



Lipid and fatty acid/alcohol compositions of the subarctic copepods *Neocalanus cristatus* and *Eucalanus bungii* from various depths in the Oyashio region, western North Pacific



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ABSTRACT

Lipids of *Neocalanus cristatus* and *Eucalanus bungii* (C3 to adults), collected in March, May, and December from various depths (0–2000 m) were studied in the Oyashio region, western North Pacific. Total lipid and wax ester contents of younger *N. cristatus* stages increased during the development, being higher in May than in March and December. Major fatty acids of younger *N. cristatus* were 16:0, 20:5(n–3), and 22:6(n–3) and the dominant alcohols were 16:0, 16:1(n–7), 20:1(n–9)/(n–11) and 22:1(n–11). The energy-rich 20:1 and 22:1 moieties increased from the younger to the adult stages showing the importance of lipid biosynthesis which may be advantageous for successful overwintering and reproduction at depth. The 16:4(n–1) fatty acid, characteristic of a diatom diet increased in May, particularly in the younger stages. Our results suggest that the diatom-dominated feeding mode of younger *N. cristatus* during the spring bloom is important for an effective accumulation of wax esters. In contrast to *N. cristatus*, *E. bungii* accumulated substantial amounts of triacylglycerols. The total lipid and triacylglycerol content increased slightly toward the older developmental stages. The major fatty acids were 16:0, 16:1(n–7), 18:1(n–9) and (n–7), and 20:5(n–3). There was no evidence of developmental or seasonal changes in the fatty acid composition. The differences in the lipid storage modes of both copepods via wax esters or triacylglycerols are species-specific but their fatty acid compositions varied according to diet and developmental stage, especially in *N. cristatus*. These lipid characteristics are discussed in relation to reproduction, feeding modes, diapause and overwintering strategies.

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1. Introduction

An extensive area of the subarctic Pacific is known as one of the high-nutrient low-chlorophyll (HNLC) regions, however, the Oyashio region is characterized by substantial spring phytoplankton blooming and consequently high production of higher trophic levels (Taniguchi and Kawamura, 1972; Taniguchi, 1999; Saito et al., 2002). In the ecosystem of the Oyashio region, large calanoid copepods are important constituents of zooplankton comprising 80–90% of the zooplankton biomass (Vinogradov, 1970; Ikeda et al., 2008). These calanoid copepods are major prey for pelagic fishes (Takeuchi, 1972; Taka et al., 1982; Odate, 1994; Yamamura et al., 2002), marine mammals (Nemoto, 1963) and sea birds (Hunt et al., 1998), and are therefore regarded as key organisms in this region. In terms of biomass in the Oyashio region, *Neocalanus cristatus* is the most abundant copepod, followed by *Eucalanus bungii* (Ikeda et al., 2008). However, these two large copepods are known to have extremely different life history

features. *N. cristatus* spawns below a depth of 500 m, mainly during autumn and winter. The eggs and hatched nauplii ascend to the surface and develop through copepodite stages 1 (C1) to 5 (C5) in mid-March to June mainly during the spring diatom bloom period. The C5 copepodids migrate to deeper layers in July and August, and then enter diapause before the final molt to adult females or males (Kobari and Ikeda, 1999). In contrast, *E. bungii* spawns in the surface layer during the spring bloom, develops to the C5 stage and descends gradually to the deeper layers to enter diapause. The individuals which overwintered as C5 molt to adults, then ascend to the surface for spawning, whereas younger individuals overwinter in diapause at C4 stage and molt to adults the following year (Tsuda et al., 2004; Shoden et al., 2005).

In the Oyashio region, both copepods are known to accumulate and biosynthesize substantial quantities of lipids in the surface layer mainly during the spring bloom season, and undergo winter diapause in deeper layers (Ikeda et al., 1990; Kobari and Ikeda, 1999; Tsuda et al., 2001; Shoden et al., 2005) similar to other copepods in cold waters (Dalsgaard et al., 2003; Lee et al., 2006). The major lipid components of the older stages of *N. cristatus* are wax esters with the important

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long-chain monounsaturated fatty acid and alcohol moieties, 20:1 and 22:1, whereas *E. bungii* contains substantial quantities of triacylglycerols with the major fatty acids 16:0, 16:1 (n = 7) and 20:5 (n = 3) (Saito and Kotani, 2000). These differences in lipid and fatty acid compositions are due to the different life histories of the copepod species as shown for other cold and temperate water copepods investigated by Kattner and Krause (1987) and Kattner et al. (1994). However, the process of lipid accumulation and differences in lipid and fatty acid and alcohol compositions of these two copepods related to their life cycles and seasonal environmental changes in the Oyashio region are still unknown.

In this study, we investigated lipid and fatty acid/alcohol compositions of various developmental stages (C3 to adult females and males) of *N. cristatus* and *E. bungii*, which were collected during different seasons at various depths in the Oyashio region. Our objective was to obtain a detailed lipid and fatty acid/alcohol data set of these copepods to get a better understanding why these copepods synthesize and accumulate different storage lipid classes with individual compositions. We discuss the role of these storage lipids in view of life history and feeding behavior and during growth and diapause which has been identified as important question in the frame of the “perspectives on marine zooplankton lipids” (Kattner et al., 2007). Besides the general importance of lipids we hypothesize that for overwintering at diapause both, wax esters and triacylglycerols, are suitable energy reserves although triacylglycerols are described to have less effective storage properties.

2. Materials and methods

2.1. Field sampling

Samples were obtained from the Oyashio region off SE Hokkaido (Fig. 1) during 2006. Copepods were collected during the pre-diatom bloom (10–12 March), post-bloom (23–26 May) and early winter (15–17 December) season from the TS ‘Oshoro Maru’ (March and December) and RV ‘Tansei Maru’ (May). Sampling was performed by vertical tows with a closing net (mouth diameter 80 cm, mesh size

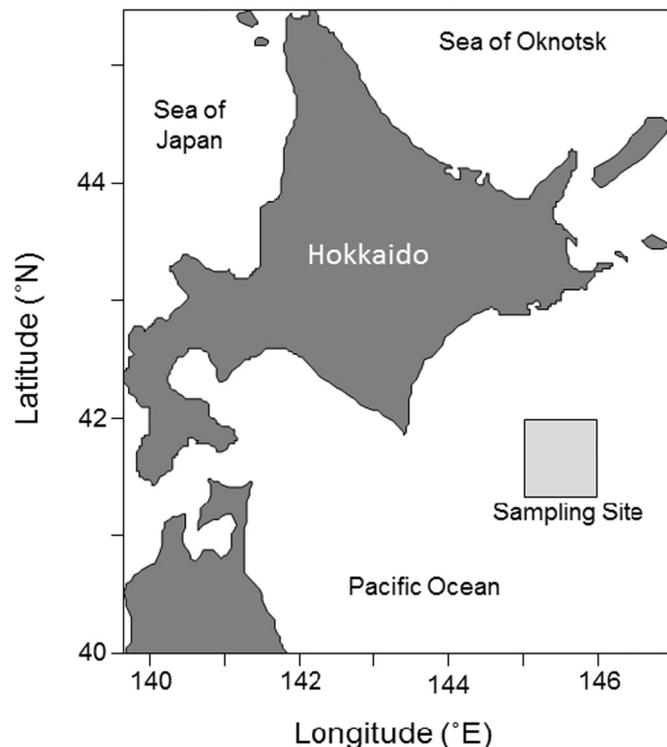


Fig. 1. Location of the sampling site (shaded square) off southeast Hokkaido, western North Pacific Ocean.

0.33 mm (Kawamura, 1968)) at the surface (0–250 m depth), middle layer (250–500 m) and deeper layer (below 500 m, Table 1). After each net retrieval, the contents were gently transferred into plastic containers (2–5 L) filled with chilled seawater. Undamaged *N. cristatus* and *E. bungii* adults (females and males) and copepodite stages (C3–C5) were sorted under a dissecting microscope.

N. cristatus is known to accumulate substantial amounts of lipid during the C5 stage (Ikeda et al., 1990). To study this lipid accumulation in detail, we classified the C5 specimens of *N. cristatus* into four categories depending on the degree of lipid storage and sampling depth, as described by Kobari and Ikeda (1999): “Transparent (C5T)” no lipid deposit; “Solid (C5S)” conspicuous lipid storage; “Intermediate (C5I)” between “C5T” and “C5S;” and “Diapause (C5D)” substantial lipid storage with the entire body colored dark red. The *E. bungii* C3–C5 copepodids were divided into “upper (U)” and “lower (L)” specimens according to sampling depth. After classification, all samples, 3 to 15 replicates each (Table 1), were immediately stored in glass vials (8 mL volume) at -85°C for further analysis. In total, 106 samples of *N. cristatus* and 107 samples of *E. bungii* were divided into 36 categories (Table 1), and were analyzed.

2.2. Lipid analysis

In the laboratory, the frozen samples were lyophilized for 48 h, and dry mass (DM) was determined gravimetrically. Total lipid (TL) was extracted from the freeze-dried samples with a mixture of dichloromethane:methanol (2:1 by volume), essentially after Folch et al. (1957), and was also measured gravimetrically.

Lipid classes were analyzed using high-performance thin layer chromatography (HPTLC) and densitometry (Olsen and Henderson, 1989; Böer et al., 2005). Briefly, pre-coated HPTLC silica gel 60 plates (20 × 10 cm, Merck) were spotted with the lipid samples and standard mixtures (phosphatidylcholine, cholesterol, oleic acid, triolein, 1-O-alkyldiacylglycerol ethers, and oleic acid palmityl ester) using a CAMAG Linomat IV autosampler. The separation of lipid classes was performed in a CAMAG horizontal chamber with hexane:diethyl ether:acetic acid (80:20:2 by volume). Lipid classes were visualized by submerging the plate in manganese (II)-chloride (4 H₂O), methanol, and sulfuric acid reagent in a CAMAG immersion device for 5 s followed by combustion at 120 °C for 20 min. The quantification was performed with a TLC Scanner (CAMAG 3) at 550 nm using the win-CATS software. Concentrations were calculated on the basis of the total lipid mass.

The fatty acid and alcohol compositions were analyzed by gas-liquid chromatography (Kattner and Fricke, 1986; Böer et al., 2005). Fatty acids from the total lipid extracts were converted to their methyl esters by transesterification in methanol containing 3% concentrated sulfuric acid at 80 °C for 4 h. After extraction with hexane, fatty acid methyl esters and free fatty alcohols were simultaneously analyzed with a gas chromatograph (Hewlett-Packard 6890 N) equipped with a DB-FFAP fused silica capillary column (30 m length, 0.25 mm inner diameter and 0.25 μm film thickness) using temperature programming (160–240 °C at 4 °C min⁻¹, hold time 15 min). Class-VP software (Shimadzu) was used for recording and integration. Fatty acids and alcohols were identified with standard mixtures and if necessary by GC-MS (Kattner et al., 1998). A total of 28 components (21 fatty acids and 7 fatty alcohols) were identified.

2.3. Statistical analyses

Seasonal differences in total lipid content and lipid classes of each developmental stage (category) were tested using the Student's *t*-test or one-way ANOVA followed by the Tukey–Kramer test. The fatty acids/alcohols data contains a large number of variables (28 fatty acids/alcohols of 36 copepod categories). To condense the original fatty acids/alcohols data into a smaller set of new composite dimension with a minimum loss of information, principal component analysis

Table 1

Neocalanus cristatus and *Eucalanus bungii*. Summary of sampling data in the Oyashio region, western subarctic Pacific Ocean in 2006. For *N. cristatus*: T: “Transparent” no lipid deposit; S: “Solid” conspicuous lipid storage; I: “Intermediate” between T and S; D “Diapause” substantial lipid storage with the entire body colored dark red. For *E. bungii*: U: “upper”; L: “lower” according to sampling depth.

Species	Stage	Month	Depth (m)	No. replicates	Inds./sample	Category code
<i>Neocalanus cristatus</i>	C3	March	0–200	3	100	N-C3-3
		March	0–250	4	25	N-C4-3
	C4	May	0–250	8	17–25	N-C4-5
		March	0–250	3	8	N-C5T-3
	C5T	May	0–250	3	8	N-C5T-5
		December	500–1000	6	8	N-C5T-12
	C5I	March	0–250	4	8	N-C5I-3
		May	0–250	4	7	N-C5I-5
	C5S	December	500–1000	10	7–8	N-C5I-12
		March	0–250	5	5	N-C5S-3
	C5D	May	0–250	4	3	N-C5S-5
		December	500–1000	8	2–3	N-C5S-12
	F	March	500–2000	7	2–3	N-C5D-3
		May	500–2000	10	3	N-C5D-5
	M	December	500–2000	5	2–3	N-C5D-12
		March	500–2000	9	2–3	N-F-3
	C3U	May	500–2000	4	2	N-F-5
		March	500–2000	4	2–3	N-M-3
	C3L	May	500–2000	4	2	N-M-2
		December	500–2000	3	2	N-M-12
	C4U	March	250–500	5	100–150	E-C3U-3
May		0–250	5	150	E-C3U-5	
C4L	December	500–2000	7	150	E-C3L-12	
	March	250–500	6	50–60	E-C4U-3	
C5U	March	1000–2000	3	50–60	E-C4L-3	
	May	500–2000	6	42–56	E-C4L-5	
C5L	December	500–2000	8	50	E-C4L-12	
	March	250–500	7	22–40	E-C5U-3	
F	May	0–250	12	21–30	E-C5U-5	
	March	1000–2000	3	17–20	E-C5L-3	
M	May	500–2000	8	7–28	E-C5L-5	
	December	500–2000	15	25	E-C5L-12	
C3U	March	0–250	5	20–25	E-FU-3	
	May	0–250	5	7–20	E-FU-5	
C4U	December	500–2000	6	10–15	E-FL-12	
	March	250–500	6	20–25	E-MU-3	

(PCA) was conducted (McCarigal et al., 2002) on the percentage contributions of fatty acids and alcohols for each sample using Primer 5 software (version 5.2.9, PRIMER-E Ltd.).

3. Results

3.1. Total lipid content and lipid class composition

The total lipid content of the C3 stages of *N. cristatus* was 14.0% DM (specimens found only in March). The transparent C5T specimens were low in lipids with only 8.4% DM (collected in December at depth) and 12.9% DM (May at surface; 0–250 m). The adult and C5D diapause specimens were all from depth and lipid-rich. C5D stages had up to 51.8% lipid of DM (December) and 53.6% DM (March). The lipid content of the females was lower (38.7% DM in May, and 39.7% DM in March) than that of adult males (42.0% DM in December) (Table 2, Fig. 2). In May, the total lipid content of the C4, C5T, C5I and C5S stages from the surface were significantly higher than in any other season within its category (Student's *t*-test, $p < 0.05$ for C4, one-way ANOVA and subsequent Tukey–Kramer test, $p < 0.05$ for C5T, C5I and C5S, Table 2, Fig. 2), whereas the total lipid content of adult females in May was significantly lower than that observed in March (Student's *t*-test, $p < 0.05$). No seasonal difference was observed for the C5D stages or adult males (one-way ANOVA, $p > 0.05$).

The wax ester content (% of total lipid) of the C4 stages of *N. cristatus* in March was 48.4% and increased to 71.2% in May. In May similar high wax ester levels were found in the C5T stages although belonging to the transparent specimens. In March and December these C5 stages had less than 50% wax esters. All other C5 stages had high wax ester contents of

about 80% independent of the classification to “Solid”, “Intermediate” and “Diapause” as well as of depth. The same holds true for the adult stages (Table 2). Despite these small seasonal differences found for the C5S and C5D stages, the values observed in December (85.8% and 85.5%, respectively) were significantly higher than those observed in March and May (Tukey–Kramer test, $p < 0.05$, Table 2). No significant seasonal differences in wax ester content were observed for females and males (Table 2).

Overall, the total lipid content of *E. bungii* was clearly lower than that of *N. cristatus*. The lipid content of the C3 stages was around 20% DM increasing substantially until the C5L stage and decreasing slightly in females and males (Table 3, Fig. 2). The lowest total lipid content was found in the C3L stages in December (15.3% DM), and highest in the C5L stages in May (39.6%), however, the differences were smaller than those in *N. cristatus* (8.4–53.6%DM, Table 2, Fig. 2). In May, the total lipid contents were significantly higher than in other seasons for stages C3U, C5U, C5L and females (Student's *t*-test or Tukey–Kramer test, $p < 0.05$). A substantial percentage of triacylglycerols were stored in *E. bungii*, but no wax esters were detected (Table 3). The triacylglycerol contents increased toward the older stages from 47.1% (C3U in March) to 82.6% of total lipids (C5L in May). In May, triacylglycerols were significantly higher than in March and December for stages C3U, C5U, C5L and adult females (Student's *t*-test or Tukey–Kramer test, $p < 0.05$, Table 3), whereas the values for stage C4L were similar to those in March.

3.2. Fatty acid and alcohol composition

A total of 21 fatty acids and 7 fatty alcohols were identified for 36 copepod categories. This data set was subjected to a principal component

Table 2
Neocalanus cristatus. Dry mass, lipid mass, lipid % of dry mass (%DM) and wax esters % of total lipid (%TL) of each category (mean \pm SD). Superscript numbers show rank of between-stages differences examined by student *t*-test or Tukey–Kramer test; NS: no significant ($p \geq 0.05$). For abbreviations refer to Table 1.

Stages	Category code	Dry mass (mg ind. ⁻¹)	Lipid mass (mg ind. ⁻¹)	Lipid (%DM)	Lipid class (%TL)				
					Wax esters	Triacylglycerols	Free fatty acids	Sterols	Polar lipids
C3	N-C3-3	0.11 \pm 0.004	0.016 \pm 0.002	14.0 \pm 1.1	52.5 \pm 0.9	8.7 \pm 0.8	1.8 \pm 0.6	8.2 \pm 0.3	28.9 \pm 0.6
C4	N-C4-3	0.36 \pm 0.05	0.049 \pm 0.01	13.5 \pm 1.0 ⁽¹⁾	48.4 \pm 4.5 ⁽²⁾	6.1 \pm 0.9	2.8 \pm 1.6	7.9 \pm 0.8	34.8 \pm 3.6
	N-C4-5	0.73 \pm 0.08	0.18 \pm 0.02	24.6 \pm 2.5 ⁽²⁾	71.2 \pm 2.7 ⁽¹⁾	–	3.9 \pm 3.3	3.5 \pm 0.5	21.4 \pm 1.9
C5T	N-C5T-3	1.31 \pm 0.11	0.13 \pm 0.02	10.3 \pm 1.1 ⁽¹⁾ (2)	32.8 \pm 2.1 ⁽³⁾	1.7 \pm 0.8	2.6 \pm 0.1	13.1 \pm 0.6	49.9 \pm 1.6
	N-C5T-5	1.16 \pm 0.04	0.15 \pm 0.03	12.9 \pm 2.2 ⁽¹⁾	76.3 \pm 1.7 ⁽¹⁾	–	–	2.8 \pm 0.0	20.8 \pm 1.7
	N-C5T-12	1.39 \pm 0.06	0.12 \pm 0.02	8.4 \pm 1.9 ⁽²⁾	49.1 \pm 6.4 ⁽²⁾	4.4 \pm 0.8	–	7.2 \pm 1.0	38.5 \pm 4.6
C5I	N-C5I-3	2.06 \pm 0.08	0.39 \pm 0.03	18.7 \pm 1.3 ⁽²⁾	77.0 \pm 1.4 ^(NS)	1.1 \pm 0.4	1.0 \pm 0.4	5.1 \pm 0.2	15.9 \pm 1.0
	N-C5I-5	2.01 \pm 0.29	0.55 \pm 0.11	27.2 \pm 2.5 ⁽¹⁾	78.7 \pm 3.2 ^(NS)	0.8 \pm 1.7	1.6 \pm 2.3	2.6 \pm 0.5	16.3 \pm 2.6
	N-C5I-12	2.21 \pm 0.19	0.54 \pm 0.13	23.9 \pm 3.8 ⁽¹⁾ (2)	78.0 \pm 2.0 ^(NS)	1.8 \pm 0.4	0.4 \pm 0.9	3.6 \pm 0.6	16.2 \pm 1.8
C5S	N-C5S-3	4.14 \pm 0.22	1.81 \pm 0.12	43.8 \pm 1.1 ⁽²⁾	83.9 \pm 1.0 ⁽²⁾	1.4 \pm 0.5	1.4 \pm 0.9	2.6 \pm 0.1	10.7 \pm 0.8
	N-C5S-5	8.02 \pm 1.25	4.16 \pm 0.73	51.7 \pm 1.8 ⁽¹⁾	83.1 \pm 1.4 ⁽²⁾	5.9 \pm 1.0	–	1.7 \pm 0.4	9.4 \pm 0.3
	N-C5S-12	5.20 \pm 0.40	2.46 \pm 0.30	47.3 \pm 2.4 ⁽²⁾	85.8 \pm 0.7 ⁽¹⁾	3.1 \pm 1.2	–	1.9 \pm 0.2	9.2 \pm 0.5
C5D	N-C5D-3	6.63 \pm 0.51	3.56 \pm 0.36	53.6 \pm 2.7 ^(NS)	84.4 \pm 1.7 ⁽¹⁾	3.8 \pm 1.1	1.1 \pm 0.6	1.9 \pm 0.5	8.8 \pm 0.8
	N-C5D-5	7.23 \pm 0.41	3.83 \pm 0.46	52.8 \pm 4.1 ^(NS)	79.6 \pm 4.5 ⁽²⁾	7.2 \pm 1.4	1.3 \pm 2.3	1.9 \pm 0.3	9.9 \pm 1.4
	N-C5D-12	7.15 \pm 1.79	3.72 \pm 1.05	51.8 \pm 1.8 ^(NS)	85.5 \pm 2.5 ⁽¹⁾	4.6 \pm 1.7	0.1 \pm 0.3	1.9 \pm 0.4	8.0 \pm 0.8
F	N-F-3	6.55 \pm 0.60	3.48 \pm 0.35	53.1 \pm 2.1 ⁽¹⁾	84.9 \pm 1.4 ^(NS)	2.7 \pm 0.6	0.2 \pm 0.3	2.6 \pm 0.2	9.5 \pm 0.9
	N-F-5	7.22 \pm 1.12	2.80 \pm 0.64	38.7 \pm 5.8 ⁽²⁾	80.5 \pm 3.2 ^(NS)	4.2 \pm 1.5	2.0 \pm 2.5	2.1 \pm 0.2	11.1 \pm 1.3
M	N-M-3	5.77 \pm 0.63	2.29 \pm 0.28	39.7 \pm 3.1 ^(NS)	85.1 \pm 1.7 ^(NS)	2.6 \pm 1.2	0.1 \pm 0.2	2.1 \pm 0.2	10.0 \pm 0.9
	N-M-5	5.20 \pm 1.05	2.20 \pm 0.70	41.8 \pm 6.2 ^(NS)	85.7 \pm 4.3 ^(NS)	0.8 \pm 1.6	–	1.5 \pm 0.1	11.9 \pm 4.2
	N-M-12	3.30 \pm 1.20	1.40 \pm 0.59	42.0 \pm 2.5 ^(NS)	85.9 \pm 0.2 ^(NS)	2.2 \pm 0.2	–	1.0 \pm 0.0	10.9 \pm 0.0

analysis (PCA). As a result of the PCA (Fig. 3), the first principal component (PC1) accounted for 51.8% of the variance, and the second principal component (PC2) for 21.5%. Hence, the two principal components explained 73.3% of the total variance. The PCA produced four multivariate groups in the two-dimensional score plot (Fig. 3A). The PC1 particularly distinguished *E. bungii* (group Eb) from *N. cristatus*, and PC2 subdivided *N. cristatus* into three groups (Nc-1 to Nc-3). For each category (Table 1) mean fatty acid and alcohol data were calculated according to four groups displayed in Fig. 3A, and the means of each group were presented in Table 4. Group Nc-1 was composed of the younger stages (C3–3 and C4–3), both collected in March. Group Nc-2 consisted of C4 to C5I stages collected in March (C5T and C5I), May (C4, C5T and C5I) and

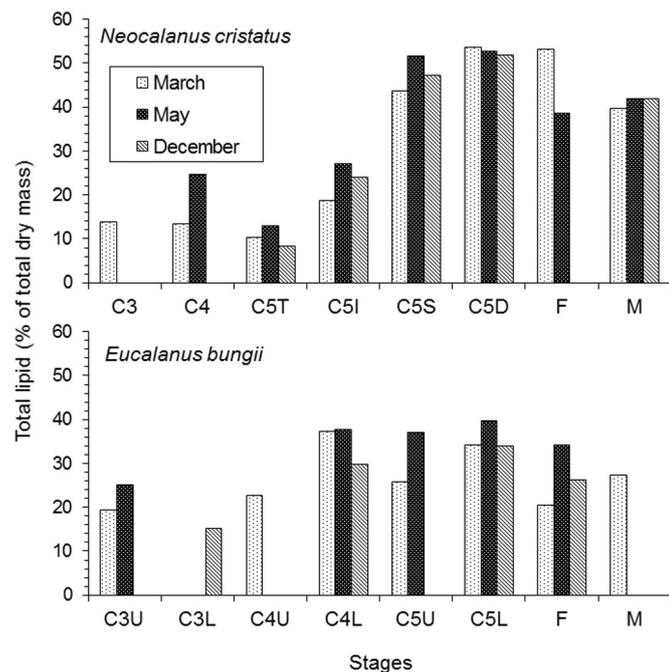


Fig. 2. *Neocalanus cristatus* and *Eucalanus bungii*. Developmental and seasonal variations of total lipid contents (% of dry mass) from C3 to adult female (F) and male (M). For *N. cristatus*: T: “Transparent”, S: “Solid”, I: “Intermediate” and D: “Diapause”. For *E. bungii*: U: “upper” and L: “lower” depth (see Table 1).

December (C5T). Group Nc-3 included the older stages (C5S to adult), irrespective of sampling season.

The eigenvector plot (Fig. 3B) suggested that the most important fatty acids and fatty alcohols for the differentiation of samples along PC1 were the 16:1(n–7), 18:1(n–9) and 18:1(n–7) fatty acids and the long-chain fatty alcohols (20:1 and 22:1). The character of the fatty acid/alcohol composition of *E. bungii* (group Eb) was determined by the amount of the same components, i.e. 16:1(n–7), 16:0, 18:1(n–9) and 18:1(n–7) were greater than those of the group Nc-1 to 3, and the absence of any fatty alcohols (Table 4). For *N. cristatus* (group Nc-1 to 3), the long-chain fatty alcohols, 20:1, composed of similar percentages of the n–11 and n–9 isomer, and 22:1(n–11) increased sharply from 14.5 and 16.7% (Nc-1) to 29.8 and 50.9%, respectively.

Important fatty acids along PC2 were the 20:1 and 22:1 isomers, 16:4(n–1), 20:5(n–3) and 22:6(n–3) as well as short-chain (C14–C18) alcohols. The 20:1 and 22:1 fatty acid isomers of *N. cristatus* increased from 1.0 and 0.6% (group Nc-1) to 14.0 and 24.2% (group Nc-3), respectively (Table 1). In particular, 20:1(n–9) and 22:1(n–11) increased strongly from 0.1 and 0.1% (Nc-1) to 8.4 and 20.6% (Nc-3), respectively. The increase of these long-chain monounsaturated fatty acids is significantly correlated with the increases in wax esters (Fig. 4). The diatom marker fatty acids 16:4(n–1) also increased with stage, from 0.9 (Nc-1) to 3.8% (Nc-3) (Table 4). The developmental and seasonal changes of the 16:4(n–1) fatty acid are displayed in Fig. 5. The proportions of 16:4(n–1) were greater in May than in March and December, particularly for the younger developmental stages. The polyunsaturated fatty acids 20:5(n–3) and 22:6(n–3) decreased from 32.3 and 28.7% to 20.4 and 6.3%, respectively, from the younger to the older stages (Table 4), whereas all short-chain moieties (C14–C18 alcohols) decreased as development progressed.

As an index of diatom feeding, the proportion of the 16:4(n–1) fatty acid of *E. bungii* was also generally highest in May, the post-bloom season (Fig. 5). The females and C5 stages exhibited higher proportions than those of younger stages (C3 to C4).

4. Discussion

It is well known that large calanoid copepods in the Oyashio region of the subarctic North Pacific store substantial quantities of lipids. A number of studies have addressed the relationship between copepod life history and lipid storage in *N. cristatus* (Kobari and Ikeda, 1999),

Table 3

Eucalanus bungii. Dry mass, lipid mass, lipid % of dry mass (%DM) and triacylglycerols % of total lipid (%TL) of each category (mean \pm SD). Superscript numbers show rank of between-stages differences examined by student *t*-test or Tukey–Kramer test. For abbreviations refer to Table 1.

Stages	Category code	Dry mass (mg ind. ⁻¹)	Lipid mass (mg ind. ⁻¹)	Lipid (%DM)	Lipid class (%TL)		
					Triacylglycerols	Sterols	Polar lipids
C3	E-C3U-3	0.044 \pm 0.002	0.009 \pm 0.001	19.4 \pm 1.9 ⁽²⁾	47.1 \pm 3.3 ⁽²⁾	11.1 \pm 0.8	41.8 \pm 2.6
	E-C3U-5	0.050 \pm 0.005	0.013 \pm 0.001	25.1 \pm 1.6 ⁽¹⁾	64.0 \pm 5.0 ⁽¹⁾	5.3 \pm 1.1	30.7 \pm 4.1
	E-C3L-12	0.046 \pm 0.002	0.007 \pm 0.001	15.3 \pm 1.5 ⁽³⁾	49.6 \pm 2.7 ⁽²⁾	7.9 \pm 0.5	42.4 \pm 2.5
C4	E-C4U-3	0.14 \pm 0.02	0.03 \pm 0.005	22.8 \pm 2.2 ⁽³⁾	54.1 \pm 8.4 ⁽²⁾	9.7 \pm 2.5	36.2 \pm 6.2
	E-C4L-3	0.25 \pm 0.01	0.09 \pm 0.01	37.2 \pm 2.1 ⁽¹⁾	81.6 \pm 0.7 ⁽¹⁾	4.7 \pm 0.2	13.7 \pm 0.6
	E-C4L-5	0.29 \pm 0.01	0.11 \pm 0.02	37.6 \pm 6.4 ⁽¹⁾	82.0 \pm 2.1 ⁽¹⁾	3.3 \pm 0.8	14.6 \pm 1.3
	E-C4L-12	0.21 \pm 0.02	0.06 \pm 0.01	29.9 \pm 2.4 ⁽²⁾	78.0 \pm 1.5 ⁽¹⁾	4.0 \pm 0.2	17.9 \pm 1.3
C5	E-C5U-3	0.35 \pm 0.10	0.09 \pm 0.02	25.8 \pm 2.3 ⁽³⁾	76.4 \pm 3.1 ⁽³⁾	4.9 \pm 0.5	18.7 \pm 2.6
	E-C5U-5	0.70 \pm 0.04	0.26 \pm 0.03	37.1 \pm 3.0 ^{(1) (2)}	82.1 \pm 1.5 ^{(2) (3)}	3.4 \pm 0.5	14.5 \pm 1.3
	E-C5L-3	0.31 \pm 0.09	0.10 \pm 0.02	34.1 \pm 3.2 ^{(1) (2)}	76.6 \pm 3.3 ⁽¹⁾	5.0 \pm 0.3	18.4 \pm 3.0
	E-C5L-5	0.70 \pm 0.05	0.28 \pm 0.01	39.6 \pm 2.5 ⁽¹⁾	82.6 \pm 1.0 ⁽¹⁾	3.2 \pm 0.5	14.2 \pm 1.1
	E-C5L-12	0.62 \pm 0.04	0.21 \pm 0.03	33.9 \pm 3.2 ⁽²⁾	80.1 \pm 1.2 ⁽²⁾	3.5 \pm 0.3	16.4 \pm 1.0
F	E-F-3	0.57 \pm 0.09	0.12 \pm 0.01	20.4 \pm 2.4 ⁽³⁾	64.3 \pm 3.0 ⁽²⁾	7.8 \pm 0.9	28.0 \pm 2.3
	E-F-5	1.25 \pm 0.06	0.43 \pm 0.03	34.1 \pm 1.5 ⁽¹⁾	80.2 \pm 2.7 ⁽¹⁾	3.4 \pm 0.1	16.4 \pm 2.5
	E-F-12	1.12 \pm 0.05	0.29 \pm 0.02	26.2 \pm 2.1 ⁽²⁾	77.0 \pm 1.9 ^{(1) (2)}	3.4 \pm 0.3	19.7 \pm 1.8
M	E-M-3	0.48 \pm 0.04	0.13 \pm 0.01	27.3 \pm 1.5	74.9 \pm 2.1	5.6 \pm 0.9	19.5 \pm 1.4

N. plumchrus/flemingeri (Tsuda et al., 2001), *Eucalanus bungii* (Tsuda et al., 2004; Shoden et al., 2005), and *Metridia pacifica/okhotensis* (Padmavati et al., 2004). These copepods accumulate oil as droplets or in an oil sac after the spring bloom season in the Oyashio region, and use their lipid stores for overwintering and reproduction and probably also for buoyancy. Although these earlier studies have documented the lipid deposition and how it corresponds to copepod development, the amount of stored lipids has been mainly described by visual determination, i.e., the size of the oil droplet in the body (Ikeda et al., 1990; Tsuda et al., 2001; Shoden et al., 2005). Gravimetric analysis of lipids in copepods revealed that total lipid contents ranged from 12 to 70% of body dry mass (Nakai, 1942; Saito and Kotani, 2000; Kotani, 2006). The lipid class and fatty acid/alcohol compositions of these copepods were also described by Saito and Kotani (2000) and Kotani (2006). They analyzed the lipid components of C4 to adult stages collected in April–May (Saito and Kotani, 2000) and October (Kotani, 2006), however, the changes of lipid accumulation and components associated with habitat depth could not be considered because the samples were collected from only one depth stratum (0–400 or 500 m). In the present study, we have succeeded to collect a data set of lipid and fatty acid/alcohol compositions of different lipid deposition types from various depth strata during the periods of higher phytoplankton biomass (March and May, i.e., pre- and after-blooming) and lower biomass (December) in the Oyashio region.

The total lipid and wax ester contents of *N. cristatus* increased markedly during C5 stage (Table 2, Fig. 2). Ikeda et al. (1990) and Kobari and Ikeda (1999) also revealed that lipid storage of *N. cristatus* increased at stage C5 just prior to entering diapause, similar to *N. plumchrus*, the western subarctic Pacific congener (Tsuda et al., 2001). Other studies have reported that copepods living in polar and subpolar regions store substantial quantities of lipids in oil sacs during the older stages of their life history. For example, the total lipid content of *Calanus finmarchicus* from the North Sea (% of body dry mass) increased exponentially from the C1 to the C5 stages (Kattner and Krause, 1987). The accumulation of lipids by boreal copepods is a general adaptation to the large fluctuations in seasonal food supply (Lee et al., 1971; Sargent and Henderson, 1986; Conover, 1988).

In the Oyashio region, the highest primary production occurs between April and May (Kasai, 2000; Saito et al., 2002). During this bloom period, the main population of *N. cristatus* undergoes a rapid development to stage C5 at the surface, and then migrates in summer/autumn to deeper layers (below 500 m) to undergo diapause. Overwintering C5 specimens develop to adults, spawn during autumn to winter at depth and lipid-rich eggs and nauplii ascend to the surface along with their development in spring (Kobari and Ikeda, 1999; Tsuda

et al., 2004). Thus, *N. cristatus* depends on the lipids stored during the previous spring bloom for somatic maintenance and reproduction as do other *Neocalanus* species in the subarctic Pacific Ocean (Fulton, 1973; Miller et al., 1984; Saito and Tsuda, 2000).

The long-term lipid deposits are stored as wax esters in many boreal and deep-living zooplankton species (Lee et al., 2006). The wax ester storage is especially pronounced in herbivorous zooplankton and related to the fatty acid and alcohol compositions, i.e., the biosynthesis and accumulation of the 20:1 and 22:1 moieties exclusively produced by herbivorous calanoid copepods (Kattner and Krause, 1989; Kattner and Hagen, 1995; Falk-Petersen et al., 2009). The energy content of the stored lipid is maximized by increasing the chain length of the constituent fatty acids and alcohols (Albers et al., 1996; Scott et al., 2002). These wax ester moieties exponentially increased from the younger to the older stages with the wax ester content in *N. cristatus* (Fig. 4). This was especially pronounced in the alcohol composition where the proportions of the 20:1 and 22:1 alcohols increased from 44 to 80%. These energy-rich compounds are strongly biosynthesized and accumulated during the development and are probably a prerequisite to sustain the metabolic activities for overwintering and reproduction since the spawning of *N. cristatus* in deep sea is completely dependent on lipid storage (Tsuda et al., 2004).

It is important to mention that *N. cristatus* synthesizes considerable amounts of the 20:1(n–11) fatty acid and especially the corresponding alcohol, both occurring in a similar percentage as the 20:1(n–9) isomer. Saito and Kotani (2000) reported even a predominance of this 20:1(n–11) isomer as alcohol moiety in both *N. cristatus* and *N. flemingeri*. In contrast to these Pacific species, the 20:1(n–11) isomer is almost absent from the North Atlantic, Arctic and Antarctic herbivorous *Calanus* and *Calanoides* species, instead the 20:1(n–9) is a major alcohol (Kattner et al., 1994; Falk-Petersen et al., 2009). In these species the 20:1(n–11) isomer is almost completely elongated to the 22:1(n–11) fatty acid and then reduced to the corresponding alcohol (Kattner and Hagen, 1995). The reason for this difference in the biosynthesis and fatty acid pathways between the Atlantic and Pacific Ocean calanoid copepods is still unknown. There is no energetic advantage in the change of the double bond position in a molecule.

No seasonal variation in the content of total lipids or wax esters was observed in the lipid-rich older stages of *N. cristatus*, however, the levels in the younger stages (C3–C5T) in May were higher than in March and December, i.e., lipid deposition begins already at younger stages during the spring bloom. These lipid storage features are different to other boreal calanoid copepods; most of them start storing lipids immediately prior to maturation (Lee et al., 1972; Kattner and Krause, 1987). Tsuda et al. (2001) reported that *N. flemingeri* starts to accumulate lipids as

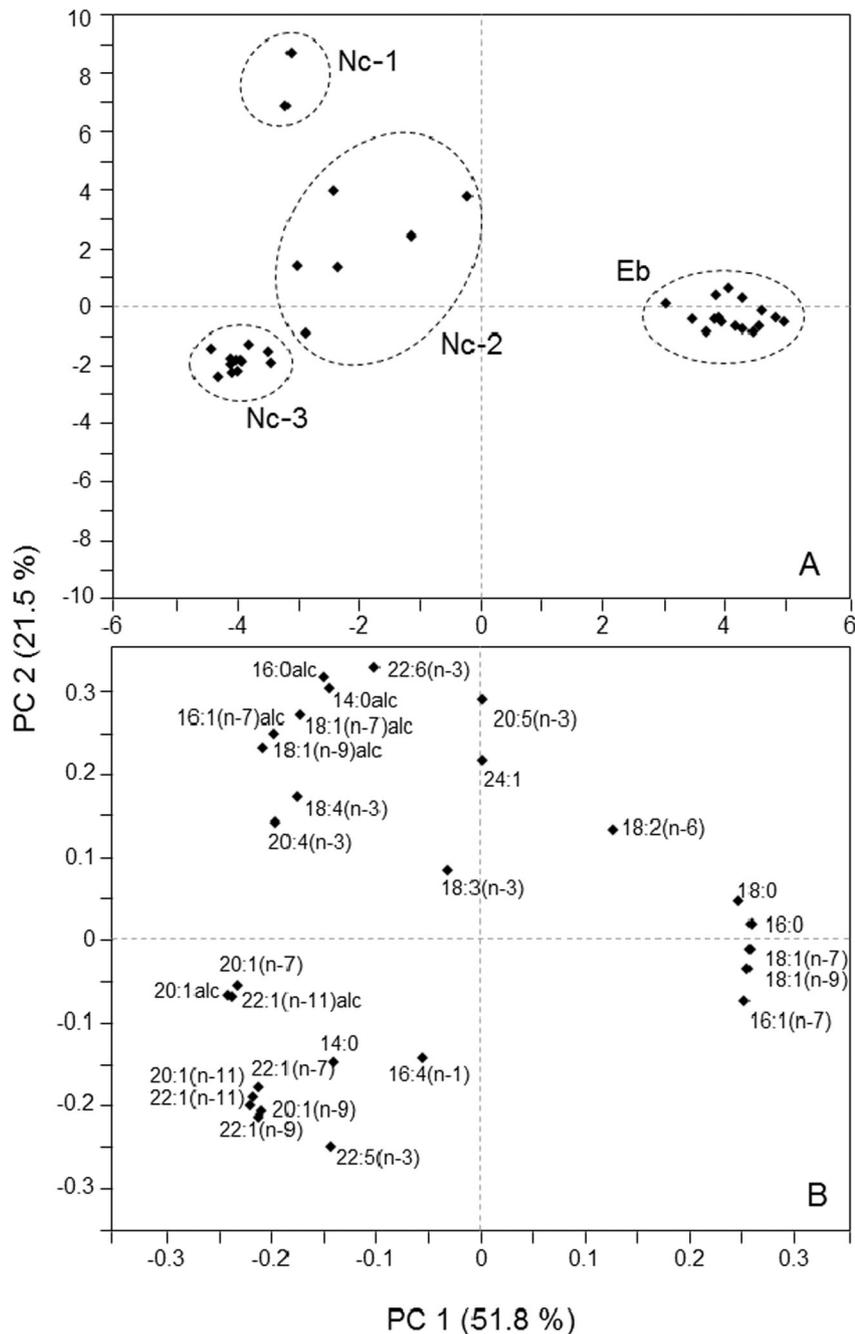


Fig. 3. Principal component analysis (PCA) based on the fatty acid and alcohol composition of 36 samples shown in Table 1. A: Principal component plot. Four multivariate groups were also shown (Nc; *Neocalanus cristatus*, Eb; *Eucalanus bungii*). B: Eigenvector plot of fatty acids and alcohols (alc).

early as the C2 stage, unlike the congeneric *N. plumchrus* which accumulates lipids during the C5 stage. The authors suggested that these differences in lipid accumulation are due to different life history strategies. *N. flemingeri* grows during winter and spring when less food is available than during the spring bloom; thus, early lipid accumulation is an adaptive strategy for survival during periods of insufficient nutrient availability for their development. On the other hand, the *N. plumchrus* development occurs in the peak or declining season of the spring bloom period when it progresses rapidly through the C1–C4 stages without lipid accumulation. Tsuda et al. (2004) revealed that C2–C4 individuals of *N. cristatus* had an oil sac or only droplets, but it rarely exceeded 10% of the prosome length, and lipid storage was mainly observed in the C5 stage. C1 individuals showed higher lipid storage, however, this storage is considered to originate from egg yolk (Saito and Tsuda, 2000) although the lipid composition of egg yolk is still

unknown. Despite the absence of lipid data for the stages preceding C3, the results from our study suggest that the timing of lipid accumulation in *N. cristatus* is probably somewhere between that of these two congeneric copepods.

In the HNLC regions of North Pacific, *N. cristatus* mainly grazes on microzooplankton and sinking particles (Dagg, 1993; Gifford, 1993). Thus, *N. cristatus* is a non-specific grazer throughout the year, consuming prey proportional to its abundance (Kobari et al., 2003). In contrast to the open ocean regime, the periodic diatom spring bloom in the Oyashio region is the dominant food resource until May; thereafter other organisms such as microzooplankton is the major food item (Kobari et al., 2003). The wax ester content of *N. cristatus* increased with increasing proportions of the 16:4(n-1) fatty acid during the younger developmental stages. Our results suggest that active feeding on diatoms by the younger stages during the bloom season promotes

Table 4

Compositions of major fatty acids and alcohols of *Neocalanus cristatus* (Group Nc-1; younger stages, Group Nc-2; middle stages, Group Nc-3; older stages) and fatty acids of *Eucalanus bungii* (Group Eb). Mean \pm SD, mass percent of total fatty acids or alcohols. n: number of samples in each group as defined by cluster and PCA analyses. Each sample is a mean of 3–15 individual samples (see Table 1).

Group (n)	<i>Neocalanus cristatus</i>			<i>Eucalanus bungii</i>
	Nc-1 (2)	Nc-2 (6)	Nc-3 (11)	Eb (16)
Fatty acids				
14:0	6.4 \pm 1.7	6.8 \pm 1.3	9.0 \pm 2.7	6.4 \pm 1.3
16:0	12.9 \pm 0.3	12.4 \pm 2.0	7.7 \pm 1.0	26.0 \pm 2.0
16:1(n-7)	1.6 \pm 0.3	3.5 \pm 0.9	3.3 \pm 0.5	16.7 \pm 1.8
16:4(n-1)	0.9 \pm 0.2	2.4 \pm 2.2	3.8 \pm 1.8	2.5 \pm 1.7
18:0	1.1 \pm 0.1	1.2 \pm 0.3	0.6 \pm 0.2	2.1 \pm 0.4
18:1(n-9)	1.1 \pm <0.1	2.4 \pm 1.4	1.2 \pm 0.3	7.1 \pm 1.0
18:1(n-7)	1.3 \pm <0.1	1.6 \pm 0.3	0.7 \pm 0.1	6.0 \pm 0.9
18:2(n-6)	0.7 \pm <0.1	0.8 \pm 0.3	0.4 \pm 0.2	0.9 \pm 0.3
18:3(n-3)	0.4 \pm 0.2	0.2 \pm 0.2	0.2 \pm 0.1	0.2 \pm 0.2
18:4(n-3)	7.2 \pm 1.6	2.5 \pm 1.0	3.4 \pm 0.8	1.4 \pm 0.7
20:1(n-11)	0.1 \pm 0.2	2.3 \pm 1.4	4.6 \pm 1.4	0.2 \pm 0.1
20:1(n-9)	0.1 \pm 0.1	2.6 \pm 1.7	8.4 \pm 2.5	0.2 \pm 0.1
20:1(n-7)	0.7 \pm <0.1	1.0 \pm 0.6	1.0 \pm 0.2	0.1 \pm <0.1
20:4(n-3)	1.0 \pm 0.1	0.6 \pm 0.2	0.7 \pm 0.2	0.4 \pm 0.1
20:5(n-3)	34.3 \pm 2.2	23.6 \pm 9.3	20.4 \pm 3.1	22.0 \pm 2.6
22:1(n-11)	0.1 \pm <0.1	8.9 \pm 6.9	20.6 \pm 3.3	0.4 \pm 0.1
22:1(n-9)	0.2 \pm 0.2	1.3 \pm 0.9	3.1 \pm 0.6	0.4 \pm 0.1
22:1(n-7)	<0.1 \pm <0.1	0.2 \pm 0.1	0.5 \pm 0.2	<0.1 \pm <0.1
22:5(n-3)	0.5 \pm <0.1	1.4 \pm 0.7	2.5 \pm 0.5	1.4 \pm 0.3
24:1	3.0 \pm 1.3	3.5 \pm 2.1	1.4 \pm 0.9	1.8 \pm 0.6
22:6(n-3)	26.3 \pm 1.4	20.8 \pm 8.2	6.3 \pm 0.8	4.1 \pm 1.6
Fatty alcohols				
14:0alc	4.8 \pm 2.8	1.3 \pm 0.9	0.8 \pm 0.3	
16:0alc	27.0 \pm 11.1	7.7 \pm 4.2	5.0 \pm 1.1	
16:1(n-7)alc	23.0 \pm 6.4	14.4 \pm 5.7	8.3 \pm 1.7	
18:1(n-9)alc	10.6 \pm 1.7	7.4 \pm 1.2	4.3 \pm 0.9	
18:1(n-7)alc	3.3 \pm 2.0	1.4 \pm 0.9	0.8 \pm 0.2	
20:1alc ^a	14.5 \pm 9.3	22.6 \pm 3.9	29.8 \pm 8.4	
22:1(n-11)alc ^b	16.7 \pm 18.3	45.2 \pm 8.5	50.9 \pm 12.0	

^a Composed of similar proportions of the n-11 and n-9 isomer.

^b Including minor proportions of the n-9 isomer.

effective accumulation of wax esters for the following diapause and reproduction of *N. cristatus*. With increasing proportions of the 16:4(n-1) fatty acid the wax ester content of *N. cristatus* increased during the younger developmental stages. In addition, the high proportion of the 16:1(n-7) alcohol as wax ester moiety, again dominant in the younger stages, shows the importance of diatom feeding since the corresponding fatty acid, one of the predominant fatty acids of diatoms, is the precursor of this alcohol. This high conversion of the 16:1(n-7) fatty acid to an alcohol is another special feature of *N. cristatus* not found in other calanoid copepods.

In contrast, the lipid accumulation of *E. bungii* is totally different compared with that of the *Neocalanus* copepods. *E. bungii* accumulated substantial amounts of triacylglycerols, but no wax esters. The total lipid and triacylglycerol content increased slightly toward the older

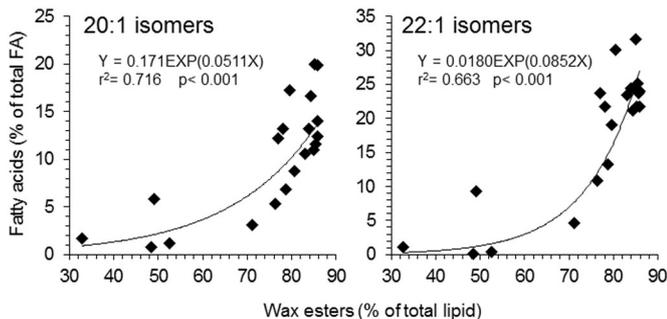


Fig. 4. *Neocalanus cristatus*. Correlations between wax esters and 20:1 and 22:1 fatty acid isomers. Replicates of each category were averaged.

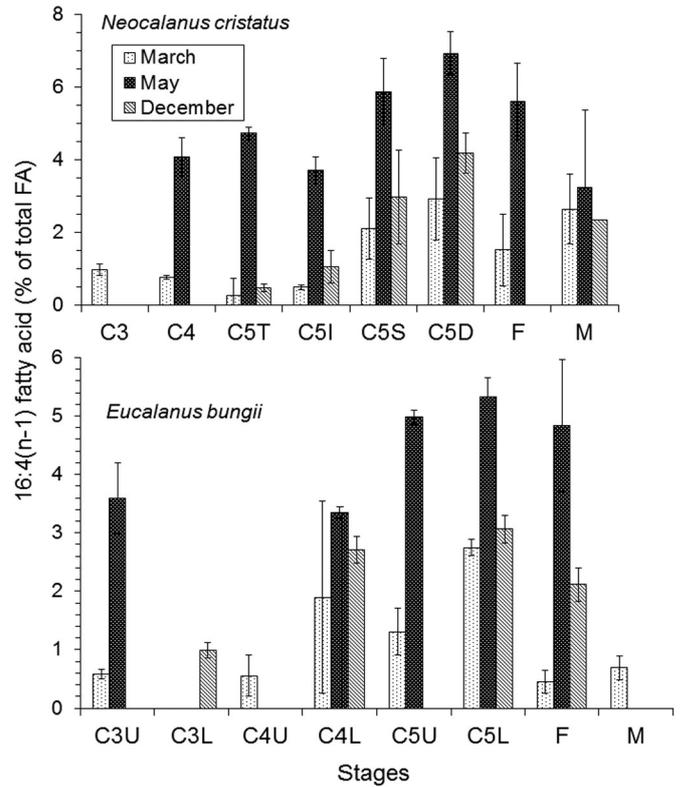


Fig. 5. *Neocalanus cristatus* and *Eucalanus bungii*. Developmental and seasonal variations of the 16:4(n-1) fatty acid (% of total fatty acid), as a diatom marker. For stages refer to Table 1.

stages, but without clear developmental and seasonal differences. This pattern of lipid accumulation points to an opportunistic rather than herbivorous feeding behavior and seems not beneficial for boreal and deep-living diapausing copepods. Ohtsuka et al. (1993) analyzed the gut content of *E. bungii* from Chukchi Sea and northern Bering Sea, and suggested that *E. bungii* is not only herbivorous, but also coprophagous and saprophagous, i.e., a real opportunistic feeder. Kotani (2006) followed up on this hypothesis and suggested that copepods storing triacylglycerols as reserve lipids may overwinter without a period of dormancy. However, it is evident that *E. bungii* migrates deeper than 500 m and undergoes diapause from autumn until early spring (Miller et al., 1984; Tsuda et al., 2004; Shoden et al., 2005; Yamaguchi et al., 2011). All specimens from December and also some from the other seasons in our study were collected deeper than 500 m (Table 1). The fatty acids of *E. bungii* were composed of large proportions of the diatom markers, 16:1(n-7), 20:5(n-3) and 16:4(n-1), suggesting that this copepod depends on phytoplankton rather than on other organisms as major food resource in the diatom-dominated environment of the Oyashio region. This is supported by the report that diatoms were the dominant food source of *E. bungii* until May (Kobari et al., 2003) and that more than 80% of the carbon ingested was obtained from the large diatom *Thalassiosira* spp. in the Oyashio region (Takahashi and Ide, 2011). The storage of triacylglycerols in combination with the absence of long-chain monounsaturated fatty acids is regarded to be disadvantageous when overwintering at depth. There are also no clear differences in lipids between the surface and deep-living C5 stages and females of *E. bungii*. The congeneric *E. californicus*, being also in diapause as adults and C5, also stores triacylglycerols (Ohman et al., 1998); unfortunately, its fatty acid composition is still unknown. There are no unique structural characteristics of particular fatty acids in *E. bungii* such as the long-chain monounsaturated fatty acids in the triacylglycerol-storing Antarctic copepods *Calanus propinquus* and *C. similimus* (Kattner et al., 2012) which may help to cope with the

diapause period. Thus, a lower metabolic activity, perhaps in combination with a higher water content in the body tissue of *E. bungii* may enable the copepod to survive diapause.

E. bungii reproduces at the surface during the spring bloom period (Tsuda et al., 2004; Shoden et al., 2005). Active feeding on diatoms by the females during this period is strongly supported by higher proportion of diatom fatty acid marker 16:4(n-1) than during other seasons (Fig. 5). The phytophagy-dominated feeding mode of *E. bungii* satisfies the energy requirement for reproduction at the surface. Indeed, the egg production rate was found to be affected by the amount of phytoplankton in the local environment (Takahashi and Ide, 2011).

Our results support that the extent and success of reproduction of *E. bungii* is probably not determined by the amount of stored lipid, but by feeding on phytoplankton, i.e., the extent of primary production. In contrast, the fecundity of *N. cristatus* is related to their lipid storage, i.e., the extent of primary production during the previous year (Tsuda et al., 2004). Thus, we conclude that the differences in lipid storage modes at first reflect the characteristics of reproduction and secondly the overwintering strategies. The life history pattern of *N. cristatus* is almost identical everywhere in the subarctic North Pacific Ocean despite the environmental differences (Tsuda et al., 2004). It has been generally accepted that substantial storage of wax esters with long-chain monounsaturated moieties is an adaptation to a pronounced seasonality of food supply (Lee et al., 2006). This lipid accumulation mode of *N. cristatus* supports the copepod to maintain the stable life history patterns that do not depend on the environmental changes widespread in the subarctic North Pacific Ocean. On the other hand, *E. bungii* has different life history patterns between the Oyashio region and eastern subarctic gyre of North Pacific, i.e., timing of the life cycle in the Oyashio region is two months earlier than that in the eastern gyre (Miller et al., 1984; Tsuda et al., 2004). But this difference can be simply explained by the difference of the timing of peak primary production in the two areas (Tsuda et al., 2004). Triacylglycerols, the principal lipids stored by *E. bungii* and other copepods, are more rapidly turned over than wax esters (Lee et al., 2006). Thus, the energy supplied by triacylglycerols is probably more suited to flexible life history features as it is for *E. bungii* and other triacylglycerol-storing copepods.

The differences in lipid and fatty acid accumulation by both copepods demonstrate the variable mechanisms of energy acquisition and overwintering which also reflect different survival strategies developed during the evolutionary history. The storage of either wax esters or triacylglycerols of these copepods in the North Pacific Ocean is a general species-specific feature as also reported from other copepods (e.g. Hagen et al., 1995) and not dependent on local conditions. The same holds true for the developmental lipid class accumulation from the young to the C5 and adult stages. Triacylglycerols seem to be a viable alternative to overwinter in diapause probably less strictly timed than for wax ester storing copepods. This is in accordance with the “plastic life history” of *E. bungii* where the timing and duration of the diapause dependent more on its nutritional status. The individual fatty acid and alcohol compositions varied more due to feeding condition and developmental stage, especially in *N. cristatus*. The difference in fatty acid and alcohol compositions between the closely related *Neocalanus* and *Calanus* species from the Pacific and Atlantic Oceans are surprising and not explainable by energetic advantages by one or the other. Additional studies with special regard on the depth distribution in combination with lipid compositions of these major copepods would enable us to better understand their life history features and to evaluate trophodynamics in the pelagic ecosystem of the western North Pacific Ocean.

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References

- Albers, C.S., Kattner, G., Hagen, W., 1996. The compositions of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: evidence of energetic adaptations. *Mar. Chem.* 55, 347–358.
- Böer, M., Gannefors, C., Kattner, G., Graeve, M., Hop, H., Falk-Petersen, S., 2005. The Arctic pteropod *Clione limacina*: seasonal lipid dynamics and life-strategy. *Mar. Biol.* 147, 707–717.
- Conover, R.J., 1988. Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. *Hydrobiologia* 167–168, 127–142.
- Dagg, M.J., 1993. Sinking particles as a possible source of nutrition for the large calanoid copepod *Neocalanus cristatus* in the subarctic Pacific Ocean. *Deep-Sea Res.* 40, 1431–1445.
- Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. *Adv. Mar. Biol.* 46, 225–340.
- Falk-Petersen, S., Mayzaud, P., Kattner, G., Sargent, J.R., 2009. Lipids and life strategy of Arctic *Calanus*. *Mar. Biol. Res.* 5, 18–39.
- Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226, 497–509.
- Fulton, J., 1973. Some aspects of the life history of *Calanus plumchrus* in the Strait of Georgia. *J. Fish. Res. Board Can.* 30, 811–815.
- Gifford, D.J., 1993. Protozoa in the diets of *Neocalanus* spp. in the oceanic subarctic Pacific Ocean. *Prog. Oceanogr.* 32, 223–237.
- Hagen, W., Kattner, G., Graeve, M., 1995. On the lipid biochemistry of polar copepods: compositional differences in the Antarctic calanoids *Euchaeta antarctica* and *Euchirella rostromagna*. *Mar. Biol.* 123, 451–457.
- Hunt, G., Russell, R.W., Coyle, K.O., Weingartner, T., 1998. Comparative foraging ecology of planktivorous auklets in relation to ocean physics and prey availability. *Mar. Ecol. Prog. Ser.* 167, 241–259.
- Ikeda, T., Hirakawa, K., Kajihara, N., 1990. Some characteristics of a cold water copepod *Calanus cristatus* from regions of the Japan Sea covered by the Tsushima warm current. *Bull. Jpn. Sea Natl. Fish. Res. Inst.* 40, 51–65.
- Ikeda, T., Shiga, N., Yamaguchi, A., 2008. Structure, biomass distribution and trophodynamics of the pelagic ecosystem in the Oyashio region, western subarctic Pacific. *J. Oceanogr.* 64, 339–354.
- Kasai, H., 2000. Seasonal change of nutrients and primary production in the Oyashio region. *Bull. Plankton Soc. Jpn.* 47, 116–118.
- Kattner, G., Fricke, H.S.G., 1986. Simple gas-liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. *J. Chromatogr.* 361, 263–268.
- Kattner, G., Hagen, W., 1995. Polar herbivorous copepods – different pathways in lipid biosynthesis. *ICES J. Mar. Sci.* 52, 329–335.
- Kattner, G., Krause, M., 1987. Changes in lipids during the development of *Calanus finmarchicus* s.l. from copepodid I to adult. *Mar. Biol.* 96, 511–518.
- Kattner, G., Krause, M., 1989. Seasonal variations of lipids (wax esters, fatty acids and alcohols) in calanoid copepods from the North Sea. *Mar. Chem.* 26, 261–275.
- Kattner, G., Graeve, M., Hagen, W., 1994. Ontogenetic and seasonal changes in lipid and fatty acid/alcohol compositions of the dominant Antarctic copepods *Calanus propinquus*, *Calanoides acutus* and *Rhinocalanus gigas*. *Mar. Biol.* 118, 637–644.
- Kattner, G., Hagen, W., Graeve, M., Albers, C., 1998. Exceptional lipids and fatty acids in the pteropod *Clione limacina* (Gastropoda) from both polar oceans. *Mar. Chem.* 61, 219–228.
- Kattner, G., Hagen, W., Lee, R.F., Campbell, R., Deibel, D., Falk-Petersen, S., Graeve, M., Hansen, B.W., Hirche, H.J., Jónasdóttir, S.H., Madsen, M.L., Mayzaud, P., Müller-Navarra, D., Nichols, P., Paffenhöfer, G.A., Pond, D., Saito, H., Stübing, D., Virtue, P., 2007. Perspectives on marine zooplankton lipids. *Can. J. Fish. Aquat. Sci.* 64, 1628–1639.
- Kattner, G., Graeve, M., Hagen, W., 2012. Energy reserves of Southern Ocean copepods: triacylglycerols with unusually long-chain monounsaturated fatty acids. *Mar. Chem.* 138–139, 7–12.
- Kawamura, A., 1968. Performance of Peterson type closing net. *Bull. Plankton Soc. Jpn.* 15, 11–12.
- Kobari, T., Ikeda, T., 1999. Vertical distribution, population structure and life cycle of *Neocalanus cristatus* (Crustacea: Copepoda) in Oyashio region, with note on its regional variations. *Mar. Biol.* 134, 683–696.
- Kobari, T., Tsuda, A., Shinada, A., 2003. Functional roles of interzonal migrating mesozooplankton in the western subarctic Pacific. *Prog. Oceanogr.* 57, 279–298.
- Kotani, Y., 2006. Lipid content and composition of dominant copepods in the Oyashio waters analyzed by the thin layer chromatography flame ionization detection method. *Plankton Benthos Res.* 1, 85–90.
- Lee, R.F., Nevenzel, J.C., Paffenhöfer, G.A., 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. *Mar. Biol.* 9, 99–108.
- Lee, R.F., Nevenzel, J.C., Paffenhöfer, G.A., 1972. The presence of wax esters in marine planktonic copepods. *Naturwissenschaften* 59, 406–411.
- Lee, R.F., Hagen, W., Kattner, G., 2006. Lipid storage in marine zooplankton. *Mar. Ecol. Prog. Ser.* 307, 273–306.
- McCarigal, K., Cushman, S., Stafford, S., 2002. *Multivariate Statistics for Wildlife and Ecology Research*. Springer, Berlin Heidelberg New York.
- Miller, C.B., Frost, B.W., Batchelder, H.P., Clemons, M.J., Conway, R.E., 1984. Life histories of large, grazing copepods in a subarctic ocean gyre: *Neocalanus plumchrus*, *Neocalanus cristatus* and *Eucaulus bungii* in the Northeast Pacific. *Prog. Oceanogr.* 13, 201–243.

- Nakai, Z., 1942. The chemical composition, volume, weight and size of the important marine plankton. J. Oceanogr. Soc. Jpn. 1, 45–55.
- Nemoto, T., 1963. Some aspects of the distribution of *Calanus cristatus* and *C. plumchrus* in the Bering Sea and its neighbouring waters, with reference to the feeding of baleen whales. Sci. Rep. Whales Res. Inst. Tokyo 17, 157–170.
- Odate, K., 1994. Zooplankton biomass and its long-term variation in the western North Pacific Ocean, Tohoku Sea area, Japan. Bull. Tohoku Natl. Fish. Res. Inst. 56, 115–173.
- Ohman, M.D., Drits, A.V., Clarke, M.E., Plourde, S., 1998. Differential dormancy of co-occurring copepods. Deep-Sea Res. II 45, 1709–1740.
- Ohtsuka, S., Ohaye, S., Tanimura, A., Fukuchi, M., Hattori, H., Sasaki, H., Matsuda, O., 1993. Feeding ecology of copepodid stages of *Eucalanus bungii* in the Chukchi and northern Bering Seas in October 1988. Proc. NIPR Symp. Polar Biol. 6, 27–37.
- Olsen, R.E., Henderson, R.J., 1989. The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. J. Exp. Mar. Biol. Ecol. 129, 189–197.
- Padmavati, G., Ikeda, T., Yamaguchi, A., 2004. Life cycle, population structure and vertical distribution of *Metridia* spp. (Copepoda: Calanoida) in the Oyashio region (NW Pacific Ocean). Mar. Ecol. Prog. Ser. 270, 181–198.
- Saito, H., Kotani, Y., 2000. Lipids of four boreal species of calanoid copepods: origin of monoene fats of marine animals at higher trophic levels in the grazing food chain in the subarctic ocean ecosystem. Mar. Chem. 71, 69–82.
- Saito, H., Tsuda, A., 2000. Egg production and early development of large subarctic copepods *Neocalanus cristatus*, *N. plumchrus* and *N. flemingeri*. Deep-Sea Res. I 47, 2141–2158.
- Saito, H., Tsuda, A., Kasai, H., 2002. Nutrient and plankton dynamics in the Oyashio region of the western subarctic Pacific Ocean. Deep-Sea Res. II 49, 5463–5486.
- Sargent, J.R., Henderson, R.J., 1986. Lipids. In: Corner, E.D.S., O'Hara, S.C.M. (Eds.), The Biological Chemistry of Marine Copepods. Clarendon, Oxford, pp. 59–108.
- Scott, C.L., Kwasniewski, S., Falk-Petersen, S., Sargent, J.R., 2002. Species differences, origins and functions of fatty alcohols and fatty acids in the wax esters and phospholipids of *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* from Arctic waters. Mar. Ecol. Prog. Ser. 235, 127–134.
- Shoden, S., Ikeda, T., Yamaguchi, A., 2005. Vertical distribution, population structure and life cycle of *Eucalanus bungii* (Copepoda: Calanoida) in the Oyashio region, with notes on its regional variations. Mar. Biol. 146, 497–511.
- Taka, S., Kitakata, M., Wada, T., 1982. The relations between thesauria, *Cololabis saira* (Brevort) and the food organisms on, especially *Calanus plumchrus*, in the southeast waters of Kuril Islands during the summer. Bull. Hokkaido Reg. Fish. Res. Lab. 47, 41–55.
- Takahashi, K., Ide, K., 2011. Reproduction, grazing, and development of the large subarctic calanoid *Eucalanus bungii*: is the spring diatom bloom the key to controlling their recruitment? Hydrobiologia 666, 99–109.
- Takeuchi, I., 1972. Food animals collected from the stomachs of three salmonid fishes (*Oncorhynchus*) and their distribution in the natural environments in the northern North Pacific. Bull. Hokkaido Reg. Fish. Res. Lab. 38, 1–119.
- Taniguchi, A., 1999. Differences in the structure of the lower trophic levels of pelagic ecosystems in the eastern and western subarctic Pacific. Prog. Oceanogr. 43, 289–315.
- Taniguchi, A., Kawamura, T., 1972. Primary production in the Oyashio Region with special reference to the subsurface chlorophyll maximum layer and phytoplankton-zooplankton relationships. In: Takenouti, A. (Ed.), Biological Oceanography of the Northern North Pacific Ocean. Idemitsu Shoten, Tokyo, pp. 419–431.
- Tsuda, A., Saito, H., Kasai, H., 2001. Life history strategies of subarctic copepods *Neocalanus flemingeri* and *N. plumchrus*, especially concerning lipid accumulation patterns. Plankton Biol. Ecol. 48, 52–58.
- Tsuda, A., Saito, H., Kasai, H., 2004. Life histories of *Eucalanus bungii* and *Neocalanus cristatus* (Copepoda: Calanoida) in the western subarctic Pacific Ocean. Fish. Oceanogr. 13, 10–20.
- Vinogradov, M.E., 1970. Vertical Distribution of the Oceanic Zooplankton. Israel Program for Scientific Translations, Jerusalem.
- Yamaguchi, A., Ohgi, T., Kobari, T., Padmavati, G., Ikeda, T., 2011. Phenology in large grazing copepods in the Oyashio region, western subarctic Pacific. Bull. Fish. Sci. Hokkaido Univ. 61, 13–22.
- Yamamura, O., Honda, S., Shida, O., Hamatsu, T., 2002. Diets of walleye pollock *Theragra chalcogramma* in the Doto area, northern Japan: ontogenetic and seasonal variations. Mar. Ecol. Prog. Ser. 238, 187–198.