NOTE

Positional Distribution of DHA in Triacyl-*sn*-glycerols of *Artemia* Nauplii Enriched with Salmon Roe Polar Lipids-Containing Fish Oil

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Edited by T. Itoh, Kitasato Univ., and accepted November 11, 2004 (received for review September 13, 2004)

Abstract: Positional distribution of fatty acids in triacyl-*sn*-glycerols (TAG) has been investigated for *Artemia franciscana* nauplii used in marine fish larviculture as an important live food. For enhancement of essential highly unsaturated fatty acids, the nauplii were enriched for 4 to 24 h with two emulsions containing fish roe polar lipids (PL), (i) a mixture of bonito head oil TAG and salmon roe PL concentrate (88:12, wt/wt) and (ii) an unmixed salmon roe PL concentrate. In TAG isolated from all enriched *Artemia* nauplii, docosahexaenoic acid (DHA) was preferentially esterified in the *sn*-3 position (0.7-22.9 mol%) followed in sequence by the *sn*-2 (0.1-7.5 mol%) and *sn*-1 (0.0-4.6 mol%) positions. This distribution pattern was the same as previously observed for enrichment with bonito head oil TAG free from salmon roe PL. Addition of fish roe PL to DHA-rich fish oil enricher does not seem to practically affect the distribution pattern of DHA characteristic of *Artemia* nauplii.

Key words: *Artemia*, docosahexaenoic acid (DHA), triacyl-*sn*-glycerol, polar lipid, positional distribution, enrichment

1 Introduction

Artemia nauplii are used in aquaculture as an important live food for marine fish larvae. However, they are deficient in essential n-3 highly unsaturated fatty acids (HUFA), icosapentaenoic acid (IPA) and docosahexaenoic acid (DHA) in particular. In order to ensure successful production of fish larvae, the nauplii are enriched with n-3 HUFA-containing materials prior to being fed to fish larvae (1-3). Marine lipids are typical enrichers of n-3 HUFA for *Artemia* nauplii (e.g., 4-8).

During the enrichment of *Artemia* nauplii, DHA mainly increase in triacyl-*sn*-glycerols (TAG) of the nauplii (7-10). Recently, it was reported that TAG of the

enriched *Artemia* nauplii contained DHA in the *sn*-3 position at a concentration much higher than those in the *sn*-1 and *sn*-2 positions (11,12). This distribution pattern was different from that of marine zooplankton that may form natural diet of marine fish (13). However, previous studies were limited to the nauplii enriched with purified fish oil TAG (11,14) or fatty acid ethyl esters (EE) (11,12,15).

In the present study, *Artemia* nauplii enriched with two emulsions containing fish roe polar lipids (PL), (i) bonito head oil TAG mixed with salmon roe PL concentrate and (ii) unmixed salmon roe PL concentrate. McEvoy *et al.* (16) reported that the use of herring roe PL along with DHA-rich tuna orbital oil has advantage

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of nutritionally favorable HUFA valance as well as of their nature such as emulsifier and antioxidant. The aim of the present paper was to supply new data on positional distribution of n-3 HUFA in TAG of the enriched *Artemia* nauplii and to discuss whether the fish roe PL practically affect the distribution pattern of DHA in *Artemia* nauplii TAG.

2 Experimental

2.1 Fish Roe PL and Fish Oil TAG Used for Enrichment

Fish roe PL used for the *Artemia* enrichment were extracted from salmon roe ("sujiko" in Japanese) and concentrated by a solvent partition procedure using hexane, ethanol and water (17). DHA-rich fish oil TAG were isolated from industrial bonito head oil by column chromatography on Silicagel 60 (Merck, Darmstadt, Germany) with hexane/diethyl ether for elution (18). Fatty acid compositions of the PL concentrate and TAG are shown in **Table 1** together with major lipid classes in the PL concentrate. Preparation procedure and detailed lipid profile of these lipids were described in our previous papers (17,18).

Artemia nauplii were enriched with the bonito head oil TAG mixed with salmon roe PL concentrate (88:12, wt/wt) (TAG + PL) or unmixed salmon roe PL concentrate. The proportions of TAG and PL in the former were due to the experiment of McEvoy *et al.* (16), where herring roe PL (12 wt%) were mixed to tuna orbital oil (88 wt%). For the TAG + PL enrichment, the bonito head oil TAG (0.88 g) and salmon roe PL concentrate (0.12 g) were homogenized with 40 ml of distilled water by two-blade blender at 20,000 rpm for 1 min, and for the PL enrichment, the salmon roe PL concentrate (1.0 g) was homogenized in the similar manner. Resulting emulsions were immediately used for *Artemia* enrichment.

2.2 Enrichment of *Artemia* Nauplii

Enrichment was carried out with 24-h-old nauplii of *Artemia franciscana* (INVE, Mountain Green, UT) at a density of 100 nauplii/mL in a 10-L tank containing 10 L of well-aerated 20 ‰ salinity artificial seawater at 20 °C (11). The TAG + PL or PL emulsions were added to the tank at the lipid concentration of 100 mg/L. Enriched *Artemia* nauplii samples (approximately 2 L of the rearing seawater) were collected on a nylon mesh

at 4, 18 and 24 h, washed in distilled water, and stored at −30°C prior to lipid analysis within 24 h. Initial samples (0 h) were also collected just before the start of the enrichment. The 24-h enrichment was carried out in triplicate (n = 3), and each sample was subjected to lipid analysis.

2.3 Lipid Analysis

Lipid analysis of the *Artemia* nauplii was carried out by the procedure previously applied to the analysis of *Artemia* nauplii (11,12) and rotifers (19). Total lipids (TL) were extracted by the method of Bligh and Dyer (20). TAG were separated from other lipids by preparative thin-layer chromatography on Silicagel 60G plates (0.5 mm thickness; Merck). Fatty acid methyl esters were prepared from the TAG by transesterification on sodium methoxide/methanol catalyst, and analyzed by gas-liquid chromatography on an Omegawax 320 capil-

Table 1 Fatty Acid Composition of Salmon Roe Polar Lipids (PL) Concentrate and Bonito Head Oil Triacyl-*sn*-glycerols (TAG) Used for Enrichment of *Artemia* Nauplii (Mol%).

Fatty acid	Salmon roe PL conc. ^{a)}	Bonito head oil TAG ^{b)}
14:0	1.1	3.2
16:0	14.2	12.3
iso-17:0	1.9	7.2
16:2n-4+n-3	0.2	1.9
16:3n-4	—	1.0
18:0	12.1	1.8
18:1n-9	9.6	15.8
18:1n-7	3.6	2.8
18:2n-6	1.7	2.4
18:4n-3	0.1	1.1
20:1n-9	1.2	1.0
20:4n-6	2.4	2.1
20:5n-3	14.2	8.1
22:5n-6	—	1.6
22:5n-3	3.6	1.5
22:6n-3	26.9	29.3
Others (<1%)	5.6	6.9

^{a)} Reported in reference (17). Lipid classes reported were phosphatidylcholines (80%), phosphatidylethanolamines (7%), phosphatidylinositols (6%), sterols (3%), free fatty acids (3%), and monoacylglycerols (1%).

^{b)} Reported in reference (18).

lary column (Supelco, Bellefonte, PA) and a flame ionization detector. Column temperature was programmed from 180 to 240°C (1°C/min).

Positional distribution of fatty acids in the TAG was also determined by stereospecific analysis previously described (11,12,19). The TAG (5 mg) were partially degraded with ethyl magnesium bromide, and then the resulting 1-, 2- and 3-monoacyl-*sn*-glycerols (MAG) were isolated by chiral-phase high performance liquid chromatography (HPLC) as their bis-3,5-dinitrophenylurethane derivatives. A column of Sumichiral OA-4100 (Sumitomo Chemical, Osaka, Japan) was used for isolation of *sn*-1- plus *sn*-2-MAG and *sn*-3-MAG fractions, and then one of Sumichiral OA-4000 for separation of *sn*-1-MAG and *sn*-2-MAG fractions. Both HPLC separations were done with hexane/dichloromethane/ethanol (40:12:3, v/v/v) as mobile phase at -10°C. Constituent

fatty acids of each fraction were directly analyzed by reversed-phase HPLC on a Capcellpak C18 UG120 S-3 column (Shiseido, Tokyo, Japan) with a linear gradient elution system of 70% acetonitrile/30% water to 100% acetonitrile generated over 90 min. Fatty acid compositions of the *sn*-1, *sn*-2 and *sn*-3 positions were calculated from the results.

3 Results and Discussion

3.1 TAG Content and Fatty Acid Composition of TAG

Table 2 shows the TL and TAG contents and fatty acid composition of TAG in the *Artemia* nauplii. The content of TAG was 6.1% (initial), 7.3-10.4% (TAG + PL enrichment) and 3.3-6.1% (PL enrichment) on dry weight base. The TAG content increased in the nauplii

Table 2 Contents of Total Lipids (TL) and Triacyl-*sn*-glycerols (TAG) and Fatty Acid Composition of TAG in the Enriched *Artemia* Nauplii.

	Initial	Enriched with TAG+PL ^{a)}			Enriched with PL ^{b)}		
	0 h (n=4)	4 h (n=1)	18 h (n=1)	24 h (n=3)	4 h (n=1)	18 h (n=1)	24 h (n=3)
Content (% dry weight base)							
TL	15.6 ± 1.3	17.7	21.8	17.8 ± 2.1	16.6	14.4	12.6 ± 0.6
TAG	6.1 ± 0.9	7.3	10.4	7.7 ± 1.0	6.1	4.7	3.3 ± 0.2
Fatty acid composition of TAG (Mol%)							
14:0	0.7 ± 0.6	1.3	1.0	1.0 ± 0.0	1.3	1.3	1.3 ± 0.0
iso-15:0	1.7 ± 0.1	1.4	0.9	0.9 ± 0.0	1.7	1.7	1.7 ± 0.1
16:0	12.6 ± 0.3	11.8	10.5	10.6 ± 0.2	12.4	13.0	13.0 ± 0.3
16:1n-9	1.4 ± 0.1	1.2	1.1	1.0 ± 0.1	1.4	1.3	1.3 ± 0.1
16:1n-7	6.6 ± 0.1	6.8	5.7	5.3 ± 0.2	6.5	6.2	5.7 ± 0.3
16:2n-6	1.1 ± 0.0	1.0	0.8	0.7 ± 0.0	1.1	1.2	1.2 ± 0.0
17:1n-8	1.1 ± 0.0	1.1	1.0	1.0 ± 0.0	1.1	1.0	1.0 ± 0.0
18:0	2.8 ± 0.1	2.6	2.9	3.3 ± 0.2	2.8	3.5	4.1 ± 0.4
18:1n-9	27.0 ± 0.6	26.4	26.3	23.9 ± 0.7	26.2	27.3	26.5 ± 0.2
18:1n-7	5.6 ± 0.0	5.4	5.1	5.1 ± 0.1	5.5	5.6	5.4 ± 0.2
18:2n-6	4.4 ± 0.1	4.2	3.7	3.6 ± 0.1	4.4	4.2	4.0 ± 0.1
18:3n-3	21.1 ± 0.4	17.6	13.3	13.0 ± 0.1	22.2	20.4	19.4 ± 1.2
18:4n-3	2.1 ± 0.1	1.9	1.4	1.3 ± 0.1	2.3	1.7	1.5 ± 0.2
20:4n-6	0.5 ± 0.0	0.9	1.5	1.1 ± 1.0	0.6	0.6	0.8 ± 0.1
20:5n-3	1.8 ± 0.0	3.3	3.4	7.1 ± 0.3	2.0	2.2	2.7 ± 0.5
22:6n-3	0.0 ± 0.0	4.5	11.4	11.3 ± 0.9	0.3	0.5	1.0 ± 0.4
Others (<1%)	9.4	8.6	10.0	9.9	8.2	8.2	9.4

Mean ± standard deviation (n=3 or 4).

^{a)} Bonito head oil TAG + salmon roe polar lipids concentrate (88:12, wt/wt).

^{b)} Salmon roe polar lipids concentrate (100%).

enriched with TAG + PL, whereas decrease of it was observed for the PL enrichment. In accordance with the changes in TAG content, the TL content also changed.

Concentrations of DHA (22:6n-3) and IPA (20:5n-3) increased in the TAG during both enrichments. With the TAG + PL enrichment, DHA and IPA increased from 0.0 and 1.8 mol% (0 h) to 11.3 and 7.1 mol% (24 h), respectively. In contrast, remarkable decrease of 18:3n-3 was observed during this enrichment (from 21.1 to 13.0 mol%). With the PL enrichment, DHA and IPA increased to 1.0 and 2.7 mol%, respectively, although these levels were not very higher than those of the initial nauplii (0 h).

3·2 Positional Distribution of Fatty Acids in TAG

Table 3 shows the positional distribution of fatty acids in TAG of the *Artemia* nauplii. Under the analytical conditions used in this study, IPA was not clearly resolved from 16:2n-6. However, content of 16:2n-6 was lower than that of IPA in all of the enriched *Artemia* nauplii (**Table 2**). DHA was separated from other major components.

DHA in each position increased in both of the enrichments. In the *Artemia* nauplii enriched with TAG + PL, concentrations of DHA in the *sn*-1, *sn*-2 and *sn*-3 positions were 1.7, 3.4 and 8.9 mol% (4 h), 4.6, 6.9 and 22.9 mol% (18 h), and 4.5, 7.5 and 22.9 mol% (24 h), respectively. In all of the enrichment periods, DHA was esterified in the *sn*-3 position in much preference to the *sn*-1 and *sn*-2 positions. Between the latter two positions, DHA was higher in the *sn*-2 position. With the PL enrichment, this fatty acid was found in the *sn*-1, *sn*-2 and *sn*-3 positions at the concentrations of up to 0.3, 0.6 and 2.3 mol% (24 h), respectively. Distribution pattern of DHA in this enrichment was similar to that observed for the TAG + PL enrichment.

IPA with inseparable 16:2n-6 was found in the *sn*-1, *sn*-2 and *sn*-3 positions at concentrations of 4.2, 11.2 and 8.2 mole% (TAG + PL, 24 h), and 1.5, 4.8 and 5.2 mole% (PL, 24 h), respectively. With the TAG + PL enrichment (24 h), IPA content in TAG was 10-fold higher than that of 16:2n-6 (**Table 2**; 7.1 mol% IPA versus 0.7 mol% 16:2n-6). IPA in this *Artemia* nauplii sample seems to be preferentially esterified in the *sn*-2 position followed by the *sn*-3 and *sn*-1 positions.

The most prominent polyunsaturated fatty acid in the initial nauplii, 18:3n-3, was very high in the *sn*-2 posi-

tion (30.8-53.4 mol%) in both of the initial and enriched *Artemia* nauplii. Distribution pattern of DHA in the enriched nauplii was not similar to that of 18:3n-3.

3·3 Comparison with Previous Data on DHA Distribution

Recent papers reported the positional distribution of DHA in *Artemia* nauplii enriched with fish oil TAG or fatty acid EE (11,12,14,15). When TAG of the enriched *Artemia* nauplii were subjected to regiospecific analysis in order to differentiate the *sn*-1,3 and *sn*-2 positions, DHA was observed in the *sn*-1,3 position higher than in the *sn*-2 position (14,15). When TAG were subjected to stereospecific analysis, this fatty acid appeared to be esterified in the *sn*-3 position in preference to the *sn*-1 and *sn*-2 positions (11,12). The TAG of *Artemia* nauplii enriched with bonito head oil TAG (24 h) contained DHA in the *sn*-1, *sn*-2 and *sn*-3 positions at the concentrations of 7.3, 10.4 and 35.0 mol%, respectively (11). Similar distribution pattern was found for shorter period enrichment, i.e. 4-h enrichment, 1.5, 2.4 and 7.6 mol% and 18-h enrichment, 5.8, 8.9 and 31.4 mol% in the *sn*-1, *sn*-2 and *sn*-3 positions, respectively (11).

In the present study, *Artemia* nauplii were enriched with bonito head oil TAG mixed with salmon roe PL concentrate. The bonito head oil TAG were the same one used in the previous study (11). The enrichment of *Artemia* nauplii was also carried out under the same conditions except for no use of gelatin as an emulsifier of TAG. DHA in the enriched *Artemia* nauplii appeared to be preferentially esterified in the *sn*-3 position followed by the *sn*-2 and *sn*-1 positions. This result was similar to the distribution pattern found in the previous enrichment without salmon roe PL. Therefore, the salmon roe PL concentrate added to bonito head oil TAG does not seem to affect the positional distribution pattern of DHA in the enriched *Artemia* nauplii.

Phospholipids have no fatty acid in the *sn*-3 position. DHA in fish roe phosphatidylcholines, major lipid class in fish roe PL, is generally esterified in the *sn*-2 position of their glycerol backbones (1). However, it is not probable that this distribution pattern hold for TAG of the enriched *Artemia* nauplii. In the present study, unmixed salmon roe PL concentrate was also used for the enrichment of *Artemia* nauplii. High preference of DHA in the *sn*-3 position was also found in this enrichment.

3·4 DHA Distribution Characteristic of *Artemia* Nauplii

Previously, McEvoy *et al.* (16) enriched *Artemia* nauplii with tuna orbital oil mixed with herring roe PL (88:12, wt/wt). After 18-h enrichment, DHA and IPA contents found in the enriched nauplii were 19.4 and

10.8 mg/g (dry weight base), respectively, corresponding to 14.1 and 7.8 % of total fatty acids. The ratio of DHA/IPA (1.8) was similar to those (approximately 2.0) found in yolk of wild fish eggs and in copepods, natural prey of most marine fish larvae (1,3,16). Based on the results of **Table 2**, DHA and IPA in the *Artemia* nauplii enriched with TAG + PL were calculated to be

Table 3 Positional Distribution of Fatty Acids in Triacyl-*sn*-glycerols (TAG) of the Enriched *Artemia* Nauplii (Mol%).

		14:0	16:0	16:1n-7 +20:4n-6	18:0 +20:1	18:1	18:2n-6	18:3n-3	20:5n-3 +16:2n-6	22:6n-3	Others
Initial <i>Artemia</i> nauplii											
0 h (n=4)	<i>sn</i> -1	1.0 ± 0.2	23.7 ± 2.2	5.3 ± 1.2	4.8 ± 1.9	45.5 ± 3.2	2.5 ± 0.4	5.6 ± 2.8	1.2 ± 0.3	0.0 ± 0.0	10.5
	<i>sn</i> -2	0.2 ± 0.1	4.4 ± 2.9	7.5 ± 1.5	1.3 ± 1.9	19.3 ± 1.8	6.8 ± 1.4	47.4 ± 7.5	2.8 ± 1.7	0.0 ± 0.0	10.4
	<i>sn</i> -3	2.7 ± 0.2	9.5 ± 0.8	8.6 ± 0.5	4.2 ± 0.7	32.7 ± 1.3	4.0 ± 1.4	11.6 ± 3.3	4.7 ± 1.3	0.1 ± 0.1	22.0
	Total	1.3 ± 0.1	12.6 ± 0.2	7.1 ± 0.1	3.5 ± 0.3	32.6 ± 0.4	4.4 ± 0.1	21.0 ± 0.5	2.9 ± 0.1	0.0 ± 0.0	14.6
Enriched with TAG+PL ^{a)}											
4 h (n=1)	<i>sn</i> -1	0.8	20.9	5.1	3.7	45.2	2.3	3.9	2.3	1.7	14.1
	<i>sn</i> -2	0.5	5.0	8.9	0.3	18.5	5.3	41.3	5.4	3.4	11.4
	<i>sn</i> -3	2.6	8.9	9.0	6.8	31.0	5.2	7.6	5.3	8.9	14.7
	Total	1.3	11.8	7.6	3.5	31.7	4.2	17.7	4.3	4.5	13.4
18 h (n=1)	<i>sn</i> -1	0.7	16.2	5.4	6.5	48.3	2.5	3.7	2.5	4.6	9.8
	<i>sn</i> -2	0.7	9.1	8.7	0.9	19.7	4.9	31.9	5.3	6.9	12.1
	<i>sn</i> -3	1.7	6.1	7.7	4.4	25.0	3.7	5.4	4.8	22.9	18.3
	Total	1.0	10.5	7.2	4.0	31.4	3.7	13.3	4.2	11.4	13.3
24 h (n=3)	<i>sn</i> -1	0.7 ± 0.1	15.7 ± 0.3	4.8 ± 0.4	7.6 ± 0.4	45.4 ± 1.4	2.5 ± 0.5	3.2 ± 0.1	4.2 ± 0.5	4.5 ± 0.6	11.4
	<i>sn</i> -2	0.6 ± 0.1	9.6 ± 0.5	7.7 ± 1.0	1.0 ± 0.0	16.9 ± 0.5	5.0 ± 0.0	30.8 ± 0.4	11.2 ± 0.3	7.5 ± 0.6	9.7
	<i>sn</i> -3	1.6 ± 0.1	6.1 ± 0.1	6.9 ± 1.0	4.5 ± 0.3	23.8 ± 0.7	3.2 ± 0.3	4.8 ± 0.1	8.2 ± 0.3	22.9 ± 2.4	18.1
	Total	1.0 ± 0.0	10.6 ± 0.2	6.4 ± 0.8	4.4 ± 0.2	29.0 ± 0.8	3.6 ± 0.1	13.0 ± 0.1	7.8 ± 0.3	11.3 ± 0.9	12.9
Enriched with PL ^{b)}											
4 h (n=1)	<i>sn</i> -1	0.9	25.5	4.8	6.3	45.1	3.5	4.4	1.0	0.0	8.5
	<i>sn</i> -2	0.0	2.5	8.0	0.2	17.9	8.0	53.4	4.4	0.1	5.5
	<i>sn</i> -3	3.1	9.4	8.5	4.2	32.0	1.8	9.2	3.9	0.7	27.2
	Total	1.3	12.4	7.1	3.6	31.7	4.4	22.2	3.1	0.3	13.9
18 h (n=1)	<i>sn</i> -1	0.9	26.3	4.3	7.6	45.1	2.3	3.2	0.9	0.0	9.5
	<i>sn</i> -2	0.0	3.1	7.8	0.5	20.3	8.9	48.0	4.8	0.2	6.4
	<i>sn</i> -3	3.2	10.2	8.2	5.3	34.2	1.3	8.2	4.3	1.4	23.7
	Total	1.3	13.0	6.8	4.4	32.9	4.2	20.4	3.4	0.5	13.1
24 h (n=3)	<i>sn</i> -1	0.9 ± 0.1	23.3 ± 1.9	4.1 ± 0.5	8.2 ± 1.4	40.9 ± 1.3	3.0 ± 0.6	6.1 ± 3.4	1.5 ± 0.3	0.3 ± 0.3	11.7
	<i>sn</i> -2	0.7 ± 0.4	5.2 ± 1.9	8.0 ± 0.3	1.0 ± 0.8	21.1 ± 1.5	5.6 ± 0.7	40.3 ± 5.5	4.8 ± 1.2	0.6 ± 0.1	12.7
	<i>sn</i> -3	2.5 ± 0.5	10.2 ± 0.9	7.5 ± 0.0	5.8 ± 0.7	33.8 ± 0.5	3.6 ± 1.5	11.6 ± 5.9	5.2 ± 0.4	2.3 ± 0.9	17.5
	Total	1.3 ± 0.0	13.0 ± 0.3	6.5 ± 0.2	5.0 ± 0.4	32.0 ± 0.1	4.0 ± 0.1	19.4 ± 1.2	3.8 ± 0.5	1.0 ± 0.4	13.9

Mean ± standard deviation (n=3 or 4).

^{a)} Bonito head oil TAG + salmon roe polar lipids concentrate (88:12, wt/wt).

^{b)} Salmon roe polar lipids concentrate (100 %).

11.9 and 3.5 mg/g (18 h) and 8.7 and 5.5 mg/g (24 h), respectively. These levels were about one half of those reported (16), probably resulting from differences not only in lipid classes analyzed (TL *versus* TAG) but also in enrichment protocols, e.g., age of the initial nauplii, temperature of the rearing seawater, dose of enricher, and the number of dosing times. However, the DHA/IPA ratio around 2.0 could be achieved in the present study. When calculated from **Table 2**, the ratio was 3.4 (18 h) and 1.6 (24 h).

The *Artemia* nauplii enriched with the herring roe PL-containing tuna orbital oil were recognized to be nutritionally closer to wild prey than those enriched with other diet (1,3,16). The enriched *Artemia* nauplii led to satisfactory (though not necessary optimal) survival, growth, pigmentation and metamorphosis of turbot larvae (3). On the other hand, including the present study, there have been no data showing that DHA is preferentially esterified in the *sn*-2 position of *Artemia* TAG. This is opposite to TAG of natural marine fish eggs or marine zooplankton where DHA is highest in the *sn*-2 position (13). Even though fish roe PL rich in DHA in the *sn*-2 position are used as a part of HUFA enricher, *Artemia* nauplii seem to incorporate DHA into the *sn*-3 position of their TAG. Therefore, positional distribution of DHA in TAG of *Artemia* nauplii is strongly characterized by high preference in the *sn*-3 position to the other two positions.

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