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Effect of Dietary *Artemia* Nauplii Enriched with Fish Oil, on Survival, Growth and Biochemical Analysis of Mullet, *Mugil Cephalus*, Larvae.

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ABSTRACT

The effects of dietary *Atemia* nauplii enriched with fish oil (rich in both n-3 and n-6 long chain fatty acids) on survival, growth and biochemical analysis of mullet, *Mugil cephalus*, larvae were studied. Two levels of fish oil (2 and 4 g /million *Artemia*) were applied for two different exposure periods (6 or 12 h) for the newly hatched brine shrimp *Artemia* and compared with normal *Artemia* (without additives)in feeding mullet larvae.Final body weight, survival rate, weight gain and specific growth rate significantly ($p<0.05$) increased with increasing the level of emulsified fish oil used to treat *Artemia* and with decreasing the exposure time to the emulsified fish oil. Total lipid significantly increased ($p<0.05$) with increasing the amount of emulsified fish oil used to treat *Artemia* and with decreasing the exposed time of the emulsified fish oil. Unsaturated fatty acids and EPA/DHA ratio significantly increased ($p<0.05$) with increasing the amount of emulsified fish oil used to treat *Artemia* and with decreasing the exposure time of the emulsified fish oil. Consequently growth and survival rate of mullet larvae increased significantly ($p<0.05$). ARA, EPA, and DHA increased significantly ($p<0.05$) with increasing the amount of the emulsified fish oil used to treat *Artemia* and with decreasing the exposure time of the emulsified fish oil giving better survival and growth of the *Mugil cephalus* larvae. Larvae at all treatments exhibited signs of stress through the weaning period, especially in the untreated aquaria. According to the results of the present work it was recommended that mullet larvae, *Mugil cephalus*, fed treated *Artemia* with 4g fish oil/million *Artemia* for 6 hours exposure time showed the best survival rate and higher growth before weaning stage.

Keywords: *Artemia* nauplii, enrichment, survival, biochemical analysis, mullet, larvae.

INTRODUCTION

In the Mediterranean Sea, there has been a decline in fry availability of some mullet species in recent years due to pollution and over-fishing of parent stocks: *M. cephalus*, for instance, has shown a decrease in number (Boglione *et al.*, 2006).

The aquaculture industry relies on the use of live food organisms (copepods, rotifers, or brine shrimp) for the successful rearing of marine fish and shrimp larvae. Nauplii of the brine shrimp *Artemia* are widely used, In order to enhance the nutritional value of the nauplii, several enrichment techniques have been developed (Han *et al*., 2005).

Determining the larval dietary requirement for essential fatty acids is an important step in the development of marine fish culture for any species (Izquierdo, 1996 and Takeuchi, 1997). Eicosapentaenoic acid (20:5 n-3, EPA), docosahexaenoic acid (22:6 n-3, DHA), and arachidonic acid (20:4 n-6, ARA) are all considered essential for marine fish and must be provided in the diet (Bransden *et al*., 2005).

Live food such as rotifers and *Artemia* that are often used as primary feeds for marine fish larvae generally lack these essential fatty acids, and therefore, these prey must be supplemented or enriched to met the needs of the growing larvae (Sargent *et al*., 1999).

However, the larvae fed on rotifers and *Artemia* containing a low level of n-3 HUFA showed poor growth, high mortality and high rate of deformity. Enrichment of live food for marine fish larvae with n-3 HUFA has been shown to improve growth and survival by many researchers (Gapasin *et al*., 1998; Liu, 1998 and Robin, 1998).

Various enrichment emulsions have been formulated differing in the fatty acids composition of their triglycerides, vitamins E and C have been incorporated in booster formulations that increase the level of ascorbic acid (AA) in *Artemia* to 2000-ppm (Sorgeloos *et al*., 2001), which offer more possibilities to satisfy the needs of different species and help to reduce problems related to disease, stress resistance, malformation, and pigmentation in numerous fish species (Sorgeloos *et al*., 2001).

Ogata *et al*., (2004), studied the fatty acid composition of five candidate aquaculture species and indicated that, broodstock management and larviculture technologies based on the nutritional traits specific to tropical species should be developed in order to establish a stable fry supply and availability. So this study was

undertaken to study the effect of feeding enriched *Artemia* with emulsified fish oil on survival and growth performance of mullet larvae according to the biochemical characteristics.

MATERIALS AND METHODS

Experimental design

This experiment was carried out in Marine Fish Laboratory (MFL) of the Faculty of Agriculture (Saba Basha), Alexandria University throughout the period of November to early October 2005.

The newly hatched *Artemia* nauplii were used to feed the fish larvae in this experiment after they were enriched by different treatments in a factorial design using fish oil level as a factor with two exposure time to the fish oil as follows:

- T1: Untreated *Artemia*.
- T2: 2 g emulsified fish oil for 6 h.
- T3: 2 g emulsified fish oil for 12 h.
- T4: 4 g emulsified fish oil for 6 h.
- T5: 4 g emulsified fish oil for 12 h.

Mugil cephalus larvae were collected from El-Maadia coast, Behyra governorate eastern Alexandria. Acclimatized for seven days by reducing salinity 1ppt daily according to El-Dahhar (2000).

The experiment was carried out at five treatments, triplicate glass aquaria (30 L), 15 larvae for each aquaculture with initial BW 0.045 g and total length of about 1.5cm. 500 individual *Artemia*/L was used to feed fish.

Brackish water, aeration, light and temperature (22-23^0 C) were adjusted. Fish feeding, aquaria management and zymogene addition (4% of the diet) were controlled according to El-Dahhar (1999).

Feeding stages through this experiment

1-First 28 days, the larvae were fed enriched *Artemia* (500 ind. /L/day)

2-The next 14 days, *Artemia* were replaced with artificial food gradually to reach 40% of the larval BW, and then continue for the last 14 days.

Diet formulation and preparation

The larvae were fed throughout the period of this study with a diet containing 44.8% dietary crude protein and 495.79 kcal energy/100g. The composition and chemical analysis of the diet are given in Table (1).

Mixtures were homogenized in a food grinder Model 5 NFGA (Kitchen Aid St. Joseph, MI 49085 USA. Zymogene, vitamins and minerals mixture and ascorbic acid were added to the diet after adding the boiling water.

Table 1: ***The composition and chemical analysis of the experimental diet used during the study to feed larvae.***

Ingredients.	Content %
Wheat flour.	**20.1**
Powder milk.	**17.8**
Boiled egg.	**17.4**
Fish meal.	**39.0**
Fish oil.	**1.8**
Vitamin & Mineral mix*.	**0.8**
Ascorbic acid.	**0.4**
CMC.**	**2.7**
Proximate Analysis (%dry matter basis)	
Dry mater	**69.43**
Crude protein	**44.80**
Crude fat	**9.71**
Crude fiber	**0.00**
Carbohydrate	**36.38**
Ash	**9.11**

****Vitamin and mineral mixture/2.5kg premix: Vitamin A, 12 million IU; Vitamin D_3 2 g; Vitamin E, 10 g; Vitamin k, 2 g; Vitamin B_1, 1 g; Vitamin B_6, 1.5 g; Vitamin B_{12}, 10 mg; Vitamin B_2, 4 g; Pantothinic acid, 10 g; Nicotinic acid, 20 g; Folic acid, 1000 mg; Biotin, 50 mg; Choline chloride, 500 g; I, 1g; Iron, 30g; Mn, 55g; Zn, 55g; Selenium,1 g. (P Faezer Co.)***

*****CMC: Carboxi -Methyl-Cellulose*.**

Artemia hatching

Artemia cysts were collected (El-Naser Saline Co., Burg El-Arab Saline Sector, Alexandria, Egypt) and hatched with the addition of saline water, then the density of the *Artemia* nauplii was estimated. Emulsification of the fish oil was carried out using (% wet weight) 50.0% fish oil, 49.9% warm water and 0.1% emulsifier (Lecithin).

Biochemical analysis

Larvae samples was weighed to the nearest ±0.0001 mg, frozen then dried at 60 ºC overnight. Moisture contents were kept frozen until further analysis (AOAC, 1984).

Total lipid contents were determined according to Folch *et al.* (1957). Fatty acids contents were prepared as fatty acid methyl esters from lipid according to Radwan (1978).

Statistics

Factorial design was performed on the related between different treatments. Differences in larval survival, weight gain, specific growth rate, condition factor, moisture, lipids and fatty acids contents through the experiments were tested using an ANOVA. Data are expressed as mean±standard error. In all cases, variable were transformed as needed in order to meet the maintenances requirements of mullet larvae according to Gatline *et al*., (1986). Defferences were considered significant for ($P<0.05$).

RESULTS

Growth performance

Larval growth rate significantly ($P<0.05$) increased through the 2^{nd}, 7^{th} and 8^{th} weeks (wk) as indicated in Figures 1 and 2. While increasing the exposure time of *Artemia* to the

emulsified fish oil increased the growth rate of these larvae significantly ($P<0.05$) through the 5th and 7th wk. Using the enriched *Artemia* with increasing levels of fish oil from zero to two and to four grams of fish oil to feed the larvae increased body weight (BW) significantly ($P<0.05$) with a highest values of 0.161±0.017 and 0.182±0.024 g for those fed *Artemia* enriched by 4 g fish oil enriched *Artemia* in the 7th and 8th wk, respectively. Starting from the 4th wk using the *Artemia* enriched with 4 g oil gets the highest larval BW having the values of 0.098±0.008, 0.110±0.011, 0.138±0.013, 0.161±0.017 and 0.182±0.024 for weeks 4th, 5th, 6th, 7th and 8th, respectively.

Duration of treating *Artemia* with the fish oil also have a significant ($P<0.05$) effect on the larval BW with higher values of 0.107±0.007 and 0.141±0.015 g for those fed enriched *Artemia* for 12 h in the 5th and 7th wk. Generally, using the enriched *Artemia* with the emulsified fish oil for 12 h to feed the larvae gets the higher larval BW starting from the 3rd wk until the last week (wk 8). The values were found to be 0.072±0.006, 0.096±0.006, 0.107±0.007, 0.131±0.010, 0.141±0.015, and 0.145±0.022 g, respectively. But the values of BW of the larvae fed the enriched *Artemia* with the fish oil for 6 h were found to be 0.063±0.004, 0.091±0.003, 0.092±0.003, 0.119±0.005, 0.118±0.006 and 0.130±0.013 from 3rd wk until the last week (8th wk), respectively.

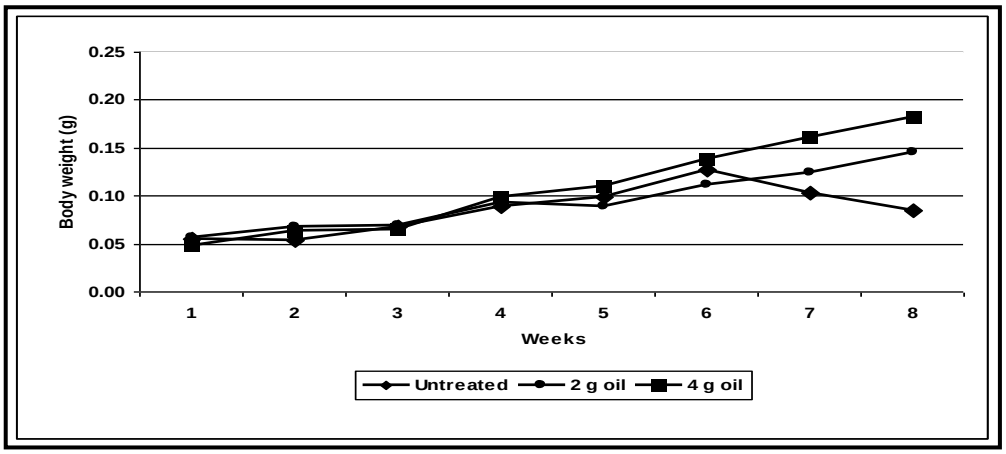

Figure 1: ***The relationship between time (weeks) and means of body weight (g) of flathead mullet, Mugil cephalus, larvae fed Artemia enriched with 0, 2 and 4 g emulsified fish oil.***

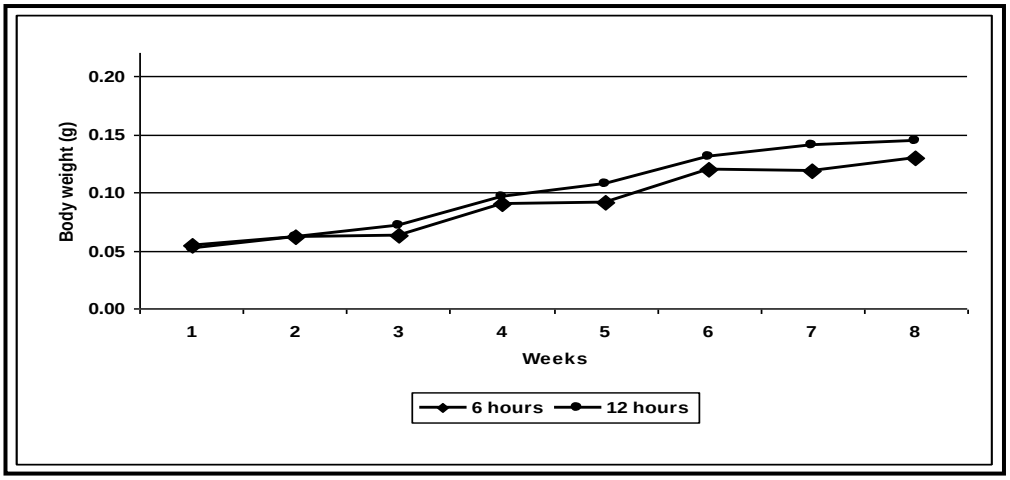

Figure 2: ***The relationship between time (weeks) and means of body weight (g) of flathead mullet, Mugil cephalus, larvae fed Artemia enriched with emulsified fish oil for 6 or 12 hour.***

There was a significant interaction ($P<0.05$) between the two factors (amount of emulsified fish oil and the *Artemia* exposure time to the fish oil) in the 2nd and 8th wk. The highest value 0.071±0.005 g was detected with 2 g fish oil for 12 h treatment through the 2nd wk, while it reached to 0.224±0.030 g with 4 g fish oil for 6 h exposure time at the end of this experiment (8th wk). However, 4 g of fish oil for 6 h was the best treatment starting from the 4th wk until the end of the experiment. The weekly values of larval BW fed *Artemia* treated with this treatment were 0.108±0.015, 0.125±0.018, 0.149±0.026, 0.190±0.022 and 0.224±0.030 from the 4th wk until the last week, respectively.

Survival rate %

Table 2 indicated that, replacing the amount of fish oil from zero to four and two grams used to enrich *Artemia*, increased survival rate (%) significantly ($P<0.05$) after weaning. On the other hand, increasing the time of *Artemia* expose to the fish oil from 6 to 12 h decreased survival rate (%) insignificantly ($P<0.05$) before and after weaning. The higher values were detected with the larvae fed enriched *Artemia* with fish oil for 6 h after weaning.

Interaction between the amount of emulsified fish oil and time of expose of *Artemia* to the fish oil significantly ($P<0.05$) affected the larval survival rate (%) after weaning. The highest values were found with 2 g fish oil for 6 h treatment before and after weaning.

Table 2: ***Average of survival rate (%) of flathead mullet, Mugil cephalus, larvae fed for eight weeks at five groups of treated Artemia with emulsified fish oil (2 and 4 g/million Artemia) for 6 or 12 hour during the experiment, before and after weaning.***

Oil conc. gram	Exposure time hour	Survival%*	
		Before weaning	After weaning
0	0	86.67±7.70	15.56±2.22^{d}
2	6	95.56±2.22	73.33±7.70^{a}
	12	86.67±3.85	48.89±2.22^{bc}
4	6	73.33±20.37	31.11±2.22^{c}
	12	93.33±0.00	66.67±20.37^{ab}
Pooled means			
0		86.67±4.87	15.56±1.41^{y}
2		91.11±2.81	61.11±6.51^{x}
4		83.33±10.15	48.89±12.13^{x}
	6	88.89±2.72	43.70±9.57
	12	85.19±7.10	40.00±8.96

Values represent means ± (SE) of N = 3 replicates, each contain 15 fish/treatment.
Means in the same column not shearing the same superscript are significantly different (P<0.05).
****. Survival % = (final no. of fish/initial no. of fish) x 100.***

Weight gain

Weight gain (WG) of the larvae fed the five treatments of this experiment is shown in Tables 3& 4. Increasing the amount of fish oil from zero to two and four grams used to enrich *Artemia* , increased larval WG significantly (P<0.05) before and after weaning. increasing the time of *Artemia* exposure to fish oil from 6 and 12 hours also increased larval WG insignificantly (P<0.05) after weaning. The higher values were found with larvae fed with enriched *Artemia* for 12h before and after weaning.

Interaction between the amount of emulsified fish oil and duration of *Artemia* exposure to the fish oil significantly (P<0.05) affected the larval WG before and after weaning. The highest values were found with 4 g fish oil for 6 h before and after weaning.

Specific growth rate (SGR %/d)

SGR%/d of the larvae fed the five treatments (Tables 3& 4) indicated that increasing the amount of fish oil from zero to two and four grams used to enrich *Artemia*, increased larval SGR(%/d) significantly (P<0.05) before and for after weaning. Increasing the time of *Artemia* exposure to the fish oil from 6 and 12 hours, caused an increase in the larval SGR(%/d) significantly (P<0.05) before and after weaning. The higher values were found with larvae fed with enriched *Artemia* for 12h before and after weaning.

Interaction between the amount of emulsified fish oil and time of expose the *Artemia* to the fish oil significantly (P<0.05) affected the larval SGR(%/d) before and after weaning. The highest values were found with 4 g fish oil for 6 h before and after weaning.

Condition factor (K)

Generally, insignificant (P<0.05) effect was achieved in the larval condition factor at the end of the experiment. However, increasing the amount of fish oil to enrich *Artemia* from zero to two and four grams increased K. While, increasing the time of *Artemia* exposure to the fish oil from 6 to 12 h almost didn't record any differences in the K.

Interaction between the amount of emulsified fish oil and time of exposure of *Artemia* to the fish oil insignificantly (P>0.05) affected the larval K. The highest value was found with 4 g fish oil for 6 h treatment as indicated in Table (4).

Chemical composition of Mugil cephalus larvae

Moisture contents

Generally, at the end of this experiment insignificant (P>0.05) effect was achieved in the larval moisture content (%). However, the comparison between the amounts of fish oil to enrich *Artemia* of two, four and zero grams, find that larval moisture content decreased from 74.11±0.66 to 73.69±1.04 and to 72.08±1.20 %, respectively. Also, increasing the time of *Artemia* exposure to the fish oil from 6 and 12 hours decreased larval moisture content from 73.95±0.95 to 72.64±0.77%, respectively.

Interaction between the amount of emulsified fish oil and time of expose *Artemia* to the fish oil insignificantly (P < 0.05) affected the larval moisture content. The highest value was found with the larvae fed *Artemia* enriched with 4 g fish oil for 12 h., it was found to be 75.13±0.52% as indicated in Figure (3).

Table 3*: Average of weight gain (g) and specific growth rate (SGR%/day) of flathead mullet, Mugil cephalus, larvae fed for eight weeks at five groups of treated Artemia with emulsified fish oil (2 and 4 g/million Artemia) for 6 or 12 hour during the experiment, before weaning.*

Oil conc. gram	Exposure time hour	Average±SE	
		WG (g)*	SGR%/day**
0	0	0.033±0.005^{b}	1.77±0.49^{b}
2	6	0.037±0.006^{b}	1.86±0.32^{b}
	12	0.036±0.010^{b}	1.73±0.43^{b}
4	6	0.062±0.004^{a}	3.38±0.74^{a}
	12	0.037±0.003^{b}	1.98±0.13^{b}
Pooled means			
0		0.033±0.003^{y}	1.77±0.31^{y}
2		0.037±0.005^{y}	1.80±0.24^{y}
4		0.050±0.006^{x}	2.68±0.46^{x}
	6	0.035±0.004^{h}	1.83±0.19^{h}
	12	0.044±0.005^{g}	2.34±0.38^{g}

Values represent means ± (SE) of N = 3 replicates, each contain 15 fish/treatment.
Means in same column not shearing the same superscript are significantly different (P<0.05).
****. Weight gain (WG) = final weight - initial weight.***
*****. Specific growth rate (SGR%/d) = [(Ln final body weight - Ln initial body weight) / No of days] X 100.***

Total lipid contents

Figure (3) indicated that significantly (p<0.05) increasing the amount of fish oil from zero to two and to four grams used to enrich *Artemia*, increased larval lipid contents from 10.37±0.21 to 13.70±1.11 and to 17.27±1.09 %, respectively. Increasing the time of *Artemia* exposure to the fish oil from 6 and 12 hours also increased larval lipid contents significantly (P<0.05). The higher value was found with larvae fed with enriched *Artemia* for 12 h being 14.98±1.58 %.

Interaction between the amount of emulsified fish oil and time of *Artemia* exposure to the fish oil significantly (p<0.05) affected the larval lipid content % at the end of this experiment. The highest value 18.94±0.16% was found with 4 g fish oil for 6 h treatment.

Fatty acids contents

The concentrations of saturated fatty acids (SFAs), unsaturated fatty acids (UFAs) (monounsaturated fatty acids (MUFAs), omega-6 highly unsaturated fatty acids (n-6 HUFAs) and omega-3 highly unsaturated fatty acids (n-3 HUFAs)) of the larvae fed

Table 4: ***Average of weight gain (g), condition factor (K) and specific growth rate (SGR%/day) of flathead mullet, Mugil cephalus, larvae fed for eight weeks at five groups of treated Artemia with emulsified fish oil (2 and 4 g/million Artemia) for 6 or 12 hour during the experiment, after weaning.***

Oil conc. gram	Exposure time hour	Average±SE		
		WG(g)*	K**	SGR%/d***
0	0	0.029±0.006^{d}	0.89±0.10	0.81±0.26^{d}
2	6	0.070±0.003^{c}	0.93±0.15	1.48±0.11^{c}
	12	0.107±0.010^{b}	1.11±0.22	1.86±0.16^{b}
4	6	0.178±0.017^{a}	1.19±0.30	2.99±0.37^{a}
	12	0.089±0.014^{bc}	1.06±0.31	1.80±0.18^{b}
Pooled means				
0		0.029±0.004^{z}	0.89±0.06	0.81±0.17^{z}
2		0.089±0.009^{y}	1.02±0.13	1.67±0.12^{y}
4		0.134±0.022^{x}	1.12±0.19	2.39±0.33^{x}
	6	0.075±0.013	1.02±0.12	1.49±0.20^{h}
	12	0.093±0.023	1.01±0.11	1.76±0.35^{g}

Values represent means ± (SE) of N = 3 replicates, each contain 15 fish/treatment.
Means in same column not shearing the same superscript are significantly different (P<0.05).
1. Weight gain (WG) = final weight - initial weight. 2. Condition factor (K) = (BW/TL^{3}) x 100.
3. Specific growth rate (SGR%/d) = [(Ln final body weight - Ln initial body weight) / No of days] x 100.

on the five treatments of the experiment are shown in Tables 5, 6, 7 and 8. Obviously, SFAs decreased significantly (P<0.05) with increasing the amount of fish oil used to enrich *Artemia* from zero to two and to four grams. Also, it decreased significantly (P<0.05) with increasing the time of *Artemia* exposure to fish oil from 6 to 12 h. Interaction between the two factors (the amount of fish oil and time of *Artemia* exposure to fish oil) was significant (P<0.05). The highest value of SFAs was observed with the larvae fed untreated *Artemia*. SFAs decreased as the amount of fish oil increased and the time of *Artemia* exposure to fish oil increased. The lowest value of the SFAs was observed with the larvae fed *Artemia* enriched with 4 g of fish oil for 12h (Tables 5 & 6).

The concentration of UFAs is generally increased significantly (P<0.05) in the larval body with increasing the amount of fish oil used to enrich *Artemia* from zero to two and to four grams, respectively. Also the

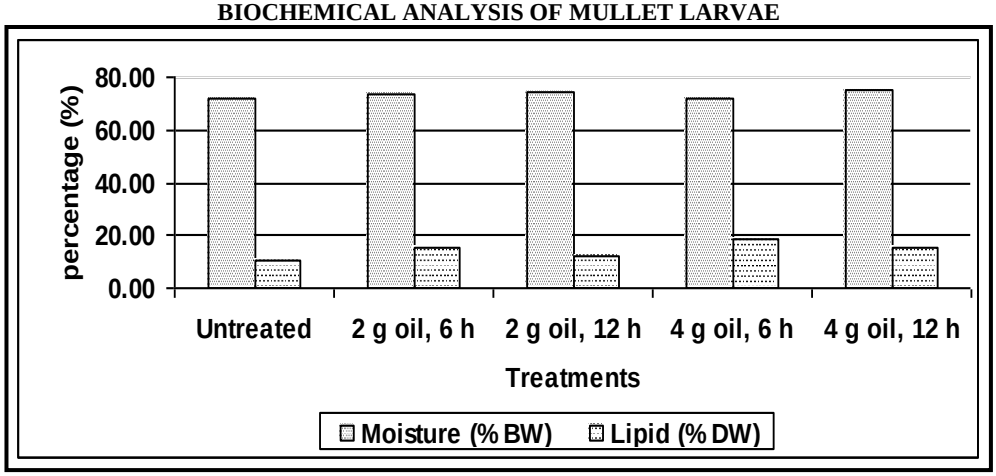

Figure 3*: Average of moisture and lipid (% of the dry weight) of flathead mullet, Mugil cephalus, larvae fed for eight weeks at five groups of treated Artemia with emulsified fish oil (2 and 4 g/million Artemia) for 6 or 12 hour at the end of the experiment.*

values of UFAs increased with increasing the time of *Artemia* exposure to fish oil from 6 to 12 h, respectively. The interaction between the fish oil and time of expose *Artemia* to fish oil was significant ($P<0.05$). The best value was found with the larvae fed *Artemia* enriched with 4 g fish oil for 12 h.

MUFAs reached the lowest value with the larvae fed untreated *Artemia*, while they increased significantly ($P<0.05$) with increasing the amount of the fish oil to 2 g. But it decreased when the fish oil increased to 4 g. Also the values of MUFAs increased with increasing the time of expose *Artemia* to fish oil from 6 to 12 h. The higher concentrations of MUFAs were achieved when the larvae fed *Artemia* enriched with 2 g fish oil for 12 h (Table 5 and 7).

Omega-6 HUFAs increased significantly ($P<0.05$) with increasing the amount of fish oil used to enrich *Artemia* from two to four grams, but the highest value was achieved with untreated *Artemia*. However, they decreased significantly with increasing the time of *Artemia* exposure to the fish oil from 6 to 12 h. Interaction between the two factors (the amount of fish oil and time of *Artemia* exposure to fish oil) was significant ($P<0.05$). The highest value was found with the larvae fed *Artemia* enriched with 4 g of fish oil for 6 h (Table 5 and 8).

Omega-3 HUFAs had almost the same rhythm of the MUFAs. There was a significant decrease ($P<0.05$) in the n-3 HUFAs with increasing the amount of fish oil from two to four grams. While they increased with increasing the time of expose *Artemia*

Table 5: ***Average of fatty acids contents % of flathead mullet, Mugil cephalus, larvae fed for eight weeks at five groups of treated Artemia with emulsified fish oil (2 and 4 g/million Artemia) for 6 or 12 hour during the experiment.***

Oil conc.	Exposure time	Fatty acids % of the total					
		∑ Saturated	∑Unsaturated				EPA/DHA
gram	hour		∑Mono	∑n-6 HUFAs*	∑n-3 HUFAs	Total	
0	0	45.84±0.40^{a}	19.89±0.46^{e}	10.93±0.06^{bc}	23.34±0.22^{e}	54.16±0.74^{e}	0.53±0.38^{e}
2	6	38.05±0.32^{b}	23.65±0.37^{c}	6.45±0.04^{d}	31.89±0.25^{c}	62.00±0.66^{d}	0.57±0.25^{d}
	12	31.16±0.24^{d}	27.41±0.24^{a}	0.98±0.02^{e}	40.45±0.24^{a}	68.84±0.50^{bc}	0.61±0.10^{c}
4	6	35.59±0.39^{c}	21.16±0.38^{d}	12.24±0.09^{a}	31.01±0.23^{d}	64.41±0.70^{c}	0.62±0.47^{b}
	12	27.98±0.38^{e}	24.92±0.35^{b}	7.53±0.09^{c}	39.56±0.30^{b}	72.02±0.74^{a}	0.65±0.21^{a}
Pooled means							
0		45.84±0.40^{x}	19.89±0.46^{z}	10.93±0.06^{x}	23.34±0.22^{z}	54.16±0.74^{z}	0.53±0.38^{z}
2		34.58±0.28^{y}	25.53±0.31^{x}	3.72±0.03^{z}	36.17±0.25^{x}	65.42±0.58^{y}	0.59±0.18^{y}
4		31.79±0.39^{z}	23.26±0.37^{y}	9.89±0.09^{y}	35.29±0.27^{y}	68.22±0.72^{x}	0.64±0.34^{x}
	6	36.82±0.36^{g}	22.41±0.38^{h}	6.35±0.08^{g}	31.45±0.24^{h}	63.21±0.64^{h}	0.60±0.36^{h}
	12	29.57±0.31^{h}	26.17±0.30^{g}	4.26±0.06^{h}	40.01±0.27^{g}	70.52±0.62^{g}	0.63±0.16^{g}

Values represent means ± (SE) of N = 2 replicates, each contain 15 fish/treatment.
Means in same column not shearing the same superscript are significantly different (P<0.05).
****HUFAs = Highly unsaturated fatty acids; defined as fatty acids with 20 or more carbon atoms and 2 or more double bonds.***

to the fish oil from 6 to 12 h. There were interaction effects on the n-3 HUFAs of larval body content between the amount of the fish oil and the exposure time. The highest value was detected with the larvae fed on *Artemia* enriched with 2 g fish oil for 12 (Tables 5 & 7). The ratio between eicosapentaenoic acid (C20:5, n-3) to docosahexaenoic acid (22:6, n-3) (EPA/DHA) in the larvae fed on the five treatments is shown in Table 5. Increasing the amount of fish oil from zero to two and to four grams used to enrich *Artemia*, increased the ratio of EPA/DHA significantly ($p<0.01$). However, increasing the time of *Artemia* exposure to the fish oil from 6 and 12 hours also increased the ratio of EPA/DHA significantly ($P<0.05$) at the end of this experiment. There was a significant interaction ($P<0.05$) between the two factors (amount of fish oil and the time of *Artemia* exposure to the fish oil). The highest

Table 6*: Average of saturated fatty acids % of the total fatty acids of flathead mullet, Mugil cephalus, larvae fed for eight weeks at five groups of treated Artemia with emulsified fish oil (2 and 4 g/million Artemia) for 6 or 12 hour during the experiment.*

Oil conc. gram	Exposure time hour	Fatty acids*							
		C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C22:0	∑ Saturated
0	0	10.55±0.05^{a}	1.80±0.01^{a}	32.15±0.24^{a}	0.77±0.01^{a}	0.58±0.08^{b}	nd^{o}	nd^{o}	45.84±0.40^{a}
2	6	5.29±0.04^{b}	0.89±0.00^{c}	30.88±0.20^{b}	0.65±0.02^{d}	0.29±0.06^{d}	nd^{o}	nd^{o}	38.05±0.32^{b}
	12	nd^{o}	nd^{o}	29.60±0.22^{c}	0.56±0.01^{e}	nd^{o}	nd^{o}	1.00±0.01^{a}	31.16±0.24^{d}
4	6	5.25±0.03^{c}	1.78±0.01^{b}	26.79±0.19^{d}	0.75±0.04^{b}	0.78±0.10^{a}	0.25±0.02	nd^{o}	35.59±0.39^{c}
	12	nd^{o}	0.88±0.00^{d}	25.51±0.30^{e}	0.66±0.00^{c}	0.49±0.05^{c}	0.25±0.02	0.19±0.01^{b}	27.98±0.38^{e}
Pooled means									
0		10.55±0.05^{x}	1.80±0.01^{x}	32.15±0.24^{x}	0.77±0.01^{x}	0.58±0.08^{x}	nd^{o}	nd^{o}	45.84±0.40^{x}
2		2.65±0.02^{y}	0.45±0.00^{z}	30.24±0.11^{y}	0.60±0.02^{z}	0.14±0.03^{y}	nd^{o}	0.50±0.01^{x}	34.58±0.28^{y}
4		2.63±0.02^{y}	1.33±0.01^{y}	26.15±0.25^{z}	0.71±0.03^{y}	0.64±0.08^{x}	0.25±0.02	0.10±0.01^{y}	31.79±0.39^{z}
	6	5.27±0.04	1.34±0.01^{g}	28.80±0.20^{g}	0.70±0.03^{g}	0.54±0.08^{g}	0.13±0.01	nd^{o}	36.82±0.36^{g}
	12	nd^{o}	0.44±0.00^{h}	27.56±0.26^{h}	0.61±0.01^{h}	0.25±0.03^{h}	0.13±0.01	0.60±0.01	29.57±0.31^{h}

Values represent means ± (SE) of N = 2 replicates, each contain 15 fish/treatment.
Means in same column not shearing the same superscript are significantly different (P<0.05).
**** Fatty acids; C14:0 (Myristic acid), C15:0 (Pentadecanoic acid), C16:0 (Palmitic acid), C17:0 (Heptadecanoic acid), C18:0 (Stearic acid), C20:0 (Arachidic acid), C22:0 (Behenic acid).***
^{o} nd= not detected.

value for the ratio of EPA/DHA was found with larvae fed *Artemia* enriched with 4 g fish oil for 12 h.

DISCUSSION

Lipids are known to provide a large portion of the metabolic energy needed during the early developmental stages of fishes (Sargent, 1995).Vetter *et al*. (1983) found that lipids provide over 98% of the energy utilized during development of red drum eggs. Similarly, in the yolksac larvae of dolphin, gross energy requirements were supplied primarily by endogenous lipid sources (Ostrowski and Divakaran, 1991).

In the present study, many differences were detected in fatty acids concentration in species *Mugil cephalus* and *Liza ramada*. This discrepancy in the fatty acids composition may be related to physiological requirement of fish. Many authors observed the relation between major biochemical components and gonadal maturation of fish (Chaturvedi *et al*., 1976; Sivakami *et al*., 1986 and El-Boray, 1997).

Corraze and Kaushik (1999) stated that; fish are characterized by higher proportion of n - 3 PUEAs,

Table 7: ***Average of monounsaturated fatty acids % of the total fatty acids of flathead mullet, Mugil cephalus, larvae fed for eight weeks at five groups of treated Artemia with emulsified fish oil (2 and 4 g/million Artemia) for 6 or 12 hour during the experiment.***

Oil conc. gram	Exposure time hour	Fatty acids*						
		C14:1	C15:1	C16:1	C17:1	C18:1	C20:1	∑Mono
0	0	3.13±0.01^{e}	0.46±0.03^{d}	11.91±0.11	0.81±0.01^{b}	3.59±0.30^{e}	nd^{o}	19.89±0.46^{e}
2	6	5.21±0.02^{c}	2.25±0.05^{b}	11.01±0.08	0.40±0.01^{d}	4.77±0.21^{c}	nd^{o}	23.65±0.37^{c}
	12	7.29±0.00^{a}	4.05±0.01^{a}	10.12±0.01	nd^{o}	5.96±0.22^{a}	nd^{o}	27.41±0.24^{a}
4	6	4.64±0.00^{d}	0.27±0.06^{e}	10.21±0.10	0.86±0.02^{a}	4.29±0.18^{d}	0.89±0.02	21.16±0.38^{d}
	12	6.72±0.01^{b}	2.06±0.00^{c}	9.32±0.12	0.46±0.01^{c}	5.48±0.20^{b}	0.89±0.01	24.92±0.35^{b}
Pooled means								
0		3.13±0.01^{z}	0.46±0.03^{z}	11.91±0.11^{x}	0.81±0.01^{x}	3.59±0.30^{z}	nd^{o}	19.89±0.46^{z}
2		6.25±0.01^{x}	3.15±0.03^{x}	10.57±0.05^{y}	0.20±0.01^{y}	5.36±0.22^{x}	nd^{o}	25.53±0.31^{x}
4		5.68±0.01^{y}	1.17±0.03^{y}	9.77±0.11^{z}	0.66±0.02^{x}	4.89±0.19^{y}	0.89±0.02	23.26±0.37^{y}
	6	4.93±0.01^{h}	1.26±0.06^{h}	10.61±0.09^{g}	0.63±0.02^{g}	4.53±0.20^{h}	0.45±0.01	22.41±0.38^{h}
	12	7.01±0.01^{g}	3.06±0.01^{g}	9.72±0.07^{h}	0.23±0.01^{h}	5.72±0.21^{g}	0.45±0.01	26.17±0.30^{g}

Values represent means ± (SE) of N = 2 replicates, each contain 15 fish/treatment.
Means in same column not shearing the same superscript are significantly different (P<0.05).
**** Fatty acids; C14:1(Myristoleic acid) n-5, C15:1(cis10-pentadecenoic acid) n-5, C16:1(Palmitoleic acid) n-7, C17:1(Heptadecenoic acid) n-7, C18:1(Oleic acid) n-9, C20:1(Eicosenoic acid) n-9.***
^{o} nd= not detected.

which had direct implication of fatty acids requirements. In this study, fatty acid composition was examined for total fatty acids (TFA) for the both species *Mugil cephalus* and *Liza ramada*, the palmitic acid (16:0) was considered the highest value among the saturated fatty acid for the both species, *Mugil cephalus* and *Liza ramada*, being 16.3 and 13.3% of the TFA, respectively, these data were in agreement with the results detected by Şengör *et al*. (2003). However, the major fatty acids indicated is palmitoleic (C16:1n-7), oleic (C18:1n-9), palmitic (16:0), and linoleic (C18:2n-6) acids of striped mullet ova, *Mugil cephalus* were 19.1, 17.5, 16.3, and 14.7 % of the TFA, respectively. These results agreed with the results reported by Lu *et al.* (1979). They reported that the major saturated and unsaturated fatty acids of salted mullet roe were C16:0, C16:1, and C18:1, respectively. These results are in agreement with those mentioned by Şengör *et al*. (2003). They reported that the major fatty acids of raw and

Table 8: Average of highly unsaturated fatty acids % of the total fatty acids of flathead mullet, *Mugil cephalus*, larvae fed for eight weeks at five groups of treated *Artemia* with emulsified fish oil (2 and 4 g/million *Artemia*) for 6 or 12 hour during the experiment.

Oil conc. gram	Exposure time hour	Fatty acids*						
		C18:2	C18:3	C20:2	C20:3	C20:4	C20:5	C22:6
0	0	8.70±0.05^{b}	21.34±0.00^{e}	1.42±0.01^{a}	0.23±0.00^{b}	0.59±0.00^{e}	0.69±0.06^{e}	1.31±0.16^{e}
2	6	4.84±0.02^{d}	29.73±0.00^{c}	0.71±0.01^{c}	0.11±0.01^{d}	0.79±0.00^{d}	0.78±0.05^{d}	1.38±0.20^{d}
	12	nd^{o}	38.12±0.01^{a}	nd^{o}	nd^{o}	0.98±0.02^{c}	0.88±0.02^{c}	1.45±0.21^{c}
4	6	9.90±0.05^{a}	28.31±0.01^{d}	0.97±0.00^{b}	0.25±0.02^{a}	1.12±0.02^{b}	1.03±0.07^{b}	1.67±0.15^{b}
	12	5.82±0.05^{c}	36.69±0.01^{b}	0.26±0.01^{d}	0.14±0.01^{c}	1.32±0.02^{a}	1.13±0.05^{a}	1.74±0.24^{a}
Pooled means								
0		8.70±0.05^{x}	21.34±0.00^{z}	1.42±0.01^{x}	0.23±0.00^{x}	0.59±0.00^{y}	0.69±0.06^{z}	1.31±0.16^{z}
2		2.42±0.01^{z}	33.93±0.01^{x}	0.35±0.01^{z}	0.06±0.01^{y}	0.88±0.01^{y}	0.83±0.21^{y}	1.42±0.21^{y}
4		7.86±0.05^{y}	32.5±0.01^{y}	0.62±0.01^{y}	0.20±0.01^{x}	1.22±0.01^{x}	1.08±0.06^{x}	1.71±0.20^{x}
	6	7.37±0.03 ^{g}	29.02±0.01 ^{h}	0.84±0.01 ^{g}	0.18±0.02 ^{g}	0.96±0.01 ^{h}	0.91±0.06 ^{h}	1.53±0.17 ^{h}
	12	2.91±0.03 ^{h}	37.41±0.01 ^{g}	0.13±0.01 ^{h}	0.07±0.01 ^{h}	1.15±0.02 ^{g}	1.01±0.04 ^{g}	1.60±0.23 ^{g}

Values represent means ± (SE) of N = 2 replicates, each contain 15 fish/treatment.
Means in same column not shearing the same superscript are significantly different (P<0.0).
**** Fatty acids; C18:2 (Linoleic acid) n-6, C18:3 (Linolenic acid) n-3, C20:2 (Eicosadienoic acid) n-6, C20:3 (Mead acid) n-6, C20:4 (Arachidonic acid) ARA n-6, C20:5 (Eicosapentaenoic acid) EPA n-3, C22:6 (Docosahexaenoic acid) DHA n-3.***
^{o} nd= not detected.

beeswaxed caviar oils were C16:0, C16:1, C16:2, C18:1 and C18:4. Other unsaturated fatty acids contents of *Mugil cephalus* ova, in the present study, such as linolenic (C18:3n-3), DHA (C22.6n-3), ARA (C20:4n-6), docosadienoic (C22:2n-6), eicosadienoic (20:2n-6), and EPA (C20:5n-3) acids were significantly high (4.9, 4.7, 2.4, 2.3, 1.4, and 1.3 % of the TFA, respectively).

Newly hatched *Artemia* nuaplii enriched with the essential fatty acids (EFAs); decosahexaenoic acid (DHA) and ecosapenteanoic acid (EPA) improved larval performance in striped bass and palmetto bass (Tuncer and Harrell, 1992), cod (Takeuchi *et al*., 1994), striped jack (Takeuchi *et al*., 1996), summer flounder (Baker *et al*., 1998), milkfish (Gapasin and Duray, 2001), Japanese flounder (Kim *et al*., 2002), cobia (Faulk and Holt, 2003), mangrove red snapper; rabbitfish; coral trout; and striped jack (Ogata *et al*.,

2004), and yellowtail snapper (Faulk *et al*., 2005).

The good growth and feed efficiency of fish fed the diet containing squid liver oil, indicated that juvenile Japanese flounder require n-3 HUFA for normal growth like other marine fish such as gilthead sea bream (Ibeas *et al*., 1996). Studies on EFA nutrition for Japanese flounder larvae show significant differences in growth between the larvae fed *Artemia* enriched with oleic acid, EPA, or DHA (Furuita *et al*., 1998 & 1999). In addition, it has been reported that development of the brain, occurrence of albinism, and salinity tolerance were influenced by dietary n-3 HUFA in larval Japanese flounder (Furuita *et al*., 1998 & 1999). These suggest that dietary fish oil (source of HUFA) is essential for normal growth and development of larval *Mugil cephalus*.

ARA, EPA, and DHA are effective for good growth and survival of *M. cephalus* larvae; however, the effect of these acids on survival is better than on growth (WG or SGR%/d). This is in agreement with study on the flatfish species (Gapasin and Duray, 2001).

Research on the nutritional requirements of marine fishes indicted that the ratios of individual PUFAs such as DHA:EPA, EPA:ARA, and n-3:n-6 PUFA, as well as the absolute concentration of these fatty acids, play an important role in egg quality and larval growth and survival (Izquierdo, 1996; Sargent *et al*., 1997 and Faulk and Holt, 2003). This finding is in agreement with our study, which indicted that the survival and growth of *M. cephalus* larvae significantly increased with the increasing of EPA: DHA, EPA: ARA, and n-3: n-6. Also, other studies confirmed that EPA: ARA is a specific indicator for marine fish larval diets (Faulk and Holt, 2003). In the present study the optimal dietary ratio of EPA:ARA is approximately 0.9:1 in *M. cephalus* larvae. Sargent *et al*. (1999) investigated the nutritional requirements of sea bass, halibut, and turbot larvae. They suggested that the optimal dietary ratio of EPA:ARA is approximately 1:1 in sea bass larvae but may be as high as 10:1 for turbot and halibut.

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تأثير التغذيه علىالأرتيميا المدعمه بزيت السمك على حيوية ونمو والتحاليل البيوكيميائيه ليرقات أسماك البورى.

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تمت دراسة تأثير تغذية يرقات أسماك البورى على الأرتيميا المدعمة بزيت السمك الذى يتميز بمحتواه من الأحماض الدهنية الغير مشبعة طويلة السلسلة (أوميجا ٣ وأوميجا ٦). وقد تم إثراء الأرتيميا بمستويين من زيت السمك (٢، ٤ جم زيت سمك/مليون أرتيميا) وذلك لفترتين زمنيتين من التعرض (٦ أو ١٢ ساعه) ثم المقارنة مع الأرتيميا فى المجموعة الضابطة بدون تعريض للزيت. وقد لوحظ زيادة معنويه ($P<0.05$) لكل من الوزن النهائى و معدل الإعاشة بزيادة تركيز الزيت وبإنخفاض فترة تعرض الأرتيميا للزيت. لوحظ زيادة معنويه ($P<0.05$) فى معدلات تركيز الدهون الكلية و مع زيادة تركيز الزيت المستخدم فى الإثراء لمدة ٦ ساعات.ولوحظ أيضا زيادة معنوية ($P<0.05$) لمعدل الإعاشة والنمو بزيادة تركيزكلا من الأحماض الدهنية غير المشبعة ونسبة EPA:DHA و ذلك بزيادة تركيز الزيت الى ٤جم و إنخفاض فترة تعرض الأرتيميا للزيت الى ٦ ساعات. كما لوحظ زيادة الأحماض الدهنية DHA, EPA, ARA معنوياً بزيادة تركيز الزيت و انخفاض فترة تعرض الأرتيميا للزيت الى ٦ ساعات و الذى أعطى كذلك أفضل معدل إعاشة و نمو ليرقات البورى.خلال أخذ الملاحظات اليومية لوحظ وجود انخفاض فى معدلى النمو و الإعاشة خلال فترة الفطام خاصة فى الأحواض غير المعاملة.مما سبق يمكن التوصية باستخدام زيت السمك فى إثراء الأرتيميا لتحسين معدلى النمو و الإعاشة ليرقات البورى، وذلك بتركيز ٤ جم/مليون أرتيميا لمدة تعرض ٦ ساعات.وخاصة فى مرحلة زيادة الغذاء.