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Effects of the Supplementation of a Lipid Emulsion Diet of *Tetraselmis suecica* for Juvenile *Tapes Philippinarum*

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Hatchery reared juvenile *Tapes philippinarum* were grown for 4 weeks. *T. philippinarum* was fed *Tetraselmis suecica* (*T.s.*) at a feeding ration of 0.5%, 1.0% and 1.5% algae (dry weight) per wet weight per day. The control diet, a mixture of *Isochrysis galbana* (Clone T-Iso) and *T. suecica* (1 : 1 on dry weight basis), is known to support good growth. *Tetraselmis suecica* is deficient in docasahexaenoic acid (22 : 6 (n–3)) but is rich in ecosephantaenoic acid (20 : 5 (n–3)). The algal diet was supplemented with a DHA-rich emulsion (Em50D) at a concentration of 50% of the dry weight of the algal diet. The growth and fatty acid composition of *T. philippinarum* were used as parameters to evaluate the effect of the lipid supplementation. The supplementation of emulsion Em50D improved very little the growth of *T. philippinarum*. From the second week onwards, the growth of *T. philippinarum* few 0.5% or 1.0% *T. suecica* with or without lipid supplementation was lower than the growth of the animals fed the control diet. The clam which received an algal diet supplemented with emulsion Em50D had a significant higher lipid content than the animals fed solely on algae. There was no direct relation between the lipid content of the *T. philippinarum* and it growth. Starved clams lost 30% lipid compared to the control fed algae and 26% of the initial lipid reserves. This result is focused that the supplementation of emulsion Em50D, with a DHA content of 45.03%, resulted in a sharp increase of the DHA levels in the clams with 21.88%, 21.19% and 20.29% at a feeding ration of 0.5%, 1.0% and 1.5% respectively. The results suggested that EPA could fulfill the (n–3) HUFA requirements of juvenile *T. philippinarum* but a mixture of EPA and DHA seemed to be a little better than only EPA.

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INTRODUCTION

Intensive rearing of bivalves has so far relied on the production of large amounts of micro-algae, which is labour intensive and requires specialized facilities[1-4]. Furthermore, culture crashes and temporal variations in the algal food value still pose problem for any aquaculture operation on developing on mass culture of unicellular algae[5, 6]. Due to the scarcity of information on bivalves remain highly unpredictable. This has resulted in the development of artificial diets such as dried heterotrophically grown algae[7-10] and has presented algal pests, micro-encapsulated diets and yeast based diets to on site algal production either as a supplement or as the main food source. Although information of bivalves nutrition is still limited but several studies[11-16] indicate the importance of lipid quality and quantity in early life stages as a source of energy and essential fatty acids. Studies utilizing more than one species of algae are difficult to interpret because it is impossible to control for interspecific differences in digestibility, toxicity and cell size[17]. This has inspired the development of artificial diets that provide specific lipid supplements during broodstock conditioning, larval and early post-larval rearing. Several studies have revealed that algal cultures and artificial diets may be an important source of bacterial contamination[18, 19]. However, very little information has been reported so far about the microbial load in the shell fish culture[20].

In the present study lipid emulsions were used to supplement an algal diet of *Tetraselmis suecica (T.s.)* with lipids. The effect of lipid supplementation on the growth of *Tapes philippinarum* and the change of fatty acid profile in *Tapes philippinarum* were investigated. In addition, the possible effect of this artificial diet on the bacterial load in the marine water was assessed.

EXPERIMENTAL

Juvenile Manila clams (*Tapes philippinarum*) were provided by Tinamenor S.A., Span and Guernsey Ltd., U.K. The seeds were transported in a refrigerated styrofoam box from the hatchery to the laboratory. They were acclimated gradually to the experimental temperature.The animals were cultured in SL aquaria each provided with a 350 m mesh; silo was submerged partially in seawater. Recirculation of water was maintained through an air water drift. An additional aeration pipe was installed in each aquarium to keep the feed in suspension. Cultures were kept in a thermostatic water bath at 21°C. The animals were stocked at an initial wet weight of 0.75 g per silo. The total live weight per silo was determined and enough animals were removed to restore the weight to the initial weight (0.75 g) prior to returning the clams to the culture system. The seed was fed a weight specific daily ration twice a day. The daily growth rate (DGR) data of the clams were taken into account to adjust the daily amount of food. The DGR was calculated from the weekly increase of total wet weight (WW) per silo. The daily ration was calculated as % dry weight (DW) of the algal diet per WW of clams. Stock cultures (algae) were kept in 20 mL test tubes at 18°C, under continuous illumination and used as starter cultures for the inoculation of 500 mL Erlenmyer flask. The Erlenmyer flasks were filled with 300 mL seawater (filtered through 0.22 micrometer filter) provided with nutrients and autoclaved (30 min at 1 atm). Vitamins were added after cooling. Inoculation of

the Ernelmyers flasks was done under aseptic conditions. Ernelmyers flasks were kept at 18°C, under constant illumination and without aeration (the suspension was shaken every day). The cell concentration of the cultures was determined with a haematocytometer. Counting was done for 3 samples with 2 counts for each sample. Lipids were extracted from the sample and also the sample fatty acids were analysed.

The uptake and assimilation of the emulsion were verified analytically by determining the fatty acid composition and the total lipid content of the animals and the diets. Furthermore, the dietary requirements for (n–3) HUFA (especially DHA and EPA) were examined by the effect of (n–3) HUFA supplementation of *T.s.* diet containing large amount of EPA but no DHA. To evaluate the effect of a 50% lipid supplementation at 3 different weight specific feeding rations, the growth rate of the animals in the various treatments was compared with the growth rate of the animals fed on a mixed algal diet which is known to support good growth. The experiment was designed with eight treatments (diets) and each treatment had three replicates (aquaria). The characteristics of each treatment are shown in Table-1. The effect of different feeding rations of *T.s.* ranging from 0 to 1.5% DW, WW/day with or without 50% lipid were compared. For the adjustment of feeding to growth, a growth rate of 10% was assumed for all treatments during the first week of the experiment. For the remaining three weeks the daily rations were compared for each treatment on the basis of DGR of the previous week. The relative dailygrowth rate (rel DGR) for each treatment was calculated using the following equation, based on the DGR of the animals fed with the mixed algal diet (DGR_{mix}).

$$\text{Rel DGR } (\%) = DGR_x\ (\%)\ [DGR_{mix}\ (\%)^{-1}] \times 100$$

where DGR_x = DGR of treatment x (x = 1 to 8)

DGR_{mix} = DGR of treatment (mix).

The rel DGR indicates the extent the growth was deviated from the mixed diet, since the mixed diet of *Isochrysis galbana* and *T.s.* considered as a good diet for juvenile *Tapes philippinarum*.

TABLE-1
TREATMENTS AND THEIR CHARACTERISTICS USED IN LIPID SUPPLEMENTATION TEST WITH *T. Philippinarum*

Treatment	Diets characteristics
Max	*I. galbana* + *T. suecica* (1 : 1) (1)
Starv	Starvation
0.5% Tetra	*T. suecica* (0.5%)
0.5% Tetra + Em50D	*T. suecica* (0.5%) + 50% lipid (Em50D)
1% Tetra	*T. suecica* (1.0%)
1% Tetra + Em50D	*T. suecica* (1.0%) + 50% lipid (Em50D)
1.5% Tetra	*T. suecica* (1.0%)
1.5% Tetra + Em50D	*T. suecica* (1.5%) + 50% lipid (Em50D)

The figures between brackets indicate the feeding ration; the lipid supplements (Em50D) are expressed as % of the dry weight of algal feed.

The experimental emulsion Em50D (prepared by INVE Aquaculture N.V.S.A. Belgium contained 50% lipid (on WW basis), water, emulgators, antioxidants, preservatives and lip soluble vitamins A, C, D and E at a concentration of 0.18%, 0.08%, 0.013% and 0.32% respectively. Summary of various diets and their effect on the lipid content of *T. philippinarum* seed is given in Table-2. At the end of the experiment, the animals were starved for 1 day to clear their gut. After harvesting and measuring the live wet weight, the animals were freezedried for 24 h. The animals of each aquarium were pulverized in a motor. The samples from each aquarium were taken to determine the DW (24 h, 60°C) and the ash content (24 h, 450°C) was calculated as the different ash free dry weight (AFDW) and also was calculated as the difference between the DW and ash weight. The rest of the animals from the same aquarium were weighted in an 11 mL test tube, 4 mL chloroform/methanol (2 : 1) + BHT was added, the tubes were flushed with N_2 and stored at –20°C until further analysis. The DGR, the total lipid content and the fatty acid composition of animals were used as parameters to evaluate the effect of lipid supplementation to an algal diet. The algal species used in this experiment were sampled twice a week. They were concentrated by centrifugation. The algal pellet was resuspended in 0.5 M ammonium formate and transferred to 50 mL tubes. After centrifugation, the ammonium formate was discarded and the tubes were flashed with N_2 and stored at –40°C. At the end of the experiment, the samples were freeze-dried for 24 h. Three subsamples of each algae were taken for DW and AFDW determinations.

TABLE-2
SUMMARY OF THE VARIOUS DIETS AND THEIR EFFECT ON THE LIPID CONTENT OF *T. Philippinarum* SEED

Treatment	Lipid content	
	mg/g DW*	mg/g AFDW†
Mix	8.32 ± 0.38[a1]	81.54 ± 4.46[a]
Starv	5.89 ± 0.58[b]	73.87 ± 5.07[a]
0.5%*Tetra*	6.79 ± 0.40[a]	76.59 ± 5.51[a]
0.5% *Tetra* + Em50D	10.88 ± 0.12[c]	97.75 ± 6.47[b]
1.0% *Tetra*	9.95 ± 0.30[a]	85.28 ± 1.05[ab]
1.0% *Tetra* + Em50D	15.02 ± 0.55[d]	136.55 ± 4.98[c]
1.5% *Tetra*	12.61 ± 0.27[e]	114.67 ± 2.49[d]
1.5% *Tetra* + Em50D	17.60 ± 0.35[f]	159.97 ± 3.19[e]

*mg lipid per g dry weight followed by the standard deviation within brackets.

†mg lipid per g ash free dry weight followed by the standard deviation within brackets.

‡Initial lipid content of the animals = 8 mg per g dry weight.

[1]The values are the average of 3 replicates ±SD.

Values with the same superscript letter in each column are not significantly different ($P < 0.05$).

Statistical analysis of the data was performed by the analysis of variance (ANOVA) and Tukey HSD multiple range tests ($P \leq 0.5$). The homogeneity of the variances of means was checked by Cochrane's and Hartley's tests.

RESULTS AND DISCUSSION

The DGR calculated weekly from the change in weight of the total biomass per aquaria are presented in Table-3. No mortality was observed over the experimental period of 4 weeks. Lipid supplementation improved the DGR of the clams in all rations of *T.s.*, but significant effects were only detected in week 1 when supplemented at a feeding ration of 0.5%. The DGR of the control treatment in week 1 was relatively low, due to contamination in the *Isochrysis galbana* culture. The highest DGR of 9.07% was achieved in the second week of experiment for the ration of 1.5% supplemented with lipids. It was significantly different from the DGR of all the other treatments except for ration 1.5% without lipid supplementation. The relative DGR (rel DGR) is shown graphically in Fig. 1. From the second week onwards, the rel DGR for the animals fed 0.5% and 1% *T.s.* with or without lipid supplementation was lower than 1.5%. Except for week 4, the rel DGR of all treatments at 1.5% with/without lipid supplementation were same or higher than the control one.

TABLE-3
DAILY GROWTH RATE (DGR) OF *T. Philippinarum* SEED FED VARIOUS ALGAL RATIONS OF *T. Suicica* WITH OR WITHOUT 50% LIPID (EMULSION EM50D) SUPPLEMENTATION (% DAY^{-1})

Treatment	Week 1	Week 2	Week 3	Week 4
Mix	4.81 ± 0.65^{a}	7.63 ± 0.33^{a}	6.82 ± 0.37^{a}	6.41 ± 0.80^{a}
Starv	0.48 ± 0.68^{c}	0.80 ± 0.25^{e}	0.18 ± 0.45^{d}	0.00 ± 0.35^{d}
0.5% Tetra	2.78 ± 0.49^{d}	3.44 ± 0.21^{b}	2.50 ± 0.38^{b}	1.84 ± 0.05^{b}
0.5% Tetra + Em50D	4.40 ± 0.14^{a}	4.06 ± 0.02^{b}	3.12 ± 0.56^{b}	$2.50 \pm 0.08b^{b}$
1% Tetra	4.95 ± 0.24^{a}	5.70 ± 0.46^{c}	4.80 ± 0.22^{c}	3.83 ± 0.15^{c}
1% Tetra + Em50D	5.59 ± 0.49^{ab}	6.66 ± 0.01^{c}	5.40 ± 0.40^{c}	4.19 ± 0.63^{c}
1.5% Tetra	6.12 ± 0.42^{b}	$8.14 \pm 0.48a^{d}$	6.86 ± 0.48^{a}	5.90 ± 0.21^{a}
1.5% Tetra + Em50D	6.66 ± 0.02^{b}	$9.07 \pm 0.68d^{d}$	6.95 ± 0.35^{a}	6.05 ± 0.21^{a}

Data represent the mean of 3 replicates ± SD.

Superscripts in the same column indicate means which do not differ significantly ($P \leq 0.5$).

The supplementation of emulsion Em50D significantly increased the total lipid content (mg, g^{-1} DW) of the clams for all feeding rations compared to the animals that received only the algal diet of the same ration. There was no significant difference in the lipid content (mg, g^{-1} DW) between the control treatment and the treatments of 0.5% and 1.0% ration without lipid supplementation. The lipid content in the treatment of 1.5% ration without lipid supplementation was significantly higher than the lipid content of the animals from the control

treatment. Supplementing the algal diet at a ration of 0.5%, 1.0% and 1.5% increased the lipid content of the seed with 60%, 51% and 40% respectively. The lipid content of clams fed 1.5% ration without lipid supplement was significantly higher than the content in the control treatment but lipid content of the clams fed 0.5% and 1.0% ration without lipid supplementation did not show any significant difference. Starve spat exhibited significantly lower lipid content compared to the fed ones (Table-3).

The lipid content of the animals that received emulsion Em50D increased significantly with increasing algal ration. When the lipid content (mg) of the clams was measured per gram of ash free dry weight (AFDW), there were no significant differences among the treatments of mixture, starvation, 0.5%, *T.s.* and 1.0% *T.s.* The lipid content (mg, g^{-1} AFDW) of the animals fed 1.0% ration was significantly lower than the lipid content fed of 0.5% supplement with lipid. The lipid supplementation resulted in a significant increase of the lipid content in all the tested feeding rations. Similar to the lipid content expressed in mg g per g DW, the lipid content expressed in mg per g AFDW significantly increased when 50% lipid was supplemented at a higher feeding ration. The lipid content inclined with 27.6%, 60% and 39.5% at a 0.5%, 1.0% and 1.5% ration respectively. Data for the fatty acid composition of the diet and initial samples of juvenile the clamps of different treatments are shown in Tables 4 and 5 and Figs. 2, 3, and 4. The proportion of the fatty acids 16 : 0, 16 : 1 (n-3), 16 : 4 (n-3), 18 : 1 (n-3), 18 : 1 (n-9), 18 : 1 (n-7) 18 : 2 (n-6), 18 : 3 (n-3), 18 : 4 (n-3), 20 : 2 (n-6), 20 : 3 (n-6), 20 : 3 (n-3), 20 : 4 (n-3), 20 : 5 (n-3) increased when the feeding ration was raised from 0.5% to 1.5% in content; the fatty acid level of 15 : 1 (n-7), 15 : 1 (n-5), 18 : 0, 20 : 4 (n-6) and the fatty acid with C > 22 (except for 22 : 4 (n-6) and 22 : 3 (n-3) which were zero) decreased significantly by increasing the feeding ration of *T.s.* from 0.5 to 1.5%. In general feeding ration in the lipid supplement treatments showed similar evolution for the corresponding fatty acids though the changes in the levels of 18 : 1 (n-7), 20 : 2 (n-6) and 22 : 5 (n-6) were not significant and the proportions of 20 : 1 (n-11), 20 : 1 (n-9) and 22 : 4 (n-6) decreased significantly by increasing ration from 0.5% to 1.5%. Feeding on *T.s.* with 50% lipid supplementation, clams exhibited different fatty acid composition compared to those fed solely *T.s.* The lipid emulsion containing high percentage of DHA (45.03%) drastically increased the DHA level in the animals with 21.88%, 21.91% and 20.29% at the feeding ration of 0.5%, 1.0%, and 1.5% respectively. Although the lipid emulsion contained reasonable amount of EPA (6.93%), the EPA level increased significantly as a result of lipid supplementation. Since the incline in the DHA level in the animals was attended by a reduction in the EPA levels, the DHA/EPA ratio changed from 0.60 to 3.65, from 0.26 to 2.79 and from 0.14 to 2.40 at a feeding ration of 0.5%, 1.0% and 1.5% respectively. The proportions of the fatty acids 12 : 0, 22 : 1 (n-7), 22 : 3 (n-3), 22 : 5 (n-6) and 22 : 4 (n-3) were not changed significantly by the addition of emulsion (50D) to *T.s.*

The lipid supplementation has greatly influenced the lipid content of clams in ration level. This is in agreement with the results of Hurd[21] who reported that clams fed a diet lacking in lipid supplement. In an experiment with *Ostrea edulis*, Laing and Millican[22] found that the amount of lipid accumulation during a 4 weeks

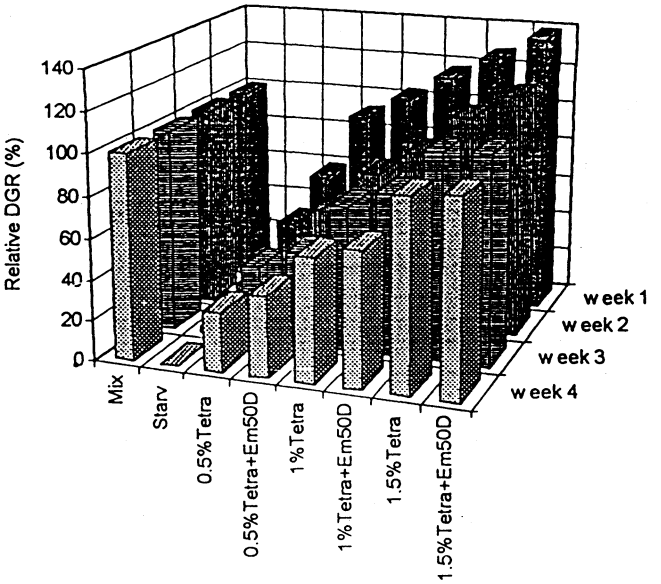

Fig. 1 Relative DGR (%) of juvenile *Tapes philippinarum* fed various algal rations with or without 50% lipid supplementation (emulsion Em50D)

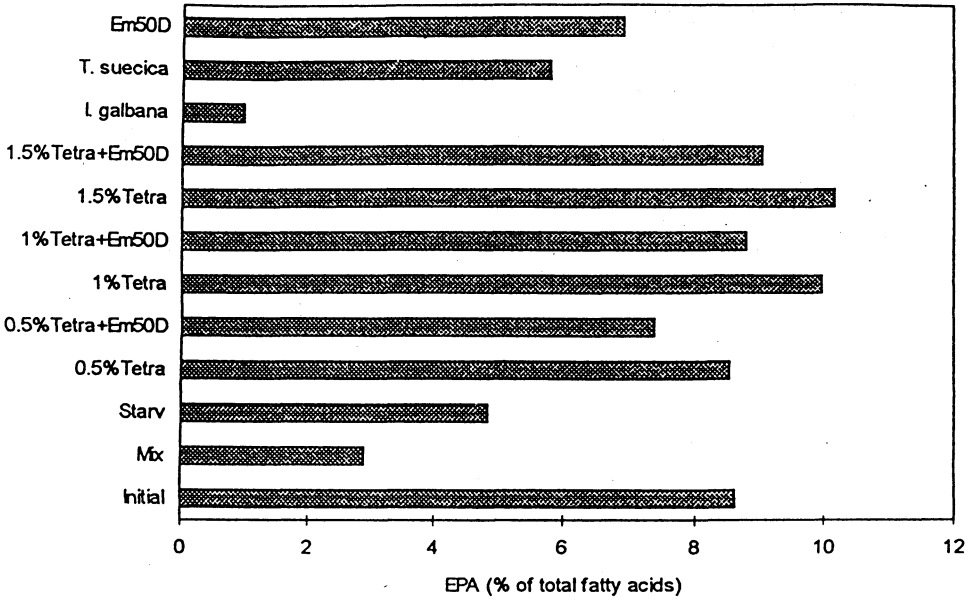

Fig. 2 EPA level in different treatments, diets and initial sample

culturing period affected their subsequent performance when they were moved to the sea and grow for 4 to 5 months. Although lipids play a major role during the development of invertebrate larvae[23], there is not necessarily a direct relationship

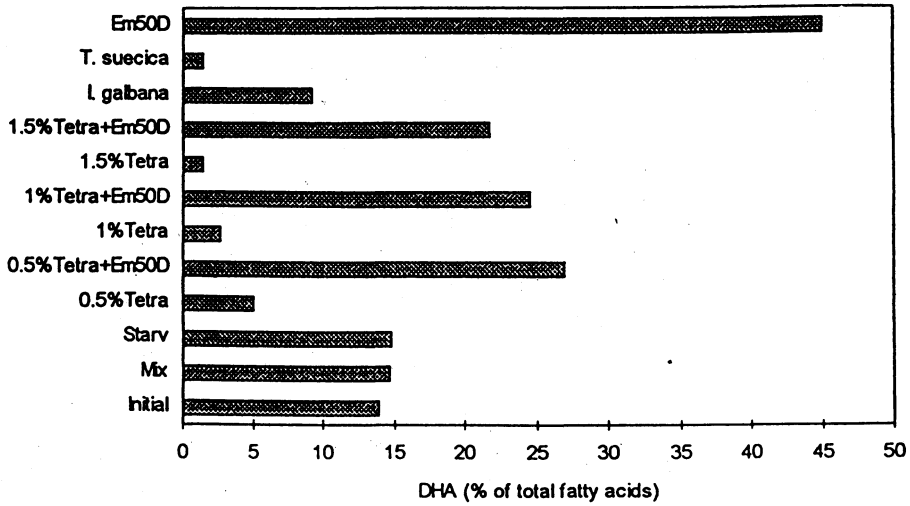

Fig. 3 DHA level in different treatments, diets and initial sample

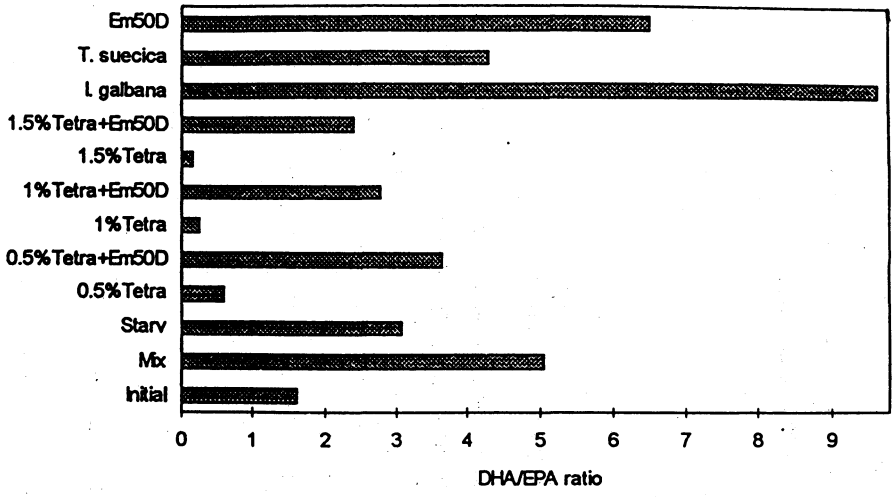

Fig. 4 DHA and EPA ratio in different treatments, diets and initial sample

between lipid and juveniles growth. The initial level of the DHA in the animals was 13.88% of total fatty acids. It increased by > 90%, > 75% and 55% in the lipid supplementation diet of 0.5%, 1.1% and 1.5% *T. s.* and increased by >15% in mixed fed animals compared to DHA of initial animals. In contrast, DHA decreased by > 60%, 80% and 85% in the animals fed 0.5%, 1.0% and 1.5% *T.s.* without lipid supplementation. Coutteau *et al.*[24] observed similar results in an experiment with *Placopecten magellanicus* where EPA and DHA concentrations increased by > 100% and 40% respectively in juveniles fed lipid emulsions as a supplement to *Isochrysis* compared to the values in the control fed algae only.

TABLE-4
FATTY ACID COMPOSITION (% OF TOTAL FATTY ACIDS) OF *T. suicica*, *I. galbana*, EMULSION 50D AND INITIAL SAMPLE OF ANIMALS USED IN LIPID SUPPLEMENTATION TEST WITH *T. Philippinarum*

Fatty acids	*T. suicica*	*I. galbana*	Emulsion	Initial
12 : 00	0.22	0.43	tr	0.81
14 : 00	2.65	19.07	0.77	1.31
15 : 00	0.42	0.13	0.1	2.34
15 : 1 (n-7)	tr	tr	tr	tr
15 : 1 (n-5)	2.24	0.81	tr	0.31
Unknown	4.28	1.43	tr	1.54
16 : 00	17.40	12.27	15.73	16.60
16 : 1 (n-7)	1.83	1.65	1.60	1.49
17 : 1 (n-9)	tr	0.36	tr	7.84
17 : 1 (n-7)	0.95	tr	tr	0.98
16 : 4 (n-3)	12.12	tr	tr	tr
18 : 00	0.54	1.08	0.90	4.49
18 : 1 (n-9)	7.34	16.92	16.90	6.54
18 : 1 (n-7)	3.14	2.21	1.13	2.51
18 : 2 (n-6)-c	5.56	5.49	2.70	1.81
18 : 3 (n-3)	16.42	9.25	0.20	2.05
18 : 4 (n-3)	7.32	8.01	0.40	2.88
20 : 1 (n-11)	tr	tr	0.10	1.53
20 : 1 (n-9)	0.74	tr	tr	1.38
20 : 2 (n-6)	0.29	0.37	tr	3.39
20 : 3 (n-6)	tr	tr	tr	0.32
20 : 4 (n-6)	0.74	0.16	0.30	2.43
20 : 3 (n-3)	tr	tr	tr	0.23
20 : 3 (n-3)	0.46	tr	0.10	tr
20 : 5 (n-3)	5.80	0.96	6.93	8.62
21 : 1 (n-7)	tr	tr	tr	1.59
21 : 1 (n-5)	tr	tr	tr	1.80
21 : 5 (n-3)	tr	tr	1.10	0.54
22 : 4 (n-6)	tr	0.18	0.10	0.72
22 : 3 (n-3)	tr	tr	tr	tr
22 : 5 (n-6)	0.35	2.76	0.90	2
22 : 4 (n-3)	tr	tr	tr	tr
22 : 5 (n-3)	tr	tr	3.13	1.18
22 : 6 (n-3)	1.35	9.26	45.03	13.88

tr ≤ 0.1%

TABLE-5
FATTY ACID COMPOSITION (% OF TOTAL FATTY ACIDS) OF *T. philippinarum* AFTER 4 WEEKS CULTURE FOR 8 DIFFERENT TREATMENTS

Fatty acid	Mix	Starv	0.50% Tetra	0.5% Tetra + Em50D	1% Tetra	1% Tetra + Em50D	1.50% Tetra	1.5% Tetra + Em50D
12 : 00	0.15a	0.33ab	0.31a	0.12ac	0.14ad	0.10cde	0.12ae	0.12ad
14 : 00	1.25	0.62a	0.30a	0.58a	0.38a	0.63a	0.31a	0.85b
15 : 00	1.11a	0.77b	1.52c	0.96d	1.47c	0.84b	1.58c	1.10ad
15 : 1 (n-7)	tr	tr a	2.41	1.08	1.21	0.36	0.47	tr a
15 : 1 (n-5)	0.32a	2.96	0.93	0.55b	0.76	0.35a	0.61b	0.20a
Unknown	1.06a	1.26a	1.19a	0.56b	1.22a	0.61b	1.26a	0.64b
16 : 00	16.25a	11.24	14.16b	12.88	17.18ac	13.86b	17.76c	14.94
16 : 1 (n-7)	0.64	1.80b	0.95	1.44ac	1.41ad	1.64ab	1.32cd	1.87b
17 : 1 (n-9)	6.60	5.15	3.65a	2.03b	4.05a	1.88b	3.94a	2.12b
17 : 1 (n-7)	0.66b	0.70b	0.88a	0.77b	0.92a	0.72b	1.03a	0.70b
16 : 4 (n-3)	0.10	0.79a	0.55b	0.31	0.60bc	0.64c	0.83a	0.73a
18 : 00	3.72	4.64a	4.70a	2.67b	3.47	2.22	2.83b	1.99
18 : 1 (n-9)	5.37b	3.99	5.20b	6.05c	6.06c	6.63a	6.26ac	7.01
18 : 1 (n-7)	3.32	5.46	4.17	4.91a	4.70a	4.93a	4.97a	4.99b
18 : 2 (n-6)-c	1.83	0.58	2.79	2.10	5.29	3.07	6.30	3.57
18 : 3 (n-3)	1.72	0.44	3.25a	1.27	5.88	2.59	7.33	3.44a
18 : 4 (n-3)	1.73	0.23	1.08	0.56	1.88	1.04	2.56	1.5
20 : 1 (n-11)	1.24	tr b	0.75a	1.96	0.76a	1.74	0.78a	1.5
20 : 1 (n-9)	2.74	1.93	2.23a	4.17	2.27a	3.81b	2.25a	3.66b
20 : 2 (n-6)	4.72a	1.98	4.49a	2.28b	4.86ac	2.54b	4.98c	2.56b
20 : 3 (n-6)	0.21b	0.23b	0.39c	0.21b	0.41c	0.33a	0.52	0.32a
20 : 4 (n-6)	2.71b	3.57	5.55	2.57ab	5.04	2.50ab	4.60	2.35a
20 : 3 (n-3)	0.57b	0.15a	0.61b	0.24a	1.01	0.45b	1.21	0.57b
20 : 4 (n-3)	tr a	0.13	0.51b	0.22	0.69	0.38ac	0.88	0.45bc
20 : 5 (n-3)	2.89	4.83	8.53a	7.40	9.95	8.80a	10.16	9.04a
22 : 1 (n-7)	2.72b	2.29c	2.44bc	1.65a	1.57a	1.14d	1.01d	0.91d
22 : 1 (n-5)	1.29a	4.11	2.99	0.65	1.46a	0.32b	0.94	0.21b
21 : 5 (n-3)	0.80	0.24a	1.35	0.36b	0.87	0.25a	0.49	0.41b
22 : 4 (n-6)	0.59	tr a	tr a	0.35a	tr a	tr a	tr a	0.21
22 : 3 (n-3)	tr a	1.47	tr a	tr a	tr a	tr a	tr a	0.00a
22 : 5 (n-6)	4.28	2.43	1.18a	0.95ab	0.53c	0.67bc	0.27c	0.59bc
22 : 4 (n-3)	0.04	0.12	tr	tr	tr	tr	tr	tr
22 : 5 (n-3)	0.45a	1.87	0.97b	2.44	0.82b	2.27	0.59a	2.03
22 : 6 (n-3)	16.63	14.84	5.11	26.99	2.63	14.54	1.42	21.71

tr ≤ 0.1%.

Identical alphabets in the same row indicate means which do not differ significantly ($P < 0.05$).

This study suggested that since 18 : 1 (n-9), 18 : 2 (n-6), 18 : 3 (n-3), did not accumulate in proportion of availability, the surplus might have been utilized for energy. Langdon and Waldock[27] in their experiment with Juvenile *C. gigus* feeding on *D. tertiolecta*, which had 88% 18 : 2 (n-6) and 31.1% 18 : 3 (n-3), growth was poor until a supplement of 22 : 6 (n-3) was fed. A similar feeding with *T.s* which had no 22 : 6 (n-3) but had 7.7% 20 : 5 (n-3), greater growth ensured than with *D. tertiolecta plus* 22 : 6 (n-3) and lipid supplementation rich 22 : 6 (n-3) to the *T.s.* fed spat resulted in a little extra growth. These findings are also similar to the results of the present study where addition of DHA with 1.5% ration of *T.s.* contributes a little extra growth, which suggest that a mixture of EPA and DHA may support better growth than individuals. In addition, it may be concluded from this experiment that the deficiency of DHA in *T.s.* is a limiting factor up to 1.0% ration of *T.s.* It appeared that EPA could fulfill the (n-3) PUFA requirements. However, the research findings of Thompson and Harrison[25] showed that increased amounts of EPA in the experimental diets of *Thalassiosira pseudonana* of different biochemical composition containing 6 to 18% of EPA were more than adequate to support the growth of larval *C. gigus.* Coutteau *et al.*[26] reported that *M. mercenaria* should significantly better the growth and survival throughout metamorphosis when the algal diet was supplemented with 50% of the DHA-rich lipid-emulsion compared to the control fed only *I. galbana* (Clone T-Iso).

The present study demonstrates that the supplementation of a DHA rich lipid emulsion has no remarkable influence on the DGR of juvenile bivalves when used to supplement *T.s.* The DGR in 1.5% ration treatment without lipid supplementation indicates that extra 1% ration (versus 0.5% tetra in mixed treatment) may replace the necessity of 0.5% *Isochrysis galbana.* It may indicate that deficiency of DHA in *T.s.* is a limiting factor up to 1.0% ration of *T.s.* showed similar pattern of DGR as mixed algal diet of 1.0% ration. It appears that EPA could fulfill the HUFA requirement of the animals when 1.5% *T.s.* was fed. Langdon and Waldock[27] found in their experiment with C either 20 : 5 (n-3) or 22 : 6 (n-3) can fulfill the requirement for higher (n-3) PUFA. One reason for the requirement of (n-3) PUFA is to maintain membrane fluidity in the relatively low and constant temperature of the marine environment[28].

The results of the experiment suggest that EPA could fulfill the (n-3) HUFA requirements of juvenile *Tapes philippinarum*, but a mixture of EPA and DHA could possibly be slightly better.

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