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A POSSIBILE EXPLANATION FOR THE DIFFERENCES IN THE FATTY ACID COMPOSITION OF FRESH-WATER AND MARINE FISHES

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The fact that the fatty acid composition of fishes is more complicated than that of land animals has already been observed long ago. On the other hand characteristic differences exist in the fatty acid composition of fresh-water and marine fisches. These differences were formulated first by LOVERN (1932) and his observations were confirmed with some corrections also by other authors (ACKMAN 1967). The differences noted by LOVERN were as follows:

"1. The oleic-linoleic acid group percentage is considerably higher in fresh-water fish fats,

2. the C_{20}- and C_{22} groups present in cosiderably smaller amounts in fresh-water fish fats,

3. palmitoleic acid is a more important component of all fresh-water fish fats than in marine ones."

The correction concerns mainly the eicosa-, and docosapolyenoic fatty acids: there are marine fishes with low (KARKHANIS and MAGAR, 1955), and fresh-water fishes with high level of these fatty acids in their lipids (GRUGER et al. 1964).

These observations were done about the total lipids of the animals which are however a mixture of different compounds (phospholipids, triglycerides etc.) of different fatty acid composition. For instance the level of palmitoleic acid in the triglycerides is usually high while in phospholipids is low. For that reason it seemed interesting to investigate if the fatty acid composition of triglycerides and phospholipids, obtained from marine and fresh-water animals, follows the above characteristics. In the present paper the fatty acid composition of fresh-water and marine algae, planktonic crustaceans and fishes is compared and an attempt has been made to throw some light on the significance of the different members of the aquatic food chains in the formation of the characterristic fatty acid composition of fishes.

Material and methods

The fatty acid composition of *Diatomea vulgare*, a mixed diatom probe, *Scenedesmus obtusiusculus*, *Chlorochloster terrestris* and *Chlorella vulgare* was investigated in this laboratory. The diatoms were collected in Lake Balaton, the green algae obtained from a large-scale cultures. The fatty acid composition of *Diatomea vulgare* and *Chlorella vulgare* has already been published (FARKAS, 1970a). One part of the data concerning the fatty acid composition of diatoms and green algae were obtained fom the literature (SCHLENK et al. 1960; KLENK et al. 1963; KATES and VOLCANI, 1966; ACKMAN and TOCHER; 1968, REITZ et al. 1967; CHUECAS and RILEY, 1969).

The fresh-water copepods were collected at different regions of Hungary. Data on the fatty acid composition of some of them *(Eudiaptomus gracilis, Mesocyclops leukarti)* have already been published (FARKAS, 1970a, b). while for others *(Arctodiaptomus bacillifer, Myxodiaptomus kupelwieseri, Acantocyclops vernalis, Cyclops vicinus, Cyclops strenuus, Thermocyclops crassus)* will be published later. Some of the marine copepods were collected by the author from the White Sea *(Pseudocalanus elongatus, Calanus glacialis, Metridia longa)* and from the Black Sea *(Acartia clausi)* other species were sampled at the Scottish Marine Biological Station *(Calanus finmarchicus)* or along the Equator (mixed marine copepod probe). These animals were fixed in a mixture of chloroform—methanol 2 : 1 and sealed under nitrogen during transport. The data concerning the fatty acid composition of *Acartia clausi, Acartia tonsa, Calanus helgolandicus* and *Tremora longicornis* were taken from the literature (LASKER and THEILACKER, 1962; ACKMAN and HOOPER, 1970; JEFFRIES, 1970).

Detailed fatty acid composition of the fresh-water fish, *Alburnus lucidus*, has already been published elsewhere (FARKAS, 1970a) the fatty acid composition of herring was investigated by ACKMAN (1967).

Only the analytical data obtained by methods equivalent to those used in this study, were taken over from the literature.

The fats were extracted and purified as described by BLIGH and DYER (1959) from freshly collected specimens. Between the collection and the start of the extraction usually no more than 60 minutes have passed. When the animals were collected far from the laboratory they were fixed in the above extraction mixture.

The triglycerides and phospholipids were separated by thin-layer chromatography on Silicagel G (Merck) plates using petroleum aether: diethyl aether : acetic acid = 70 : 30 : 1 as solvent. The phospholipids were scraped inmediately after the development — before evaporating of the solvent and the triglycerides after detection by Rhodamin B, into ampoules containing 5% hydrochloric acid in absolute methanol. The transesterification was performed in the presence of the Silicagel under CO_2 at 80 °C.

A CHROM III IKZ instrument, equipped with flame ionization detector was employed for analysing the fatty acid compositions. The column was 6-feet long and filled with 15% EGSS-Y on 100—120 mesh Gas Chrom P. All the probes were rechromatographed after hydrogenation. The quantification was made by triangulation technic and the results were corrected for the chain lenght distribution of the hydrogenated (FARQUHAR et al. 1959) samples.

Results

Fig 1. shows the average fatty acid composition of green algae and diatoms. Characteristic differences can be found between the fatty acid composition of the members of the two graet taxonomic group. While in the fats

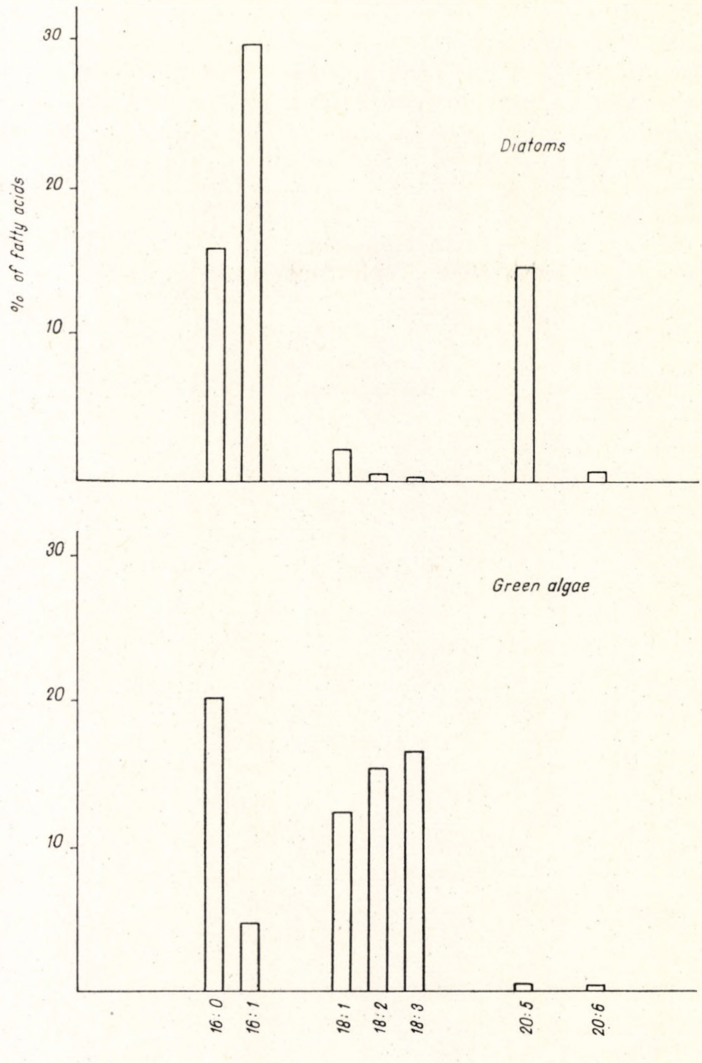

Fig. 1. Average fatty acid composition of diatoms and green algae. The values were calculated from the analytical data of the following species: diatoms; *Asterionella japonica, Bidulphya sinensis, Chaetoceras septentrionale, Cyclotella cryptica, Diatomea vulgare, Ditylum brightwelli, Lauderi borealis, Navicula pelliculosa, Nitschia angularis, Nitschia chlosterium, Nitschia thermalis, Phaeodactylum tricornutum, Sceletonema costata, Thalassiosira fluviatilis, mixed diatom probe from the Lake Balaton,*
green algae; *Chlamidomonas* sp., *Chlamidomonas reinhardi, Chlorochloster terrestris, Chlorella pyrenoidosa, Chlorella vulgare, Dunaliella primoleata, Scenedesmus obliqus, Scenedesmus quadricaudata*

of diatoms the level of palmitoleic acid exceeds that of palmitic acid, the fats of green algae are richer in palmitic acid than in palmitoleic acid. Moreover, the fats of diatoms are poor in lincleic and linolenic and rich in eicosapentaenoic acids. In the fats of green algae however the levels of octadecapolyenoic acids are high but only few eicosapentaenoic acid can be detected. The only common feature between the two groups is the low level of docosahexaenoic acid. The above differences seem to be independent from the biotop: the fats of marine green algae are rich in linoleic and linolenic acids (Jezyck and Penincak, 1966; Ackman et al. 1970) and in the fats of fresh-water diatoms the level of these fatty acids is not higher than in their marine relatives (Kates and Volcani, 1966; Farkas, 1970a, b).

Comparing the fatty acid compositions of the higher members of the aquatic food chains with that of phytoplankton *(Figs. 2, 3)* the following characteristics can be observed:

a) In the marine food chain the level of palmitoleic acid reduces already on the level of planktonic crustaceans,

b) in the members of marine food chain the level of linoleic and linolenic acid is similar to that in diatoms and in members of fresh-water food chain to that in green algae,

c) in the fats of both fresh-water and marine copepods significant amount of docosahexaenoic acid is accumulating,

d) in the fats of fishes the level of eicosapentaenoic and docosahexaenoic acid is not higher than in that of planktonic crustaceans.

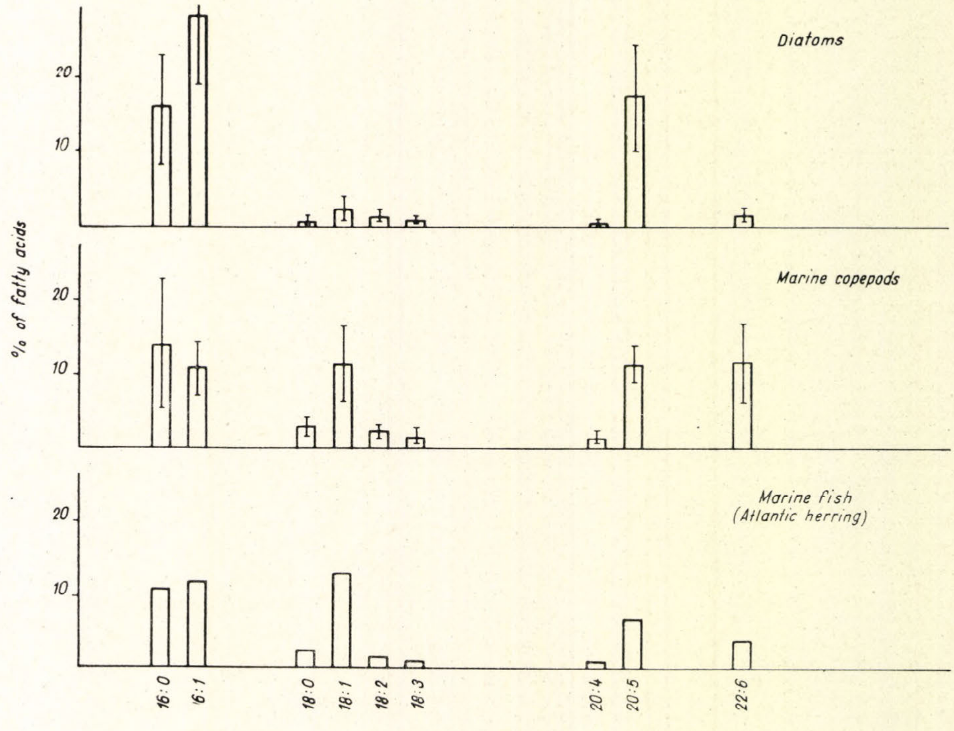

Fig. 2. Distribution of the most important fatty acids in the members of the marine food chain

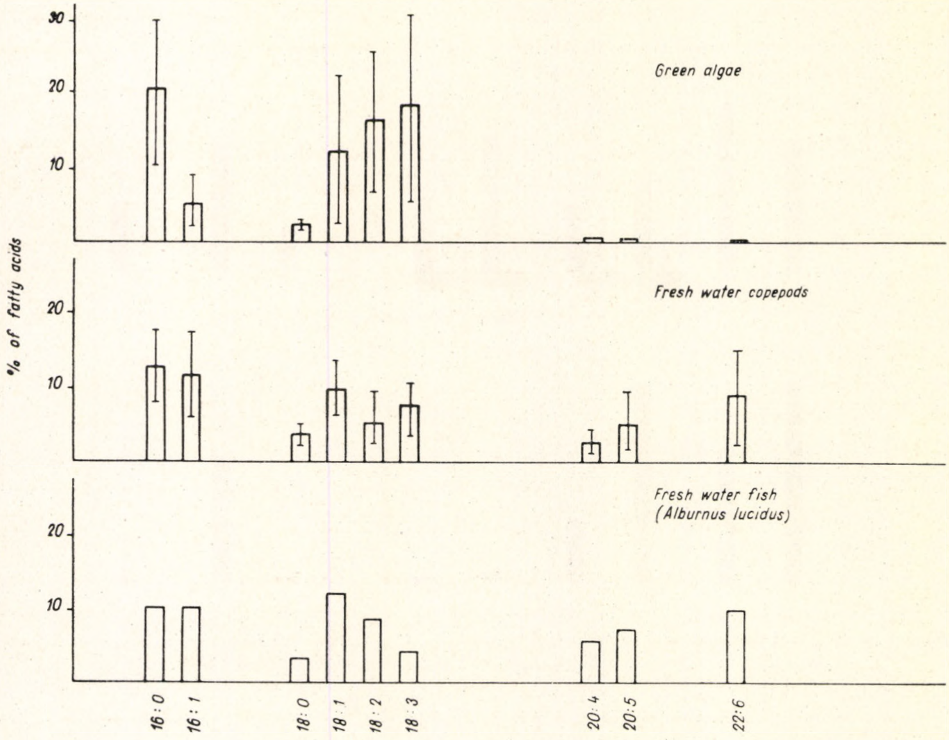

Fig. 3. Distribution of the most important fatty acids in the members of the fresh-water food chain

Separating the triglycerides and phospholipids from the fats of planktonic crustaceans it was found that eicosa-, and docosapolyenoic fatty acids accumulate in the phospholipids and octadecapolyenoic acids in the triglycerides *(Fig. 4)*. It is remarkable that while in the case of octadecapolyenoic fatty acids the phospholipids (and of course also the triglycerides) can be well distinguished in the investigated fresh-water *(Eudiaptomus gracilis)* and marine *(Calanus finmarchicus)* species, regarding the eicosapentaenoic and docosahexaenoic acids no substantial difference exists between the two animals.

Further investigations seem to indicate that the environmental temperature may be one of the most important factors controlling the fatty acid composition of phospholipids. In the phospholipids of the fresh-water copepod, *Cyclops vicinus,* the level of docosahexaenoic acid becomes about three times higher with decreasing water temperature *(Fig. 5)*. Essentially the same is found in the case of marine copepods too. In the phospholipids of *Pseudocalanus elongatus* originating from the regions of the polar circle, the docosahexaenoic acid was found in the same amount as in that of *Cyclops vicinus* collected in winter, and this value was nearly three times higher than in copepods collected about the Equator. The palmitoleic and oleic acids behaved in marine species similarly as in *Cyclops vicinus.*

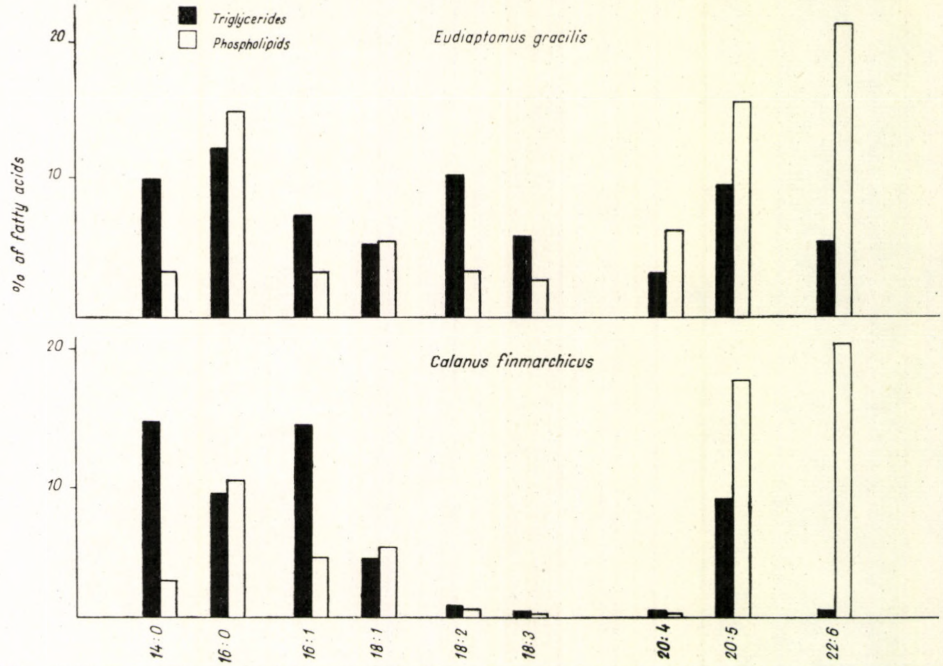

Fig. 4. Fatty acid composition of triglycerides and phospholipids of a fresh-water *(Eudiaptomus gracilis)* and a marine *(Calanus finmarchicus)* copepod

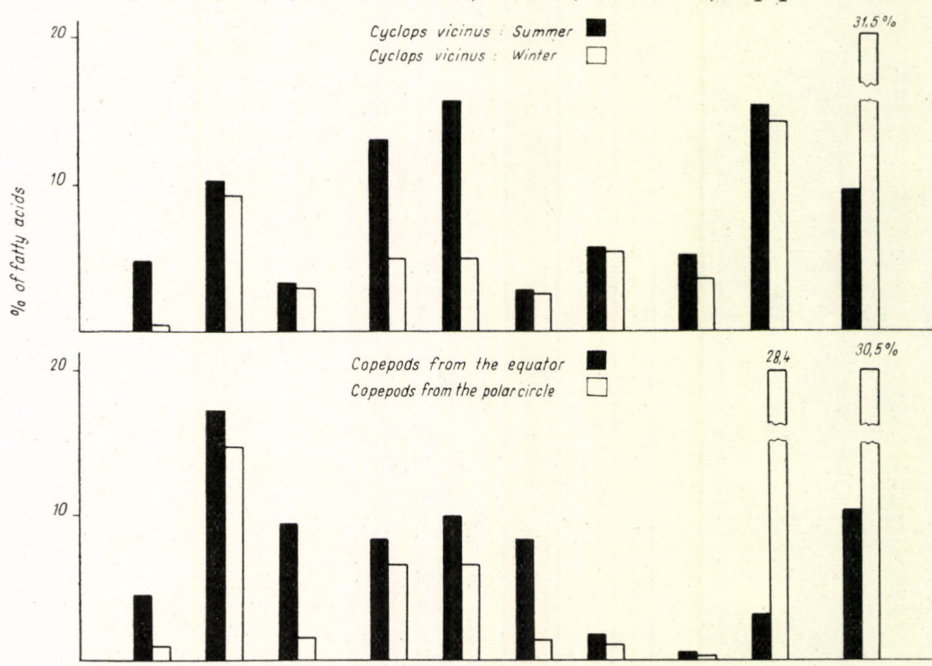

Fig 5. Phospholipid fatty acid composition and environmental temperature in fresh-water and marine copepods

Discussion

Published data show that the species composition of the phytoplankton of the seas and fresh-water bodies is different. The phytoplankton of the former is characterized in the first place by members of Bacillariophyceae and Dinoflagellata (McConnaughey, 1970), in fresh-water bodies however the green and blue-green algae are predominating. In the seas the later have only local and temporal significance, while in fresh-water bodies especially in winter times the diatoms may obtain a considerable role.

If we compare the fatty acid composition of the members of the food chain phytoplankton, crustacea plankton, fish, on the fats of fishes the peculiarities which originate directly from the phytoplankton and those appearing in the higher trophic levels can be well recognized. There is no doubt that planktonic crustaceans may modify the fatty acid composition of the fat of algae. In the fats of marine copepods the level of palmitoleic acid decreases and that of oleic acid increases in comparison to the diatoms. The situation in fresh-water copepods is apparently inverse: the level of the palmitoleic acid increases, seemingly to the expense of the palmitic acid. This fact concerns however only the total fat i.e. the fat which was taken up by fishes with the planktonic crustaceans. As *Fig. 4* clearly indicates, the triglycerides of marine copepods, regarding the the relation of plamitic to palmitoleic acid, still preserve the peculiarities of the fat of diatoms and the same is true also for the triglycerides of fresh-water copepods and the fats of green algae. Thus the changes observed in the level of palmitic and palmitoleic acid are to be attributed rather to differences in the proportion of triglycerides and phospholipids in the fat probe than to desaturation processes taking place in crustaceans. As a matter of fact the level of palmitoleic acid increases only at the level of the fishes, and in the triglycerides of plankton-feeding fishes it can reach or even surpass that of palmitic acid (Farkas, 1970a). In the triglycerides of fresh-water fishes the accumulation of palmitoleic acid is not more extensive than in triglycerides of marine fishes and in many instances it does not exceed the values obtained for these animals (Ackman, 1967). Also in the triglycerides of marine fishes the level of palmitoleic acid exceeds that of palmitic acid, but this is probably still the characteristic of diatom fats.

The investigations have also shown that the docosapolyenoic and in the case of fresh-water bodies also the eicosapolyenoic fatty acids enter into the food chain at the level of planktonic crustaceans. The low level of long chain polyenoic fatty acids in the fats of green algae suggests that these organisms are capable to synthetize these fatty acids only at a very limited rate. Also the diatoms seem to be unable for synthetizing docosahexaenoic acid in a considerable amount, though it seems that the chain elongating and desaturating reaction in this group goes to the eicosapentaenoic acid. The docosahexaenoic acid appears first in the phospholipids of planktonic crustaceans and it is highly interesting that its level is a function of the environmental temperature. In spite of the fact that the food of different crustaceans maydiffer considerably, the content of docosahexaenoic acids in phospholipids may almost be equal. The best explanation of this phenomenon is probably that the copepods assure the functioning of their membranes at low temperatures with the aid of these low melting fatty acids.

It was proved that also fishes are capable of producing long chain fatty acids from linoleic and linolenic acids (MEAD et al. 1960; KLENK and KREMER, 1960) but it is quite probable that the bulk of these fatty acids originate in the phospholipids of planktonic crustaceans. In the phospholipids of fishes the level of docosahexaenoic acid does not increase further than in the phospholipids of planktonic crustaceans, there is an increase however in the triglyceride docosahexaenoic acid content (FARKAS, 1970a). This is probably to be traced back to the fact that on the course of the intestinal resorbtion of the fats, one part of the monoglycerides formed from the phospholipids will be consumed for the formation of triglycerides. Thus in the triglycerides of fishes consuming phospholipids, poor in long chain polyunsaturated fatty acids, less fatty acids of this kind are to be expected, than in those of which food is rich in these fatty acids. Perhaps this is the reasos for the low level of eicosa-, and docosapolyenoic fatty acids in fishes living in subtropic seas (KARKHANIS and MAGAR, 1955) and that in the fats of tropic fresh-water fishes less long chain fatty acids were observed (PATHAK and REDDY, 1962) than in that of fishes from the Lake Balaton (FARKAS and HERODEK, 1967).

What neither the planktonic crsutaceans nor the fishes are able to produce are the linoleic and linolenic acids. Regarding the level of these fatty acids the marine fishes follow the fats of diatoms and the fresh-water fishes the fats of green algae. LOVERN (1934) and more recently KREPS et al. (1969) have shown that in the fats of Salmonidae on the course of passing from marine to fresh-water life, octadecapolyenoic acids are accumulating. This fact is obviously connected with differences in the species composition of phytoplankton of the two habitats and emphazises the importance of planktonic algae in the formation of one of the most important differences between the fatty acid composition of fresh-water and marine fishes.

Summary

Considering the data concerning the metabolism of fatty acids in aquatic animals and comparing the fatty acid compositions in different levels of aquatic food chains for the differences observed in the fatty acid composition of fresh-water and marine fishes the following explanation is offered:

1. The differences in the levels of linoleic and linolenic acids can be traced back to the differences in species composition of phytoplankton in seas and inland waters. In marine phytoplankton predominate the diatoms with low level of linoleic and linolenic acids, in fresh-water bodies however the green and blue-green algae with high levels of both fatty acids. The distribution of these fatty acids in the fats of herbivoreous planktonic crustaceans shows the same pattern as in their food.

2. Although both marine and fresh-water fishes were shown to be able to desaturate and elongate linoleic and linolenic acids to long chain polyunsaturated fatty acids, the bulk of these fatty acids originates in lower trophic levels. Some planktonic crustacean species are able to regulate the levels of these fatty acids in their phospholipids according to the environmental temperature. Decrease in the environmental temperature results in an increase of eicosa-, and docosapolyenoic fatty acids in both fresh-water and marine crustaceans. The distribution of long chain polyunsaturated fatty acids in

the triglycerides of marine and fresh-water fishes is similar to that in their natural focd.

3. Planktonic crustaceans and fishes can dehydrogenate saturated fatty acids of both endogenous and exogenous origin. This process may modify the ratio of saturated to monounsaturated fatty acids in higher trophic levels as compared to the phytoplankton.

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KÍSÉRLET AZ ÉDES- ÉS TENGERVÍZI HALAK ZSÍRSAVÖSSZETÉTELÉBEN KIMUTATOTT KÜLÖNBSÉGEK ÉRTELMEZÉSÉRE

Farkas Tibor

Összefoglalás

Figyelembe véve a vízi szervezetek zsíranyagcseréjére és zsírsavösszetételére vonatkozó irodalmi és saját adatainkat, valamint az édes- és tengervízi halak zsírsavösszetételében talált különbségeket a következőkre vezetjük vissza:

1. A linol- és a linolénsav szintjében megfigyelt különbségeket az édesvizek és tengerek phytoplanktonjának faji összetételében megmutatkozó eltérések okozzák. A tengerekben a Bacillariophycea rend tagjai dominálnak, melyekben alacsony, az édesvizekben a Chlorophyta és Cyanophyta rend tagjai, melyekben magas a 18 : 2 és 18 : 3 zsírsavak szintje. A linol- és linolénsav megoszlása a herbivora planktonrákok és planktonevő halak zsírjában hasonló a phytoplanktonban talált értékekhez.

2. Ámbár mind a tengeri, mind az édesvízi halak képesek linol- és linolénsavból hosszú szénláncú polyen zsírsavakat képezni, ezen zsírsavak főtömege az alacsonyabb tropikus szinteken képződik. Bizonyos planktonrákok eicosa és docosapolyen zsírsavakat halmoznak fel phospholipidjeikben és a felhalmozás mértéke a környezet hőmérsékletének a függvénye. A környezet hőmérsékletének csökkenésével párhuzamosan növekszik a hosszú szénláncú polyen zsírsavak szintje mind az édesvízi, mind a tengeri rákok phospholipidjeiben.

3. A palmitin-palmitoleinsav aránya a planktonrákok és planktonevő halak trigliceridjeiben lényegében a phytoplankton zsírjához igazodik. Zöld algákkal táplálkozási kapcsolatban levő szervezetekben a palmitoleinsav szintje alacsonyabb, kovamoszatokkal táplálkozási kapcsolatban levő szervezetekben pedig magasabb, mint a palmitinsavé.