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The Embryonic Development of Black Sea Turbot (*Psetta maxima* Linnaeus, 1758) Eggs in Different Incubation Temperatures and Salinities

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Abstract

In this research, the effects of different temperatures (8, 10, 12, 14, 16 and 18°C) and salinities (5, 10, 15, 18, 20, 25, 30, 35 and ‰40) on the development of turbot (*Psetta maxima*) eggs were investigated in triplicates. Broodstock produced in CFRI were used in this research. Fertilization, hatching, anomaly rate and larvae length were measured. Time dependent embryonic development were observed.

Embryonic development was completed in each temperatures. It is observed that water temperature has significant effects on fertilization, hatching, anomaly and larvae length ($P<0.05$). Regression analysis showed that there was a negative correlation between the embryonic development and the water temperature of incubation. Time for hatching at 8 and 18°C were 2552 hour/degree and 1350 hour/degree, respectively. Better fertilization rate were obtained in salinities of ‰18, ‰20 and ‰25, respectively. Fertilization was not succeeded in the salinity values of 5‰ and ‰10. The anomaly rate in the salinity values of 15‰ and 40‰ was higher then in accordance with other salinity values.

According to result of this study, to get high fertilization and haching rate and low abnormality for Black Sea Turbot, water temperature and water salinity were found as 14°C and ‰18, respectively.

Key Words: Black sea turbot, embryonic development, water temperature of incubation, salinity, Karadeniz Kalkan Balığı, Embriyonik gelişim, İnkübasyon su sıcaklığı, Tuzluluk

Introduction

Turbot (*Psetta maxima* Linnaeus, 1758) is a flatfish species in Europe, which is increasingly being cultured in China since its introduction in 1992 (FAO, 2010). It is farmed on the Atlantic coast of Europe as well as on the Pacific coast of Asia including China, Korea and Japan (Ma et al., 2013). In the North Sea, turbot (*Scophthalmus maximus*) represent highly valuable species in commercial fisheries (Kerby et al., 2013). There are several studies on culture conditions of this species. However there is little published data on the water parameters requirement of embryonic development for turbot.

Temperature is the main environmental factor management of the development of fish eggs. It determines certain morpological feature, hatching rate, embryonic development and the behaviour of larvae. The

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temperature requirement varies among species and even for various development stages (e.g. spawning, embryonic larval development) of given species (El- Hakim et al., 2009). Salinity can affect yolk utilisation and larval growth and survival by influencing the amount of energy needed for osmoregulation (Howell et al., 1991).

The aim of this study was to investigate the effects of different temperatures (8, 10, 12, 14, 16 and 18°C) and salinities (5, 10, 15, 18, 20, 25, 30, 35 and ‰40) on the development of turbot (*Psetta maxima*) eggs. From the egg stock randomly and fertilized in different temperatures in three separate series of experiments. Fertilization, hatching, anomaly rate and larvae length of these series were measured. Time dependent embryonic development was observed.

Material and Method

5 female (6-year-old, mean length: 58.9±3.34cm, mean weight: 4599.8±821.25g) and 10 male (4-year-old, mean length: 53.9±7.43cm, mean weight: 3220.9±852.51g) Black Sea Turbot (*Psetta maxima* Linnaeus, 1758) broodstocks which were produced in sea fish hatchery of Trabzon Central Fisheries Research Institute (CFRI) were used in the present study. Broodstocks were kept in 1 FRP adaptation tanks (2×4×0,5 m) that were equally divided into 4. Water temperature was 10 °C initially and increased 0,5°C every day until 14 °C. Water was filtered with a mechanical filter containing anthracite and sand that is 0,8 mm in diameter and finally with a 5µ cartridge filter it was exposed to ultraviolet light. The water was exposed to UV light with 5µ and 1µ cartridge filters one more time before its use for incubation. Maturity control and stripping operation were both made on a special stripping table. 1,5 mm inner diameter cannulas were used to take oocyte samples from female gonads.

Hormone Application

LHRH-a hormone was applied to females which have oocyte diameter larger than approximately 400µm. After calculating the hormone amount (range 100 µg kg− 1) according to the weight of the fish, female turbots were induced to spawn using intramuscular pellet implants which has 0,5mm in inner diameter containing LHRH-a. On the other hand, males were not applied with hormone since enough amount of sperm can be obtained. After all, eggs were matured one week after the hormone application to females and matured eggs were stripped.

Broodstock Management

The males were checked and mature ones were picked for artificial fertilization. Little amount of semen was added with a drop of seawater and sperm movements were observed under a x100 zoom microscope. First, the sperm was sucked by a sterilized syringe, which has a 10cm. transparent plastic pipe, during the process of stripping. Then, it (The sperm) was kept in an iced polystyrene box until fertilization under the temperature of 4ºC.

Matured eggs of 4 female fish were stripped in a box about one week after the hormone application and consequently 2650 g of eggs were collected in a pool. 100 of these eggs were randomly selected and their diameters were measured under the microscope.

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Egg Fertilizing

In this study, embryonic development was synchronously observed in two stages. For the first stage, it was observed in stabilized salinity (‰18) and different temperatures (8, 10, 12, 14, 16 ve 18°C). Enough amount of egg and sperm was taken from the stock and fertilized artificially with wet fertilizing method (Chereguini et al., 1999; Maslova, 2002; Kjørsvik et al., 2003; Aydın, 2008).

For the second stage, it was observed in stabilized temperatures (14ºC) and different salinities (‰5, ‰10, ‰15, ‰18, ‰20, ‰25, ‰30, ‰35, ‰40). In order to observe the embryonic development; firstly the egg and secondly the sperm were put in sequence in each water salinity concentrations and fertilized.

The fertilization time is considered as the moment when the sperm unite the eggs.

Incubation of Eggs

In order to observe the embryonic development of turbot eggs in different temperatures, 3000 fertilized eggs were put in 3 beakers (1 lt.), and beakers were put in the incubators which had adjusted temperatures different temperatures for each. To determine fertilization rate, hatching rate and abnormalities, ≈100 eggs were randomly placed in 3 beakers (250 ml) in different temperatures for each, as well.

Salinity adjustment was made to observe the effect of salinity difference on the egg development. Sea salt was used to increase salinity and filtered freshwater was used to decrease. The salinity was measured by refractometer. Approximately 100 eggs were randomly placed in 3 beakers (250 ml) in different salinities for each. To observe the time dependent embryonic development of turbot eggs for each different salinity, 3000 fertilized eggs were put in 3 beakers (1 lt.) (Nissling et al., 2006).

Anti-bacterial substance (Streptomycin 0,05g/l) was added into the incubating water to eliminate bacterial reproduction. The incubation water was refreshed daily with reserved water that has the same properties, at the ratio of 2/3. Dead eggs and larvae were taken out from the beaker daily (Nissling et al., 2006; Aydın, 2008).

Calculating the Rate of Fertilizing, Hatching and Abnormalities

Transparent and clear eggs were put in each beaker to determine fertilizing, hatching and abnormalities rates. If cell division could be seen on eggs, they were recorded as fertilized; on the other hand if cell division could not be seen, they were recorded unfertilized eggs (Howell et al., 1991, Aydın, 2008). Fertilized egg number was determined via microscopic observation in 2-64 blastomer stages.

Fertilizing rate was calculated with fertilized egg number to total egg number (Howell et al., 1991; Nissling et al., 2006; Aydın, 2008). Also, hatching rate was calculated with yolk-sac larvae number to fertilized egg number.

The morphologically deformed larvae were determined as abnormal larvae and abnormality was calculated with abnormal larvae number to total larvae number. Calculations were indicated with "%".

Observing Embryonic Development in Different Temperatures

To observe the embryonic development of turbot eggs that were incubated in different temperatures (8, 10, 12, 14, 16, 18°C) and with ‰ 18 of salinity which is natural for Black Sea, pictures of eggs were taken once in 30

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minutes for the first 16 hours then in various time periods. The pictured eggs were remarked via determining embryonic development stages in different temperatures. The temperature values of normally accomplished embryonic development were determined.

Observing Embryonic Development in Different Salinity Values

Embryonic development of turbot fish egg was determined in 14ºC via using 9 different salinity values (‰5, ‰10, ‰15, ‰18, ‰20, ‰25, ‰30, ‰35, ‰40). ‰18 valued water, which is natural for Black Sea, was taken as control group among treatment groups. The photographs of embryonic development were taken at certain intervals and salinity values of accomplished embryonic development were determined. The differences between salinity values were determined with revealing fertilizing rate, hatching rate and abnormalities.

Statistical Analysis of the Data

The comparison of fertilizing, hatching and abnormality rates in different temperature and salinity values was made by variance analysis (ANOVA). The difference was tested by Tukey. The data was analysed with Statistica 7,0 and Excel 2007.

Results

As a result, the average diameter of eggs was found 1.09 mm±0.03 mm (min: 1.02 mm, max: 1.15 mm) by the measurements on the samples from egg stock that was set up to use in this experiment. Additionally, the eggs contain pelagic, translucent oil globule.

Embryonic Development at Different Incubation Temperatures

Water temperatures were determined as 8,08°C±0,06°C at 8°C, 10,07°C±0,06°C at 10°C, 12,06°C±0,05°C at 12°C, 14,07°C±0,05°C at 14°C, 16,08°C±0,08°C at 16°C and 18,10°C±0,07°C at 18°C during the study where embryonic development was observed. Fertilizing rate, hatching rate, abnormality and size of prelarvae were checked in sequence for an embryonic development observation. In addition to these, all embryonic development phases were observed under the microscope and created *Table 1*. Embryonic development phases, observed in the egg, were photographed as seen in *Fig. 1*.

The highest fertilizing rate was confirmed in 14 °C and the lowest was in 8 °C. Significant decrease was seen on fertilizing rates according to increased or decreased temperatures ($P<0.05$). The most successful hatching rate was at 14 °C and the least was at 18 °C. There was a significant decline for hatching rates as increasing or decreasing the incubation water temperature compared to 14 °C ($P<0.05$). There were also notochord and tail deformations of prelarvae post hatching (Fig. 2). 8 °C and 18 °C both have the highest abnormality rates. Significant rise of abnormalities were recorded upwards and downwards from 14 °C, which eggs were incubated ($P<0.05$). The difference between the prelarvae lengths of the hatching temperatures 12°C, 14°C, 16°C, 18°C and the temperatures 8°C, 10°C was significantly important ($p<0.05$) (Table 2). There was negative linear relation between incubation temperature and hatching time. The more the temperature rises, the less the hatching rate lasts.

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Embryonic Development at Different Salinities

9 different salinity values were used to observe embryonic development as 5‰, 10‰, 15‰, 18‰, 20‰, 25‰, 30‰, 35‰, 40‰. 18‰ valued water, which is natural for Black Sea, was determined as the control group. The differences among salinities were determined by calculating the rate of fertilization, hatching and abnormalities. Besides, the effect of salinity over egg floatability was also recorded.

During the experiment, the fertilizations were not come to fruition for Black Sea Turbot both at 5‰ and 10‰ salinities. On the other hand, the highest peak of hatching was at 18‰ salinity. The difference between other salinity values was significantly important ($P<0.05$). The more the salinity decreases, the hatching rate went quite low. And 15‰ salinity kept the lowest rate of hatching. 18‰ salinity was also the lowest of values to have abnormalities. The abnormality rate increased with the rise and fall of salinity according to 18‰ value. Most of the abnormalities screened at 15‰ salinity and the differences of abnormalities between other values were found significantly important ($P<0.05$). The differences between the average larvae size during hatching at all salinity values were found insignificant ($P>0.05$) (Table 3). No difference was recorded about hatching times because the eggs were incubated at the same temperature (14°C) in different salinities. The hatchings occurred after 116 hours at all salinities in which fertilizations happened.

It is widely known that seawater has effects on floatability of pelagic fish eggs. Black Sea Turbot eggs, which have pelagic properties, sank to the bottom at 5‰, 10‰, 15‰ salinities; wallowed in water body at 18‰, 20‰ salinities; and all eggs concentrated on the surface at 25‰, 30‰, 35‰ and 40‰ salinities (Fig. 3).

Discussion

Hara et al., (2002) reported that the approximate fertilization rate of Black Sea Turbot is 39.5% according to their research performed between the years 1998 and 2001. Çolak (2002) detected the fertilization rate as 80% during his research in which he used the method of dry fertilization on eggs that were stripped from turbots kept at 13-15°C water temperature. Aydın & Şahin (2011) stated that the fertilization rate changed between 9.1% and 97.7% on their research in which they used the method of wet fertilization with 10 turbots from the wild, at a 14°C stabilized water temperature. According to the results of the present study, the highest fertilization rate is determined as 90.83%±0.29% at 14°C. So, this is in accordance with the literature. The second highest fertilization rate after 14°C was 88.76%±0.29% at 16°C. The fertilization rates were reported as 71.04%±0.56% and 73.62%±0.198% in sequence at the experimental groups of 8°C and 10°C temperatures and these are also detected as the lowest fertilization performance groups. With the information gathered in this study, it can be said that the optimal fertilization for turbots actualizes at 14°C temperature.

A successful embryonic development brings along high amount of hatching. Incubation water temperature is the most important agent that influences the hatching rate as it influences the fertilization rate (Iglesias et al., 1995). Aydın (2008) reported that the overall hatching rate was approximately 70.8% on his study over 5 different broodstocks at 14°C. Çolak (2002) found the hatching rate of fertilized eggs as 64% at 16°C. The highest hatching rate acquired in the present study at 14°C with 76.96±0.50%. The second highest hatching rate achieved after 14°C was at 12°C with 67.68±0.539%. And the lowest hatching rate detected as 33.46±0.60% at 18°C temperature. Significant declines were observed for hatching rates according to increasing or decreasing of 14°C incubation water temperature. Since high temperature accelerates embryonic

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development (Jones, 1972; Kuhlmann & Quantz, 1980; Devauchelle et al., 1988) there were some problems about fulfilling the embryonic development, in addition to this some larvae died particularly during gastrula phase. So, the lowest hatching rate was recorded at 18°C. In the light of such information; the temperature of water to be used for fertilization process may be above optimum but it can be said that optimum water temperature should be provided for both successfully fulfilling the embryonic development and hatching at will.

Spectrova (1974) and Person-Le Ruyet (2002) notified that lengths of recently hatched turbot prelarvae were 2.5-3.1 mm in their researches. Çolak (2002) stated that prelarvae which he obtained from turbot eggs, incubated at 16°C are 2.7-3 mm. Çiftçi et. al., (2002) also stated that lengths of prelarvae hatched at 14°C are 2.5 mm. In present study; the lengths of recently hatched prelarvae were recorded respectively according to different temperatures 2.52mm±0.014mm at 8°C, 2,53mm±0.011mm at 10°C, 2.65mm±0.015mm at 12°C, 2.68mm±0.015mm at 14°C, 2.69mm±0.017mm at 16°C and 2.69mm±0.014mm at 18°C. The more the temperature decreased below 12°C, the lengths of prelarvae were significantly shorter. As a result of these, it can be said that incubation water temperature directly affects the lengths of recently hatched prelarvae, too.

Abnormal larvae rate can be evaluated by successful hatching and quality criteria (Maslova, 2002). Also, it is known that the temperature which is far from optimum limit on fish culture conditions and salinity value cause notochord deformations (Fırat et. al., 2006). For present study, the lowest abnormality rate detected as 5.20±0.354% at 14°C. The highest abnormality rates were 1, 6.65±0.75% at 8°C and 15.50±0.79% at 18°C. The abnormal hatching increased as getting distant from optimum water temperature. Notochord deformations were frequently seen especially at 8°C in which embryonic development takes a longer time; and at 18°C in which embryonic development is rapid depending on the water temperature. This study shows that prelarvae deformations increase if embryonic development is long or rapid.

The resut that the incubation process and embryonic development of turbot eggs are largely affected by water temperature is stated by Jones (1972), Kuhlmann & Quantz (1980) and Devauchelle et al. (1988). In present study 2-cell blastomer phase has started approximately 4 hour and 40 minutes after hatching time based on 8°C of water temperature. Cell divisions were quite undetermined each of 2-cell blastomer phase and 4, 8, 16-cell blastomer phases. Besides, asymmetric and different magnitude cell divisions, separated cell divisions and cells with uncertain cell membrane were frequently occurred. Valin and Nisling (1998), and Aydın (2008) stated that it was a symptom of sensitiveness to encounter different magnitude cell divisions, separated cell divisions and cells with uncertain cell membrane in their researches about egg quality of Atlantic Turbot and Black Sea Turbot. So, in this study, it can be said that water temperature is the reason of that sensitiveness.

Aydın (2008) identified that there is no relation between blastomer morphology and fertilization but there is a relation between blastomer morphology and hatching on his research on Black Sea Turbot eggs. The group with normal blastomer divisions had higher hatching rate than those with irregular blastomer divisions. Present study got a result in why prelarvae of fertilized Black Sea Turbot eggs got influenced from abnormalities of blastomer morphology at 8°C incubation water temperature. Blastomer abnormalities observed at 10°C shared similarity with blastomer abnormalities at 8°C temperature. Likewise, the eggs that had abnormal blastomer divisions were tensely discovered in the embryonic development observation at 18°C. Although there were no uncertain cell divisions at 18°C, 8°C and 10°C, there were frequent discrete cell divisions. Cell divisions

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accelerated as the incubation water temperature increased, thus it made formation of abnormal blastomers inevitable. Thereupon, the lowest hatching occurred at 18°C. Similarly, as a result of abnormal blastomer divisions at 8°C and 10°C, in which blastomer divisions were rather slow, were the temperatures that the other lowest hatching rate occurred. Abnormal blastomer divisions were lesser at 12°C and 16°C than those are supposed to be seen at lower or higher temperatures. At 14°C blastomer abnormalities can be reported as scarce. The success of hatching rate at 14°C also supports this situation. Nissling et al. (2006), specified that every species have an optimum specific temperature value for embryonic development, hatching time and larval development. In fact, hatching rates showed difference from each other at tested water temperatures. According to present study the optimum incubation water temperature is 14°C for Black Sea Turbot. Although the best fertilization, hatching and less abnormality rates were seen at this temperature, 12°C as the lowest and 16°C as the highest can be preferred for Black Sea Turbot in case the optimum conditions could not be provided. Devauchelle et al. (1988) expressed that the upper limit of water temperature is 16.5-17°C for quality embryonic development and embryonic and larval deformations increased at higher temperatures. The results of present study show similarities with that research as well.

Incubation time of turbot eggs realized according to different water temperatures like; 16°C/4 days, at 13-15°C/1350h-1830h, at 10°C/2450h was notified by Spectrova (1974) and Person-Le Ruyet (1990). In present study, fertilized Black Sea Turbots realized prelarvae hatchings as; 8°C/2552h (13,5 days), 10°C/2415h (10,6 days), 12°C/1948h (6,7 days), 14°C/1617 (4,8 days), 16°C/1475h (3,8 days) and 18°C/1350h (3,1 days). Incubation water temperature is able to increase or decrease the embryonic development speed. Hatching time decreases when you increase incubation water temperature by the optimum temperature. In light of this information, it can be said that present study supports the literature.

Nissling et. al. (2006), reported that salinity has negative effects on fertilization rate, sperm mobility and egg development in their research at Baltic Sea. In present study, fertilization wasn't realized at 5‰ and 10‰ salinities in a test that was done for different salinity values on Black Sea Turbot eggs. The lowest salinity value was 15‰ that fertilization realized among testing groups with a rate of 69.80%. Nissling et al. (2006), stated that the lowest salinity was 5.5-6‰ that turbot eggs can be fertilized in Baltic Sea. This state in the Baltic Sea is originated from the ambiance. Because turbots in Baltic Sea are adapted to brackish water and their eggs are demersal (Kuhlmann & Quantz, 1980). Black Sea's natural salinity rate is 18‰ and the fertilization rate in this salinity realized as 78.29%. The fertilization rates of 78.03% at 20‰ salinity and 77.35% at 25‰ salinity gave the results closest to the fertilization rate at 18‰ salinity. On the other hand, fertilization rates of 74.92% at 30‰, 69.01 at 35‰ and 55.48% at 40‰ salinities were the lowest of all. The optimum fertilization salinity rates for Black Sea Turbots were found as 18‰, 20‰ and 25‰ in present study. Kuhlmann & Quantz (1890) found the optimum fertilization rate for Turbots in Baltic Sea as 15‰-20‰. So, within this perspective, it also can be said that present study supports the literature.

Iglesias et al. (1995) also stated that the most important factor for obtaining a higher hatching rate from turbot eggs is the characteristic properties of sea water which is used for the incubation, as well as the egg quality and proper hygienic circumstances. Accomplishment of specie's lifecycle under aquaculture conditions is closely related to the water temperature and salinity variance that specie can tolerate. Nissling et al. (2006) notified that low or high salinity value decreases the hatching rate in their research on different salinities, using turbot

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eggs in Baltic Sea. In present study hatching of prelarvae is on the peak with a rate of 75.99% in 18‰ salinity, which realized at 14°C, among salinities that fertilizations were successful. With the result of this study, we can claim that 18‰ salinity is the optimum value for Black Sea Turbot, in which the highest hatching revealed. And the lowest hatching rate is 33.57% at 15‰ salinity that the lowest value of which fertilization success was possible. As a result of present study, it can be said that low or high salinity affects the hatching of turbot eggs in a negative way.

Rosenthal & Alderdice (2976), Devauchelle et al. (1988) suggested that adverse salinity conditions (low or high) influences turbot eggs, hence they cause some kind of abnormalities on embryonic development. They also stated that hatching is influenced negatively, and also larvae reflect some abnormalities as a result of abnormal embryonic development. In present study the amount of15‰ salinity, in which the lowest of hatching revealed, had the highest abnormality rate with 10.92%. The abnormality rate of other salinity values were as; 4.32% at 18‰, 4.73% at 20‰, 4.78% at 25‰, 4.76% at 30‰ and 5.55% at 35‰.

The difference among approximate prelarvae length of the hatchings from fertilized eggs in different salinities was found insignificant as a result of present study ($p>0.05$). These prelarvae lengths are; 2.68mm at 15‰ salinity, 2.68mm at 18‰, 2.68mm at 20‰, 2.69mm at 25‰, 2.69mm at 30‰, 2.68mm at 35%, and 2.69 at 40‰. According to what Iglesias et al. (1995) stated, length of larvae, egg diameter and egg efficiency is related to returning of yolk sac to the body back. Both of these are a function of incubation temperature. With this test it was determined that prelarvae lengths, which were obtained from Black Sea Turbot eggs at different salinity but stable temperature, are the same. Thereby, it can be said that the main factor is the incubation water temperature which influences the length of prelarvae of turbots during hatching.

Devauchelle et al. (1988) implied that the salinity has an influence on submerging or floating, development, hatching rate of turbot eggs and loss of them throughout incubation time besides salinity also influences larval deformations. In present study, Black Sea turbot eggs submerged at 5‰, 10‰ and 15‰ salinities. At 18‰ and 20‰ salinities, eggs were pelagic in the water mass. At 25‰, 30‰, 35‰ and 40‰ salinities all eggs were floating on the water surface. Nissling et al. (2006) reported that salinity must be approximately 21‰ for floating of turbot eggs in Baltic Sea. Bagge (1981) also reported that the turbot eggs in the Baltic Sea develop in a demersal way at 6-8‰ salinity. Within this perspective, we can say that present study supports the literature.

Consequently, similar to most of the fish species, there may be a lot of reasons for early period embryonic development disorders for Black Sea Turbots but salinity and temperature are the two most important of those. Present study would lend assistance to make turbot aquaculture widespread with contributing the entrepreneurs who plan to cultivate turbots at several regions of Turkey which have different water temperature and salinity.

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Table 1. Embryonic development of Black Sea Turbot (*Psetta maxima*) eggs at different temperatures.

Phase	Temperature					
	8ºC	10 ºC	12 ºC	14 ºC	16 ºC	18 ºC
Fertilization	00:00	00:00	00:00	00:00	00:00	00:00
2-cell Blastomer	04:40	03:10	02:45	02:15	01:45	1:35
4-cell Blastomer	06:20	04:20	04:00	03:25	02:35	2.05
8-cell Blastomer	07:50	05:20	04:45	04:15	03:15	2:50
16-cell Blastomer	10:35	07:50	06:15	05:00	03:55	3:30
32-cell Blastomer	12:55	10:10	07:50	06:25	04:40	4:10
Morula	32:10	18:15	10:05	09:15	06:40	6:15
Blastula	55:10	31:50	21:40	16:00	07:40	7:05
Ecto- Gastrula	74:05	54:05	30:35	25:45	23:00	17:50
Meso- Gastrula	97:50	77:10	42:50	30:25	27:10	21:05
Embryo Formation	121:55	100:40	50:50	36:45	30:40	25:20
Neurula	144:40	115:15	61:10	44:45	37:40	28:20
Endo- Neurula	152:55	124:15	66:30	52:30	42:10	30:20
Embryonic Phase I	177:45	139:35	118.15	72:50	60:05	42:20
Embryonic Phase II	224:25	172:15	129:55	92:30	69:45	50:25

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Embryonic Phase III	271:00	198:15	137:10	99:50	75:45	56:00
Larval Phase	290:30	213:20	146:15	106:40	82:30	63:10
Hatched (50%)	305:35	229:45	153:30	112:40	88:40	73:20
Hatched (100%)	319:05	241:50	162:40	115:50	92:20	75:00
Time-Level	2552	2415	1948	1617	1475	1350
Day	13,2	10,6	6,7	4,8	3,8	3,1

Table 2. The fertilization rate, hatching rate, abnormalities and size of prelarvae, at different temperatures.

	8 °C	**10 °C**	**12 °C**	**14 °C**	**16 °C**	**18 °C**
Fertilization rate	71.00±0.56	73.62±0.20	84.68±0.22	90.83±0.29	88.76±0.29	85.32±0.72
Hatching rate	40.28±0.31	49.85±0.59	67.68±0.54	76.96±0.50	57.41±0.60	33.46±0.60
Larva Abnormalities	16.65±0.75	10.67±0.59	6.89±0.81	5.20±0.35	6.14±0.74	15.50±0.79
Length of prelarva	2.52±0.01	2,53±0,01	2,65±0,01	2,68±0,01	2,69±0,02	2,69±0,01

Table 3. The fertilization rate, hatching rate, abnormalities and size of prelarvae, at different temperatures.

	‰15	**‰18**	**‰20**	**‰25**	**‰30**	**‰35**	**‰40**
Fertilization Rate	69.80±0.74	78.29±0.47	78,03±0.47	77.35±0.73	74.92±0.36	69.01±0.72	55.48±0.75
Hatching Rate	33.57±0.91	75.99±0.66	71,30±0.38	71.85±0.65	69.32±0.97	66.0±0.58	53.15±0.71
Larvae Abnormalities	10.92±1.91	4.32±0.40	4.73±0.41	4.78±0.43	4.76±48	5.55±0.47	8.49 5±0.57
Prelarvae Size	2.68±0.02	2.68±0.02	2.68±0.02	2.69±0.02	2.69±0.02	2.68±0.02	2.69±0.02

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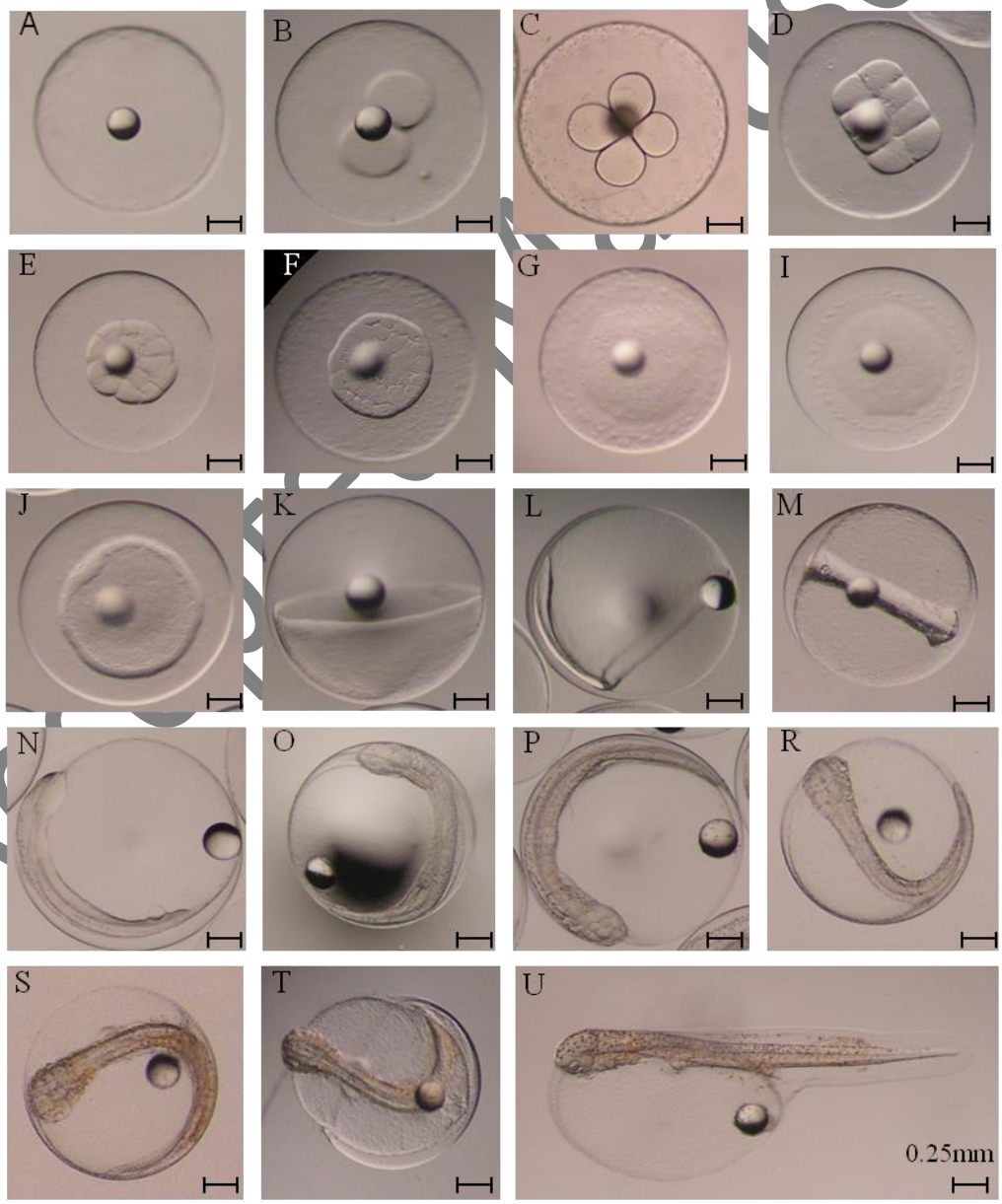

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Figure 1. Embryonic development phases of Black Sea Turbot (*Psetta maxima*) eggs. A- Oosperm, B- 2-cell blastomer, C- 4-cell blastomer, D- 8-cell blastomer, E- 16-cell blastomer, F- 32-cell blastomer, G- Morula phase, I- Blastula phase, J- Ecto-gastrula, K- Meso-gastrula, L- Embryo formation, M- Neurula, N- Endo-neurula, O- Embryonic phase-I, P- Embryonic phase-II, R- Embryonicphase-III, S- Larval phase, T- Hatched (50%). U-Hatched (100%)

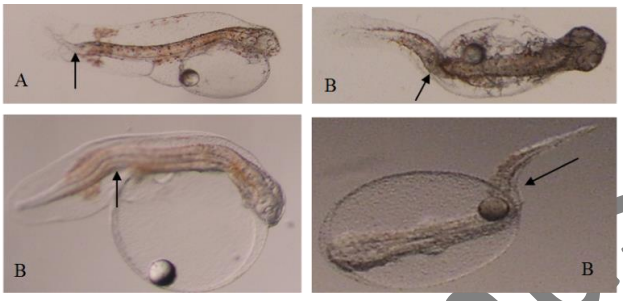

Figure 2. (A) tail and (B) notochord deformations of prelarvae after hatching

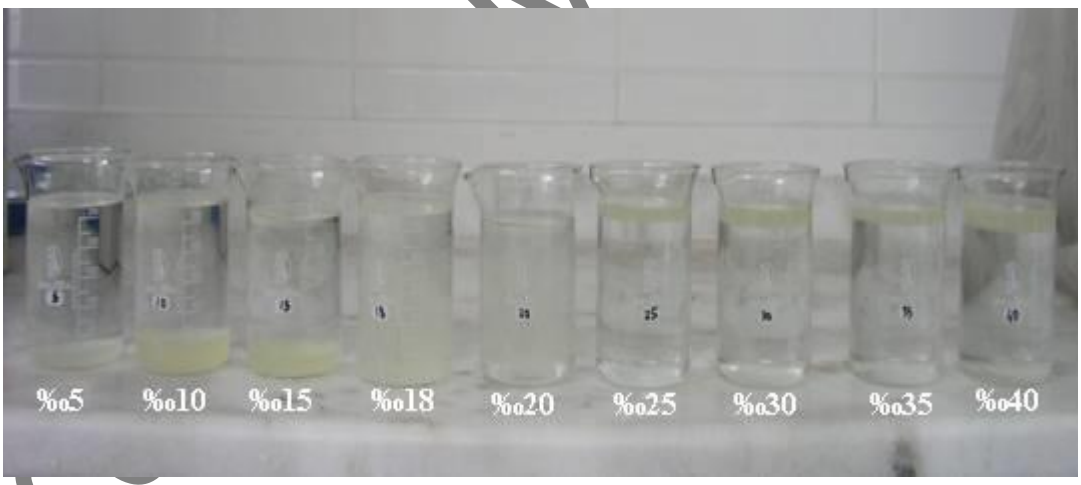

Figure 3. Floating and sinking conditions of Black Sea Turbot eggs in different salinities.