The effect of environmental temperature on the fatty acid composition of crustacean plankton

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SUMMARY Measurements of the iodine value made over a period of three years demonstrate a regular yearly cycle in the composition of the fat of crustacean plankton in Lake Balaton. The melting point of the lipid of the planktonic copepods remained during the whole year somewhat lower than the water temperature. The proportions of the C_{20}–C_{22} polyunsaturated acids in the planktonic crustaceans increased with decreasing temperature and in some species exceeded the values characteristic of marine animals. In fresh-water fish, kept at room temperature and fed on freshly collected fresh-water plankton, the lipids formed in winter resembled marine fish oil.

In the crustaceans raised on algae containing no fatty acids longer than C_{18}, the C_{20}–C_{22} acids always appeared as major components of the copepods.

FISH FATS ARE characterized by high proportions of C_{20-22} highly unsaturated fatty acids. It was demonstrated as early as 1932 (1) that marine fish contain significantly more C_{20-22} than do fresh-water fish. From that time on the "fresh-water" and "marine" indications came into general use for types of fatty acid composition, but no explanation has been offered for this difference. The question is in close correlation with the origin of C_{20-22} fatty acids present in fish.

Recently isotope studies (2) demonstrated the synthesis in fish of C_{20-22} polyenoic acids from exogenous precursors of linoleic and linolenic types; however, earlier feeding experiments (3) indicated that the greater majority of such polyenoic acids derived from food. If this is the case, the problem is why the food of marine fish contains greater amounts of C_{20-22} than that of fresh-water fish. The food chain of water ecosystems can be simplified to algae, crustaceans, and fish. Some data (4) have been obtained from one marine species and a small sample of fresh-water species indicating that differences exist in the fatty acid composition of marine and fresh-water crustaceans similar to those of marine and fresh-water fish. The problem of the two types of fatty acid composition has now been shifted from the level of fish down to crustaceans. The authors investigated previously the fatty acid composition of the fresh-water crustacean plankton (5, 6) and found that with lowering of the environmental temperature the amount of C_{20-22} gradually increases and the fatty acid composition becomes similar to that of marine animals.

This observation led to the assumption that the environmental temperature is one of the factors responsible for the differences in fatty acid composition between marine and fresh-water animals. In this connection data are presented here for crustaceans sampled from cold and warm seas. The authors' previous observations were made on the mixed crustacean plankton of a fresh-water lake. Now the seasonal cycle of the fatty acid composition of several selected species will be demonstrated. The biological significance of the high amount of C_{20-22} acids in crustaceans needs some interpretation. It seemed probable that these highly unsaturated acids may have a role in assuring the liquid state of the stored lipids. This hypothesis is investigated now by comparing the melting point of lipids with the environmental temperature in different seasons. As to the origin of C_{20-22} acids present in crustaceans some uncertainty remained after the previous work. In the few investigated unicellular alga species these acids are never present in significant amounts, but it is practically impossible to control the fatty acids of all the organisms which could serve as food of crustaceans under natural conditions. It seemed therefore desirable to investigate this problem in feeding experiments under laboratory conditions.

MATERIALS AND METHODS

The planktonic crustaceans are small animals, a few millimeters in length. They obtain their food, consisting

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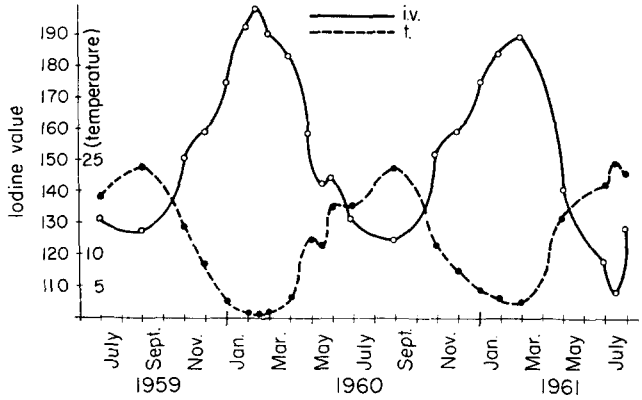

FIG. 1. Changes in iodine value of the fat of the crustacean plankton and of the water temperature in Lake Balaton, 1959–61.

of unicellular algae, chiefly by filtration. The main body of crustacean plankton is composed of two groups of lower crustaceans. These are the cladocerans and copepods, two groups which show significant differences in their morphology, behavior, and distribution. The copepods are represented both in fresh water and sea by a large number of species and individuals, while the role of cladocerans is inferior in the sea.

The mixed crustacean plankton was collected with a No. 6 net. First we analyzed it as a whole. Later we succeeded in separating the two main groups, the cladocerans and copepods. After the water containing the collected material had been vigorously aerated, the cladocerans—all of them possessing a hydrophobic cuticula—adhered to the surface and could be quantitatively removed. The isolation of the single species in each case required laborious procedures, worth trying only if the species desired was abundant in the sample. In this manner, we obtained from the mixed crustacean plankton 30–50 mg, and from the selected species 1–3 mg, of fat. The marine crustaceans we received in glass vials, filled with 70% ethanol, sealed after flushing with CO_2. The crustacean fed on algae were kept in 100-liter aquaria. The algae originated from pure cultures. Under these conditions the cladocerans propagated quickly and many generations were fed on algal diet before analysis. The copepods were kept on algae from the nauplius stage on and were analyzed when adult. Our experiments with fish started with newly born guppies, *Lebistes reticulatus*. They were raised for 2 months on living crustacean plankton, from Lake Balaton, as their only food. Every 3rd day, fresh crustaceans were added in a quantity just sufficient to last until the next feeding.

TABLE 1 SEASONAL CHANGES IN THE FATTY ACID COMPOSITION OF THE MIXED CRUSTACEAN PLANKTON OF LAKE BALATON

Date of Sampling	Water Temperature	Fatty Acid Composition*				
		C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
	°			%		
1959 July 14	23.0	4.5	25.5	38.7	16.0	15.3
Aug. 24	23.5	1.0	27.6	39.7	15.5	15.8
Oct. 4	12.5	5.4	20.5	37.7	20.6	15.8
Nov. 14	7.2	8.1	18.2	32.6	20.6	20.5
Dec. 14	2.3	8.1	14.0	38.9	17.6	21.4
1960 June 1	17.0	5.7	26.2	37.1	17.7	13.3
Aug. 29	24.5	5.4	25.5	34.4	13.3	11.4
Oct. 24	13.5	7.8	22.0	37.0	16.2	17.0
Dec. 20	4.5	7.7	12.0	35.0	21.7	23.6
1961 June 13	20.5	2.6	44.6	38.2	9.5	5.1
July 3	24.9	—	37.2	43.5	11.8	7.5
July 13	22.6	1.4	29.0	42.1	12.8	14.7
Oct. 11	15.4	7.2	28.2	35.5	16.6	12.5
Nov. 10	9.0	13.1	8.5	34.7	21.2	22.5
Nov. 21	7.6	7.1	15.1	38.3	18.1	21.4

* After hydrogenation.

All samples were ground with anhydrous sodium sulfate and extracted three times with petroleum ether (boiling range 40–70°), for 15 min under reflux, using 30 ml petroleum ether per g sample. The extraction and subsequent procedures were carried out in a strictly inert atmosphere. The lipids were dissolved in petroleum ether and stored in a refrigerator at −20°, sealed in glass vials after flushing with CO_2.

On an aliquot of the total lipid fraction, we measured the iodine value, using Kaufmann's semimicro method (7). Another portion was hydrogenated in the presence of a palladium catalyst. The fatty acid mixture obtained after hydrogenation was analyzed by paper chromatography according to Kaufmann (7). To detect the spots, copper acetate–rubeanic acid reagent was used. The quantitative evaluation was performed photometrically. The results were converted into percentages by weight. We determined the accuracy of this technique several times by the use of model fatty acid mixtures and the results corresponded to those postulated by Seher (8). Each sample was chromatographed on three independent occasions and the deviations never exceeded 5%.

For melting point measurements we employed the Kofler-type microscope, with a temperature-conditioned sample holder. A 2–3 mg sample of fat was placed on the slide, cooled to a temperature 20° below the expected melting point and afterward heated at a speed of 1° per min. The melting point was taken as the temperature at which the minute roughness of the surface of the fat droplet disappeared. This change was rapid and easily observed and the results from this technique were reproducible to within ±1°.

RESULTS

As a preliminary assay of the suspected seasonal changes, iodine value measurements were performed on

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TABLE 2 SEASONAL CHANGES IN THE FATTY ACID COMPOSITION OF SELECTED CRUSTACEAN SPECIES

Species	Place	Date	Water Temperature	C_{14}	Fatty Acid Composition* C_{16}	C_{18}	C_{20}	C_{22}
Eudiapt. gracilis (Copepoda)	Lake Balaton	June 62	22.5	—	34.2	35.5	16.8	13.5
		Sept. 62	18.0	7.4	29.9	28.5	15.4	18.8
		Nov. 62	10.1	16.4	20.8	30.9	12.9	19.0
		Nov. 62	2.8	9.7	13.8	36.9	19.9	19.7
Cyclops vicinus (Copepoda)	Lake Balaton	Oct. 62	16.2	11.5	29.9	27.3	13.7	17.6
		Oct. 62	15.4	7.8	27.6	28.1	17.7	18.8
		Nov. 62	9.4	10.7	22.8	25.9	16.7	23.9
		Nov. 62	7.0	8.4	16.7	33.3	16.2	25.4
		Nov. 62	2.8	6.1	15.6	36.3	16.2	25.8
Cyclops vicinus (Copepoda)	Lake Belsötó	Sept. 60	23.5	—	20.5	37.0	19.4	23.1
		Oct. 60	13.9	—	22.2	24.9	21.2	31.7
		Dec. 60	5.0	—	18.4	22.7	23.0	35.9
		June 61	20.5	—	37.0	29.3	15.0	18.7
		July 61	21.5	—	21.9	40.9	21.6	15.6
		Nov. 61	9.0	—	29.3	30.3	19.6	20.8
Daphnia cucullata (Cladocera)	Lake Balaton	June 61			36.4	42.6	15.8	5.2
		Aug. 61	24.0	9.0	31.6	39.7	14.8	4.9
		Oct. 61	16.2	11.5	28.9	38.9	15.3	5.4
		Oct. 61	15.4	13.4	25.4	40.5	11.7	9.0
		Nov. 61	9.4	14.7	22.8	42.9	9.6	10.0
		Nov. 61	9.2	16.3	17.3	42.2	15.0	9.2
		Feb. 62	0.0	10.8	22.9	40.7	13.3	12.3
Daphnia magna (Cladocera)	Lake Belsötó	June 61	20.5	—	57.3	35.0	7.7	—
		Nov. 61	9.0	—	37.5	54.7	7.8	—

* After hydrogenation.

the fat of crustacean plankton. The results are presented in Fig. 1. The equation of the regression line calculated from these data is:

$$\text{Iodinevalue (I.V.)} = 188.8 - (3.03 \pm 0.21)t.$$

The deviation of the regression coefficient from 0 is very highly significant ($P < 0.001$).

It seemed probable that the chemical changes indicated by the iodine values result in a relatively constant physical state of the planktonic crustacean fat throughout the year. In order to characterize this state, melting point measurements were carried out on the fat of planktonic copepods sampled from Lake Balaton in 1962. The results were as follows (date, mean water temperature in period immediately preceding measurement, mp): 9 August, 22.8°, 19°; 11 September, 20.3°, 17°; 9 October, 16.2°, 12°; 15 October, 15.3°, 12°; 29 October, 15.1°, 11°; 6 November, 9.4°, 8°; 13 November, 9.2°, 5°; 4 December, 2.6°, 2°. It should be pointed out that in these crustaceans the fat is stored extracellularly in the so-called oil sacs. We believe that the physical state of the fat, stored in this way, corresponds fairly well to that observed under the microscope, through the structural lipids, also extracted, may cause some smaller deviations. It appears that the melting points of the fats of planktonic crustaceans are always just below the environmental temperature.

In some previous work (5, 6), paper chromatography of the hydrogenated fatty acids of crustacean plankton showed in one cooling period of Lake Balaton an increase of the proportion of C_{20-22} acids. Similar observations made in two subsequent years are shown together with the earlier results in Table 1.

The gas–liquid chromatography (GLC) analysis of one of the samples revealed the following composition (as percentages of the total peak areas).[1] 14:0 = 22.5%, 16:0 = 11.6%, 16:1 = 2.5%, 16:2 = 0.4%, 18:0 = 0.7%, 18:1 = 2.9%, 18:2 = 9.8%, 18:3 = 9.0%, 18:4 = 14.4%, 18:? = 2.7%, 20:4 = 4.3%, 20:5 = 9.6%, 22:5 = 2.3%, 22:6 = 7.2. According to these more detailed data, the C_{20-22} acids of crustacean plankton are indeed tetra-, penta- and hexaenoic acids, a result analogous to that obtained from an analysis of the C_{20-22} acids of fish (9).

Recently we succeeded in selecting some individual crustacean species in quantities large enough for analysis. The results are presented in Table 2. The fatty acid composition of each species proved to be different

[1] The apparatus employed was a Wheelco Model 10 (Barber-Colman Co.) with a 6 ft, 6 mm i.d. column of ethylene glycol succinate polyester, 16% on 80–120 mesh silanized Chromosorb at 185–200°. Compounds were identified by comparison of their retention times with those of known standards. Areas were measured by triangulation. Fatty acids are designated by chain length and number of double bonds.

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TABLE 3 FEEDING EXPERIMENTS WITH WATER ORGANISMS

Sample	Aquarium Temperature	Iodine Value	Fatty Acid Composition*				
			C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
	°				%		
Algal diet (*Scenedesmus obtusiusculus*)	—	—	—	47.4	52.6	—	—
Cladocerans:							
Daphnia magna	ca. 20	—	—	42.5	57.4	—	—
Daphnia cucullata	20	—	—	44.4	52.4	3.2	—
Moina rectirostris	20	—	—	46.3	50.8	2.9	—
Copepods:							
Mesocyclops leuckarti	20	—	—	39.9	42.4	7.7	10.0
Cyclops vicinus	20	—	6.9	19.4	39.8	18.3	15.6
Acanthocyclops viridis	20	—	—	32.0	30.0	20.2	17.8
Algal diet (*Chlorella pyrenoidosa*)	20	121.5	—	35.7	64.3	—	—
Daphnia magna	25	127.7	—	38.9	61.1	—	—
Daphnia magna	10	173.2	—	32.6	67.4	—	—
Fish fed on crustacean plankton: *Lebistes reticulatus*							
Summer	22		6.4	31.9	27.8	12.2	21.7
Winter	22		4.2	14.7	20.0	25.3	35.8

* After hydrogenation.

and was modified differently by the changes of water temperature. No C_{20-22} acids were detectable in *Daphnia magna* and the amount of these acids in *Daphnia cucullata* showed only a slight increase as the temperature decreased. The *Daphniae* belong to the cladocerans. On the other hand in the two copepod species *Eudiaptomus gracilis* and *Cyclops vicinus* there appeared to be a definite increase in C_{20-22} acids at lower temperatures. From a biological point of view these two copepod species are better adapted to the cold environment than the two cladoceran species.

The (hydrogenated) fatter acid compositions of two marine copepod species from different seas proved to be:

Paracalanus parvus from Bay of Naples: $C_{14} = 6.8\%$, $C_{16} = 56.3\%$, $C_{18} = 27.2\%$, $C_{20} = 4.7\%$, $C_{22} = 5.0\%$. *Calanus finmarchicus* from the North Sea: $C_{14} = 6.8\%$, $C_{16} = 29.4\%$, $C_{18} = 22.0\%$, $C_{20} = 23.6\%$, $C_{22} = 18.2\%$.

It is too early to consider the problem settled on the basis of this single comparison, but the results indicate that the temperature effect is as easily detectable in different seas as it is during a seasonal temperature change in a fresh-water lake.

As a first step in clarifying the mechanism of the temperature effect, the origin of the fatty acids present in crustaceans must be investigated. For this reason we started with feeding experiments under controlled conditions (See Table 3). Three Cladocera and three Copepoda species were raised in aquaria on unicellular green algae *Scenedesmus obtusiusculus*. The fat of the cladocerans remained similar to that of their food. In the copepods, on the other hand, the C_{20-22} acid group appeared always as the major component. It seems plausible that the copepods obtained these acids by elongation of the linoleic and linolenic acids according to the divinyl methane rhythm, as demonstrated in vertebrates (10, 11). The temperature effect was also investigated under aquarium conditions, but for technical reasons only on *Daphnia magna* (see again Table 3). Two groups were kept at 20° and 10°, respectively. In this case *Chlorella pyrenoidosa* (green algae) served as food. Again there were no C_{20-22} acids in the *Daphnia magna*, but at the lower temperature the proportion of C_{18} increased. The iodine value difference between the crustaceans at the two temperatures corresponded to the equation given above for natural conditions, though the absolute values are somewhat higher. We assume that all planktonic crustaceans react to decreasing temperature by accumulation of polyenoic acids, but in some species the C_{18}, rather than the C_{20-22}, polyenoic acids are of greater importance in this process.

The characteristic fatty acid compositions of different planktonic crustaceans permit a simple and natural test of the effect of diet on the fats of fish. In aquaria at 20° two groups of guppies were raised on summer and winter plankton respectively. The fatty acid compositions were as follows:

Fish fed on summer plankton: $C_{14} = 6.4\%$, $C_{16} = 31.9\%$, $C_{18} = 27.8\%$, $C_{20} = 12.2\%$, $C_{22} = 21.7\%$.

Fish fed on winter plankton: $C_{14} = 4.2\%$, $C_{16} = 14.7\%$, $C_{18} = 20.0\%$, $C_{20} = 25.3\%$, $C_{22} = 35.8\%$.

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In this experiment, the seasonal difference detected in the crustacean plankton manifests itself clearly in fish, although the quantity of C_{20-22} was higher in the fat of both groups of fish than in the dietary fat.

DISCUSSION

On the basis of the above results, it is suggested that the main pathway for the biogenesis of the C_{20-22} fatty acids in aqueous ecosystems is as follows. The algae produce large amounts of C_{16-18} polyenes. The crustaceans, mainly the copepods, elongate these acids to C_{20-22}. The fish readily take up the largest part of the C_{20-22} fatty acids from the crustaceans and store it. The significance of the chain elongation for the crustaceans is that the C_{20-22} highly unsaturated acids ensure a low melting point for the extracellularly stored lipids. With reference to the lipids in fish, we believe that it is not the salinity of the body of water, but its size that is the determining factor. The characteristic fatty acid composition accepted for marine fish is based on species caught in the comparatively cold North Sea, where the fat of the crustacean plankton must be very rich in highly unsaturated fatty acids. As one consequence of smaller dimensions, most fresh-water lakes become considerably warmer in summer. As the temperature decreases later in the year, the feeding activity of fish declines and even stops, so that their food, consisting largely of summer plankton, is very poor in the longer-chain fatty acids. Our recent results on marine crustaceans and the data found in the literature concerning fish from warm seas (12) fit into the picture we have already suggested (13). As a second consequence of the smaller dimensions in fresh water the importance of the food originating on land, shore, and bottom increases, and this kind of food is poor in polyenes.

The different distribution of the cladocerans and copepods in fresh water and the seas might be a further factor contributing to the formation of marine and fresh-water fatty acid types of fish oils (13).

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