

## Effects of enriched *Artemia urmiana* with HUFA on growth, survival, and fatty acids composition of the Persian sturgeon larvae (*Acipenser persicus*)

Hafezieh M.<sup>1,2\*</sup>; Mohd Salah Kamarudin S.<sup>2</sup>; Che Rose Bin Saad<sup>2</sup>;  
Mostafa Kamal Abd Sattar<sup>2</sup>; Agh N.<sup>3</sup>; Valinassab T.<sup>1</sup>;  
Sharifian M.<sup>1</sup> and Hosseinpour H.<sup>4</sup>

Received: June 2008

Accepted: January 2009

### Abstract

Recently, the nutritional requirements of marine finfish larvae have received considerable attention, and studies have shown that docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and arachidonic acid (ARA) affects the growth and survival of marine finfish larvae. This study investigated the effects of different *Artemia* enrichments containing variable amounts of DHA and EPA on the growth and survival of larval Persian sturgeon (*Acipenser persicus*). Four different *Artemia* enrichments ICES30/4 (with 20.90mg/g DW DHA and 6.29mg/g DW EPA), Sturgeon Ovary Oil (SOO) (with 2.76mg/g DW DHA and 7.55mg/g DW EPA), Cod Liver Oil (CLO) (with 7.64mg/g DW DHA and 11.39mg/g DW EPA) and Linseed Oil (LO) (with 0.00mg/g DW DHA and 0.03mg/g DW EPA) in seventy five aquaria (each 45 liter, with three replicates per treatment) were used. The resultant *Artemia* contained a different concentration of DHA (0.00-5.99mg/g DW) and EPA (0.69-4.97mg/g DW). Larvae were fed with *Artemia* from 3 to 20 days after active feeding at 250 prey l<sup>-1</sup>. Results showed that there were significant differences between treatments regarding to the total length and wet weight but no significant differences were found in dry weight between the larvae reared on different treatments. However, larvae reared on LO were significantly higher (in weight) than larvae reared on ICES30/4 and SOO. Larval survival on the SOO enriched *Artemia* (93.3±1.6) at 20<sup>th</sup> day was significantly higher than other treatments. Our results showed a positive effect of *Artemia* DHA proportions on growth and survival of the Persian sturgeon, and demonstrated that larvae of this species require a high ratio of dietary DHA to EPA.

**Keywords:** Persian sturgeon larvae, Fatty acid composition, Enrichments, *Artemia urmiana*

1- Iranian Fisheries Research Organization, P.O.Box: 14155-6116 Tehran, Iran

2- Faculty of Agriculture, University of Putra, No. 43400, Serdang, Slangor, Malays

3- Artemia and Aquatic Animal Research Center, Urmia University, P.O.Box: 57153-165 Urmia, Iran

4- Main Office of Education and Teaching of area 5, P.O.Box: 14156-1435 Tehran, Iran

\*Corresponding author's email: jhafezieh@yahoo.com

## Introduction

Lipids and fatty acids are major sources of metabolic energy during the embryonic and pre-feeding larval stages in fish. At hatch, yolk-sac larvae have high levels of these energy sources, but they are dramatically reduced during the endogenous feeding stage (Evans *et al.*, 2000). Thus, start-feeding larvae require a live food that provides sufficient levels of these energy sources. Studies have shown that essential fatty acids (EFA), such as docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6) are also important in larval fish nutrition (Takeuchi, 1997; McEvoy *et al.*, 1998; Estevez *et al.*, 1999; Sargent *et al.*, 1999). These fatty acids, as components of phospholipids (PL), are critical structural and physiological components of the cell membranes of most tissues. However, the live feeds commonly used for the first-feeding larval stages, such as rotifers and *Artemia*, are naturally poor in these fatty acids, so enrichment of live foods with lipids rich in EFA is necessary to achieve better growth and survival through metamorphosis (Rainuzzo *et al.*, 1997). Recently, absolute and relative levels of DHA, EPA, and ARA in the diets of marine fish larvae have received considerable attention (Sargent *et al.*, 1999; Harel *et al.*, 2002; Bell & Sargent, 2003). DHA, which has a competitive relationship with EPA, is particularly important for normal neural development and function, including that of retina and brain (Sargent *et al.*, 1999). Studies have shown that the DHA requirement in the diet differs among fish

species, especially in cold-water fish species such as yellowtail flounder (*Limanda ferruginea*) and the Atlantic halibut (*Hippoglossus hippoglossus*), which require high levels of dietary DHA (McEvoy *et al.*, 1998; Copeman *et al.*, 2002). However, Planas and Cunha (1999) reported that turbot larvae (*Scophthalmus maximus*) require lower levels of DHA in their diet for better growth and survival. Other specific benefits of feeding DHA-enriched diets to fish larvae include successful metamorphosis, reduced pigmentation problems, enhanced vision capabilities, improved neural development and stress resistance (Watanabe, 1993).

The Persian sturgeon (*Acipenser persicus*) is the first rehabilitated species in the Caspian Sea (Anon., 2003). In order to develop this species, a consistent production of juvenile fish must be achieved. Understanding the nutritional requirements of early larval sturgeon, especially of EFA such as DHA and EPA, is important for successful mass production. This study investigated the effect of DHA level and DHA/EPA ratio in four *Artemia* enriched diets on growth and survival of the Persian sturgeon larvae.

## Materials and methods

Four different enrichment oils were used: ICES30/4, Sturgeon Ovary Oil (SOO), Cod Liver Oil (CLO) and Linseed Oil (LO). The fatty acids in ICES (a commercial emulsion) 30/4, SOO, and CLO were composed of 23.69%, 3.13%, and 8.11% DHA and 7.12%, 9.13%, and 12.10% EPA in total fatty acid,

respectively (Table 1). The LO contained very low proportions of EPA and no DHA content.

*Artemia urmiana* were enriched with ICES30/4, SOO, CLO, and LO in 12L vessels at a density of 300 *Artemia* nauplii L<sup>-1</sup>. The resultant *Artemia* were designated as enriched *Artemia* 1 (EA1), enriched *Artemia* 2 (EA2), enriched *Artemia* 3 (EA3), and enriched *Artemia* 4 (EA4). Each batch of *Artemia* was enriched at 0.08g of enrichment material L<sup>-1</sup> of *Artemia* culture for 24h. Enrichment diets were divided into three portions and added at times: 0:00 and 12:00. Water temperature and salinity for enrichment were 22°C and 32ppt, respectively. Samples of 24h enriched *Artemia* were taken from each enrichment vessel for lipid analysis (Emadi, *et al.*, 2005).

The sleeping stage larvae from Shahid Beheshti Fish Rearing and Propagation Complex were moved to *Artemia* Research Center in Urmia University in plastic bag containing 1:3 water and 2:3 oxygen. After adaptation (20 min/°C), larvae moved in two tanks (1000L) with suitable water flow for 4 days. Water temperature was maintained at 20°C. After adaptation and start to active feeding, 20250 larvae (250 larvae L<sup>-1</sup>) were transferred to each of the seventy-five 45L rectangular glass aquaria (three replicates per treatment) that were randomly placed in a thermo-regulated water bath. This was considered day 1 of the experiment.

The water temperature in the experimental tanks was maintained at 20°C and monitored twice daily. A flow through water system was provided at an initial flow of 150ml.min<sup>-1</sup>.

Dissolved oxygen was monitored weekly, and remained at 9mg.l<sup>-1</sup>. Light was provided around the clock at 1000 lux. Larvae were fed four times a day with *Artemia* enriched (ES1 for ICES30/4, ES2 for SOO, ES3 for CLO and ES4 for LO enrichment, one treatment with three replications as the control group were fed with *Artemia* non-enriched). Each experimental tank was aerated, which ensured a homogenous distribution of prey within the tank (Bessonart *et al.*, 1999).

Five larvae (i.e., 15 per treatment) were randomly sampled for morphometric measurements from each experimental tank on 1, 8, 13, and 20 days. Larval length was measured with 0.001 scales and then placed on a 1.0cm<sup>2</sup> pre-weighed aluminum foil. The foils were dried at 60°C for 48h. Foils were then stored in a desiccators and weighed again (Copeman *et al.*, 2002). Survival at 20 days was determined by counting all larvae from each tank.

Lipid samples and lipid analysis triplicate samples, consisting of approximately 10mg dry weight of larvae, were taken from each tank after hatching and after 20 days (end of the experiment). Samples were placed directly in chloroform and stored under nitrogen 20°C, until extraction.

Lipids were extracted in chloroform/methanol according to Parrish (1999), using a modified Folch procedure (Folch *et al.*, 1957). Lipid classes were determined using thin layer chromatography with flame ionization detection (TLC/FID) as described by Parrish (1987). Extracts were spotted on silica gel-coated Chromarods, and a three-stage development system was used to separate lipid classes.

The first separation consisted of a 25 min and a 20 min development in 99:1:0.05 hexane/diethyl ether/formic acid. The second separation consisted of a 40 min development in 80:20:1 hexane/diethyl ether/formic acid. The last separation consisted of two 15 min developments in 100% acetone followed by two 10 min developments in 5:4:1 chloroform/methanol/water. After each separation, the rods were scanned, and the three chromatograms were combined using T-Data Scan software (RSS, Bemis, Tennessee, USA). The signal detected in mv was quantified using lipid standards (Sigma).

Fatty acid methyl esters (FAME) were prepared by transesterification with 10% boron trifluoride (BF<sub>3</sub>) in methanol at 85°C for 1.5 h (Morrison & Smith, 1964).

A Varian model 3400 Gas Chromatograph (GC) equipped with a Varian 8100 Auto-sampler was used for fatty acid analysis (Varian, California, and USA). An Omegawax 320 column, 30m long, 0.32mm, i.d., 0.25µm film thickness (Supelco, Bellefonte, Pennsylvania, USA) was used for separations. Hydrogen was used as the carrier gas, and the flow rate was set at 2ml.min<sup>-1</sup>. The column temperature profile

was as follows: 65°C for 0.5 min, hold at 195°C for 15 min after ramping at 40°C.min<sup>-1</sup>, and hold at 220°C for 0.75 min after ramping at 2°C.min<sup>-1</sup>. The injector temperature was increased from 150°C to 250°C at 200°C.min<sup>-1</sup>. Peaks were detected by flame ionization with the detector held at 260°C. Fatty acid peaks were integrated using Varian Star Chromatography Software (version 4.02), and were identified with reference to available standards (PUFA 1, 3 and 37 Component FAME Mix, Supelco Canada, Ontario, Canada) (Copeman *et al.*, 2002).

All data were tested for normality to satisfy the assumptions of ANOVA. Two-way ANOVA were used to determine the statistical significance of treatment on dry weight and standard length of sturgeon larvae. One-way ANOVA with the Duncan multiple comparison tests were used to compare differences in survival of larvae, lipid class and fatty acid composition of *Artemia* and larvae between treatments. Differences were considered significant at the P<0.05 level.

**Table 1: The fatty acid composition (mg.g<sup>-1</sup> dry weight) of the enrichment oils**

Sample	ICES30/4	CLO	SOO	LO
C20:4n6 (ARA)	0.78	5.61	5.00	0.78
C20:5n3 (EPA)	6.29	11.39	7.55	0.03
C22:6n3 (DHA)	20.90	7.64	2.76	0.00
DHA/EPA	3.32	0.67	0.36	0.00
Σ Saturated	32.17	27.13	28.80	25.69
Σ Monoenes	21.87	35.93	41.28	20.17
Σ n-6 HUFA	0.78	5.61	5.00	0.78
Σ n-3 HUFA	27.19	19.03	10.31	0.03
ω -3/ω-6	34.85	3.39	2.06	0.038

## Results

The total protein content of enriched *Artemia* in all treatments was significantly higher ( $P < 0.037$ ) than in the initial *Artemia* (control), and in ICES30/4, it was significantly higher than that in the SOO, CLO and LO treatments (Table 2). The total lipid content of *Artemia* increased in all treatments after 24h enrichment, but it was significantly higher ( $P < 0.011$ ) in the ICES30/4 (20.87%) and LO (19.45%) treatments than that in the SOO and CLO treatments. The protein/lipid ratio of the enriched *Artemia* in all groups was lower than that in the initial *Artemia*. However, CLO and SOO showed a significantly higher ratio than them in ICES30/4 and LO.

All treatments resulted in higher levels of EPA than DHA except for ICES30/4. The highest levels of EPA and DHA in ICES30/4 treatment were 4.97 and 5.99, respectively. *Artemia* enriched with ICES30/4, SOO and CLO had significantly higher DHA/EPA ratios than *Artemia* enriched with LO and Control ( $P < 0.021$ ).

Initial and final length and wet weight of sturgeon larvae during first three days were  $19.0 \pm 0.03$  mm,  $21.2 \pm 0.04$  mm and  $33.0 \pm 2.34$  mg,  $46.8 \pm 3.03$  mg, respectively. The effects of enrichment with ICES30/4 and SOO on length of sturgeon larvae were significant ( $P < 0.034$ ). From the 8<sup>th</sup> day to the end of the experiment, larvae reared on ICES30/4 and SOO were significantly larger than larvae reared on other treatments (Tables 3 to 5).

Enrichment had no significant effect on the dry weight but had significant effect on the wet weight of sturgeon larvae. ICES30/4 performed the highest WW in sturgeon larvae

in the 8<sup>th</sup> day but there were not significant between ICEA30/4 and SOO during the 8<sup>th</sup> till 20<sup>th</sup> days (Tables 3 to 5).

ICES30/4 and SOO enrichment had a significant effect on the survival rate of larval sturgeon. At the end of experimental period (day 20) SOO (93.3%) and CLO (91.7%) enrichment had higher survival rate than the other treatments (Table 6). Cochran's test for variance outliers (Kanji, 1994) was used to determine outliers in the data, and a significant critical value ( $P < 0.05$ ) was found for the SOO and CLO survival data. When the data were analyzed after removing this outlier, larval survival in the SOO and CLO treatments were significantly higher ( $P < 0.019$ ), while no significant difference was found among the other three treatments.

The total protein content of enriched sturgeon larvae in all treatments were significantly lower ( $P < 0.047$ ) than in the control, but in ICES30/4 (69.62%) and LO (68.93%) were significantly higher than in the SOO and CLO treatments (Table 6). The total lipid content of sturgeon larvae increased only in SOO treatment (22.14%) which was significantly different with other treatments ( $P < 0.021$ ). The protein/lipid ratio of the enriched sturgeon larvae in CLO was the highest (5.84) while between controls, ICES30/4 and LO treatments there were not significant differences. SOO treatment had the lowest protein/lipid ratio (3.06).

There were no significant differences between controls, CLO and LO treatments in ARA ( $P > 0.067$ ) but the amount of this fatty

acid were higher than ICES30/4 and SOO treatments (Table 6). ICES30/4 was the only oil which can improve the DHA amount ( $2.75 \pm 0.08$ ) in larvae compares to the control, other treatments resulted in lower levels of EPA than the control. Although, there were significant differences between groups regarding to EPA ( $P < 0.043$ ) but none of the treatments could improve

levels of EPA in sturgeon larvae compare to the control ( $2.97 \pm 0.12$ ).

The ratio of DHA/EPA was highest in ICES30/4 (1.11), and had significant difference with other treatments and control which they did not have any significant difference between them.

**Table 2: Average total protein, lipid (% DW), DHA and EPA Fatty acid composition (mg/g DW) and DHA: EPA ratios in all enriched *Artemia* and control**

	Control	EA1	EA2	EA3	EA4
Protein	55.12 $\pm$ 3.02 <sup>a</sup>		59.90 $\pm$ 0.65 <sup>c</sup>	58.43 $\pm$ 1.40 <sup>b</sup>	58.17 $\pm$ 1.20 <sup>b</sup>
Lipid	16.79 $\pm$ 0.70 <sup>a</sup>		20.87 $\pm$ 0.55 <sup>d</sup>	18.86 $\pm$ 0.62 <sup>b</sup>	19.45 $\pm$ 0.72 <sup>c</sup>
Protein/Lipid	3.28 <sup>b</sup>		2.87 <sup>a</sup>	3.09 <sup>b</sup>	2.99 <sup>a</sup>
C22:6n3 (DHA)	0.00 <sup>a</sup>		5.99 $\pm$ 0.06 <sup>c</sup>	0.69 $\pm$ 0.05 <sup>b</sup>	0.70 $\pm$ 0.09 <sup>b</sup>
C20:5n3 (EPA)	0.82 $\pm$ 0.1 <sup>b</sup>		4.97 $\pm$ 0.26 <sup>e</sup>	1.71 $\pm$ 0.12 <sup>c</sup>	2.55 $\pm$ 0.06 <sup>d</sup>
DHA/EPA	0.00 <sup>a</sup>		1.20 <sup>d</sup>	0.40 <sup>c</sup>	0.29 <sup>b</sup>

In each row, superscript letters demonstrate the significant differences.

**Table 3: Total length (mm), wet and dry weight (mg) of sturgeon larvae in day 8**

Treatments	TL	DW	WW
Control	23.7 $\pm$ 0.3 <sup>a</sup>	9.8 $\pm$ 0.4 <sup>a</sup>	81.7 $\pm$ 2.5 <sup>b</sup>
ES1	25.1 $\pm$ 0.5 <sup>c</sup>	9.8 $\pm$ 2.1 <sup>a</sup>	87.3 $\pm$ 1.00 <sup>c</sup>
ES2	24.0 $\pm$ 0.7 <sup>b</sup>	9.4 $\pm$ 1.6 <sup>a</sup>	80.1 $\pm$ 7.4 <sup>b</sup>
ES3	23.7 $\pm$ 0.5 <sup>a</sup>	9.7 $\pm$ 1.5 <sup>a</sup>	75.1 $\pm$ 8.9 <sup>a</sup>
ES4	24.3 $\pm$ 0.7 <sup>b</sup>	9.2 $\pm$ 1.2 <sup>a</sup>	80.3 $\pm$ 3.8 <sup>b</sup>

In each row superscript letters demonstrate the significant differences.

**Table 4: Total length (mm), wet and dry weight (mg) of sturgeon larvae in day 13**

	TL	DW	WW
Control	32.0 $\pm$ 0.7 <sup>b</sup>	18.7 $\pm$ 0.5 <sup>a</sup>	162.2 $\pm$ 2.4 <sup>a</sup>
ES1	32.2 $\pm$ 1.2 <sup>b</sup>	19.0 $\pm$ 3.7 <sup>a</sup>	174.8 $\pm$ 16.2 <sup>b</sup>
ES2	32.3 $\pm$ 0.8 <sup>b</sup>	18.4 $\pm$ 2.4 <sup>a</sup>	171.3 $\pm$ 2.1 <sup>b</sup>
ES3	32.5 $\pm$ 0.3 <sup>b</sup>	18.2 $\pm$ 0.9 <sup>a</sup>	163.9 $\pm$ 5.4 <sup>a</sup>
ES4	31.7 $\pm$ 0.9 <sup>a</sup>	8.8 $\pm$ 0.4 <sup>a</sup>	158.3 $\pm$ 3.4 <sup>a</sup>

In each row superscript letters demonstrate the significant differences.

**Table 5: Total length (mm), wet and dry weight (mg) and survival rate (%) of sturgeon larvae in day 20**

	TL	DW	WW	Survival
Control	40.3±2.1 <sup>a</sup>	29.3±4.3 <sup>a</sup>	297.3±31.4 <sup>a</sup>	87.9±2.3 <sup>a</sup>
ES1	41.3±2.1 <sup>b</sup>	30.5±6.9 <sup>a</sup>	307.3±53.9 <sup>b</sup>	88.0±0.4 <sup>a</sup>
ES2	41.6±1.0 <sup>b</sup>	30.9±4.2 <sup>a</sup>	309.9±25.9 <sup>b</sup>	93.3±1.6 <sup>b</sup>
ES3	39.9±0.9 <sup>a</sup>	28.0±1.9 <sup>a</sup>	292.1±10.8 <sup>a</sup>	91.7±0.8 <sup>b</sup>
ES4	40.2±1.1 <sup>a</sup>	31.5±4.6 <sup>a</sup>	302.4±28.1 <sup>a</sup>	87.2±0.2 <sup>a</sup>

In each row superscript letters demonstrate the significant differences

**Table 6: Average total protein, lipid (% DW), ARA, DHA and EPA Fatty acid composition (mg/g DW) and DHA: EPA ratios in of all enriched sturgeon larvae and control**

	Control	ES1	ES2	ES3	ES4
Protein	69.93±3.21 <sup>c</sup>	69.62±2.10 <sup>c</sup>	67.90±3.10 <sup>a</sup>	68.00±3.10 <sup>a</sup>	68.93±3.21 <sup>b</sup>
Lipid	17.37±0.33 <sup>b</sup>	16.65±0.18 <sup>b</sup>	22.14±1.18 <sup>c</sup>	11.63±0.40 <sup>a</sup>	15.40±0.26 <sup>b</sup>
Protein/Lipid	4.02 <sup>b</sup>	4.18 <sup>b</sup>	3.06 <sup>a</sup>	5.84 <sup>c</sup>	4.47 <sup>b</sup>
C20:4n6 (ARA)	0.47±0.07 <sup>b</sup>	0.98±0.11 <sup>a</sup>	0.98±0.12 <sup>a</sup>	1.53±0.20 <sup>b</sup>	1.35±0.10 <sup>b</sup>
C22:6n3 (DHA)	2.30±0.17 <sup>b</sup>	2.75±0.08 <sup>c</sup>	1.44±0.24 <sup>a</sup>	2.28±0.18 <sup>b</sup>	1.48±0.10 <sup>a</sup>
C20:5n3 (EPA)	2.97±0.12 <sup>c</sup>	2.46±0.09 <sup>b</sup>	1.87±0.21 <sup>a</sup>	2.90±0.13 <sup>b</sup>	2.17±0.15 <sup>b</sup>
DHA/EPA	0.77 <sup>a</sup>	1.11 <sup>b</sup>	0.77 <sup>a</sup>	0.78 <sup>a</sup>	0.68 <sup>a</sup>

In each row superscript letters demonstrate the significant differences

## Discussion

A relationship between DHA levels and DHA/EPA ratios of *Artemia*, and the growth and survival of sturgeon larvae was found in the present study. Takeuchi *et al.* (1994) investigated the effect of DHA levels of rotifers on the growth, survival rate, and abnormalities of larval cod (*Gadus macrocephalus*), and suggested that the appropriate level of DHA that should be

contained in the rotifers was around 1% DW. In their study, any amount higher than 1% DHA resulted in a high percentage of abnormal fish, together with high mortality. However, in our study, larval sturgeon fed SOO containing 1.44±0.24 DW of DHA had higher growth and survival rate than larvae fed with ICES30/4 with 2.75±0.08 DW of DHA. Although Sargent *et al.*

(1999) suggested that species-specific requirements for DHA exist among marine finfish larvae but several other studies suggested that much higher levels of DHA (or n-3 highly unsaturated fatty acids e HUFA) could reduce larval survival (Planas & Cunha, 1999). Izquierdo *et al.* (1992) showed that, in larval Japanese flounder (*Paralichthys olivaceus*), lower (or higher) DHA content (1.5%) of *Artemia* did not affect survival, but larvae were significantly larger when fed *Artemia* containing a higher percentage of DHA (up to 3.5%). However, Salhi *et al.* (1994), in their study with gilthead sea bream (*Sparus aurata*), showed that larvae fed with a lower DHA micro diet (>0.5%) had a significantly lower survival than larvae fed with a higher DHA micro diet (1.2-1.3%). They suggested that the growth of larvae was affected by a combination of DHA content and total dietary lipid. In our study, however, the SOO (1.44±0.24 DW of DHA) treatment gave a significantly higher survival than the ICES30/4 (2.75±0.08 DW of DHA) but total dietary lipid was higher in SOO than ICES30/4.

Rodriguez *et al.* (1997) reported that a higher DHA/EPA ratio during the rotifer stage improved the growth and survival of gilthead sea bream. Copeman *et al.* (2002) found that yellowtail flounder fed high DHA/EPA (8:1) had a higher growth and survival than those fed a DHA/EPA ratio of 1.9:1. However, there was no significant difference in the growth of Japanese flounder and turbot larvae when they were fed with different dietary ratios of DHA and EPA (Estevez *et al.*, 1999; Furuita *et al.*, 1999). Harel *et al.* (2002) investigated the

effect of commercial enrichment materials on early development of three larval fish. They reported no significant difference in growth between striped bass (*Morone saxatilis*) and gilthead sea bream larvae fed with *Artemia* enriched with Algamac 2000 or PL-Cr (DHA-rich phospholipids extract of *Cryptocodinium sp.*). However, the growth of halibut larvae fed *Artemia* enriched with DHA Selco was lower than the growth of larvae fed with PL-Cr. Our studies also showed that sturgeon larvae fed low DHA/EPA diets (SOO) showed better growth and survival than those fed high DHA/EPA diets (ICES30/4). On the other hand, sturgeon larvae fed LO, which almost equivalent level of DHA had compared with SOO, had a lower survival than those fed SOO treatments. All these studies, including the present study, suggested the existence of species-specific requirements for the DHA/EPA ratio for growth and survival of marine finfish larvae.

The lipid composition of eggs/yolk has been suggested as an indicator for determining the nutritional requirements of first-feeding larvae. Typically, a dietary DHA/EPA ratio of 2:1 is found in marine species, and has been suggested as adequate for larval feeding (Tocher & Sargent, 1984; Sargent *et al.*, 1999). However, in our experiment, growth and survival of larval sturgeon improved with increasing ratio. Similar to other experiments (Tocher & Sargent, 1984), newly hatched cod larvae in our experiment had a DHA/EPA ratio of 2:1. DHA/EPA ratio of larval cod increased as the larvae grew, irrespective of the rotifer enrichment. However, increase in the DHA/EPA ratio was significantly higher in the ICES30/4

treatment, which yielded not better growth than the other two treatments. From our results, it seems that larval sturgeon requires a lower DHA: EPA ratio than some other marine finfish species. Copeman *et al.* (2002) suggested that larval yellowtail flounder require higher dietary DHA levels for better growth. Thus, our results indicated that the presence of high DHA and lower EPA levels in the diet may not be important for better growth of sturgeon larvae. Watanabe (1993) suggested that the DHA content of Atlantic cod larvae could be reduced rapidly during larval development after hatching. In our study, the DHA levels of the Persian sturgeon larvae in CLO treatment did not change compared with the initial value. Meanwhile, the DHA levels of larval sturgeon fed with SOO and LO were lower than the initial value, suggesting that the DHA levels of sturgeon larvae should be kept close to the initial levels for better larval growth, and that can be accomplished by feeding diets with a relatively high DHA level and high DHA/EPA ratio. Copeman *et al.* (2002) found that supplementing diets with high EPA levels was not effective for the growth of yellowtail flounder. Similarly, in our experiment, EPA levels of larvae enriched with SOO was very low ( $1.87 \pm 0.21$ ) in all treatments and had effect on the survival of sturgeon larvae.

Recently, studies have indicated that arachidonic acid (ARA) levels in marine fish larvae may be important for stress tolerance, pigmentation, growth, and survival (Bell & Sargent, 2003). In particular, the competitive interactions between EPA and ARA are important in the formation of eicosanoids (Harel & Place, 2003). In our study, ARA

levels in larvae were higher in all treatments than in their respective diets. Thus, larval cod appear to have the ability to selectively incorporate dietary ARA into their body tissues. Similarly, Copeman *et al.* (2002) found that yellowtail flounder larvae have the ability to increase the dietary ARA levels in the body tissue in spite of lower dietary ARA levels (as low as 2.2% of total fatty acid). Zheng *et al.* (1996) reported that prey enriched with higher ARA provided no improvement in survival for Pacific cod larvae. Similarly, in our studies, ARA levels in *Artemia* did not affect growth and survival of the Persian sturgeon. However, previous studies showed that dietary ARA levels are important for improved growth and survival in gilthead sea bream (Bessonart *et al.*, 1999; Koven *et al.*, 2001).

Sargent *et al.* (1999) suggested that both the concentration and ratio, not only between DHA and EPA, but also between EPA and ARA, are important in larval marine fish nutrition. Thus, it appears that the ARA levels in diet have a species-dependent effect on sturgeon fish. Chemical composition of the enrichment diets used in our experiment differed not only in essential fatty acids, but also in phosphor-lipids, proteins, and micro-nutrients. Although our results showed that DHA, EPA, and DHA:EPA ratio had significant effects on the growth, survival, and composition of larval sturgeon, differences in other nutrients could have also affected our results.

In conclusion, high dietary lipid appears to be effective in improving the nutritional value of *Artemia* for the improvement of growth and survival of sturgeon larvae.

## Acknowledgements

We would like to express our thanks to Iranian Fisheries Research Organization (IFRO) for financial supporting of this project and Shahid Beheshti Fish Rearing and Propagation Complex for providing sturgeon larvae.

## References

- Anonymous, 2003.** Report on sturgeon fishes stock assessment in Iran. Propagation and culture deputy, Iranian Fisheries Organization. 78P. (in Persian).
- Bell, J.G. and Sargent, J.R., 2003.** Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture*, **218**:491-499.
- Bessonart, M., Izquierdo, M.S., Salhi, M., Hernandez-Cruz, C.M., Gonzalez, M.M. and Fernandez-Palacios, H., 1999.** Effect of dietary arachidonic acid levels on growth and survival of gilthead sea bream (*Sparus aurata* L.) larvae. *Aquaculture*, **179**:265-275.
- Copeman, L.A., Parrish, C.C. Brown, J.A. and Harel, M., 2002.** Effects of docosahexaenoic, eicosapentaenoic and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): live food enrichment experiment. *Aquaculture*, **210**:285-304.
- Emadi, H., Nouri F., Hafezieh, M., Agh, N and Abrishamkar, S., 2005.** Optimal condition for enrichment of juvenile *Artemia urmiana* using cod liver oil. *Journal of Marine Science and Technology*, **1**(1).
- Evans, R.P., Zhu, P., Parrish, C.C. and Brown, J.A., 2000.** Lipid and amino acid metabolism during early development of marine fish. *In*: Seafood in health and nutrition-transformation in fisheries and aquaculture: Global Perspectives, (F. Shahidi ed.). Science Tech Publishing Company, St. John's, Newfoundland. pp.477-493.
- Estevez, A., McEvoy, L.A., Bell, J.G. and Sargent, J.R., 1999.** Growth, survival, lipid composition and pigmentation of turbot (*Scophthalmus maximus*) larvae fed live-prey enriched in arachidonic and eicosapentaenoic acids. *Aquaculture*, **180**:321-343.
- Folch, J., Lees, M. and Sloane-Stanley, G.H., 1957.** A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **22**:497-509.
- Furuita, H., Konishi, K. and Takeuchi, T., 1999.** Effect of different levels of eicosapentaenoic and docosahexaenoic acid in *Artemia* nauplii on growth, survival and salinity tolerance of larvae of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, **170**:59-69.
- Harel, M., Koven, W., Lein, I., Bar, Y., Behrens, P., Stubblefield, J., Zohar, Y. and Place, A.R., 2002.** Advanced DHA, EPA and ARA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture*, **213**:347-362.
- Harel, M. and Place, A.R., 2003.** Tissue essential fatty acid composition and competitive response to dietary manipulations in white bass (*Morone chrysops*), striped bass (*M. saxatilis*) and hybrid striped bass (*M. chrysops* x *M. saxatilis*). *Comparative Biochemistry and Physiology, Part B*. **135**:83-94.
- Izquierdo, M.S., Arakawa, T., Takeuchi, T., Haroun, R. and Watanabe, T., 1992.** Effect of n-3 HUFA levels in *Artemia* on growth of larval Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, **105**(1):73-82.

- Kanji, G.K., 1994.** 100 Statistical Tests. Sage Publications, London, UK. 216P.
- Koven, W., Barr, Y., Lutzky, S., Ben-Atia, I., Weiss, R., Harel, M., Behrens, P. and Tandler, A., 2001.** The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead sea bream (*Sparus aurata*) larvae. *Aquaculture*, **193**:107-122.
- McEvoy, L.A., Naess, T., Bell, J.G. and Lie, T., 1998.** Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: A comparison with fry fed wild copepods. *Aquaculture*, **163**:237-250.
- Morrison, W.R. and Smith, L.M., 1964.** Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride methanol. *Journal of Lipid Research*, **5**:600-608.
- Parrish, C.C., 1987.** Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. *Canadian Journal of Fisheries and Aquatic Sciences*, **44**:722-731.
- Parrish, C.C., 1999.** Determination of total lipid, lipid classes and fatty acids in aquatic samples. *In: Lipids in Freshwater Ecosystems*. pp.4-12. (eds. M.T. Arts and B.C. Wainman). Springer-Verlag, New York, USA. 157P.
- Planas, M. and Cunha, I., 1999.** Larviculture of marine fish: Problems and perspectives. *Aquaculture*, **177**:171-190.
- Rainuzzo, J.R., Reitan, K.I. and Olsen, Y., 1997.** The significance of lipids at early stages of marine fish: A review. *Aquaculture*, **155**:103-115.
- Rodriguez, C., Perez, J.A., Diaz, M., Izquierdo, M.S., Fernandez-Palacios, H. and Lorenzo, A., 1997.** Influence of EPA/DHA ratio in rotifers on gilthead sea bream (*Sparus aurata*) larval development. *Aquaculture*, **150**:77-89.
- Salhi, M., Izquierdo, M.S., Hernandez-Cruz, C.M., Gonzalez, M. and Fernandez-Palacios, H., 1994.** Effect of lipid and n-3 HUFA levels in micro diets on growth, survival and fatty acid composition of larval gilthead sea bream (*Sparus aurata*). *Aquaculture*, **124**:275-282.
- Sargent, J., Bell, J.G., McEvoy, L.A., Tocher, D. and Estevez, A., 1999.** Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, **177**:191-199.
- Takeuchi, T., Zheng, F., Takeuchi, T., Yosheda, M., Hirokawa, J. and Watanabe, T., 1994.** Nutritive value of DHA-enriched rotifer for larval cod. *Nippon Sustainable Aquaculture*, **60**:641-652.
- Takeuchi, T., 1997.** Essential fatty acid requirements of aquatic animals with emphasis on fish larvae and fingerlings. *Fisheries Science*, **5**:1-25.
- Tocher, D.R. and Sargent, J.R., 1984.** Analysis of lipids and fatty acids in ripe roes of some northwest European marine fish lipids. *Journal of the World Aquaculture Society*, **7**:492-499.
- Watanabe, T., 1993.** Importance of docosahexaenoic acid in marine larval fish. *Journal of the World Aquaculture Society*, **24**:152-161.
- Zheng, F., Takeuchi, T., Yosheda, K., Kobayashi, M., Hirokawa, J. and Watanabe, T., 1996.** Requirement of larval cod for arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid using enriched *Artemia* nauplii. *Nippon Suisan Gakkaishi*. **62**:669-676.

## اثرات غنی‌سازی آرتمیا با اسیدهای چرب فوق غیراشباع بر رشد، بازماندگی و ترکیبات اسیدهای چرب لارو ماهی خاویاری ایرانی (*Acipenser persicus*)

محمود حافظیه<sup>۱\*</sup>؛ محمد صالح کامارودین<sup>۲</sup>؛ چي رز بن سعد<sup>۲</sup>؛  
مصطفی کمال عبد ستار<sup>۲</sup>؛  
ناصر آق<sup>۳</sup>؛ تورج ولي نسب<sup>۱</sup>؛ منصور شریفیان<sup>۱</sup> و حمیرا  
حسین‌پور<sup>۴</sup>

تاریخ دریافت: خرداد ۱۳۸۷ تاریخ پذیرش:

بهمن ۱۳۸۷

### چکیده

نیازهای غذایی لارو ماهیان باله دار دریایی بسیار به تازگی مورد توجه قرار گرفته است و مطالعات به تاثیر دوکوزاهگزانوئیک اسید (DHA)، ایکوزاپنتانوئیک اسید (EPA) و آراشیدونیک اسید (ARA) بر رشد و بازماندگی لارو این ماهیان اشاره دارد. در این آزمایش اثرات غنی سازیهای مختلف آرتمیا محتوی مقادیر مختلف EPA و DHA بر رشد و بازماندگی لارو ماهی خاویاری ایرانی مورد مطالعه قرار گرفته است. چهار ماده غنی‌ساز تجاری آرتمیا شامل ICES30/4 (با ۲۰/۹۰ میلی‌گرم DHA در گرم وزن خشک و ۶/۲۹ میلی‌گرم EPA در گرم وزن خشک)، روغن تخمدان ماهی خاویاری (با ۲/۷۶ میلی‌گرم DHA در گرم وزن خشک و ۷/۵۵ میلی‌گرم EPA در گرم وزن خشک)، روغن کبد ماهی کاد (با ۷/۶۴ میلی‌گرم DHA در گرم وزن خشک و ۱۱/۳۹ میلی‌گرم EPA در گرم وزن خشک)، و روغن بذر کتان (با ۰/۰۰ میلی‌گرم DHA در گرم وزن خشک و ۰/۰۳ میلی‌گرم EPA در گرم وزن خشک) در هفتاد و پنج تانک مستطیلی ۴۵ لیتری با سه تکرار برای هر تیمار مورد استفاده قرار گرفتند. نتایج آرتمیا نشان از تفاوت غلظتهای مختلف DHA (از صفر تا ۵/۹۹ میلی‌گرم در هر گرم وزن خشک) و EPA (از ۰/۶۹ تا ۴/۹۷ میلی‌گرم در هر گرم وزن خشک) دارد. لارو ماهی خاویاری پس از شروع تغذیه فعال به تعداد ۲۵۰ عدد در هر تانک و از روز سوم تا بیستم توسط آرتمیای غنی شده تغذیه شدند. در پایان آزمایش نتایج نشان داد در خصوص طول کل و وزن تر اختلاف معنی‌دار بین تیمارها وجود دارد ولی هیچ اختلافی در وزن خشک بین آنها مشاهده نگردید. با این وجود، لاروهایی که با روغن بذرتان تغذیه شدند، اختلاف معنی‌داری (در وزن تر) نسبت به لاروهایی که با ICES30/4 و روغن تخمدان ماهی خاویاری تغذیه شدند، نشان دادند. میزان بقا لاروهای تغذیه شده از آرتمیا غنی شده با روغن تخمدان ماهی خاویاری (۹۳/۳ ± ۱/۶) در روز بیستم بیشتر از بقیه تیمارها بدست آمد. نتایج این تحقیق نشان می‌دهد که میزان DHA در رشد و بازماندگی لارو ماهی خاویاری ایرانی اثر مثبتی و نسبت بالای DHA/EPA برای این ماهی لازم است.

۱ - موسسه تحقیقات شیلات ایران، تهران صندوق پستی: ۱۴۱۵۵-۶۱۱۶

۲ - دانشکده کشاورزی دانشگاه پوترای مالزی، شماره ۴۳۴۰۰، سردانگ، سلانگور، مالزی

۳ - مرکز تحقیقات آرتمیا و سایر آبزیان دانشگاه ارومیه، صندوق پستی: ۵۷۱۵۳-۱۶۵

۴ - اداره کل آموزش و پرورش منطقه ۵ تهران صندوق پستی: ۱۴۱۵۶-۱۴۳۵

\* پست الکترونیکی نویسنده مسئول: jhafezieh@yahoo.com

کلمات کلیدی: ماهی خاویاری ایرانی، ترکیبات اسیدهای چرب، غنی سازی، آرتمیا ارومیان