Effects of ICES30/4-enriched *Artemia urmiana* nauplii on growth, survival, salinity tolerance and fatty acid composition of *Acipenser persicus* larvae

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Abstract:
Effects of *Artemia urmiana* enriched with ICES30/4 as a commercial emulsion of highly unsaturated fatty acid (HUFA) on growth, survival, salinity tolerance and fatty acid composition of the Persian sturgeon larvae were evaluated. Artemia enriched for two different time periods (12 and 24 hours) and non-enriched artemia were fed to *A. persicus* larvae (average initial wet weight 46.80± 2.03mg) during 20 days. The n3-HUFA content ranged from 0.82±0.08 mg /g DW in non-enriched artemia to 7.69±0.2 mg/g DW in enriched artemia during 24 h with ICES30/4 and ranged from 3.20±0.30 mg/g DW in sturgeon fish larvae fed with non-enriched artemia to 5.98±0.43 mg/g DW in fish larvae fed with 24 h-artemia enriched with ICES30/4 after 20 days. The n3-HUFA content significantly increased compared to 12 h enrichment period both in artemia and sturgeons fed with these artemia (p<0.05). A significant increase in survival percentages and salinity tolerance were observed between fish larvae fed with enriched and non-enriched artemia (p<0.05), while growth rate did not significantly differ among the treatments (p>0.05).

Keywords: *Artemia urmiana*, Persian sturgeon (*Acipenser persicus*), Enrichment, Growth, Survival performance, Fatty acids composition

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Introduction

Rearing of larval fish is the most critical stage in the production cycle for many species. The primary problem in rearing relates to the transitional period from endogenous to exogenous food resources and thus to adequate feed supply (Leger *et al*., 1986; Abi-Ayad and Kestemont, 1994; Noori *et al*., 2011; Achionye-Nzeh, 2012). A readily available diet which has a high nutritional quality and is easily accepted and digested by the larval fish is essential to success (Kim *et al*., 1996; Okunsebor and Ayuma, 2011). Dietary lipids play an important role in fish nutrition for provision of both essential fatty acids (EFA) and energy. Dietary lipids also assist in the absorption of fat-soluble nutrients (Sargent *et al*., 1999; Abedian Kenari and Naderi, 2015).

Live prey organisms, especially zooplankton, are generally used as initial larval food for certain species of fish (Leger *et al*., 1986). Being naturally low in EPA, commonly used live foods for first feeding of larvae, such as rotifer and artemia, have to be enriched with lipids rich in EFA prior to feeding (Copeman *et al*., 2002; Shakourian *et al*., 2011).

It has been suggested that white sturgeon may require both n_3 and n_6 fatty acids based on growth and the $20{:}3n_9/20{:}4n_6$ and $20{:}3n_9/22{:}6n_3$ ratio in liver phospholipids (Hung and Deng, 2002). *Artemia* sp., however poor in EFA, is one of the most important starter live foods in sturgeon larviculture in Iran. Therefore, artemia EFA- enrichment as a live food is needed to meet the requirements of sturgeon for EFA.

Some methods have significantly enhanced EFA level in *Artemia* sp. all tested in 24 h exposed enrichment period (Sundbom and Vrede, 1997; Von Elert, 2002; Ravet *et al*., 2003, Bahadir Koca *et al*., 2015), but no study exists on the effects of EFA-enriched artemia on the performance of Persian sturgeon larvae. This study presents a specific approach to enrich artemia with ICES30/4 as a commercial emulsion with high HUFA contents and hope to find the shortage time for enriching (INVE Company, Belgium). The study also aims at evaluating the role of HUFA in the first feeding of the Persian sturgeon larvae and their effects on the growth performance and body composition of the larvae.

Material and methods

Sturgeon larvae (8 days post hatch) were obtained from the Shahid Beheshti Sturgeon Hatchery Center in Rasht, Iran. The larvae were fed with artemia nauplii (Instar 1) for three days before the main experiment. The larvae with initial wet weight of 46.80± 3.03mg and total length of 21.2±0.04 mm were randomly distributed in 12 groups of 250 individuals per rectangular elliptic fiberglass tanks of 25 L each (10 larvae/L). Each tank was supplied with water via 0.5 inch PVC pipe at a flow rate of 0.8 L/min. Water was continuously aerated (compressed air)

to keep oxygen levels close to 7.4±0.3 mg/L (n=12). The tank outlet and inlet was protected by a 250µm net screen. Water quality was checked periodically; pH was about 7.8±0.02, and temperature was 20±5˚C.

Artemia urmiana nauplii were hatched in Artemia and Aquatic Animal Research Center, Urmia University, from the Urmia lake batch under standard condition. Enrichment solution was prepared with ICES30/4 emulsion polysorbate (Tween 80, Merck) and freshwater according to Ako *et al*. (1994). In this method, first, 5mL polysorbate was added to 50mL freshwater and mixed carefully, then 50mL ICES30/4 was added to the solution and mixed; 0.3-0.5mL of the final solution as an enrichment solution was used for 1 L of the incubator. *A. urmiana* at a density of 300 individual/L were enriched in an incubator at a temperature of about 20˚C according to the methods described by Von Elert (2002) and two different enrichment exposure times were employed (12 and 24 hours). The solutions were prepared daily in order to maintain the quality at comparable levels throughout the experiment.

Three different artemia enrichment treatments were tested (analysis was performed in duplicate or triplicate): (I) non-enriched artemia, (II) 12 h-enriched artemia with ICES30/4, and (III) 24h-enriched artemia with ICES30/4.

Sturgeon fish larvae were fed with *A. urmiana* nauplii 4 times per day (Kolkovski *et al*., 2000). The survival rate in each treatment was calculated, based on counting the number of dead larvae. The wet weight of larvae was measured by randomly sampling of 10 larvae in each replicate on the 8th, 13_{th} and 20th days. Growth indexes of fish were calculated based on the following equations (Lee *et al*., 2003).

After 20 days of larval rearing, tolerances of larvae against salinity were measured. Artificial sea water (6, 12 and 18 g/L) was made by adding marine salt into the water in order to evaluate larvae resistance against salinity stress (Kolkovski *et al*., 2000). From each tank, 30 larvae were collected and transferred into the small baskets inside the aquarium. The larval survival was computed by counting of the dead larvae after 1, 2, 4, 8, 12, 24, 36, 48 and 72 hours of salinity stress test.

$$\text{Weight gaing}(\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weith}} \times 100 \quad (1)$$

$$\text{Specific Growth Rate (SGR)} = \frac{\text{In (final weith)} - \text{In (initial weight)}}{\text{Days}} \times 100 \quad (2)$$

Enough samples of artemia and 10 larvae from each replicate were collected and kept in a freezer before analyzing. Extraction of lipid from artemia and fish larvae was implemented through saponification with 2g NaOH in 100ml methanol according to Folch *et al*. (1957). Fatty acid methyl esters were prepared by transesterification with borontrifluoride (BF3) in methanol (Metcalfe and Schmitz, 1961). Fatty acids methyl esters were measured in a DANI-1000 gas chromatograph with flame ionization detector. The column was BPX 70 with a capillary column of 30 m length and ID of 0.25mm. The carrier gas was helium at a pressure of 100 K Pa. Detector and injector temperatures were 260 and 250˚C, respectively. The thermal gradient was 183˚C for 10 min, and then increased by 4˚C/min to 260˚C and this temperature was held for 20 min. The fatty acids were quantified by comparing areas of their peaks with a peak of an internal standard, (C18:0). The peaks of fatty acids were analyzed by connecting the GC to a personal computer and utilizing Millennium software. Peak identification was performed by means of standard sample.

To analyze results, one-way ANOVA was applied and the mean comparison was completed through LSD test at reliability level of 5%. All variances were checked for normality and homogeneity. Data analysis was carried out in SPSS software (release 14.0).

Results

Result on the fatty acid profile of non-enriched and enriched artemia for 12 and 24 hours are shown in Table 1. Eicosapentaenoic acid (EPA, 22:5n3) content in non-enriched artemia was about 0.82±0.11 mg/g DW and docosahexanoic acid (DHA, 22:6n3) was not detected. After enrichment with ICES30/4 for 12 and 24 hours, EPA content increased to about 2.33±0.09, 3.69±0.11mg/g DW of artemia, respectively. The highest n3-HUFA concentration was observed in 24 hours enriched artemia (7.69±0.2 mg/g DW). Table 2 shows the fatty acid composition of sturgeon larvae fed with enriched and non-enriched artemia. Content of n3-HUFA in larvae fed with the enriched artemia was improved accordingly with increments of the enrichment times. This means that larvae with the highest n3-HUFA (5.98±0.15 mg/g-^{1}DW) were those that have been fed artemia enriched diet for 24 hours.

The wet weight of the sturgeon after the 1_{st}, 8_{th}, 13_{th} and 20th day of rearing are shown in Fig. 1. No significant differences were found among treatment's growth rate (p>0.05). Nevertheless, the average growth rate in larvae fed with artemia during 24 h enrichment was higher than the others. Table 3 shows significant differences between survival percentages of the fish larvae fed with non-enriched and enriched artemia (p<0.05).

Table 1: Average fatty acid content in *Artemia urmiana* before and after enrichment in two different times 12 and 24 hours (in mg per dry gram of artemia)*.

Fatty acids artemia enrichment	Non-enriched	ICES30/4 12h	ICES30/4 24h
14:0 (**ns**)	1.42	1.01	1.05
14:1n5	1.16 ^{c}	1.00 ^{b}	0.87 ^{a}
15:0 (**ns**)	0.27	0.32	0.75
15:1n	1.54 ^{b}	1.20 ^{a}	0.96 ^{a}
16:0 (**ns**)	12.21	12.16	12.32
16:1(n-7) (**ns**)	3.34	3.23	3.37
17:0 (**ns**)	1.95	1.45	1.73
17:1n7	2.44 ^{c}	0.91 ^{a}	1.08 ^{b}
18:0	4.90 ^{a}	5.38 ^{b}	6.18 ^{c}
18:1(n9) (**ns**)	18.56	16.74	17.64
18:1(n7)	2.86 ^{a}	3.42 ^{b}	4.31 ^{c}
18:2(n6)-cis (**ns**)	8.25	7.47	7.03
18:3(n3) (**ns**)	29.12	25.49	28.66
20:1(n9) (**ns**)	0.26	n.d	n.d
20:2(n6) (**ns**)	0.33	0.26	0.30
20:3(n3) (**ns**)	0.22	n.d	0.35
ARA 20:4(n6)	0.61 ^{b}	0.47 ^{a}	0.70 ^{c}
EPA 20:5(n3)	0.82 ^{a}	2.33 ^{b}	3.69 ^{c}
DHA 22:6(n3)	n.d ^{a}	1.87 ^{b}	4.00 ^{c}
ΣSFA (**ns**)	21.39	20.32	22.03
ΣUFA(**ns**)	69.51	64.39	72.96
ΣPUFA	39.61 ^{a}	37.89 ^{a}	44.73 ^{b}
Σn3-HUFA	0.82 ^{a}	4.20 ^{b}	7.69 ^{c}

*Values are expressed as the means of two replicates; n.d = not detected; (ns)= not significant. Numbers within the same row with different superscripts are significantly different (p<0.005).
ΣSFA= total saturated fatty acid
ΣUFA= total unsaturated fatty acid
ΣPUFA= total polyunsaturated fatty acid
Σn3-HUFA= total n3 highly unsaturated fatty acid

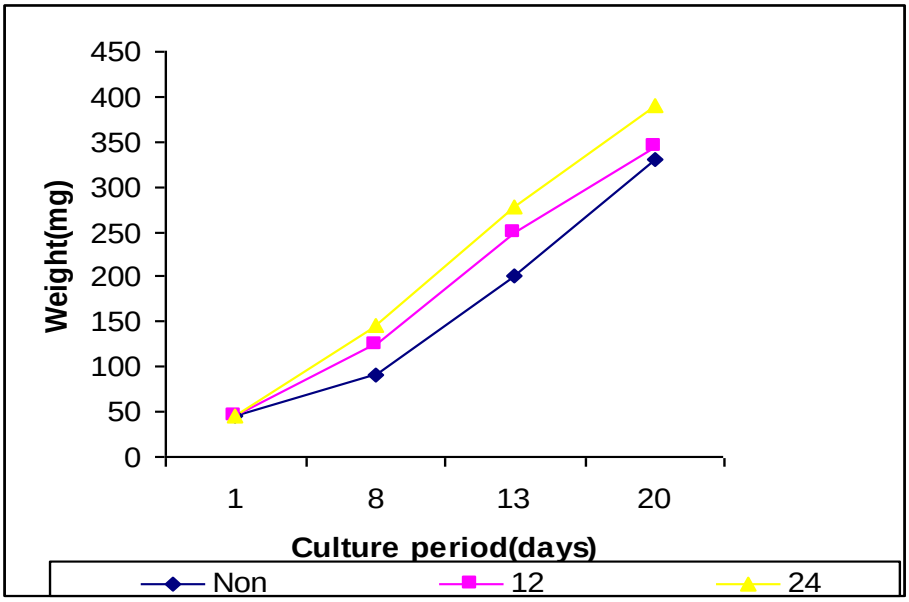

Figure 1: Average wet weight of the Persian sturgeon larvae during 20 days, fed with non-enriched, 12 and 24 h-enriched artemia.

Table 2: Average fatty acid content in the Persian sturgeon larvae at the end of the experiment (in mg per dry gram)*.

Fatty acids artemia enrichment	Non-enriched	ICES30/4 12h	ICES30/4 24h
14:0 (**ns**)	0.77	0.72	0.58
14:1n5	1.78 [b]	0.64 [a]	0.34 [a]
15:0 (**ns**)	0.35	0.42	n.d
15:1n	0.82 [b]	0.74 [b]	0.47 [a]
16:0	13.09 [a]	16.88 [b]	15.13 [b]
16:1(n-7)	4.23 [a]	4.07 [a]	7.65 [b]
17:0	2.18 [c]	1.29 [b]	0.85 [a]
17:1n7	0.40 [a]	1.41 [b]	1.30 [b]
18:0 (**ns**)	11.11	9.67	10.57
18:1(n9)	20.17 [c]	13.75 [a]	15.65 [b]
18:1(n7) (**ns**)	4.50	4.81	4.28
18:2(n6)-cis (**ns**)	4.40	3.72	3.07
18:3(n3)	9.42 [c]	7.31 [b]	5.62 [a]
20:1(n9)	2.41 [c]	1.95 [b]	n.d [a]
20:2(n6) (**ns**)	0.34	n.d	0.40
20:3(n3)	1.62 [b]	0.89 [a]	0.89 [a]
ARA 20:4(n6)	1.47 [a]	2.78 [b]	3.02 [b]
EPA 20:5(n3)	1.90 [a]	2.72 [b]	3.05 [b]
DHA 22:6(n3)	1.30 [a]	2.01 [b]	2.93 [c]
ΣSFA (**ns**)	27.50	28.98	27.13
ΣUFA	54.76 [b]	46.80 [a]	48.67 [a]
ΣPUFA	22.86 [c]	21.38 [b]	18.98 [a]
Σn3-HUFA	3.20 [a]	4.73 [b]	5.98 [c]

*Values expressed are the means of two replicates. Numbers within the same row with different superscripts are significantly different (p<0.005).
n.d.=not detected.
(ns)=not significant

Table 3: Average growth and survival of the Persian sturgeon larvae fed with artemia enriched at various levels of time* after 20 days.

Growth parameter	non-enriched	artemia enrichment times	
		12 hours	24 hours
Initial total length (mm)	21.20± 0.1	22.02±0.2	21.00±0.5
Final total length (mm) (**ns**)	40.30±2.10	42.28±1.34	41.13±0.16
Weight gain (g) (**ns**)	535.2±10.8	580.19±10.0	565.24 ±13.8
SGR (**ns**)	11.30±1.70	12.14±0.18	12.30±0.17
Survival Rate (%)	80.90±2.31[a]	88.83±0.73 [b]	91.53±0.31 [c]

*Mean±Sd. of three replicates. Numbers within the same row with different superscripts are significantly different (p<0.05).
(ns)= not significant

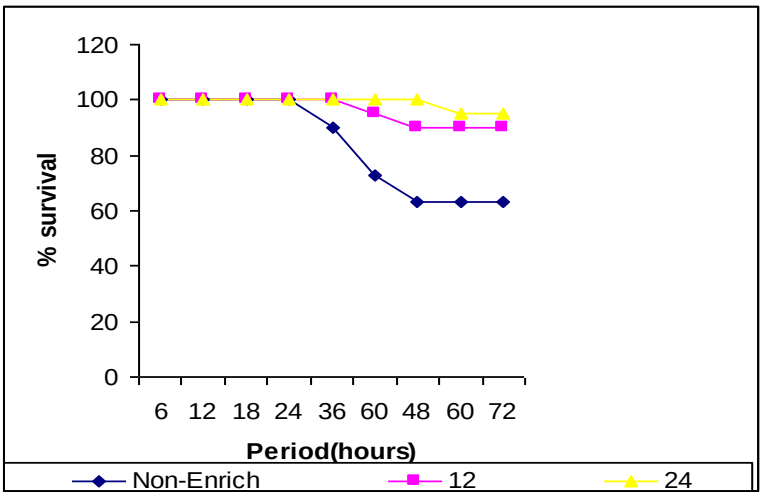

Figure 2: Average survival rate (%) of the Persian sturgeon larvae in 6 ppt salinity.

The highest survival percentage was observed in the larvae fed with 24 hours enriched artemia.

The results of salinity tolerance in 6 ppt salinity are shown in figure 2. The highest survival rate was observed in those larvae fed with 24 h-enriched artemia. All sturgeon fish larvae were killed and exposed to 12 and 18 ppt salinity. No significant differences was seen between enriched and non-enriched treatments (p<0.05), but this difference was significant between 12-hour and 24 hour enrichment treatments (p<0.05). The highest and lowest tolerances were observed in 24-hours enriched and non enriched artemia, respectively.

Discussion

Many different methods have been used for enrichment of live foods (Coutteau and Sorgeloos, 1997; Weers and Gulati, 1997; Von Elert, 2002; Ravet *et al*., 2003; Abedian Kenari and Naderi, 2015).

Arachidonic acid content in artemia nauplii improved significantly (p<0.05) when they enriched with ICES30/4 (Table 1). Different authors reported increasing ARA contents in artemia strains, when they were enriched with fish oils (Kolkovski *et al*., 2000; Koven *et al*., 2001; Brandsen *et al*., 2005; and Achionye Nzeh *et al*., 2012).

The EPA and DHA of artemia were increased with the increment of enrichment times (Table 1). Similar results were achieved for DHA/EPA and ω3/ω 6 ratios. These results were in agreement with Cho *et al*. (2001) who reported when artemia nauplii enriched with ω–yeast, the amount of HUFA fatty acids in artemia nauplii tended to increase. Still, this was different with the work of Sundbom and Vrede (1997), Von Elert (2002), Ravet *et al*. (2003) who suggested DHA could be converted to EPA. The genetical differences may be the reason which must be focused in future studies.

It is worth stating that both EPA and DHA play critical role in survival of fish larvae and specially sea fishes (Watanabe *et al*., 1983; Dhert *et al*., 1990; Sorgeloos *et al*., 1991; Sorgeloos

and Leger, 1992; Kraul *et al*., 1993; Watanabe, 1993; Bahadir Koca *et al*., 2015). We also observed that feeding of the Persian sturgeon larvae with HUFA-enriched artemia resulted in survival improvement compared to those fed with non-enriched artemia.

Growth rate of sturgeon larvae was not affected by enrichment (Table 3 and Fig. 1), which was in agreement with Cynthia *et al*. (2005) who did not find any relationship between Algamac 2000 enrichment and cobia (*Rachycentron canadum*) larval growth.

The results of this study proved that feeding the Persian sturgeon with live food containing high n3-HUFA content increased larval salinity tolerance (Fig. 2). The competitive interactions between EPA and AA are important in the formation of eicosanoids. Eicosanoids are a group of biologically active molecules, once known as local hormones, which include prostaglandins, thromboxanes, and leukotrienes (Sargent, 1995). Several studies have demonstrated that prostaglandins (PGs) are involved in the control of osmoregulatory processes and the regulation of the stress induced hypothalamus–pituitary–interrenal(HPI) axis, which facilitates the release of cortisol, the main corticosteroid in teleost fish (Gupta *et al*., 1985; Wales, 1988). The relationship between feeding of larvae with HUFA-enriched artemia and enhancing resistance against environmental stress has been reported in other species of fish (Dhert *et al*., 1990; Ako *et al*., 1994). Since the content of all such acids increases due to enrichment, it shows that fatty acid plays an important role in resistance against stress (Lavens and Sogeloos, 1996).

According to the results of this study, artemia HUFA level was enhanced by increasing the enrichment time and, therefore, resulted in a better larval development, stress resistance and production enhancement of Persian sturgeon.

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References

Abedian Kenari, A. and Naderi, M., 2015. Effects of enriched *Artemia* by fish and soybean oils supplemented with vitamin E on growth performance, lipid peroxidation, lipase activity and fatty acid composition of Persian sturgeon (*Acipenser persicus*) larvae.

Abi-Ayad, A. and Kestemont, P., 1994. Comparison of the nutritional status of goldfish (*Carassius auratus*) larvae fed with live, mixed, or dry diet. *Aquaculture*, 128, 163-176.

Achionye_Nzeh, C.G., Olumuji, K. and Bello, N., 2012. Effects of Artemia nauplii and formulated diet on growth and survival pf larvae and post larvae of *Clarias gariepinus*

(L). *International Journal of Life Science and Pharma Research*, 2(**2**), 29-34.

Ako, H., Tamaru, C.S., Bass, P. and Lee, C.S., 1994. Enhancing the resistance to physical stress in larvae of *Mugil cephalus* by the feeding enriched *Artemia* nauplii. *Aquaculture*, 122**,** 81-90.

Bahadir Koca, S., Uzunmehmetoglu, O.Y. and Yazicioglu, B., 2015. Effects of enriched artemia on growth and survival of juvenile freshwater crayfish (*Astacus leptodactylus* Esch. 1823). *Iranian Journal of Fisheries Sciences*, 14(**1**), 45-56.

Bransden, M.P., Battaglene, S.C., Morehead, D.T., Dunstan, G.A. and Nichols P.D., 2005. Effect of dietary 22:6n-3 on growth, survival and tissue fatty acid profile of striped trumpeter (*Latris lineata*) larvae fed enriched artemia. *Aquaculture*, 243, 331-344.

Cho, S.H., Hur, S.B. and Jo, J.Y., 2001. Effect of enriched live feeds on survival and growth rates in larvae Korean rockfish, *Sebastes schlegeli Hilgendorf*. *Aquaculture Research*, 32, 199-208.

Copeman, L.A., Parrish, C.C., Brown, J.A. and Harel, M., 2002. Effect of DHA, EPA and ARA on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): A live food enrichment experiment. *Aquaculture*, 210, 285-304.

Coutteau, P. and Sorgeloos, P., 1997. Manipulation of dietary lipids, fatty acids, and vitamins in zooplankton cultures. *Freshwater Biology*, 38, 501-512.

Cynthia, K., Faulk, G. and Holt, J., 2005. Advances in rearing cobia *Rachycentron canadum* larvae in recirculating aquaculture systems: Live prey enrichment and greenwater culture. *Aquaculture*, 249, 231-243.

Dhert, P., Lavens, P., Duray, M. and Sorgeloos, P., 1990. Improved larval survival at metamorphosis of Asian seabass (*Lates calcarifer*) using omega 3-HUFA-enriched live food. *Aquaculture*, 90, 63-74.

Folch, J., Lees, M. and Stanely. G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biology Chemistry*, 226, 497-509.

Gupta, O.P., Lahlou, B., Botella, J. and Porthé-Nibelle, J., 1985. *In vivo* and *in vitro* studies on the release of cortisol from interrenal tissue in trout. I: effects of ACTH and prostaglandins. *Journal of Experimental Biology*, 43, 201–212.

Hung, S.S.O. and Deng, D.F., 2002. Sturgeon, *Acipenser* spp. *In*: C.D. Webster and C.E. Lim (eds). Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing. pp. 344-358.

Kim, J., Massee, K.G. and Hardy, W.H., 1996. Adult *Artemia* as food for first feeding coho salmon

(*Oncorhynchus kisutch*). *Aquaculture*, 144, 217-226.

Kolkovski, S., Czesny, S., Yackey, C., Moreau, R., Cihla, F., Mahan, D. and Dabrowski, K., 2000. The effect of vitamin C and E in (n-3HUFA-enriched *Artemia* nauplii on growth, survival, and stress resistance of freshwater walleye (*Stizostedion vitreum*) larvae. *Aquaculture Nutrition*, 6, 199-20.

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.

Kraul, S., Ako, H., Brittain, K., Cantrell, R. and Nagao, T., 1993. Nutritional factors affecting stress resistance in the larval *Coryphaena hippurus*. *Journal of World Aquaculture Society*, 24, 186-193.

Lavens, P. and Sorgeloos, P., 1996. Manual on production and use of live food for aquaculture. FAO. 375P.

Lee, S.M., Lee, J.H. and Kim, K.D., 2003. Effect of dietary essential fatty acids on growth, body composition, and blood chemistry of juvenile starry flounder (*Platichthys stellatus*). *Aquaculture*, 225, 269-281.

Leger, P., Bengston, D.A., Simpson, K.L. and Sorgeloos, P., 1986. The use and nutritional value of *Artemia* as a food source. *Oceanography Marine Biology*, 24, 521-623.

Metcalfe, L.D. and Schmitz, A.A., 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. *Analytical Chemistry*, 33, 363-364.

Noori F, Takami, G.A., Van Speybroeck M., Van Stappen, G., Shiri-Harzevili, A.R. and Sorgelous, P., 2011. Feeding *Acipenser persicus* and *Huso huso* larvae with *Artemia urmiana* nauplii. *Journal of Applied Ichthyology*, 27, 781-783.

Okunsebor, S.A. and Ayuma, V., 2011. Growths, survival rate and condition factor of *Heteroclaris hatchinling* fed cultured *Moina micra*, Shell free Artemia and combination of both as starter feed Livestock Research for rural Dev. 23(**3**).

Ravet, J.L., Brett, M.T. and Muller-Navarra, D.C., 2003. A test of the role of polyunsaturated fatty acids in phytoplankton food quality for *Daphnia* using liposome supplementation. *Limnology and Oceanography*, 48, 1938-1947.

Sargent, J.R., 1995. Origins and functions of egg lipids: Nutritional implications. *In*: N.R. Bromage and J.R. Roberts (eds.), Brood stock Management and Egg and Larval Quality. Oxford University Press, Cambridge, UK. pp. 353–372.

Sargent, J.R., McEvoy, L.A. and Bell, J.G., 1999. Requirements, presentation, and source of polyunsaturated fatty acid in marine

fish larvae feed. *Aquaculture*, 177, 191-199.

Shakourian, M., Poukazemi, M., Sadati, M.A., Hassan, M.H.S., Pourali, H.R. and Ashdati, H.R., 2011. Effects of Replacing live food with formulated diets on growth and survival rates of Persian sturgeon (*Acipenser persicus*) larvae. *Journal of Applied Ichthyology*, 27(**2**), 771-774.

Sorgeloos, P. and Leger, Ph., 1992. Improved larviculture outputs of marine fish, shrimp, and prawn. *Journal of World Aquaculture Society*, 23, 251-264.

Sorgeloos, P., Lavens, P., Leger, Ph. and Tackaert, W., 1991. State of the art in larviculture of fish and shellfish. *In*: P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier (eds), Larvi '91- Fish & Crustacean Larviculture Symposium. *European Aquaculture Society*, 15, 3-5.

Sundbom, M. and Vrede, T., 1997. Effects of fatty acid and phosphorus content of food on growth, survival, and reproduction of *Daphnia*. *Freshwater Biology*, 38, 665-674.

Von Elert, E., 2002. Determination of limiting polyunsaturated fatty acid in *Daphnia galeata* using a new method enriches food algae with single fatty acid. *Limnology and Oceanography*, 46, 1764-1773.

Wales, N.A.M., 1988. Hormone studies in *Myxine glutinosa*: effects of the eicosanoids arachidonic acid, prostaglandin E1, E2, A2, F2α, thromboxane B2 and of indomethacin on plasma cortisol, blood pressure, urine flow and electrolyte balance. *Journal of Comparative Physiology*, 158B, 621–626.

Watanabe, T., 1993. Importance of docosahexanoic acid in marine larvae fish. *Journal of World Aquaculture Society*, 24, 152-161.

Watanabe, T., Kitajima, C. and Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: A review. *Aquaculture*, 34, 115-143.

Weers, P.M.M. and Gulati, R.D., 1997. Effect of the addition of polyunsaturated fatty acids to the diet on the growth and fecundity of *Daphnia galeata. Freshwater Biology*, 38**,** 721-729.