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Enrichment strategies for *Artemia* using emulsions providing different levels of $n-3$ highly unsaturated fatty acids

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Abstract

Three separate enrichment trials compared the $n-3$ highly unsaturated fatty acid (HUFA) levels in *Artemia franciscana* nauplii (Great Salt Lake, USA) as a function of the $n-3$ HUFA level in the emulsion (30% and 50%), the enrichment dose (0.05, 0.1, 0.2, 0.3 and 0.4 g l^{-1}) and the degree of dilution of a high HUFA emulsion with an $n-3$ HUFA-free emulsion (100/0, 50/50, 25/75, 12.5/87.5 and 0/100, HUFA-rich/HUFA-free, w/w). The standard emulsions were distributed by the International Council for the Exploration of the Sea, Working Group on the Mass Rearing of Juvenile Fish (ICES). After the 48-h enrichment, the content of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and total $n-3$ HUFA increased to 23.1, 46.4 and 74.3 mg g^{-1} DW, respectively, in *Artemia* enriched with ICES 50 (50% $n-3$ HUFA) and to 14, 41.4, 59.9 mg g^{-1} DW in nauplii enriched with ICES 30 (30% $n-3$ HUFA). After the 12-h enrichment, the highest enrichment level of DHA, EPA and total $n-3$ HUFA was observed at the 0.4 g l^{-1} dose (12.7, 30.2 and 45.3 mg g^{-1} DW, respectively). After the 24-h enrichment, the highest enrichment levels were, however, found at the 0.3 g l^{-1} dose, i.e., respectively, 28.9, 53.2 and 85.6 mg g^{-1} DW as compared to 21.7, 51.1 and 75.9 mg g^{-1} DW at the 0.4 g l^{-1} dose. After 12 h enrichment, no significant difference in $n-3$ HUFA levels was found among the 100/0, the 50/50 and the 25/75 treatments. After 24 h enrichment, the content of DHA in nauplii of the 100/0 treatment was twice that of the 50/50 treatment (20.2 vs. 9.3 mg g^{-1} DW), which was in turn five times higher than in the 12.5/87.5 treatment.

Keywords: *Artemia*; $n-3$ Highly unsaturated fatty acid (HUFA); Docosahexaenoic acid (DHA); Eicosapentaenoic acid (EPA); Enrichment

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1. Introduction

The brine shrimp *Artemia* is widely used as a live food organism for marine fish and crustacean larvae in commercial hatcheries. Freshly hatched *Artemia* nauplii contain very low levels of $n-3$ highly unsaturated fatty acids (HUFA), being especially deficient in docosahexaenoic acid (DHA). The importance of dietary DHA for proper development of marine fish larvae has been documented repeatedly (Kanazawa, 1993; Watanabe, 1993; Reitan et al., 1994). In order to overcome the lack of $n-3$ HUFA in *Artemia* nauplii, various enrichment techniques have been developed to enhance their $n-3$ HUFA content. The degree of success in modifying the fatty acid profile of the nauplii has been shown to be influenced by the type of the enrichment diet, the enrichment conditions and the *Artemia* strain itself. Examples of practical and experimental enrichment diets are unicellular algae (Watanabe et al., 1980), emulsion (Léger et al., 1987; McEvoy et al., 1996), liposomes (Ozkizilick and Chu, 1994) and microencapsulated diets (Southgate and Lou, 1995). The lipid sources in these diets differ in lipid class composition (McEvoy et al., 1996; Tocher et al., 1997), $n-3$ HUFA content (Dhert et al., 1993; Evjemo et al., 1997) and DHA/eicosapentaenoic acid (EPA) ratio (Naess et al., 1995; Evjemo et al., 1997). Differences in enrichment conditions are related to the salinity of the culture medium, the concentration of experimental emulsion (Rees et al., 1994), the enrichment duration (Narciso et al., 1999) and the temperature following starvation (Danielsen et al., 1995; Triantaphyllidis et al., 1995; Evjemo et al., 1997). Also, the species and geographical origin of the *Artemia* affect the success of the enrichment procedure (Triantaphyllidis et al., 1995; Evjemo et al., 1997). The lack of a standardized enrichment strategy mostly renders a comparison of results obtained in the large number of *Artemia* enrichment studies impossible (Coutteau and Mourente, 1997).

The present study evaluates differences in the $n-3$ HUFA incorporation in *Artemia* nauplii related to the enrichment strategies. These differed in the $n-3$ HUFA content of the diet, the supplied dose and the dilution of $n-3$ HUFA-rich by $n-3$ HUFA-free emulsion.

2. Materials and methods

2.1. Hatching of Artemia cysts

Artemia franciscana cysts (ARC No: 1320, INVE Aquaculture, Belgium) from Great Salt Lake (UT, USA) were used in each of the three experiments. The cysts (4 g l^{-1}) were disinfected with a hypochlorine solution of 200 μg l^{-1} for 20 min before hatching. After washing with tap water to remove the remaining hypochlorite, the cysts (2 g l^{-1}) were incubated in filtered seawater (0.45 μm cartridge filter) at 28°C under continuous aeration and light. After hatching, the nauplii (more than 90% Instar I) were separated from the empty cyst shells and transferred to 2-l glass tubes (cylindroconical shape) in a water bath at 28°C with continuous aeration from the bottom of cone using an additional airstone to keep oxygen levels above 5–6 mg l^{-1}.

2.2. Enrichment experiments

The standard enrichment emulsions (International Council for the Exploration of the Sea, Working Group on the Mass Rearing of Juvenile Fish; ICES, 1994) contained 30% and 50% of total $n-3$ HUFA (percentage dry matter) with DHA/EPA ratio of 0.73 and 0.84, respectively (ICES 30/0.6/C: ICES 30 and ICES 50/0.6/C: ICES 50, respectively) and an emulsion devoid of $n-3$ HUFA (ICES 0/−/C: ICES 0). The experimental emulsion contained (percentage wet weight) lipids (62%), water (30%), emulsifiers, antioxidants and liposoluble vitamins. The fatty acid compositions of the ICES emulsions are shown in Table 1. Temperature and salinity during the enrichment were 28°C and 34 g l^{-1}. Each enrichment experiment was performed in triplicate cones.

Table 1
The fatty acid composition (mg g^{-1} dry weight) of ICES experimental emulsions containing approximately 50%, 30% and no $n-3$ HUFA (ICES 50, ICES 30 and ICES 0, respectively)

	ICES 50/0.6/C	ICES 30/0.6/C	ICES 0/−/C
12:0	13.1	24.8	485.7
14:0	2.5	58.6	149.4
16:0	21.0	136.6	86.8
16:1$n-7$	6.4	67.6	1.1
18:0	22.4	19.2	25.2
18:1$n-7$	12.6	38.0	1.5
18:1$n-9$	63.5	92.7	64.7
18:2$n-6$	37.6	44.7	43.6
19:0	1.3	nd[b]	nd
18:3$n-3$	9.8	10.6	4.3
18:4$n-3$	6.5	16.3	nd
20:0	5.3	1.2	nd
20:1$n-9$	28.7	6.6	nd
20:2$n-6$	2.6	1.3	nd
20:3$n-6$	1.2	0.8	nd
20:4$n-6$	10.4	9.5	nd
20:4$n-3$	11.0	6.3	nd
20:5$n-3$	231.4	147.9	nd
21:5$n-3$	13.3	6.3	nd
22:5$n-6$	7.7	3.9	nd
24:0	5.9	nd	nd
22:5$n-3$	50.5	11.9	nd
22:6$n-3$	193.6	107.5	nd
Σ Saturated[a]	76.1	254.2	748.0
Σ Monoenes	125.5	211.2	66.6
Σ $n-6$ PUFA	61.1	63.8	43.6
Σ $n-3$ PUFA	513.7	317.8	4.3
Σ $n-3$ HUFA[c]	497.4	290.9	nd
DHA/EPA	0.84	0.73	0

[a] Sums include minor fatty acid not shown in table.
[b] nd: not detected.
[c] $\geq$ 20:3$n-3$.

Table 2
Fatty acid composition (mg g^{-1} dry weight) of freshly hatched *A. franciscana* nauplii (t_0) and after 24 and 48 h enrichment with emulsions ICES 50 and ICES 30. Data represent means (SD) ($n = 3$) except for ICES 30 enrichment ($n = 2$). Different superscripts within each enrichment time indicate a significant difference ($P < 0.05$) between both types of emulsions (ANOVA, Tukey's HSD)

	Freshly hatched	24-h Enriched		48-h Enriched		Effect of enrichment time[a]	
		ICES 30	ICES 50	ICES 30	ICES 50	ICES 30	ICES 50
14:0	1.4	3.3 (0.5)^{A}	1.2 (0.0)^{B}	3.2 (0.5)^{A}	1.0 (0.1)^{B}	–	–
16:0	18.5	22.8 (2.6)^{A}	15.9 (1.5)^{B}	18.4 (3.4)	11.8 (1.1)	–	–
16:1$n-7$	5.7	11.6 (1.0)	5.0 (0.5)	11.9 (0.4)	5.4 (0.4)	–	–
18:0	7.3	10.2 (1.2)	9.1 (0.6)	12.0 (0.6)^{A}	9.4 (0.4)^{B}	–	–
18:1$n-7$	13.8	15.8 (1.3)^{A}	12.9 (0.9)^{B}	18.5 (0.3)^{A}	13.7 (0.8)^{B}	*	–
18:1$n-9$	27.6	37.0 (1.1)^{A}	29.8 (1.5)^{B}	43.7 (1.1)^{A}	34.1 (1.6)^{B}	*	*
18:2$n-6$	9.9	11.1 (0.3)^{A}	8 (0.5)^{B}	13.7 (0.1)^{A}	8.6 (0.5)^{B}	**	–
19:0	nd[b]	0.3 (0.1)	0.4 (0.1)	0.2 (0.1)	0.1 (0.1)	–	–
18:3$n-3$	38.4	31.9 (1.6)	30.7 (2.4)	26.9 (1.0)	29.3 (2.5)	*	–
18:4$n-3$	4.9	2.7 (0.3)^{A}	3.1 (0.0)^{B}	2.9 (0.0)	2.6 (0.5)	–	–
20:0	0.2	0.6 (0.1)	0.5 (0.0)	0.6 (0.1)	0.6 (0.0)	–	–
20:1$n-9$	0.7	1.3 (0.7)	2.7 (0.5)	1.6 (0.3)	4.1 (0.2)	–	*
20:3$n-6$	0.1	0.2 (0.1)	nd	0.3 (0.1)	0.2 (0.0)	–	–
20:4$n-6$	1.8	2.5 (0.1)	2.4 (0.2)	3.6 (0.2)	3.5 (0.1)	–	**
20:4$n-3$	0.6	0.9 (0.1)	0.8 (0.3)	1.3 (0.5)	0.9 (0.1)		
20:5$n-3$	8.8	29.6 (0.8)	33.7 (2.1)	41.4 (1.2)	46.4 (3.4)	**	**
21:5$n-3$	0.3	0.8 (0.2)	0.4 (0.1)	0.7 (0.1)	0.9 (0.2)	–	–
22:5$n-6$	nd	0.3 (0.1)	0.3 (0.1)	0.5 (0.2)	0.9 (0.1)	–	–
24:0	nd	1.4 (0.2)	1.3 (0.4)	1.5 (0.1)	0.8 (0.1)	–	–
22:5$n-3$	nd	2.6 (0.1)	3.1 (1.2)	4.0 (0.5)^{A}	6.1 (0.3)^{B}	–	*
22:6$n-3$	0.1	11.2 (0.7)^{A}	16.0 (1.5)^{B}	14.0 (0.1)^{A}	23.1 (0.5)^{B}	*	***
Σ Saturated[c]	30.1	41.1 (0.5)^{A}	29.8 (1.2)^{B}	41.0 (1.6)^{A}	25.9 (2.4)^{B}	–	–
Σ Monoenes	52.7	67.9 (4.3)	50.4 (0.1)	77.1 (6.2)	57.7 (0.5)	–	–
Σ $n-6$ PUFA	12.4	14.8 (1.7)^{A}	11.1 (0.5)^{B}	18.2 (0.1)^{A}	13.2 (2.3)^{B}	***	–
Σ $n-3$ PUFA	53.7	78.7 (6.8)	87.8 (2.6)	91.2 (6.5)	109.3 (5.4)	–	–
Σ $n-3$ HUFA[d]	10.4	44.1 (3.4)^{A}	54.0 (1.2)^{B}	61.4 (3.7)	77.4 (3.4)	*	**
DHA/EPA	0.01	0.4 (0.0)	0.5 (0.0)	0.3 (0.0)	0.5 (0.0)	–	–

[a] $P > 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
[b] nd: not detected.
[c] Sums include minor fatty acid not shown in table.
[d] $\geq$ 20:3$n-3$.

2.3. Experiment 1

Emulsions ICES 50 and ICES 30 were used for comparing their HUFA enrichment efficiency. The emulsions were fed (0.2 g l^{-1}) 0, 12, 24 and 36 h after hatching, i.e., t_0, t_{12}, t_{24} and t_{36}. The *Artemia* nauplii were transferred to clean water after 24 h enrichment before the additional enrichment. Samples were harvested at t_0, t_{12}, t_{24}, t_{36} and t_{48}.

2.4. Experiment 2

Different doses of ICES 50 were fed to evaluate effects of feeding concentration. Five concentrations (0.05, 0.1, 0.2, 0.3 and 0.4 g l^{-1}) were tested. The emulsion was fed at the beginning of enrichment (t_0) and after 12 h (t_{12}). Samples were taken at t_0, t_3, t_6, t_{12} and t_{24}.

2.5. Experiment 3

Two ICES emulsions (ICES 50 and ICES 0, free of $n-3$ HUFA) were mixed and fed at t_0 and t_{12} (0.2 g l^{-1}). The mixture ratios of ICES 50 to ICES 0 were 100/0, 50/50, 25/75, 12.5/87.5 and 0/100%, respectively. Samples were taken at t_0, t_{12} and t_{24}.

2.6. Fatty acid analysis

The *Artemia* nauplii were analyzed by a direct transmethylation method according to Lepage and Roy (1984). The samples (250 mg wet weight) were rinsed with tap water to

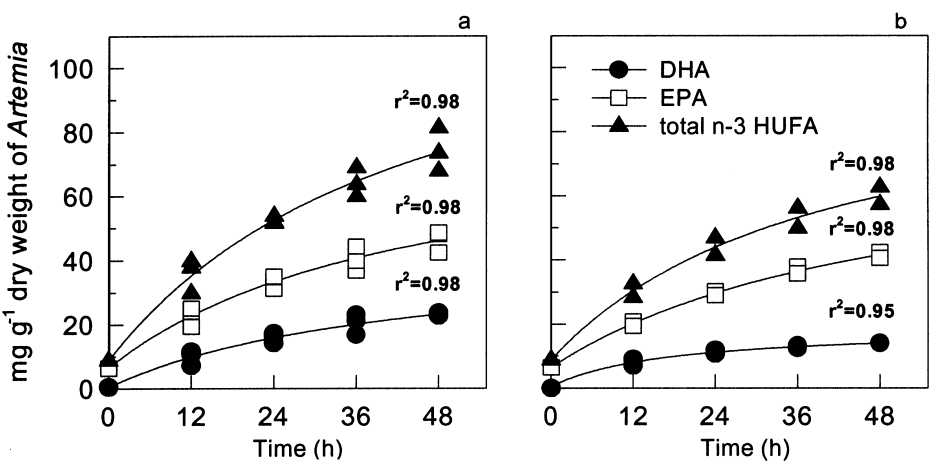

Fig. 1. Changes in the contents (mg g^{-1} dry weight) of DHA, EPA and total $n-3$ HUFA in *A. franciscana* freshly hatched nauplii during 48 h enrichment with ICES 50 (a) and ICES 30 (b) in relation to enrichment time.

Table 3
Fatty acid composition (mg g^{-1} dry weight) of freshly hatched *A. franciscana* nauplii (t_0) and after 12 and 24 h enrichment with different feeding concentrations of ICES 50. Data represent means (SD) ($n = 3$). Different superscripts within each enrichment period indicate a significant difference ($P < 0.05$) according to the enrichment doses (ANOVA, Tukey's HSD)

	Freshly hatched	12 h Enriched					24 h Enriched				
		0.05	0.1	0.2	0.3	0.4	0.05	0.1	0.2	0.3	0.4
14:0	1.4	1.4 (0.1)	1.4 (0.2)	1.2 (0.0)	1.3 (0.1)	1.3 (0.1)	1.0 (0.1)	1.2 (0.2)	1.3 (0.2)	1.2 (0.2)	1.0 (0.1)
16:0	19.4	17.3 (0.8)	18.1 (1.3)	17.1 (1.8)	17.4 (1.4)	16.7 (0.6)	13.5 (1.4)	15. 6 (0.8)	16.3 (1.2)	14.9 (1.1)	16.2 (1.1)
16:1$n-7$	7.4	6.4 (0.4)	6.8 (0.8)	6.1 (0.6)	6.0 (0.8)	6.1 (0.2)	4.7 (0.1)	5.6 (0.3)	5.7 (0.7)	5.1 (0.4)	5.7 (0.3)
18:0	7.4	8.5 (1.1)	9.3 (1.4)	9.0 (0.6)	9.0 (0.8)	8.0 (0.3)	8.2 (1.1)	9.2 (0.9)	9.0 (0.2)	9.1 (0.4)	9.6 (0.4)
18:1$n-7$	14.2	14.4 (0.6)	14.4 (0.4)	14.4 (1.8)	14.0 (1.5)	13.7 (0.6)	11.5 (1.3)	13.6 (0.5)	13.8 (1.0)	13.3 (0.8)	14.3 (1.3)
18:1$n-9$	34.8	32.6 (0.4)	33.3 (0.4)	33.1 (2.3)	35.7 (0.9)	35.2 (0.1)	28.2 (3.1)^{A}	31.0 (1.4)^{A}	33.7 (04)^{A}	33.3 (1.3)^{A}	36.3 (3.0)^{B}
18:2$n-6$	10.1	8.6 (0.2)^{A}	9.3^{AB} (0.2)	9.6 (0.5)^{B}	10.3 (0.2)^{BC}	10.3 (0.1)^{CD}	7.3 (0.6)^{A}	9.0 (0.2)^{B}	9.4 (0.3)^{BC}	9.5 (0.2)^{BC}	10.7 (0.8)^{C}
19:0	tr[a]	0.2 (0.0)	tr	0.2 (0.1)	tr	tr	tr	tr	tr	0.2 (0.1)	tr
18:3$n-3$	40.0	32.1 (0.2)	34.3 (1.2)	32.2 (3.8)	33.2 (0.6)	33.2 (0.3)	25.6 (0.6)	27.9 (1.1)	30.6 (1.9)	27.5 (0.3)	28.0 (2.6)
18:4$n-3$	5.0	3.2 (0.2)	3.5 (0.1)	3.2 (0.6)	3.7 (0.2)	3.6 (0.1)	2.0 (0.3)^{A}	2.4 (0.2)^{AB}	2.9 (0.5)^{B}	2.8 (0.2)^{AB}	2.8 (0.1)^{AB}
20:0	0.2	0.2 (0.1)	0.3 (0.0)	0.3 (0.0)	0.4 (0.1)	tr	0.3 (0.1)	0.3 (0.1)	0. 3 (0.2)	0.3 (0.1)	0.2 (0.1)
20:1$n-9$	0.7	0.2 (0.1)	0.2 (0.1)	0.3 (0.1)	0.3 (0.0)	0.3 (0.1)	0.2 (0.0)	0.3 (0.1)	0.4 (0.2)	0.3 (0.1)	0.6 (0.1)
20:3$n-6$	tr	tr	tr	0.2 (0.1)	tr	tr	tr	tr	0.2 (0.0)	0.3 (0.2)	0.2 (0.1)
20:4$n-6$	1.8	2.0 (0.1)^{A}	2.2 (0.7)^{AB}	2.7 (0.4)^{B}	2.6 (0.1)^{AB}	2.6 (0.0)^{AB}	2.2 (0.1)^{A}	2.6 (0.2)^{AC}	3.0 (0.5)^{AB}	3.7 (0.1)^{B}	3.6 (0.2)^{BC}
20:4$n-3$	0.6	0.7 (0.1)	0.6 (0.3)	1.3 (0.1)	1.2 (0.2)	1.4 (0.2)	0.6 (0.1)^{A}	1.0 (0.3)^{A}	1.4 (0.5)^{A}	1.8 (0.3)^{A}	2.1 (0.0)^{B}
20:5$n-3$	8.9	13.6 (0.2)^{A}	19.4 (0.6)^{B}	27.2 (1.6)^{B}	29.1 (1.0)^{CD}	30.2 (1.1)^{D}	18.1 (1.9)^{A}	29.4 (1.3)^{AB}	41.0 (4.8)^{B}	53.2 (1.6)^{C}	51.1 (2.8)^{C}
21:5$n-3$	0.3	0.5 (0.2)	0.5 (0.2)	0.9 (0.1)	0.5 (0.1)	0.8 (0.1)	0.4 (0.5)	tr	0.6 (0.3)	1.2 (0.1)	0.8 (0.3)
22:5$n-6$	nd[b]	tr	0.3 (0.1)	0.3 (0.1)	0.2 (0.1)	tr	0.2 (0.1)	0.2 (0.1)	tr	tr	tr
24:0	nd	0.6 (0.2)	0.6 (0.0)	0.5 (0.1)	0.5 (0.2)	0.4 (0.0)	0.9 (0.1)	0.9 (0.3)	1.1 (0.1)	1.2 (0.3)	0.8 (0.2)
22:5$n-3$	nd	0.4 (0.2)^{A}	1.4 (0.2)^{AB}	3.6 (0.1)^{BC}	3.1 (0.2)^{CD}	3.1 (0.2)^{D}	1.3 (0.2)^{A}	3.0 (0.3)^{AB}	4.6 (0.0)^{ABC}	7.0 (0.7)^{C}	6.0 (0.4)^{BC}
22:6$n-3$	0.2	1.8 (0.1)^{A}	4.9 (0.3)^{B}	10.4 (1.0)^{C}	12.2 (0.5)^{CD}	12.8 (1.0)^{D}	4.4 (0.4)^{A}	10.5 (1.7)^{B}	23.1 (0.0)^{C}	28.9 (2.4)^{D}	21.7 (0.3)^{C}
Σ Saturated[c]	30.7	30.8 (3.5)	34.4 (5.8)	30.7 (1.4)	35.0 (5.4)	28.5 (1.3)	26.6 (3.2)	29.9 (4.6)	30.8 (2.9)	28.4 (2.5)	29.6 (1.4)
Σ Monoenes	59.9	57.5 (1.2)	59.0 (4.3)	57.6 (2.8)	60.0 (3.7)	60.1 (3.9)	48.0 (2.3)^{A}	54.4 (3.4)^{A}	56.9 (4.7)^{A}	56.5 (5.4)^{A}	60.7 (4.6)^{B}
Σ $n-6$ PUFA	12.6	11.1 (0.5)^{A}	12.4 (0.6)^{B}	13.1 (0.1)^{BC}	14.2 (0.1)^{C}	13.6 (0.3)^{BC}	10.0 (1.2)^{A}	12.3 (0.4)^{B}	13.0 (0.7)^{B}	14.1 (0.3)^{BC}	15.6 (2.7)^{C}
Σ $n-3$ PUFA	55.5	52.3 (1.0)^{A}	66.0 (1.4)^{B}	78.9 (3.5)^{C}	83.5 (2.2)^{C}	85.6 (1.1)^{C}	53.0 (0.5)^{A}	75.4 (3.1)^{B}	106.2 (4.2)^{C}	122.9 (5.0)^{C}	112.5 (1.7)^{C}
Σ $n-3$ HUFA[d]	10.5	17.0 (0.5)^{A}	28.2 (0.9)^{B}	43.5 (3.9)^{C}	46.6 (2.9)^{CD}	48.8 (1.8)^{D}	24.8 (2.5)^{A}	45.1 (2.9)^{B}	72.7 (4.4)^{C}	92.6 (5.9)^{D}	81.7 (5.3)^{C}
DHA/EPA	0.02	0.13 (0.0)^{A}	0.25 (0.0)^{B}	0.38 (0.1)^{C}	0.42 (0.0)^{C}	0.42 (0.0)^{C}	0.24 (0.0)^{A}	0.36 0.0)^{B}	0.56 (0.1)^{C}	0.54 (0.0)^{C}	0.42 (00)^{B}

[a] tr: < 0.1 mg g^{-1} dry weight.
[b] nd: not detected.
[c] Sums include minor fatty acid not shown in table.
[d] $\geq 20:3n-3$.

remove the residual emulsion. The internal standard was eicosadienoic acid (20:2$n-6$) for analyzing the *Artemia* nauplii and 23:0 for the emulsions. Fatty acid methyl esters (FAME) were separated by gas chromatography with on-column injection into a Chrompack CP 9001 with a polar column (BPX 70). The carrier gas was hydrogen and detection mode FID. The Maestro Chrompack program was used to integrate the chromatogram.

2.7. Statistical analysis

Data represent means of triplicate analyses, except for the fatty acid enrichment with ICES 30/0.6/C in Table 2 ($n=2$). Data are analyzed by a one-way ANOVA followed by Tukey's Honest Significant Difference test ($P<0.05$) (Sokal and Rohlf, 1981).

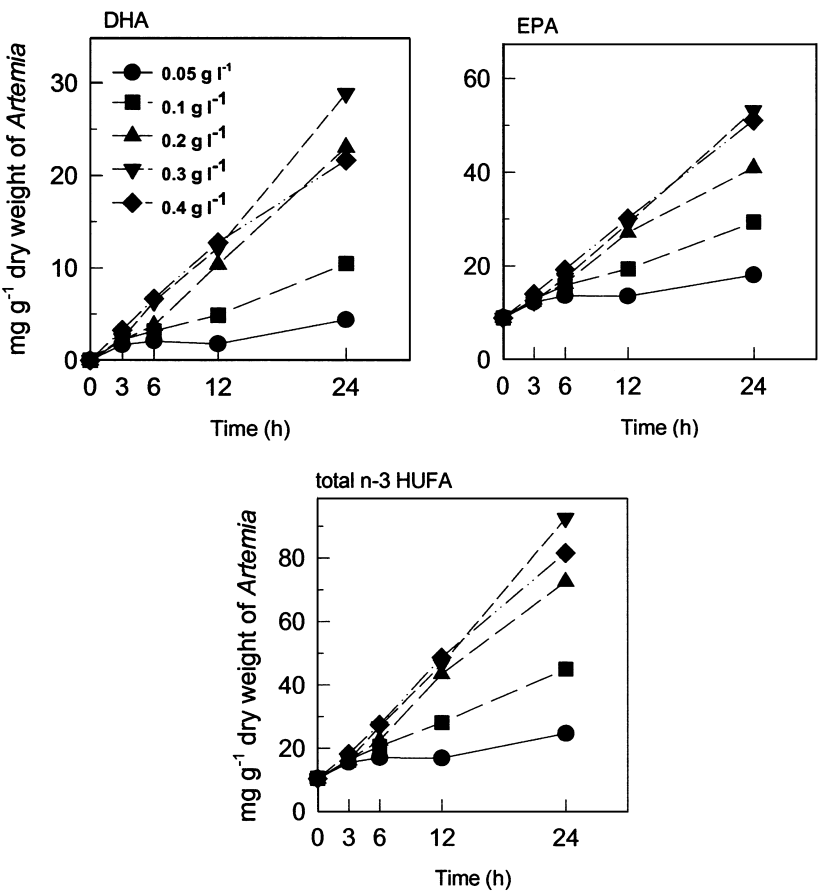

Fig. 2. Changes in the contents (mg g^{-1} dry weight) of DHA, EPA and total $n-3$ HUFA in *A. franciscana* freshly hatched nauplii during 24 h enrichment with ICES 50 in various feeding concentrations.

Table 4
Fatty acid composition (mg g^{-1} dry weight) of freshly hatched *A. franciscana* nauplii (t_0) and after 12 and 24 h enrichment with various ratios of ICES 50 and ICES 0 emulsions. Data represent means (SD) ($n = 3$). Different superscripts within each enrichment period indicate a significant difference ($P < 0.05$) according to the ratio of ICES 50/ICES 0 (ANOVA, Tukey's HSD)

	Freshly hatched	12 h Enriched					24 h Enriched				
		100/0	50/50	25/75	12.5/78.5	0/100	100/0	50/50	25/75	12.5/78.5	0/100
14:0	1.7	1.7^{A} (0.3)	4.3 (0.7)^{AB}	6.9 (1.0)^{BC}	8.2 (1.2)^{C}	8.2 (1.6)^{C}	1.7 (0.2)^{A}	4.3 (0.8)^{AB}	7.7(0.6)^{BC}	7.7 (0.7)^{BC}	9.5 (1.9)^{C}
16:0	18.6	20.5 (4.6)	24.6 (4.1)	25.7 (3.5)	27.2 (4.4)	25.4 (4.5)	21.0 (2.4)	23.1 (3.7)	25.3 (4.4)	21.5 (3.4)	24.7 (4.3)
16:1$n-7$	7.3	7.1 (1.7)	7.8 (1.3)	7.6 (0.9)	7.7 (1.3)	7.1 (1.0)	7.4 (0.8)	6.6 (0.8)	6.5 (1.0)	5.2 (1.2)	6.0 (0.7)
18:0	6.8	10.5 (1.5)	11.0 (1.6)	10.9 (1.8)	11.5 (1.7)	10.9 (2.1)	11.7 (1.6)	13.1 (2.1)	13.1 (2.6)	10.7 (2.2)	12.8 (2.3)
18:1$n-7$	14.0	17. 5 (3.0)	18.4 (3.2)	17.9 (2.1)	18.5 (2.9)	16.5 (3.2)	19.1 (2.8)	18.4 (2.6)	19.0 (2.6)	14.8 (2.8)	17.8 (3.0)
18:1$n-9$	33.0	38.9 (7.5)	42.5 (6.7)	43.1 (6.9)	45.0 (7.8)	42.0 (8.0)	42.5 (7.1)	42.5 (6.7)	43.1 (6.7)	34.0 (7.2)	38.3 (5.7)
18:2$n-6$	9.4	11.1 (2.2)	13.4 (2.1)	14.4 (2.3)	15.6 (3.1)	14.9 (3.1)	11.7 (1.9)	13.7 (2.2)	15.5 (2.6)	12.8 (2.7)	15.1 (2.4)
19:0	nd[a]	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	tr	tr	tr	tr	tr	tr
18:3$n-3$	37.8	40.2 (6.7)	42.6 (7.5)	42.7 (6.5)	45.0 (8.1)	43.5 (8.1)	38.8 (6.8)	36.8 (6.6)	37.4 (7.1)	30.8 (7.5)	37.3 (6.0)
18:4$n-3$	4.6	4.5 (0.6)	4.7 (0.6)	4.9 (0.6)	5.1 (0.8)	4.9 (0.8)	4.0 (0.6)	3.6 (0.5)	3.6 (0.6)	2.8 (0.5)	3.9 (0.5)
20:0	0.2	tr	tr	tr	tr	nd	tr	tr	tr	tr	nd
20:1$n-9$	0.7	1.2 (0.1)	1.2 (0.0)	1.2 (0.1)	1.2 (0.1)	1.0 (0.1)	1.8 (1.0)	1.7 (0.0)	1.3 (0.1)	0.9 (0.2)	0.9 (0.1)
20:3$n-6$	tr[b]	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
20:4$n-6$	1.7	3.1 (0.5)	3.0 (0.5)	2.7 (0.5)	2.7 (0.4)	2.3 (0.3)	4.9 (0.9)^{A}	4.0 (0.6)^{A}	3.2 (0.6)^{AB}	2.2 (0.6)^{B}	2.1 (0.3)^{B}
20:4$n-3$	0.6	0.9 (0.1)	1.1 (0.1)	1.0 (0.1)	0.9 (0.1)	0.9 (0.1)	1.3 (0.1)	1.1 (0.2)	0.9 (0.2)	0.6 (0.1)	0.6 (0.1)
20:5$n-3$	8.9	25.9 (4.2)^{A}	26.2 (4.5)^{A}	20.9 (3.6)^{AB}	17.2 (2.1)^{AB}	10.6 (1.8)^{B}	47.1 (2.5)^{A}	37.6 (1.9)^{A}	28.4 (2.7)^{AB}	16.1 (3.1)^{BC}	9.5 (1.3)^{C}
21:5$n-3$	0.3	0.6 (0.1)	0.6 (0.1)	0.6 (0.1)	0.4 (0.1)	0.2 (0.1)	0.7 (0.2)	0.9 (0.3)	0.5 (0.0)	0.2 (0.1)	0.3 (0.1)
22:5$n-6$	nd	0.4 (0.1)	tr	tr	tr	nd	0.6 (0.2)	0.4 (0.1)	0.3 (0.1)	tr	nd
24:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
22:5$n-3$	nd	1.1 (0.3)^{A}	1.2 (0.2)^{A}	0.8 (0.0)^{AB}	0.4 (0.0)^{BC}	tr^{C}	2.9 (0.9)^{A}	2.0 (0.2)^{AB}	1.3 (0.1)^{BC}	0.5 (0.1)^{CD}	tr^{D}
22:6$n-3$	nd	7.1 (1.8)^{A}	6.3 (2.0)^{A}	4.1 (0.8)^{AB}	2.1 (0.2)^{BC}	0.2 (0.1)^{C}	20.2 (4.4)^{A}	9.3 (1.5)^{B}	5. 4 (1.3)^{BC}	1.9 (0.3)^{CD}	0.1 (0.0)^{D}
Σ Saturated[c]	28.7	35.5 (3.5)^{A}	47.3 (3.4)^{AB}	55.1 (3.8)^{AB}	60.5 (4.2)^{B}	58.9 (4.9)^{B}	37.3 (4.4)	47.2 (2.6)	58.5 (3.4)	52.8 (5.5)	62.5 (5.0)
Σ Monoenes	59.0	68.2 (6.5)	73.7 (5.6)	73.5 (5.2)	76.4 (6.4)	58.9 (5.7)	74.2 (5.0)	72.5 (5.1)	73.4 (5.3)	57.5 (5.6)	65.8 (4.7)
Σ $n-6$ PUFA	11.7	13.7 (2.7)	17.2 (2.7)	17.9 (3.0)	18.9 (3.3)	16.9 (3.4)	18.0 (3.0)	17.2 (2.4)	19.7 (2.7)	15.4 (3.2)	17.6 (3.1)
Σ $n-3$ PUFA	52.8	81.3 (7.1)	83.1 (6.2)	75.4 (5.7)	71.8 (5.6)	61.0 (5.6)	116.1 (3.3)^{A}	91.9 (3.1)^{AB}	78.2 (4.3)^{AB}	53.3 (5.4)^{B}	52.2 (4.0)^{B}
Σ $n-3$ HUFA[d]	10.4	36.6 (4.1)^{A}	35.8 (5.9)^{A}	27.8 (4.3)^{AB}	21.6 (2.4)^{BC}	12.5 (2.6)^{C}	73.3 (5.2)^{A}	51.5 (2.0)^{AB}	37.2 (2.9)^{BC}	19.7 (2.8)^{C}	11.0 (1.5)^{C}
DHA/EPA	0	0.27 (0.0)^{A}	0.24 (0.0)^{A}	0.20 (0.0)^{AB}	0.12 (0.0)^{B}	0.02 (0.0)^{C}	0.43 (0.1)^{A}	0.25 (0.0)^{B}	0.19 (0.0)^{BC}	0.12 (0.0)^{C}	0 (0.0)^{D}

[a] nd: not detected.
[b] tr: < 0.1 mg g^{-1} dry weight.
[c] Sums include minor fatty acid not shown in table.
[d] $\geq$ 20:3$n-3$.

3. Results

3.1. Experiment 1

The results of the fatty acid enrichment kinetics in the nauplii when feeding emulsions ICES 50 and ICES 30 are shown in Fig. 1. The initial values of DHA, EPA and total $n-3$ HUFA in the freshly hatched *Artemia* nauplii were 0.1, 8.8 and 10.4 mg g^{-1} DW. The respective levels increased to 11.2, 29.6, 44.1 mg g^{-1} DW after 24 h enrichment with ICES 30 and to 16.0, 33.7 and 54.0 mg g^{-1} DW after 24 h enrichment with ICES 50. The additional enrichment (t_{24}–t_{48}) significantly increased the respective contents to 14.0, 41.4 and 61.4 mg g^{-1} DW with ICES 30 and up to 23.1, 46.4 and 77.4 mg g^{-1} DW with ICES 50 (Table 2). The time course changes represented in Fig. 1 show a leveling off in the rate of $n-3$ HUFA incorporation.

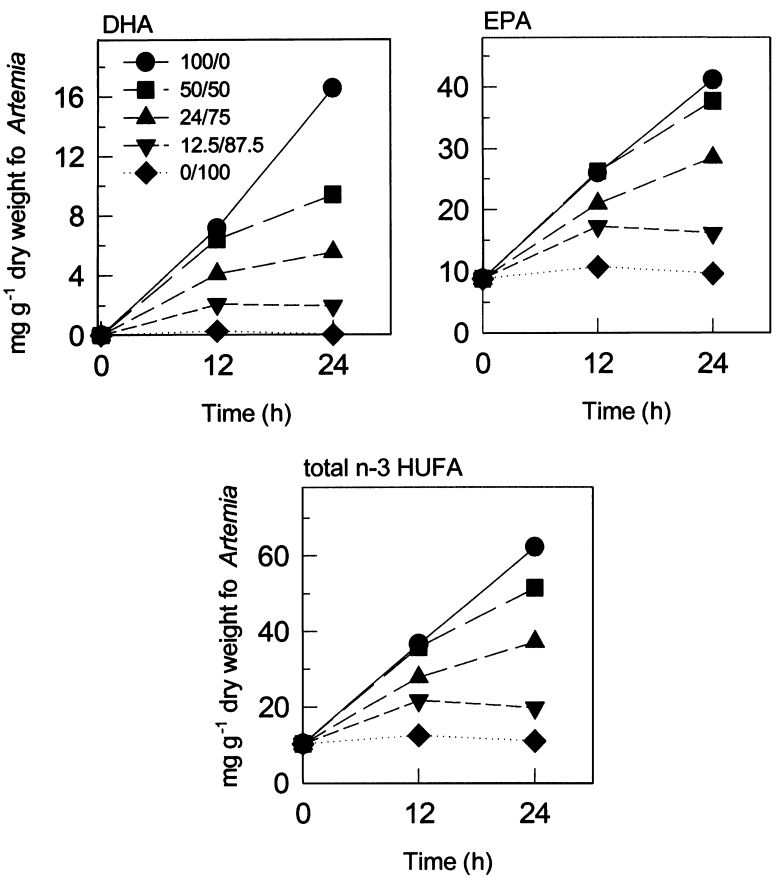

Fig. 3. Changes in the contents (mg g^{-1} dry weight) of DHA, EPA and total $n-3$ HUFA in *A. franciscana* freshly hatched nauplii during 24 h enrichment with various mixture ratios of ICES 50/ICES 0.

3.2. Experiment 2

The fatty acid enrichment results when supplying varying doses of ICES 50 are shown in Table 3 and Fig. 2. During the first enrichment (t_0–t_{12}), the highest $n-3$ HUFA levels were found in nauplii fed the highest concentrations, without significant difference ($P < 0.05$) between the 0.3 and 0.4 g l^{-1} doses. After the second enrichment (t_{12}–t_{24}), the DHA level in nauplii fed the 0.3 g l^{-1} dose (28.9 mg g^{-1} DW) was significantly higher than that of nauplii fed the 0.4 g l^{-1} dose (21.7 mg g^{-1} DW) which in turn was similar to the DHA level in nauplii of the 0.2 g l^{-1} treatment (23.1 mg g^{-1} DW). This was not the case for EPA values which were the highest in nauplii of the 0.3 and 0.4 g l^{-1} concentrations.

3.3. Experiment 3

Table 4 and Fig. 3 show the modifications in the fatty acid profiles following the enrichment with different mixtures of ICES 50 and ICES 0. During the first enrichment period (t_0–t_{12}), *Artemia* nauplii had a DHA, EPA and total $n-3$ HUFA content of 7.1, 25.9 and 36.6 mg g^{-1} DW, respectively, in the 100/0 treatment (100% ICES 50 and 0% ICES 0), similar to the levels of, respectively, 6.3, 26.2 and 35.8 mg g^{-1} DW in the 50/50 treatment (50% ICES 50 and 50% ICES 0). After the second enrichment (t_{12}–t_{24}), the amounts of DHA, EPA and total $n-3$ HUFA were 20.2, 47.1 and 73.3 mg g^{-1} DW in the 100/0 treatment, as compared to only 9.3, 37.6 and 51.5 mg g^{-1} DW in 50/50 treatment. From the 0/100 treatment (solely fed ICES 0), the levels of DHA, EPA and total $n-3$ HUFA in *Artemia* nauplii were not statistically different ($P > 0.05$) from those in the freshly hatched *Artemia* nauplii.

4. Discussion

The present study used standards of emulsified lipids providing different $n-3$ HUFA levels for examining the kinetics and level of essential $n-3$ HUFA incorporation in *A. franciscana*. In order to facilitate comparisons among studies using enriched *Artemia* nauplii for determining $n-3$ HUFA requirements of various marine fish species, the Working Group on Mass Rearing of Larval and Juvenile Marine Fish of ICES has developed a series of standard emulsions.

Although of prime interest for studies on $n-3$ HUFA requirements of marine fish larvae, few studies have expressed the HUFA content of enriched *Artemia* in quantitative amounts. The highest DHA value detected in the present study after 24 h enrichment was 28.9 mg g^{-1} DW, using the 50% $n-3$ HUFA emulsion at 0.3 g l^{-1}. This is intermediate between the value of 36 mg DHA g^{-1} DW found by Evjemo et al. (1997) and the value of 21.0 mg DHA g^{-1} DW of 24-h enriched *A. franciscana* reported by Coutteau and Mourente (1997) both using an ICES emulsion containing 30% $n-3$ HUFA (0.3 and 0.2 g l^{-1}, respectively), but with a DHA/EPA ratio of 4 instead of the present ratio of 0.8. The discrepancies between the latter values underline

the importance of standardizing enrichment protocols in order to facilitate comparisons among different enrichment studies.

The geographical origin of the *Artemia* species, the enrichment diet and the enrichment conditions (initial development stage of nauplii, enrichment time, dose and type of emulsion) are the most obvious factors known to influence the enrichment results (Léger et al., 1987). However, other unknown factors seem to interfere. In the present study, for instance, one treatment was precisely repeated in each of the enrichment trials, i.e., a 24-h enrichment with ICES 50 at a dose of 0.2 g l^{-1}. Despite the fact that the *A. franciscana*, of same origin and batch, were hatched, enriched, sampled and analyzed under identical conditions, a discrepancy in enrichment levels was noted among the three trials with levels ranging from 16 to 23.1 mg g^{-1} DW for DHA, from 33.7 to 47.1 mg g^{-1} DW for EPA and 52.5 to 73.3 mg g^{-1} DW for $n-3$ HUFA.

The fatty acid level in the *Artemia* nauplii is the result of rapid and complex metabolic processes of absorption, incorporation into body lipids and catabolism. It is well documented in marine vertebrates such as fish that $n-3$ HUFA levels correlate well with dietary $n-3$ HUFA supply (Sargent et al., 1993). Also in the present study, within each enrichment trial, differences in the amount of exogenously supplied DHA were reflected in the nauplii whole body. The $n-3$ HUFA metabolism in *A. franciscana* contrasts, however, with that in most marine vertebrates, and even marine invertebrates such as copepods, in that they appear to store DHA principally in the triglyceride fraction from where it is very rapidly catabolized during starvation (Barclay and Zeller, 1996; Coutteau and Mourente, 1997; Estévez et al., 1998; Navarro et al., 1999). The similar or even lower DHA level when supplying a concentration of 0.4 g l^{-1} as compared to, respectively, 0.2 or 0.3 g l^{-1} in the present study suggests the catabolism of DHA by the *Artemia* also during the feeding process. Using radiolabelled DHA, Navarro et al. (1999) demonstrated that almost 20% of the ^{14}C DHA radioactivity was recovered in EPA after a 24-h *Artemia* enrichment. For present EPA levels, it was not possible to differentiate whether they originated from retroconversion of DHA or directly from the diet. But, the leveling off of DHA uptake at the high dose of 0.4 g l^{-1} was also seen for EPA, showing a regulatory mechanism for the maximum uptake of both $n-3$ HUFA. A possibility not verified here is that at the high doses autoxidation may have caused a reduction of the available HUFA in the enrichment medium, despite the antioxidant-protection of the ICES emulsions (McEvoy et al., 1995).

Summarizing the present data on improvement of the nutritional value of *Artemia* nauplii for marine larviculture, it was seen that higher DHA values were obtained with ICES 50 than with an ICES 30 $n-3$ HUFA emulsion at an optimal feeding concentration of 0.2–0.3 g l^{-1} and without any dilution with a HUFA-free emulsion. A 24-h enrichment approximately doubled total $n-3$ HUFA levels as compared to a 12-h enrichment.

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