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Comparative Study of Fatty Acid Composition of Gametes, Embryos and Larvae of Rainbow Trout, *Oncorhynchus mykiss*

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ABSTRACT

The fatty acid compositions of unfertilized eggs, semen, fertilized eggs, eyed embryo, yolk-sac larvae and absorbed yolk-sac larvae were investigated in rainbow trout (*O. mykiss*). The polyunsaturated fatty acids (PUFA) were found the highest followed by monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) in the unfertilized eggs. On the other hand, PUFA was the highest followed by SFA and MUFA in the semen. The proportions of the some fatty acids, such as C14:0, C16:1n-7, C18:0, C18:1n-9, C18:2n-6, C18:3n-3, C20:2n-6, C20:3n-6, C20:4n-6, C22:6n-3 remained stable from fertilized eggs to yolk-sac larvae, therefore no significant ($P>0.05$) difference was observed in these fatty acids between fertilized eggs and yolk-sac larvae. Nevertheless, significant ($P<0.05$) decreases were observed in C14:0, C16:1n-7, C18:1n-9, C18:2n-6, C18:3n-3, C20:1n-9, C20:2n-6, C20:3n-6 between fertilized eggs and absorbed yolk-sac larvae. Moreover, a significant ($P<0.05$) decrease was observed in n-6 value between the fertilized eggs and the absorbed yolk-sac larvae. It reduced from 17.45% at the fertilized eggs to 10.19% at absorbed yolk-sac larvae. It is considered that the reductions of C18:2n-6, C20:2n-6, C20:3n-6 caused a decrease in total n-6. On the other hand, an increase in arachidonic acid (AA) and docosahexaenoic acid (DHA) give rise to (AA)/eicosapentaenoic acid (EPA) and DHA/EPA ratios in absorbed yolk-sac larvae.

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***Oncorhynchus mykiss*, Fertilized eggs, Fatty acids, Rainbow trout, Larvae.**

INTRODUCTION

Salmonids are a family of ecologically and commercially important fish species worldwide (Harlıoğlu, 2011; Kanyılmaz, 2016). Numerous studies were carried out to investigate gamete quality and preservation (Sarvi *et al*., 2006; Niksirat *et al*., 2007a, b; Hatef *et al*., 2007, 2009), genetics (Weber *et al*., 2014; Skagemo *et al*., 2014) and diseases (Burrells *et al*., 2001; Maynard *et al*., 2016) of salmonids aiming to improve production of these valuable species in the aquaculture.

Lipids and fatty acids have significant roles in the reproduction of fish. They affect egg quality, sperm viability, spawning and survival of larvae (Yanes-Roca *et al*., 2009; Lahnsteiner *et al*., 2009). Lipids are the main sources of metabolic energy throughout the embryonic developmental stages in fish and lipid metabolism may vary between different species. For example, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) are the most important fatty acids in the total lipid of eggs of most fish and it is known that these fatty acids markedly influence the reproductive parameters in fish (Bell *et al*., 1995; Yanes-Roca *et al*., 2009). Moreover, DHA, EPA and AA are all involved in maintaining cell membrane structure integrity and function in fish (Sargent *et al*., 1999). AA, precursor of prostaglandin, stimulates ovarian and testicular steroidogenesis and is assumed to be involved in embryonic development of the immune system, hatching and early larval performance (Sorbera *et al*., 1998; Harlıoğlu, 2014).

Fish exhibit seasonal variations in their lipid metabolism particularly related to reproductive process (Bon *et al*., 1997). Konar *et al*. (1999) examined the variation of total lipid and fatty acid composition in the muscle tissues of females and males of *Copoeta trutta* and *Barbus rajanorum* during reproduction period. They observed that the amount of total lipid in muscle tissues of *Copoeta trutta ve Barbus rajanorum* increased in June, but the amount of total lipid and total fatty acid in muscle tissues in both species decreased in August.

Several studies have shown a relationship between embryo and larval quality and biochemical composition (Fraser *et al*., 1988; Abi-Ayad *et al*., 1997; Cejas *et al*., 2004; Laurel *et al*., 2010; Cruzado *et al*., 2011; Farhoudi *et al*., 2011). By studying changes in biochemical composition that occur during embryonic and early larval development, probable nutritional requirements of first feeding larvae can be estimated (Fraser *et al*., 1988). Therefore, the aim of present study was to describe and compare fatty acid composition in gametes (unfertilized

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egg and semen) as well as different developmental stages of embryo and larvae including fertilized eggs, eyed stage, yolk-sac larvae and absorbed yolk-sac larvae in rainbow trout as a highly commercial fish species.

MATERIALS AND METHODS

Gamets (eggs and semen), embryo and larvae of rainbow trout were obtained in June 2013 from a commercial trout production farm, Elazığ, Turkey. Controlled insemination and egg incubation with photoperiod manipulation (18 h dark: 6 h light) has been applied successfully in this farm since 2000. Fish were subjected to accelerated photoperiod regimes leading to earlier spawning. Fish were fed a commercial trout pellets. It included 45% protein, 13% fat, 13.14% ashes, and 89.86% dry matter. The water temperature was fluctuated between 12.2-13.5 °C, dissolved oxygen was 7.3-7.7 and pH was 7.7-7.9. Ten females and 15 males were used in the study. Average weight of females *O. mykiss* was 3.8-4.5 kg and that of males was 1.8-2.2 kg.

Fatty acid analyses

Fatty acid analyses of unfertilized eggs, semen, fertilized eggs, eyed stage, yolk-sac larvae and absorbed yolk-sac larvae were carried out in the present study. For this lipids were extracted with hexane-isopropanol (3:2 v/v; Hara and Radin, 1978). A sample of 1 g (wet weight) was homogenized with 10 ml hexane-isopropanol mixture. The homogenate was centrifuged at 5000 rpm for 5 min at 4°C and the supernatant was used for fatty acid analyses. Fatty acids in the lipid extracts were converted into methyl esters including 2% sulfuric acid (v/v) in methanol (Christie, 1992). The mixture was vortexed and then kept at 50°C for 12 h. Subsequently, after being cooled to room temperature, 5 ml of 5% sodium chloride was added and then it was vortexed. Fatty acid methyl esters were extracted with 2x5 ml hexane, treated with 5 ml 2% $KHCO_3$ solution and then the hexane phase was evaporated by the nitrogen flow and 0.5 ml fresh hexane (Christie, 1992) was added. Samples were placed in auto sampler vials.

Fatty acid methyl esters were analyzed using the Shimadzu GC-17 Ver. 3 gas chromatograph (Kyoto, Japan). 25 m long Machery-Nagel (Germany) capillary column with an inner diameter of 0.25 µm and a thickness of 25 micron film was used. Column temperature was kept at 120-220°C, injection temperature was at 240°C and the detector temperature was at 280°C. The nitrogen carrier gas flow was 1 mL/min. The fatty acids methyl esters were identified by comparison with authentic external standard mixtures analyzed under the same conditions. Data were analysed by Class GC 10 software version 2.01.

Statistical analysis

The data on the fatty acid composition obtained from rainbow trout eggs and larvae were analyzed by using one-way analysis of variance (ANOVA), followed by Duncan's new multiple range test. Differences were considered significant at $P<0.05$.

RESULTS

Total fatty acid composition (expressed as a percentage of total fatty acids) in unfertilized eggs and semen of rainbow trout is presented in Table I. The results of present study showed that in the eggs PUFA was the highest followed by MUFA and SFA. On the other hand, in the semen PUFA was the highest followed by SFA and MUFA. The amount of Σ n-6, DHA/EPA, AA/EPA was higher in the unfertilized eggs than the semen. Among the SFAs, palmitic (C16:0) and stearic (C18:0) acids were the major SFAs in the unfertilized eggs and semen of rainbow trout. In addition, oleic acid (C18:1n-9) was the predominant FA within the MUFAs in the unfertilized eggs and semen. PUFAs were higher in the semen (57%) than the unfertilized eggs (52.2%). Among the PUFAs, DHA, AA and EPA were the major PUFAs in the unfertilized eggs and semen of rainbow trout. Arachidonic acids, DHA and EPA were higher in the semen with 5%, 30.8%, 15.6%, respectively than those of the unfertilized eggs (2.9%, 25.9%, 5.2%, respectively).

Total fatty acid composition of fertilized eggs, eyed embryo, yolk-sac larvae and absorbed yolk-sac larvae are also presented in Table I. The proportions of the some fatty acids such as C14:0, C16:1n-7, C18:0, C18:1n-9, C18:2n-6, C18:3n-3, C20:2n-6, C20:3n-6, C20:4n-6, C22:6n-3 remained stable from fertilized eggs to yolk-sac larvae and no significant ($P>0.05$) differences were determined in those fatty acids between fertilized eggs and yolk-sac larvae. However, significant decreases were observed in C14:0, C16:1n-7, C18:1n-9, C18:2n-6, C18:3n-3, C20:1n-9, C20:2n-6, C20:3n-6 between fertilized eggs and absorbed yolk-sac larvae ($P<0.05$).

Increases of C16:0 and C18:0 caused significant differences in the total SFA between the fertilized eggs and the absorbed yolk-sac larvae ($P<0.05$). MUFA accounted on average of 23.3% of the total fatty acids in the fertilized eggs, 26.6% in the eyed embryo, 24.8% in the yolk-sac larvae and 18.9% in the absorbed yolk-sac larvae. It is thought that the decrease in total MUFA was due to the losses of C16:1n-7, C18:1n-9, C20:1n-9.

A significant decrease was observed in n-6, which diminished from 17.5% at the fertilized eggs to 10.2% at the absorbed yolk-sac larvae ($P<0.05$). The decrease in total n-6 was due to the losses of C18:2n-6, C20:2n-6,

Table I.- Fatty acid composition of utilized eggs, semen, fertilized eggs, eyed embryo, yolk-sac larvae and larvae after yolk-sac resorption of *O. mykis.*

Fatty acids (% of total fatty acids)	Unfertilized eggs	Semen	Fertilized eggs	Eyed embryo	Yolk-sac larvae	Larvae after yolk-sac resorption
C14:0	1.2±0.1	nd	1.2±0.1^{a}	1.3±0.1^{a}	1.2±0.1^{a}	0.6±0.0^{b}
C16:0	14.2±0.7	19.6±0.8	13.5±0.0^{c}	14.3±0.6^{b}	14.1±0.3^{bc}	17.8±0.7^{a}
C16:1n-7	2.8±0.3	nd	3.1±0.5^{a}	3.1±0.3^{a}	3.1±0.2^{a}	1.6±0.2^{b}
C18:0	6.8±1.1	5.2±0.5	6.8±1^{b}	6.5±0.7^{b}	6.1±0.1^{b}	7.8±0.5^{a}
C18:1n-9	20.7±1.5	18.2±1.3	21.5±0.7^{a}	21±2^{a}	19.5±1.1^{ab}	17.2±4.4^{b}
C18:2n-6	9.3±1.2	5.5±0.6	10.7±1.2^{a}	9.3±1.3^{a}	10.3±0.7^{a}	4.8±1.1^{b}
C18:3n-3	1±0.2	nd	1±0.2^{a}	1.2±0.1^{a}	1.2±0.0^{a}	0.4±0.0^{b}
C20:1n-9	2.3±0.0	nd	2.3±0.2^{ab}	2.5±0.1^{a}	2.1±0.1^{b}	1.4±0.2^{c}
C21:0	0.2±0.1	nd	0.2±0.0	0.2±0.0	nd*	nd
C20:2n-6	2.6±0.2	nd	2.5±0.4^{a}	2.7±0.2^{a}	2.5±0.1^{a}	1.8±0.2^{b}
C20:3n-6	1.9±1.1	nd	1.6±0.2^{a}	1.5±0.1^{a}	1.5±0.1^{a}	0.6±0.6^{b}
C20:4n-6	2.9±0.3	5.0±0.6	2.7±0.4^{b}	2.4±0.2^{b}	2.6±1.2^{b}	3.3±0.2^{a}
C22:0	0.3±0.0	nd	0.3±0.0	0.3±0.1	0.3±0.0	nd
C20:5n-3	5.2±0.7	15.6±1.7	5.4±0.8^{a}	4.9±0.2^{a}	4.9±0.2^{a}	5.2±0.2^{a}
C22:2	0.5±0.5	nd	0.3±0.1^{a}	0.38±0.0^{a}	0.5±0.1^{a}	0.4±0.0^{a}
C22:5n-3	2.9±0.2	nd	2.9±0.2^{a}	2.7±0.1^{a}	2.5±0.6^{a}	2.5±0.9^{a}
C22:6n-3	25.9±1.6	30.8±1.4	26.6±2.2^{b}	25.1±2.03^{b}	25.7±0.4^{b}	37.2±1.6^{a}
ΣSFA	22.4±1.5	24.8±0.8	21.7±0.8^{bc}	22.5±0.7^{b}	21.5±0.5^{c}	25.7±0.9^{a}
ΣMUFA	25.8±1.7	18.2±1.3	23.3±8.9^{ab}	26.6±2.2^{a}	24.8±1.2^{ab}	18.9±3.5^{b}
ΣPUFA	52.2±2	57±1.8	53.6±3.7^{ab}	49.6±4.5^{b}	51.2±1.1^{ab}	54.7±2.8^{a}
Σn-3	35±1.7	46.4±1.6	35.9±2.3^{b}	34±2.3^{b}	34.3±1^{b}	44.5±2.5^{a}
Σn-6	16.7±1.3	10.5±0.4	17.5±1.7^{a}	14.4±3.9^{b}	16.8±0.8^{ab}	10.2±0.7^{c}
n-3/n-6	2.1±0.2	4.4±0.2	2.1±0.2^{b}	2.6±1.2^{b}	2±0.1^{b}	4.4±0.4^{a}
EPA/DHA	0.2±0.0	0.5±0.1	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.1±0.0^{b}
DHA/EPA	5±0.7	2±0.3	5±0.8^{b}	5.1±0.2^{b}	5.3±0.2^{b}	7.2±0.3^{a}
AA/EPA	0.7±0.1	0.3±0.0	0.5±0.1^{b}	0.5±0.0^{b}	0.5±0.1^{b}	0.6±0.1^{a}

Superscripts in a row with different letters represent significant difference (P<0.05). Values are mean ±SD. nd, not determined.

C20:3n-6. On the other hand, an increase in AA and DHA gave place to an increase in AA/EPA and DHA/EPA ratios. The content of AA in eggs and larvae after yolk-sac resorption was 2.7% and 3.3%, respectively. The content of DHA in fertilized eggs and larvae after yolk-sac resorption was 26.58% and 37.16% respectively. Significant (P<0.05) differences was observed in DHA between fertilized eggs and absorbed yolk-sac larvae. Consequently, Σn-3 and n-3/n-6 values significantly (P<0.05) increased in absorbed yolk-sac larvae.

DISCUSSION

In the present study, more than 50% of the unfertilized eggs and semen's fatty acid were PUFA. In addition, MUFA and SFA occurred in lower quantities than PUFA in the semen and unfertilized eggs. Mansour *et al.* (2011) reported that sperm of high fertility class Arctic char, *Salvelinus alpinus* had lower concentrations of SFA and higher concentrations of unsaturated fatty acids (C20:4n-6, C22:5n-3, C22:6n-3, Σ n-3, Σ n-6). In this study, a significant amount C18:2n-6 and C20:4n-6 were present in the semen (5.5% and 5.%, respectively). Similarly, Aras *et al.* (2003) found that C18:2n-6 and C20:4n-6 was present in the semen 4.8% and 6.8%, respectively in *O. mykiss* in natural spawning season. Mansour *et al.* (2011) reported that C18:2n-6 and C20:4n-6 were present in the semen (3% and 5.4%, respectively) in high fertility class of *S. alpinus*. The significance of C20:4n-6 for sperm viability was also demonstrated in a previous study on *O. mykiss* (Lahnsteiner *et al.*, 2009). Sorensen *et al.* (1988) reported that C20:4n-6 is one of the precursors of prostaglandins which have been shown to stimulate the male sexual behavior and synchronize the process of male and female spawning.

In the present study, it was observed that total MUFA in the unfertilized eggs (25.8%) were higher quantities than that of the semen (18.2%) in *O. mykiss*. Aras *et al.*

(2003) also reported that total MUFA in the unfertilized eggs were higher (33.9%) than that of the semen (17.7%) in *O. mykiss*. Similarly, Mansour *et al*. (2011) reported 35.9% and 16.6% of total MUFA in the eggs and spermatozoa, respectively. Bell *et al*. (1997) and Sargent *et al*. (2002) reported that lipids are utilized as energy sources throughout embryogenesis. In marine and freshwater fish eggs, lipid reserves are consumed by the developing embryo, both as substrates for energy metabolism and as structural components in membrane biogenesis (Sargent, 1995).

Pickova *et al*. (1997) suggested that positively correlated DHA/EPA ratio with egg symmetry and viability. In this study, a significant amount DHA and EPA were present in the unfertilized eggs (25.9% DHA, 5.2% EPA) and semen (30.8% DHA, 15.6% EPA) in *O. mykiss*. The ratio of EPA/DHA in the present study was circa 0.20 in unfertilized egg which is in the same range (0.2) of that of *O. mykiss* in natural spawning season (Haliloğlu *et al*., 2003).

In the present study, during larval development total MUFA and total n-6 fatty acids were significantly decreased. Decrease in the levels of C16:1n-7, C18:1n-9, C18:2n-6, C20:1n-9, C20:2n-6, C20:3n-6 during development indicated that these fatty acids were consumed as energy source. In freshwater and marine fish MUFAs are a suitable energy source, mainly for organogenesis, metamorphosis and basal metabolism (Farhoudi *et al*., 2011). Similarly, embryos of Atlantic salmon (*Salmo salar*), carp (*Cyprinus carpio*), catabolize fatty acids during the last stage of embryogenesis (Cowey *et al*., 1985; Farhoudi *et al*., 2011). Cejas *et al*. (2004) reported that after hatching, during yolk resorption, the decrease in total lipid content and the changes observed in lipid class content similarly reflect the consumption and lipid mobilization by the larvae. Haliloğlu *et al*. (2003) reported that MUFAs such as C16:1n-7, C18:1n-9 were consumed in egg to fist feeding stage for energetic purposes in *O. mykiss* in natural reproduction period.

In the present study, the percentage of total n-3 fatty acids were significantly increased in the larvae after yolk-sac resorption. Particularly, the increase of C22:6n-3 (DHA) fatty acid concentration occurred in the larvae after yolk-sac resorption. During larval development C22:5n-3 and C20:5n-3 (EPA) were conserved. Similarly, Mourente and Vaquez (1996) reported that in Senegalese sole larvae, DHA was conserved and not being utilized as an energy substrate during larval development. Furthermore, Gimenez *et al*. (2008) stated that in common dentex (*Dentex dentex*) larvae, SFA and MUFA decreased along larval development, while PUFA content increased. DHA levels are relatively conserved in the early common dentex larva. Watanabe (1993), have reported that DHA acts a more important function in the enzyme activity of the cell membrane and in physiological stability than EPA has. In the present study, during larval development, of the total n-6 fatty acids, only C20:4n-6 was increased. Therefore, the ratio of AA/EPA was increased in this study and it was 0.49% in the fertilized eggs and 0.64% in the larvae after yolk-sac larvae. Gimenez *et al*. (2008) reported that these results can also be associated with the development of the immunological system later in development, where AA, and to lesser extent EPA, are eicosanoid precursors involved in immune and inflammatory responses in fish.

CONCLUSION

In conclusion, the present study indicates that *O. mykiss* obtained from photoperiod manipulation utilizes fatty acids particularly MUFAs as energy substrates during its early development stages. In addition, significant decreases occur in C14:0, C16:1n-7, C18:1n-9. Moreover, C18:2n-6, C18:3n-3, C20:1n-9, C20:2n-6, C20:3n-6 reduced from fertilized eggs to absorbed yolk-sac larvae. However, EPA, DHA and AA were conserved between fertilized eggs and absorbed yolk-sac larvae, possibly due to their importance physiological processes.

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Statement of conflict of interest

The author declare no conflict of interest regarding this paper.

REFERENCES

Aras, N.M., Haliloğlu, H.İ., Atamanalp, M. and Kocaman, E.M., 2003. Comparison of the fatty acid compositions of egg and sperms of broodstock rainbow trout (*Oncorhynchus mykiss*). *Türk. J. Vet. Anim. Sci.,* **27**: 833-837.

Abi-Ayad, S.M.E.A., Melard, C. and Kestemont, P., 1997. Effects of n−3 fatty acids in Eurasian perch broodstock diet on egg fatty acid composition and larvae stress resistance. *Aquacul. Int.,* **5**: 161-168.

Bell, J.G., Farndale, B.M., Bruce, M.P., Navas, J.M. and Carrillo, M., 1997. Effects of broodstock dietary lipid on fatty acid composition of eggs from sea bass (*Dicentrarchus labrax*). *Aquaculture,* **149**: 107-119. https://doi.org/10.1016/S0044-8486(96)01436-6

Bell, M.V., Batty, R.S., Dick, J.R., Fretwell, K., Navarro, J.C. and Sargent, J.R. 1995. Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). *Lipids,* **30**: 443-449. https://doi.org/10.1007/BF02536303

Bon, E., Corraz, G., Kaushik, S. and Menn, L., 1997. Effects of accelerated photoperiod regimes on the reproductive cycle of the female rainbow trout: I- seasonal variations of plasma lipids correlated with vitellogenesis. *Comp. Biochem. Physiol.,* **1188A**: 183-190. https://doi.org/10.1016/S0300-9629(96)00415-X

Burrells, C., Williams, P.D. and Forno, P.F., 2001. Dietary nucleotides: a novel supplement in fish feeds: Effects on resistance to disease in salmonids. *Aquaculture,* **199**: 159-169. https://doi.org/10.1016/S0044-8486(01)00576-2

Cejas, J.R., Almansa, E., Jerez, S., Bolanos, A., Felipe, B. and Lorenzo, A., 2004. Changes in lipid class and fatty acid composition during development in white seabream (*Diplodus sargus*) eggs and larvae. *Comp. Biochem. Physiol.,* **139**: 209-216. https://doi.org/10.1016/j.cbpc.2004.07.010

Cowey, C.B., Bell, J.G., Knox, D., Fraser, A. and Youngson, A., 1985. Lipid and lipid antioxidant systems in developing eggs of salmon (*Salmo salar*). *Lipids,* **20**: 567-572. https://doi.org/10.1007/BF02534281

Christie, W.W., 1992. *Gas chromatography and lipids*. The Oil Pres Glasgow, UK, pp. 302.

Cruzado, I.H., Herrera, M., Quintana, D., Rodiles, A., Navas, J.I., Lorenzo, A. and Almansa, E., 2011. Total lipid and fatty acid composition brill of eggs *Scophthalmus rhombus* L. relationship between lipid composition and egg quality. *Aquacul. Res.,* **42**: 1011-1025. https://doi.org/10.1111/j.1365-2109.2010.02684.x

Farhoudi, A., Abedian Kenari, A.M., Nazari, R.M. and Makhdoomi, C.H., 2011. Study of body composition, lipid and fatty acid profile during larval development in caspian sea carp (*Cyprinus carpio*). *J. Fish. aquat.* Sci., **6**: 417-428.

Fraser, A.J., Gamble, J.C. and Sargent, J.R., 1988. Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). *Marine Biol.,* **99**: 307-313. https://doi.org/10.1007/BF02112122

Gimenez, G., Estevez A., Henderson, R.J. and Bell, J.G., 2008. Changes in lipid content, fatty acid composition and lipid class composition of eggs and developing larvae (0–40 days old) of cultured common dentex (*Dentex dentex* Linnaeus 1758). *Aquacul. Nutr.,* **14**: 300-308. https://doi.org/10.1111/j.1365-2095.2007.00530.x

Hara, A. and Radin, N.S., 1978. Lipid extraction of tissues with a low- toxicity. *Anal. Biochem.,* **90**: 420-426. https://doi.org/10.1016/0003-2697(78)90046-5

Harlıoğlu, A.G., 2011. The influence of replacing fish meal partially in diet with soybean meal and full-fat soya on growth and body composition of rainbow trout (*Oncorhynchus mykiss*). *Pakistan J. Zool.,* **431**: 175-182.

Harlıoğlu, A.G., 2014. The importance of arachidonic acid in fish nutrition. *Yunus Res. Bull.,* **3**: 59-65.

Haliloğlu, H.İ., Aras, N.M., Yanık, T., Atamanalp, M. and Kocaman, E.M., 2003. Investigation of changes in fatty acid composition at early development stages of rainbow trout (*Oncorhynchus mykiss*). *Turk. J. Vet. Anim. Sci.,* **27**: 1105-1109.

Hatef, A., Niksirat, H., Amiri, B.M., Alavi, S.M.H. and Karami, M., 2007. Sperm density, seminal plasma composition and their physiological relationship in the endangered Caspian brown trout (*Salmo trutta caspius*). *Aquacul. Res.,* **38**: 1175-1181. https://doi.org/10.1111/j.1365-2109.2007.01784.x

Hatef, A., Niksirat, H., Mohammad, S. and Alavi, A., 2009. Composition of ovarian fluid in endangered Caspian brown trout, S*almo trutta caspius*, and its effects on spermatozoa motility and fertilizing ability compared to freshwater and a saline medium. *Fish Physiol. Biochem.*, **35**: 695-700. https://doi.org/10.1007/s10695-008-9302-6

Kanyılmaz, M., 2016. Reproductive performance of a newly described salmonid fish, Alakir Trout (*Salmo Kottelati*), a candidate species for aquaculture. *Pakistan J. Zool.*, **48**: 83-89.

Konar, V., Canpolat, A., Yılmaz, Ö. and Gürsu, F., 1999. Variation of total lipid and fatty acid amount and compositions in muscles of *Capoeta trutta* and *Barbus rajanorum* mystaceus during reproduction period. *Tr. J. Biol.,* **23**: 319-330.

Lahnsteiner, F., Mansour, N., McNiven, M. and Richardson, G.F., 2009. Fatty acids of rainbow trout (*Oncorhynchus mykiss*) semen: Composition and effects on sperm functionality. *Aquaculture*, **298**: 118-124. https://doi.org/10.1016/j.aquaculture.2009.08.034

Laurel, B.J., Copeman, L.A., Hurst, T.P. and Parrish, C.C., 2010. The ecological significance of lipid/fatty acid synthesis in developing eggs and newly hatched larvae of Pacific cod (*Gadus macrocephalus*). *Mar.*

Biol., **157**: 1713-1724. https://doi.org/10.1007/s00227-010-1445-1

Mansour, N., Lahnsteiner, F., McNiven, M.A., Richardson, G.F. and Pelletier, C.S., 2011. Relationship between fertility and fatty acid profile of sperm and eggs in Arctic Char, *Salvelinus alpinus*. *Aquaculture,* **318**: 371-378. https://doi.org/10.1016/j.aquaculture.2011.05.023

Maynard, B.T., Taylor, R.S., Kube, P.D., Cook, M.T. and Elliott, N.G., 2016. Salmonid heterosis for resistance to amoebic gill disease (AGD). *Aquaculture*, **451**: 106-112. https://doi.org/10.1016/j.aquaculture.2015.09.004

Mourente, G. and Vaquez, R., 1996. Changes in the content of lipid, lipid classes and their fatty acids of developing eggs and unfed larvae of the Senegal sole, *Solea senegalensis* Kaup. *Fish Physiol. Biochem.*, **15**: 221-235. https://doi.org/10.1007/BF01875573

Niksirat, H., Sarvi, K., Amiri, B.M., Karami, M. and Hatef, A., 2007a. *In vitro* storage of unfertilized ova of endangered Caspian brown trout (*Salmo trutta caspius*) in artificial media. *Anim. Reprod. Sci.*, **100**: 356-363. https://doi.org/10.1016/j.anireprosci.2006.08.019

Niksirat, H., Sarvi K., Amiri, B.M., Hatef, A., 2007b. Effects of storage duration and storage media on initial and post-eyeing mortality of stored ova of rainbow trout *Oncorhynchus mykiss*. *Aquaculture,* **262**: 528-531. https://doi.org/10.1016/j.aquaculture.2006.10.031

Pickova, J.R., Dutta, P.C., Larsson, P.O. and Kiessling, A., 1997. Early embryonic cleavage pattern, hatching success and egg lipid fatty acid composition: comparison between two cold stocks. *Canadian J. Fish. aquat. Sci.,* **54**: 2410-2416. https://doi.org/10.1139/cjfas-54-10-2410

Rainuzzo, J.R., Reitan, K.I. and Olsen, Y., 1997. The significance of lipids at early stages of marine fish. *Aquaculture,* **155**: 103-115. https://doi.org/10.1016/S0044-8486(97)00121-X

Sargent, J.R., 1995. In: *Broodstock management and eggs and larval quality* (eds. N.R. Bromage and R.J. Roberts), Blackwell Science, London, UK, pp. 353-372.

Sargent, J., Bell, G., McEvoy, L., Tocher, D. and Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture,* **177**: 191-199. https://doi.org/10.1016/S0044-8486(99)00083-6

Sargent, J.R., Tocher, D.R. and Bell, J.G., 2002. In: Fish nutrition (eds. J.E. Halver and R.W. Hardy), Third Edition, Elsevier Science, USA, pp. 181-257.

Sarvi, K., Niksirat, H., Amiri, B.M., Mirtorabi S.M., Rafiee G.R., Bakhtiyari, M., 2006. Cryopreservation of semen from the endangered Caspian brown trout (*Salmo trutta caspius*). *Aquaculture,* **256**: 564-569. https://doi.org/10.1016/j.aquaculture.2006.02.012

Skagemo, V., Sonesson, A.K., Meuwissen, T.H.E., Lillehammer, M. and Rye, M., 2014. The dissemination of genetic improvement in salmon production. *Aquaculture,* **422**: 78-83. https://doi.org/10.1016/j.aquaculture.2013.11.028

Sorbera, L.A., Zanuy, S. and Acrrillo, M., 1998. A role for polyunsaturated fatty acids and prostaglandins in oocyte maturation in the sea bass (*Dicentrarchus labrax*). *Ann. N.Y. Acad. Sci.,* **839**: 535-537. https://doi.org/10.1111/j.1749-6632.1998.tb10863.x

Sorensen, P.W., Hara, T.J., Stancey, N.E. and Goetz, F.W., 1988. F prostoglandins function as potent stimulants that comprise the post-ovulatory female sex pheromone in goldfish. *Biol. Reprod.*, **39**: 1039-1050. https://doi.org/10.1095/biolreprod39.5.1039

Yanes-Roca, C., Rhody, N., Nystrom, M. and Main, K.L., 2009. Effects of fatty acid composition and spawning season patterns on egg quality and larval survival in common snook (*Centropomus undecimalis*). *Aquaculture,* **287**: 335-340. https://doi.org/10.1016/j.aquaculture.2008.10.043

Watanabe, T., 1993. Importance of docosahexaenoic acid in marine larval fish. *J. World Aquacul. Soc.,* **24**: 495-501. https://doi.org/10.1111/j.1749-7345.1993.tb00004.x

Weber, G.M., Hostuttler, M.A., Cleveland, B.M. and Leeds, T.D., 2014. Growth performance comparison of intercross-triploid, induced triploid, and diploid rainbow trout. *Aquaculture,* **433**: 85-93. https://doi.org/10.1016/j.aquaculture.2014.06.003