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Effects of Photoperiod Regime on Growth and Efficiency of Yolk Utilization in Yolk-Sac Fry of Rainbow Trout (*Oncorhynchus mykiss*)

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ABSTRACT

In this study, growth and yolk-sac conversion efficiency were determined in yolk-sac fry of rainbow trout (*Oncorhynchus mykiss*) under five different photoperiod regimes. The newly hatched yolk-sac fry were exposed to an ambient photoperiod (control 41°N) and four different fixed photoperiods 16h L — 8h D, 8 h L — 16h D, 24h L (continuous light), and 24h D (continuous dark). The initial size of yolk-sac fry were significantly same, however, the total length and wet weight of the yolk-sac fry at 618 degree-days (swim-up stage/at the end of study) differed significantly among all treatments. The best growth and yolk-sac absorption was found in 24h D compared to all treatments. After 24h D treatment, the second best growth and yolk-sac absorption was observed in 24h L treatment. Moreover, excluding 24h D treatment, it was found that growth rate increase with increasing in photoperiod regime. Water content of the yolk-sac fry was significantly same in all treatments during the study.

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Authors' Contribution
NB designed the study and wrote the manuscript. FDS performed the experimental work and collected the data.

Key words
Fish growth, Light, Photoperiod, Salmonidae

INTRODUCTION

In developing culture techniques, the primary concern is to adopt an environmental regimen that minimize the yolk-sac fry mortality and enhance yolk conversion efficiency to produce the largest possible first-feeding larvae that generally maximize the initial feeding success (Hansen, 1985; Hansen and Moller, 1985) because transition from endogenous to exogenous feeding is a crucial step and those fail to start initial feeding successfully will be quickly died due to the starvation (Morretti, 1999; Başçınar, 2010).

There is some evidence that extrinsic factors such as photoperiod, temperature, and food availability are particularly important in the growth of teleost fish that stimulate appetite and improves the food conversion (Thorpe *et al.*, 1989; Imsland *et al.*, 1995; Jobling and Koskela, 1996; Taylor *et al.*, 2006). The artificially extended photoperiod has been used to increase the feed conversion to improve the fish growth rate. Several studies demonstrated that extended photoperiod regime plays a major role in the stimulation of a series of changes that included feeding intake, feed conversion, and reproductive function without any adverse effects (Whitehead *et al.*, 1978; Saunders *et al.*, 1985; Boeuf and Bail, 1999; Biswas *et al.*, 2004; Önder *et al.*, 2016).

The influence of extended photoperiod has been observed in rainbow trout (*Oncorhynchus mykiss*), a cultivated species of the family Salmonidae, to enhance their growth rate and reduce the production time (Ergün *et al.*, 2003; Taylor *et al.*, 2005; Taylor *et al.*, 2006). Several other studies have shown that the artificial photoperiod also influence the spawning time (Bromage *et al.*, 1992; Davies *et al.*, 1999), immune system (Leonardi and Klempau, 2003) and hematological parameters (Valenzuela *et al.*, 2008) of rainbow trout. The light intensity also affects the growth of rainbow trout and under high intensity (*e.g.*, 100 lux vs. 1600 lux) rainbow trout appeared to be more active resulting improved growth (Cho, 1992).

Though the effects of different photoperiod regimes have been successfully demonstrated in young and adult rainbow trout, but the possible effect of the photoperiod manipulations on the growth of yolk-sac fry of rainbow trout has not been evaluated. To our knowledge, this is the first study demonstrating the effects of photoperiod regime on growth, development, and yolk utilization of rainbow trout during their yolk utilization phase. The result of this study will assess to explore the proper hatchery management skills of rainbow trout during their larval stage.

MATERIALS AND METHODS

Fish and laboratory conditions

Eggs were stripped from six females and fertilized with milt obtained from three males at the trout hatchery

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in the Faculty of Marine Sciences, Karadeniz Technical University, Trabzon. After fertilization, the eggs were incubated in a vertical incubator with a slow upwelling inflow provided with complete darkness.

Artificial photoperiod regimes

After hatching, about 6000 yolk-sac fry were randomly selected and equally distributed into five groups (having triplicate sub-groups) using glass aquarium for each treatment (10 L). Each group was exposed to different photoperiod regimes. There were five light treatments: ambient photoperiod (control 41° N), 24h dark (24h D), 24h light (24h L), 8h light and 16 h dark (8h L — 16h D), and 16 h light and 8 h dark (16h L — 8h D). Luminous intensity was set to 1000 lux. The aerated fresh water in the batches was circulated and 50% of water was replaced daily. The temperature was measured three times a day (at 8^{30}, 12^{30} and 16^{00}) with a digital thermometer. Dead larvae were removed twice a week.

Sampling of larvae

The sampling commenced at 5 day intervals from hatching after 349th degree-day (DD=water temperature, °C, x age, days post hatch) and repeated at 395, 440, 484, 529, 574 and 618 degree-days. Ten yolk-sac fry were randomly sampled at each sampling period, *i.e*. a total of 350 larvae used during the study. Sampled yolk-sac fry were anesthetized in a benzocaine solution (20 mg/l) and then preserved in 10% formaldehyde (Dumas *et al*., 1995). After a minimum interval of three weeks, fixed yolk-sac fry were dissected to separate the yolk sac from body. Body and yolk were dried separately at 60°C for 48h and weighed individually after 48h (Hansen, 1985).

Measurements

Yolk sac efficiency (*YCE*), dry yolk sac consumption rate (*YCR*; mg/day), daily length and (*LGR*; mm/day) weight (*WGR*, mg/day) growth rates, and development index (K_D) were calculated through the following formulas:

1. $YCE = (L_t - L_0) / (Y_0 - Y_t)$
2. $YCR = (Y_0 - Y_t) / t$
3. $LGR = (L_t - L_0) / t$
4. $WGR = (W_t - W_0) / t$
5. $K_D = 10 \times (Wet\ weight^{1/3}) / length$

Where
L_0 is dry larval length at time zero, L_t is dry larval length at time t, W_0 is dry larval weight at time zero, W_t is dry larval weight at time t, Y_0 is yolk sac dry weight at time zero, Y_t is yolk sac dry weight at time t, and t is number of day.

Statistical analyses

The data were analyzed with analysis of variance (ANOVA), Tukey test and regression analysis by using MINITAB® and MS EXCEL® software. The significance of all slopes (Ho: b=0) and regressions were tested at the 0.05 probability (Hodson and Blunt, 1986), and statistical significance of differences among regression evaluations and slope values were determined with analysis of covariance (Zar, 1999).

RESULTS

The mean diameter of rainbow trout eggs were 5.3±0.2 mm. The hatching was started at 334 and ended at 364 degree-days. The mean length and maximum wet weight of initially hatched yolk-sac larvae were 17.17±0.012 mm and 70.46±2.88 mg, respectively. The water temperature was 9.7±0.74°C during the study. During the experiment the mortality rates were less than 5% in all groups.

The yolk-sac fry length and wet weight was observed to increase for the first few days at all light treatments attending a maximum value of length and weight, and then started to decrease (Figs. 1 and 2). Significant differences in mean wet weight of all light treatments were found among the yolk-sac fry with a pattern of increase from 349 to 574 degree-days in all light treatments except in 16h L — 8h D and 24h D groups where the increment in wet weight was occurred till 529 degree-days and 618 degree-days respectively (Table I).

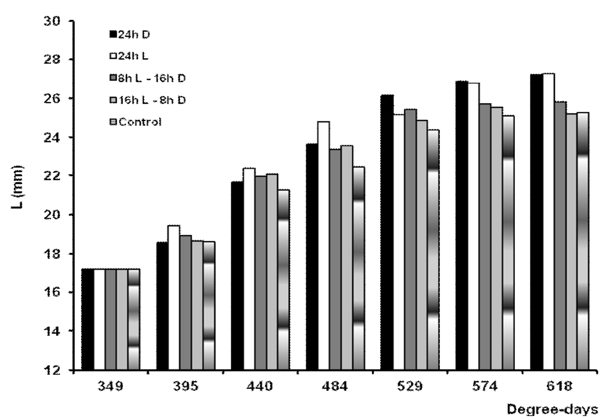

Fig. 1. Growth in length of rainbow trout yolk-sac fry at five different photoperiod treatments.

The dry yolk sac weight was continuously decreasing by each day due to its absorption by yolk-sac fry (Fig. 3). The *YCE* and K_D were significantly different between all light treatments. The maximum *YCE* and K_D values were found in 24h D group. The second maximum values of these parameters were seen in 24h L (Table II). The lowest values of *YCE* and K_D were found in 8h L—16h D group.

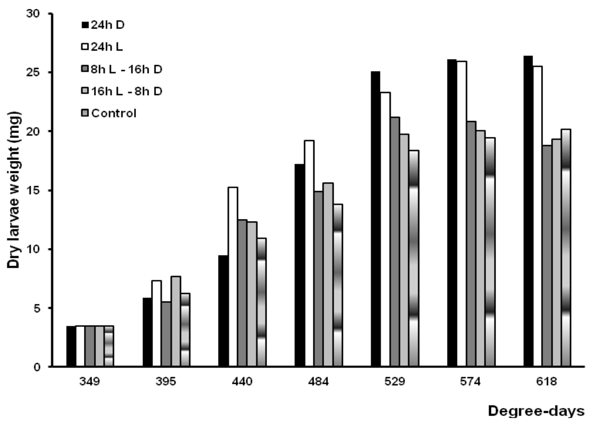

Fig. 2. Dry larvae body weights of rainbow trout yolk-sac fry at five different photoperiod treatments.

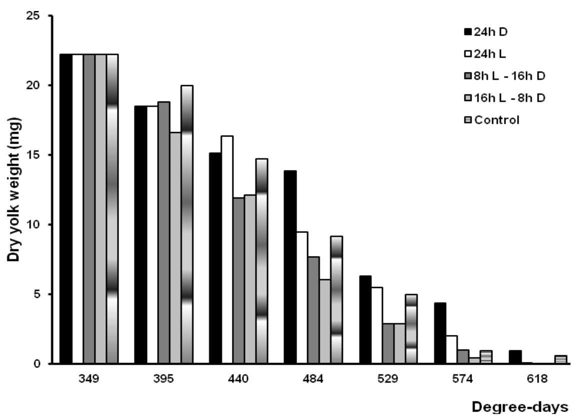

Fig. 3. Dry yolk sac weights of rainbow trout yolk-sac fry at five different photoperiod treatments.

The 24h D treatment displayed the lowest *YCR* value and the maximum value of *WGR*. The lowest values of *LGR* and *WGR* were observed in the control group. The water content of yolk-sac fry was significantly same in all treatments ($p > 0.05$).

The dry yolk-sac fry weight and dry yolk-sac weight was plotted against degree-days to examine their relationship. The relationship of dry yolk-sac fry weight and dry yolk sac weight to degree-days were linear and the regression was highly significant (Table III). The comparison of regression slope (*b*) of different light treatments showed that highest value of *b* was displayed by 24h D treatment followed by 24h L treatment that has the second highest value of *b*.

DISCUSSION

Contrary to expectation, the yolk-sac fry held in 24h D treatment displayed the better growth rate than those held at different light treatments. It was observed that yolk-sac fry length and wet weight increased for the first few days and then directly started to decline once attending a maximum value of length and weight. Such increment and then decline may suggest that those obtaining maximum length and weight earlier will have smaller length and weight at the end of complete yolk-sac absorption. The increment in length and weight gain of yolk-sac fry in 24h D treatment was continued till 618 degree-days. Moreover, it can be seen in Table I that the yolk-sac fry of 24h D group had the maximum wet weight of yolk-sac fry at 618 degree-days. According to Peterson *et al.* (1996) increase in yolk-sac fry length during yolk utilization phase is a biologically important aspect and the most of this increase occurred at post-anal part of the body that reflects the development of locomotory capability.

Using the *YCE*, *YCR*, and $K_{D,}$ as development indicators, the faster development rate was found in 24h D treatment. After 24h D treatment, the second best development rate was observed in 24 h L treatment. Excluding 24h D treatment, it was found that development rate increase with increasing photoperiod regimes (Table II). The value of *b* in regression analysis also increases with increasing in photoperiod regime, showed the highest *b* in 24 h L treatment. Puvanendran and Brown (2002) also reported that at 24h L (continouse light) photoperiod, the Atlantic cod (*Gadus morhua*) yolk-sac fry grew better compared to 18h L — 06h D and 12h L — 12h D light treatments. Fuchs (1978) also observed better growth rate of yolk-sac fry sole (*Solea solea*) under a continuous photoperiod. Thus, generally the teleost fish larvae showed best growth rate under continuous light. However, Barahona-Fernandes (1979) and Villamizar *et al.* (2009) obtained a maximum growth rate of yolk-sac fry of sea bass (*Dicentrarchus labrax*) under an 18h L — 6h D photoperiod and found that the continuous light (24h L) photoperiod did not give the best growth rate in larval sea bass.

The *YCE* and K_D values in control treatments are closely similar to those reported by Başçınar, (2010) for rainbow trout yolk-sac fry (control group in Başçınar, 2010 study). The water content of yolk-sac fry was significantly similar in all treatments and are closely similar to those previously reported by Başçınar (2010).

Table I.- Maximum yolk-sac fry wet weight of rainbow trout from 349 to 618 degree-days (DD).

Day (DD)	24h D	24h L	8h L — 16h D	16h L — 8h D	Control	*P value*
0 (349)	70.46 ± 2.88	70.46 ± 2.88	70.46 ± 2.88	70.46 ± 2.88	70.46 ± 2.88 [a]	n.s.
5 (395)	100.44 ± 2.85[c]	95.81 ± 2.14 [c]	76.77 ± 2.84 [a]	84.87 ± 3.91 [b]	79.65 ± 2.17 [ab]	<0.05
10 (440)	125.78 ± 3.35 [b]	117.79 ± 1.15 [b]	107.72 ± 3.05 [a]	106.51 ± 4.12 [a]	107.21 ± 4.21 [a]	<0.05
15 (484)	139.71 ± 4.62 [c]	130.77 ± 2.27 [c]	111.37 ± 4.54 [a]	120.60 ± 2.87 [b]	110.28 ± 5.00 [a]	<0.05
20 (529)	151.43 ± 4.96 [d]	142.01 ± 2.70 [c]	127.50 ± 4.93 [b]	122.67 ± 3.50 [ab]	118.72 ± 3.96 [a]	<0.01
25 (574)	158.70 ± 2.38 [c]	154.27 ± 1.38[c*]	130.33 ± 2.98 [b*]	122.32 ± 3.86 [a]	121.59 ± 5.04 [a*]	<0.01
30 (618)	160.78 ± 3.16 [c*]	147.06 ± 1.75 [b]	117.43 ± 4.83 [a]	117.64 ± 3.35 [a]	115.83 ± 5.96 [a]	<0.01

Table II.- Summary of the parameters *YCE, YCR, LGR, WGR*, K_D, and water content of rainbow trout yolk-sac fry held under different photoperiod regimes.

Parameter	24 h D (30th day)	24 h L (25th day)	8 h L — 16H D (25th day)	16h L — 8h D (20th day)	Control (25th day)	*p value*
YCE	0.94 ± 0.12[c]	0.87 ± 0.07[c]	0.60 ± 0.10[a]	0.73 ± 0.15[b]	0.67 ± 0.13[ab]	< 0.05
YC (mg/day)	0.82 ± 0.08[a]	1.02 ± 0.09[b]	1.03 ± 0.10[b]	1.14 ± 0.13[c]	1.01 ± 0.11 [b]	< 0.05
LGR (mm/day)	0.33 ± 0.03[ab]	0.40 ± 0.05[c]	0.35 ± 0.05[a]	0.38 ± 0.06[b]	0.32 ± 0.05[a]	< 0.05
WGR (mg/day)	3.01 ± 0.49[c]	3.06 ± 0.40[c]	1.88 ± 0.58[a]	2.61 ± 0.70[b]	1.82 ± 0.63[a]	< 0.01
K_D	1.98 ± 0.06[b]	1.93 ± 0.04[b]	1.89 ± 0.06[a]	1.96 ± 0.03[b]	1.92 ± 0.05[ab]	< 0.05
Water content*	82.95 ± 0.99	81.90 ± 0.69	83.23 ± 1.41	81.57 ± 0.99	83.21 ± 0.97	>0.05

*water content was 63.3% at hatch

Table III.- Relationship* between degree-days and dry yolk-sac fry weight, and degree-days and dry yolk sac weight.

Treatment	*a*	*b*	R^2	*p value*
Relationship between degree-days and dry yolk-sac fry weight				
24 h D	-32.01	0.099	0.89	<0.0001
24 h L	-25.87	0.088	0.91	<0.0001
8 h L-16h D	-19.11	0.068	0.79	<0.0001
16h L- 8h D	-16.82	0.063	0.83	<0.0001
Control	-19.31	0.067	0.85	<0.0001
Relationship degree-days and dry yolk sac weight.				
24 h D	61.97	-0.099	0.83	<0.0001
24 h L	58.08	-0.095	0.90	<0.0001
8 h L-16h D	53.95	-0.091	0.87	<0.0001
16h L- 8h D	50.39	-0.086	0.89	<0.0001
Control	53.88	-0.089	0.88	<0.0001

*Based on linear regression; *a*, intercept; *b*, slope

CONCLUSIONS

In conclusion, the yolk-sac fry showed the best growth rate in continuous dark (24h D) and continuous light (24 h L) while changing in photoperiod regime (*i.e.*, 8h L — 16H D, 16h L — 8h D) doesn't support best growth rate of rainbow trout yolk-sac fry. Sudden light changes might cause stress in the rainbow yolk-sac fry that results in poor growth rate. Though in continues dark or light rainbow trout fry showed the best growth rate, but compare to continues light, the maximum yolk-sac fry growth was found in continues dark; that leaves a question with why? This question may be answered by Migaud that "the small fish are more sensitive to light than their elder siblings simply through the greater transmission of light through the pineal window (6% at 600 nm in post-smolts vs. 3% in adult salmon" (as cited in Taylor *et al.*, 2006). But it should be clarified with further experiments.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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