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Sources of cyclopropanoid fatty acids in the mummichog *Fundulus heteroclitus*

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Abstract

The saturated fatty acids in lipids of the mummichog *Fundulus heteroclitus* (L.) contain the characteristic bacterial *cis*-9,10-methylenehexadecanoic and *cis*-9,10-methyleneoctadecanoic acids in addition to straight-chain, iso, anteiso and multi-branched fatty acids. To ascertain whether the cyclopropanoid fatty acids (CFA) might be used as a marker for bacterial components in the mummichog diet, the saturated fatty acids of detritus were compared with those of fish intestines and their contents, and with those of eviscerated fish. The relative amounts of individual fatty acids in each sample indicated that microorganisms or invertebrate animals that consume microorganisms constitute a part of the mummichog's diet. A similar analysis of anterior and posterior sections of the fish intestines showed a higher concentration of CFA in the posterior section, suggesting that at least some of the cyclic acids might originate from the resident bacterial gut population. When a group of mummichogs was maintained on a beef-liver diet devoid of cyclic acids, carcass lipids were not depleted of CFA. Instead, an increase in concentration was observed. It was concluded that the intestinal microflora have a role in supplying CFA to mummichog lipids. Since the overall results indicate that these acids are derived from commensal bacteria as well as from bacteria associated with the diet, it is not possible to use such compounds as food-chain markers.

Introduction

In river and coastal ecosystems a variety of fish subsist on diets containing detritivores. Some species also ingest substantial amounts of detritus (Darnell, 1964; Nixon and Oviatt, 1973; Peters and Kjelson, 1973; Kneib and Stiven, 1978). Detritus is rich in microbial growth and several studies of detritivores in streams (Nelson and Scott, 1962; Ladle *et al.*, 1972; Kostalos, 1976), estuaries (Odum and de la Cruz, 1963; Fenchel, 1970; Odum, 1970) and the marine environment (Newell, 1965; Fenchel, 1969; Moriarty, 1976) have suggested that bacteria provide the main food component. Adherent bacteria ingested with decaying phytoplankters (Kogure *et al.*, 1982) can form a further, though probably less substantial, dietary component. Marine invertebrates in particular can use microorganisms for an important part of their diet (Rieper, 1978) and the role of the plant detrital microflora in food chains has been recognized in recent years by numerous investigators (Mann, 1972; Peters and Kjelson, 1973; Schultz and Quinn, 1973; Landry, 1977; Chervin, 1978; Moore, 1978; Paerl, 1978; Lee, 1978). However, the contribution of microorganisms varies between species (Baker and Bradnam, 1976) and remains difficult to assess. Among potential markers that might be used to detect and possibly quantify a bacterial input, the characteristic fatty acids of microbial lipids are worthy of investigation because of the high precision of modern analytical procedures for this group of substances.

Among the fatty acids that may be used as bacterial markers, those containing a cyclopropane ring are of particular interest. They are formed by adding a methyl group across the double bond of monoenoic acids (Law, 1971) and are more stable than their precursors while contributing similar properties to the lipid into which they are incorporated. They have also been identified in a wide variety of microorganisms (Goldfine, 1972) but are not present in all species (Ifkovits and Ragheb, 1968). Cyclopropanoid fatty acids (CFA) are found in the seed oils of certain higher plants, notably of the *Malvaceae* (Smith *et al.*, 1961) but their distribution in the plant kingdon is limited (Yano *et al.*, 1972) and only the seed oil of *Byrsocarpus coccineus* has so far been reported to contain one of the better known bacterial cyclopropanoids, lactobacillic acid (Spencer *et al.*, 1979). Other known sources where

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bacteria were discounted as the responsible agent are protozoa of the genus *Crithidia* (Meyer and Holz, 1966), the millipede *Graphidostreptus tumuliporus* (Oudejans *et al.*, 1971) and the rumen of sheep (Body, 1972). In the latter two organisms, the structures of the cyclopropanoid acids differed from those found in bacterial lipid. Thus the presence of CFA in animals is *prima facie* evidence of a role for bacteria in the nutrition of the organism. There are no reports in the literature of CFA having been used to trace food chains in fish, although they have been included among bacterial markers in sediments (Perry *et al.*, 1979).

For investigation we selected the mummichog *Fundulus heteroclitus* (L.), a common euryhaline and eurythermal cyprinodont that inhabits estuaries, marshes and coastal streams of the eastern seaboard of North America from Mexico to the Gulf of Saint Lawrence (Bigelow and Schroeder, 1963). This is a stomachless fish with a relatively short and featureless digestive tract (Babkin and Bowie, 1928); it can absorb food with similar efficiency across a range of temperatures (Targett, 1979). The diet of mummichogs has been reported to consist primarily of detritus (Darnell, 1964; Jeffries, 1972) and several studies (Nixon and Oviatt, 1973; Challis, 1976; Patriquin and Butler, 1976) have indicated that detritus is a dietary component. In other studies a range of marine invertebrates has been identified in the gut contents and the normal diet has been considered to be mainly animal in origin (Fritz, 1974; Kneib and Stiven, 1978). In laboratory studies, Prinslow *et al.* (1974) found that detritus added to the diet of mummichogs did not contribute to growth of the fish. Whether the role of detritus in the mummichogs' diet is a direct or an indirect one via detritivorous invertebrates, it is clear that these fish are an important factor in the transport of energy from marshes to the deep-water estuary consumers (Valiela *et al.*, 1977; Weisberg and Lotrich, 1982).

Analysis of the fatty acids of mummichog lipid provided the first reported evidence that CFA are present in fish (Cosper and Ackman, 1983). The lipid of juveniles contained a higher proportion of these compounds than did the lipid of adults. Examination of the gut contents showed that the immature fish also had a larger proportion of detritivorous crustaceans in their diet. Additional evidence favouring a dietary origin for the CFA was provided by a comparison of males and females. Female fish in the sample were substantially larger than males and gut content analysis showed that the diet of the females differed in that it contained a large amount of plant material, consistent with an intake of detritus. The females also showed a higher proportion of CFA and branched-chain acids in their carcass lipid.

Although these results suggest that the CFA originate from bacteria via a food chain with detritus and detritivores as probable components, the possibility that they are formed by commensal bacteria, which may differ in numbers as changes occur in the diet, must also be considered. To determine whether the presence of CFA in body lipids of the mummichog can be taken as evidence of a detrital component in the food chain, we have compared the saturated fatty acid (SFA) contents of lipids in the eviscerated carcass of *Fundulus heteroclitus* with that of fore- and hind-gut sections of the intestines, and with that of detritus collected from the sampling site. We have also examined the lipids of fish held in captivity for an extended period on a diet of beef liver devoid of CFA.

Material and methods

Sample collection

Mummichogs were collected from a channel running through a marsh near Lawrencetown, Nova Scotia, Canada. The fish were captured with a minnow seine and transported in oxygenated plastic containers to an aquarium through which sea water of approximately 32‰ S was circulated at 20 °C. A marked difference in size between males and females was observed. In a batch of 165 females and 67 males, the largest female weighed 32.0 g and was 126 mm in length while the largest male weighed only 8.5 g and was 91 mm in length. Fish sacrificed on the day of capture were collected during the summer months (May–August). A total of 526 mummichogs with an average weight of 3.80 g were also collected in October. These fish were held in captivity in an aquarium containing water circulated from the bottom of Halifax harbour at its existing temperature. They were initially fed ground liver at the rate of 60 mg per fish per day; the ration was gradually increased to twice this amount as the fish adjusted to the diet. After five months, 19 fish with an average wet weight of 2.63 g were sacrificed, eviscerated and the SFA of their carcass lipid was analysed.

Where freshly caught fish were analysed for lipid SFA, they were eviscerated on the day of capture. The carcasses of sacrificed or captured fish were normally stored for lipid extraction by quick-freezing at −40 °C. They were glazed with ice after the initial freezing process. The term carcass used here refers to fish that have been eviscerated and have had the head removed. In experiments where the intestines and contents were analysed, the gut was removed during evisceration, separated where necessary into foregut and hindgut regions, and stored at −40 °C for analysis.

Detritus was collected in glass jars at the same time and from the same location as a sample of fish was taken. It was frozen at −40 °C until analysed.

Extraction and esterification of lipids

Samples were extracted and the lipid fraction was recovered by the method of Bligh and Dyer (1959). Lipid samples were transesterified with methanol and boron trichloride as described by Cosper and Ackman (1983), using a modified procedure of Brian *et al.* (1972).

Chromatography

Fatty acid esters were fractionated by thin-layer chromatography on silicic acid impregnated with silver nitrate (Paradis and Ackman, 1977). The zone containing saturated fatty acid esters was scraped from the plate and extracted successively with petroleum ether, petroleum ether-chloroform (1:1) and chloroform. The sample recovered by evaporating the pooled extracts was analysed on a Perkin-Elmer Model 990 gas chromatograph fitted with a flame ionization detector. The resolution required for separating and identifying minor components was achieved by coupling in tandem two open-tubular stainless-steel columns, each 50 m long and 0.25 mm internal diameter, coated with butanediol succinate (BDS) polyester liquid phase. The column temperature was 150 °C; that of the injection port and manifold assemblies was 250 °C. Helium was used as the carrier gas.

Identification of fatty acids

The principal fatty acids and some CFA were identified by comparing retention times with those of authentic standards. Assignment of peaks for *cis*-9,10-methylenehexadecanoic acid (cyc-17:0) and *cis*-9,10-methyleneoctadecanoic acid (cyc-19:0) was verified by signal enhancement when samples in which such peaks were detected were co-injected with methyl esters of authentic cyc-17:0 or cyc-19:0. In addition, each of the esterified samples was treated with urea according to the procedure of Ackman and Hooper (1968) to remove long-chain aliphatic compounds from solution as urea complexes. The failure of urea to form complexes with compounds tentatively identified from retention times as branched-chain and cyclic fatty acids provided supporting evidence for the structure assignment.

To identify cyc-17:0 and cyc-19:0 unequivocally, esterified fatty acid samples were also examined on a Finnigan model 9610 gas chromatograph-mass spectrometer system. A single open-tubular stainless steel column (50 m × 0.25 mm) coated with BDS was used with helium at 50 psig as the carrier gas. The operating conditions were: column temperature 170 °C, injection port 200 °C, manifold assemblies 200 °C. Reference samples of cyc-17:0 and cyc-19:0 esters were first examined to determine the most intense characteristic ions and the mass spectrometer was programmed in a jump-scan multiple-ion detection (MID) mode to scan for only 12 relevant masses. The data were recorded as a reconstructed ion chromatogram (RIC). Characteristic mass peaks for cyc-17:0 were observed at m/z 250, 208 and 194; for cyc-19:0 the characteristic mass peaks were at m/z 278 and 236.

Quantitative measurements of the amount of each fatty acid present in the mixture of methyl esters chromatographed were obtained by converting values for gas-chromatograph recorder peak area to weight percentages, as described by Ackman (1972).

Spearman rank correlation coefficients were calculated as described by Snedecor (1956).

Results

Saturated fatty acids in lipids from carcass and intestines

An analysis of the SFA fraction of lipids extracted from the carcass and from the combined intestines and intestinal contents of freshly caught fish is shown in Table 1. The Spearman ϱ value of 0.95, calculated by comparing the rank order of straight-chain fatty acids in the two samples, indicates considerable similarity in these components. However, there was almost 1.40 times as much hexadecanoic (16:0) acid in carcass as in intestinal lipid, whereas the intestines contained lipid with higher proportions of tetradecanoic (14:0) and octadecanoic (18:0) acids. When values for the iso- and anteiso-series of branched-chain acids are compared, the ϱ value of 0.97 also indicates marked similarity. However, the content of these acids in the carcass lipid was generally lower than that in the intestinal lipid. This is reflected in the overall composition of the two samples (Table 2). The lipid from both sources contained CFA but here also there was less in that from the carcass.

Closely associated with the peaks for cyc-17:0 and cyc-19:0 in the chromatogram were additional distinct peaks with retention times suggestive of isomers. Whereas cyc-17:0 and cyc-19:0 were identified by comparison of retention times with those of authentic standards and by testing for peak enhancement when a mixture of standards and sample was chromatographed, the appropriate standards needed to identify isomeric compounds in this way were not available.

To determine whether CFA were uniformly distributed along the length of the gut, the intestines from a sample of freshly caught fish were subdivided as closely as possible into two parts of equal length. Because of the marked difference in size between the sexes, male and female fish were treated separately. Analyses of SFA in the lipid from anterior and posterior intestines are given in Table 1. Except for the values for 16:0 and 18:0 in the posterior male intestine, the concentrations of the principal fatty acid components were quite similar. Among the minor components, the branched-chain acids were also present in similar amounts. The concentration of longer-chain acids in lipid from the anterior section was somewhat lower than in that from the posterior section; a more distinct trend in this direction was observed for the CFA (Table 2).

Saturated fatty acids in detritus

The SFA fraction of detritus collected from the same location and at the same time as the sample of male and female mummichogs described above differed markedly in composition from either the carcass or intestines of the fish

Table 1. Relative amounts (weight %) of saturated fatty acids in lipid from *Fundulus heteroclitus*, detritus and beef liver

Fatty acid	Sample								
	Freshly caught fish		Intestines (males)		Intestines (females)		Detritus	Captive fish	Beef liver
	Carcass	Intestines	Anterior	Posterior	Anterior	Posterior		(carcass only)	
12:0	0.02	0.28	0.19	0.33	0.34	0.27	2.42	0.14	0.03
Iso-13:0	0.09	0.06	0.05	0.06	0.08	0.06	0.26	0.04	0.01
Anteiso-13:0	0.02	0.03	0.02	0.03	0.04	0.03	0.16	0.05	0.01
13:0	0.11	0.18	0.14	0.19	0.22	0.15	0.96	0.06	0.06
3,7,11-Trimethyl-dodecanoic acid	0.04	0.04	–	–	–	–	0.27	–	–
Iso-14:0	0.09	0.24	0.22	0.25	0.28	0.21	1.44	0.08	0.06
Anteiso 14:0	–	0.02	0.02	0.02	0.09	0.02	–	–	0.01
14:0	1.92	4.84	5.53	4.87	4.95	4.07	13.0	4.06	1.28
4,8,12-Tetramethyltri-decanoic acid	0.05	0.03	0.03	0.04	0.04	0.02	0.13	0.18	0.04
9-Methyltetra-decanoic acid	0.05	0.02	0.02	0.02	0.04	0.02	0.29	0.05	0.02
10-Methyltetra-decanoic acid	0.05	0.08	0.07	0.08	0.12	0.08	0.96	0.07	0.02
Iso-15:0	0.85	1.56	1.57	1.62	1.79	1.35	4.73	0.16	0.31
Anteiso-15:0	0.39	0.80	0.78	0.82	0.91	0.72	1.63	0.27	0.27
15:0	2.55	2.58	2.41	2.84	2.64	2.56	3.43	2.94	0.67
cis-7,8-Methylenetetra-decanoic acid*	0.05	0.06	0.10	0.07	0.01	0.06	0.58	0.02	–
Iso-16:0	0.50	0.80	0.78	0.90	0.83	0.76	1.07	0.51	0.25
Anteiso-16:0	–	–	–	–	–	–	2.32	0.07	0.05
16:0	63.1	44.4	43.4	49.3	43.1	42.4	41.6	62.2	40.4
Pristanic acid	0.13	0.07	0.05	0.08	0.07	0.08	2.53	0.05	0.02
9-Methylhexadecanoic acid	0.42	0.55	0.69	0.55	0.53	0.50	5.05	0.33	–
10-Methylhexadecanoic acid	0.15	0.10	0.09	0.12	0.12	0.10	0.59	0.21	0.09
Iso-17:0	1.35	2.05	1.93	2.09	2.12	2.14	0.93	1.37	0.65
Anteiso-17:0	0.67	1.08	0.97	1.03	1.18	1.16	0.66	0.78	0.69
17:0	3.83	5.04	5.04	5.08	5.04	4.91	1.43	2.99	1.57
Phytanic acid	0.03	–	–	–	–	–	0.06	0.23	1.41
cis-7,8-Methylene-hexadecanoic acid*	0.10	0.12	0.11	0.12	–	–	–	0.09	–
cis-9,10-Methylene-hexadecanoic acid	0.12	0.20	0.02	0.12	0.27	0.37	0.67	0.16	–
cis-11,12-Methylene-hexadecanoic acid*	0.05	0.10	0.13	0.06	0.05	0.20	–	0.11	–
cis-13,14-Methylene-hexadecanoic acid*	0.02	0.04	0.08	0.03	–	0.09	–	–	–
Iso-18:0	0.37	0.66	0.62	0.65	0.65	0.68	0.05	0.58	0.16
Anteiso-18:0	0.06	0.11	0.08	0.11	0.10	0.14	–	0.13	0.04
18:0	20.2	28.9	30.5	24.2	30.2	31.1	5.52	18.6	50.9
10-Methyloctadecanoic acid	0.06	0.12	0.09	0.14	0.07	0.21	0.72	–	–
12-Methyloctadecanoic acid	0.08	0.08	0.04	0.07	0.01	0.15	0.06	0.08	0.09
Iso-19:0	0.67	0.82	0.65	1.00	0.57	1.12	0.44	0.87	0.04
Anteiso-19:0	0.23	0.30	0.22	0.31	0.27	0.41	–	0.33	0.09
19:0	0.71	1.20	1.08	1.20	1.18	1.35	0.06	0.86	0.23
cis-9,10-Methylene-octadecanoic acid	0.05	0.08	0.14	0.14	0.03	0.08	0.22	0.15	–
cis-11,12-Methylene-octadecanoic acid*	0.08	0.14	–	0.21	0.06	0.14	–	–	–
cis-13,14-Methylene-octadecanoic acid*	0.20	0.28	–	–	0.29	0.28	–	–	–
Iso-20:0	0.01	0.04	0.02	–	0.01	0.13	1.72	0.04	–
20:0	0.46	1.30	1.42	1.21	1.26	1.35	1.71	0.70	0.15

* Tentative identification

Table 2. Sample size, lipid content and relative amounts (weight %) of saturated fatty acids grouped in homologous series

Group	Sample*								
	Freshly caught fish		Intestines (males)		Intestines (females)		Detritus	Captive fish	Beef liver
	Carcass	Intestines	Anterior	Posterior	Anterior	Posterior		(carcass only)	
Number of fish	44	44	30	30	20	20	–	19	–
Weight of sample (g)	124	8.1	2.2	4.0	3.0	7.0	41.4	50	50.5
Weight of lipid (g)	1.42	0.22	0.06	0.11	0.15	0.11	0.029	0.73	2.29
Straight-chain fatty acids	92.9	89.4	89.7	89.4	90.0	88.3	70.1	92.6	95.3
Iso and anteiso branched-chain fatty acids	5.34	8.65	8.27	8.90	7.83	8.91	16.0	5.08	2.79
Other branched-chain acids	0.81	0.94	1.00	0.98	0.89	1.06	7.67	0.74	0.22
Isoprenoid acids	–	–	0.08	0.12	0.11	0.10	2.99	0.53	0.80
Cyclopropanoid fatty acids**	0.14	0.28	0.16	0.22	0.30	0.45	0.89	0.31	–
Total	99.2	99.4	99.2	99.6	99.1	98.8	97.7	99.3	99.1

* Same sample as in Table 1. Deficits from 100% in the totals are accounted for by acids with less than 12 carbons and by minor compounds not positively identified

** Only *cis*-9,10-methylenehexadecanoic and *cis*-9,10-methyleneoctadecanoic acids are included

(Tables 1 and 2). A ϱ value of 0.57 was calculated by comparing the rank order of straight-chain fatty acids in eviscerated mummichogs and detritus; the value for intestines and detritus was 0.70. When the rank order of branched-chain acids in eviscerated fish and detritus was compared, the ϱ value was 0.33; for intestines and detritus, it was 0.38.

All of the samples contained both cyc-17:0 and cyc-19:0; the concentrations of these acids were highest in the lipid from detritus and lowest in that from the mummichog carcasses. This was also true of the iso- and anteiso-series and of the other singly-branched acids. The isoprenoid acids were well represented in the detritus sample with pristanic acid the main component. Much smaller amounts were present in the intestinal lipid and in the lipid from eviscerated mummichogs.

Saturated fatty acids of captive fish

The compositions of the SFA from mummichogs kept in captivity on a beef-liver diet and from a sample of the beef liver on which they were fed are given in Table 1 and summarized by fatty acid groups in Table 2.

The ϱ value of 0.93 calculated from a rank-order comparison of saturated straight-chain fatty acids from the liver-fed mummichog sample and from mummichogs freshly caught from the natural habitat indicates that their relative composition was similar. In the concentrations of the major acids, the straight-chain acid fraction of captive fish was much closer in composition to that of freshly caught fish than to that of beef liver. For example, the 18:0 acid constituted 50.9% of the SFA in beef liver but only 18.6% in the fish fed beef liver. The 16:0 acid represented 40.4% of the SFA in beef liver in contrast to 62.2% in captive mummichogs. The ϱ value of 0.82 calculated by comparing the rank-order of iso- and anteiso-fatty acids in captive and freshly caught fish indicates greater differences in the branched-chain than in the straight-chain fatty acid contents. However, the relative overall concentrations of branched-chain acids were quite similar and differed appreciably from that of beef-liver lipid. Only in the presence of phytanic acid, which is not a normal constituent of the mummichog carcass, was the influence of the diet apparent. A relatively high concentration of CFA was present in lipid from the captive fish. This did not come from beef liver since neither cyc-17:0 nor cyc-19:0 was detected among its lipid fatty acids.

Discussion

The most abundant fatty acid in the eviscerated mummichog was 16:0. The amount (65% of total SFA) is similar to that found in many other fish (Ota and Yamada, 1974). It is also consistent with the general similarity in SFA composition of marine teleosts (Ito and Fukuzumi, 1962), with 16:0 always the largest component (Ackman and Sipos, 1965). The abundance of 18:0, the next largest component in mummichog SFA, may reflect the high level of phospholipid in these fish (Cosper, 1981). A correlation between high phospholipid content and preponderance of 18:0 over 14:0, which is more commonly the second most abundant SFA in animal fat, has been established for the Newfoundland capelin (Ackman *et al.*, 1969) in which the carcasses were mostly muscle with cellular phospholipid accounting for more than 0.5% of the tissue content. This high phospholipid level would also account for the low content of shorter straight-chain acids in eviscerated fish relative to the amounts in their intestines and in detritus.

Branched-chain and cyclopropanoid fatty acids were present as minor components in mummichog lipid. Similar amounts of the iso- and anteiso-fatty acids have been found in other fish lipids (Ackman and Sipos, 1965; Ackman and Hooper, 1968). These substances have been considered as bacterial markers (Cooper and Blumer 1968; Kline, 1978) and are particularly abundant in the lipid of ruminants (Smith and Duncan, 1979; Smith *et al.*, 1979). Although they are also found in some other organisms, including marine phytoplankton (Chuecas and Riley, 1969), their concentration in bacteria is usually much higher than elsewhere (Dees and Moss, 1975; Kentaro and Kitamikado, 1978). Thus the larger proportion of these acids in lipid from the mummichog intestines and detritus than in carcass lipid may be attributed to the presence of microorganisms. It should be noted that the mummichog lipid contained iso- and anteiso-19:0 fatty acids. These have seldom been reported as lipid components, but amounts comparable to those present in mummichogs were recently found in two species of Australian molluscs (Johns *et al.*, 1980), which possessed enteric microorganisms that aided in digestion of cellulose. It is likely that the presence of these acids in both mummichogs and molluscs was associated with microbial activity.

The concentrations of CFA in mummichog lipid were comparable to those of the iso- and anteiso-acids. Since lipid from the intestines contained more CFA than did lipid from eviscerated fish and detritus collected in the fishes' natural habitat was even richer in these components, the results were consistent with a dietary intake of detritus-borne bacteria. The types of CFA in the intestinal and carcass lipid of mummichogs appeared to be more diverse than in detritus where only *cis*-7,8-methylenetetradecanoic, cyc-17:0 and cyc-19:0 acids were detected. However, the identification of CFA other than cyc-17:0 and cyc-19:0 is only tentative and, because cyc-19:0 fed to mummichogs is metabolized to products corresponding to the tentatively identified CFA (Cosper, 1981), differences in CFA composition cannot be regarded as evidence against a detrital origin for these compounds.

Fatty acids with a mid-chain methyl group are common in bacterial lipid and are sufficiently characteristic to have been used as bacterial markers (Cranwell, 1974). The presence of these compounds in mummichog carcass lipid thus implicates bacteria in the fish's nutrition. Of the series of 9-, 10- and 12-methyl fatty acids detected, 9-methylhexadecanoic acid was the most abundant. This may reflect the particularly high concentration of this component in detritus.

Isoprenoid alkanoic acids are commonly present in marine oils, although the concentration can vary considerably (Blumer *et al.*, 1964; Ackman *et al.*, 1971). They are formed in animals by metabolism of chlorophyll and other substances with isoprenyl chains and are produced by some microorganisms. If they originate in the food chain, they can participate in the dietary fatty acid pool. The low level of isoprenoid acids in the mummichog samples relative to those in detritus raises a question about the contribution of detritus to the nutrition of fish used in this study. Gut analyses (Cosper, 1981) indicated that much of the diet consisted of invertebrates that assimilate substantial amounts of detrital microorganisms (e.g. Fenchel, 1970; Baker and Bradnam, 1976). The transfer of lipid from invertebrates in the diet has been shown to account for the presence of odd-chain fatty acids in the smelt *Osmerus mordax* (Paradis and Ackman, 1976) and is probably an important avenue for incorporation of detrital fatty acid components. The extent to which particular fatty acids from the detrital bacteria are incorporated into fish lipid may differ according to their history in the food chain. The relative importance of the indirect route of fatty acid incorporation into the body tissue of mummichogs is suggested by the higher CFA content of lipid from juveniles, which had a predominantly crustacean diet (Cosper and Ackman, 1983).

Concentrations of CFA and branched-chain acids were higher in lipid samples obtained from the posterior than in those from the anterior section of the mummichog intestine. Since the concentrations of straight-chain SFA did not vary in the two lipid samples, the higher concentration of cyclic and branched-chain acids in the posterior section can be attributed to an unequal distribution of microorganisms. Some fish are known to have a significant microbial flora in the digestive tract (Colwell, 1962). In the herbivorous minnow *Orthodon microlepidotus,* this flora has been shown to increase from foregut to hindgut (Kline, 1978). The higher concentration of CFA in the hindgut of mummichogs may thus indicate that the CFA are from a natural and permanent bacterial gut population. However, differences in the concentrations of CFA and branched-chain acids from the two gut sections were relatively small and might be due to other factors. In particular, differences in the digestibility of bacteria relative to associated dietary components would affect the CFA concentration in different regions of the intestine. As noted by others (Radtke and Dean, 1979; Targett, 1979), digestion in the relatively short mummichog intestine takes place under alkaline conditions and there are no well defined peptic glands, so food conversion may be relatively inefficient.

Mummichogs maintained for a prolonged period on a CFA-free, beef-liver diet were generally similar to those captured from their natural habitat in the proportions of major straight-chain SFA in their carcass lipid. The minor differences noted may well have been due to the lower temperature experienced by the captive fish (Kemp and Smith, 1970; Farkas and Csengeri, 1976). There was also little difference in the total amount of iso- and anteiso-fatty acids. Of particular importance, however, was the slightly higher concentration of cyc-17:0 in the fish fed beef liver and the substantial increase in the concentration of cyc-19:0 compared with the freshly caught fish. As a result, the total content in the lipid from the fish held in captivity on the beef liver diet approximately doubled. Since the beef liver lacked CFA, the increase was probably due to the development of a bacterial flora specifically

adapted to this diet. The microflora in the intestines of the prawn *Penaeus japonica* is reported to change in genera, numbers and physiological activity with age and diet (Yasuda and Kitao, 1980). When the fish *Ctenopharyngodon idella* and *Tinca tinca* were maintained on an artificial diet, the microbial flora of the intestine showed less species variation but higher physiological activity than the microbial flora of fish given a natural diet. The artificial diet increased proteolysis and other hydrolytic action. The total number of microorganisms increased with increasing amount of food consumed (Lubianskiene *et al.*, 1977). Thus some of the differences in CFA and branched-chain acid composition of lipid from the various mummichog samples could have arisen from differences in the types of microorganisms colonizing their intestinal tracts.

The results from this experiment establish that a dietary intake of bacteria is not necessary to maintain the concentration of CFA in mummichog lipid. Since the CFA content is depleted by turnover unless the acids are constantly replenished (Cosper, 1981), we conclude that mummichogs possess an intestinal microflora from which CFA are incorporated into body lipids. The combined evidence from the present studies suggests that the small amounts of CFA detected in the wild mummichog body lipid originate from both dietary sources and the activity of commensal bacteria in the intestine. Because of the latter contribution and the likelihood that the relative amounts from each source vary with diet, the presence of CFA in the lipids of this fish cannot safely be used to indicate a bacterial component in the food chain.

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