Trophic Dynamics of Copepods in the Strait of Georgia

by

Rana El-Sabaawi
BSc, University of Western Ontario, 1999
MSc, University of British Columbia, 2002

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Abstract

Although food quality is thought to play an important role in the survival of marine copepods, the extent of natural variability in food quality remains poorly characterized. Here I characterize the different scales at which food quality varies in copepods of the Strait of Georgia, British Columbia, Canada. Significant interannual variability occurs in the diet of *Neocalanus plumchrus* in the Strait of Georgia. Between 2001-06 the fatty acid profiles of *N. plumchrus* switched from omnivorous, oceanic signatures to herbivorous, diatom-dominated signatures. An index of food quality (DHA/EPA) is strongly correlated to the abundance of diapausing *N. plumchrus*, suggesting that the relative proportion of essential fatty acids provided by dinoflagellates and diatoms are related to the survival of this species. Combined fatty acid and stable isotope analysis indicated that the spring calanoid copepods of the Strait of Georgia occupy three trophic positions: *Eucalanus bungii* is herbivorous, *Calanus marshallae* and *N. plumchrus* are omnivorous, while *Euchaeta elongata* is carnivorous. Oceanic conspecifics of Strait of Georgia copepods experience a more omnivorous diet, as indicated by the presence of higher proportions of flagellate and carnivory markers, and lower proportions of diatom-based markers in their fatty acids. Despite spatial differences in the quality of their diets, the relative trophic positions of these copepods are constant as indicated by their stable isotope signatures. There is a correlation between the trophic information provided by

stable isotopes and fatty acids. However, stable isotopes are not sensitive enough to capture the range of dietary variability observed in fatty acids, and fatty acids do not always provide reliable markers of carnivory and trophic position. Over the span of a season, copepods can utilize a wide range of dietary items including diatoms, flagellates, bacteria, detritus and microzooplankton. Copepods can switch from herbivory to carnivory in response to declining chlorophyll concentrations after the spring bloom, and are occasionally able to utilize detrital and bacterial sources. I conclude that the quality of copepod diets in the SoG varies on interannual, interspecific and seasonal scales. The implications of these findings are discussed in relation to ecosystem models of the area, and to copepod physiology.

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Dedication

This thesis is dedicated to my grandparents, Ramziah and Hussein, and my niece and nephew, Nusayba and Khaled.

Chapter1: Introduction

1.1 General Introduction

Copepods represent a key trophic link in marine ecosystems. Not only are they important prey items for higher trophic levels, they are also important mediators of vertical carbon flux in many parts of the ocean. Copepods have the ability to exploit a wide range of prey including phytoplankton, bacteria, marine snow, copepod nauplii, eggs and microzooplankton (Kleppel et al. 1991, Kleppel 1993). Different aspects of dietary quality (e.g. diet composition, nitrogen and carbon content per unit diet) have been shown to strongly affect copepod growth and reproduction both in the field and the laboratory (Arendt et al. 2005, Jónasdóttir et al. 1994). The evidence for this aspect of copepod nutrition remains controversial and inconsistent, however, and is thus far from being resolved. Changes in patterns of copepod feeding have also been shown to affect the quality of diet available to higher consumers, and the abundance of phytoplankton and microzooplankton in the ocean (St. John et al. 2001, Stibor et al. 2004).

This thesis aims to characterize the degree of omnivory in the calanoid copepods of the Strait of Georgia, Canada. In particular, this thesis assesses the different scales of variability in copepod omnivory (interannual, interspecific and seasonal) by using a combination of stable isotopes and fatty acids. The goal of this work is to provide dietary information which can assist in the development of ecosystem models for the region (and other similar coastal systems), which to date, have assumed that copepods are largely

herbivorous, utilizing only phytoplankton (e.g. Li et al. 2000, Parsons and LeBrasseur 1970). Biochemical indices of trophic dynamics can provide information on how the composition of copepod diets change seasonally and interannually, and can help to assess whether changes in dietary availability cause copepods to switch their foraging tactics. Understanding the extent to which copepods utilize the different sources of carbon available to them is crucial to our understanding of ecosystem function because variability in copepod feeding can alter patterns of carbon transfer in marine ecosystems.

1.2 Copepod omnivory: prevalence and characterization

Copepod omnivory depends on mandible morphology, foraging tactics and prey availability. Carnivorous copepods have lightly-packed spiny mouth parts suitable for puncturing prey, while herbivorous copepods have densely-packed setose mouth parts better suited for crushing diatoms. Omnivores have a morphology that is somewhere in between these extremes (Mauchline 1998). The distance between setae in the mouthparts determines the range of particle sizes that copepods can filter and ingest, and is usually wider in omnivorous copepods than in herbivorous species (Michels and Schnak-Shiel 2005). Herbivorous and omnivorous copepods use chemoreception to detect food particles, which they capture by generating feeding currents, while carnivorous species employ mechanoreception and hunt actively for their prey (Jiang and Osborn 2004). However, morphology alone does not predict trophic classification of copepods because many herbivorous and omnivorous species have also been shown to be opportunistic

carnivores (e.g. Landry 1981). Although some copepods are able to actively select their food based on the size of the particle ingested, mechanoreception of prey movement, or on chemosensory recognition of biochemical cues (Poulet and Marsot 1978, Atkinson 1995), in others the quality of the ingested diet mirrors local phytoplankton composition (e.g. Stevens et al. 2004a).

Food quality varies significantly in the oceans. Large gradients in phytoplankton community composition and nutrient limitation occur over geographic and temporal scales. Coastal margins tend to be more productive than oceanic gyres, but the latter usually contain high concentrations of microzooplankton, which have been shown to be quite nutritious for copepods (Klein-Breteler et al. 1999). The degree of omnivory in copepods is best described by methods which can quantify or assess the contribution of different dietary sources to the overall nutrition of the animal. Traditional methods of assessing dietary source and trophic position have relied on gut content analysis (Mauchline 1998, Perissinotto et al. 2000). Such techniques can be misleading, however, because organisms with harder shells take longer to digest than do soft-shelled organisms, and therefore tend to be over-represented in the guts of their predators (Perissinotto et al. 2000, Gurney et al. 2001, Schmidt et al. 2006). In recent years, the characterization of omnivory in copepods has benefited from the use of biochemical tracers of dietary quality such as stable isotopes and fatty acids, which provide information on assimilated diets, rather than ingested diets (Minagawa and Wada 1984, Dalsgaard et al. 2003).

1.2.1 Stable isotopes as trophic tracers

The use of stable isotopes of nitrogen and carbon ($\delta^{15}N$ and $\delta^{13}C$, respectively) in tracing trophic dynamics has become quite common (Gannes et al. 1997, Post 2002a). This is based on the observation that $\delta^{15}N$ is progressively enriched in a predictable fashion with increasing trophic level (by ~ 3.5 ‰), whereas $\delta^{13}C$ does not vary appreciatively between diet and consumer (McConnaughey and McRoy 1979, Minagawa and Wada 1984). Therefore, $\delta^{15}N$ can be used to calculate trophic position, whereas $\delta^{13}C$ can be used to assess the contribution of different dietary sources to the diet of an animal. Trophic position (Δ) is often measured as $\Delta = (\delta^{15}N_{consumer} - \delta^{15}N_{prey})/3.5 + \lambda$, where $\delta^{15}N_{consumer}$ is the stable nitrogen signature of the consumer, $\delta^{15}N_{prey}$ is the nitrogen signature of the prey (also known as the dietary baseline) and λ is the trophic position of that prey (Post 2002a).

The use of stable isotopes to study trophic dynamics is attractive because they are relatively inexpensive, easy to measure, and because their use allows the quantification of ecological concepts (e.g. foodchain length), which can then be tested statistically or experimentally (e.g. Post 2002b). Although stable isotopes have been used extensively to monitor trophic dynamics in the oceans (e.g. Sato et al. 2002), the interpretation of stable isotope data can be hindered by several technical issues. For example, while the average trophic fractionation is ~ 3.5 ‰, a wide range of variability in trophic fractionation is often observed in nature (Vander Zanden and Rasmussen 2001). Moreover, the accurate assessment of Δ depends on accurate measurements of $\delta^{15}N_{prey}$ on a time-scale relevant to

the consumer's turnover rate (Post 2002a). For example, in order to calculate the trophic position of a herbivorous copepod, the "baseline" isotopic signature of local phytoplankton community is required. However, pure phytoplankton samples are impossible to obtain in the field because they are difficult to separate from other organic particles of similar size. Instead, particulate organic matter (POM) is often sampled as a phytoplankton surrogate. However, POM is composed of a heterogeneous mixture of organic materials, and only in high chlorophyll regions does it approach the true signature of pure phytoplankton. In addition, POM measurements (which are often made at the same time that zooplankton are sampled) may not represent a true baseline for zooplankton because POM turns over on shorter time-scales than do mesozooplankton.

Another problem with the use of stable isotopes is that physiology can have a strong impact on the isotopic composition of an animal, regardless of its trophic position or dietary history. For example, lipids are isotopically lighter than other tissues because the enzymes involved in lipid synthesis fractionate in favour of lighter isotopes (Deniro and Epstein 1978, Post et al. 2007). Therefore, samples with variable lipid content can display differences in $\delta^{13}C$ that are unrelated to feeding history. This is especially true for high latitude copepods which often store large lipid reserves (e.g. Sato et al. 2002). To date, there is no consensus on whether stable isotope samples should be corrected for lipid content. However, samples with heterogeneous lipid content can be standardized using either chemical extraction, which physically removes lipids from the sample, or via mathematical modelling based on statistical relationships between stable isotopes and proxies of lipid content (e.g. C/N ratios) (McConnaughey and McRoy 1979, Post et al.

2007). Regardless of the problems associated with using stable isotopes, they have proven quite useful in elucidating trophic dynamics in the oceans, especially when used in conjunction with other tracers such as fatty acids (e.g. Alfaro et al. 2006).

1.2.2 Fatty acids as trophic tracers

The use of fatty acids as trophic tracers in marine copepods has also become increasingly popular (Dalsgaard et al. 2003). Fatty acids are carbon rich molecules that are required for structural support and energy storage. Marine copepods have a limited capacity to synthesize fatty acids, however, and as such have to rely on their diet to supply them with many of these important compounds (Graeve et al. 2005, Bell et al. 2007). Phytoplankton, microzooplankton, bacteria and terrestrial detritus all contain fatty acid markers which are retained by their copepod predators, and which can therefore be used to qualitatively assess the sources of diets (Dalsgaard et al. 2003).

All organisms are able to synthesize fatty acids through the enzyme acetyl-coA, also known as fatty acid synthetase I (unless otherwise noted, all information for this paragraph was summarized from Dalsgaard et al. 2003). The most common product of this pathway is palmitic acid (16:0[1]), but saturated fatty acids up to 20 carbons are also produced. From these saturates, almost all organisms are able to produce monounsaturated fatty acids which contain a single double bond. End products of this

[1] The naming convention of fatty acids employed here is X:Yn-Z, where X is the number of carbon atoms, y is the number of double bonds, and Z is the position of the double bond from the carboxyl end of the molecule.

pathway are usually taxon-specific, and can thus be used to infer trophic relations. For example, some calanoid copepods (e.g *Neocalanus* and some *Calanus* spp.) produce monunsaturated fatty acids containing 20-22 carbons in length, which can be used to infer feeding on copepods by fish (Saito and Murata 1998, Saito and Kotani 2000, Scott et al. 2002). Only plants and phytoplankton are able to extend this pathway to synthesize the polyunsaturated fatty acids that are required for growth and reproduction in all animals. Patterns of fatty acid synthesis are dictated by evolution, therefore phytoplankton and plants that share a common ancestor usually produce similar fatty acids. For example, green algae contain high proportions of 18:3n-3 and 18:3n-6, which are also a significant component of the lipids of terrestrial plants. Some animals are capable of interconverting polyunsaturated fatty acids, but the rates associated with this process are not high enough to supply growth requirements (Graeve et al. 2005, Bell et al. 2007).

Diatoms are the most important phytoplankton group in coastal systems, and usually support productive and efficient foodwebs. However, in recent years the quality of diatoms as a diet for copepods has been questioned (Ianora et al. 2003). In the laboratory, diatom-rich diets have been shown to hinder hatching success and naupliar viability (Ianora et al. 1996). However, it remains unclear why diatom ingestion does this. Some studies have suggested that nutrient-limited diatoms produce polyunsaturated aldehydes that are toxic to copepods (Tosti et al. 2003). Others have suggested that diatoms are deficient in certain essential nutrients that are required for growth and reproduction (Arendt et al. 2005). The extent to which the diatom effect occurs in the field is poorly-

understood, and what research there has been on the issue has provided inconclusive (and often contradictory) data (Irigoien et al. 2002).

Diatoms are often characterized by high concentrations of 16:1n-7, 20:5n-3 and polyunsaturated fatty acids which contain 16 carbons (16PUFA)(Thompson et al. 1992, Viso and Marty 1993). The fatty acid 20:5n-3, also called eicosapentaenoic acid (or EPA) is an essential fatty acid required for copepod growth and reproduction (Brett and Müller-Navarra 1997)(Diagram 1). Dinoflagellates are another important group of phytoplankton in coastal regions. Some dinoflagellates form blooms which can be either toxic or noxious to higher trophic levels. The ingestion of toxic dinoflagellates is harmful to copepods (e.g. Ianora et al. 2004), but dinoflagellates are also rich in the fatty acid 22:6n-3 (also known as docosahexaenoic acid or DHA)(Diagram 1), which is an essential fatty acid required by copepods for structural support and reproduction (Thompson et al. 1992, Viso and Marty 1993, Arendt et al. 2005).

The relative proportion of DHA to EPA is often used as an indicator of the relative proportion of dinoflagellate to diatoms in copepod diets (Dalsgaard et al. 2003). Because DHA is preferentially retained by higher trophic levels in marine foodwebs, it can also be used to infer trophic position (e.g. Veefkind 2003). Recent studies have shown this ratio to be positively related to several indices of copepod reproduction and growth, suggesting that a balance between these two essential fatty acids in the diet has to be achieved for the survival of copepods (e.g. Arendt et al. 2005). The proportion of DHA to EPA in the polar lipids of marine organisms is homeostatic under tight genetic control, and is thought

to affect the organism's mobility, nerve function and membrane stability at low-temperature (Albers et al. 1996, Scott et al. 2002 and references therein).

In general, flagellates are rich in polyunsaturated fatty acids containing 18 carbons (18PUFA)(Viso and Marty 1993). The ratio of 16PUFA to 18PUFA is thus used to indicate the relative proportion of diatoms to flagellates in the diet (e.g. Alfaro et al. 2006). However, because some algae share a common ancestor with terrestrial plants, 18PUFA markers can also be found in terrestrial detritus (Budge and Parrish 1998). Marine bacteria have been shown to contain high levels of branched (*iso* and ante-*iso*) saturated and monounsaturated fatty acids containing 15-17 carbon atoms (Kaneda 1991). The proportions of these markers in the lipids of copepods can be used to indicate feeding on bacterial aggregates, marine snow or bacterivorous microzooplankton (e.g. Stevens et al. 2004b,c). Several markers of carnivory also exist, and those are discussed in the introduction to Chapter 3.

Preparing fatty acid samples for analysis is expensive and time-consuming. Because the fatty acid content of a single copepod is often below the level of detection for most instruments, many individuals have to be pooled for each replicate. Therefore, sample number is limited by both the availability of copepods and by the expense of sample processing. Moreover, the signatures of some trophic markers can be obscured by either copepod physiological processes or by contamination from other sources. For example, although the fatty acid marker 16:1n-7 is commonly used as a diatom marker, it is a bi-product of *de novo* fatty acid synthesis created by the desaturation and elongation of 16:0.

Therefore, for 16:1n-7 to be effective as a diatom maker, it either has to be expressed in relation to 16:0 or the use of the marker has to be restricted to samples of the same species. To make matters more complicated, this marker is also produced by bacteria, which can confound diatom signatures in oligotrophic regions of the ocean (e.g. Stevens et al., in press). In summary, the interpretation of fatty acid trophic markers can be subjective and requires great care. Despite problems associated with using fatty acids as trophic tracers, however, they have proven quite useful in characterizing omnivory in marine and freshwater zooplankton.

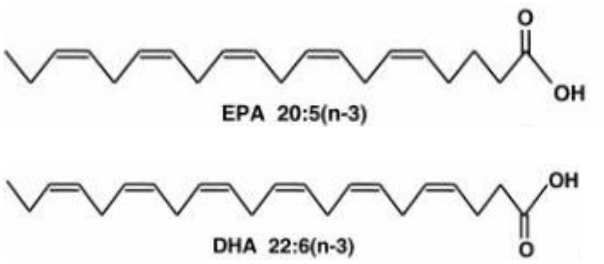

Diagram 1: The polyunsaturated fatty acids EPA (produced by diatoms) and DHA (produced by dinoflagellates). Pictures courtesy of Dr. P. Mansour at www.atse.org.au

1.3 Ecological setting: The Strait of Georgia and the STRATOGEM project

The Strait of Georgia (SoG) is a semi-enclosed estuarine system which lies between mainland British Columbia and Vancouver Island. It is a very productive ecosystem, and a feeding ground for a variety of economically important fishes (Ketchen et al. 1983). Chemical, biological and physical attributes in the SoG are strongly seasonal. Surface

temperatures in the region vary between ~ 6^{o}C (winter) to ~ 18^{o}C (summer), however, a significant warming trend has also been observed in the region, with temperatures rising by 0.024^{o}C Y^{-1} (Masson and Cummins 2007). Surface circulation in the SoG is dominated by freshwater input from the Fraser River (LeBlond 1983). Discharge of the Fraser is also seasonal, with the peak usually occurring in May or June (Morrison and Foreman 2005). Early in the spring, the Fraser River is nutrient-rich, but as spring progresses its waters become nutrient-depleted. However, shear forcing between the Fraser plume and deep, nutrient-rich oceanic waters results in the injection of nutrients into the surface layer, causing the edge of the plume to be more productive than the surrounding waters (Yin et al. 1997a). Surface salinity in the SoG varies from <20 PSU during periods when Fraser discharge is high, to ~ 32 PSU when Fraser discharge is low (LeBlond 1983). Over 30% of the particulate organic carbon in the SoG is introduced by the Fraser River, as is a large fraction of dissolved organic carbon (Johannessen et al. 2003). Most of the dissolved organic carbon is metabolized within the SoG, however, studies attempting to correlate patterns in bacterial activity within the SoG to Fraser River input have largely failed (Albright 1983, Johannessen et al. 2003).

Biomass production in the SoG is seasonal, with peak chlorophyll concentrations usually occurring in late March or early April (Harrison et al. 1983). The timing, peak and composition of the spring phytoplankton bloom vary considerably from year to year (Stockner et al. 1979, Bornhold 2000). Timing of the bloom depends on nutrient concentration and light levels, and can be accurately predicted from wind speeds and cloud cover (Allen SE, in prep). The bloom is largely composed of centric, chain-forming

diatoms (e.g. *Skeletonema costatum*, *Thalassiosira*, *Chaetoceros*) as well as large flagellates and ciliates (Harrison et al. 1983). Toward the early summer chlorophyll concentrations decrease as the phytoplankton bloom becomes nitrate limited, although occasional, short-lived summer blooms are often observed. The summer phytoplankton community is composed of small flagellates, dinoflagellates and microzooplankton, and is supported by regenerated nutrients (Price et al. 1985). Occasionally, harmful algal blooms are also observed in the area. A smaller autumn bloom, composed of small centric diatoms, occurs in September supported by nutrients that are injected into the surface layer during autumn storm activity, but is usually light-limited (Harrison et al. 1983). In the winter, the SoG phytoplankton community is composed of heterotrophic nanoflagellates, small flagellates and microzooplankton (Koeller et al. 1979). During this time storm and wind activity result in the mixing of nutrient-rich water into the surface layer, but the production of large diatoms is limited by light availability and cold temperatures (Harrison et al. 1983).

Peak mesozooplankton biomass in the SoG also occurs in the spring, and is typically dominated by the calanoid copepod *Neocalanus plumchrus* (Harrison et al. 1983, Diagram 2). *N. plumchrus* is widely-distributed in the North Pacific, and is thought to be an important prey item for higher trophic levels. Nauplii of *N. plumchrus* are produced at depth in the later winter or early spring (Mackas et al. 1998). Their arrival at the surface broadly coincides with the onset of spring phytoplankton production, but mismatch with this food source is known to occur (Bornhold 2000). By the time they reach the surface, *N. plumchrus* nauplii have molted to the first copepodite stage (CI). During feeding, these

copepodites then molt through four stages, accumulating biomass and lipids along the way. At stage CV, when the copepodites have accumulated sufficient lipid stores, they descend to a depth of ~ 400 m in the SoG, where they spend the remaining summer, autumn and early winter in a state of diapause (Fulton 1973). In early winter, CV copepodites emerge from diapause, molt into adults, and begin reproduction (Campbell et al. 2004).

N. plumchrus is unique in the SoG because it is the only copepod species in which reproduction occurs at depth, independent of ambient food concentrations. In other overwintering copepods such as *Calanus marshallae* and *Eucalanus bungii*, reproduction occurs at the surface during the spring bloom (Harrison et al. 1983). However, the survival of *N. plumchrus* copepodites is related to food composition, in the sense that they obtain their maximal body ration while feeding on a mixture of large diatoms and flagellates, rather than a diet composed exclusively of either item, or of smaller versions of these items (Parsons et al. 1969). *N. plumchrus* has been shown to achieve near maximum survival and growth rates at food concentrations typical of spring bloom conditions in the SoG (Parsons et al. 1969).

Other important spring copepods in the SoG include *E. bungii*, *C. marshallae*, *Calanus pacificus*, *Metridia pacifica* and *Pseudocalanus* spp. Recent evidence suggests that the spring biomass of *M. pacifica* and *C. pacificus* in the SoG can reach levels equivalent to (or even higher than) *N. plumchrus* (Sastri et al. in prep). The remainder of the year is dominated by small copepods, krill, jellies and occasionally, amphipods. In general, most

of our knowledge of the SoG zooplankton community is qualitative, with the exception of *N. plumchrus*. Very little is known about standing stocks, life histories or feeding habits of many SoG zooplankton species. Recently, zooplankton biomass in the region has been shown to be significantly correlated to fisheries catch (Ware and Thomson 2005). Therefore, the lack of information about SoG zooplankton may hinder regional fisheries management efforts.

Attempts to link physical forcing and biological production in the SoG have generally focused on factors that control primary production. Early studies in the region suggested that the spring phytoplankton bloom was driven by nutrients introduced by the Fraser River (Yin et al. 1995, and references therein). Later studies showed that significant phytoplankton blooms occur well after the river nutrients have been depleted (Yin et al 1995). It is now believed that entrainment of oceanic nitrate into the plume of the Fraser is responsible for maintaining a high level of production in the late spring (Yin et al 1995, 1997a). Theoretical models indicate that oceanic fluxes out-weigh Fraser-driven fluxes in the SoG (Mackas and Harrison 1997). Although mixing through Juan de Fuca Strait causes 70-90% of oceanic nutrients to exit the system before reaching the SoG, estuarine circulation through Juan de Fuca is still a major source of nutrients to the SoG (Pawlowicz 2001, Mackas and Harrison 1997). Variability in wind mixing and *N. plumchrus* grazing were initially suggested as mechanisms by which the timing of the spring phytoplankton bloom was regulated (Yin et al. 1996, 1997b,c), however, recent studies have shown that light and nutrients are responsible for initiating the bloom (Allen SE, in prep).

Compared to primary production, little is known about how physical forcing might affect zooplankton dynamics in the region. Bornhold (2000) showed that between 1969 and 1998, the timing of *N. plumchrus* emergence advanced by ~ 1 day per year, most likely in response to rising temperatures. Li et al. (2000) used a Nutrient-Phytoplankton-Zooplankton model to test the effects of physical forcing on zooplankton dynamics in the region. They concluded that estuarine circulation did not affect zooplankton significantly, and that zooplankton standing stocks were sensitive to factors that might influence their rate parameters. Unlike neighbouring regions where routine zooplankton sampling programs have been in place for many years (e.g. west Vancouver Island, Oregon upwelling, Ocean Station P), no consistent zooplankton monitoring took place in the SoG until the late 1990s. Therefore, until recently, our understanding of biophysical coupling in the SoG was limited by the lack of data.

Most of the data in this thesis were collected in conjunction with the Strait of Georgia Ecosystem Monitoring Project (STRATOGEM), which took place between 2002-05. The goals of STRATOGEM were to develop a better understanding of physical-biological coupling mechanisms in the SoG, and to develop a conceptual model of how (and why) primary productivity and zooplankton biomass fluctuate interannually. The field component of STRATOGEM consisted of two types of sampling. The first involved a series of monthly cruises on board the CCGS *Siyay* hovercraft, during which basic physical, biological and chemical parameters were measured at 9 stations usually within one tidal cycle. The second part of the field component involved underway measurements

of fluorescence, nitrate, temperature and salinity recorded by BC Ferries running the Tsawwassen – Nanaimo and Horseshoe Bay-Nanaimo routes (more information available at www.stratogem.ubc.ca).

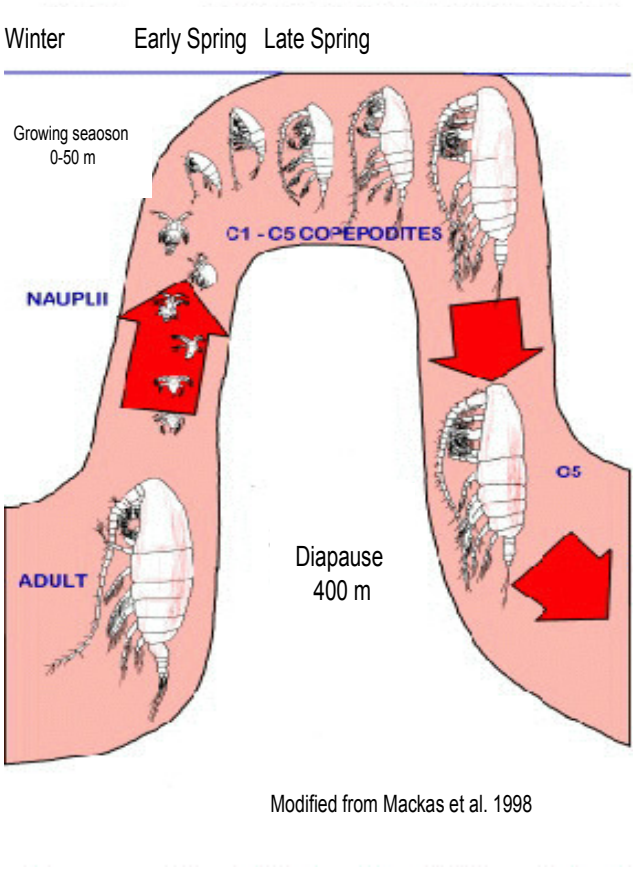

Diagram 2: Life cycle of *Neocalanus plumchrus* in the Strait of Georgia. Modified from Mackas et al. 1998.

1.4 Thesis objectives and structure

The three primary objectives of this thesis are:

1) To characterize interannual variability in the diet of *Neocalanus plumchrus* (Chapter 2)

Significant interannual variability in the timing, abundance and composition of the spring phytoplankton bloom occurs in the SoG. However, nothing is known about how this variability affects calanoid copepods. This chapter documents a 6-year time series of fatty acid data from *N. plumchrus*, and shows how variability in the composition of the spring phytoplankton bloom in the SoG is reflected in the fatty acids of this copepod. It also shows that an index of food quality (DHA/EPA) is potentially related to the survival of this species in the SoG. This chapter has been submitted to Marine Ecology Progress Series, and has been presented in several conferences. This study represents the longest time-series of fatty acid data collected from a single copepod species to date.

2) To characterize interspecific, interannual and geographic variability in the diets and trophic positions of SoG spring calanoid copepods (Chapter 3)

In the SoG, spring mesozooplankton biomass is dominated by a few species of large calanoid copepods. The goals of this chapter are to characterize dietary variability and trophic positions of four species of spring copepods from the SoG, using a combination of fatty acids and stable isotopes. This work indicates that copepods of the SoG occupy three trophic positions, and that significant interannual variability occurs in their diets. It also highlights the advantages of using a combination of biochemical indices in elucidating trophic dynamics in marine copepods. This chapter is also currently in review in Marine Ecology Progress Series.

3) To characterize seasonal variability in the feeding patterns of calanoid copepods in the SoG (Chapter 4)

In this chapter two different approaches are used to characterize the annual cycle of trophic dynamics in copepods: a taxonomic approach which classifies copepods into different trophic groups (based on published experiments and morphological attributes), and a biochemical approach which involves analyzing fatty acids and stable isotopes of an abundant omnivorous copepod (*Metridia pacifica*). This chapter presents one of the first characterizations of the copepod trophic cycle in the SoG in more 30 years, and shows that copepods are able to utilize a wide range of diet including phytoplankton, detritus, microzooplankton, and possibly, bacterial aggregates. The implication is that the path of energy transfer in the SoG ecosystem varies dramatically over the year. This paper will be submitted as the second paper in a package of two papers on copepod composition in the SoG, the other is currently in preparation by Sastri et al.

The thesis concludes with a brief summary section in which I synthesize the findings of Chapters 2-4, and in which I offer suggestions for future research and application.

1.5 Statement of authorship

I am the first author on all three manuscripts produced by this thesis. I designed the studies, collected and analyzed the samples, and analyzed and interpreted the data. Dr. John Dower and Dr. Asit Mazumder are co-authors on all of them. They provided

editorial input and advice on sample analysis and interpretation. Dr. Martin Kainz is a co-author on the first two manuscripts – he provided editorial input and advised on data interpretation. Dr. Akash Sastri is a co-author on the third paper because it is part of a collaborative effort to analyze the STRATOGEM data set.

Chapter 2: Interannual variability in fatty acid composition of the copepod *Neocalanus plumchrus* (Marukawa) in the Strait of Georgia, British Columbia (Canada)

2.1 Introduction

Phytoplankton vary widely in their composition and nutritional content, causing the quality of copepod diets to vary significantly in the oceans (Jónasdóttir et al. 2005, Klein-Breteler et al. 2005). Understanding the extent of natural variability in food quality is important because poor food quality affects the growth, production and reproduction of copepods and fish (e.g. Müller-Navarra et al. 2000, St. John et al. 2001, Arendt et al. 2005). In recent years the use of fatty acid trophic tracers has greatly enhanced our ability to characterize natural variability in the quality of food available for copepods. Marine copepods are incapable of synthesizing the majority of fatty acids required for growth and reproduction and, as such, need to acquire them from their diet (Bell et al. 2007). Phytoplankton produce taxon-specific fatty acids, which are retained in their zooplankton predators, and which can be used as qualitative tracers of dietary source (Dalsgaard et al. 2003). Diatoms, for example, are characterized by high concentrations of eicosapentaenoic acid (EPA, 20:5n-3), 16:1n-7 and the presence of polyunsaturated fatty acids (PUFA) containing 16 carbon chains (16PUFA), whereas dinoflagellates are characterized by high concentrations of the essential PUFA docosahexaenoic acid (DHA, 22:6n-3), and PUFA containing 18 carbons (18PUFA, specifically, 18:5n-3)(Thompson et al. 1992, Viso and Marty 1993, Graeve et al. 1994a, Stevens et al. 2004b, Graeve et al. 2005). Essential PUFA such as DHA and EPA are important for the physiology of marine

copepods, and have been shown to affect the efficiency of energy transfer in foodwebs (Müller-Navarra et al. 2000, St. John et al. 2001). Other examples of fatty acid trophic markers for phytoplankton, microzooplankton, bacteria and calanoid copepods are provided in Table 1.

The use of fatty acid trophic markers in calanoid copepods has been verified in the laboratory and the field, and has succeeded in establishing trophic relations among different species of copepods and across large spatial gradients (e.g. Graeve et al. 1994a, Stevens et al. 2004b,c, Graeve et al. 2005). Most studies have focused on either spatial trends within a single region, or short-term temporal trends, usually on the scale of a single year (e.g. Stevens et al. 2004b, Lischka and Hagen 2007). In contrast, interannual variability in dietary quality remains poorly documented, but has been suggested to play a role in controlling population dynamics of marine organisms on longer time-scales (Kattner et al. 1994, Litzow et al. 2006). Here, I use fatty acid trophic markers to characterize interannual variability in the diet of an important calanoid copepod from a productive and highly variable coastal ecosystem over a period of 6 years.

The Strait of Georgia (SoG) is a highly productive coastal ecosystem on the west coast of Canada. Biological production in the SoG is highly seasonal, with peak biomass and production of zooplankton and phytoplankton occurring in the spring (Harrison et al. 1983). The spring mesozooplankton biomass is dominated by *Neocalanus plumchrus*, a large, lipid-storing calanoid copepod (Harrison et al. 1983). Copepodites of *N. plumchrus* appear early in the spring bloom, and while feeding, molt through five copepodite stages

becoming progressively larger and accumulating large lipid stores (Evanson et al. 2000). Later in the spring, when it reaches stage CV, *N. plumchrus* descends to a depth of ~ 400 m where it overwinters until it molts into the adult stages in the winter (Fulton 1973, Campbell et al. 2004). Fatty acid signatures of overwintering *Neocalanus* spp. have been used to infer the quality of the diet they have experienced during the previous spring (Evanson et al. 2000, Saito and Kotani 2000).

Although the composition of the spring phytoplankton bloom in the SoG varies significantly from year to year, the extent to which variability in dietary quality affects *N. plumchrus* has never been studied (Stockner et al. 1979). During the spring bloom, the SoG phytoplankton community progresses from a flagellate-dominated winter community, to a diatom-dominated spring community, and the diet available to *N. plumchrus* is thus usually composed of a mixture of both those types of phytoplankton (Harrison et al. 1983). *N. plumchrus* copepodites have been shown to achieve their optimal body ration while feeding on a mixture of large diatoms and flagellates, rather than a diet composed exclusively of either item (Parsons et al. 1969). In addition, variation in the relative composition of diatoms and dinoflagellates in the diet has been shown to affect the retention of the essential PUFA EPA and DHA in copepods (Graeve et al. 1994a, Stevens et al. 2004b).

My goal is to characterize the range of interannual variability in the diet of *N. plumchrus*, and to link it to environmental parameters and population dynamics. I test whether the relative abundance of the essential fatty acids EPA and DHA varies with changes in

phytoplankton composition in the lipid profiles of diapausing and actively-feeding animals. I also consider two years of fatty acid data from *N. plumchrus* and its congener *N. cristatus* at Ocean Station P (OSP, 50°N 145°W) in the Northeast subarctic Pacific to more clearly characterize regional geographic variability in the diet of *Neocalanus* spp. This study represents the longest time series of fatty acid composition in calanoid copepods reported to date.

2.2 Materials and Methods

2.2.1 Field methods

N. plumchrus from the SoG were collected from a single station located in the deepest pocket in the Strait, at 49°15'N, 123°45'W (Fig 1). Sampling was conducted in May (2003-06) or in the fall (2001-02) of each year when the *N. plumchrus* SoG community was composed entirely of overwintering CVs. Copepods collected during the autumn were not expected to have different lipid profiles than those collected in the summer because only a small fraction of wax esters is consumed between those months, and fatty acids are consumed in proportion to each other (Evanson 2000, Campbell et al. 2004). For fatty acid analysis, *N. plumchrus* were sorted from 0-400 m vertical net tows collected with a SCOR net (0.57 m diameter, 236 μm mesh, towed at 0.5 m s^{-1}). Each replicate contained ~10-30 animals, and the number of replicates for a given date ranged from 1-7, depending on the availability of animals. Sorted samples were stored in cryovials at -80°C until analysis. Samples from OSP (*N. plumchrus* and *N. cristatus*) were

collected from 0-1000 m in September of 2001 and 2005 using the same sorting and storage procedures.

Phytoplankton community composition and *N. plumchrus* abundance data from the Strait of Georgia Ecosystem Monitoring Program (STRATOGEM, www.stratogem.ubc.ca) were used to contextualize the fatty acid profiles of *N. plumchrus*. The STRATOGEM project ran from April 2002 to June 2005, and included monthly to bimonthly sampling from several stations in the SoG including 49°15'N, 123°45'W (Pawlowicz et al. in prep). Phytoplankton samples were collected at the chlorophyll maximum (~ 5 m) during the spring blooms of 2003-05 and once in the spring bloom of 2002 (Sastri and Dower, submitted). Samples were preserved in Lugol's solution, identified under an inverted compound microscope and converted to biomass (Sournia 1978). Phytoplankton were divided into four taxonomic groups: diatoms, dinoflagellates, flagellates (containing cryptophytes, euglenoids and all other flagellates) and photosynthetic ciliates (*Mesodinium rubrum*). For each cruise, the Shannon-Weiner (S-W) diversity index was calculated as $H' = -\Sigma p_i \ln p_i$, where p_i is the proportion of each taxonomic group as a total of the biomass (Zar 1984). Unfortunately, phytoplankton composition data were unavailable from 2001 or 2006.

The SoG *N. plumchrus* population was sampled at least monthly to track seasonal and interannual trends in abundance (expressed as animals per m^2). Samples were collected using the same net as previously described, and preserved in 5% buffered formalin. Samples from 2006 were collected from the same station using ships of opportunity. Only

those abundance estimates that correspond to the dates when fatty acids are analyzed were reported. *N. plumchrus* abundance data from 2001-02 were taken from Campbell et al. (2004).

In order to assess how diatoms and flagellate fatty acid markers in *N. plumchrus* varied in relation to phytoplankton composition during copepod development, sampling of SoG *N. plumchrus* abundance, fatty acid profiles of animals and phytoplankton community composition was conducted over four cruises during the spring bloom of 2005, in conjunction with the STRATOGEM project. Copepod fatty acid samples were processed as previously described, but with a larger number of *N. plumchrus* per replicate (~ 30-60 animals per replicate) to account for the low body mass of younger stages.

2.2.2 Laboratory methods

In the lab, animals were freeze-dried (<40^{o}C for 48 hours), weighed, and placed in 2 ml HPLC-grade chloroform. The samples were flushed with nitrogen gas, sealed with Teflon®-lined caps, wrapped in Teflon® tape and stored at -80^{o}C until extraction. Fatty acid extractions followed Parrish et al. (1999) and Kainz et al. (2004). Briefly, the samples were sonicated and vortexed three times in a 4:2:1 chloroform: methanol:water mixture. The extraction took place on ice and under N_2 gas to limit possible sample degradation. The organic layers were pooled, and the extracts capped in a N_2 atmosphere, sealed and stored at -80^{o}C to prevent degradation. Fatty acids were analyzed as methyl esters (FAME) that were prepared by trans-esterfying the lipid extract in 14% BF3-CH3OH at 85^{o}C for 1 hour (Kainz et al. 2004).

Esterified fatty acids were analyzed using a gas chromatograph (Varian CP-3800) equipped with a flame ionization detector, and a Suppelco 2560 capillary column (100 m, 0.25 mm inner diameter and 0.2 μm film thickness). Unmethylated tricosonic acid was used as an internal standard to check the combined efficacy of the extraction and methylation. Fatty acids were identified as FAME by comparing retention times against those of a commercial standard (37-component FAME mix, Suppelco 47885-U). Fatty acids not included in this standard were verified on a Varian 2000 GC/mass spectrometer, with reference to Ackman (1986). The extraction and methylation efficiency was >90%, and the coefficient of variation among multiple injections of the same standard was <5%. All fatty acid data were reported as % of total fatty acids. The trophic and dietary tracers used in this study are summarized in Table 1.

2.2.3 Statistical analysis

Both nonparametric multivariate and parametric univariate analyses were used to explore underlying structures in the data, and to test the significance of differences in fatty acid markers. Nonparametric multivariate analyses were performed using PRIMER (version 5), following Clarke (1993)(details of the analysis are in Appendix 1). A Bray-Curtis dissimilarity matrix was constructed using raw, untransformed, fatty acid data (Bray and Curtis 1957). The resultant groupings were visualized via Multidimensional Scaling (MDS) and average-neighbour clustering using rank similarities. Stress values <0.20 were considered robust following the recommendation of Clarke (1993). Analysis of similarity (ANOSIM) was performed to test the statistical significance of groupings,

followed by Similarity Percent (SIMPER) analysis to assess the contributions of individual fatty acids to the observed clustering pattern. Subsequently, one-way ANOVA was performed on arcsine transformed data (tested for normality and equal variance) to test the significance of fatty acid trophic markers. A Tukey-Kramer post hoc analysis was applied to compare means when ANOVA was significant. Samples from 2001-02 were excluded from the ANOVA because they consisted of only single values without replicates. OSP animals from both 2001 and 2005 were combined for the ANOVA because they were not significantly different. Univariate analyses were performed using JMP (version 6), following Zar (1984).

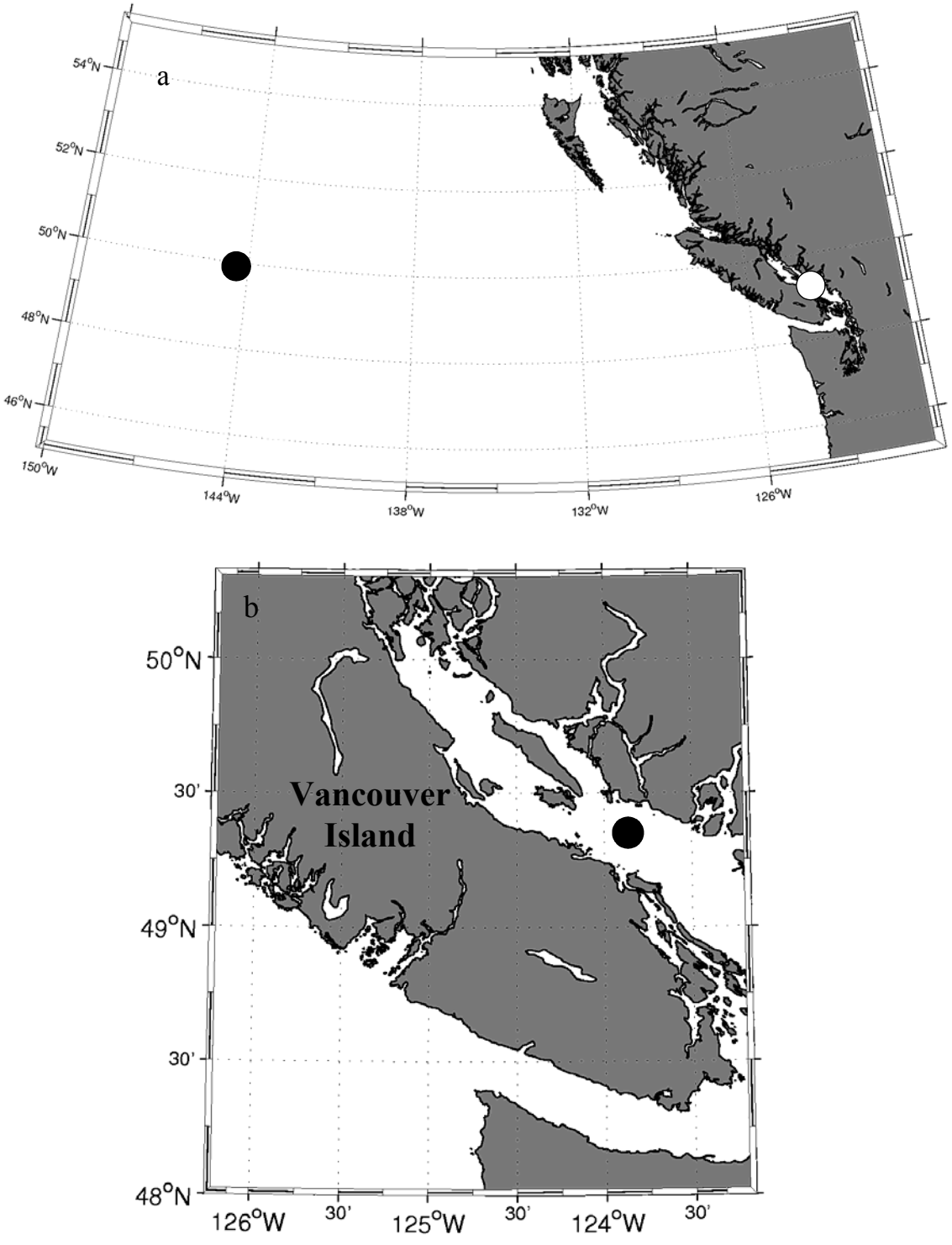

Figure 1: (a) Maps showing location of sampling sites in the Strait of Georgia (white circle), Station S4-1, 49°15'N 123°45'W) and in the Northeast subarctic Pacific (black circle, Ocean Station P, 50°N 145°W) (b) close up of Strait of Georgia location (black circle)

Table 1: Summary of trophic and dietary fatty acid markers discussed in Chapter 2.

Marker	Source	Reference
16:1n-7	Diatoms	Graeve et al. (1994a), Viso and Marty (1993)
EPA	Diatoms	Viso and Marty (1993), Graeve et al. (1994a), Graeve et al. (2005)
16PUFA[1]	Diatoms	Thompson et al. (1992), Graeve et al. (1994a), Graeve et al. (2005)
DHA	Dinoflagellates	Viso and Marty (1993)
18PUFA[2]	Flagellates	Thompson et al. (1992), Viso and Marty (1993)
DHA/EPA	Dinoflagellate/diatoms, carnivory	Budge and Parrish (1998)
16PUFA/18PUFA	Diatoms/flagellate	Budge and Parrish (1998), Alfaro et al. (2006)
18:2n-6	Terrestrial detritus or green algae	Dalsgaard et al. (2003)
15:0 + 17:0[3]	Bacteria	Kaneda et al. (1991)
18:1n-9/18:1n-7	Omnivory or Carnivory	Stevens et al. (2004b,c)
20-22MUFA[4]	Wax ester synthesis	Sargent and Whittle (1981)
22MUFA/20MUFA[5]	Calorific value	Scott et al. (2002)

[1]includes all PUFA containing 16 carbon atoms [2]includes all PUFA containing 18 carbon atoms [3]includes *iso* and ante-*iso* branched chains containing 15-17 carbons [4]includes all monounsaturated fatty acids containing 20 or 22 carbon atoms (20:1n-9, 20:1n-11, 22:1n-9, 22:1n-11) [5]is the sum of 22:1n-9 and 22:1n-11 over the sum of 20:1n-9 and 20:1n-11.

2.3 Results

2.3.1 Patterns in *N. plumchrus* abundance and phytoplankton composition

The abundance of *N. plumchrus* in the SoG declined by ~ 87%, from > 8000 animals per m^2 in 2001 to ~ 1000 animals per m^2 in 2006 (Fig 2). Diatoms were almost always the dominant phytoplankton group during the spring bloom, but the composition of the phytoplankton community varied significantly from year to year (Fig 3a). The 2002 bloom was dominated by diatoms and ciliates, the 2003 bloom was dominated by diatoms and dinoflagellates, while the blooms of 2004-05 were dominated primarily by diatoms. Additionally, the concentration of diatoms increased considerably between 2002-05, causing the maximum proportion of diatom carbon to total flagellate carbon to increase progressively, from ~ 200x in the spring blooms of 2002-03 to more than 800x in the spring bloom of 2005 (Fig 3b). As a result, the average S-W index during the time when *N. plumchrus* was actively feeding was lower in 2004-05 (~ 0.20) than in 2002-03 (~ 0.55), indicating that the developing *N. plumchrus* copepodites encountered a more homogeneous, diatom-dominated diet in 2004-05 compared to 2002-03 (Fig 3c).

2.3.2 Fatty acids

Fatty acid profiles of *Neocalanus* spp. from the SoG and OSP were dominated by the saturated fatty acids 14:0, 16:0, and the essential PUFA EPA and DHA. MDS ordination (overlaid with cluster analysis) separated the data into three statistically different groups (Fig 4, Table 2). Cluster 1 included *N. plumchrus* and *N. cristatus* from OSP, plus the SoG *N. plumchrus* from 2001. Cluster 2 contained SoG *N. plumchrus* from 2002-2004, while Cluster 3 encompassed SoG *N. plumchrus* from 2005-06. Clusters 1 and 3 were the

most separated (Global R = 0.771), while Clusters 1 and 2, and 2 and 3 displayed a higher degree of overlap (Global R = 0.660 and 0.595, respectively). Clusters 2 and 3 were separated from Cluster 1 primarily on the basis of the proportions of 20-22MUFA and 18PUFA (which were higher in oceanic than in coastal copepods). Cluster 3 was further separated from cluster 2 by having high proportions of diatom markers (EPA and 16PUFA, Table 3, Appendix 1). 16PUFA were composed predominantly of 16:4n-1 while concentrations of 16:4n-3 were very low (data not shown). On the other hand 18PUFA were composed predominantly of 18:4n-3, while concentrations of 18:3n-3 and 18:3n-6 were low, and 18:5n-3 was undetected in any of the samples (data not shown).

Diatom markers (EPA, 16:1n-7 and 16PUFA) were relatively high t in SoG *N. plumchrus* from 2005-06, and relatively low in oceanic specimens (Table 4, Fig 5a). Flagellate markers (DHA and 18PUFA) were high in oceanic samples and low in the SoG (Table 4, Fig 5b). The green algal/terrestrial detritus marker 18:2n-6 was significantly higher in 2004-05 than in any other year in SoG, and was low in OSP samples (Fig 5b). The ratio of 16PUFA to 18PUFA (indicating the relative proportion of diatoms to flagellates in the diet) was highest in 2005 and 2006, and lowest in oceanic animals, while the opposite was generally true for DHA/EPA ratios (which indicate the relative proportions of dinoflagellate to diatom in the diet)(Fig 6). Bacterial markers were higher in oceanic animals than in coastal animals, with the exception of 2004, and there were no significant differences in the omnivory index 18:1n-9/18:1n-7 among any of the samples (Fig 7). 20-22MUFA were significantly higher in oceanic samples than in coastal samples (where

there were also no significant differences between years), and the ratio of 22MUFA/20MUFA in *N. cristatus* was significantly higher than all *N. plumchrus* (Fig 8).

2.3.3 The spring bloom of 2005

During the spring bloom of 2005 a major collapse in the population of *N. plumchrus* was observed in late March (between Julian days 60 and 80), during which the abundance of actively feeding copepods declined from ~ 35 animal per m^3 to ~ 4 animals per m^3 (Fig 9a). This collapse coincided with a spike of diatom production in which diatom carbon was ~ 1000x more abundant than flagellate and ciliate carbon (Fig 9b). During this time the proportion of diatoms in the diet of developing *N. plumchrus* copepodites also increased, as evidenced by a decrease in the DHA/EPA ratio and an increase in the 16PUFA/18PUFA ratio in the animals. The former was caused by a decrease in the proportions of DHA, while the latter was caused by the preferential accumulation of diatom markers compared to flagellate markers (Fig 9c,d,e).

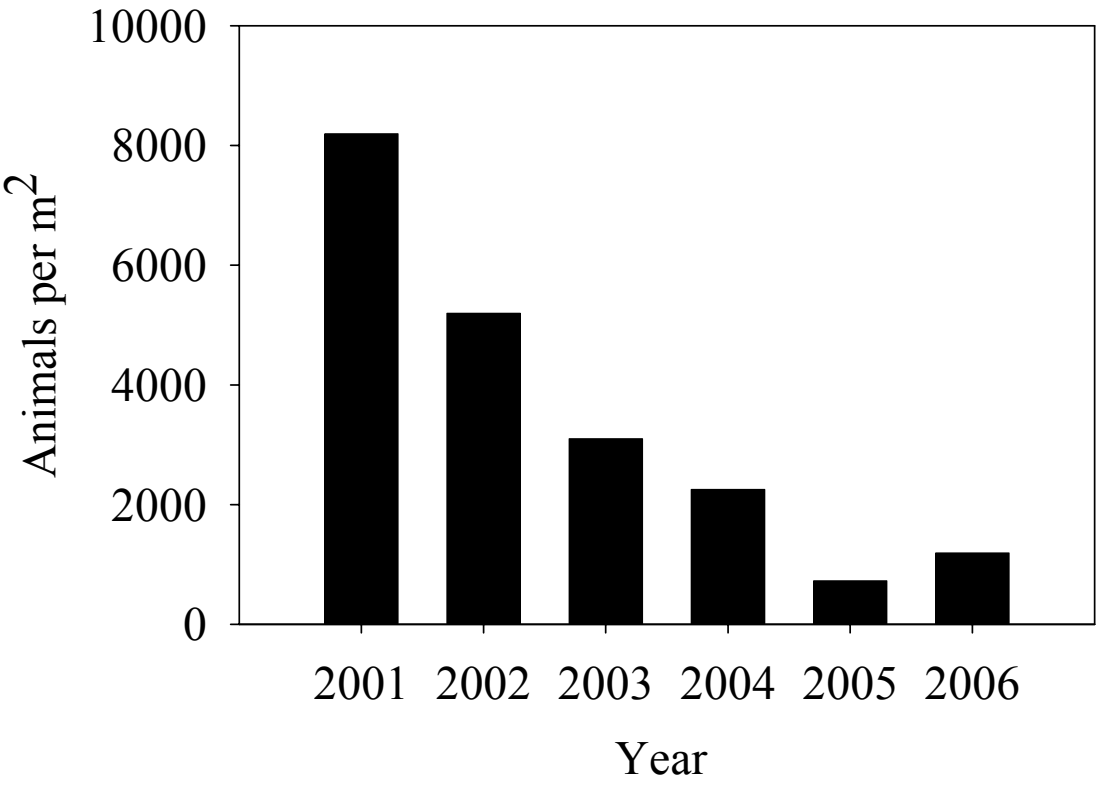

Figure 2: Depth-integrated abundance of diapausing *Neocalanus plumchrus* (animals per m^2) from 0-400 m in the Strait of Georgia between 2001-2006.

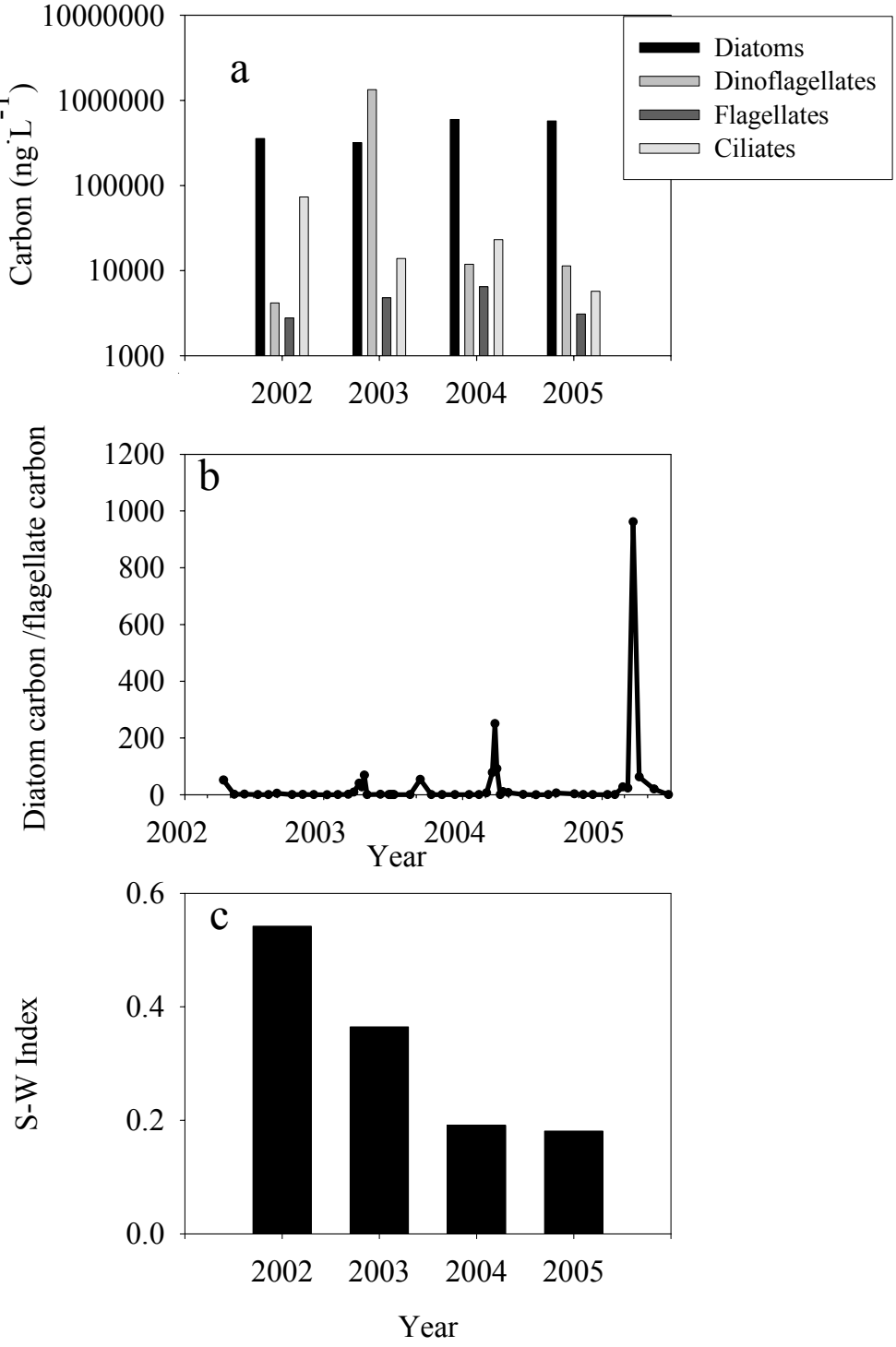

Figure 3:(a) The average composition of the spring phytoplankton blooms in 2002 (n=1), 2003 (n=8), 2004 (n=8) and 2005 (n=4) (b) the proportions of diatoms to flagellates at the peak of the phytoplankton from each year (c) the Shannon-Weiner index of group diversity averaged over spring blooms in 2002 (n=1), 2003 (n=8), 2004 (n=8) and 2005 (n=4)

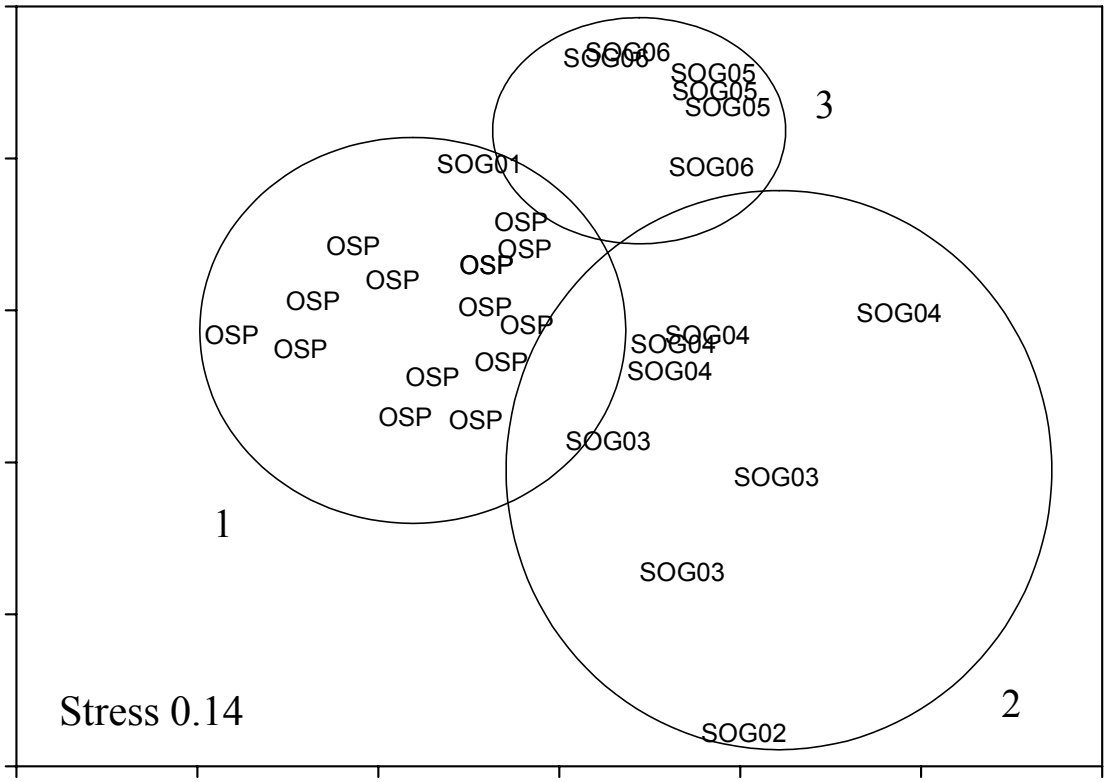

Figure 4: Multidimensional scaling ordination of a Bray-Curtis dissimilarity matrix calculated from raw untransformed proportional fatty acid data. Animals from the Strait of Georgia are indicated as SoG followed by year of sampling (e.g. SoG01 is *Neocalanus plumchrus* from 2001) and Ocean Station P animals are indicated as OSP regardless of their species and year of sampling. The circles represent groupings revealed by rank-based average-neighbour clustering performed on the same matrix. Significant differences and degree of overlap are indicated in Table 2.

Table 2: Analysis of Similarity (ANOSIM) between the 3 clusters discovered with multidimensional scaling analysis and clustering shown in Fig. 4. Each value is represented as the Global R statistic (significance level). Cluster 1 contains all oceanic *Neocalanus* spp. and *Neocalanus plumchrus* from the Strait of Georgia from 2001. Cluster 2 contains all Strait of Georgia animals from 2002-04. Cluster 3 contains Strait of Georgia animals from 2005-06.

	Cluster 1	Cluster 2	Cluster 3
Cluster 1		.66 (P =0.001)	.771 (P =0.001)
Cluster 2	.66 (P =0.001)		.595 (P =0.002)
Cluster 3	.771 (P =0.001)	.595 (P =0.002)	

Table 3: Similarity Percent (SIMPER) analysis used to asses the contribution of individual fatty acids to the 3 clusters discovered by multidimensional scaling analysis and clustering as shown in Fig. 4. All values are expressed in % fatty acid. Av % comp refers to the average composition of the tracer present in each cluster. Cum. % refers to the cumulative dissimilarity explained by the tracer (also see Appendix 1)

Clusters 1 and 2		Cluster1	Cluster 2	
Average dissimilarity	Fatty acid	Av % comp	Av % comp	Cum.%
26.75%	20-22MUFA	13.84	4.47	21.05
	18PUFA	9.92	3.91	34.61
	14:0	19.16	20.86	47.22
	16:0	12.48	16.11	59.34
	18:2n-6	1.64	6.15	69.41
	16PUFA	2.73	4.84	76.95
	EPA	11.4	10.3	84.18
	DHA	7.21	6.86	89.83
	18:1n-9/18:1n-7	3.34	4.02	93.25
		Cluster 1	Cluster 3	
Clusters 1 and 3	Fatty acid	Av % comp	Av % comp	Cum.%
Average dissimilarity	16PUFA	2.73	9.57	16
25.92%	20-22MUFA	13.84	7.82	30.57
	18PUFA	9.92	3.59	44.27
	14:0	19.16	24.71	56.47
	18:2n-6	1.64	6.11	67.86
	EPA	11.4	16.3	78.55
	DHA	7.21	4.13	85.22
	15+17	2.68	0.71	89.46
	18:1n-9/18:1n-7	3.34	1.54	93.48
		Cluster2	Cluster 3	
Clusters 2 and 3	Fatty acid	Av % comp	Av % comp	Cum.%
Average dissimilarity	EPA	10.3	16.3	15.5
23.00%	14:0	20.86	24.71	30.74
	16:0	16.11	12.32	44.41
	16PUFA	4.84	9.57	56.09
	18:2n-6	6.15	6.11	66
	20-22MUFA	4.47	7.82	74.81
	DHA	6.86	4.13	82.18
	18:1n-9/18:1n-7	4.02	1.54	88.5
	16:1n-7	1.88	3.41	92.3

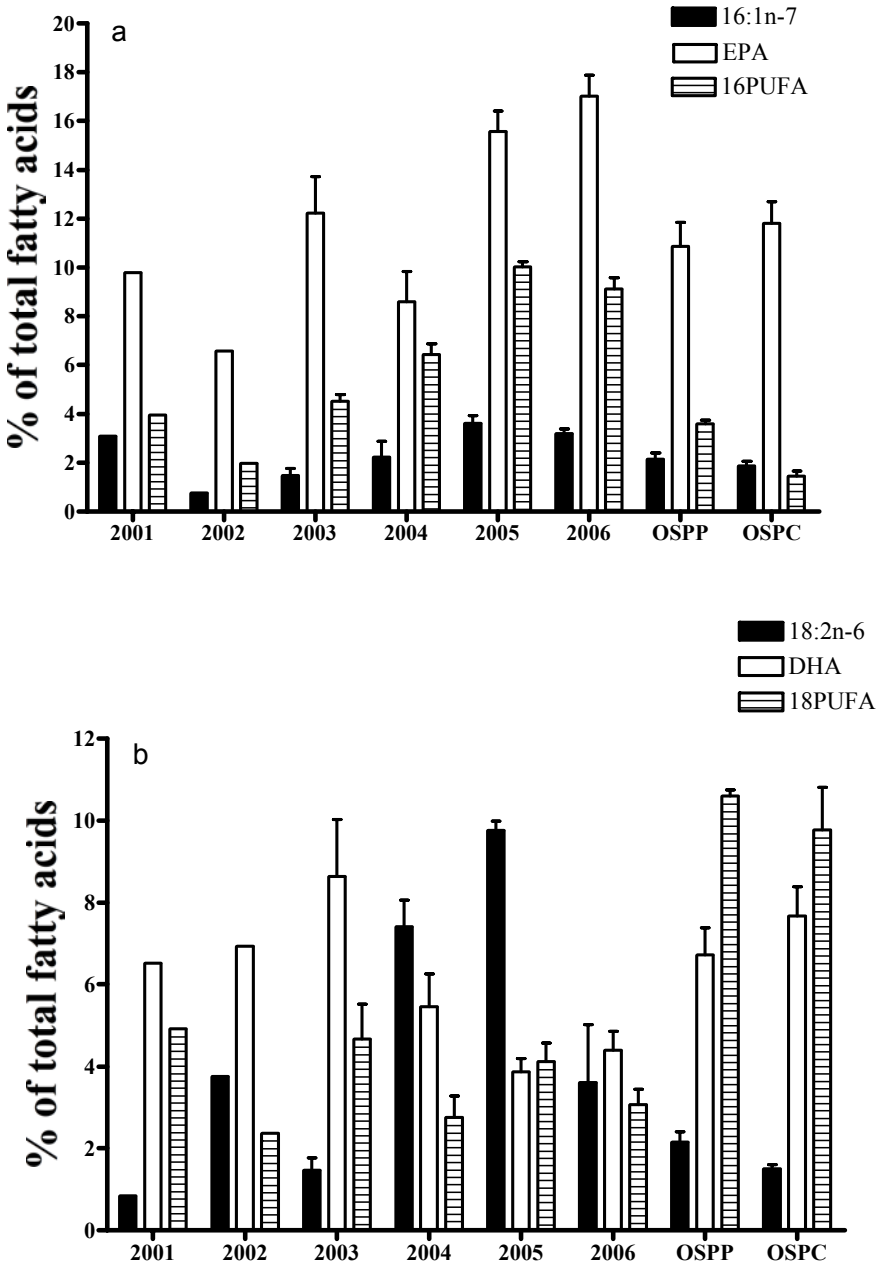

Figure 5. (a) The distribution of diatom markers calculated from fatty acid profiles of *Neocalanus plumchrus* from the Strait of Georgia between 2001 and 2006 (designated by year) and from *Neocalanus plumchrus* (OSPP) and *Neocalanus cristatus* (OSPC) from Ocean Station P (b) The distribution of flagellate markers calculated from fatty acid profiles of these same animals. Degree of significance is indicated in Table 4. Error bars represent 1 standard deviation from the mean.

Table 4: Summary of statistical analyses used to test the significance of differences in the concentrations of major fatty acids between years and regions. Different letters designate years or organisms that are found to be significantly different by Tukey-Kramer post hoc analysis. Order of letters corresponds to average level of tracer. P is the level of significance discovered using Analysis of Variance (ANOVA).

	SoG (year)				OSP (organism)		
Tracer	2003	2004	2005	2006	*plumchrus*	*cristatus*	P
14:0	BC	BC	A	AB	AB	C	0.0002
16:0	A	B	B	B	B	B	0.001
16:1n-7	BC	BC	A	AB	C	C	0.0004
EPA	A	C	A	A	BC	B	0.0001
DHA	A	CD	D	D	AB	BC	0.006
16PUFA	BC	BC	A	AB	CD	D	0.0009
18PUFA	B	B	B	B	A	A	0.0001
DHA/EPA	A	A	B	B	A	A	0.0001
16PUFA/18PUFA	C	B	AB	A	D	D	0.0001
15+17	D	BC	D	CD	B	A	0.0001
18:2n-6	C	BC	AB	D	D	D	0.0001
18:1n-9/18:1n-7	A	A	A	A	A	A	0.06
20-22MUFA	C	C	C	BC	B	A	0.001
22MUFA/20MUFA	B	B	B	B	B	A	0.001
Number of samples	3	4	3	3	7	7	

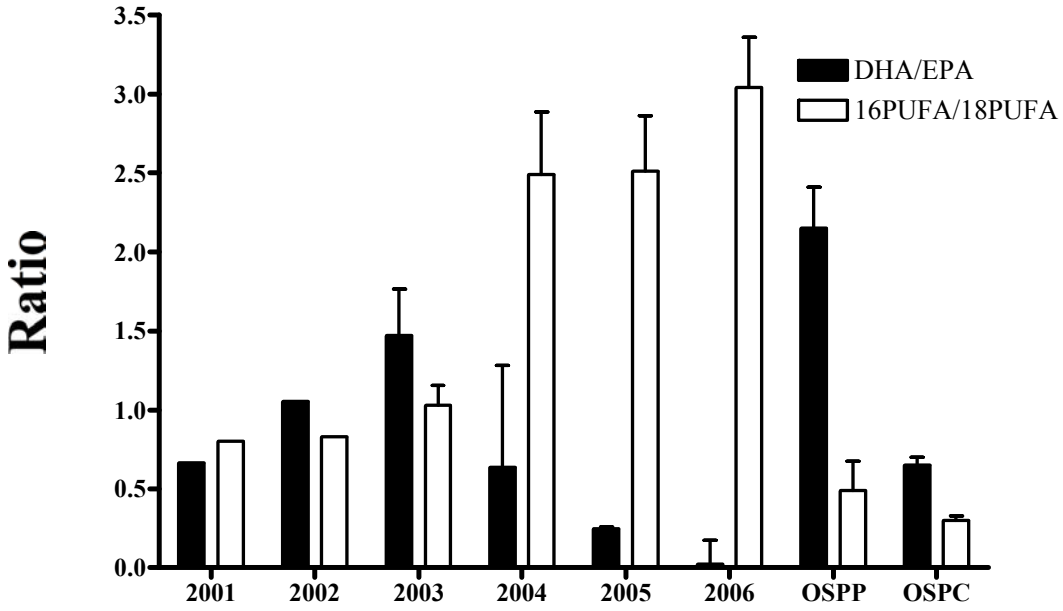

Figure 6: Fatty acid markers indicating the proportion of diatoms to flagellates calculated from fatty acid profiles of *Neocalanus plumchrus* from the Strait of Georgia between 2001 and 2006 (designated by year) and from *Neocalanus plumchrus* (OSPP) and *Neocalanus cristatus* (OSPC) from Ocean Station P. Degree of significance is indicated in Table 4. Error bars represent 1 standard deviation from the mean.

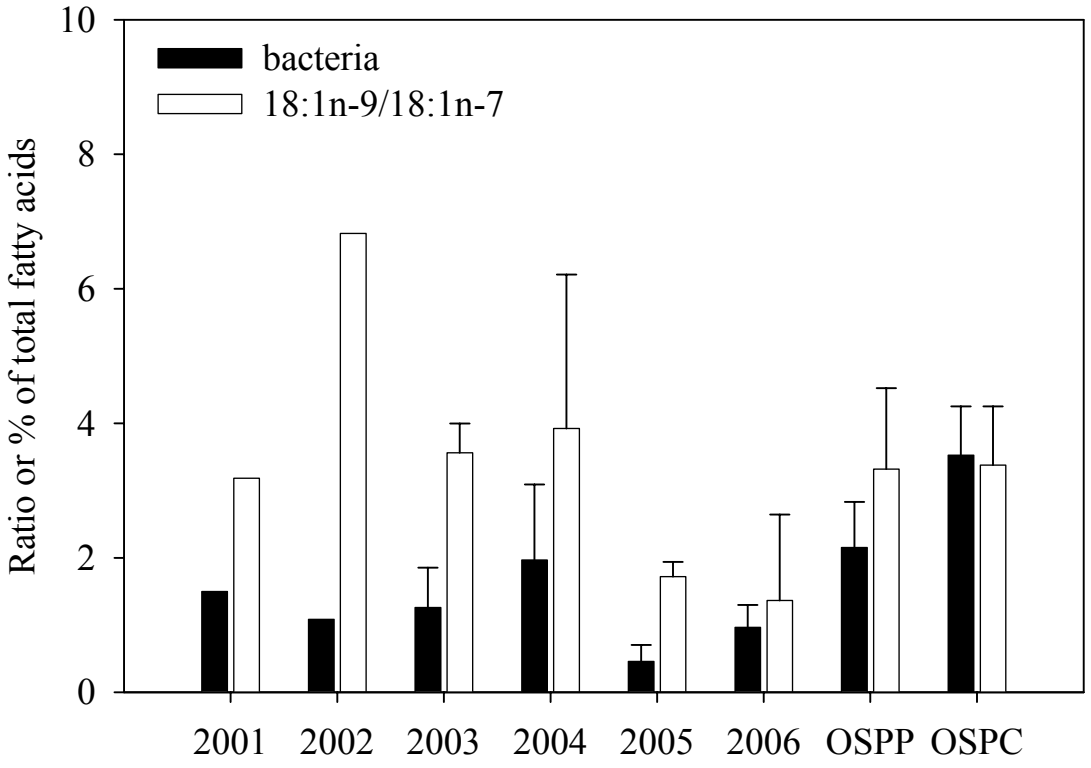

Figure 7: Fatty acid markers representing bacterial markers and omnivory markers calculated from fatty acid profiles of *Neocalanus plumchrus* from the Strait of Georgia between 2001 and 2006 (designated by year), and from *Neocalanus plumchrus* (OSPP) and *Neocalanus cristatus* (OSPC) from Ocean Station P. Degree of significance is indicated in Table 4. Error bars represent 1 standard deviation from the mean.

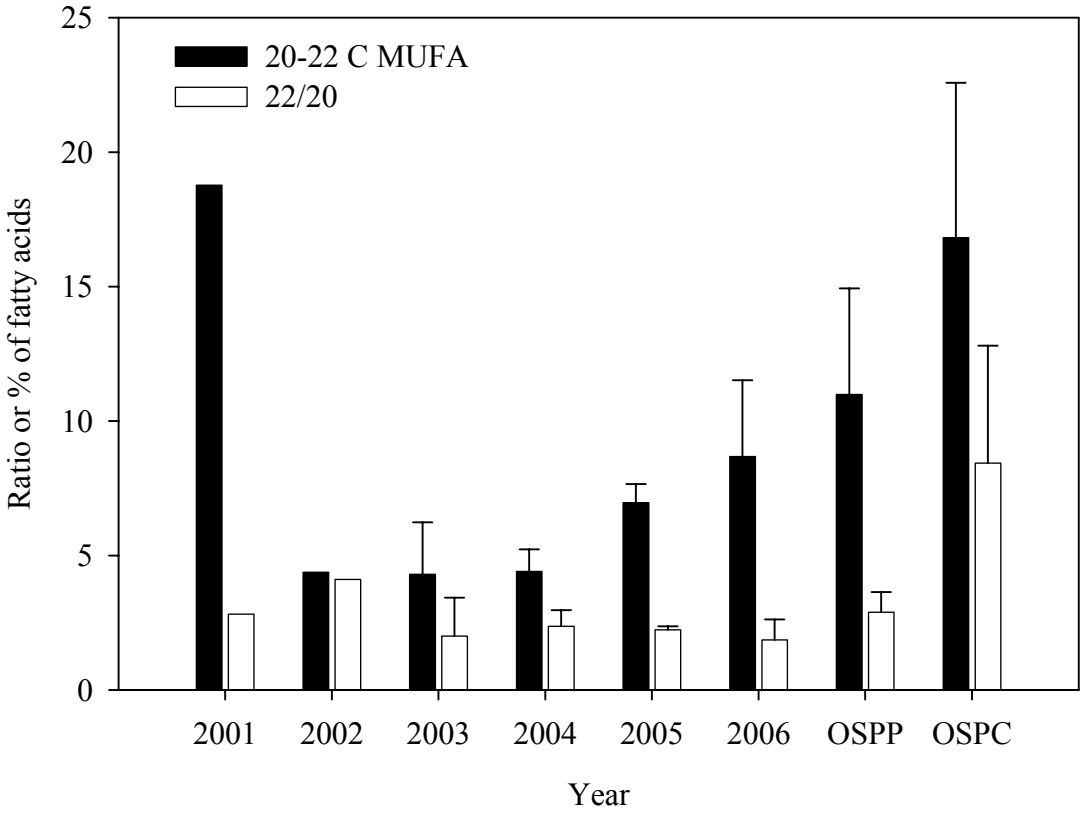

Figure 8: Fatty acid markers denoting wax ester synthesis calculated from fatty acid profiles of *Neocalanus plumchrus* from the Strait of Georgia between 2001 and 2006 (designated by year), and from *Neocalanus plumchrus* (OSPP) and *Neocalanus cristatus* (OSPC) from Ocean Station P. 20-22 is the sum of all MUFA containing between 20 and 22 carbons, and 22/20 is (22:1n-9+22:1n-11)/(20:1n-9+20:1n-11). Degree of significance is indicated in Table 4. Error bars represent 1 standard deviation from the mean.

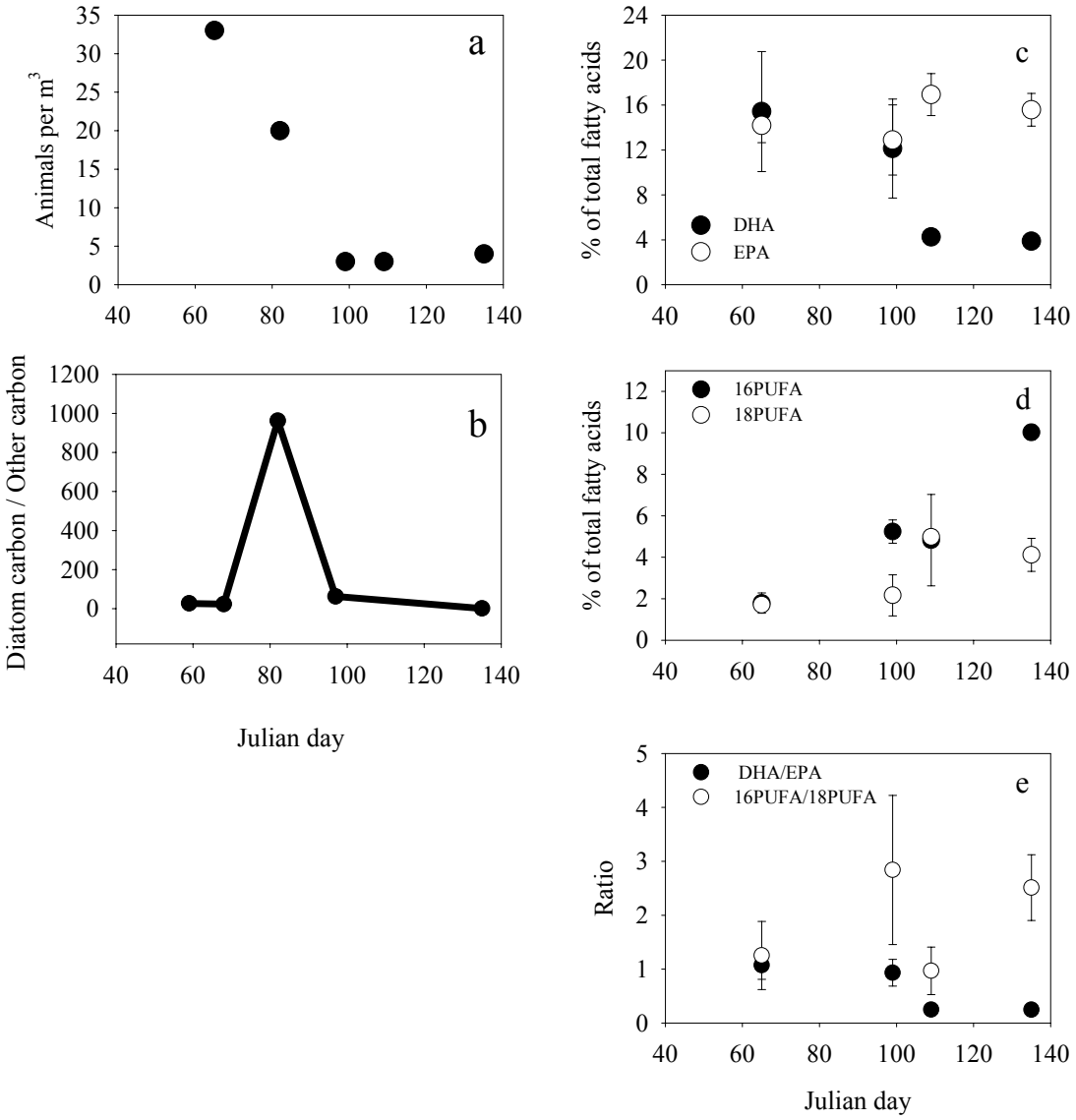

Figure 9: A collapse in the abundance of *Neocalanus plumchrus* in the SoG during the spring bloom of 2005 coincides with (a) a high pulse of diatom carbon over other phytoplankton carbon, and (b) with an increase in contribution of diatoms to the diet as denoted by increasing diatom fatty acids in the lipids of *N. plumchrus* (c, d, e).

2.4 Discussion

2.4.1 General findings and geographic variability

My results show significant spatiotemporal variability in the composition of *Neocalanus*. The SoG and OSP represent two very different feeding environments; the SoG is a highly productive, diatom-based system while OSP is a high-nutrient low-chlorophyll region where iron limits the production of large diatoms, and where the phytoplankton community is composed primarily of small flagellates, cyanobacteria and small diatoms (Harrison et al. 1983, 2004). Grazing experiments have shown that *Neocalanus* spp. feed primarily on ciliates at OSP, with only a minor contribution from phytoplankton (Dagg 1993). My fatty acid data support these observations: *N. plumchrus* from the SoG is primarily herbivorous, with high proportions of diatom-based fatty acid markers, whereas animals from OSP contain much higher proportions of flagellate and bacterial markers, suggesting omnivorous feeding. Low ratios of 18:1n-9/18:1n-7 in the lipid of *Neocalanus* spp., regardless of their origin, suggests that carnivory is not an important feeding habit for this genus (Hagen et al. 1995). My fatty acid profiles from coastal *N. plumchrus* agree with the findings of Lee (1974), which is the only other report of complete fatty acid profiles isolated from whole copepods of this genus.

Wax ester synthesis is evidenced by the presence of 20-22MUFA, which are synthesized *de novo*, and which can be used as a proxy for wax ester concentrations when combined with their corresponding fatty alcohols (Sargent and Whittle 1981, Kattner and Hagen 1995, Saito and Kotani 2000). The relative proportion of these MUFA is generally higher

in oceanic *Neocalanus* spp. than in coastal animals, and is within the range of proportions reported from the wax esters of other *Neocalanus* spp. (Lee et al. 2006). Thus, oceanic *Neocalanus* spp. are higher in calorific content than their coastal congeners, which in turn suggests that they are more adapted to extreme fluctuations in food availability (Scott et al. 2002). Longer-chained MUFA have been shown to contain a higher calorific content than short-chained MUFA; *N. cristatus* from OSP displays the highest proportion of 22MUFA to 20MUFA indicating that it is more adapted to extreme fluctuations in food availability than *N. plumchrus* (Scott et al. 2002). There are no clear interannual patterns in 20-22MUFA in the SoG.

2.4.2 Interannual variation in the diet of *N. plumchrus* from the SoG

Over the course of this study, the abundance of *N. plumchrus* in the SoG declined significantly, and a collapse of *N. plumchrus* occurred in spring bloom of 2005 while the developing copepodites stages were feeding (Fig 2, Fig 9a). Recent findings suggest that the 2005 collapse of *N. plumchrus* in the SoG coincided with the failure of the developing CII copepodites to molt to CIII, while copepods that made it through this crash continued to develop normally (Sastri and Dower, submitted). Total chlorophyll concentrations in 2005-2006 were not significantly different than in previous years suggesting that the collapse of *N. plumchrus* did not occur because of food limitation *per se* (Pawlowicz et al. in prep). It is also unlikely that the decline in *N. plumchrus* biomass was the result of increasing predation pressure. Between 2001-2005, stocks of major predators in the area (e.g. salmon, herring, hake and birds) did not increase appreciably (Perry 2006), and

potential macrozooplankton predators, such as chaetognaths and jellies declined (El-Sabaawi, unpublished data). 2005 is now widely recognized as having been an unusual year in the Northeast Pacific: the SoG experienced unusually warm deep water temperatures (though surface temperatures were not warmer than in previous years), and low zooplankton biomass anomalies were observed all the way from Oregon to the west coast of Vancouver Island (Mackas et al. 2006, Masson and Cummins 2007).

Between 2001 and 2006, the diet of *N. plumchrus* in the SoG shifted from OSP-like omnivory (in 2001) to moderate herbivory (between 2002-04), and finally, to intense herbivory dominated by diatom markers (2005-2006, Fig 4). The increasing proportion of diatoms relative to flagellates in the diet of *N. plumchrus* in the SoG was signified by the increase of the ratio of 16PUFA/18PUFA and a decrease in the ratio of DHA/EPA, both corresponding to increasing ratios of diatoms to flagellates in the water column. The flagellate marker 18PUFA did not show any significant trend over time in the SoG, indicating that similar proportions of flagellates were consumed in each year. However, DHA was low in 2004-2006 suggesting that even though flagellates had been consumed, they were likely not as rich in essential fatty acids as in previous years (Fig 5). The high proportion of 18:2n-6 in 2004-2005 relative to other years indicates that animals were supplementing their diet with green algae or on terrestrial detritus. Therefore, between 2001-06 not only did the contribution of diatoms in the diet of *N. plumchrus* increase, but the contribution of dinoflagellates decreased in favour of green algae. These patterns correlate well with patterns of phytoplankton composition (Fig 3a). In 2002-2003, high

DHA/EPA ratios in *N. plumchrus* coincide with high proportions of diatoms to either ciliates and dinoflagellates, respectively.

The highest values of 16PUFA/18PUFA and the lowest values of DHA/EPA occurred in 2005 and 2006; years when *N. plumchrus* biomass in the SoG was at its lowest. Interestingly, this is not the first incident in which low *N. plumchrus* biomass coincided with low DHA/EPA ratios in the SoG. The fatty acids of diapausing *N. plumchrus* were measured during a similar decline which occurred in 1996-97 (Evanson et al. 2000, Bornhold 2000). In 1996, a "good year" in terms of *N. plumchrus* biomass, DHA/EPA was ~ 1.2, comparable to my values from 2002. In 1997, a "poor year" for *N. plumchrus* biomass, DHA/EPA was ~ 0.3, comparable to my values from 2005-06. Regression analysis of *N. plumchrus* abundance on DHA/EPA using all the data gathered in this study, plus data from Evanson (2000) and Bornhold (2000) shows a strong relationship between those two parameters (Fig 10; $R^2 = 0.68$, $p<0.001$), indicating that a decline in the survival of *N. plumchrus* is likely linked to an imbalance of DHA to EPA availability or retention.

The idea of an "optimal ratio" of dietary DHA/EPA is already well established in the aquaculture, zooplankton and fisheries literature (e.g. Arendt et al. 2005, Jónasdóttir et al. 2005). The physiological basis for this are unclear, but DHA and EPA have been shown to be required for different physiological processes in mud crabs where DHA is required for molting and EPA is required for maintaining general physiological function (Kobayashi et al. 2000). The correlation between molting failure in *N. plumchrus* and low

DHA/EPA ratios in the animals during the spring bloom of 2005 supports these observations. However, the fact that *N. plumchrus* copepodites which survived the crash continued to molt and develop normally despite low DHA/EPA suggests that the somatic requirement of DHA and EPA differs among stages, and that older copepodite stages are more capable of buffering poor food quality than are younger copepodite stages, as recently suggested by Koski et al. (2006). Further experiments are needed to test the effect of varying proportions of diatom- and dinoflagellate-derived essential fatty acids on the somatic development of *N. plumchrus* at different copepodite stages. Nonetheless, the correlation between the abundance of *N. plumchrus* and DHA/EPA suggests that an all-diatom diet decreases the survival of *N. plumchrus* in the SoG.

As in other temperate coastal systems, the pathway from diatoms to copepods to juvenile fishes in the SoG is thought to be one of the most efficient and productive trophic pathways, especially during the spring (Parsons et al. 1969, Parsons and LeBrasseur 1970, Harrison et al. 1983). However, recent studies have suggested that diatoms may have been ascribed a more important dietary role than they warrant, and that omnivory is common and even beneficial to copepods (Kleppel 1993, Paffenhöffer et al. 2005). Some studies have shown that diatoms impede several aspects of copepod reproduction (Jónasdóttir et al. 1998, Ianora et al. 2003), either by the presence of toxic polyunsaturated aldehyde compounds, or by the absence of certain essential fatty acids that are required for copepod growth (such as DHA), but which are provided by non-diatom phytoplankton (Jónasdóttir et al. 1998 and references therein). Whether diatoms are actually harmful to copepods or whether they are simply poor quality food, especially

when they are not supplemented by other phytoplankton, is still under investigation (Paffenhöffer et al. 2005).

The correlation between DHA/EPA and abundance of *N. plumchrus* does not conclusively implicate poor food quality as the cause of decline of this copepod. However, it underscores how little we know about this aspect of copepod nutrition. My results add strength to the call for more thorough experimentation on trophic dynamics, specifically the physiological thresholds of essential fatty acids that are required for somatic growth and reproduction of copepods (Ianora et al. 2003). My results also show the benefit of including routine fatty acid measurements in long-term zooplankton monitoring projects not just in highlighting physiological factors that contribute to population dynamics, but also as a means of generating testable hypotheses about the effect of food quality on somatic growth.

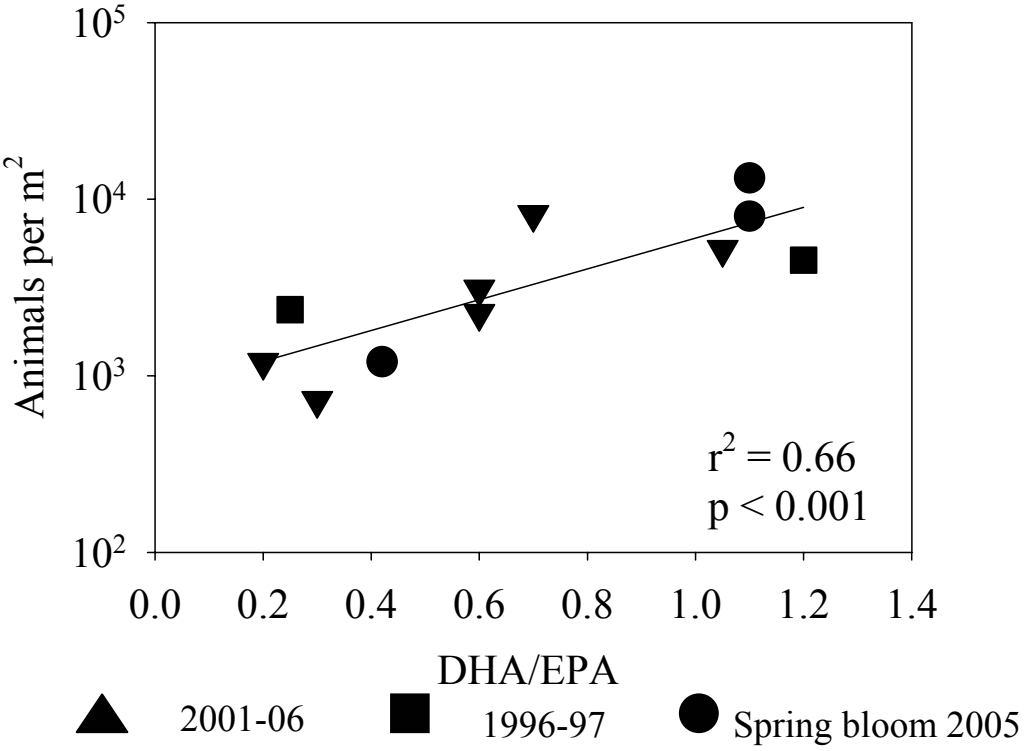

Figure 10: The correlation between *Neocalanus plumchrus* abundance (animals per m^2) and the ratio of DHA/EPA in diapausing animals from 2001-06 (triangles), actively feeding animals from the spring bloom of 2005 (circles) and from Bornhold (2000) and Evanson et al. (2000) in 1996-97 (squares).

Chapter 3: Characterizing the trophic positions of calanoid copepods in the Strait of Georgia: insight from stable isotopes and fatty acids

3.1 Introduction

Copepods, an important component of marine ecosystems, are capable of utilizing a wide range of diets (Kleppel 1993). The trophic flexibility of omnivorous organisms can act as a stabilizing force in aquatic ecosystems (Sprules and Bowerman 1988). However, omnivory also affects foodchain length, and can change the quality of food available to higher consumers (Hairston and Hairston 1993, St. John et al. 2001, Post and Takimoto 2007). Changes in feeding patterns of copepods have been shown to trigger trophic cascades and affect the growth of larval fish (St. John et al. 2001, Stibor et al. 2004). As such, considerable effort has been expended in attempting to characterize the quality of copepod diets and their trophic position in the ocean (e.g. Graeve et al. 1994b; Stevens et al. 2004a).

In the Strait of Georgia (SoG), a productive coastal ecosystem on the west coast of Canada, biomass production is highly seasonal with the peak occurring in the spring (Harrison et al.1983). The spring phytoplankton bloom is consumed by a few species of large calanoid copepods (*Neocalanus plumchrus*, *Calanus marshallae* and *Eucalanus bungii*), which account for ~ 90% of the total spring mesozooplankton biomass, and which represent key prey items for higher trophic levels in the area (Krause and Lewis

1979, Harrison et al. 1983, Pawlowicz et al. in prep). The spring phytoplankton bloom in the SoG varies considerably in magnitude, timing and composition, causing the quality and quantity of food available to these copepods to vary considerably from year to year (Stockner et al. 1979). To date, however, the degree of omnivory in the diets of these copepod species and their trophic positions in the SoG have not been assessed, and although they are widely distributed in the Northeast Pacific, little is known about geographic variability in their diets.

Here, I characterize the trophic positions and dietary quality of these species, as well as *Euchaeta elongata* (a carnivorous spring calanoid), over three years in the SoG and at Ocean Station P (OSP), an oceanic station in the Northeast Pacific. To do so I use a combination of stable isotopes and fatty acids. The use of nitrogen stable isotopes ($\delta^{15}N$) to calculate trophic position is based on the fact that nitrogen isotopes display a constant level of enrichment (~ 3.5 ‰ per trophic level) with trophic level (Minagawa and Wada 1984). Stable carbon isotopes ($\delta^{13}C$) do not display a significant enrichment between trophic levels, and as such are useful in assessing the sources of carbon in foodwebs (McConnaughey and McRoy 1979). However, whereas $\delta^{15}N$ provide relatively accurate information about trophic level, $\delta^{13}C$ is sometimes an ambiguous tracer of food source, especially when different dietary sources have a similar $\delta^{13}C$. The use of fatty acid trophic markers (FATMS) is based on the premise that phytoplankton, microzooplankton, and bacteria all produce taxon-specific fatty acids which are retained by their predators, and which can thus be used to qualitatively assess the relative trophic position and dietary quality (Dalsgaard et al. 2003). The efficacy of FATMS in copepods has been validated

in the laboratory and the field, and several indices of copepod omnivory are available (Graeve et al. 1994a, Stevens et al. 2004b,c, Graeve et al. 2005). High proportions of 18:1n-9 to 18:1n-7 denote carnivory in copepods, amphipods and krill (Hagen et al. 2007, Stevens et al. 2004b, Nyssen et al. 2005, Schmidt et al. 2006). Because carnivorous zooplankton often have higher proportions of polar lipids, which are rich in polyunsaturated fatty acids (PUFA), than do herbivorous crustacean zooplankton, the proportion of PUFA to saturated fatty acids (SFA) can also be used as an index of carnivory (Cripps and Atkinson 2000, Stevens et al. 2004b). Yet another index of carnivory is the ratio of docosahexaenoic acid to eicosapentaenoic acid (22:6n-3/20:5n-3, DHA/EPA)(Dalsgaard et al. 2003). DHA is also an important component of polar lipids, and is highly conserved in marine foodwebs (Scott et al. 2002, Veefkind 2003). However, this ratio also reflects the relative proportions of dinoflagellate to diatoms in the diets of herbivorous and omnivorous copepods because dinoflagellates are rich in DHA, whereas diatoms are rich in EPA (Viso and Marty 1993). This is also an example of how fatty acid indices of carnivory and trophic level are sometimes confounded by contamination from other sources (Dalsgaard et al. 2003, and references therein).

The combination of FATMS and stable isotopes (recently dubbed "the 2-dimensional approach to trophic dynamics") has proven to be useful in deciphering trophic relations among amphipods, krill and across ecosystems (Nyssen et al. 2005, Alfaro et al. 2006, Perga et al. 2006, Schmidt et al. 2006). Because FATMS are more specific to dietary source than are $\delta^{13}C$, they can alleviate some of the ambiguities that can result from using stable isotopes on their own, particularly in cases where the differences between $\delta^{13}C$ of

different carbon sources are small. Thus, combining both techniques provides a method of testing whether foods of different quality modify trophic interactions within a community, an aspect of trophic ecology that remains poorly understood. To the best of my knowledge, this study represents the first attempt to use this combined technique to investigate trophic dynamics in copepods.

3.2 Methods

3.2.1 Field methods

Sampling took place between 2004-06 in conjunction with the Strait of Georgia Ecosystem Monitoring Project (STRATOGEM, www.stratogem.ubc.ca) and using ships of opportunity. Zooplankton samples were collected from a single station (49°15'N 123°45'W) in the SoG in late April or early May, after the spring bloom. At this time *N. plumchrus*, *E. bungii* and *C. marshallae* have begun to overwinter between 300-400 m (Krause and Lewis 1979, Harrison et al. 1983, Campbell et al. 2004). Zooplankton were sampled using a SCOR net (236 µm mesh, 0.57 m diameter) towed vertically from 0-400 m at 0.5 $m.s^{-1}$. Copepods for fatty acid analyses were sorted shortly after the net was collected, while animals for stable isotope analysis were sorted from partially thawed bulk samples (initially frozen at -80°C) in the laboratory. *N. plumchrus* and *C. marshallae* were sampled as stage CV, while *E. bungii* and *E. elongata* were sampled at stage CV females. Fatty acid replicates contained 10-50 animals, and the number of replicates ranged from 1-4 depending on the availability of animals. Sorted samples were stored in cryogenic vials at -80°C until the time of analysis. In 2005, fatty acid profiles and stable isotopes of copepods from SoG were compared to animals collected at OSP (50°N

145°W). Samples from OSP (*N. plumchrus*, *E. elongata* and *E. bungii*) were collected from 0-1000 m in September of 2005 using the same sorting and storage procedures.

To provide a baseline of stable isotope signatures on a scale relevant to copepod feeding, several samples of particulate organic matter (POM) were collected using Niskin bottles deployed at the chlorophyll maximum (~5 m) over the duration of the spring bloom (early March – late April, n=3-4) from each year. Approximately 1 L of water was passed through 200 μm mesh to remove small copepods, and then filtered onto pre-combusted 47 mm GF/F filters (450°C for 4 hours). Filters were examined under a dissecting microscope to remove any detritus or mesozooplankton, and then frozen at -20°C until analysis. Due to logistical constraints, baseline POM samples were not collected at OSP.

3.2.2 Laboratory methods

3.2.2.1 Fatty acids

In the lab, animals were freeze-dried (<40°C for 48 hours), weighed, and placed in 2 ml HPLC-grade chloroform. Sample analysis followed the protocol outlined in Chapter 2. All fatty acid data are reported as % of total fatty acids. All fatty acid indices used in this study are summarized in Table 5.

3.2.2.2 Stable isotopes

Animals were pooled to create ~1 mg of dry weight per replicate (3 replicates per analysis, 2-30 animals per replicate). Animals were rinsed to remove carbonates and salts, and then dried at 60°C for 48 hours. Dried samples were weighed and packed in tin capsules, and analyzed using a Europa Hydra 20/20 continuous flow isotope mass

spectrometer coupled with a Europa ANCA-SL elemental analyzer (to convert organic carbon and nitrogen into CO_2 and N_2 gasses, respectively) at the UC Davis stable isotope facility (Davis, CA). Isotopic abundances are expressed in δ notation, $\delta = ((R_{sample}/R_{standard})-1)*1000$, where R is the ratio of the heavier isotope to the lighter isotope. Standards for $\delta^{13}C$ and $\delta^{15}N$ were PeeDee Belemnite carbonates and atmospheric nitrogen, respectively.

To remove the bias associated with heterogeneous lipid content, 22 samples of copepods with a range of C/N ratios from 4 to 16 were extracted to remove lipids prior to stable isotope analysis, following Post et al. (2007). Samples were extracted overnight in 8 ml 2:1 chloroform:methanol solution at room temperature. After extraction the samples were rinsed several times in chloroform to remove excess lipids, and then dried and packaged for stable isotopes analysis. The relationship between extracted samples and unextracted samples was found to be:

$$\delta^{13}C_{extracted} = -1.85 + (0.38*C/N) + \delta^{13}C_{original} \qquad (r^2 = 0.60 \text{ and } p < 0.001)$$

where $\delta^{13}C_{extracted}$ represents the sample after extraction, and $\delta^{13}C_{original}$ is the sample before extraction. Standardization of $\delta^{13}C$ to lipid content using this equation resulted in $\delta^{13}C$ values that were within 0.5 ‰ of those that were extracted chemically. The C/N of extracted samples was ~ 4, and the $\delta^{13}C_{extracted}$ did not vary significantly with more extensive methods of lipid isolation, suggesting that lipid removal was complete.

POM filters were dried at 60°C for 48 hours. POM samples were not acidified before stable isotope analysis because preliminary analysis indicated that carbonate did not significantly affect the $\delta^{13}C$ of POM (Appendix 2). Trophic position (Δ) was expressed as $\Delta = (\delta^{15}N_{copepod} - \delta^{15}N_{POM})/3.5 + 1$, where Δ is a continuous measure of trophic position, $\delta^{15}N_{copepod}$ is the $\delta^{15}N$ of the copepod, $\delta^{15}N_{POM}$ is the $\delta^{15}N$ of POM averaged over the entire spring bloom, and 3.5 is the degree of nitrogen fractionation (in ‰) observed for each trophic level (Minagwa and Wada 1984, Post 2002a).

3.2.3 Statistical analyses

To assess the degree of dissimilarity between fatty acid samples, average-neighbour cluster analysis was conducted on a Bray-Curtis dissimilarity matrix based on raw fatty acid data expressed as % of total fatty acids (Bray and Curtis 1957). The contribution of individual fatty acids to these clusters was tested using Similarity Percent (SIMPER) analysis. Correlation between FATMS and stable isotopes was tested using Spearman product-moment correlation. ANOVA, followed by Tukey-Kramer post-hoc analysis was conducted on arcsine-transformed data to assess the significance of the differences between individual FATMS or stable isotopes only in cases where the number of replicates was >3. Clustering and SIMPER analyses were performed using Primer software following Clarke et al. (1993), while ANOVA was performed using SigmaStat (version 5) following Zar (1984).

Table 5: Trophic and dietary fatty acid markers discussed in Chapter 3

Marker	Source	Reference
DHA/EPA	Dinoflagellates/diatoms, carnivory	Budge and Parrish 1998
16PUFA/18PUFA[1]	Diatoms/flagellates	Budge and Parrish 1998, Alfaro et al. 2006
18:2n-6	Terrestrial detritus or green algae	Dalsgaard et al. 2003
15:0 + 17:0[2]	Bacteria	Kaneda et al. 1991
PUFA/SFA[3]	Carnivory	Stevens et al. 2004b
18:1n-9/18:1n-7	Carnivory or omnivory	Stevens et al. 2004b
D/F[4]	Diatoms/flagellates	Adapted from Dalsgaard et al. (2003)

[1]16PUFA includes all PUFA containing 16 carbon atoms, and 18PUFA includes all PUFA containing 18 carbon atoms [2]includes *iso* and ante-*iso* branched chains containing 15-17 carbons [3]PUFA is the sum of all polyunsaturated fatty acids whereas SFA is the sum of all saturated fatty acids [4] D refers to the sum of all diatom markers (16:1n-7+ 20:5n-3 and 16PUFA) whereas F refers to the sum of all flagellate markers (22:6n-3, 18:2n-6, 18PUFA).

3.3 Results

3.3.1 General patterns in SoG copepods

Cluster analysis on FATMS separated the SoG copepods into three groups: Cluster 1 contained *E. elongata*, cluster 2 contained *N. plumchrus* and *C. marshallae*, while cluster 3 contained *E. bungii* (Fig 11). *E. elongata* was characterized by high concentrations of DHA, EPA and 18:1n-9 and low concentrations of 14:0, while *C. marshallae* and *N. plumchrus* were characterized by high concentrations of 14:0, EPA, 16PUFA and 18PUFA. *E. bungii* was characterized by high concentrations of 16:0, 18:1n-7, EPA, 16PUFA and 18:1n-9 (Table 6, Appendix 3).

Stable isotopes separated copepods from 2004-05 into three groups: *E. elongata*, *C. marshallae* and *N. plumchrus,* and *E. bungii* (Fig 12). However, in 2006 these groupings were less distinct (Fig 12e,f). Patterns observed in stable isotope mixing diagrams were caused by the presence of distinct trophic levels as reflected in $\delta^{15}N$ and, to a lesser extent, by differences in food source as reflected by $\delta^{13}C$ (Fig 12). On average, *E. elongata* displayed the highest $\delta^{15}N$ and Δ ($\delta^{15}N \sim 10.81 - 14.87$ ‰ and $\Delta \sim 3.4\text{-}1.2$). *N. plumchrus* and *C. marshallae* displayed intermediate values ($\delta^{15}N \sim 9.42\text{-}10.71$ ‰ and $\Delta \sim 2.57\text{-}1.51$) and *E. bungii* displayed the lowest values ($\delta^{15}N \sim 7.38\text{-}8.47$ ‰ and $\Delta \sim 1.22\text{-}1.57$). Standardizing $\delta^{13}C$ to lipid content changed the stable isotope mixing diagrams significantly. Lipid-standardized $\delta^{13}C_{extracted}$ were on average ~ 3 ‰ heavier than $\delta^{13}C_{original}$ and minimized variability among species. On average, *E. elongata* displayed the lightest $\delta^{13}C_{extracted}$ (~ -19.00 to-21.70 ‰) and *N. plumchrus* the heaviest (~ -16.00 to -17.00 ‰).

3.3.2 Correlations between FATMS and stable isotopes

With a few exceptions, FATMS generally correlated very well with stable isotopes. Three fatty acid markers of carnivory (18:1n-9/18:1n-7, DHA/EPA, PUFA/SFA) correlated significantly with $\delta^{15}N$ and Δ (Table 7). However, the correlation between $\delta^{15}N$ and FATMS was only significant when all species were grouped. Although the correlation between Δ and DHA/EPA, 18:1n-9/18:1n-7 and PUFA/SFA also fell apart when some species were examined individually, it remained strong in others. For example, Δ was significantly correlated with DHA/EPA in *E. elongata*, with 18:1n-9/18:1n-7 in *C. marshallae* and *N. plumchrus*, and with PUFA/SFA in *E. bungii*. Standardizing $\delta^{13}C_{original}$ for lipids improved the correlation between $\delta^{13}C_{copepod}$ and FATMS of dietary quality (DHA/EPA, 16PUFA/18PUFA and D/F), suggesting that heavy $\delta^{13}C_{extracted}$ was correlated with high proportions of diatoms in the fatty acids of these animals (Table 8).

3.3.3 Interspecific differences in trophic position and dietary quality

Among the species considered *E. elongata* displayed the most carnivorous signatures (i.e. higher ratios of DHA/EPA, 18:1n-9/18:1n-7, PUFA/SFA)(Fig 12, 13). There were no consistent differences between *C. marshallae, E. bungii* or *N. plumchrus* in any of the omnivory markers. However, the ratios of DHA/EPA and 18:1n-9/18:1n-7 tended to be lower in *E. bungii* than in either *N. plumchrus* or *C. marshallae*, suggesting that *E. bungii* was more herbivorous than the other two. Furthermore, *E. elongata* usually displayed much lower proportions of 16PUFA/18PUFA and D/F than did the other species (Fig 13). In 2005, *E. elongata* displayed very high 16PUFA/18PUFA, but the proportion of 16PUFA was just as low in 2005 as in 2004 and 2006 (~ 1.97± 1.0 % in 2005 compared

to an average of ~ 1% in 2004 and ~ 2 % in 2005). There were no consistent patterns in 16PUFA/18PUFA or D/F between *N. plumchrus*, *C. marshallae* and *E. bungii*, although the latter tended to have higher proportions of these indices than the other two. There were no observable differences in the proportion of bacterial markers among the different species.

3.3.4 Interannual differences in trophic position and dietary quality

Interannual differences between species can be seen in the clustering pattern observed in Fig 11. Within the larger cluster, *N. plumchrus* and *C. marshallae* from 2004, 2005 and 2006 clustered distinctly, suggesting that fatty acid composition varied between years. *N. plumchrus* consumed progressively more diatoms between 2004-06, as evidenced by the rise of D/F, 16PUFA/18PUFA and a decline in DHA/EPA over that period (Fig 14a). No significant interannual differences were observed in 18:1n-9/18:1n-7, PUFA/SFA or in bacterial markers in the fatty acids of this species. Though DHA/EPA and D/F both pointed to increased diatom consumption by *E. bungii* between 2004-06, 16PUFA/18PUFA displayed the opposite pattern (Fig 14b). There were no apparent differences in FATMS of *C. marshallae* between 2004 and 2006, but 2005 appeared to have been a year of high diatom consumption, as evidenced by low DHA/EPA, high 16PUFA/18PUFA and D/F (Fig 14c). No interannual differences were observed in *E. elongata* (Fig 11, Fig 14d).

3.3.5 Geographic variability in trophic position and dietary quality

Copepods from OSP displayed more omnivorous FATMS than did copepods from the SoG (Table 9). In 2005, OSP copepods were characterized by lower proportions of diatom- derived polyunsaturated fatty acids (16PUFA and EPA) and slightly higher proportions of DHA than at SoG. Whereas *N. plumchrus* and *E. elongata* from OSP displayed high proportions of 18PUFA, the opposite was true for *E. bungii*. Both *E. bungii* and *N. plumchrus* displayed higher proportions of 18:2n-6 in the SoG than at OSP. The $\delta^{15}N$ of *N. plumchrus* was significantly lower at OSP (~ 7.9 ‰) than at SoG (~ 10.9 ‰)($p < 0.05$). There were no significant regional differences in $\delta^{15}N$ of either *E. elongata* or *E. bungii*, but $\delta^{13}C_{extracted}$ were significantly lighter at OSP than at SoG for all species ($p < 0.02$, Figure 15).

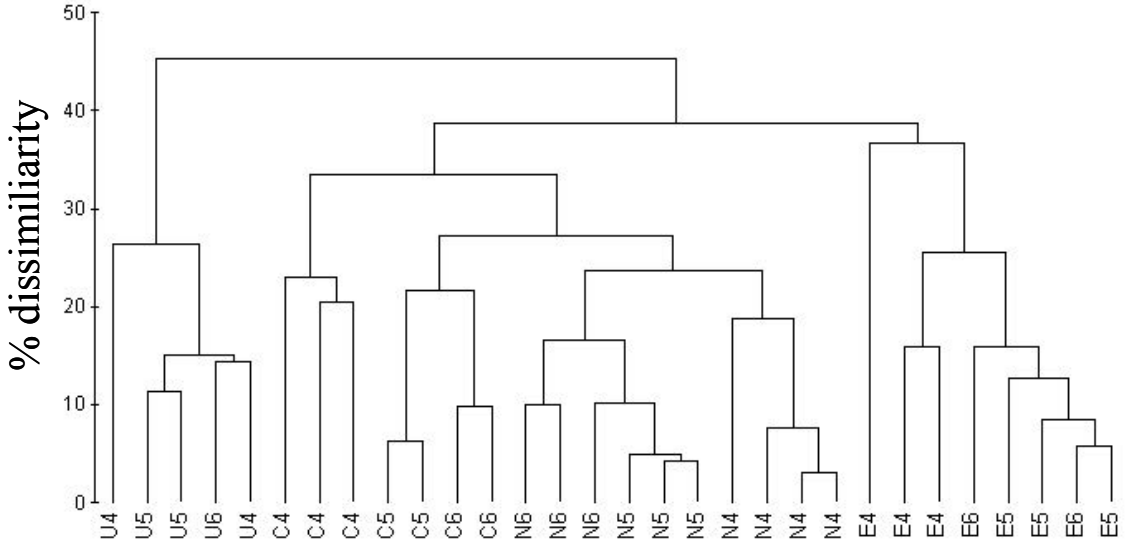

Figure 11. Average-neighbour cluster analysis based on a Bray-Curtis dissimilarity matrix of raw, untransformed fatty acid data (expressed in % of total fatty acids) of copepods collected in May of 2004-06. The letters denote copepod species (E is *Eucalanus bungii*, C is *Calanus marshallae*, N is *Neocalanus plumchrus* and U *is Euchaeta elongata*), whereas the numbers denote years in which copepods were sampled (i.e. 2004, 2005, 2006).

Table 6. Similarity Percent (SIMPER) analysis used to asses the contribution of individual fatty acids to the 3 clusters revealed by the average-neighbour cluster analysis shown in Fig 1. All values are expressed in % of total fatty acids. Av % comp refers to the average composition of the tracer present in each cluster. Av. Sim refers to the average similarity contributed by the fatty acid. Sim/SD is the ratio of similarity to standard deviation. Contrib% is the contribution to the fatty acid to the overall similarity, and cum.% is additive overall similarity (Appendix 3).

Cluster	Similarity	Fatty acid	Av.% comp.	Av.Sim	Sim/SD	Contrib%	Cum.%
Euchaeta elongata	80.4	DHA	19.3	23.8	12.6	29.6	29.6
		EPA	14.5	16.8	12.8	20.9	50.5
		16:00	9.8	11.9	11.5	14.8	65.3
		18:1n-9	12.4	10.6	1.2	13.2	78.4
		16:1n-7	4.7	5.2	7.5	6.5	84.9
		14:00	2.3	2.6	4.4	3.3	88.2
		18:00	2.2	2.3	6.0	2.9	91.1
Neocalanus plumchrus		14:00	17.8	16.5	2.8	22.1	22.1
Calanus marshallae	74.4	EPA	14.1	13.3	4.0	17.9	40.1
		16:00	10.3	10.6	4.7	14.3	54.3
		16PUFA	7.3	6.9	2.5	9.3	63.6
		18:2n-6	6.9	6.2	1.9	8.3	72.0
		DHA	5.3	5.0	3.6	6.7	78.7
		16:1n-7	4.7	4.0	2.2	5.4	84.1
		18PUFA	3.8	3.7	3.7	4.9	89.0
		18:1n-9	3.5	2.2	1.4	2.9	91.9
Eucalanus bungii	76.9	16:00	21.4	20.7	6.2	27.0	27.0
		EPA	13.9	12.2	2.6	15.9	42.9
		16:1n-7	8.1	6.9	2.2	8.9	51.8
		16PUFA	7.0	6.5	3.8	8.5	60.3
		18:1n-9	7.8	6.5	3.2	8.5	68.8
		18:1n-7	8.7	6.0	3.0	7.8	76.5
		18:2n-6	5.3	5.1	3.8	6.6	83.1
		14:00	4.8	4.2	2.8	5.4	88.5
		18PUFA	4.2	3.2	5.1	4.1	92.7

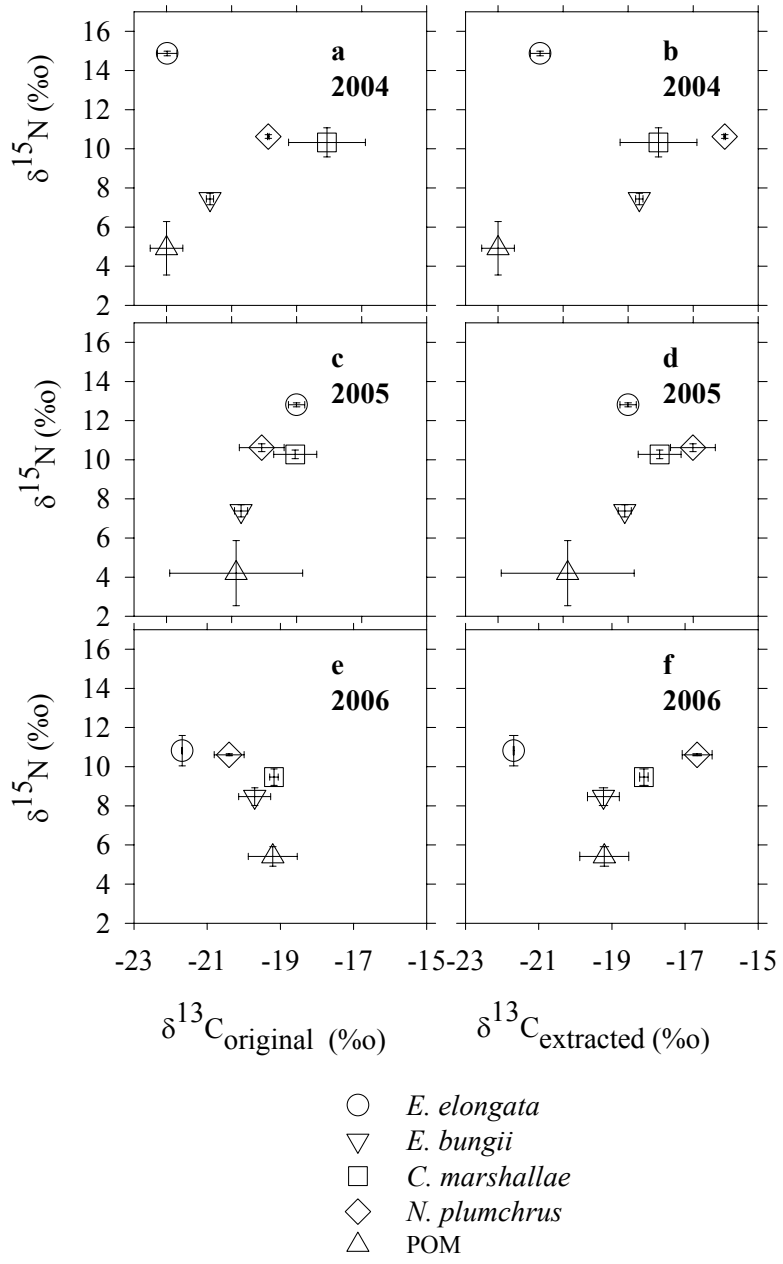

Figure 12. Isotope mixing diagrams of nitrogen stable isotopes ($\delta^{15}N$) and carbon stable isotopes ($\delta^{13}C$) of copepods collected in May of 2004-06. Panels (a, c, e) show original $\delta^{13}C$ data, and panels (b,d,e) show lipid-standardized signatures. Samples are represented as mean ± 1 standard deviation (n=3).

Table 7. Correlations between trophic position indices of stable isotopes ($\delta^{15}N$ of copepods) and fatty acid trophic markers, when all species are pooled, or separated on the basis of the clustering pattern revealed in Figure 11. The coefficient r represents Pearson product-moment correlation coefficient, and P is the degree of significance ($\alpha = 0.05$). Statistically significant values are in bold typeface.

		All species pooled		*Euchaeta elongata*		*Eucalanus bungii*		*Neocalanus plumchrus* and *Calanus marshallae*	
Parameter	FATM	r	P	r	P	r	P	r	P
$\delta^{15}N$	DHA/EPA	**0.8125**	**0.0001**	0.475	0.1962	-0.0945	0.8239	0.08495	0.7473
	18:1n-9/18:1n-7	**0.6064**	**0.0001**	-0.421	0.2602	0.1744	0.6536	0.4053	0.0952
	PUFA/SFA	**0.4775**	**0.0019**	-0.5286	0.0717	0.3677	0.1651	-0.4116	0.051
Δ	DHA/EPA	**0.563**	**0.0002**	**0.9187**	**0.0002**	-0.0235	0.4671	0.107	0.3357
	18:1n-9/18:1n-7	**0.5036**	**0.0009**	0.2448	0.2628	0.53043	0.0831	**0.7345**	**0.0003**
	PUFA/SFA	**0.3348**	**0.023**	-0.2275	0.0815	**0.7995**	**0.0044**	0.1588	0.0557

Table 8. Correlation between fatty acid trophic markers and stable isotope markers of dietary quality ($\delta^{13}C$) when all species are pooled, and when separated on the basis of the clustering pattern revealed in Fig 11. $\delta^{13}C_{original}$ represents the $\delta^{13}C$ of copepods before lipid standardization, whereas $\delta^{13}C_{extracted}$ represents the $\delta^{13}C$ of copepods after lipid standardization. The coefficient r represents the Pearson product-moment correlation coefficient, whereas P is the degree of significance at $\alpha = 0.05$. Significant values are in bold typeface.

		All species pooled		*Euchaeta elongata*		*Eucalanus bungii*		*Neocalanus plumchrus* and *Calanus marshallae*	
Parameter	FATM	r	P	r	P	r	P	r	P
$\delta^{13}C_{original}$	DHA/EPA	-0.3127	0.0714	-0.034	0.4653	**-0.6386**	**0.0321**	0.336	0.0862
	16PUFA/18PUFA	0.2711	0.0551	**0.9245**	**0.0002**	**-0.6139**	**0.0394**	-0.282	0.1282
	D/F	0.1553	0.183	0.3901	0.1497	0.7025	0.174	-0.1476	0.2795
$\delta^{13}C_{extracted}$	DHA/EPA	**-0.5484**	**0.0003**	0.1708	0.3302	0.7493	0.01	0.3135	0.1026
	16PUFA/18PUFA	**0.3963**	**0.0084**	**0.9835**	**0.0001**	**0.9082**	**0.0004**	**0.6817**	**0.0009**
	D/F	**0.3351**	**0.029**	0.2059	0.09553	0.0955	0.4043	0.2192	0.191

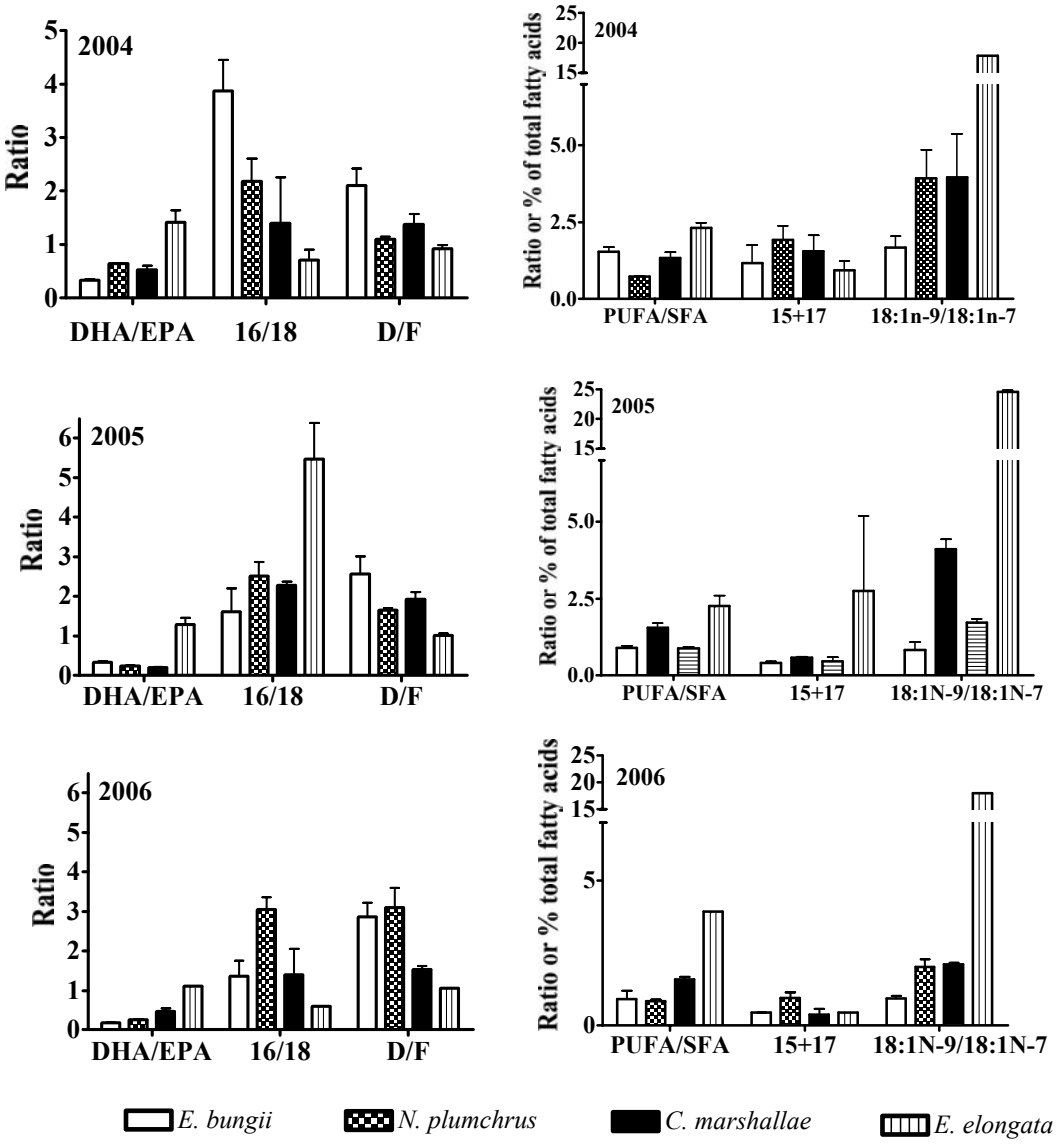

Figure 13. Interspecific differences in fatty acid trophic markers of dietary quality and trophic position in copepods from the Strait of Georgia between 2004-06. Fatty acid trophic markers are summarized in Table 5. Error bars represent 1 standard deviation from the mean (n=2-4).

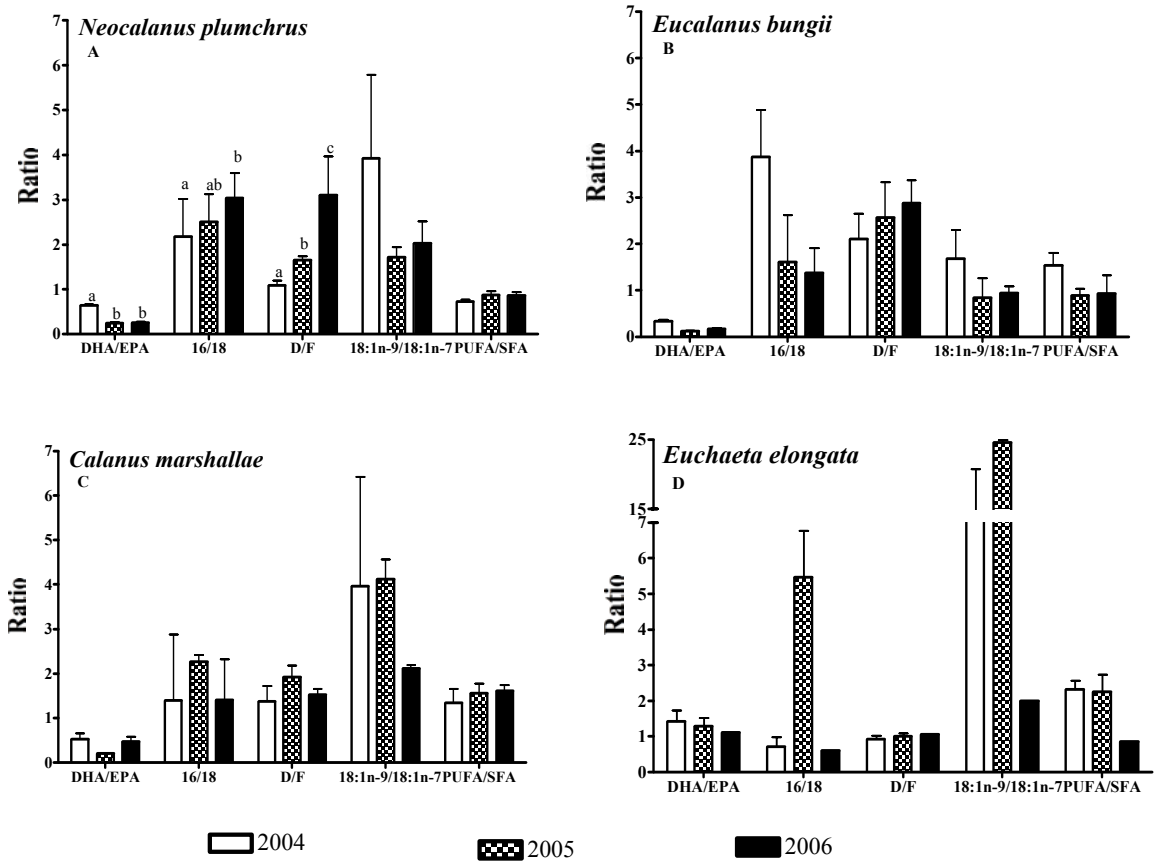

Figure 14. Interannual variability in the fatty acid trophic markers of *Neocalanus plumchrus*, *Eucalanus bungii*, *Calanus marshallae* and *Euchaeta elongata* between 2004-06. Fatty acid trophic markers are summarized in Table 5. Error bars represent 1 standard deviation from the mean (n=2-4). Bars topped by different letters are statistically significant ($P < 0.05$) using ANOVA and Tukey-Kramer post hoc analysis. ANOVA was performed only in cases where all groups contained more than 3 replicates.

Table 9: Spatial differences in fatty acid profiles of *Eucalanus bungii*, *Euchaeta elongata* and *Neocalanus plumchrus* from the Strait of Georgia (SoG) and Ocean Station P (OSP) in 2005. Data are expressed as average % of total fatty acids ± 1 standard deviation.

	Eucalanus bungii		*Euchaeta elongata*		*Neocalanus plumchrus*	
Fatty acid	SoG	OSP	SoG	OSP	SoG	OSP
14:0	6.1 ± 0.9	10.7 ± 0.3	2.0 ± 0.5	2.5 ± 0.2	26.0 ± 1.0	21.2 ± 2.7
15+17	0.4 ± 0.1	1.1 ± 0.0	2.8 ± 2.3	1.0 ± 0.4	0.5 ± 0.2	2.1 ± 0.8
16:0	25.3 ± 2.1	25.7 ± 1.0	9.0 ± 2.2	4.4 ± 1.0	11.5 ± 0.2	12.9 ± 1.4
16:1n-7	10.7 ± 0.9	8.4 ± 0.6	3.8 ± 2.9	9.8 ± 1.0	3.6 ± 0.5	1.6 ± 0.4
16PUFA	5.9 ± 0.2	1.7 ± 0.1	2.0 ± 1.3	0.4 ± 1.3	10.0 ± 0.4	4.8 ± 5.1
18:0	2.6 ± 0.3	1.6 ± 0.1	2.5 ± 0.9	0.6 ± 0.5	0.7 ± 0.0	1.2 ± 0.3
18:1n-7	9.0 ± 5.1	3.0 ± 0.2	0.7 ± 0.3	0.7 ± 2.5	0.6 ± 0.2	0.5 ± 0.1
18:1n-9	6.1 ± 0.1	4.0 ± 0.2	16.8 ± 3.9	24.8 ± 9.1	0.9 ± 0.2	2.0 ± 0.4
18:2n-6	5.9 ± 1.3	3.3 ± 0.1	1.3 ± 0.3	1.7 ± 0.2	9.8 ± 0.4	1.7 ± 0.2
18PUFA	5.9 ± 5.5	2.0 ± 0.1	0.3 ± 2.1	4.0 ± 1.2	4.1 ± 0.8	10.7 ± 1.6
20:1n-9	0.4 ± 0.1	0.2 ± 0.0	0.6 ± 0.3	1.2 ± 2.1	1.2 ± 0.5	0.8 ± 0.5
20:1n-11	0.1 ± 0.1	0.1 ± 0.0	0.4 ± 1.0	2.4 ± 0.4	3.1 ± 0.2	5.8 ± 4.5
22:1n-9	0.1 ± 0.0	0.2 ± 0.0	1.1 ± 1.1	0.2 ± 0.3	0.1 ± 0.0	0.2 ± 2.6
22:1n-11	0.1 ± 0.1	0.1 ± 0.0	0.6 ± 0.9	2.0 ± 0.2	2.6 ± 0.3	5.0 ± 3.2
EPA	16.6 ± 0.5	15.4 ± 0.8	14.5 ± 4.7	6.6 ± 4.3	15.6 ± 1.5	8.9 ± 1.3
DHA	2.0 ± 0.2	4.9 ± 0.3	18.7 ± 3.6	12.2 ± 1.4	3.9 ± 0.6	5.5 ± 0.5
16/18	1.6 ± 1.0	1.1 ± 0.1	5.5 ± 3.1	0.1 ± 0.3	2.5 ± 0.6	0.7 ± 0.6
DHA/EPA	0.1 ± 0.0	0.3 ± 0.0	1.3 ± 0.3	1.8 ± 0.3	0.2 ± 0.0	0.6 ± 0.1
18:1n-9/18:1n-7	0.8 ± 0.4	1.5 ± 0.0	24.8 ± 8.0	37.2 ± 9.8	1.7 ± 0.2	4.1 ± 0.9
Diatoms	33.8 ± 1.4	25.7 ± 1.3	20.4 ± 4.2	16.9 ± 4.6	29.6 ± 1.3	15.4 ± 4.6
Flagellates	13.8 ± 4.6	10.3 ± 0.5	20.3 ± 3.3	17.9 ± 2.4	17.8 ± 1.3	18.1 ± 2.0
PUFA/SFA	0.9 ± 0.1	0.7 ± 0.1	2.3 ± 0.8	2.7 ± 0.2	0.9 ± 0.1	0.8 ± 0.2

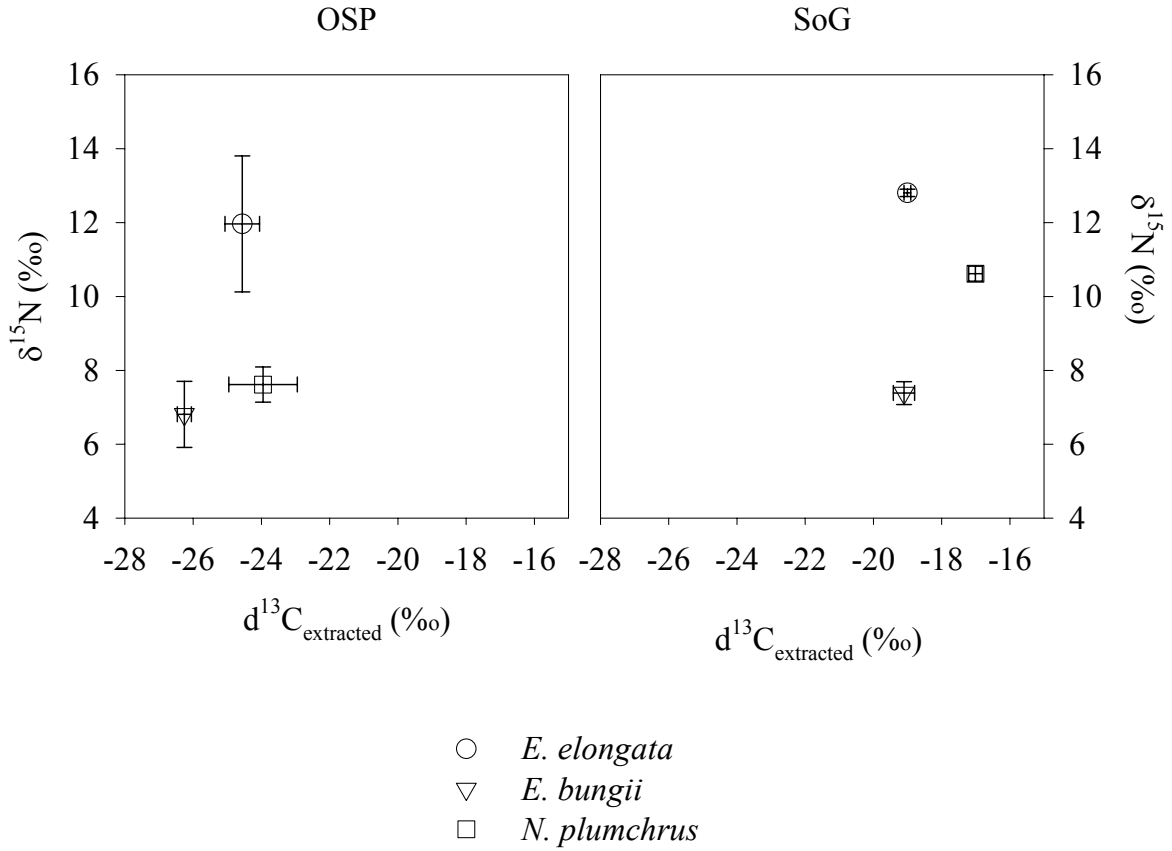

Figure 15. Spatial comparison of isotope mixing diagrams of copepods from the Strait of Georgia (SoG) and Ocean Station P (OSP). Samples are represented as mean ± 1 standard deviation (n=3). The $\delta^{13}C$ signatures were standardized for lipid content. All SoG samples were collected in May 2005, and all OSP samples were collected in September 2005.

3.4 Discussion

3.4.1 Interspecific, interannual and geographic variability in food quality

The fatty acid profiles of the copepod species examined here provide information on their overwintering strategies, their relative trophic positions and the quality of their diets. *N. plumchrus* and *C. marshallae* store their lipids as wax esters which are characterized by high proportions of the fatty acids 20:1n-9, 20:1n-11, 22:1n-9 and 22:1n-11 (Sargent and Whittle 1981). Consequently, *C. marshallae* and *N. plumchrus* contain higher proportions of these fatty acid markers than either *E. elongata* or *E. bungii* (Table 6). *E. bungii* stores its lipids as triacylglycerols characterized by high proportions of 18:1n-9, 18:1n-7 and 16:1n-7 fatty acids, which are abundant in its fatty acid profile (Lee 1974, Saito and Kotani 2000). Although most *Euchaeta* spp. are presumed to store wax esters, it is difficult to judge this from their fatty acid profiles alone because their storage signatures are often influenced by the storage signatures of their prey (Hagen et al. 1995).

Both fatty acids and stable isotopes suggest that in the SoG *E. elongata* is consistently carnivorous, while *E. bungii* is herbivorous and *N. plumchrus* and *C. marshallae* are omnivorous. *N. plumchrus*, *E. bungii* and *C. marshallae* also display interannual variability in their FATMS, which is demonstrated in the cluster analysis (Fig 11), and which in the case of *N. plumchrus* is statistically significant (Fig 14). Between 2004-06, the diets of *N. plumchrus*, and to a lesser extent *E. bungii*, became progressively more diatom rich. The diet of *C. marshallae* was more diatom rich in 2005 than in either 2004 or 2006. Recent data suggests that taxonomically, the 2004 spring phytoplankton bloom was more diverse than in 2005 (which was almost exclusively dominated by diatoms),

suggesting that the fatty acid profiles of these copepods may reflect phytoplankton concentrations in the water column (El-Sabaawi et al, Chapter 2). The divergence in dietary quality between *C. marshallae*, *E. bungii* and *N. plumchrus* in 2006 could be due to variability in the timing of feeding and overwintering. No discernable interannual differences were detected in *E. elongata* in the cluster analysis, suggesting that interannual variability in the signatures of this copepod is relatively minor. Hagen et al. (1995) suggested that in this genus lipids are metabolized quickly thereby obscuring small differences in dietary quality.

Unlike the diatom-rich SoG, the protist assemblage at OSP is composed of small flagellates, microzooplankton and cyanobacteria (Harrison et al. 2004). As expected, *E. bungii*, *N. plumchrus* and *E. elongata* all display more omnivorous signatures at OSP than they do in the SoG. However, the results of this spatial study should be interpreted with caution, because 2005 appears to have been an unusual year in the SoG, and because SoG *N. plumchrus* can experience a wide range of dietary quality, which can overlap with oceanic conditions in some years (El-Sabaawi et al, submitted). It is perhaps not surprising that the $\delta^{15}N$ signatures of copepods at OSP overlap with those in the SoG because, despite differences in the protist assemblage between the two sites, the range of $\delta^{15}N_{POM}$ at OSP (0-7 ‰) is only slightly lighter than in the SoG (1.5 – 8 ‰)(Needoba et al. 2006, Johannessen et al. 2005). Though both *N. plumchrus* and *E. bungii* display relatively high FATMS of carnivory at OSP, neither approaches the trophic position of *E. elongata*, indicating that neither species has changed its foraging strategy to accommodate a more oceanic diet. Some copepods have been shown to switch foraging

tactics from herbivory (mainly filter feeding) to carnivory (raptorial feeding and active hunting) depending on the availability of food (Landry 1981, also see Chapter 4), whereas others do not (Sommer et al. 2005a, Sommer et al. 2005b). An alternative explanation is that the high proportions of 18:1n-9 in OSP copepods reflect feeding on prymnesiophytes, which can be rich in this fatty acid (Dalsgaard et al. 2003). However, prymnesiophytes are also rich 18PUFA, but no enrichment in this marker is evident in OSP copepods. The $\delta^{13}C_{extracted}$ signatures of copepods from OSP are significantly lighter than those from the SoG, suggesting that SoG copepods consume a more diatom-rich diet than their oceanic counterparts. Because diatoms can utilize the isotopically-heavy bicarbonate ion as a carbon source, diatoms are often heavier in $\delta^{13}C$ than are flagellates (Fry and Wainright 1991).

3.4.2 The 2-dimensional approach in copepods: strengths and weaknesses

I found significant correlation between FATMS and stable isotopes, in agreement with other studies that have used this combined approach (Nyssen et al. 2005, Alfaro et al. 2006). High contributions of diatoms in the FATMS of copepods (as evidenced by low DHA/EPA and high 16PUFA/18PUFA) were correlated with heavier $\delta^{13}C_{extracted}$ when all of the species were combined in the correlation analysis (Table 8). However, despite the general correlation, $\delta^{13}C_{extracted}$ did not capture the general trends observed in FATMS in the SoG. For example, although FATMS of SoG *N. plumchrus* indicated a significant shift towards diatom-based herbivory over the duration of this study, no significant differences were observed in $\delta^{13}C_{extracted}$ for this species. One reason that $\delta^{13}C$ may have failed to capture differences in food sources observed in the FATMS between years in the

same species is that changes in the proportions of diatoms and flagellates in the diet may not have been large enough to induce a change in $\delta^{13}C_{extracted}$. Even across two substantially different environments such as OSP and SoG, the $\delta^{13}C_{extracted}$ of these copepods varied by < 5.00 ‰.

This study also highlights the importance of lipid extraction in the interpretation of stable isotope data. Before lipid standardization, my *E. bungii* samples displayed a lighter $\delta^{13}C$ signal than did either *C. marshallae* or *N. plumchrus* – a superficial interpretation based on this pattern would suggest that *E. bungii* was feeding on more flagellates than either *C. marshallae* or *N. plumchrus*. However, after lipid standardization, all three species showed similar $\delta^{13}C$ signatures, suggesting that they were feeding on the same pool of carbon, which was reflected in their FATMS. Thus, standardizing copepod samples for lipids is extremely important for the trophic interpretation of stable isotope data.

However, one problem with using stable isotopes to study trophic dynamics is that there are no standardized protocols for lipid extraction or sample preparations (Post et al. 2007). The significant correlation between $\delta^{15}N$, Δ and FATMS of carnivory indicate that all three indices convey the same information about the relative trophic positions of these copepods. The $\delta^{15}N$ of copepods is a product of the signature of the diet, as well as trophic fractionation (Minagawa and Wada 1984). Therefore, on its own $\delta^{15}N$ is not a good indicator of absolute trophic position, because differences in baseline signatures can vary from year to year, potentially obscuring the trophic component of $\delta^{15}N$. The measure of Δ used here is perhaps a crude measure of absolute trophic position, since it

assumes that all of the species are feeding from the same pool of phytoplankton collected at the chlorophyll maximum. However, the Δ values reported here are very similar to those measured using $\delta^{15}N$ of amino acids from the same genera in the California Current (Landry 2007). At the very least, my estimates of Δ represent one method of removing the effects of baseline variability from the $\delta^{15}N$ signature.

When the correlations between Δ and FATMS are examined by species, they do not systematically break down as did the correlation between $\delta^{15}N$ and FATMS. Rather, for each group Δ correlates significantly with some indices of carnivory, but not for others. It is not surprising that DHA/EPA correlates significantly with Δ in carnivorous *E. elongata*, but not in the herbivorous *E. bungii*, *C. marshallae* or *N. plumchrus*. In herbivorous copepods, DHA/EPA varies with ambient proportions of diatoms and flagellates, in which case interannual variability in the relative proportions of these phytoplankton groups can cause DHA/EPA to vary independently of trophic position. Both *E. elongata* and *E. bungii* synthesize 18:1n-9 in addition to acquiring it from their diet. Standardizing 18:1n-9 to 18:1n-7 is believed to account for *de novo* synthesis, but recent evidence suggests that 18:1n-7 can also vary with diet, especially in regions where bacterial input is high (Stevens et al. 2004b,c). Therefore, in species where this particular *de novo* synthesis pathway is active (such as *E. elongata* and *E. bungii*), the trophic signature of 18:1n-9/18:1n-7 can become confounded. The implication is that although all of the carnivory indices investigated here provide a consistent picture of relative trophic position, caution must be employed when using such tracers to indicate absolute trophic

positions within the same genera, or among copepods with different pathways of *de novo* fatty acid synthesis.

3.4.3 Conclusions

As foodweb tracers of trophic dynamics, stable isotopes and fatty acids each have their strengths and weaknesses. On their own, stable isotopes supply relatively accurate information about relative and even absolute trophic position if variability in the dietary baseline is accounted for, but often fail to capture subtleties of dietary source captured by fatty acid indices. The use of stable isotopes to trace changes in dietary sources should be limited to situations where differences in these sources are expected to be substantial. On their own, fatty acids supply information on relative trophic position and food quality, but cannot provide absolute trophic position, and some indices of trophic position may be confounded by the products of *de novo* fatty acid synthesis. Applying both techniques together provides a more accurate picture of trophic dynamics because their strengths are complementary. Using this 2-dimensional approach, I have shown that copepods in the SoG occupy three trophic positions and that, despite geographic variability in their dietary quality, they are able to maintain their relative trophic position, suggesting that changes in dietary quality do not result in a change in foraging tactics.

Chapter 4: The seasonal cycle of copepod trophic dynamics in the Strait of Georgia

4.1 Introduction

Characterizing energy flows is central to our understanding of marine ecosystems. Within a typical marine ecosystem, numerous trophic pathways can interact, increasing the complexity of energy flow (e.g. Bode et al. 2004). The complexity and interconnectedness of marine foodwebs are thought to be facilitated by omnivory, which allows organisms to utilize a wide range of diets, thereby increasing ecosystem stability (e.g. Fagan 1997). Copepods, which are the primary link between primary producers and secondary consumers in the oceans, are omnivores capable of utilizing a wide range of allochthonous and autochthonous carbon sources (Kleppel 1993). Copepod omnivory can lead to changes in the trophic level of apex predators, which affects the efficiency by which energy is transferred to higher consumers (Hairston and Hairston 1993). Changes in copepod feeding have also been shown to be important in structuring lower trophic levels (Stibor et al. 2004, Vadstein et al. 2004), and changes in the dietary quality of copepods resulting from their omnivory can affect the survival of their predators (e.g. St John et al. 2001).

In high-latitude coastal ecosystems, the availability of food for copepods is highly seasonal. Periods of high primary and secondary productivity typically occur when phytoplankton are dominated by diatom blooms, while periods of low productivity are characterized by the increased importance of the microbial loop. However, some studies

have suggested that even during the spring the microbial loop can represent an important component of copepod feeding (Vargas et al. 2007). Coastal ecosystems are also exposed to significant input of terrestrial detritus, which can provide an important source of allochthonous energy for the system. Mechanisms that control food supply (such as the timing of the spring bloom and the input of rivers) are typically closely tied to changes in climate and physical forcing, and as such can vary dramatically from year to year.

The Strait of Georgia (SoG), a productive coastal ecosystem on the west coast of Canada, is an important region for a variety of commercial and recreational fisheries (Ketchen et al 1983). Zooplankton biomass is strongly correlated to fisheries yield in the region (Ware and Thomson 2005), and copepods are the dominant group of zooplankton (Legare 1954, Harrison et al. 1983). In SoG the spring is dominated by large, primarily herbivorous copepods, whereas the summer, autumn and winter communities are dominated by smaller herbivorous and omnivorous copepods (Harrison et al. 1983). Phytoplankton shift from a highly productive spring assemblage dominated by large diatoms and flagellates, to a low productivity winter community dominated by small flagellates and microzooplankton (Stockner et al. 1979). Small, “mini-blooms” of phytoplankton are observed in the summer, and are thought to be important for maintaining high zooplankton biomass throughout the summer (Harrison et al. 1983, Pawlowicz et al. in prep). A small peak of chlorophyll biomass, typically composed of small centric diatoms, is sometimes observed in the autumn (Harrison et al. 1983).

The Fraser River, which peaks in June, introduces a significant amount of allochthonous detrital carbon into the SoG basin (Johannessen et al. 2005). Whether this represents a significant nutritional subsidy to the mesozooplankton of the region is unknown (Johannessen et al. 2003). The Fraser also introduces a significant amount of dissolved organic matter, which is metabolized in the SoG basin (Johannessen et al. 2003). Though bacterial biomass is insufficient to fully support copepod growth in the region, it may provide a dietary supplement to copepods either through direct ingestion of large aggregates or by indirect assimilation of bacterivorous flagellates and microzooplankton (Seki and Kennedy 1969).

Although the input of inorganic nutrients by the Fraser tends to be small compared to oceanic sources, shear forcing induced by the Fraser River plume has been shown to stimulate the entrainment of oceanic nutrients from below the mixing layer into the surface layer (Yin et al. 1995, 1997a). The entrainment of oceanic nutrients into the surface of the SoG is thought to be responsible for maintaining high levels of primary production around the edges of the plume (Yin et al. 1997a). Therefore, high Fraser River discharge can indirectly stimulate the input of autochthonous (oceanic) nutrients into the SoG.

This study represents the first to attempt to describe how trophic dynamics and dietary quality of copepods vary seasonally in the SoG, and to assess the importance of allochthonous carbon sources to the region. Previous attempts to study trophic dynamics in the SoG have assumed that copepods are strictly herbivorous, or that they are unable to

utilize allochthonous carbon (Parsons and LeBrasseur 1970). I discuss the relevance of these findings to current understanding of copepod dynamics in the SoG, and to modelling efforts in the region.

4.2 Methods

4.2.1 Standing stocks

Sampling was conducted in conjunction with the STRATOGEM project (www.stratogem.ubc.ca). Mesozooplankton were sampled from 49°15'N 123°45'W using a SCOR-type net (236 μm white mesh, 0.57 m diameter mouth, equipped with a TSK flowmeter) towed vertically from 100 m to the surface at ~ 1 $m \cdot s^{-1}$. Samples were preserved in 5% buffered formalin, and identified to species and stage levels whenever possible. Total mesozooplankton and copepod biomass ($mg \cdot m^{-3}$) were estimated using length:weight regressions from the Institute of Ocean Sciences zooplankton database (Sidney, BC). Trends in community composition, species-specific abundances and life history from the 2002-05 STRATOGEM data set are discussed by Sastri et al. (in prep). Copepods were further classified as omnivorous, herbivorous, carnivorous or detritivorous based on Mauchline (1998), with one notable exception. In his compilation, Mauchline (1998) classified *Calanus* spp. as herbivorous, but there is considerable evidence for omnivory in this genus (Landry 1981). Therefore, both *C. marshallae* and *C. pacificus* were classified as omnivorous here.

Phytoplankton samples were collected from the chlorophyll maximum (~ 5m), preserved in Lugol's solution, identified to species level, and converted to carbon units following

(Sournia 1978). Chlorophyll was extracted from water collected from 0, 5 and 10 m using 5L Niskin bottles, and analyzed fluorometrically following Parsons et al. (1984). Approximately 200 ml of seawater was filtered through polycarbonate filters (0. 2 μm, 47 mm). Samples were extracted overnight in HPLC-grade acetone at -20^{o}C. Chlorophyll fluorescence was measured with a Turner A10 fluorometer, and phaeopigments corrected for with 10% HCL. Chlorophyll concentrations were integrated over the depths sampled using trapezoidal integration. Nitrate concentrations (μM) were measured from samples collected at 5 m using 5L Niskin bottles. A ~ 30 ml sub-sample was collected, and filtered through a pre-combusted 25 mm GF/F filter (at 450^{o}C for 4 hours). The filtrate was analyzed using a Bran and Luebbe AutoAnalyzer (model 3). Surface salinity (PSU) was measured using a Sea-Bird SBE 19Plus conductivity-temperature-depth profiler.

4.2.2 Biochemical sampling

To assess how copepod diets and trophic positions changed seasonally, the fatty acids and stable isotopes of the copepod *Metridia pacifica* (Stage CV females) were analyzed. Measuring these parameters on a bulk sample of all copepods is logistically impossible because it is difficult to separate copepods from other mesozooplankton of the same size (e.g. small krill, pteropods, etc), and because diatom chains represent a significant source of contamination in zooplankton nets during the spring (Campbell and Dower, in press). In addition, it has been shown that δ^{15}N of bulk mesozooplankton samples is only weakly correlated with actual trophic structure, and is strongly affected by the degree of nutrient limitation in the water column (Bode and Alvarez-Ossorio 2004). *M. pacifica* was chosen as a representative copepod because it is multigenerational and feeds year-round in the

SoG, and because its prosome length is close to the average size of all the copepod species in the SoG (Sastri et al. in prep). *Metridia* is a truly omnivorous genus with the ability to filter a wide size range of particles (Sullivan et al. 1975). *M. pacifica* CV females from 200 m SCOR net casts towed at a rate 0.5 m. s^{-1} were sorted using wide-mouthed glass pipettes.

4.2.3 Fatty acid analysis

Fatty acids can be used to infer trophic relations in copepods because copepods acquire many of the fatty acids required for their growth and reproduction from their diets (Graeve et al. 2005). Major dietary items contain group-specific fatty acids which can be used to trace their contribution to copepod diets (Dalsgaard et al. 2003). For fatty acid analysis one sub-sample of pooled *M. pacifica* females (each containing 70-100 animals) was analyzed per sampling date. Fatty acid samples were analyzed following the protocol outlined in Chapter 2. All fatty acid data are reported as % of total fatty acids. The fatty acid trophic markers (FATMS) used here are summarized in Table 10.

To assess the underlying structure in the fatty acid data a combination of Multidimensional Scaling (MDS) and average-neighbour clustering (based on rank similarities) was applied to a Bray-Curtis dissimilarity matrix constructed using raw, untransformed, fatty acid data (Bray and Curtis 1957, Clarke 1993). MDS stress values <0.20 were considered robust (Clarke 1993). Similarity Percent (SIMPER) analysis was used to assess the contributions of individual fatty acids to the observed clustering patterns.

4.2.4 Stable isotope analysis

For stable isotopes, 2-3 replicates (each containing ~ 15 animals, average dry weight ~ 0.7 mg per sample) were analyzed as described in Chapter 3. Isotopic abundances are expressed in δ notation, $\delta = ((R_{sample}/R_{standard})-1)*1000$, where R is the ratio of the heavier isotope to the lighter isotope. Standards were PeeDee Belemnite carbonates and atmospheric nitrogen for $\delta^{13}C$ and $\delta^{15}N$ respectively. Stable isotope samples were standardized for lipid content as described previously (Chapter 3).

Samples of particulate organic matter (POM) were collected from the chlorophyll maximum as a measure of copepod dietary baseline. Approximately 1L of water was filtered through a 47 mm pre-combusted GF/F filter. Samples were analyzed as described in Chapter 3. Trophic position (Δ) was expressed as $\Delta = (\delta^{15}N_{copepod} - \delta^{15}N_{POM})/3.5 +1$, where Δ is a continuous measure of trophic position, $\delta^{15}N_{copepod}$ is the $\delta^{15}N$ of the copepod, $\delta^{15}N_{POM}$ is the $\delta^{15}N$ of POM averaged over the entire spring bloom, and 3.5 is the degree of trophic nitrogen fractionation (in ‰) observed for each trophic level (Minagwa and Wada 1984, Post 2002a). $\delta^{13}C_{copepod}$ and $\delta^{13}C_{POM}$ refer to the isotopic carbon signatures of copepods and particulate organic matter, respectively (as do $\delta^{15}N_{copepod}$ and $\delta^{15}N_{POM}$ for nitrogen).

Table 10. Summary of dietary fatty acid markers discussed in Chapter 4.

Marker	Diet	Reference
DHA/EPA[1]	Dinoflagellates/diatoms, Carnivory	Budge and Parrish (1998)
16PUFA/18PUFA[2]	Diatoms/ flagellates	Budge and Parrish (1998)
18:2n-6	Terrestrial detritus or green algae	Dalsgaard et al. (2003)
15:0 + 17:0[3]	Bacteria	Kaneda et al. (1991)
18:1n-9/18:1n-7	Carnivory or omnivory	Stevens et al. (2004b,c)
18:1n-7	Bacteria or *de novo* synthesis	Stevens et al. (2004b,c)
18:3n-3, 18:3n-6	Terrestrial detritus or green algae	Budge et al (2001), Dalsgaard et al (2003)
PUFA/SFA[4]	Carnivory	Stevens et al. (2004b,c)
22:0 and 24:0	Riverine or terrestrial detritus	Budge et al (2001)

[1] EPA is eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3) [2] 16PUFA includes all polyunsaturated fatty acids (PUFA) containing 16 carbon atoms, while 18PUFA includes all PUFA containing 18 carbon atoms [3]includes *iso* and ante-*iso* branched chains containing 15-17 carbons [4]SFA is the sum of all saturated fatty acids

4.3 Results

In 2004, peak mesozooplankton biomass occurred on April 6th (33.68 mg·m^{-3}), while the maximum copepod biomass occurred on March 29th (21.46 mg·m^{-3}). The lowest mesozooplankton and copepod biomass was recorded on January 14th (4.11 mg·m^{-3} and 1.92 mg·m^{-3}, respectively, Fig 16). Copepods constituted 37-68% of the total mesozooplankton biomass in the upper 100 m (Fig 16). In 2005, maximum mesozooplankton biomass (32.54 mg·m^{-3}) coincided with the maximum copepod biomass (15.69 mg·m^{-3}) a week earlier than in 2004 (Fig 16). In 2005, copepods represented 28 - 48% of the total mesozooplankton biomass in the upper 100 m. Chlorophyll concentration was highest in the spring and lowest in the winter, ranging between 28.5 µg·L^{-1} (April 6th 2004) to 0.28 µg·L^{-1} (Feb 2nd 2005). In both years peak copepod biomass coincided with the peak chlorophyll concentration.

The majority (75-90%) of the copepod biomass was made up by omnivorous species (Fig 17). The proportion of herbivorous copepods increased during the spring (to ~ 35% of the biomass in 2004, 21% of the biomass in 2005), often overlapping with the peak chlorophyll concentration. In the summer, the proportion of carnivores was higher than the proportion of herbivores (10-15% compared to 0-10%), coinciding with relatively low chlorophyll concentrations. The spring phytoplankton community was composed primarily of diatoms (1x10^6 to 1.5x10^6 ng C·L^{-1}), whereas the summer assemblage was characterized by flagellates and ciliates and low concentrations of diatoms (Fig 17b). A fall diatom bloom was observed in the autumn of 2004. Detritivores appeared to be relatively unimportant in SoG, and constituted less than 0.05% of copepod biomass.

MDS ordination coupled with average-neighbour cluster analysis separated the fatty acid data into three groups (Fig 18). Group 1 contained a single winter sample (Feb 28, 2004), characterized by high proportions of the bacterial fatty acid marker (18:1n-7), and by proportions of DHA, EPA, 16PUFA and 18PUFA. Group 2 contained mid- to late-spring bloom samples (late April to mid May), which were characterized by low proportions of DHA, 18:1n-9 and 18PUFA and by high proportions of EPA, 16:1n-7 and 18:2n-6. Group 3 contained summer, winter and autumn samples, as well as a few early spring bloom samples, and was characterized by high proportions of 18:1n-9, DHA, 18PUFA and EPA (Table 11, Appendix 4).

The $\delta^{15}N$ of particulate organic matter ($\delta^{15}N_{POM}$), which was used as a measure of dietary baseline to calculate the Δ of *M. pacifica*, ranged between 1 ‰ in winter and 8 ‰ in spring (Fig 19a). $\delta^{15}N_{POM}$ displayed the opposite trajectory of nitrate concentrations, which ranged between 0.2 μM in June 2004 and 29.1 μM in January 2004. $\delta^{15}N_{copepods}$ ranged from 8-11 ‰ over the study, and was often decoupled from variability in $\delta^{15}N_{POM}$. The Δ of *M. pacifica* was low in the spring (2 – 2.5), coinciding with periods of high chlorophyll biomass, whereas high Δ (~ 3.00) was observed in the late summer, autumn and winter; coinciding with periods of low chlorophyll production (Fig 19b). In terms of interannual variability, Δ was lower in the spring and summer of 2005 (~ 2.00) than the spring and early summer of 2004 (~ 2.5). Two FATMS of trophic position (DHA/EPA and 18:1n-9) displayed similar patterns to Δ (Fig 19c), whereas a third fatty acid marker

of trophic position (PUFA/SFA) did not vary significantly across both years (data not shown).

$\delta^{13}C_{POM}$ ranged from -18 to - 26 ‰, with heavy values generally coinciding with high concentrations of diatoms (Fig 20a). $\delta^{13}C_{copepod}$ ranged from -19 ‰ in the spring to -24‰ in the summer of 2005 (Fig 20a). Patterns in $\delta^{13}C_{copepod}$ were similar to those of $\delta^{13}C_{POM}$ in the spring, but were slightly decoupled during the summer. Two FATMS of dietary quality, DHA/EPA (which indicates the relative proportion of dinoflagellates to diatoms), and 16PUFA/18PUFA (which indicates the relative proportion of diatoms to flagellates in copepod diets), varied in opposite trajectories, and reflect patterns seen in diatom concentrations. However, the peak of 16PUFA/18PUFA, and the minimum of DHA/EPA occurred ~ 6 weeks after the highest concentration of diatom carbon was measured (Fig 20b).

The fatty acid 18:2n-6, which denotes the presence of detritus or green algae, accounted for up to ~ 6% of the fatty acids of *M. pacifica*. Lower proportions of 18:2n-6 in *M. pacifica* fatty acids generally occurred during periods when surface salinity was high, suggesting that, in this case, 18:2n-6 might have represented an oceanic flagellate source rather than a riverine detrital source (Fig 21a). The fatty acids 22:0 and 24:0 often indicate terrestrial input in estuaries. Whereas 22:0 was often below detection limit, 24:0 reached ~ 4% of the total fatty acids of *M. pacifica* in June of 2004, coinciding with the lowest measured surface salinity in the area (< 22 PSU)(Fig 21a). In the rest of the samples the proportion of 24:0 was very low (< 1%). Bacterial sources indicated by the

trophic marker (15:0 + 17:0) were found at concentrations < 3% of total fatty acids, and displayed maximal proportions in May of 2004 and 2005 (Fig 21b). The fatty acid marker 18:1n-7, which denotes either bacteria or *de novo* fatty acid synthesis, reached maximum proportions (~ 10% of total fatty acids) in Feb 2004, but was generally low and invariable for the rest of the year.

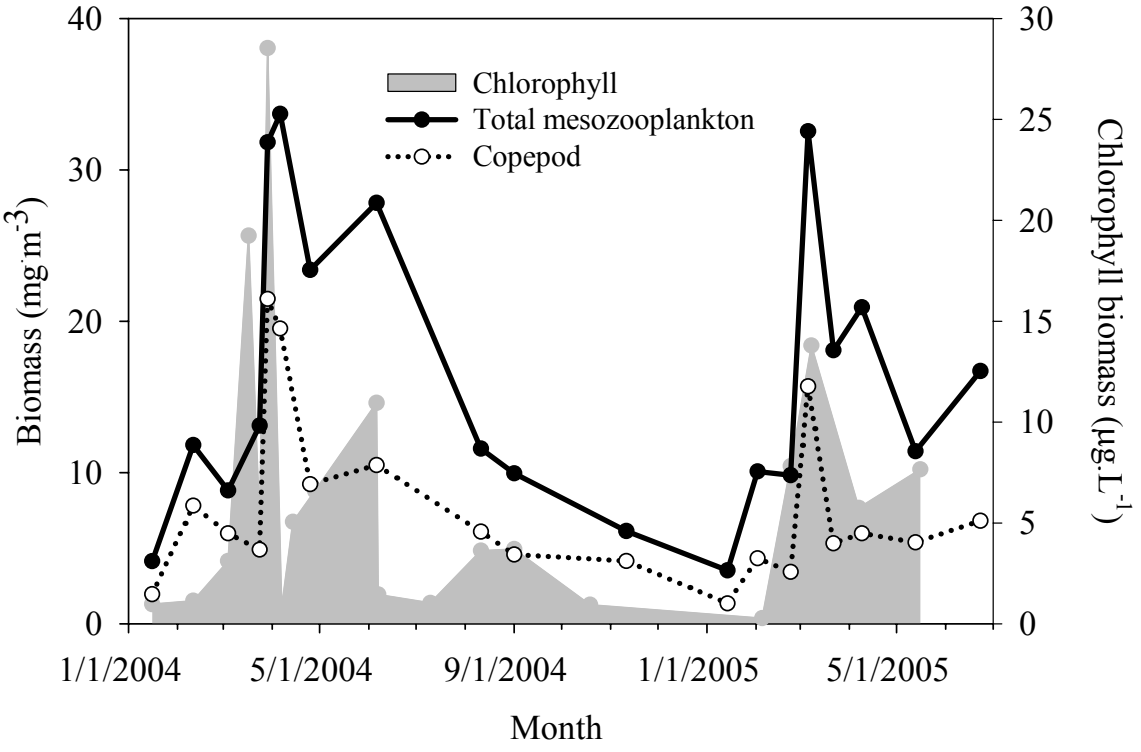

Figure 16: Total mesozooplankton and copepod biomass ($mg \cdot m^{-3}$), and depth-integrated chlorophyll concentrations ($\mu g \cdot L^{-1}$) in the Strait of Georgia between January 2004-June 2005.

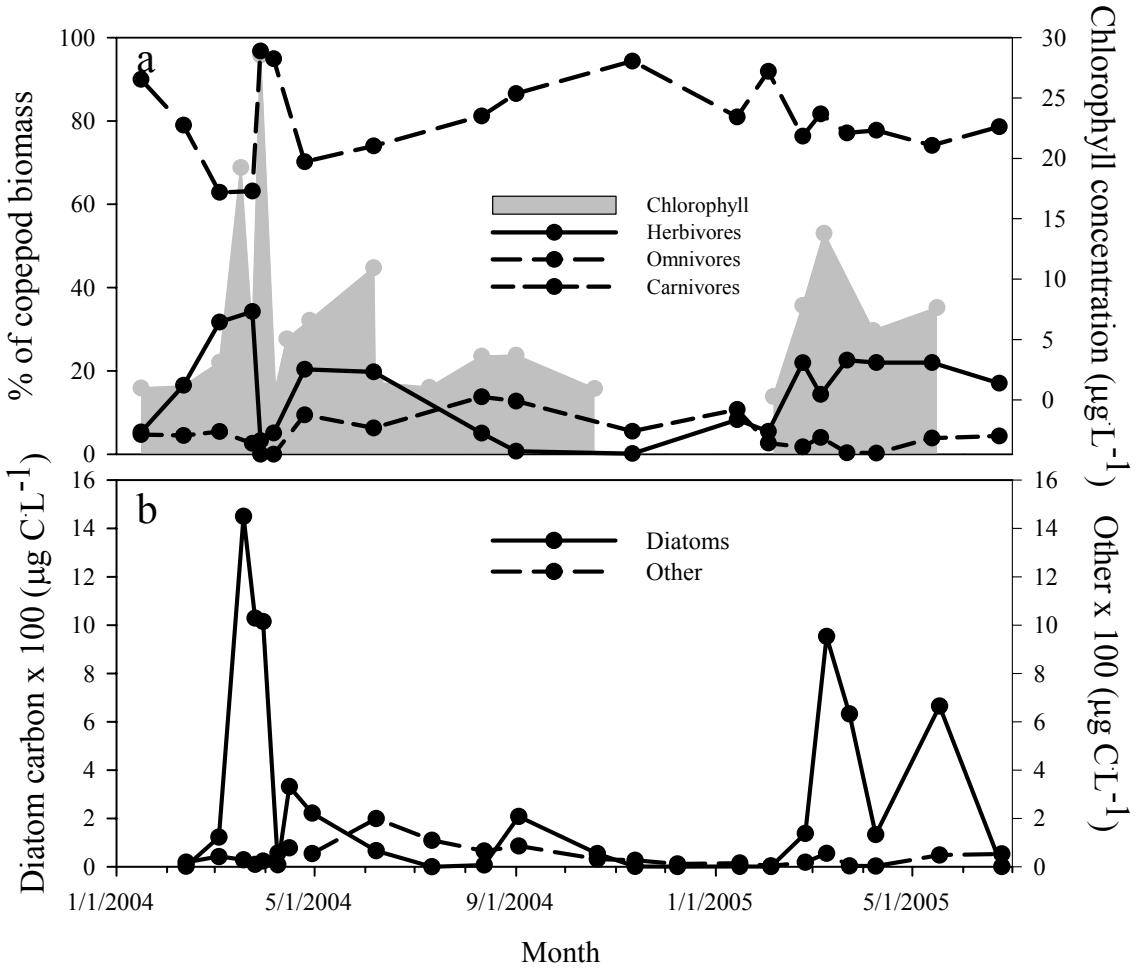

Figure 17 (a) The seasonal cycle of copepod trophic modes in the Strait of Georgia and (b) major phytoplankton groups (µg C·L^{-1}). "Other" represents ciliates, flagellates and dinoflagellates.

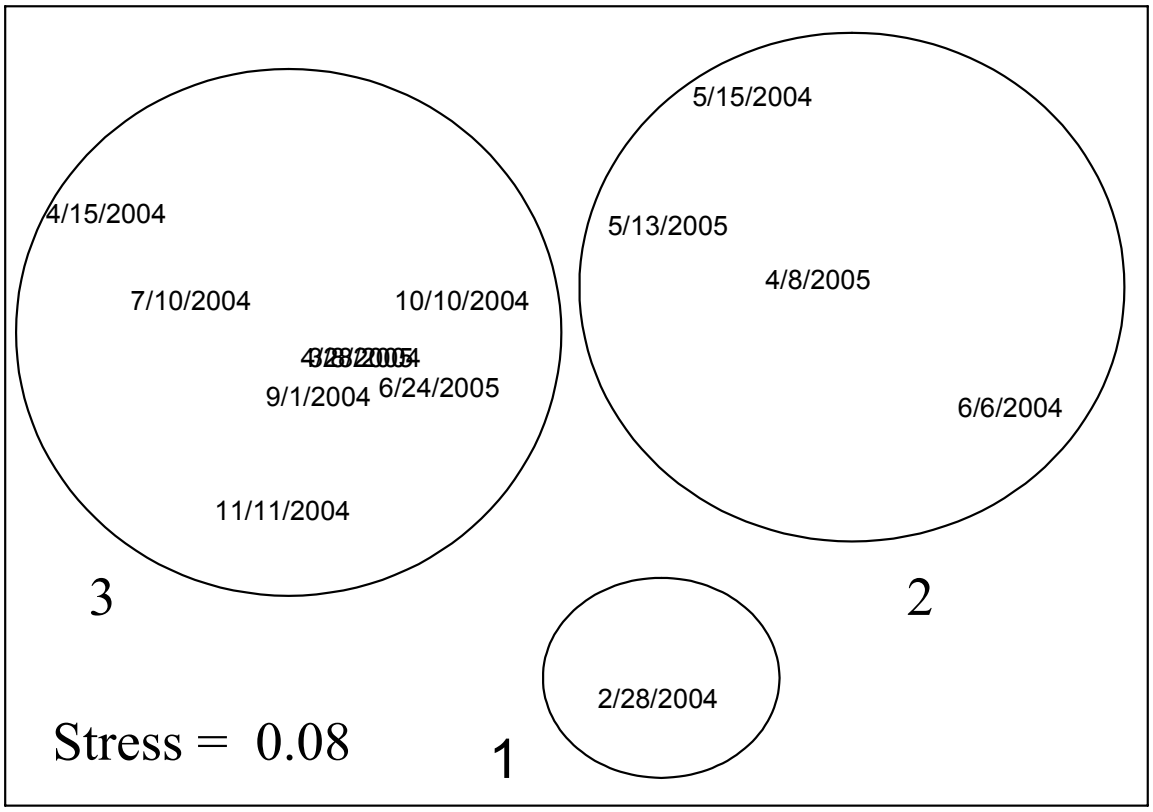

Figure 18. Multidimensional scaling (MDS) ordination performed on a Bray-Curtis dissimilarity matrix based on raw, untransformed fatty acid data of *Metridia pacifica* females collected in the Strait of Georgia. The circles represent the clustering patterns found using hierarchical average-neighbour cluster analysis based on rank similarities of the same dissimilarity matrix. SIMPER analysis performed on these groups is reported in Table 11.

Table 11. Similarity Percent (SIMPER) analysis used to assess the contribution of individual fatty acids to the 3 clusters revealed by the multidimensional scaling analysis and clustering shown in Figure 18. Values are expressed in % fatty acid. Av % comp refers to the average percent composition of the tracer present in each cluster (Appendix 4).

Clusters	Average dissimilarity	Fatty acid	Cluster 1	Cluster 3	
1 and 3	24.8		Av % Comp	Av % Comp	Cum.%
		18:1n-7	15.8	2.8	30.1
		18:1n-9	1.6	8.1	45.1
		16:0	11.9	17.6	58.2
		EPA	16.8	18.8	65.4
		DHA	12.2	13.8	71.8
		14:0	4.1	6.0	76.2
		18PUFA	5.9	4.3	80.4
		16PUFA	3.6	1.9	84.4
		16:1n-7	8.5	6.9	88.1
		18:0	1.8	2.9	90.7

Clusters	Average dissimilarity	Fatty acid	Cluster 1	Cluster2	
1 and 2	27.5		Av % Comp	Av % Comp	Cum.%
		18:1n-7	15.8	2.3	28.2
		DHA	12.2	6.3	40.6
		14:0	4.1	10.0	52.9
		16:0	11.9	15.6	60.6
		16:1n-7	8.5	11.1	66.2
		18PUFA	5.9	3.4	71.4
		18:0	1.8	3.9	76.4
		18:1n-9	1.6	3.5	80.4
		EPA	16.8	15.5	84.1
		16PUFA	3.6	2.3	86.9
		18:2n-6	3.9	3.6	89.8
		15+17	0.7	2.0	92.5

Clusters	Average dissimilarity	Fatty acid	Group 3	Group 2	
2 and 3	22.6		Av % Comp	Av % Comp	Cum.%
		DHA	13.8	6.3	19.4
		18:1n-9	8.1	3.5	31.3
		16:1n-7	6.9	11.1	42.2
		14:0	6.0	10.0	52.6
		EPA	18.8	15.5	63.1
		16:0	17.6	15.6	72.1
		18:0	2.9	3.9	77.9
		18PUFA	4.3	3.4	82.1
		18:2n-6	2.9	3.6	85.3
		24:0	0.0	1.0	87.9
		18:1n-7	2.8	2.3	90.2

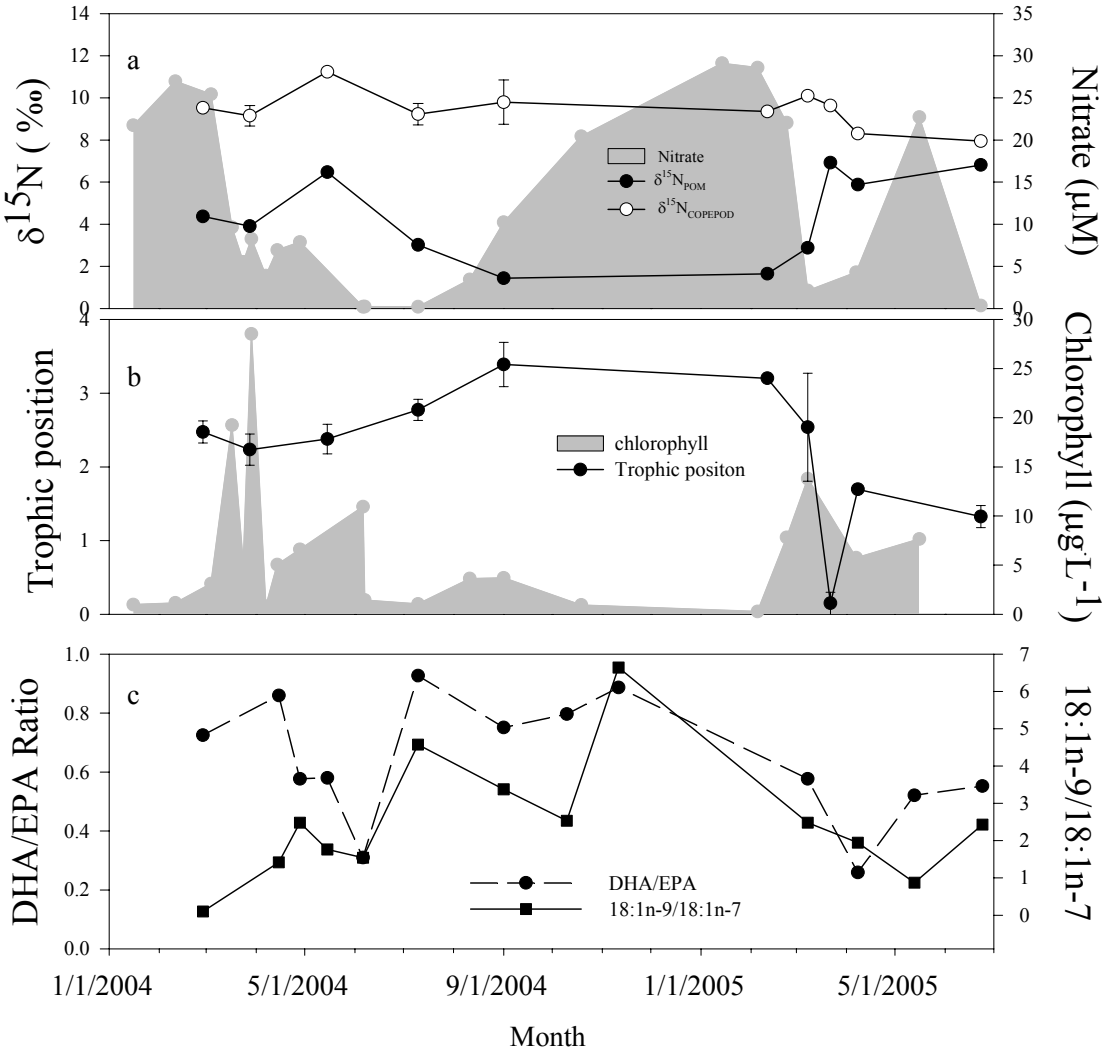

Figure 19. Seasonal variability in the trophic position of *Metridia pacifica*. Panel (a) represents the cycle of $\delta^{15}N_{POM}$, $\delta^{15}N_{copepod}$ (both in ‰) and nitrate concentration (µM) at 5 m. Panel (b) represents the cycle in the trophic position of *M. pacifica* (Δ) and chlorophyll concentrations ($\mu g \cdot L^{-1}$) and panel (c) represents the seasonal cycle in two fatty acid trophic markers of trophic position (DHA/EPA and 18:1n-9/18:1n-7)

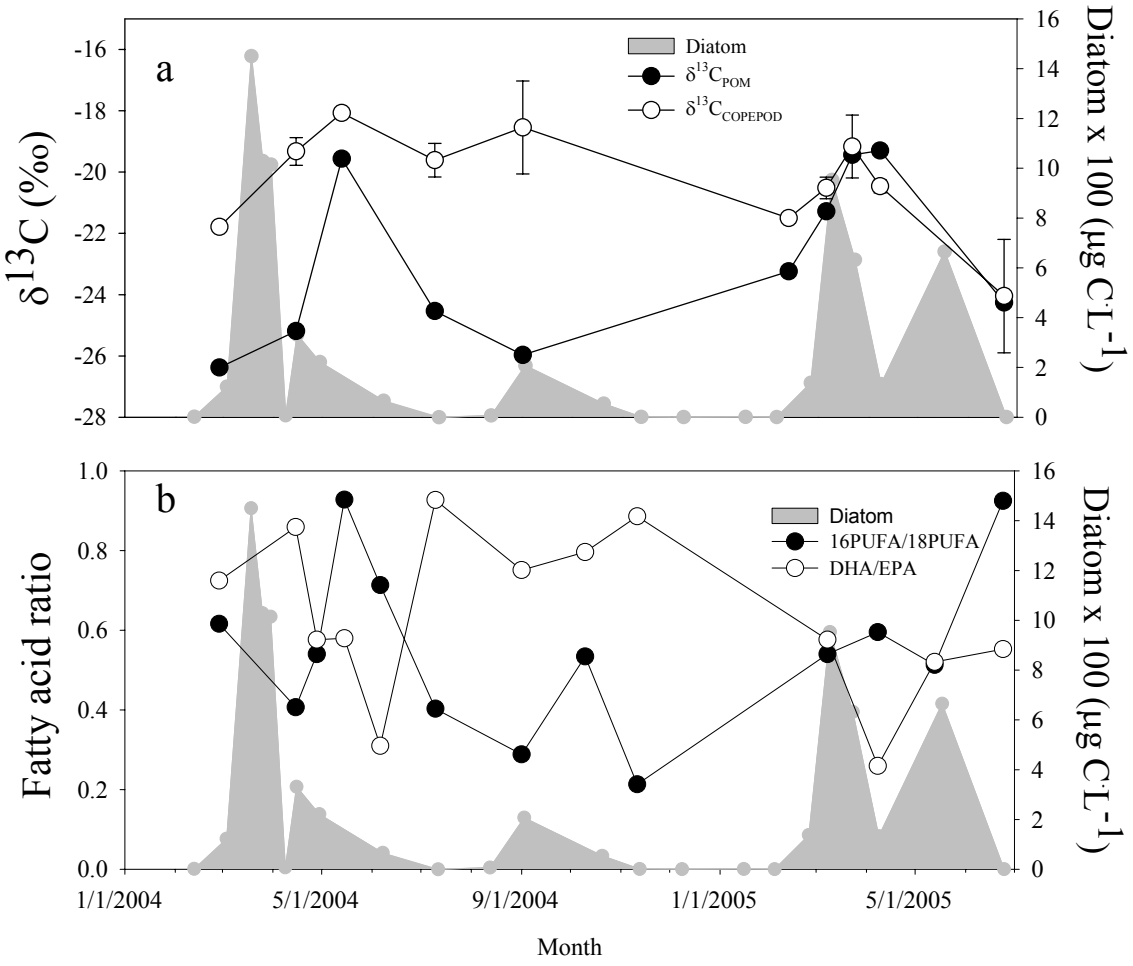

Figure 20. Seasonal variability in the contribution of phytoplankton to the diet of *Metridia pacifica*. (a) Variability in $\delta^{13}C_{POM}$, $\delta^{13}C_{copepod}$ (both in ‰) and diatom concentration at 5 m (µg·L^{-1}) . (b) The seasonal cycle of two fatty acid trophic markers of food quality (16PUFA/18PUFA and DHA).

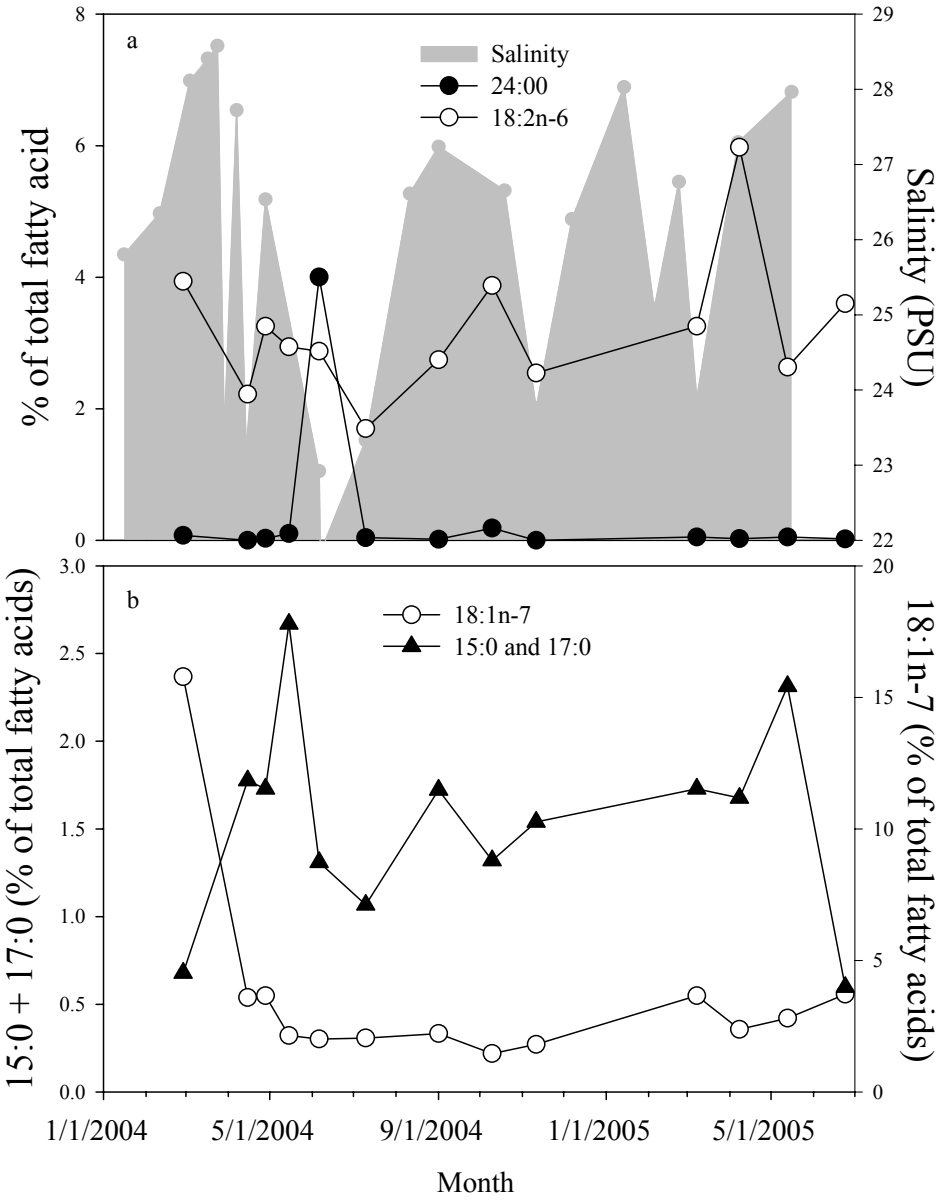

Figure 21. Seasonal variability in the contribution of detrital and bacterial sources to the diet of *Metridia pacifica*. (a) Seasonal variability in the fatty acid trophic markers 18:2n-6 and 24:0 overlaid on surface salinity (PSU). (b) Seasonal variability in the bacterial fatty acid markers 15:0 +17:0 and 18:1n-7

4.4 Discussion

There is considerable seasonal variability in the biochemical composition of copepods, and the sources of food that they receive in the SoG. My results suggest two different pathways of energy transfer that alternate in significance over the year. The spring pathway is highly productive, supporting high mesozooplankton biomass and larger copepod species (such as *N. plumchrus* and *Eucalanus bungii*). The base of this pathway is composed primarily of diatoms and few flagellates. During this time, the copepod assemblage is dominated by herbivorous and omnivorous copepods (*N. plumchrus*, *E. bungii*, *M. pacifica*, *Calanus marshallae* and *Calanus pacificus*)(Sastri et al, submitted). Omnivorous copepods (such as *M. pacifica*) feed herbivorously in the spring as indicated by relatively low trophic positions, high proportions of diatom fatty acid trophic markers, and low proportions of carnivory markers (Fig 19,20).

Peaks of 16PUFA/18PUFA and minima of DHA/EPA occur a few weeks after maximal diatom concentrations. It has been previously shown that the time it takes for copepods to display changes in the fatty acid signatures of their diets varies considerably across genera and also depends on physiological condition (Graeve et al. 1994a, 2005, Stevens et al. 2004b). Nonetheless, these patterns coincide with periods when $\delta^{13}C_{copepod}$ is relatively heavy, indicating that animals are utilizing diatom carbon (Fry and Wainright 1991). The importance of a flagellate component in this pathway is demonstrated by the relatively high proportions of the flagellate marker 18:2n-6 in the lipids of *M. pacifica* during this time (Table 11). These findings are supported by previous grazing experiments which demonstrated that in the spring, *N. plumchrus* obtains its maximum

body ration in the SoG when feeding on a combination of diatoms and flagellates (Parsons et al. 1969).

The second energy transfer pathway dominates from the summer to winter. During this time, the dominant copepod species (e.g. *Oithona*, *Pseudocalanus*) are typically smaller than those found in spring, and the proportion of carnivorous copepods is higher than that of herbivorous copepods. Stable isotopes and fatty acid data indicate that omnivorous copepods feed more carnivorously during this time (Fig 19). Although winter grazing experiments have not yet been conducted in the SoG, supporting evidence for this pathway can be found in neighbouring Saanich Inlet, where copepods have been shown to feed primarily on small flagellates and microzooplankton during the winter (Koeller et al. 1979).

These findings are similar to the seasonal trophic dynamics of *Pseudocalanus* spp. in an Arctic Fjord (Lischka and Hagen 2007). In the spring, the fatty acid profiles of this copepod are characterized by high proportions of diatom fatty acid markers, whereas the remainder of the year is characterized by high proportions of flagellate markers, and high carnivory indices. Peters et al. (2006) detected a similar pattern in *Pseudocalanus* spp. from the Baltic Sea, but with increased ingestion of cyanobacteria in the summer.

Recent evidence suggests that the trophic position of omnivorous intermediate consumers affects total foodchain length (Post 2002b, Post and Takimoto 2007). In this case, the spring diatom-supported foodchain in the SoG is shorter than the winter, flagellate-

supported foodchain. Shorter foodchains have been shown to be more efficient than longer ones, resulting in the transfer of more energy from primary producers to secondary consumers (Hairston and Hairston 1993). Indeed, the spring diatom foodchain in the SoG supports much higher biomass and larger copepods than does the winter flagellate foodchain. However, recent evidence also suggests that dietary diversity during the spring is important for the survival of herbivorous copepods in the region (El-Sabaawi et al, Chapter 2).

When chlorophyll concentrations become low in the summer, *M. pacifica* switches from herbivory to carnivory. Other summer copepod species common in the SoG (e.g. *Acartia* spp., *Pseudocalanus* spp. and *C. pacificus*) are also known to switch from herbivory to carnivory when phytoplankton concentrations become too low to support copepod biomass (Landry 1981, Gifford and Dagg 1988, Lischka et al. 2007). Landry (1981) suggested that a similar switch from herbivory to carnivory by *Calanus pacificus* at the end of the spring phytoplankton bloom allows coastal ecosystems to maintain high zooplankton biomass without depleting diminishing phytoplankton stocks. Therefore, the switch in the feeding mode after the spring bloom is an example by which copepod omnivory can regulate ecosystem stability. Another example of how patterns of trophic dynamics can maintain ecosystem stability is evident in the rise of carnivorous copepod biomass in the summer. These copepods are thought to alleviate grazing on phytoplankton by grazing on the nauplii and copepodites of herbivorous copepods.

The role of allochthonous carbon in marine ecosystems is currently under debate (Huxel et al. 2002). In some estuaries, terrestrial detritus is an important source of energy to the system (Moore et al 2004). In others, terrestrial detritus is not consumed directly by animals, but rather, stimulates ecosystem metabolism by fuelling bacterial production, which is then consumed by microzooplankton, thus providing an indirect source of energy to higher consumers (Sobczak et al 2005, Martineau et al 2004).

The Fraser River introduces a substantial amount of detritus and dissolved organic carbon into the SoG basin (Johannessen et al. 2003, 2005). Peak Fraser discharge typically occurs in June, but varies dramatically from year to year. Deciphering the use of detrital sources by copepods in the SoG using stable isotopes is problematic because riverine signatures ($\delta^{13}C$ -25 ‰ and $\delta^{15}N$ 1.5 ‰, Johnannessen et al. 2005) overlap with signatures commonly reported for flagellates in marine coastal systems. The fatty acid trophic markers 18:3n-3, 18:3n-6 and 18:2n-6 are often associated with terrestrial detritus, but can also be abundant in some algal groups (Dalsgaard et al. 2003). The fatty acids 22:0 and 24:0 represent a terrestrial signature that is not found in any other group of phytoplankton (Budge et al. 2001). Both were present at very low proportions in the fatty acids of *M. pacifica*, except for June 2004 when surface salinity was very low and the proportion of 24:0 reached 5% of total fatty acids (Fig 21b). Therefore, copepods in the SoG can occasionally take advantage of terrestrial detritus.

An alternative pathway by which riverine detrital input can subsidize copepod growth is by stimulating bacterial production. Almost all of the dissolved organic carbon brought in

by the Fraser is metabolized within the SoG basin (Johnannessen et al 2003). Seki and Kennedy (1969) suggested that bacteria can provide a significant dietary subsidy for SoG copepods, especially in the winter. Albright (1977) noted that SoG bacterial production peaks in both the summer and in fall, but was unable to correlate it to Fraser input directly, and suggested that phytoplankton production (as well as riverine input) can regulate bacterial production in the SoG. Peak bacterial marker concentration (15:0 + 17:0) occurred in May, in agreement with Albright's observations, suggesting that copepods do indeed consume bacteria or bacterivorous protozoans, at least in the summer. However, the proximity of this peak to the spring phytoplankton bloom suggests that it is not stimulated by the Fraser, but by the decay of the spring bloom. The fatty acid 18:1n-7 is sometimes cited as a bacterial marker in copepods, but is also involved in *de novo* fatty acid synthesis (Stevens et al. 2004b). With the exception of 1 sample collected in the winter of 2004, where 18:1n-7 reached 15% of total fatty acids, the proportion of 18:1n-7 in the fatty acids of *M. pacifica* remained constant over time. Though the presence of 18:1n-7 at high proportions in the winter supports the suggestions of Seki and Kennedy (1969), variability in the *de novo* fatty acid synthesis cannot be ruled out (Stevens et al. 2004b).

4.5 Ecological implications for the Strait of Georgia

I have shown that trophic dynamics vary over time in the SoG. To date, efforts to model the SoG planktonic community have focused only on spring time dynamics, and assumed that copepods feed only on phytoplankton (e.g. Li et al. 2000). My results suggest that

copepods are capable of exploiting a much wider range of dietary items, including bacteria and terrestrial detritus. Though the spring trophic pathway in the SoG is certainly very productive, it is also very short in duration. The summer-autumn-winter pathway predominates for most of the year, and supports a significant fraction of the zooplankton biomass in the region. Therefore, increased understanding of trophic flow during these periods of low productivity is crucial to our understanding of this commercially important ecosystem.

Chapter 5: Conclusions and Future Research

5.1 The utility of fatty acid measurements in zooplankton time-series

In Chapter 2 I showed that the diet of *Neocalanus plumchrus*, the biomass dominant copepod in the Strait of Georgia (SoG), has the potential to vary significantly between years. This data set represents the longest time-series of fatty acids collected from a single copepod species. It also highlights the utility of fatty acids as an index of food quality in *N. plumchrus*, and supports the recent call to include routine lipid measurements in zooplankton time-series (Kattner et al. 2007). Other indices of food quality (e.g. nitrogen, phosphorous, amino acids) should be included as well because evidence suggests that they can play important roles in the growth and survival of marine organisms (Malzahn et al. 2007, Mitra and Flynn 2005).

The most intriguing finding of Chapter 2 is the significant correlation between DHA/EPA and the abundance of *N. plumchrus*, suggesting that the balance of these essential fatty acids is related to the survival of this copepod in the SoG. The DHA/EPA ratio is known to be related to the reproduction and growth of copepods, fish, shrimp and clams (Arendt et al. 2005, Jónasdóttir et al. 2005, Budge and Parrish 1998). A natural follow up to my work would be to elucidate the physiological basis of this correlation through experimentation, and more extensive field monitoring. Raising *N. plumchrus* in the laboratory is difficult because it is an annual species, but copepodites and gravid females can be easily collected from the field and maintained under laboratory conditions (e.g.

Campbell et al. 2004). Therefore, testing the effects of food quality on the survival of *N. plumchrus* copepodites can be accomplished in a laboratory setting. This could involve collecting CI copepodites from the SoG in late February, incubating them on a variety of diets, and monitoring how efficiently the copepods can molt and accumulate biomass under each dietary condition. The experimental treatments could contain diatoms (both nutrient-limited and nutrient-replete), dinoflagellates, microzooplankton and mixed cultures. Another possibility would be to directly manipulate concentrations of essential fatty acids through the use of liposomes (Buttino et al. 2006a). Liposomes are lipid vesicles, which are synthesized in the laboratory to contain pure emulsions of a single fatty acid, and which are about the same size as a phytoplankton cell. Although the use of liposomes in biological oceanography is relatively new, they have already shown great promise in preliminary studies of copepod physiology (Buttino et al. 2006b).

Another way by which the DHA/EPA ratio can influence copepod survival is by modulating egg production, hatching success or naupliar survival. As shown in Chapters 2 and 3, a natural gradient of food quality exists in the diet of *N. plumchrus* across large geographic scales. Interestingly, a doubling of the DHA/EPA ratio is also observed across this gradient. Fluorescent probes (such as TUNEL and ANEXIN) that can rapidly assay copepod naupliar viability could be used to assess whether this natural gradient in DHA/EPA translates into different rates of naupliar survival (Ianora 2007). For example, gravid *N. plumchrus* females could be collected from both Ocean Station P (in February) and the SoG (in December), and then incubated at the same temperature until they produce eggs. Once eggs are produced in the lab, egg production estimates can be made,

and newly-emerged nauplii can be assayed for viability and deformity, thus providing a first-order estimate of whether differences in food quality translate into differences in reproductive success.

5.2 The recent decline of *Neocalanus plumchrus* and its possible effect on the Strait of Georgia ecosystem

The biomass of *N. plumchrus* has been shown to fluctuate considerably throughout its range. For instance, in the Oyashio region of the North Pacific, decadal patterns in *N. plumchrus* biomass mirror those of the Pacific Decadal Oscillation (Tadokoro et al. 2005). The interplay between low food concentrations and high sardine biomass during regimes of low *N. plumchrus* biomass is thought to be the primary mechanism behind this variability. Interannual variability in the biomass of *N. plumchrus* in the Eastern Japan Sea is thought to be caused by variability in mixed layer stratification, whereby a more stratified mixed layer limits phytoplankton productivity by decreasing nutrient concentrations in the mixed layer (Chiba and Saino 2003). Although interannual variability in the biomass of *N. plumchrus* has not been assessed at Ocean Station P, it is now known that the timing of this species is advancing (Mackas et al. 1998). Systematic and consistent sampling of *N. plumchrus* in the SoG only started in 1996 (Bornhold 2000, Campbell et al. 2004). Therefore, our ability to understand the mechanisms driving decadal variability in this species in the SoG is currently hindered by the lack of data. However, the 1996-2007 data set may allow for a preliminary assessment.

Given that the extent to which *N. plumchrus* biomass in the SoG varies has not yet been evaluated, there is no context in which to place the recent decline observed between 2001-06. Although a similar collapse of *N. plumchrus* is thought to have occurred in 1997 (Bornhold 2000), the population seems to have recovered by 1998. However, the most recent samples from 2007 were comparable in abundance to those from 2005 and 2006, indicating that the population has not yet recovered (Dower et al., unpublished data). Stockner et al. (1979) also reported a similar decline in *N. plumchrus* biomass in 1976, but the recovery was not reported. An important thing to keep in mind is that both Bornhold (2000) and Stockner et al. (1979) did not enumerate *N. plumchrus* directly, but estimated its biomass from the total biomass of zooplankton measured between 0 and 20 m. Gardner (1977) suggested that high survival of diapausing *N. plumchrus* in the SoG is correlated to high deep water temperature, but asserted that this correlation may not be physiological in nature, but rather a result of covariance between physiologically-unrelated factors. Deep water in the SoG was warmer in 2005 and 2006 than in previous years, but this did not translate into increased *N. plumchrus* biomass (Masson and Cummins 2007).

Neocalanus spp. are an important source of food for higher trophic levels. In addition, ontogenetic migration of *Neocalanus* spp. is thought to contribute to vertical carbon flux in the Pacific (Bradford-Grieve et al. 2001, Kobari et al. 2003). Kobari et al. (2003) estimated that the ontogenetic flux of *N. plumchrus* to the deeper oceans is almost equivalent to that generated by primary production. Therefore, the loss of *N. plumchrus* from the SoG may have geochemical, as well as ecological implications.

5.3 The combined use of stable isotopes and fatty acids as trophic tracers

Chapter 3 demonstrates how the combination of fatty acids and stable isotopes can be useful in studying trophic relations among marine copepods. Stable isotopes supply information on trophic position, but fail to capture subtle changes in dietary source. Fatty acids provide information on dietary sources, but provide inconsistent information on trophic position. This research represents the first time that this combined approach has been used to elucidate trophic dynamics in copepods, although it has already been shown to be effective for krill and amphipods (Nyseen et al. 2005, Schmidt et al. 2006). A natural follow up to this work would be to extend this 2-dimensional approach to other zooplankton groups in the SoG, or even to whole ecosystem studies in the region.

Non-copepod groups such as amphipods, krill and pteropods have received little attention in the SoG, but data from the STRATOGEM project indicate that they may be key groups in the region (Pawlowicz et al. in prep). Amphipods are important copepod predators in the SoG (Haro-Garay 2003). The biomass of copepods and amphipods cycles out of phase in the SoG, but the mechanism behind this pattern remains unclear (Haro-Garay 2007). Krill are an important source of food for higher trophic levels in the ocean. Although krill can utilized a wide range of prey items their growth rates are known to depend on the quality of their diet (Pond et al. 2005). Thus far, almost all studies of krill physiology have been limited to polar regions or the Oregon upwelling zone, with little work done elsewhere. Any studies of krill in the SoG will therefore enhance our knowledge of physiology and ecology of this group on a large scale.

This thesis highlights how different copepod species respond to variability in dietary quality. The fatty acid profiles of *N. plumchrus* largely reflect ambient protist composition. *Euchaeta elongata* is consistently carnivorous, regardless of its feeding environment. *Metridia pacifica* has the ability to switch from herbivory (during periods when chlorophyll concentrations are high), to carnivory (when chlorophyll concentrations are low). These findings have several implications for ecosystem studies and trophic ecology. The isotopic signatures of copepods are commonly used as dietary baselines for whole ecosystem trophic studies employing stable isotopes (e.g. Bode et al. 2007). Therefore, the choice of copepod species can affect the interpretation of ecosystem data. Recent evidence suggests that the biomass of copepod species that can switch between herbivory and omnivory (such as *M. pacifica* and *Calanus pacificus*) is increasing in the SoG, whereas the biomass of filter-feeding copepods (such as *N. plumchrus* and *E. bungii*) is decreasing (Sastri et al. in prep). Dietary switching in copepods is believed to promote ecosystem stability because it allows copepods to continue feeding at optimal rates when phytoplankton concentrations become low, without depleting phytoplankton stocks completely (Landry 1981 and references therein). Therefore, the increased biomass of diet-switching species could indicate that the SoG may be becoming more stable. Also, both *M. pacifica* and *C. pacificus* are multigenerational species which are found in SoG surface waters year-round. They are larger than what has commonly been accepted as summer species in the SoG (e.g. *Pseudocalanus* spp). Therefore, their increase might represent a temporal expansion in high-quality diets available to higher consumers, which may have previously been limited to the spring.

5.4 Seasonality in copepod diets

Chapter 4 shows that the trophic structure and dietary sources of copepods vary seasonally in the SoG. During the spring, the copepod assemblage is characterized by the presence of large herbivorous copepods, whereas the remainder of the year is characterized by the presence of small carnivorous copepods. Omnivorous copepods are common all year long, but feed herbivorously in the spring, and carnivorously from the summer to the winter. Occasionally copepods are able to utilize river-born detrital particles. Indices of bacterial ingestion are higher in the early summer, suggesting feeding on bacterial aggregates, bacterivorous flagellates or microzooplankton.

The findings of Chapter 4 are relevant to modelling efforts in the SoG, which have thus far assumed that copepods are primarily herbivorous. By using a coupled biophysical model, Li et al. (2000) showed that SoG zooplankton biomass is strongly affected by factors which control their biological rate parameters. Increases in zooplankton growth or mortality rates can radically change the characteristics of the model results, and therefore, the overall system. By their own admission, however, the model of Li et al. (2000) is overly simplistic because it only includes zooplankton feeding on a single food source, and because it completely ignores the life history of *N. plumchrus*. Improving ecosystem models of the SoG is a central goal of the STRATOGEM project. My findings suggest that variability in food quality may play a key role in regulating copepod biomass in the SoG. The experiments that I suggested above would aid in quantifying the effect of food quality on copepod survival in the SoG, and eventually (for models to be predictive), the effect of food quality on copepod dynamics must be accounted for.

Fortunately, the inclusion of parameters related to food quality has become more common in ecosystem models. Functional forms describing zooplankton selectivity or diet switching were reviewed by Tian (2006). Gentleman et al. (2003) reviewed the assumptions implicit in functional forms of zooplankton feeding on different types of prey. Recently Mitra (2006) introduced a model by which zooplankton switch diets when confronted with poor quality prey. Mazzochi et al. (2006) used an individual-based model to show that diet composition has a strong effect on the growth rates of *Temora* spp. Pierson et al. (2007) used a numerical model to show that a collapse in a generation of *Calanus pacificus* in Dabob Bay was not directly linked to diatom ingestion. Roelke (2000) showed that copepod production was directly controlled by a threshold of food quality under algal bloom conditions in a model that can easily be adapted to simulate diatom blooms in the SoG.

However, accounting for the effects of dietary quality in ecosystem models can potentially increase model complexity, increase computing time, decrease model stability and increase the difficulty in teasing out the mathematics from the biology (Flynn 2005). Therefore, a balance between model complexity and realism should be achieved in order for any SoG ecosystem model to be useful.

5.5 Looking beyond the Strait of Georgia: why does food quality matter?

One of the most important problems facing biological oceanographers is assessing the factors that relate to fish recruitment in the ocean. In particular, the question of how variability in zooplankton biomass and composition affects fish recruitment remains contentious. Most attempts to assess the role of zooplankton in the survival of young fish have focused on correlating bulk biomass (or abundances) of zooplankton to fish recruitment. Until recently, the role of the zooplankton quality on the survival and recruitment of fish has been largely ignored, primarily because it is usually assumed that zooplankton can buffer poor dietary quality. However, it is becoming increasingly clear that the total abundance of food available to predators does not matter if food quality is low. For example, Malzahn et al. (2007) have recently suggested that food quality effects (e.g. poor nitrogen and phosphorous in the diet) can transfer up the foodchain to higher trophic levels, causing variability in fish biomass that is decoupled from the biomass of their prey. The availability of essential fatty acids has been recently suggested to play a role in fish community changes observed over decadal scales in the North Pacific (Litzow et al. 2006). The DHA/EPA ratio has also been shown to be extremely important in the early stages of marine fishes (Rainuzzo et al. 1997): low DHA/EPA can create structural imbalances in larval and juvenile fishes, which can translate to impaired growth and reduced survival probability.

If limitations of food quality can override food biomass, then correlating bulk zooplankton biomass to fish biomass alone may not be appropriate if the goal is to understand factors which affect recruitment. In general, most zooplankton time-series involve measurement of either biomass or abundance, both of which are vulnerable to biases caused by unquantified advection, patchiness and to variability resulting from different forms of sampling. The inclusion of biochemical indices in zooplankton time-series can add valuable information about the potential of food quality to affect higher trophic levels.

Another factor which can cause variability in fish recruitment is the degree of mismatch between the emergence of fish larvae and their prey (Cushing 1990). Temporal advances in the phenology of zooplankton is one way by which rising ocean temperatures have affected zooplankton stocks in the ocean (Mackas et al. 2007). Temporal advance in the timing of peak zooplankton biomass can therefore lead to decoupling between fish and their prey, which can potentially lead to lower fish recruitment in a warmer ocean. Studies have suggested that the concentrations of food can override predator-prey mismatch (Durant et al. 2005), and here I would like to suggest that this can be extended to food quality as well. Between 1996-2006, a wide range of mismatch between *N. plumchrus* and phytoplankton was observed (Table 12). 1996, 2004 and 2006 were years where observed *N. plumchrus* biomass was different than what might be expected based on the match/mismatch hypothesis. For example, in 2006 there was an almost perfect match between zooplankton and phytoplankton, but the quality of the diet as indicated by

low DHA/EPA (0.26) was poor, which may have hindered the survival of that cohort. The interpretation of these data is, of course, open to a great deal of subjectivity. For example, the effect of egg production and naupliar survival on total *N. plumchrus* biomass in any given year is unknown. In addition, the extent to which DHA/EPA of female *N. plumchrus* hinders egg production and naupliar survival is unknown. The goal of this preliminary analysis is not to assert mechanisms, but to point to the necessity of increasing our understanding of how food quality varies in the ocean, and how this variability can affect the survival of marine organisms.

Table 12: The range of mismatch between *N. plumchrus* and peak spring chlorophyll concentrations in the Strait of Georgia, between 1996-2006. Bolded years demonstrate years where predictions of *N. plumchrus* success contradict predictions from the match/mismatch hypothesis, suggesting that food quality (as indicated by the DHA/EPA ratio of *N. plumchrus*) may override the effect of mismatch between predator and prey.

Year	Match or mismatch[1]	DHA/EPA[2]	Biomass[3]	Reference
1996	**Mismatch**	**High**	**High**	**Bornhold 2000**
1997	Mismatch	Low	Low	Bornhold 2000
2002	Match	High	Very High	STRATOGEM
2003	Match	High	High	STRATOGEM
2004	**Mismatch**	**High**	**High**	**STRATOGEM**
2005	Mismatch	Low	Low	STRATOGEM
2006	**Match**	**Low**	**Low**	**STRATOGEM**

[1] match is determined by the distance between peak *N. plumchrus* biomass and peak chlorophyll concentration. Peak *N. plumchrus* occurs when all animals are in stage CIII (Sastri et al. in prep). Peak chlorophyll concentrations were determined by Susan Allen (UBC) based on observations from the ferry-mounted chlorophyll fluorometers employed by STRATOGEM. [2] High DHA/EPA is presumed to be above > 0.3 because that is the level at which *N. plumchrus* was observed to collapse in 2005 [3] High biomass is designated in years where *N. plumchrus* abundance > 3000 animals per m^2.

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Appendix 1: Details of SIMPER analysis from Chapter 2

Group 1 and 2
Average dissimilary 26.75%

Species	Group 1 Av.Abund	Group 2 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
total MUFA	13.8	4.5	5.6	1.7	21.1	21.1
18(PUFA)	9.9	3.9	3.6	2.2	13.6	34.6
14	19.2	20.9	3.4	1.4	12.6	47.2
16:00	12.5	16.1	3.2	1.3	12.1	59.3
18:2n-6	1.6	6.2	2.7	2.7	10.1	69.4
16(PUFA)	2.7	4.8	2.0	1.4	7.5	77.0
20:5n-3	11.4	10.3	1.9	1.4	7.2	84.2
22:6n-3	7.2	6.9	1.5	1.4	5.7	89.8
18:1n-9/18:1n-7	3.3	4.0	0.9	1.3	3.4	93.3

Group 1 and 3
Average dissimilary 25.92%

Species	Group 1 Av.Abund	Group 3 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
16(PUFA)	2.7	9.6	4.2	5.9	16.0	16.0
total MUFA	13.8	7.8	3.8	1.4	14.6	30.6
18(PUFA)	9.9	3.6	3.6	2.6	13.7	44.3
14	19.2	24.7	3.2	1.2	12.2	56.5
18:2n-6	1.6	6.1	3.0	1.7	11.4	67.9
20:5n-3	11.4	16.3	2.8	1.8	10.7	78.6
22:6n-3	7.2	4.1	1.7	1.8	6.7	85.2
sum 15+17	2.7	0.7	1.1	1.9	4.2	89.5
18:1n-9/18:1n-7	3.3	1.5	1.0	1.5	4.0	93.5

Group 2 and 3
Average dissimilarity 23.00%

Species	Group 2 Av.Abund	Group 3 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
20:5n-3	10.30	16.30	3.57	1.75	15.50	15.50
14	20.86	24.71	3.50	2.04	15.23	30.74
16	16.11	12.32	3.15	1.29	13.68	44.41
16(PUFA)	4.84	9.57	2.69	3.08	11.68	56.09
18:2n-6	6.15	6.11	2.28	1.82	9.91	66.00
total MUFA	4.47	7.82	2.03	1.64	8.81	74.81
22:6n-3	6.86	4.13	1.70	1.26	7.38	82.18
18:1n-9/18:1n-7	4.02	1.54	1.45	1.48	6.31	88.50
16:1n-7	1.88	3.41	0.88	2.02	3.81	92.30

Appendix 2: Effect of acidification on $\delta^{13}C$ and $\delta^{15}N$ signatures of particulate organic matter

$\delta^{15}N_{original}$ (‰)	$\delta^{15}N_{acidified}$ (‰)	acidified-original	$\delta^{13}C_{original}$ (‰)	$\delta^{13}C_{acidified}$ (‰)	acidified-original
3.02	2.09	0.93	-24.53	-24.84	0.30
6.65	5.19	1.46	-24.29	-25.06	0.77
7.87	5.52	2.35	-23.28	-23.59	0.31
7.41	7.68	-0.27	-22.87	-23.43	0.56
8.20	6.42	1.78	-22.64	-23.09	0.45
5.94	5.11	0.84	-19.32	-19.49	0.17
	Average	1.18+/-0.9		Average	.43+/-0.21

Filters of particulate organic matter collected over a range of depths (0-400) were cut into 2 equal parts. One side was acidified with a few drops of 10% HCL. After the acid dried off, samples were dried and packed as described in chapter 3. Acidification led to a dramatic and inconsistent alteration in the $\delta^{15}N$ signature, whereas the alteration in the $\delta^{13}C$ was minor, and generally consistent.

Appendix 3: Details of SIMPER analysis from Chapter 3

	E. bungii	*N. plumchrus* and *C. marshallae*				
Fatty acid	Av.% comp.	Av.% comp.	Av.Diss	Diss/SD	Contrib%	Cum.%
14.0	4.8	17.8	7.5	2.0	19.5	19.5
16.0	21.4	10.3	6.5	2.1	16.7	36.2
18:1n-7	8.7	1.0	4.4	1.2	11.5	47.6
18:1n-9	7.8	3.5	3.3	1.3	8.6	56.2
20:5n-3	13.9	14.1	3.2	1.3	8.4	64.6
16:1n-7	8.1	4.7	2.6	1.6	6.7	71.2
18:2n-6	5.3	6.9	1.7	1.5	4.5	75.7
22:6n-3	2.6	5.3	1.6	1.2	4.2	80.0
16(PUFA)	7.0	7.3	1.6	1.2	4.2	84.1
18(PUFA)	4.2	3.8	1.2	0.9	3.0	87.2
20:1n-9	0.5	2.3	1.1	1.0	2.9	90.1
Average dissimilarity	38.70					

	E. bungii	*E. elongata*				
Fatty acid	Av.% comp.	Av.% comp.	Av.Diss	Diss/SD	Contrib%	Cum.%
22:6n-3	2.6	19.3	10.2	11.2	22.9	22.9
16.0	21.4	9.8	6.9	2.4	15.6	38.5
18:1n-9	7.8	12.4	4.8	1.9	10.7	49.1
18:1n-7	8.7	1.3	4.5	1.1	10.1	59.2
16(PUFA)	7.0	1.6	3.4	1.9	7.5	66.7
20:5n-3	13.9	14.5	2.7	1.3	6.1	72.8
16:1n-7	8.1	4.7	2.5	1.7	5.5	78.3
18:2n-6	5.3	1.4	2.4	2.5	5.4	83.7
18(PUFA)	4.2	1.0	1.9	1.1	4.4	88.0
14.0	4.8	2.3	1.5	1.6	3.5	91.5
Average dissimilarity	45.50					

	N. plumchrus and *C. marshallae*	*E. elongata*				
Fatty acid	Av.% comp.	Av.% comp.	Av.Diss	Diss/SD	Contrib%	Cum.%
14.0	17.8	2.3	9.5	2.6	21.0	21.0
22:6n-3	5.3	19.3	8.8	5.0	19.3	40.2
18:1n-9	3.5	12.4	6.2	1.8	13.7	53.9
16(PUFA)	7.3	1.6	3.6	2.3	8.0	61.9
18:2n-6	6.9	1.4	3.6	2.4	7.8	69.7
20:5n-3	14.1	14.5	2.7	1.5	6.0	75.7
18(PUFA)	3.8	1.0	1.8	1.9	3.9	79.7
16.0	10.3	9.8	1.4	1.8	3.1	82.8
20:1n-9	2.3	2.2	1.4	1.2	3.1	85.9
16:1n-7	4.7	4.7	1.3	1.5	2.9	88.7
22:1n-9	1.7	0.7	1.0	1.1	2.3	91.0
Average dissimilarity	45.50					

Appendix 4: Details of SIMPER analysis from Chapter 4

Fatty acid	Cluster 1	Cluster 3				
	Av % Comp	Av % Comp	Av.Diss	Diss/SD	Contrib%	Cum.%
18:1n-7	15.8	2.8	7.2	12.7	30.1	30.1
18:1n-9	1.6	8.1	3.6	2.5	15.1	45.1
16:0	11.9	17.6	3.1	6.4	13.1	58.2
EPA	16.8	18.8	1.7	1.6	7.2	65.4
DHA	12.2	13.8	1.5	1.0	6.5	71.8
14:0	4.1	6.0	1.0	1.1	4.3	76.2
18PUFA	5.9	4.3	1.0	1.8	4.2	80.4
16PUFA	3.6	1.9	0.9	2.7	4.0	84.4
16:1n-7	8.5	6.9	0.9	1.4	3.8	88.1
18:0	1.8	2.9	0.6	2.3	2.6	90.7
Average dissimilarity	24.8					

Fatty acid	Cluster 1	Cluster2				
	Av % Comp	Av % Comp	Av.Diss	Diss/SD	Contrib%	Cum.%
18:1n-7	15.8	2.3	7.8	27.4	28.2	28.2
DHA	12.2	6.3	3.4	3.0	12.4	40.6
14:0	4.1	10.0	3.4	3.1	12.3	52.9
16:0	11.9	15.6	2.1	1.0	7.7	60.6
16:1n-7	8.5	11.1	1.5	2.2	5.5	66.2
18PUFA	5.9	3.4	1.5	2.0	5.3	71.4
18:0	1.8	3.9	1.4	0.7	5.0	76.4
18:1n-9	1.6	3.5	1.1	2.0	4.0	80.4
EPA	16.8	15.5	1.0	0.8	3.7	84.1
16PUFA	3.6	2.3	0.8	1.8	2.9	86.9
18:2n-6	3.9	3.6	0.8	2.8	2.8	89.8
15+17	0.7	2.0	0.8	2.2	2.7	92.5
Average dissimilarity	27.5					

Fatty acid	Group 3	Group 2				
	Av % Comp	Av % Comp	Av.Diss	Diss/SD	Contrib%	Cum.%
DHA	13.8	6.3	4.37	1.96	19.35	19.35
18:1n-9	8.1	3.5	2.7	1.86	11.96	31.31
16:1n-7	6.9	11.1	2.45	2.79	10.84	42.15
14:0	6.0	10.0	2.37	2.02	10.49	52.64
EPA	18.8	15.5	2.37	1.4	10.49	63.12
16:0	17.6	15.6	2.03	1.75	9	72.12
18:0	2.9	3.9	1.31	0.87	5.79	77.91
18PUFA	4.3	3.4	0.95	1.46	4.22	82.13
18:2n-6	2.9	3.6	0.7	1.04	3.12	85.25
24:0	0.0	1.0	0.6	0.6	2.67	87.92
18:1n-7	2.8	2.3	0.52	1.66	2.31	90.23
Average dissimilarity	22.6					