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Interannual variability in fatty acid composition of the copepod *Neocalanus plumchrus* in the Strait of Georgia, British Columbia

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ABSTRACT: Although food quality is thought to play an important role in the survival of marine organisms, the extent of natural variability in food quality over long time scales remains poorly characterized. We present a 6 yr time series of fatty acid data from the calanoid copepod *Neocalanus plumchrus*, an important contributor to mesozooplankton biomass in the Strait of Georgia and the northeast Pacific Ocean. Fatty acid profiles indicate significant spatiotemporal differences in the diet of this copepod. Spatially, oceanic specimens display fatty acid signatures characteristic of omnivorous copepods while coastal animals display primarily herbivorous, diatom-based signatures. Temporally, the fatty acid profiles of coastal *N. plumchrus* shifted from an omnivorous oceanic diet to an herbivorous, diatom-based diet between 2001 and 2006. The ratio of diatom to flagellate fatty acid markers increased over time, peaking from 2005 to 2006. The composition of flagellate markers also changed from primarily dinoflagellate markers (rich in docosohexaeonic acid) to green algal markers (poor in this essential fatty acid). The diet of *N. plumchrus* as deduced from fatty acids correlates with phytoplankton community composition. The abundance of coastal *N. plumchrus* in the Strait of Georgia was strongly correlated with the ratio of docosahexaeonic acid to eicosapentaeonic acid in the lipids of these copepods. We also discuss the potential for an imbalance of essential fatty acids supplied by a diatom-rich diet to limit the growth and survival of *N. plumchrus* in the Strait of Georgia.

KEY WORDS: Copepod · Diapause · Diet · Docosahexaeonic acid · Eicosapentaeonic acid · DHA · EPA · Fatty acids · Food quality · *Neocalanus*

INTRODUCTION

Phytoplankton and other protists vary widely in their composition and nutritional content, causing the quality of copepod diets to vary significantly in the oceans (Jonasdottir et al. 2005, Klein-Breteler et al. 2005). Understanding the extent of natural variability in food quality is important because poor food quality can affect 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,

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which are retained in their zooplankton predators and can be used as qualitative tracers of dietary source (Dalsgaard et al. 2003). Diatoms, for example, are characterized by high concentrations of eicosapentaeonic acid (EPA, 20:5n-3), 16:1n-7 and by the presence of polyunsaturated fatty acids (PUFA) containing 16 carbon chains (16PUFA), whereas dinoflagellates are characterized by high concentrations of the essential PUFA docosahexaeonic acid (DHA, 22:6n-3) and PUFA containing 18 carbons (18PUFA, specifically, 18:4n-3) (Thompson et al. 1992, Viso & Marty 1993, Graeve et al. 1994, 2005, Stevens et al. 2004a). Essential PUFAs such as DHA and EPA are important for the physiology of marine copepods and have been shown to affect the efficiency by which energy is transferred in food webs (Müller-Navarra et al. 2000, St. John et al. 2001). Other examples of fatty acid trophic markers of 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 and within different species of copepods and across large spatial gradients (e.g. Graeve et al. 1994, 2005, Stevens et al. 2004b). 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 & 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 long time scales (Kattner et al. 1994, Litzow et al. 2006). Here, we 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 yr.

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 mezozooplankton 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 5 copepodite stages (CI–CV) 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 (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 & 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 *Neocalanus 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 usually composed of a mixture of both 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). Variation in the relative composition of diatoms and dinoflagellates in the diet have been shown to affect the retention of the essential PUFAs EPA and DHA in copepods (Graeve et al. 1994, Stevens et al. 2004a).

The goal of the present study was to characterize the range of interannual variability in the diet of *Neocalanus plumchrus* and to link it to environmental parameters (phytoplankton composition) and population dynamics (patterns in copepod abundance). We tested whether the relative abundance of the essential fatty acids EPA and DHA varied with changes in phytoplankton composition of the lipid profiles of diapausing and actively feeding copepods. We also considered 2 yr 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 Ocean to more clearly characterize regional geographic variability in the diet of *Neocalanus* spp. The present study represents the longest time series of fatty acid composition in calanoid copepods reported to date.

MATERIALS AND METHODS

Field methods. ***Interannual patterns in copepod fatty acids and phytoplankton composition:*** *Neocalanus plumchrus* from the SoG were collected from a single station located in the deepest pocket in the SoG, at 49° N, 123° W (Fig. 1). Sampling was conducted in May (2003–2006) or in the fall (2001–2002) of each year when the *N. plumchrus* SoG community was composed almost entirely of overwintering stage CV copepods. These copepods feed only in the spring and spend the rest of year in a state of diapause, subsisting on their lipid reserves. Copepods collected during the autumn were not expected to have different lipid profiles than those collected in the summer because they have not ingested anything new, and because only a small fraction of wax esters is consumed between those months and fatty acids are consumed in proportion to each other (Evanson et al. 2000, Campbell et al. 2004). For

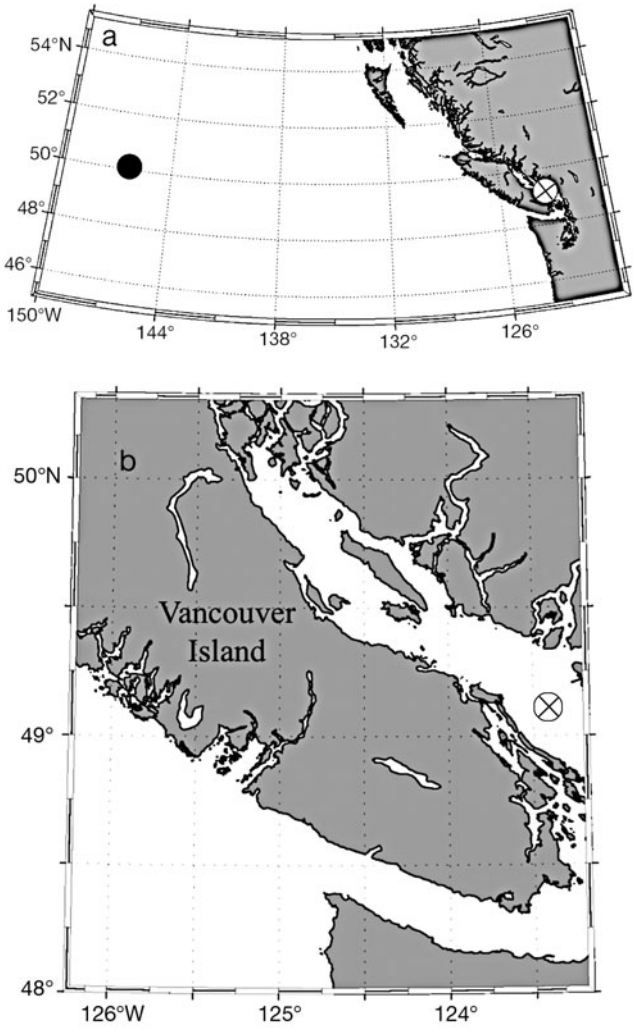

Fig. 1. (a) Sampling sites in the Strait of Georgia (⊗, Station S4-1, 49° N 123° W) and the northeast subarctic Pacific (●, Ocean Station P, 50° N 145° W). (b) Close-up of Strait of Georgia location (⊗)

fatty acid analysis, *N. plumchrus* were sorted from 0 to 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 to 30 copepods, and the number of replicates for a given date ranged from 1 to 7, depending on the availability of copepods. Sorted samples were stored in cryovials at −80°C until analysis. Samples from OSP (*N. plumchrus* and *N. cristatus*) were collected from 0 to 1000 m in September of 2001 and 2005 using the same sorting and storage procedures.

Phytoplankton community composition and *Neocalanus plumchrus* abundance data from the Strait of Georgia Ecosystem Modelling Study (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 (R. Pawlowicz et al. unpubl. data). Phytoplankton samples were collected at the chlorophyll maximum (~5 m) during the spring blooms of 2003–2005 and once in the spring bloom of 2002 (Sastri & Dower 2009). Samples were preserved in Lugol's, identified under an inverted compound microscope and converted to biomass (Sournia 1978). Phytoplankton were divided into 4 taxonomic groups: diatoms, dinoflagellates, flagellates (containing cryptophytes, euglenoids and all other flagellates) and photosynthetic ciliates *Mesodinium rubrum*. For each cruise, the Shannon-Weiner 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.

As part of STRATOGEM, the SoG *Neocalanus plumchrus* population was sampled at least monthly to track seasonal and interannual trends in abundance (expressed in copepods m^{-2}). Here we report *N. plumchrus* abundance data which correspond to the dates from which fatty acids were analyzed. *N. plumchrus* abundance data from 2001 to 2002 were taken from Campbell et al. (2004). 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 on ships of opportunity.

Fatty acid dynamics during the spring bloom of 2005: To assess how diatoms and flagellate fatty acid markers in *Neocalanus plumchrus* varied in relation to phytoplankton composition during active copepod development, sampling of SoG *N. plumchrus* abundance, fatty acid profiles of copepods and phytoplankton community composition was conducted over 5 cruises during the spring bloom of 2005 (over 80 d), in conjunction with the STRATOGEM program. Copepod fatty acid samples were processed as previously described, but with a larger number of *N. plumchrus* per replicate (~30 to 60 copepods per replicate) to account for the low body mass of juvenile stages. Phytoplankton composition was measured as previously described.

Laboratory methods. In the lab, animals were freeze-dried (<40°C for 48 h), 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°C until extraction.

Fatty acid extractions were based on the protocols of Parrish (1999) and Kainz et al. (2004). The samples were sonicated and vortexed 3 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 in a single tube, and the extracts capped in under N_2 gas,

sealed and stored at –80°C to prevent degradation. Fatty acids were analyzed as methyl esters prepared by trans-esterfying the lipid extract in 14% BF3-CH3OH at 85°C for 1 h (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, 0.2 µm film thickness). Unmethylated tricosonic acid (23:00) was used as an internal standard to check the combined efficacy of the procedure. Fatty acid methyl esters were identified by comparing retention times against those of a commercial standard (37-component FAME mix, Supelco 47885-U). Fatty acids not included in this standard were verified using mass spectra (Varian 2000 GC/mass spectrometer) following Ackman (1991). 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 % total fatty acids. The trophic and dietary tracers used in the present study are summarized in Table 1.

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). All statistical analyses were performed on fatty acid data expressed as % total fatty acids. A Bray-Curtis dissimilarity matrix was constructed using raw, untransformed fatty acid data (expressed in % total fatty acids) (Bray & Curtis 1957). The resultant groupings were visualized via multi-dimensional scaling (MDS) ordination and average neighbour clustering using rank similarities. Stress values <0.20 were considered robust following the recommendation of Clarke (1993). Analysis of similarities (ANOSIM) was performed to test the statistical significance of groupings, followed by similarity percentage (SIMPER) analysis to assess the contributions of individual fatty acids to the observed clustering pattern. Subsequently, 1-way ANOVA was performed on arcsine-transformed percentage data (tested for normality and equal variance) to test the significance of those fatty acids trophic markers that SIMPER showed to be important. A Tukey-Kramer *post hoc* analysis was applied to compare means when ANOVAs were significant. Samples from 2001 to 2002 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).

RESULTS

Patterns in *Neocalanus plumchrus* abundance and phytoplankton composition

The abundance of SoG *Neocalanus plumchrus* declined by ~80%, from >8000 ind. m^{-2} in 2001 to <2000 ind. m^{-2} in 2006 (Fig. 2). Diatoms were consistently the most dominant phytoplankton group during the spring bloom, but the composition of the phytoplankton community varied considerably from year to year (Fig. 3a). The concentration of diatoms increased

Table 1. Summary of trophic and dietary fatty acid tracers discussed in the present study

Tracer	Diet	Source
16:1n-7	Diatoms	Graeve et al. (1994), Viso & Marty (1993)
EPA	Diatoms	Viso & Marty (1993), Graeve et al. (1994, 2005)
16PUFA[a]	Diatoms	Thompson et al. (1992), Graeve et al. (1994, 2005)
DHA	Dinoflagellates	Viso & Marty (1993)
18PUFA[b]	Flagellates	Thompson et al. (1992), Viso & Marty (1993)
DHA/EPA	Flagellates to diatom, carnivory	Budge & Parrish (1998)
16PUFA/18PUFA	Herbivory, diatoms to flagellates	Mayzaud et al. (1989), Budge & Parrish (1998)
18:2n-6	Terrestrial plants/green algae	Dalsgaard et al. (2003)
15:0 + 17:0[c]	Bacteria	Kaneda (1991)
18:1n-9/18:1n-7	Bacteria and omnivory	Stevens et al. (2004a,b)
20-22MUFA[d]	Wax ester synthesis	Sargent & Whittle (1981)
22MUFA/20MUFA[e]	Calorific value	Scott et al. (2002)

[a]Includes all PUFA containing 16 carbon atoms
[b]Includes all PUFA containing 18 carbon atoms
[c]Includes *iso* and anti-*iso* branched chains containing 15–17 carbon atoms
[d]Includes all monounsaturated fatty acids containing 20 or 22 carbon atoms (20:1n-9, 20:1n-11, 22:1n-9 and 22:1n-11)
[e]Sum of 22:1n-9 and 22:1n-11 over the sum of 20:1n-9 and 20:1n-11

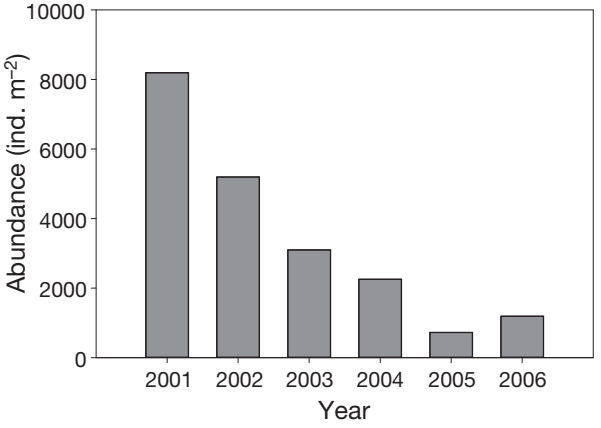

Fig. 2. *Neocalanus plumchrus*. Abundance of diapausing copepods m^{-2} from 0 to 400 m depth in the Strait of Georgia between 2001 and 2006. Data from 2001–2002 are from October, 2003–2006 data are from May

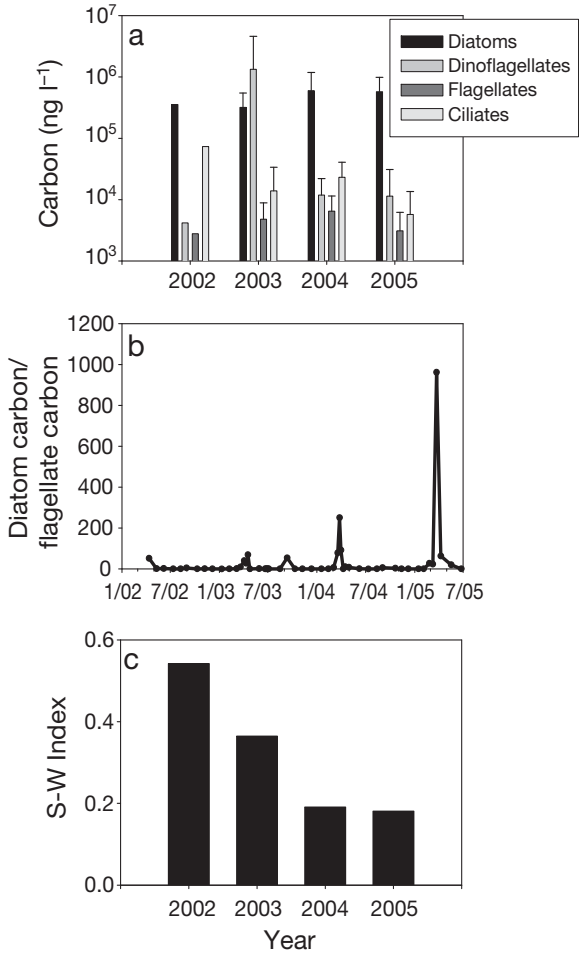

Fig. 3. Spring phytoplankton blooms in 2002 (n = 1), 2003 (n = 8), 2004 (n = 8) and 2005 (n = 4). (a) Composition; (b) proportion of diatoms to flagellates at the peak of the phytoplankton from each year; (c) Shannon-Wiener index (H′) of group diversity averaged over spring blooms

considerably between 2002 and 2005, causing the maximum proportion of diatom carbon to total flagellate carbon to increase progressively, from ~200× in the spring blooms of 2002–2003 to more than 800× in the spring bloom of 2005 (Fig. 3b). As a result, the average Shannon-Wiener index during the time when *N. plumchrus* was actively feeding was lower in 2004–2005 (~0.20) than in 2002–2003 (~0.55), indicating that the developing *N. plumchrus* copepodites encountered a more homogeneous, diatom-dominated diet in 2004–2005 than in 2002–2003 (Fig. 3c).

Fatty acids

Fatty acid profiles of *Neocalanus* spp. from the SoG and OSP were dominated by the saturated fatty acids 14:0 and 16:0 and the essential PUFAs EPA and DHA. MDS ordination (overlaid with cluster analysis) separated the data into 3 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 to 2004, while Cluster 3 encompassed SoG *N. plumchrus* from 2005 to 2006. Clusters 1 and 3 were the most separated (Global R = 0.771), while Clusters 1 & 2 and 2 & 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) and

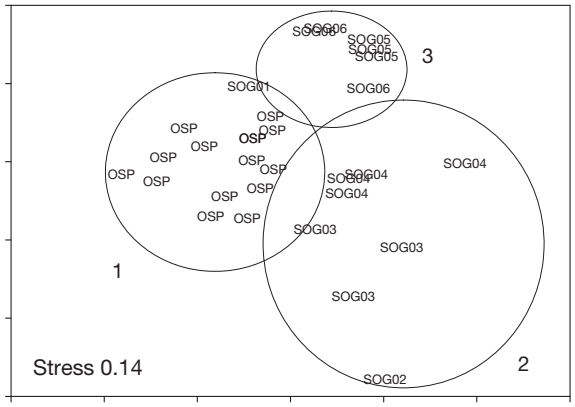

Fig. 4. Multidimensional scaling ordination of a Bray-Curtis dissimilarity matrix calculated from raw, untransformed proportional fatty acid data. The degree of stress (0.14) is within the range of values recommended by Clarke (1993) for robust groupings. Copepods from the Strait of Georgia are indicated as SoG followed by year of sampling (e.g. SOG01 is *Neocalanus plumchrus* from 2001) and Ocean Station P copepods are indicated as OSP regardless of species. 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 similarities (ANOSIM) between the 3 clusters discovered with multidimensional scaling analysis and clustering as shown in Fig. 4. Each value is represented as the Global R statistic (p-value). Cluster 1 contains all oceanic *Neocalanus* spp. and *N. plumchrus* from the Strait of Georgia from 2001. Cluster 2 contains all Strait of Georgia copepods from 2002–2004. Cluster 3 contains Strait of Georgia copepods from 2005–2006

	Cluster 1	Cluster 2	Cluster 3
Cluster 1		0.66 (0.001)	0.771 (0.001)
Cluster 2	0.66 (0.001)		0.595 (0.002)
Cluster 3	0.771 (0.001)	0.595 (0.002)	

by 16PUFA and EPA (which displayed the opposite trend, Table 3). Cluster 3 was further separated from Cluster 2 by having high proportions of diatom markers (EPA and 16PUFA, Table 3). 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 highest in SoG *Neocalanus plumchrus* from 2005 to 2006 and lowest in oceanic copepods (Table 4). Flagellate markers (DHA and 18PUFA) were high in oceanic samples and low in the SoG, with the exception of 2003 (Table 4). The green algal/terrestrial detritus marker 18:2n-6 was higher in 2004–2005 than in any other year in SoG, and was low in OSP samples (Table 4). 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 copepods, while the opposite was generally true for DHA/EPA ratios (which indicate the relative proportions of dinoflagellates to diatoms in the diet) (Table 4). Bacterial markers were higher in oceanic copepods than in coastal copepods, 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 (Table 4). This index has been found to be elevated in carnivorous copepods and those feeding on microzooplankton (Stevens et al. 2004a, El-Sabaawi et al. 2009). 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 was highest in *N. cristatus*.

The spring bloom of 2005

During the spring bloom of 2005 a major collapse in the population of *Neocalanus plumchrus* was observed in late March (between Julian Days 60 and 80), during which the abundance of actively feeding copepods declined from ~35 to ~4 m^{-3} (Fig. 5a). This collapse coincided with a spike of diatom production in which diatom carbon was ~1000× more abundant than flagellate and ciliate carbon (Fig. 5b). 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 copepods. 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. 5c–e).

Table 3. Similarity percent (SIMPER) analysis used to assess 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. Avg. % comp: average composition of the tracer present in each cluster; Cumul.%: cumulative dissimilarity explained by the tracer

Fatty acid	Avg. % comp	Avg. % comp	Cumul. %
Clusters 1 and 2 (average dissimilarity 26.75%)			
	Cluster 1	Cluster 2	
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
Clusters 1 and 3 (average dissimilarity 25.92%)			
	Cluster 1	Cluster 3	
16PUFA	2.73	9.57	16
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
Clusters 2 and 3 (average dissimilarity 23.00%)			
	Cluster 2	Cluster 3	
EPA	10.3	16.3	15.5
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

Table 4. Composition of fatty acid trophic markers in *Neocalanus* spp. from the Strait of Georgia and Ocean Station P (OSP). Composition expressed in % total fatty acids; data are mean (SD). Uppercase letter superscripts designate years or organisms that are found to be significantly different by ANOVA performed on arc-transformed data, followed by Tukey-Kramer *post hoc* analysis ($p < 0.05$). Order of letters corresponds to average level of tracer. Values not followed by letters were not tested using ANOVA. OSPP: *N. plumchrus*; OSPC: *N. cristatus*; n: number of samples

Fatty acid tracer	2001	2002	2003	2004	2005	2006	OSPP	OSPC
14:0	22.9	26	18.6 (1.7)^{BC}	18.2 (3.1)^{BC}	25.8 (1)^{A}	23.7 (0.5)^{AB}	21.9 (2.1)^{AB}	15.1 (4.7)^{C}
16:0	13.6	26.8	15.6 (4.6)^{A}	10.2 (1.6)^{B}	11.5 (0.2)^{B}	13.2 (0.3)^{B}	13 (1.2)^{B}	11.6 (1.2)^{B}
18:0	0.9	2.8	1.6 (0.7)	1.3 (0.3)	0.7 (0)	1.1 (0.2)	1.1 (0.3)	1 (0.4)
sum 15+17[a]	1.5	1.1	1.3 (0.6)^{D}	2.0 (1.1)^{BC}	0.5 (0.2)^{D}	1.0 (0.3)^{CD}	2.2 (0.6)^{B}	3.5 (0.7)^{A}
16:1n-7	3.1	0.8	1.5 (0.6)^{BC}	2.2 (0.1)^{BC}	3.6 (0.5)^{A}	3.2 (0.3)^{A}	2.1 (0.7)^{C}	1.8 (0.5)^{C}
16PUFA[b]	4.0	2.0	4.5 (1.7)^{BC}	6.4 (0.8)^{BC}	10 (0.4)^{A}	9.1 (0.8)^{AB}	3.6 (0.4)^{CD}	1.4 (0.5)^{D}
EPA	9.8	6.6	12.2 (2.9)^{A}	8.6 (2.2)^{C}	15.6 (1.5)^{A}	17 (1.5)^{A}	10.9 (2.6)^{BC}	11.8 (2)^{B}
18PUFA[c]	4.9	2.4	4.7 (1.7)^{A}	2.8 (0.9)^{CD}	4.1 (0.8)^{D}	3.1 (0.6)^{D}	10.6 (1.5)^{AB}	9.8 (2.3)^{BC}
DHA	6.5	6.9	8.6 (2.8)^{A}	5.5 (1.4)^{CD}	3.9 (0.6)^{D}	4.4 (0.8)^{D}	6.7 (1.6)^{AB}	7.9 (1.6)^{BC}
18:1n-9	2.3	2.8	3.0 (1.6)	4.0 (1.1)	0.9 (0.2)	1.8 (1.8)	1.8 (0.3)	1.7 (0.2)
20:1n-9	5.3	0.7	0.5 (0.7)	0.4 (0.5)	1.2 (0.5)	2.3 (1.3)	1.3 (0.9)	2.4 (1)
20:1n-11	4.2	0.9	1.0 (1.3)	1.5 (0.3)	3.1 (0.2)	2.6 (2.2)	4.5 (3.6)	1.8 (1.5)
22:1n-9	1.4	0.6	1.6 (1.8)	2.5 (0.9)	0.1 (0)	3.8 (3.2)	0.9 (2.0)	0.9 (0.8)
22:1n-11	7.9	2.2	1.1 (1.5)	0 (0.1)	2.6 (0.3)	0 (0)	4.3 (2.4)	11.7 (5.1)
18:2n-6	0.8	3.8	5.5 (0.7)^{C}	7.4 (1.4)^{BC}	9.8 (0.4)^{AB}	2.5 (3.6)^{D}	1.8 (0.2)^{D}	1.5 (0.2)^{D}
16PUFA/18PUFA	0.8	0.8	1.0 (0.2)	2.5 (0.7)	2.5 (0.6)	3.0 (0.6)	0.5 (0.5)	0.2 (0.1)
DHA/EPA	0.7	1.1	0.7 (0.1)^{A}	0.6 (0.1)^{A}	0.3 (0.1)^{B}	0.3 (0.1)^{B}	0.6 (0.1)^{A}	0.7 (0.1)^{A}
18:1n-9/18:1n-7	3.2	6.8	3.6 (0.4)^{A}	3.9 (2.3)^{A}	1.7 (0.2)^{A}	1.4 (1.3)^{A}	3.3 (1.1)^{A}	3.4 (0.9)^{A}
20-22 MUFA	18.7	4.3	4.3 (1.9)^{C}	4.4 (0.8)^{C}	7 .0(0.7)^{C}	8.7 (2.8)^{BC}	11 (3.7)^{B}	16.8 (5.6)^{A}
22MUFA/20MUFA	1.0	1.7	1.8 (0.2)^{B}	1.3 (0.6)^{B}	0.6 (0.1)^{B}	0.9 (0.8)^{B}	1 (0.4)^{B}	3.8 (1.3)^{A}
n	1	1	3	4	3	3	7	7

[a]Includes *iso* and anti-*iso* branched chains containing 15 or 17 carbon atoms
[b]Includes all PUFA containing 16 carbon atoms
[c]Includes all PUFA containing 18 carbon atoms

DISCUSSION

General findings and geographic variability

Our results show significant spatiotemporal variability in the fatty acid composition of *Neocalanus* spp. The SoG and OSP represent 2 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 the phytoplankton 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). Our 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). Our fatty acid profiles from coastal *N. plumchrus* are in agreement with the findings of Lee (1974), 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 can be used as a proxy for wax ester concentrations when combined with their corresponding fatty alcohols (Sargent & Whittle 1981, Kattner & Hagen 1995, Saito & Kotani 2000). The relative proportion of these MUFAs is generally higher in oceanic than in coastal *Neocalanus* spp., and 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 low food concentrations (Scott et al. 2002). Longer-chained MUFAs have been shown to contain a higher calorific content than short-chained MUFAs; *N. cristatus* from OSP displays the highest proportion of 22MUFA to 20MUFA indicating that it has a higher calorific value than *N. plumchrus* at OSP (Scott et al. 2002). Though there are no clear interannual patterns in 20-22MUFA in the SoG, there was a higher contribution of longer-chained MUFA in 2003–2004 compared to 2005–2006 (Table 3), indicating that copepods

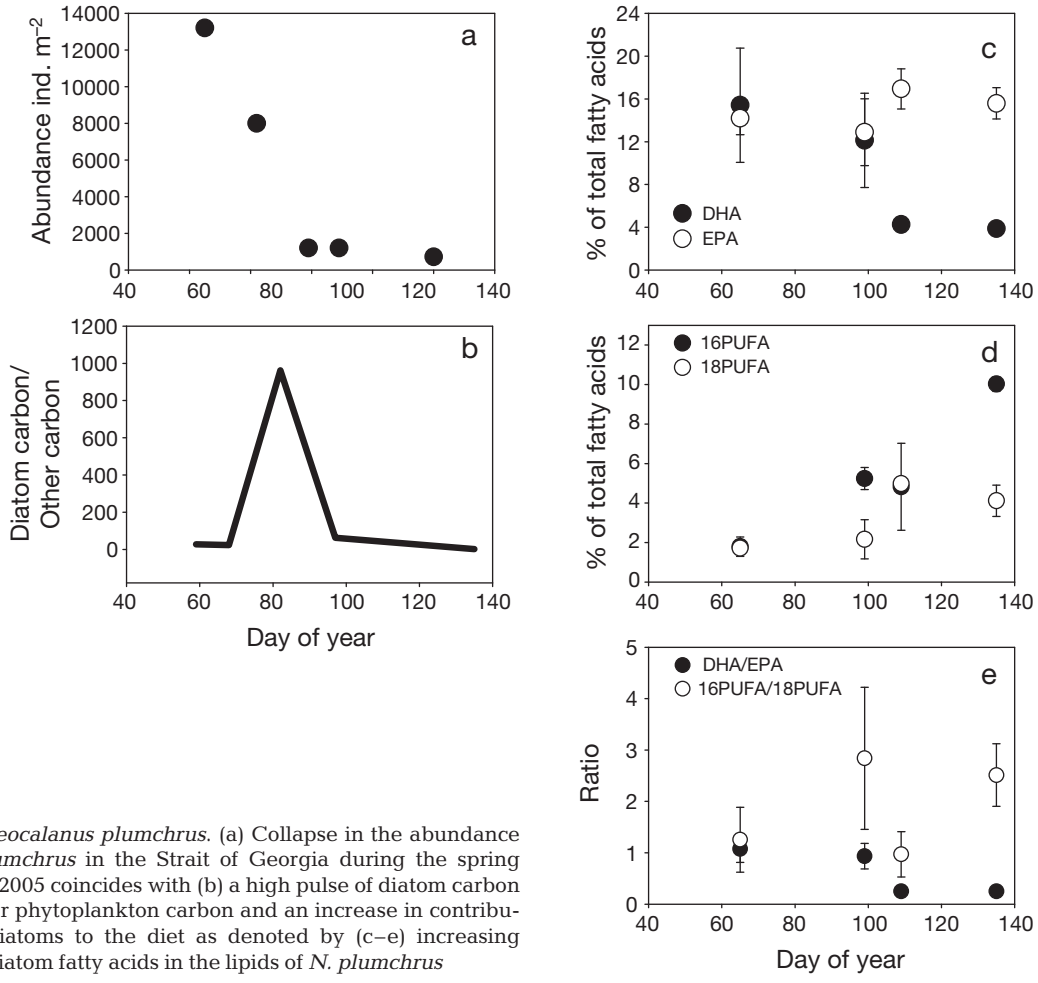

Fig. 5. *Neocalanus plumchrus*. (a) Collapse in the abundance of *N. plumchrus* in the Strait of Georgia during the spring bloom of 2005 coincides with (b) a high pulse of diatom carbon over other phytoplankton carbon and an increase in contribution of diatoms to the diet as denoted by (c–e) increasing diatom fatty acids in the lipids of *N. plumchrus*

from 2005–2006 contained more calorific content. This is consistent with values in copepods living under poor food conditions.

Interannual variation in the diet of *Neocalanus plumchrus* from the SoG

Over the course of the present study, the abundance of *Neocalanus 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 (Figs. 2 & 5a). 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 & Dower 2009). 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* (R. Pawlowicz et al. unpubl. data). It is also unlikely that the decline in *N. plumchrus* biomass was the result of increasing predation pressure. Between 2001 and 2005, stocks of major predators in the area (e.g. salmon, herring, hake and birds) did not increase appreciably (DFO 2006), and potential macrozooplankton predators, such as chaetognaths and gelatinous zooplankton, declined (R. El-Sabaawi unpubl. data). The year 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 & Cummins 2007).

Between 2001 and 2006, the diet of *Neocalanus plumchrus* in the SoG shifted from OSP-like omnivory (in 2001) to moderate herbivory (between 2002 and 2004), and finally, to intense herbivory dominated by diatom markers (2005–2006, Fig. 4). The increasing proportion of diatoms over flagellates in the diet of *N. plumchrus* in the SoG was signified by the increase of

the 16PUFA/18PUFA ratio and a decrease in the DHA/EPA ratio, both corresponding to increasing ratios of diatoms to flagellates in the water column. The flagellate marker 18PUFA did not show any significant trend with time in the SoG, indicating that similar total concentrations 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 (Table 4). 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 terrestrial detritus. Therefore, between 2001 and 2006, not only did the contribution of diatoms in the diets of *N. plumchrus* increase, but the contribution of dinoflagellates decreased in favor of green algae. These patterns correlate more or less with patterns of phytoplankton composition (Fig. 3), though it is important to note that the phytoplankton composition data reported here represent a few sparse sampling points over the spring bloom, which may vary on short time scales. In general, there were no differences in total lipids per copepod observed over the length of the present study (R. El-Sabaawi unpubl. data), and Campbell et al. (2004) observed no significant interannual variability in the concentrations of storage lipids between diapausing animals from 2001 to 2003.

The highest values of 16PUFA/18PUFA and the lowest values of DHA/EPA occurred in 2005 and 2006, years when *Neocalanus 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 1996–1997 (Bornhold 2000, Evanson et al. 2000). In 1996, a good year in terms of *N. plumchrus* biomass, the DHA/EPA ratio was ~1.2, comparable to our values from 2002. In 1997, a poor year for biomass, the DHA/EPA ratio was ~0.3, comparable to our values from 2005 to 2006. Regression analysis of *N. plumchrus* abundance on DHA/EPA ratios using all the data gathered in the present study plus data from Evanson et al. (2000) and Bornhold (2000) shows a strong relationship between those 2 parameters ($R^2 = 0.6$, $p < 0.001$, Fig. 6), indicating that a decline in the survival of *N. plumchrus* is 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 (Arendt et al. 2005, Jonasdottir et al. 2005). The physiological basis for this is unclear, but DHA and EPA have been shown to be required for different physiological processes in shrimp, where DHA is required for molting and EPA is required for

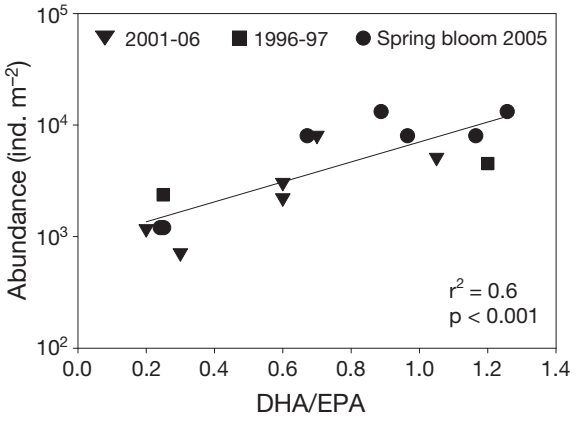

Fig. 6. *Neocalanus plumchrus*. Relationship between *N. plumchrus* abundance (copepods m^{-2}) and the ratio of DHA/EPA in (▼) diapausing copepods from 2001–2006, (●) actively feeding copepods from the spring bloom of 2005 and (■) diapausing copepods from 1996–1997 (Bornhold 2000, Evanson et al. 2000). DHA and EPA are essential fatty acids required for copepod growth and reproduction. Optimal dietary DHA/EPA have been shown to be required for the growth and reproduction of copepods, larval fishes and clams

maintaining general physiological function (Kobayashi et al. 2000). The correlation between molting failure in *Neocalanus plumchrus* and low DHA/EPA ratios in the copepods during the spring bloom of 2005 supports these observations. However, the fact that *N. plumchrus* copepodites that survived the crash continued to molt and develop normally despite low DHA/EPA ratios 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 Koshki et al. (2006). The quality of diet beyond the collapse may have also improved, enhancing the survival of remaining *N. plumchrus*. Further experiments are needed to test the effect of varying proportions of diatom- and flagellate-derived fatty acids on the somatic development of *N. plumchrus* at different copepodite stages. Nonetheless, the correlation between *N. plumchrus* abundance and DHA/EPA ratios suggests that an all-diatom diet decreases the survival of *N. plumchrus* in the SoG.

As in other coastal temperate 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 & 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). Some studies have shown that diatoms impede several aspects of copepod reproduction

(Jonasdottir et al. 1998, Ianora et al. 2003), either by the presence of toxic polyunsaturated aldehyde compounds or by the absence of essential fatty acids that are required for copepod growth (such as DHA) (Jonasdottir 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 very much under investigation (Ianora et al. 2003). Certainly, diatoms typically dominate the phytoplankton community during the spring bloom periods in the SoG, however, they are typically present with other phytoplankton groups (Stockner et al. 1979), which would allow for a diverse copepod diet. Our evidence suggests that in 2004–2005, the diversity of dietary items available to *N. plumchus* declined compared to 2002–2003. Recent evidence also suggests that the overwhelming diatom community in the spring of 2005 may have been nutrient-limited, which would exacerbate feeding on such a homogenous diet (Sastri & Dower 2009).

The correlation between DHA/EPA ratios and abundance of *Neocalanus 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. Our 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). Our 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.

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