CHAPTER – VII

IMPACT OF MICROALGAL HUFA CONTENT ON LARVAL HUFA COMPOSITION AND PERFORMANCE

Mass cultured micro algae are the primary food source for shrimp larvae, as early larval stages (zoeal stages) rely on cultured algae as sole food. Attempts to replace live feed with artificial diets resulted in slower larval growth (Galgani and Aquacop, 1988; Kurmaly *et al.,* 1989b). Algae appears to contain substances that trigger enzyme activity (gut enzyme stimulant), resulting in better digestion (Amjad *et al.,* 1993; Jones *et al.,* 1993, 1997; Le Vay *et al.,* 1993; Rodriguez *et al.,* 1994; Kumlu and Jones, 1995).

Although many species of microalgae suitable for mass-culturing are being used as food in mariculture, not all produce good growth and survival in all species (Webb and Chu, 1983; Enright *et al.,* 1986). Microalgae must possess a number of key attributes to be useful in aquaculture. The algae must be of an appropriate size for ingestion, e.g. from 1 to 15 μm for filter feeders; 10 to 100 μm for grazers (Webb and Chu, 1983; Kawamura *et al.,* 1988; Jeffrey, *et al.*, 1992) and readily digested. They must have rapid growth rates, be amenable to mass culture, and also be stable in culture to fluctuations in temperature, light and nutrients as may occur in hatchery systems. Finally, algae must have good nutrient composition and free from toxins that might be transferred up in the food chain.

Microalgae contains protein, lipid, carbohydrate, vitamins, minerals and specific nutrients such as essential fatty acids, amino acids etc. (Brown *et al.*, 1997). Crustacean larval development encompasses a sequence of moult and intermoult periods during which the feeding regime appears to be the determinant of success (Ouellet *et al.*, 1992). Among the various nutrients supplied by microalgae, the fatty acids have recently attracted much attention. Study has shown that the fatty acid composition of the shrimp larvae is directly related to the fatty acid content of the diet (Mourente *et al.*, 1995).

Microalgae have been noted to be an excellent source of fatty acid (Cohen *et al.*, 1995; Borowitzka, 1998). In fact, oil extracts from algae containing long-chain polyunsaturated fatty acids (LC-PUFA) are already in use as nutritional supplements in human infant formula (Cohen *et al.*, 1995).

The importance of HUFAs (Highly unsaturated fatty acids) in larval fish and crustacean nutrition has been extensively investigated during the past twenty years (Halver, 1980; Lall, 2000). Among the HUFAs, the eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) have been shown to be essential for a variety of molluscs, prawn and fish larvae and may be essential to other marine animals too (Castell *et al.*, 1986; Volkman *et al.*, 1992; Sargent *et al.*, 1999).

It has been demonstrated that shrimps have a limited ability to synthesize *de novo* the n-6 and n-3 families of fatty acids, including the polyunsaturated linoleic (18:2n-6, LOA) and linolenic (18:3n-3, LNA) acids. They also have a limited ability to elongate and desaturate these polyunsaturated fatty acids (PUFA) to HUFA such as arachidonic acid (20:4n-6, ARA), EPA and DHA (Kanazawa *et al.*. 1979a,b,c; Kayama *et al.*, 1980).

Diets enriched with n-3 HUFA (mainly EPA and DHA) have resulted in increased growth and survival of shrimp larvae (Sorgeloos and Leger, 1992).

In recent years, there has also been raising interest in ratios of DHA/EPA in diets of marine larvae (Watanabe, 1993; McEvoy *et al.,* 1996). Thinh *et al.* (1999) analysed 13 species of marine microalgae for their fatty acid content and its resultant effect on EPA/DHA ratio of *Artemia*- fed on these algae.

Thus, it is prudent that a combination of algae be selected to feed larvae such that it satisfies the requirement for the vital HUFAs (mainly EPA and DHA) and improves the growth and survival. Studies by various authors have given an idea regarding the biochemical composition of commonly maricultured microalgae (Volkman *et al.,* 1989, 1992; Dunstan *et al.,* 1994). According to the authors, diatoms, eustigmatophytes, cryptomonads, rhodophytes and some prymnesiophytes are rich sources of EPA (7-34%) and prymnesiophytes and cryptomonads are relatively rich in DHA (0.2 -11%).

Evaluation of a particular microalgae or mixture of algae for the culture organisms have been widely reported for fishes (Caers *et al.,* 2003), molluscs (Whyte *et al.,* 1989; Delaunay *et al.,* 1993; Khardin *et al.,* 2003) and shrimp larvae (Chu, 1989; D'Souza and Loneragan, 1999; D'Souza and Kelly, 2000; D'Souza *et al.,* 2002). To support the objective of obtaining microalgae with high quantity of required fatty acid, technology also has been developed to manipulate the microalgae (James *et al.*, 1989; Dunstan *et al.*. 1993; Day and Tsavalos, 1996; D'Souza and Kelly, 2000).

In the present study, larval rearing of *Fenneropenaeus indicus* was carried out using two microalgae with distinctly different fatty acid profile, one of them selected for its high EPA content and the other for its DHA. The treatments included larvae reared feeding only one of the two algae and those fed both in combination. The objectives included analyzing the effect of treatments on the EPA, DHA and other fatty acid composition of larvae and its possible effect on larval survival and growth.

MATERIALS AND METHODS

Microalgae culture

The algae selected for the experiments were *Chaetoceros muelleri* Lemmermann 1898 (= *C. gracilis,* CS 176, size 5-8 µm, Bacillariophyceae) and *Isochrysis* sp. Parke (clone. T.Iso, Tahiti strain, CS 177, size 3x5 µm, Prymnesiophyceae). Starter algae were maintained in f_2 medium (Guillard and Ryther, 1962). The starter cultures were maintained axenically in batch culture method and mass cultures maintained semi-continuously in 500 litre capacity photobioreactors (Solar Components Corp., USA).

Nutrient recipe for the tubular reactors composed of skelon (Taiwanese compost mixture), commercial urea (46% prilled N_2), sodium orthophosphate ($Na_2H_2PO_4$) and EDTA. The above ingredients were added at a rate of 1.5 g, 20 g, 0.5 g and 1 g for 100 litres of culture volume respectively. Through out the experimental period, the algal concentration in cultures were checked three times a day using improved Neubauer haemocytometer.

PLATE - 4

Semi-continuous microalgal culture in photobioreactors

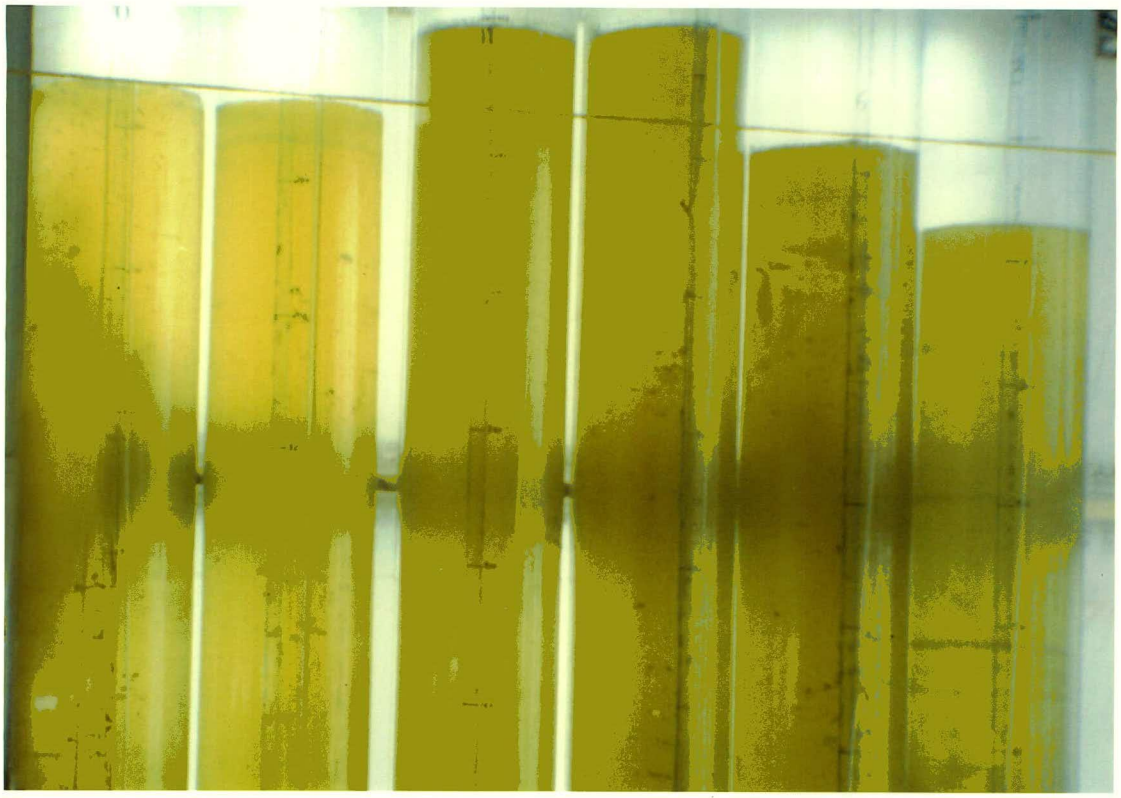

PLATE - 4

Larval rearing

In the larval rearing tanks (1000 l), the larvae at nauplii 5 stage were stocked at 100 larvae/l density. The different larval tanks were fed either *Chaetoceros* (at a concentration of 1 x 10^5 cells/ml) or *Isochrysis* (1x10^5) or a combination of the above two (1:2 by dry weight) reaching a final concentration of 1 x 10^5 cells/ml. The larval density was checked every 4 hours with a haemocytometer and the needed quantity was pumped again to maintain the required concentration. Four tanks were run for each treatment. Once the larvae reach mysis 1 stage, the larval total length was measured for each treatment. No *Artemia* or artificial feeds were used in the experiments. The percentage larval survival was also estimated at the end of the experiment.

Fresh filtered , UV-treated seawater was added to the larval tanks for the initial two days (to reach 100 % tank volume). Tanks with zoea 3 and mysis 1 stages were given 30% and 50% water exchange respectively.

For dry weight estimation, the algae from 100ml volume, filtered and retained on the pre-weighed glass-fibre were used. The salt on the filtrate was removed by rinsing the filter with 2M of ammonium formate. The filter was dried in an oven at 105° C and reweighed.

Development index was calculated using the formula given by Villegas and Kanazawa (1979). Development index = A/total number of larvae staged, where A=Σ (stage value x number of larvae at that stage). The stage values assigned were 1, 2, 3 and 4 for Z1, Z_2, Z_3 and M_1 respectively. Therefore, higher the proportion of advanced stage larvae in the treatment, the higher the development index. From each replicate 75 larvae were sampled randomly for the assessment of larval stage. Staging of larvae was

according to Silas *et al.* (1978). Survival was assessed by five random samples from different parts of the tanks with 500 ml beaker.

Biochemical analysis

For fatty acid analysis of microalgae, algal cells were harvested from 200 ml of each culture by filtering through pre-combusted (450 °C; 16 h) glass-fibre filters (Whatman GF/F, 47 mm). The filters with algae were placed in polypropylene cryogenic vials and immediately frozen by immersion in liquid nitrogen. The larval samples were thoroughly washed with sterile filtered seawater, freeze dried and stored. All samples were stored in liquid nitrogen until analysis.

Lipid extraction was by following the protocol of Bligh and Dyer (1959). The procedures involved in fatty acid analysis have been explained in chapter 1.

Data analysis

The statistical significance between treatments were tested by one way Analysis of Variance (ANOVA). When significance was noticed comparison of means was by Tukey's significant test. The percentage survival values were arcsine transformed.

RESULTS

Algae dry weight and fatty acid composition

The dry weight (pg/cell) of *Chaetoceros* and *Isochrysis* was 64.3 and 28.4 respectively. Fatty acid analysis of *Chaetoceros*, *Isochrysis* and a mixture of both the microalgae are presented in Table 25. Fatty acid composition of *Chaetoceros* showed high content of 14:0, 16:0, 16:1 n-7, 16:3 n-4 and 20:5 n-3. *Isochrysis* was rich in 14:0.

Table 25. Fatty acid composition, as percentage of total fatty acids of the microalgae and their mix used to feed larvae in the experiments.

Fatty acid	*Chaetoceros muelleri*	*Isochrysis* sp. (T.Iso)	Algal mix
C14:0	19.2 ± 0.9	26.5 ± 1.5	21.1 ± 0.6
C 16:0	10.8 ± 0.4	12.3 ± 1.7	11.7 ± 1.3
C18:0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
C 16:1 n-7	25.1 ± 1.1	3.8 ± 1.2	16.1 ± 0.9
C 18:1 n-9	1.9 ± 0.4	12.0 ± 3.3	7.8 ± 2.1
C 16:2 n-7	4.2 ± 0.4	0.8 ± 0.4	2.0 ± 1.0
C 16:3 n-4	9.1 ± 0.9	0.5 ± 0.2	4.2 ± 0.6
C18:2 n-6	1.1 ± 0.3	4.5 ± 1.1	3.0 ± 0.3
C18:3 n-3	0.3 ± 0.0	4.7 ± 0.7	2.9 ± 1.0
C18:4 n-3	0.7 ± 0.2	14.4 ± 0.9	8.1 ± 1.7
C20:2 n-6	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C20:3 n-3	0.9 ± 0.3	0.1 ± 0.0	0.3 ± 0.0
C20:3 n-6	1.4 ± 0.7	tr	0.5 ± 0.1
C20:4 n-3	0.7 ± 0.4	0.1 ± 0.0	0.3 ± 0.1
C20:4 n-6 (ARA)	0.7 ± 0.1	tr	0.4 ± 0.2
C20:5 n-3 (EPA)	11.2 ± 1.3	0.3 ± 0.2	5.9 ± 0.8
C22:5 n-3	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
C22:6 n-3 (DHA)	0.4 ± 0.1	9.2 ± 2.1	3.9 ± 0.6
Σ PUFA	18.03 ± 2.4	25.10 ± 2.7	21.00 ± 3.1
(n-3) HUFA	12.60 ± 2.0	9.70 ± 1.8	10.30 ± 1.2
(n-6) HUFA	0.70 ± 0.1	tr	0.40 ± 0.2

Values are means of triplicate analysis ± S.D; tr=trace levels (less than 0.1% of total fatty acids); PUFA =≥16:2: HUFA = ≥20:4 ; Total fatty acids do not add up to 100 as minor components are not included.

16:0, 18:1 n-9, 18:4 n-3 and 22:6 n-3. *Chaetoceros* was deficient in 22:6 n-3 and *Isochrysis* in 20:5 n-3. One of the important fatty acid namely arachidonic acid (20:4 n-6) was present in a meager quantity in *Chaetoceros* and was found only in traces (<0.1%) in *Isochrysis*.

The *Chaetoceros* when compared to *Isochrysis*, recorded lesser total values for saturated fatty acids (30.4 vs 39.1) and polyunsaturated fatty acids (18.03 vs 25.1), but had a higher monounsaturated (27 vs 15.8) and HUFA (13.3 vs 9.7). HUFA n3/n6 ratio was also higher in *Chaetoceros*. Noticeably, n-6 HUFA was found only in traces in *Isochrysis*. The mix of both the algae resulted mostly in intermediate values. Interestingly, among the algal samples, the HUFA n3/n6 ratio was highest in mixed algal sample (25.75).

Fatty acid composition of the different larval samples from the treatments is given in table 26. The Z_1 samples from all three treatments were rich in the vital HUFAs namely EPA and DHA.

In the larval development there was a gradual reduction in EPA, DHA and ARA content of larvae even with those fed with algae rich in a particular nutrient (Table 26). However, the quantity of particular HUFA in M_1 sample was directly related to the concentration of same in the algae it was fed. The M_1 from tanks fed with *Chaetoceros* had higher amount of EPA. Comparatively, the rate of reduction of EPA in *Chaetoceros* (EPA rich)-fed larvae was higher than reduction of DHA in *Isochrysis* (DHA rich)-fed larvae, indicating faster utilization of EPA. The ARA content was found to be little higher in M_1 samples from mixed algae-fed tanks.

Table. 26. Fatty acid profile of the different larval stages of *P .indicus* fed *Chaetoceros* or *Isochrysis* or their mix

Fatty acid	*Chaetoceros muelleri*				*Isochrysis* sp.				Mixed algae			
	Z_1	Z_2	Z_3	M_1	Z_1	Z_2	Z_3	M_1	Z_1	Z_2	Z_3	M_1
C14:0	2.4±0.1	3.7±1.3	4.1±0.7	5.7±0.3	0.9±0.2	3.4±0.1	4.3±0.4	5.9±0.5	3.4±0.0	3.7±0.2	4.3±0.4	5.1±0.6
C16:0	16.1±1.3	17.3±0.9	18.7±1.1	18.9±1.3	15.3±0.9	17.0±0.3	19.2±0.3	19.7±0.9	15.4±0.6	16.0±1.0	18.4±0.5	16.6±1.1
C18:0	7.3±2.0	7.1±1.0	6.7±0.9	6.1±0.5	6.3±0.6	6.5±0.4	6.5±0.7	6.2±0.6	7.6±0.3	7.4±0.8	7.0±0.6	7.0±0.8
C16:1 n-7	5.6±1.1	6.3±0.5	6.8±0.5	6.5±0.3	4.3±0.3	4.9±0.9	3.9±0.2	3.8±0.3	4.6±0.1	5.2±0.2	5.9±0.3	5.9±0.6
C18:1 n-7	4.3±0.7	4.0±0.4	5.6±0.4	5.9±0.6	5.4±0.3	6.1±0.5	6.4±0.9	6.7±0.4	4.8±0.6	5.1±0.3	5.0±0.7	5.3±0.9
C18:1 n-9	7.1±0.6	5.9±0.3	5.5±1.0	5.1±0.6	8.2±1.0	5.9±0.6	5.3±0.7	4.7±1.3	5.3±0.6	4.6±0.3	4.4±0.4	4.1±0.3
C16:2 n-7	0.1±0.0	0.1±0.0	0.3±0.1	0.3±0.1	tr	tr	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
C 16:3 n-4	4.1±0.6	4.3±0.2	4.3±0.3	4.0±0.3	tr	tr	tr	tr	1.3±0.3	0.9±0.2	0.7±0.1	0.7±0.4
C18:2 n-6	1.7±0.3	2.9±0.1	2.7±0.2	3.3±0.9	3.1±0.2	3.7±0.3	3.4±0.2	3.5±0.2	2.2±0.0	2.9±0.6	3.1±0.3	3.4±0.9
C18:3 n-3	0.3±0.1	1.1±0.0	1.0±0.2	2.7±0.2	0.7±0.1	1.4±0.3	2.9±0.7	3.6±0.7	0.7±0.0	2.2±0.0	3.1±0.1	3.4±0.6
C18:4 n-3	0.3±0.0	0.3±0.1	0.1±0.0	0.1±0.0	2.1±0.6	2.3±0.2	2.0±0.1	1.8±0.2	1.9±0.6	2.3±0.6	2.7±0.6	3.0±0.4
C20:2 n-6	0.6±0.2	0.5±0.2	0.6±0.1	0.6±0.2	0.7±0.2	0.7±0.4	0.4±0.0	0.3±0.1	0.5±0.2	0.4±0.2	0.4±0.0	0.6±0.2
C20:3 n-3	tr	tr	0.1±0.0	0.1±0.0	0.1±0.0	tr	tr	tr	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
C20:3 n-6	0.1±0.0	0.1±0.0	0.3±0.0	0.3±0.0	0.2±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1	0.2±0.0	0.3±0.1	0.2±0.1
C20:4 n-3	0.4±0.2	0.6±0.2	0.6±0.3	0.4±0.1	0.2±0.0	0.2±0.1	0.1±0.0	tr	0.4 ±0.1	0.6±0.1	0.5±0.2	0.5±0.2
C20:4 n-6	3.7±0.4	2.6±0.6	1.8±0.6	1.6± 0.3	3.1±0.3	2.6±0.4	2.1±0.7	1.1±0.3	3.9±0.6	3.0±0.3	2.3±0.3	1.9±0.2
C20:5 n-3	16.1±0.4	14.7±0.5	13.8±0.3	13.0±0.5	14.7±0.4	12.3±0.9	9.1±1.2	6.6±0.7	14.9±0.8	13.0±0.7	10.9±0.8	10.9±0.3
C22:5 n-3	0.2±0.0	0.2±0.0	0.1±0.0	0.1±0.0	0.3±0.1	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
C22:6 n-3	14.2±1.1	12.1±1.0	11.2±0.4	9.0±0.6	15.0±0.4	14.7±0.6	13.9±0.9	13.4±0.3	15.3±0.9	14.1±1.1	12.7±0.6	11.6±0.7
Σ PUFA	7.2±1.0	9.3±0.5	9.4±0.7	11.4±1.4	6.9±1.0	8.2±1.0	8.9±0.8	9.4±1.0	7.0±1.0	9.1±1.7	10.5±1.0	11.4±2.1
Σ n-3 HUFA	30.9±1.4	27.6±1.4	25.7±0.8	22.5±0.9	30.2±0.7	27.4±1.1	23.2±1.7	20.1±0.8	30.7±1.4	27.8±1.6	24.1±1.1	23.1±1.0
Σ n-6 HUFA	3.7±0.4	2.6±.0.6	1.8±0.6	1.6±0.3	3.1±0.3	2.6±0.4	2.1±0.7	1.1±0.3	3.9± 0.6	3.0±0.3	2.3±0.3	1.9±0.2

Values are means of triplicate analysis ± S.D; tr=trace levels (less than 0.1% of total fatty acids); PUFA = ≥16:2 < 20:4 ; HUFA = ≥20:4 ; Total fatty acids do not add up to 100 as minor components are not included.

Other fatty acids which recorded decreasing concentration with larval development include 18:0 and 18:1 n-9. Rest of the fatty acids exhibited an increasing trend, when larvae were fed with algae rich in a particular fatty acid or showed a slight decline when the algae it was fed was deficient in them. Comparison between treatments for the vital HUFAs in M_I showed that *Chaetoceros*-fed larvae had the highest amount of EPA. Meanwhile, the larvae were significantly poor ($P<0.05$) in DHA than the other two treatments. The ARA value of M_I of the same treatment (*Chaetoceros*-fed) was slightly higher than larvae from *Isochrysis* treatment, but not significant ($P>0.05$). *Isochrysis*-fed larvae at M_I stage had only good quantity of DHA, significantly ($P<0.05$) higher from *Chaetoceros*-fed larvae but not from M_I fed mixed algae Mixed algae-fed myses, recorded EPA value not significantly different from *Chaetoceros*-fed larvae. It had DHA value closer to *Isochrysis* -fed larvae and highest ARA value.

Larval rearing experiments

The survival from Z_I to M_I stage was higher with the mixed algae treatment followed by *Chaetoceros*-fed larvae and then by *Isochrysis*-fed larvae (Table 27). However, the values did not show any statistically significant difference ($P>0.05$). The total length of M_I larvae also exhibited a similar trend, but all three treatments differed significantly from each other. The development index value of the mixed algae fed larvae differed significantly from other treatments ($P<0.05$). However, the value from the other two treatments failed to show any significance between them ($P>0.05$).

A comparative analysis of HUFA composition of M_I fed with different algae or algal combinations is given in table 28. It can be seen that the mixed algae-fed larvae had a medium quantity of EPA, (significantly higher than *Isochrysis*-fed larvae; $P<0.05$). medium amount of DHA and the highest ARA quantity.

Table 27. The survival till mysis 1, total length of mysis 1 and development index of *F. indicus* zoeae fed with EPA rich microalgae (*Chaetoceros*) or DHA rich microalgae (*Isochrysis*) or their combination.

Treatment	Survival % (Z_I-M_I)	Total length of M_I (mm)	Development Index
Chaetoceros	89.3 ± 1.44^a	4.44 ± 0.029^a	1.93 ± 0.01^a
Isochrysis	83.7 ± 1.44^a	4.27 ± 0.025^b	1.86 ± 0.02^a
Algal mix	90.7 ± 1.06^a	4.55 ± 0.011^c	1.98 ± 0.01^b

Means ± S.E., with different superscript in the same column differ significantly ($P<0.05$)

Table 28. Major HUFA composition of mysis 1 larvae fed with two different microalgae and their mix.

Treatment	Fatty acid content (% TFA) and related ratio values				
	EPA	DHA	ARA	EPA/DHA	Σ n-3 HUFA
Chaetoceros	13.0 ± 0.5^a	9.0 ± 0.6^a	1.6 ± 0.3^a	1.46 ± 0.06^a	22.5 ± 0.9^a
Isochrysis	6.6 ± 0.7^b	13.4 ± 0.2^b	1.1 ± 0.3^a	0.49 ± 0.04^b	20.1 ± 0.8^b
Algal mix	10.9 ± 0.3^a	11.6 ± 0.7^b	1.9 ± 0.2^a	0.94 ± 0.02^c	23.1 ± 1.0^{ab}

Values are means ± S.D; Means within the same column having different superscript are significant ($P<0.05$)

DISCUSSION

Biochemical composition of various microalgae have been analysed and reported (Brown and Jeffery, 1992; Brown *et al.,* 1997; Brown, 2002). The nutritional value of algae is related to its biochemical composition and among the nutrients lipid and fatty acids are considered vital (Watanabe *et al.*, 1983; Shamsudin, 1992) especially when fed to early developing larvae.

As unsaturated fatty acids (UFA) are considered essential for the initial larval stages (Jones *et al.,* 1979) more attention has been turned towards content of such fatty acids in micro algae (Volkman *et al.,* 1989, 1992; Dunstan *et al.,* 1993; Harel *et al.*, 2002). Later studies evaluated the algae based on its PUFA content and analysed the effect of feeding such algae on the biochemical composition of animal tissues ,survival and growth (Barclay and Zeller, 1996; Caers *et al.,* 2003). Similar works were also carried out with shrimp larvae (D'Souza and Loneragen, 1999; D'Souza and Kelly, 2000).

In this study, the analysis of fatty acid composition of the two micro algae confirmed the differences in the biochemical composition between algae from different classes. While the diatom exhibited the dominance of EPA, the flagellate contained higher amount of DHA.

Only limited studies have dealt with the fatty acid composition of various shrimp larval stages (Ward *et al.,* 1979; Teshima and Kanazawa, 1982; Cahu *et al.,* 1988; D'Souza and Kelly, 1999; Gonzalez-Felix *et al.*, 2002). In the present study, among the two dominant monounsaturates 16:1 n-7 was noticed to increase with larval development while the 18:1 n-9 showed a decrease in level. Nevertheless. greater difference in

quantity of both monounsaturates at the algae level was not indicated in the larval biochemical composition. This suggests that most of the nutrient had come from the egg and gets retained till the zoea stage. The reduction in 18:1 n-9 indicates its requirement for the developing larvae. Mourente *et al.* (1995) also reported that the 18:1 n-9 level did not increase from zoea to mysis stages of *Melicertus kerathurus*.

The PUFA, 18:3 n-3 though was low in *Chaetoceros*, developing larvae accumulated the same rapidly. The same fatty acid was suggested to influence the development rate of *Penaeus semisulcatus* larvae by D'Souza and Kelly (1999). Thus, it could be assumed that the accumulation of 18:3 n-3 by *Chaetoceros*-fed larvae was sufficient enough to avoid remarkable difference in the development rate between them and 18:3 n-3 rich *Isochrysis*-fed larvae. Noticeably, the mixed-algae-fed larvae had comparatively higher quantity of same PUFA from early stage itself, suggesting the possible role of same in influencing development index (DI). The increment in the larval content of 18:3 n-3 compared to declining EPA and DHA supports the greater nutritive value of EPA and DHA versus 18:3 n-3, as suggested previously (Guary *et al.*, 1976; Kanazawa *et al.*, 1979a,b; Xu *et al.*, 1994b).

In general, it has been proved that HUFAs have high nutritional value than PUFAs (Cahu *et al.*, 1986; 1994; Teshima *et al.*, 1992; Gonzalez-Felix *et al.*, 2003). Though the total n-3 HUFA content differed with the three algal combinations, it did not seem to affect its content in zoeae. However, at mysis 1 stage, the larvae fed *Isochrysis* indicated lower content of n-3 HUFA. As it has been suggested, that higher n-3 HUFA could improve the growth and survival of larvae (Sorgeloos and Leger, 1992), this could be a reason for lower growth and survival obtained with *Isochrysis*. In *M. kerathurus*, Mourente *et al.* (1995) reported that all fatty acids increased in content (in terms of pg

fatty acid/μg dry weight) with larval development, but observed decrease after M_1 stage. This contradicts with the present results, where the reduction occurred from zoea stage itself.

The present results corroborate with the results of Cahu *et al.* (1988) in *F. indicus*. Using a flagellate (*Pavlova lutheri,* containing good amount of EPA and DHA and lesser ARA) as the live feed these authors reported that both EPA and DHA were present in equal amounts in neutral and phospholipids of eggs and their level became lower and lower during larval development. The difference or rather delay in decline of HUFA with Mourente *et al.* (1995) could not be explained as the data were not provided for individual stages but as an average of all substages. Moreover, the feeding regime also included rotifer at Z_3 stage. Species-specific differences, broodstock feeding, initial nutrient storage in egg and nauplii, all could have also influenced the decline.

The fatty acid composition of developing larvae in this study also confirmed that the same was influenced by the composition of diet as it had been reported previously for shrimp (Cahu *et al.,* 1988). D'Souza and Loneragan (1999) tried four different algae for the shrimp larvae and noticed that the algae which gave the best results had higher levels of EPA and ARA.

From the present results, the mediocre performance of larvae fed DHA-rich, *Isochrysis* suggest that the high amount of DHA in the algal diet alone was not sufficient for the performance of larvae. This agrees with the results of D'Souza and Loneragan (1999). It may be that the limited quantity of DHA in *Chaetoceros* added to large proportions of DHA in nauplii was sufficient to meet the larval requirements for their

development to M_1. Meanwhile, results also indicate that ratio of EPA and DHA in larvae could have influenced the larval performance.

As seen from table 28, the EPA/DHA ratio was close to 1 with mixed algae-fed tanks, while other two were at significant ($P<0.05$) extremes. Thus, a ratio value closer to 1 in the larval body seems to perform better. There seems to be saturation level for EPA as noticed with *Chaetoceros*-fed larvae. Elevated dietary EPA relative to DHA was postulated to have a negative impact on fish larval neural function and thus growth and survival (Rodriguez *et al.*, 1997). The results in turn also suggest the equal quantity requirement for EPA and DHA in the larval tissue.

The importance of n-6 fatty acid ARA in larval performance could not be pointed out from these experiments, as the larvae did not differ in their content appreciably. Even then, the larvae which gave better results (mixed algae) had slightly higher levels of ARA than the poor performers and there was reduction in its quantity with larval development. Cahu *et al.* (1988) noticed that only during the Z_2 and Z_3 stages the ARA exhibited decline and then increased.

The higher impact of different microalgae on development than survival was also noticed by D'Souza and Kelly (2000). The authors proposed that the effect was not only due to the fatty acids but also influenced by other nutrient components like protein, carbohydrates etc. However, in another experiment, where number of microalgae were tried for various shrimps, D'Souza and Loneragen (1999) noticed that the lipid and carbohydrate compositions of the algae had little or no effect on the lipid and carbohydrate compositions of the larvae or their performance. Interestingly, the larvae

that performed best had significantly more lipid and carbohydrate than those that performed worst.

The results from the present study also support the generally accepted and proven fact that mixed algae diets could improve the performance of larvae by providing a more comprehensive range of fatty acids (Kumlu and Jones, 1993; D'Souza and Loneragan, 1999). Moreover, it is important to note that the biochemical composition of algae differs depending upon the culture conditions, age of culture and mode of culture (Brown *et al.*, 1992). Studies have reported even the same strain showing difference in its fatty acid content, related to light regime (Brown *et al.*, 1996), growth phase (Dunstan *et al.*, 1993), light intensity (Thompson *et al.*,1993), culture media (Ben Amotz *et al.*, 1985), temperature (Thompson *et al.*, 1992), pH (Guckert and Cooksey, 1990), and season (Pernet *et al.*, 2003). Moreover, many of the changes were said to be species-specific.

It is recommended that individual analysis of the chosen algae is carried out and decided for use based upon the complementary nature of fatty acids mainly for PUFA and HUFA. The recent objective for the selection of microalgae based on fatty acid composition targets not only growth and survival but also the resultant effect on immune functions of the animal (Delaporte *et al.*, 2003). The following conclusions could be drawn from the present experiment,

1) All vital HUFAs namely EPA, DHA and ARA in their order of importance showed a decreasing trend during larval development, confirming their need for development,

2) The reduction of a particular fatty acid in larval stages during development was directly related to the presence of the same in the diet.

3) Other than the HUFAs, the quantity of 18:1 n-9 and 18:3 n-3 in the larvae also could affect the development,

4) The larvae fed mixed algae diet with balanced HUFA nutrition resulted in better growth and survival. A HUFA n3/n6 ratio value close to 1 seems to give better results,

5) The ARA also (though could not be proved) could influence the development.

6) The study further emphasizes the importance of considering the relative amounts of DHA, EPA and ARA simultaneously in shrimp larval feed as it has been demonstrated for fish (McEvoy *et al.*, 1998; Sargent *et al.*, 1999).