Assessing diet and nutritional value of small pelagic copepods through lipid analysis

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Abstract

Copepods, a major group of crustacean zooplankton, occupy a central role within marine food webs. Connecting primary producers to higher trophic levels and influencing global biogeochemical cycles, variations in community composition can have lasting impacts on the marine environment. Small marine copepods often greatly exceed the abundance of large-bodied copepods, yet historically they have been overlooked. As the ocean warms, conditions may favour an increasing abundance of smaller copepod species. Therefore, understanding the ecology of small-bodied copepods and their effect on the ecosystem is crucial for estimations of how climate change may affect the marine ecosystem. In June, July, August, and October 2020, copepod samples were collected at five on-shelf stations, two off-shelf stations, one oceanic station, and an intertidal beach along the west coast of Vancouver Island. Through lipid extraction and quantification, and visualization with multivariate non-metric multidimensional scaling, I observed differences in lipid classes and fatty acid profiles among large-bodied oceanic, small-bodied oceanic, and small-bodied intertidal copepod species. Small oceanic copepods all had relatively similar total lipid masses but differed based on their storage lipid. While *Aetideus divergens, Pseudocalanus minutus, Pseudocalanus mimus,* and *Clausocalanus lividus* stored wax esters similarly to the large-bodied copepod species, *Acartia longiremis* and *Mesocalanus tenuicornis* both stored triacylglycerols, which are associated with more active year-round life cycles and higher metabolic rates. Intertidal copepod *Tigriopus californicus* had the lowest total lipid mass. Three significantly distinct feeding strategies were observed among species, with the large-bodied copepod species grouping together, and the small-bodied copepod species diverging into two groups that corresponded to the storage lipid distinctions. Almost all small copepod species were observed to have higher proportions of bacterial fatty acid markers

than did the large-bodied copepods, indicating they are more connected to the microbial loop than the omnivorous-herbivorous large-bodied copepods. My research contributes to our understanding of the ecology and life strategies of these small-bodied copepod species, and also provides some of the first lipid analysis for certain species.

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Introduction

The importance of zooplankton within the marine ecosystem

Zooplankton are an incredibly diverse group of mesoplankton with a wide range of body morphologies. Occupying a central role within marine food webs, they are major primary consumers - connecting primary producers, such as phytoplankton and cyanobacteria, to higher trophic levels, such as fish, marine mammals, and seabirds (El-Sabaawi et al. 2009, Winder & Jassby 2011). Zooplankton biomass, abundance, community composition, and diet are influenced by a variety of abiotic and biotic factors, such as environmental variation in temperature (Mackas & Galbraith 2002) or the amount and type of primary productivity (Durbin et al. 1983, Hairston & Hairston 1993). The response of zooplankton to this variability directly dictates the energy available to higher marine predators; therefore, the abundance and production of zooplankton is a strong indication of the overall productivity of the marine ecosystem as a whole. The energy transferred from zooplankton affects all aspects of the marine environment: from affecting the recruitment of commercially important fish (Alvarez-Fernandez et al. 2015, Peterson & Schwing 2003), to influencing the success of planktivorous and piscivorous marine birds (Kitaysky & Golubova 2000), or even direct effects on large marine mammals such as certain species of whales (Greene & Pershing 2004). Given this disproportionate effect, knowledge of how zooplankton vary in distribution and composition is thus vital to a broader, in-depth understanding of their impact on the overall health and sustainability of marine food webs in both the short and long term.

While often classified taxonomically, or simply as being either gelatinous or crustacean, several studies have categorized zooplankton using functional traits (Pomerleau et al. 2015, Venello et al. 2021). Functional diversity is a useful tool to investigate how communities diverge

in structure and function rather than how they differ taxonomically (Pomerleau et al. 2015). Functional traits employed to categorize zooplankton into different groups are phenotypic or behavioural characteristics that relate to feeding and reproduction patterns, as well as to other life history traits. Feeding strategy is a noteworthy consideration as it illustrates how an organism feeds and the mechanisms by which it selects appropriate prey, thus determining which nutrients and biomolecules are incorporated into its body. These life history traits vary among zooplankton groups and are shown to differ even among species of zooplankton within the same sub-group (Venello et al. 2021).

Copepods and the effect of omnivory

Copepods are the dominant group of crustacean zooplankton and directly affect the energy flow available to higher marine predators through variations in their feeding strategy (herbivory, omnivory, carnivory) and dietary intake throughout the year (Pomerleau et al. 2015). Herbivorous copepods consume a range of different primary producers such as diatoms, dinoflagellates, cyanobacteria, and prymesiophytes. Omnivorous copepods tend to supplement this herbivorous diet by feeding on microheterotrophs, such as protozoans or ciliates (Kleppel 1993). There are also several species of copepods that are solely carnivorous, feeding on fish eggs or copepod nauplii (Kleppel 1993, Turner et al. 1985). Additionally, a third of all copepod species survive parasitically on fish or invertebrates (Humes 1994).

Omnivory among copepods is dependent both on physical features, such as mandible gnathobase morphology (Michels & Schnack-Schiel 2005), and environmental factors, such as prey availability (Kleppel 1993). Copepod species that exhibit omnivory have a mandibular morphology lying somewhere on the spectrum between the setose mouthparts used by

herbivorous copepods to crush diatoms and the spiny mouthparts used by carnivorous copepods to skewer and capture prey (Michels & Schnack-Schiel 2005, Romano et al. 1999). This flexibility in diet has a significant impact on the energy and nutrition available to apex marine predators (Hairston and Hairston 1993) and has also been shown to have a stabilizing influence on marine food webs (Long et al. 2011, Sprules & Bowerman 1988). Within copepod species, feeding types vary and can include active ambush feeding, passive ambush feeding, current feeding, or cruise feeding (defined by Kiørboe 2011). As well, copepods differ in reproductive modes, such as broadcast spawning or egg-brooding sacs (Kiørboe & Sabatini 1994), and lifecycle strategies, such as diapause and overwintering generally exhibited by larger species of mid- to high-latitude copepods (Conover 1988). Collectively, such differences in feeding, reproduction, and life cycle dictate the energy stores and resources available to a given species to support its particular lifestyle.

Copepods can also play an important role as indicators of climate change as they are sensitive to environmental variation (Richardson 2008). Copepods are poikilothermic, meaning that their physiological processes are highly sensitive to temperature. They are also short-lived, with lifespans often under a year, thus monitoring programs can observe direct coupling of changes in climate and zooplankton population dynamics (reviewed by Hays et al. 2005). This is an important aspect of zooplankton monitoring, as changes in zooplankton abundance and biomass can be used to help understand how climate change may affect productivity in the future.

Small marine copepods: their fundamental role in marine food webs

Small planktonic marine copepods (~1-3 mm in length) are the most abundant metazoans on Earth (Turner 2004). Although their overall biomass is often much less than larger zooplankton species, their abundance has been found to greatly exceed that of larger copepods (Middelbo et al. 2019, Turner & Dagg 1983). Despite this, their tiny size has led them to be largely overlooked and very little is known about many of these species and their overall importance and contribution to marine ecosystems. Small copepods are consumed by a wide range of predators – including larval and juvenile fish, carnivorous zooplankton, and even larger species of copepods (Lough & Mountain 1996, Turner 2004). To overcome this high vulnerability to predation, small-bodied copepods have evolved highly productive lifestyles and reproductive strategies (Kiørboe & Sabatini 1994, Uye et al. 2002, Turner 2004). Small copepods also perform essential roles within global biogeochemical cycles (Turner 2004), such as the ocean's biological carbon pump which copepods largely affect through their diet and feeding behaviour (Koski et al. 2017, Turner 2015). By feeding on phytoplankton and other particulate organic matter, copepods transport carbon to the deep ocean via diel vertical migration, fecal pellets, or as carcasses (Koski et al. 2017, Turner 2015). Small copepods are recently being considered as principal players influencing the vertical flux of carbon (Koski et al. 2020).

Historically, small copepods have been overlooked primarily due to inadequate sampling. For many years the mesh in nets used for zooplankton collection was too coarse to collect these small zooplankton, so small-bodied copepod abundance went largely unnoticed (Dugas & Koslow 1984, Nielsen & Andersen 2002). Nielsen and Andersen (2002) found that total copepod biomass in a Norwegian fjord was underestimated by as much as 65% when sampling with a 200

µm WP2 net versus estimates from Niskin bottle samples concentrated on finer 45 µm nets. Another source of the under-recognition of small copepods has been the low temporal resolution of most sampling programmes. Many small copepod species vary in abundance seasonally, often increasing in abundance when the larger species of copepods diapause for the winter (Arendt et al. 2013). Therefore, the lack of frequent sampling programmes contributed to small, seasonally dominant copepod species being overlooked.

Some of the small copepod species found off the west coast of Vancouver Island (WCVI) include *Acartia longiremis, Aetideus divergens, Clausocalanus lividus, Mesocalanus teniucornis, Pseudocalanus minutus,* and *Pseudocalanus mimus.* These species have been grouped into zoogeographic categories of 'subarctic', 'boreal shelf', 'southern', and 'exotic southern' copepods (Mackas & Galbraith 2002) (Table 1). Southern copepod species are associated with warm seawater temperatures and are normally abundant off the California coast. Boreal shelf copepods are associated with cooler ocean temperatures and are found mainly on the continental shelf extending from central Oregon to the Bering Sea. Subarctic oceanic copepods are copepods with a distinctive lifecycle, with a brief spring to early summer growing season followed by a migration down to diapause. Exotic southern taxa are either absent or incredibly unusual in British Columbia samples as these are rare warm-water species. These species have also been categorized into functional feeding groups based on functional traits including feeding strategy – such as active ambush omnivores versus omnivore-herbivores (Venello et al. 2021) (Table 1).

Table 1. Functional feeding groups and zoogeographic categorization of copepod species as defined by [1]Venello et al. (2021) and [2]Mackas & Galbraith (2002). AAO: active ambush omnivore, OH: omnivore-herbivore.

Size	Species	Length (mm)[1]	Functional feeding group[1]	Zoogeographic category[2]
Small-bodied copepod species	*Aetideus divergens*	1.80	AAO	-
	Acartia longiremis	1.07	AAO	Boreal shelf
	Clausocalanus lividus	1.15	OH	Exotic southern
	Mesocalanus tenuicornis	2.10	AAO	Southern
	Pseudocalanus mimus	1.52	OH	Boreal shelf
	Pseudocalanus minutus	1.46	OH	Boreal shelf
Large-bodied copepod species	*Calanus marshallae*	3.70	OH	Boreal shelf
	Neocalanus cristatus	9.23	OH	Subarctic
	Neocalanus plumchrus	4.98	OH	Subarctic

West coast of Vancouver Island: trends and effects of environmental and seasonal variation on oceanic copepods

The WCVI is a highly productive area of the Pacific Ocean, situated in the transition zone between the equatorward-flowing upwelling California Current to the south and the poleward-flowing downwelling Alaska Current to the north (Kämpf & Chapman 2016). The degree to which either current prevails varies both seasonally and annually due to differences in the strength of northwesterly winds, the Coriolis effect, and broader oceanographic trends such as the El Niño-Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (Thomson 1981, Richardson 2008). These seasonal variations dictate the diet and biomolecules available to copepods to incorporate into their bodies and thus the efficiency of trophic energy transfer to higher trophic levels (El-Sabaawi et al. 2010, Legendre & Rassoulzadegan 1995). During the highly productive spring phytoplankton bloom off the WCVI, copepods often feed on diatoms, a

major group of algae. The food chain during this time is much shorter as diatoms are larger and richer in fatty acids than most other species of phytoplankton. Therefore, the transfer of energy to higher predators is highly efficient, supporting the increased biomass and success of predators (El-Sabaawi et al. 2010, Legendre & Rassoulzadegan 1995). When the diatom bloom and overall productivity slow and dinoflagellates (smaller, less nutritious phytoplankton) begin to dominate, copepods must supplement their diet with additional prey sources such as cyanobacteria and protozoans to fulfill their dietary requirements (El-Sabaawi et al. 2010, Legendre & Rassoulzadegan 1995). This makes the overall trophic transfer to higher predators less efficient.

The Pacific Ocean is influenced by several different environmental variation events, importantly warm water intrusions such as the ENSO phenomenon and 'the Blob'. ENSO is a warm, nutrient-poor regional ocean current that can vary on timescales ranging from months to decades and can linger along the west coast of North America (reviewed by Timmermann et al. 2018). 'The Blob' was a marine heatwave that occurred throughout the North Pacific Ocean and reached the offshore waters west of the WCVI in late 2014 (Bond et al. 2015). This persistent marine heatwave coincided with an especially strong ENSO event from 2015-2016 (Tseng et al. 2017). Studies have shown that the WCVI copepod community varies in turn with these environmental trends with warm-water lipid-poor smaller southern species being transported poleward during periods of low upwelling and cold-water lipid-rich northern species being transported equatorward during periods of upwelling in the spring and summer (Mackas et al. 2001, Mackas et al. 2007).

Understanding dietary dynamics of animal populations using lipid and fatty acid analysis

A detailed understanding of the dietary dynamics of animal populations is a key tool for many observational ecologists. What an animal is eating can tell scientists not only how the organism or species is using its resources, but also where they fit into the food web (Kelly & Scheibling 2012). In the marine environment, classical methods of diet analysis, such as gut content analysis or the direct observation of feeding, are logistically challenging (if not impossible) as many of the animals are so small (Kelly & Scheibling 2012). As such, marine scientists have developed techniques, such as lipid analysis, to allow them to indirectly infer what is happening - both in the trophic interactions of organisms as well as in their diets (Kelly & Scheibling 2012, Pomerleau et al. 2015). Lipid analysis is one of the most commonly used methods for dietary assessment in marine studies as fatty acids (FA) have been shown to be reliable dietary tracers to characterize zooplankton diet (Kelly & Scheibling 2012, Lee et al. 1971a, reviewed by Dalsgaard et al. 2003). Lipid analysis has the advantage of supplying longer-term dietary information than the snapshot in time that classical methods might provide (Iverson et al. 2004). When zooplankton feed, they directly incorporate the species-specific FAs of their prey into their bodies (Lee et al. 1971b, reviewed by Dalsgaard et al. 2003). Thus, quantifying FA and lipid profiles provides an accurate way to observe the dietary composition of organisms. Whereas FAs provide a way to deduce the source of nutrients an organism has fed on, examining lipid classes can further reveal how such nutrients are processed and stored.

Lipids are a heterogeneous group of hydrophobic biomolecules that are of biological significance. Present in all living organisms, they play several biological roles such as energy source and storage, membrane integrity, buoyancy, and cell signaling (Couturier et al. 2020). Within marine species there are 16 identified marine lipid classes (Parrish 1988), which can be

classified as either storage lipids (triacylglycerols and wax esters) or structural lipids (phospholipids). FAs are constituents of these lipid types. Storage lipids function as the main energy stores for zooplankton, whereas structural lipids are essential components of membranes. FAs have been used to investigate aquatic and marine environments for over 50 years. The wealth of observational FA analysis and laboratory feeding studies have provided marine ecologists with an effective guide to ascertaining which markers and tracers are indications of which diets (reviewed by Dalsgaard et al. 2003).

Phytoplankton, the base of all pelagic marine food webs, have distinct FA compositions. Essential fatty acids (EFA), FAs that are crucial for proper organism functioning but cannot be synthesized by most organisms at a rate to match their requirements (Parrish 1999), include docosahexaenoic acid (DHA, 22:6ω3) and eicosapentaenoic acid (EPA, 20:5ω3). EFAs are more or less exclusively synthesized by phytoplankton, plants, and macrophytes within the marine ecosystem (Dalsgaard et al. 2003) and, since other marine organisms cannot synthesize ω-3 and ω-6 polyunsaturated fatty acids (PUFA) *de novo* (Parrish 1999), they must acquire these from their diet. Therefore, when looking at zooplankton lipid profiles, EFA and FA tracers can be used to qualitatively investigate dietary quality and feeding strategy.

FA composition can be affected by a multitude of factors other than diet; for example, the developmental or reproductive stage the organism is at (Galloway & Winder 2015) or even temperature and salinity (Hazel & William 1990). Quantifying FA and lipid profiles thus provides an accurate mechanism to deduce the dietary intake of organisms, as well as providing information about the physiological condition and habitat of the organism.

Principle diatom biomarkers are EPA, 16:1ω7, 16:4ω1, and 16- and 20- PUFAs (Kelly & Scheibling 2012, Stevens et al. 2004a). Principle dinoflagellate markers are DHA, 18:4ω3, and

18- and 22- PUFAs (Kelly & Scheibling 2012, Stevens et al. 2004a). Prymesiophyte markers are 18:4ω3 and 18:5ω3 (Dalsgaard et al. 2003). Biomarkers for bacteria are typically odd and/or branched FAs (OBFA) (Budge and Parrish 1998) and 18:1ω7 (Stevens et al. 2004b). The DHA/EPA (dinoflagellates/diatom) ratio is a useful index for carnivory (Dalsgaard et al. 2003) with a higher DHA/EPA ratio indicating higher instance of carnivory. Carnivory is also indicated by the presence of 18:1ω9 (Stevens et al. 2004a).

Objectives and Hypotheses

In this study, I will determine differences in lipid and fatty acid (FA) composition of small-bodied and large-bodied copepod species. I will also determine how abundance of these species vary over a 30-year period. These differences are expected to relate to variations in diet, life history, and local oceanographic conditions.

Objective #1: Variation in small copepod species nutrition and diet

My first objective was to examine lipid class and FA profiles of several species of small copepods to determine whether diet and nutritional values varied among different species. It was hypothesized that diet and nutritional values will vary among species as they likely exhibit different life histories and feeding strategies. This examination focused on the species *Pseudocalanus minutus, Pseudocalanus mimus, Acartia longiremis, Mesocalanus tenuicornis, Aetideus divergens,* and *Clausocalanus lividus.*

Objective #2: Variation in small copepod nutrition and diet among different environments

My second objective was to compare the FA and lipid profiles of small pelagic copepod species to the FA and lipid profile of *Tigriopus californicus*, an intertidal harpacticoid copepod species similar in size. This was done to determine whether the feeding environment (open water pelagic versus coastal splash pool) plays a role in determining FA and lipid profiles. It was hypothesized that there will be a difference between the diet and nutritional values of intertidal copepods and pelagic copepods due to the variance in environment. The coastal splash pool is a highly productive environment with constant input of nutrients and fast turnover, meaning that *T. californicus* will predictably have less overall storage lipid.

Objective #3: Small copepod nutrition and diet compared to larger copepod species

My third objective was to compare the FA and lipid profiles of small pelagic copepod species to those of larger pelagic copepod species, which are already well described in the literature (El-Sabaawi et al. 2009, El-Sabaawi et al. 2010). These large-bodied copepod species include *Neocalanus plumchrus, Neocalanus cristatus,* and *Calanus marshallae.* It was hypothesized that larger-bodied copepod species will utilize diet and energy sources differently than small copepods due to differences in life history and strategy. It was also expected that large-bodied copepods would have a greater total lipid mass than small-bodied copepods.

Objective #4: Compare abundance trends over a 30-year period

My final objective was to examine how the abundance of these species varied off the west coast of Vancouver Island (WCVI) over a 30-year period using the zooplankton database maintained by the Department of Fisheries and Oceans. There is evidence that with warming ocean trends the WCVI copepod community may shift away from northern, cold-water species that are nutritious prey sources for higher trophic levels towards a higher abundance of either lipid-poor southern and/or smaller bodied copepods that are less nutritious (Mackas et al. 2007, Galbraith et al. 2016). Thus, examining abundance trends will further allow me to investigate how environmental change affects this critically important group of species. It was expected that there will be variations in the presence and dominance of copepod species as a result of fluctuating environmental factors, such as ocean temperature, over the 30-year period considered in this study.

Materials and Methods

Zooplankton collection

Zooplankton lipid samples were collected at five on-shelf stations, two off-shelf stations, one oceanic station, and an intertidal beach (Figure 1) along the west coast of Vancouver Island (WCVI) and in the Strait of Georgia. Samples were collected in June and July 2020 on the La Perouse monitoring program and in August 2020 on the Line P monitoring program in partnership with the Department of Fisheries and Oceans (DFO) and the Canadian Coast Guard. *Tigriopus californicus* samples were collected from Baynes Beach in October 2020 (Figure 1).

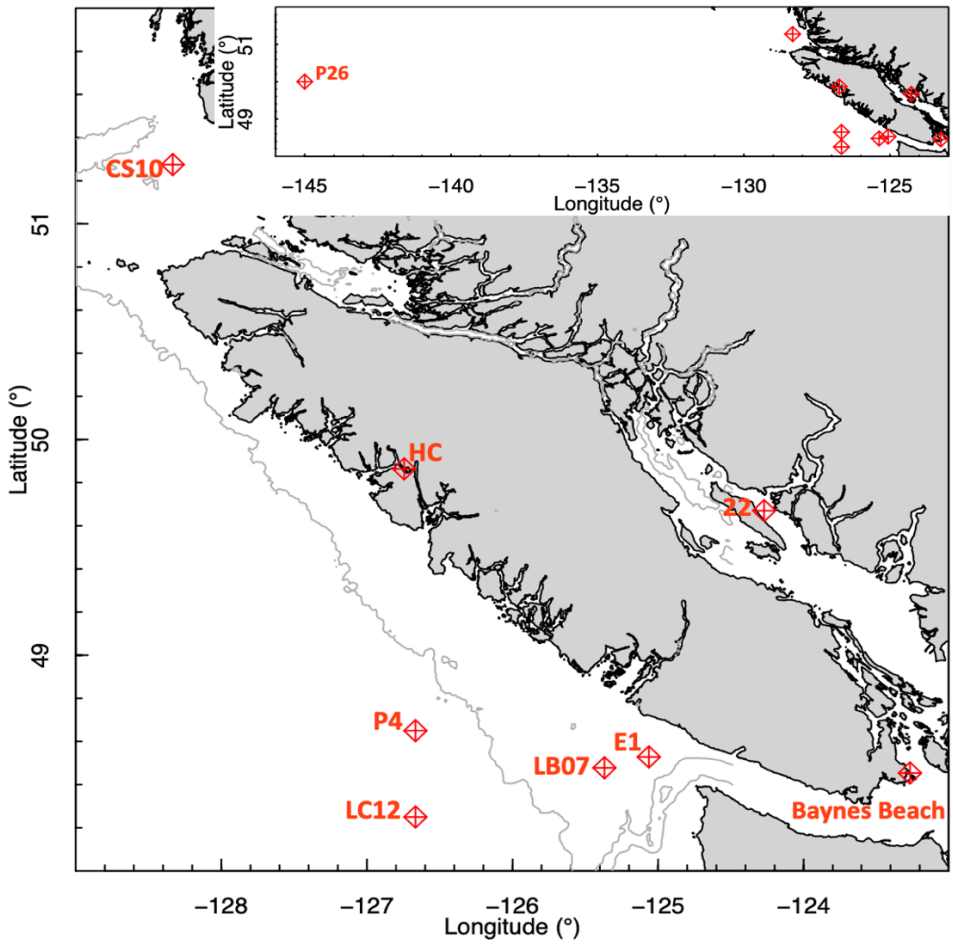

Figure 1. Map of the west coast of Vancouver Island, outlining the La Perouse, Line P, and intertidal locations where copepod samples for lipid analysis were collected. 200 m shelf break is indicated by the grey line.

Oceanic zooplankton species were collected with a vertical net haul (VNH), a 236 μm mesh Bongo net with a diameter of 0.56 m was towed vertically between zero to 250 m depth, or from zero to 10 m off the seafloor at stations that had depths <250 m. If zooplankton catches were sparse, additional deep tows were performed. The specimens were collected from the cod-end and sorted on board by species into lipid-cleaned pre-weighed glass vials. They were then transferred to -80˚C storage.

T. californicus seston samples were collected from Baynes Beach by suctioning both water and *T. californicus* copepods from intertidal splash pools. The samples were then filtered onto pre-weighed ashed 47 mm GF/F filters using a vacuum pump. For *T. californicus* sample Tig 1, Tig 2, Tig 3, and Tig 4 specimens were retained on a 250 μm sieve and backwashed onto the pre-weighed ashed 47 mm GF/F filters. For sample Tig 5, specimens were collected on a 125 μm sieve. Samples were then transferred to -80˚C storage.

Table 2. Descriptions of the copepod species, stations, dates, and depths sampled for lipids on the west coast of Vancouver Island in June, July, August, and October 2020.

Station	**Date (2020)**	**Cruise**	**Copepod samples**	
			Depth (m)	**Species**[1]
22	18 July	La Perouse	0-144	Cm, Np, Pmt
LB07	23 June	La Perouse	0-59	Al, Cm, Np, Pm
LC12	24 June	La Perouse	0-103	Mt, Np
E1	29 June	La Perouse	0-44	Ad, Pm
HC	29 June	La Perouse	0-99	Ad, Pmt
CS10	6 July	La Perouse	0-33	Al, Cm, Pm
P4	14 Aug	Line P	0-250	Al, Cm, Mt, Pmt
P26	20 Aug	Line P	0-250	Cl, Nc
Baynes Beach	22 Oct	-	-	Tc

[1]Ad = *Aetideus divergens*, Al = *Acartia longiremis*, Cl = *Clausocalanus lividus*, Cm = *Calanus marshllae*, Mt = *Mesocalanus tenuicornis*, Nc = *Neocalanus cristatus*, Np = *Neocalanus plumchrus*, Pm = *Pseudocalanus mimus*, Pmt = *Pseudocalanus minutus*, Tc = *Tigriopus californicus*

Further sample information and station coordinates are located in Table 24A and Table 25A.

Oceanographic data collection

Hydrographic data and water samples were collected using a CTD rosette at stations 22, LB07, LC12, E1, HC, and CS10 as part of the La Perouse monitoring program in June and July 2020. No CTD profile data are available for stations P4 and P26 as the data from the Line P monitoring program in August 2020 had not been processed at the time of writing this thesis.

Temporal patterns in zooplankton abundance

The species-specific abundance data used in this analysis were extracted from the zooplankton database maintained by DFO (Institute of Ocean Sciences, Sidney, BC, Canada). Southern shelf zooplankton samples were collected during DFO's long-term La Perouse monitoring cruises in April, May and June from 1980 to 2018. VNHs were conducted using a 236 μm mesh Bongo net from near bottom to sea surface (when bottom depth was < 250 m) on the continental shelf and upper slope and from 250 m to surface at off-shelf deeper locations. Samples were identified and enumerated to species.

Species-specific abundances (abundance per square meter) were calculated from raw counts of copepods using a subsampled fraction of the original sample, corrected for water volume and depth of tow. The abundance numbers for each species, per month and year, were summed and log transformed (Log10 (abundance + 0.001)) (as per Mackas et al. 2001). Log-scaling allows for easier comparison of abundance anomalies as an anomaly of +1 for a given species will represent a tenfold higher abundance than the climatological mean. Conversely, an anomaly of -1 represents a species abundance that is only 10% of the climatological average (as per Galbraith et al. 2016).

Following procedure from Venello et al. (2021), to calculate anomaly I averaged the log-transformed abundance values by months (April, May, and June). In order to establish if the abundance varied significantly from normal, a climatological mean (1980-2016) was calculated by taking the average of the monthly log-transformed data. The climatological mean was subtracted from the monthly abundance data for April-June, and then averaged per year to give annual spring abundance anomalies.

A note about the taxonomy of the genus Pseudocalanus

Pseudocalanus species are some of the most abundant small copepods off the WCVI in the DFO zooplankton database. In 1989 however, it was determined that there were actually four *Pseudocalanus* species present off the WCVI: *Pseudocalanus minutus, P. mimus, P. newmani* and *P. moultoni.* Since then, the abundance of the two species considered in this analysis, *P. minutus* and *P. mimus*, have been tracked separately. However, as there is no way to distinguish between the *Pseudocalanus* species in the database before 1989, in this analysis pre-1989 abundance of *Pseudocalanus* is a sum of multiple species. Following 1989, only abundance of *P. minutus* and *P. mimus* were summed.

Dry mass analysis prior to lipid extraction

Copepod samples were removed from the -80˚C freezer and thawed. With pre-weighed measurements of vials, the wet mass of the copepods was determined using a Mettler analytical single-pan H5 balance (±0.1 mg). In the case of the *T. californicus* samples, copepod wet mass was measured in combination with pre-weighed filter mass. Samples were then freeze-dried for 24 hours and re-weighed to determine their dry mass. Vials were placed under nitrogen gas and

capped with Teflon®-lined caps and Teflon® tape. Samples were stored at -80˚C until lipid extraction.

Lipid extraction

In total, 27 copepod samples, 1 blank sample, and 1 seston sample were prepared for lipid extraction following Parrish (1999), as modified from Folch et al. (1957). Freeze-dried samples were removed from the -80˚C freezer. Using 0.5 ml chloroform-extracted water, the samples were left to rehydrate for 2 minutes before 2 ml of HPLC-grade chloroform and 1 ml of ice-cold HPLC-grade methanol were added. Samples were ground using a Teflon® rod which was washed back into the sample vial with a further 1 ml of ice-cold 2:1 chloroform:methanol and 0.5 ml of chloroform-extracted water. The sample was recapped and vortexed, sonicated for 4 minutes, then centrifuged for 3 minutes at 1,500 rpm. The lower organic layer was removed using a double pipetting technique and placed in an extract vial under nitrogen gas. The smaller pipette was washed back into the original sample vial using 1.5 ml of chloroform on the outside and 1.5 ml of chloroform on the inside of the pipette. This procedure was repeated thrice more. After the final wash, the long pipette was washed into the extract vial with 3 ml of chloroform on the inside of the pipette and 3 ml of chloroform on the outside of the pipette. Sample vials were evaporated to dryness under nitrogen gas and then recapped. The samples were sealed with Teflon® tape and stored in the -20˚C freezer before being sent for analysis at the Aquatic Research Cluster within the Memorial University of Newfoundland (MUN).

Total lipid, fatty acid derivative preparation, and fatty acid determination

Lipid classes and fatty acids (FA) were analysed at the Aquatic Research Cluster at MUN. Lipid classes were determined by thin-layer chromatography using an Iatroscan Mark VI

TLC-FID (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan), silica-gel coated Chromarods, and a three-step development method (Parrish 1987). Lipid extracts were transesterified using the Hilditch reagent (H_2SO_4/MeOH) method of Toyes-Vargas et al. (2020) to make fatty acid methyl ester (FAME) derivatives. Using gas chromatography, FA peaks were identified using comparison of retention times to commercial standards (BAME, Supleco 27, Pufa No 1, and Pufa No 3). All FA data are reported as percentages of the total FAs in the sample. Saturated fatty acids (SFA) were characterized by the absences of double bonds within the lipid molecule, monounsaturated fatty acids (MUFA) with one double bond, dienes with two double bonds, and polyunsaturated fatty acids (PUFA) by the presence of three or more double bonds present within the lipid molecule (Parrish 1999). The summed primary dinoflagellate markers, listed as '∑dino' (Tables 6 to 8), are 18:4ω3 and 22:6ω3 (DHA) (Kelly & Scheibling 2012). The summed primary diatom markers, listed as '∑diatom' (Tables 6 to 8), are 16:1ω7 and 20:5ω3 (EPA) (Kelly & Scheibling 2012). The DHA/EPA (dinoflagellates/diatom) ratio is used as an index for carnivory (Dalsgaard et al. 2003). Odd numbered and/or branched fatty acids (OBFA) is the sum of bacterial markers (Budge and Parrish 1998).

Statistical analyses

The total lipid (TL) in each sample was standardized using the total number of individuals in the sample and the wet mass, reported as grams of TL per grams of wet mass. Upon receiving data back from MUN, it was found that samples 420, 486, and 415 experienced obvious signs of contamination by hydrocarbons (HC). In order to correct for this, I averaged the peak area of HC present for all uncontaminated samples of small copepod species and used this as the HC value for 420, 486, and 415. Differences in TL among large-bodied, small-bodied, and

intertidal copepods were determined using a Kruskal-Wallis test in conjunction with a Dunn's test.

Non-metric multi-dimensional scaling (NMDS) analysis using a Bray-Curtis dissimilarity matrix was conducted using the 'vegan' package in R (R Core Team 2020, Oksanen et al. 2019) to visualize FA profiles for the various copepod species. FAs determined to be strong biomarkers were included in analysis. A square root transformation was applied to all FA data used in the NMDS. NMDS creates an ordination of samples within a 2-dimensional coordinate frame based on a similarity matrix, with greater proximity of samples indicating greater similarity (Clarke 1993). The "goodness of fit" of the ordination is evaluated via a stress coefficient, which represents the amount of disagreement in configuration between the two axes represented. A stress value <0.1 indicates a good ordination with no risk of deducing false inferences (Clarke 1993). Significance of FA vectors was determined using the envfit function in R-vegan (Oksanen et al. 2019).

Clusters were identified using hierarchical average-neighbour cluster analysis based on rank similarities of the same Bray-Curtis dissimilarity matrix. Using these cluster designations, ellipses were added to the NMDS plots at the 95% confidence level. ADONIS, a permutational multivariate analysis of variance (PERMANOVA), and a pairwise-ADONIS was used to test whether the identified clusters of copepod FA compositions differed significantly. Similarity percentage (SIMPER) analysis was used to assess the contributions of individual FAs to the clusters in order to determine which FAs accounted for the most similarity. Differences in total mass of FA, EPA, and DHA were determined using Kruskal-Wallis tests in conjunction with Dunn's tests

Results

Oceanographic conditions at time of sampling

Sea surface temperatures (SST) (2 m depth) in June and July 2020 at La Perouse stations 22, CS10, HC, E1, LB07, and LC12 were 16.5, 14.3, 13.4, 11.0, 13.4, and 14.8˚C, respectively (Figure 2). Station 22 had the warmest SST, with temperature ranging from 8.9-16.7˚C within the water column, whereas station E1 had the coldest SST, with temperature ranging from 6.9-11.0˚C within the water column. Stations CS10, HC, LB07 and LC12 had temperatures ranging from 9.7-14.3, 9.3-13.4, 7.0-13.4 and 8.3-14.8˚C, respectively. Stations 22 and HC had sharp thermoclines, with temperature dropping rapidly whereas stations CS10, E1, LB07, and LC12 exhibited a more gradual decrease in temperature. At station 22 the thermocline reached low temperatures at around 50 m depth, whereas station HC reached low temperatures much shallower near 25 m depth.

Using fluorescence as a proxy for chlorophyll, there were observable differences in both the amount and depth of the chlorophyll maximum. Stations CS10 and LC12 both had fluorescence peak at around 35 m depth, whereas the other stations peaked near the surface and steadily declined. Station E1 had the lowest fluorescence ranging from 0.11-1.38 μg/L and station LB07 had the highest at 0.11-8.8 μg/L. Stations 22, CS10, HC, and LC12 had fairly similar amounts of fluorescence ranging from 0.13-5.55, 0.35-3.05, 0.11-5.90 to 0.07-4.1 μg/L, respectively.

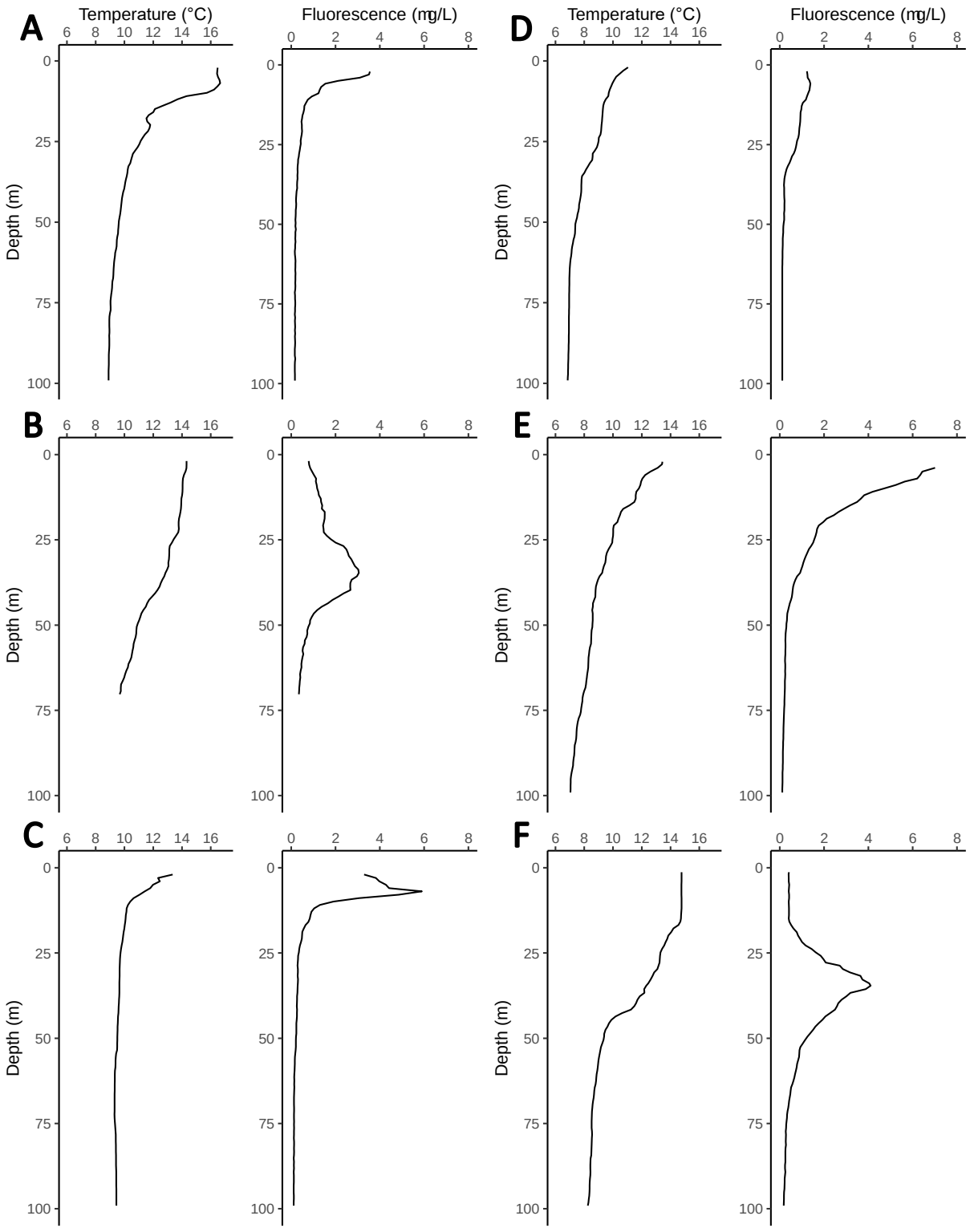

Figure 2. Stations sampled in June and July 2020 along the WCVI on the La Perouse monitoring cruise. Temperature (˚C) and fluorescence (µg/L), as a proxy for chlorophyll, profiles are shown with depth (m) for stations (A) 22, (B) CS10, (C) HC, (D) E1, (E) LB07, (F) LC12. Depth is scaled to 100 m to better represent surface variation.

Abundance anomalies

Calanus marshallae, *Neocalanus cristatus*, *Neocalanus plumchrus*, *Mesocalanus tenuicornis*, and *Acartia longiremis* show high variability in abundance anomalies with periods of both positive and negative anomalies (Figure 3). 'Subarctic' *Neocalanus* species abundance anomaly trends were broadly similar to most 'boreal shelf' copepod species, such as *C. marshallae* and *A. longiremis*. *C. marshallae* and *A. longiremis* displayed highly similar trends in abundance anomalies. *C. marshallae* experienced more negative anomalies over time, with few years post-1990 exhibiting positive anomalies. Post-1998 *Pseudocalanus* species experienced several strong positive anomalies for abundance that remained relatively constant until 2015. 'Southern' copepod *M. tenuicornis* had positive abundance anomalies during years 'boreal shelf' and 'subarctic' species showed negative anomalies. *Clausocalanus lividus* and *Aetideus divergens* only appeared once or twice throughout the time series.

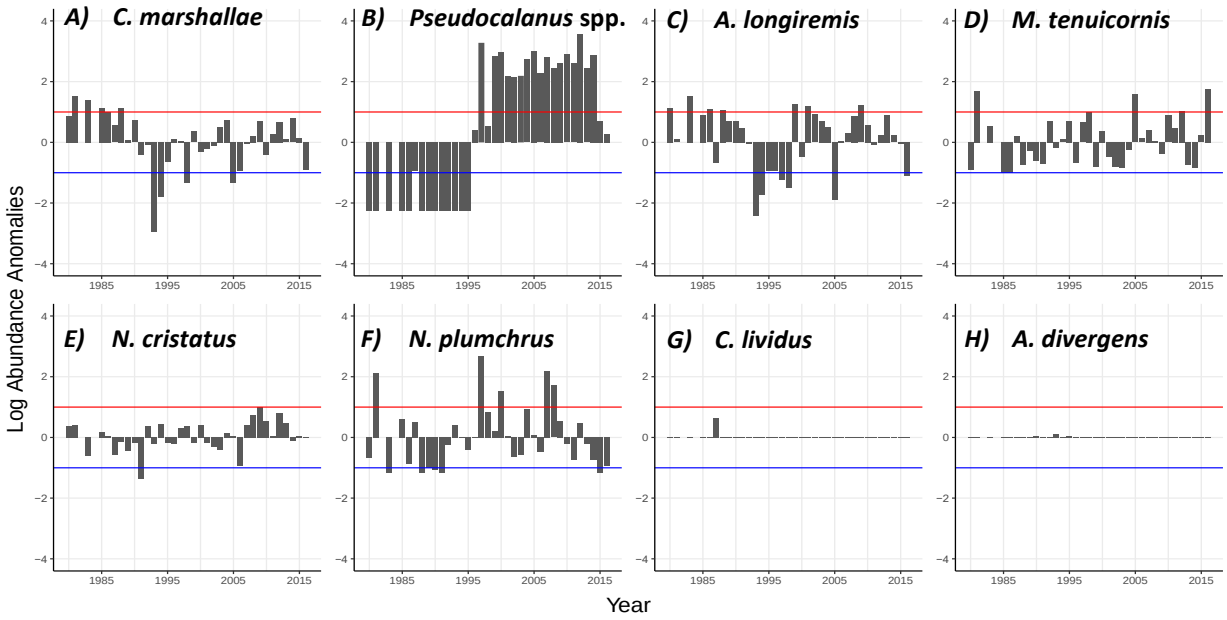

Figure 3. Annual spring (April-June) species abundance anomalies sampled on the southern shelf off the west coast of Vancouver Island from 1980 to 2016: (A) *Calanus marshallae*, (B) *Pseudocalanus* spp., (C) *Acartia longiremis*, (D) *Mesocalanus tenuicornis*, (E) *Neocalanus cristatus*, (F) *Neocalanus plumchrus*, (G) *Clausocalanus lividus*, (H) *Aetideus divergens*. Red line denotes strong positive anomaly threshold (>1) and blue line denotes strong negative anomaly threshold (<1).

Lipid class comparison

Overall, total lipid (TL) mass ranged from 0.05 to 337.26 g g^{-1} WM (Figure 4). Large-bodied copepod species had higher TL overall. The majority of TL in *C. marshallae, N. cristatus,* and *N. plumchrus* was composed of steryl and wax esters (>50%) (Table 3). Small-bodied marine copepods' TL mass ranged from 1.1 to 72.22 g g^{-1} WM. Lipid class composition split the small copepod species into two main groups, with some using steryl and wax esters as their main storage lipids, and others using triacylglycerols (Table 3). *A. divergens, P. minutus, P. mimus,* and *C. lividus* all had wax ester present in varying quantities. *M. tenuicornis* and *A. longiremis* both had higher amounts of triacylglycerols and phospholipids. *T. californicus* had very low TL mass overall at 0.05 g g^{-1} WM. The majority of *T. californicus* lipid class composition was dominated by triacylglycerols (22.20±9.35%) and phospholipids (46.48±2.86%). The TL class composition of *T. californicus* was similar to the splash pool seston.

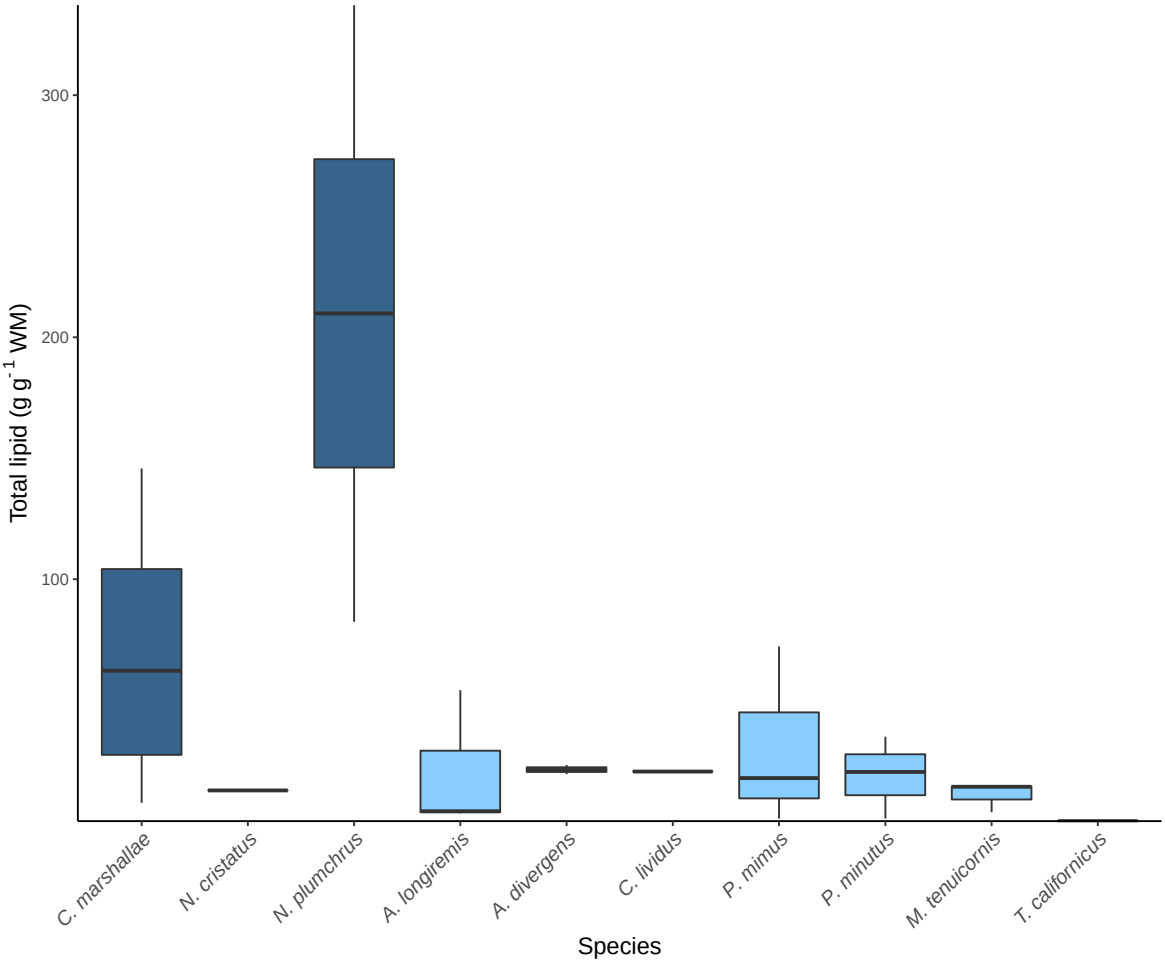

Figure 4. Total lipid mass (g g^{-1} WM) of individual copepod species. Large-bodied copepod species are represented by dark blue, small-bodied copepod species in light blue, and intertidal copepod species *T. californicus* in grey.

Table 3. Lipid class composition (% ± standard deviation) of copepod species and splash pool seston.

Lipid Class (%)	*C. marshallae* (n=4)	*N. plumchrus* (n=2)	*N. cristatus* (n=1)	*P. minutus* (n=3)	*P. mimus* (n=3)	*C. lividus (n=1)*	*M. tenuicornis* (n=3)	*A. longiremis* (n=3)	*A. divergens* (n=2)	*T. californicus* (n=5)	*Splash pool seston* (n=1)
Hydrocarbons	0.18±0.31	3.24±3.24	7.35	0.00	24.81±32.76	0.00	22.67±32.06	1.82±2.57	1.05±1.05	2.01±1.89	3.09
Steryl Esters/ Wax Esters	50.83±29.37	83.98±0.92	59.56	32.18±22.83	9.83±9.44	22.47	0.12±0.17	0.00	19.51±1.25	6.41±4.43	0.00
Ethyl Esters	0.00	0.00	0.00	0.00	0.00	17.64	0.00	0.00	0.00	0.00	0.00
Methyl Esters	0.16±0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl Ketones	3.55±5.98	0.00	0.00	11.62±8.32	10.29±11.04	6.96	0.19±0.27	3.50±1.76	1.89±1.89	0.34±0.22	0.21
Methyl ketones	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glyceryl Ethers	2.16±2.08	1.01±0.14	1.46	2.71±3.84	0.00	0.00	0.00	0.84±1.19	2.14±2.14	0.00	0.00
Triacylglycerols	3.41±2.00	1.47±0.04	3.24	3.91±2.77	3.34±2.80	4.07	2.85±2.38	1.18±1.67	9.95±0.68	22.20±9.35	11.28
Free Fatty Acids	3.10±1.44	1.06±0.76	1.97	0.07±0.07	0.17±0.24	1.95	0.00	5.00±7.08	2.08±2.08	3.05±1.73	1.31
Alcohols	0.24±0.26	0.67±0.16	0.29	1.07±1.52	9.27±7.91	1.10	2.10±1.51	0.85±1.20	14.81±2.67	2.03±2.87	1.99
Sterols	1.71±1.30	0.97±0.37	2.18	0.64±0.91	0.30±0.43	0.77	1.07±0.88	0.00	4.86±4.86	1.59±0.98	4.53
Diacylglycerols	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acetone Mobile Polar Lipids	5.78±1.26	1.83±0.72	2.49	21.65±13.46	21.78±10.92	6.44	20.96±8.30	30.49±11.80	31.64±3.58	15.89±7.52	25.46
Phospholipids	28.78±33.20	5.78±2.32	21.44	26.13±23.76	20.20±6.79	38.61	50.03±30.73	56.33±20.28	12.08±4.98	46.48±2.86	52.12

TL was found to differ significantly between species of large-bodied, small-bodied, and intertidal copepods ($p<0.001$) (Table 4). The groups of copepods that were significantly different from one another were the large-bodied oceanic copepods and the intertidal copepod, *T. californicus*, ($p<0.001$); as well as the small-bodied oceanic copepods and the intertidal copepod ($p=0.014$) (Table 5). Kruskal-Wallis and Dunn's tests were done to test for significant differences among all species of copepod and found significant difference between *C. marshallae* and *N. plumchrus* to *T. californicus* (Table 17A; Table 18A).

Table 4. Results of individual Kruskal-Wallis tests to determine whether total lipid mass (g g^{-1} WM) of copepod species differed significantly between large-bodied copepods, small-bodied copepods, and intertidal copepod species. p-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

	Comparison	**p-value**	**p adj.**
Total lipid (g g^{-1} WM)	Large-bodied, small-bodied, intertidal	<0.001	**<0.001**

Table 5. Results of Dunn's tests to determine which groups of copepod species, large-bodied, small-bodied, or intertidal, were significantly different from each other in total lipid mass (g g^{-1} WM). p-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

	Comparison	**Z**	**p-value**	**p adj.**
Total lipid (g g^{-1} WM)	Large-bodied - Intertidal	3.82	<0.001	**<0.001**
	Large-bodied - Small-bodied	1.71	0.088	0.263
	Intertidal - Small-bodied	-2.82	0.0048	**0.014**

Fatty acid composition of copepod species

The highest fatty acids (FA) across all species of copepod were 16:0, 20:5ω3, 18:0, 22:6ω3, 18:1ω9, and 14:0. Relatively high amounts of diatom markers 20:5ω3 (EPA, >26%) and 16:1ω7 (>6%) were present in the large-bodied copepod species *C. marshallae* (Table 6). *N. cristatus* had, on average, higher amounts of dinoflagellate markers than diatom markers with

18:4ω3 (>4%) and 22:6ω3 (DHA, >13%). *N. plumchrus* had high amounts of diatom marker 20:5ω3 (EPA, >15%). Small-bodied copepods exhibited greater variability in diets (Table 7). Almost all species of small copepod had higher proportions of odd-numbered and/or branched chain bacterial fatty acid markers (OBFA) making up their diet, with *P. mimus* and *P. minutus* having the highest OBFA values (>19%) and *C. lividus* and *M. tenuicornis* having the lowest OBFA values (<6%). *A. divergens* and *C. lividus* both have high amounts of diatom marker 20:5ω3 (EPA, >8%) and dinoflagellate marker 22:6ω3 (DHA, >12%). These species had the highest DHA/EPA ratios with 1.41±0.27% and 1.71% respectively. They also had the highest amounts of 18:1ω9 (>15%). The intertidal copepod, *T. californicus,* (Table 8) had similar amounts of dinoflagellate and diatom markers with the highest percentage of composition being 22:6ω3 (DHA, >11%). *T. californicus* also had high OBFA (>7%) and a DHA/EPA ratio of 1.56±0.85%.

Table 6. Fatty acid composition, shown as percentages of total fatty acids (% ± standard deviation), of large-bodied copepods *Calanus marshallae, Neocalanus cristatus,* and *Neocalanus plumchrus.* Fatty acids included are >1% in all samples[1].

Fatty acids (%)	*C. marshallae* (n=4)	*N. plumchrus* (n=2)	*N. cristatus* (n=1)
14:0	9.24±1.77	5.79±5.53	9.75
TMTD[2]	0.06±0.11	0.00	0.00
ι15:0	0.34±0.12	0.08±0.08	0.78
αι15:0	0.15±0.09	0.03±0.02	0.36
15:0	0.67±0.31	0.17±0.11	1.23
ι16:0	1.14±0.47	0.19±0.18	0.52
16:0	14.89±11.59	12.83±4.45	12.01
16:1ω11	0.02±0.01	0.09±0.06	0.02
16:1ω9	0.25±0.05	0.30±0.30	0.65
16:1ω7	6.45±1.35	1.66±1.05	1.84
ι17:0	0.33±0.11	0.07±0.07	0.67
αι17:0	0.30±0.02	0.31±0.03	0.23
16:2ω6	0.87±0.17	0.47±0.41	0.47
17:0	0.38±0.44	0.40±0.33	0.56
16:3ω4	0.93±0.27	0.69±0.46	0.72

ι18:0	0.38±0.59	0.03±0.01	0.64
16:4ω3	0.10±0.07	2.24±2.20	0.07
16:4ω1	1.84±1.09	3.33±0.16	0.20
18:0	1.68±1.39	9.59±8.86	1.12
18:1ω9	2.78±0.38	0.36±0.36	2.64
18:1ω7	0.70±0.36	12.17±11.89	0.70
18:1ω6	0.01±0.01	2.57±2.55	0.03
18:1ω5	0.26±0.09	0.20±0.15	0.63
18:2ω6	0.70±0.26	0.41±0.20	0.68
18:2ω4	0.12±0.04	0.08±0.07	0.09
18:3ω3	0.51±0.21	0.07±0.07	0.48
18:4ω3	2.62±0.84	1.45±1.42	4.57
20:1ω11	0.36±0.13	1.99±1.83	2.55
20:1ω9	4.46±2.84	1.09±0.29	2.11
20:4ω6	0.59±0.14	3.81±3.59	0.52
20:4ω3	0.71±0.11	0.38±0.38	1.02
20:5ω3	26.98±7.99	11.69±11.69	21.74
22:0	0.11±0.06	0.16±0.04	0.20
22:1ω11(13)	5.27±3.34	2.86±2.75	8.30
22:1ω9	0.45±0.30	0.23±0.06	0.70
21:5ω3	0.32±0.04	0.05±0.05	0.43
23:0	0.05±0.03	0.04±0.04	0.06
22:4ω6	0.10±0.06	1.61±1.61	0.05
22:5ω6	0.43±0.37	0.52±0.44	0.21
22:4ω3	0.03±0.03	0.02±0.02	0.06
22:5ω3	1.50±0.44	12.40±12.18	1.76
24:0	0.04±0.08	0.28±0.01	0.00
22:6ω3	7.88±4.87	4.10±1.33	13.69
24:1	0.59±0.07	0.42±0.18	1.00
Σ SFA	27.53±11.71	29.72±7.85	25.18
Σ MUFA	22.45±7.62	25.02±9.52	23.06
Σ Diene	2.08±0.57	1.35±0.00	1.67
Σ PUFA	45.29±5.47	43.13±17.12	46.88
Σ dino[3]	10.50±4.47	5.55±0.08	18.26
Σ diatom[4]	33.42±9.27	13.35±12.74	23.58
DHA/EPA	0.42±0.43	0.06±0.06	0.63
OBFA[5]	3.77±1.18	1.38±0.07	5.09

[1] Proportional percentage fatty acid data <1% within samples were excluded: 14:1,15:1, αι16:0, 16:1ω5, 16:3ω3, 17:1, 18:1ω3, 18:2α, 18:2β, 18:3ω4, 18:3ω6, 18:4ω1, 18:5ω3, 19:0, 19:3ω6, 20:0, 20:1ω7, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 20:3 ω6, 21:00, 22:1ω7, 22:2ω6, 22:3ω3, 2:2NMIDa, 2:2NMIDb, pristanic, phytanic

[2] TMTD is trimethyltridecanoic acid

[3] 18:4ω3 and 22:6ω3 (DHA)

[4] 16:1ω7 and 20:5ω3 (EPA)

[5] ι15:0, αι15:0, 15:1, ι16:0, αι16:0, ι17:0, αι17:0, 17:0, 17:1

Table 7. Fatty acid composition, shown as percentages of total fatty acids (% ± standard deviation), of small-bodied oceanic copepod species *Aetideus divergens*, *Acartia longiremis*, *Clausocalanus lividus*, *Mesocalanus tenuicornis*, *Pseudocalanus mimus*, and *Pseudocalanus minutus*. Fatty acids included are >1% in all samples[1].

Fatty acids (%)	*A. divergens* (n=2)	*A. longiremis* (n=3)	*C. lividus* (n=1)	*M. tenuicornis* (n=3)	*P. mimus* (n=3)	*P. minutus* (n=3)
14:0	1.66±0.26	4.93±2.01	2.16	4.63±3.40	2.54±0.59	2.99±1.89
TMTD[2]	0.46±0.29	0.15±0.22	0.24	0.00	1.30±0.45	1.21±0.75
ι15:0	1.88±1.69	1.16±1.00	0.36	0.49±0.36	1.39±1.28	0.26±0.26
αι15:0	0.88±0.75	0.48±0.21	0.24	0.40±0.28	0.74±0.61	0.12±0.14
15:0	1.75±1.32	1.73±0.47	0.45	1.48±1.03	1.61±1.00	0.55±0.60
ι16:0	1.30±0.67	0.79±0.66	0.11	0.49±0.34	5.11±1.63	11.10±7.59
16:0	14.75±3.13	48.59±6.50	19.82	41.78±17.1	31.05±16.7	25.34±23.9
16:1ω11	0.00	0.00	0.00	0.03±0.04	0.00	0.00
16:1ω9	2.67±2.28	1.29±1.82	0.19	0.36±0.29	1.96±1.94	0.08±0.11
16:1ω7	4.52±0.17	0.48±0.59	1.32	0.65±0.26	3.59±2.24	3.77±2.55
ι17:0	1.26±0.36	1.05±1.04	0.88	0.51±0.37	1.31±0.41	0.34±0.10
αι17:0	3.72±3.66	1.80±2.38	0.14	0.21±0.02	2.50±3.28	0.16±0.12
16:2ω6	0.76±0.19	0.21±0.29	0.00	0.01±0.02	0.60±0.48	0.68±0.47
17:0	0.19±0.19	0.87±0.35	0.92	2.04±0.95	0.62±0.37	0.62±0.88
16:3ω4	0.47±0.11	0.04±0.05	0.41	0.18±0.13	0.79±0.31	1.09±0.67
ι18:0	0.00	0.22±0.31	0.36	0.13±0.18	7.29±9.59	6.27±8.87
16:4ω3	0.00	0.00	0.81	1.62±2.29	0.29±0.35	0.00
16:4ω1	0.08±0.08	0.37±0.34	0.11	0.63±0.83	0.54±0.45	1.29±0.93
18:0	6.41±1.23	22.47±6.94	4.99	17.39±5.16	11.30±7.11	9.42±9.29
18:1ω9	15.01±1.02	0.89±0.78	20.25	6.97±8.27	4.65±2.81	12.36±9.91
18:1ω7	3.38±0.25	0.21±0.29	0.66	1.63±2.05	0.72±0.46	0.38±0.27
18:1ω6	0.00	0.00	0.05	0.00	0.02±0.03	0.02±0.02
18:1ω5	0.10±0.05	0.00	0.08	0.07±0.07	0.21±0.15	0.18±0.14
18:2ω6	3.72±0.95	0.44±0.08	1.78	0.73±0.46	0.58±0.24	0.39±0.21
18:2ω4	0.60±0.4	0.06±0.08	0.00	0.01±0.01	0.17±0.17	0.10±0.07
18:3ω3	0.69±0.04	0.00	1.08	0.25±0.35	0.36±0.31	0.20±0.21
18:4ω3	0.79±0.31	0.53±0.74	3.82	0.37±0.52	1.12±0.90	1.41±1.07
20:1ω11	0.66±0.11	0.23±0.33	0.91	0.20±0.20	0.22±0.16	0.68±0.57
20:1ω9	1.02±0.46	0.16±0.23	0.84	0.39±0.28	0.42±0.6	0.25±0.30
20:4ω6	0.75±0.18	0.12±0.17	0.39	2.67±3.67	0.24±0.29	0.26±0.19
20:4ω3	0.72±0.07	0.74±0.45	2.63	0.29±0.21	0.80±0.10	0.56±0.25
20:5ω3	9.01±1.20	1.48±1.98	8.79	1.59±2.07	5.55±3.65	8.65±6.12
22:0	0.54±0.11	0.56±0.73	0.00	0.15±0.14	0.17±0.18	0.09±0.12
22:1ω11(13)	0.07±0.07	0.00	0.00	0.18±0.18	0.04±0.05	0.57±0.81
22:1ω9	0.90±0.75	0.26±0.36	0.00	0.15±0.12	0.00	0.00
21:5ω3	0.15±0.15	0.00	0.32	0.02±0.02	0.07±0.10	0.23±0.18
23:0	0.21±0.21	1.02±1.21	0.00	0.06±0.05	0.04±0.06	0.00
22:4ω6	0.40±0.25	0.00	0.71	0.94±1.07	0.52±0.12	0.51±0.17
22:5ω6	0.41±0.13	2.13±1.06	5.15	0.66±0.76	2.14±1.19	2.39±0.39

22:4ω3	0.17±0.17	0.08±0.12	0.00	0.10±0.14	0.08±0.11	0.47±0.58
22:5ω3	1.12±0.57	0.57±0.80	0.21	0.36±0.44	0.87±1.04	0.37±0.30
24:0	0.07±0.07	0.23±0.16	0.00	0.19±0.02	0.21±0.16	0.00
22:6ω3	12.99±4.07	1.83±1.71	15.06	6.97±5.12	4.10±3.00	2.46±197
24:1	0.61±0.42	0.00	0.50	0.23±0.17	0.00	0.27±0.30
Σ SFA	27.04±2.65	81.63±9.34	29.40	68.22±22.6	49.58±24.1	40.79±35.2
Σ MUFA	30.58±2.46	3.80±2.67	26.07	11.20±11.3	12.54±8.16	18.68±12.3
Σ Diene	5.24±1.17	0.86±0.59	1.78	1.02±0.63	1.35±0.81	1.53±1.00
Σ PUFA	27.78±4.76	8.22±5.48	40.66	17.24±12.5	17.98±8.25	20.77±11.0
Σ dino[3]	13.78±4.39	2.35±2.42	18.88	7.34±5.26	5.22±3.71	3.87±3.03
Σ diatom[4]	13.53±1.37	1.96±2.57	10.11	2.24±2.29	9.14±5.88	12.42±8.61
DHA/EPA	1.41±0.27	0.32±0.45	1.71	16.77±22.4	0.51±0.36	0.19±0.15
OBFA[5]	11.33±7.22	8.10±4.55	3.62	5.88±3.32	20.82±8.55	19.45±10.1

[1] Proportional percentage fatty acid data <1% within samples were excluded: 14:1,15:1, αι16:0, 16:1ω5, 16:3ω3, 17:1, 18:1ω3, 18:2α, 18:2β, 18:3ω4, 18:3ω6, 18:4ω1, 18:5ω3, 19:0, 19:3ω6, 20:0, 20:1ω7, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 20:3 ω6, 21:00, 22:1ω7, 22:2ω6, 22:3ω3, 2:2NMIDa, 2:2NMIDb, pristanic, phytanic

[2] TMTD is trimethyltridecanoic acid

[3] 18:4ω3 and 22:6ω3 (DHA)

[4] 16:1ω7 and 20:5ω3 (EPA)

[5] ι15:0, αι15:0, 15:1, ι16:0, αι16:0, ι17:0, αι17:0, 17:0, 17:1

Table 8. Fatty acid composition, shown as percentages of total fatty acids (% ± standard deviation), of small intertidal copepod species *Tigriopus californicus* and splash pool seston. Fatty acids included are >1% in all samples[1].

Fatty acids (%)	*T. californicus* (n=5)	*Splash pool seston* (n=1)
14:0	3.15±0.49	3.29
TMTD[2]	0.00	0.00
ι15:0	1.79±0.17	1.50
αι15:0	1.37±0.35	0.54
15:0	1.04±0.15	0.60
ι16:0	0.53±0.23	0.26
16:0	26.97±11.06	25.01
16:1ω11	0.02±0.02	1.61
16:1ω9	0.38±0.19	0.43
16:1ω7	6.31±3.03	12.02
ι17:0	0.74±0.14	0.70
αι17:0	0.55±0.09	0.20
16:2ω6	1.00±0.53	0.64
17:0	1.41±0.25	0.33
16:3ω4	0.95±0.24	0.66
ι18:0	0.00	0.00
16:4ω3	0.05±0.03	0.09
16:4ω1	0.19±0.09	0.06
18:0	10.27±9.39	2.14
18:1ω9	6.69±3.16	5.19
18:1ω7	4.19±2.13	6.05
18:1ω6	0.00	0.00

18:1ω5	0.36±0.28	1.13
18:2ω6	1.86±0.76	2.50
18:2ω4	0.08±0.10	0.00
18:3ω3	1.53±1.06	6.73
18:4ω3	1.16±1.56	10.63
20:1ω11	0.41±0.60	4.10
20:1ω9	0.42±0.23	0.00
20:4ω6	1.17±0.60	0.80
20:4ω3	0.80±0.42	0.35
20:5ω3	6.00±3.05	7.19
22:0	0.43±0.23	0.01
22:1ω11(13)	0.19±0.12	0.02
22:1ω9	0.03±0.04	0.11
21:5ω3	0.82±0.90	0.85
23:0	0.16±0.08	0.00
22:4ω6	0.04±0.06	0.00
22:5ω6	1.13±0.31	0.00
22:4ω3	0.06±0.09	0.00
22:5ω3	0.40±0.23	0.04
24:0	0.76±0.45	0.23
22:6ω3	11.76±6.59	2.30
24:1	0.00	0.00
Σ SFA	44.47±21.4	31.78
Σ MUFA	20.16±9.37	32.01
Σ Diene	3.17±1.43	3.14
Σ PUFA	27.04±11.8	29.86
Σ dino[3]	12.93±6.72	12.92
Σ diatom[4]	12.31±6.00	19.22
DHA/EPA	1.56±0.85	0.32
OBFA[5]	7.99±1.46	19.22

[1] Proportional percentage fatty acid data <1% within samples were excluded: 14:1,15:1, αι16:0, 16:1ω5, 16:3ω3, 17:1, 18:1ω3, 18:2α, 18:2β, 18:3ω4, 18:3ω6, 18:4ω1, 18:5ω3, 19:0, 19:3ω6, 20:0, 20:1ω7, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 20:3 ω6, 21:00, 22:1ω7, 22:2ω6, 22:3ω3, 2:2NMIDa, 2:2NMIDb, pristanic, phytanic

[2] TMTD is trimethyltridecanoic acid

[3] 18:4ω3 and 22:6ω3 (DHA)

[4] 16:1ω7 and 20:5ω3 (EPA)

[5] ι15:0, αι15:0, 15:1, ι16:0, αι16:0, ι17:0, αι17:0, 17:0, 17:1

Fatty acid non-metric multi-dimensional scaling analysis

Non-metric multi-dimensional scaling (NMDS) analysis of fatty acid composition gave a stress value of 0.076 (Figure 5). Each of the 22 vectors represents a FA with the exception of PUFA, which represents the sum of FAs with 3 or more double bonds, and OBFA, which represents the sum of odd-numbered and/or branched fatty acids, that are synthesized only by bacteria (Wilson et al. 2010, Dalsgaard et al. 2003). Of those 22 vectors, 15 were found to be

significant ($p < 0.05$) (Table 9). All vectors were included on the NMDS figure (Figure 5). The data separated into 3 distinct clusters determined by the cluster dendrogram (Figure 7A). Large-bodied copepod species, except for one sample of *N. plumchrus*, were tightly grouped as Cluster 2 (Figure 5), which was strongly associated with vectors representing FAs characteristic of a predominantly diatom diet, such as 16:1ω7, 20:5ω3, and 16-PUFA. 18:4ω3, a FA predominantly found in dinoflagellates, also associates with Cluster 2. The small-bodied copepods separated into two clusters with more range, Clusters 1 and 3. Cluster 3, mainly samples of *A. divergens, C. lividus, P. mimus, P. minutus,* and *T. californicus,* associated with 22:6ω3 and 18-PUFA, vectors representative of a dinoflagellate diet. The data exhibit a large range across the NMDS1 axis with Cluster 1 spread out the furthest. Cluster 1, mainly samples of *A. longiremis*, *M. tenuicornis* and single samples (n=1) of other small copepod species, associated with 16:0 and 18:0.

Table 9. All NMDS loading vectors (fatty acids), r^2-values and p-values listed for NMDS1 and NMDS2. Significant vectors ($p < 0.05$) are shown in bold. Vectors refer to individual fatty acids, with the exception of the OBFA and PUFA which were grouped to produce a stronger signal. OBFA represents the sum odd-numbered and/or branched chain bacterial fatty acid markers and PUFA represents fatty acids with three or more double bonds.

Vectors	NMDS1	NMDS2	r^2	p-value
14:0	**-0.166**	**-0.986**	**0.602**	**0.001**
16:0	**0.978**	**-0.207**	**0.892**	**0.001**
16:1ω7	**–0.954**	**–0.299**	**0.515**	**0.001**
16:3ω4	**–0.873**	**–0.488**	**0.590**	**0.001**
16:4ω3	**–0.022**	**1.000**	**0.646**	**0.001**
16:4ω1	–0.985	0.172	0.204	0.064
18:0	**0.932**	**0.362**	**0.933**	**0.001**
18:1ω9	**–0.521**	**0.853**	**0.410**	**0.008**
18:1ω7	**–0.063**	**0.998**	**0.5933**	**0.001**
18:2ω6	**–0.505**	**0.883**	**0.235**	**0.038**
18:3ω3	–0.872	0.289	0.158	0.139
18:4ω3	**–0.745**	**–0.667**	**0.556**	**0.002**

20:4ω6	**–0.0815**	**0.997**	**0.708**	**0.001**
20:4ω3	–0.815	–0.579	0.169	0.124
20:5ω3	**–0.628**	**–0.778**	**0.789**	**0.001**
22:4ω6	**–0.028**	**1.000**	**0.721**	**0.001**
22:5ω6	0.744	0.668	0.073	0.387
22:4ω3	0.983	–0.182	0.104	0.243
22:5ω3	–0.398	–0.917	0.247	0.059
22:6ω3	**–0.635**	**0.773**	**0.467**	**0.002**
OBFA	–0.136	0.991	0.013	0.862
PUFA	**–0.979**	**–0.204**	**0.859**	**0.001**

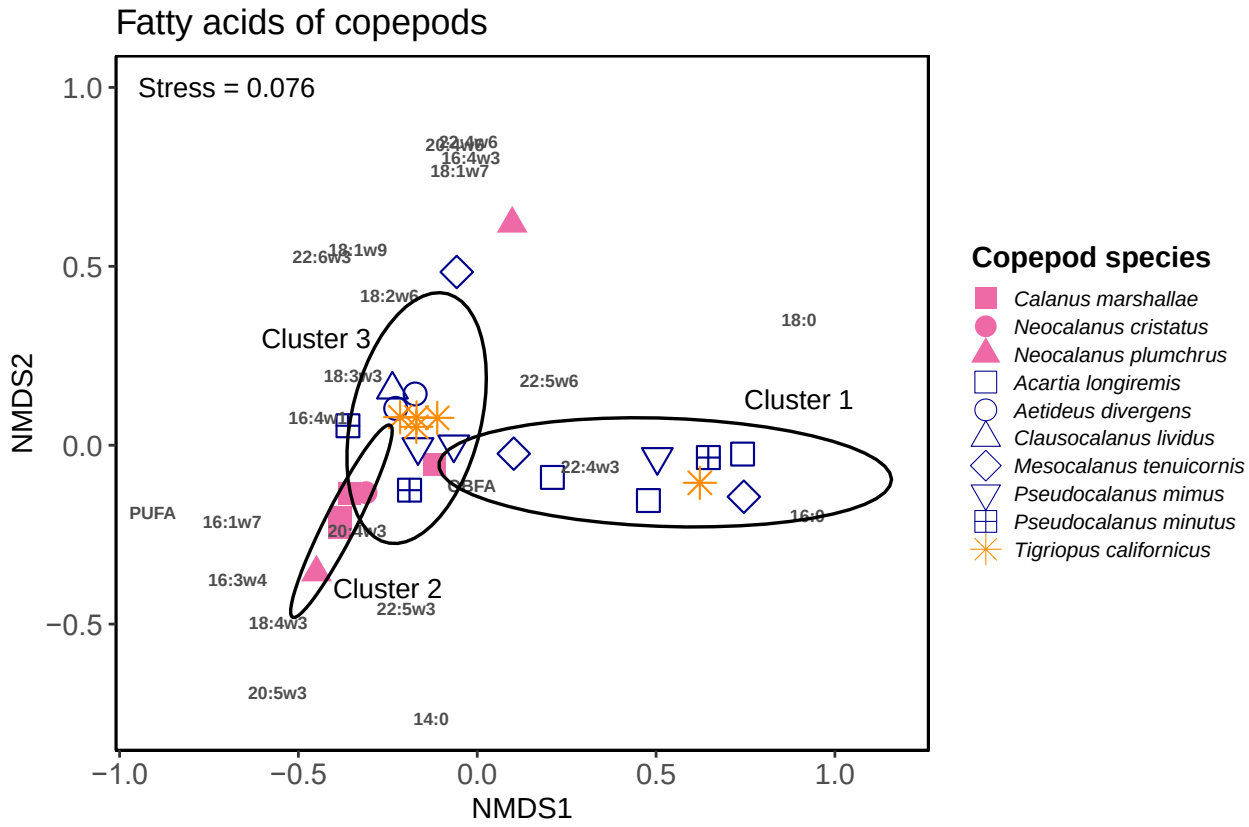

Figure 5. Non-metric multi-dimensional scaling plot to visualize the underlying structure of fatty acid profiles of copepod species. A Bray-Curtis dissimilarity matrix was used to construct the plot with a stress value of 0.076. Ellipses represent three separate clusters of samples determined using hierarchical average-neighbour cluster analysis. Large-bodied copepod species are pink, small-bodied copepod species are blue, and intertidal copepod species is yellow.

An ADONIS test found FA composition of copepods differed significantly amongst clusters (r^2=0.39, p=0.001) (Table 10). Cluster 2 and 3 were significantly different (r^2=0.36, p=0.003), as were Cluster 2 and 1 (r^2=0.78, p=0.003) and Cluster 3 and 1 (r^2=0.57, p=0.003) (Table 11).

Table 10. Results of an ADONIS test to determine the significance of clusters produced by hierarchical cluster analysis. A p-value <0.05 is considered significant and is shown in bold.

	Df	**Sum of Sqs**	**Mean Sqs**	**F Model**	**r^2**	**p-value**
Cluster	1	0.37	0.37	16.23	0.39	**0.001**
Residuals	25	0.57	0.023		0.61	
Total	26	0.94			1.00000	

Table 11. Results of a pairwise-ADONIS test to determine which copepod species clusters were significantly different from each other in terms of fatty acid composition. P-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

Pair	**Df**	**Sum of Sqs**	**F Model**	**r^2**	**p-value**	**p adj.**
2 vs 3	1	0.22	9.60	0.36	0.001	**0.003**
2 vs 1	1	0.73	38.95	0.78	0.001	**0.003**
3 vs 1	1	0.72	27.02	0.57	0.001	**0.003**

Similarity percentage (SIMPER) analysis was used to determine the contribution of individual FAs to the three significantly different NMDS clusters. Only FAs that contributed to a cumulative sum of ~75% were included (Table 12; Table 13; Table 14). The main FAs driving differences in FA composition between Cluster 2 and Cluster 3 were PUFAs, 20:5ω3 (EPA), 16:0, OBFA, 18:1ω9, 14:0, and 22:6ω3 (DHA) (Table 12). PUFA, EPA, and 14:0 were highest in Cluster 2 at 50.47, 27.90, and 10.36%, respectively. 16:0, OBFA, 18:1ω9, and DHA were highest in Cluster 3 at 18.8, 12.02, 11.03, and 10.90%, respectively. The differences in FA composition between Cluster 2 and Cluster 1 were driven by PUFA, 16:0, EPA, and 18:0 (Table 13). Cluster 2 had highest percentage of PUFA and EPA at 50.47 and 27.90%, respectively. Cluster 3 had the highest percent of 16:0 and 18:0 at 51.65 and 21.77%, respectively. The FAs

responsible for differences between Cluster 3 and Cluster 1 were 16:0, PUFA, 18:0, 18:1ω9, DHA, and OBFA (Table 14). Cluster 3 had highest percentage of PUFA, 18:1ω9, DHA, and OBFA at 30.37, 11.03, 10.90, and 21.02%, respectively. Cluster 1 had highest percentages of 16:0 and 18:0 at 51.65% and 21.77%, respectively.

Table 12. Results of an average similarity percentage (SIMPER) analysis used to determine the contribution of individual fatty acids (FA) to Cluster 2 and Cluster 3 revealed by cluster analysis. Only fatty acids that contribute to a cumulative sum of ~75% are included.

				Cluster 2	**Cluster 3**		
FA	**Avg.**	**SD**	**Ratio**	**Avg. a (%)**	**Avg. b (%)**	**Cumulative Sum**	**Marker**
PUFA[1]	0.079	0.029	2.78	50.47	39.37	17.90	General condition
20:5ω3	0.079	0.026	3.09	27.90	8.04	35.63	Diatom
16:0	0.040	0.023	1.72	9.01	18.81	44.70	N/A
OBFA[2]	0.038	0.039	0.97	3.19	12.02	53.24	Bacteria
18:1ω9	0.036	0.024	1.46	2.36	11.03	61.23	Carnivory
14:0	0.032	0.0068	4.65	10.36	2.37	68.35	N/A
22:6ω3	0.025	0.018	1.45	6.48	10.90	0.74	Dino

[1]Fatty acids with three or more double bonds
[2]Odd-numbered and/or branched chain bacterial fatty acid markers

Table 13. Results of an average similarity percentage (SIMPER) analysis used to determine the contribution of individual fatty acids (FA) to Cluster 2 and Cluster 1 revealed by cluster analysis. Only fatty acids that contribute to a cumulative sum of ~75% are included.

				Cluster 2	**Cluster 1**		
FA	**Avg.**	**SD**	**Ratio**	**Avg. a (%)**	**Avg. b (%)**	**Cumulative Sum**	**Marker**
PUFA[1]	0.18	0.033	5.48	50.47	7.61	24.92	General condition
16:0	0.18	0.033	5.58	9.01	51.65	49.71	N/A
20:5ω3	0.11	0.025	4.66	27.90	1.18	65.31	Diatom
18:0	0.09	0.029	3.13	0.90	21.77	77.50	N/A

[1]Fatty acids with three or more double bonds
[2]Odd-numbered and/or branched chain bacterial fatty acid markers

Table 14. Results of an average similarity percentage (SIMPER) analysis used to determine the contribution of individual fatty acids (FA) to Cluster 3 and Cluster 1 revealed by cluster analysis. Only fatty acids that contribute to a cumulative sum of ~75% are included.

				Cluster 3	Cluster 1		
FA	**Avg.**	**SD**	**Ratio**	**Avg. a (%)**	**Avg. b (%)**	**Cumulative Sum**	**Marker**
16:0	0.15	0.025	3.24	18.81	51.65	25.54	N/A
PUFA[1]	0.10	0.035	2.89	30.37	7.61	43.17	General condition
18:0	0.067	0.033	2.01	7.09	21.77	54.86	N/A
18:1ω9	0.046	0.029	1.59	11.03	0.82	62.84	Carnivory
22:6ω3	0.042	0.023	1.81	10.90	1.78	70.14	Dino
OBFA[2]	0.038	0.033	1.15	12.02	8.46	76.79	Bacteria

[1]Fatty acids with three or more double bonds
[2]Odd-numbered and/or branched chain bacterial fatty acid markers

Total fatty acid, EPA, and DHA

Overall, total fatty acid (TFA) ranged from 0.02 to 180.81 g g^{-1} WM (Figure 6). Large-bodied copepod species had the highest overall TFA, except for *N. cristatus*. Small copepod species had similar levels of TFA, ranging from 0.4 to 29.48 g g^{-1} WM. *T. californicus* had the lowest mean TFA at 0.28 g g^{-1} WM. Of the small-bodied copepods, *A. longiremis* had the highest mean TFA at 11.30 g g^{-1} WM. Large-bodied copepod species *N. plumchrus* and *C. marshallae* generally had the highest mean absolute amounts of EPA at 18.80 and 11.42 g g^{-1} WM, respectively. *M. tenuicornis*, *A. longiremis*, and *T. californicus* exhibited the lowest mean amounts of EPA at 0.14, 0.037 and 0.022 g g^{-1} WM, respectively. DHA was lot more consistent, ranging from 0-4.45 g g^{-1} WM. *C. lividus* had the highest amounts of DHA among small copepods at 1.96 g g^{-1} WM.

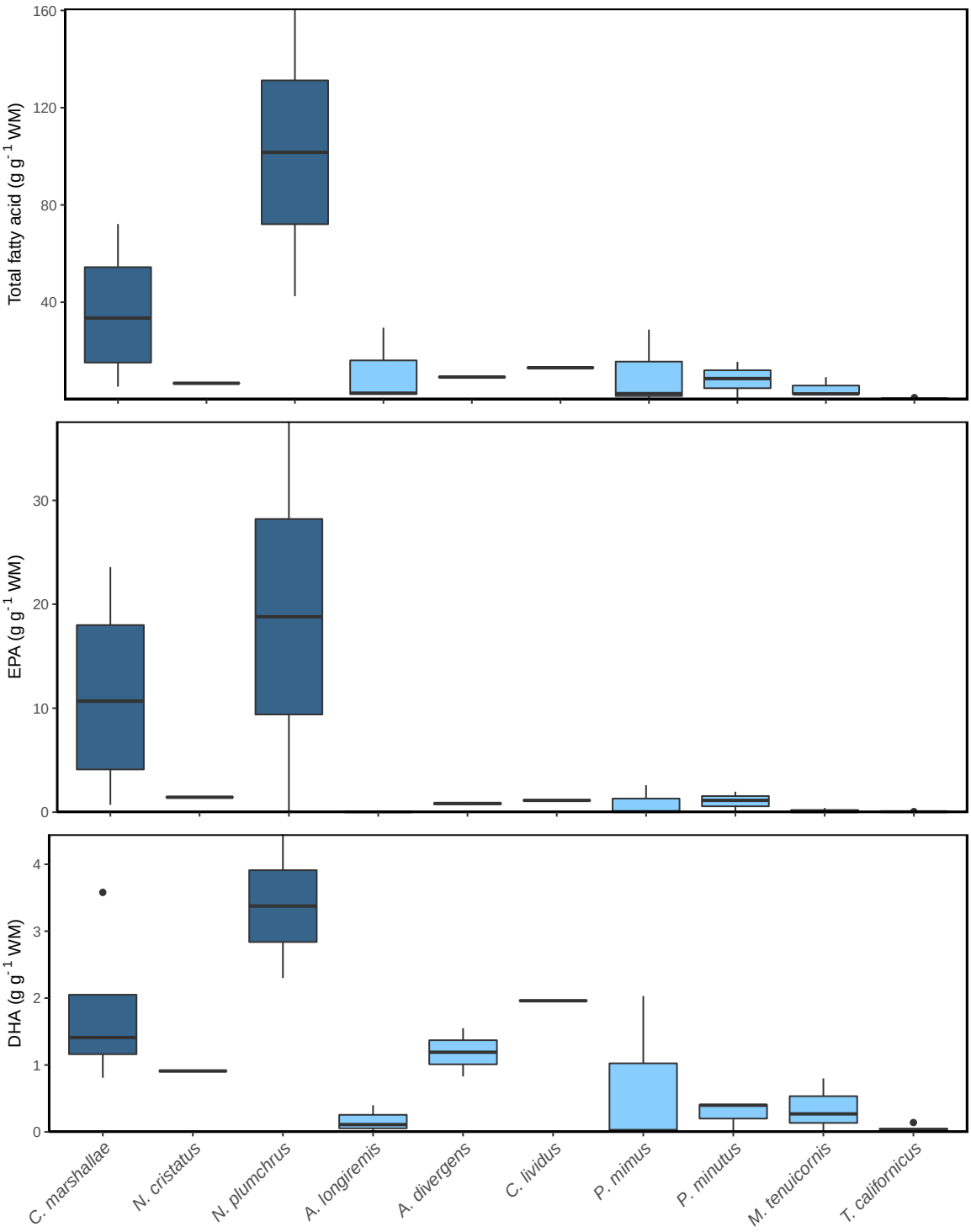

Figure 6. Boxplots showing A) the total fatty acid (g g^{-1} WM), B) EPA (g g^{-1} WM), and C) DHA (g g^{-1} WM) of fatty acid samples extracted from species of copepods. EPA and DHA are the fatty acids 20:5ω3 and 22:6ω3, respectively. Black circles represent outliers. Large-bodied copepod species are represented by dark blue, small-bodied copepod species in light blue, and intertidal copepod species *T. californicus* in grey.

Clusters determined from NMDS testing were found to have a significant difference in the absolute amount of TFA ($p=0.024$), EPA ($p<0.001$) and DHA ($p=0.0017$) (Table 15). Cluster 1 and Cluster 2 were found to be significantly different in TFA, EPA, and DHA (Table 16). Cluster 3 and Cluster 2 were found to be significantly different in EPA only. Kruskal-Wallis and Dunn's tests were done to test for significant differences among all species of copepod and found significant difference between *C. marshallae* and *N. plumchrus* to *T. californicus* (Table 19A; Table 21A).

Table 15. Results of individual Kruskal-Wallis tests used to determine whether total fatty acid mass (g g^{-1} WM), total EPA mass (g g^{-1} WM) or total DHA mass (g g^{-1} WM) differed significantly among clusters determined from the NMDS plot. EPA and DHA are the fatty acids 20:5ω3 and 22:6ω3, respectively. P-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

	p-value	**p adj.**
Total fatty acid (g g^{-1} WM)	0.024	**0.024**
Total EPA (g g^{-1} WM)	<0.001	**<0.001**
Total DHA (g g^{-1} WM)	0.0017	**0.0017**

Table 16. Results of individual Dunn's tests to determine which clusters were significantly different from each other in terms of total fatty acid mass (g g^{-1} WM), total EPA mass (g g^{-1} WM) or total DHA mass (g g^{-1} WM). EPA and DHA are the fatty acids 20:5ω3 and 22:6ω3, respectively. P-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

	Comparison	**Z**	**p-value**	**p adj.**
Total fatty acid (g g^{-1} WM)	Cluster 1 – Cluster 3	-20.52	0.60	1.000
	Cluster 1 – Cluster 2	-2.59	0.0096	**0.029**
	Cluster 3 – Cluster 2	-2.39	0.017	0.050
Total EPA (g g^{-1} WM)	Cluster 1 – Cluster 3	-2.15	0.032	0.095
	Cluster 1 – Cluster 2	-3.96	<0.001	**<0.001**
	Cluster 3 – Cluster 2	-2.50	0.012	**0.037**
Total DHA (g g^{-1} WM)	Cluster 1 – Cluster 3	-2.35	0.019	0.057
	Cluster 1 – Cluster 2	-3.50	<0.001	**0.0013**
	Cluster 3 – Cluster 2	-1.84	0.066	0.20

Discussion

Abundance anomalies

Surface ocean temperature strongly influences the composition of copepod assemblages along the west coast of Vancouver Island (WCVI) (Venello et al. 2021, Galbraith et al. 2016, Mackas et al. 2007). Venello et al. (2021) looked at how abundance varied on the southern shelf of the WCVI among copepods grouped as either active-ambush predators, including *Aetideus divergens, Acartia longiremis,* and *Mesocalanus tenuicornis*, or omnivore-herbivores, such as *Clausocalanus lividus, Pseudocalanus mimus, Pseudocalanus minutus, Calanus marshallae, Neocalanus cristatus*, and *Neocalanus plumchrus.* Finding no strong positive or negative anomalies in abundance, Venello et al. (2021) concluded that the species within these groups likely exhibit some functional redundancy to mitigate the effects of environmental change. However, in this thesis, when examining individual species of copepods within those functional groups, there were observable differences in abundance trends, indicating variability in life histories and environmental preferences (Figure 3).

Pseudocalanus species, species that typically dominate the relative abundance off the WCVI (Mackas et al. 2007, El-Sabaawi et al. 2010), experienced the strongest positive abundance anomalies across all species post-1998. *C. marshallae* and *A. longiremis*, species who are also common off the WCVI (Mackas et al. 2007, Peterson & Schwing 2003), had similar trends to *Pseudocalanus* species but exhibited more variability with both positive and negative anomalies. These species are 'boreal shelf' copepods and, as the samples included in the analysis were exclusively from the southern shelf of the WCVI, their contribution to community assemblage is significant. *N. cristatus* and *N. plumchrus*, larger 'subarctic' copepods, have similar trends in abundance anomalies, indicating they have similar life history patterns and

tolerances to environmental stress. *M. tenuicornis* is a 'southern' copepod that showed increases in abundance following warm water events when 'boreal shelf' and 'subarctic' species declined. C. *lividus,* an 'exotic southern' copepod, and *A. divergens,* only appear once or twice across the 30-year period for the shelf stations considered in this analysis.

Major fluctuations in abundance can be linked with observed El Niño-Southern Oscillation (ENSO) events, especially the 1982-1983, 1997-1998, and 2015-2016 occurrences. The 2015-2016 ENSO event, in conjunction with the marine heatwave known as 'the Blob' (Bond et al. 2015), caused overall lower abundance among species, especially among boreal shelf and subarctic copepod species. As northern species abundance declines during warm-water ENSO events, southern copepod species abundance is often observed to increase (Galbraith et al. 2016, Mackas et al. 2007). *M. tenuicornis,* a southern copepod favouring warmer waters, had overall higher abundance as other species declined. Similar responses were also observed following the 1997-1998 ENSO event. *C. lividus* is classified as an exotic southern species and typically found offshore (Mackas & Galbraith 2002). As the abundance data considered in the analysis was for on-shelf stations off the WCVI, these rare appearances may only reflect sampling strategy.

Looking within functional groups may provide insight into how much variability there is among species. As large-bodied, northern copepods are negatively impacted by warm-water events, smaller copepod species will likely increase in abundance as ocean temperatures rise. This may result in serious repercussions on the community structure of the WCVI because when small-bodied species of copepod dominate the community, nutrient recycling, carbon transfer, and trophic energy flux are weakened (Kattner & Hagen 2009, Lee et al. 2006). Increased

occurrences of smaller southern species, that have lower lipid stores and wax ester than the large northern species (Mackas et al. 2004, Kattner & Hagen 2009), may have a similar effect.

Total lipid and lipid classes

Total lipid (TL) mass was highest for large-bodied copepod species *C. marshallae* and *N. plumchrus* (Figure 4). While I could find no TL mass values for *C. marshallae* reported in the literature, the TL mass of *N. plumchrus* was lower than previously reported values for the Pacific (Lee & Hirota 1973, Evanson et al. 2000). These cold-water, northern copepod species have to store more lipid to support both diapause and their annual reproductive cycles (Evanson et al. 2000). Their main storage lipids were wax esters (WE); these are common for many boreal and arctic calanoid copepods (Miller et al. 2017) and agree with previous findings in the literature (Lee et al. 2006). Lipid storage as WE is hypothesized to be an adaptation to herbivorous life cycles in temperate and polar systems with seasonal pulses of phytoplankton productivity, such as the WCVI (Miller et al. 2017). While there is considerable interspecific variation in the proportion of WE, there is also intraspecific variability (Miller et al. 2017) (Table 3; Table 4; Table 5). The developmental stage of a copepod will have a large effect on the observed lipid profiles. The last juvenile stage of the copepod life cycle (stage V) is often heavy in lipid, especially WEs, as they descend to the deep water to diapause (Kattner & Hagen 2009). When stage V juveniles moult into adult stage VI to reproduce, they use their lipid stores as fuel to reproduce (Kattner & Hagen 2009). If a sample contains a stage VI copepod female, there is the possibility that she was already ‘spent’, having used up all her lipid stores. This is likely a major reason behind some of the variability observed among samples of large copepods, such as *C. marshallae.*

The sample of *N. cristatus* had extremely low TL mass, which does not match the literature values previously determined (Lee & Hirota 1973, Saito & Kotani 2000). However, as there was only one sample of *N. cristatus,* it is hard to deduce the reasoning behind this unlikely value. One possibility could be the rupturing of oil sacs during capture, where the WE is stored in *N. cristatus*; though, there is no evidence to suggest the copepods were damaged. The sample of *N. cristatus* was a stage V copepod (Table 24A) which typically signifies that the copepod should have large lipid stores to support itself through diapause and reproduction (Kattner & Hagen 2009). *N. cristatus* however has been observed to have several different stage V forms (Kobari & Ikeda 1999). In a study conducted in the Oyashio region of the North Pacific, Kobari and Ikeda (1999) classified *N. cristatus* stage V into three different forms: 'solid' stage V that are well-developed with obvious lipid storage, 'transparent' stage V that are poorly developed and have no lipids, and 'intermediate' stage V that falls in between. 'Transparent' stage V *N. cristatus* were found to have very similar values of TL mass to the value reported in this thesis (Yamada et al. 2016). While Kobari and Ikeda (1999) reported transparent-type stage V individuals to be more common after August, this could potentially be another explanation for the low TL mass value observed.

All small-bodied oceanic copepods had relatively similar levels of TL mass (Figure 4). Upon investigation of lipid classes however, the small copepods diverged into two groups that differed in their storage lipid (Table 3). *A. longiremis* and *M. tenuicornis* both stored triacylglycerols (TAG) whereas *A. divergens, P. minutus, P. mimus,* and *C. lividus* utilized WE at their primary storage lipid. The form of storage lipid used by a copepod reveals much about its ecology and metabolism. Whereas WEs are an energy reserve associated with large herbivorous cold-water species that undergo diapause and have annual or biannual lifecycles, TAGs are more

commonly observed in warm water or small copepod species as a quick fuel source (Kattner & Hagen 2009, Lee et al. 2006). Since species from warmer ecosystems are able to feed year-round, their life cycles tend to be shorter and they do not undergo diapause. This results in smaller lipid stores in copepods from these systems (Lee et al. 2006). Additionally, *M. tenuicornis* and *A. longiremis* are both classified as active-ambush omnivores (Venello et al. 2021), favouring life strategies that include opportunistic feeding behaviour with higher metabolic rates and lower lipid accumulation (Peters et al. 2007).

The splash-pool harpacticoid copepod *T. californicus* exhibited the lowest TL mass values (Figure 4) which can be largely attributed to its lifecycle. Growing in splash pools above the high tide line, their environment constantly fluctuates in both temperature and salinity. Additionally, they are often at risk of population extinctions via desiccation (Lear & Oppenheimer 1962). Due to the high variability within their environment, *T. californicus* has adapted a very rapid life cycle, ranging from 21-30 days, that enables them to reproduce year-round (Powlik et al. 1997). The lipid class breakdown of the splash pool seston sample and *T. californicus* shows that there is twice the amount of TAG in the copepod than the seston sample, meaning *T. californicus* is using and storing TAG to fuel its active lifestyle (Table 3).

The proportion of phospholipids, which are structural membranes, is less variable among species than are storage lipids (Table 3). The copepod species that utilize TAG as their main storage lipid, *A. longiremis* and *M. tenuicornis*, have higher phospholipid percentages. This is possibly because they have less storage lipid contributing to their overall TL (Kattner & Hagen 2009). Phospholipids often contain high levels of the essential fatty acids (EFA) 20:5ω3 (EPA) and 22:6ω3 (DHA) (Kattner & Hagen 2009, Lee et al. 2006).

Fatty acid composition of species

The fatty acid (FA) composition of the copepods examined in this study manifested into three significantly different feeding strategies (Figure 5). Cluster 1 contained most of the *A. longiremis* and *M. tenuicornis* samples, and single samples (n=1) of *T. californicus, P. minutus,* and *P. mimus.* Compared to Cluster 2 and Cluster 3, Cluster 1 had much lower PUFA values overall and high values of 16:0 and 18:0. *M. tenuicornis,* a southern species, and *A. longiremis,* a boreal shelf species, were both noticeably different from the other small copepods in this study in that they used TAG as their main storage lipid. They also contained high proportions of phospholipid (Table 3). Phospholipid FA composition in copepods is dominated by EPA, DHA, and 16:0 (Kattner and Hagen 2009), which may explain the high values of 16:0 observed, though there are variations in phospholipid FA composition among species. 16:0 is also a principal component of TAG (Albers et al. 1996). The single sample of *T. californcius* present in Cluster 1 contained much smaller individuals than the other samples (Table 24A). The splash pool where *Tigriopus* samples were retrieved may have had *T. californicus* copepods that were in different stages of their life cycles, resulting in this specific sample being compositionally different in terms of FAs. The single occurrence of *P. minutus* in Cluster 1 was collected at station P4, where samples of *M. tenuicornis* and *A. longiremis* were also collected. As the corresponding oceanographic data for P4 are not yet available, it is impossible to investigate what the oceanic conditions were like and if P4 was food limited in August 2020. The sample of *P. mimus* observed in Cluster 1 was collected at station E1 which had the overall lowest chlorophyll values. Thus, the low values of PUFA we see for Cluster 1 (Table 13; Table 14) could in part be caused by lack of access to nutritious or sufficient prey sources.

Cluster 2 was composed of almost all of the large-bodied copepod samples (Figure 5). The copepods in Cluster 2 had the highest amounts of diatom markers, 20:5ω3 (EPA) and 16:1ω7, and dinoflagellate marker 18:4ω3. These species, *N. plumchrus, N. cristatus,* and *C. marshallae,* are large, primarily herbivorous copepods that store lipid as WE for diapause and reproduction. They are also comparatively much higher in PUFA than are smaller species of copepod (Table 6; Table 7), presumably making them a more nutritious food source for higher-level consumers than PUFA-poor small copepods. The FA composition observed corresponds to values presented in the literature (Lee et al. 2006, El-Sabaawi et al. 2009).

Cluster 3 was composed of the small-bodied copepods *T. californicus*, *P. minutus, P. mimus, C. lividus,* and *A. divergens.* Almost all species of small-bodied copepods had higher proportions of OBFA making up their diet than did the large-bodied copepods (Table 7; Table 12). *P. mimus* and *P. minutus* had the highest levels of OBFA, indicating either bacteria, bacterivorous ciliates, and/or marine snow are important food sources for those species. OBFA values are not reported in the literature for these small copepod species. When OBFA values are reported for copepod species, they are for large-bodied copepods whose relative amounts of OBFA are typically small (Stevens et al. 2004a, Stevens et al. 2004b, El-Sabaawi et al. 2009). Thus, observing these high OBFA values in small-bodied copepods is a significant finding.

Carnivory, indicated by the presence of 18:1ω9, was much higher among the small-bodied copepods of Cluster 3 than the large-bodied copepods of Cluster 2 (Table 12). This indicates that the small-bodied copepods are able to feed more carnivorously as compared to the omnivorous-herbivorous large-bodied copepods of Cluster 2. Similarly, 18:1ω9 was much higher in Cluster 3 than Cluster 1, signifying differences among these two clusters of small copepod species (Table 14). Except for *M. tenuicornis,* DHA/EPA ratios were relatively similar across all

species (Table 6; Table 7; Table 8). *M. tenuicornis* had a DHA/EPA ratio of 16.77±22.4% (Table 7) which is the result of one irregular sample of *M. tenuicornis* having very little EPA. While unlikely that *M. tenuicornis* would have this DHA/EPA ratio, the sample was left in as there are no values in the literature to compare with for this species.

Absolute amounts of total fatty acid, EPA, and DHA

Total fatty acid (TFA) was highest in large-bodied copepod species, relating largely to their high levels of TL mass, excluding *N. cristatus* (Figure 6). *N. cristatus* had a low TFA mass corresponding to its low TL mass. As previously discussed, this could in part be due to the 'transparent' stage V phenomenon (Kobari & Ikeda 1999). Kobari and Ikeda (1999) observed that transparent-type stage V *N. cristatus* copepods were predominant when their environment was food limited and the copepods were poorly nourished, typically occurring following the summer growing season. The single sample of *N. cristatus* studied in this thesis was collected at station P26 in August 2020. Station P26 is located 1400 km off the coast (Figure 1) and typically exhibits trends of low productivity where smaller flagellates dominate (Peña & Varela 2007). The FA composition of *N. cristatus* had high values for dinoflagellate markers, 22:6ω3 and 18:4ω3 (Table 6), and had the highest DHA mass among large and small copepods (Figure 6). The low TL and TFA mass and higher dinoflagellate markers trend are similarly observed in the sample of *C. lividus* which was also collected at station P26. Therefore, the conditions at P26 may have also been what affected this anomalous *N. cristatus* sample.

Clusters determined from NMDS testing were found to have a significant difference in the absolute amount of TFA, EPA, and DHA. This indicates that these variables are differing due to something other than taxonomy, such as environmental condition or life strategy. As values of

TFA, EPA, and DHA mass for these copepod species off the WCVI have not yet been reported in the literature, limited comparisons can be made. For all the large-bodied copepod species, total DHA mass was observed to be lower than total EPA mass, which corresponds to recent findings by Stevens et al. (unpublished data). DHA mass appears to be less variable across species than EPA mass, with only one significant difference found between Cluster 1 and Cluster 2 (Table 16). As DHA is a dinoflagellate marker, its consistency among species could indicate that all species likely have a proportion of flagellates either supplementing or comprising the majority of their diet. The higher values of EPA in large-bodied copepod species indicates that diatoms are a major contributor to the large-copepod diet, but less important for smaller copepods.

Study limitations and future pursuits

In this study there were limited samples of several species, notably *N. cristatus* and *C. lividus* which had only one sample each. To present a more comprehensive analysis of these species, especially small copepod species about which much less is known, this study should be repeated with increased sample numbers per species to account for interspecific and intraspecific variation amongst species. For certain species of small copepods, such as *M. tenuicornis, A. divergens*, and *C. lividus*, there are little to no lipid and FA composition data available in the literature. Thus, any studies concerning the diet and life histories of these species would contribute greatly to our understanding of what influences affects them.

Species responses to increasing temperature is another important field of research. In the future, the oceans will experience new combinations of temperature, salinity, pH, and productivity. Shifts towards ocean warming and increased stratification will not only alter zooplankton community assemblages but the very base of the marine food web. Under warmer,

more stratified conditions, primary producer composition is expected to shift towards lower-lipid smaller cyanobacteria and dinoflagellates, which will likely directly impact the copepod community (Sommer et al. 2002, Peña et al. 2019). Studies have previously shown that copepod community assemblage has a strong impact on marine processes (Koski et al. 2017, Turner 2015) and consumer survival (Alvarez-Fernandez et al. 2015, Kitaysky & Golubova 2000). Shifts in community assemblage away from larger, lipid-rich copepod species, such as *C. marshallae* and *N. plumchrus*, has been directly linked to decline in fish recruitment success, such as the commercially and ecologically important Atlantic cod (Beaugrand et al. 2003) and Pacific salmon (Peterson & Schwing 2003). Despite their size, we know that small copepods are significant players within the ecosystem. With important influences on the biological carbon pump (Koski et al. 2020) and the stability and success of marine food webs (Long et al. 2011, Alvarez-Fernandez et al. 2015), solely studying the ecology and responses of large-bodied copepod species may lead to erroneous estimations of how climate change will affect the marine environment. With warming ocean conditions possibly favouring smaller copepod species (Sommer et al. 2002, Galbraith et al. 2016, Mackas et al. 2007), our understanding of these assemblages is crucial for the future. Clearly, more work needs to be undertaken on these often-dominant small copepod species about which so little is known.

Conclusions

Objective #1: Variation in small copepod species nutrition and diet

Small oceanic copepod species can be divided into two distinctly different groups in terms of lipid and fatty acid composition, indicating differences in life histories and feeding strategies. Differences were largely driven by the form of storage lipid utilized by the copepods, which reflected their ecology and metabolism. Species that stored triacylglycerols likely required a faster energy source to fuel higher metabolism, more opportunistic feeding, or a more active reproductive cycle than species storing wax esters. Two significantly different feeding strategies were observed among small copepod species. The group comprised mainly of *A. longiremis* and *M. tenuicornis* had higher proportions of 16:0 and 18:0. The other group comprised of *P. minutus, P mimus, A. divergens, C. lividus*, and *T. californicus*, had higher proportions of PUFA and 18:1ω9.

Objective #2: Variation in small copepod nutrition and diet among different environments

The intertidal harpacticoid copepod, *T. californicus*, had a very low total lipid mass which can be largely attributed to its environment. Living in highly productive tidal splash pools, *T. californicus* has a much more rapid life cycle than marine species, resulting in lower lipid storage. *T. californicus* stores triacylglycerols as its main storage lipid to fuel its active life cycle. The fatty acid composition of intertidal and small-bodied oceanic copepods was similar, with *T. californicus* associating with the small oceanic copepods that had high proportions of DHA, PUFAs, and 18:1ω9.

Objective #3: Small copepod nutrition and diet compared to larger copepod species

Large-bodied oceanic copepods differed significantly in fatty acid composition when compared to small-bodied copepods, indicating differences in feeding strategies and life histories. Wax esters were found to be present in much higher quantities in the large-bodied omnivorous-herbivorous copepods than the small-bodied copepods, likely as an adaptation to sustain themselves through diapause and their annual reproductive cycles. Almost all species of small copepods were found to have a higher proportion of bacterial fatty acid markers than large-bodied copepods, indicating that they are more connected to the microbial loop. Conversely, large-bodied copepods were richer in diatom and dinoflagellate markers, in addition to total PUFA.

Objective #4: Compare abundance trends over a 30-year period

Abundance anomalies were found to be strongly influenced by temperature and warm water events on the west coast of Vancouver Island. Subarctic and boreal shelf species biomass anomaly trends were broadly similar, indicating they likely have similar life history patterns and tolerances to environmental stress. Large, lipid-rich cold-water species were overall negatively impacted by warm-water events. Following El Niño events, southern species were observed to experience increases in abundance as subarctic and boreal shelf species declined.

References

Albers CS, Kattner G, Hagen W (1996) The composition of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: evidence of energetic adaptations. Marine Chemistry 55: 347–358

Alvarez-Fernandez S, Licandro P, van Damme CJG, Hufnagl M (2015) Effect of zooplankton on fish larval abundance and distribution: a long-term study on North Sea herring (*Clupea harengus*). ICES Journal of Marine Science 72: 2569–2577

Arendt KE, Juul-Pedersen T, Mortensen J, Blicher ME, Rysgaard S (2013) A 5-year study of seasonal patterns in mesozooplankton community structure in a sub-Arctic fjord reveals dominance of *Microsetella norvegica* (Crustacea, Copepoda). Journal of Plankton Research 35: 105–120

Beaugrand G, Brander KM, Lindley JA, Souissi S, Reid PC (2003) Plankton effect on cod recruitment in the North Sea. Nature 426: 661–664

Bond AR, Cronin MF, Freeland H, Mantua N (2015) Causes and impacts of the 2014 warm anomaly in the NE Pacific. Geophysical Research Letters 42: 3414–3420

Budge SM, Parrish CC (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Organic Geochemistry 29: 1547–1559

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117–143

Conover RJ (1988) Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. Hydrobiologia 167: 127–142

Couturier LIE, Michel LN, Amaro T, Budge SM, da Costa E, De Troch M, Di Dato V, Fink P, Giraldo C, Le Grand F, Loaiza I, Mathieu-Resuge M, Nichols PD, Parris CC, Sardenne F, Vagner M, Pernt F, Soudant P (2020) State of art and best practices for fatty acid analysis in aquatic sciences. ICES Journal of Marine Science 77: 2375–2395

Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46: 225–340

Dugas JC, Koslow JA (1984) *Microsetella norvegica*: a rare report of a potentially abundant copepod on the Scotian Shelf. Marine Biology 84: 131–134

Durbin EG, Durbin AG, Smayda TJ, Verity PG (1983) Food limitation of production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. Limnology and Oceanography 6: 1199–1213

El-Sabaawi R, Dower JF, Kainz M, Mazumder A (2009) Characterizing dietary variability and trophic positions of coastal calanoid copepods: insight from stable isotopes and fatty acids. Marine Biology 156: 225–237

El-Sabaawi R, Sastri AR, Dower JF, Mazumder A (2010) Deciphering the seasonal cycle of copepod trophic dynamics in the Strait of Georgia, Canada, using stable isotopes and fatty acids. Estuaries and Coasts 33: 738–752

Evanson M, Bornhold E, Goldblatt R, Harrison P, Lewis A (2000) Temporal variation in body composition and lipid storage of the overwintering, subarctic copepod *Neocalanus plumchrus* in the Strait of Georgia, British Columbia (Canada). Marine Ecology Progress Series 192: 239–247

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226: 497–509

Galbraith M, Young K, Perry I (2016) Zooplankton along the BC continental margin 2015. Canadian Technical Report of Fisheries and Aquatic Sciences 2179: 64–71

Galloway AWE, Winder M (2015) Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. PLOS one 10: 1–23

Greene C, Pershing AJ (2004) Climate and the conservation biology of North Atlantic right whales: the right whale at the wrong time? Frontiers in Ecology and the Environment 2: 29–34

Hairston NG, Hairston NG (1993) Cause-effect relationships in energy flow, trophic structure, and interspecific interactions. The American Naturalist 142: 379–411

Hays GC, Richardson AJ, Robinson C (2005) Climate change and marine plankton. TRENDS in Ecology and Evolution 20: 337–334

Hazel JR, Williams EE (1990) The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Progress in Lipid Research 29: 167–227

Humes AG (1994) How many copepods? Hydrobiologia 292: 1–7

Iverson SJ, Field C, Bowen WD, Blanchard W (2004) Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs 74: 211–235

Kämpf J, Chapman P (2016) The California Current Upwelling System. In: Upwelling Systems of the World. pp. 97-160

Kattner G, Hagen W (2009) Lipids in marine copepods: latitudinal characteristics and perspective to global warming. In: Arts MT, Brett MT, Kainz MJ (eds), Lipids in Aquatic Ecosystems. pp 257–280

Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series 446: 1–22

Kiørboe T (2011) How zooplankton feed: mechanisms, traits and trade-offs. Biological Reviews 86: 311–339

Kiørboe T, Sabatini M (1994) Reproductive and life cycle strategies in egg-carrying cyclopoid and free-spawning calanoid copepods. Journal of Plankton Research 16: 1353–1366

Kitaysky AS, Golubova EG (2000) Climate change causes contrasting trends in reproductive performance of planktivorous and piscivorous alcids. Journal of Animal Ecology 69: 248–262

Kleppel GS (1993) On the diets of calanoid copepods. Marine Ecology Progress Series 99: 183–195

Kobari T, Ikeda T (1999) Vertical distribution, population structure and life cycle of *Neoalanus cirstatus* (Crustacea: Copepoda) in the Oyashio region, with notes on its regional variations. Marine Biology 134: 683–696

Koski M, Boutorh J, de la Rocha C (2017) Feeding on dispersed vs. aggregated particles: the effect of zooplankton feeding behavior on vertical flux. PLOS one 12: 1–17

Koski M, Valencia B, Newstead R, Thiele C (2020) The missing piece of the upper mesopelagic carbon budget? Biomass, vertical distribution and feeding of aggregate-associated copepods at the PAP site. Progress in Oceanography 181: 1–9

Lear DW, Oppenheimer C (1962) Consumption of microorganisms by the copepod *Tigriopus californicus*. Limnology and Oceanography 7: lxiii-lxv

Lee RF, Hirota J (1973) Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. Limnology and Oceanography 18: 227–239

Lee RF, Hirota J, Barnett AM (1971a) Distribution and importance of wax esters in marine copepods and other zooplankton. Deep-Sea Research 18: 1147–1165

Lee RF, Nevenzel JC, Paffenhöfer GA (1971b) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Marine Biology 9: 99–108

Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Marine Ecology Progress Series 307: 273–306

Legendre L, Rassoulzadegan F (1995) Plankton and nutrient dynamics in marine waters. Ophelia 41: 153–172

Long ZT, Bruno JF, Duffy JE (2011) Food chain length and omnivory determine the stability of a marine subtidal food web. Journal of Animal Ecology 80: 586–594

Lough RG, Mountain DG (1996) Effect of small-scale turbulence on feeding rates of larval cod and haddock in stratified water on Georges Bank. Deep-Sea Research II 43: 1745–1772

Mackas DL, Galbraith M (2002) Zooplankton community composition along the inner portion of Line P during the 1997-1998 El Niño event. Progress in Oceanography 54: 423–437

Mackas DL, Thomson RE, Galbraith M (2001) Changes in zooplankton community of the British Columbia continental margin, 1985-1999, and their covariation with oceanographic conditions. Canadian Journal of Fisheries and Aquatic Sciences 58: 685–702

Mackas DL, Peterson WT, Zamon JE (2004) Comparisons of interannual biomass anomalies of zooplankton communities along the continental margins of British Columbia and Oregon. Deep-Sea Research II 51: 875–896

Mackas DL, Batten S, Trudel M (2007) Effects on zooplankton of a warmer ocean: Recent evidence from the Northeast Pacific. Progress in Oceanography 75: 223–252

Michels J, Schnack-Schiel SB (2005) Feeding in dominant Antarctic copepods–does the morphology of the mandibular gnathobases relate to diet? Marine Biology 146: 483–495

Middelbo AB, Møller EF, Arendt KE, Thyrring J, Sejr MK (2019) Spatial, seasonal and inter-annual variation in abundance and carbon turnover of small copepods in Young Sound, Northeast Greenland. Polar Biology 42: 179–193

Miller JA, Peterson WT, Copeman LA, Du X, Morgan CA, Litz MNC (2017) Temporal variation in the biochemical ecology of lower trophic levels in the Northern California Current. Progress in Oceanography 155: 1–12

Nielsen TG, Andersen CM (2002) Plankton community structure and production along a freshwater-influenced Norwegian fjord system. Marine Biology 141: 707–72

Oksanen J, Guillaume Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MH, Szoecs E, Wagner H (2019) Vegan: Community Ecology Package. R package version 2.5-4

Parrish CC (1987) Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Canadian Journal of Fisheries and Aquatic Sciences 44: 722–731

Parrish CC (1988) Dissolved and particulate marine lipid classes: a review. Marine Chemistry 23: 17–40

Parrish CC (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: Arts MT, Wainman BC (eds), Lipids in freshwater ecosystems. pp 4–20

Peña MA, Varela DE (2007) Seasonal and interannual variability in phytoplankton and nutrient dynamics along Line P in the NE subarctic Pacific. Progress in Oceanography 75: 200–222

Peña MA, Nemcek N, Robert M (2019) Phytoplankton responses to the 2014-2016 warming anomaly in the northeast subarctic Pacific Ocean. Limnology and Oceanography 64: 515–525

Peters J, Dutz J, Hagen W (2007) Role of essential fatty acids on the reproductive success of the copepod *Temora longicornis* in the North Sea. Marine Ecology Progress Series 341: 153–163

Peterson WT, Schwing FB (2003) A new climate regime in northeast Pacific ecosystems. Geophysical Research Letters 30: 1–4

Pomerleau C, Sastri AR, Beisner B (2015) Evaluation of functional trait diversity for marine zooplankton communities in the Northeast subarctic Pacific Ocean. Journal of Plankton Research 37: 712–726

Powlik JJ, Lewis AG, Spaeth M (1997) Development, body length, and feeding of *Tigriopus californicus* (Copepoda, Harpacticoida) in laboratory and field populations. Crustaceana 70: 324–343

Richardson AJ (2008) In hot water: zooplankton and climate change. ICES Journal of Marine Science 65: 279–295

R Core Team (2020) R: A language and environment for statistical computing.

Romano P, Feletti M, Mariottini GL, Carli A (1999) Ecological and nutritional implications of the mandibular structure in the Antarctic calanoid copepod *Metridia gerlachei* Giesbrecht, 1902: an ultrastructural study. Polar Biology 22: 7–12

Saito J, Kotani Y (2000) Lipids of four boreal species of calanoid copepods; origin of monoene fats of marine animals at higher trophic levels in the grazing food chain in the subarctic ocean ecosystem. Marine Chemistry 71: 69–82

Sommer U, Stibor H, Katechakis A, Sommer F, Hansen T (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production: primary production. Hydrobiologia 484: 11–20

Sprules WG, Bowerman JE (1988) Omnivory and food chain length in zooplankton food webs. Ecology 69: 418–426

Stevens CJ, Deibel D, Parrish CC (2004a) Species-specific differences in lipid composition and omnivory indices in Arctic copepods collected in deep water during autumn (North Water Polynya). Marine Biology 144: 905–915

Stevens CJ, Deibel D, Parrish CC (2004b) Incorporation of bacterial fatty acids and changes in a wax ester-based omnivory index during a long-term incubation experiment with *Calanus glacialis* Jaschnov. Journal of Experimental Marine Biology and Ecology 303: 135–156

Thomson RE (1981) Oceanography of the British Columbia coast. Dept. of Fisheries and Oceans, Ottawa

Timmermann A, An SI, Kug JS, Jin FF, Cai W, Capotondi A, Cobb KM, Lengaigne M, McPhaden MJ, Stuecker MF, Stein K, Wittenberg AT, Yun KS, Bayr T, Chen HC, Chikamoto Y, Dewitte B, Dommenget D, Grothe P, Guilyardi E, Ham YG, Hayashi M, Ineson S, Kang D, Kim S, Kim WM, Lee JY, Li T, Luo JJ, McGregor S, Planton Y, Power S, Rashid H, Ren HL, Santoso A, Takahashi K, Todd A, Wang G, Wang G, Xie R, Yang WH, Yeh SQ, Yoon J, Zeller E, Zhang X (2018) El Niño-Southern Oscillation complexity. Nature 559: 535–545

Toyes-Vargas EA, Parrish CC, Viana MT, Carreón-Palau L, Magallón-Servín P, Magallón-Barajas FJ (2020) Replacement of fish oil with camelina (*Camelina sativa*) oil in diets for juvenile tilapia (var. GIFT *Oreochromis niloticus*) and its effect on growth, feed utilization and muscle lipid composition. Aquaculture 523: 1–11

Tseng YH, Ding R, Huang X (2017) The warm Blob in the northeast Pacific - the bridge leading to the 2015/16 El Niño. Environmental Research Letters 12: 1–9

Turner JT (2004) The importance of small planktonic copepods and their roles in pelagic marine food webs. Zoological studies 43: 255–266

Turner JT (2015) Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. Progress in Oceanography 130: 205–248

Turner JT, Dagg MJ (1983) Vertical distribution of continental shelf zooplankton in stratified and isothermal waters. Biological Oceanography 3: 1–40

Turner JT, Tester PA, Hettler WF (1985) Zooplankton feeding ecology. A laboratory study of predation on fish eggs and larvae by the copepods *Anomalocera ornata* and *Centropages typicus*. Marine Biology 90: 1–8

Uye S, Aoto I, Onbé T (2002) Seasonal population dynamics and production of *Microsetella norvegica*, a widely distributed but little-studied marine planktonic harpacticoid copepod. Journal of Plankton Research 24: 143–153

Venello TA, Sastri AR, Galbraith MD, Dower JF (2021) Zooplankton functional group responses to environmental drivers off the west coast of Vancouver Island, Canada. Progress in Oceanography 190: 1–14

Wilson SE, Steinberg DK, Chu FLE, Bishop JKB (2010) Feeding ecology of mesopelagic zooplankton of the subtropical and subarctic North Pacific Ocean determined with fatty acid biomarkers. Deep-Sea Research I 57: 1278–1294

Winder M, Jassby AD (2011) Shifts in zooplankton community structure: implications for food web processes in the upper San Francisco Estuary. Estuaries and Coasts 34: 675–690

Yamada Y, Nishida S, Graeve M, Kattner G (2016) Lipid and fatty acid/alcohol compositions of the subarctic copepods *Neocalanus cristatus* and *Eucalanus bungii* form various depths in the Oyashio region, western North Pacific. Comparitive Biochemistry and Physiology B 198: 57–65

Appendix A: Cluster dendrogram and additional statistical tests

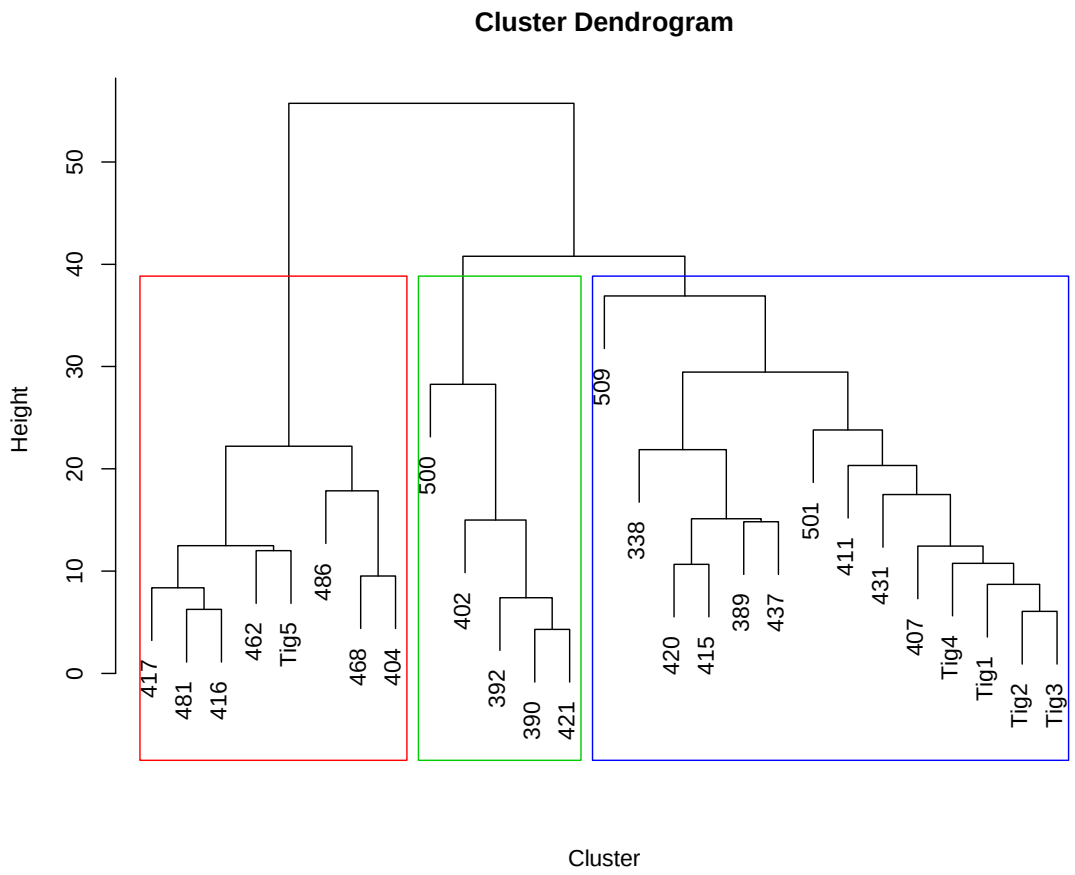

Figure 7A. Cluster dendrogram produced using hierarchical average-neighbour cluster analysis based on rank similarities of a Bray-Curtis dissimilarity matrix of fatty acid data. Numbers represent arbitrary sample numbers. Cluster 1 is depicted in red, Cluster 2 is depicted in green, and Cluster 3 is depicted in blue. Clusters were determined to be significantly different by an ADONIS test (Table 10).

Table 17A. Results of individual Kruskal-Wallis tests used to determine whether total lipid mass (g g^{-1} WM) differed significantly among large-bodied copepod species, small-bodied copepod species, and all copepod species. P-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

	Comparison	**p-value**	**p adj.**
Total lipid (g g^{-1} WM)	Large-bodied copepod species	0.39	0.39
	Small-bodied copepod species	0.061	0.061
	All species	0.040	**0.040**

Table 18A. Results of a Dunn's test to determine which species were significantly different from each other in terms of total lipid mass (g g^{-1} WM). Only species that were significantly different are shown. P-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

Comparison	Z	p-value	p adj.
C. marshallae - T. californicus	3.30	<0.001	**0.044**
N. plumchrus - T. californicus	3.40	<0.001	**0.030**

Table 19A. Results of individual Kruskal-Wallis tests used to determine whether total fatty acid mass (g g^{-1} WM), total EPA mass (g g^{-1} WM) or total DHA mass (g g^{-1} WM) differed significantly among large-bodied, small-bodied, and intertidal copepod groups. EPA and DHA are the fatty acids 20:5ω3 and 22:6ω3, respectively. P-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

	p-value	p adj.
Total fatty acid (g g^{-1} WM)	<0.001	**<0.001**
Total EPA (g g^{-1} WM)	0.032	**0.032**
Total DHA (g g^{-1} WM)	0.0031	**0.0031**

Table 20A. Results of individual Dunn's tests to determine which groups of copepod species, large-bodied, small-bodied, or intertidal were significantly different in terms of total fatty acid mass (g g^{-1} WM), total EPA mass (g g^{-1} WM) or total DHA mass (g g^{-1} WM). EPA and DHA are the fatty acids 20:5ω3 and 22:6ω3, respectively. P-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

	Comparison	Z	p-value	p adj.
Total fatty acid (g g^{-1} WM)	Large – Intertidal	3.88	<0.001	**<0.001**
	Large – Small	2.03	0.43	0.13
	Intertidal – Small	-2.60	0.0093	**0.028**
Total EPA (g g^{-1} WM)	Large – Intertidal	2.44	0.015	**0.044**
	Large – Small	2.13	0.033	0.10
	Intertidal – Small	-0.88	0.38	1.00
Total DHA (g g^{-1} WM)	Large – Intertidal	3.18	0.0015	**0.0044**
	Large – Small	2.69	0.0071	**0.021**
	Intertidal – Small	-1.22	0.22	0.66

Table 21A. Results of individual Kruskal-Wallis tests used to determine whether total fatty acid mass (g g^{-1} WM), total EPA mass (g g^{-1} WM) or total DHA mass (g g^{-1} WM) differed significantly among species. P-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

	p-value	**p adj.**
Total fatty acid (g g^{-1} WM)	0.043	**0.043**
Total EPA (g g^{-1} WM)	0.19	0.19
Total DHA (g g^{-1} WM)	0.049	0.05

Table 22A. Results of a Dunn's test to determine which species were significantly different from each other in terms of total fatty acid mass (g g^{-1} WM). Only species that were significantly different are shown. P-values were adjusted using the Bonferroni method; a p adj. value <0.05 is considered significant and is shown in bold.

Comparison	**Z**	**p-value**	**p adj.**
C. marshallae - T. californicus	3.35	<0.001	**0.036**
N. plumchrus - T. californicus	3.33	<0.001	**0.039**

Appendix B: Fatty acid biomarker abbreviations and species samples

Table 23A. Fatty acid biomarker abbreviations used in Tables 6 to 8. db = double bond.

Biomarker abbreviation	Sum fatty acids
ΣSFA	Sum of saturated fatty acids (0 dbs)
ΣMUFA	Sum of monounsaturated fatty acids (1 db)
ΣDiene	Sum of diunsaturated fatty acids (2 dbs)
ΣPUFA	Sum of polyunsaturated fatty acids (≥3 dbs)
ΣDino	18:4ω3 + 22:6ω3 (DHA)
ΣDiatom	16:1ω7 + 20:5ω3 (EPA)
OBFA	ι15:0, αι15:0, 15:1, ι16:0, αι16:0, ι17:0, αι17:0, 17:0, 17:1

Table 24A. Descriptions of copepod species samples, including station, depth, date, and reproductive stage. ‘f’ denotes female stage VI, ‘v’ denotes juvenile stage V.

Species	Sample	Station	Depth	Date	Cruise	Stage
Acartia longiremis	486	LB07	0-59	23/06/2020	2020-005	f
	404	CS10	0-33	06/07/2020	2020-005	f
	462	P4	0-250	14/08/2020	2020-008	f
Aetideus divergens	407	E1	0-44	29/06/2020	2020-005	v/f
	415	HC	0-99	29/06/2020	2020-005	v/f
Calanus marshallae	390	22	0-144	18/06/2020	2020-005	v
	392	P4	0-250	14/08/2020	2020-008	v
	411	CS10	0-33	06/07/2020	2020-005	f
	421	LB07	0-59	23/06/2020	2020-005	v
Clausocalanus lividus	431	P26	0-250	20/08/2020	2020-008	f
Mesocalanus tenuicornis	501	LC12	0-103	24/06/2020	2020-005	f
	417	LC12	0-103	24/06/2020	2020-005	f
	468	P4	0-250	14/08/2020	2020-008	f
Neocalanus cristatus	402	P26	0-250	20/08/2020	2020-008	v
Neocalanus plumchrus	500	22	0-144	18/06/2020	2020-005	v
	509	LC12	0-103	24/06/2020	2020-005	v
Pseudocalanus mimus	420	LB07	0-59	23/06/2020	2020-005	f
	416	E1	0-44	29/06/2020	2020-005	f
	437	CS10	0-33	06/07/2020	2020-005	f
Pseudocalanus minutus	388	HC	0-99	29/06/2020	2020-005	v
	389	22	0-144	18/06/2020	2020-005	v
	481	P4	0-250	14/08/2020	2020-008	v
Tigriopus californicus	Tig 1	Baynes Beach	-	22/10/2020	-	-
	Tig 2	Baynes Beach	-	22/10/2020	-	-

Tig 3	Baynes Beach	-	22/10/2020	-	-
Tig 4	Baynes Beach	-	22/10/2020	-	-
Tig 5	Baynes Beach	-	22/10/2020	-	*

*Tig 5 sample had smaller *T. californicus* individuals than other samples

Table 25A. Station latitude and longitude.

Station	**Latitude**	**Longitude**
22	49.67	-124.2717
E1	48.478	-125.36833
HC	48.25	-126.66667
CS10	51.275	-128.33333
LB07	48.528333	-125.06333
LC12	50	-145
P4	48.65	-126.66667
P26	49.863783	-126.7441
Baynes Beach	48.4537	-123.266