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SHORT COMMUNICATION **KISA ARAŞTIRMA**

Out-of-season spawning of mountain trout (*Salmo macrostigma* Duméril, 1858) through artificial photoperiod manipulation

Yapay fotoperiyot uygulaması ile dağ alabalığından (*Salmo macrostigma*, Dumeril, 1858) mevsim dışı döl alımı

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Abstract: The effects of artificial photoperiod manipulation on spawning time and hatchery success of 3+ old mountain trout (*Salmo macrostigma*) broodstock were investigated. Broodstock fish in the photoperiod group were exposed to constant long days (18L:6D), from February 1st until May 31st, 2013 and then to constant short days (8L:16D) starting from June 1st, 2013. The fish in the control group were exposed to natural light-dark regimes. Out-of-season milt was observed in males starting from August 15th. Out-of-season spawning of females started in August 30th and reached its peak level in September 13th. Mean values of absolute fecundity, relative fecundity, and egg size were found to be 1242.48±382.55 eggs/fish, 1148.76±336.91 eggs/kg and 4.14±0.19 mm respectively. Mean fertilization, eyeing, and hatching rates of out-of-season eggs were determined as 56.77%, 20.81%, and 11.95% respectively. Regardless of the fact that the mean fecundity of females in the photoperiod group and hatching success (egg diameter, fertilization, eyeing, and hatching rates) of out-of-season eggs were lower than those in the control group, the overall results demonstrate that that regulating spawning time and out-of-season spawning of mountain trout by the use of artificial photoperiod is possible.

Keywords: *Mountain trout, salmo macrostigma, photoperiod, out-of-season spawning*

Öz: Bu çalışmada yapay fotoperiyot uygulamasının 3+ yaşlı Dağ alabalığı (*Salmo macrostigma*) damızlıklarının üreme dönemi ve kuluçka başarısı üzerindeki etkileri araştırılmıştır. Çalışmada fotoperiyot grubunu oluşturan balıklar, 01 Şubat – 31 Mayıs 2013 tarihleri arasında sürekli uzun gün (18A:6K), 01 Hazirandan itibaren ise sürekli kısa gün (8A:16K) uygulamasına tabi tutulmuştur. Kontrol grubunu oluşturan balıklar ise doğal aydınlık-karanlık döngüsüne tabi tutulmuştur. Yapay fotoperiyot döngüsüne tabi tutulan erkek damızlıklardan 15 Ağustos'tan itibaren sperma/süt gözlenirken, dişi balıklardan ilk yumurta alımı 30 Ağustos'ta gerçekleşmiş ve 13 Eylül'de pik noktaya ulaşmıştır. Fotoperiyod uygulaması ile mevsim dışı yumurta veren dişi balıkların ortalama mutlak yumurta verimi 1242,48±382,55 adet/balık, oransal yumurta verimi 1148,76±336,91 adet/kg, ortalama yumurta çapı ise 4,14±0,19 mm olarak bulunmuştur. Sağılan mevsim dışı yumurtaların ortalama döllenme, gözlenme ve açılma oranı sırasıyla %56,77, %20,81 ve %11,95 olarak saptanmıştır. Fotoperiyot grubundaki dişi damızlıkların ortalama yumurta verimi ile mevsim dışı elde edilen yumurtaların kuluçka başarısı (yumurta çapı, döllenme, gözlenme ve açılma oranları) kontrol grubundakine göre daha düşük olmasına rağmen genel olarak elde edilen sonuçlar yapay sürekli uzun ve kısa gün uygulaması ile *Salmo macrostigma*'nın üreme mevsimin düzenlenmesini ve mevsim dışı yumurta sağımının mümkün olduğunu göstermektedir.

Anahtar kelimeler: *Dağ alabalığı, salmo macrostigma, fotoperiyot, mevsim dışı döl alımı*

INTRODUCTION

Photoperiod is defined as changes from day to night and seasonal changes in day lengths which are generated by the rotation of the earth around its axis and around the sun. Photoperiod is the most reliable external sign in nature that shows the time of year (Gwinner, 1986, Duinker, 1996). The feeding and reproductive activities of living organisms as well as annual rhythm of physiological events are synchronized with photoperiod, especially in temperate and high-altitude regions (Randall et al., 1998; Bromage et al., 2001; Dey et al., 2005). According to Boeuf and Bail, (1999) the basic rhythms of nature such as day and seasons depend on the variability of the day length, and many animal species, including fishes, exhibit a 24-hour cycle in their activities. Although temperature, precipitation, food, and environmental factors, such as pheromones, affect spawning, but spawning time for most fishes is determined by seasonal changes in day length. According to many researchers, daylight duration has an important effect on "time perception" (zeitgeber) of fishes and affects many physiological activities of many fish species (Boeuf and Bail, 1999; Purchase et al., 2000; Duinker, 1996;

Bromage et al., 2001). Seasonally changing day length (bright time) and water temperature are the main environmental factors which synchronize spawning time in temperate-zone species including species of the Salmonidae family (Pavlidis et al., 1992; Taylor et al., 2008; Wang et al., 2010; Wilkinson et al., 2010; Migaud et al., 2010).

Thanks to the impact of photoperiod on living organisms, nowadays artificial photoperiod (light-dark) applications have become an important tool for broodstock management, and by using different protocols, eggs can be stripped out of season by advancing or delaying the spawning time of many aquatic species, particularly species of the Salmonidae family. By use of artificial photoperiod regimes eggs and fry/juveniles can be obtained from broodstocks at different periods of the year or throughout the year, and producers do not have to rely only on one cycle of spawning based on the natural reproductive season. Manipulation and management of spawning season of fish species by use of artificial photoperiod regimes provide many opportunities in terms of farm management and production planning. In this regards instead of a single spawning cycle during the natural spawning season of the species, spreading/extending the spawning period year round facilitates multiple use of existing physical infrastructure, e.g., hatchery facilities (incubators, tanks) and also labor. On the other hand as far as marketing is concerned, out of season and year round production of eggs and juveniles ensures the continuity of supply of marketable fish year round in commercial aquaculture business.

Today, the presence of *Salmo trutta* has been recorded throughout Europe, North Africa, Middle East, and Western Asia (Sedgwick, 1982). Mountain trout (*Salmo macrostigma*, Dumeril 1858), is found naturally in many rivers from West to East and North to South of Turkey (Geldiay and Balık, 2002; Kocabas, 2009). Excessive fishing pressure on adult individuals, especially during the spawning season, and damming of the rivers, the natural living areas of mountain trout, has had a negative impact on recruitments and natural population of mountain trout in Turkey (Korkmaz, 2005). Mountain trout is among native trout sub-species for which stock enhancement schemes through release of hatchery-raised fry is being implemented. Public hatcheries and particularly those belonging to Ministry of Forestry and Water Affairs (Nature Protection and National Parks Directorate) are mandated to produce hatchery-raised mountain trout fry for release in to natural habitats of this species.

Public hatcheries responsible for stock enhancement and release of hatchery-raised fry usually accommodate a large number of broodstocks of wild trout belonging to different sub-species. The natural spawning period of these sub-species are very close to each other and concentrated between September and January. Therefore, labor intensive hatchery operations such as stripping a large number of broodstock, incubation of eggs, and feeding of the fry should be managed during a short period of time. Moreover, existing physical infrastructures in hatcheries (e.g., water, incubators, such as larval tank) can also be a constraint to manage hatchery operations for thousands of broodstock and thus hinder the production of healthy fry for release. Regulating spawning time of broodstock by photoperiod manipulation can be, therefore, a useful tool for managing hatchery operations more efficiently both in terms of labor and infrastructure in hatcheries where broodstocks of multi trout- sub-species have to be handled.

There are many studies on the regulation of reproduction time and out of season spawning in many species belonging to the family Salmonidae, especially for Rainbow trout by using artificial photoperiod regimes (Pavlidis et al., 1992; Randal and Bromage, 1998; Holcombe et al., 2000; Bromage et al., 2001; Davies and Bromage, 2002; Bonnet et al., 2007a; Pornsoping et al., 2007; Bonnet et al., 2007b; Wilkinson et al., 2010; Fjelldal et al., 2011). However, as far as literature reviews reveal out of season spawning of mountain trout by use of artificial photoperiod has not been studied. The objective of this study was, therefore, to investigate the possibility of obtaining out of season eggs and fry from 3+ old mountain trout (*Salmo macrostigma*) broodstock by use of artificial photoperiod manipulation (long-days) and evaluate their hatchery performance under actual conditions of public hatchery engaged in stock enhancement scheme (Kadincik stream/Mersin).

MATERIALS AND METHODS

This study was carried out in a public trout hatchery (Bahçe Wild Trout Hatchery) belonging to Ministry of Forestry and Water Affairs Ministry, 7. Regional Directorate (Mersin/Çamlıyayla: N:37.23 E:34.62). A total of 160, 3+ old mountain trout broodstock fish (*Salmo macrostigma*), including 120 female and 40 males were used. Initial mean length, weight, and condition factor of male and female broodstocks are given in Table 1 and Table 2.

Table 1. Initial total length (cm), live weight (g), and condition factor of female mountain trout broodstock used in the experiment

	Photoperiod group	Control group
Total length (cm)	36.58 ± 3.25	36.22 ± 3.25
min - max	29 – 46	28 - 45
Live weight ± sd (g)	652.55 ± 211.33	641.60 ± 184.16
min - max	255-1302	318-1182
Condition factor ± sd	1.29 ± 0.19	1.32 ± 0.13
min - max	1.01 - 1.77	1.06 - 1.62

Table 2. Initial total length (cm), live weight (g), and condition factor of male mountain trout broodstock used in the experiment

	Photoperiod group	Control group
Total length ± sd (cm)	36.40 ± 5.42	37.00 ± 3.16
min - max	25 - 44	32 - 44
Live weight ± sd (g)	721.80 ± 281.09	747.55 ± 185.90
min - max	218 - 1277	374 - 1154
Condition factor ± sd	1.42 ± 0.13	1.45 ± 0.16
min - max	1.24 - 1.74	1.25 - 1.77

The experiment was conducted indoor using four green fiberglass breeding tanks, 2 m x 2 m x 1 m. Tanks in the photoperiod group were placed in the “Photoperiod room” isolated from external light, while the control group tanks placed in the same building were positioned to be exposed to natural photocycle cycle. As light source, an 80-watt spiral white light was placed at 1 m above the water surface of photoperiod tanks. Light intensity was arranged and measured as 680 lux at the water surface (Davies and Bromage, 2002; Pornsoping et al., 2007). An EMT-445 light meter was used to measure the light intensity, and a LUTRON LX-101 was used as an automatic timer to regulate the light-dark cycles. Vertical flow incubators were used for the incubation of fertilized eggs.

During the first two months, 6 mm commercial trout feed containing 41% protein and 24% fat were used to feed the broodstock. Thereafter, through the end of the trial, 9 mm commercial trout feed containing 39% protein and 24% fat were used.

Experiment setup and procedures

Experimental tanks were arranged in two parallels groups, i.e., photoperiod and control group. Each experimental tank was stocked with 30 females and 10 male broodstock fish, keeping the female-to-male ratio as 3:1 (Pavlidis et al., 1992). Stocking density in all tanks was set to 7-8 kg/m^3 (Laird and Needham, 1991).

Utmost attention was given to the distribution of broodstock fish among experimental tanks to avoid any significant differences between mean initial weights and condition factors of fish in two groups. In fact, one-way ANOVA and Independent Sample T-Test did not reveal any statistically significant differences between the photoperiod and control group in terms of the mean initial weights and condition factors of the broodstocks ($P > 0.05$). Fish were adopted to experimental conditions for one week.

The experiment started on February 1st, 2013, with constant long-day application (16L:08D) to the photoperiod group. Constant long-day regime continued until May 31st. Starting from June 1st, constant short day (08L: 16D) regime was implemented.

At the beginning of the experiments broodstock were fed twice daily (morning and evening) by hand. Daily feeding rate was 1.0% of body weight. In line with industry practices, the daily feeding rate was reduced to 0.6% of body weight starting from July (Personal Interview: Atilla ERTÜRK).

Broodstock from the photoperiod and control group were examined for eggs and milt by hand in a week starting from August 15th (Kurtoglu et al., 1998). Reproductive/mature fish were anesthetized by 2-phenoxyethanol anesthetic (0.08-0.5 mg/L) prior to striping to minimize stress and prevent any damage (Borski and Hodson, 2003). The day when eggs were collected from majority of mature female fish was defined as the peak spawning time in the photoperiod group (Pavlidis et al., 1992).

The photoperiod group was terminated after the last broodstock was examined for mature eggs on October 26th. The control group was terminated on December 27th after the latest eggs were collected from females during their natural spawning season. The experiment ended on February 5th, 2014, when all eggs collected throughout the experiment were hatched.

Fertilized eggs were treated with 1-2 mg/L formaldehyde every two days for disinfection (Bohl, 1982; Baur and Rapp, 1988; Schlotfeldt and Alderman, 1995).

Evaluation Criteria

Absolute and relative egg fecundity was used in determining the amount of eggs stripped from female broodstock. The volumetric method was used for determining the amount of stripped eggs (Bromage et al., 1992; Kurtoglu et al., 1998; Karatas, 2005). Absolute and relative fecundity was calculated by using the following formula (Pavlidis et al., 1992):

Absolute egg fecundity = Number of eggs/Number of fish

Relative egg fecundity (unit/kg) = Number of eggs/Fish weight (kg)

Egg size (diameter) and hatchery success criteria which include fertilization, eyeing, and hatching rates were used to assess the quality of collected eggs (Noori et al., 2010). A 0.01-mm precision caliper was used for measuring the size (diameter) of the stripped eggs (Rahbar et al., 2011).

Experimental fish were not sacrificed for egg collection. Eggs and milt were collected by striping. Gonadosomatic index (GSI) was calculated only for female broodstock based on pre-spawning(striping) and post spawning weights according to Heinimaa and Heinima (2004) using the following formula;

$$GSI = (W_G/W_B)x100$$

Condition factor (K) calculated according to (Akhan, 2010) as following;

$$CF = (BWx100)/L^3$$

Dead eggs were collected and counted by using volumetric method throughout the incubation period, and the fertilization, eyeing, and hatching rates were calculated as percentage (Baki et al., 2011).

Fertilization rate was determined as following (Suziki and Fukuda, 1971; Yanık and Aras, 1994).

Fertilization ratio (%) = (Number of fertilized eggs/Total number of eggs) x 100

Eyeing and fertilization rates were calculated from Kötzner (1978), Refstie (1978), and Yanık and Aras (1994), as following:

Survival at eyeing ratio (%) = (Number of surviving eggs/Total number of eggs) x 100

Opening ratio (%) = (Number of larvae /Total number of eggs) x 100

A one-way ANOVA test was performed for statistical evaluation of the initial mean weights of broodstock and compliance testing of distribution. Normality test and Spearman correlation analysis were performed for the statistical assessment of the relationship between the weight-height of the broodstock and fecundity and egg diameter. An Independent Sample T-Test was used for the comparison of mean absolute and relative egg fecundity, egg diameter, fertilization, eyeing and hatching rates between photoperiod and control group. SPSS 11.5 software was used for statistical analysis.

RESULTS

Thirteen females and 2 male broodstock in the photoperiod group died during the experiment. The first out of season eggs in the photoperiod group was observed and stripped at August 30th, from 1 broodstock. Collection of out of season eggs from 42 females continued throughout September and early October. The peak spawning time was September 13th, when eggs were stripped from 47.6% of the females (Table 3). No eggs were observed in the remaining 5 female broodstock in the photoperiod group. Out of season milt was first observed in 12 male broodstock on August 15th and in all remaining 18 males throughout August and early September (Table 4).

Table 3. Breakdown of spawning time of females in the photoperiod group

Spawning date	Number of fish stripped	Spawned fish(%)*
30.08.2013	1	2,4
06.09.2013	2	4,8
13.09.2013	20	47,6
21.09.2013	5	11,9
05.10.2013	14	33,3

*Spawning fish x 100 / Total spawning fish

Table 4. Breakdown of males with milt (observed) in the photoperiod group

Spawning check dates	Number of males with milt	Males with milt(%)
15.08.2013	12	% 67
21.08.2013	2	% 11
30.08.2013	3	% 17
06.09.2013	1	% 5

Seven female and 1 male broodstock in the control group died during the experiment. Egg maturation was first observed in 5 of the broodstock during their natural spawning season on December 19th. Eggs maturation and stripping continued on December 24th (13) and December 27th (30) (Table 5). While eggs were collected from 48 female broodstock in control group, no mature eggs were identified in the remaining 5 females. Milt was first observed on December 11th in 5 male broodstock in the control group. Milt from the remaining 14 males was collected throughout December.

Table 5. Breakdown of spawning time of females in the control group

Spawning dates	Spawned number of fish	Spawned fish rate (%)*
19.12.2013	5	10.4
24.12.2013	13	27.1
27.12.2013	30	62.5

*Spawning fish x 100 / Total spawning fish

It is worth mentioning that the spawning period in the control group (December) corresponds to the natural spawning period of mountain trout in Kadıncık stream (Çamlıyayla/Mersin) and under the conditions of hatchery in which this experiment was conducted.

Mean absolute and relative fecundity and egg diameters for female broodstock in the photoperiod and control group are given in Table 6. Accordingly, absolute and relative fecundities and egg diameters of females in the photoperiod group were lower than those in the control group. In terms of mean absolute and relative fecundities and egg diameters, the differences between the two groups were found to be statistically significant ($P<0.05$).

Correlation analysis showed that there was a positive, moderate linear correlation ($r=0.577$; $P<0.001$) between the individual weights and absolute fecundity of the broodstock in the photoperiod group. However, a positive and low-grade relationship ($r=0.490$; $P=0.001$) was found between the length and absolute fecundity. Furthermore, there was a positive low-grade relationship ($r= 0.436$; $P=0.004$) between the weight and egg diameter of broodstock, but no linear relationship was determined between the length and egg diameter, and egg diameter and absolute fecundity ($P>0.05$).

A positive and low-grade relationship ($r=0.456$; $P<0.001$) was also detected between the weight and absolute fecundity of the female broodstock in the control group. There was a positive low-grade relationship ($r=0.467$; $P<0.001$) between length and absolute fecundity, but no significant relationship was found between the weight or length and egg diameter of the fishes ($P>0.05$).

Table 6. Fecundity and egg diameter of females in photoperiod and control groups

	Photoperiod Group	Control Group
Total Length ± sd (cm)	41.73 ± 2.96	45.14 ± 2.54
min - max	36 - 48	40 – 50
Live Weight ± sd	1096.48 ± 220.52	1394.27 ± 234.39
min - max	722 - 1754	1097-1965
Absolute fecundity ± sd [1]	1242.48 ± 382.55	1902.50 ± 681.90
min - max	288 -1944	240 -3000
Relative fecundity ± sd [2]	1148.76 ± 336.91	1347.63 ± 446.84
min - max	214 -1849	203 -2198
Egg diameter ± sd (mm)	4.14 ± 0.19	4.72 ± 0.30
min - max	3.874 - 4.858	4.329 - 5.431

[1] Number of eggs /Fish , [2] Number of eggs / kg

The fertilization, eyeing, and hatching rates of the out of season eggs collected from the broodstock in the photoperiod group were determined as 56.77±6.78, 20.81±7.92, and 11.95±4.88%, respectively. For the control group the fertilization, eyeing, and hatching rates were found to be 58.71 ± 9.30, 34.75 ± 10.08, 20.31 ± 7.14%, respectively (Table 7).

Table 7. Hatching success of mountain trout eggs (%)

	Photoperiod Group	Control Group
Number of eggs	52 184	91 320
Fertilization rate ± Sd (%)	56.77 ± 6.78	58.71 ± 9.30
min - max	47.19 - 69.70	31.00 - 69.91
Eyeing rate ± Sd (%)	20.81 ± 7.92	34.75 ± 10.08
min - max	12.91 - 28.81	13.95 - 56.58
Hatching rate ± Sd (%)	11.95 ± 4.88	20.31 ± 7.14
min - max	8.58 - 18.15	7.75 - 32.55

No statistically significant difference between the photoperiod and control group was found in terms of the mean fertilization rate (P>0.05). However, the difference between eyeing and hatching rates were found to be statistically significant (P>0.05).

The eyeing time of out of season eggs collected from photoperiod group took 21 days while the hatching took 14 days in the following eyeing stage. For the control group, the eyeing was observed on 23rd day and hatching in the following 17 days (Table 8).

Table 8. Incubation period of mountain trout eggs (Sd: Standard deviation)

	Photoperiod Group	Control Group
Eyeing:		
Days	21	23
Degree-days ± Sd [1]	237.81 ± 5.10	236.06 ± 7.33
°C	11.0 – 11.9	10.1 - 10.6
Hatching:		
Days	14	17
Degree-days ± Sd [1]	155.45 ± 7.39	172.45 ± 8.44
°C	10.7 – 11.6	10.2 - 10.5

[1] The hatching time of the eggs placed in different trays were observed separately. Standard deviation is calculated according to eyeing and hatching period of eggs in different trays.

DISCUSSION

The reproductive cycle of female fish is divided into three oocyte developmental stages namely: (1) the first phase where the primary oocytes develop and grow, (2) vitellogenesis where energy and materials accumulate into oocytes for embryonic development, and (3) ovulation and spawning stages where eggs mature. It is known that photoperiod plays a crucial role in all the three stages in temperate regions (Wang et al., 2010). The first phase is usually triggered by photoperiod and/or a change in water temperature. Although photoperiod has a moderate impact on the third stage, Wang et al. (2010), reported that increased duration of darkness is needed for the final stage of gametogenesis.

The results obtained in this study clearly show that constant long-days followed by short-days cycles have had an impact on the reproductive physiology of the mountain trout and further reveal that regulating spawning time and collection of out of season eggs and milt by use of artificial photoperiod is possible for mountain trout broodstocks. Compared to their natural spawning period in Kadıncık stream (Çamlıyayla/Mersin), spawning time in female and male mountain trout broodstock was advanced by 3 months in this study by exposing broodstock fish to artificial long days (18L:6D) between February 1st and May 31st and to short days (8L:16D) starting from June 1st.

Since authors have not come across studies on regulating spawning time of mountain trout by use of artificial photoperiod regimes in literature reviews, findings of this study were compared with those conducted on other Salmonidae species or any work on spawning characteristics of mountain trout in wild.

As stated before, mean absolute and relative fecundity values of females in the photoperiod group producing out of season eggs were found to be lower than those of the control-group where spawning took place within natural spawning season of mountain trout in Kadıncık stream. Similarly, mean egg diameter in photoperiod was lower than that of the control group. These differences in mean values between the two groups were found to be statistically significant (P<0.05). When compared to other studies, the mean absolute and relative fecundities of the female broodstock producing out of season eggs in this study were also lower than those reported for wild mountain trout. While Kocabas (2009) reported the mean relative fecundity value of 2403 ± 953 eggs/kg for mountain trout in wild, Erer (2004) documented mean absolute and relative fecundity values of 7 930 eggs/female and 1322 ± 233 eggs/kg, respectively. For mountain trout broodstock, Baki et al. (2011) reported mean absolute and relative fecundity values as 1757 ± 85 eggs/fish 1432 ± 34 eggs/kg respectively. The mean diameter of out of season eggs in this study were also below the values for wild mountain trout found by Kocabas, (2009) as 4.30 ± 0.52 mm.

It should be underlined that the values reported by these researchers for both absolute and relative egg fecundities and egg diameters are those obtained from wild fish during their natural spawning season. As mentioned earlier findings of this study are for out of season eggs where spawning time of female broodstocks was advanced by 3 months compared to their natural spawning season. Therefore, gametogenesis and especially the vitellogenesis phase were accelerated and shortened.

Indeed, it is well documented by many studies conducted on rainbow trout that delaying or advancing spawning time by photoperiod application could affect the dynamics and time of gametogenesis (Bon et al 1997; Bon et al., 1999; Bonnet et al., 2007b; Sarameh et al., 2013). Bon et al. (1997) emphasized that the eggs obtained by photoperiod manipulation from rainbow trout were smaller than the eggs stripped during the natural spawning period. Bromage et al. (2001) also reported that advancing spawning time by the photoperiod application resulted in smaller eggs. Other researchers have also documented that advancing spawning by use of artificial photoperiod regimes have resulted in smaller egg size in Rainbow trout (Nomura, 1962; Buss, 1982; Duston and Bromage, 1988). Bon et al. (1999) emphasized that GTH-I levels in the fishes exposed to accelerated photoperiod were higher during vitellogenesis, so a decrease in egg diameter was not related to the decrease in the GTH-I level in the plasma but instead was associated with the disruption of egg growth in the further stages of vitellogenesis. Davies and Bromage (2002) stated that the reason for obtaining smaller eggs when compared to eggs obtained in the normal reproduction season was result of shortening of vitellogenesis stage by use of artificial photoperiod regimes.

In this study mean fertilization, eyeing, and hatching rates of the out of season eggs obtained by photoperiod application was found to be lower than that of the control group, and the difference between the groups in terms of parameters was statistically significant. It is well acknowledged that egg quality is influenced by environmental factors and husbandry practices (Bobe, 2015; Yevtushenko and Sherelo, 2016). Many researchers emphasize that the quality of the out of season eggs and therefore the hatching success can be affected by artificial photoperiod application (Holcombe et al., 2000; Bonnet et al., 2007a; Bonnet et al., 2007b). Bonnet et al. (2007a) stated that advancing the reproduction stage of rainbow trout by artificial photoperiod regimes can negatively affect the egg quality of the broodstock. Bonnet et al. (2007b) have also documented that the quality of the eggs obtained from Rainbow trout broodstock exposed to artificial long-short-day-photoperiod was affected by photoperiod. According to the same researchers, while the eyeing rate of eggs obtained from Rainbow trout broodstock during their natural spawning time (control group) was 93.3%, eyeing rate of out of season eggs varied between 38.0% and 49.0%. Holcombe et al. (2000) reported that the photoperiod application may affect egg quality in the stage of egg yolk formation (vitellogenesis). Similarly Carillo et al. (1989) found that artificial photoperiod may have a negative impact on egg quality, hatching success and also on survival rate at first feeding stage of fry and that this may vary depending on the light regime applied.

In this context, lower egg fecundity of the female broodstock exposed to artificial photoperiod and lower hatchery success of out of season eggs in the photoperiod group in comparison to control group can be possibly explained by shortened duration of egg formation stage and negative impacts on egg quality due to use of artificial photoperiod.

Regardless of the fact that the absolute and relative fecundity values of female broodstock in the photoperiod group and egg diameter, fertilization, eyeing, and hatching rates of the out of season eggs were lower than those in the control group, the overall results demonstrate that it was possible to advance the spawning time of mountain trout by use of artificial long and short-day regimes. In this regard the findings of this study shed light on possibility of using artificial photoperiod regimes for regulating spawning time of this endangered sub-species in public hatcheries responsible for stock enhancement by release of hatchery-raised fry. Moreover, regulating spawning periods would facilitate better brood stock management and more efficient use of hatchery facilities.

Nevertheless, it would be useful to investigate different photoperiod protocols, i.e., light-dark cycles to identify the best application/protocol in order to improve fecundity, egg quality, and hatching success of out of season eggs in mountain trout.

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