

The Effect Of Water Temperature On Spawning Out Of Season In Rainbow Trout, *Oncorhynchus Mykiss*

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Abstract

Salmonid culture is a production sector with widespread throughout the world. Different stocks of rainbow trout spawn in certain months of the year in hatcheries. This seasonality of spawning imposes considerable constraints on trout farming because the consequent restrictions on the supply of eggs and fry make it difficult for on-growing farm to maintain a continuity of production of table-size fish throughout the year. Hatcheries should artificially control the spawning time of their broodfish so that batches of eggs and fry might be produced all year round. The modification of spawning time are possible using manipulation of photoperiod. This series of studies were carried out in order to identify the effects of varying water temperature on the ovulation of rainbow trout be exposed to photoperiodic manipulation. This study was carried out in two different facilities. In the first facility, the photoperiod regime was only consisted of constant short days (LD 6:18). The water temperature had been changed between 7.5 and 15 °C during the the experiment in this facility. In the second facility, it had been changed between 11 – 25.5 °C. The broodstock was seperated into two different group. These photoperioded regims consist of constant short days(LD 6:18) and altered photoperiod (LD 18:6 from February until May, then LD 12:12 from May until June, then LD 6:18 from June until November) which are acted to advanced spawning. In both experiments, photoperiod regimes changed the maturation time regardless of water temperature. However, when the water temperature is high, modulating effects of the temperature on the maturation and ovulation time were observed. When the spawning time modified to June – August, ovary growth and egg survival rate had been reduced by the temperature effect (>14 °C). It was found that the egg quality decreased when the water temperature was 17 °C although the eggs could be removed from the fish. Based on the

these results, the role of water temperature in determining spawning time should take into account, especially in conjunction with photoperiodic manipulation.

Keywords: Photoperiod, Rainbow trout, water temperature, ovulation, egg

1. INTRODUCTION

Despite the reproduction periods of trouts spreads of each month of the year, generally it occurs between 6-8 weeks in the autumn-winter seasons. To be able to control the reproduction period, It is important to change some environmental factors such as temperature and day length. Due to the seasonal egg production, sustainability of trout farming is eliminated. Therefore, egg production should be provided in all seasons and as a result continuously production of market size fish must be guaranteed. Despite of using hormonal applications as LHRH, HCG or pituitary extracts for spawning, this methods provide only 2-3 weeks progress but the produced gametes have poor quality. Photoperiod applications are used more effectively to change the timing of reproduction (Bromage, et al., 1984; Duston and Bromage, 1986). It is possible to take the spawning time 6 months earlier or later with the photoperiod applications (Bromage, et al., 1993). However, the water temperature that is effective on spawning should be considered along with applications of photoperiod. There are studies that report the effects of temperature on the egg ovulation and spawning times (Morrison and Smith, 1986; Nakari, et al., 1987; Johnstone, et al., 1992; Taranger, et al., 1999). High temperatures cause on the eggs over-maturity. Suitable water temperatures encourage ovulation (Taranger and Hansen, 1993).

Trout farming is made in many parts of the world's water sources. It is possible that the changing climatic conditions affects on the water temperature. The effect of the temperature on the commercial trout stocks, can emerge in the form of reproductions limitation. This series of studies were carried out in order to identify the effects of varying water temperature on the ovulation of rainbow trout be exposed to photoperiodic manipulation.

2. Materials and methods

This study was carried out on the commercial rainbow trout broodstock populations (*Oncorhynchus mykiss*) in two different farms. Broodstock fish were fed with 1% rate of broodstock-feed (Pinar-Çamli Fish Feed, Turkey).

Trial 1

The first trial was conducted in a private trout farm, that had 20 tons/year production capacity and was placed in Izmir/Turkey, in winter and summer seasons. In the size of 12 m³(6 x 2 x 1 m), three raceway type concrete pools were used. The used water was spring water and annual temperature variation took place between 7.5 - 15 °C. After two-year-old broodstock fish spawned at the beginning of December with the natural illumination cycle, constant illumination program was applied as 6-hours light / 18 hours dark (6L:18D). The daylight (> 20 000 lx) was used in the light period. The concrete pools were covered with black tarpaulin in the dark period. In this study, 60 female and 20 male fish were used. The first trial started in January and lasted for 396 days.

Trial 2

The first trial was conducted in a commercial trout farm, that had 300 tons / year market size trout production capacity and placed in Aydın/Turkey, in winter and summer seasons. The broodstock fish were obtained from the fish that grown in this farm. The photoperiod group of constant short day (CSD) (6L:18D); The group of constant illumination program and variable photoperiod (VP); group of fish that spawned naturally in the summer (SNS) were 3-years-old. The broodstock fish were used at their second spawning season. In the group of CSD, 500 female fish and 200 male were used. In the group of VP, 500 female and 200 male fish were used. And in the group of SNS 200 female and 100 male fish were used. The CSD and VP were kept in the 44x2,5x0,7 m sized concrete pools as mixed; SNS were kept in 30x5x1,3 m sized soil pools as mixed. The concrete pools were covered with a light-proof tarpaulin. For lighting, 100-watt light bulbs were used in order to achieve a minimum of 40 lux light on the water. After the eggs of the broodstock fish in the CSD were taken in the winter period, 18D:6L constant light program was applied to the from 1st of February. The fish in the VP was exposed to the 18L:6D from February until May; the 12L:12D from May until June; and to the 6L:18D after June until November (Bromage and Cumarantunga, 1988). The second trial started in December and lasted for 300 days. The broodstock fish were fed according to the water temperature 1-2 times in a day ad-libitum with Bagci Commercial Fish Feed. The feed was formulated to contain 16-18% crude oil, 40-45% crude protein, 2% cellulose and 3500-4050 k.cal. kg-1 energy. Ovulation time was followed by controlling the fish 2 times in a week and the ovulated fish were allocated and prepared for milking.

2.1. Statistics

When samples complied with the criteria for parametric testing, Egg diameters were compared using Student's t-test. Kruskal-Wallis test (in Trial 1) and The Mann-Whitney U-test (in Trial 2) were used to compare the fertilization rates, hatching rates and survival rates. All analyses were performed by using SPSS 15.0 version.

Results

Trial 1

Spawning profiles

The broodstock fish that had natural reproduction time in December, started spawning in June as a results of constant short day photoperiod regime. The fish spawned 5 months earlier than normal period. Maximum number of spawning fish was observed in July. The response of fish to the implementation of photoperiod, was measured as 75%. When the constant short day photoperiod regime (6L:18D) resumed to apply, 53,3 percentage of the same fish spawned for the second time in February. Thus, two times spawning of the fish was provided in 14 months. The response of the natural and constant short day (6L:18D) photoperioded fish to the spawning times in the different temperature showed no significant difference ($p > 0.05$).

Egg quality, fecundity and size

In the first and second spawning; the obtained, fertilisation, hatching and survival rates of the natural and constant short day (6L:18D) photoperioded eggs were different statistically ($p < 0.05$). The egg diameters were found different in all groups. It was seen that when the fish spawned earlier time, egg diameters became smaller.

Trial 2

Spawning profiles

The stream water, supplied with the artesian from the dam, was used in the farm. The water temperature increased rapidly when the dam water level decreased in July. It was measured high water temperature (20 - 25.7 C) and lower oxygen saturation (4.8 - 5 ppm) between July and October. This period was reported the most important critical production period of the farm. In the constant short day photoperiod group (CSD), ovulation and removal of the eggs started at the beginning of June and lasted June 30. Response of the photoperioded fish was observed as 17.8% . Egg removal of the naturally spawning in the summer group (SNS) was made in June and July when the 16L:8D. Response of the fish to the photoperiod application was measured 62%. The fish under the variable photoperiod (VP); were exposed 18L:6D from February until May, 12L:12D from May until June, from June 6L:18D. In this group the spawning period-expected in September was not seen due to the high water temperature. All the broodstock fish died. Those fish were cut and controlled. Ovulation of the eggs was observed. Avarage weight of broodstock fish was measured as 2246 ± 413 gr and the obtained mean egg weight per broodstock fish was detected as 307 ± 119 gr. Due to there was no sperm observation from the male broodstock, fertilisation could not be processed. Gonadosomatic index (GSI) was detected as 13.9%. Responses to spawning time in the different temperature and season of the experimental groups; constant short day (6L:18D) photoperiod, variable light photoperiod and naturally spawning in the summer group; were found statistically different (p<0.05).

Egg quality, fecundity and size

Hatching and survival rates were considered as quality criteria for the fish eggs. In the group of CSD, the fertilisation, hatching and survival rates was found respectively, 52,2 ± 1,7%; 24,1 ± 1,1%; 11,3 ± 0,8% ; and in SNS group, 35,4 ± 1,1%; 17,3 ± 0,8%; 8,3 ± 0,5% . Fertilisation, hatching and survival rates of Constant short day (6L:18D) photoperioded and naturally spawned in summer fish, were found statistically different (p<0.05). Egg diameters were detected similar in all groups and smaller in the early spawnings.

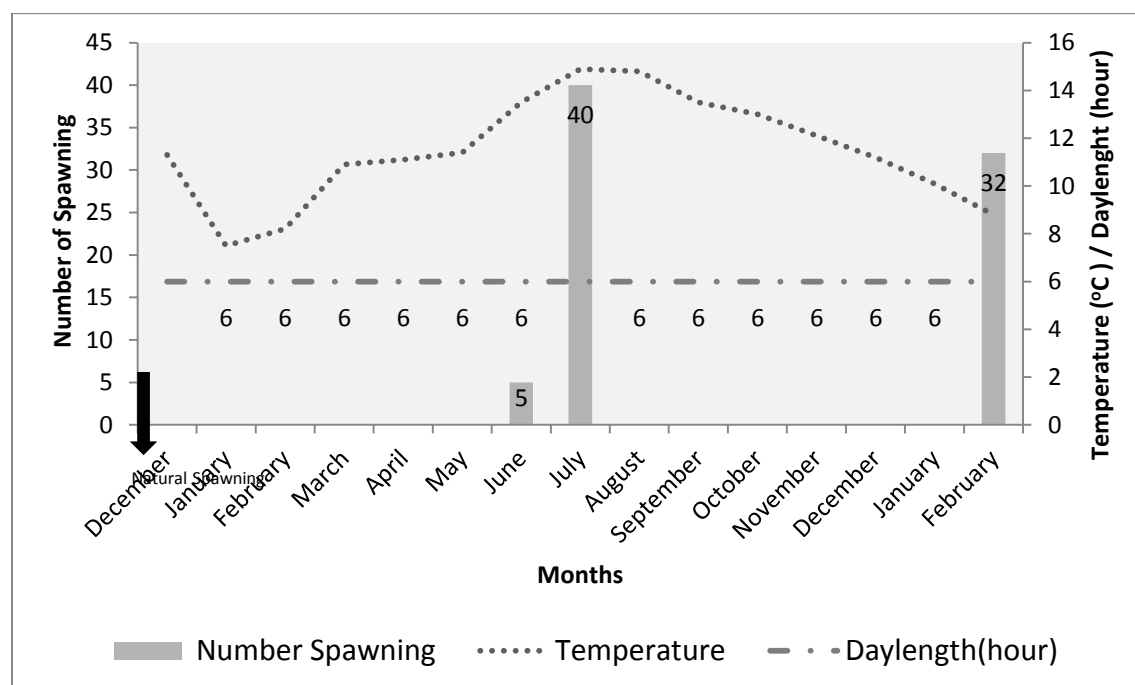


Figure 1. The effects of constant photoperiods(CSD) on the timing of spawning in the rainbow trout (Trial.1)

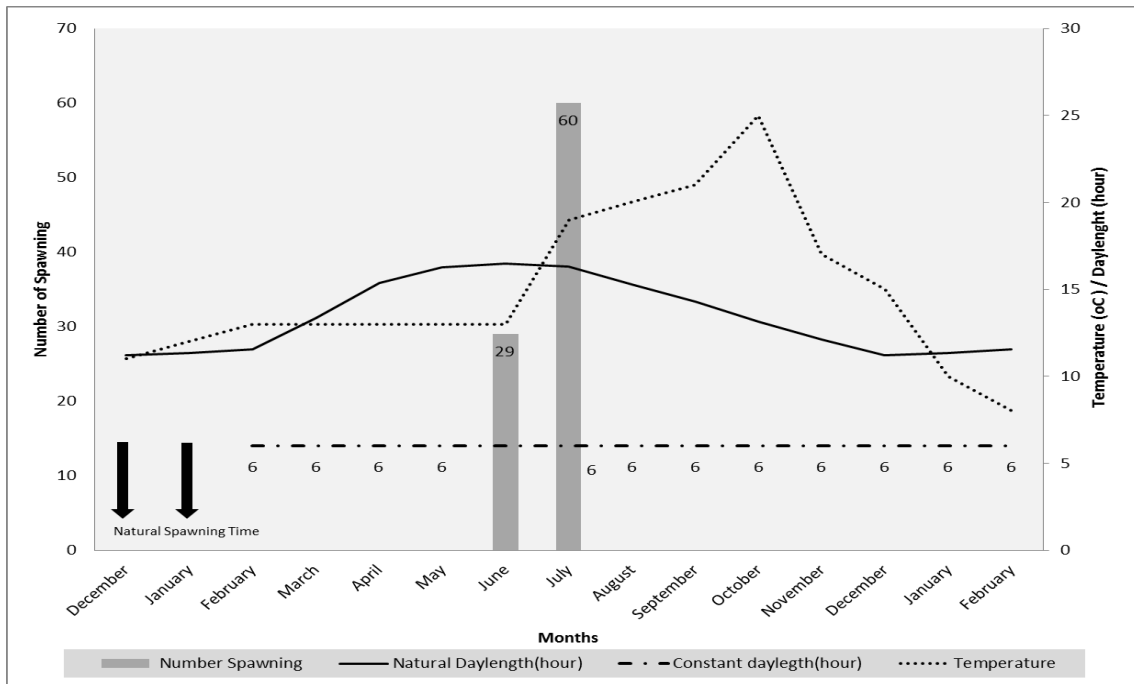


Figure 2. The effects of constant photoperiods(CSD) on the timing of spawning in the rainbow trout(Trial 2)

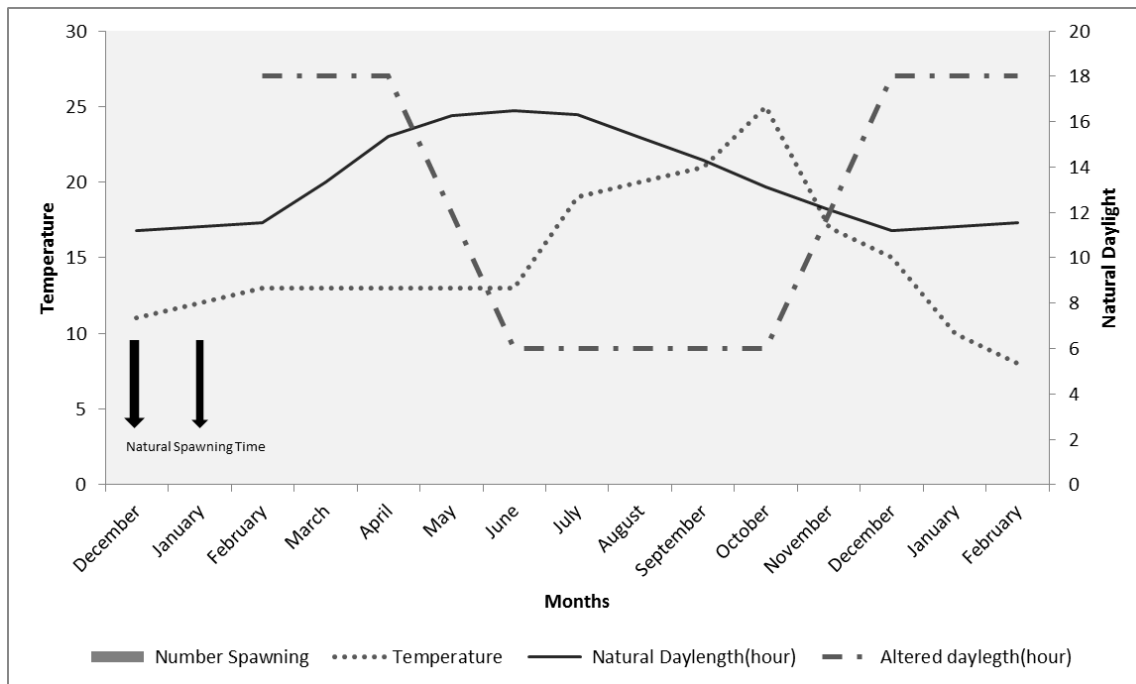


Figure 3. The effects of variable photoperiod (VP) on the timing of spawning in the rainbow trout(Trial 2)

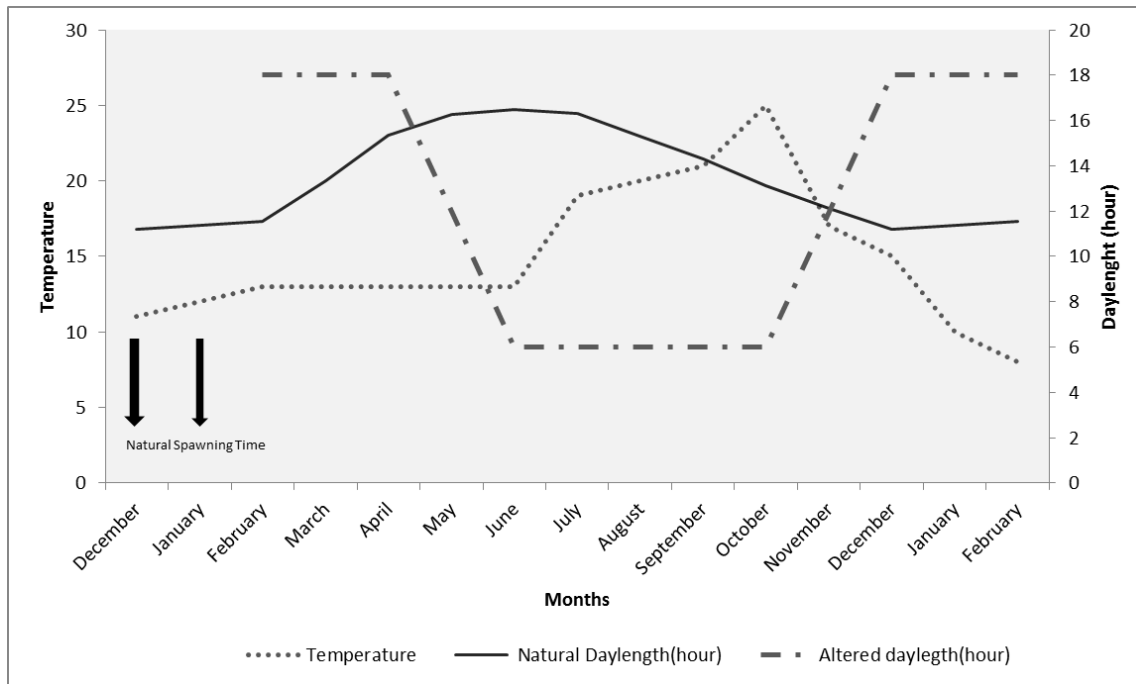


Figure 4. The naturally spawning in the summer group (SNS) in the rainbow trout(Trial 2)

Table 4. Total and Relative Fecundity, fish weight, egg diameter, fertilisation rate, hatching rate, and egg survival rates for broodstock in the two experimental groups in Trial 1

Groups	Total Fecundity	Fish weight	Relative Fecundity	Egg diameter	Fertilisation Rate	Hatching Rate	Survival Rate
	(eggs/fish)	(kg)	(eggs/kg fish)	(mm)	(%)	(%)	(%)
Natural Spawning	2659 ± 100 (60)	1552 ± 42	2034 ± 95	5,6 ± 0,1	91,8 ± 1,5	64,9 ± 1,4	38,8 ± 1,3
Constant Short Day First Spawning	3897 ± 1252 (45)	1950 ± 297	1975 ± 492	5,0 ± 0,2	71,2 ± 1,72	50,1 ± 2	28,6 ± 1,6
Constant Short Day Second Spawning	6459 ± 1051 (32)	3390 ± 345	1905 ± 295	5,4 ± 0,3	87,4 ± 1	83,9 ± 1,4	63,6 ± 1,45

Table 5. Total and Relative Fecundity, fish weight, egg diameter, fertilisation rate, hatching rate, and egg survival rates for broodstock in the two experimental groups in Trial 2

Groups	Total Fecundity	Fish weight	Relative Fecundity	Egg diameter	Fertilisation Rate	Hatching Rate	Survival Rate
	(eggs/fish)	(kg)	(eggs/kg fish)	(mm)	(%)	(%)	(%)
Summer Naturally Spawning	2848 ± 583 (124)	400 ± 157,4	2067 ± 460	4,2 ± 0,1	35,4 ± 1,1	17,3 ± 0,8	8,3 ± 0,5
Constant Short Day First Spawning	2480 ± 450 (89)	1759 ± 250	1411 ± 264	4,24 ± 0,1	52,2 ± 1,7	24,1 ± 1,1	11,3 ± 0,8
Altered Photoperiod	-	2243 ± 449	GSI: %13,9	-	-	-	-

2.2. Discussion

2.2.1. Effects of photoperiod and temperature on spawning time

In the trials, spawning time was observed 5 months earlier (in July) than normal period by applying constant short day photoperiod regime to the trouts that naturally spawned in December. After the first spawning in the July, it is recorded that the same fish spawned second time with 2 months delay (in February) compared to normal period. It was noticed that when the constant short day photoperiod method was applied, there was only 2 week difference between the different fishes in Trial 1 and Trial 2. Broodstock fish reached ovulation later in the cold water. However, the maximum number of spawned fish were in the same month (July). Thus, in the both trials showed significant effect of photoperiod on rainbow trout's maturation and spawning. On the other hand, there was not a significant effect of temperature on maturation and spawning time. Nevertheless, modulatory effects of the water temperature were observed. Davies and Bromage (2002) found similar results about the water temperature and reported the water temperature affected on spawning and ovulation. They also mentioned that ovulation period spreaded and was delayed at lower temperatures. There are lot of study about delaying the spawning by the cold water (Morrison and Smith, 1986; Nakari, et al., 1988; Johnstone, et al., 1992). But the influence mechanism of temperature on physiological maturation has not been understood yet. According to the some studies, in salmon and the trout, low temperature levels cause delaying on spawning as a result of reducing the accumulation of vitellogenin at the last stage of the formation of vitellogenic.

In the Trail 2, it was determinated that high temperature levels inhibited maturation, although spawning was expected in September. The other studies on salmonids, concluded that high temperature levels inhibited ovulation (Breton, et al., 1983; Pankhurst and Thomas, 1998; Chmlevsky, 1999; King and Pankhurst, 1999). It was added that steroid formation with the high temperature could delay the spawning (Kime, 1980). But, still, the influence mechanism of the high temperature on the final maturation and spawning time of salmonids could not be determinated (Davies and Bromage, 2002). In the Trail 2, it was observed that, spawning success reduced when the water temperature exceeded 14 °C. If the temperature reached 17 °C suddenly, the eggs could be removed but there were problems on ovarium maturation and ovulation. Since the water temperature levels reached 19 – 20 °C in spawning season (September), the fish under the variable photoperiod (VP) died before ovulation. Pankhurst et al.(1996), reported that ovaries of trout were functional below 15 °C, but not at the level of 18-20 C°. Davies and Bromage (2002), found that ovarian problems were decreased by 16 °C water temperature level before ovulation. In the Trial 1, as a result of constant short day application, 75% of the experimental fish spawned in the first spawning, and 53.6% in the second. In the Trial 2, constant short day regime, 17.8% of fish spawned although naturally spawned group in the summer. This result can be connected with both temperature and photoperiod's combine effect.

2.2.2. The effect of water temperature on egg quality

In the Trial 1, by decreasing the water temperature in December, in the natural and constant short day groups survival rates were better than the high temperature seasons. But in the Trial 2, the survival rates of the eggs and the fries decreased because of the high temperature. Although egg diameters was expected to be bigger according to fish weight, eggs became smaller in the constant short day photoperioded groups, since the spawning period was taken early. According to earlier studies (Buss, 1982; Bromage and Cumaranatunga, 1988) vitellogenesis period was short by taking the spawning period earlier, and so the egg

diameters became smaller. It was reported that there was no correlation between egg diameter and survival rates in trout. The natural cycle eggs and the photoperioded eggs had same quality (Springate and Bromage, 1985). In Trial 1, Decreasing in the egg quality was observed by increasing the water temperature to 14.9 °C in June-July compared with 11 °C in December. In the Trial 2, a significant reduction in egg quality was appeared at the 16.6 °C temperature level in June-July. GSI was measured as 13.9% in September when the temperature level reached 17 – 19 °C. Although abdomen part of the fish was soft, the eggs could not be removed and most of the brood stock fish died. Overly-ripe eggs in the ovary was observed in some individuals. There is a red spot in the over-ripe trout eggs (Craik and Harvey, 1984). The studies about the over-ripe trout egg (Breton, et al., 1983; Springate, et al., 1984; Billard, 1985), were related with the eggs that remained in the abdominal cavity after ovulation and transformed to over-ripe. Since the eggs were released but not absorbed back, it was expressed this eggs did not fit with atretic eggs (Bromage and Cumaranatunga, 1988). It is know that high temperature levels increase the over-ripening rate of ovulated eggs. This was explained with disintegration of the non-functional eggs that were exposed high temperature before ovulation (Billard, 1985). Despite the water temperature was 16 - 17 °C, the eggs and sperm were removed. But, hatching and fry survival rates decreased considerably. In addition, at the high temperatures, lack of sperm was observed in male brood stock. It is necessary to consider this restriction, for out of season spawning. It was found that when the water temperature was below 14 °C, the last maturation and non-functional ovarias was prevented and were consistent with survival rates. Davies and Bromage (2002) reported that values below 16 °C before ovulation, reduced the ovary problems.

3. Regulation of Photoperiod Practices

In the Trial 1 and Trial 2, it was detected that when the seasonal water temperatures were occurred within the acceptable limits; there would be no problem on the maturation and ovulation in the photoperiod applications early-spawning. According to the literature on the introduction part, long-short photoperiod application can take spawning time 6 months early since December. In the trials, it was observed that constant short day photoperiod groups spawned in July. When the 18L:6D photoperiod regime applied, the spawning period was taken 3 month early. But, number of the spawning fish and the survived fry decreased in summer. Since the high water temperature, some brood stock fish died. In summer, naturally ovulated fish (twice in a year), spawned in a high number (62%) in June and July. However, in this period, the water temperature was between 14.6 °C and 16.6 °C; very significant declines on fertilisation, hatching and survival were observed.

Consequently, according to this study, that examined the effects of photoperiod and water temperature on spawning and fry survival, it was reported photoperiod was a basic environmental factor. It was detected that the water temperature had a modulatory role on performance. In addition, It was observed that water temperature had significant effects on maturation process, spawning time, ovulation and egg quality of the commercial rainbow trout stocks.

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