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Lipid class composition of benthic-pelagic fishes (Cottocomephorus, Cottoidei) from Lake Baikal

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Abstract

A study was made of the lipid class composition in liver, gonads and red and white muscle of two endemic Baikal fish species *Cottocomephorus grewingki* and *Cottocomephorus inermis*. The main lipid classes were triacylglycerols and phospholipids. The highest levels of triacylglycerol (81.8%) were found in the liver of *C. inermis* males, and of phospholipid (38.3%) – in males testes of both species. The main phospholipid classes were phosphatidylcholine and phosphatidylethanolamine, which varied in examined organs and tissues in both fish species from 44.3 to 78.7%, and from 16.8 to 42.1%, respectively.

Introduction

Cottocomephorus grewingki (Dybowski) and *Cottocomephorus inermis* (Jakowlew) are endemic Baikalian benthic-pelagic species belonging to the family *Cottidae* (Sideleva 1982). *C. grewingki* is a gregarious planktophage, 80–90% of its food ration being copepods (Koryakov 1972). Up to the late 1960 decade *C. grewingki* is a commercial fish. *C. inermis* is a predator, 65% of its food ration being *Macrohectopus branickii* (Amphipoda) and 35% – young pelagic cottoid fishes (Gurova and Pastukhov 1974). *C. grewingki* and *C. inermis* lack swim bladder; they represent two of the four species of cottoid fishes, living in the pelagic zone. It is known that buoyancy of fish without swim bladder depends on the lipid content in their body as lipids play an important role in decreasing the body's density (DeVries and Eastman 1978; Eastman and DeVries 1982; Clarke et al. 1984). Although specialization of cottoid fish to live in pelagic zone due to their morphological adaptation has already been considered (Sideleva and Kozlova 1989; Sideleva et al. 1992), we know little about the lipid content and composition in these fish. Thus, in this paper the results of an examination of the lipid class composition and of the phospholipids organs and tissues of *C. grewingki* and *C. inermis* are presented.

Materials and methods

Animals

C. grewingki and *C. inermis* were caught using bottom gill nets in the southern portion of the Lake Baikal basin. Samples were taken during the sexual maturation period of December 1989. Ten males and females of each species were analyzed. Samples of liver, gonads and red and white muscles were taken from each fish, homogenized and put into sample bottles in 20 vol of chloroform:methanol (2:1).

Lipid extraction and analysis

Total lipid were extracted using the method of Folch et al. (1957). The extract was concentrated and dried under vacuum in a rotary evaporator for gravimetrical weight measurement. Lipid class composition was analysed by a thin-layer chromatography method on the 'Silufol' – UV-254 plates followed by densitometry (Kopytov 1985). For the analysis of phospholipid composition 10 samples of lipid extracts of each tissues from 10 fish were combined into a single sample and determined by a thin-layer microchromatography method (Svetashev and Vaskovsky 1972).

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Table 1. Phospholipid class composition of tissues from two species of cottoid fish from Lake Baikal

Tissues	Phospholipid classes (% total phospholipid)*						
	PC	LPC	PE	PI	PS	SPM	DPG
Cottocomephorus grewingki (females)							
Liver	66.7	–	22.4	4.0	1.3	4.1	1.5
Ovaries	78.7	–	16.8	0.9	trace	1.9	1.7
Red muscle	53.3	–	35.3	2.2	1.0	1.0	7.2
White muscle	59.0	–	29.8	2.7	2.3	3.4	2.8
Cottocomephorus grewingki (males)							
Liver	44.3	–	27.0	7.9	4.9	8.3	7.6
Testes	54.4	0.7	24.4	3.8	5.8	6.9	4.0
Red muscle	51.7	–	35.2	2.5	1.4	1.8	7.3
White muscle	58.0	–	30.4	4.2	1.5	2.0	3.9
Cottocomephorus inermis (females)							
Liver	59.4	–	28.2	3.2	2.4	3.8	2.9
Ovaries	58.0	–	33.7	2.7	1.3	1.7	2.7
Red muscle	55.6	–	33.1	2.0	1.0	0.3	7.9
White muscle	53.0	–	34.5	2.8	2.9	3.7	3.1
Cottocomephorus inermis (males)							
Liver	53.7	–	32.1	5.5	2.8	2.3	3.7
Testes	54.8	–	39.5	0.9	0.9	3.2	0.7
Red muscle	50.8	–	42.1	1.3	0.6	0.8	4.3
White muscle	60.8	–	26.8	2.9	2.1	1.3	6.1

*PC = phosphatidylcholine; LPC = lysoPC; PE = phosphatidylethanolamine; PI = phosphatidylinositol; PS = phosphatidylserine; SPM = sphingomyeline; DPG = diphosphatidylglycerol; – = not detected.

Results and discussion

Phospholipid class composition

Table 1 shows phospholipid composition of liver, gonads, red and white muscles of females and males *C. grewingki* and *C. inermis*. The principal phospholipids are phosphatidylcholine and phosphatidylethanolamine. The content of phosphatidylcholine in the organs and tissues of both species varied from 44 to 79%, and that of phosphatidylethanolamine from 16 to 42%. Phosphatidylinositol, phosphatidylserine, sphingomyelin and diphosphatidylglycerol contents was much lower (lower than 8%). From our data we deduce that the phospholipid class composition of *C. grewingki* and *C. inermis* corresponds to available literature data about content of phospholipid classes in the fish tissues. It is known from the literature that the phosphatidylcholine is the dominant phospholipid. For example, in ovaries of Atlantic herring, *Clupea harengus*, the polar lipids consist 90% of phosphatidylcholine (Tocher et al. 1985) and in ovaries of Atlantic salmon, *Salmo salar*, the phosphatidylcholine is 94% (Cowey et al. 1985). In different organs and tissues of bogue, *Boops boops*, (liver, head, skin, muscles) the phosphatidylcholine varied from 36 to 59% (Kapoulas and Miniadis-Meimaroglou 1985). Phosphatidylcholine serves not only structural function in the cells, but it is an essential component, along with phosphatidylethanolamine in many catabolic systems. Phosphatidylethanolamine is the second most important phospholipid quantitatively. For example, in ovaries of whitefish, *Coregonus albula*, the phosphatidylethanolamine ranged from 15 to 26% (Lizenko 1980) and in organs and tissues of bogue from 24 to 34% (Kapoulas and Miniadis-Meimaroglou 1985).

The other classes of phospholipids are present in small amounts. Diphosphatidylglycerol is a component of mitochondrion respiratory systems. The red muscles of both studied species are characterized by the highest content of diphosphatidylglycerol (7%) (Table 1) in comparison with white muscle, liver and gonads. Its higher content in the red muscle indicates that the processes of oxidation take place on a higher level in red muscle than in white muscle. This coincides with the opinion of some authors (quoted from Love 1970) who believe that red muscles are characterized by a high rate of aerobic metabolism. Therefore, it is characteristic of red muscle that not only the content of lipids is higher but their utilization is more intensive in comparison with white muscle. The phosphatidylinositol and sphingomyelin are basically part of the nervous tissues. The appearance of the sphingomyelin and the increase of its content in the brain of animals indicates a progressive line in the evolutionary processes (Kreps 1981).

Table 2. Lipid content (± SD) of tissues from two species of cottoid fish from Lake Baikal

Tissue	Total lipids (% fresh wt)
Cottocomephorus grewingki (females)	
Liver	5.6 ± 0.7
Ovaries	6.3 ± 0.7
Red muscle	2.4 ± 0.2
White muscle	1.4 ± 0.1
Cottocomephorus grewingki (males)	
Liver	6.7 ± 0.3
Testes	2.6 ± 0.1
Red muscle	3.0 ± 0.1
White muscle	1.6 ± 0.1
Cottocomephorus inermis (females)	
Liver	11.7 ± 1.1
Ovaries	2.7 ± 0.2
Red muscle	4.3 ± 0.3
White muscle	1.5 ± 0.03
Cottocomephorus inermis (males)	
Liver	19.5 ± 1.3
Testes	2.3 ± 0.1
Red muscle	4.7 ± 0.5
White muscle	1.4 ± 0.1

Lipid content and lipid class composition

In fish, lipids are important biochemical components which perform energy and structural functions. Fish, like other animals, accumulate lipids in their bodies. *C. grewingki* and *C. inermis* belong to the group of medium fat fishes having an average lipid content of 2–8% (Kozlova 1997). The lipid content and lipid class composition of liver, gonads and red and white muscles from the two freshwater cottoid fish are shown in Tables 2 and 3.

Eight lipid fractions: phospholipids, monoacylglycerols, diacylglycerols, sterols, free fatty acids, triacylglycerols, an unidentified fraction, and sterols esters were found. The qualitative composition of the fractions is similar in all organs and tissues examined, while the total contents of lipid differ. The lipid class composition of both species is dominated by triacylglycerols and phospholipids, but triacylglycerols are always predominant. The content of triacylglycerols is twice the content of phospholipids in liver and white muscle, and five times higher in red muscle. Individual quantitative compositions of liver, ovaries and testes, red and white muscles lipids of both fish species are due to morphofunctional features of this tissues.

The liver and muscles in fish serve as a fat depot as well as performing other physiological functions, and the liver is the main lipid storage organ in the body of many fish species. As an example, the liver of cod, *Gadus morhua*, contains up to 67% of lipids (wet weight), whereas in muscles the lipid is $\leq$ 1% (Jangaard et al. 1967). In the liver of many freshwater fish species (northern pike, *Esox lucius*, Atlantic salmon, *Salmo salar*, perch, *Perca fluviatilis*, roach, *Leucisus rutilis*, brook trout, *Salvelinus fontinalis*) the content of lipids is much lower (5–10%), but it never exceeds the content in the two types of muscles (Kinsella et al. 1977; Gunstone et al. 1978; Medford and Mackay 1978; El-Sayed et al. 1984; Puustinen et al. 1985). *C. grewingki* and *C. inermis* have relatively large livers (3–7% relative to body mass depending on physiological state) which is an attribute derived from their benthic origin. The liver lipid of *C. inermis* (11.7% of wet weight in females and 19.5% in males) is 2–3 times higher than that of *C. grewingki*. As shown earlier (Kozlova 1997), the liver of *C. grewingki* performed mainly in a metabolic role, whereas in *C. inermis* if served also as a storage site for lipid reserves. Therefore, in *C. inermis* liver the triacylglycerols are predominant, reaching 82% of total lipid in males of this species. In the liver of *C. grewingki* the content of triacylglycerols was only 50%, and the phospholipids content 28%.

Gonads synthesize sexual hormones, and their lipid content is dependent upon the stage of sexual

Table 3. Lipid class composition (± SD) tissues from two species of cottoid fish from Lake Baikal

Tissue	Lipid class composition (% total lipid)*							
	PL	MAG	DAG	Sterols	FFA	TAG	Unidentified fraction	SE
Cottocomephorus grewingki (males)								
Liver	28.1 ± 3.5	1.8 ± 0.1	2.9 ± 0.2	11.4 ± 1.4	3.7 ± 0.5	49.7 ± 5.2	2.9 ± 0.3	1.4 ± 0.1
Testes	38.3 ± 2.5	trace	4.7 ± 0.6	28.6 ± 1.4	6.4 ± 0.8	13.6 ± 0.8	4.7 ± 0.6	3.2 ± 0.5
Red muscle	15.5 ± 1.7	trace	1.2 ± 0.2	4.7 ± 0.4	1.1 ± 0.1	75.5 ± 2.2	1.2 ± 0.1	0.8 ± 0.1
White muscle	29.3 ± 1.7	trace	1.6 ± 0.3	8.0 ± 0.9	1.5 ± 0.1	56.2 ± 2.0	2.1 ± 0.1	1.2 ± 0.1
Cottocomephorus inermis (males)								
Liver	8.8 ± 1.3	trace	1.1 ± 0.1	6.0 ± 0.7	1.0 ± 0.1	81.8 ± 2.5	1.3 ± 0.2	trace
Testes	38.3 ± 1.9	trace	trace	33.4 ± 1.6	5.0 ± 0.4	17.0 ± 1.5	4.0 ± 0.4	1.8 ± 0.2
Red muscle	23.2 ± 1.6	trace	trace	13.5 ± 1.2	2.6 ± 0.3	53.9 ± 1.9	4.8 ± 0.4	1.7 ± 0.1
White muscle	23.9 ± 1.4	trace	trace	16.9 ± 1.2	1.1 ± 0.1	49.4 ± 1.5	7.5 ± 0.7	1.5 ± 0.1
Cottocomephorus inermis (females)								
Liver	11.0 ± 0.6	trace	1.7 ± 0.3	7.5 ± 0.6	2.1 ± 0.5	75.8 ± 2.0	1.8 ± 0.3	0.8 ± 0.1
Ovaries	23.4 ± 1.4	trace	1.2 ± 0.1	25.7 ± 1.8	8.3 ± 0.6	28.8 ± 3.2	11.7 ± 1.2	3.9 ± 0.5
Red muscle	24.2 ± 1.5	trace	trace	12.1 ± 0.9	2.6 ± 0.3	55.5 ± 4.5	3.6 ± 0.4	1.9 ± 0.9
White muscle	29.5 ± 1.5	trace	trace	13.4 ± 0.6	1.9 ± 0.1	48.1 ± 1.6	5.9 ± 0.8	1.6 ± 0.4

*PL = phospholipid; MAG = monoacylglycerol; DAG = diacylglycerol; FFA = free fatty acid; TAG = triacylglycerol; SE = sterol ester.

cycle and the sex of fish (Newsome and Leduc 1975; Nefedova and Lizenko 1978; Vuorela et al. 1979). As a rule, the ovaries of fish accumulate much more stored lipids, particularly triacylglycerols, than the testes, because the provision of energy for the offspring and depends mainly on their way of life, feeding level and especially on the reproductive ecology of each species. For example, in such fish species as pike, roach and bream, *Abramis brama*, the ovarian lipid content was 3–5% of wet weight, whereas in salmonid fishes it is 9–10% (Lizenko 1980). The females of *C. grewingki* and *C. inermis* spawn eggs under stones, and the males guard the nest for the whole period of embryogenesis. The lipid content in the gonads of *C. grewingki* and *C. inermis* is not high, 2–6%. Ovaries and testes differ from other organs in the higher content of sterols (from 25 to 33% of total lipids). Triacylglycerols are predominant in the ovaries (28%), whereas phospholipids are more important in the testes (38%), as qualitative characteristic of most fish testes lipids.

In the red and white muscles of many fish species significant reserves of lipids are accumulated and processes of lipolysis also take place. The lipid content in the red muscle of *C. grewingki* and *C. inermis* ranged from 2.4 to 4.7%, whereas in the white muscle – from 1.4 to 1.6%. The content of triacylglycerols also was higher in the red muscle than in the white muscle. Although the lipid content in white muscle is lower, this tissue also takes an active part in lipid accumulation. The relationship of red and white muscle mass differs in different fish species depending on the level of swimming activity. Taking into account that the red muscle forms only 7% of body weight in both fish species, and white muscle –32% in *C. grewingki* and 29% in *C. inermis* (personal unpublished data), the main reserves of lipids and, particularly triacylglycerols, are accumulated in white muscle. A similar phenomenon was found in other fish species, for example, in scorpion fishes, *Scorpaena porcus* (Shchepkin 1971), pollack, *Pollachius virens* (Storozhuk 1975) and horse mackerels, *Trachurus trachurus* (Dobrusin 1978).

C. grewingki and *C. inermis* belong to the group of secondary pelagic fishes (that also includes 2 typically pelagic species, *Comephorus baicalensis* and *Comephorus dybowskii*) which form part of the unique open water ecosystem of Lake Baikal (Taliev 1955; Koryakov 1972; Sideleva 1982; Sideleva and Kozlova 1989). The specific origin and living conditions of these species favoured the appearance of various adaptations both at organism and population levels. As all the species examined lack a swim bladder, they needed a series of adaptations for living in the pelagic zone, formed in several ways including increased buoyancy due to an increased lipids content. Of the 4 species, only one reached neutral buoyancy, due to a very high lipid content (about 40% wet weight) (Starikov 1977), namely, *Comephorus baicalensis*. As an adaptation for life in the pelagic zone, *C. grewingki* has a stable lipid content in the body (9% wet weight) throughout the year cycle, spawning period being an exception (2–3%) when the fish are tied to the bottom (Kozlova 1977). *C. inermis* differs from *C. grewingki* in having higher level morphological adaptations to living in the pelagic zone, and hence changes in the fish's lipid contents depend distinctly on their physiological state.

Triacylglycerols are one of universal forms of stored lipids used mainly for various energetic needs. In an overwhelming majority of fish, triacylglycerols are a dominant fraction of neutral lipids (Lee et al. 1975; Henderson and Tocher 1987; Neighbors 1988; Eastman 1988; Vlieg and Body 1988). Lipid class composition of *C. grewingki* and *C. inermis* and *C. baicalensis* and *C. dybowskii* (Morris 1984; Ju et al. 1997) have confirmed that these fish species store considerable quantities of triacylglycerols (over 90% in *C. baicalensis*). Wax esters, one of the functions of which is to enhance buoyancy, are absent from Lake Baikal's hydrobionts (Morris 1983), so for these organisms triacylglycerols may perform a similar function to wax esters in marine meso- and bathypelagic organisms.

Drawing an analogy between the two coldwater ecosystems – Lake Baikal and the Antarctic – one may see that in both cases, the food abundance in the pelagic zone is a major factor determining the species of originally benthic families (*Cottoidei* in Lake Baikal and *Notothenioidei* in the Antarctic) to occupy the water column. The cottoid fishes, *C. grewingki* and *C. inermis* examined here are similar to cryopelagic notothenioid *Pagothenia borchgrevinki* that have adapted to living in the water column, in lipid content in white muscle and liver (1.91 and 7.64% wet weight, respectively) (Clarke et al. 1984). Thus, further studies of lipids in bladderless fish of Lake Baikal as one direction of their adaptation to a life in the pelagic zone will eventually help understand their evolutionary processes in the lake.

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