JOURNAL OF OLEO SCIENCE
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J. Oleo Sci., Vol. **53**, No. 1, 45-51 (2004)

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NOTE

Lipid Profile of Krill *Euphausia pacifica* Collected in the Pacific Ocean near Funka Bay, Hokkaido, Japan

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Edited by T. Itoh, Kitasato Univ., and accepted September 4, 2003 (received for review June 5, 2003)

Abstract: This paper reports the total lipid content, lipid class composition, and fatty acid composition of the krill *Euphausia pacifica* collected in the northwestern Pacific Ocean near Funka Bay, Hokkaido, Japan. The krill were caught in spring, summer, and winter in three consecutive years, 2000-2002. Lipid content of the *E. pacifica* samples was ranged from 5.1 to 11.6% on the basis of dry weight. Major lipid classes determined for the samples of 2002 were triacylglycerols (TAG) (3.4-27.3%), free fatty acids (FFA) (6.9-22.2%), sterols (5.4-12.9%), phosphatidylethanolamines (PE) (3.4-17.5%), and phosphatidylcholines (PC) (36.2-53.8%). All of the samples subjected to fatty acid analysis were high in 16:0 (19.0-24.5% of total fatty acids), 18:1n-9 (7.5-10.0%), 18:1n-7 (6.3-8.1%), 20:5n-3 (IPA) (15.3-24.7%), and 22:6n-3 (DHA) (8.4-20.7%). The lipids of *E. pacifica* were found to be generally rich in PC and in IPA and DHA. Lipid content and proportion of TAG were higher in the spring samples than in the summer samples. The summer samples were higher in the concentration of DHA in total fatty acids.

Key words: *Euphausia pacifica*, lipid content, lipid composition, fatty acid composition

1 Introduction

Euphausia pacifica is one of the most common krill species in subarctic waters of the North Pacific Ocean and has huge biomass. The krill is an important link in the marine food chain and is also a potentially important marine bioresource for human use. Recently, Saito *et al*. (1) detailed the lipid profile of this species caught in the two locations of the northwestern Pacific Ocean off the coasts of Sanriku and Onagawa, Japan. The lipids of their samples were characterized by high contents of phospholipids (PL), high concentrations of icosapentaenoic acid (IPA) and docosahexaenoic acid (DHA) in PL, and low concentration of DHA in triacylglycerols (TAG). Lipid contents were varied in the catching seasons and locations. However, available information on the lipids of *E. pacifica* is very limited. The same authors pointed out that only four previous papers (2-5) described the lipid profile of this species including fatty acid composition.

In the present study, lipid content, lipid class composition, and fatty acid composition of new samples of *E. pacifica* have been determined. The samples were collected in the Pacific Ocean near Funka Bay, Hokkaido, Japan in three consecutive years. There has been no report on the lipid profile of *E. pacifica* in this area. The aim of this study is to provide the analytical data as fundamental information on the lipids of *E. pacifica*.

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2 Experimental

2·1 Materials

E. pacifica were collected in the northwestern Pacific Ocean near the mouth of Funka Bay, Hokkaido, Japan (42˚00′N, 141˚20′E) in the years 2000-2002. The catching dates are shown in **Table 1**. The *E. pacifica* were collected with modified-IKMT net or FMT net at the depth 10-35 m, except for the samples of November 18, 2002 collected at 45-110 m depth. The collected *E. pacifica* were classified based on their body size, and the samples with total body lengths more than 13 mm were stored at −18℃ for lipid analysis.

2·2 Lipid Analysis

Lipid extraction was carried out in 2002 at the dates

Table 1 Lipid Content and Lipid Class Composition of *Euphausia pacifica* (Total Body Length, ≧13 mm) Collected in the Pacific Ocean near Funka Bay, Hokkaido, Japan.*

Date of collection	Sex	Numbers of samples	Lipid content on dry base (wt%)[a]	Lipid class composition (%)[b]						
				SE+WE	TAG	FFA	ST	PE	PC	Others
2000*[1]										
April 26	Mixture[c]	n=1	8.9†	1.7	0.2	78.8	6.4	1.5	5.9	5.5
June 12	Mixture	n=1	9.5†	0.2	1.0	83.1	5.5	1.3	4.3	4.6
July 15	Mixture	n=1	8.0	1.6	9.2	75.2	6.2	1.1	4.5	2.2
August 7	Mixture	n=1	5.1	1.5	2.8	75.1	8.4	1.6	8.0	2.6
December 18	Mixture	n=1	6.2	0.0	2.8	77.8	9.3	1.8	4.8	3.5
2001*[1]										
April 18	Mixture	n=1	8.0	1.4	5.9	76.3	5.0	0.3	3.3	7.8
May 14	Mixture	n=1	9.5†	0.9	10.2	74.3	4.9	0.7	2.3	6.7
June 27	Mixture	n=3[d]	7.6†	2.0	9.5	61.5	6.4	1.9	11.1	7.6
July 30	Male	n=2[d]	5.1	0.3	3.8	53.6	12.3	6.3	19.1	4.6
July 30	Female	n=2[d]	7.0†	0.7	8.7	55.1	9.0	4.1	18.0	4.4
August 21	Male	n=1	6.4	0.6	2.9	63.4	11.7	4.3	10.6	6.5
August 21	Female	n=1	5.7	0.2	3.0	61.9	10.8	4.6	14.4	5.1
2002										
May 21*[2]	Male	n=1	8.8†	0.1	22.9	12.0	5.7	7.5	46.7	5.1
May 21*[2]	Female	n=1	10.3†	1.6	27.3	10.7	6.4	5.7	44.3	4.0
May 21*[2]	Mixture	n=1	11.6†	2.0	25.5	22.2	5.4	3.4	36.2	5.3
August 17*[3]	Male	n=1	5.6†	0.8	3.4	9.3	12.0	17.5	52.5	4.5
August 17*[3]	Female	n=1	6.2†	1.0	7.0	11.0	12.9	14.5	47.5	6.1
August 17*[3]	Mixture	n=1	7.5†	0.0	6.4	9.7	11.9	13.0	53.8	5.2
November 18*[4]	Male	n=1	8.0	0.0	19.3	6.9	8.0	8.2	53.7	3.9
November 18*[4]	Female	n=1	8.4†	3.1	15.1	6.9	6.0	12.2	48.7	8.0

*All of the lipid analyses including extraction of total lipids from *E. pacifica* were carried out in 2002. Extraction dates were May 2–June 30*[1], July 3*[2], September 5*[3] and December 11*[4], 2002.

SE, sterol esters; WE, wax esters; TAG, triacylglycerols; FFA, free fatty acids; ST, sterols; PE, phosphatidylethanolamines; and PC, phosphatidylcholines.

[a] Percentages determined by gravimetry on the basis of dry matter weights. †The value with obelisk is mean of dupricate determinations for each sample.

[b] Peak area percentages obtained by TLC–FID (means of triplicate determinations).

[c] Mixture of males and females with unknown proportions.

[d] Data are expressed as mean value of the two or three samples.

presented in **Table 1**. The *E. pacifica* sample (0.5 g, approximately 10-16 individuals) was lyophilized for 5.5 h, and total lipids (TL) were immediately extracted from the dry matter by a procedure based on the method of Bligh and Dyer (6). Lipid contents were determined gravimetrically.

Lipid class composition was analyzed by thin-layer chromatography-flame ionization detection (TLC-FID) with Iatroscan TH-10 and Chromarod S-III (Iatron Laboratories, Tokyo, Japan). After chloroform solutions of the TL (3 mg/mL, 4 μL) were applied, the rods were developed by chloroform/methanol/water (80:30:3, v/v/v) to a distance of 4 cm from origin, and then by benzene/chloroform/acetic acid (90:10:1, v/v/v) to 10.5 cm from origin.

Fatty acid methyl esters were prepared by heating the TL with 7% BF_3-methanol for 1 h at 100℃ in a screw-capped test-tube under nitrogen. Methyl esters were purified by TLC on a Silicagel GF plate (Analtech, Newark, USA) with toluene for development (7). The fatty acid methyl esters were analyzed by gas-liquid chromatography with a Shimadzu GC-17A instrument (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a Supelcowax 10 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Supelco, Bellefonte, USA). The column temperature was isothermal at 200℃. The injector and detector temperatures were 250 and 260℃, respectively. The carrier gas was hydrogen (95 kPa). Peak area percentages were obtained with a Shimadzu C-R6A integrator.

3 Results

3·1 Lipid Content

Lipid contents of the *E. pacifica* samples were ranged from 5.1 to 11.6 wt% on the dry matter base (**Table 1**). The females tended to be higher in the TL contents than the males, with one exception of the samples of August 21, 2001. The TL contents of the April and May samples were higher than those in the August samples, i.e., 8.9% vs. 5.1% (2000), 8.0 and 9.5% vs. 6.4 and 5.7% (2001), and 8.8-11.6% vs. 5.6-7.5% (2002). The December or November samples contained TL at the levels higher than the preceding August samples, i.e., 6.2% vs. 5.1% (2000) and 8.0 and 8.4% vs. 5.6-7.5% (2002). **Figure 1** shows the changes in lipid content of *E. pacifica* during 2000-2002. It is apparent that the lipid content was highest in spring every year and decreased during spring to summer. After summer, the lipid content started to increase toward the next spring.

3·2 Lipid Class Composition

Lipid class compositions of the *E. pacifica* samples were determined as shown in **Table 1**. In the 2000 and 2001 samples extracted after very long period of storage (9-25 months), extremely high proportions of free fatty acids (FFA) were observed in the TL. In the 2002 samples extracted after 20-40 days of storage, percentages of FFA were 6.9-22.2% and phosphatidylcholines (PC) were the most abundant lipid class (36.2-53.8% of the TL). Other major lipid classes were TAG (3.4-27.3%), phosphatidylethanolamines (PE) (3.4-17.5%),

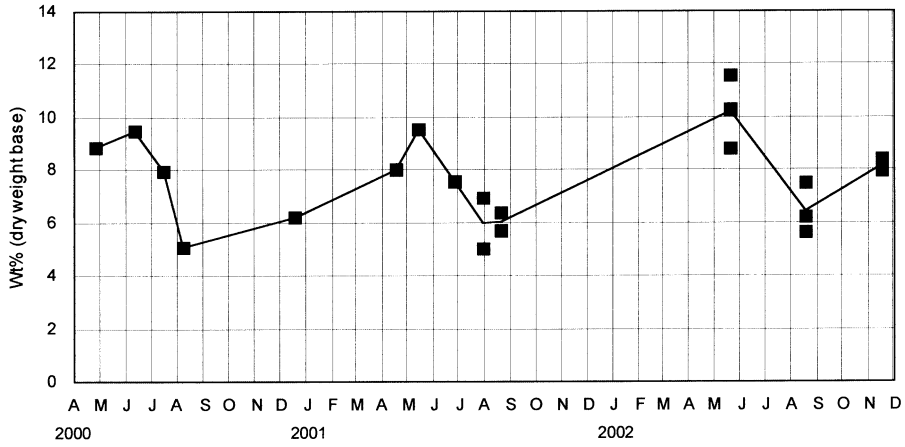

Fig. 1 Changes in Total Lipid Content of *Euphausia pacifica* Collected in the Pacific Ocean near Funka Bay, Hokkaido, Japan (Wt%, Dry Weight Base).

and sterols (ST) (5.4-12.9%). Sterol esters + wax esters were relatively low (up to 3.1%). TAG were highest in the samples of May 21 (22.9-27.3%) and lowest in those of August 17 (3.4-7.0%). In November 18, TAG were middle (15.1 and 19.3%). TAG were rich in spring and decreased during spring to summer. After summer, this lipid class started to increase in *E. pacifica*.

3·3 Fatty Acid Composition of TL

E. pacifica collected in spring (April or May) and summer (August) of 2000 and 2001, and all samples of 2002 were subjected to fatty acid analysis of TL. **Table 2** shows the fatty acid compositions of these samples. Major fatty acids at more than 10% of total fatty acids in most samples were 16:0 (19.0-24.5%), IPA (16.2-24.7%) and DHA (8.4-20.7%). Other major fatty acids found at more than 5% were 14:0 (2.5-6.2%), 16:1n-7 (3.6-11.4%), 18:1n-9 (7.5-10.0%), and 18:1n-7 (6.3-8.1%). Of these fatty acids, DHA showed remarkable differences in its concentrations between the spring and summer samples. The concentrations of DHA in the spring samples were 8.4-11.0%, whereas those in the summer samples were 15.7-20.7%. This fatty acid was higher in the summer samples. The winter samples of 2002 contained DHA at the concentrations of 14.0 and 14.3%, which were lower than those in the preceding summer samples. In contrast, IPA tended to be higher in the spring samples (18.0-24.7%) than in the summer samples (15.3-20.0%). Similarly, 16:1n-7 was also higher in the spring (6.4-11.3%) than in the summer (3.6-7.2%). In general, fatty acids of the *E. pacifica* samples were rich in n-3 HUFA, and concentration of DHA was especially high in the summer samples.

4 Discussion

In the present study, lipid profile of *E. pacifica* was revealed for the samples collected in the Pacific Ocean near Funka Bay. According to the analytical data, lipid profile of the *E. pacifica* samples can be summarized as follows. (i) The lipid content was 5.1-11.6% on dry weight base. (ii) The lipids were generally rich in PC. (iii) The lipids were also rich in n-3 HUFA (IPA and DHA). (iv) The lipids of spring and summer differed in lipid content (spring > summer), proportion of TAG (spring > summer), and concentration of DHA (spring < summer).

As pointed out by Saito *et al*. (1), there have not been many reports on the lipids of *E. pacifica*. However, fragmentary information is available from the previous studies on this species varied in catching-areas, seasons, and years. The lipid content previously presented on dry weight base were 7.02 and 7.85% in the samples landed on Senzaki and Kesennuma, Japan (8), and 23.8 and 18.9% in the samples collected off the coasts of Eureka and Washington, USA (9). Lipid contents observed in the present study were closer to the Japanese samples. In the present study, lipid contents on wet weight base were significantly influenced by the amount of drain-water. However, observed values, 1.1-3.2% on wet weight base (not shown in **Table 1**), were similar to those of the previous samples collected in Japan, i.e., 0.6-6.2% (1), 1.1-3.5% (4), 1.3 and 1.4% (5), and 1.02-1.49% (8).

Representative lipid classes of *E. pacifica* were reported as follows: TAG (1.0-29.5%), FFA (4.9-30.1%), ST (7.7-20.6%), PE (18.3-43.9%), and PC (12.2-57.7%) (off Sanriku and Onagawa) (1); TAG (1 and 21%), FFA (9 and 23%), ST (9 and 10%), and PL (59 and 65%) (Kesennuma and off Kushiro) (5); TAG (18.5-21.9%), FFA (19.2-41.9%), ST (9.7-15.8%), and PL (8.0-21.2%) (the Sea of Japan) (4); and neutral lipids (TAG, FFA and ST) (66.5%), PE (4.5%), and PC (21.0%) (off Eureka) (3). The percentages of wax esters often combined with sterol esters were varied from trace amount to 10.8% (1,4,5). The present results for the samples collected in 2002 were similar to those in the former two reports described above (1,5). Saito *et al*. (1) described that high PL contents may be a characteristic of *E. pacifica* lipids. The result for the samples collected near Funka Bay supports this view.

In contrast, the samples of 2000 and 2001 showed extremely high proportion of FFA in the TL. It is probable that this resulted from hydrolysis of other lipid classes during much longer period of storage prior to lipid extraction. Furthermore, postmortem lipolytic changes of krill are reported to be very rapid. Antarctic krill (*Euphausia superba*) after 72 h of storage at 3℃ contained about 20% less PL, including half of the initial PC and 6% increase of FFA (10). In this respect, lipid class compositions of the 2002 samples and those in some previous reports may also contain the results of such rapid hydrolysis during the storage and any treatment at ambient temperature.

Fatty acids most abundant in TL of *E. pacifica* were reported as follows: in the samples of the Pacific Ocean

off Japan, 16:0 (20.1 and 18.3%), 18:1 (16.4 and 7.0 %), IPA (25.9 and 20.5%), and DHA (14.7 and 12.8%) (2,8); and in the samples off Eureka and Washington, 16:0 (28.5 and 22.8%), 18:1 (15.1 and 13.9%), IPA (30.9 and 27.9%), and DHA (6.4 and 16.1%) (9). The present samples also contained these fatty acids at the concentrations not very different from those in the previous two reports, indicating that IPA and DHA were rich in *E. pacifica*.

Some previous papers reported fatty acid composi-

Table 2 Fatty Acid Composition of Total Lipids in *Euphausia pacifica* Collected in the Pacific Ocean near Funka Bay, Hokkaido, Japan (wt%).

	2000		2001			2002							
	Apr 26	Aug 7	May 14	Aug 21		May 21			Aug 17			Nov 18	
Fatty acid	Mix.	Mix.	Mix.	Male	Female	Male	Female	Mix.	Male	Female	Mix.	Male	Female
14:0	2.5	4.8	4.6	3.5	4.9	4.7	4.2	4.4	2.8	3.3	3.1	6.2	4.6
iso-15:0	0.1	0.2	0.3	0.1	0.2	0.4	0.3	0.4	0.2	0.2	0.3	0.6	0.4
15:0	0.2	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.6	0.6	0.6	0.8	0.7
16:0	22.4	21.3	22.3	19.7	20.2	21.2	21.3	24.5	19.0	21.2	19.8	20.9	21.9
16:1n-7	8.5	5.7	11.4	5.5	7.2	7.9	7.9	6.4	3.6	4.5	4.7	4.0	4.3
16:1n-5	0.2	0.3	0.4	0.2	0.3	0.4	0.4	0.3	0.2	0.2	0.3	0.5	0.5
iso-17:0	0.4	0.3	0.4	0.4	0.4	0.6	0.4	0.4	0.4	0.4	0.4	0.5	0.6
anteiso-17:0	0.2	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.3
16:2n-4+phytanic	2.5	2.3	3.1	0.9	1.7	2.9	3.5	1.6	0.8	0.9	1.0	1.5	2.0
17:0	0.2	0.4	0.2	0.5	0.5	0.3	0.3	0.2	0.6	0.6	0.6	0.6	0.5
16:3n-4+17:1	0.6	0.6	0.9	0.5	0.6	0.8	0.8	0.7	0.6	0.7	0.6	0.9	0.8
iso-18:0	0.2	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.5	0.5
16:4n-1	0.4	0.2	0.9	0.1	0.3	0.6	0.8	0.6	0.1	0.1	0.1	0.2	0.3
18:0	1.5	1.4	1.3	1.6	1.5	1.1	1.3	1.3	1.4	1.4	1.4	1.2	1.3
18:1n-9	8.1	9.9	7.5	8.4	7.8	7.5	8.0	10.0	8.8	9.1	9.1	9.2	8.8
18:1n-7	8.1	6.4	6.8	7.6	6.7	7.0	6.7	7.6	7.1	6.8	6.9	6.3	6.9
18:1n-5	0.2	0.2	0.3	0.2	0.1	0.3	0.3	0.4	0.2	0.2	0.2	0.2	0.3
18:2n-6	1.1	2.7	1.4	2.3	2.0	1.6	1.4	1.3	2.7	2.7	2.7	2.5	2.3
19:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.3	0.2
18:3n-3	0.3	1.7	0.8	0.8	1.0	1.0	0.9	0.8	1.8	1.9	1.8	1.9	1.6
18:4n-3	1.0	2.0	2.3	0.6	0.8	2.2	2.1	1.8	1.2	1.2	1.2	3.6	2.8
20:1n-11+20:1n-13	0.2	0.2	0.3	0.1	0.2	0.4	0.3	0.3	0.2	0.2	0.2	0.5	0.4
20:1n-9	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.7	0.3	0.3	0.3	0.3	0.3
20:1n-7	0.2	0.1	0.2	0.1	0.1	0.2	0.3	0.2	0.1	0.1	0.1	0.2	0.2
20:2n-6	0.1	0.2	0.1	0.3	0.2	0.2	0.1	0.2	0.3	0.2	0.3	0.2	0.2
20:4n-6	1.6	2.1	0.8	3.1	3.0	2.5	2.0	1.0	3.5	3.2	3.2	2.0	1.8
20:4n-3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
20:5n-3	24.7	15.3	20.2	19.6	20.0	20.2	20.9	18.0	18.3	17.7	17.7	16.2	17.4
22:1n-9	0.1	0.2	0.1	0.1	0.2	0.4	0.4	0.3	0.0	0.1	0.1	0.0	0.0
22:3n-6	0.9	0.3	0.6	0.3	0.3	0.5	0.6	0.4	0.2	0.2	0.3	0.3	0.4
22:5n-6	0.2	0.2	0.1	0.3	0.2	0.1	0.1	0.1	0.3	0.2	0.4	0.2	0.2
22:5n-3	0.4	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.2	0.3
22:6n-3	10.4	16.3	8.4	19.0	15.7	10.8	10.1	11.0	20.7	18.3	19.1	14.0	14.4
24:1n-9	0.0	0.2	0.0	0.1	0.0	0.0	0.1	0.5	0.2	0.1	0.1	0.0	0.0
Others (<0.2 %)	1.8	2.5	2.3	2.1	1.9	2.4	2.4	2.8	2.2	1.9	2.0	2.8	2.7

See **Table 1** for details of the *E. pacifica* samples.

tions of major lipid classes of *E. pacifica*. Concentration of IPA was reported to be 5.1-23.2% in TAG (1,5), 19.0-42.2% in PC (1,3), and 12.8-25.9% in PE (1,3). DHA were 1.5-6.3% in TAG (1,5), 9.4-25.3% in PC (1,3), and 24.8-42.2% in PE (1,3). The most recent analysis (1) showed that IPA and DHA were dominant fatty acids in PL and that markedly higher level of DHA was found in PE. DHA was relatively low in TAG. In the present study, fatty acid compositions of lipid classes were not determined. However, samples with higher proportions of PE and PC (e.g., August 17, 2002) contained DHA at the concentrations higher than those with lower proportions of these lipid classes (e.g., May 21, 2002) (**Table 2**). This result suggests that the concentration of DHA was higher in PE and/or PC than in TAG of the *E. pacifica* collected near Funka Bay. In contrast, IPA was somewhat higher in the samples with lower proportions of PE and PC. For more clarity, fatty acids need to be analyzed for each of the lipid classes.

The previous papers reported seasonal variations of lipid content in *E. pacifica* (1,4). The spring samples (March, April, and May) were reported to be higher in lipid content than the samples of other seasons (February, June, October, and December) (1,4). Such high lipid content in the spring samples probably resulted from increases in dietary phytoplankton in northern sea area in spring and accumulation of lipid reserves for spawning, as pointed out previously (1,4). The present study also showed the similar tendency (**Fig. 1**). Mayzaud *et al*. (11) investigated seasonal variations in the lipid and fatty acid composition of the krill *Meganyctiphanes norvegica* from the Ligurian Sea. The seasonal changes in TL showed maximum accumulation in early summer (June) and minimum levels in early winter. In the same area, the phytoplankton maximum was recorded in April, and zooplankton biomass was also increasing. Maximum zooplankton biomass was generally recorded in spring and early summer (May). Summer and autumn months (July to December) were characterized by low biomass of phytoplankton and low to intermediate concentrations of micro- and mesozooplankton. Thus, the increase in lipid content observed in June was related to the April-May abundance of both primary and secondary products. In contrast, Saether *et al*. (12) reported that the lipid content of three common species of North Atlantic krill including *M. norvegica* was highest in autumn and winter months and lowest in the spring. The same authors, however, described that the three species accumulated lipids during the summer, autumn, and winter when food is abundant and depleted their lipid reserves during the late winter and spring when food is scarce. It is probable that the highest lipid accumulation observed for the spring samples of *E. pacifica* is also closely related to food supply. Higher proportions of TAG in the spring samples of 2002 also seems to be related to food supply and consequent depot lipid accumulation.

Higher concentrations of DHA in TL of the summer samples seem to result from low proportions of TAG containing low levels of this acids as mentioned above. Similar changes were observed for DHA of the krill *M. norvegica* from Ligurian Sea (11). In this krill, concentration of DHA in TL was highest in early winter minimum in the TL content. Although DHA were one of the dominant fatty acids in both TAG and PL, higher concentration was observed in PL rather than in TAG. Seasonal changes in fatty acid composition of TL in *M. norvegica* were related to the succession of populations with varying levels of TAG.

In general, lipid profile of *E. pacifica* collected in the Pacific Ocean near Funka Bay was not very different from that fragmentarily reported for the same species. On the other hand, the present study has an advantage that the *E. pacifica* samples were collected in the long term period (three years) in one area. As far as the authors know, there has been no report describing the lipids of *E. pacifica* collected for more than ten months. Without catching-area variations, the present study could confirm the lipid profile of *E. pacifica* and especially its seasonal changes. During spring to summer, contents of TL and TAG decreased and concentration of DHA in TL increased in *E. pacifica*. From the result of this study, it is apparent that these seasonal changes are repeated every year in the same area.

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