Technical Report

Fatty acid composition of zooplankton prey for juvenile salmonids in Puget Sound

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Executive summary

Diets rich in essential fatty acids (EFAs) are critical for the nutritional physiology of many fish, particularly during juvenile life stages. EFAs can be limiting in aquatic food webs because they are only synthesized by algae and must be acquired by zooplankton and benthic invertebrates to transfer up the food chain.

Recent studies in the Pacific Northwest show strong correlations between marine survival of salmon and zooplankton community composition, but weaker correlations with zooplankton biomass, indicating that prey composition and quality may be critically important to juvenile salmon.

We sampled zooplankton in different basins of Puget Sound from March to October 2017 to determine the dietary quality, in terms of EFA and energy content, of zooplankton prey for juvenile salmon and to gain insight into the lower trophic level food web of Puget Sound using fatty acid trophic biomarkers.

The percent composition of fatty acids (FAs) differed among broad zooplankton taxonomic groups. There also was high within-group variation in FAs, which was mostly related to differences among genera/species. The within-taxa variation potentially reflects differences in feeding habits, as we found that many taxa varied in their abundance of diatom biomarkers.

We found significant differences in the total FA and energy content among zooplankton taxa. Gammarid amphipods, and especially *Cyphocaris challengeri*, contained the highest total FA content (a proxy for lipid content) (119 ± 79 µg FA mg DW^{-1}, mean ± SD), followed by euphausiids (krill) (86 ± 56 µg FA mg DW^{-1}), and hyperiid amphipods (71 ± 28 µg FA mg DW^{-1}), mysids (61 ± 14 µg FA mg DW^{-1}), fish larvae (60 ± 20 µg FA mg DW^{-1}), crab larvae (59 ± 40 µg FA mg DW^{-1}) and copepods (56 ± 39 µg FA mg DW^{-1}). Krill and *C. challengeri* were also among the taxa with the highest energy content (20,624 ± 1,176 J/gDW and 19,733 ± 1,896 J/gDW), and crabs had the lowest (16,287 ± 1,124 J/gDW). Shrimp had the lowest FA content (44 ± 19 µg FA mg DW^{-1}), and thus were relatively poor-quality prey items, although they had the highest energy content (20,926 ± 2,294 J/g DW).

Similar to the total FA content, amphipods also had a high EFA content. Amphipods, fish, mysids, and krill were rich in EPA (eicosapentaenoic acid, 20:5ω3) and DHA (docosahexaenoic acid, 22:6ω3). The Hyperiidae amphipods and cephalopods were an especially good source of the omega-6 fatty acid ARA (arachidonic acid, 20:4ω6), while copepods lacked ARA almost entirely.

We also found some seasonal variation in the EFA content of the most abundant taxa. The EFA content in the amphipods *C. challengeri* and *Themisto pacifica* dipped during July. The EFA content of Cancridae zoeae and megalopae increased from March to September, while the krill *Euphausia pacifica* did not show seasonal trends in EFA content.

The amphipod *C. challengeri* displayed some spatial variation in the EFA content while *T. pacifica* did not. The EFA content of Cancridae megalopae was very similar among different basins of Puget Sound, but Cancridae zoeae seemed to have lower EFA content in South Sound and San Juan Islands compared to Central Basin, Bellingham Bay, and Admiralty Inlet. Unresolved differences among crab species may have contributed to the variability. The krill *E. pacifica* did not vary in their EFA content among basins.

From the taxa analyzed, hyperiid and gammarid amphipods were the highest quality prey items, followed by mysids, crab larvae, krill, and fish, whereas the EFA content of copepods and shrimp was markedly lower.

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Introduction

One of the crucial steps in the salmon life cycle is their transition from the freshwater to the marine environment. Growth during the early marine period has been deemed "critical" for overall marine survival of salmon with faster growing individuals having higher survival (Beamish et al. 2004, Duffy & Beauchamp 2011). The diet of juvenile salmonids in Puget Sound consists mostly of zooplankton, including euphausids, crab larvae, hyperiid and gammarid amphipods, larval fish, and large copepods (Simenstad 1982, Daly et al. 2009, Duffy et al. 2010). There is considerable inter-annual, seasonal, and spatial variability in the growth and survival of juvenile salmon in Puget Sound that is thought to be related to the availability and quality of their prey. Furthermore, coho salmon (*Oncorhynchus kisutch*) and the ESA-listed Chinook salmon (*Oncorhynchus tshawytscha*) currently suffer from low survival in Puget Sound (Zimmerman et al. 2015, Ruff et al. 2017). Pacific herring (*Clupea pallasii*) and surf smelt (*Hypomesus pretiosus*), which utilize similar food resources as juvenile salmon, have also declined over the past 40 years (Greene et al. 2015). Salmon survival has been linked to continental-scale variability in climate patterns and ocean conditions along the west coast of North America (Mantua et al. 1997, Kilduff et al. 2014), but the mechanisms linking these patterns to survival are still poorly known. Changes in zooplankton community composition, which affect both the quantity and quality of salmon prey resources, has been suggested as a possible mechanism (Keister et al. 2011).

The essential fatty acid (EFA) composition of dietary items is important for determining the quality of food resources because EFAs play a critical role in the early development, growth and survival of many fish (Sargent et al. 1999a, Sargent et al. 1999b, Rainuzzo et al. 1997). In particular, the omega-3 (ω3) and omega-6 (ω6) EFAs; eicosapentaenoic acid (EPA; 20:5ω3), docosahexaenoic acid (DHA, 22:6ω3), and arachidonic acid (ARA, 20:4ω6), are the most important dietary constituents for juvenile salmonid development (Sargent et al. 1999a, Sargent et al. 1999b, Ruyter et al. 2000, Tocher 2003, Glencross 2009). EFA deficiencies can result in severe problems such as impaired vision and reduced foraging success (Bell et al. 1995, Tocher 2003). EFAs are only synthesized *de novo* by phytoplankton (Brett and Müller-Navarra 1997) so they must bio-accumulate up the food chain to consumers (Fraser et al. 1989, Tocher et al. 1998, Ravet et al. 2010, Strandberg et al. 2015). Thus, for juvenile salmonids, the fatty acid composition of their zooplankton prey may be a critical factor determining their well-being, growth, and survival.

In addition to being essential nutritional components, fatty acids can be used as dietary tracers (Dalsgaard et al. 2003). The FA composition of zooplankton and fish indicates feeding history that is integrated over days to weeks (St. John and Lund 1996, Brett et al. 2009b), and therefore provides *in situ* information on feeding history while avoiding some of the methodological problems associated with using gut content analyses to discern feeding history (Nielsen et al. 2018). Fatty acids also provide important insights into the primary producers that most strongly support upper trophic level production (Ravet et al. 2010, Galloway et al. 2014, Strandberg et al. 2018). Although consumer fatty acid composition often has strong taxon-specific characteristics (Persson & Vrede 2002, Budge et al. 2002, Richoux 2011, Hiltunen et al. 2015), it can also be altered by changing feeding patterns based on the availability of nutritious phytoplankton at the base of the food web (e.g., diatoms and cryptophytes), or switching from herbivory to omnivory or carnivory when necessary (Landry 1981, Vargas et al. 2006). Zooplankton that obtain most of their energy and lipids

via the classic diatom pathway will have a high proportion of the diatom biomarker FAs such as 16:1ω7, certain 16 carbon chain (C_{16}) PUFAs (e.g., 16:4ω1, 16:2ω4, and 16:3ω4), and EPA in their lipids (Dalsgaard et al. 2003, Hirche et al. 2003, Stevens et al. 2004, Jonasdottir 2019). In contrast, zooplankton which obtain their resources via a protozoan-intercepted pathway should have a strong signal of the monounsaturated FA (MUFA) 18:1ω9 and a high ratio between 18:1ω9 and 18:1ω7 in their FAs (Dalsgaard et al. 2003, Hirche et al. 2003, Stevens et al. 2004). A high 18:1ω9 to 18:1ω7 ratio has also been linked to carnivory in marine crustaceans (Falk-Petersen et al. 2000, Stevens et al. 2004). A 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). Consumption of carbon of terrestrial origin by zooplankton can be clearly tracked by the much lower ω3:ω6 ratios of terrestrial plants and the very characteristic long-chain saturated fatty acids (SAFAs) that are part of the cuticular waxes present in leaves (Brett et al. 2009a, Taipale et al. 2015). Bacteria contain iso- and anteiso branched fatty acids and odd-chain SAFA which generally are absent in phytoplankton (Kaneda 1991, Dalsgaard et al. 2003). A variety of additional biomarkers are useful to characterize dinoflagellates-cryptophytes (e.g., 18:4ω3, DHA), green algae (e.g., 18:2ω6, 18:3ω3), and other phytoplankton groups (Dalsgaard et al. 2003, Brett et al. 2009b, Jonasdottir 2019).

The transfer of fatty acids up the food chain from a diatom bloom to copepod zooplankton to fish larvae was clearly demonstrated in a mesocosm study of larval herring (Fraser et al. 1989). Thus, environmental changes that affect phytoplankton species composition can result in changes in food quality that transfer up the food web (Fraser et al. 1989, St. John and Lund 1996, Rossi et al. 2006, Daly et al. 2010, Vargas et al. 2010). Because salmon are very rich in EFA and have high dietary FA requirements, they are especially sensitive to changes in diet quality (Sargent et al. 1999b). This indicates that ocean conditions may affect salmon growth and survival through changes in prey quality, which may vary with climate through changes in circulation, primary production, temperatures, etc. Similar conclusions on the importance of prey quality to fish have been drawn in several other studies worldwide. Litzow et al. (2006), for example, hypothesized that climate-driven changes in fish community assemblages are biochemically-controlled through changes in dietary availability of essential fatty acids in the Gulf of Alaska and Bering Sea ecosystems. Quantifying differences in the quality of prey items and the variation in quality seasonally and spatially is a first step towards understanding these important food web links to salmon growth and survival.

The primary objective of this study was to determine taxonomic, spatial, and temporal variability in the quality of juvenile salmon prey items in Puget Sound and gain insights into the characteristics of lower trophic levels using fatty acid biomarkers. We collected zooplankton samples in different basins of Puget Sound during the main juvenile salmon out-migration period of March to October and analyzed the fatty acid composition of 286 samples covering ~60 zooplankton taxa. In addition, we measured the energy content of 48 samples covering 13 taxa. Here we present this unique dataset and provide the first information on the FA and EFA content of juvenile salmon prey items in Puget Sound, and their spatial and temporal variability.

Methods

Samples for zooplankton and larval and small juvenile fish were collected from March to October 2017 in the following basins of Puget Sound and adjacent waters: Whidbey Basin, Central Basin, South Sound, Hood Canal, Admiralty Inlet, San Juan Islands, and Bellingham Bay (Figure 1). A complete list of zooplankton and larval fish species can be found in the Supplemental Data file. Samples from each basin were obtained for abundance and biomass as part of a regular, bi-weekly monitoring program; additional samples for lipid and calorimetry analyses were collected opportunistically as time and personnel allowed. Samples collected for lipid analysis were prioritized to address three main objectives: 1) assess as many important juvenile salmon prey taxa as possible (taxonomic coverage), 2) assess spatial variability across multiple basins within a short period (spatial coverage), and 3) assess temporal variability by obtaining samples from within the same region across months (seasonal coverage).

Zooplankton were sampled using 60-cm diameter, 335-µm mesh bongo nets equipped with a flow meter and towed obliquely over the upper 30 m of the water column (the depth over which juvenile salmon feed during daytime). For zooplankton species abundance and biomass estimation, samples were preserved in 5% buffered formalin and returned to the laboratory for taxonomic analysis. Formalin-preserved samples for biomass estimation were quantitatively counted and identified to species and life stage using methods described in Keister et al. (2017). The lengths of taxa that vary greatly within a life stage were measured. Biomass (in carbon) of large taxa was calculated from densities either using length:dry weight or length:carbon relationships reported in the literature (e.g., Lavaniegos and Ohman 2007, Webber and Roff 1995, Williams and Robins 1979), and, for small organisms, from carbon conversions by species and life stage taken from the literature. Where literature conversions were reported in dry weight (DW) rather than carbon values, data were converted to carbon weight by assuming carbon content of 45%.

Tows were repeated to collect organisms for lipid and energy content analyses, towing deeper in the water column as time allowed. For these, the contents of the nets were gently rinsed into coolers, chilled with ice blocks, and bubbled to keep zooplankton alive until sorted at the University of Washington later in the same day. Taxa were sorted by gently selecting live individuals under the microscope, quickly double-dipping them in tap water to remove exterior salt, and grouping them by species (or genus/family where species identification was not practical) into cryovials to obtain an estimated 1 mg dry weight per sample for lipid analysis, or 50 mg dry weight per sample for calorimetry. At each step, organisms were kept chilled to prevent mortality and break-down of lipids. Twelve broad taxonomic groups containing ~60 taxa were collected (see Supplemental Table 1) and kept frozen (-80°C) until analyses.

Lipids were extracted using a modified Folch method (Folch et al. 1957) from freeze-dried (0.5-8.0 mg) zooplankton samples (n = 286). All used glass-ware was pre-combusted at 450°C for 4h and solvent-rinsed. Lipids were extracted twice with 2:1 (by volume) chloroform:methanol and Milli-Q water was added to remove non-lipid components. The combined organic phases were evaporated under a constant stream of N_2 gas. Once evaporated, toluene followed by 1% H_2SO_4 in methanol were added to resuspend the fatty acids. The sample was flushed with N_2 gas and then heated in a 90°C water bath for 90

minutes to methylate the fatty acids. The produced fatty acid methyl esters were extracted twice with *n*-hexane. The combined organic extractions were then concentrated under a constant stream of N_2 gas and transferred to GC-vials for gas chromatography analysis.

The fatty acid methyl esters were analyzed using a Gas Chromatograph coupled with a Flame Ionization Detector (GC-FID, Hewlett Packard HP6890) with an Agilent DB-23 column (30 m * 0.25 mm* 0.15 µm). Helium was used as a carrier gas with an average velocity of 25 cm s^{-1}. The oven program was as follows: 50°C for 1 min, 10°C min^{-1} to 100°C, 2°C min^{-1} to 140°C, 1°C min^{-1} to 180°C and held for 5 min, 2°C min^{-1} to 200°C, and finally 10°C min^{-1} to 240°C and held for 5 min. Fatty acid peaks in the chromatographs were identified by comparing retention time with reference standards (FAME 37 mix and GLC-68D) and a subset of samples run on a gas chromatograph mass spectrometer (GC-MS Shimadzu QP2010 Plus) under the same method and analytical conditions. Sample fatty acid concentrations were quantified using a dilution series of the GLC-68D standard (Nu-Check-Prep).

Prey energy densities were measured from samples frozen immediately after capture or following live-sorting. Approximately 2 g of fresh or thawed sample material was used for each sampling unit in order to ensure that sufficient dry mass remained to form sample pellets of 0.1g (range 0.05–0.15 g). The samples were oven-dried in pre-weighed tins at 60ºC for at least 48 h until constant dry weight was achieved. Prior to drying, the blotted wet weight was recorded for samples of Pacific Herring juveniles, but not for other taxa. Dried samples were ground to a fine powder with a mortar and pestle, homogenized, and pressed into pellets. The pellets were weighed to the nearest 0.001g and combusted in a Parr 6725 semi-micro bomb calorimeter to measure the energy density (J/g dry weight). Calibration of the calorimeter was checked by combusting standard pellets of benzoic acid, and the final energy values were adjusted for the length of uncombusted fuse wire remaining. Data are reported in J per g DW including ash, and the percent of DW that was ash is also provided in the Supplemental Data file. Data on Pacific Herring is also available as J/gWW.

We used non-metric multidimensional scaling (NMDS) to explore differences in the fatty acid percent composition among samples. Variables (fatty acids) that correlated strongly with the NMDS axes ($r > 0.6$) are presented as vector overlays. This "global" (all taxa) NMDS is also shown in additional figures to highlight intra-group differences by plotting the markers with higher taxonomic resolution. Permutational multivariate analysis of variance (PERMANOVA) was used to test the differences in fatty acid percent composition among broad taxonomic groups. PERMANOVA was run with unrestricted permutation of raw data and type III sum of squares, and the "taxonomic group" was treated as a fixed factor. All multivariate analyses were performed on arcsine square root transformed proportion data using Euclidean distance as the distance measure. The data did not follow normal distributions even when transformed and thus to study the quality of food items we used the non-parametric Kruskal-Wallis H-test to examine differences among broad taxonomic groups in the total (sum FA µg mg DW^{-1}), essential fatty acid content (µg EPA/DHA/ARA mg C^{-1}) and energy density (J/gDW). The groups that had only 1-2 replicate samples (ostracods, polychaetas, pteropods, and cephalopods) were left out of the statistical analyses but were included in the figures. The multivariate statistics were performed with PRIMER 6.1.15 with the PERMANOVA+ add-on, and the other statistical analyses were carried out with IBM SPSS 24.

Results

The highest zooplankton carbon biomasses were observed in May and June at Bellingham Bay (>40 mg C m^{-3}) (Supplemental Figures S1-S7). The biomass peaks during summer were < 20 mg m^{-3} in other stations. Crab larvae dominated the zooplankton community during summer at most stations, while hyperiid amphipods, shrimp, and copepods were also common. The biomass of krill, gammarid amphipods, mysids, and fish larvae were generally low. Those taxa are typically too deep to be sampled by the 0-30 m oblique tows during the day.

We were able to quantify 45 different fatty acids in the zooplankton of Puget Sound. The results are presented as the mass fraction of DW (µg FA mg DW^{-1}), mass fraction of carbon (µg FA mg C^{-1}), and as a proportion of total fatty acids (%) in Supplemental Data file. The fatty acids that contributed >0.5% are also presented in Table 1. The fatty acid percent composition differed among the broad taxonomic groups of zooplankton (PERMANOVA, $F_{7,271}$ = 24.803, p = 0.001, Figure 2), and the taxonomic group explained 42% of the variation in the data. The broad taxonomic groups all differed from each other (pairwise comparisons, t = 1.899-9.110, p < 0.01 in all). The average proportion of PUFA varied from 38% in the gammarid amphipods to 63% in pteropods (Figure 3). The gammarid amphipods contained a higher share of MUFAs, and more specifically 18:1ω9, than the other taxa (Figure 2, Table 1).

Many of the broad taxonomic groups also had high within-group variation that was mostly related to differences among species and genera. The gammarid amphipod *Cyphocaris challengeri* contained a very high proportion of 18:1ω9 (26.8 ± 5.6%), while the other gammarid amphipods (mainly *Calliopius pacificus*) had a lower level of 18:1ω9 (15.8 ± 4.3%) (Supplemental Table 1, Figure 4). The Hyperiid amphipods could be separated into low ARA taxa (the genera *Themisto* and *Primno* with 2.4 ± 2.2% of ARA) and high ARA taxa (*Hyperoche* and *Hyperia* with 10.0 ± 1.7% of ARA) (Supplemental Table 1, figure 5). Only five copepod samples were analyzed, and these exhibited high variation in fatty acid composition with *Neocalanus plumchrus* having higher values for diatom biomarker fatty acids (16:2ω4 and 16:3ω4) than *Calanus* spp. and *Epilabidocera amphitrites* (Supplemental Table 1). Crab larvae also exhibited variation in diatom biomarker fatty acids with higher levels in the Pinnotherid crab *Fabia subquadrata* than in most other taxa (Figure 6). In shrimp, *Pasiphaea pacifica* had a different fatty acid composition from the other taxa, specifically a higher proportion of 18:1ω9 (Figure 7). Crangonidae shrimps showed high levels of diatom biomarkers in some samples, and high proportions of ARA, DHA, and bacterial markers in others. In krill, *Euphausia pacifica* had a similar fatty acid composition to *Thysanoessa* spp. (Figure 8). Furthermore, the total fatty acid content of krill correlated strongly with the proportion of SAFA (r = 0.761, n = 45), and especially with 14:0 (r = 0.661) and 16:0 (r = 0.663), which indicates that they accumulate these SAFA in their lipid stores.

Total fatty acid content (as µg mg DW^{-1}) (a proxy for lipid content) was highest in gammarid amphipods (Kruskal-wallis H=54.542, p<0.001, n = 279, Figure 9). Krill, hyperiid amphipods, and mysids had a lower fatty acid content than gammarids, but higher than shrimp (Figure 9). Crab larvae had an intermediate fatty acid content; lower than gammarids and krill, but similar to mysids, hyperiids, and fish larvae.

Similar to the total fatty acid content, the content of EFAs (EPA, DHA, ARA) was highest in the gammarid and hyperiid amphipods followed by mysids, larval fish, krill, and crab larvae (Table 2, Figure 10). Cephalopods (Octopoda) and pteropods (*Limacina helicina*) had a very high EFA content, but both had only two replicates analyzed due to their relative scarcity in samples. The EFA content also varied among taxa within the groups: e.g., the amphipods of the family Hyperiidae had a higher ARA content than *Themisto pacifica*, another hyperiid amphipod (Figure 10). *T. spinifera* had higher EPA+DHA and ARA content than the other krill taxa. Also, Paguridae zoeae had a higher EFA content than the other crab zoeae. The ratio between DHA and EPA varied among the taxa and especially crab zoeae and megalopae had a low amount of DHA compared to EPA while larval Pacific herring exhibited the highest DHA:EPA ratios in our data (Figure 11). The larval fish samples also had very high variation in their DHA:EPA ratios.

We also studied the spatial and temporal variation in the EFA content of the most abundant taxa in our samples: Cancridae zoeae and megalopae, the amphipods *Themisto pacifica* and *Cyphocaris challengeri*, and the krill *Euphausia pasifica*. In both amphipod species the EFA content dropped in July and increased after that (Figure 12). *C. challengeri* exhibited some variation in EFA content among the basins of Puget Sound, although some basins had a low number of samples analyzed, while *T. pacifica* did not exhibit substantial spatial variation in EFA content (Figure 13). The Cancridae crab larvae showed an increase in EFA content from March to September (Figure 14). There were no spatial trends in the EPA+DHA content of Cancridae megalopae; Cancridae zoeae tended to have higher EPA+DHA content in Admiralty Inlet, Bellingham Bay, and Whidbey Basin than in South Sound or San Juan Islands, although we had a low number of replicate samples from some basins (Figure 15). Cancridae ARA content did not exhibit similar spatial trends. The spatial and temporal trends in krill EFA content were minor (Figures 16, 17).

The energy content per g DW of zooplankton and juvenile fish measured by bomb calorimetry differed across taxonomic groups (Figures 18 and 19) and differed from the patterns in total fatty acid content. Fish, gammarid amphipods, euphausiids, mysids, and shrimps all had similar energy content when grouped across species (Figure 18); crab larvae were distinctly lower energy per g DW (Kruskal-Wallis, $H=14.562$, $p=0.006$, $n = 47$) , but also had higher %Ash Weight (~20% compared to 10-19% for other taxa; Supplemental Data) indicating a higher proportion of indigestible exoskeleton material. Among the different species, the shrimp *Pasiphaea pacifica* had the highest energy content of the taxa we measured, followed by the euphausiid, *Euphausia pacifica* (Figure 19). The fish all had fairly high energy content, and all species of crab were much lower.

Discussion

We found significant differences in the percent fatty acid composition of the key zooplankton prey for juvenile salmonids in Puget Sound. In addition to broad taxonomic groups differing in fatty acids, we also found high within-group variation at the species/genera level. This is consistent with the findings of several other studies that taxonomic differences are the main driver of variation in consumer fatty acid composition (Budge et al. 2002, Persson & Vrede 2002, Richoux 2011, Hiltunen et al. 2015). These differences can arise from phylogenetically driven physiological differences (e.g., in the ability to modify dietary fatty acids) among taxa

or from the utilization of different food sources. For instance, copepods and cladoceran zooplankton have different responses to dietary availability of long chain (e.g., 22 carbon chain length; C_{22}) PUFA: copepods accumulate C_{22} PUFA to their tissues, while cladocerans convert C_{22} PUFA to C_{20} PUFA (Persson & Vrede 2006, Burns et al. 2011, Strandberg et al. 2014). Experiments with marine copepods and *Daphnia* have demonstrated that zooplankton fatty acid composition can also be greatly modified by their diets (Graeve et al. 1994, Brett et al. 2006).

In our data, the diatom fatty acid biomarkers (14:0, 16:1ω7, 16:3ω4, and EPA) and the "flagellate-/animal-origin" biomarkers (ARA, DHA, 18:1ω9) were important in separating the broad groups in the NMDS figure. Differences in fatty acid composition of closely related species are more likely due to differences in feeding habits. We found differences in fatty acid composition within genera/species that belong to the same broad taxonomic group, for example family-level differences in crab larvae were related to the abundance of diatom biomarkers. Especially the zoeae of the Pinnotheridaen crab *Fabia subquadrata* seems to be dependent on diatoms as a food source, which is also reflected in their low DHA:EPA ratios compared to the other crab larvae. The gammarid amphipod *C. challengeri* contained high amounts of 18:1ω9. The high 18:1ω9 seems to be characteristic of gammarid amphipods, as it has been found in other studies of both marine and freshwater gammarid amphipods (Graeve et al. 2001, Daly et al. 2010, Salonen et al. *in press*). However, *C. challengeri* from Puget Sound seems to differ from the gammarid amphipod *Atylus tridens* on the Washington coast by having a much higher proportion of DHA (13.8 ± 5.4% vs. 5.8 ± 0.6%) (Daly et al. 2010). We found krill to accumulate SAFA as a storage lipid (total fatty acid content correlated strongly with proportion of SAFA). This is supported by the finding of Saito et al. (2002) who showed *Euphausia pacifica* have up to 39% of SAFA in their storage lipid triacylglycerols (TAGs). The Antarctic krill *Euphausia suberba* also accumulate the SAFAs 14:0 and 16:0 together with 18:1ω9 in TAG (Hagen et al. 2001). We also found high variation in diatom markers of copepods. *Neocalanus plumchrus* in Strait of Georgia exhibits high temporal and spatial variation in diatom biomarker fatty acids reflecting a shift in diet between diatoms and flagellates (El-Sabaawi et al. 2009).

Interestingly, the ARA content varied greatly among the hyperiid amphipods in our data. The amphipods of the family Hyperiidae (the genera *Hyperoche* and *Hyperia*) contained on average 10% of ARA, and are thus a very good source of ARA in juvenile fish diets. A much lower proportion of ARA was found in *Hyperia medusarum* on the Washington coast (Daly et al. 2010). ARA is the major precursor for eicosanoids, localized hormones, in fish, and this molecule is important for salmon smoltification and adaptation to sea water (Sargent et al. 1999, Bell et al. 1997). Diets deficient in ω-6 PUFA lead to abnormal pigmentation in Chinook salmon (Nicolaides & Woodall 1962). However, Huang et al. (2008) found no differences in Chinook salmon growth, survival, or osmoregulation with various levels of ω-6 and ω-3 PUFA.

In addition to absolute amounts, the ratios of EFAs are important for fish growth and survival (Sargent et al. 1999). Daly et al. (2010) reported that all the invertebrate prey in salmon diets analyzed from coastal Washington contained DHA:EPA ratios from 0.19 to 0.75, with the highest ratio in crab larvae. In contrast, we found significantly higher DHA:EPA ratios in salmon prey; the crab larvae had the lowest DHA:EPA ratio in our dataset (0.39) with other taxa ranging from 0.64 to 1.23 (excluding taxa with n = 1-2). A higher proportion of EPA

leading to these lower DHA:EPA in Washington coast invertebrates ratios could indicate higher reliance on a diatom-based food chain on the coast. In fish, the optimal ratios of EPA, DHA and ARA are likely species-specific (Sargent et al. 1999). In larvae of yellowtail flounder, growth and survival was positively correlated with DHA:EPA ratios up to ~8 (Copeman et al. 2002), whereas in Pacific cod, optimal growth was attained with DHA:EPA ratio ~1 (Copeman & Laurel 2010). Chinook salmon on the Washington coast contained DHA:EPA ratios of >1 (Daly et al. 2010) and >2 (Litz et al. 2017a), and from Fraser river estuary ~3 (Mjaavatten et al. 1998), potentially reflecting a higher demand for DHA than EPA in juvenile Chinook. However, a laboratory study by Litz et al. (2017b) found no difference in growth of Chinook salmon with dietary DHA:EPA ratios of 0.6-1.5. It seems that the zooplankton eaten by juvenile salmon in Puget Sound and on the Washington coast differ in fatty acid composition. The total proportion of EFA in invertebrate prey was similar in Daly et al. (2010) and our data, but the ratios of EFAs differed, potentially resulting in differences in food quality.

We did not measure lipid percentage per DW (lipid%) in the zooplankton samples, but instead used the total fatty acid content (sum FA µg mg DW^{-1}) as a proxy for lipid content because fatty acids are more pertinent to fish nutrition and energetics. Lipids are a heterogeneous group of compounds that are both insoluble in water and soluble in non-polar solvents; lipids include fatty acids, wax esters, sterols, pigments, and some vitamins (Gurr et al. 2002). The lipid% is usually determined gravimetrically by extracting the tissue with powerful solvents and then weighing the extract after the solvent has evaporated. This is nearly impossible to accomplish with high accuracy on small samples (such as is often the case with zooplankton). Also, some of the compounds extracted this way play no direct role in fish physiology, except as a potential energy source (e.g., plant pigments and some phytosterols); others play important physiological roles even though their concentrations may be very low (e.g., some vitamins and sterols). Typically, when ecologists think of lipids they are actually envisioning "edible" fat and oils, which are almost entirely esters of glycerol linked to fatty acids (Gurr et al. 2002). For example, in common triglycerides, fatty acids account for ≈ 95% of the mass of the overall molecule (Gurr et al. 2002). To transform the total fatty acid content to lipid%, it is important to know what kind of lipid molecules the species contains. In freshwater fish, total fatty acid content is highly correlated with lipid% and fatty acids comprise ~70% of lipids (Ahlgren et al. 1996), but similar conversion factors have not yet been determined for zooplankton. The analysis of fatty acids by gas chromatography will also yield more detailed information on the nutritional quality of food items (e.g., EFA content) that could be employed in food web studies.

The energy density values we measured are in agreement with previously published literature values (Duffy et al. 2010, Litz et al. 2019 and references therein). The amphipods, larval fish, euphausiids and shrimps had a relatively higher energy density than crab larvae. We found a mismatch between total lipid (measured as sum FA µg mg DW^{-1}) and energy density values: shrimps, which had a low lipid content, were among the most energy dense prey items.

The EFA content of food (as a mass fraction of carbon or DW) is an important factor for fish nutrition (Sargent et al. 1999a, Sargent et al. 1999b, Ruyter et al. 2000, Tocher 2003, Glencross 2009). We found significant differences in the EFA content of key prey taxa of juvenile salmonids in Puget Sound. Gammarid and hyperiid amphipods were the highest quality taxa in our dataset, followed by mysids, crab larvae, krill, and fish larvae while the

quality of copepods and shrimp was markedly lower. Schabetsberger et al. (2003) found that juvenile salmon in the Columbia river plume fed selectively on large and pigmented prey, such as hyperiid amphipods, euphausiids, and fish larvae, while negatively selecting for shrimp and small copepods. Thus, our data indicate that while selecting for large and pigmented prey, the juvenile salmonids are also selecting for prey that are high quality in terms of their fatty acid content. Fish contained the highest EFA content (mg g WW^{-1}) in a study of salmon prey items on the Washington coast (Daly et al. 2010). In that study, *Cancer magister* larvae were considered the highest quality prey taxa of the invertebrates while hyperiids were poor quality taxa (Daly et al. 2010), highlighting differences in prey quality between Puget Sound and the coastal Washington.

We found spatial and temporal differences in EFA content of some zooplankton taxa. The amphipods *T. pacifica* and *C. challengeri* had a drop in their EFA content in July, which might reflect differences in their prey composition or decreased prey abundance during that time. It could also be related to the life-cycle of these amphipods. The spatial differences in EFA content were minor in our data compared with the differences among taxonomic groups or months. However, we were only able to evaluate this for a few taxa since we had limited spatial coverage for most taxa. Cancridae zoeae did exhibit spatial variation in their EFA content: they seemed to have lower EFA content in the South Sound and San Juan Islands compared to the Central Basin, Bellingham Bay, and Admiralty Inlet. Crab larvae are particularly important prey for juvenile Chinook salmon during their critical growth period in June-July after shifting from shoreline to epi-pelagic habitats (Duffy et al. 2010). During this period, juvenile Chinook increase their body mass by severalfold, and their body mass at the end of this period is highly positively correlated with overall marine survival to the returning adult stage 2-4 years later (Duffy and Beauchamp 2011). The copepod *Neocalanus plumchrus* in Strait of Georgia has been found to exhibit both temporal and spatial variation in EFA content (El-Sabaawi et al. 2009). Variation in lipid content and fatty acid composition of copepods is also prominent in Northern California Current (Miller et al. 2017). Arctic/Antarctic zooplankton (especially copepods) exhibit higher seasonal variation in their lipid composition and content than was found for our data in Puget Sound (Lee et al. 2006). Puget Sound is a temperate estuary with less pronounced seasonal variation in primary production compared to higher latitudes, so lower seasonality in lipid types and storage would be expected in most taxa. However, we also did not sample during the late fall and winter, which might have revealed larger differences in fatty acid composition (Miller et al. 2017).

Conclusions

Our data shows that the nutritional quality of juvenile salmon prey taxa varies markedly in Puget Sound. Amphipods exhibited high lipid, EFA, and energy content, and thus were the highest quality prey items. Crab larvae, which are among the most important prey of juvenile salmon in Puget Sound, had an intermediate EFA content and the lowest energy density of the studied taxa. Copepods tend to have low energy density (Litz et al. 2019) and were low in EFA in our study, indicating that their nutritional value is lower than that of the other prey taxa. Despite their high energy density, the shrimps had low lipid and EFA content, highlighting how different measures of food quality may not always agree. We also found

some spatial and temporal variation in zooplankton EFA content. Overall, our dataset encompasses ~60 zooplankton taxa that juvenile salmon prey upon and provides baseline information on the nutritional quality of key prey and trophic relationships in Puget Sound. In the long-term, the results of this study could lead to more sensitive indicators of salmon survival, which incorporate both zooplankton species composition and the fatty acid composition of important prey taxa. We anticipate that such an indicator would correlate more strongly with salmon survival than those currently in use, thereby enabling more accurate forecasts of future returns.

Tables & Figures

Table 1. The percent fatty acid composition (mean ± SD) by broad taxonomic group ($n \geq 5$) for all fatty acids that contribute ≥0.5% to the total fatty acids.

Fatty Acid	Copepods (n = 5)	Crab larvae (n = 79)	Fish larvae (n = 19)	Gammarids (n = 43)	Hyperiids (n = 40)	Euphausiids (n = 45)	Mysids (n = 8)	Shrimps (n = 40)
14:0	5.5 ± 5.6	3.7 ± 1.9	2.0 ± 1.1	2.6 ± 1.2	3.0 ± 1.1	3.7 ± 1.7	3.0 ± 2.1	2.2 ± 1.1
16:0	22.4 ± 3.8	17.7 ± 3.3	22.9 ± 2.4	19.3 ± 2.3	15.5 ± 3.8	22.8 ± 2.9	22.5 ± 1.2	20.5 ± 2.5
16:1ω7	4.2 ± 1.9	6.9 ± 3.4	3.3 ± 1.6	3.6 ± 2.0	3.1 ± 2.2	4.7 ± 2.2	4.1 ± 1.2	4.8 ± 1.9
16:2ω4	1.0 ± 0.8	0.8 ± 0.5	0.6 ± 0.2	0.5 ± 0.3	0.7 ± 0.4	1.1 ± 0.6	0.5 ± 0.3	0.4 ± 0.3
17:0	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.2	0.4 ± 0.3	0.8 ± 0.5	0.3 ± 0.1	0.6 ± 0.2	0.8 ± 0.4
16:3ω4	1.4 ± 1.7	0.7 ± 0.8	0.2 ± 0.2	0.5 ± 0.5	0.3 ± 0.4	0.7 ± 0.5	0.4 ± 0.4	0.2 ± 0.4
16:4ω1	0.4 ± 0.4	1.2 ± 1.3	0.3 ± 0.1	0.5 ± 0.3	0.6 ± 0.4	0.9 ± 0.6	0.7 ± 0.6	0.5 ± 0.5
18:0	4.0 ± 1.8	9.5 ± 3.4	7.0 ± 2.4	3.0 ± 2.3	5.7 ± 2.5	3.0 ± 1.5	3.7 ± 0.9	7.5 ± 2.7
18:1ω9	2.7 ± 1.0	6.2 ± 2.0	7.4 ± 3.2	23.7 ± 7.2	11.7 ± 2.1	9.1 ± 2.0	9.0 ± 1.7	8.2 ± 3.7
18:1ω7	1.9 ± 0.6	6.8 ± 1.8	4.0 ± 1.3	2.7 ± 1.1	3.6 ± 1.1	6.2 ± 1.3	3.6 ± 0.9	7.2 ± 1.9
18:2ω6	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.4	1.6 ± 1.5	1.1 ± 0.7	1.3 ± 0.6	0.8 ± 0.2	0.9 ± 0.4
18:3ω3	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.3	0.8 ± 0.4	0.9 ± 0.5	0.9 ± 0.7	0.5 ± 0.2	0.6 ± 0.4
18:4ω3	1.4 ± 0.5	1.3 ± 1.0	1.1 ± 1.0	1.1 ± 0.5	1.7 ± 1.1	1.9 ± 1.6	1.0 ± 0.3	0.7 ± 0.6
20:1ω9	0.6 ± 0.3	0.9 ± 0.3	0.7 ± 0.6	1.7 ± 1.1	1.3 ± 0.7	0.4 ± 0.5	1.1 ± 0.6	0.7 ± 0.7
20:1ω7	0.2 ± 0.1	0.8 ± 0.2	0.2 ± 0.1	0.3 ± 0.3	0.7 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.4
20:4ω6	0.3 ± 0.1	1.2 ± 0.5	1.6 ± 1.3	1.6 ± 1.3	3.8 ± 3.6	1.3 ± 0.5	1.4 ± 0.6	1.7 ± 1.0
20:5ω3	19.3 ± 4.5	24.4 ± 4.5	16.6 ± 3.9	15.4 ± 5.8	18.5 ± 2.5	21.7 ± 2.9	26.4 ± 4.7	22.1 ± 3.4
21:5ω3	0.5 ± 0.1	0.7 ± 0.3	0.4 ± 0.2	0.4 ± 0.3	0.3 ± 0.2	0.7 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
22:5ω3	0.7 ± 0.2	0.6 ± 0.3	2.0 ± 1.0	0.7 ± 0.4	0.9 ± 0.7	0.5 ± 0.2	0.5 ± 0.1	0.6 ± 0.3
22:6ω3	23.8 ± 8.2	9.3 ± 3.6	22.9 ± 7.6	13.5 ± 4.8	18.2 ± 3.8	14.6 ± 4.9	14.4 ± 2.2	13.8 ± 4.2

Table 2. Differences in essential fatty acid content (µg mg C^{-1} of EPA, DHA, and ARA) among broad taxonomic groups according to Kruskal-Wallis H-test. Different letters denote significant difference in posthoc test. For cephalopods, ostracods, pteropods, and polychaetes, *n* = 1-2 so they were excluded from the statistical comparisons. See Table 1 for *n* in other groups.

	Kruskal-Wallis									
	H	*p*	Copepods	Mysids	Crab larvae	Shrimps	Euphausiids	Gammarids	Hyperiids	Fish larvae
EPA (20:5ω3)	65,042	<0.001	bc	a	a	c	b	a	a	b
DHA (22:6ω3)	130,134	<0.001	bc	bc	c	d	b	a	a	a
ARA (20:4ω6)	125,418	<0.001	d	b	b	c	b	a	a	b

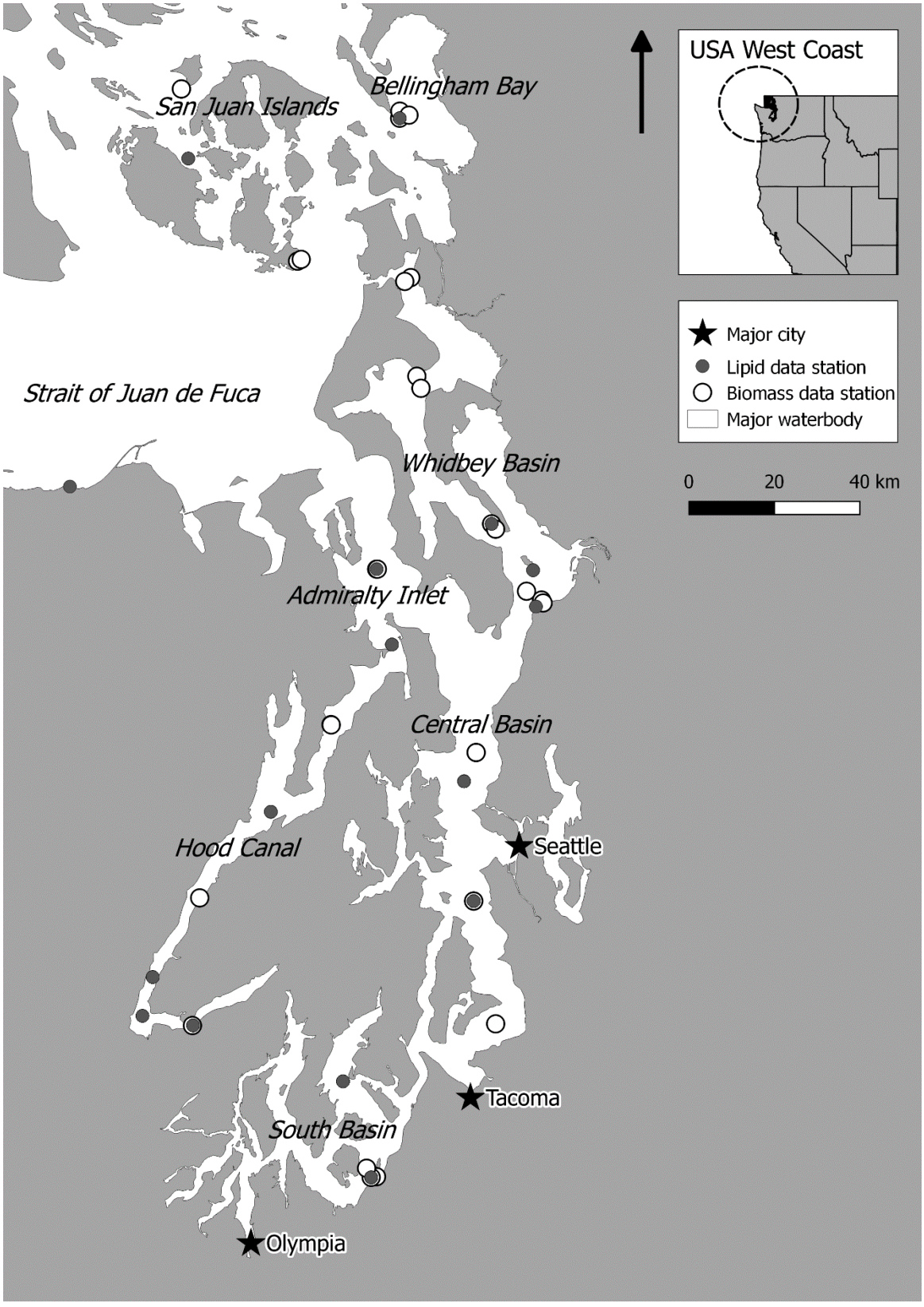

Figure 1. Zooplankton sampling stations in Puget Sound and adjacent waters.

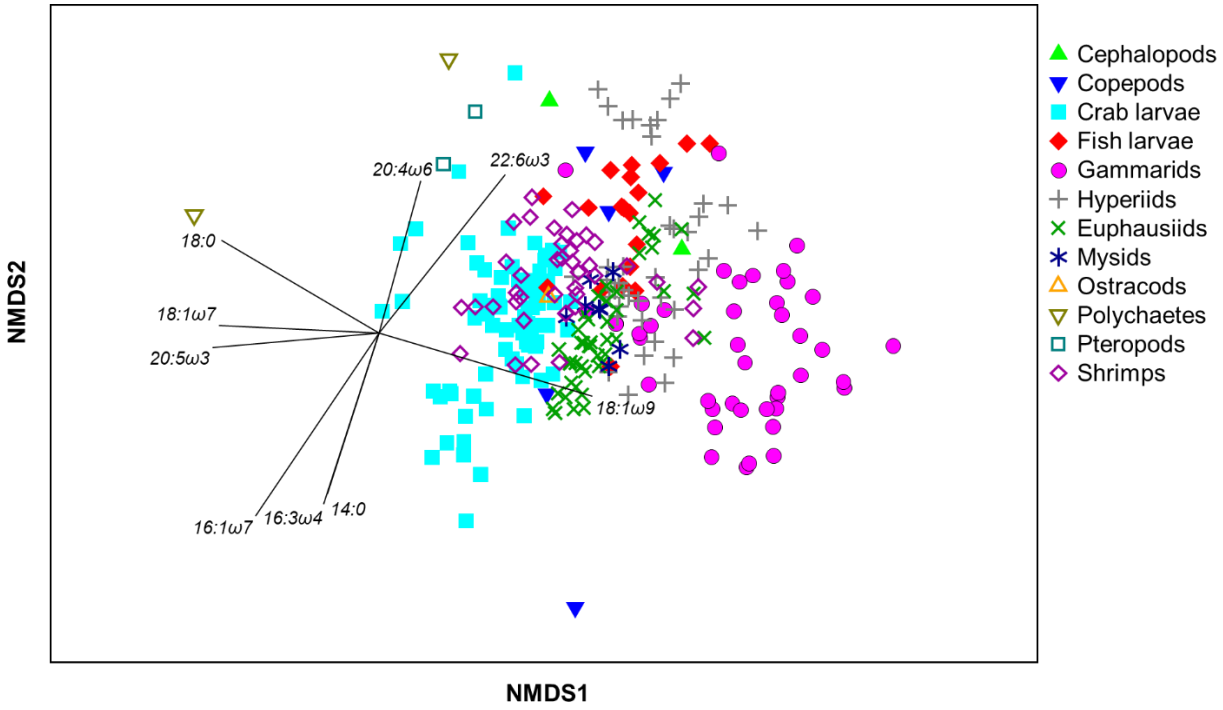

Figure 2. NMDS figure of percent (%) fatty acid composition in broad taxonomic groups of zooplankton in Puget Sound and adjacent waters in March-October 2017. The fatty acids that most strongly correlated (r>0.6) with the axes are presented as vectors. Total n=286. The stress for 2-D ordination was 0.17. Figures 4-8 show gammarid, hyperiid, crab, shrimp, and euphausiids groups in greater detail.

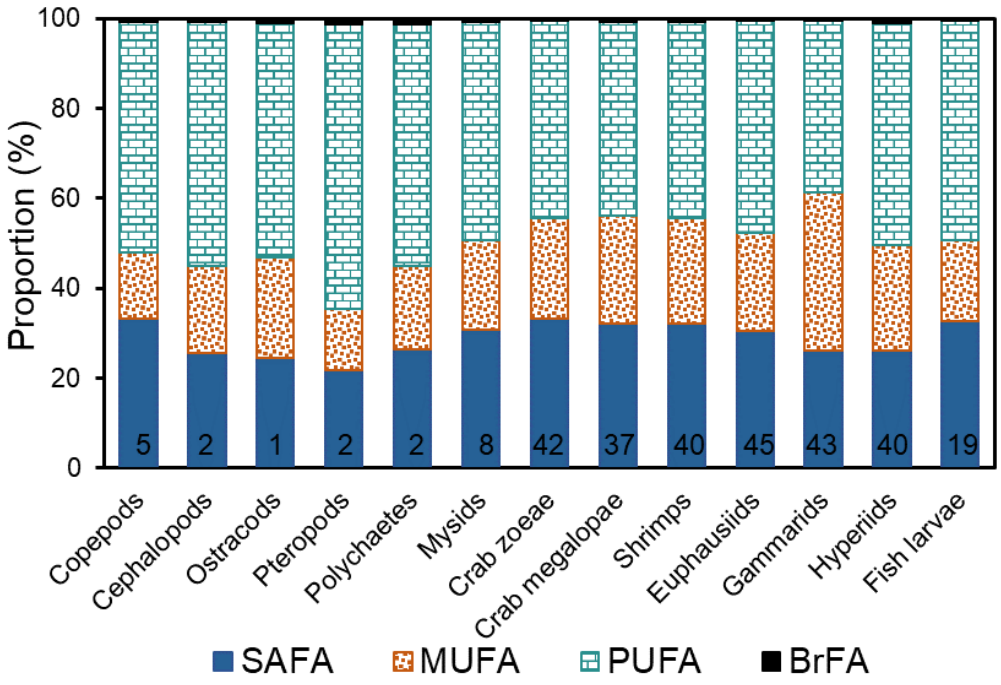

Figure 3. Percent (%) of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and iso- and anteiso-branched fatty acids (BrFA) of total fatty acids in broad taxonomic groups of zooplankton. The number in the bars indicates *n*.

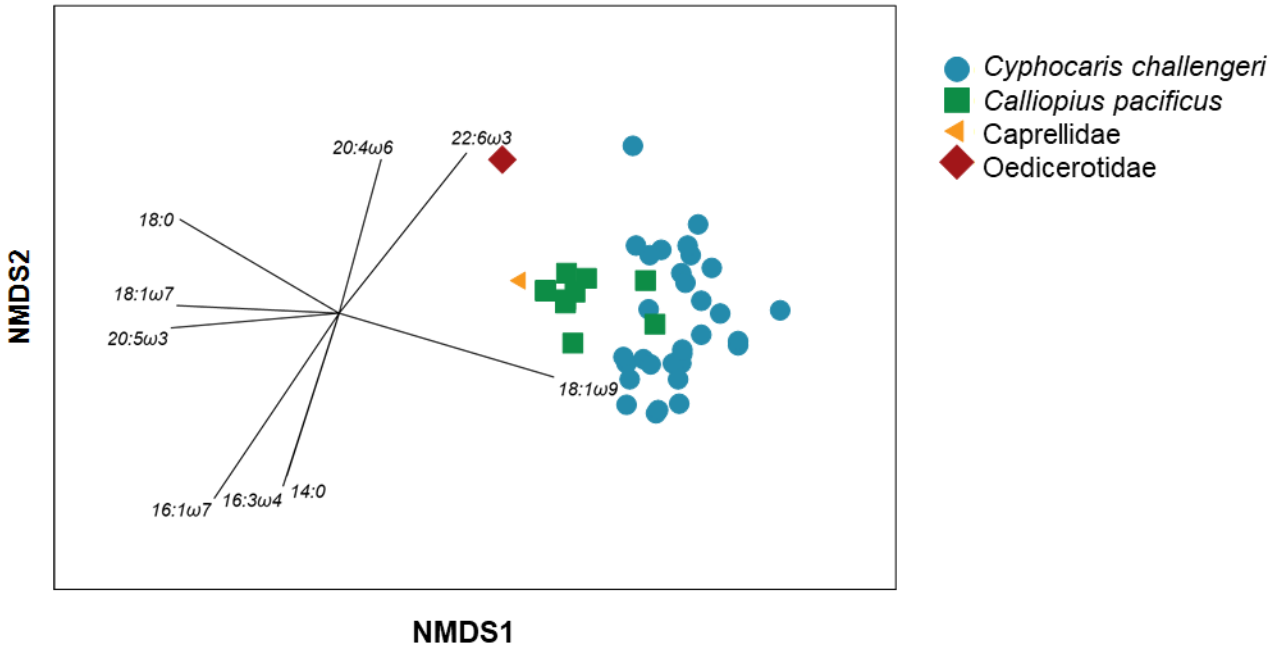

Figure 4. NMDS figure of percent (%) fatty acid composition of gammarid amphipods.

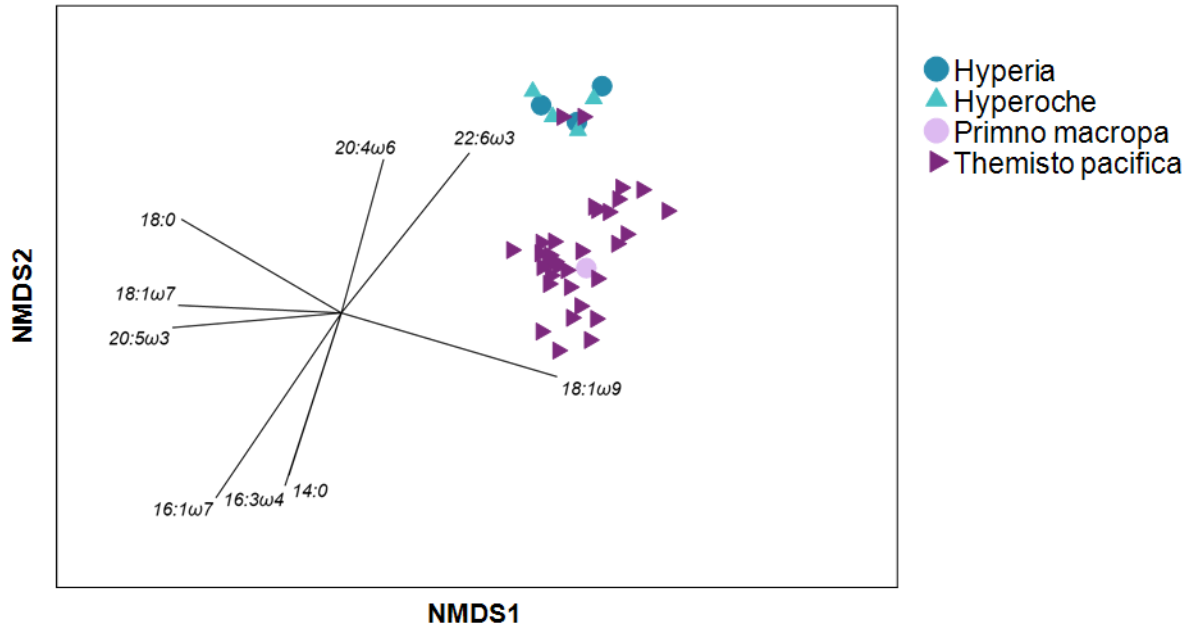

Figure 5. NMDS figure of percent (%) fatty acid composition of hyperiid amphipods.

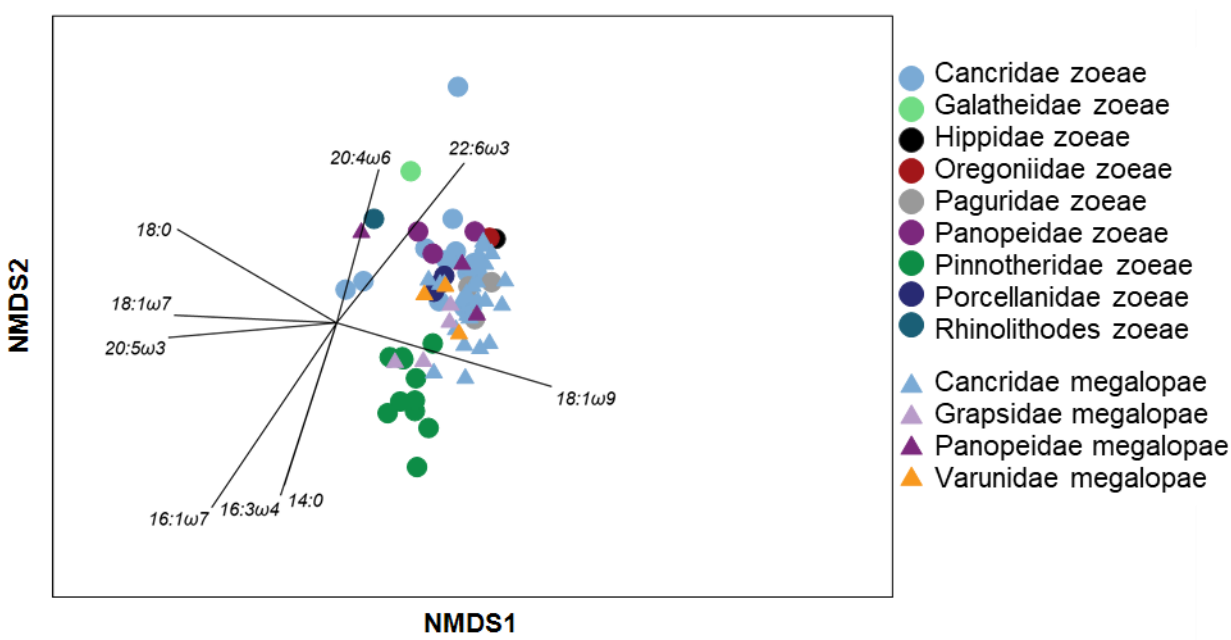

Figure 6. NMDS figure of percent (%) fatty acid composition of crab zoeae (circles) and megalopae (triangles).

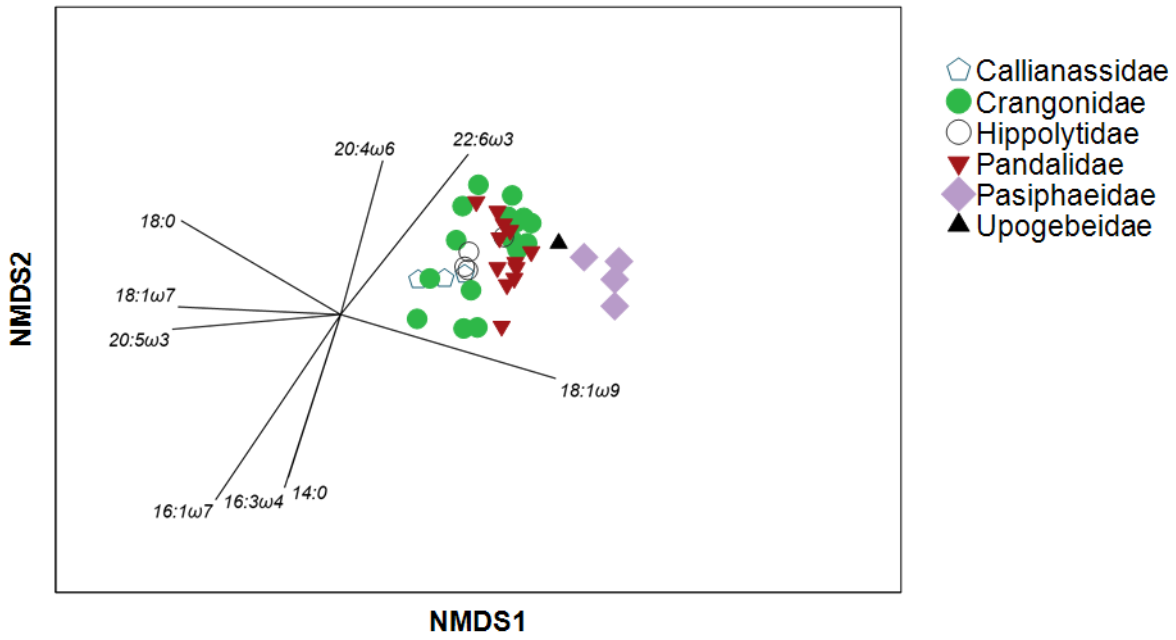

Figure 7. NMDS figure of percent (%) fatty acid composition of shrimp.

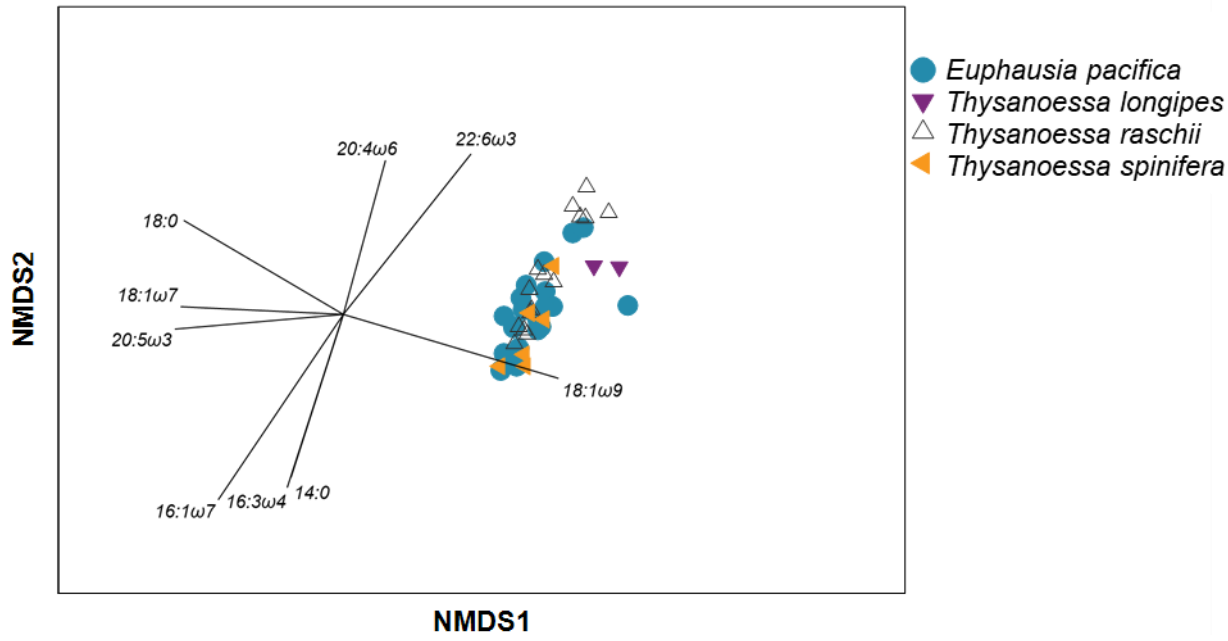

Figure 8. NMDS figure of percent (%) fatty acid composition of euphausiids.

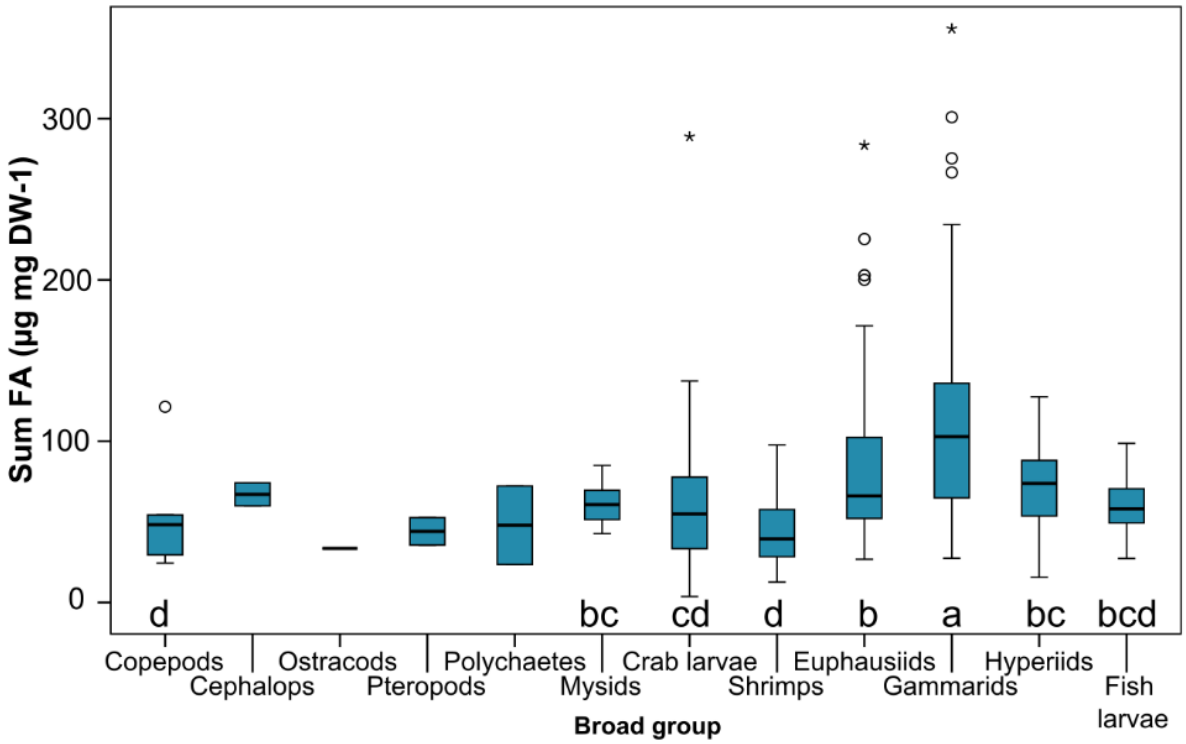

Figure 9. Total fatty acid content (a proxy of lipid content) of broad taxonomic groups of zooplankton in Puget Sound and adjacent waters in March-October 2017. The box represents the 25th and the top 75th quartile, while the line is the median. The whiskers represent the maximum and minimum values, omitting the outliers (circles; more than 1.5 box lengths from the median) and extreme values (asterisks; more than 3 box lengths from the median). Different letters denote significant difference in Kruskal-Wallis posthoc test. For cephalopods, ostracods, pteropods, and polychaetes *n* = 1-2 and they were excluded from the statistical comparisons. See Table 1 for *n* in other groups.

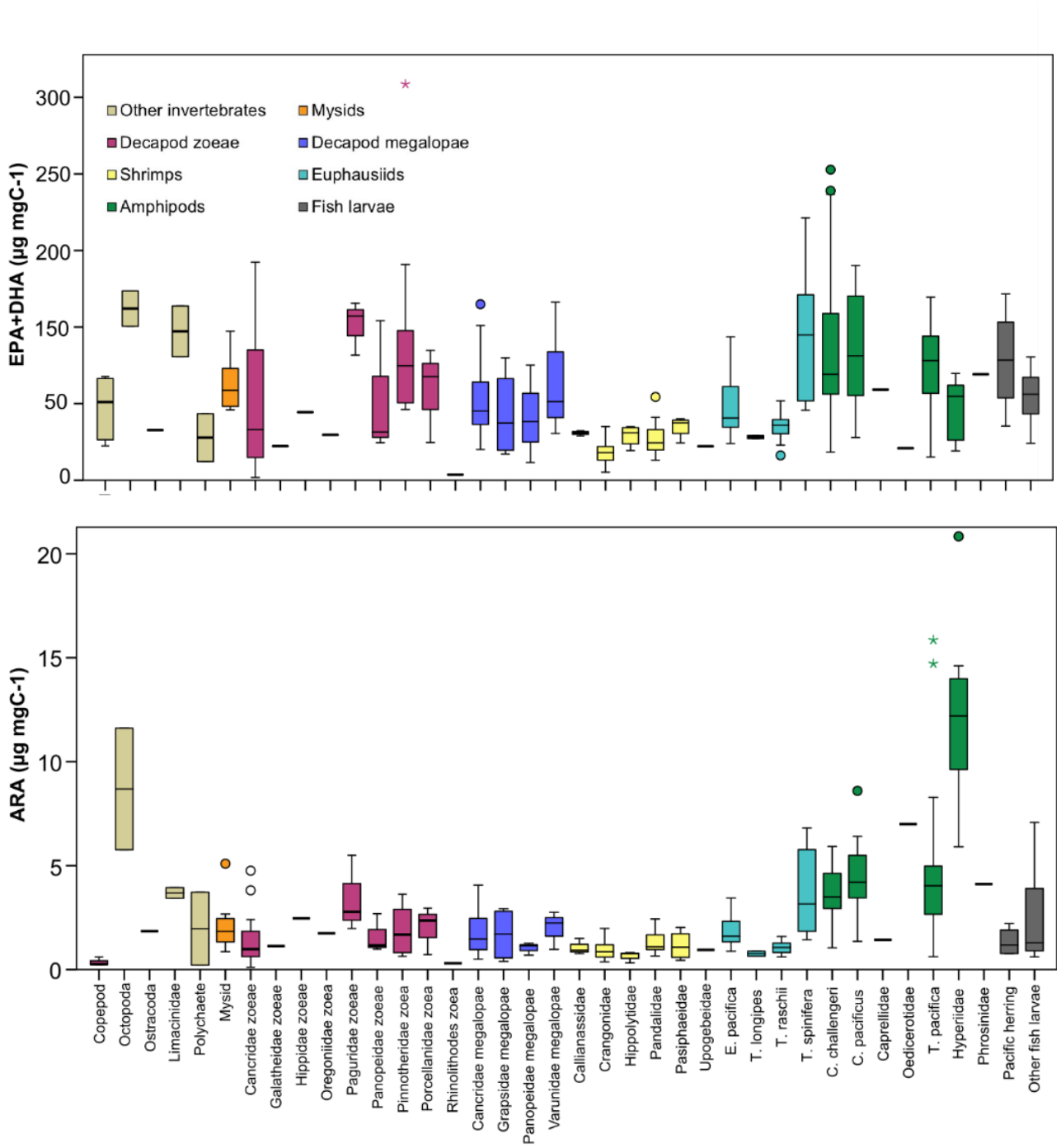

Figure 10. EPA + DHA, and ARA content (µg mg C^{-1}) in zooplankton.

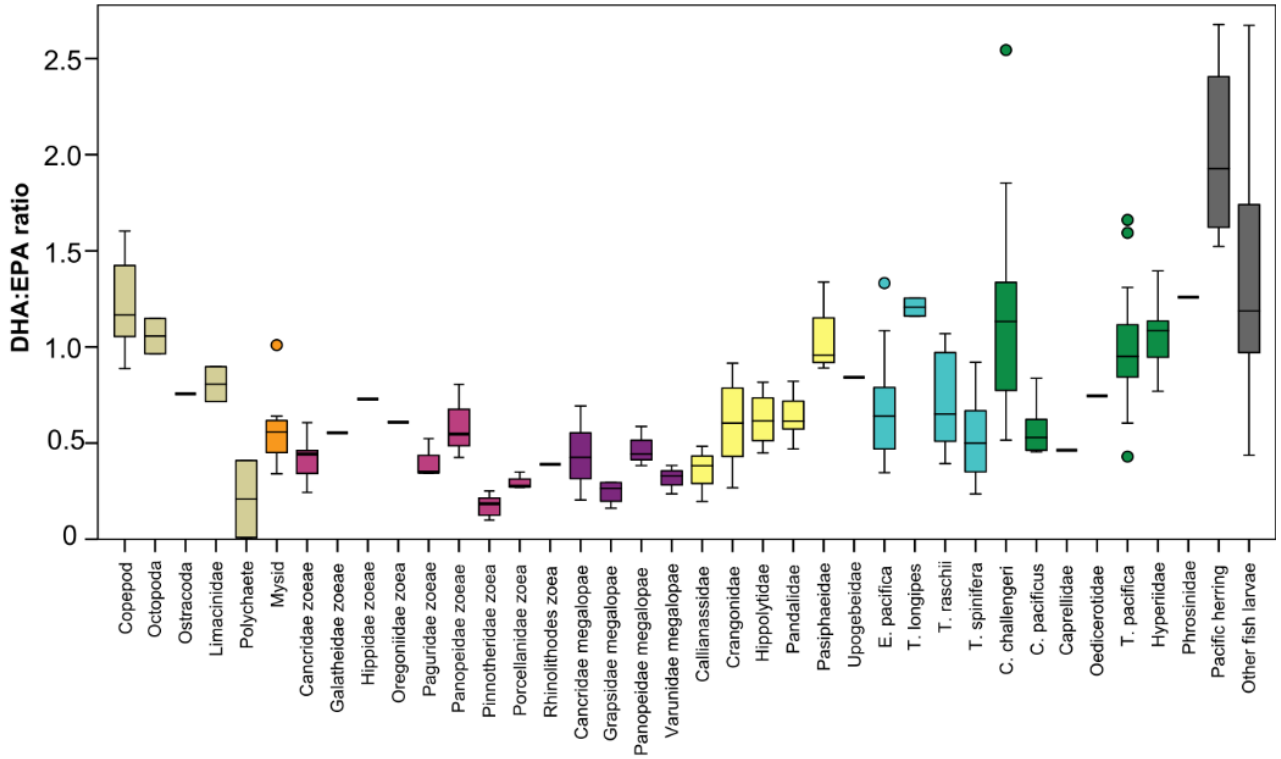

Figure 11. The ratio of DHA:EPA in zooplankton.

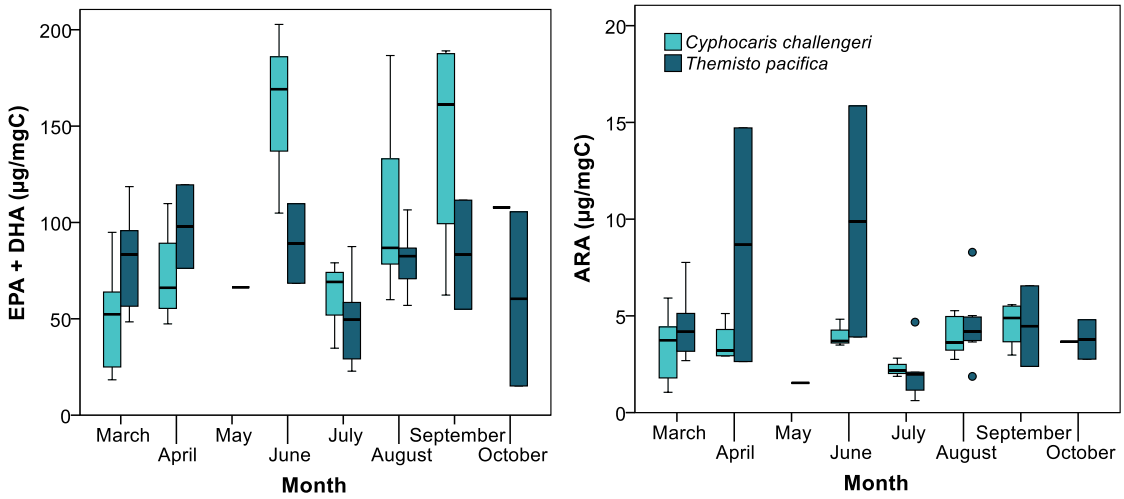

Figure 12. The monthly EPA+DHA and ARA content (µg mg C^{-1}) in the hyperiid amphipod *Themisto pacifica* and the gammarid amphipod *Cyphocaris challengeri*. Note the different y-axis scales.

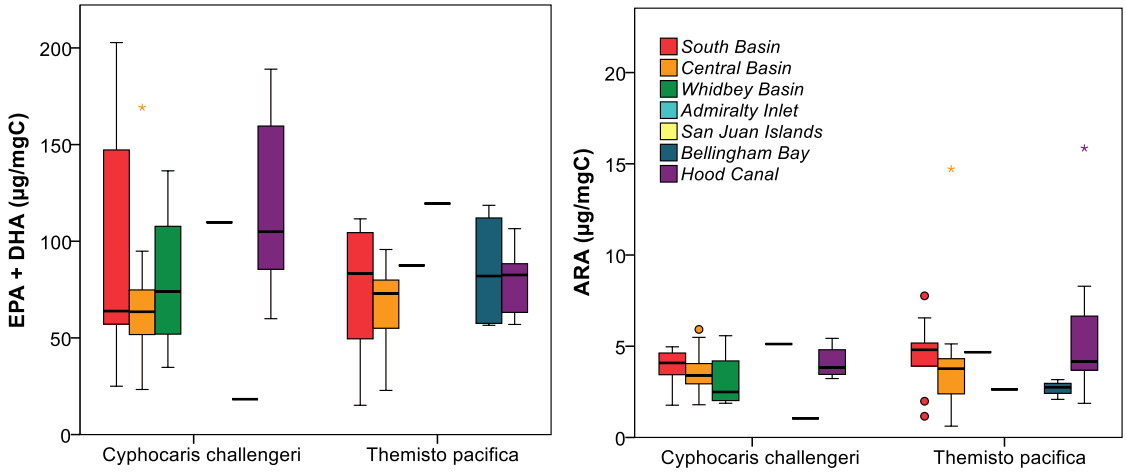

Figure 13. The EPA+DHA and ARA content (µg mg C-1) of the hyperiid amphipod *Themisto pacifica* and the gammarid amphipod *Cyphocaris challengeri* in basins of Puget Sound. Note the different scales on Y-axis.

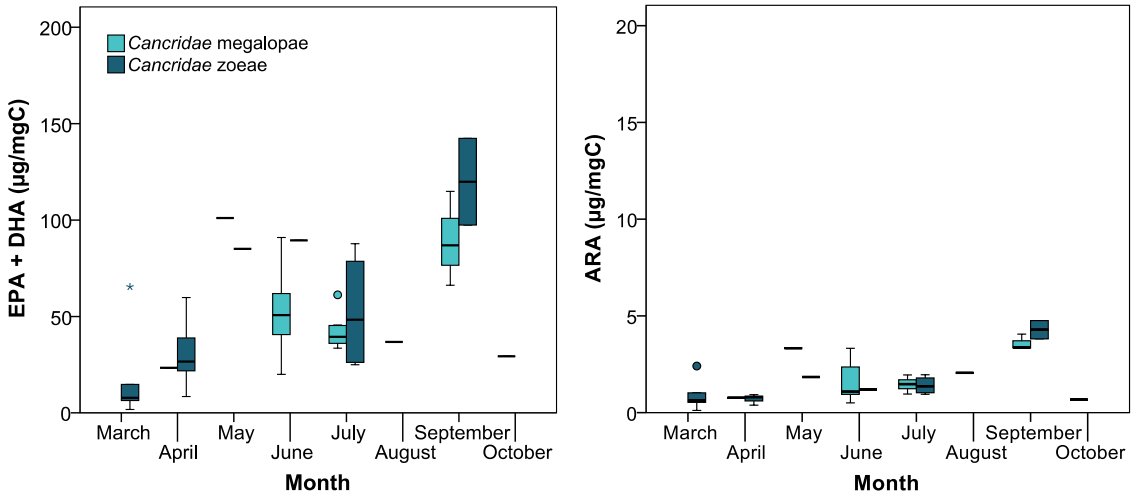

Figure 14. The monthly EPA+DHA and ARA content (µg mg C^{-1}) of Cancridae zoeae and megalopae in Puget Sound in 2017. Note the different scales on Y-axis.

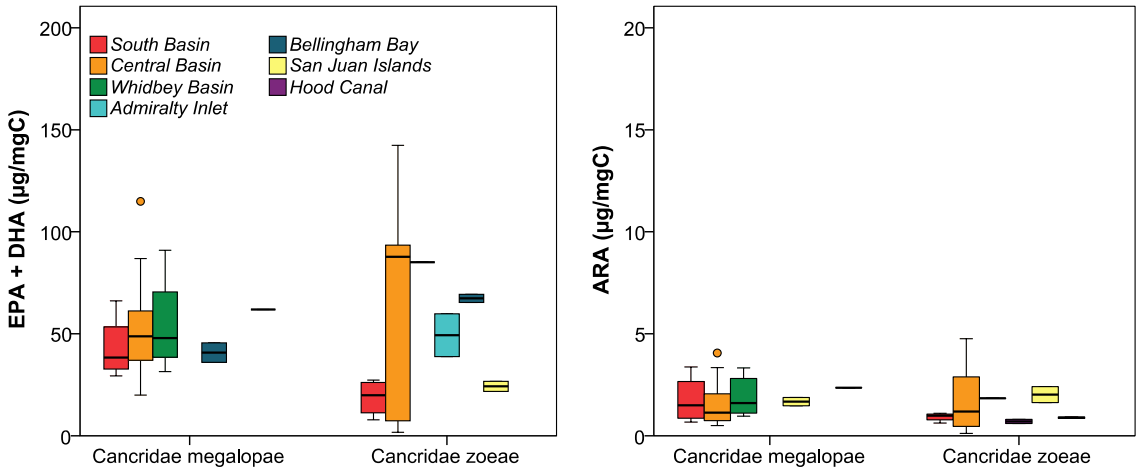

Figure 15. The EPA+DHA and ARA content (µg mg C-1) of Cancridae zoeae and megalopae in basins of Puget Sound in 2017. Note the different scales on Y-axis.

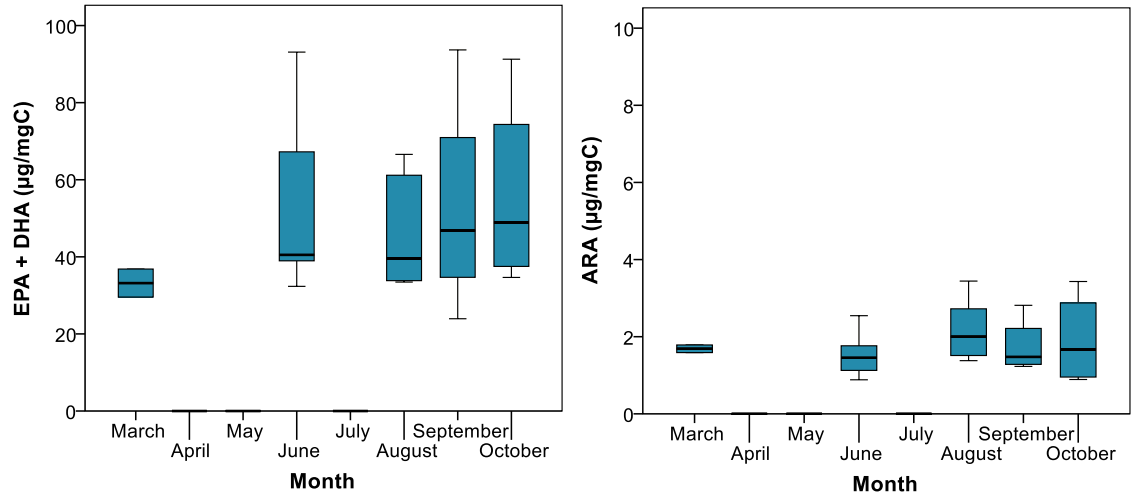

Figure 16. The monthly EPA+DHA and ARA content (µg mg C^{-1}) of the krill *Euphausia pacifica* in Puget Sound in 2017. Note the different scales on Y-axis.

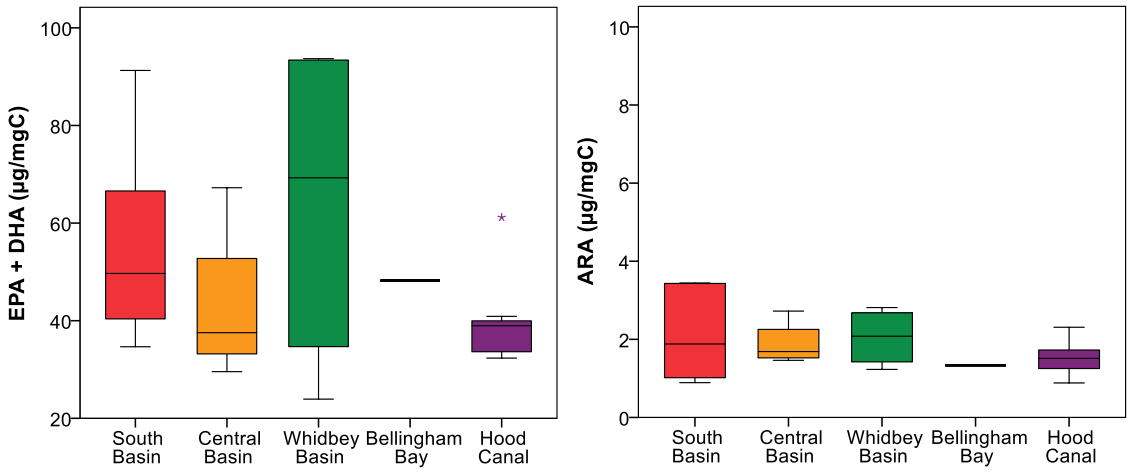

Figure 17. The EPA+DHA and ARA content (µg mg C^{-1}) of the krill *Euphausia pacifica* in basins of Puget Sound in 2017. Note the different scales on Y-axis.

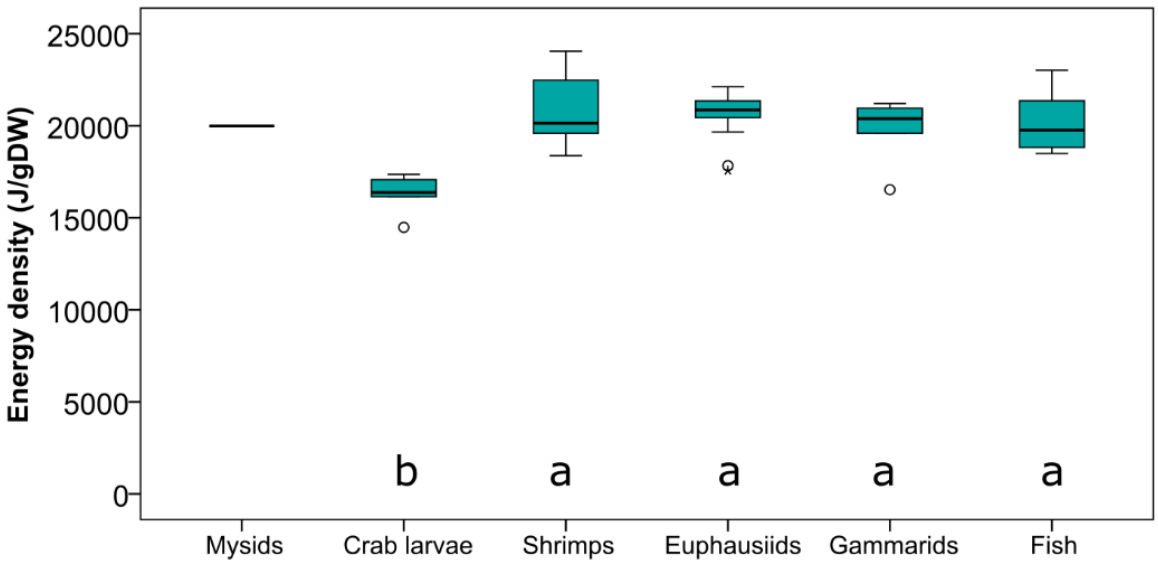

Figure 18. Energy content (J/g·DW) of broad taxonomic groups of zooplankton and fish collected from Puget Sound in 2017. Different letters denote significant difference in Kruskal-Wallis posthoc test.

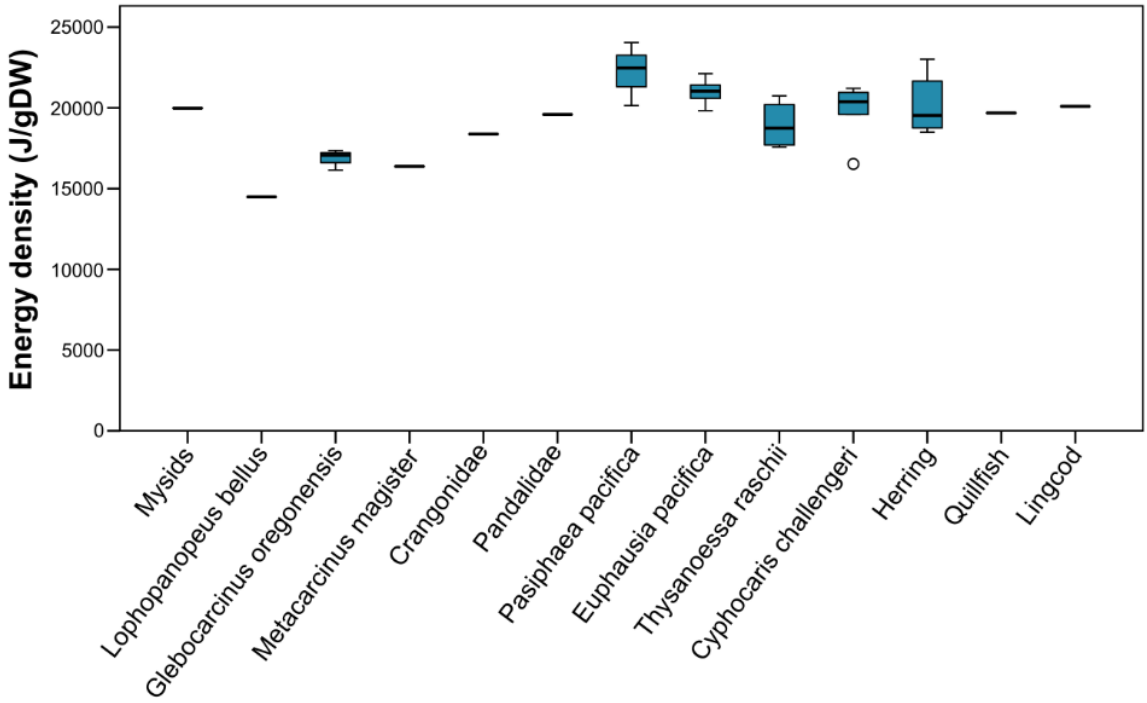

Figure 19. Energy content (J/g·DW) of zooplankton and fish collected from Puget Sound in 2017.

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