Lipid and Fatty Acids of Three Edible Myctophids, Diaphus watasei, Diaphus suborbitalis, and Benthosema pterotum: High Levels of Icosapentaenoic and Docosahexaenoic Acids

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Abstract: The fatty acid compositions of the total lipids of three edible deep-sea fishes, Diaphus watasei, Diaphus suborbitalis, and Benthosema pterotum, were compared with those of a highly migratory fish, Katsuwonus pelamis, to clarify their lipid characteristics and nutritional value as seafood. The mean lipid contents in the three myctophids were markedly higher than that of K. pelamis. All three myctophids had medium levels of 20:5n-3 (icosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA) in their lipids, similar to those in surface pelagic fishes. The actual EPA and DHA amounts of the three myctophid fishes were higher than were those of K. pelamis. Therefore, the nutritional values of the myctophids as source for EPA and DHA are better than that of K. pelamis, and this finding may introduce a new resource of healthy marine food from these under-utilized deep-sea fish species.

Key words: deep-sea fish, docosahexaenoic acid, fish lipid, icosapentaenoic acid, myctophid, polyunsaturated fatty acid

1 Introduction

Lanternfish belongs to the family Myctophidae, Myctophiformes, which is one of the most widespread and plentiful of deep-sea families, similar to typical deep-sea pelagic families, such as bristlemouth (Gonostomatidae, Stomiiformes) and ridgeheads (Melampidae, Stenoberyciformes). Myctophids are widespread, occurring abundantly in boreal to tropical meso- and bathypelagic depths (200–2,000 m) worldwide. They feed primarily on zooplankton, such as copepods, euphausiids, and other small pelagic crustaceans, while they are prey to oceanic dolphins and seals, and large pelagic animals. In the northwestern Pacific Ocean, three edible deep-sea fishes, Diaphus watasei, Diaphus suborbitalis, and Benthosema pterotum, mostly distribute in temperate to tropical zone.

It is generally known that all marine fishes characteristically amass various sorts of long-chain n-3 polyunsaturated fatty acids (PUFA) in their lipids, such as docosahexaenoic acid (DHA, 22:6n-3) and icosapentaenoic acid (EPA, 20:5n-3). There are much attention for the lipids and fatty acids of seawater fishes, with growing recognition of the beneficial uses of dietary fish oils for humans. However, compared with many reports on the fatty acid determination of pelagic surface animal species, little information has been available on the detailed lipid class and fatty acid compositions of deep-sea fish species (13 species of Myctophids). Despite a great deal of attention being paid to the ecological and biological aspects of myctophids in the northwestern Pacific Ocean (for Benthosema pterotum; for D. watasei and Diaphus suborbitalis etc.; for B. pterotum; for B. watasei etc.), few reports of studies of the chemical components of the flesh of myctophids for the nutritive value have been published (for 22 species of myctophids; for 13 myctophid species; for 3 myctophid species; for 2 myctophid species). Furthermore, only a few papers (for B. pterotum) have presented analyses.

Abbreviations: DHA, docosahexaenoic acid; DMOX, 4,4-dimethyloxazolone; EPA, icosapentaenoic acid; GC/MS, gas chromatography/mass spectrometry; MUFA, monounsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acids; TAG, triacylglycerols; TFA, total fatty acids; TL, total lipids.
of the fatty acid composition of the three edible myctophids, *D. watasei* (Watase's lanternfish), *D. suborbitalis*, and *B. pterotum* (Skinnycheek lanternfish).

In order to clarify their lipid characteristics and to introduce a new resource of healthy marine food rich in n-3 PUFA in preparation for a future population explosion, detailed analyses of fatty acid compositions of the three edible myctophids (*Diaphus watasei* Jordan and Starks, *Diaphus suborbitalis* Weber, and *Benthosema pterotum* Alcock) were investigated in the present study, compared with that of skipjack tuna, *Katsuwonus pelamis* Linnaeus.

### 2 Material and methods

#### 2.1 Materials

The biological data of three myctophids, *D. watasei*, *D. suborbitalis*, and *B. pterotum*, are listed in Table 1. Three types of myctophids (samples 1–11) were collected from October 2011 to November 2012 in Suruga Bay and the Sea of Enshu Nada on Honshu Island in Japan. *K. pelamis* (sample 12) came from the Pacific Ocean (off Sanriku coast) and were purchased at a recognized commercial wholesale market in Yokohama ("Nanbu wholesale market") in September 2011. After their biological data were determined, the specimens were immediately frozen at −40°C.

#### 2.2 Lipid extraction and analysis of lipid classes

The whole body of each individual of the two myctophids (*D. suborbitalis*, and *B. pterotum*) and the ordinary muscle of *D. watasei* and *K. pelamis* were homogenized in a mixture of chloroform and methanol (2:1, v/v, Table 2), and a portion of each homogenized sample was extracted according to the Folch procedure.[21] The extracted crude lipids of the three myctophids were separated into neutral and polar lipids by Sep-Pak silica cartridges (Waters, Milford, MA, USA). Neutral lipids were separated using thin-layer chromatography (TLC) on Silica gel 60 plates (Merck) with hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as the developing solvent. Spots were visualized by spraying copper acetate/phosphoric acid solution and successive heating at 130°C for 20 min. The chromatograph was scanned with public domain software (Scion Image, Scion, USA).[22]

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Date</th>
<th>Locality</th>
<th>Replicate animals</th>
<th>Length (mm)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>October 13, 2011</td>
<td>Enshu Nada</td>
<td>3</td>
<td>8.6 ± 0.7</td>
<td>7.9 ± 2.1</td>
</tr>
<tr>
<td>1</td>
<td>November 30, 2011</td>
<td>Suruga Bay</td>
<td>4</td>
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</tr>
<tr>
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<td>March 22, 2012</td>
<td>Suruga Bay</td>
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<td>12.8 ± 0.7</td>
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<td>Suruga Bay</td>
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<td>September 10, 2012</td>
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</tr>
<tr>
<td>2</td>
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<td>Suruga Bay</td>
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<td>5.7 ± 0.2</td>
<td>2.5 ± 0.3</td>
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<td>4</td>
<td>September 20, 2011</td>
<td>Off Sanriku coast</td>
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<td>3552.0 ± 102.5</td>
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Lipid and Fatty Acids of Three Edible Myctophids, Diaphus watasei, Diaphus suborbitalis, and Benthosema pterotum: High Levels of Icosapentaenoic and Docosahexaenoic Acids


Table 2 Lipid classes of whole body of the three myctophids and ordinary muscles in Katsuwonus pelamis.

<table>
<thead>
<tr>
<th>Lipid classes</th>
<th>Sample No.</th>
<th>Replications*</th>
<th>Lipid content for lipids</th>
<th>Replications*</th>
<th>Lipid classes</th>
<th>Wax esters</th>
<th>Sterol esters</th>
<th>Diacylglycerol esters</th>
<th>TAG*</th>
<th>Sterols</th>
<th>Diacyl glycerol</th>
<th>Free fatty acids</th>
<th>PE**</th>
<th>Other minor phospholipids</th>
<th>PC**</th>
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<td></td>
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<td>7.5 ± 1.9</td>
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<td>2.8 ± 0.2</td>
<td>12.3 ± 0.2</td>
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<td>D. suborbitalis</td>
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<tr>
<td>B. pterotum</td>
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<td>9.2 ± 0.7</td>
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<td>68.8 ± 0.8</td>
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<td>19.4 ± 0.5</td>
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<tr>
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<td>4.2 ± 0.9</td>
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<td>1.9 ± 0.2</td>
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<td>1.9 ± 0.2</td>
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<td>3.6 ± 0.2</td>
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<td>1.8 ± 0.4</td>
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<td>7.8 ± 0.2</td>
<td>2.5 ± 0.1</td>
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<td>7.8 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>10.6 ± 0.3</td>
<td>27.1 ± 0.6</td>
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<td>Katsuwonus pelamis</td>
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<td>2.0 ± 0.2</td>
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<td>0.0 ± 0.0</td>
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<td>61.3 ± 1.7</td>
<td>4.1 ± 0.4</td>
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<td>2.9 ± 0.2</td>
<td>4.3 ± 1.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Sources are expressed as weight percent of total lipids (n = 4-6).

b TAG, PE, and PC (a) myctophid, (b) Katsuwonus pelamis, and phosphatidylcholine, respectively.
c Wax ester fraction and PE fraction of the three myctophids contain all other neutral and polar lipids, respectively.

d Individual lipids from each lipid class, such as the phospholipid class, were qualitatively identified with standards by comparing the Rf values using thin-layer chromatography (Merck and Co. Ltd., Kieselgel 60, thickness of 0.25 mm for analysis). All sample lipids were dried under argon at room temperature and stored at −40°C under argon.

2.3 Nuclear magnetic resonance (NMR) spectroscopy and determination of lipid classes
Spectra were recorded on a GSX-270 NMR spectrometer (JEOL Co. Ltd., Tokyo, Japan) in the pulsed Fourier transform mode at 270 MHz in deuteriochloroform solution using tetramethylsilane as an internal standard.

2.4 Preparation of methyl esters and gas-liquid chromatography (GLC) of esters
Each crude lipid of three myctophids was converted to fatty acid methyl esters by direct transesterification with methanol containing 1% concentrated hydrochloric acid for 2.5 hr. Individual components of TAG, PE, and PC fractions of K. pelamis were converted to fatty acid methyl esters by direct transesterification with methanol containing 1% concentrated hydrochloric acid under reflux for 1.5 hr, as previously reported, to produce the fatty acid methyl esters.

The composition of the fatty acid methyl esters was determined by gas-liquid chromatography (Table 3). Analysis was performed on HP-6890 (Hewlett Packard Co. Ltd., Yokogawa Electric Corporation, Tokyo, Japan) gas chromatographs equipped with an Omegawax-250 fused silica capillary column (30 m × 0.25 mm i. d.; 0.25 µm film, Supelco Japan Co. Ltd., Tokyo, Japan). The temperatures of the injector, the FID detector, and the column were held at 230, 240, and 215°C, respectively, and the split ratio was 1:76. Helium was used as the carrier gas at a constant inlet rate of 0.7 mL/min. Quantitation of individual components was performed by means of HP ChemStation System (A. 06 revision, Yokogawa HP Co. Ltd., Tokyo, Japan) electronic integrators.

2.5 Preparation of 4,4-dimethyloxazoline derivatives (DMOX) and analysis of DMOX by gas chromatography-mass spectrometry (GC/MS)
The DMOX derivatives were prepared by adding an excess amount of 2-amino-2-methyl propanol to a small amount of fatty acid methyl esters in a test tube under an argon atmosphere. The mixture was heated at 180°C for 18 hr. Analysis of the DMOX derivatives was performed by a HP G1800C GCD Series II (Hewlett Packard Co., Yokogawa Electric Corporation, Tokyo, Japan) GC/MS equipped with the same capillary used for determining the respective fatty acids with the HP WS (HP Kayak XA, G1701BA version, PC workstations). The temperatures of the injector and the column were held at 230 and 215°C, respectively. The split ratio was 1:76, and the ionization voltage was 70 eV. Helium was used as the carrier gas at a constant inlet rate of 0.7 mL/min. The fatty acid methyl esters were identified by comparing the methyl ester and DMOX derivative mass spectral data obtained by GC/MS. The DMOX derivatives were identified by comparing the mass spectral data obtained with authentic samples.

2.6 Structural elucidation of the DMOX derivatives of the fatty acids in myctophid lipids
Representative chromatograms of the DMOX derivatives of the B. pterotum lipids and the MS charts of the major fatty acids are displayed in Figs. 2-4 with the chromatogram of DMOX derivatives of the myctophid lipids (Fig. 1). Each MS spectrum was obtained every 0.009 min (Figs. 2-4).
For example, a spectrum at 42.697 min (Scan No. 5512) in Fig. 3 is one of the representative spectra of 22:5\(\Delta_4,7,10,13,16\)-22:5 because 22:5\(\Delta_4,7,10,13,16\)-22:5 was detected near 42.67 min as one peak in the chromatogram (Fig. 1). More than 150 spectra \(M_{-383}\) were obtained by scanning the peak \(41.5-43.0\) min. In Fig. 3 (42.697 min, Scan No. 5512), MS peaks of a DMOX derivative of 20:5\(\Delta_4,7,10,13,16\)-22:5 are \(M_{-383}, 368, 354, 340, 326, 312, 298, 286, 272, 258, 246, 232, 218, 206, 192, 178, 166, 152, 138, 126,\) and 113 (base peak), and six pairs of the peaks (M-383/M-368, M-368/M-354, M-354/M-340, M-340/M-326, M-326/M-312, M-312/M-298, M-298/M-286) are respectively reflected by 10 double bonds: \(\Delta_4\) (n-6), \(\Delta_7\) (n-9), \(\Delta_10\) (n-12), \(\Delta_13\) (n-15), and \(\Delta_14\) (n-18).

2.7 Statistical analyses

More than two experimental replications \((n=2-4)\) in Table 2 of lipid classes of samples were completed for each lipid class. For all samples of fatty acid determination by gas-liquid chromatography, more than two replications \((n=2-8)\) in Table 3 were made. Significant mean differences were determined using a one-way analysis of variance (ANOVA). Tukey’s multiple procedure was used to compare the differences among mean values. Differences were regarded as significance level of \(p<0.05\).
Fig. 1 The chromatogram of the DMOX derivatives of the fatty acids in *B. pterotum* lipids. Analysis of the DMOX derivatives was performed on a HP G1800C GCD Series II gas chromatograph mass spectrometer and a 6890N Network GC-System (5973N Mass Selective Detector, Agilent Technology Co., Yokogawa Electric Corporation) equipped with the same capillary columns for determining the fatty acids with an HP WS (HP Kayak XA, G1701BA version, PC workstations). The temperatures of the injector and the column were held at 230 and 215°C, respectively. The split ratio was 1:75, and the ionization voltage was 70 eV, respectively. Helium was used as the carrier gas at a constant inlet rate of 0.7 mL/min. Each MS spectrum was obtained by every 0.009 min.

Fig. 2 The MS peaks (16.965 min, Scan No. 1128) of a DMOX derivative of 20:1n-11 (Δ9-20:1) are M+·363, 348, 334, 320, 306, 292, 278, 264, 250, 236, 222, 208, 196, 182, 168, 154, 140, 126, and 113 (base peak) are respectively reflected by a double bond: Δ-9 (n-11).
Fig. 3 The MS peaks (42.697 min, Scan No. 5512) of the DMOX derivative of 22:5n-6 (Δ4,7,10,13,16-22:5) are M-383, 368, 354, 340, 326, 312, 298, 286, 272, 258, 246, 232, 218, 206, 192, 178, 166, 152, 138, 126, 113 (base peak) and four pairs of the peaks (M-298/M-286, M-258/M-246, M-218/M-206, M-178/M-166, and M-138/M-126) are respectively reflected by five double bonds: Δ-16 (n-6), Δ-13 (n-9), Δ-10 (n-12), Δ-7 (n-15), and Δ-4 (n-18).

Fig. 4 The MS peaks (52.166 min, Scan No. 6797) of the DMOX derivative of 22:6n-3 (Δ4,7,10,13,16,19-22:6) are M-381, 366, 352, 338, 326, 312, 298, 286, 272, 258, 246, 232, 218, 206, 192, 178, 166, 152, 138, 126, and 113 (base peak), and six pairs of the peaks (M-338/M-326, M-298/M-286, M-258/M-246, M-218/M-206, M-178/M-166, and M-138/M-126) are respectively reflected by six double bonds: Δ-19 (n-3), Δ-16 (n-6), Δ-13 (n-9), Δ-16 (n-6), and Δ-19 (n-3).
3 Results
3.1 Lipid content of the three myctophids and K. pelamis

The biological data of the three myctophid and K. pelamis samples are listed in Table 1 with other relevant data. As shown in Table 2, the lipid contents of the samples (sample Nos. 1-5: 2.7-13.1% for D. watasei, sample Nos. 6-8: 4.3-6.7% for D. suborbitalis, and sample Nos. 9-11: 3.6-9.2% for B. pterotum) were markedly higher than that of the highly migratory fish (sample No. 12: 2.0% for K. pelamis). The lipid class compositions of the three myctophid and K. pelamis are also shown in Table 2. TAG (for D. watasei: 39.0-82.4% of total lipids, TL, for D. suborbitalis: 54.7-64.9% of TL, B. pterotum: 50.2-68.8% of TL, and K. pelamis: 61.3% of TL) was the major component in all four fish lipids. As for other major classes, medium levels of glycerophospholipids were observed (for D. watasei: 12.3-29.7%, for D. suborbitalis: 15.1-24.9%, B. pterotum: 19.4-27.1%, and K. pelamis: 11.2%), while wax esters, sterols and diacylglycerols were found as minor components in all fish lipids. All fish lipids mainly contained glycerol derivatives (TAG, diacylglycerols, glycerophospholipids) and the total proportion of these derivatives reached over 70%.

3.2 Fatty acid composition in the three myctophid and K. pelamis lipids

The major fatty acids in the three myctophid lipids are shown in Tables 3. Seven dominant fatty acids (roughly more than 3% of total fatty acids: TFA) of the samples were found; 14:0, 16:0 and 18:0 as saturated fatty acids, 16:1n-7 and 18:1n-9 as monounsaturated fatty acids (MUFA), 20:5n-3 (EPA) and 22:6n-3 (DHA) as n-3 polyunsaturated fatty acids (PUFA) with noticeable levels (roughly more than 1% of TFA) of six fatty acids; 18:1n-7, 20:1n-9, 22:1n-11, 24:1n-9, 20:4n-6, and 22:5n-3 (docosapentaenoic acid). The same seven dominant fatty acids in the K. pelamis muscle lipids were also found (Table 4); 14:0, 16:0, 18:0, 16:1n-7, 18:1n-9, EPA, and DHA with noticeable levels of similar six fatty acids; 18:1n-7, 20:1n-9, 18:2n-6, 20:4n-6, 22:4n-6, and 22:5n-3. Although very similar kinds of the major fatty acids were found in both the lipids between the three deep-sea fishes and the pelagic surface fish, slightly different from the fatty acid profiles of the myctophids and K. pelamis lipids. Higher levels of DHA in K. pelamis lipids (28.8%) were found, while the myctophids lipids contain its lower levels (6.9-18.5%).

3.3 Actual PUFA levels in the tissues of the three myctophids and K. pelamis

Actual PUFA levels in the fish tissues (mg/100g of tissue) were obtained by multiplying the fatty acid percentage of TFA and the lipid contents. For example, in the October sample of D. watasei (sample No. 1), the numerical value of the mean EPA percentage in the TFA was 4.3% (Table 4), and that of the mean lipid contents was 2.7% (Table 3). Therefore, the actual EPA value in the tissue was 114.5 mg/100 g of the tissue of the March sample of D. watasei. Almost all the actual EPA levels of the three myctophids (95.8-1046.2 mg/100 g of tissue) were higher than was that of K. pelamis (111.6 mg/100 g of tissue), except for sample No. 2 (November samples) of D. watasei (95.8 mg/100 g of tissue). Similarly, the actual DHA levels of the three myctophids (401.4-1393.8 mg/100 g of tissue) were mostly higher than was that of K. pelamis (517.3 mg/100 g of tissue), except for the two cases, according to the lower lipid contents of D. watasei (sample No. 1: 412.1 mg/100 g of tissue of the October sample and sample No. 2: 401.4 mg/100 g of tissue of the November sample).

4 Discussion
4.1 Lipid content of the three myctophids and K. pelamis

The high levels of the lipid contents of the three myctophid (Table 2) are similar to those in other myctophid species. In contrast, the lipid content of K. pelamis was very low, similar to that in other highly migratory fish species, which migrate over wide areas. These findings suggest that myctophids mostly accumulate prey lipids in their body with efficient diel migration. In the neutral lipids, TAG was the major component in all four fish lipids, with low levels of wax esters. This implies that almost all myctophids contain glycerol derivatives similar to surface fish species and that only a few species include wax esters as major lipid components. This phenomenon supports the report of Neighbors, which states that wax esters are not important in deep-sea fishes. Wax esters are probably only one of the depot lipids in deep-sea organisms as well as TAG, and are interchangeable with TAG as an energy source. In contrast, some deep-sea fishes, such as castor oil fishes, have high levels of wax esters and these fishes cause seborrhea and diarrhea (contents of wax esters in TFA, Lepidocycium flavobrunneum: 89.4% and Ruettus pretosus: 89.3%). In polar lipids, medium levels of phospholipids, which are probably important as tissue membrane lipids, were observed in both deep-sea and surface fish species. High levels of TAG in the three edible myctophids, D. watasei, D. suborbitalis, and B. pterotum, show a typical lipid profile of a diel migratory myctophid, similar to those in other species.

4.2 Fatty acid composition in the three myctophids and K. pelamis

In the two Diaphus spp. lipids, higher levels of MUFA
(total MUFA levels: 39.1–53.6% for D. watasei, and 35.1–40.5% for D. suborbitalis in Table 3) were found, while those in the B. pterotum lipids contained lower levels of total MUFA (22.9–27.6%), which is similar to that in K. pelamis (total MUFA levels: 21.1%). In particular, characteristically high levels of long-chain (LC-) MUFA, such as 20:1n-9, 22:1n-11, and 24:1n-9, were found in the two Diaphus spp. lipids. Although the total MUFA levels of the B. pterotum lipids were low, noticeable levels of the LC-MUFA were found in the B. pterotum lipids (0.8–1.3% for 20:1n-9, 0.9–1.5% for 22:1n-11, and 0.8–1.1% for 24:1n-9), similar to those in the two Diaphus spp. lipids (1.9–3.8% for 20:1n-9, 0.5–6.2% for 22:1n-11, and 0.5–1.5% for 24:1n-9). This trend differed from the negligible levels of LC-MUFA in the lipids of K. pelamis (0.5% for 20:1n-9, 0.2% for 22:1n-11, and 0.7% for 24:1n-9). The noticeable levels of LC-MUFA in all three myctophids lipids are similar to those in other deep-sea fishes reported previously²⁷⁻²⁸.

In contrast, higher levels of LC-PUFA, particularly DHA, were only observed in K. pelamis. Similar to the dominant DHA in other highly migratory fishes, such as tuna species (for Thunnus obesus,¹³; for Thunnus thynnus,¹³; for Thunnus albacares,¹¹,²⁹; for Thunnus alalunga,²⁹; for Euthunnus affinis,³⁰; for Thunnus tonggol,¹⁴), DHA was the major fatty acid in the K. pelamis lipids. Similar levels of EPA in both the different types of fishes (2.2–10.8% for the three deep-sea myctophids and 5.1% for the surface fish, K. pelamis) were observed. However, the levels of DHA in the K. pelamis lipids (31.5%) were markedly higher than were those in the three myctophid lipids (6.9–18.5% for the three myctophids). This shows a conclusive difference between surface migratory fishes and other fishes³⁰, in particular deep-sea fishes. K. pelamis may rapidly use MUFA as an energy source during wide and rapid migration and concentrate essential DHA (structural fatty acid) in its muscle, while depot MUFA (storage fatty acids), which is an important energy source for deep-sea existence were accumulated in the myctophids, which efficiently use minimum energy during their vertical migration, similar to other deep-sea fishes³⁰.

4.3 Actual high levels of EPA and DHA in the three myctophids and promising source of healthful marine food

There are many health benefits related to the intake of marine foods rich in n-3 PUFA. In particular, EPA plus DHA are most important elements related to the benefits of marine food consumption, including the prevention of various circulatory diseases, neurodevelopment in infants, and Alzheimer’s disease in aged people¹¹,¹²,³¹. In general, highly migratory fishes, such as K. pelamis, are considered one of the most practical sources of n-3 PUFA. These tuna species have recently been consumed excessively (canned, frozen, and fresh) and the numbers of many tuna species have been reduced by overfishing. Although they have high percentages of DHA in their lipids, the lipid contents and actual PUFA percentages in their tissue are not as high as in their lipids. The actual PUFA levels in fish tissues are important for practical use as food. In the present study, we found that the three edible myctophids are effective seafood sources of PUFA. Except for one case, almost all of the actual EPA levels of the three myctophids were higher than was that of K. pelamis. Similarly, except for two cases, the actual DHA levels of the three myctophids were mostly higher than was that of K. pelamis. For example, in the June sample of D. watasei (sample No. 5), the EPA plus DHA level (1421.5 mg/100 g of tissue) was more than two times that in K. pelamis (628.9 mg/100 g of tissue). The actual EPA plus DHA levels in the three myctophids (497.2–2440.0 mg/100 g of tissue) were mostly higher than was that in K. pelamis (628.9 mg/100 g of tissue). Except for D. watasei, which has already been used in numerous areas in Japan, two myctophids, D. suborbitalis and B. pterotum, were eaten at only a limited number of local places, and almost no myctophids and other deep-sea fishes are generally utilized, although they may be edible and rich in PUFA. The high nutritional values of the three edible myctophids observed in the present study may serve a promising and practical source of a new marine food for easily obtaining high levels of n-3 PUFA. They will also be a good, novel source of healthy marine food, rich in EPA and DHA. Perhaps they will open new possibilities for three-dimensional deep-sea fishing in preparation for a future population explosion.

Supporting Information
The material is available free of charge via the Internet at http://dx.doi.org/10.5650/jos.ess13224

Acknowledgments
The authors thank Mr. Akihito Takashima for his skilled technical assistance. H. S. and K. K. performed all aspects of the study including the design of the experiments, analyzing the data, and writing the manuscript. S. H. assisted in collecting samples, measuring the biological data, and analyzing the data.

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