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to their wax esters

by M. Kayama

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Studies on the Lipids of Micronektonic Fishes caught in Sagami and
Suruga Bays, with Special Reference to Their Wax Esters

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The so-called micronekton is an intermediate group between the nekton and the plankton from the view-points of swimming ability as well as of its body size. Most of the micronektonic fishes have been known to undertake diurnal vertical migration and dwell in the meso- and bathypelagic zones of ocean. Fifteen species belonging to four families, Gonostomatidae (A), Sternoptychidae (B), Chauliodontidae (C) and Myctophidae (D), were collected from the surface to about 2000 m depths in Sagami and Suruga Bays by ORI-plankton net and Isaacs-Kidd midwater trawl at night.

The lipid contents of the micronektonic fishes on the wet weight basis were 2.1% to 6.0% in A, 1.4% in B, 2.4% in C, and 2.0% to 11.9% in D. The wax esters in their lipids were 13.0% to 69.8% in Gonostomatinae (sub-family of A) and over 50% in most of the genus *Cyclothone*, 15.3% and 6.1% in Maurolicinae (sub-family of A), 4.2% in B, 7.2% in C, and 1.7% to 67.1% in D. As the representative example of micronektonic fish, *Cyclothone atraria* was chosen and its various tissue lipids were analyzed. The fat-filled swim-bladder contained the highest amounts of lipids (52.1%), in which 78.3% were the wax esters.

The wax esters consist mainly of C₁₀ to C₁₁ with even carbon chain-lengths. The major esters are C₁₁, C₃₆, C₃₈, C₄₀ and C₄₂, and also a trace amounts of odd numbered esters exist. The acid moiety of wax esters is dominantly monoenoic acids and docosahexaenoic acid, while the alcohol moiety contains mainly C_{16:0}, C_{18:1}, C_{20:1} and C_{22:1}, as much as 80% in the sum of four components. Comparing the wax ester distribution of micronektonic fish with that of mullet roe, the former ranges rather longer esters because of major moiety of longer chain alcohols in its esters.

Although the relation between the micronektonic fishes and their wax ester contents in the lipids has not been fully understood yet, the bathypelagic nonmigrants, such as the genus *Cyclothone*, contain larger quantities of wax esters than the surface and midwater migrants which contain less than 30% and contain a few per cent in most of them.

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1. Introduction

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It has been thought that the distribution of wax esters in ocean fish oil was limited to specific types of fishes. As referred to in a previous report ¹, these are Chlamydoselachus anguineus, Ruvettus pretiosus*, Podpmea longipes, Lepidocybium flavo-brunneum, a species of Coelacanth and Kuroo-matoudai**. Wax esters are more particularly found in the ovary oil of Mugil cephalus, Gourami, Meruru-sa**, Theragra chalcogramma, and Scomber scombrus (mackerel).¹

The ocean extends horizontally and also vertically and the ecological division of living things in the ocean has been recognized as falling into groups according to both vertical and horizontal directions. Until recently ocean animals were roughly divided into three groups according to swimming ability; nekton such as bonito, plankton, and benthos such as shellfishes. However, recent progress in ecology has revealed that there exists a rather large group of animals which fall between the first two groups mentioned above; this new group has not been the object of much research. Not only their swimming abilities but also their body sizes are intermediate between the two groups. Recently this group of animals has been called, in general, micronekton ²⁻⁴. During the search for fishes suitable for biochemical research on the wax esters of fish, it was found that some Myctophidae, which belong to the micronektonic group, contain a quantity of wax esters in their lipids ⁵. This paper reports the results of the investigation of the lipids, with emphasis on the wax esters, of the micronektonic fishes caught in Sagami and Suruga Bays. The study of lipids of the ecologically similar micronektonic shrimps (which live in the meso- and bathypelagic zones of the ocean and were caught in the same Bays) will be reported in the following paper ⁶.

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A part of future ocean development includes a plan to develop the meso- and bathypelagic fishes as a food resource by expanding the fishing

Translator's note: *a species of Scombroproops boops.

**as transliterated; no translation found

grounds in a vertical direction. Then mixed fishing of those fishes containing non-glyceride lipids⁷ such as was esters, etc. could be carried out. Therefore, the investigation of the wax ester contents of these fishes is considered to be important. Moreover, most of these micronektonic fishes have been known to undertake diurnal vertical migration and perform an important role for the circulation of substances of the upper and lower zones of the ocean. The investigation of their lipids could contribute to the elucidation of these specific ecological phenomena.

2. Experimental

2.1 Fishes Examined

The micronektonic fishes used for analysis were collected mainly at night (May 25 - June 3, 1971, and June 9 - June 13, 1973) by the Tokyo University Oceanographic Institute's ship Tansui Maru in Sagami and Suruga Bays using a large type ORI-plankton net (diameter 160 cm, length 750 cm, mesh 0.33 X 0.33 mm) and an Isaacs-Kidd midwater trawl at depths ranging from the surface to about 2000 m. A total of fifteen fishes were collected and they are: five specimens of Gonostomatinae of the Gonostomatidae species, one specimen of Maurolicinae of the same species, one specimen each of Sternoptychidae and Chauliodontidae, seven specimens of Myctophidae; the taxonomic system for these specimens is shown in Figure 1.

The year of collection, the number of fishes examined, the average body weight, and the average standard body lengths are shown in the left hand portion of Table 1. These micronektonic fishes, except for the female *Gonostoma gracile* and the *Chauliodus sloani*, are less than 10 cm in length and as their name indicates, they are small fish.

2.2 Extraction of Fish Lipids

The lipids were extracted according to the method of Bligh and Dyer⁸ using separate extractions for each individual fish specimen. In the *Cyclothone atraria* case, the lipids were extracted from the individual tissues and then the comparison studies were made.

2.3 Thin Layer Chromatography and Densitometry

2.4 The Separation of Lipid Class and Gas Chromatography

These experimental methods of lipid analysis were reported in a previous paper¹.

3. Results and Discussion

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3.1 The Lipid Content and Lipid Class Composition of Micronektonic Fishes

The lipid contents (wet weight %) and the analytical results of the lipid class compositions of individual fishes are shown in the right hand side of Table 1.

Stenobranchius nannochir and *Lampanyctus macropterus* contained lipids which were about 11% of their body weights but the others contained less than 10%. The lipid content varied significantly with the species of fish: in *Gonostomatidae* it was 2.1-6.0%; *Sternoptyx diaphana* of *Sternoptychidae* contained the lowest value of 1.4%; in *Chauliodus sloani* of the *Chauliodontidae* species it was 2.4%; in *Myctophidae* the values were 2.0-11.9%.

When the lipid class composition is surveyed, the following general trend is observed: in the lipids of high wax ester content, the content of triglyceride is low and conversely for low wax ester content, triglyceride is a main component¹. Free fatty acids, sterol and phospholipids are also main components but their contents vary greatly with the fish species. When the wax ester contents were specially examined, rather high amounts were found in *Gonostomatinae*, genus *Cyclothone*: *Cyclothone atraria* 69.8%

and 58.4%; *Cyclothone pseudopallida* 53.5% and 44.8%; *Cyclothone alba* 54.0% and 45.5%; *Cyclothone pallida* 34.0% and 46.8%. Since *Gonostoma gracile* of the same subspecies undergoes ex reversal during its growth⁴, the wax ester contents of both male and female species were obtained based on the standard body length and these were 20.0% and 15.2%; the female has a slightly higher wax ester content. The amount in *Maurollicus m lleri* of the *Maurollicinae* subspecies caught in 1971 was 15.3% and the 1973 specimen contained 6.1%. Thus, even in the same fish, the content varies considerably according to the year in which they were caught. The *Sternoptyx diaphana* (*Sternoptychidae* species) wax ester content of 4.2% and the *Chauliodus sloani* (*Chauliodontidae* species) of 7.2% were rather low amounts. The contents were over 10% in *Myctophum nitidulum* (*Myctophidae* species) 10.6% and *Myctophum asperum* 17.2%; it was low in *Diaphus glandulifer* 3.7% and 6.5% *Diaphus fulgens* 4.4%, , *Diaphus latus* 1.7%; the latter are of the genus *Diaphus*. *Stenobranchius mannochir* contained a very high wax ester of 67.1% p.438 as well as a high lipid volume; in *Lampanyctus macropterus*, in spite of its high lipid content, the wax ester content was only 3.7%. The above results show that the wax ester contents vary significantly by micro-nektonic fish species.

3.2 Wax Ester Composition of Micronektonic Fishes

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Of the fifteen kinds of micronektonic fishes, the wax esters were isolated from the fishes with high wax ester contents; isolations were from *Cyclothone atraria*, *Cyclothone pseudopallida*, *Cyclothone alba*, *Cyclothone pallida*, and *Stenobranchius nanno chir*; and since there were enough sample oils, wax esters were also isolated from *Gonostoma gracile* and *Lampanyctus macropterus*. The results of gas chromatographic analyses are shown in Table 2.

The chain lengths of all the sample wax esters were from C_{30} to C_{44} and the even numbered esters such as C_{34} , C_{36} , C_{38} , C_{40} and C_{42} were the main components; trace amounts of the odd numbered esters were also detected. This is in contrast to the mullet roe in which the wax ester distribution was C_{26} to C_{40} with the C_{32} and C_{34} esters being the main components ¹.

3.3 Fatty Acid and Alcohol Composition of the Wax Esters

After saponification of the wax esters with sodium metal in ethanol and then with sodium hydroxide ⁵, the resulting fatty acids were esterified to the methyl esters and the resulting alcohols were acetylated; both were then analyzed by gas chromatography. The results are shown in Table 3.

The main composition of the fatty acids was $C_{16:0}$, the monoenoic acids $C_{16:1}$, $C_{18:1}$, $C_{20:1}$, and the highly unsaturated fatty acid $C_{22:6}$. For the alcohols the main components were $C_{16:0}$, $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$.

In contrast to mullet roe in which the main alcohol component was $C_{16:1}$ ¹, the micronektonic fishes have much longer monoenoic alcohols and as a result the esters have longer chains. If these fishes produce alcohols by direct reduction of the fatty acids ⁹, the reduction of the monoenoic acids $C_{16:0}$, $C_{18:1}$, $C_{20:1}$, and $C_{22:1}$ to their corresponding alcohols would predominate.

3.4 The Composition of Lipids of Individual Tissues, Particularly of the Swim-Bladder of *Cyclothone atraria*

As a representative species of the micronektonic fishes of Sagami and Suruga Bays, *Cyclothone atraria* was chosen and its various tissue lipid compositions are shown in Table 4.

The lipid contents are: in viscera 12.5%, in muscle 4.4%, and in gonads 3.6%; however, in the swim-bladder it was a very high 52.1%. In every case

the wax ester is the main component, and in the case of the swim-bladder lipid a high of 78.3% of wax ester was found. Triglyceride, free fatty acids, sterol and phospholipids were the other main components.

Marshall ² has made comparison studies on the structures of fish swim-bladders; he reported that the tissue of the swim-bladder is more developed with depth in going from the epipelagic to the meso- and bathypelagic zones. However, some species of the bathypelagic zone lack the swim-bladder altogether and in some others the swim-bladder is filled with fat instead of gas. He discusses the relationship between the water pressure and the swim-bladder, particularly the mechanism of its buoyancy. It is interesting that the swim-bladder of *Cyclothone atraria* is filled with fat; it is 80% occupied by the low specific gravity wax esters of the lipid class.

3.5 Micronektonic Fishes and Wax Esters

Lipids are a major energy reserve of the fish and generally tri-glyceride is its main component. However, micronektonic fishes have a quantity of wax esters. The epipelagic zone (where photosynthesis takes place) is abundant with living things and triglyceride is the main lipid component of fish which live there. However, in the meso- and bathypelagic zones, the amount of living things is diminished and in order to adjust to such environment the energy reserve is more specific. Although the energy reserve systems differs in going from triglyceride to wax esters, as was seen in plankton *Calanus helgolandicus* ¹⁰, micronektonic fishes will utilize both for the energy resource while giving preference to the former. It can be considered that storing the low specific gravity wax ester lipids is similar to the case of squalene in the liver oil of selachii (subspecies) ¹¹; it has the effect of reducing the energy consumption for the fish in order

to gain buoyancy; and for fish which have lost the function of gas control of the swim-bladder under high water pressure, it is reasonable that the main lipid component is wax esters.

If these fifteen specimens of micronektonic fishes found in Sagami and Suruga Bays are classified according to Kawaguchi's ecological classification⁴ of surface migrant, midwater migrant and midwater nonmigrant groups while observing the relationship to their wax ester contents, the following is noted: the four species of the genus *Cyclothone* belonging to the nonmigrant group and *Stenobranchius nonnochir* which is in between the latter two species contain more than 50% wax esters; however, which which belong to the first two groups have less than 20% wax ester amounts. Since the many ecological properties are mixed and complex, it is difficult p.440 to generalize these relationships at the present stage; but it might be possible to clarify these when more detailed data are accumulated.

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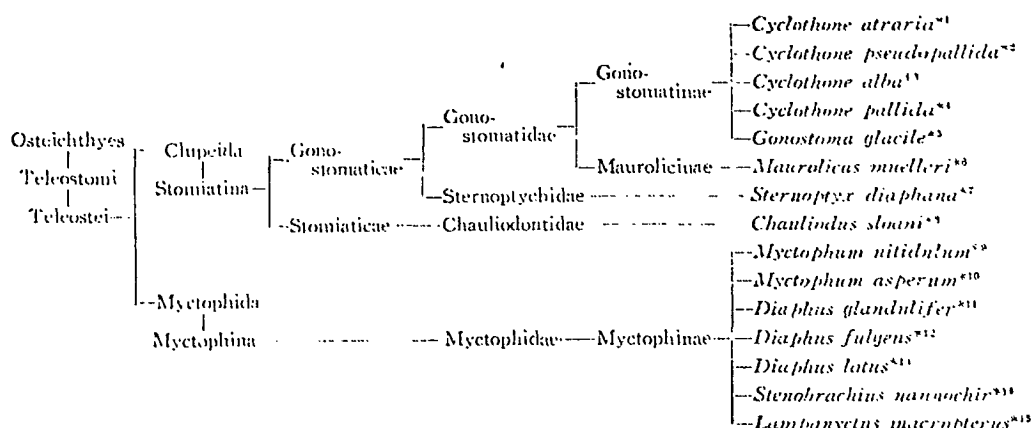


Fig.-1 Taxonomic scheme for micronektonic fishes caught in Sagami and Suruga Bays.

*1, Ouhadaka; *2, Hiiro-nhadaka; *3, Yukionhadaka; *4, Usuonhadaka; *5, Yokaeso; *6, Kyuriso; *7, Muneso; *8, Houraeso; *9, Susukihadaka; *10, Arabadaka; *11, Senhadaka; *12, Kobihadaka; *13, Hirohadaka; *14, Sekkihadaka; *15, Katahadaka in Japanese.

Table-1 Biological measurement, lipid content, and lipid class composition of micronektonic fish caught in Sagami and Suruga Bays.

Specimen	No. of specimens	Average		Lipid content (wet%)	Lipid class (%)							
		B.W. (g)	B.L. (cm)		W.E. (St.E.)	DAGE	Trig.	FFA	F. Alc.	St.	P. Gly.	Phos.
<i>Cyclothone atraria</i> (71)	10	0.47	4.4	5.4	69.8	7.1	16.0	1.2	—	2.1	—	3.8
<i>Cyclothone atraria</i> (73)	20	0.13	3.4	4.0	58.4	—	17.7	12.0	2.8	5.0	1.9	2.2
<i>Cyclothone pseudopallida</i> (71)	40	0.05	2.4	4.3	53.5	7.9	22.4	Tr.	—	3.6	2.0	10.6
<i>Cyclothone pseudopallida</i> (73)	25	0.04	2.2	2.5	41.8	—	27.8	11.3	—	8.8	3.6	3.7
<i>Cyclothone alba</i> (71)	50	0.04	2.0	5.3	54.0	—	29.3	Tr.	—	3.6	—	13.1
<i>Cyclothone alba</i> (73)	30	0.04	2.0	2.1	45.5	—	27.2	11.9	2.2	7.6	2.7	2.9
<i>Cyclothone pallida</i> (71)	15	0.10	2.8	2.3	34.0	5.7	44.6	2.4	—	3.7	2.0	7.6
<i>Cyclothone pallida</i> (73)	7	0.03	1.3	2.5	46.8	2.0	14.4	11.2	10.8	8.0	3.0	3.8
<i>Gonostoma gracile</i> ♂ (71)	3	2.90	10.7	2.5	20.0	8.7	51.2	6.7	—	4.1	2.3	7.0
<i>Gonostoma gracile</i> ♀ (73)	2	1.65	9.4	6.0	13.0	4.0	64.5	9.8	Tr.	4.5	2.5	1.7
<i>Gonostoma gracile</i> ♂ (71)	6	0.50	6.3	2.9	15.2	8.7	47.0	6.7	—	6.5	3.4	12.5
<i>Maurolicus muelleri</i> (71)	50	0.25	2.5	5.5	15.3	—	54.1	6.0	—	7.6	3.0	14.0
<i>Maurolicus muelleri</i> (73)	10	0.58	3.6	5.6	6.1	—	75.3	8.4	—	7.3	1.4	1.5
<i>Sternoptyx diaphana</i> * (73)	2	0.20	1.4	1.4	4.2	—	18.4	13.2	6.6	18.1	3.3	36.2
<i>Chauliodus sloani</i> * (71)	2	8.80	12.8	2.4	7.2	3.8	43.9	11.8	—	10.5	8.2	14.9
<i>Myctophum nitidulum</i> (71)	5	1.00	4.3	3.8	10.6	—	45.6	12.8	—	8.0	6.7	16.3
<i>Myctophum asperum</i> * (71)	1	1.10	3.4	2.0	17.2	—	37.9	21.9	—	11.2	4.2	7.6
<i>Diaphus glandulifer</i> (71)	15	1.30	4.6	7.9	3.7	—	51.9	13.0	9.4	9.9	—	12.1
<i>Diaphus glandulifer</i> (73)	3	2.20	5.6	3.5	6.5	—	62.6	15.2	—	9.1	4.3	2.3
<i>Diaphus fulgens</i> * (71)	3	3.20	4.9	7.1	4.4	2.8	65.7	9.4	—	6.6	4.4	6.7
<i>Diaphus latus</i> (71)	2	0.70	3.5	8.7	1.7	—	67.6	9.6	—	8.1	5.4	7.6
<i>Stenobrachius nannochir</i> * (71)	1	1.30	4.8	11.4	67.1	—	6.3	6.5	3.2	6.1	4.7	6.1
<i>Lampanyctus macropterus</i> (71)	3	3.80	7.6	11.9	3.7	4.5	65.2	7.1	—	7.5	6.6	5.4

* These were specimens without sex.

Abbreviations: B.W., body weight; B.L., standard body length; W.E., wax esters; St. E., sterol esters; DAGE, diacylglycerol ethers; Trig., triglycerols; FFA, free fatty acids; F. Alc., free fatty alcohols; St., sterols; P. Gly., partial glycerols; Phos., phospholipids.

Table-2 Chain-length composition of wax esters obtained from micronektonic fish lipids.

Wax ester (Carbon chain-length)	<i>Cyclothone atraria</i>		<i>Cyclothone pseudopallida</i>		<i>Cyclothone alba</i>		<i>Cyclothone pallida</i>		<i>Gonostoma gracile</i>		<i>Stenobranchius nannochir</i>		<i>Lampanyctus macropterus</i>	
	F.A.	Alc.	F.A.	Alc.	F.A.	Alc.	F.A.	Alc.	F.A.	Alc.	F.A.	Alc.	F.A.	Alc.
30	0.1	Tr.	3.3	—	2.5	—	2.1	—	1.8	—	1.6	—	1.1	—
31	Tr.	—	—	—	—	—	—	—	—	—	—	—	—	—
32	6.4	Tr.	21.0	Tr.	11.2	Tr.	7.2	Tr.	6.8	Tr.	7.0	Tr.	8.7	Tr.
33	0.6	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
34	11.7	Tr.	14.4	Tr.	24.6	Tr.	15.9	Tr.	18.7	Tr.	13.3	Tr.	2.9	Tr.
35	0.6	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
36	20.4	Tr.	16.6	Tr.	17.3	Tr.	15.4	Tr.	17.8	Tr.	15.6	Tr.	12.5	Tr.
37	0.6	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	—	—	—	—	—	—
38	20.1	Tr.	18.8	Tr.	18.0	Tr.	12.1	Tr.	11.7	Tr.	20.0	Tr.	21.8	Tr.
39	Tr.	—	Tr.	—	Tr.	—	—	—	—	—	—	—	—	—
40	17.0	Tr.	18.4	Tr.	15.2	Tr.	24.5	Tr.	20.1	Tr.	19.7	Tr.	28.7	Tr.
41	0.8	Tr.	Tr.	Tr.	Tr.	Tr.	—	—	—	—	—	—	—	—
42	14.9	Tr.	4.8	Tr.	6.9	Tr.	15.8	Tr.	16.7	Tr.	14.0	Tr.	16.3	Tr.
44	6.8	Tr.	2.7	Tr.	4.3	Tr.	7.0	Tr.	6.4	Tr.	8.8	Tr.	8.0	Tr.
46	Tr.	—	Tr.	—	Tr.	—	Tr.	—	—	—	—	—	—	—

Table-3 Fatty acid and alcohol composition of wax esters obtained from micronektonic fish lipids.

Chain length : unsaturation	<i>C. atraria</i>		<i>C. pseudo-pallida</i>		<i>C. alba</i>		<i>C. pallida</i>		<i>G. gracile</i>		<i>S. nannochir</i>	
	F.A.	Alc.	F.A.	Alc.	F.A.	Alc.	F.A.	Alc.	F.A.	Alc.	F.A.	Alc.
12:0	—	Tr.	—	0.1	Tr.	0.3	—	—	Tr.	Tr.	Tr.	Tr.
13:0	—	Tr.	—	0.1	—	0.2	—	—	—	—	—	—
14:0	7.9	1.3	3.5	8.9	3.4	4.4	2.3	2.0	1.8	3.8	2.0	6.2
14:1	—	0.2	—	0.7	0.6	0.6	—	1.2	0.5	0.7	—	0.5
15:0	2.9	0.2	1.2	0.9	—	1.0	0.5	1.8	Tr.	1.0	1.2	0.7
16:0	3.2	7.2	6.6	28.3	7.4	27.3	5.0	16.8	3.3	37.7	0.7	28.1
16:1	8.0	1.8	10.9	3.0	10.2	3.5	9.5	3.6	8.6	4.9	10.0	3.5
17:0	0.7	0.4	Tr.	0.6	1.9	1.6	Tr.	1.7	0.5	1.1	Tr.	0.4
17:1	—	0.4	2.4	0.5	—	1.3	1.1	1.9	—	1.0	2.5	0.4
18:0	Tr.	1.2	2.3	2.3	0.6	6.0	1.5	2.6	0.7	3.6	—	2.2
18:1	11.0	6.5	25.0	3.9	34.9	10.9	23.7	2.7	27.4	11.1	17.6	5.0
18:2	2.1	0.7	6.8	0.4	2.6	1.2	2.5	0.4	3.5	1.0	4.2	0.2
18:3	Tr.	—	3.9	—	1.4	—	3.2	—	0.9	—	7.0	—
18:4	3.6	—	Tr.	—	0.6	—	Tr.	—	0.3	—	Tr.	—
20:0	—	0.4	—	0.8	—	1.5	—	0.2	—	0.6	—	0.4
20:1	29.0	28.0	9.9	14.5	7.9	13.7	7.0	18.3	6.8	11.9	15.9	15.7
20:3 (22:1)	16.0	—	11.9	—	13.2	—	11.2	—	7.1	—	21.2	—
20:4	Tr.	—	Tr.	—	Tr.	—	Tr.	—	Tr.	—	Tr.	—
20:5	5.4	—	1.0	—	1.4	—	1.1	—	2.2	—	1.1	—
21:1	—	0.4	—	0.4	—	Tr.	—	1.6	—	Tr.	—	0.3
22:1	—	45.2	—	30.8	—	23.6	—	41.5	—	20.1	—	35.1
22:3	Tr.	—	5.8	—	2.7	—	4.4	—	2.4	1.1	Tr.	1.3
22:4	Tr.	—	Tr.	—	3.4	—	1.1	—	0.9	—	Tr.	—
22:5	1.9	—	Tr.	—	2.2	—	1.6	—	11.8	—	Tr.	—
22:6	8.3	—	5.8	—	5.6	—	24.3	—	21.3	—	16.6	—
24:1	—	5.9	—	3.8	—	2.9	—	3.7	—	Tr.	—	Tr.

Abbreviations as for Table-1.

Table-4 Lipid class composition of various tissues with special reference to fat-filled swimbladder separated from *Cyclothone atraria*.

Tissue	Lipid content (%)	Lipid class (%)							
		W.E. (St. E.)	DAGE	Trig.	FFA	F. Alc.	St.	P. Gly.	Phos.
Viscera	12.5	56.0	2.0	21.0	8.0	3.0	4.0	—	6.0
Muscle	4.4	59.0	2.0	12.0	11.0	4.0	6.0	—	6.0
Fat-filled swim-bladder	52.1	78.3	—	12.8	3.3	0.8	1.9	1.9	1.0
Gonad	3.6	45.9	—	39.1	8.1	0.6	1.7	2.5	2.1

Abbreviations as for Table-1.

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