



Effect of Different Photoperiod Regimes on Sperm Quality, Fecundity and Fertilization in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

The present study was carried out to determine effect of different photoperiod regimes on sperm quality parameters, ovulation/spermiation time and hatchery performance of rainbow trout (*Oncorhynchus mykiss*) broodstock. The designation was done as combination of different long and short photoperiod regimes such as: 18L:6D and 18D:6L (group I); 14L:10D and 14D:10L (group II) and natural lighting (control group). All treatments were carried out as three replications at each group.

As a result, the highest mean spermatozoa motility (83.0±2.1 %) and motility period (67.2±6.3 s) were determined in control group. It was determined that the longest ovulation was occurred in female rainbow trout broodstock at 265 days in group I. Although the highest mean absolute egg productivity was determined as 3654.7±298.3 eggs/fish in group I, the highest mean relative egg productivity was determined as 137.3±24.5 eggs/kg in control group. Furthermore, the highest mean egg diameter (4.6±0.1 mm) and fertilization rate (87.0±2.5 %) were determined in control group. Statistical analyses revealed that spermatozoa motility, spermatozoa motility period and spermatozoa density positively correlated with fertilization rate in all photoperiod regimes (P>0.05). On the other hand, semen volume and semen pH negatively correlated with fertilization rate in all photoperiod regimes (P>0.05). It is interesting to note that only statistically important positive correlation was determined between relative fecundity and fertilization rate in 18L:6D/18D:6L photoperiod regime (r=0.452, P<0.05).

Consequently, results revealed that combined long and short artificial photoperiod regimes can advance ovulation and spermiation and also can effect gamete quality and hatchery performance of rainbow trout during out-of-season spawning.

Keywords: photoperiod, sperm, ovulation, fertilization, *Oncorhynchus mykiss*.

Farklı Fotoperiyot Rejimlerinin Gökkuşuğu Alabalıklarında (*Oncorhynchus mykiss*) Sperma Kalitesi, Fekundite ve Fertilizasyon Üzerine Etkisi

Özet

Sunulan bu çalışma, farklı fotoperiyot rejimlerinin gökkuşuğu alabalığı (*Oncorhynchus mykiss*) damızlıklarının sperma kalite parametreleri, ovulasyon/spermiasyon süreleri ve kuluçka performansları üzerine olan etkilerini belirlemek amacıyla yürütülmüştür. Bu çalışma, farklı uzun ve kısa fotoperiyot rejimlerinin kombinasyonu (Grup I: 18A:6K ve 18K:6A; Grup II: 14A:10K ve 14K:10A ve doğal aydınlanma (kontrol grubu)) şeklinde planlanmıştır. Bütün uygulamalar her grupta üç tekrarlolu olacak şekilde yürütülmüştür.

Çalışma sonuçlarına göre, en yüksek ortalama spermatozoa motilitesi (%83,0±2,1) ve motilite süresi (67,2±6,3 s) kontrol grubunda belirlenirken, dişi gökkuşuğu alabalığı damızlıklarında en uzun ovulasyon I. grupta 265 günde gerçekleşmiştir. Her ne kadar en yüksek ortalama mutlak yumurta verimi 3654,7±298,3 yumurta/balık olarak I. grupta belirlense de, en yüksek ortalama relatif yumurta verimi 137,3±24,5 yumurta/kg olarak kontrol grubunda belirlenmiştir. Ayrıca, en büyük ortalama yumurta çapı (4,6±0,1 mm) ve en yüksek fertilizasyon oranı (%87,0±2,5) kontrol grubunda belirlenmiştir. İstatiksel analizler, tüm fotoperiyot rejimlerinde; spermatozoa motilitesi, spermatozoa motilite süresi ve spermatozoa yoğunluğu ile fertilizasyon oranları arasında pozitif korelasyon olduğunu göstermiştir (P>0,05). Diğer taraftan, tüm fotoperiyot rejimlerinde sperma hacmi ile sperma pH'sı, fertilizasyon oranları ile negatif korelasyon göstermiştir (P>0,05). İstatiksel olarak önemli tek pozitif korelasyon, relatif fekundite ile fertilizasyon arasında 18A:6K/18K:6A fotoperiyot rejiminde belirlenmiştir (r=0,452; P<0,05).

Sonuç olarak, bu çalışmanın sonuçları üreme mevsimi dışında kombine uzun ve kısa yapay fotoperiyot rejim uygulamalarının, gökkuşuğu alabalıklarında ovulasyon ve spermiasyon sürelerini erkene çekebileceğini ve gamet kalitesi ile kuluçka performansını etkileyebileceğini ortaya koymuştur.

Anahtar Kelimeler: Fotoperiyot, sperma, ovulasyon, fertilizasyon, *Oncorhynchus mykiss*.

Introduction

The environmental control of reproduction in fish has great interest for the development of aquaculture industry (Wang *et al.*, 2010). Photoperiod and water temperature show their effect on gonadal development through endocrine system that controls reproduction. Although water temperature can effect reproductive development and spawning, photoperiod has been accepted as the most important factor synchronizing sexual maturation and reproduction in fish (Bromage *et al.*, 2001).

On the other hand, in spite of well establishing of water temperature effect over the initiation and modulation of reproductive development (Davies and Bromage, 2002; Gillet, 1991), little is known about influence of photoperiod on gonadal development and gamete quality in salmonid fish. In general, photoperiod manipulations have been successfully applied to improve growth of larvae and juveniles and also to get fish to the commercial weight as soon as possible in aquaculture.

It has been known that photoperiod is involved for the regulation of annual rhythms in teleost fishes (Handeland and Stefansson, 2001). In fish farms, photothermal programs are commonly used to control reproductive cycle and to obtain larvae all year round (Wang *et al.*, 2010). The ability to control spawning has enabled supplies of seed for fish farms during the year (Bromage and Roberts, 1995). Grow-out fish farms are better able to meet the market requirements for year-round production of market-sized fish by exploiting such supplies of seed.

In aquaculture, most researches examining photoperiod and reproduction have focused on shifting spawning period by compressing duration of the reproductive cycle to advance or delay the spawning season (Davies and Bromage, 2002; Carillo *et al.*, 1989; Scott *et al.*, 1984). By increasing photoperiod over one month period, it is possible to advance spawning period in different cultured fish species. From this point of view, rainbow trout culture requires controlling their reproductive cycle so that an out-of-season spawning is achieved. Therefore, induction of the reproductive cycle should be done irrespective of season (Migaud *et al.*, 2004).

Research on multiple batch spawning of Nile tilapia (*Oreochromis niloticus* L.), has shown that long photoperiods (18L:6D) promote general spawning performance in female broodstock compared to short (6L:18D) and normal (12L:12D) days (Campos-Mendoza *et al.*, 2004). In rainbow trout, Bromage *et al.* (1984) and Scott *et al.* (1984) determined that constant long days or continuous light advanced spawning. In addition, Davies *et al.* (1999) found that combination of long and short photoperiod regimes can advance or delay maturation and also spawning in rainbow trout.

Despite widespread acknowledgment of the importance of photoperiod in the control of

reproduction in fish, there is lack of information about influence of long and short photoperiod regimes on spermiation, sperm quality and hatchery performance of rainbow trout broodstock to obtain summer egg. Therefore, the present study was conducted to investigate the influence of different combined long and short photoperiod regimes on egg and sperm quality, spawning and also fertilization efficiency of rainbow trout broodstock for the out-of-season spawning.

Materials and Methods

Broodstock Management and Experimental Design

The experiment was carried out in hatchery unit of the Mer-Su trout farm (Mugla, Turkey) using broodstock ponds (55 m³). Each photoperiod regime was applied as triplicate at 3 concrete ponds and the broodfish were randomly distributed into the each 9 concrete ponds at density of 30 fish (male and female ratio was 1:2) and also allowed to acclimate for two weeks. Following acclimation period, broodfish were starved for 24 h, and their body weights were measured after anaesthetizing with 0.1 ppm quinaldine (Merck). The initial mean (\pm SEM) body weights were 415.7 \pm 20.0 g for male and 2370.5 \pm 35.7 g for female broodfish. Each concrete ponds were supplied with filtered freshwater at 7 L/min and water temperature was 9 \pm 2°C during the experimental period. The broodfish were fed *ad libitum* twice daily with a commercial diet (Bioaqua, Çamlı Yem, İzmir, Turkey) containing 44% crude protein and 18% crude lipid during light phase only.

Each experiment ponds were illuminated with two 160-W fluorescent tubes suspended 50 cm above of the water surface and isolated from other ponds. Light intensity was maintained at 1500 lx on the water surface during light phase and control ponds were left uncovered. In order to provide gonadal development and obtain summer egg, broodfish were exposed to combined artificial photoperiods and natural light-dark cycle (control group). The photoperiod regimes were arranged as 18 h light : 6 h dark (18L:6D), 6 h light : 18 h dark (6L:18D), 14 h light : 10 h dark (14L:10D) and 10 h light : 14 h dark (10L:14D) using 24-h timers. Each photoperiod regime was started at 09:00 hour.

After spawning of rainbow trout broodstock (between November and December), first group broodfish were exposed to 18L:6D photoperiod regime for 4 months beginning on 5 January 2012. Following, on 6 May 2012, the same group broodfish were exposed to 6L:18D photoperiod regime for 3 months. At the same dates and periods, second group broodfish were exposed to 14L:10D photoperiod regime and then exposed to 10L:14D photoperiod regime. Natural photoperiod regime was applied to the control group and each photoperiod regime was

carried out as triplicate through experimental period.

Gamete Collection

Before each manipulation, fish were anesthetized in 0.1 ppm quinaldine (Argent Laboratories, Redmond, Washington, USA). Females were checked for ovulation 2 times a week. Upon detection of the ovulation, eggs were collected into 100 mL sterile plastic tubes by manual stripping. Eggs that well rounded and transparent were used for fertilization. At each egg collection day, after removing of urine from the urinary bladder, sperm samples were carefully collected into 50 ml sterile plastic tubes by manual pressure on the abdomen of mature males. Following collection of the gametes, adult male and female fish were placed into 250-L tank containing clean water and having extensive aeration for approximately 10 min for recuperation and then returned to their original ponds.

Semen Quality, Fecundity and Egg Size

Semen volume was determined volumetrically using graduated tubes. Spermatozoa motility was evaluated by placing about 10 μ l semen on a glass microscope slide and adding 100 μ l activation solution (0.3% NaCl). The samples were examined with a dark field light microscope (Olympus, Tokyo, Japan) at 40x magnification and motility was expressed as the percent of spermatozoa that were motile. Each motility determination was performed in triplicate for each semen sample. Same person conducted all sperm motility observations in order to decrease degree of variation among observers. Spermatozoa motility period was determined using a sensitive chronometer (1/100) starting simultaneously with addition of activation solution into the sample until spermatozoa maintained forward swimming activity.

Spermatozoa density was determined by the hemacytometric method. For this aim, sperm was diluted (1/1000) with Hayem solution (5 g Na_2SO_4 , 1 g NaCl, 0.5 g HgCl_2 and 200 ml bicine) and a droplet of the diluted sperm was placed on Thoma's hemocytometer (TH-100, Hecht-Assistent, Sondheim, Germany) slide (depth 0.1 mm) with a coverslip and counted using dark-field microscope. After a few minutes (to allow sperm sedimentation), the number of spermatozoa was counted with a phase contrast microscope (Olympus, Tokyo, Japan) at 100x magnification and expressed as $\times 10^9 \text{ mL}^{-1}$. Semen pH was measured using digital pH meter (Model GLP 21, Crison, Spain) within 30 min of sampling. Semen colour was evaluated visually immediately following collection. Fecundity and egg size were evaluated from 180 females. Fecundity was calculated according to volumetric method described by Alvarez-Lajonchere (1982) and egg size was determined by using a sensitive micrometer (Tronic,

Melbourne, Australia) at 0.01 mm sensitivity.

Fertilization

Fertilization was carried out with sperm and egg pools of broodstock exposed to same photoperiod regimes. All fertilization trials were done as 3 replicates in dishes with 5 mL of eggs (150 ± 10 eggs for each group). The dry fertilization technique was used and the insemination dosage was 2×10^5 spz/egg for each fertilization experiment. Sperm obtained from each fish was poured onto the eggs separately and gently mixed about 20 s. One minute later, 20 mL fertilization solution (3 g urea, 4 g NaCl and 1 L distilled water) was added and incubated about 20 min. Following fertilization, the eggs were rinsed with hatchery water and each group eggs were incubated separately in a vertical egg incubator trays. Fertilization rate was determined as the percentage of eyed eggs about 40 days later following fertilization.

Statistical Analysis

Statistical analyses were performed with SPSS 10 for Windows statistical software package. Motility data were normalized through arcsine transformation. Correlations between spermatological parameters, fecundity, egg size and fertilization rates were determined using Pearson's correlation test. Results are presented as mean \pm SEM and significance was considered at the level of $\alpha=0.05$.

Results

Physical spermatological parameters of the male broodstock and also fecundity, fertilization and hatching rates of the female broodstock exposed to different photoperiod regimes were shown in Table 1 and 2 respectively. During all the out-of spawning season, over 80% of the males were spermiated. It is interesting to note that only significant differences were determined in term of spermatozoa motility between experimental groups ($P < 0.05$). Semen colour was determined as milky white in all samples.

All the adult female rainbow trouts spawned (100%) in the control group, while the number that spawned in the other treatments ranged from 62% in group I to 84% in group II. It was determined that the longest ovulation period was occurred in female rainbow trout broodstock at 265 days in group I. The majority of eggs were obtained during one month (between August 20 and September 17).

Statistical analysis shows that only statistically important positive correlation was determined between relative fecundity and fertilization rate in 18L:6D/18D:6L photoperiod regime ($r=0.452$, $P < 0.05$). Correlations between fertilization rates and sperm quality parameters are shown in Tables 3 - 8.

Table 1. Physical sperm quality parameters of the male rainbow trout (*Oncorhynchus mykiss*) broodstock exposed to different photoperiod regimes

Groups	Weight (g)	Length (cm)	Semen Volume (ml)	Spermatozoa Motility (%)	Spermatozoa Motility Period (s)	Spermatozoa Density ($\times 10^9$ /ml)	Semen pH
I	467.0 \pm 64.7 ^a	35.4 \pm 1.7 ^a	9.1 \pm 1.2 ^a	70.0 \pm 2.5 ^a	63.0 \pm 0.8 ^a	11.3 \pm 0.5 ^a	8.3 \pm 0.02 ^a
II	444.4 \pm 45.9 ^a	32.3 \pm 1.6 ^a	8.5 \pm 0.6 ^a	79.0 \pm 2.3 ^b	62.4 \pm 1.5 ^a	9.7 \pm 0.3 ^a	8.4 \pm 0.08 ^a
Control	386.1 \pm 6.5 ^a	31.7 \pm 2.0 ^a	7.7 \pm 1.4 ^a	83.0 \pm 2.1 ^b	67.2 \pm 6.3 ^a	10.7 \pm 0.6 ^a	8.4 \pm 0.06 ^a

Values are means \pm S.E.M.; means in the same column with different superscript are significantly different ($P < 0.05$) from each other.

Table 2. Fecundity parameters, fertilization and hatching rates of the female rainbow trout (*Oncorhynchus mykiss*) broodstock exposed to different photoperiod regimes

Groups	Weight (g)	Length (cm)	Relative Egg Fecundity (eggs/kg)	Absolute Fecundity (eggs/fish)	Egg Diameter (mm)	Fertilization Rate (%)	Hatching Rate (%)
I	2361.0 \pm 18.5	53.5 \pm 1.6	124.8 \pm 23.8	3654.7 \pm 298.3	4.4 \pm 0.1	85.3 \pm 1.8	68.0 \pm 2.0
II	2480.5 \pm 15.6	51.4 \pm 1.5	132.4 \pm 14.5	3508.4 \pm 162.0	4.37 \pm 0.1	84.0 \pm 2.0	67.3 \pm 2.1
Control	2339.5 \pm 20.5	53.3 \pm 2.0	137.3 \pm 24.5	3559.9 \pm 254.7	4.6 \pm 0.1	87.0 \pm 2.5	72.6 \pm 2.3

Values are means \pm S.E.M.

Table 3. Correlations between sperm quality parameters and fertilization rates of male rainbow trout (*Oncorhynchus mykiss*) broodstock exposed to 18L:6D/18D:6L photoperiod regime (Group I)

	Semen Volume (ml)	Spermatozoa Motility (%)	Spermatozoa Motility Period (s)	Spermatozoa Density ($\times 10^9$ /ml)	Semen pH
Spermatozoa Motility (%)	-0.530				
Spermatozoa Motility Period (s)	-0.030	0.097			
Spermatozoa Density ($\times 10^9$ /ml)	0.460	-0.497	-0.095		
Semen pH	0.211	-0.364	-0.469	0.176	
Fertilization Rate (%)	-0.214	0.033	0.222	0.143	-0.691*

*Correlation is significant at the 0.05 level.

Table 4. Correlations between sperm quality parameters and fertilization rates of male rainbow trout (*Oncorhynchus mykiss*) broodstock exposed to 14L:10D/14D:10L photoperiod regime (Group II)

	Semen Volume (ml)	Spermatozoa Motility (%)	Spermatozoa Motility Period (s)	Spermatozoa Density ($\times 10^9$ /ml)	Semen pH
Spermatozoa Motility (%)	0.425				
Spermatozoa Motility Period (s)	-0.448	-0.589			
Spermatozoa Density ($\times 10^9$ /ml)	0.011	0.277	-0.391		
Semen pH	-0.598	-0.282	0.110	0.052	
Fertilization Rate (%)	-0.185	0.002	0.059	0.440	-0.096

Table 5. Correlations between sperm quality parameters and fertilization rates of male rainbow trout (*Oncorhynchus mykiss*) broodstock exposed to natural photoperiod regime (Control group)

	Semen Volume (ml)	Spermatozoa Motility (%)	Spermatozoa Motility Period (s)	Spermatozoa Density ($\times 10^9$ /ml)	Semen pH
Spermatozoa Motility (%)	0.535				
Spermatozoa Motility Period (s)	-0.025	0.167			
Spermatozoa Density ($\times 10^9$ /ml)	0.037	0.067	0.059		
Semen pH	0.237	-0.104	0.107	-0.203	
Fertilization Rate (%)	-0.111	0.202	0.524	0.415	-0.072

Table 6. Correlations between fecundity parameters, fertilization and hatching rates of female rainbow trout (*Oncorhynchus mykiss*) broodstock exposed to 18L:6D/18D:6L photoperiod regime (Group I)

	Relative Fecundity (eggs/kg)	Absolute Fecundity (eggs/fish)	Egg Diameter (mm)
Absolute Fecundity (eggs/fish)	0.367		
Egg Diameter (mm)	0.026	0.276	
Fertilization Rate (%)	0.452*	0.309	0.188

*Correlation is significant at the 0.05 level.

Table 7. Correlations between fecundity parameters, fertilization and hatching rates of female rainbow trout (*Oncorhynchus mykiss*) broodstock exposed to 14L:10D/14D:10L photoperiod regime (Group II)

	Relative Fecundity (eggs/kg)	Absolute Fecundity (eggs/fish)	Egg Diameter (mm)
Absolute Fecundity (eggs/fish)	0.332		
Egg Diameter (mm)	-0.322	-0.606*	
Fertilization Rate (%)	-0.094	-0.172	0.152

*Correlation is significant at the 0.05 level.

Table 8. Correlations between fecundity parameters, fertilization and hatching rates of female rainbow trout (*Oncorhynchus mykiss*) broodstock exposed to natural photoperiod regime (Control group)

	Relative Fecundity (eggs/kg)	Absolute Fecundity (eggs/fish)	Egg Diameter (mm)
Absolute Fecundity (eggs/fish)	0.364		
Egg Diameter (mm)	0.169	-0.128	
Fertilization Rate (%)	-0.035	0.167	0.242

Discussion

In aquaculture, availability of eggs and fry all year round is very important in order to optimize profitability of fish farming. Therefore, artificial induction of spawning and controlled reproduction of cultured species seem necessary for fish farms dealing with species that can not spawn in captivity. Thus, the hatchery is obliged to keep its own broodstock and to manipulate the reproductive cycle to extend availability of seed for the out-of spawning season.

Different culture techniques and methods have been applied in aquaculture. Among these applications, photoperiod techniques have great importance to increase somatic growth and to control reproduction. It has been well known that most researches applying different photoperiod regimes, have been focused to improve growth parameters in different fish species. As a result of these studies, it was determined that increasing of light period had a positive affect on feeding and growth of fish (Kissil *et al.*, 2001; Rad *et al.*, 2006).

On the other hand, many researches have stated that photoperiod is the most important environmental factor affecting reproductive activity in rainbow trout (Scott *et al.*, 1984). Photoperiod applications to control reproduction were carried out over 24-h cycles of variable light/dark regimes. For this aim, it has been known that 18L:6D, 16L:8D, 14L:10D and

24L:0D photoperiod regimes have been widely applied in aquaculture (Whitehead and Bromage, 1980; Bromage *et al.*, 1984).

In this study, it was determined that ovulation can occurs out-of spawning season when the photoperiod regimes were changed. According to Bromage *et al.* (1984), ovulation can be delayed about 4 months following exposure to constant short day lighting and then sudden long day lighting. Similarly, Burlier and Billard (1984) determined that ovulation can be delayed about 2 months changing long day lighting to constant short day lighting. In addition, Okumuş (2002) indicated that it is possible to advance spawning about 3-4 months at constant short days lighting following constant long day lighting.

In this research, ovulation was occurred at 265 days in group I whereas at 160 days in group II when female adult rainbow trout broodstock exposed to constant long and short day lighting respectively. Finding of this research is accordance with results of Randall and Bromage (1998) that indicated spawning was occurred in August applying constant long (18L:6D) and following short day photoperiod regimes (6L:18D) in rainbow trout. Similarly, Şen (2013) indicated *Salmo trutta macrostigma* spawned 3 months earlier than normal spawning season by applying constant long day lighting (18L:6D) for 4 months following constant short day lighting (8L:16D).

It is interesting to note that absolute fecundity was determined highest in group I rather than others. It shows that 18L:6D/18D:6L photoperiod regime has positive effect to increase absolute fecundity. On the other hand, determination of low relative fecundity in group I indicates that body weights of these broodfish higher than that of other groups. Regarding in terms of absolute and relative fecundity, differences between experimental groups can be explained by age and weight of the broodstock. According to Johnson and Johnson (1999), fecundity increases depending on increase at weight and length of the broodstock.

Findings of this study demonstrate that egg size was smaller than that of obtained normal spawning season. It might be due to shortening of the vitellogenesis because of artificial photoperiod. Statistical analyses revealed the existence of positive correlations between egg size and fertilization rate.

The mean semen volumes of all experimental groups in this study was lower from most of other results reported by McNiven *et al.* (1993), Lahnsteiner *et al.* (1993) and also Geffen and Evans (2000) for rainbow trout. On the other hand, according to results of the present study it seems that photoperiod regimes have affected semen volume positively when compared with the control group. The differences between experimental groups may be due to feeding conditions, water quality and also light/dark cycle. Buyukhatipoglu and Holtz (1984) found that semen volume is significantly higher at the beginning of the spawning season than later periods.

The spermatozoa motility and its duration have great influence on successful fertilization. However, spermatozoa motility varies in vigor and duration not only among males but also within individual males depending on ripeness (Tekin *et al.*, 2003). Mean spermatozoa motility of rainbow trout varied between 70.0±2.5% and 83.0±2.1% among the experimental groups and also positively correlated ($P>0.05$) with the fertilization rate. Most studies on fish species have shown that duration and motility of semen may vary seasonally (Benau and Terner, 1980).

Spermatozoa density may also effect the fertilization rate (Aas *et al.*, 1991). Findings on spermatozoa density varied between ($9.7\pm 0.3\times 10^9$ ml⁻¹ and $11.3\pm 0.5\times 10^9$ ml⁻¹) and corroborated with the results of Munkittrick and Moccia (1987) and McNiven *et al.* (1993) but not with the findings of Buyukhatipoglu and Holtz (1984). The differences may be due to dilution ratio, age or spawning season. The mean semen pH in our study was generally confirmed by Piironen (1985) and Munkittrick and Moccia (1987).

Fertilization procedure was carried out using 2×10^5 spermatozoa per egg as stated by Munkittrick and Moccia (1984). The results regarding fertilization rates rather higher than findings of Wheeler and Thorgard (1991) and Lahnsteiner *et al.* (1997) but lower than findings of Pornsoping *et al.* (2007) and Samarin *et al.* (2008). The fertilization results can be

explained by positive relationships of sperm motility, spermatozoa/egg ratio and egg size with the fertilization ratio.

In conclusion, the present study showed that photoperiod regimes have an significant effect on spermatozoa motility. Results revealed that spermatozoa motility, motility period and spermatozoa density positively correlated with fertilization rate in all photoperiod regimes ($P>0.05$). In addition, this study demonstrated possibility of obtaining summer egg by applying long and short photoperiod regimes under hatchery conditions.

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