A COMPARATIVE STUDY ON COMPOSITION AND DYNAMICS OF PHOSPHOLIPIDS IN FISH

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Hossam Mohamed Kotkat

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Contents

ABBREVIATIONS

AA	Arachidonic acid
ANS	8-anilinonaphthalino sulphate
Cl	Cardiolipin
CO	Cyclooxegenase
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
GLC	Gas liquid chromatography
HPLC	High performance liquid chromatography
LO	Lipooxegenase
LTB_4	Leukotriene B_4
NL	Neutral lipid
n-3 FA/n-6 FA	Ratio of linoleic to linolenic fatty acids
n-3 PUFA	Linolenic acid family
n-6 PUFA	Linoleic acid family
PA	Phosphatidic acid
PC	Phosphatidyl-choline
PE	Phosphatidyl-ethanolamine
PG	Phosphatidyl-glycerol
PGI	Prostaglandins I_3
PGI_3	Prostacyclins
PI	Phosphatidyl-inositol

PL (TPL)	Phospholipid (total phospholipid)
PS	Phosphatidyl-serine
PUFA	Polyunsaturated fatty acid
Sat.FA/Unsat.FA	Ratio of saturated to unsaturated fatty acids
SM	Sphingomyelin
TG	Triglyceride
TL	Total lipid
TLC	Thin-layer chromatography
TXA_2	Thromboxane
UI	Polyen index
VLDL	Very low density lipoprotein

INTRODUCTION

Lipids not only are nutriment of highest calorific value (transport form of metabolic fuel), which are significant energy resources of living organism, but also as main structure components of biomembranes. Some substances classified among the lipids (unsaturated fatty acids) have intense biological activity, physiological function and precursors for several chemical compounds (prostanoides biosynthesis). According to their structure unsaturated fatty acids are divided into families of monounsaturated (n-7 and n-9 fatty acids) diunsaturated and polyunsaturated (n-3 and n-6 fatty acids) ones.

Vertebrates are unable to synthesise fatty acids with double bonds more distal from the carboxyl end of the fatty acid than the ninth carbon atom. Thus, linoleic acid (18:2 n-6) is an essential fatty acid; it cannot be synthesized and it must be ingested in the principal polyunsaturated fatty acid (PUFA) in oil from plant seed (e.g. corn oil and safflower oil). It is characterized as an n-6 or omega-6 fatty acid. In both animals and humans, linoleic acid can undergo elongation and desaturation to yield arachidonic acid (20:4 n-6). There is another important dietary class of PUFA, of which α-linolenic acid (18:3 n-3) is the parent compound. Like linoleic acid, α-linolenic acid is also an essential fatty acid (Neuringer and Conner, 1986). Both n-6 (AA) and n-3 (EPA + DHA) are important precursors of eicosanoids. Their absence from the food is accompanied with severe metabolic lesions. Fish need for their normal growth n-3 fatty acids.

Linolenic acid is present in green leaves, and is associated with the chloroplast. Elongation and further desaturation of alpha-linolenic acid occurs in animals and rarely in humans to yield eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Tinoco,

1982). Some plants oils, notably linseed and soybean oils, contain appreciable amounts of α-linolenic acid while marine phytoplankton and zooplankton are rich sources of the longer n-3 PUFA such as EPA and DHA. EPA and DHA is abundant in also marine animals (Bang et al., 1976, Hirai et al., 1980 and Kromhout et al., 1985).

In developed industrial societies, nutritional deficiency diseases are unusual and the life expectancy, which is continually improving, reflects the availability of a relatively safe and nutritious food supply. Many of the contemporary health problems are chronic, age-related physiopathologies (Cardiovascular disease, cancer, obesity, hypertension, diabetes, arthritis, osteoporosis, etc.) that are influenced by dietary habits and nutrient imbalances (Kinsella, 1988).

Epidemiological, clinical and animal nutritional studies indicate that the consumption of n-3 PUFA in sea foods and fish oils is associated with a reduced incidence of coronary heart disease, cancer and several inflammatory diseases (Leaf and Weber, 1988). Dietary n-3 PUFA can depress plasma lipoproteins, especially very low density lipoprotein (VLDL) and triglycerides by inhibiting triglyceride and apoprotein synthesis. They also replace arachidonic acid (AA) in phospholipid pools with EPA and DHA. The n-3 PUFA of fish oil competitively inhibit cyclooxygenase and lipooxygenase and reduce eicosanoid synthesis, particularly thromboxane (TXA_2) and leukotriene B_4 (LTB_4), by platelets and macrophages. Omega - 3 fatty acids not only modify platelet functions and eicosanoid synthesis, but also affect many other mechanisms involved in the pathogenesis of atherosclerosis, in addition increased the deformability of erythrocytes and have antiaggregatory, antithrombotic and antiinflammatory properties (Haines et al., 1986, Albrink et al., 1986 and Kasim et al.,1988).

The amount of DHA necessary to maintain normal levels of liver and brain phospholipids is 11 mg/kg daily. The amount needed to prevent cardiovascular disease in the elderly is about 800-1100 mg/d of α-linolenic acid and 300-400 mg/d of EPA + DHA. The profound triacylglycerol-lowering effects of fish or fish diets rich in these n-3 fatty acids is now an established fact and these fatty acids are clearly of clinical significance and therapeutic potential as concerning the avoidance of coronary heart disease and hypertriglyceridaemia (Phillipson et al., 1985).

Numerous factors may influence the amount of omega - 3 fatty acids in fish. The following study will focus on some of these factors, first of all on temperature, and diet.

The aims of the present study

1. **To** compare the fatty acid, polar head group, and molecular species compositions of freshwater, and marine fish adapted seasonally or during evolution to different temperatures.
2. **To** follow the changes in the above lipid parameters during short-term thermal adaptation in a representative fish species.
3. **To** study the possible relationship between feeding habit and liver phospholipid composition in selected freshwater fish species.
4. **To** follow the possible changes in liver phospholipid composition in the course of aging in common carp (Cyprinus carpio L.).

LITERATURE SURVEY

2.1. Biological function of omega - 3 fatty acids

Dietary trials aimed at reducing the risk of cardiovascular disease have emphasized the importance of ingesting marine oil and fish products that are rich in n-3 PUFA and poor in n-6 fatty acids. The incidence of myocardial infarction and coronary atherosclerosis among Greenland Eskimos is low. This has been attributed to the protective effect of a diet rich in fatty fish and marine mammals (Dyerberg et al., 1978). Therefore, n-3 PUFA, particularly EPA and DHA, are subjected to extensive research (Siess et al.,1980).

The n-3 PUFA were shown to inhibit of increase of the AA and prostanoids derived from AA. When n-3 fatty acids are ingested, some of the AA in the membrane phospholipids will be replaced by EPA and to lesser extent by DHA. Prostaglandins of the three series will then be formed from EPA (Preston, 1982). TXA_3 is much less potent than TXA_2, but prostacyclins PGI_3 is as active in vasodilatation as PGI_2.

In addition to a prolongation of the bleeding time, there is substantial agreement that platelet aggregation to adenosine diphosphate, epinephrine and collagen is inhibited, that TXA_2 production is decreased, that the whole blood viscosity is reduced and that the plasma cholesterol and triglyceride levels are decreased when sufficient n-3 fatty acids are included in the diet (Kromhout et al., 1985). Moreover, leucotriene B_5 formed from EPA is less chemotactic than leucotrine B_4 by formed from AA. Thus it also have importance in reducing inflammatory processes. Role of EPA in arterycoronary disease has been emphased by several authors (Lewis and Austen 1981, Corey et al., 1983).

The hypolipidaemic effect of the n-3 PUFA may involve several mechanisms. These include suppression of hepatic fatty acid, TG, and apoprotein synthesis, diminution of VLDL

secretion, and enhanced low density lipoprotein (LDL) receptor-mediated removal. They may increase lipoprotein lipase activity and improve cholesterol excretion via bile acids (Leaf and Weber, 1988).

In summary, dietary n-3 PUFA reduce AA levels of cells (platelet, macrophages, neutrophils and monocytes) with concurrent replacement with EPA and/or DHA, thereby decreasing the availability of AA for eicosanoid synthesis. Both EPA and DHA are effective inhibitors of cyclooxygenase (CO) and lipoxygenase (LO) and when released from phospholipid pools they effectively inhibit the amounts of eicosanoids produced.

2.2. Fish as main sources for omega - 3 fatty acids

At present, fish are the only source of n-3 long-chain PUFA, although extensive research in being carried out to reveal alternative source of this fatty acids. Among this, mass production of certain bacteria (Martin et al., 1981 and Dicken and Thompson 1982), filamentous fungi (Nichols et al 1982) or diatoms are the possible candidates. The advantage of this approach could be that these organisms could be produced by biotechnological ways (fermentation). The annual production of fish oils is roughly 1 x 10^6 metric tons and for a long time this will be only source of EPA and DHA. Northern hemisphere fish such as cod, menhaden, pilchards and herring are all rich in n-3 fatty acids, (Ackman, 1982). EPA and DHA are present in much higher concenterations in fish oils than in oils of vegetable origin. Fats from freshwater fish differ from those of marine origin in that they generally contain more C_{16} and C_{18} monoenoic acids and less C_{20} and C_{22} monoenoic acids. Also the ratio of n-3 to n-6 fatty acids is lower in fat from freshwater fish than in marine fish (Gunstone, 1989). Thus fresh-water fish, investigated till now, are less important in supplying the

increased needs for n-3 polyenes. But it cannot be excluded, that new fresh-water fish species will appear in this field. Among them herbivorous fish seem to be a good candidates. Silver carp, for instance have been reported as a good candidate in this respect (Farkas and Roy, 1989)

2.2.1. Lipids in invertebrates and fish of different origin

Fish lipids similar to marine and fresh-water crustaceans are known for the complexity of their fatty acid composition. The lipids of marine and freshwater fish have certain characteristics in fatty acid composition (Lovern, 1951). These distinctions are based mainly on the chain lengths rather than any other fundamental properties. Studies on marine fish (Brockerhoff et al., 1964) have shown that the triglycerides of these animals contain the PUFA mainly eicosapentaenoic 20:5 (EPA) and docosahexaenoic 22:6 (DHA) predominantly bound to the β-hydroxyl group of glycerol.

The most characteristic features in freshwater fish are the higher levels of C_{16} and C_{18} acids and lower levels of C_{20} and C_{22} acids as compared to marine fish (Ackman, 1967). It has been observed that these differences are based mainly on the chain length rather than any other fundamental properties and are due to the dietary fat rather than habitat (Ackman, 1967). Herring, salmon, Baltic herring, turbot and trout are the species most rich in EPA (Puustinen et al., 1985).

The freshwater fish generally contain a lower proportion of n-3 PUFA than marine fish (Monsen, 1985). The lipids of edible proteins of freshwater fish are rich in palmitic (16:0), palmitoleic (16:1 n-7) and oleic acid (18:1 n-9), while the total PUFA is lower in their muscle than in the muscle of marine fish (Kayama et al., 1986). The lipids of cultured

animals have higher levels of n-6 fatty acids and lower levels of n-3 fatty acids and (n-3) / (n-6) ratios as compared with their wild counterparts (Chanmugam et al., 1986). Thus, all freshwater species with the exception of rainbow trout, had n-3 PUFA contents lower than 10-15% while the highest proportion of n-3 PUFA in the marine finfish were found in southern blue whiting (48.5 %) and the lowest in organ roughly was (0.9%), in the liver due to its euryhaline nature tolerating salinity fluctuations (Vlieg and Body, 1988).

In general, all freshwater fish have a fair percentages of AA and linoleic acid as compared with marine fish (Chetty et al., 1989).

The lipid composition of invertebrates on which the aquatic food chain is based is influenced by several factors, including diet, gametogenesis and also environmental temperature. The n-3 polyenoic fatty acids are present in rather high concentrations in the marine copepods calanus sp., (Farkas et al., 1988) as well as in some fresh-water copepods, such as cyclopida and calanida. The fresh-water cladocera, serving also as food for several fish, are rich sources of EPA,but not DHA (Farkas, 1970 b).

2.2.2. Effect of geographical distribution (tropical and cold seas)

Vast numbers of the world's population live in tropical or temperate climates and consume fish not common in colder water. Tropical fish oils have been found to be relatively saturated (Gopakumar and Nair 1972). Interest in the lipids from fish caught in tropical water was stimulated by the recent report by O'Dea and Sinclair (1982) showing that fatty acids in lipid extracts from these fish contain levels of AA (20:4 n-6) ranging from 5 to 14% of the total fatty acids, in addition to the expected n-3 fatty acids.

Fish caught in temperate southern Australian waters are also a rich source of n-6

PUFA (Gibson et al 1984). Malaysian fish generally contain high levels of saturated fatty acids (range 36-55%) and variable amounts of monosaturates, unlike fish caught in cold northern hemisphere waters, which contain AA (range 2-12%) in addition to the expected EPA (range 1-13%) and DHA (range 6.6-40.4%) (Gibson et al., 1984). Gibson (1985) has shown that the fatty acids of many Australian fish, particularly those from tropical waters, contain higher levels of n-6 fatty acids than those of fish from the northern hemisphere. Subsequent investigations confirmed elevated levels of AA in some tropical species (Fogerty et al., 1986). Temperate Australian fish were rich in palmitic acid (range 16.0-25.0%) and DHA (range 15.0-53.0%). There were variable amounts of AA (range 1.0-15.0%) and EPA (range 3.0-23.0 %) (Dunstan et al., 1988). Total lipids of the Norwegian Atlantic halibut (Hippoglossus hippoglossus) were dominated by neutral lipids, which comprised approximately 75% of the lipid fraction, and these fractions contained high concentrations of 18:1 fatty acid, whereas the polar lipid fraction had a high content of n-3 fatty acids 20:5 and 22:6 (Haug et al., 1988). In contrast with cold seawater species, many fish from warm and temperate water contain considerable amounts of n-6 fatty acids (Ackman, 1989). The amount of n-6 fatty acids was greater than in marine fish from cold water. In contrast n-3 fatty acids were the major PUFA in fish species from the Persian Gulf (Agren et al., 1991).

2.2.3. Environmental temperature and lipids

One of the most important factors affecting metabolism and composition of lipids in fish is the environmental temperature. Some of fish live under changing thermal conditions. Temperature may changing diurnally, seasonally, or suddenly within the same day-time. But even those fish living under constant thermal conditions are exposed to variation of

temperature for instance during vertical migration.

Seasonal acclimatization of eurythermal ectotherms involves compensatory responses to changes in temperature. These responses are aimed at maintaining functional integrity and proper physicochemical properties of biomembranes. Several studies on fish have demonstrated a clear seasonal variation in fatty acid composition and physical state of membrane lipids as revealed recently by Hazel (Hazel, 1984). However, fatty acid compositions and extent of response with fluidity to temperature may change from tissue to tissue or membrane type to membrane type. Cossins and Prosser, (1978) have shown that sarcoplasmic reticulum dose not respond to thermal variation while mitochondria gave the most pronounced response in the same fish. Sardinella longiceps responded with an increase in the level of DHA and a decrease in the saturated fatty acids when the temperature fell from 30°C to 25°C from summer to winter (Gopakumar and Nair, 1973).

At the level of cell metabolism, temperature-related changes were found in the synthesis of fatty acids, the ascorbic acid content of the blood and renal tissues and membrane phospholipids (Farkas and Csengeri, 1976; Wodtke, 1981a).

The inhibition of the immune response to low temperatures in poikilothermic was suggested by Nybelin (1968), or blockage of specific single or multiple points in the afferent or efferent immune system. Most warmwater fish have an optimal immune response at temperatures between 20°C and 30°C, whereas coldwater species respond best at temperatures between 10°C and 15°C (Avtation, 1969).

Abruzzini et al., (1982) showed that in pike (Lagodon rhomboides) temperature changes affect the fluidity of the lymphocyte plasma membrane, leading to the conclusion that the process of homoviscus adaptation first described by Sinensky (Sinensky, 1974) for E.coli

of these membranes may be an important regulatory aspect of immunity.

Several considerations suggest that lipids or fatty acids may be transferred seasonally, not only from somatic to ovarian tissue, but also from neutral lipid to polar lipid compartments (Nelson and Mcpherson, 1986). If north temperate fish do accumulate significant amounts of polar lipids in their ovaries and muscle over winter, and concurrently deplete them from somatic tissues, then seasonal transfers of fatty acids may occur between neutral lipids and polar lipids. Furthermore, PUFA may be preferentially more abundant in polar lipids than in neutral lipids (Henderson and Tocher, 1987). There do not appear to be large seasonal transfers of fatty acids from somatic tissues (liver, muscle and adipose tissue) to ovarian tissues or from neutral to polar lipids in female pike (Schwalme and Mackay, 1992).

2.2.4. Adaptation of fish to different environmental temperatures

The environmental temperature is an important factor in the life of plants and animals. Over 20 years ago it was known that in fish, subjected to different environmental temperatures, many alterations occur in their fatty acid metabolism; there is an increase in the ratio of PUFA to saturated fatty acids in membrane lipids in response to lowered ambient temperature. Kayama and Tscuchiya (1962) found that the lipids of guppy (Lebistes reticulatus) contained higher percentages of palmitic and stearic acids at high temperature than at low temperature, whereas the levels of palmitoleic, oleic and docosahexaenoic acids showed an opposite picture.

The results of Johnston and Roots (1964) suggested that prolonged cold exposure leads to an accumulation of long-chain PUFA in the phospholipids of fish. Knipprath and

Mead (1966) observed that muscle and liver lipids from rainbow trout (Salmo gairdneri) contain more PUFA at low temperature than at higher temperature.

The fluid properties of membrane lipids are known to affect a variety of cellular functions (Singer, 1974) and there is a growing body of evidence which suggests membrane fluidity control. Since the physical properties of membrane lipids are strongly temperature-dependent, a useful approach to the study of the biological roles of membrane fluidity and its regulation is to examine the effects of temperature acclimatization on membrane lipid components. Many organisms are known to respond to changes in environmental temperature by changing the composition of their membrane lipids and, in fact, this appears to be a primary mechanism for the regulation of membrane fluidity (Sinensky, 1974).

In previous studies of the temperature acclimatization of membrane lipids in Tetrahymena, it was found that membrane fluidity is regulated primarily through the activity of phospholipid fatty acid desaturation (Fukushima et al., 1976). Wodtke (1978) found that the phospholipid fatty acid from liver mitochondria in carp acclimatized at a high environmental temperature (32°C) had a lower amount of n-3 and a lower ratio of unsaturated fatty acids, but a higher amount of n-6 monounsaturated fatty acids. At low acclimatization temperature there was a decreased ratio of the linoleic to the linolenic family. Further, Schünke and Wodtke (1983) reported that at low acclimatization temperature (10°C) the ratios of phospholipid/protein were identical in the rough and the smooth endoplasmic reticulum of carp liver microsomes, but that at a high acclimatization temperature (30°C) the ratio of phospholipid/protein was lower and the activities of glucose-6-phosphatase, cytochrome b_5 and cytochrome P-450 were higher. It was also suggested that the unsaturated fatty acids are the major determinants of the membrane fluidity in fish (Cossins, 1983).

An increased acyl chain unsaturated in cell membrane phospholipids is one mechanism that has been identified as an adaptation to lower environmental temperature in fish (Hazel, 1984). Hazel and Carpenter (1985) showed rapid changes in the phospholipid composition of rainbow trout gill tissue acclimatized at 5°C and 20°C for a period up to 28 days.

The metabolic reactions involved in the changes in fatty acid composition of phospholipids in poikilothermic organisms acclimatized to extreme temperature have not been completely elucidated. Recently, a temperature-controlled metabolic heterogeneity of the molecular species of different phospholipids has been observed in trout (Hazel and Zerba, 1986; Abdul Malak et al., 1989), suggesting a significant difference in the level of 20:5 n-3 fatty acid between gill, kidney, intestine and liver PC in rainbow trout acclimatized to 11°C and 21°C; these differences correlate with a modification of the molecular species distribution in the PC in these organs. The lower incubation temperature decreased the relative net incorporation of all the ^{14}C-labelled PUFA into PC and increased the relative incorporation of the PUFA into the other phosphoglycerides, especially PE.

2.2.5. Composition of lipids in relation to feeding habit

The precursors of n-3 and n-6 long-chain PUFA are formed by the members of phytoplankton. Animals cannot synthesize these fatty acids, but elongate and desaturate them when taken-up with the food (Spielmann et al., 1988). The capacity to form PUFAs varies from species to species.

One of the most studied areas is the effect of the food quality on the lipid composition and metabolism of different fish species. Csengeri et al., (1978) found that the fatty acid

composition of the food was reflected in the tissue fatty acid composition in common carp and silver carp. At the same time, Csengeri et al., (1978 b) found that the fatty acid composition and fat content of the diet influence the formation of fatty acids in carp liver. The main sources of plant material in the diet of herbivorous fish are most likely to be the green algae, diatoms and higher plants. Among fish feeding on higher plants, (grass carp), the digestion efficiency varies greatly. Herbivorous fish generally have a low digestion efficiency when fed natural diets as compared to carnivorous fish (Brett and Groves, 1979).

The n-3 PUFA such as EPA and DHA are passed through the pelagic food chain from the phytoplankton to accumulate in higher order carnivores (Sargent and Whittle, 1981). Rady and Matkovics (1984) studied the effect of the feeding habits on the brain lipid metabolism. It was found that there are differences between the brain lipid fractions; their fatty acid compositions depend on the fish species and diet: herbivorous fish have a higher level of 22:6 n-6 than omnivorous fish. Tocher and Sorgent (1984) carried out experiments on the ripe roes of marine fish. The results indicated that the highest total lipids and neutral lipids were found in the sand eel and capelin, and the highest polar lipids in the herring, cod, haddock, whiting, saithe, sand eel and capelin. The omnivorous species of bony fish contain more AA (mean 10%) than the carnivorous bony fish (mean 5%) (Dunstan et al., 1988). Grass carp readily elongated and desaturated dietary linolenic acid into chain PUFA (Cai and Curtis, 1989).

The highest food consumption, growth rate and n-3 fatty acid (especially 18:3 n-3) concentrations in muscle and liver occurred in grass carp fed the aquatic plants Elodea (Cai and Curtis, 1990).

2.2.6. Lipids in fish in relation to aging

Study of the aging process may be pursued in a wide variety of fields, ranging from the chemical, to the social, and from a consideration of those changes associated with the development of the very young to the degenerative changes that become dominant in the very old. Of particular interest to the biochemist is molecular aging, that is the irreversible changes occurring with time in molecules that ordinarily have a very low turnover rate. It seems evident that these changes are of paramount importance in the degenerative processes since the molecules involved are necessarily those that ensure the continuity of the tissue and the organism.

Very little biochemical information is available in the area of development of the membrane of mammalian tissue, and this situation is especially true for fish. Dallner et al., (1965) studied the development of rabbit liver microsome membrane through assessment of the composition of protein and non-protein components, as well as by the study of the activities of various microsomal enzymes during development. Dallner et al. (1965 b) investigated the fatty acid compositions of rat liver microsomal total lipids at the ages of 0 days, +5 days and +90 days, and experienced large deviations between the three ages studied. Miller and Cornatzer (1966) showed a progressive increase in concentration (μg phospholipid phosphorus/mg protein) of microsomal (PC), (PE) and phosphatidyl inositol (PI) during development (-12, -9, 0, +2, +9 and +14 days of age). Baldwin and Conatzer, (1969) reported that palmitoleic acid demonstrates a uniform decrease during early development in the total lipids and both PC and PE, but in the 112-day animal the amount is only slightly lower than that observed for the earliest prenatal animal studied.

In general, the total lipid content decreases gradually during embryogenesis and in

particular during larval development, and lipid is utilized from both polar and neutral fractions throughout the development period (Tocher et al., 1985). It has also been found that, throughout all the development period of herring (before fertilization, after fertilization, and during subsequent embryonic and early development), the fatty acid composition of the total lipids remains essentially constant (Tocher et al., 1985). The accumulation of 20:4 n-6 and 22:6 n-3 during development was noted in adult fish (Pagliarani et al., 1986), rats and monkeys (Neuringer and Connor, 1986), mammalian brain (Anderson and Connor, 1988) and chickens (Anderson et al., 1989). The amount of DHA was much higher than that of EPA, especially in older fish, and the ability to modify the fatty acids develops with age in Vendace (Muje et al., 1989).

It was found that the egg dry mass, lipid and carbon content all decreased during development as reserves were utilized, and at the same time both the water and mineral ash content increased (Clark et al., 1990). In the total lipids, the saturated fatty acids generally decreased and the monounsaturated fatty acids increased from larvae to adults, but the proportions of PUFA in individual phosphoglycerides were generally highest in the juvenile stage and lowest in adult fish (Mourente and Tocher,1992).

MATERIALS AND METHODS

3.1. Collection of fish

The following animals were used in this study:

Species	Origin	Temperature
Marine fish		
Mugil cephalus	North Egypt	20-25°C
Clupea harengus	Baltic Sea	5-7°C
Fresh-water fish		
Oreochromis niloticus	River Nile Egypt	20-25°C
Acerina cernua	North Finland	7-10°C
Esox lucius	Hungary	25°C
Cyprinus carpio	Hungary	20-22°C
Hypothalmichthys molitrix	Hungary	20-22°C
Silrus glanis	Hungary	20-22°C
Sprague Dawley rats (200-250 gm)	E F Fa Credo,L, Arbreste, France	

For the experiments, fish were collected from their natural habitats, the water temperature varied according to the season.

The animals used in the experiments were somatically healthy. No external or internal parasites were found during veterinary investigation.

3.1.1. Warm adapted fish

Marine fish (Mugil cephalus) was collected on the north coast-line of the Mediterranean Sea over an extent about 40 km. The freshwater fish, Tilapia (Oreochromis niloticus L.), the most common species in the inland fisheries of Egypt, was collected in monthly samples from March 20, 1992. The water temperature was near 19°C in both cases;

the surface temperature was 24°C. Liver tissues were fixed in chloroform-methanol (2:1 v/v) containing 0.01% BHT and frozen until transferred to the laboratory.

3.1.2. Cold adapted fish

Marine fish (Clupea harengus) was collected from Baltic Sea and the freshwater fish (Acerna cernua) was collected on the north of Finland on March 20, 1991. The water temperature was 7°C-10°C. The liver tissues were fixed in chloroform-methanol (2:1 v/v) containing 0.01 % BHT and frozen until transferred to the laboratory.

3.1.3. Environmental temperature (seasonal variation)

1.2 - 1.7 kg sexually mature pike (Esox lucius L.) were collected from different fish ponds of the Fishery Research Institute at Szarvas. The water temperature in these ponds varied between 2-3°C and 25-30°C in winter and summer respectively. The animals were brought to the laboratory alive and killed immediately.

3.1.4. Adaptation of fish to temperature (under experimental conditions)

In other experiments 1.2 -1.7 kg winter acclimatized pike (Esox lucius L.) (obtained from the Fishery Research Institute at Szarvas) were kept in a thermostated aquarium supplied with aerated recirculated water in a laboratory at 25°C. The water temperature was lowered by 0.5°C/hr from 25°C to 5°C. During acclimatization to the warm 25°C ± 0.5°C and cold 5°C ± 0.5°C temperatures, a constant photoperiod (15 hr of light) was maintained.

3.1.5. Relation of feeding habit to fatty acid composition

Sexually mature common carp (Cyprinus carpio L.) weighing 500-700 g, as omnivorous fish, silver carp (Hypothalmichthys molitrix) weighing 1200-1500 g, as herbivorous fish, and cat fish (Silrus glanis L.) weighing 1000-1200 g, as carnivorous fish, were collected from their natural habitats around the Fisheries Research Institute at Szarvas, Hungary. The actual temperature at the time of collection was 18°C. The animals were brought to the laboratory alive in nylon bags, under continuous aeration, and acclimatized for at least 7 days in well-aerated tap-water at 19-20°C.

3.1.6. Fatty acid composition in relation to aging

Carp (Cyprinus carpio L.) of different ages, 1, 2 and 3 years, weighing 50 g, 200 g and 800 g, respectively, were obtained from a nearby fish farm (Fehértó, Szeged, Hungary) on May 10, 1992. The water temperature in these ponds was 19°C ± 1°C.

The animals of one year were not separated as to sex, because the sex organs had not become active. The livers were rapidly excised and weighed, and the gall bladders were removed as soon as possible.

3.2. Maintenance of fish

Carp and pike were maintained in well-aerated tap-water at the experiment temperature of 18-20°C for at least 7 days before the experiment begin, and they were starved until utilization.

3.3. Collection and preparation of tissues

The animals were killed by vertebral rupture, and their organs were rapidly removed and cleaned from accessory connective and adipose tissues and washed with Tris-sucrose solution to remove the blood. The wet weights of the tissues were measured.

3.4. Lipid analysis

3.4.1. Extraction of total lipids

Liver tissue was collected from treated and control fish. The total lipids were extracted according to Folch et al. (1957). The tissue was homogenized with a 2:1 /v/v/ chloroform - methanol mixture to a final volume 20 times the weight of the wet tissue sample.

The homogenization was carried out in a Potter-Elvehjem or a Biomix homogenizer (MTA, KUTESZ, Budapest, Hungary). About 3 min was sufficient for complete homogenization. The homogenate was filtered through a fat-free paper into a glass-stoppered vessel.

Washing of crude extract. The crude was mixed thoroughly with 20% of its volume of either water or an adequate solution (0.1 M KCl), and the mixture was allowed to separate into two phases, without interfacial fluff, either by standing or by centrifugation. As much of the upper phase as possible was removed by aspiration, and removal of the solutes was completed by washing the interface several times with small amounts of chloroform:methanol:water (50:50:1) in such a way as not to disturb the lower phase.

Finally, the lower phase was stored, after bubbling N_2 gas or CO_2 into the lipid solvent and the further addition of 0.01% BHT as antioxidant, to prevent peroxidation of lipids and fatty acids. It was stored in a sealed tube at -25°C in a deep freezer until assayed.

3.4.2. Separation of phospholipids

Phospholipid pools were purified by silicic acid column chromatography, using chloroform to elute neutral lipids and methanol for phospholipids (Leray et al., 1987).

3.4.3. Separation of phospholipid subfractions

Phospholipids were subfractionated by thin-layer chromatography (TLC) according to Fine and Sprecher (1982) on pre-coated G-60 Silica gel-TLC plates (F254, E. Merck, Darmstadt, Germany), with a mixture of chloroform : methanol : petroleum ether : acetic acid : boric acid (40:20:30:10:1.8) (v/v) as mobile phase. After development, the plates were dried in a stream of CO_2 and lipid classes were identified by spraying with a solution of 8-anilinonaphthalino sulphate (ANS) in 50% methanol. In other cases the air-dried chromatogram was exposed to iodine vapours for visualization of the different PL subfractions such as LPC, SM, PC, PS, PI, PE, PG, PA and CL, respectively. The spots were identified using authentic standards (Serdary Res. Lab. Bellefonte Cal. USA.) The spots were detected under UV at 254 nm and removed for quantitative determination of phosphorus according to Rouser et al. (1970). At first they were digested using 0.5 ml HCL for 15 min at 180°C and then 0.6 ml $HCLO_4$ was added and kept at 180°C for 60 min. Reaction was stopped by adding 3 ml distilled water to it. After that 0.5 ml of 2.5% ammonium molybdate was added to it followed by addition of 0.5 ml of 10% ascorbic acid. The mixture was

shaken thoroughly and incubated in a water bath at 100°C for 5 minutes. Absorbance was measured at 790 nm and phosphorus content was calculated in accordance with the standard.

3.4.4. Determination of fatty acid compositions

The spots were transferred into screw capped tubes and transferred in the presence of dry methanol containing 5% HCl at 80°C under CO_2 or N_2 for 2.5 hrs.

The methyl esters of the fatty acids were extracted from the esterification mixture after diluting it with an equal volume of distilled water and petroleum ether. The petroleum ether phase was removed in a small conical tube and the solvent was evaporated under CO_2. The methyl ester residue was then taken up in a small amount of benzen.

3.4.5. Gas chromatography of fatty acid methyl esters

Segregation of the fatty acids was performed on 10% FFAP on 80-100 mesh Supelcoport (Supelco, Bellefonte PA) in a 2 m long column (2 mm i.d.). A Hitachi model 263-80 gas chromatograph connected to a Hitachi model 263-80 data processor was used for analysis.

Methyl esters were also separated using a Hewlett-Packard 5890 II equipped with a capillary column coated with SP 2330 of 0.25 m thickness (0.25 mm I. D. x 30 mi CPS-Li Quadrex, New Haven, CT,U.S.A.). High-purity nitrogen was applied as carrier gas with a flow rate of 230 KPa. Hydrogen was used at 100 KPa and 280 KPa. The dual column system was programmed from 160°C to 200°C to give partial separation of 18:3 (n-3) and 20:1 (n-9) at a rate of 1°C/min. The detector temperature and injector temperature were 250°C and 230°C, respectively. The peaks were identified by means of primary and

secondary standards and by plotting log relative elution temperature versus the number of carbon atoms (Schmit and Wynner, 1966). The percentage composition was calculated as a weight percentage (w/w %) using a Hewlett-Packard 3396 A integrator. All peaks between myristic acid (14:0) and DHA (22:6 n-3) were include in the calculations.

3.4.6. Separation of molecular species, quantitation by high performance liquid chromatography (HPLC)

200-300 μg of phospholipids was resolved into phospholipid classes by TLC on silica gel HR using a solvent chloroform : methanol : distilled water [65:25:4 (v/v)] (Anderson et al., 1969). PE and PC were visualized by spraying the plate with ANS as described before. Spots on the chromatoplate corresponding to PC and PE were scraped from the plate and BHT (250 μg) was added to the scrapings as antioxidant (Miljanch et al., 1979). The phospholipid classes were then extracted from the gel scrapings with two washing of 5 ml of chloroform-methanol (1:1 v/v), followed by one wash with 5 ml of chloroform-methanol-water (65 : 45 : 12) and once more with 5 ml of chloroform-methanol (1/1 v/v) (Louie et al., 1988). The combined washes were subsequently evaporated under an inert atmosphere of N_2 in rotatory evaporator.

The individual phospholipid classes were hydrolysed with phospholipase C from Bacillus cereus for PE and from Clostridium perferinges for PC Sigma Chemicals, St. Louis, MO) by a modification of the method of Takamura et al. (1986). The purified phospholipid class was dispersed by bath sonication in 1.25 ml of 10 mM Tris-HCl (pH 7.5) containing 30 mM H_3BO_3 and 10 ml $CaCl_2$. Sixty units of phospholipase C and 2 ml of diethyl ether were added to the mixture and vortexed. Complete hydrolysis was achieved by maintaining

the suspension overnight at room temperature under constant agitation by magnetic stirring. Hydrolysis at higher temperature was avoided since PUFA are susceptible to oxidative degradation. The aqueous phase was extracted twice with diethyl ether and the combined ether extracts were dried under argon or N_2.

Preparation of dinitrobenzoyl derivatives:

The benzoate derivatives of diacylglycerols were prepared immediately by a modification of the procedure described by Blank et al., (1984). The diacylglycerol thus prepared and 25 mg of dinitrobenzoyl chloride (DNBC) were dried for 30 min in a desiccator. The mixture was dissolved in 0.5 ml of dry pyridine and heated in a sealed vial at 60°C for 10 min. Then 0.5 ml of distilled water was added, and the solution was heated at 60°C for a further 10 min. After 2.0 ml of 0.1 N HCl had been added, the product was extracted three times with 1.5 ml of n-hexane. The combined extracted was dried under a N_2 flow to remove pyridine, the residue was dissolved in 2.0 ml of n-hexane, and this solution was washed with equal volumes of 0.1 N HCl (three times), 0.1 N $NaHCO_3$, and 1 N NaCl. Then 2.0 ml of distilled water was added and the product was extracted with 2.0 ml of n-hexane. The diacylglycerol-benzoates were separated into subclasses (alkenylacyl, alkylacyl and diacyl) by TLC on silica gel HR in a solvent system of toluene-hexane-diethyl ether (50 : 45 : 4 by volume). After solvent development, the chromatoplate was visualized by UV light. The diacylglycerobenzoate (DGB) fraction was scraped from the plate into 2 ml of chloroform-methanol (3:1 v/v). The solvent was evaporated in vacuum, the DGB were redissolved in a known volume of hexane, and an aliquot was taken for determination.

Separation of DGB derivatives was made by high-performance liquid chromatography HPLC (Water associates, model 440) on a Nucleosyl C-18 column

(5 μm, 4 mm i.d. X 250 mm) using acetonitrile-2-propanol (80 : 20 v/v, of HPLC grade Carlo Erba, Milano, Italy) as mobile phase with a (flow rate of 1.0 ml/min pressure, 54 kg/cm^2, temperature, 25°C). Samples were dried in a N_2 flow to remove n-hexane, then residue was dissolved in acetonitrile, and a 20 : 100 μl aliquot of the solution was loaded to the liquid column (using a 20 μl loop). The peaks were recorded at 254 nm and calculated using a data processor (Hitachi, Model 263-80) connected with HPLC.

Identification of molecular species was accomplished by using authentic standards 16:0 / 22:6, 18:0 / 22:6, 16:0 / 20:4, 18:0 / 20:5, 16:0 / 18:1, 18:1 / 18:1 and 18:0 / 18:1, as well as on the basis of their relative retention time of Blank et al. (1984).

3.4.7. Fluorescence anisotropy measurements

The fluorescent probes used was 1,6-diphenyl-1,3,5-hexatriene (DPH; Sigma Chemical Co., St. Louis, Mo) as described by Montaudon et al. (1984) one μl of the DPH solution 4 X 10^6 mM in tetrahydrofurane was added to a chloroform solution of 250 μg phospholipid. The chloroform was carefully removed in vacuum. 4 ml of tris HCl buffer, pH 7.4, was added to the phospholipid film and the mixture was vortexed for 5 minutes followed by sonication in a both type sonicator for 20 minutes. The resulting vesicle suspension was used for the measurements.

Steady-state fluorescence anisotropy polarization was measured with a computer-controlled thermostatable Hitachi MPF-2A spectrophotofluorimeter. The sample was excited with vertically polarized light at 366 nm with a slit width of 12 nm, and the vertically ($\mathbf{I_v}$) and horizontally ($\mathbf{I_h}$) polarized emitted light was read at 430 nm using a slit width of 18 nm. The standard error in separate determinations was always less than 0.005. Fluorescence

anisotropy was calculated according to the formula:

$$R_{ss} = I_{(vv)} - Z \bullet I_{(vh)} / I_{(vv)} + 2Z \bullet I_{(vh)}$$

where $Z = 0.92$, calculated according to the formula $Z = I_{(vh)} / I_{(vh)}$, in which vv and vh are the fluorescence intensities measured with the emission analyzer parallel or perpendicular, respectively, to the polarization of the detection system for vertically and horizontally polarized light. These measurements were based on the work of Kuhry et al. (1985), who showed that both the fluorescence intensity and steady-state anisotropy (R_{ss}) were influenced by the scatter in such a way that :

$I_{(measured)} = I_{(fluorescence)} + I_{(catter)}$ and

$R_{s(measured)} = f R_{s(fluorescence)} + (1 - f) . R_{s(scatter)}$ where

f (a balanced fluorescence intensity factor) $= I_{fluorescence} / I_{fluorescence} + I_{scatter}$.

Higher values of anisotropy represent more ordered, less fluid structures.

3.6. Chemicals

Silica gel Go TLC plates were obtained from E. Merck (Darmstadt, F.R.G.). Sphingomyelin (SPH), phosphatidyl inositol (PI), and cardiolipin (CL) were purchased from Koch-Light Laboratories (Colnbrook, Bucks, U.K.) 1-Palmitoyl-2-oleoyl-phosphatidyl-choline (POPC), 1-Palmitoyl-2-oleoyl-phosphatidyl-ethanolamine (POPE), dioleoyl phosphatidyl ethanolamine (DOPE), phosphatidyl serine, and lysolecithin were obtained from Sigma Chemicals Co. (St. Louis, Mo). Authentic fatty acid methyl esters were purchased from Sigma Chemicals Co. (Supelco Inc. Bellefonte, AP). Methanol, chloroform, acetonitrile

(HPLC grade), 2-propanol (HPLC grade), diethyl ether, light petroleum (b.p. 35-60°C) and n-hexane were obtained from SDS (Peypin, France). Phospholipase C X III (from Bacillus cereus) was obtained from Boehringer Mannheim (Mannheim,F.R.G.), 1,2-dipalmitoyl-sn-glycerol, 1,2-dioleoyl-sn-glycerol, 1-steroyl 2-arachidonoyl-sn-glycerol from Sigma (St. Louis, Mo, USA), 3,5-dinitrobenzoyl chloride (DNBC) from Dojindo Laboratories (Kumanato, Japan), pyridine (silylation grade) from Pierce Chemical Co. (Rockford, Illinois), diphenylhexatriene (DPH) and series of n-(9-anthroxyloxy) stearic and palmitic acids were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL).

All other chemicals were of reagent grade.

3.7. Statistical analysis

Differences in the fatty acid compositions of tissue phospholipids were evaluated by using the Student t-test. Acyl indexes were calculated from the fatty acid composition data, and the values obtained were subjected to analysis of variance followed by determination of significant differences and multiple regression.

RESULT AND DISCUSSION

4.1. Fatty acid composition of rat and fish

Total lipids and lipid fractions (PL, NL, PC and PE) of the liver tissue of fish and rats were analyzed by gas liquid chromatography (GLC) and capillary chromatography to determine the quantitative and qualitative differences between the two animals. Figures 1 a and b show representative GLC tracings of fatty acids from fish and rat liver PL.

4.1.1. Fatty acid composition of fish

Fatty acids originating either from polar lipids (PL) or from neutral lipids (TG) of a fish (pike) and rat are compared in Tables 1 and Fig. 2.

Generally, most of the fatty acids seemed to be unsaturated, belonging to the n-6 and n-3 families. The most abundant fatty acids appeared to be palmitic (16:0), oleic (18:1 n-9), AA (20:4 n-6), EPA (20:5 n-3) and DHA (22:6 n-3). The n-3 fatty acids predominated in all of the fish species investigated, comprising almost 50% of the total polyenes. Of these fatty acids, 22:6 n-3 comprised (10-30%) of the total. There were clear differences between the different fish species. 20:5 n-3 was the second most abundant PUFA in almost all species. Carp was an exception in this respect.

With regard to n-6 PUFA, fish contained more than 10% of total fatty acids. The major n-6 fatty acid present was AA, the rest of the n-6 series including 18:2, 20:2, 20:3 and 22:4.

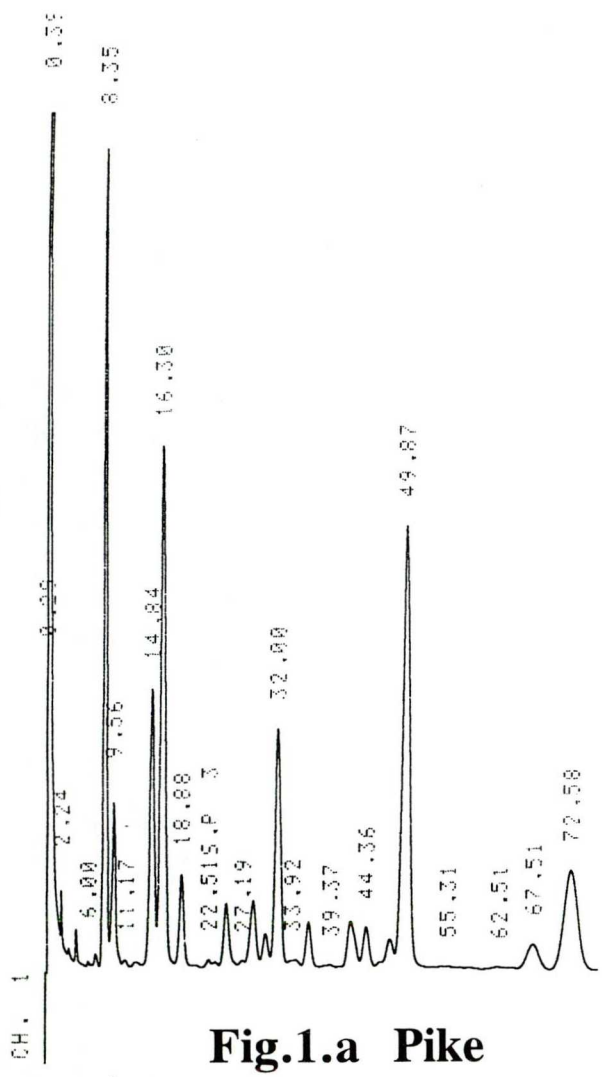

Fig.1.a Pike

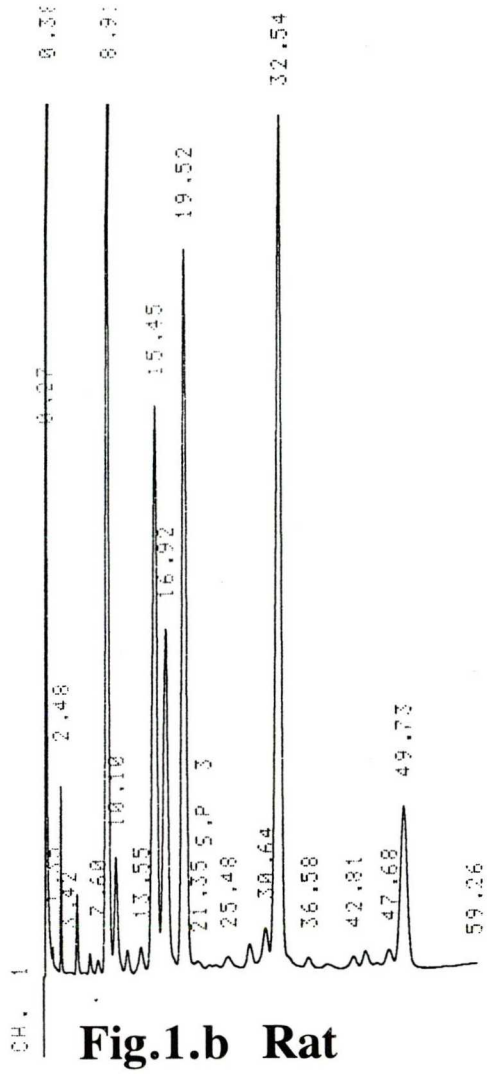

Fig.1.b Rat

Separation of liver phospholipid fatty acids of pike and rat by gas liquid chromatography

Retention time	Fatty acid	Retention time	Fatty acids
8.35	16:0	8.91	16:0
9.56	16:1 n-7	10.10	16:1 n-7
14.84	18:0	15.45	18:0
16.30	18:1 n-9	16.92	18:1 n-9
18.88	18:2 n-6	19.52	18:2 n-6
22.51	18:3 n-3	22.93	18:3 n-3
32.0	20:4 n-6	32.54	20:4 n-6
36.34	20:5 n-3	36.58	20:5 n-3
49.87	22:6 n-3	49.73	22:6 n-3

Table 1

Comparative investigation on liver fatty acid compositions of rats (homoiothemic animals) and pike (poikilothermic)

Fatty acid	TL		NL		TPL		PC		PE	
	Rat	Pike	Rat	Pike	Rat	Pike	Rat	Pike	Rat	Pike
14:0	0.96 ± 0.24	1.46 ± 0.25	1.17 ± 0.16	1.97 ± 0.22	0.77 ± 0.17	1.30 ± 0.19	0.50 ± 0.18	0.85 ± 0.19	0.62 ± 0.07	0.66 ± 0.49
16:0	21.64 ± 2.06	18.72 ± 2.03	25.09 ± 1.87	20.87 ± 2.20	19.05 ± 1.42	22.48 ± 1.10	18.99 ± 2.91	27.44 ± 0.27	19.49 ± 1.69	15.40 ± 3.55
16:1 n-7	3.59 ± 1.31	10.75 ± 2.79	11.94 ± 2.60	9.98 ± 0.83	2.22 ± 0.71	9.46 ± 0.30	3.15 ± 1.25	7.20 ± 0.51	1.00 ± 0.01	2.80 ± 2.35
18:0	9.91 ± 2.08	3.75 ± 1.62	3.03 ± 0.76	3.88 ± 0.44	16.21 ± 2.03	2.87 ± 0.29	11.07 ± 0.23	1.77 ± 0.40	16.18 ± 1.40	3.36 ± 1.04
18:1 n-9	9.32 ± 0.60	17.15 ± 3.48	22.31 ± 0.58	17.49 ± 3.57	7.17 ± 1.50	13.90 ± 0.83	8.09 ± 0.67	11.37 ± 0.67	6.51 ± 1.78	10.42 ± 1.59
18:2 n-6	21.55 ± 1.40	8.67 ± 2.99	25.97 ± 5.67	8.53 ± 1.46	16.25 ± 2.03	6.40 ± 1.69	23.66 ± 4.11	5.54 ± 0.96	10.54 ± 1.58	4.55 ± 1.05
18:3 n-3	0.14 ± 0.09	2.90 ± 0.39	0.26 ± 0.28	3.52 ± 0.55	trace	2.32 ± 0.12	trace	2.45 ± 0.28	trace	1.29 ± 0.14
18:4 n-3	0.26 ± 0.11	0.82 ± 0.24	---	0.76 ± 0.43	0.30 ± 0.17	0.56 ± 0.12	0.38 ± 0.10	0.55 ± 0.13	0.31 ± 0.10	---
20:2 n-6	0.36 ± 0.24	0.65 ± 0.23	0.39 ± 0.18	0.42 ± 0.08	0.44 ± 0.21	0.46 ± 0.13	0.57 ± 0.16	0.14 ± 0.00	0.46 ± 0.18	---
20:3 n-6	0.67 ± 0.17	0.71 ± 0.26	0.35 ± 0.06	0.55 ± 0.02	0.83 ± 0.27	0.39 ± 0.10	0.62 ± 0.19	0.24 ± 0.00	0.42 ± 0.04	0.13 ± 0.00
20:4 n-6	23.58 ± 1.57	5.86 ± 1.25	4.24 ± 1.07	6.75 ± 0.86	26.19 ± 2.34	6.12 ± 0.42	26.03 ± 2.43	4.19 ± 0.42	30.81 ± 0.44	9.81 ± 2.25
20:5 n-3	0.39 ± 0.17	5.55 ± 1.36	0.61 ± 0.08	5.86 ± 0.87	0.41 ± 0.26	5.37 ± 0.66	0.52 ± 0.32	5.37 ± 0.66	0.37 ± 0.02	9.74 ± 0.15
22:4 n-6	0.33 ± 0.03	1.12 ± 1.49	---	0.54 ± 0.47	0.66 ± 0.20	0.41 ± 0.35	0.10 ± 0.03	0.18 ± 0.13	0.52 ± 0.10	0.82 ± 0.38
22:5 n-3	0.54 ± 0.15	2.14 ± 1.12	0.31 ± 0.12	1.77 ± 0.56	0.70 ± 0.23	1.51 ± 0.07	0.39 ± 0.11	1.55 ± 0.54	1.13 ± 0.28	1.35 ± 0.09
22:6 n-3	5.57 ± 0.53	18.16 ± 3.20	2.63 ± 1.39	16.91 ± 1.20	6.63 ± 0.75	25.65 ± 1.18	5.87 ± 0.40	30.86 ± 0.91	11.32 ± 0.59	39.99 ± 2.05

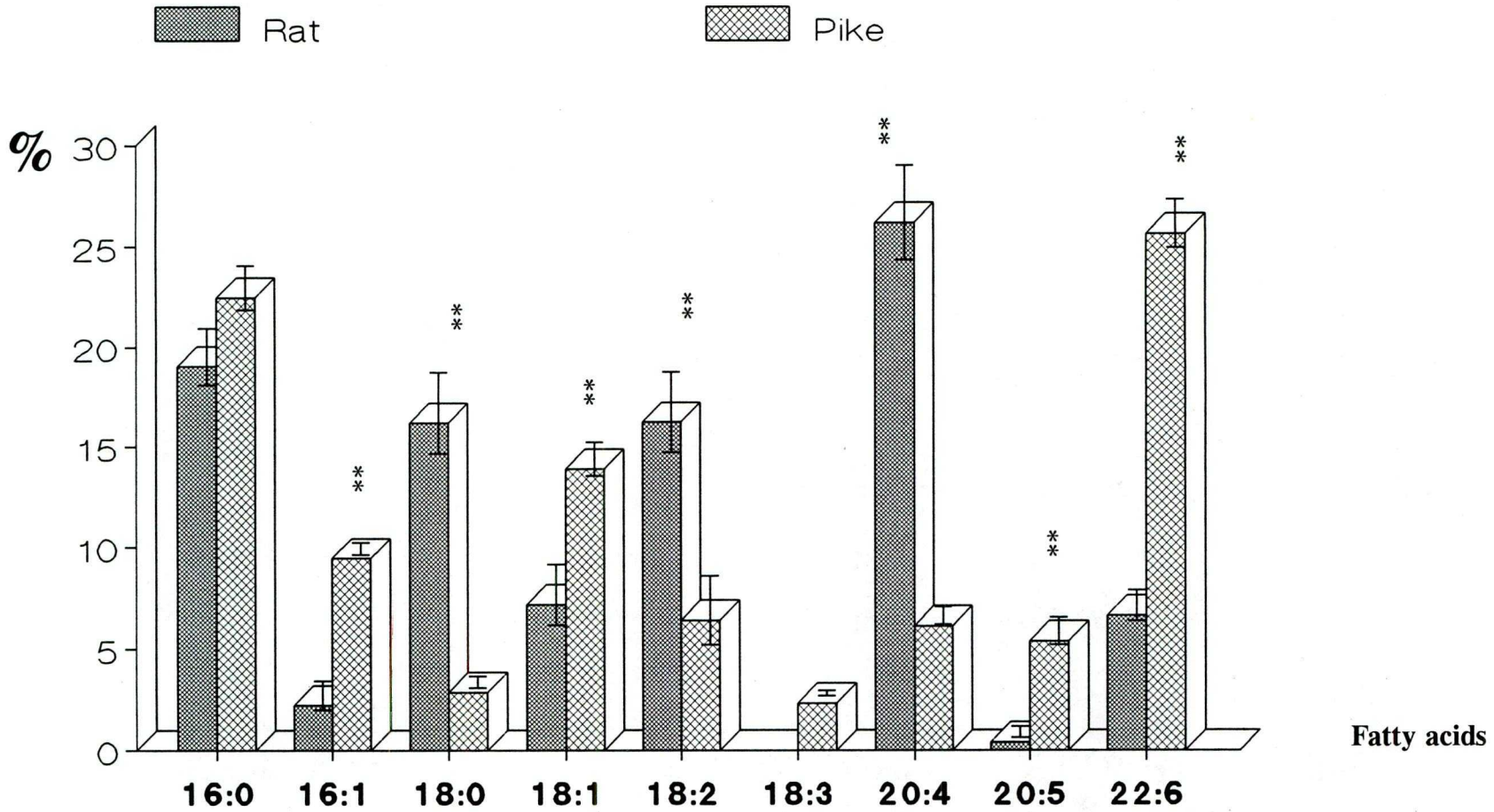

Figure 2 **Comparative investigation on liver fatty acid compositions of total phospholipids of rats (homoiothemic animals) and pike (poikilothermic) [N = 3]** * $P < 0.01$ ** $P < 0.001$

Figure 2.a

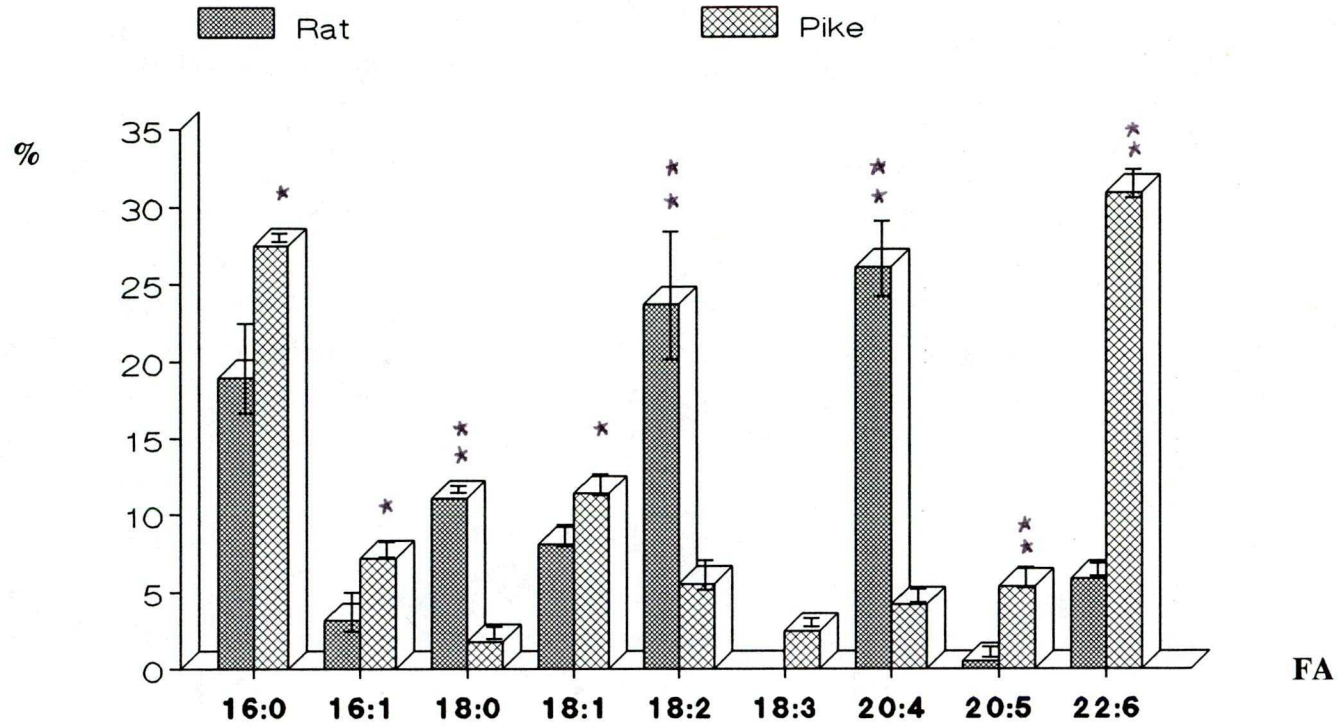

Figure 2.b

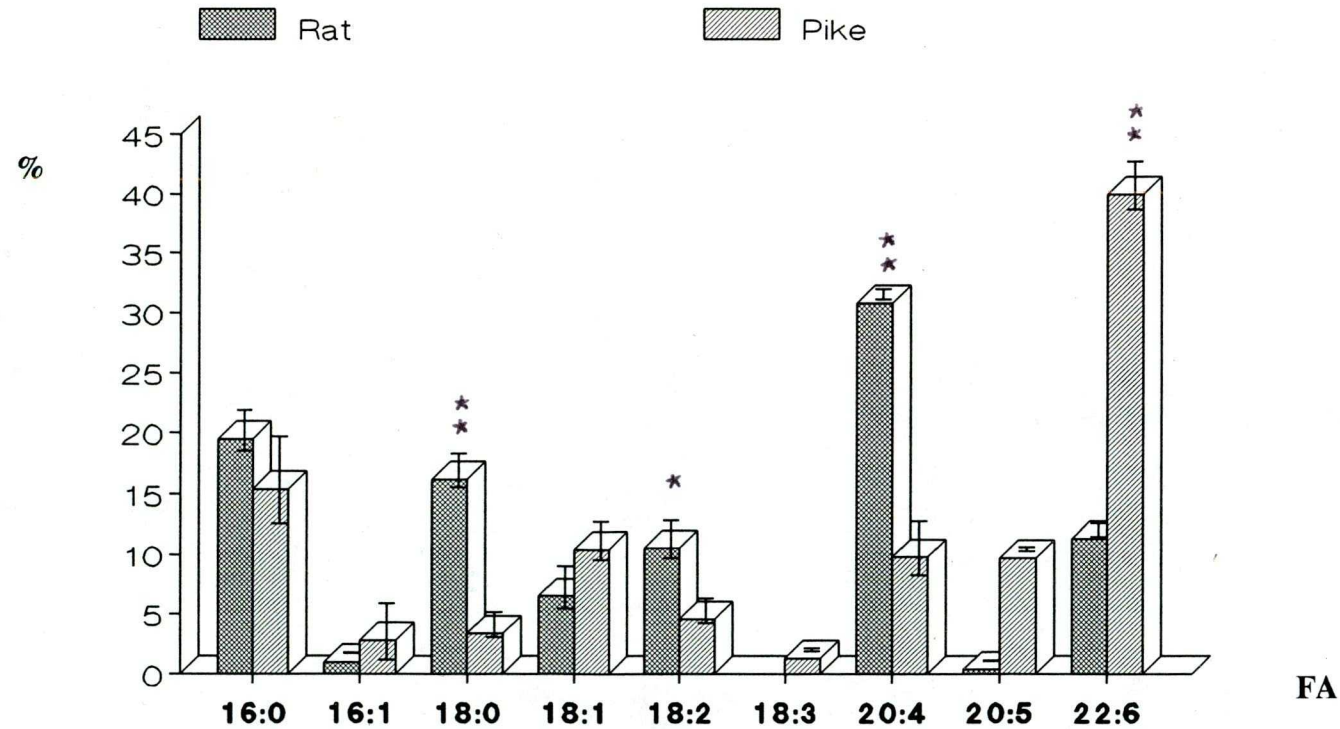

Figure 2.a,b

Comparative investigation on liver fatty acid compositions of rat and pike [N=3]

2.a Phosphatidylcholine **2.b phosphatidylethanolamine**

* $P < 0.01$ **$P < 0.001$

Palmitic (16:0) and octadecaenoic (18:1 n-9) acids were the major saturated and monounsaturated fatty acids. The amount of palmitoleic acid (16:1 n-7) was the same as that of octadecaenoic acid. Eicosenoic (20:1) and decosaenoic (22:1) acids are rather common in marine fish. Fish lipids contain smaller amounts of fatty acids of the linoleic family (linoleic acid and AA) as compared to the fatty acids of the linolenic type. It has been proposed that the linoleic type acids may not be essential for the metabolism of fish, or may be needed in very small quantities (Brenner et al., 1963). The fatty acids of the linolenic family can probably replace them in many or all of their functions (Richardson et al., 1961).

Polar lipids, the PL, of fish are rich in saturated and polyenoic fatty acids; monoenoic acids are present only in small proportion. Approximately 75% of the saturated fatty acids are composed of palmitic acid (16:0), which is usually the major fatty acid in fish PL. Higher amounts of EPA and DHA were detected in fish phospholipids. These results are compatible with the previous suggestion that fish are able to concentrate fatty acids of the linolenic acid type and use them for metabolic functions, and that linoleic-type acids can at least partially replace them. Thus, PUFA are important structural and functional components and are not utilized for energy.

In contrast with polar lipids, the neutral lipids, the TG, are rich in saturated fatty acids (mainly myristic acid, palmitic acid and stearic acid) and are more devoted to energy than the polyunsaturated ones. Monounsaturated acids, which constitute about one quarter of the total fatty acids, have palliative structural and functional roles. The neutral lipids of fish contained much less PUFA.

4.1.2. Polar head group composition of fish phospholipids

PC and PE are the major polar lipid subclass. They are characterized by a high percentage of PUFA and n-3 fatty acids and low levels of linoleic acid and AA. PE is more unsaturated than is PC (Table 2).

It has been demonstrated that fish follow the structural pattern typical for most animals, in that the PUFA are preferentially located in the β-position of glycerol (Brockerhoff and Hoyle, 1963). To explain this phenomenon of specific fatty acid distribution, it was suggested that the typical TG structure may originate in plankton and then be retained through the fish food chains (Brockerhoff et al., 1964).

4.1.3. Fatty acid composition of rat liver lipids

Liver, total lipids and lipid fractions from both young and old rats were analyzed . A representative GLC tracing is given in Fig. 1. A comparison of Fig.1a and Fig.1b shows that rats and fish liver lipids are qualitatively similar. This might perhaps be due to that the fact liver cells perform the same function in both animal species. However, there are significant quantitative differences between the two animals.

We can conclude, therefore, that fish are not greatly different from rats in the metabolism of fatty acids, although the degree and rates at which these fatty acids are formed and metabolized might be different. The higher level of n-3 fatty acids in fish might be explained in that their food chain is based on green algae and diatoms, which are rich either in 18:3 n-3, the precursor of all n-3 long-chain polyunsaturates, or in 20:5 n-3 (diatoms), which can easily be converted to 22:6 n-3. It must also be kept in mind that fish are

Table 2

Polar lipid class contents of rat and pike liver

Polar lipid classes %	Rat	Pike
Lysophosphatidylcholine	1.79 ± 0.57	4.86 ± 0.33
Sphingomyelin	2.39 ± 0.39	4.50 ± 1.78
Phosphatidylcholine	30.48 ± 4.87	58.82 ± 0.54
Phosphatidylinositol	13.80 ± 2.52	5.32 ± 1.01
Phosphatidylserine	4.30 ± 1.26	1.60 ± 0.51
Phosphatidylethanolamine	28.63 ± 4.49	19.93 ± 0.25
Phosphatidylglecerol	7.88 ± 1.68	1.78 ± 0.49
Phosphatidic acid	7.14 ± 2.26	2.88 ± 0.16
Cardiolipin	3.59 ± 3.31	0.32 ± 0.08

poikilothermic (cold blooded) animals and their fatty acid composition is influenced by the water temperature (Farkas et al., 1980), while rats have a constant body temperature and their lipid metabolism is not affected by the environmental temperature.

There is some evidence suggesting that fatty acids of linoleic type are of less importance for fish than for rats, and acids of linolenic type probably replace them in many of their functions.

Interestingly, rat liver phospholipids were more rich in PE and more poor in PC than fish liver phospholipids (Table 2). We cannot give any explantation to this, presently.

4.2. Fatty acid composition in fish in relation to geographical dimensions

To have an insight into the roles of lipid and fatty acid composition in the metabolism of fish in relation to environmental temperature, fish originating from different geographical regions, and thus adapted to contrasting temperatures, were also investigated. At the same time, by comparison of the fatty acid compositions of freshwater and marine fish, the effect of the environmental habitat could also be investigated. In this thesis, the liver fatty acid and PL molecular species compositions of representative species of warm-adapted freshwater (Oreochromis niloticus) and marine (Mugil cephalus) fish are compared with those of representative cold-adapted freshwater (Acerina cernua) and marine (Clupea harengus) fish. From the published data (Wodtke, 1978; Hazel and Zebra, 1986; Hazel et al., 1987 and Farkas and Roy 1989) and logical considerations, it can be expected that fish inhabiting a cold-adapted environment have a more unsaturated fatty acid composition, regardless of the water type (freshwater or marine), than those inhabiting a warmer environment.

It can also be postulated that a given fatty acid composition characterizes a given environmental temperature, provided that only the fatty acid unsaturation controls the membrane physical state.

Table 3 suggests that there are no significant differences in liver total PL fatty acid compositions between freshwater and marine fish from the same thermal environment, but at least with freshwater fish the effect of temperature is clearly visible.

As concerns n-6 PUFA, marine and freshwater fish contained more than 10% of total fatty acids. The major n-6 fatty acid present was AA; other fatty acids of the n-6 series present included 18:2 (n-6), 20:2 (n-6), 20:3 (n-6) and 22:4 (n-6).

Table 3

Fatty acid composition (weight %) of liver of warm and cold-adapted freshwater and marine fish

Fatty acids	TPL				PC				PE			
	WAF		CAF		WAF		CAF		WAF		CAF	
	O.n.	M.c.	A.c.	Cl.h.	O.n.	M.c.	A.c.	Cl.h.	O.n.	M.c.	A.c.	Cl.h.
16:0	24.41 ± 3.58	23.58 ± 1.85	21.79 ± 1.09	16.33 ± 1.72	28.15 ± 4.50	24.43 ± 1.41	23.59 ± 1.99	19.87 ± 1.13	16.84 ± 3.35	12.93 ± 2.32	13.59 ± 1.68	9.89 ± 1.19
16:1 n-7	6.34 ± 0.32	6.98 ± 1.06	5.63 ± 1.98	1.95 ± 0.12	6.13 ± 0.99	5.41 ± 1.19	5.43 ± 2.95	3.04 ± 0.27	1.93 ± 0.67	5.70 ± 1.75	2.30 ± 0.74	1.63 ± 0.64
18:0	5.64 ± 1.12	5.66 ± 1.11	5.25 ± 2.55	5.93 ± 0.32	4.43 ± 1.29	5.54 ± 1.21	3.71 ± 2.19	4.24 ± 0.99	11.57 ± 1.32	8.26 ± 1.51	6.64 ± 2.83	5.43 ± 0.44
18:1 n-9	10.99 ± 0.43	8.03 ± 1.21	14.37 ± 3.52	12.83 ± 1.49	10.06 ± 1.57	7.03 ± 1.10	12.83 ± 2.98	10.63 ± 2.44	12.76 ± 2.13	9.09 ± 1.59	15.55 ± 1.35	15.59 ± 1.24
18:2 n-6	2.68 ± 0.69	1.62 ± 0.72	0.77 ± 0.11	1.16 ± 0.20	2.92 ± 0.41	2.07 ± 0.36	0.71 ± 0.19	0.73 ± 0.24	2.18 ± 0.32	3.31 ± 0.56	1.21 ± 0.88	0.81 ± 0.18
18:3 n-3	1.04 ± 0.79	0.98 ± 0.35	0.25 ± 0.07	0.59 ± 0.18	0.76 ± 0.24	0.48 ± 0.31	0.28 ± 0.02	0.32 ± 0.13	0.22 ± 0.05	0.98 ± 1.13	0.27 ± 0.02	0.50 ± 0.09
20:4 n-6	7.35 ± 0.19	5.02 ± 0.63	4.24 ± 0.75	5.20 ± 0.69	5.31 ± 0.80	5.15 ± 0.91	3.58 ± 0.74	4.45 ± 1.16	10.26 ± 1.33	8.23 ± 1.28	5.53 ± 1.12	5.12 ± 0.67
20:5 n-3	9.61 ± 2.85	10.46 ± 2.48	13.62 ± 3.45	15.33 ± 0.52	15.32 ± 4.71	12.66 ± 3.69	15.38 ± 3.65	22.69 ± 0.94	8.72 ± 0.44	9.03 ± 1.63	15.11 ± 5.03	16.26 ± 2.42
22:5 n-3	7.94 ± 0.42	6.64 ± 1.15	3.14 ± 1.05	1.03 ± 0.33	6.13 ± 0.14	5.37 ± 0.21	1.57 ± 0.74	0.65 ± 0.10	4.40 ± 0.92	5.83 ± 0.62	1.53 ± 0.56	1.22 ± 0.27
22:6 n-3	19.27 ± 1.18	24.58 ± 2.58	29.14 ± 3.64	30.41 ± 2.24	17.00 ± 1.49	27.35 ± 1.31	31.45 ± 5.24	29.77 ± 2.80	28.56 ± 2.96	30.11 ± 2.10	37.24 ± 3.24	32.85 ± 1.63
n-3 / n-6	3.15 ± 0.34	4.53 ± 0.59	8.30 ± 2.65	7.49 ± 0.80	3.89 ± 0.72	4.98 ± 0.37	10.42 ± 2.30	10.53 ± 1.72	2.86 ± 0.16	3.24 ± 0.27	7.89 ± 0.21	8.61 ± 0.82
Sat / unsat	0.46 ± 0.05	0.45 ± 0.02	0.37 ± 0.06	0.33 ± 0.04	0.51 ± 0.08	0.46 ± 0.02	0.38 ± 0.02	0.33 ± 0.01	0.41 ± 0.09	0.31 ± 0.07	0.26 ± 0.05	0.21 ± 0.02
UI	276.24 ±29.12	288.37 ± 7.16	303.89±33.24	303.93± 8.81	269.07±36.45	303.88±16.23	317.00±12.02	325.18 ±15.35	281.86±39.52	308.99±17.41	334.18±6.38	325.35 ±2.35

WAF: warm-adapted fish, **CAF**: cold-adapted fish, **O.n.**: Oreochromis niloticus, **M.c.**: Mugil cephalus, **A.c.**: Acerina cernua, **Cl.h.**: Clupea harrengus

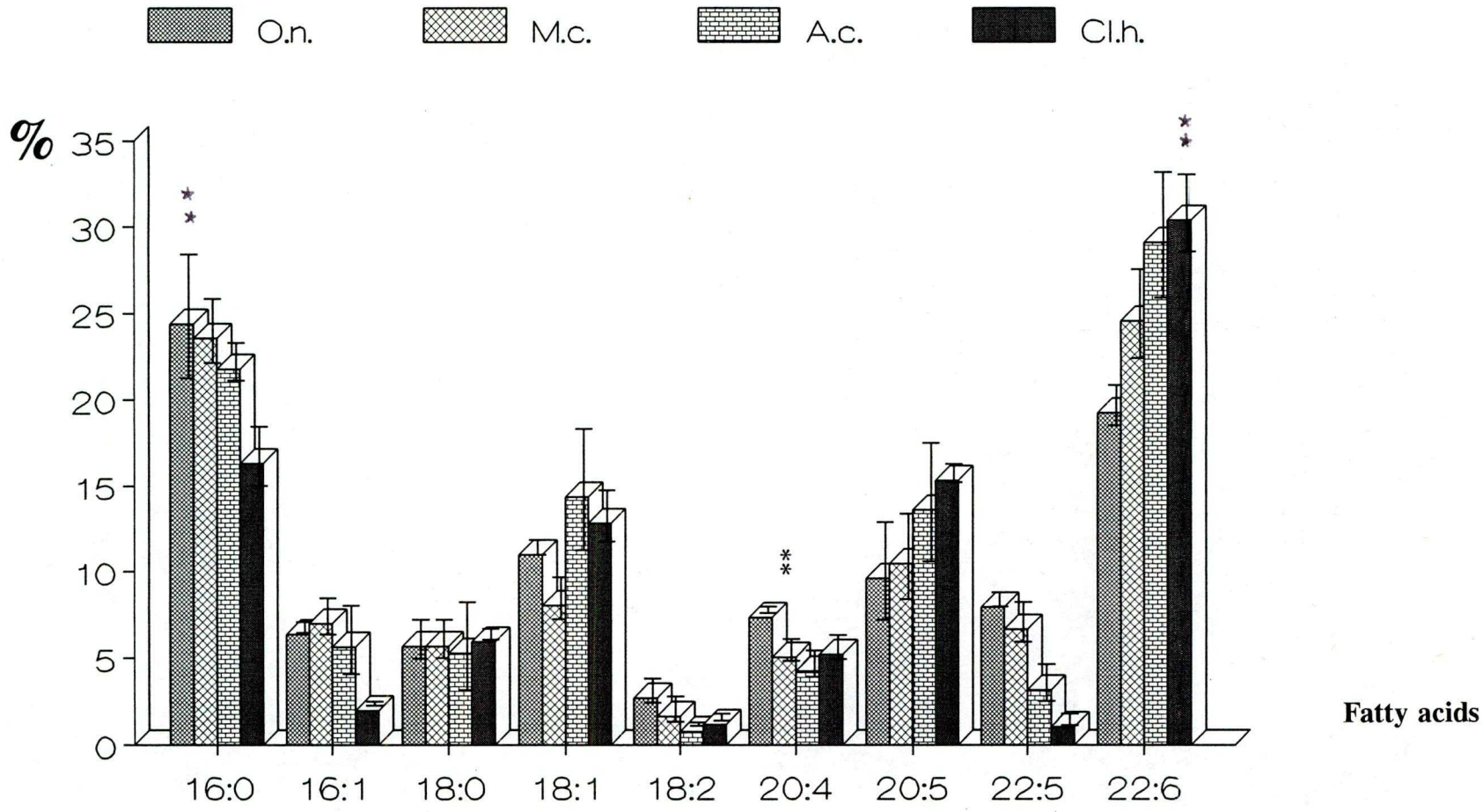

Figure 3 **Fatty acid composition of total phospholipids (weight %) of liver of warm and cold-adapted freshwater and marine fish [N = 3]**

* $P < 0.01$ ** $P < 0.001$

Figure 3.a

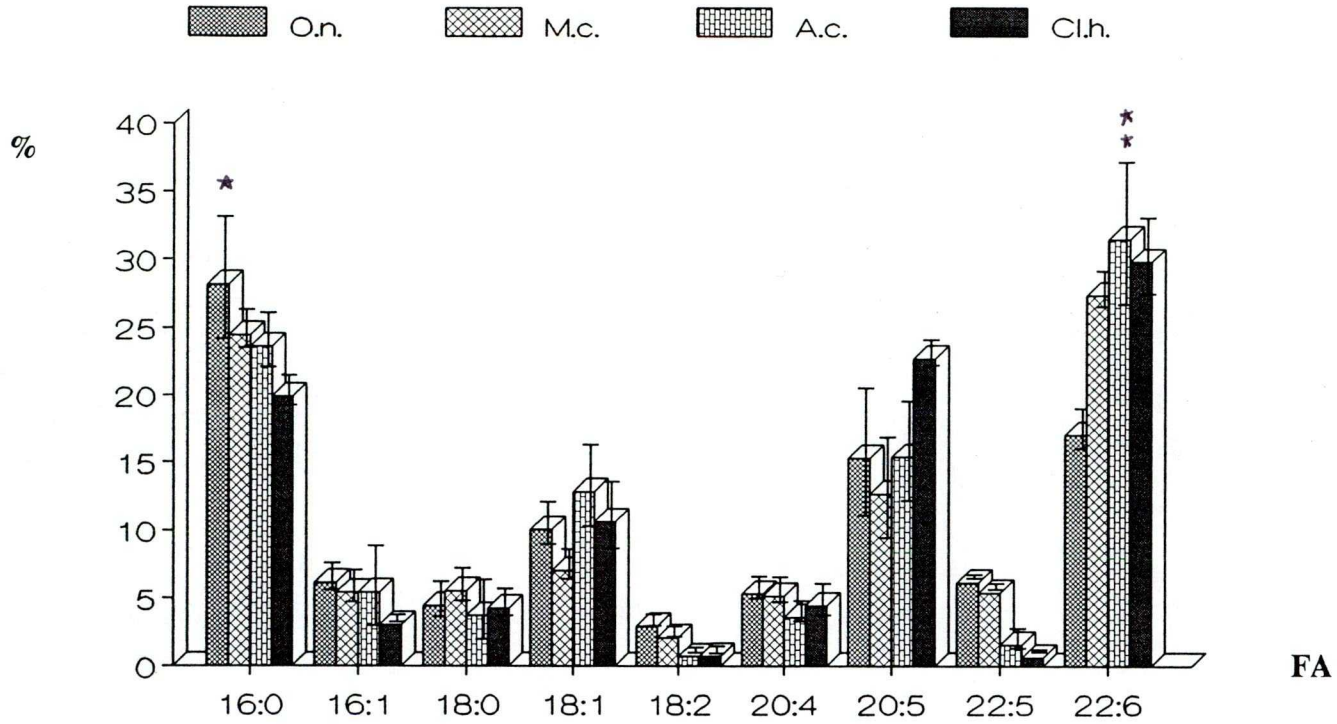

Figure 3.b

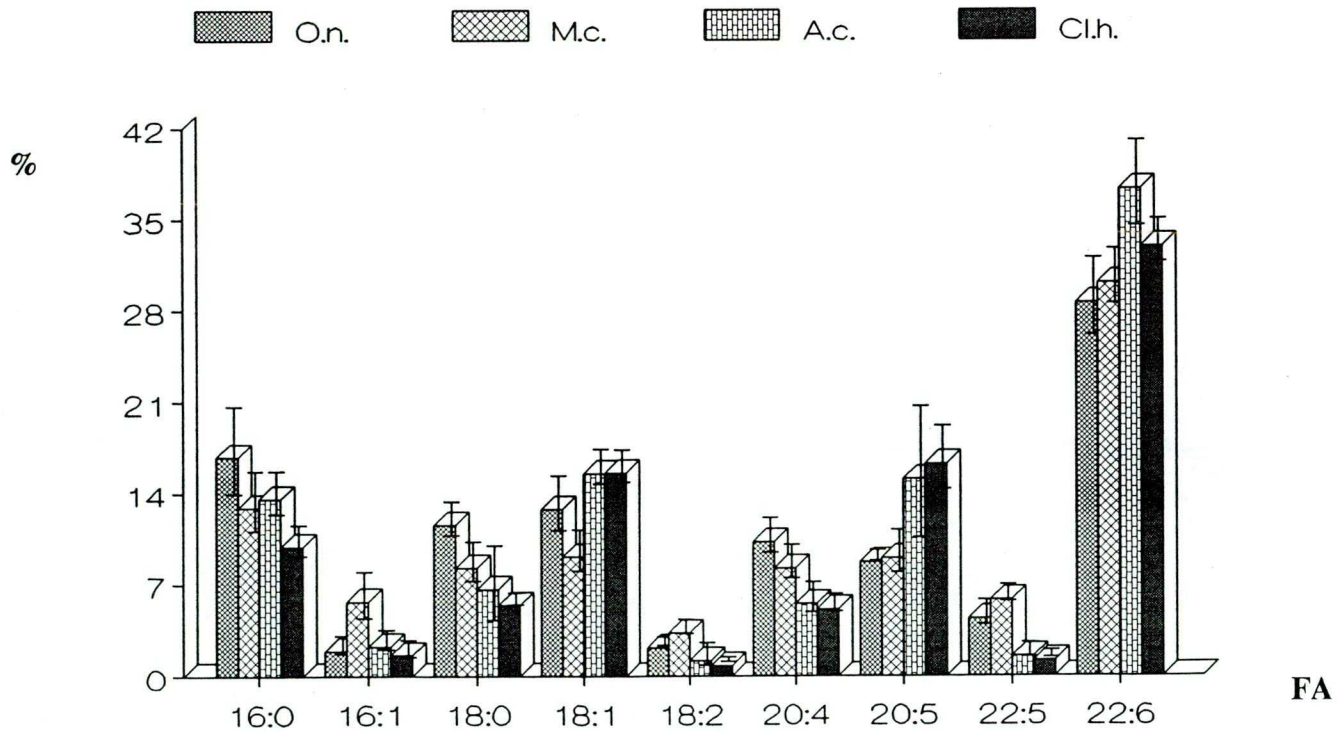

Figure 3.a,b

Fatty acid composition of liver of warm and cold-adapted freshwater and marine [N=3]

3.a Phosphatidylcholine **3.b phosphatidylethanolamine**

* $P < 0.01$ **$P < 0.001$

The n-3 PUFA comprised more than 50% of the total fatty acids. The ratio of n-3 / n-6 was higher in marine fish than in freshwater fish.

Effect of thermal adaptation on phospholipid fatty acid composition

1. Fatty acid compositions in warm-adapted fish:

Lipid levels and fatty acid composition may vary with species, sex, age, season of the year, food availability salinity, and water temperature (Stansby, 1981; Monsen, 1985). In attempts to try to explain the different fatty acid and PL compositions in freshwater fish and marine fish, it has been speculated that the marine fish originating from higher altitudes contain higher concentrations of polyunsaturated long-chain fatty acids in order to maintain the functional integrity of the biomembranes.

In the TPL fraction, 20:5 (n-3) and 22:6 (n-3) are present in high quantities, but interestingly their levels are almost identical and the same is true also for saturates and monoenes Table 3. The level of 22:6 n-3 was somewhat lower in the warm-adapted fish Oreochromis niloticus, but we are not sure that, is a general phenomenon. Against this are the data obtained for the warm-adapted pike (Table 7): this fish showed levels of 22:6 as high in summer as in winter.

Fatty acid composition of major individual phospholipids

The PE fraction was expected to be considerably more unsaturated than the PC fractions. In contrast with this expectation, these were no significant differences, with the exception of Oreochromis niloticus, between the two PL classes in general, or between the fish originating from different geographical locations and thermal environments. This

observation is interesting and argues against the role of 22:6 n-3 in thermal adaptation. 22:6 n-3 is a very low-melting fatty acid and one might expect that control of the membrane physical state may vary with its level. However, in fish adapted to contrasting temperatures this might not be the case. It is probable that the control of membrane fluidity takes place at some other level.

Omega-3 PUFA in fish lipids are of considerable dietary interest with respect to the prevention of circulatory diseases (Kinsella, 1989). The two major (n-3) PUFA from fish species are EPA and DHA. Cultured freshwater fish generally contain lower proportions of (n-3) PUFA than marine fish, which confirms the finding of Monsen (1985). As already mentioned, this result may be due to the fact that the marine food chain is based on diatoms rich in long-chain polyunsaturates, while the freshwater food chain is based on green algae or an artificial diet such as corn, rich in 18:2 (n-6) and 18:3 (n-3).

2. Fatty acid composition of cold-adapted fish

The fatty acid distributions in the total and major individual PL of marine fish (Clupea harengus) from the Baltic Sea and of freshwater fish (Acerina cernua) from North Finland, adapted to 5-10°C, are listed in Table 3. The fatty acids in the total PL of these cold-adapted fish seemed to be more unsaturated than in warm-adapted fishes, as evident from higher DHA levels and also from lower Sat./Unsat. ratios. It was interesting to note that the level of 18:1 n-9 in PE was higher in both cold-adapted fish species than in their counterpartner, while that of 18:0 was lower, thus giving rise to lower 18:0 / 18:1 ratios. This might be traced back to a more intensive conversion of 18:0 → 18:1. Some experimental data show a higher activity of stearic acid-CoA desaturase in fish exposed to reduced temperatures

(Lynch and Thompson, 1988). It is probable that this oleic acid is directed first of all into PE.

It has been proposed that the physical properties of the individual fatty acids to affect the physical properties of the acyl lipids which make up the lipid bilayer, the most commonly demonstrable effect being the phase transition, or the temperature at which the membrane changes from a liquid state to a rigid crystalline one (Stubbs and Smith, 1984). The phase transition has been shown to decrease with the degree of unsaturation due to the presence of cis double bonds, which disrupt the close packing that is possible with saturated chains. The presence of even a single double bond is sufficient to exert a profound influence on the physical properties of the acyl lipid (Chetty et al., 1989).

Additional evidence that fish regulate the physical state of their PL very precisely according to temperature comes from observations made on animals exposed to different temperatures. Cossins (1983) demonstrated that membranes of an Antarctic fish are more fluid than those in tropical fish. On the basis of this observation and the differences in fatty acid composition in the total PL of warm and cold-adapted fish investigated here, the same can be expected in our case. However, this point should be controlled by more direct investigations.

3. Polar head group composition

PC predominated the polar lipid fraction in both the warm and the cold-water fish (46.2% and 83.6%, respectively). The increased level of PE in cold-water species, seen here with freshwater fish from Finland, has also been noted with some other species (Cossins, 1977; Farkas et al., 1984) (Table 4). It has been proposed that for the stability of the bilayer

Table 4

Polar lipid class compositions of cold and warm-adapted fish

Polar lipid classes %	Warm-water fish Oreochromis niloticus	Cold-water fish Acerina cernua
Lysophosphatidylcholine	1.32 ± 0.269	3.32 ± 1.954
Sphingomyelin	4.43 ± 0.396	5.42 ± 0.705
Phosphatidylcholine	79.56 ± 5.729	50.05 ± 4.683
Phosphatidylinositol	1.51 ± 0.993	1.45 ± 1.144
Phosphatidylserine	0.73 ± 0.593	1.42 ± 0.288
Phosphatidylethanolamine	10.24 ± 3.571	34.73 ± 5.008
Phosphatidylglycerol	0.97 ± 0.799	1.11 ± 0.669
Phosphatidic acid	0.85 ± 0.624	0.91 ± 0.330
Cardiolipin	0.26 ± 0.074	1.23 ± 0.157

at reduced temperature an increased level of wedge-shaped PL (PE in this case) is required (Wieslander et al., 1980). Increase of the number of wedge-shaped molecules, however, is one of the ways to achieve stability of the bilayer in the cold. An alternative way is to incorporate more unsaturated fatty acids into PE. An elevated level of 18:1 in cold-adapted fish might serve this purpose.

4. Major molecular species in liver phosphatidylethanolamine and phosphatidylcholine of representative cold and warm-adapted freshwater and marine fish

Fig. 4 shows a representative HPLC tracing of PE obtained from cold-adapted fish (Acerina cernua). By this technique at least 15-20 species can be detected, but due to the overlapping of some peaks, the true figure is even higher. Table 5 gives the distribution of the separated species in PC and PE of representative warm-adapted marine and freshwater fish.

PC and PE were found to consist of six major molecular species: 18:1 / 22:6; 16:0 / 22:6; 16:0 / 20:4; 18:1 / 22:6; 16:0 / 22:6 and 18:0 / 20:5, making up two-thirds of the sample. In the warm-adapted marine PE, there are small amounts of 20:4 n-6 and higher amounts of 20:5 n-3 as compared with PC. Interestingly, dipolyunsaturated species (22:6 / 22:6; 20:5 / 22:6, etc.) were also present. Disaturated species (16:0 / 16:0; 14:0 / 16:0) were not detected.

In the warm-adapted freshwater fish (Oreochromis niloticus), the major molecular species of diacyl-PC was 16:0 / 22:6 (42.7%), followed by 18:1 / 22:6 (13.4%). Other species, including 20:4 / 22:6; 16:0 / 20:4; 18:0 / 20:5; 18:0 / 22:6 and 16:0 / 18:1, were

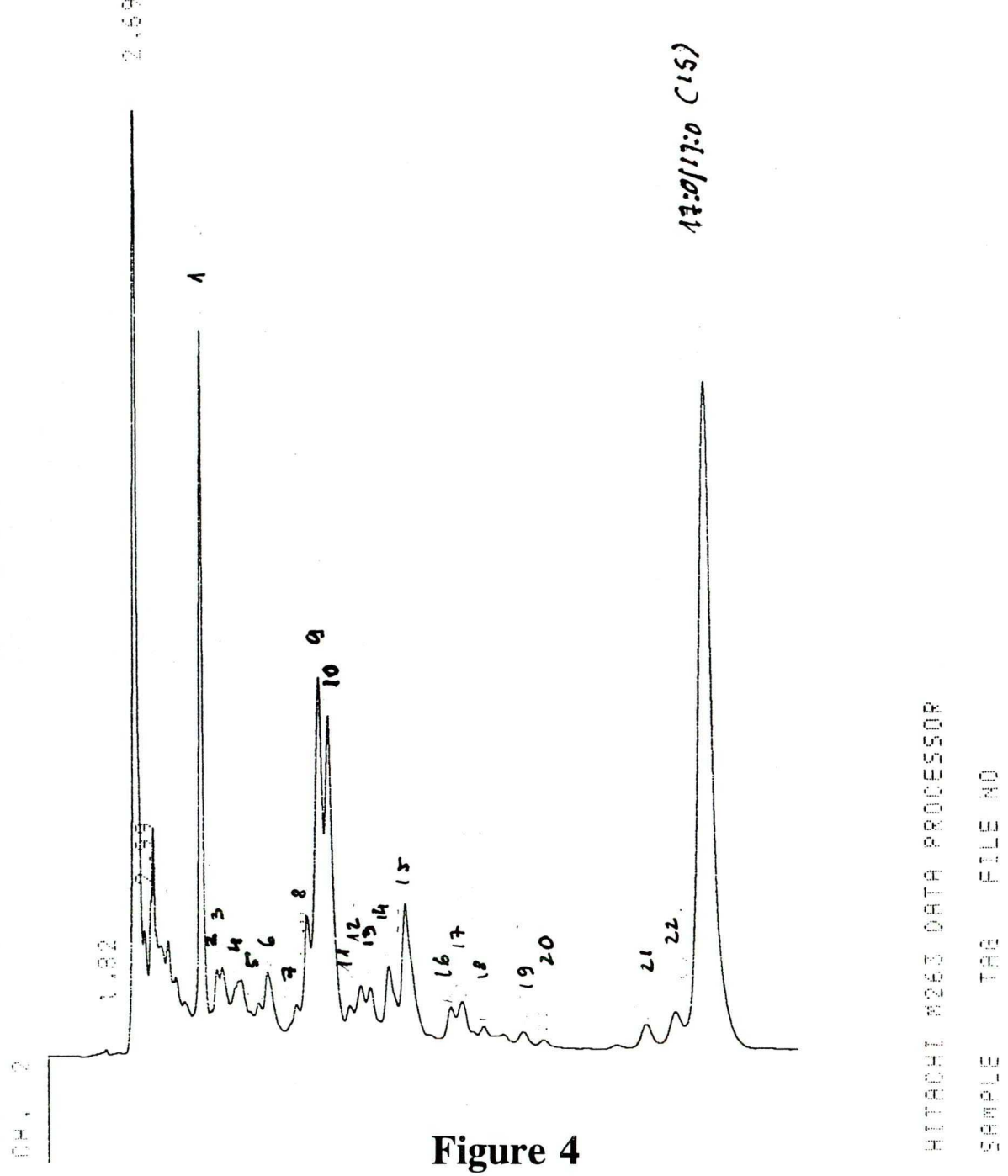

Figure 4

HPLC tracing of phosphatidylethanolamine from liver of <u>Acerina</u> <u>cernua</u>

1 18:4/20:5	2 18:4/22:6	3 20:5/22:6
4 22:6/22:6	5 ?	6 22:5/22:6
7 16:0/18:4	8 18:1/20:5	9 18:1/22:6
10 16:0/22:6	11 16:/22:5	12 18:0/20:5
13 18:0/22:6	14 18:0/20:4	15 18:0/16:1
16 16:1/20:1	17 ?	18 18:1/18:1
19 16:0/18:1	20 16:0/16:0	21 ?
22 ?		

Table 5

Major molecular species composition of phosphatidylethanolamine and phosphatidylcholine in liver of warm-adapted freshwater and marine fish

Molecular species	PE		PC	
	O.n.	M.c.	O.n.	M.c.
20:5 / 22:6	---	1.57	1.38	0.70
22:6 / 22:6	0.48	0.91	0.43	0.70
20:5 / 20:5	---	1.68	2.17	0.82
20:4 / 22:6	---	3.19	4.26	1.55
16:1 / 22:6	6.07	1.02	1.26	2.22
16:0 / 18:0	0.76	1.57	2.35	1.38
18:1 / 20:5	1.15	3.00	2.22	4.04
18:1 / 22:6	6.07	12.57	13.42	15.73
16:0 / 22:6	14.41	19.76	42.69	18.02
16:0 / 20:5	2.76	3.37	1.96	3.55
	---	3.94	2.59	6.49
16:0 / 20:4	6.33	7.36	4.37	4.96
18:0 / 20:5	7.62	15.66	5.14	5.60
18:1 / 18:2	1.79	8.27	2.81	5.29
18:0 / 22:6	19.87	1.68	3.11	7.0
16:0 / 18:2	---	---	1.41	2.92
14:0 / 16:0	---	---	0.74	---
18:0 / 20:4	16.60	---	1.59	1.91
18:0 / 16:1	1.60	5.66	0.56	2.11
18:1 / 18:1	---	---	0.53	---
16:0 / 18:1	2.94	5.66	3.53	2.11
16:0 / 16:0	---	---	1.02	0.87

O.n.: Oreochromis niloticus M.c.: Mugil cephalus

present between 3 and 5%. The level of dipolyunsaturated species was considerably higher in PE.

In the cold-adapted marine fish (Clupea harengus), PE was found to comprise four main molecular species, with 18:1 / 20:5, 18:1 / 22:6, 16:0 / 22:6, 18:0 / 22:6 and 18:0 / 20:4 making up two-thirds of the sample, the last species contributing one-third of the PE (Table 6). While a further 8 minor species were detected, 16:0 / 18:1 (5.8%) and 18:0 / 20:5 (4.3%) were the only other species present at greater than 2.6%. It was assumed in all the separations here that the more unsaturated fatty acid was at position 2. Of the 8 minor species detected, the most interesting were the di PUFA species 20:5 / 20:5, 20:5 / 22:6, 22:6 / 20:5 and 22:6 / 22:6 (total 2.4%), with the last species being the most important.

In the cold-adapted marine fish, there is a low amount of 20:4 (n-6) as compared with freshwater fish and warm-adapted marine fish.

The most interesting difference between cold and warm-adapted fish, irrespective of their origin, is a significantly higher level of 18:1 / 22:6 species in PE. In the former (Table 3) it was demonstrated that the PE from cold-adapted fish is richer in 18:1 than that from warm-adapted fish. Moreover, Table 4 indicates that the level of PE is higher in the cold-adapted fish as compared with the warm-adapted ones. Considering these data, it can be proposed that 18:1 / 22:6 in the PE plays a crucial role in adaptation to cold. Introduction of a cis double bond into the Sn-1 position of PC greatly increases the conicity of the molecule. Thus, cold-adapted fish species increase not only the relative amount of this PL, but also the molecular shape (conicity) during (evolutionary) adaptation to low temperature. This might have consequences in terms of thermal adaptation.

Table 6

Major molecular species composition of phosphatidylethanolamine and phosphatidylcholine in liver of cold-adapted freshwater and marine fish

Molecular species	PE		PC	
	A.c.	Cl.h.	A.c.	Cl.h.
18:4 / 20:5	14.10	0.67	1.75	0.43
18:4 / 22:6	1.66	1.07	1.41	0.24
20:5 / 22:6	1.83	2.03	1.12	2.41
22:6 / 22:6	1.55	4.52	0.78	7.03
20:4 / 20:4	0.72	0.45	2.68	0.86
	2.27	0.73	1.82	1.88
18:1 / 20:5	5.36	6.53	5.69	4.57
18:1 / 22:6	17.02	27.53	22.22	26.29
16:0 / 22:6	16.04	26.97	30.61	23.94
16:0 / 22:5	1.35	4.16	1.28	2.70
18:0 / 20:5	2.68	2.61	2.65	0.32
18:0 / 22:6	2.58	3.89	3.39	3.02
18:0 / 20:4	4.31	3.60	1.79	2.34
18:0 / 16:1	6.58	5.20	2.65	4.07
16:0 / 20:1	2.31	3.17	1.96	5.32
	2.85	0.86	0.88	4.35
18:1 / 18:1	1.00	0.66	0.73	0.64
16:0 / 18:1	1.09	1.76	9.21	9.36
16:0 / 16:0	0.57	0.75	0.53	0.83

A.c.: Acerina cernua **Cl.h.:** Clupea harengus

4.3. Effect of seasonal variations of temperature on the lipid composition and physical state in pike liver

To study in detail how the environmental temperature affects the PL fatty acid composition and physical state, pike, a fish active in a wide range of temperatures (5-25°C) was selected. This fish feeds and even breeds in cold seasons. In an attempt to answer this question, two approaches were selected. In one approach, the fatty acid compositions of cold and warm-adapted fish were investigated. It was assumed that these fish mimic the responses given by fish adapted to cold and warm environments. In another approach, warm-adapted pike were exposed to 5°C by a controlled cooling of the aquarium temperature, to follow the changes in fatty acid, polar head group composition, fluidity and molecular species composition of selected PL from the liver.

Seasonal changes (winter and summer) did not bring about marked differences in the fatty acid concentrations of total lipids and total PL and their major subclasses (Table 7). Although some minor differences in levels of some individual fatty acids can be observed, for instance in summer, the increase in environmental temperature led to increases in the levels of 18:0 and 20:4, whereas the levels of monounsaturated and polyunsaturated acids showed an opposite tendency. As seen with fish adapted to temperature, 22:6 was not affected by the adaptation temperature in pike. This is in contrast with published results on other freshwater fish and calls attention to possible inter-species differences between the different fish. It must also be considered that pike feeds actively in winter too, and its food might also be rich in polyenes such as 22:6, due to an active adaptation of fatty acid composition to temperature.

Table 7

Major fatty acids in total lipids and total and individual phospholipids in liver of winter and summer-adapted pike

Fatty acids	TL		TPL		PC		PE	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
14:0	1.46 ± 0.25	0.56 ± 0.15	1.30 ± 0.19	0.64 ± 0.10	0.85 ± 0.19	0.33 ± 0.15	0.66 ± 0.49	1.37 ± 0.43
16:0	18.72 ± 2.03	18.42 ± 0.80	22.48 ± 1.10	22.22 ± 1.41	27.44 ± 0.27	25.15 ± 1.94	15.40 ± 3.55	14.50 ± 1.41
16:1 (n-7)	10.75 ± 2.79	8.49 ± 1.32	9.46 ± 0.30	5.11 ± 1.10	7.20 ± 0.51	4.28 ± 0.61	2.80 ± 2.35	1.41 ± 0.28
18:0	3.75 ± 1.62	6.21 ± 0.75	2.87 ± 0.29	4.73 ± 0.84	1.77 ± 0.40	2.39 ± 0.39	3.36 ± 1.04	6.01 ± 0.31
18:1 (n-9)	17.15 ± 3.48	19.41 ± 0.60	13.90 ± 0.83	9.73 ± 0.25	11.37 ± 0.67	11.26 ± 0.48	10.42 ± 1.59	10.29 ± 1.21
18:2 (n-6)	8.67 ± 2.99	5.48 ± 0.62	6.40 ± 1.69	3.73 ± 0.70	5.54 ± 0.96	2.75 ± 0.69	4.55 ± 1.05	4.72 ± 0.79
18:3 (n-3)	2.90 ± 0.39	2.26 ± 0.26	2.32 ± 0.12	1.44 ± 0.37	2.45 ± 0.28	1.35 ± 0.37	1.29 ± 0.14	1.70 ± 0.48
20:3 (n-6)	0.71 ± 0.26	0.55 ± 0.21	0.39 ± 0.10	0.37 ± 0.21	0.24 ± 0.00	0.22 ± 0.03	0.13 ± 0.00	0.41 ± 0.17
20:4 (n-6)	5.86 ± 1.25	8.50 ± 0.82	6.12 ± 0.42	10.69 ± 1.27	4.19 ± 0.42	8.57 ± 0.95	9.81 ± 2.25	10.99 ± 1.03
20:5 (n-3)	5.55 ± 1.36	6.43 ± 3.11	5.37 ± 0.66	6.93 ± 0.79	5.37 ± 0.66	9.54 ± 0.72	9.74 ± 0.15	6.64 ± 0.69
22:4 (n-6)	1.12 ± 1.50	1.56 ± 0.39	0.41 ± 0.35	2.56 ± 0.56	0.18 ± 0.13	1.69 ± 0.64	0.82 ± 0.38	2.46 ± 0.29
22:5 (n-3)	2.14 ± 1.12	3.63 ± 0.65	1.51 ± 0.07	3.22 ± 0.22	1.55 ± 0.54	3.48 ± 0.60	1.35 ± 0.09	2.49 ± 0.48
22:6 (n-3)	18.16 ± 3.20	16.24 ± 1.49	25.65 ± 1.18	26.82 ± 2.43	30.86 ± 0.91	26.40 ± 2.22	39.99 ± 2.05	35.55 ± 2.99
(n-3) / (n-6)	1.84 ± 0.34	1.69 ± 0.10	2.66 ± 0.44	2.20 ± 0.21	4.06 ± 0.16	3.15 ± 0.32	3.53 ± 0.68	2.50 ± 0.11
Sat. / Unsat.	0.32 ± 0.05	0.33 ± 0.02	0.36 ± 0.02	0.38 ± 0.04	0.43 ± 0.00	0.39 ± 0.05	0.23 ± 0.04	0.28 ± 0.03
UI	242.46 ± 19.62	239.89 ± 1.34	260.21 ± 8.55	298.38 ± 15.75	278.78 ± 3.30	299.75 ± 13.20	364.96 ± 15.93	325.32 ± 25.93

4.3.1. Polar head group composition of phospholipids

The PL composition of liver tissue of pike exposed to seasonal variations shows the same general pattern as that observed with fish adapted to contrasting temperature (Table 8). As shown in this Table, there is a significantly higher percentage of PE in the winter pike as compared with the summer.

These data confirms speculation that restructuring of the polar head group composition of PL is a general phenomenon. Observations made on trout (Hazel and Carpenter, 1985) suggest that this reaction is rather rapid and is triggered immediately after the onset of a drop in the environmental temperature. From the data presented in Table 7, however, it does not follow that the accumulation of PE in response to cold acclimatization is accompanied by the accumulation of 18:1.

4.3.2. Physical state of phospholipids in cold, and warm-adapted pike

In another series of experiments, the fluorescence anisotropy of DPH embedded into PL vesicles was determined from a different set of pikes. A temperature scan between 5°C and 30°C was made and the values are represented as a function of 1/T. Figure 5 shows that PL vesicles obtained from the cold-adapted pike have a lower r value at any temperature measured than those from warm-adapted ones. This is interpreted as suggesting that membranes in cold-adapted fish are more fluid than those in warm-adapted ones. The homeoviscous efficacy, which is the extent by which the membrane fluidity compensates for temperature changes, is far from 100% (30-40%). A 30-40% homeoviscous efficacy has been measured for other fish species too (Cossins and Prosser, 1982).

Table 8

Polar lipid class contents of winter and summer-adapted pike liver

Polar lipid classes %	Winter 5°C	Summer 25°C
Lysophosphatidylcholine	4.86 ± 0.33	2.62 ± 0.38
Sphingomyelin	4.50 ± 1.78	6.12 ± 0.07
Phosphatidylcholine	58.82 ± 0.54	64.52 ± 0.11
Phosphatidylinositol	5.32 ± 1.01	6.45 ± 0.18
Phosphatidylserine	1.60 ± 0.51	1.98 ± 0.16
Phosphatidylethanolamine	19.93 ± 0.25	13.40 ± 0.46
Phosphatidylglecerol	1.78 ± 0.49	0.91 ± 0.27
Phosphatidic acid	2.88 ± 0.16	2.44 ± 0.10
Cardiolipin	0.32 ± 0.08	1.56 ± 0.21

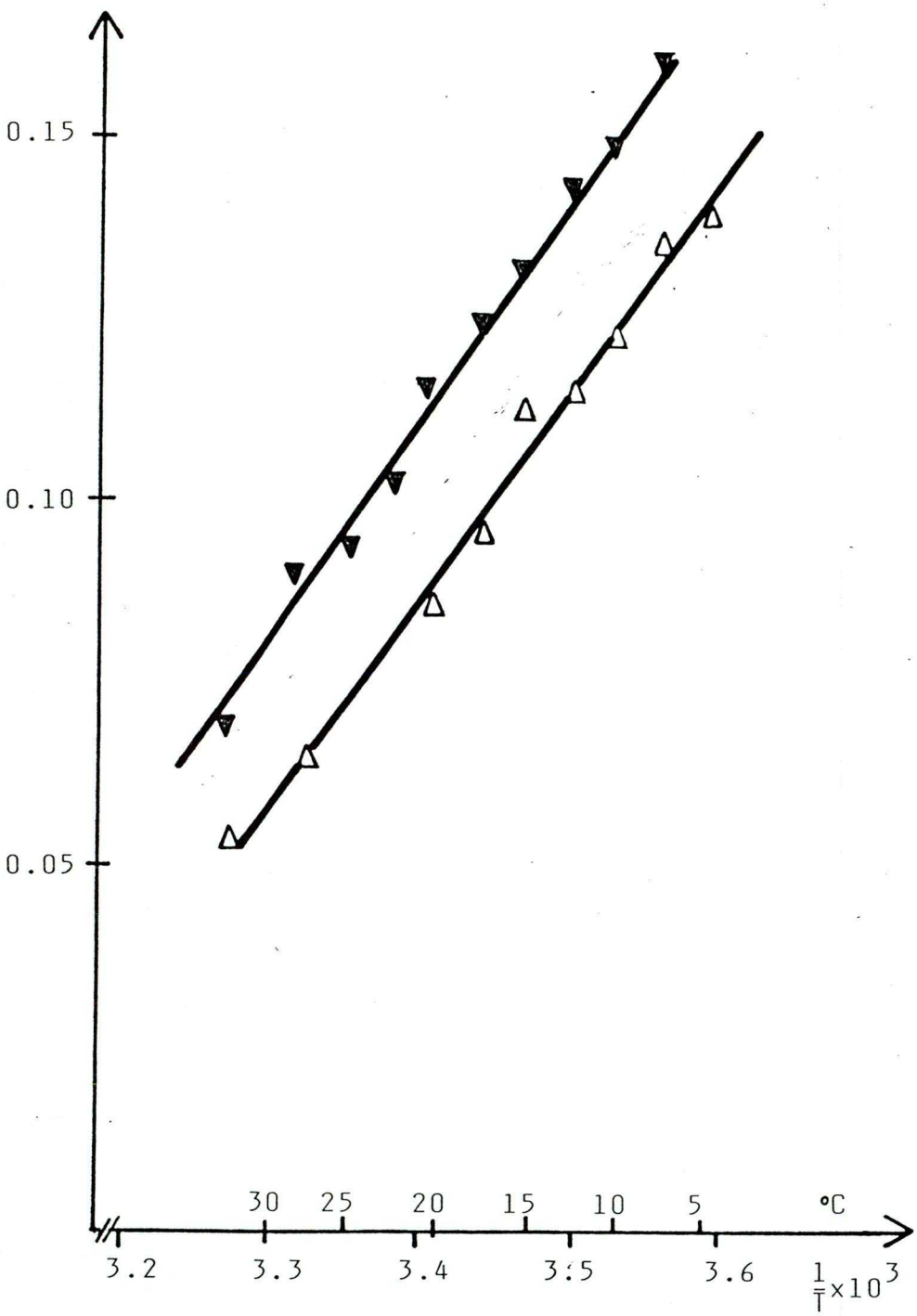

Figure 5

DPH FLUORESCENCE POLARISATION OF PHOSPHOLIPIDS VESICLES OBTAINED FROM LIVERS OF WARM ADAPTED (▼-▼) AND COLD ADAPTED (Δ-Δ) PIKE

The most interesting conclusion which can be drawn from this experiment is that this change in the physical state of the membrane PL may take place without spectacular modification of the total PL fatty acid composition (Table 7). Thus, it is quite clear that the overall fatty acid composition plays only a minor, or no role in controlling the physical state of the PL membranes. It can be postulated that adaptation of the physical state of membranes to temperature takes place either at the level of polar head group composition or at the level of the molecular architecture of the component PL.

Although PE has been shown to accumulate during cold adaptation in several poikilotherms (Neas and Hazel, 1985; Momchilova et al., 1986) the contribution of the type of the head group can be questioned. The positive charge in the N^+ of PE contracts rather than disturbs the membrane packing due to its interaction with the neighbouring phosphate-ester (P=O). In fact, the phase transition temperature of PE with the same fatty acid composition is higher than that of PC.

4.3.3. Molecular species composition in relation to thermal adaptation:

To resolve these contradiction between Tables 7 and 8 and Fig. 5, the molecular species composition of PC and PE from Table 5 confirm, that adaptation may take place at the level of the molecular architecture of certain PL. Table 9 gives the molecular species composition of PC and PE.

20-25 molecules could be separated by HPLC, but the true figure in this case too is certainly higher, due to several overlapping species. As seen with species adapted to cold and warm environments, the most important change that could be observed is an increase in the

Table 9

Major molecular species in liver phosphatidylethanolamine and phosphatidylcholine of summer and winter pike

Molecular species	PE		PC	
	Summer 25°C	Winter 5°C	Summer 25°C	Winter 5°C
22:6 / 22:6	5.01	1.22	2.32	0.78
22:5 / 22:6	4.45	1.98	---	2.90
20:5 / 22:6	7.31	5.47	2.38	0.65
18:1 / 20:5	4.91	4.88	---	2.14
18:1 / 22:6	12.82	16.05	10.32	6.85
16:0 / 22:6	21.89	33.06	24.23	38.32
16:0 / 20:4	---	---	4.89	3.14
18:1 / 16:1 ± 20:1 / 22:5	---	---	7.51	4.67
18:1 / 18:2	4.48	2.81	11.74	5.29
16:0 / 18:2	6.52	7.20	4.28	2.33
18:0 / 20:4	7.87	7.08	4.62	4.82
X	5.32	3.73	---	---
18:0 / 22:6	8.16	7.27	6.67	8.26
14:0 / 16:0 ± 20:0 / 20:5	4.42	---	3.15	4.35
18:1 / 18:1	1.53	1.12	2.47	1.18
16:0 / 18:1	3.90	3.35	12.24	13.49
16:0 / 16:0	1.43	---	3.48	0.82
X	---	0.43	---	---
X	---	0.87	---	---
X	---	0.53	---	---

level of 18:1/22:6 PE species. The accumulation of 18:1/22:6 PE in the temperature-down-shifted pike liver is exactly in accord with observations made on summer and winter-adapted pike and warm and cold-adapted freshwater and marine fish Tables (5 and 6). Although we do not have data on the thermotropic phase transition temperature of this PL, it is evident that introduction of a cis-Δ9 double bond into the Sn-1 position of the molecule renders this species more fluid than, for instance, is 16:0/22:6. This speculation is based on observations made on PC. The thermotropic phase transition temperature of 16:0 / 16:0 drops from 41.5°C to -12°C when one palmitic acid is replaced with oleic acid to give 16:0 / 18:1 PC. A further cis double bond in position 1 (18:1 / 18:1 PC) brings about a further reduction of this temperature to -20°C. This double bond increase also causes a favourable change in the anisotropy of DPH shown in Fig.5, due to the elevated level of 18:1 / 22:6 PE species. An increase from 21 to 33% of 16:0 / 22:6 in the PE of winter adapted pike might also have contributed to this response.

4.4. Effects of down-shift of temperature on pike liver lipid

To have information on these questions, summer-adapted pike were exposed to a quite fast drop in temperature.

The acclimation temperature brought about marked differences in the fatty acid patterns of total lipid, PL and major individual PL from pike liver. The data in Table 10 (Fig. 6, 6 a,b) support earlier observations regarding the differences in fatty acid compositions of total lipid and PL, as well as of PC and PE. It is evident from the data presented here that the pike liver under the present experimental conditions follows the trends in the previous studies. A decrease in environmental temperature leads to a lower level of palmitic acid (16:0) in TPL, PC and PE, and an increase in the level of AA (20:4 n-6) in PC. The level of oleic acid (18:1 n-9) was significantly increased in TPL and PE on down-shift of the temperature, but decreased in PC. At the same time, the level of some PUFA increased. The most marked increase was observed with DHA (22:6 n-3), which increased from 15 to 30% in TL, 30 to 45% in TPL, 11 to 47% in PC and 42 to 48% in PE. This animal did not change the level of EPA (20:5 n-3). It was found that decrease in environmental temperature reduces the ratio of saturated to unsaturated fatty acids.

The fatty acid composition in fish evolutionarily or seasonally adapted to temperature represents a steady-state in adaptational processes and might well be traced to similar changes at the lower level of the food chain. These data, though informative, do not tell anything about the individual responses of sudden changes in temperature. Fish living in temperate climates are exposed to such changes. The temperature might change diurnally, or rapidly

Table 10

Fatty acid composition of total phospholipids and individual phospholipids of pike liver controlled to shift-down of temperature in vivo

Fatty acid Temp. °C	TL		TPL		PC		PE	
	25°C	25→5°C	25°C	25→5°C	25°C	25→5°C	25°C	25→5°C
14:0	1.66	1.46	0.93	1.09	0.05	0.46	0.31	0.60
16:0	14.73	14.92	19.71	6.72	31.94	9.71	19.80	9.31
16:1 (n-7)	10.83	6.09	6.34	5.52	3.89	2.63	1.85	2.83
18:0	3.55	5.54	3.09	2.92	4.66	5.19	4.47	2.61
18:1 (n-9)	18.98	20.58	13.60	16.01	23.33	12.72	8.74	15.17
18:2 (n-6)	9.11	4.81	6.09	4.19	10.41	2.96	2.74	3.21
18:3 (n-3)	3.73	1.16	2.58	1.10	0.49	0.51	0.69	0.65
18:4 (n-3)	1.85	0.78	1.15	0.43	1.22	0.64	0.40	--
20:2 (n-6)	1.72	0.78	1.07	0.71	0.77	0.69	0.41	--
20:3 (n-6)	0.39	0.57	0.29	0.41	0.89	0.82	--	--
20:4 (n-6)	7.00	5.86	8.56	8.27	6.95	9.04	10.07	10.20
20:4 (n-3)	3.67	1.38	1.91	1.02	0.07	1.13	--	--
20:5 (n-3)	5.53	3.48	6.57	4.28	3.09	4.37	5.51	3.74
22:4 (n-6)	0.82	0.76	1.58	1.25	0.38	1.18	1.74	2.17
22:5 (n-3)	1.92	1.44	1.42	0.58	0.43	0.60	1.08	1.34
22:6 (n-3)	14.49	30.37	30.09	45.49	11.43	47.28	42.15	48.14
Sat. / unsat.	0.25	0.28	0.23	0.12	0.58	0.18	0.33	0.14
(n-3) / (n-6)	1.64	3.02	2.48	3.57	0.86	3.71	3.33	3.46
UI	241.38	284.98	316.16	376.98	174.38	388.13	354.25	390.09

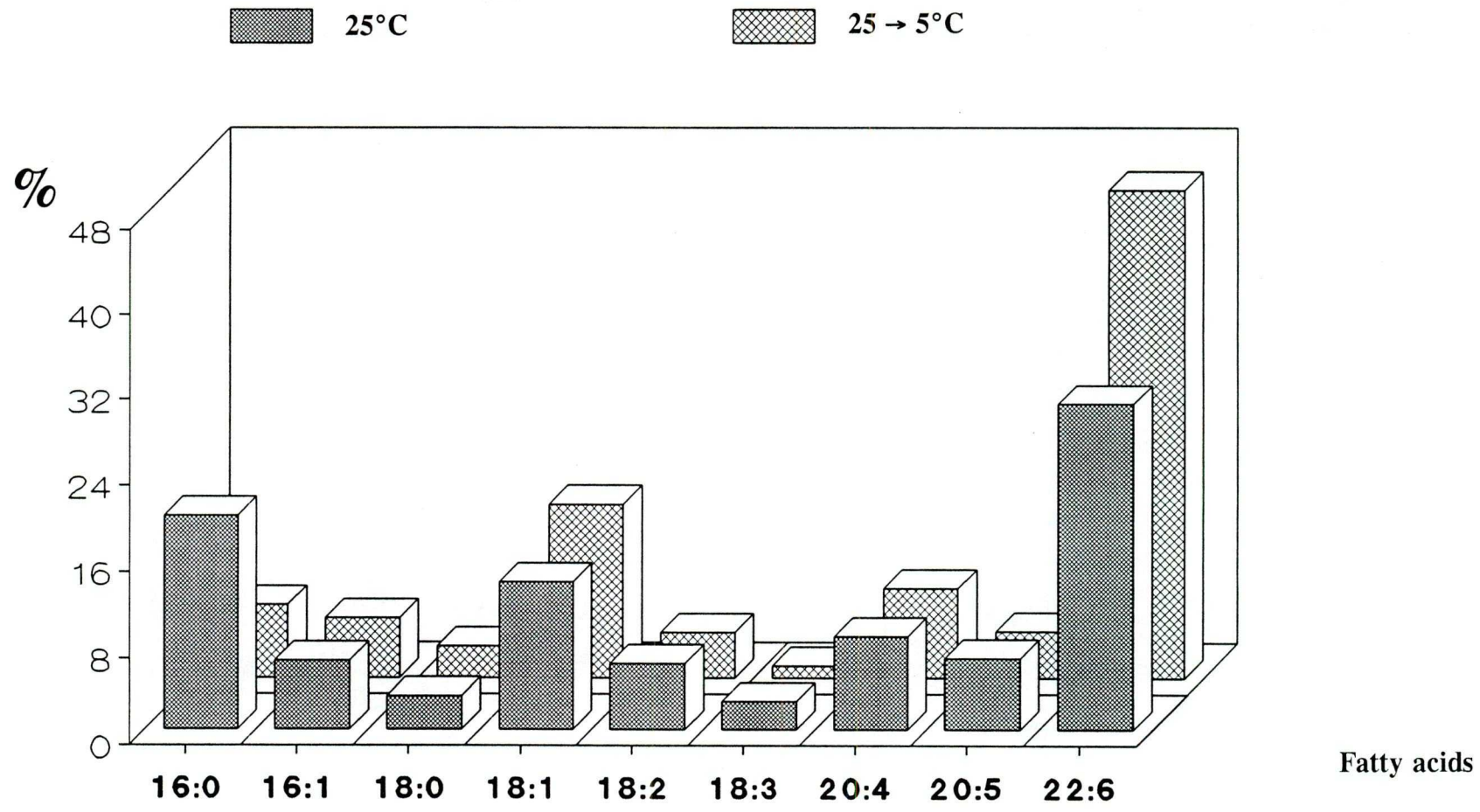

Figure 6 **Fatty acid composition of total phospholipids of pike liver controlled to shift-down of temperature in vivo**

Figure 6.a

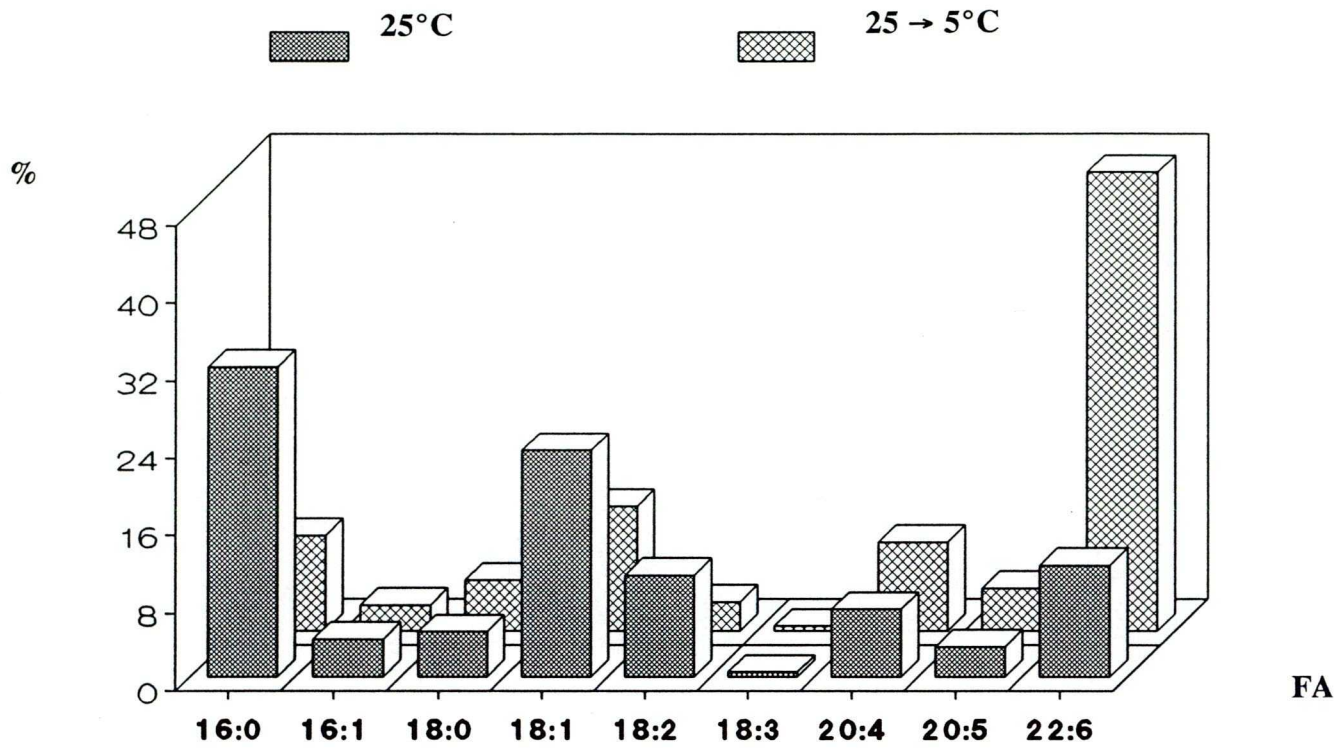

Figure 6.b

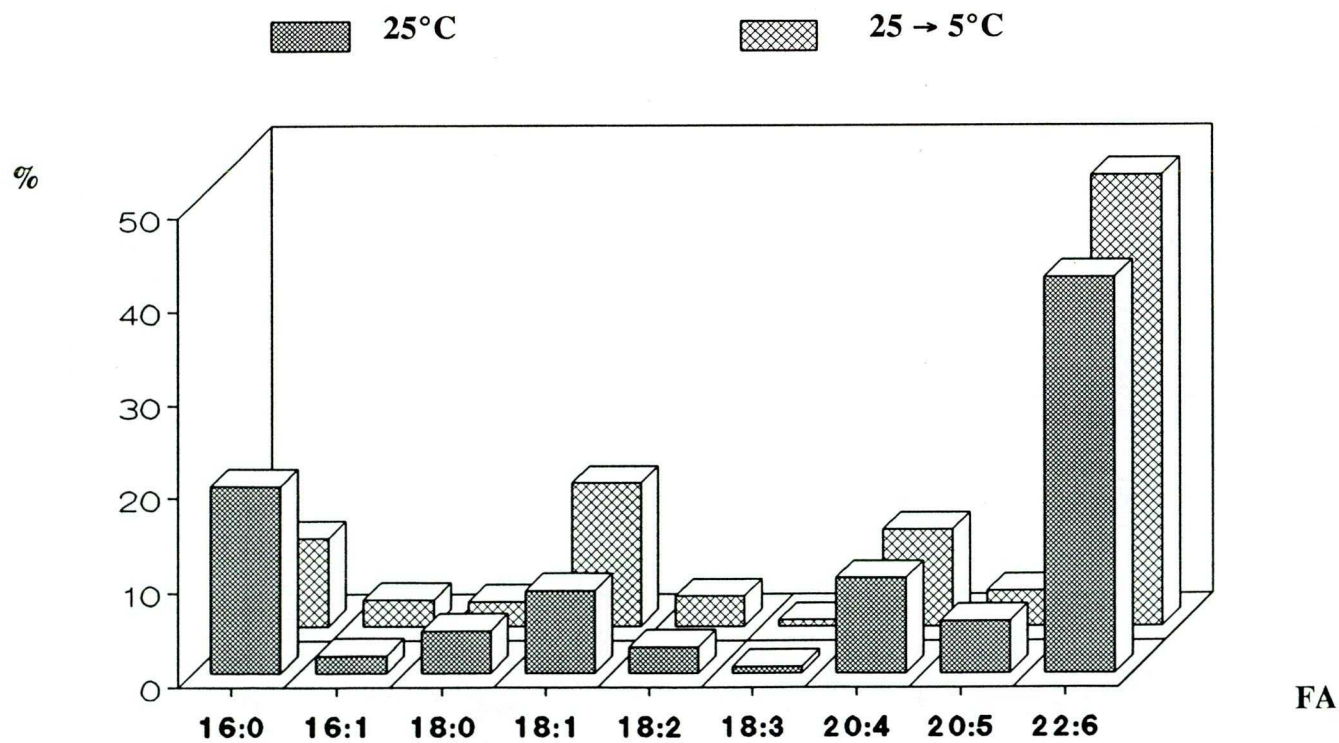

Figure 6.a,b **Fatty acid compositions of major individual phospholipids of pike liver controlled to shift-down of temperature in vivo**
6.a Phosphatidylcholine **6.b phosphatidylethanolamine**

during the same period, or fish inhabiting deep waters are exposed to such changes during their vertical migration.

Phospholipid composition

Thermal acclimatization has a major effect upon the PL class distribution of the pike liver. As shown in Table 7, a down-shift of the temperature brought about changes in PL compositions, and this response was in agreement with that observed with thermally adapted pike (Table 11 and Fig. 7). The membranes of cold-adapted (acclimatized) poikilotherms routinely possess higher proportions of PE and lower proportions of PC than membranes at higher growth temperatures (Hazel, 1979 a). Furthermore, only the winter-active species displayed increased proportions of PE. In our experiment, the level of PE also increased during a down-shift of the temperature, with a concomitant decrease in PC, the ratio of PC to PE thereby decreasing considerably.

Molecular species composition of PC and PE in relation to down-shift of temperature

To see whether the rapid changes observed in fatty acid and polar head group composition are reflected in the fine composition and architecture of the individual PL, the molecular species analysis was extended to temperature-shifted pike. The data are presented in Table 12. The major molecular species of diacyl-PE in the cold was 18:1 / 22:6 (21.8 %), followed by 16:0 / 22:6 (15.9 %). Other species included 20:5 / 20:5, 20:5 / 22:6,

Table 11

Effect of down-shift of temperature on polar head group composition in pike liver phospholipids

POLAR LIPID CLASSES %	25°C	25→5°C
Lysophosphatidylcholine	4.76	2.31
Sphingomyelin	12.32	9.77
Phosphatidylcholine	40.15	35.39
Phosphatidylinositol	1.83	4.06
Phosphatidylserine	2.32	3.32
Phosphatidylethanolamine	26.26	32.39
Phosphatidylglycerol	4.15	5.43
Phosphatidic acid	4.10	4.79
Cardiolipin	4.10	2.58

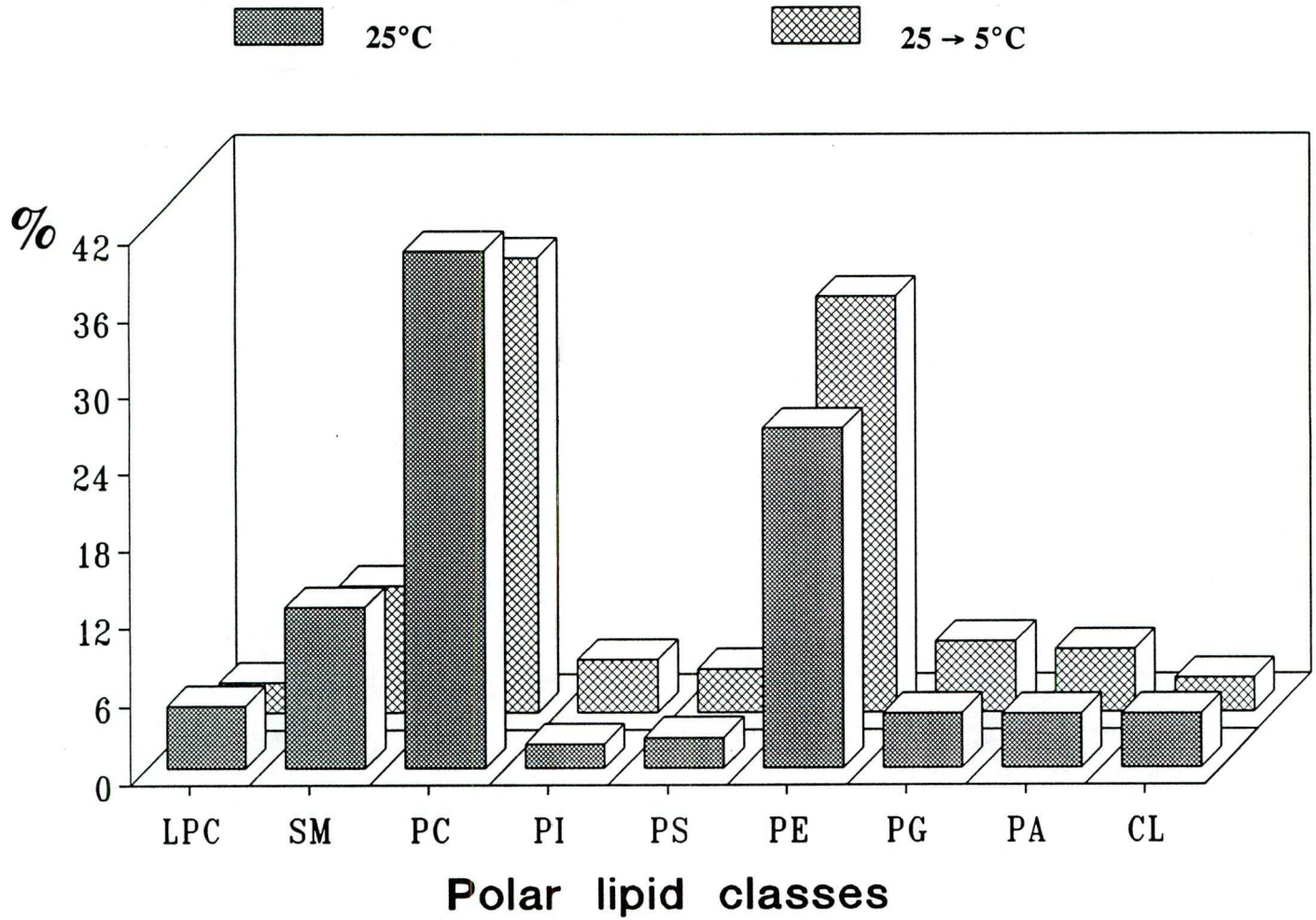

Figure 7 **Effect of down-shift of temperature on polar head group composition in pike liver phospholipids**

Table 12

Major molecular species in liver phosphatidylethanolamine and phosphatidylcholine of pike controlled to down-shift temperature

Molecular species	PE		PC	
	25°C→25°C	25°C→5°C	25°C→25°C	25°C→5°C
22:6 / 22:6	---	---	1.17	---
20:5 / 20:5	1.07	4.57	---	---
20:5 / 22:6	Trace	4.42	---	0.96
22:4 / 22:6	Trace	4.43	---	1.19
X	1.16	---	3.44	---
22:5 / 22:6	---	1.70	---	---
20:4 / 22:6 ± 16:1 / 20:5	2.30	5.49	2.83	3.10
22:4 / 22:6	1.13	2.66	---	---
16:0 / 18:4	Trace	5.80	---	---
18:1 / 22:6	11.24	21.77	8.24	13.24
16:0 / 22:6	42.36	15.90	31.17	26.45
16:0 / 20:4	2.67	2.68	3.91	5.04
18:1 / 16:1 ± 20:1 / 20:5	2.00	5.49	5.12	0.62
18:1 / 18:2	4.10	2.81	6.30	6.33
18:0 / 18:2	---	---	9.59	11.73
16:0 / 18:2	5.40	---	1.79	---
14:0 / 16:0 ± 16:0 / 22:6	4.35	2.35	2.91	10.44
18:0 / 20:4	15.30	1.50	4.55	3.22
18:1 / 18:1	0.47	---	1.43	3.63
16:0 / 18:1	1.27	0.70	14.60	13.17
16:0 / 16:0	4.70	4.27	1.58	0.87
16:0 / 18:0	0.55	---	---	---
18:0 / 20:1	1.76	---	---	---
X	---	---	0.47	---
X	---	---	0.37	---

22:4 / 22:6, 20:4 / 22:6, 18:1 / 16:1 and 16:0 / 16:0, which were present between 4 and 5%. Another 7 were minor species.

In the warm-adapted pike, the major PE species were 16:0 / 22:6 and 18:0 / 20:4 (42.4% and 15.3%, respectively), while monoenoic docosahexaenoic species, 18:1 / 22:6 comprised 11.2%. It was most important to observe that the down-shift of temperature led to an accumulation of 18:1 / 22:6 PE, thus mimicing the evolutionary or seasonally response. Another interesting feature is the rapid accumulation of di-polyunsaturated PE species, an effect also observed with evolutionarily and seasonally adapted fish (Table 8) These PL have a very low thermotropic phase transition temperature (mostly around -40°C). Only a small increase in their proportion might favourably affect membrane fluidity in response to cold. Thus, both 18:1 / 22:6 PE and the di-polyunsaturated PE species in combination with disaturated ones might effectively control membrane fluidity.

In addition to these factors, the sterol to phospholipid ratio is also considered to be factor controlling membrane fluidity. In our study, however, this is disregarded, since we did not extend the investigations to that level.

Diacyl-PE contains a much higher proportion of polyenoic species than diacyl-PC. Presence of a small amount of more unsaturated species of diacyl-PE in the cold-adapted species disproportionately affects the physical state of dispersions of PL membranes. Therefore, high unsaturated species of PE could be of importance in maintaining the morphology of the cell membranes (Robinson et al., 1986).

4.5. Effect of diet on fatty acid composition of fish

According to the literature, part of the long-chain PUFA of fish lipids may be derived from the diet but, they may be depleted from the body in a short time if the diet does not contain these acids (Kaneko et al., 1967). Fish are able to form these fatty acids from linoleic and linolenic acids by the same biochemical pathway as do mammals (Knipparth and Mead, 1966; Farkas and Csengeri, 1976), but the formation of these fatty acids is relatively slow. Furthermore, a definite amount of linolenic acid in the diet is essential for fish (Reiser et al., 1963; Castell et al., 1972). Conceivably, the feed being fed to fish may have an influence on their lipid metabolism and the fatty acid composition of fish and the measured fatty acid compositions are the resultants of dietary and biosynthetic process. Evidently, a high intake of long-chain PUFA suppresses the biosynthetic processes, and over a certain level of intake this predominates the fatty acid compositions.

To examine the dietary effects, the fatty acid compositions of omnivorous, carnivorous and herbivorous fish were studied. To exclude the thermal effects, only warm-adapted fish, as represented in Table 13, were investigated. The carp were partly maintained on an artificial diet cereals and corn, while the catfish and the silver carp consumed only a natural diet.

Table 13

Species	Feeding habit
Cyprinus carpio L.	Omnivorous
Hypophthalmichthys molitrix V.	Phytoplankton
Silrus glanis L.	Carnivorous

4.5.1. Fatty acid composition of feeds

The artificial food was a mixture of cereal meals (wheat and corn). The natural food was composed of different plankton organisms. Mainly phytoplankton (green algae and diatoms) organisms were found in the intestine of silver carp (Csengeri et al., 1978 b). The artificial foods contained three major fatty acids. Linoleic acid (18:2 n-6) had the highest level, followed by oleic acid (18:1 n-9) and palmitic acid (16:0) (Table 14), while the natural foods contained higher amounts of n-3 PUFA and low amounts of saturated fatty acids (Table 14).

4.5.2. Fatty acid composition of fish

4.5.2.1. Total phospholipids

The fatty acid compositions of the total polar lipids are listed in Table 15 (Fig.8). There are considerable differences between the fish species, reflecting the differences in their diets. This is especially true for linoleic acid (18:2 n-6) , AA, EPA and DHA. The share of monoenes was elevated in omnivorous fish, suggesting their ability to introduce the n-9 double bond (Δ9 desaturase) in stearic acid formed from dietary carbohydrates (Blond et al., 1981), but this fatty acid has also been recognized as a biochemical indicator of an increased intake of carbohydrate (Csengeri et al., 1978).

The total n-6 PUFA were significantly higher ($P > 0.05$), but the total n-3 PUFA and the (n-3)/(n-6) ratio were significantly lower ($P < 0.05$) in omnivorous fish as compared with carnivorous and herbivorous fish. The omnivorous fish received an artificial diet of corn or wheat, with relatively much 18:2 n-6. A low level of n-3 long-chain PUFA might be

Table 14

Fatty acid compositions of experimental diets (potential food)

Fatty acids	Natural food		Artificial food		
	Green algae	Diatoms	Common carp fed	Wheat	corn
14:0	2.2	7.7	1.0	0.25	0.17
16:0	6.1	7.6	23.3	17.86	11.80
16:1 n-7	9.7	11.4	3.8	0.63	0.68
18:0	1.6	4.1	7.2	2.04	2.48
18:1 n-9	17.2	8.6	30.6	16.16	30.51
18:2 n-6	12.1	6.5	21.8	54.72	50.59
18:3 n-3	21.0	3.4	4.1	6.30	2.56
18:4 n-3	1.2	1.7	2.3	0.78	0.64
20:2 n-6	1.1	1.2	0.4	0.71	---
20:3 n-6	3.4	---	---		
20:3 n-3	---	---	---		
20:4 n-6	0.9	1.8	2.5		
20:4 n-3	---	---	0.1		
22:5 n-3	1.8	25.8	1.7		
22:4 n-6	---	0.4	0.2		
22:5 n-6	---	---	0.4		
22:5 n-3	---	---	---		
22:6 n-3	---	1.6	---		

Table 15

Comparative study on total and individual phospholipid fatty acids from liver of different freshwater fish in relation to feeding habit

Fatty acids	TPL			PC			PE		
	Herbivorous	Omnivorous	Carnivorous	Herbivorous	Omnivorous	Carnivorous	Herbivorous	Omnivorous	Carnivorous
	Silver carp	Common carp	Cat fish	Silver carp	Common carp	Cat fish	Silver carp	Common carp	Cat fish
14:0	0.45 ± 0.37	0.54 ± 0.16	0.78 ± 0.25	0.45 ± 0.17	0.60 ± 0.07	0.29 ± 0.10	0.44 ± 0.02	0.06 ± 0.04	0.13 ± 0.03
16:0	22.00 ± 3.69	16.68 ± 0.66	17.07 ± 1.14	22.28 ± 5.21	21.43 ± 2.28	19.83 ± 3.32	16.67 ± 1.74	15.93 ± 3.86	9.98 ± 1.57
16:1 (n-7)	2.96 ± 0.36	10.14 ± 1.26	4.24 ± 1.88	3.10 ± 0.48	9.63 ± 2.34	3.88 ± 1.65	1.98 ± 0.14	5.42 ± 0.94	0.78 ± 0.61
18:0	5.13 ±1.03	5.27 ± 0.40	12.73 ± 3.20	4.19 ± 0.79	3.93 ± 0.25	6.35 ± 1.99	8.84 ± 1.24	11.24 ± 1.08	21.44 ± 2.81
18:1 (n-9)	12.10 ± 2.84	18.60 ± 2.95	13.5 ± 2.65	11.57 ± 2.14	17.37 ± 1.36	14.77 ± 1.06	7.12 ± 1.12	10.18 ± 6.59	5.71 ± 1.63
18:2 (n-6)	0.83 ± 0.27	6.41 ± 0.73	6.42 ± 0.55	0.68 ± 0.24	4.28 ± 0.28	4.47 ± 2.41	0.30 ± 0.01	3.31 ± 0.26	2.67 ± 1.08
18:3 (n-3)	0.90 ± 0.44	---	1.00 ± 0.50	1.22 ± 0.44	---	0.66 ± 0.32	0.79 ± 0.50	---	0.12 ± 0.06
20:3 (n-6)	0.20 ± 0.03	1.40 ± 0.32	1.09 ± 0.22	0.30 ± 0.10	1.64 ± 0.59	1.03 ± 0.39	0.21 ± 0.01	1.24 ± 0.10	0.89 ± 0.60
20:4 (n-6)	8.08 ± 2.54	15.85 ± 1.25	11.12 ± 1.52	8.45 ± 2.87	17.93 ± 1.14	10.47 ± 2.30	9.32 ± 0.96	22.36 ± 1.57	16.23 ± 4.19
20:5 (n-3)	8.06 ± 2.80	0.03 ± 0.01	6.20 ± 1.20	9.02 ± 3.22	---	12.90 ± 4.06	10.28 ± 0.73	---	7.88 ± 0.56
22:4 (n-6)	2.94 ± 0.62	6.29 ± 0.62	1.75 ± 0.72	2.37 ± 0.95	3.31 ± 2.00	1.03 ± 0.40	3.28 ± 0.91	7.15 ± 3.76	1.64 ± 0.28
22:5 (n-3)	0.93 ± 0.46	0.45 ± 0.29	1.58 ± 0.64	2.10 ± 1.62	0.12 ± 0.00	1.73 ± 0.23	2.97 ± 0.76	0.57 ± 0.05	1.79 ± 0.65
22:6 (n-3)	33.84 ± 2.45	16.38 ± 4.18	21.21 ± 3.98	33.21 ± 2.52	17.56 ± 2.85	20.87 ± 0.57	36.24 ± 4.87	20.41 ± 4.88	28.97 ± 2.16
(n-3) / (n-6)	3.74 ± 0.79	0.53 ± 0.16	1.49 ± 0.20	4.09 ± 0.77	0.61 ± 0.15	2.13 ± 0.28	3.85 ± 0.66	0.59 ± 0.18	1.83 ± 0.30
Sat. / unsat.	0.38 ± 0.05	0.29 ± 0.01	0.46 ± 0.09	0.37 ± 0.08	0.35 ± 0.05	0.38 ± 0.11	0.35 ± 0.05	0.38 ± 0.07	0.48 ± 0.10
UI	316.27 ± 19.98	236.47 ± 25.81	254.03 ± 2.89	322.58 ± 22.01	235.88 ± 5.53	276.98 ± 29.04	352.51 ± 25.39	260.77 ± 33.8	308.51 ± 9.74

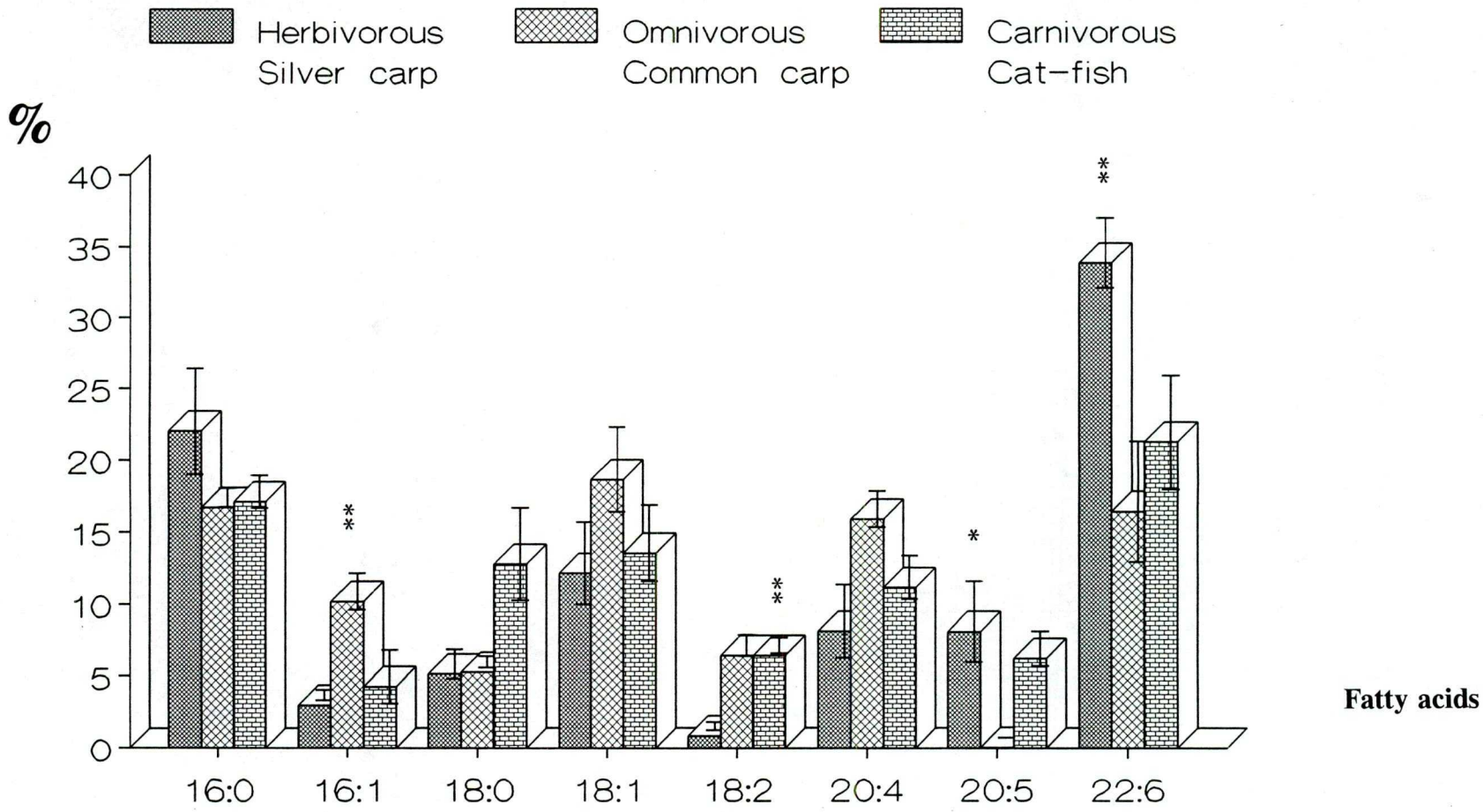

Figure 8 **Comparative study on total phospholipid fatty acids from liver of different freshwater fish in relation to feeding habit [N = 3]**

* $P < 0.01$ ** $P < 0.001$

Figure 8.a

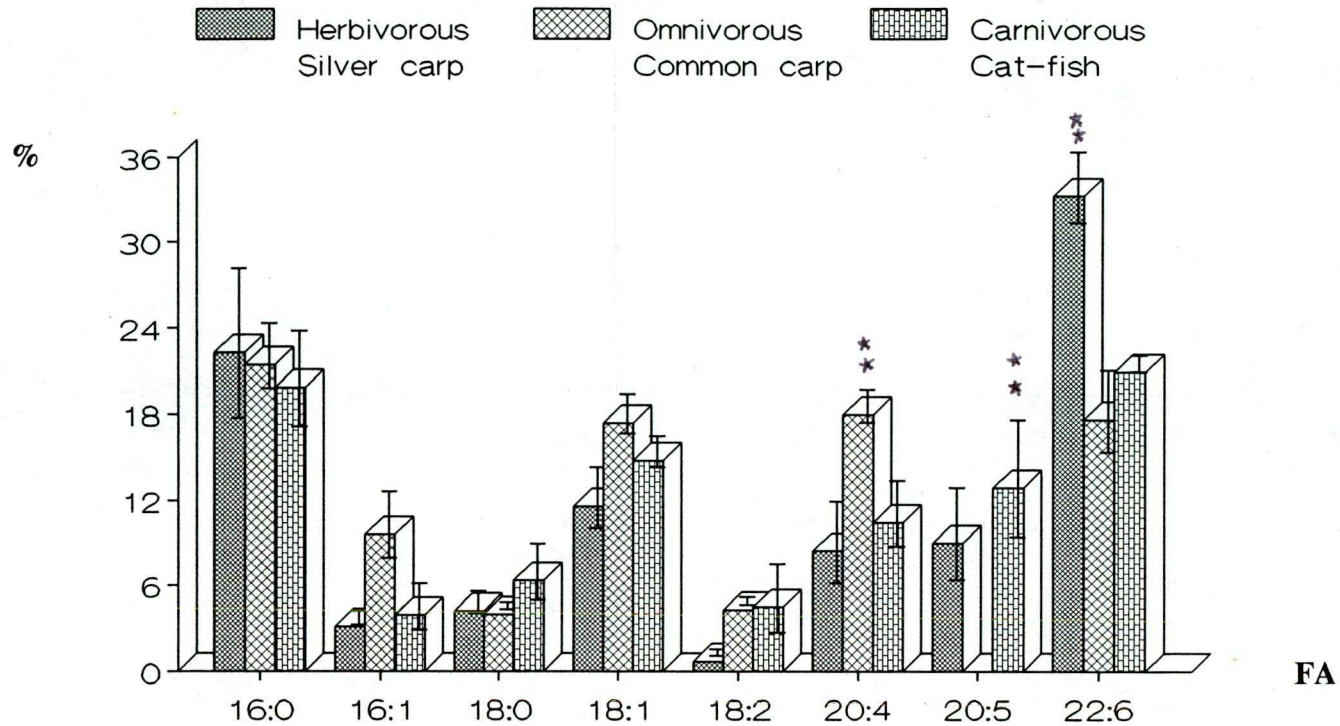

Figure 8.b

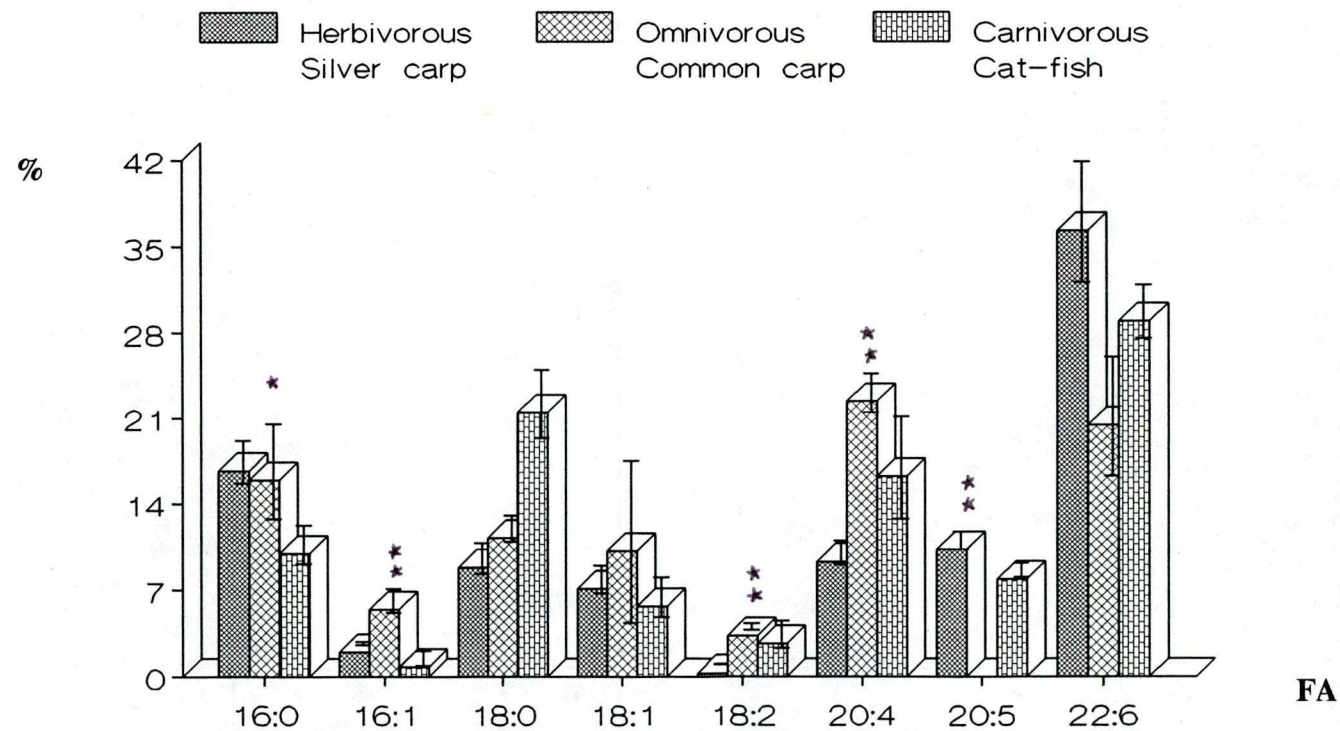

Figure 8.a,b Comparative study on major individual phospholipid fatty acids from liver of different freshwater fish in relation to feeding habit [N=3]

8.a Phosphatidylcholine 8.b phosphatidylethanolamine

* $P < 0.01$ **$P < 0.001$

explained by the absence or scarcity of 18:3 n-3 in their diet. On the other hand, the percentage of total n-3 PUFA, and predominantly EPA and DHA was higher in the herbivorous fish. It might be pointed out that lipids of plant sources are rich in 18:3 n-3 (green algae) and 20:5 n-3 (diatoms) (Table 14).

Therefore, the most notable differences between omnivorous and herbivorous or carnivorous fish was in the precursor for especially 18:3. This might have led to a lower level of n-3 polyenes. The high level of 18:1 n-9 in the cereals explains the high level of AA in carp.

4.5.2.2. Fatty acid composition of phosphatidylcholine

The data presented in Table 15 (Fig. 8 a) showed that the carnivorous fish have the highest level of saturated fatty acids (primarily 16:0 and 18:0), omnivorous fish contain a higher amount of monoenoic, while the herbivorous contain higher amounts of 20:5 n-3 and 22:6 n-3.

The data show that the examined carp readily took up the supplementary artificial food, which can be characterized by a high carbohydrate content and a low proportion of fat. The food intake from the artificial food can be reflected by an elevated level of oleic acid in the liver of these fish. As compared with fish fed on natural food or with usual fish feeds, the lipids of these species contained large proportions of eicosa- and, in particular, docosapolyenoic fatty acids.

There is no doubt that the precursors of these fatty acids (linoleic and linolenic acids) are synthesized in algae and fish need algal lipids directly (Castell et al., 1972). This phenomenon was explained by the fact that the formation of the enzymes involved in de novo

fatty acid synthesis showed an increased rate under the effect of food (Danaldson, 1973). The AA content of the liver is almost the same for the three species, although it is higher in the common carp, but lower in the silver carp and cat fish (22% versus 9-16%). This may be due to the differences in the lipid metabolism of these fish, rather than the differences in the fatty acid composition of the diet. In spite of the high linoleic acid content of the feeds offered, the herbivorous fish lipids contained a low proportion of AA. This could be explained by the observation that, if the levels of linoleic and linolenic acid were too high in the diet, a small amount of AA was synthesized from linoleic acid in certain fish (Reiser et al., 1963).

4.5.2.3. Phosphatidylethanolamine

PE was more unsaturated, exhibited higher proportions of 20:5 n-3 and 22:6 n-3 and had a lower saturated to unsaturated fatty acid ratio than those for PC. The herbivorous fish contained more PUFA (70%) and had a lower proportion of saturated fatty acids (20%). This fish also had an active Δ 6 desaturase, which transforms of linoleic acid into γ-linolenic acid and is very sensitive to metabolic and endocrine regulation (Blond et al., 1981).

However, since the fatty acid composition reflects the diet, it may be possible to increase the level of n-3 PUFA in the lipid of cultured fish by dietary manipulation. Increasing the n-3 fatty acid content of cultured fish diet could result in higher n-3 PUFA levels and n-3 / n-6 ratios, and may enhance the marketability of aquaculture products (Olsen and Ringo, 1992).

In the PE of herbivorous fish, level of linolenic acid 18:3 (n-3) was also low, whereas 22:6 (n-3) was significantly increased. This low level in fish can be explained either by a low

dietary intake or by its intensive conversion to 22:6 n-3. In fact, diatoms, the major food of silver carp (Farkas, 1970; Farkas and Roy, 1989), are rather poor in 18:3 n-3, but rich in 20:5 n-3. In the same fish, a low amount of AA can be explained by the observation that, if the level of linoleic acid was too high in the diet, only a small amount of AA was synthesized from it (Reiser et al., 1963).

4.5.2.4. Phospholipid polar head group composition

Table 16 shows the PL polar head group composition of the liver of some fish with different feeding habits. PC and PE were the major polar lipids. Carnivorous fish contained similar amounts of PC and PE (32%), and slightly small proportions of SM and PI, the PS / PS ratio was 6.1%. Omnivorous and herbivorous fish had a lower PS / PI ratio (0.8% a nd 0.7%, respectively), but had similar contents of PC, PE and SM (40-41%, 23.5-31.5% and 7.1-7.2%, respectively).

Although there were apparent differences in the PC and PE levels, it is not clear that these were due to their feeding habit. These PL are constituents of different cellular membranes, the compositions of which might be under strict control, in contrast with the acyl chains, which can be modified exogenously (Farkas et al., 1984).

Table 16

Polar lipid content of freshwater fish liver in relation to feeding habit

Polar lipid classes %	Herbivorous Silver carp	Omnivorous Common carp	Carnivorous Catfish
Lysophosphatidylcholine	6.05 ± 0.95	3.04 ± 0.50	5.39 ± 0.59
Sphingomyelin	7.25 ± 1.82	7.13 ± 1.12	5.54 ± 1.08
Phosphatidylcholine	40.88 ± 3.08	41.21 ± 6.55	37.66 ± 3.02
Phosphatidylinositol	4.86 ± 1.39	6.79 ± 1.47	1.94 ± 0.77
Phosphatidylserine	3.55 ± 1.82	5.47 ± 2.43	5.39 ± 1.00
Phosphatidylethanolamine	23.49 ± 2.20	31.45 ± 3.36	31.72 ± 1.20
Phosphatidylglycerol	6.52 ± 1.38	4.41 ± 1.14	5.24 ± 0.47
Phosphatidic acid	4.94 ± 0.73	0.68 ± 0.35	3.29 ± 0.06
Cardiolipin	2.44 ± 0.35	0.17 ± 0.02	3.84 ± 0.37

4.6. Effect of fish age on fatty acid composition

4.6.1. Total fatty acids of phospholipids

Several data have shown that the membrane lipid composition changes during the aging progress (Fillerup and Mead, 1966; Linz, 1968; Tocher et al., 1985). The activity of different desaturases also declines (Phillips et al., 1969). Thus the saturated fatty acids tend to predominate. This results in a loss of membrane fluidity and is associated with altered membrane functions, etc. Although this phenomenon has been studied in detail in mammals no similar data are available on fish.

In the total polar lipids, the proportion of saturated (primarily 16:0) was significantly decreased with aging of fish, while stearic acid (18:0) was increased (Table.17). Of the monoenoic acids primarily 16:1 n-7 and 18:1 n-9, the former remained unaffected, while the latter was increased in the 2-year-old animals.

The highest concentration of unsaturated fatty acids was found in the early stage of development. This is not surprising. There is intensive membranogenesis in young animals, and this progress requires more unsaturated fatty acids such as AA.

The lower concentrations of unsaturated fatty acids, found in the 3-year-old animals, may be due to a change in diet. The percentage of total n-3 PUFA, predominantly 20:5 and 22:6, remained relatively constant during aging of fish. It is probable that the level of these acids is determined by the diet.

4.6.2. Phosphatidylcholine (PC)

The proportions of total saturates and monoenes were greater than in total polar lipids

Table 17

Fatty acid composition of total and individual phospholipids in relation to aging of common carp (Cyprinus carpio L.)

Fatty acids	TPL			PC			PE		
	One year	Two year	Three year	One year	Two year	Three year	One year	Two year	Three year
14:0	0.72 ± 0.28	3.24 ± 1.25	0.83 ± 0.08	0.79 ± 0.08	0.87 ± 0.21	0.99 ± 0.39	0.15 ± 0.04	0.13 ± 0.02	0.12 ± 0.02
16:0	26.30 ± 1.73	21.00 ± 1.41	20.15 ± 0.95	29.67 ± 1.74	24.93 ± 0.45	24.43 ± 2.92	15.28 ± 0.28	15.06 ± 0.91	14.44 ± 0.97
16:1 (n-7)	5.92 ± 0.19	5.44 ± 1.40	5.09 ± 0.66	6.59 ± 1.44	6.98 ± 0.60	6.84 ± 0.21	0.98 ± 0.08	1.68 ± 0.76	0.79 ± 0.25
18:0	8.40 ± 1.43	12.65 ± 1.57	13.3 ± 2.46	4.17 ± 0.99	5.45 ± 0.42	4.49 ± 0.62	15.55 ± 3.77	14.13 ± 0.61	17.13 ± 2.62
18:1 (n-9)	13.09 ± 2.06	17.21 ± 0.50	14.76 ± 1.13	12.27 ± 2.43	15.39 ± 1.96	13.62 ± 2.12	9.16 ± 1.63	10.18 ± 1.06	8.19 ± 1.21
18:2 (n-6)	2.08 ± 0.26	2.51 ± 0.26	1.68 ± 0.26	2.18 ± 0.19	2.17 ± 0.62	2.22 ± 0.43	0.77 ± 0.32	1.99 ± 0.04	0.97 ± 0.38
18:3 (n-3)	0.22 ± 0.04	0.19 ± 0.05	0.22 ± 0.11	0.21 ± 0.02	0.21 ± 0.05	0.34 ± 0.08	0.33 ± 0.21	0.13 ± 0.06	0.31 ± 0.22
20:3 (n-6)	0.85 ± 0.08	0.92 ± 0.13	0.95 ± 0.13	0.89 ± 0.11	1.47 ± 0.42	1.18 ± 0.45	0.72 ± 0.11	0.83 ± 0.10	0.53 ± 0.37
20:4 (n-6)	12.64 ± 0.87	10.36 ± 0.62	9.96 ± 1.28	11.74 ± 0.89	10.32 ± 2.04	8.58 ± 0.52	19.55 ± 0.53	16.37 ± 2.16	12.61 ± 0.62
20:5 (n-3)	1.53 ± 0.98	0.79 ± 0.23	1.94 ± 0.10	1.85 ± 1.66	1.82 ± 0.48	3.47 ± 0.26	3.23 ± 1.19	4.62 ± 0.51	3.71 ± 1.46
22:4 (n-6)	1.91 ± 0.61	0.88 ± 0.55	2.38 ± 0.28	1.32 ± 0.74	2.01 ± 0.41	0.91 ± 0.74	3.17 ± 0.85	0.99 ± 0.27	2.88 ± 1.22
22:5 (n-3)	2.70 ± 0.21	0.44 ± 0.39	2.21 ± 0.15	2.75 ± 0.35	1.17 ± 0.35	1.57 ± 0.29	3.23 ± 0.48	0.54 ± 0.25	4.74 ± 2.45
22:6 (n-3)	23.12 ± 2.09	22.80 ± 2.56	25.98 ± 3.37	24.48 ± 3.58	26.36 ± 0.82	29.89 ± 5.61	26.77 ± 4.62	32.72 ± 0.77	32.69 ± 2.39
(n-3) / (n-6)	1.58 ± 0.28	1.53 ± 0.01	2.03 ± 0.40	1.71 ± 0.30	1.84 ± 0.30	2.54 ± 0.23	1.41 ± 0.34	1.88 ± 0.23	2.42 ± 0.35
Sat. / Unsat.	0.56 ± 0.01	0.59 ± 0.05	0.53 ± 0.05	0.53 ± 0.01	0.45 ± 0.02	0.43 ± 0.07	0.45 ± 0.07	0.42 ± 0.01	0.47 ± 0.08
UI	244.93±10.36	221.8 ±18.39	253.07±14.19	250.86±17.88	256.07 ±9.04	275.48±30.22	301.77±31.41	278.39 ±56.1	316.46±18.5

(Table 17). The saturates in PC decreased significantly from the first year to the third year, probably due to the conversion of stearic acid to its unsaturated analogue. The proportion of total n-3 PUFA was higher in the adults than in the young (Table 17). The changes in n-3 PUFA were mainly due to fluctuations in the percentage of 22:6 n-3.

*4.6.3. **Phosphatidylethanolamine (PE)***, was more unsaturated and exhibited highest proportions of 22:6 n-3 and 20:5 n-3 than PC; both initially increased on increase in the age of the fish (Table 17). The proportions of total saturates and monoenes tend to be unaffected during the aging of fish. There were no statistical significant changes in either the total or the individual polar lipids between young and old fish.

The proportion of 20:4 n-6 is higher in PE than in PC in all ages in carp liver. In rats, the levels of 20:4 and other polyenes are also higher in PE than in PC. PE is a conic-shaped molecule and its conicity is also dependent on the component fatty acids. These conic-shaped molecules, due to their easy conversion from a bilayer to non-bilayer structure, might play an important role in membrane dynamics and functions. Whether AA is of dietary origin or was formed by the fish requires further investigations. Most probably, a large proportion of the AA is of biosynthetic origin, because zooplankton is relatively poor in this fatty acid (Farkas, 1970).

4.6.4. Polar lipid head group composition

Fig.9 shows that PC was the major PL (52.12% - 67.39% of the total lipids) followed by PE (15.34 - 31.82%) and LPC (0.9-2.7%). It was interesting to observe that the level of PC decreased with increasing age, mostly at the expense of PE. In contrast,

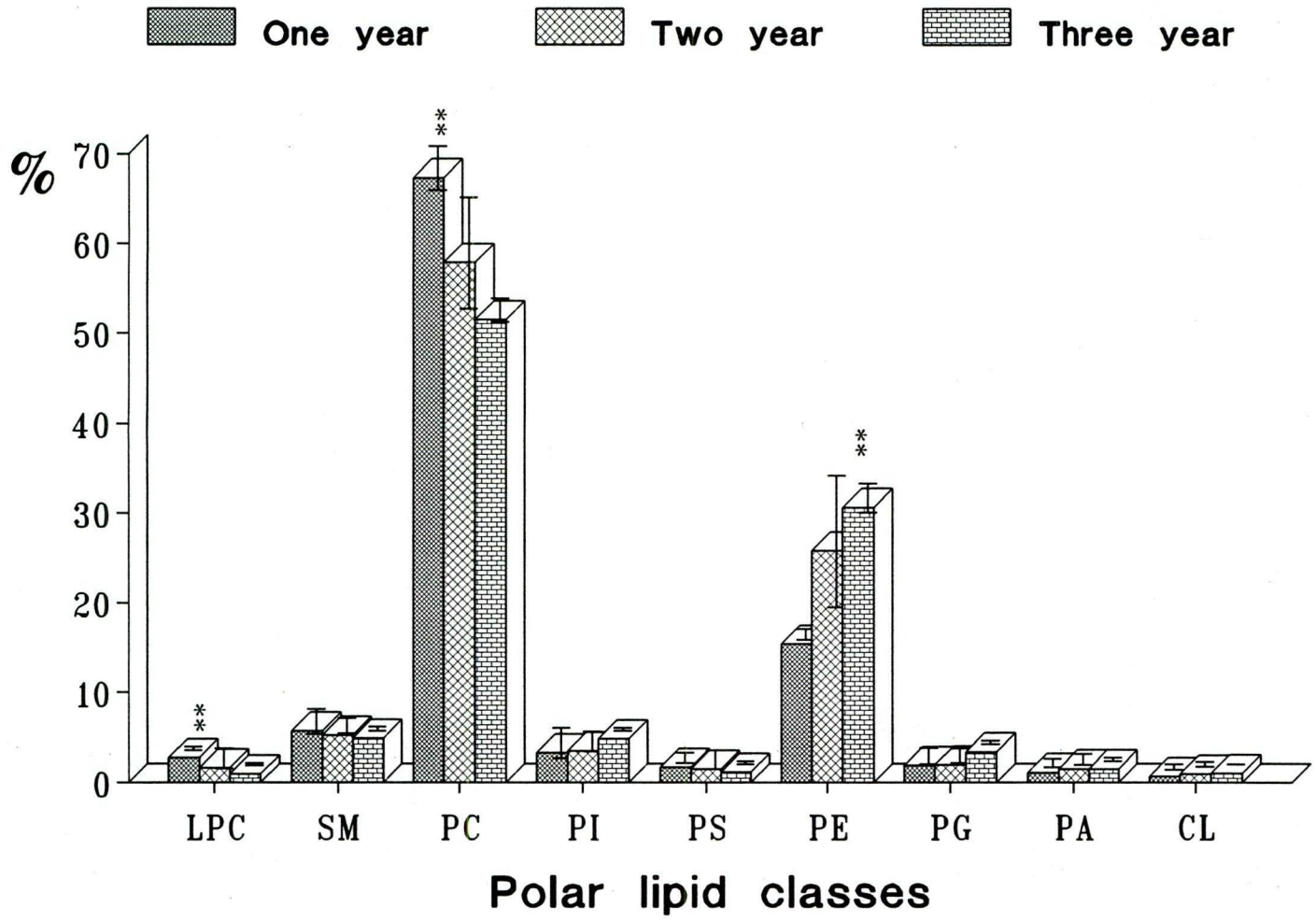

Figure 9 **Polar lipid content in relation to aging of common carp**

[N = 3]

* P < 0.01 ** P < 0.001

the proportions of PS, PI and phosphatidic acid remained relatively constant throughout aging.

These PL are the major factors controlling membrane fluidity and linked functions. As mentioned, the thermotropic phase transition temperature of PE is always higher than that of PC with identical fatty acid composition. The increase in PE during aging might result in more rigid membranes. In fact, rigidification of these structures during aging has been observed in several systems, but this might also be attributed to increased sterol to PL ratios. However, more direct investigations must be performed to check this speculation.

SUMMARY AND CONCLUSION

Investigations were carried out on the composition of the liver lipids of fish inhabiting waters of different temperatures and on fish with different feeding habit /or different ages. These investigations revealed the following findings:

1. Fish and rat phospholipids do not differ qualitative as far as the fatty acid compositions are concerned. However, rat liver phospholipids are more poor in n-3 type polyunsaturated than fatty acids from the fish liver phospholipids. In addition rat liver phospholipids contain phosphatidylethanolamine in a higher concentration than do fish liver phospholipids. Lower level of polyunsaturates and higher level of phosphatidylethanolamine in rat liver phospholipids might render these structures more rigid, which is, indeed, expected from a homoiothermic animal.

2. The Total phospholipids from cold-adapted marine and freshwater fish (Clupea harengus and Acerina cernua) were more unsaturated than those their warm-adapted counterpartners. The effect of the environmental temperature on fatty acid composition was more pronounced for the freshwater fish than the marine ones. Phosphatidylethanolamine tended to accumulate in cold-adapted species. Comparison of the molecular species composition of purified phosphatidylcholine and phosphatidylethanolamine the revealed the accumulation of 18:1 / 22:6 species in the later.

It is concluded that:

a) An increased number of conic-shaped molecules such as PE contributes greatly to the stability of membranes in the cold.

b) Not only the former, but also the conicity might be of importance in this process.

3. The seasonal changes in the phospholipid fatty acid composition of a freshwater fish (pike) which is active in summer and winter paralleled the changes demonstrated for freshwater and marine fish habiting subtropical and arctic waters. The same was true for the molecular species composition of purified PC and PE. The effect of temperature on membrane fluidity was demonstrated by using a DPH fluorescence polarization technique. The fluorescence anistoropy of DPH embedded into phospholipid vesicles prepared from cold-adapted pike was significantly lower than in phospholipid vesicles from warm-adapted pike.

4. The effect of temperature on the fatty acid composition of phospholipids was also demonstrated under experimental conditions. A down-shift of the temperature brought about an increase in unsaturation, and the accumulation of PE and 18:1 / 22:6 species. As found with fish adapted during evolution or seasonally to different temperatures.

5. A correlation was found between the fatty acid composition of feed and lipid metabolism of the fish. The proportion of total n-6 PUFA was higher, while the total n-3 PUFA, the n-3 / n-6 were significantly lower in omnivorous fish as compared with carnivorous and herbivorous fish. The percentages of n-3 PUFA and especially 22:6 n-3 and 20:5 n-3 were higher in the herbivorous fish, while

carnivorous fish exhibited the highest level of saturated fatty acids.

6. A study was made of the changes in fatty acid and polar head group composition in relation to fish aging. Phospholipids from young carp (Cyprinus carpio L.) tender to be more unsaturated than in older ones. This might be explained by a more intensive membranogenesis in the young animals. PC and PE were the major components in the PL fraction. It was interesting to observe that PC declined with fish aging, whereas PE increased with aging. These PL are the major components controlling the membrane fluidity, and increased PE during aging might result in rigid membranes, a characteristic of aged membranes.

REFERENCES

Abdul Malak N., Brichon G., Meister R. and Zwingelstein G.(1989) Environmental temperature and metabolism of the molecular species of phosphatidylcholine in the tissues of the rainbow trout. Lipids 24, 318-324.

Abruzzini A. F., Ingram L. O. and Clem L. W. (1982) Temperature mediated processes in teleost immunity homeoviscous adaptation in teleost lymphocytes. Proc. Soc. Exp. Biol. Med. 169, 12-18.

Ackman R. G. (1967) Characteristics of the fatty acid composition and biochemistry of some fresh-water fish oils and lipids in comparison with marine oils and lipids. Comp. Biochem. Physiol 22, 907-922.

Ackman R. G. (1982) Fatty acid composition of fish oil. In: Barlow S.M. Stansby ME (Editors), Nutritional evaluation of long-chain fatty acids in fish oils. Academic Press, London pp, 25-88.

Ackman R. G. (1989) Nutritional composition of fats in sea foods. Prog. Food Nutr. Sci. 13, 161-241.

Albrink M. J., Ullrich I. H., Blehschmidt N. G., Mike P. and Rogers J. S. (1986) The beneficial effect of fish oil supplements on serum lipids and clotting function of patients with type II diabetes mellitus (Abstract). Diabetes 35 (suppl.1): 43 A

Anderson G. J. and Connor W. E. (1988) Uptake of fatty acids by the developing rat brain. Lipids 23, 286-290.

Anderson G. J., Connor W. E., Corliss J. D. and Lin D. S. (1989) Rapid modulation of the n-3 docosahexaenoic acid level in the brain and the retina of the newly hatched chick. J. Lipid Res. 30, 433-441.

Anderson R. E., Maude M. B. and Feldman G. L. (1969). Lipids of ocular tissues. I. The phospholipids of mature rabbit and bovine lens. Biochem. Biophys. Acta 187, 345-53.

Agren J. J., AL-Amad H. and Hánninen O. (1991) Fatty acid content and composition of five fish species from the Persian Gulf. Comp. Biochem. Physiol. 100B, 339-341.

Avtation R. R. (1969) Temperature effect on antibody production and immunological memory in carp (Cyprinus carpio L.) immunized against bovine serum albumin (BSA). Immunology 17, 927-931.

Baldwin J. and Cornatzer W. E. (1969) Liver lipid during development. Lipids 3, 361-367.

Bang H. O., Dyerberg J. and Hjϕne N. (1976) The composition of food consumed by greenland Eskimos. Acta Med. Scand 200, 69-73.

Blank M. L., Ribinson M., Fitzgerald V. and Synder F. (1984) Novel quantitative method for determination of molecular species of phospholipids and diglycerides. J. Chromatogr. 298, 473-482.

Blond J. P., Lemarchal P. and Spielmann D. (1981) comparative desaturation of linoleic and dihomo-γ- linolenic acids by homogenates of human liver in vitro. CR Acad. Sci. (D) Paris, 292, 911-914.

Brenner R. R., Vazza V. D. and DE Tomás M. E. (1963) Effect of a fat-free diet and of different dietary fatty acids (palmitate, oleate and linoleate) on the fatty acid composition of freshwater fish lipids. J. of Lipid Res. 4, 341-345.

Brett J. R. and Groves T. D. D. (1979) Physiological energetics. In: V. S. Hoar and D. J. Rondall (Editors) Fish Physiology 8. Academic Press, New York, NY. pp. 279-352.

Brockerhoff H. and Hoyle R.J. (1963) On the structure of the depot fats of marine fish and mammals. Arch. Biochem. Biophys. 102, 452-455.

Brockerhoff H., Hoyle J. and Ronald K. (1964) Retention of the fatty acid distribution pattern of a dietary triglycerides in animal. J. Biol. Chem. 239, 735-739.

Brocherhoff H., Yurkwski M., Hoyle R.J. and Ackman R.G. (1964) Fatty acid distribution in lipids of marine plankton. J. Fish Res. BD. Canada, 21, 1379-1383

Cai Z. and Curtis L. R. (1989) Effects of diet on composition, growth fatty acid composition of young grass carp. Aquaculture 81, 47-60.

Cai Z. and Curtis L. R. (1990) Effects of diet and temperature on food composition, growth rate and tissue fatty acid composition of triploid grass carp. Aquaculture 88, 313-327.

Castell J. D., Sinnhuber R.O., Wales J. H. and Lee D. J. (1972) Essential fatty acids in the diet rainbow trout (Salmo gairdneri). Growth, food conversion and grass deficiency symptoms. J. Nutrition, 102, 77-86.

Chanmugam P., Boudreu M. and Hwang D. H. (1986) Differences in the n-3 fatty acid contents in pond-reared and wild-fish and shell-fish. J. Food Sci., 51, 1556-1557

Chetty N., Reavis S. C., Immelman A. R., Atkinson P. M. and Vanas J. G. (1989) Fatty acid composition of some south African freshwater fish. SAMT. 76, 368-370.

Clarke A., Brown J. H. and Holmes L. J. (1990) The biochemical composition of eggs from Macrobrachium Rosenberg II in relation to embryonic development. Comp. Biochem. Physiol. 96B, 505-511.

Corey E. J., Chuam Shih. and Cashman J. R. (1983) Docosahexaenoic acid is a strong inhibitor of prostaglandins but not leucotriene biosynthesis. Proc. Natl. Acad. Sci. USA. 80, 3581-3584.

Cossins A. R. (1977) Adaptation of biological membranes to temperature. The effect of temperature acclimation of gold-fish, upon the viscosity of sympatosomal membranes. Biochem. Biophys. Acta 470, 395-411.

Cossins A. R. (1983) Adaptive responses of fish membranes to altered environmental temperature Biochem. Soc. Trans 11, 332-333.

Cossins A. R. and Prosser C. L. (1978) Evolutionary adaptation of membranes to temperature. Proc. Natl. Acad. Sci. USA. 75, 2040-2043.

Cossins A. R. and Prosser C. L. (1982) Variable homoviscous responses of different brain membranes of thermally-acclimated goldfish. Biochem. Biophys. Acta 687, 303-309.

Csengeri I., Farkas T., Moyoros F., Oláh J. and Rágyanszki M. (1978) Fatty acids composition of liver and muscle tissues of silver carp (Hypophthalamichthys molitrix V.) and pig head (Hypophthalamichthys nobilis R.) in relation to diet. Aquacultura. Hungarica (Szarvas) 1, 35-43.

Csengeri I., Farkas T., Moyoros F., Oláh J. and Szalay M. (1978 b) Effect of feeds on the fatty acid composition of carp (Cyprinus carpio L.) Aquacultura Hungarica (Szarvas), 1, 24-34.

Dallner G., Siekevitz P. and Palade G. E. (1965) Synthesis of microsomal membranes and their enzymatic constituents in developing rat liver. Biochem. Biophys. Res. Commun. 20, 135-141.

Dallner G., Siekevitz P. and Palade G. E. (1965 b) Phospholipids in hepatic microsomal membranes during development. Biochem. Biophys. Res. Commun. 20, 142-148.

Danaldson W. E. (1973) Glucose stimulation of fatty acid desaturation in liver of newly-hatched chicks. Biochem. Biophys. Acta, 316, 8-12.

Dickens B. F. and Thompson G. A. Jr. (1982) Phospholipid molecular species alterations in microsomal membranes as an initial key step during cellular acclimation to low temperature. Biochemistry 21, 3604-3611.

Dunstan G. A., Sinclair A. J., O'Dea K. and Naughton J. M. (1988) The lipid content and fatty acid composition of various marine species from southern Australian costal waters. Comp.. Biochem. Physiol. 91B, 165-169.

Dyerberg J., Bang H. O., Stoffersen E., Moncada S. and Yane J. R. (1978) Eicosapentaenoic acid and prevention of atherosclerosis. Lancet ii, 117-119

Farkas T. (1970) The dynamic of fatty acids in the aquatic food chain phytoplankton, zooplankton, fish. Annal. Biol. Tihany, 37, 165-176.

Farkas T. (1970 b) Investigation on fats fresh-water crustaceans. I. Fatty acid composition of lipids obtained from Eudiaptomus gracilis G. O. Sars (Cladocera) Acta Biol. Acad. Sci. Hung. 21, 225-233.

Farkas T. and Csengeri I. (1976) Biosynthesis of fatty acids by the carp (Cyprinus carpio L.) in relation to environmental temperature. Lipids 11, 401-407.

Farkas T., Csengeri I., Majoros F. and Olah J. (1980) Metabolism of fatty acids in fish III combined effect of environmental temperature and diet on formation and deposition of fatty acid in carp, (Cyprinus carpio L.) Linnaeus 1758. Aquaculture 20, 29-40.

Farkas T., Nemecz G. Y. and Csengeri I. (1984) Differential response of lipid metabolism and membrane physical state by an activity and passively overwintering planktonic crustacean. Lipids 19, 436-442.

Farkas T. and Roy R. (1989) Temperature mediated restructuring of phosphatidylethanolamine in livers of freshwater fishes. Comp. Biochem. Physiol. 93B, 217-222.

Farkas T., Storebakken T. and Bhosle N. B. (1988) Composition and physical state of phospholipids in calanoid compepods from India and Norway. Lipids 23, 619-622.

Fillerup D. L. and Mead J. F. (1966) The lipids of the aging human brain. Lipids 2, 295-298.

Fine J.B. and Specher H. (1982) Unidimensional thin layer chromatography of phospholipids on boric acid impregnated plates. J. Lipids Res. 13, 660-663.

Fogerty A. C., Evans A. J., Ford G. L. and Kennett B. H. (1986) Distribution of n-6 and n-3 fatty acids in lipid classes in Australian fish. Nutr. Reports International. 33, 777-786.

Folch J., Lees M. and Sloane Stanley G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509

Fukushima H., Martin C.E., Iida H., Kitajima Y., Thompson Jr. G. A. and Nozawa Y. (1976) Changes in membrane lipid composition during temperature adaptation by thermotolerant strain of Tetrahymena pyriformis. Biochem. Biophys. Acta 431, 165-179.

Gibson R.A. (1985) Sources of arachidonic acid and eicosapentaenoic acids in Australian and Asian fish. Proc. Second Int. Congr. On essential fatty acids, prostaglandins and leucotrienes. London, pp. 43-44.

Gibson R.A., Kneebone R. and Kneebone G.M. (1984) comparative levels of arachidonic acid and eicosapentaenoic acid in Malaysian fish. Comp. Biochem. Physiol. 78C, 325-328.

Gopakumar K. and Nair M. R. (1972) Fatty acid composition of eight species of Indian marine fish. J. Sci. Fd Agric. 23, 493-496.

Gopakumar K. and Nair M. R. (1973) Lipid composition of fives species of Indian prawns. J. Sci. Fd Agric. 26, 319-325.

Gunston F. D. (1989) Fatty acids in fish oils and their analysis by gas chromatography. Br. J. Clin. Pract. 81, 15-19.

Haines A. P., Sanders T.A.B., Imeson J. D., Mahler R. F., Martin J., Mistry M., Vickers M. and Wallace P. G. (1986) Effect of a fish oil supplement on platelet function haemostatic variables and albuminuria in insulin dependent diabetics thromb. Res. 43, 643-655.

Haug T., Ringϕ E. and Petersen G. W. (1988) Total lipid and fatty acid composition of polar and neutral lipids in different tissues of Atlantic halibut (Hippoglossus hippoglossus L.) Sarsia, 73, 163-168.

Hazel J. R. (1979 a) The influence of thermal acclimation on membrane lipid composition of rainbow trout liver. Am. J. Physiol. 236, 91-101.

Hazel J. R. (1984) Effects of temperature on the structure and metabolism of cell membranes in fish. Am. J. Physiol. 246, R 460-R 470.

Hazel J. R. and Carpenter R. (1985) Rapid changes in the phospholipid composition of gill membranes during thermal acclimation of the rainbow trout, Salmo gairdneri. J. comp. Physiol. B. 155, 597-602.

Hazel J. R., Hagar A. F. and Prouitt N. L. (1987) Temperature dependence of phospholipids deacylation / reacylation in isolated hepatocyte of thermally acclimated rainbow trout (Salmo gairdneri). Biochem. Biophys. Acta 918, 149-158.

Hazel J. R. and Zebra E. (1986) Adaptation of biological membranes to temperature: molecular species compositions of phosphatidylcholine and phosphatidylethanolamine in mitochondrial and microsomal membranes of liver from thermally-acclimated rainbow trout. J. Comp. Physiol B. 156, 665-674.

Henderson R. J. and Tocher D. R. (1987) The lipid composition and biochemistry of fresh-water fish. Prog. Lipid. Res. 26, 281-347.

Hirai A., Hamazaki T. and Terano T. (1980) Eicosapentaenoic acid and platelet function in Japanese. Lancet 2, 1132-1133.

Johnston P. V. and Roots B. I. (1964) Brain lipid fatty acids and temperature acclimation. Comp. Biochem. Physiol. 11, 303-309.

Kaneko T., Takeuchi S., Ichi Higashi H. and Kikuchi T. (1967) Effect of dietary lipids on fish under cultivation. III Effect of methyl esters of highly unsaturated fatty acids methyl linoleate and methyl palmitate on the fatty acid composition of rainbow trout flesh lipids. Bull. Jap. Soc. Sci. Fish., 33, 47-55.

Kasim S. E., Stern B., Khilnani S., Mclin P., Baciorowshi S. and Jen K.L.C (1988) Effects of omega-3 fish oils on lipid metabolism, glyceric control and blood pressure in type II diabetic patients. J. Clin. Endocrinol Metab. 67, 1-5

Kayama M., Hirata M. and Hisai T. (1986) Effect of water temperature on the desaturation of fatty acids in carp. Bull Japan.Soc. Sci. Fish. 52, 833-857.

Kayama M. and Tscuchiya Y. (1962) Possible conversion pathway of polyunsaturated acid in fish. Tohoky J. Agric. Res. 13, 229-235.

Kinsella J. E. (1988) Food lipids and fatty acids. Food Technology 42, 124-127.

Kinsella J. E. (1989 a) n-3 polyunsaturated fatty acid requirements. Proceeding MIT conference on fish oil. M. Karel and R. lees editors. Marcel Dekker. NY.

Knipparth W. G. and Mead J. F. (1966) Influence of temperature on the fatty acid pattern of Mosquito fish (Gambuzia affinis) and guppy (Lebistes reticulatus). Lipids 1, 113-117.

Kromhout D., Bosschieter E. B. and Coulander C.D.L. (1985) The inverse relation between fish consumption and 20 year mortality from coronary heart disease. N Engl. J. Med. 312, 1205-1209.

Kuhry J. G., Duportail G., Bronner C.H. and Laustriat G. (1985) Plasma membrane fluidity measurements on whole living cells by fluorescence anisotropy of trimethyammonium diphenyl-hexatriene. Biochem. Biophys. Acta 845, 60-67.

Leaf A. and Weber P. C. (1988) Cardiovascular effects of n-3 fatty acids. N. Engl. J Med. 318, 549-571.

Leray C., Pelletier X., Hemmendinger S. and Cazenare J. P. (1987) Thin-layer chromatography of human platelet phospholipids with fatty acid analysis. J. Chromatogr. 420, 411-416.

Lewis R. A. and Austen K. F. (1981) Mediation of local homeostasis and inflammation by leukotriens and other mast cell-dependent compounds. Nature London 293, 103-108.

Linz P. H. (1968) Variation in plasma lipids with age and sex in a hypertriglyceridemic rat. Lipids 4, 384-387.

Louie K., Wiegand D. R. and Anderson R. E. (1988) Docosahexaenoate containing molecular species of glycerophospholipids from frog rod outer segments show different rates of biosynthesis and turnover. Biochemistry 27: 9014-9020.

Lovern J. A. (1951) "Biochemistry of fish" P. 49 Biochemical Cambridge University press.

Lynch D. V. and Thompson G. A. (1988) Enzymic pathway in the regulation of membrane fluidity. In physiological Regulation of Membrane Fluidity Edited by Aloia R. C., Curtain C.C. and Gordon L.M. pp. 323-360 Alan R. Liss, New York.

Martin Ch. R., Siegel D. and Aarson L. R. (1981) Effects of temperature acclimation on Neurospora phospholipids: Fatty acid desaturation appears to be a key element in modifying phospholipid fluid properties. Biochem. Biophys. Acta 665, 399-407.

Miljanich G. P., Sklar L. A., White D. L. and Dratz E. A. (1979) Diasturated and di polyunsaturated phospholipids in the bovine retinal rod outer segment disc membrane. Biochem. Biophys. Acta 552, 294-306.

Miller J. E. and Cornatzer W. E. (1966) Studies on liver phosphatidylcholine: II Effect of sex, age and species differences on phosphatidylcholines from liver mitochondria and microsome. Lipids 4, 102-108.

Momchilova A.B., Petkova O. H. and Konmanov K. S. (1986) Phospholipid compositions influence phospholipase A_2 activity in rat liver plasma membranes. Int. J. Biochem. 18, 945-952.

Monsen E.R. (1985) In: NIH Launching major research program on fish and health. Food Chemical news 34-39: 6-8

Montaudon D., Robert J. and Canguilhem B. (1984) Fluorescence polarization study of lipids and membranes prepared from brain hemispheres of a hibernating mammal. Biochem. Biophys. Res. Commun. 119, 396-400.

Mourente G. and Tocher D. R. (1992) Lipid class and fatty acid composition of brain lipids from Atlantic herring (Clupea harengus) at different stages of development. Marine Biology 112, 553-558.

Muje P., Agren J.J., Lindovist O. V. and Hanninen O. (1989) Fatty acid composition of vendace (Coregonus albula L.) muscle and its plankton. Feed. Comp. Biochem. Physiol. 92B, 75-79.

Neas N. P. and Hazel J. R. (1985) Phospholipase A_2 from liver microsomal membranes of thermally acclimated rainbow trout. J. exp. Zool. 233, 51-60.

Nelson G. B. and Mcpherson R. (1986) A composition of seasonal lipid changes in two populations of brook char (Salvelinus fontinalis). Am. Midl Nat 117, 139-147.

Neuringer M. and Connor W. E. (1986) n-3 fatty acid in the brain and retina evidence for their essentiality. Nutrition Rev 44, 285-294.

Nichols P. D., Klumpp D. W. and Johns R.B. (1982) Lipid components of the sea grasses Posidonia australis and Heterozostera tasmanica as indicators of carbon source. Phytochemistry 21, 1613-1621

Nybelin O. (1968) The influence of temperature on the formation of agglutinins in fish. Bull. Off. Int. Epizoot 69, 9-10.

O'Dea K. and Sinclair A. J. (1982) Increased proportion of arachidonic acid in plasma lipids after two week on a diet of tropical sea-food. Am. J. Clin. Nutr. 36, 868-872.

Olsen R. E. and Ringo E. (1992) Lipids of Arctic of charr Salvelinus alpinus (L.) II Influence of dietary fatty acids on the elongation and desaturation of linoleic and linolenic acid. Fish physiol. Biochem. 9, 393-399.

Pagliarani A., Pirini M., Trigari G. and Ventrella. (1986) Effect of diet containing different oils on brain fatty acid composition in sea bass (Dicentrarchus labrax L.). Comp. Biochem. Physiol. 83B, 277-282.

Phillips G. P., Dodge J. T. and Howe C. (1969) The effect of aging of human red cells in vivo on their fatty acid composition. Lipids 4, 544-549.

Phillipson B. E., Rothrock D. W., Connor W. E., Harris W. S. and Illingworth D. R. (1985) Reduction of plasma lipids, Lipoproteins and apoproteins by dietary fish oils in patients with hypertriglyceridemia. New Engl. J. Med. , 312, 1210-1215.

Preston F. E. (1982) Platelets and prostaglandins. In: Roath S, ed Topical Reviews in Haematology, 2 Bristol: Wright PSG. 140-159.

Puustinen T., Punnonen K. and Uotila P. (1985) The fatty acid composition of 12 north-European fish species. Acta. Med. Scand 218, 59-62.

Rady A. A. R. and Matkovics B. (1984) Comparative investigations of brain lipids of fishes having different types of feeding behaviour. Abstracts of presentation of the (Adv. in liquid chromatography Szeged)pp. 109-115

Reiser R., Stevenson B., Kayama M., Choudhury R. B. R. and Hood D. W. (1963) The influence of dietary fatty acids and environmental temperature on the fatty acid composition of teleost fish. J. Am. Oil Chemist. Soc. 40, 507-513.

Richardson T., Tapped A. L. and Gruger E. H. (1961) Essential fatty acids in mitochondria. Jr. Arch. Bichem. Biophys. 94, 1-3.

Robinson M., Blank M. L. and Snyder F. (1986) Highly unsaturated phospholipid molecular species of rat erythrocyte membranes. Selective incorporation of arachidonic acid into phosphoglycerides containing polyunsaturation in both acyl-chains. Arch. Biochem. Biophys. 250, 271-279.

Rouser G., Fleischer S. and Yamamto A. (1970) Two-dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus and analysis of spots. Lipids 5, 494-496.

Sargent J. R. and Whittle K. J. (1981) Lipids and hydrocarbons in the marine food web. In: A R Longhurst (Editor), Analysis of Marine Ecosystems, Academic Press, London, pp, 491-533.

Schmit J. A. and Wynne R. B. (1966) Relative elution temperature: A simple method for

measuring peak retention in temperature programmed gas chromatography. J. Gas Chromatog. 4, 325-328.

Schünke M. and Wodtke E. (1983) Cold-induced increase of Δ9 and Δ6-desaturase activities in endoplasmic membranes of carp liver. Biochem. Biophys. Acta 734, 70-75.

Schwalme K. and Mackay W. C. (1992) Seasonal changes in the neutral and polar lipid fatty acid content of female northern pike (Esox lucius L.). Canadian J. of Zoology 70, 280-287.

Siess W., Roth O., Scherer B., Kurzmann I., Bohlig B. and Weber P. C. (1980) Platelet membrane fatty acids, platelet aggregation and thromboxane formation during a mackerel diet. Lancet 1, 441-444.

Sinensky H. (1974) Homeoviscous adaptation of homeostatic process that regulates the viscosities of membrane lipids in Escherchia coli. Proc. Natl. Acad. Sci. USA 71, 522-525.

Singer S. J. (1974) The molecular organization of membrane. Ann. Rev. Biochem 43, 805-833.

Spielmann D., Bracco U., Traltler H., Crozier G., Holman R., Ward M. and Cotter R. (1988) Alternative lipid to usual n-6 PUFAs: γ-linolenic acid, α-linolenic acid, stearic acid, EPA, Et. Session VII Supplement printed in USA. 12, 1115-1225.

Stansby M. E. (1981) Reliability of fatty acids purporting to represent composition of oil from different species of fish. Journal of the American Oil Chemists. Society. 58, 13-16.

Stubbs C. D. and Smith A. D. (1984) The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochem. Biophys. Acta 779, 89-137.

Takamura H., Narita H., Vrade R. and Kito M. (1986) Quantitative analysis of polyenoic phospholipid molecular species by high performance lipid chromatography. Lipids 21, 356-361.

Tinoco J. (1982) Dietary requirements and functions of α-linolenic acid in animals. Prog. Lipid Res. 21, 1-45.

Tocher D. R., Fraser A. J., Sargent J. R. and Gamble J. C. (1985) Lipid class composition during embryonic and early larval development in Atlantic herring (Clupea harengus L.). Lipids 20, 84-89

Tocher D. R. and Sargent J. R. (1984) Analysis of lipids and fatty acids in ripe rose of some north west European marine fish. Lipids, 19, 492-499.

Vileg P. and Body D. R. (1988) Lipid contents and fatty acid composition of some New Zealand fresh-water finfish and marine fish, shell-fish and roes. New Zealand Journal of Marine and fresh-water Res. 22, 151-162.

Wieslander A., Christiansen A., Rilfors A.L. and Lindbrom H. (1980) Lipid bilayer stability in membranes. Regulation of lipid composition in Acehloplasma laidlawii as governed by molecular shape. Biochemistry 19, 3650-3655.

Wodtke E. (1978). Lipid adaptation in liver mitochondrial membrane of carp acclimated to different temperature. Biochem. Biophys. Acta 529, 280-291.

Wodtke E. (1981 a) Temperature adaptation of biological membranes. The effects of acclimation temperature on the unsaturation of the main neutral and changed phospholipids in mitochondrial membrane of the carp (Cyprinus carpio L.). Biochem. Biophys. Acta 640, 698-709.