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The Effects of Fatty Acid Composition of Black Sea Turbot Egg (*Psetta maxima* Linnaeus, 1758) from Different Breeding Periods on the Egg and Larval Quality

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ABSTRACT: In this study, determination of the parameters that affect the egg quality of Black Sea turbot (*Psetta maxima*) were aimed. Therefore the fatty acid compositions, fertilization, hatching, and survival rates of the eggs of different spawning seasons from the 4 eight-year-old Black Sea turbot were compared.
Fertilization rates of first, midst and the end of the spawning seasons were determined as 54.91±5.90%, 60.36±1.61%, 71.69±1.56%. Hatching rates were determined as 34.67%, 42.22% and 84.41%. The differences between the spawning seasons were significant in terms of fertilization and hatching rates ($p<0.05$). Total saturated fatty acids (SFA) were calculated as 23.65 ± 0.44%, 18.89 ± 0.49% and 22.77 ± 0.56% in first, midst and last of the spawning seasons and the difference between first and the midst seasons were significant ($p<0.05$). Total polyunsaturated fatty acids were determined as 22.78 ± 0.27%, 20.44 ± 0.30%, 21.21 ± 0.57%, respectively and the differences between season were determined as statistically significant ($p<0.05$).
As a result, when the fertilization and hatching ratios were considered as egg quality criteria, the eggs taken in the last season may have high quality.

Key words*: Turbot (Psetta maxima), egg quality, fatty acid composition, fertilization rate, hatching rate*

Farklı Üreme Periyotlarındaki Karadeniz Kalkan Balığı (Psetta maxima Linnaeus, 1758) Yumurtalarının Yağ Asidi Kompozisyonunun Yumurta ve Larva Kalitesi Üzerine Etkisi

Bu çalışmada, Karadeniz kalkan balığında (*Psetta maxima* Linnaeus, 1758) yumurta kalitesini etkileyen faktörlerin belirlenmesi amaçlanmıştır. Bu sebeple, 8 yaşında, 4 adet damızlık Karadeniz kalkan balığının üreme döneminin farklı evrelerine ait yumurtaların, yağ asidi kompozisyonları, döllenme ve larva çıkış oranları ve yaşama oranları karşılaştırılmıştır.
Üreme sezonunun başı, ortası ve sonundaki yumurta partilerinin döllenme oranları sırasıyla, %54.91±5.90, %60.36±1.61, %71.69±1.56 olarak tespit edilmiştir. Larva çıkış oranları ise sırasıyla, %34.67, %42.22 ve %84.41 olarak belirlenmiştir. Üreme sezonu boyunca döllenme oranları ve larva çıkış oranları istatistiksel olarak farklılık göstermiştir ($p<0.05$). Toplam doymuş yağ asit (SAFA) miktarları sırasıyla, %23.65±0.44, %18.89±0.49 ve %22.77±0.56 olarak saptanmış, ilk ve orta partiler arasındaki farkın istatistiki olarak önemli olduğu belirlenmiştir ($p<0.05$). Toplam çoklu doymamış yağ asitleri (PUFA) miktarı sırasıyla %22.78±0.27, %20.44±0.30, %21.21±0.57 olarak tespit edilmiş ve partiler arasındaki farkın istatistiki olarak önemli olduğu ($p<0.05$) görülmüştür. Sonuç olarak döllenme ve larva çıkış oranı yumurta kalite kriteri olarak değerlendirildiğinde sezon sonunda bırakılan yumurtaların yüksek kalitedeki yumurtalar olduğu söylenebilir.

Anahtar kelimeler: *Kalkan balığı (Psetta maxima), yumurta kalitesi, yağ asidi kompozisyonu, döllenme oranı, larva çıkış oranı*

1. Introduction

The most commonly traded fish species in the Black Sea are; haddock, red mullet and turbot (Polat, 2011). Turbot is the most valuable of among these. Turbot (*Scophthalmus maximus*) are summer-spawner flat fish which release their eggs in batches over a period of 2-3 months in approximately 10 weeks (McEvoy et al., 1993).

Pelagic turbot eggs are smooth and global, their perivitelline space is narrow, vitelluses are homogenous and they also contain a posterior positioned oil globule. The incubation period of eggs changes according to the water temperature: 9-10 days in 10^{o}C, 7 days in 12^{o}C and 5 days in 14^{o}C. The total length of yolk-sac larvae are generally 2.14-2.80 mm.

It is seen in the researches, which have been made to determine how essential lipids and fatty acids are for embryonic and larval development of sea fish; phospholipids have an important role in embryogenesis and pre-larvae of halibut, turbot and sea bass (Özgür, 2009).

Obtaining egg and larvae in highest quantity and quality is the major aim of fish production. Parameters of egg quality are defined as egg properties that have an important role over egg fertilization, embryonic development and larvae survival rate (Bromage et al., 1992).

The main criteria to determine the egg quality are fertilization and hatching ratio however, those are not enough for determination. It has been stated that, the total lipid content of eggs has also a positive correlation between fertilization and hatching rates (Zhukinsky and Kim, 1981). Therefore, in this research, fatty acid composition was used as main parameter for determination of egg quality. Furthermore fertilization, hatching, survival and abnormality rates of larvae were observed.

In this study, it is aimed to define the factors that affect egg quality of Black Sea Turbot to help aquaculture save time and money by obliterating low quality egg batches. Herewith, the process of Black Sea Turbot production accelerates.

2. Material and Method

This research was performed in the laboratory of Central Fisheries Research Institute, Ministry of Food, Agriculture and Livestock. 4 female, 8-year-old, (63.73±1.59 cm, 5374.25±439.97 g) and 15 male, 5-year-old, (46.57±1.29 cm, 1898.16±182.81 g) Black Sea Turbot were used in the study. The fish were obtained from the hatchery of the institution.

LHRH-a hormone was applied to females that have oocytes larger than 400 μm. The appropriate pellet hormone (LHRH-a: 100 μg/kg fish) was injected into the pre-dorsal muscle by a metal equipment that has a 0.5 mm inner radius (Çiftçi et al 2002; Aydın, 2008; Polat, 2011).

Male fish were not exposed to hormone since they have enough sperms. On the other hand, hormone injected female fish were culminated in mature eggs after a week and mature eggs were stripped (Polat, 2011).

Stripped eggs were fertilized based on the wet method (Chereguini et al., 1999; Maslova, 2002; Kjørsvik et al., 2003; Aydın, 2008). In the name of determining the fertilization, hatching rate and abnormalities; 100 eggs were placed in 3 different beakers (500 ml each) from every batch during the breeding season.

The incubation of the eggs took place in an optimal temperature maintaining incubator at 14^{o}C. Approximately 100 eggs in beaker were monitored and fertilization rate was calculated

as the ratio of fertilized eggs to total number of eggs (Howell et al., 1991; Nissling et al., 2006; Aydın, 2008; Polat, 2011). Hatching rate was calculated by dividing the yolk-sac larvae number to fertilized egg number. Morphological deformation observed in larvae is defined as abnormal larvae and this is also calculated with abnormal larvae number to total larvae number. All these calculations are termed in percent (%).

Unfertilized eggs were stripped from the broodstock to the tubes (50ml.). The egg samples were kept for fatty acid analysis at -80 ^{o}C. This process was sustained along the breeding season.

The egg samples taken during the breeding season (early, mid and late batches) were transformed into fatty acids methyl esters in compliance with the Soxhlet System (AOAC, 1995) and fatty acid compounds were determined in a gas chromatography.

Minitab 15 statistical software was used for data analysis. Analyses of variances were performed by using *one-way ANOVA* and differences between the means were tested by using Tukey's test.

3. Results and Discussion

Fatty acids in eggs are used as energy source and as structural member for embryos and larvae development. While a part of fatty acids join the structural lipids in larvae tissue, the other part of them are catabolized (Wiegand, 1996). Catabolized n-3 PUFA and SFA during the first periods of development are utilized for energy production in the later phases (Falk-Peterson et al., 1989).

In the name of examining the factors that affect the Black Sea Turbot egg quality, the eggs (taken from the early, mid and late batches) were taken during the breeding season, were determined in point of fatty acids composition. The total amount of SFA in the samples were found as 23.65±0.44%, 18.89±0.49% and 22.77±0.56% respectively for the early, mid and the late batches and the differences between the early season and mid-season was found statistically significant ($p<0.05$). In respect to this result, palmitic acid (C16:0) was found to have the highest amount among SFA acids (Table 1). Palmitic acid (C16:0), which is the main structure member of phospholipids and plays and important role in membrane formation through the embryogenesis, is a dominant fatty acid in SFA's (Dantagnan et al., 2007). Besides, Vázquez et. al., (1994) report that C16:0 is used as an energy source during hatching. Silversand et. al., (1996) examined the fatty acid compositions of wild and cultured turbot in the name of estimating the nutritional requirements during embryo and pre-larvae development. They reported that fatty acid values in wild and cultured turbot eggs are consequently as follows: SFA (2.7±0.2 %, 3.5±0.2 %), palmitic acid (16.1±0.3%, 14.9±0.5%), stearic acid (4.0±0.5%, 2.8±0.1%). They also reported that palmitic acid amount is higher than other SFAs. Palmitic acid ratios in similar studies found to be supportive to our findings.

The total amount of MUFA was found 26.05±0.30%, 24.53±1.61%, 25.16±0.76% respectively for the early, mid and late batches and the differences between the batches were found statistically insignificant ($p>0.05$). As seen in Table-1 the highest amount of MUFA was oleic acid (18:1ω9) and there were no difference during the season. MUFA types as 16:1 n-7, 18:1 n-7 and 18:1 n-9 are used as energy source during development period from fertilized egg until first nutrition phase of post larvae (Vázquez et. al., 1994). On the other hand, McEvoy et. al., (1993) took samples from turbot egg during early, mid and late breeding seasons and they examined fatty acids compositions of eggs. They only determine

the values of oleic acid (C18:1ω9), palmitoleic acid (C16:1), eicosanoid acid (C20:1), erucic acid (C22:1n9) and nervonic acid (C24:1n9) from MUFA, according to the fatty acids data analysis. They also discovered while amount of C18:1n9, C16:1 and C20:1 are low during the late season, only C18:1ω9 is high during mid-season, among the MUFA. According to the output data of research; oleic acid (C18:1n9) value are significantly higher than all other values MUFA. Present study proves that amount of MUFA are higher than other fatty acid groups. It can be told that; the egg contains high amount of energy for embryonic development. Therefore, it supports the information in literature that MUFAs are energy sources.

Table 1. The amount of fatty acids determined during the season in Black Sea Turbot. (%)

FATTY ACIDS	**EARLY**	**MID**	**LATE**
C14:0	2.82±0.13^{a}	2.73±0.12^{a}	2.82±0.17^{a}
C16:0	14.53±0.32^{a}	11.67±0.47^{b}	14.50±0.46^{a}
C18:0	3.37±0.23^{a}	2.98±0.33^{a}	3.14±0.21^{a}
C20:0	2.49±0.03^{a}	1.16±0.05^{b}	1.96±0.16^{c}
C24:0	0.44±0.02^{a}	0.35±0.02^{b}	0.35±0.02^{b}
ΣSFA	**23.65±0.44^{a}**	**18.89±0.49^{b}**	**22.77±0.56^{a}**
C16:1	6.72±0.12^{a}	5.56±0.19^{b}	6.39±0.28^{a,b}
C18:1ω9	18.16±0.37^{a}	17.81±1.47 ^{a}	17.76±0.63 ^{a}
C20:1	0.46±0.01^{a}	0.59±0.02^{b}	0.38±0.04^{a}
C22:1ω9	0.35±0.01^{a}	0.30±0.02^{b}	0.29±0.03^{a,b}
C24:1ω9	0.37±0.01^{a}	0.27±0.01^{b}	0.33±0.01^{a}
ΣMUFA	**26.05±0.30^{a}**	**24.53±1.61^{a}**	**25.16±0.76^{a}**
C18:2ω6	3.22±0.13^{a}	3.02±0.08 ^{a}	3.17±0.16 ^{a}
C20:4ω6	1.21±0.09 ^{a}	1.18±0.03 ^{a}	1.33±0.06 ^{a}
C18:3ω3	1.81±0.19^{a}	1.62±0.05 ^{a}	1.88±0.12 ^{a}
C20:5ω3	3.27±0.11 ^{a}	2.75±0.12^{b}	3.07±0.14 ^{a,b}
C22:6ω3	13.29±0.03 ^{a}	11.87±0.30 ^{b}	11.76±0.31 ^{a,b}
ΣPUFA	**22.78±0.27^{a}**	**20.44±0.30^{b}**	**21.21±0.57^{a,b}**
DHA/EPA	**4.08±0.12^{a}**	**4.36±0.30^{a}**	**3.86±0.18^{a}**
ΣPUFAω6	4.42±0.13^{a}	4.20±0.08^{a}	4.49±0.10^{a,b}
ΣPUFAω3	18.36±0.15^{a}	16.24±0.22^{b}	16.71±0.48^{a,b}
n-3/n-6	**4.16±0.08^{a}**	**3.87±0.02^{b}**	**3.72±0.07^{b}**

SFA= Saturated fatty acids (%)
MUFA=Monounsaturated fatty acids (%)
PUFA=Polyunsaturated fatty acids (%)
Each value may express average ± standard error.
The expressed values with different superscripts in the same line are statistically different from each other ($p<0.05$).

The amount of DHA (C22:6n3) and EPA (C20:5n3) in the PUFA were determined and DHA/EPA ratio was calculated respectively for the early, mid and late batches. The DHA/EPA ratio was calculated as 4.08±0.12%, 4.36±0.30%, 3.86±0.18% for the early, mid and late batches respectively and the differences between the batches were found to be

statistically insignificant ($p>0.05$) (Table 1). DHA, which is highly found in marine fish as essential fatty acids, is notified effective for brain and nerve system development (Evans et. al., 1996). Therefore, it can be said that DHA plays an important role in embryonic development and larvae period (Furuita et. al., 2006). DHA amount, which is highly found in egg, decreases during the development because DHA is consumed as growth and energy source in pre-larvae period (Takeuchi, 1997). Arachidonic acid (ARA) value increases when fish are exposed to extraordinary environments or situations that increase their stress levels (Yanes-Roca et. al., 2009). Present research shows no difference in arachidonic acid during the season. The reason of the stability on arachidonic acid levels might depend on the controlled hatching conditions in the facility. Watanabe (1993), specifies that DHA plays a more important role than EPA in enzyme activity and physiologic balance of cell membrane. Lack of DHA may lead to behavioral disorder in larvae (Sargent, 1995). Bell et. al., (1995) alleged that DHA is more important than EPA in lipids biochemistry function. Koven et. al., (1993) confirmed the literature with observing DHA stability while EPA loss in their research on seabream. According to the data of present study DHA is higher than EPA in PUFA. Accordingly, this study supports the literature.

Furuita et. al., (2006) stated that n-6 fatty acids have negative effect over egg quality of Japanese eel. According to the data of present study, the highest n-6 fatty acids amount, the best fertilizing and hatching rates were found at the late season. For this reason, it can be said that, there is an important relation between n-6 fatty acids and egg quality for turbot. Pickova et. al., (1997) stated that DHA/EPA ratio is directly proportionated to egg quality. Tocher, (2010) determined that n-3 HUFA necessities of larvae decrease as DHA/EPA ratio increases. Furuita et. al., (2006) stated that, there is insignificant ($p>0.05$) difference between DHA/EPA ratios of low quality eggs and high quality eggs in their research on Japanese eels.

Incubation water temperature was measured approximately 14.08±0.01°C during the experiment in which embryonic development was observed (Figure 1).

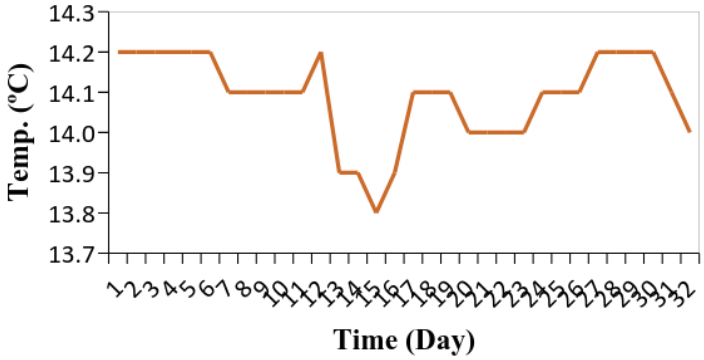

Figure 1. Incubation water temperature used in the experiment.

Embryonic development was monitored during the breeding season. According to the findings, the highest fertilization and hatching rates were seen at the end of this season (Table 2). A remarkable difference is shown between the survival rates of the larvae on the first and the third days which consume yolk-sac ($p<0.05$) (Table 2). The result of the regression analyzes shows that; larvae's growth in length was irregular until they consume their yolk-sacs ($r^2=0.28$), (Figure 2). There is not enough studies on using fertilizing rate as egg quality criteria for marine fish (McEvoy, 1984; Aydın and Polat, 2007). However, Kjorsvik et. al.,

(2003) determines a positive co-relation between fertilizing rate and hatching rate in their study over Atlantic Turbot. In contrast to this study, Aydın and Polat, (2007) determined that such a relation cannot be observed over Black Sea Turbot in their study. Evans et. al., (1996) examined the influence of fertilizing rate and larvae survival rate of first and second batches of Atlantic Halibut eggs biochemical composition. They found fertilizing rate of first batch as 56%, and second batch as 81%. Evans et. al., (1996) notified there is a positive co-relation between fertilizing rate and larvae survival rate in their study. Present study shows that late season has the highest fertilizing rate and mid-season has the highest survival rate. Yanes-Roca et. al., (2009) stated that the higher DHA amount is, the higher rational values of these three parameters (fertilizing rate, hatching rate and survival rate) in their study over *Centropomus undecimalis*. Furuita et. al., (2006) detected a positive influence of DHA amount over hatching and survival rate in their study on Japanese eel. According to the data of present study there is no relation between fertilizing rate, hatching rate and survival rate. The difference between species may cause such results.

Tablo 2. Data derived by the embryonic development observation during the breeding season.

	Early Season	**Mid-Season**	**Late Season**
Fertilization Rate (%)	54.91±5.90[a]	60.36±1.61[b]	71.69±1.56[c]
Hatching Rate (%)	34.67±5.89[a]	42.22±5.43[b]	84.41±0.98[c]
Abnormality Rate (%)	28.84±5.60[a,b]	30.92±3.46[a]	13.35±0.84[b]
Survival Rate of the Yolk-sac Consumed Larvae (%)	9.85±1.32[a]	40.45±8.17[b]	44.79±7.98[c]
Survival Rate of the 3rd day After Consuming Yolk-sac (%)	2.86±1.31[a]	12.34±1.29[b]	11.14±2.90[c]
Length of Yolk-sac Larvae (mm)	3.01±0.10[a]	3.28±0.01[b]	3.33±0.04[c]
Length of Post-Larvae (mm)	4.22±0.18[a]	3.89±0.05[b]	3.68±0.02[c]

Each value may express average ± standard error.

The expressed values with different superscripts in the same line are statistically different from each other ($p<0.05$).

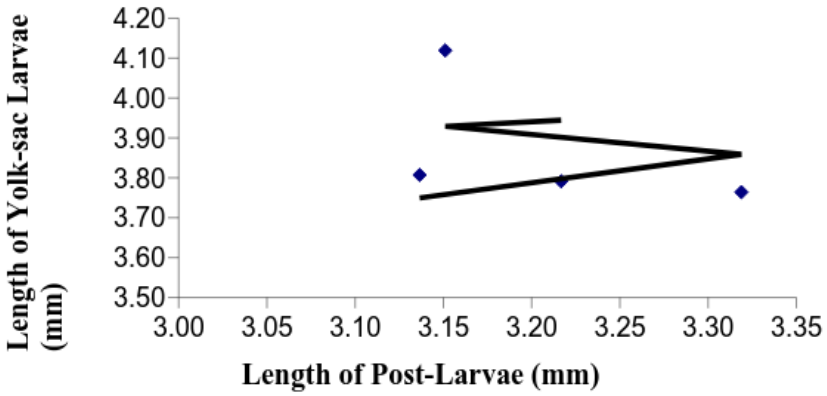

Figure 2. The regression relation among larvae lengths.

Additionally, in this study notochord and tail deformation was also observed at pre-larvae during the breeding season (Figure 3).

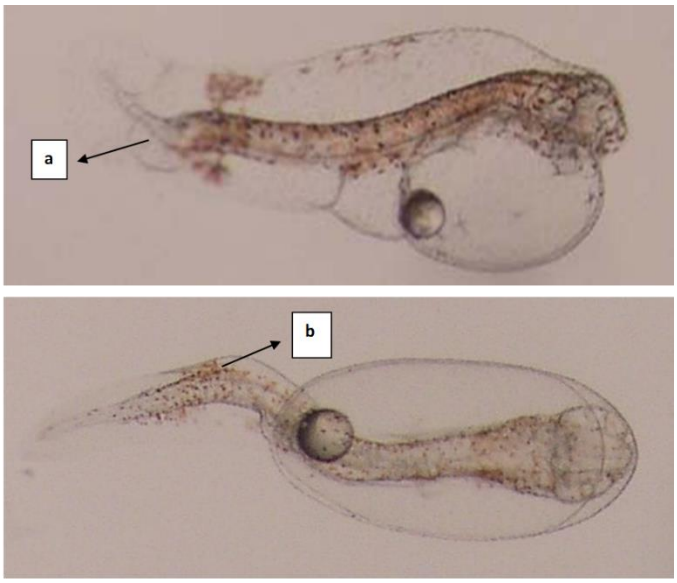

Figure 3. a) Tail deformation. b) Notochord deformation seen in pre-larvae (Original).

4. Conclusion

Finally; even the differences between fatty acid compositions of batches are found to be significant, this parameter may not be considered as egg quality criteria. However, fertilizing rate and hatching rate show a constant rise during the season. Although fertilizing and hatching rates are both at their highest peak at late season, survival rate is the highest at mid-season. For this reason, it can be said that, when fertilizing and hatching rates are considered as egg quality criteria, late season eggs are high-quality on the other hand when survival rate is considered as egg quality criteria mid-season eggs are high-quality.

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