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Effects of Selected Fatty Acids Supplementation on Growth and Fecundity in *Artemia franciscana*

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Effects of supplementing selected fatty acids on fatty acid incorporation (17 days) , and progeny production (14 days) in *Artemia franciscana* (Great Salt Lake, USA) were studied. To compare with the control four diets, which differed in fatty acid composition alone contain *Dunaliela tertiolecta* and an emulsion either rich in OA (oleic acid, 18:1n-9), ARA (arachidonic acid, 20:4n-6), EPA (eicosapentaenoic acid, 20:5n-3), or DHA (docosahexaenoic acid, 22:6n-3). Each of these emulsions was supplemented at a ratio of 20 % of the daily dose of *D. tertiolecta* (% algal dry weight). The initial OA and ARA values were 33.5 and 1.7 mg/g DW of freshly-hatched nauplii, respectively. After 11 days of feeding, these values increased to 38.8 and 7.6 mg/g DW in *Artemia* receiving the fatty acid supplement rich in each of the respective fatty acids. After 14 days, the levels were almost doubled, reaching 62.8 and 13.4 mg/g respectively. On EPA supplementation, its level after 11 days of feeding was 14.3 and 17.3 mg/g in male and female, respectively and was 16.0 and 23.1 mg/g in the male and female after 14 days, respectively. The EPA accumulated more in the body (39.1 mg/g) than in ovisac (16.9 mg/g). In the DHA supplementation group also, DHA levels after 11 days of feeding were 3.1 and 5.5 mg/g in male and female, respectively. After 14 days, the DHA level continued to increase in male, but slightly decreased to 4.6 mg/g in female. It was not richer in ovisac (2.6 mg/g) than in the remaining body of female (4.6 mg/g). In conclusion, fatty acids supplied by a lipid emulsion as a supplement to the algal diet are well incorporated in the adult *Artemia*. Apart from being an extra source of energy, these emulsions may function as source of HUFA which may play an essential role for growth and progeny production (fecundity) of *Artemia*.

Keywords: *Artemia franciscana, Dunaliela tertiolecta*, Fatty acids, Fecundity, Growth

Introduction

The dietary requirements of n-3 highly unsaturated fatty acids (HUFA), particularly docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), have been documented for a number of marine fishes (Izquierdo et al., 1992; Watanabe, 1993; Sargent et al., 1997). DHA as well as its ratio to EPA appear critical during the early larval stages, as it affects growth and survival of marine fish (Kanazawa, 1993; Watanabe, 1993; Reitan et al., 1994; Furuita et al., 1996). Related to its role as a precursor of the eicosanoids, arachidonic acid (20:4n-6, ARA) was later added to the list of dietary essential fatty acids for the marine fish (Castell et al., 1994; Estévez et al., 1999; Sargent et al., 1999).

Brine shrimp *Artemia* is widely used as a live prey in marine larviculture. It, however, does not fulfil the requirement of n-3 highly unsaturated fatty acids (HUFA) in the most marine species. In this respect, a wide range of techniques has been developed to improve nutritional value of *Artemia* nauplii (Watanabe, 1993).

In contrast to the extensive use of *Artemia* nauplii, the use of juvenile and adult *Artemia* is limited in aquaculture due to its restricted commercial availability and high cost (Léger et al., 1986). However, the nutritional value of properly fed adult *Artemia* is superior to that of freshly hatched nauplii (Léger et al., 1986). Dhont et al. (1993) indicated that adult *Artemia* size (10 15 mm) is more advantageous to be captured by predators than that of larva (400-500 μm).

Therefore, the aim of this study is effects of supplemented emulsions enriched in oleic acid (OA, 18:1n-9), n-3 HUFA, EPA, and DHA, or an n-6 HUFA, arachidonic acid (ARA, 20:4n-6), on fatty acid incorporation, growth and progeny production in *Artemia franciscana*.

Materials and Methods

Cyst hatching and inoculation

Artemia franciscana (Great Salt Lake, USA) was used in

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each of the five feeding trials. The cysts were disinfected with hyphochlorine (200 ppt; 20 min); thereafter they were incubated in filtered artificial seawater (35 g/L salinity, 28°C) under continuous aeration and light for hatching. After hatching, nauplii were separated and transferred to 800 ml cylindro-conical glass tubes at a density of 0.38 ind/ml with continuous light and aeration from the bottom of the cone.

Preparation of diets

Pure oils of DHA (>95% purity, Itochu Techno-Chemical Inc), EPA (>95% purity, Itochu Techno-Chemical Inc), OA (>95% purity) and ARA (40% purity, Martek) were mixed (Table 1) before being emulsified. Each lipid emulsion contained 60% lipid (W.W.), 30% water, 0.02% antioxidants (ethoxyquin, Sigma), 10% emulsifier (Tween 80, Sigma) and 0.1% vitamin E (Sigma, USA). The fatty acid composition of the selected emulsions was analyzed by gas-chromatography, and is given in Table 1. (McEvoy et al., 1996). *Dunaliella tertiolecta* was grown in a Walne medium supplemented with a vitamin mix in filtered natural seawater (21°C, 34 g/l).

Feeding

The animals were fed on live *D. tertiolecta* once a day following the standard feeding regime applied for the culture of *Artemia* (Coutteau et al., 1992). 20% of lipid emulsion was based on cellular dry weight of *D. tertiolecta*. Control group was fed only the algal diet.

Table 1. Composition of the lipid emulsions (% weight)[1]

	Emulsions			
	OA	EPA	DHA	ARA
OA	60.0	30.0	30.0	30.0
EPA	-	30.0	-	-
DHA	-	-	30.0	-
ARA	-	-	-	30.0
Tween 80	10.0	10.0	10.0	10.0
Vitamin E	0.1	0.1	0.1	0.1
Ethoxyquin	0.02	0.02	0.02	0.02
Water	29.8	29.8	29.8	29.8

[1]OA, oleic acid ethyl ester (98% purity). Sigma, USA, EPA, eicosapentaenoic acid ethyl ester (95% purity). Itochu Techno-Chemical Inc, Japan.
DHA, docosahexaenoic acid ethyl ester (95% purity). Itochu Techno-Chemical Inc, Japan.
ARA, arachidonic acid triacylglycerol (40% purity). Martek, USA.
Tween 80. Sigma, USA.
Vitamin E. Sigma, USA.
Ethoxyquin. Sigma, USA.

Growth

Growth was determined by measuring the body length from the tip of the head to the end of the telson with a dissecting microscope equipped with a camera lucida and a digitizing table after 17 days. Male and female individuals were measured separately from day 11. Ten females from each replicate cone were measured, while the number of males measured was proportionate to the sex ratio in the culture.

Fecundity

From day 11, the number of *Artemia* in riding position, indicating the commencement of sexual dimorphism, was monitored. Fifteen pairs per treatment group were then transferred to a beaker of 150 ml for the fecundity experiment. Fecundity, a reproductive characteristic, was evaluated from the total number of offspring, and offspring in cyst and nauplii.

Fatty acid analysis

The fatty acid composition of the *Artemia* nauplii after 14 day culture was analyzed by a direct transmethylation method following Lepage and Roy (1984). The 111internal standard was 20:2n-6. The resulting fatty acid methyl esters (FAME) were separated and identified on a Chrompack CP 9001 gas chromatograph equipped with an autosampler and a TPOCI (temperature programmable on-column injector). Samples were injected on a polar 50 m capillary column, BPX70 (SGE, Australia), with a diameter of 0.32 mm and a layer thickness of 25 m connected to a 2.5 m methyl deactivated pre-column. The carrier gas was H_2, at a pressure of 100 kPa and the detection mode FID (flame ionization detector). The oven temperature was set to increase from the initial temperature of 85 to 150°C at a rate of 30°C/min, from 150 to 152°C at 0.1°C/min, from 152 to 172°C at 0.65°C/min, from 172 to 187°C at 25°C/min and to stay at 187°C for 7 min. The injector was heated from 85°C to 190°C at 5°C/sec and stayed at 190°C for 30 min. Identification was based on standard reference mixtures (Nu-Chek-Prep, Inc., U.S.A.). Integration and calculations were made with a software program (Maestro, Chrompack).

Statistical analysis

Data represent means of triplicate except fatty acid composition (n=2). Statistical analysis of the data are performed by a one-way ANOVA (Tukey's HSD test, $P<0.05$).

Table 2. Increase in body length (mm) of *Artemia* fed on diets supplemented with emulsions enriched in different fatty acids[1]

	Culture period						
	Day 7	Day 11		Day 14		Day 17	
		Male	Female	Male	Female	Male	Female
Control	4.7 (0.7)[a]	5.9 (0.8)	6.5 (0.8)	6.6 (0.7)	6.6 (0.8)[a]	7.2 (0.8)	7.0 (1.2)[a]
OA	4.8 (0.8)[a]	6.0 (0.8)	6.8 (0.4)	6.5 (0.8)	7.3 (0.6)[ab]	7.0 (0.6)	7.7 (1.1)[ab]
EPA	5.2 (0.8)[a]	6.3 (0.6)	7.3 (1.3)	7.2 (0.7)	8.1 (0.5)[b]	8.1 (0.9)	8.6 (0.5)[b]
DHA	5.5 (0.7)[b]	6.8 (0.6)	7.0 (0.7)	7.2 (0.7)	8.1 (0.9)[b]	7.4 (0.7)	8.5 (0.9)[b]
ARA	5.2 (0.6)[a]	6.7 (0.4)	7.2 (0.7)	7.0 (0.4)	7.7 (0.6)[b]	7.2 (0.8)	7.9 (0.9)[ab]

[1]Data represent means of determinations in triplicate groups (sd). Different superscripts indicate a significant difference according to the treatment ($p<0.05$); Control=only fed algae, OA=oleic acid, 18:1n-9 or ARA=arachidonic acid, 20:4n-6 or EPA=eicosapentaenoic acid, 20:5n-3 or DHA=docosahexaenoic acid, 22:6n-3.

Results

Growth

The growth of *Artemia* fed on different diets is shown in Table 2. The DHA-rich oil treatment gave the best growth during the early developmental stages (until the 7th day). No significant difference between the lipid treatment groups was observed during later stages; however the groups fed on EPA and DHA-rich oil emulsions tended to improve growth, as compared to the control group fed only the algal diet and the OA-fed group.

Fecundity

The high fecundity promoted by the diet supplemented with ARA was not significantly different from those groups fed diet supplemented with EPA or DHA (Fig. 1). No difference in percentages of encysted offspring was observed between the different groups (Fig. 2). However, it should be noted that cyst production was high (generally more than 80 % of total offspring).

Fatty acid composition

The initial level of OA and ARA-fed groups of the freshly-hatched nauplii was 33.5 and 1.7 mg/g DW, respectively. After 11 days of feeding the lipid supplements rich in each of the respective fatty acids, these values increased to 33.8 and 7.6 mg/g, respectively. After 14 days, the respective levels was almost doubled (62.8 and 13.4 mg/g; Table 3).

In the EPA group, EPA levels after 11 days of feeding, increased from 8.4 to 14.3 and 17.3 mg/g in male and female, respectively. After 14 days, the levels slightly increased from 14.3 to 16.0 mg/g in male, but remarkably from 16.0 to 23.1 mg/g in female. In the ovisac and remaining body of female *Artemia, it* was 16.9 and 39.1 mg/g , respectively (Table 4).

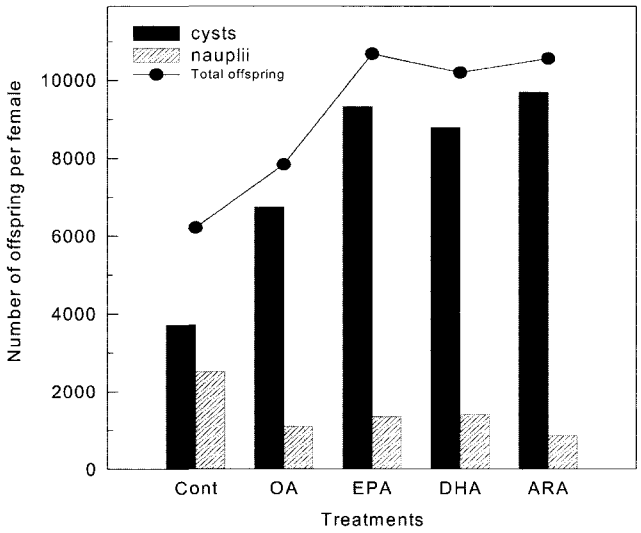

Fig. 1. Cumulative offspring production by a female *Artemia* fed on diets supplemented with OA=oleic acid, 18:1n-9 or EPA=eicosapentaenoic acid, 20:5n-3 or DHA=docosahexaenoic acid, 22:6n-3 or ARA=arachidonic acid, 20:4n-6.

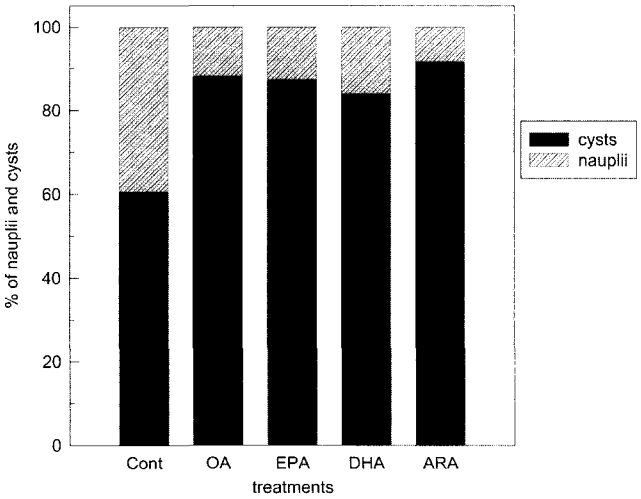

Fig. 2. Production of cyst and nauplius of *Artemia* fed on diet supplemented with Cont=only fed algae, OA=oleic acid, 18:1n-9 or ARA =arachidonic acid, 20:4n-6 or EPA=eicosapentaenoic acid, 20:5n-3 or DHA=docosahexaenoic acid, 22:6n-3.

Table 3. Fatty acid composition (mg/g dry weight) of freshly hatched *Artemia* and after 11 and 14 days fed on algal diet of *D. tertiolecta* supplemented with 20% of ARA or OA-rich emulsion[1]

	Freshly-hatched	ARA-rich Emulsion		OA-rich Emulsion	
		Day 11	Day 14	Day 11	Day 14
14:0	1.4	0.7	0.9	0.7	1.1
16:0	18.9	12.2	15.1	14.8	12.1
16:1n-7	7.4	1.6	2.8	3.3	5.3
18:0	6.8	6.1	7.7	6.1	5.1
18:1n-7	14.5	5.3	6.1	5.5	5.4
18:1n-9	33.5	21.7	45.0	38.8	62.8
18:2n-6	9.7	10.8	12.5	14.6	11.7
19:0	nd[3]	nd	Nd	nd	nd
18:3n-3	39.4	20.1	23.8	30.1	24.8
18:4n-3	4.8	1.1	1.2	2.1	2.1
20:0	0.2	0.1	0.2	0.1	0.1
20:1n-9	0.7	nd	Nd	0.1	0.1
20:3-6	0.6	0.2	0.3	0.3	0.3
20:4n-6	1.7	7.6	13.4	0.9	0.7
20:4n-3	0.6	nd	Nd	0.2	0.1
20:5n-3	8.4	0.5	0.4	0.7	0.7
21:5n-3	0.3	nd	Nd	nd	nd
22:5n-6	nd	nd	Nd	nd	nd
24:0	nd	nd	nd	nd	nd
22:5n-3	nd	nd	nd	nd	nd
22:6n-3	nd	0.2	0.2	0.3	0.1
Σ saturated	28.6	19.6	30.6	29.8	23.2
Σ monoenes	59.9	29.8	55.4	49.2	74.6
Σ n-6 PUFA	12.0	22.0	30.4	21.4	17.9
Σ n-3 PUFA	54.1	22.2	25.7	32.9	27.7
Σ n-3 HUFA[2]	9.9	0.9	0.8	0.9	1.1

[1]Sums include minor fatty acid not shown in table, n-6 PUFA: ≥18:2n-6, n-3 PUFA: ≥18:3n-3
[2]≥20:3n-3; [3]nd: not detected

After 11 days of feeding the DHA level in the DHAfed group increased from 0.0 to 3.1 and 5.5 mg/g, in male and female, respectively. However, the level slightly decreased from 5.5 to 4.6 in female on the 14th day. It was 2.6 and 4.6 mg/g in the mature ovisac and remaining body of the female, respectively. The EPA levels slightly decreased in both male and female after 14 days. It was 8.4 and 8.0 mg/g in ovisac the and remaining body of the female, respectively (Table 5).

Discussion

By providing algal diets supplemented with HUFA enriched emulsions, the present study has clearly shown the incorporation of fatty acids and their utilization for growth and progeny production in *Artemia franciscana*. Joshi and Diwan (1996) have observed that growth and fecundity of a live organism depends upon a number of environmental and physical factors, including the effect of food quantity, composition and seasonal variability. One of the most important factors is nutritional component, especially the lipids, which vary widely (Sargent, 1995). The fatty acid level in the *Artemia* nauplii results from the rapid and complex metabolic processes of absorption, incorporation into body lipids and catabolism. It is well documented in marine vertebrates like the fishes that n-3 HUFA levels correlate well with the dietary n-3 HUFA supply (Sargent et al., 1993). However, very limited information is available on the specific supplementation with emulsion containing HUFA-rich oil in a crustacean like *Artemia*. Exogenously supplied different sources of HUFA were differently incorporated into the whole body as well as ovisacc of *Artemia*. The metabolism of n-3 HUFA in *A. franciscana* differs, however from that in most marine vertebrates and invertebrates, especially the copepods, in which DHA is stored principally in the triglyceride fraction, from

Table 4. Fatty acid composition (mg/g dry weight) of freshly hatched *Artemia* and after 11 and 14 days feeding on algal diet of *D. tertiolecta* supplemented with 20% of EPA-rich emulsion[1]

	Freshly-hatched	Day 11		Day 14			
		Male	Female	Male	Female	Ovisac	Body
14:0	1.7	0.5	0.7	0.4	0.6	0.7	1.1
16:0	18.6	10.6	12.9	9.9	11.2	9.7	14.3
16:1n-7	7.3	1.9	2.3	2.2	2.9	1.3	2.1
18:0	6.8	6.3	6.7	6.4	6.1	3.3	5.0
18:1n-7	14.0	5.5	6	5.7	6.1	5.3	6.3
18:1n-9	33.0	23.9	28.5	30.8	40.4	38.5	56.6
18:2n-6	9.4	10.2	12.4	8.9	11.9	6.4	10.9
19:0	nd[3]	nd	nd	Nd	nd	nd	nd
18:3n-3	37.8	15.9	20.1	14.9	19.7	18.6	38.1
18:4n-3	4.6	1	1.4	0.9	1.7	2.0	3.5
20:0	0.2	0.5	0.4	0.3	0.3	0.2	0.2
20:1n-9	0.7	0.4	0.3	0.4	0.4	tr[4]	0.5
20:3n-6	0.1	0.2	0.2	0.1	0.2	tr	tr
20:4n-6	1.7	0.5	0.4	0.4	0.4	0.3	0.5
20:4n-3	0.6	nd	nd	nd	nd	0.2	0.5
20:5n-3	8.9	14.3	17.3	16.0	23.1	16.9	39.1
21:5n-3	0.3	nd	nd	nd	nd	tr	nd
22:5n-6	nd	nd	nd	nd	nd	tr	0.1
24:0	nd	nd	nd	nd	nd	nd	nd
22:5n-3	nd	0.1	nd	nd	nd	tr	0.1
22:6n-3	nd	0.6	0.2	0.3	0.6	0.1	0.2
Σ saturated	28.7	20.1	23.3	19.0	20.7	14.1	23.3
Σ monoenes	59.0	32.3	37.9	39.9	50.7	46.5	70.3
Σ n-6 PUFA	11.7	13.4	16.4	11.8	15.6	9.1	17.1
Σ n-3 PUFA	52.8	32.3	39.4	32.5	44.8	37.7	81.0
Σ n-3 HUFA[2]	10.4	15.4	17.9	16.6	24.1	17.2	39.8

[1]Sums include minor fatty acid not shown in table, n-6 PUFA: ≥18:2n-6, n-3 PUFA: ≥18:3n-3
[2]≥20:3n-3; [3]nd: not detected; [4]tr: <0.1 mg/g dry weight

which it is catabolized during starvation (Coutteau and Mourente, 1997; Evjemo et al., 1997; Estévez et al., 1998; Navarro et al., 1999). Clearly, the HUFA supplied as a supplement to algal diet, is well incorporated into the adult *Artemia* during the culture period. The HUFA, especially the DHA is catabolized during sexual maturation of female *Artemia.*

Numerous publications have shown that as important dietary factors, the lipid and fatty acid composition in the diet of broodstock, are known to control maturation and progeny production in many fishes. Some fish species readily incorporate dietary HUFA into eggs. HUFA with 20 or more carbon atom affect maturation of fish, directly or through their metabolites. In some fish species, HUFA in broodstock diets increases fecundity, egg quality and fertilization success. Not surprisingly, the HUFA supplementation in the algal diets of *Artemia* has induced production of a higher number of cysts.

In summary, this present study evaluated the effect of supplementing various dietary fatty acids on fatty acid incorporation, growth and progeny production characteristics in *Artemia franciscana* during a long term study. The four dietary treatments only differed in fatty acid composition. They consisted of *Dunaliela tertiolecta* supplemented with an emulsion either rich in oleic acid or in ARA, EPA or DHA. Each of these emulsions was supplemented at a ratio of 20% of the daily dose of *D. tertiolecta* (% algal dry weight). The results indicated that fatty acids supplied by a lipid emulsion as a supplement to the algal diet are well incorporated in the adult *Artemia*. Apart from being an extra source of energy, these emulsions may function as source of HUFA which may play an essential role for growth and progeny production (fecundity) of *Artemia*. Future study is necessary to examine more closely the dietary effects and fatty acid incorporation of a high EPA supply in male and female separately.

Table 5. Fatty acid composition (mg/g dry weight) of freshly hatched *Artemia* and after 11 and 14 days feeding on algal diet of *D. tertiolecta* supplemented with 20% DHA-rich emulsion[1]

	Freshlyhatched	Day 11		Day 14			
		Male	Female	Male	Female	Ovisac	Body
14:0	1.7	0.4	0.5	0.6	0.7	0.7	0.5
16:0	18.6	9.6	10.7	9.6	11.0	10.8	9.8
16:1n-7	7.3	0.3	0.5	0.4	0.5	0.4	0.5
18:0	6.8	6.0	6.3	5.8	6.0	6.1	5.6
18:1n-7	14.0	4.4	4.7	4.4	5.0	5.2	4.4
18:1n-9	33.0	15.7	19.7	18.6	25.2	30.6	22.2
18:2n-6	9.4	10.2	12.1	10.2	13.6	13.7	12.1
19:0	nd[3]	Nd	0.1	nd	0.1	0.1	0
18:3n-3	37.8	16.8	21.6	19.6	26.0	24.2	23.6
18:4n-3	4.6	1.2	1.7	1.3	2.6	3.0	2.6
20:0	0.2	Nd	nd	nd	nd	nd	nd
20:1n-9	0.7	Nd	nd	nd	nd	nd	nd
20:3n-6	0.1	Nd	nd	nd	nd	nd	nd
20:4n-6	1.7	0.1	0.1	0.1	0.1	0.1	0.1
20:4n-3	0.6	Nd	nd	0.3	0.3	0.3	0.3
20:5n-3	8.9	4.9	6.1	5.2	7.6	8.4	8.0
21:5n-3	0.3	Nd	nd	nd	nd	nd	nd
22:5n-6	nd	Nd	nd	nd	nd	nd	nd
24:0	nd	Nd	nd	nd	nd	nd	nd
22:5n-3	nd	Nd	nd	nd	nd	nd	nd
22:6n-3	nd	3.1	5.5	3.5	4.6	2.6	4.6
Σ saturated	28.7	18.1	20.7	19.7	23.5	19.5	21.6
Σ monoenes	59.0	21.3	26.4	24.4	32.3	37.1	28.6
Σ n-6 PUFA	11.7	13.5	16.5	14.3	18.8	19.1	17.6
Σ n-3 PUFA	52.8	25.9	35.0	29.5	41.2	27.2	26.2
Σ n-3 HUFA[2]	10.4	7.9	11.6	8.8	12.5	11.3	12.9

[1]Sums include minor fatty acid not shown in table, n-6 PUFA: ≥18:2n-6, n-3 PUFA: ≥18:3n-3
[2]≥20:3n-3; [3]nd: not detected

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References

Castell, J. D., J. G. Bell, D. R. Tocher and J. R. Sargent, 1994. Effects of different dietary arachidonic acid: docosahexaenoic acid ratios on phospholipid fatty acid composition and prostaglandin production in juvenile turbot (*Scophthalmus maximus*). Aquaculture, **155**: 149–164.

Coutteau, P., L. Brendonck, P. Lavens and P. Sorgeloos, 1992. The use of manipulated bakers yeast as an algal substitute for the laboratory culture of Anostraca. Hydrobiologia, **234**: 25–32.

Coutteau, P. and G. Mourente. 1997. Lipid classes and their content of n-3 highly unsaturated fatty acids (HUFA) in *Artemia franciscana* after hatching, HUFA-enrichment and subsequent starvation. Mar. Biol, **130**: 81–91.

Dhont, J., P. Lavens and P. Sorgeloos, 1993. Preparation and use of *Artemia* as food for shrimp and prawn larvae. (*in*) J.P. McVey (ed.), CRC Handbook of Mariculture, 2nd edition, Vol. 1. Crustacean Aquaculture. CRC Press, Boca Raton, Florida, pp. 61–93.

Evjemo, J. O., P. Coutteau, Y. Olsen and P. Sorgeloos, 1997. The stability of DHA in two *Artemia* species following enrichment and subsequent starvation. Aquaculture, **155**: 135–148.

Estévez, A., L. A. McEvoy and J. G. Bell and J. R. Sargent, 1998. Effects of temperature and starvation time on the pattern and rate of loss of essential fatty acids in *Arttemia* nauplii previously enriched using arachidonic acid and eicosapentaenoic acid-rich emulsion. Aquaculture. **165**: 295–311.

Estévez, A., L. A. McEvoy and J. G. Bell, 1999. Growth, survival, lipid composition and pigmentation of turbot (*Scophthalmus*

maximus) larvae fed live-prey enriched in arachidonic and eicosapentaenoic acids. Aquaculture, **180**: 321–343.

Furuita, H., T. Takeuchi, M. Toyota, S. Satoh and T. Watanabe, 1996. EPA and DHA requirements in early juvenile red seabream using HUFA enriched *Artemia* nauplii. Fish. Sci, **62**: 236–251.

Izquierdo, M. S., T. Arakawa, T. Takeuchi, R. Haroun and T. Watanabe, 1992. Effect of n-3 HUFA levels in *Artemia* on growth of laval Japanese flounder (*Paralichthys olivaceus*). Aquaculture, **105**: 73–82.

Joshi, V. P. and A. D. Diwan. 1996. Biochemical changes in the tissue of female prawn *Macrobrachium idella* (Hilgendorf, 1898) during different breeding seasons. J. Aquac. Trop, **11**: 227–251.

Kanazawa, A., 1993. Importance of dietary docosahexaenoic acid on growth and survival of fish larvae. (*in*) C. S. Lee, M. S. Su and I. C. Liao (eds.), Finfish Hatchery in Asia: Proceeding of finfish Hatchery in Asia 91. Tungkang, Taiwan. TML Conference Proceeding 3. pp. 87–95.

Léger, Ph., D. A. Bengtson, K. L. Simpson and P. Sorgeloos, 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mari. Biol. Ann. Rev, **24**: 521–623.

Lepage, G. and C. C. Roy, 1984. Improved recovery of fatty acids through direct transesterification without prior extraction or purification. J. Lipid Res. **16**: 593–600.

McEvoy, L. A., J. C. Navarro, F. Hontoria, F. Amat and J. R. Sargent, 1996. Two novel *Artemia* enrichment diets containing polar lipid. Aquaculture, **144**: 339–352.

Navarro, J. C., R. J. Henderson, L. A. McEvoy, M. V. Bell and F. Amat. 1999. Lipid conversion during enrichment of *Artemia*. Aquaculture, **174**: 155–166.

Reitan, K. I., J. R. Rainuzzo and Y. Olsen, 1994. Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae. Aquacult. Internatl., **2**: 33–48.

Sargent, J. R. 1995. Sources and functions of egg lipids: nutritional implication. (*in*) Bromage, N. R. and R. J. Roberts. (eds.), Broodstock Management and Egg and Larval Aquality. Blackwood Science, Oxford, pp. 353–372.

Sargent, J. R., J. G. Bell and L. A. McEvoy, 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture, **155**: 117–127.

Sargent, J. R., J. G. Bell, M. V. Bell, R. J. Henderson, D. R. Tocher, 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish. (*in*) Lahlou, B., P. and Vitiello, (eds.), Aquaculture: Fundamental and Applied Research. Coastal and Estuarine Studies 43. American Geophysical Union, Washington D.C., pp. 103–124.

Sargent, J. R., L. McEvoy, A. Estévez, G. Bell, M. Bell, J. Henderson and D. Tocher, 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture, **179**: 217–229.

Watanabe, T., 1993. Importance of docosahexaenoic acid in marine larval fish. J. World Aquacult. Soc., **24**: 152–161.

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