipod *Cyphocaris challenger*i, while shrimp and copepods had much lower EFA content. Crab larvae, which are important prey for juvenile salmon in Puget Sound, had intermediate EPA + DHA content and the lowest DHA/EPA ratio, and were rich in diatom biomarkers. Temporal and spatial trends in zooplankton lipids were less apparent than the taxonomic differences, although the EFA content increased from spring to summer in Cancridae zoeae and the amphipod *C. challenger*i. These results on taxon-specific EFA content provide baseline information on the nutritional quality of zooplankton that can be applied in food web models. Combining zooplankton fatty acid data (quality) with taxon-specific zooplankton biomass data (quantity) enables development of new, sensitive indicators of juvenile fish production to help assess recent declines in salmon production in the Pacific Northwest and predict future adult returns.

Keywords Lipids · EPA · DHA · ARA · Biomarkers · Food quality

Introduction

Puget Sound is a deep estuarine fjord in Washington State, USA that consists of several distinct basins separated by natural sills and narrow straits that vary in salinity, temperature,

and primary production (Moore et al. 2008). Zooplankton exhibit wide variation in biomass and community composition in Puget Sound, with differences occurring among basins, seasons, and years (Keister et al. 2017). Zooplankton, together with ichthyoplankton, are important food for many fish, including juvenile salmonids. Many stocks of anadromous Pacific salmon (*Oncorhynchus* spp.) on the west coast of the USA and Canada, and particularly from Puget Sound, have undergone steep population declines since the 1980s, including coho salmon (*O. kisutch*) and Puget Sound Chinook salmon (*O. tshawytscha*) (Zimmerman et al. 2015; Kendall et al. 2017; Ruff et al. 2017). In Puget Sound, one leading hypothesis for these declines is that the quantity or quality of important zooplankton prey has decreased over time (US Salish Sea Technical Team 2012). A recent study showed that the energy density and lipid composition of zooplankton prey varies taxonomically in the northern Salish Sea (Costalago et al. 2020; Weil et al. 2020); however, zooplankton quality

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in terms of fatty acid content has not been assessed in Puget Sound and may be quite different from other regions due to differences in oceanographic conditions, species composition, productivity, and organic matter sources.

In addition to meeting their energy demands, consumers need a balanced composition of essential nutrients in their diets to develop normally and achieve high growth rates. Many studies have demonstrated that polyunsaturated fatty acids (PUFA) are essential for the growth and development of crustaceans (Coutteau et al. 1997; Nghia et al. 2007) and fish (Ruyter et al. 2000; Glencross 2009). The omega-3 (ω -3) PUFAs eicosapentaenoic acid (EPA, 20:5 ω 3) and docosahexaenoic acid (DHA, 22:6 ω 3) and the omega-6 (ω -6) PUFA arachidonic acid (ARA, 20:4 ω 6) are considered essential fatty acids (EFAs) because they cannot be synthesized *de novo* and they are necessary for somatic growth, reproduction, immune responses, and osmoregulation of fish (Bell et al. 1997; Bell and Sargent 2003; Glencross 2009). Although the nutritional requirements of fish have mostly been studied in aquaculture settings, field studies also indicate the importance of dietary FAs; e.g., growth of herring larvae is linked to DHA availability in the field (Paulsen et al. 2014). Some freshwater and anadromous fish, including salmonids, have the ability to modify dietary PUFA, i.e., elongate and desaturate shorter-chain precursors (18:2 ω 6 and 18:3 ω 3) to ARA, EPA, and DHA (Glencross 2009); however, this modification pathway is generally inefficient, and a direct supply of dietary EFA results in higher growth rates than do the dietary short-chain precursors (Takeuchi and Watanabe 1982; Ruyter et al. 2000; Barry and Trushenski 2020). The same is true for marine zooplankton; e.g., copepods and euphausiids seem to be unable to convert short-chain precursors to EFA at physiologically significant rates (Bell et al. 2007).

Fatty acids are often used as biomarkers to investigate trophic interactions in aquatic systems because they are transferred from prey to predator tissues largely unmodified (Dalsgaard et al. 2003). Hence, certain fatty acids or their ratios may provide important insights into the resources that most strongly support upper trophic level production (Fraser et al. 1989; Galloway et al. 2014; Strandberg et al. 2018). For example, fatty acids indicative of diatoms, i. e., 16:1 ω 7, EPA, and certain ω -4 and ω -1 PUFAs (Jonasdottir 2019), have been shown to transfer through the marine food chain via zooplankton to fish (Fraser et al. 1989; St. John and Lund 1996). Dinoflagellates are characterized by 18:4 ω 3, 18:5 ω 3, and DHA (Jonasdottir 2019). The DHA:EPA ratio has been used to infer the relative importance of diatoms vs. dinoflagellates at the base of marine food webs (Dalsgaard et al. 2003). DHA and 18:4 ω 3 have also been used as trophic markers for flagellates in general (Dalsgaard et al. 2003). Allochthonous organic matter (OM), which is rich in long-chain saturated fatty acids (SAFA; e.g., 22:0, 24:0, 26:0)

originating in cuticular waxes of plant leaves (Hiltunen et al. 2019b), may contribute to basal food sources in estuarine systems. Bacteria and protozoans can also be important food resources for planktonic food webs, and iso- and anteiso-branched fatty acids and odd-chain SAFA are characteristic of bacteria (Kaneda 1991; Dalsgaard et al. 2003). In marine crustaceans, high 18:1 ω 9/18:1 ω 7 ratio has been linked to carnivory (Falk-Petersen et al. 2000; Stevens et al. 2004; El-Sabaawi et al. 2009).

Although the fatty acid composition of consumers is modified by diet, taxon-specific characteristics are also well documented (Persson and Vrede 2006; Budge et al. 2002; Hiltunen et al. 2015). For example, marine copepods are characterized by the 20 and 22 carbon chain (C_{20} and C_{22}) monounsaturated fatty acids (MUFA) and fatty alcohols they synthesize to store lipids as wax esters (Lee et al. 2006). Furthermore, the copepod *Limnocalanus macrurus*, which occurs both in fresh and brackish water environments, produces conspicuous long-chain PUFA (C_{24} and C_{26}) that are transferred to planktivorous fish (Hiltunen et al. 2014). Fatty acid profiles of prey have also been used to quantitatively infer fish diets based on their fatty acid composition (Budge et al. 2012; Happel et al. 2016).

The diet of Chinook and coho salmon during their early marine residence in Puget Sound is composed of euphausiids, crab larvae, hyperiid and gammarid amphipods, and large copepods, with an increasing proportion of 0-age fish as juvenile salmon grow larger (Simenstad 1982; Duffy et al. 2010; Gamble 2016). The potential role of prey quality in the decline of salmon production in Puget Sound has not been addressed due to lack of data on nutritional quality of zooplankton prey. As a part of the Salish Sea Marine Survival Project, we sampled zooplankton throughout Puget Sound and adjacent waters for analysis of seasonal, spatial, and taxon-specific fatty acid composition and EFA content of zooplankton. The goal of this study was to evaluate the nutritional quality of diverse zooplankton taxa based on their fatty acid content, especially in regard to the nutritional requirements of juvenile salmon, and to explore trophic linkages in the planktonic food web using fatty acid biomarkers. We hypothesized that zooplankton taxa differ in their fatty acid composition, the differences are linked to feeding modes and diet sources, and that there are differences in the quality of zooplankton as prey for higher trophic level consumers determined by their EFA content.

Methods

Zooplankton samples ($n = 274$) from the major regions of Puget Sound (Whidbey Basin, Central Basin, South Sound, Hood Canal, Admiralty Inlet) and adjacent waters

of northern Washington (San Juan Islands and Bellingham Bay) were collected for lipid analyses from March to October 2017 (Fig. 1). An additional 35 samples were collected from a few locations in June–July 2015 and 12 samples from July–September 2018 using the same methods except as noted below. Most samples were collected using 60-cm-diameter, 335- μ m mesh bongo nets towed obliquely through approximately the upper 30–180 m of the water column. Of these 321 samples, 225 were collected during the day and 96 were collected at night; nighttime samples were ideal for collecting larger taxa, such as adult euphausiids and amphipods, which are rarely in the

upper water column during the day. Some daytime tows were deep enough to collect organisms that undergo diel vertical migration during the day. Various other collection techniques were used for a minority of the samples: four crab and ostracod samples were collected with a light trap at night; 12 fish, crab, and shrimp samples were collected in the upper 3 m during the day in a surface trawl; and 7 amphipod and shrimp samples were collected during the day and at night with a 200–335- μ m mesh, 0.25-m² Hydrobios Multinet. The nets were gently rinsed into coolers, chilled with ice packs, and aerated to keep zooplankton alive until sorting later the same day or, when live sorting

Fig. 1 Map of the sampling stations for zooplankton fatty acid composition in Puget Sound and adjacent waters of northern Washington in 2017–2018

was not possible, the bulk zooplankton samples were frozen (-70°C) for up to 1 month then partially thawed to separate by taxon (this included a few samples in 2017 and all samples collected in 2015).

Zooplankton, including ichthyoplankton, sorted for fatty acid analyses were grouped by species or family where species identification was not practical. In total, 13 broad taxonomic groups containing over 60 taxa were collected and stored frozen (-70°C) until analysis. It was not practical to separate different life stages of most taxa, apart from crab zoeae, megalopae, and euphausiids (all of which were subadults or adults). Of the 20 fish samples most were larval fish, but three were juveniles and two postlarvae.

The samples were freeze-dried, weighed, and extracted with 2:1 chloroform–methanol following Folch et al. (1957). The fatty acids were transformed into fatty acid methyl esters (FAMES) by acid-catalyzed transesterification. The FAMES were dissolved in hexane, and run through a Gas Chromatograph coupled with a Flame Ionization Detector (GC-FID; Hewlett Packard HP6890) with an Agilent DB-23 column ($30\text{ m} \times 0.25\text{ mm} \times 0.15\text{ }\mu\text{m}$). Helium was used as a carrier gas with an average velocity of 25 cm s^{-1} . A subset of samples was also run with a Gas Chromatogram–Mass Spectrometer (GC–MS; 2017 samples: Shimadzu QP2010 Plus, 2015 samples: Agilent 6890 N and 5973 N) using the same column and temperature program to confirm peak identifications. FAMES were quantified using a dilution series of a fatty acid standard mixture (GLC-68D, Nu Chek Prep.). See the technical report by Hiltunen et al. (2019a) for a more detailed description of the sample collection and processing in 2017. Individual FAMES were identified based on their mass spectra.

Differences in percent fatty acid composition of zooplankton among taxa, seasons (spring: March–April, summer: May–July, fall: August–October), and regions were tested with Permutational Multivariate Analysis of Variance (PERMANOVA). Taxon, region, and season were treated as fixed factors. PERMANOVA was only performed on taxa with $n > 7$, and regions with $n > 10$ (total $n = 302$). PERMANOVA was run with 4999 permutations, type III sum of squares, and permutation of residuals under a reduced model. We used PERMDISP to test for differences in within-group multivariate dispersion to evaluate the reliability of the PERMANOVA results. Differences in fatty acid composition were visualized with a non-metric multidimensional scaling (NMDS) ordination plot. The multivariate statistics were conducted on Bray–Curtis distances. We used the sum of PUFA unique for diatoms (DIFA, i.e., the sum of 16:2 ω 4, 16:3 ω 4, 16:4 ω 1, 18:3 ω 4, 18:4 ω 1), and the 16:1 ω 7/16:0, and DHA/EPA ratios as biomarkers for diatoms, with high DIFA and 16:1 ω 7/16:0 ratios and low DHA/EPA ratios indicating high contributions from diatoms. The 18:1 ω 9/18:1 ω 7 ratio was used as index of carnivory, and the sum of odd-chain

and iso- and anteiso-branched fatty acids (BAFA) was used as an indicator of bacterial and microbial loop contributions. We evaluated the performance of different diatom indices (DIFA, 16:1 ω 7/16:0, and DHA/EPA ratios) with Pearson correlations.

We did not measure dry weights for the 2015 samples, and hence have only used these samples in the plots and analyses based on FA proportions. Thus, the reported EFA contents ($\mu\text{g mg DW}^{-1}$) are only based on 2017 samples. Differences in EPA + DHA and ARA concentrations among zooplankton taxa were tested with a non-parametric Kruskal–Wallis H test because the data were not normally distributed, even with common transformations. Kruskal–Wallis H test was also used for seasonal differences in EFA content for the five most abundant taxa in our samples: Cancridae zoeae and megalopae, the amphipods *Themisto pacifica* and *Cyphocaris challengerii*, and the euphausiid *Euphausia pacifica*. Also, Cancridae crab larvae and the euphausiid *Euphausia pacifica* were tested for differences in EFA content among life-history stages. We also compared zooplankton and ichthyoplankton EFA content to the nutritional requirements of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) proposed by the Food and Aquaculture Organization of the United Nations (FAO) (FAO 2020a,b). These threshold values are based on aquaculture studies, and therefore may not correspond directly to nutritional requirements in the field or for other salmonids, but they provide a useful baseline to compare our EFA results against. Statistical analyses were conducted with IBM SPSS 24 and PRIMER 7 with the PERMANOVA + add-on. The map was drawn with QGIS (QGIS Development Team 2016).

Results

We used PERMANOVA to investigate how taxonomic identity, season, and sampling location affected zooplankton fatty acid composition. The fatty acid percent composition differed among the broad taxonomic groups of zooplankton (PERMANOVA, $F_{6,224} = 15.465$, $p < 0.001$; Table 1 and Fig. 2), with taxonomic group explaining 41% of the variation in the data. The effects of season and region were also significant, but these variables (and their two-way interaction) only explained a small fraction of the variance in the data (Table 1), indicating that taxonomic differences were the major driver of patterns in fatty acid composition. Pairwise comparisons of the broad taxonomic groups indicated that all groups differed from each other ($t = 2.170\text{--}10.562$, $p < 0.002$ in all; Online Resource 2); however, the PERMDISP routine identified differences in dispersion within the groups (Online Resource 2). More specifically, mysids had lower dispersion than gammarids, hyperiids, and fish (probably due to a lower sample size), while dispersion was

Table 1 The effect of broad taxonomic group, season (spring, summer, fall), and region on zooplankton fatty acid composition according to PERMANOVA

Term	df	SS	MS	Pseudo-F	p	R ²
Broad group	6	13,313	2219	15.465	<0.001	41
Region	4	1201	300	2.093	0.005	3
Season	1	590	590	4.111	0.003	12
Broad group×region	29	4595	158	1.104	0.219	1
Broad group×season	11	2098	191	1.329	0.078	2
Region×season	7	1579	226	1.573	0.028	2
Broad group×region×season	16	2510	157	1.094	0.287	1

The amount of explained variation (R²) was calculated from estimates of components of variation

higher in gammarids than in shrimp and euphausiids (Online Resource 3). Mysids also overlapped significantly with the other taxa in the NMDS plot (Fig. 2), which together with large differences in dispersion makes it difficult to discern if the fatty acid composition of mysids differed from the other groups. Gammarids, however, formed a distinct group in the NMDS with little overlap with euphausiids and shrimp, indicating that the difference in fatty acid composition between these groups is reliable despite the small differences in dispersion.

A large fraction of the among- and within-group variation in fatty acid percent composition was driven by 18:1 ω 9, diatom biomarkers (16:1 ω 7, 16:4 ω 1, EPA), ARA, and DHA (Fig. 2). Gammarids, and especially *Cyphocaris challengeri*, were rich in 18:1 ω 9 (Table 2 and Online Resource 1). This was also reflected in gammarids having a very high 18:1 ω 9/18:1 ω 7 ratio. Copepods (mainly *Calanus pacificus*) had high proportions of DHA and 24:1 ω 9, and surprisingly, similar or even lower levels of C₂₀ + C₂₂ MUFA than the

other groups (Table 2). Cephalopods, polychaetes, and pteropods had > 5% C₂₀ + C₂₂ MUFA, but only two samples of each were analyzed (Online Resource 1). Of the hyperiid amphipods, the genera *Hyperoche* and *Hyperia* had higher proportions of ARA than any other taxa (10.0 $\pm$ 1.7%, mean $\pm$ SD), while *Themisto pacifica* and *Primno macropa* had a lower proportion of ARA (2.4 $\pm$ 2.1%) (Table S3). Crab larvae had the lowest proportion of DHA, a low DHA/EPA ratio, and a low carnivory index (18:1 ω 9/18:1 ω 7) (Table 2). Crab larvae were rich in diatom biomarker fatty acids, and there was also variation among taxa with the Pinnotherid crab *Fabia subquadrata* having higher values for diatom biomarkers than most other crab taxa (Online Resource 1). Among krill, *Euphausia pacifica* had a similar fatty acid composition to *Thysanoessa* spp. (comprised mainly of *T. raschii* with some *T. spinifera*). In shrimp, *Pasiphaea pacifica* had a different fatty acid composition from the other taxa, specifically a higher proportion of 18:1 ω 9 and consequently a higher carnivory index (18:1 ω 9/18:1 ω 7) (Online

Fig. 2 Non-metric multidimensional scaling (NMDS) ordination of fatty acid percent composition in Puget Sound zooplankton (n = 321). The final stress for 2-D solution was 0.18. Fatty acids that correlate (r > 0.4) with either of the axis are shown as vectors

Table 2 Fatty acid percent composition (mean $\pm$ SD) and trophic indices in broad taxonomic groups ($n > 2$) of Puget Sound zooplankton

Fatty acid	Gammarids n=46			Hyperiid n=44			Mysids n=8			Fish n=20			Crab larvae n=87			Euphausiids n=45			Copepods n=19			Shrimps n=45		
14:0	2.7	$\pm$ 1.2	3.1	$\pm$ 1.1	3.3	$\pm$ 1.9	1.9	$\pm$ 1.1	3.6	$\pm$ 1.8	3.7	$\pm$ 1.7	5.3	$\pm$ 3.0	2.3	$\pm$ 1.1								
16:0	19.2	$\pm$ 2.4	15.4	$\pm$ 3.6	21.6	$\pm$ 3.2	22.8	$\pm$ 2.3	17.5	$\pm$ 3.3	22.8	$\pm$ 2.9	18.1	$\pm$ 3.6	20.2	$\pm$ 2.5								
16:1 ω	0.2	$\pm$ 0.1	0.7	$\pm$ 0.9	0.3	$\pm$ 0.1	0.3	$\pm$ 0.2	0.3	$\pm$ 0.2	0.3	$\pm$ 0.2	0.1	$\pm$ 0.0	0.2	$\pm$ 0.2								
16:1 ω 7	3.8	$\pm$ 1.9	3.2	$\pm$ 2.1	4.5	$\pm$ 1.1	3.2	$\pm$ 1.7	6.7	$\pm$ 3.3	4.7	$\pm$ 2.2	4.9	$\pm$ 1.8	4.9	$\pm$ 1.8								
16:2 ω 4	0.5	$\pm$ 0.3	0.7	$\pm$ 0.4	0.5	$\pm$ 0.3	0.6	$\pm$ 0.2	0.7	$\pm$ 0.5	1.1	$\pm$ 0.6	1.2	$\pm$ 0.5	0.4	$\pm$ 0.3								
16:3 ω 4	0.5	$\pm$ 0.5	0.4	$\pm$ 0.4	0.4	$\pm$ 0.4	0.2	$\pm$ 0.2	0.7	$\pm$ 0.8	0.7	$\pm$ 0.5	1.4	$\pm$ 1.0	0.2	$\pm$ 0.3								
16:4 ω 1	0.5	$\pm$ 0.3	0.6	$\pm$ 0.4	0.8	$\pm$ 0.6	0.3	$\pm$ 0.1	1.2	$\pm$ 1.2	0.9	$\pm$ 0.6	1.3	$\pm$ 1.2	0.4	$\pm$ 0.5								
18:0	2.7	$\pm$ 2.0	5.6	$\pm$ 2.4	3.9	$\pm$ 1.3	7.1	$\pm$ 2.5	9.2	$\pm$ 3.4	3.0	$\pm$ 1.5	3.0	$\pm$ 1.1	7.4	$\pm$ 2.6								
18:1 ω 9	24.1	$\pm$ 6.9	11.4	$\pm$ 2.2	8.0	$\pm$ 1.6	7.6	$\pm$ 3.2	6.1	$\pm$ 2.0	9.1	$\pm$ 2.0	1.6	$\pm$ 0.9	7.7	$\pm$ 3.8								
18:1 ω 7	2.6	$\pm$ 1.1	3.5	$\pm$ 1.1	3.8	$\pm$ 1.2	4.0	$\pm$ 1.3	6.7	$\pm$ 1.8	6.2	$\pm$ 1.3	1.2	$\pm$ 0.6	7.1	$\pm$ 1.8								
18:2 ω 6	1.5	$\pm$ 1.5	1.1	$\pm$ 0.6	0.7	$\pm$ 0.2	0.7	$\pm$ 0.4	0.6	$\pm$ 0.2	1.3	$\pm$ 0.6	0.6	$\pm$ 0.2	0.8	$\pm$ 0.4								
18:4 ω 3	1.1	$\pm$ 0.5	1.7	$\pm$ 1.0	1.0	$\pm$ 0.3	1.1	$\pm$ 1.0	1.2	$\pm$ 0.9	1.9	$\pm$ 1.6	1.5	$\pm$ 1.4	0.8	$\pm$ 0.6								
20:1 ω 9	1.7	$\pm$ 1.1	1.2	$\pm$ 0.7	1.1	$\pm$ 0.6	0.7	$\pm$ 0.6	0.9	$\pm$ 0.3	0.4	$\pm$ 0.5	0.7	$\pm$ 0.8	0.6	$\pm$ 0.7								
20:4 ω 6	1.6	$\pm$ 1.3	3.6	$\pm$ 3.5	1.3	$\pm$ 0.6	1.7	$\pm$ 1.4	1.2	$\pm$ 0.5	1.3	$\pm$ 0.5	0.5	$\pm$ 0.2	1.6	$\pm$ 1.0								
20:5 ω 3	15.4	$\pm$ 5.6	18.7	$\pm$ 2.5	27.2	$\pm$ 4.8	16.3	$\pm$ 4.0	24.7	$\pm$ 4.4	21.7	$\pm$ 2.9	21.2	$\pm$ 3.6	22.8	$\pm$ 3.9								
22:1 ω 11	1.1	$\pm$ 1.4	0.2	$\pm$ 0.3	0.4	$\pm$ 0.7	0.3	$\pm$ 0.6	0.1	$\pm$ 0.1	0.2	$\pm$ 0.4	1.3	$\pm$ 1.6	0.4	$\pm$ 0.9								
22:5 ω 3	0.6	$\pm$ 0.2	0.9	$\pm$ 0.7	0.6	$\pm$ 0.2	2.1	$\pm$ 1.0	0.6	$\pm$ 0.3	0.5	$\pm$ 0.2	1.2	$\pm$ 0.6	0.6	$\pm$ 0.3								
22:6 ω 3	13.3	$\pm$ 4.5	18.6	$\pm$ 3.8	14.1	$\pm$ 2.2	23.1	$\pm$ 7.5	9.8	$\pm$ 3.8	14.6	$\pm$ 4.9	26.5	$\pm$ 6.1	13.7	$\pm$ 4.0								
24:1 ω 9	0.3	$\pm$ 0.2	0.4	$\pm$ 0.2	0.2	$\pm$ 0.1	0.9	$\pm$ 0.5	0.1	$\pm$ 0.1	0.2	$\pm$ 0.2	1.5	$\pm$ 0.5	0.3	$\pm$ 0.4								
BAFA	1.5	$\pm$ 0.8	2.6	$\pm$ 0.9	1.6	$\pm$ 0.4	1.2	$\pm$ 0.8	1.4	$\pm$ 0.5	1.2	$\pm$ 0.5	1.3	$\pm$ 0.4	1.8	$\pm$ 1.0								
DIFA	1.7	$\pm$ 1.0	1.8	$\pm$ 1.0	2.1	$\pm$ 1.2	1.3	$\pm$ 0.6	2.8	$\pm$ 2.5	2.9	$\pm$ 1.6	4.2	$\pm$ 2.5	1.2	$\pm$ 1.2								
16:1 ω 7/16:0	0.2	$\pm$ 0.1	0.2	$\pm$ 0.1	0.2	$\pm$ 0.1	0.1	$\pm$ 0.1	0.4	$\pm$ 0.2	0.2	$\pm$ 0.1	0.3	$\pm$ 0.2	0.2	$\pm$ 0.1								
18:1 ω 9/18:1 ω 7	11.9	$\pm$ 8.4	3.4	$\pm$ 1.1	2.3	$\pm$ 0.8	2.0	$\pm$ 0.8	1.0	$\pm$ 0.4	1.5	$\pm$ 0.4	1.6	$\pm$ 1.2	1.2	$\pm$ 0.9								
DHA/EPA	0.9	$\pm$ 0.4	1.0	$\pm$ 0.2	0.5	$\pm$ 0.2	1.5	$\pm$ 0.6	0.4	$\pm$ 0.2	0.7	$\pm$ 0.3	1.3	$\pm$ 0.3	0.6	$\pm$ 0.2								
20+22MUFA	4.3	$\pm$ 3.1	3.2	$\pm$ 1.5	2.4	$\pm$ 1.4	1.6	$\pm$ 1.2	2.5	$\pm$ 0.7	1.2	$\pm$ 1.0	2.9	$\pm$ 2.9	2.2	$\pm$ 1.8								

Only fatty acids with mean contribution $> 1\%$ in at least one taxa are included. See Online Resource 1 for the full FA profiles. BAFA bacterial fatty acids; sum of odd-chain, and iso- and anteiso-branched fatty acids. DIFA fatty acids unique for diatoms; sum of 16:2 ω 4, 16:3 ω 4, 16:4 ω 1, 18:3 ω 4, and 18:4 ω 1

Resource 1). Crangonidae shrimp showed high levels of diatom biomarkers in some samples, and high proportions of ARA, DHA, and bacterial biomarkers in others. Consistent with the results for the whole fatty acid profiles, we did not observe clear within-group seasonal differences in the fatty acid indices (data not shown).

Fatty acids unique for diatoms (DIFA, sum of 16:2 ω 4, 16:3 ω 4, 16:4 ω 1, 18:3 ω 4, 18:4 ω 1) formed up to 11.7% of the total fatty acids in zooplankton, and bacterial fatty acids (BAFA) up to 5.4%. Apart from a low proportion of 22:0 (0.2 $\pm$ 0.2%), we did not find long-chain saturated fatty acids indicative of terrestrial OM utilization in zooplankton (Online Resource 1). The highest DHA/EPA ratio was found in fish (1.5 $\pm$ 0.6) and the lowest in crab larvae (0.4 $\pm$ 0.2) (Table 2). The 16:1 ω 7/16:0 ratio correlated strongly with DIFA ($r = 0.631$, $p < 0.001$, $n = 321$) and the DHA/EPA ratio ($r = -0.503$, $p < 0.001$, $n = 321$). However, the values for 16:1 ω 7/16:0 were rather low in our data, with only two samples having a 16:1 ω 7/16:0 ratio > 1 .

For the 2017 samples, we estimated the total fatty acid and EPA content and potential quality of each zooplankton taxon as prey for higher trophic level consumers. Total fatty acid content (a proxy for lipid content) differed among the broad taxonomic groups (Kruskal–Wallis, $H = 56.062$, $p < 0.001$, $n = 279$, among the species with sufficient sample size available; Fig. 3). Gammarids had higher total fatty acid content (121 $\pm$ 79 $\mu\text{g mg DW}^{-1}$) than the other taxa. Hyperiid amphipods (71 $\pm$ 28 $\mu\text{g mg DW}^{-1}$) and euphausiids (86 $\pm$ 56 $\mu\text{g mg DW}^{-1}$) had lower total fatty acid content than gammarids, but higher than shrimp (44 $\pm$ 19 $\mu\text{g mg DW}^{-1}$). Fish, mysids, crab larvae, and copepods had an intermediate total fatty acid content (Fig. 3). There were significant differences in the zooplankton EPA + DHA content among the broad taxonomic groups (Kruskal–Wallis, $H = 48.69$, $p < 0.001$, $n = 279$; Fig. 4a). The highest EPA + DHA content was found in gammarid amphipods (32 $\pm$ 18 $\mu\text{g mg DW}^{-1}$, mean $\pm$ SD) while shrimp had the lowest EPA + DHA content (16 $\pm$ 7 $\mu\text{g mg DW}^{-1}$) (Fig. 4a). The hyperiid and

Fig. 3 Total fatty acid content (a proxy for lipid content) of zooplankton and ichthyoplankton in Puget Sound and adjacent waters of northern Washington from March to October of 2017. The box represents the 25th to 75th quartile of the data; line is the median; and whiskers represent the maximum and minimum values, omitting the outliers (asterisks; more than 1.5 box lengths from the median). Different letters denote significant differences in Kruskal–Wallis post-hoc test; taxa with few replicates ($n = 1–2$) were not tested for differences and individual values for these are plotted with round markers

gammarid amphipods contained significantly more ARA than the other taxa (2.2 ± 1.7 and 1.4 ± 0.6 $\mu\text{g ARA mg DW}^{-1}$, respectively) ($H = 105.07$, $p < 0.001$, $n = 279$; Fig. 4b). Copepods were especially poor in ARA with only 0.2 ± 0.1 $\mu\text{g ARA mg DW}^{-1}$.

A portion of the within-group variation in zooplankton EFA content was related to differences among species and families (Fig. 5). Among hyperiid amphipods the genera *Hyperoche* and *Hyperia* had higher ARA content than the other taxa. Of the crab larvae, *Paguridae* zoeae seemed to have higher ARA and EPA + DHA content than some of the other zoeae taxa. Among the euphausiids, *T. spinifera* had higher EFA content than the other taxa. The DHA/EPA ratio also varied among the species and families of zooplankton (Fig. 6). The *Pasiphaea pacifica* shrimp had a higher DHA to EPA ratio than other shrimp. The DHA/EPA ratio was highest in larval fish, but also varied substantially.

We compared these EFA values to the nutritional requirements of Atlantic salmon and rainbow trout in aquaculture systems proposed by FAO ($10–15$ $\mu\text{g EPA + DHA mg DW}^{-1}$, 5 $\mu\text{g ARA mg DW}^{-1}$). Excluding some crab larvae and Crangonidae shrimp, the zooplankton and ichthyoplankton taxa exceeded the minimum requirement for EPA + DHA (Fig. 5a). However, the requirement for ARA was not met in most of the taxa we sampled (Fig. 5b).

We also studied seasonal variation in EFA content in the five taxa for which we had the best temporal coverage: *Cyphocaris challengerii*, *Themisto pacifica*, *Cancridae* zoeae and megalopae, and *Euphausia pacifica*. The EFA content increased from spring to summer in *C. challengerii* (EPA + DHA: $H = 10.70$, $p = 0.003$) and *Cancridae* zoeae

(EPA + DHA: $H = 6.68$, $p = 0.04$; ARA: $H = 6.28$, $p = 0.04$), while the other taxa did not exhibit significant differences among seasons ($p > 0.05$; Fig. 7). We also investigated the differences in EFA content among life-history stages of Cancridae crab larvae and the krill *Euphausia pacifica*. The EFA content of Cancridae crab larvae was lower in the early zoea stages (I–III) than in late-stage zoea and megalopa (EPA + DHA: $H = 14.55$, $p = 0.002$; ARA: $H = 8.26$, $p = 0.04$; Online Resource 4). The EFA content of krill *Euphausia pacifica* did not differ between juveniles and adults ($p > 0.05$; Online Resource 4).

Discussion

We analyzed the fatty acid composition of zooplankton and ichthyoplankton collected from different regions of Puget Sound from March to October. There were significant differences in both fatty acid percent composition and nutritional quality (based on EFA content) of Puget Sound zooplankton. The fatty acid composition was mainly driven by taxonomic differences, which explained almost half of the variation in the data.

Temporal and spatial trends in fatty acid composition were significant but weaker than taxonomic differences, possibly because the major taxonomic groups were not systematically sampled in all regions throughout the whole time period. However, when comparing EFA content among seasons in the five most abundant taxa in our samples, *Cancridae* zoeae and the amphipod *Cyphocaris challengerii* exhibited seasonal differences, whereas *Cancridae* megalopae,

Fig. 4 **a** Eicosapentaenoic acid + docosahexaenoic acid (EPA + DHA) and **b** arachidonic acid (ARA) content ($\mu\text{g mg DW}^{-1}$) of zooplankton in Puget Sound and adjacent waters of northern Washington from March to October of 2017. Box and whisker plots as described in Fig. 3. Different letters denote significant differences in Kruskal–Wallis post-hoc test; taxa with few replicates ($n = 1\text{--}2$) were not tested for differences

T. pacifica, and *E. pacifica* did not. In most taxa, we did not separate life stages, and that potentially confounded seasonal differences as there may be dietary shifts, e.g., increased carnivory with age. Yamada et al. (2016) found differences in both lipid content and composition among life stages and seasons of copepods in the western North Pacific, and several studies in polar regions have found strong seasonality in lipids (Kattner et al. 1994; Mayzaud et al. 2011). However, consistent with our results, Costalago et al. (2020) found that season and region had statistically significant but minor effects on zooplankton fatty acid composition in the northern Salish Sea. Similarly, salmon prey taxa (juvenile fish and pelagic invertebrates) from coastal Washington differed in their fatty acid composition but there was little difference when comparing May and June samples (Daly et al. 2010).

Feeding experiments demonstrate the incorporation of diatom and dinoflagellate fatty acid biomarkers into *Calanus*

spp. tissues, indicating that fatty acids can be used as biomarkers to investigate zooplankton diets (Graeve et al. 1994). In our data, crab larvae, euphausiids, and copepods were rich in diatom biomarkers, which is consistent with herbivory and direct reliance on primary production. The high DHA/EPA ratios in copepods could also indicate high consumption of flagellates (including dinoflagellates); however, copepods only had a low proportion of another flagellate marker 18:4 ω 3, which makes this less likely. Instead, the high DHA/EPA ratio in copepods could be due to preferential retention of DHA over EPA, and thus be less affected by diet than the C_{16} and C_{18} PUFAs characteristic of diatoms. Even the carnivorous zooplankton taxa had > 1% diatom biomarkers, further indicating the importance of diatoms as a basal resource and demonstrating how these biomarkers transfer up the food chain. Bacterial fatty acids (BAFA) contributed 0.2–5.4% of zooplankton total fatty acids, with the highest values in

Fig. 5 **a** EPA+DHA and **b** ARA content ($\mu\text{g mg DW}^{-1}$) in species (or families) of zooplankton and ichthyoplankton from Puget Sound and adjacent waters of northern Washington. Number of samples (n)

in brackets after taxon name. Box and whisker plots as described in Fig. 3. The dashed lines correspond to minimum nutritional requirements of Atlantic salmon and rainbow trout proposed by FAO

hyperiid amphipods, shrimp, and mysids. BAFA are a biomarker for feeding on bacteria (or bacterivorous protozoans), which may be attached to detritus that can originate both from autochthonous and allochthonous sources (Ederington et al. 1995; Canuel 2001). Hyperiid amphipods are carnivorous as indicated by their high 18:1 ω 9/18:1 ω 7 ratio, but they seem to prey on taxa that rely more on the detrital food web than do other carnivorous taxa. Shrimp had the second lowest carnivory index and the lowest contribution of DIFA, which together with high BAFA could indicate feeding on detritus. Based on gut content analyses, *Pandalidae* shrimp larvae are omnivorous, and they may feed on phytoplankton, zooplankton, and ciliates (Ariza and Ouellet 2009). Detritus

and ciliates, together with phytoplankton and zooplankton, have also been found in the guts of mysids (Viherluoto et al. 2000). Only a low proportion of the long-chain SAFA 22:0 was found in zooplankton while 24:0, 26:0, and 28:0 were not detected, indicating that significant direct contribution of terrestrial organic matter to zooplankton production in Puget Sound was unlikely.

We found very high values of the carnivory index 18:1 ω 9/18:1 ω 7 in gammarid amphipods, and especially in *C. challengeri*. Yamada and Ikeda (2003) suggested that based on their metabolic rates and chemical composition *C. challengeri* in western subarctic Pacific Ocean are opportunistic carnivores that may also feed on zooplankton carcasses. Similar

to our results, a high proportion of 18:1 ω 9 was also found in *C. challenger* in the nearby Strait of Georgia (Costalago et al. 2020). 18:1 ω 9 is characteristic for amphipods both in marine and freshwater environments (Graeve et al. 2001; Daly et al. 2010; Salonen et al. 2019), but it is unclear whether amphipods accumulate 18:1 ω 9 from their prey (e.g., copepods) and/or synthesize it de novo.

We found that the different diatom indices (16:1 ω 7/16:0, DIFA, DHA/EPA) correlated, both in the whole dataset as well as within the broad taxonomic groups. Our findings suggest that these are robust indices of diatom-based diets across broad taxonomic groups. In general, the values for 16:1 ω 7/16:0 were rather low in our data; values > 1 were only detected in two samples of *Fabia subquadrata*. Previous studies have shown the 16:1 ω 7/16:0 ratio may reach > 2 in herbivores and carnivores that depend on a diatom-based food chain (Cripps et al. 1999; Auel et al. 2002). However, PUFA indicative of diatoms (DIFA) reached > 10% in some crab larvae, particularly in April–June, consistent with the spring diatom peak in Puget Sound. It is likely that crab zoeae progress to a more carnivorous diet as they age, which may explain their seasonal change in FAs. The phytoplankton community in Puget Sound is typically dominated by diatoms year-round. This was especially true in 2017 when dinoflagellates were scarce (PSEMP 2019). The most common diatom genera were *Chaetoceros*, *Skeletonema*, and *Thalassiosira*; genera in which 16:1 ω 7 may vary from 10 to 50% of total FA (Jonasdottir 2019). The consistently low

Fig. 7 EPA+DHA (a, c, and e) and ARA (b, d, and f) content in the amphipods *Cyphocaris challenger* and *Themisto pacifica* (a and b), Cancridae crab larvae (c and d), and the euphausiid *Euphausia pacifica* (e and f) in spring (March–April), summer (May–July), and fall (August–October). Number of samples (*n*) in brackets below season. Box and whisker plots as described in Fig. 3. Significant within taxa seasonal differences in pair-wise comparisons are denoted with different letters

values 16:1 ω 7/16:0 in zooplankton could be explained by the diatom community consisting of taxa with low 16:1 ω 7 content.

With a consistently high contribution of diatoms in the phytoplankton community, it is not surprising that there was little seasonal variation in zooplankton fatty acid composition. In general, we found that the planktonic food web in Puget Sound was highly supported by diatoms, with microbial/detrital food sources important in a few taxa, e.g., shrimp. The higher reliance on the microbial/detrital pathway correlated with lower EPA content in shrimp. Thus, our first hypothesis was supported as taxon-specific differences in fatty acid composition were linked to feeding strategies of zooplankton.

Estuarine habitats are often highly productive, and serve as important nursery and feeding areas for juvenile salmonids during their migration to the open waters of the Pacific Ocean (Simenstad et al. 1982). Prey selection by fishes is influenced by size, morphology, pigmentation, evasiveness, and availability, but the role of selection based on prey

Fig. 6 DHA to EPA ratio in species (or families) of zooplankton and ichthyoplankton from Puget Sound and adjacent waters of northern Washington. Number of samples (*n*) in brackets after taxon name. Box and whisker plots as described in Fig. 3

quality is poorly understood. Juvenile coho and Chinook salmon prey selectively on large and pigmented prey items such as hyperiid amphipods, euphausiids, crab megalopae, and fish (Schabetsberger et al. 2003). Our results indicate that amphipods (both gammarids and hyperiids) together with larval/juvenile fish, mysids, euphausiids, crab larvae, and cephalopods are a good source of EFAs in juvenile salmon diets. Crab larvae are abundant in Puget Sound, and are an important prey source for juvenile salmonids, comprising up to 75% of Chinook salmon diets in Puget Sound in early summer (Duffy et al. 2010). High consumption of crab larvae in May–July has been associated with high growth rate and survival in juvenile Puget Sound Chinook salmon (Beauchamp and Duffy 2011). Our fatty acid composition data indicate that Puget Sound crab larvae are highly reliant on diatoms, which establishes an important link between diatom production and juvenile salmon growth during their critical growth period in early summer. We also found that shrimp and copepods are lower sources of EFAs in Puget Sound, although for copepods this may be partly due to timing of the sampling and that we did not sample many of the late-stage *Calanus* that accumulate lipids for diapause. In the northern California Current, juvenile Chinook salmon exhibit negative selectivity for larval stages of the bay ghost shrimp (*Neotrypaea californiensis*) and small copepods (Schabetsberger et al. 2003). Furthermore, Fraser River Sockeye salmon juveniles select euphausiids, amphipods, decapods, terrestrial insects, fish, eggs, and cumacean prey, but not copepods (Price et al. 2013). Similar to EFA, the total FA content was highest in the gammarid amphipods, and especially in *C. challengerii*. Previous studies have found that *C. challengerii* has high lipid/fatty acid content in nearby waters of British Columbia as well (Sargent and Lee 1975; Costalago et al. 2020). Thus, it seems that salmon select high-quality prey taxa when they are available, but whether the selection is based on the food quality or dependent on other factors such as size or prey behavior remains to be explored in controlled feeding trials.

Daly et al. (2010) studied the fatty acid composition of salmon prey taxa along the coast of Washington during the summer upwelling period and found that juvenile fish contained the highest amount of EFA (ARA + EPA + DHA mg g WW⁻¹). Of the invertebrates, *Metacarcinus magister* (formerly *Cancer magister*) larvae were the only ones measured with comparable DHA content to fish (Daly et al. 2010). The hyperiid amphipod *Primno macropa* and gammarid amphipod *Atylus tridens* had similar total EFA content to crab larvae, but significantly lower DHA content. This is somewhat different from our results where hyperiids (primarily *Themisto pacifica*) and gammarids (*C. challengerii*) were richer in DHA (and total EFA) than crab larvae.

The ratios of EFAs, in addition to absolute amounts, are important for fish growth and survival (Sargent et al. 1999).

All of the invertebrate prey in salmon diets from coastal Washington contained DHA:EPA ratios from 0.19 to 0.75, with the highest ratios observed in crab larvae and euphausiids (Daly et al. 2010). In contrast, we found that crab megalopae and zoeae had lower DHA:EPA ratios (0.4 ± 0.2) than the other invertebrate taxa (0.6–1.2), similar to crab larvae in the Strait of Georgia (Costalago et al. 2020). In previous studies (Daly et al. 2010; Costalago et al. 2020) and in our study, prey fish exhibited higher DHA:EPA ratios than invertebrates: 1.0 ± 0.3 for juvenile fish in Daly et al. (2010), 1.85 for larval fish in Costalago et al. (2020), and 1.5 ± 0.6 for larval fish in our study. Similar to these fish, juvenile salmon have higher DHA:EPA ratios than invertebrates (on average > 1.5) (Mjaavatten et al. 1998; Daly et al. 2010; Litz et al. 2017a) potentially indicating higher demand for DHA than EPA. Litz et al. (2017b) found no difference in growth of Chinook salmon over dietary DHA:EPA ratios of 0.6–1.5; however, more studies are needed to elucidate the effects of different EFAs and their ratios on juvenile salmon performance. The comparison between our study and that of Daly et al. (2010) reveals dissimilarities in the potential prey quality between Puget Sound and coastal Washington, which could help explain differences in salmon performance between these areas.

We compared the EFA content of Puget Sound zooplankton and ichthyoplankton to the minimum requirements for optimal growth of Atlantic salmon and rainbow trout proposed by FAO. However, FAO does not provide values for the wild salmonid species that are native to Puget Sound, and the threshold values are based on aquaculture studies, so they may not be entirely representative of requirements of fish living in the more complex and dynamic natural environments. Despite these caveats, these values may provide insights on the approximate amounts of EFA needed by native juvenile salmonids. The EPA + DHA threshold ($10\text{--}15 \mu\text{g mg DW}^{-1}$) was exceeded in most of the zooplankton and ichthyoplankton taxa we analyzed, excluding a few crab larvae and Crangonidae shrimp. This suggests that juvenile fish that feed opportunistically on multiple taxa would easily gain enough EPA + DHA from their diet in Puget Sound. The threshold for ARA ($5 \mu\text{g mg DW}^{-1}$), on the other hand, was not met by most taxa, as only some samples of the hyperiid amphipods *Hyperia* and *Hyperoche* exceeded this threshold. However, a study on Chinook salmon juveniles found that diets lacking ARA completely (but offering 10–15% other ω -6 PUFA) did not suppress growth or survival of fish (Grant et al. 2008), indicating that the threshold for ARA likely is species-specific and may depend on the availability of other ω -6 PUFA. The role of ARA (and other ω -6 PUFA) in fish nutrition has been much less extensively studied than the ω -3 PUFA EPA and DHA, although ARA is recognized as a crucial eicosanoid precursor in

fish tissues (Bell and Sargent 2003). The ratio of ARA to EPA + DHA (or ω -6 to ω -3 PUFA) may also be important for fish performance, further complicating the estimation of threshold values, even for aquaculture systems.

Food quantity and quality influence fish performance concurrently and separating their effects can be challenging. Juvenile salmon survival in Southeast Alaska was linked with stomach fullness (food quantity) rather than prey energy density (food quality) (Weitkamp and Sturdevant 2008). Similarly, in Puget Sound, juvenile salmon growth was linked to food supply, while composite energy density of diets in July varied very little and was not linked to survival (Beauchamp and Duffy 2011). On the other hand, using a bioenergetics model, Ballantyne et al. (2003) estimated that the growth of juvenile Sockeye salmon in Lake Washington is potentially limited by low DHA in their primary zooplankton prey. Fatty acid as a component of prey quality has rarely been taken into account in field studies, likely because detailed, species-specific information on fatty acid composition of prey has not been previously available. Fatty acid composition is not the only factor determining prey quality; however, considering it in a complementary fashion with, e.g., prey quantity, energy density, costs of prey capture, and consumption may provide important insights when studying fish performance in the field.

Zooplankton play a pivotal role in transferring phytoplankton-produced EFA to fish, and long-term changes in zooplankton biomass or community composition have been suggested as possible controls on the low salmon survival in Puget Sound (Keister et al. 2011; Hertz et al. 2016). Our dataset that encompasses ~60 zooplankton taxa that juvenile salmon and numerous other fish species prey upon provides baseline information on the nutritional quality of key prey and trophic relationships in Puget Sound that can be used in modeling studies. Similar to taxon-specific differences in zooplankton energy density in the Salish Sea discovered by Weil et al. (2020), we found differences in EFA content among broad salmon prey groups and even closely related species. Thus, the nutritional quality both in terms of EFA content and energy density of zooplankton prey for higher trophic level consumers varies, indicating that changes in zooplankton community composition may have far-reaching consequences for the whole food web. In the long term, the results of this study could also lead to more sensitive indicators of salmon survival, which incorporate both zooplankton species composition and the fatty acid composition of key prey taxa.

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Declarations

Conflict of Interest The authors declare no competing interests.

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