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Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids

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Abstract

The lipid profiles of a few species of marine unicellular algae and yeast were studied with emphasis on fatty acids as part of a search for the nutritional value of plankton to the diet of marine fish larvae commonly used in marine hatcheries. The general proximate chemistry of rotifers was closely related to the proximate chemistry of the diet organism, exhibiting a higher content of protein and carbohydrate and a lower content of lipid Major lipids in all algae, yeast and rotifers comprised mono-, di- and tri-glycerides and polar lipids. The algae *Chaetoceros gracilis* Schutt, *Isochrysis galbana* Parke and their respective algae-fed rotifers exhibited higher amounts of neutral lipids, consisting mainly of cyclic and branched polyunsaturated components. Fatty acid composition of the algae was species-specific, with the highest ratio of polyethylenic to saturated and monoethylenic acid in *I. galbana* and *Phaeodactylum tricornutum* Bohlin, and the highest content (15%) of n-3 highly unsaturated fatty acids in *Nannochloropsis salina* and *P. tricornutum*. A closely mirrored distribution of the fatty acids, but with a lower amount of n-3 highly unsaturated fatty acids, was present in the respective algae-fed rotifers. Comparison of the fatty acid spectrum of *Artemia* sp. and *Euterpina acutifrons* grown in the laboratory on *I galbana* with zooplankton samples of *E. acutifrons* and *Oithona nana* collected from the sea showed a higher concentration of docosahexaenoic acid (22:6 n-3) in the naturally collected sample. The results indicate that the efficacy of the food algae *C. gracilis* and *I. galbana* in increasing the survival of fish larvae in marine hatcheries is not obvious on the sole basis of fatty acid composition.

Introduction

The nutritional quality of the food offered to cultured marine organisms is crucial during the first few weeks of larval life Most reports (Fujita, 1979, Scott and Middleton, 1979; Watanabe, 1979; Watanabe *et al.*, 1983) have suggested that lipids in general and specifically n-3 (previously termed $\omega 3$) highly unsaturated fatty acids (HUFA) have an essential role in the larvae diet

The rotifer *Brachionus plicatilis* has been used extensively as a live food for rearing fish larvae, as well as planktonic crustaceans, due to its appropriate size, rapid production rate and suitability for mass culturing under controlled conditions (Watanabe *et al*, 1983). Although rotifers have been found to synthesize some n-3 HUFA (Lubzens *et al.*, 1985), the amount accumulated is small and insufficient to meet the possible demand of the fish larvae. Fatty acids must be provided to the rotifers via their food, which in most cases is supplied by unicellular algae or baker's yeast. Since baker's yeast lacks n-3 HUFA, yeast grown rotifers are fed on algae containing these fatty acids 8 to 24 h prior to their transfer into the fish tanks (Watanabe *et al.*, 1983). The requirement of lipids is not restricted to fish larvae and crustaceans Oyster larvae (Cher and Webb, 1984) and *Crassostrea gigas* spat (Langdon and Waldock, 1981) have also shown a developmental dependence on certain algal diets containing different lipid compositions. The importance of marine unicellular algae in natural and artificial food chains comprised of algae, zooplankton and fish larvae lies in the primary property of the algae as photosynthetic first producers. The algal content is transferred directly, or indirectly through the zooplankton mediation, to the larvae.

The present communication is aimed at studying the dietary contribution of a few marine algae commonly used in mariculture in comparison to rotifers and copepods fed on a diet of algae, or collected from the sea, as a means of evaluating the possible role of different lipid components in the natural food chain, with special emphasis on fatty acids and neutral lipids

Materials and methods

Organisms

Phytoplankton. Chaetoceros gracilis Schutt, SERI # S/CHAET-1; *Chlorella stigmatophora* Butcher, UTEX # LB

993; *Nannochloropsis salina,* SERI # S/NANNO-1; *Isochrysis galbana* Parke, UTEX # LB 987; *Phaeodactylum tricornutum* Bohlin, UTEX # 640.

Yeast Saccharomyces cerevisiae.

Zooplankton. Brachionus plicatilis Muller (Lubzens *et al*, 1980); *Artemia* sp., *Euterpina acutifrons, Oitona nana* Zooplanktonic organisms were locally isolated strains.

Growth conditions

Algae Algae were cultivated on sea water medium enriched with 5 m*M* KNO_3, 0.2 m*M* KH_2PO_4, 1.5 μM $FeCl_3$, 6 μM EDTA, 2 m*M* $NaHCO_3$, 20 m*M* Tris-Cl pH 7 5, 0.25 m*M* Na_2SiO_3, and trace metal mix (McLachlan, 1973). Cells were grown in liquid medium at 22 °C under continuous illumination of cool-white and Agro-Lite Westinghouse fluorescent lamps at a light intensity of about 10 W m^{-2}. Cells were harvested by centrifugation

Yeast Common Baker's yeast was dissolved in distilled water and 1 μg was provided to the rotifers per day (Fujita, 1979).

Zooplankton. Zooplankton were bred at 22 °C in the same algal grown sea water medium and fed with late logarithmic grown algae or on yeast for several generations prior to chemical analysis. Zooplankton were sieved on 60-μm nylon nets and washed with clean sea water prior to concentrating and freezing Sea plankton were collected with 60-μm nylon nets from different locations at about 2 000 m offshore. A size fraction of 100 to 200 μm was filtered from the samples on nylon nets and assayed microscopically for species isolation prior to freezing and lyophilization

Analytical methods

Wet samples were frozen and lyophilized for chemical analysis. Ash-free dry weight was determined by ashing at 540 °C. Protein was assayed as described by Lowry *et al* (1951) or by Kochert (1978 a) after hydrolysis in 1 N NaOH for 1 h at 100 °C Total carbohydrates were analyzed by the phenol-sulfuric acid method following acid hydrolysis in 2 N HCl for 1 h at 100 °C (Kochert, 1978 b). Total lipids were extracted with methanol-chloroform-water (10.5:4 v/v/v) (Bligh and Dyer, 1959), modified as previously described (Kates, 1972). Total lipid weight was determined gravimetrically, and the lipid was fractionated on heat activated silicic acid columns (Ben-Amotz *et al.,* 1985). Total and column fractionated lipids were studied by thin layer chromatography. Fatty acid methyl esters were preparaed by esterification with BCl_3 as described recently (Miller, 1982) and analyzed on a Hewlett Packard 5890 A gas chromatograph equipped with a flame ionization detector and with a 3390 A Reporting Integrator. A Hewlett Packard Ultra # 1 fused silica capillary column of crosslinked methyl silicone, 50 m × 0.2 mm ID and 0.33-μm standard film, was used with He from 150° to 300 °C at 4 C° min^{-1} and held isothermically. The identification of the fatty acids was based on retention time relative to known standards.

Results

Proximate cellular composition

The gross chemical composition of five unicellular marine algae and of yeast was assayed in comparison to the chemical composition of *Brachionus plicatilis* fed on these diet organisms (Table 1). Most of the algae contained about 30% protein, 25% carbohydrate, 21% lipid, and a relatively

Table 1. *Brachionus plicatilis.* Chemical composition of the rotifer and its dietary algae and yeast

Species	% organic weight			
	Protein	Carbohydrate	Lipid	Unknown
Chaetoceros gracilis	36 1	49 2	20.7	–
Rotifer/*C gracilis*	32 0	44 9	20 1	3.0
Chlorella stigmatophora	30.9	20 6	20 1	28.4
Rotifer/*C stigmatophora*	28 1	10 0	7 4	54.5
Isochrysis galbana	34 9	11 0	20 1	34 0
Rotifer/*I galbana*	29 1	7.0	11 1	52 8
Nannochloropsis salina	23 3	24 4	14 5	37.8
Rotifer/*N salina*	28 0	24 1	16 4	31.5
Phaeodactylum tricornutum	35 1	24 2	20.5	20.2
Rotifer/*P tricornutum*	51 4	13 5	8 9	26 2
Yeast (*Saccharomyces cerevisiae*)	53 2	41 3	1 0	4 6
Rotifer/yeast	55 4	28 0	4 5	2 1

Each assay was done in triplicate, replicates agreed within 5% of the mean

Table 2. Lipid fractionation on unisil columns

Species	% of total lipid weight				
	Hexane	Benzene	Chloroform	Acetone	Methanol
Chaetoceros gracilis	0 8	40 6	8 6	34 1	15 9
Rotifer/*C gracilis*	0 6	27 5	11 7	43 8	16 4
Chlorella stigmatophora	2 1	6 8	35 0	52.7	3 4
Rotifer/*C stigmatophora*	2 0	8 8	23 8	25 0	40 4
Isochrysis galbana	1 5	27 4	33 1	26 3	11 7
Rotifer/*I galbana*	1 6	23.1	17 5	16 3	41 5
Nannochloropsis salina	1 3	6 4	5 1	56 1	31 1
Rotifer/*N salina*	1 2	6 6	7 1	50 4	34 7
Phaeodactylum tricornutum	0 1	8 0	31 0	31 1	29 6
Rotifer/*P tricornutum*	0 1	8 7	33.2	44 3	13 7
Yeast (*Saccharomyces cerevisiae*)	0.8	11 0	6 2	51.1	33 1
Rotifer/yeast	0 5	6 5	11 9	54 2	35 3

Percentages were determined by gravimetry

high content of an unknown fraction: *Chaetoceros gracilis* was composed of a higher percentage of carbohydrate and practically no unknown fraction. Yeast contained 53% protein, 41% carbohydrate, and a low content of lipid of around 1%. Rotifers fed on the algae and on yeast presented a closely similar chemical composition to the diet food, with the exception of the lipid content, which was lower in rotifers fed on *Chlorella stigmatophora* and *Phaeodactylum tricornutum* Rotifers fed on yeast synthesized lipids up to about 5% in comparison with 1% total lipid per yeast organic weight.

Lipid composition

The total lipid extract of each alga and of rotifers fed on the specific alga was fractionated on silicic acid with hexane, benzene, chloroform, acetone and methanol (the lipid fractionation is summarized in Table 2). The hexane eluate which contains hydrocarbons was low in all samples, similar to previous observations (Ben-Amotz *et al.*, 1985). The benzene eluate was the major fraction in *Chaetoceros gracilis,* and exceeded 40% of the total lipids in this diatom. *Isochrysis galbana* comprised relatively high amounts of lipids in the benzene eluate, identified preliminarily by thin layer chromatograph (TLC) as alkenones, and cyclic and polyunsaturated hydrocarbons. The other algae showed much lower amounts of lipids in the benzene eluate. The rotifers fed on *C. gracilis* and *I. galbana* presented similarly high amounts of neutral lipids in the benzene eluate relative to rotifers fed on the green algae and on *Phaeodactylum tricornutum.* The chloroform eluate presented a prominent content of glycerides in *Chlorella stigmatophora, P tricornutum* and in their respective rotifers The polar lipids, glycolipids in the acetone eluate and phospholipids in the methanol eluate were the major lipid fraction in all the algae, yeast and rotifers.

Fatty acids

Table 3 illustrates the fatty acid composition of the total algal and yeast lipids. The major fatty acids present in yeast were 16.0, 16:1 n-7 and 18:1, in *Nannochloropsis salina* 16.0, 16:1 n-7, 18:0 and 20.5 n-3, in *Chlorella stigmatophora* 16·0, 16:1 n-7, 18:0 and 18:1, in *Isochrysis galbana* 14 0, 16:0, 18·0, 18.1 and 18.2 n-6; in *Chaetoceros gracilis* 14:0, 16·0, 16:1 n-7 and 20.5 n-3; and in *Phaeodactylum tricornutum* 14.0, 16·0, 16:1 n-7, 16:2+3 and 20·5 n-3. The ratio of polyethylenic acids to saturated and monoethylenic acid was highest in *I galbana* and *P. tricornutum* and minimal in *C. stigmatophora* and *C gracilis* The ratio of saturated fatty acids to monoethylenic and polyethylenic fatty acids was maximal in *N. salina* and *C. gracilis* and lowest in yeast n-3 HUFA's were predominant in *N salina* and *P tricornutum.*

The fatty acid composition of the total lipids of rotifers fed on the algae or yeast is given in Table 4. The major fatty acids present in rotifers fed on yeast were 16 0, 16:1 n-7, 18:0, 18:1 and 18·3 n-3; in rotifers fed on *Nannochloropsis salina* 16:0, 16.1 n-7, 18:0, 18.1, 18 2 n-6 and 20:5 n-3; in rotifers fed on *Chlorella stigmatophora* 14:0, 16:0, 16·1 n-7, 18:0, 18:1 and 18:2 n-6; in *Isochrysis galbana* 14·0, 16·0, 16·1 n-7, 18:0, 18·1, 18 2 n-6 and 22:6 n-3; in rotifers fed on *Chaetoceros gracilis* 14:0, 16·0, 16·1 n-7 and 18:1, and in rotifers fed on *Phaeodactylum tricornutum* 14.0, 16·0, 16·1 n-7, 18:1 and 20:4 n-3. The rotifers showed the same basic profile of the fatty acids as the diet algae with a major content of C:16 and C:18 fatty acids. Long polyunsaturated fatty acids of 20.4 n-3 and 20:5 n-3 were detected in rotifers fed on *P. tricornutum;* 20:5 n-3 in rotifers fed on *N salina;* 20 4 n-3 and 20:5 n-3 in rotifers fed on *C gracilis,* and 20.4 n-3 and 22:6 n-3 in rotifers fed on *I. galbana.* Rotifers fed on yeast lacking HUFA (Table 3) showed the lowest ratio of saturated to monoethylenic and polyethylenic fatty acids. n-3 HUFA's were predominant in *P tricornutum* (13%), in *N salina* (8.5%) and in *C gracilis* (7.0%).

Table 3. Percentages of total fatty acid composition of unicellular marine algae

FAME	*Chaetoceros gracilis*	*Chlorella stigmatophora*	*Isochrysis galbana*	*Nannochloropsis salina*	*Phaeodactylum tricornutum*	*Saccharomyces cerevisiae*
14 0	7 3	0 8	22 0	4.3	7 2	1.0
16 0	34 5	16.3	9 4	25.2	17 6	13 1
16·1 n–7	39 3	13.5	4 9	32.8	33.3	37 1
16·2+3	2.9	1.5	–	0.3	10 2	–
17:0	0 7	–	–	–	0 7	0 7
18 0	2.2	22 7	10.7	13.5	4 7	5 5
18.1	0.9	29 2	20.2	4.8	2.0	40 4
18·2 n–6	0 6	2 6	19 8	–	1 1	–
18 3 n–3	0.8	5 1	4 6	1 2	1 3	–
18 4	0.8	–	3.1	0 5	1.3	–
20.1	–	0 5	–	–	–	–
20.2 n–6	–	0 9	–	–	–	–
20 4 n–3	1 4	1 9	–	0 8	–	–
20·5 n–3	4 1	1 3	–	14.8	14.7	–
22.6 n–3	–	–	3 4	–	0 3	–
Saturated	44 7	39 8	42 1	43.0	30 2	20 3
Monoethylenic	40 2	43 2	25 1	37.6	35.3	77 1
Polyethylenic	10 6	13 3	30 9	17.6	28.9	–
Unidentified	4 5	3 7	1 9	1.8	5.6	2 6
Poly/Sat+Mono	0 12	0 16	0.46	0.21	0 44	–
Sat/Mono+Poly	0 88	0 70	0.75	0.77	0.49	0 26
Σn–3 HUFA	5.5	3 2	3 4	15.6	15 0	0

Table 4. *Brachionus plicatilis* Percentages of total fatty acid composition of *B. plicatilis* grown on the indicated algae

FAME	*Chaetoceros gracilis*	*Chlorella stigmatophora*	*Isochrysis galbana*	*Nannochloropsis salina*	*Phaeodactylum tricornutum*	*Saccharomyces cerevisiae*
14:0	9.3	4.6	6 7	3.5	5 6	2.6
14·1	–	0 6	1.0	–	0 3	–
16 0	18 3	25 3	12.4	18 3	18 3	7 4
16·1 n–7	29 2	5 9	9 6	20 7	22.5	31 3
16.2+3	3 2	1 2	1 0	0 5	2 6	–
17.0	1.6	2 6	1 5	0 9	2 7	–
18 0	3 6	8 4	7.5	4 9	3 6	4 4
18 1	8 9	10 0	9 6	16 7	6 5	10.6
18.2 n–6	3 8	8.9	14 9	7 3	4 2	–
18·3 n–3	3 8	2.8	4 2	1.8	3 6	34.5
18 4	2 1	1.7	2.0	1 8	1 0	1.4
20·0	0 3	0 6	–	0 8	0 4	–
20 1	0.4	–	3 1	–	0.8	–
20:2 n–6	0 7	1.6	–	0 9	1.8	–
20.3 n–3	0 7	3 2	–	1 2	0.8	4 6
20 4 n–3	3.2	1 8	3.4	1.6	10 1	–
20 5 n–3	2.8	0 8	1.6	6.5	1 8	–
22.4 n–6	0 5	1.2		0 4	1 4	–
22 6 n–3	1 0	0 5	4.2	0 8	0 8	–
Saturated	33 1	41 9	28.1	28 4	31 1	14 4
Monoethylenic	39 0	17 1	23.3	37.4	30 4	41.9
Polyethylenic	21 3	23 1	31.3	22.8	28 1	40.5
Unidentified	6 6	18.3	17.4	11.3	11 2	3 2
Poly/Sat+Mono	0.29	0 39	0 61	0.34	0.45	0 72
Sat/Mono+Poly	0.55	1 0	0 51	0.47	0.53	0 17
Σn–3 HUFA	7.0	3 1	5 8	8.5	12.7	–

Table 5. Percentages of total fatty acid composition of laboratory grown plankton in comparison with plankton collected from the sea

FAME	Laboratory plankton grown on *Isochrysis galbana*		Sea plankton	
	Artemia sp	*Euterpina acutifrons*	*Euterpina acutifrons*	*Oithona nana*
12.0	1 2	1 2	1 2	2 9
14 0	3 3	13.7	9 3	8 2
16·0	15.5	16.5	24 5	31 4
16 1 n–7	13.3	3 4	4 7	5 4
16·2+3	–	–	3 2	–
17.0	1 5	0.6	1 4	2 1
17 1	1 8	0.4	–	–
18·0	6.5	2.7	5 4	10 9
18:1	14 3	1 0	–	8 8
18 2 n–6	28.3	33 8	6.5	2 5
18 3 n–3	4 0	4 7	2 2	6 2
18 4 n–3	1 8	7 9	4 4	–
20:3 n–3	–	1 6	–	–
20 4 n–3	0 7	–	–	–
20 5 n–3	1.4	2 7	11 1	9 9
22 1	–	–	1 2	–
22·0	1 4	–	0 5	–
22 6 n–3	–	6 0	16 1	10 6
Saturated	29.4	34 7	42 3	55 5
Monoethylenic	29.4	4 7	5 9	14.2
Polyethylenic	36 2	56 7	43 5	29 2
Unidentified	6 4	3 9	8 3	1 1
Poly/Sat + Mono	0 63	1 44	0 90	0 41
Sat/Mono + Poly	0 45	0.56	0.85	1 27
Σn–3 HUFA	2.1	8.7	27 2	20.5

The fatty acid composition of *Artemia* sp. and *Euterpina acutifrons* grown in the laboratory on *Isochrysis galbana* was compared to that of sea plankton samples collected from the Mediterranean shore (Table 5). An *Oithona nana* population was found in one sample from one location and an *E acutifrons* population in another The major fatty acids in *Artemia* sp. fed on *I. galbana* were 16:0, 16 1 n-7, 18:0, 18:1 and 18·2 n-6; in *E acutifrons* fed on *I. galbana* 14:0, 16:0, 18:2 n-6, 18:4 n-3 and 22 6 n-3; in *O. nana* collected from the sea 14:0, 16:0, 16:1 n-7, 18:0, 18:1, 18.3 and 22:6 n-3; and in *E. acutifrons* collected from the sea 14:0, 16:0, 18:0, 18·2 n-6 and 22:6 n-3 The highest total percentage of n-3 HUFA was detected in the sea plankton, with a maximal content of 16 1% in *E. acutifrons.*

Discussion

In general the chemical composition of rotifers was quite similar to that of the diet algae which they were fed. Proximate composition of carbohydrate and protein in algae, rotifers fed on algae, yeast, and rotifers fed on yeast showed close similarity in total percentage; the lipid fraction of yeast and rotifers fed on yeast was lower than that of algae and rotifers fed on algae.

The lipid composition of rotifers was largely dependent on that of their food, indicating that most of the diet lipids are incorporated and stored in the rotifer lipids. The major polar lipids of the algae assayed are those commonly found in photosynthetic eukaryotes. Although the concentration of the total phospholipids and glycolipids changed between one species to another, the relative proportions of the individual polar lipid components remained fairly constant. The rotifers exhibited closely similar proportions between polar lipids and neutral lipids as the algae, indicating lipid absorption, partial metabolism and incorporation within the rotifer.

It was interesting to note that the benzene fraction, which usually contains multibranched and/or polyunsaturated hydrocarbons, was a predominant fraction in *Chaetoceros gracilis* and *Isochrysis galbana* and in their respective rotifers. Marlowe *et al* (1984) and Ben-Amotz *et al.* (1985) have recently shown accumulation of long chain C.34–37 alkenones in *Isochrysis* species. Preliminary analysis of the benzene lipid eluate of *C. gracilis* has shown the presence of a high molecular weight (mw $\simeq$ 700) hydrocarbon as the prominent component (Ben-Amotz *et al.*, unpublished data).

A few differences have been noted in the fatty acid composition within the yeast, algae and rotifers and between the rotifers and their diet species. These are best indicated by the summary of saturated, monoethylenic, and polyethylenic acids and HUFA, and the calculated ratios (Tables 3, 4). The general trend showed that the rotifers acquire and maintain their fatty acid profile from the diet food with slight modifications.

Comparison of the laboratory grown zooplanktonic rotifers and copepods cultivated on *Isochrysis galbana* with the naturally collected copepods clearly showed high enrichment of n-3 HUFA in sea plankton The same lipid extracts of the sea collected copepods revealed in addition a higher content of neutral lipids (benzene eluate) with a few predominant high molecular weight components (Ben-Amotz, unpublished data). This may well indicate that the naturally collected sea plankton acquire their fatty acid content and neutral lipids from a variety of natural algae enriched with these lipid components

The above results indicate that the tested zooplankton cultivated under controlled conditions do not mimic the fatty acid profile of sea plankton. Natural marine collections of zooplankton are enriched with n-3 HUFA; the enrichment may be related to different phytoplankton species or to a diet on some algae containing a higher content of n-3 HUFA. A proper way to increase the level of n-3 HUFA in the diet of fish larvae growing in marine hatcheries is best achieved by selecting a variety of microalgae growing under the appropriate conditions which induce accumulation of n-3 HUFA Based on the fatty acid composition and the content of n-3 HUFA per se, the efficacy of the commonly applied algae *Chaetoceros gracilis* and *Isochrysis galbana* is not obvious One would assume that *Nannochloropsis salina* and *Phaeodactylus tricornutum* would be the best food to decrease larval fish mortality on the basis of the fatty acid composition. Nevertheless, experience accumulated in marine hatcheries shows that *P tricornutum* is inferior to *C gracilis* and *I. galbana* for fish viability with rotifers as the mediator. We speculate that neutral hydrocarbons, such as alkenones, isoprenoids and polyunsaturated hydrocarbons, are essential lipids for proper fish larvae propagation, in addition to the well documented requirement for n-3 HUFA. It may well be that neutral hydrocarbons function as natural intracellular antioxidants in preventing oxidation of fatty acids. The synergistic effect of n-3 HUFA and antioxidant hydrocarbons along the food chain provide a dietary mix essential for the fish larvae growth and development.

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