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Effects of Photoperiod Manipulation on Growth Performance and Hematological Responses of Juvenile Caspian Roach *Rutilus rutilus caspicus*

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Abstract

A 8-week trial was conducted to evaluate the effects of photoperiod manipulation on the growth performance and hematological parameters of juvenile Caspian roach, *Rutilus rutilus caspicus* (average weight 1.46 ± 0.12 g mean±SD) reared under five photoperiods (24 h Light, 24L; 18 h Light & 6 h Dark, 18L:6D; 12 h Light & 12 h Dark, 12L:12D; 6 h Light & 18 h Dark, 6L:18D; 24 h Dark, 24D) with constant light intensity 1,500 lx on the water surface. Triplicate of 20 fish were allocated into each of 15 fiberglass tanks of 50 L capacity and they were fed three times per day with the commercial feed (SFK, Co., Sari - Iran) contains 50.0% protein and 10.5% lipid. At the end of experimental period, final body weight, weight gain and specific growth rates of fish exposed to 24L were significantly higher than those of fish exposed to 12L:12D, 6L:18D and 24D ($P < 0.05$). Red blood cell and hemoglobin of fish exposed to 24L were significantly higher than those of fish exposed to 24D. No significant difference observed in hematocrit, white blood cell and plasma glucose among the different treatments groups. Therefore, these results demonstrated that the growth performance of juvenile Caspian roach can be significantly stimulated by using 24L and 18L:6D photoperiods without any measurable significant stress response such as plasma glucose concentration.

Key words: Caspian roach, *Rutilus rutilus caspicus*, Photoperiod, Growth performance, Hematological responses

Introduction

The Caspian roach, *Rutilus rutilus caspicus*, belongs to Cyprinidae is one of the most economically important and valuable telostei in the Caspian Sea. This kind of fish exist in the southern part of the Caspian Sea especially Iran's shores (Rameshguru et al., 2011). Knowledge of the optimal environmental conditions for fish growth during their early life stage is necessary to enhance the yields and reduce costs in culture production (Lambert and Dutil, 2001; Shan et al., 2008). The manipulation of environmental factors such as temperature, salinity and photoperiod currently is used to modulate fish growth in culture (Jobling, 1994). Among them, photoperiod has been used successfully to improve the larval (Hart et al., 1996), juvenile and adult growth of some species (Simensen et al., 2000; Biswas and Takeuchi, 2003; Petit et al., 2003; Biswas et al., 2005, 2006). When photoperiod is increased, fish may adjust to the new photoperiod by displaying higher

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feeding activity, growth and feed utilization (Boehlert, 1981; Woiwode and Adelman, 1991). Photoperiod manipulation can regulate physiological functions of fish such as growth, survival, gonadal maturation, reproduction (Björnsson et al., 2000; Ginés et al., 2003; Bonnet et al., 2007) and metabolism (Biswas and Takeuchi, 2003; Biswas et al., 2005, 2006; Taylor et al., 2006). In general, long photoperiod improves the performance of fish, probably because of increased feed availability (Boeuf and Le Bail, 1999). For example, photoperiods longer than that of ambient conditions increased growth of larval rabbit fish, *Siganus guttatus* (Duray and Kohno, 1988), sea bass, *Dicentrarchus labrax* (Barahona-Fernandes, 1979; Ronzani Cerqueira and Chatain, 1991), barramundi, *Lates calcarifer* (Barlow et al., 1995), and greenback flounder, *Rhombosolea tapirina* (Hart et al., 1996).

In order to improve production efficiency in hatcheries, it is important to optimize the conditions in which the fish are reared. These conditions can include the physical culture environment (temperature, salinity, light intensity and photoperiod) and general nutritional parameters such as diet composition, ration and feeding frequency (Mohseni et al*.,* 2006). The strategy of their rearing condition will help us to gain best results in their culture and can affect their growth and survival. However, there is no information on the effect of photoperiod manipulation on the growth performance of juvenile Caspian roach. Therefore, the aim of the present study was to investigate the effect of photoperiod manipulation on growth performance and hematological parameters of juvenile Caspian roach, *Rutilus rutilus caspicus*, reared in the culture tanks.

Materials and Methods

Experimental design

Juvenile Caspian roach, *Rutilus rutilus caspicus* were obtained from the Fish Nursery Center of Sijaval, Iran. At the beginning of the feeding trial, a total of 300 fish, averaging initial weight 1.46 ± 0.12 g, were carefully selected from the stock tanks and directly distributed into 15 tanks with 50 L volume of brackish water (20 fish/tank) to acclimate to the new rearing environment for 2 weeks at 12 h light: 12 h dark (12L:12D) photoperiod. After acclimation, five artificial photoperiods with three replicate were established: i) 24 h Light, 24L; ii) 18 h Light & 6 h Dark, 18L:6D; iii) 12 h Light & 12 h Dark, 12L:12D; iv) 6 h Light & 18 h Dark, 6L:18D; and v) 24 h Dark, 24D. Each set of three tanks for each photoperiod were illuminated with one 40 (W) fluorescent lamp with light intensity at 1,500 lx on the water surface. The periods of light and dark was contorted with black polyvinyl sheet on the culture tanks to inhibit light penetration from outside. During 8 weeks of feeding trial, Average of water temperature, oxygen and pH were 26.5 ± 2°C, 6.5 ± 1.1 ppm and 7.5-8, respectively. All tanks were continuously aerated using air-stones connected to an air pump, and 20% of the water was renewed daily with receiving filtered brackish water from the center tank. Fish were fed at 5% of the total stocked biomass daily with a commercial diet (containing: crude protein 50.0%, crude lipid 10.5%; SFK, Co., Sari - Iran). The total fish weight in each tank was determined every 2-week and daily feed rations were split into three equal amounts given at 09:00, 13:00 and 17:00 hours to all culture tanks.

Sampling and parameters estimation

At the termination of the feeding trial, fish were starved for 24 h and the total number and weight of fish in each tank was determined for calculation of weight gain (WG), specific growth rate (SGR), feed efficiency (FE), feed intake (FI) and survival. After obtaining the final total weight, five fish were randomly selected from each tank and blood samples were withdrawn from the caudal vein using a 26-gauge hypodermic needle on a 1-ml syringe and transferred to tubes that were kept on ice until centrifugation at 1,600 (g) for 10 min. Hematocrit (PCV) was determined using the microhematocrit method (Brown, 1980) and hemoglobin (Hb) was measured by the cyanmethemoglobin procedure using Drabkin's solution. Hb standard prepared from human blood (Sigma Chemical Co., St Louis, MO, USA) was used. Plasma glucose was measured by the enzymatic glucose-HK procedure (Sigma). We also calculated number of white blood cells (WBCs), and number of red blood cells (RBCs) according to Ranzani-Paiva et al. (2004). The rest of the fish were freeze-dried as whole body and held at −80°C until used for proximate composition analysis. The samples of diet and whole-fish body from each treatment were analyzed according to the standard methods of AOAC (2000) for moisture, protein, lipid, and ash. Moisture content of samples was estimated by drying oven at 135ºC for 2 h to constant weight. Crude protein was determined using the Kjeldahl method (N × 6.25) after acid digestion. Crude lipid was determined by soxhlet extraction using the Soxtec system 1046 (Tacator AB, Hoganas, Sweden), and ash was determined by combusting dry samples in a muffle furnace at 550 °C for 6 h.

Statistical analysis

Data were subjected to one-way analysis of variance test using Statistix 3.1 (Analytical Software, St Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference test was used to compare means. Treatment effects were considered significant at $P < 0.05$.

Results

Table 1 shows the growth performance parameters of juvenile Caspian roach exposed to different photoperiod regimes.

At the end of 8 weeks of experiment, FW, WG and SGR of fish exposed to 24L photoperiod were significantly higher than those of fish exposed under 12L:12D, 6L:18D and 24D photoperiods ($P < 0.05$). However, there were no significant differences in FW, WG and SGR among fish reared under 24L and 18L:6D photoperiods, or among those under 24D, 18L:6D, 12L:12D and 6L:18D photoperiods ($P > 0.05$). There were no significant differences in FE, FI and survival of fish among all the experimental treatments ($P > 0.05$).

Whole-body proximate composition of juvenile Caspian roach exposed to different photoperiod regimes are shown in Table 2. There were no significant differences in whole-body protein, lipid, moisture and ash contents of fish among all treatments ($P > 0.05$).

The hematological parameters of juvenile Caspian roach exposed to different photoperiod regimes are shown in Table 3. RBC of fish reared under 24L and 18L:6D photoperiods was significantly higher than those of fish exposed under

Table 1. Effect of different photoperiods on growth performance of juvenile Caspian roach *Rutilus rutilus caspicus* for 8 weeks[1]

L:D cycle[2]	IW[3]	FW[4]	WG[5]	SGR[6]	FE[7]	FI[8]	Survival (%)
24L	1.45	4.97[a]	240[a]	2.24[a]	2.46	7.10	96.0
18L:6D	1.48	4.73[ab]	224[ab]	2.12[ab]	2.48	6.57	96.3
12L:12D	1.46	4.59[b]	214[b]	2.03[b]	2.43	6.47	93.0
6L:18D	1.45	4.48[b]	206[b]	1.97[b]	2.40	6.28	95.0
24D	1.44	4.51[b]	208[b]	1.98[b]	2.48	6.13	94.0
Pooled SEM[9]	0.11	0.06	4.02	0.03	0.04	0.13	0.88

[1]Values in same column with different superscript are significantly different at $P < 0.05$.
[2]24 h Light, 24 L; 18 h Light & 6 h Dark, 18L:6D; 12 h Light & 12 h Dark, 12L:12D; 6 h Light & 18 h Dark, 6L:18D; 24 h Dark, 24D.
[3]Average initial weight.
[4]Average final weight.
[5]Weight gain (%)= (final weight – initial weight) × 100 / initial weight.
[6]Specific growth rate (%) = 100 × (ln final weight – ln initial weight)/rearing period (days).
[7]Feed efficiency (%)= 100 × (weight gain /fed intake).
[8]Feed intake (g/fish)= dry feed consumed (g) / fish number.
[9]Pooled standard error of mean: SD/√n.

Table 2. Effect of different photoperiods on whole body proximate composition (% of DM basis) of juvenile Caspian roach *Rutilus rutilus caspicus* for 8 weeks[1]

L:D cycle[2]	Crude protein (%)	Crude lipid (%)	Crude ash (%)	Moisture (%)
24L	15.83	9.84	5.28	66.68
18L:6D	15.95	9.91	5.37	66.43
12L:12D	15.77	9.95	5.40	66.39
6L:18D	15.93	9.86	5.34	66.63
24D	15.96	9.81	5.22	66.54
Pooled SEM[3]	0.04	0.03	0.03	0.05

[1]Values in same column with different superscript are significantly different at $P < 0.05$.
[2]24 h Light, 24 L; 18 h Light & 6 h Dark, 18L:6D; 12 h Light & 12 h Dark, 12L:12D; 6 h Light & 18 h Dark, 6L:18D; 24 h Dark, 24D.
[3]Pooled standard error of mean: SD/√n.

Table 3. Effect of different photoperiods on hematological parameters of juvenile Caspian roach *Rutilus rutilus caspicus* for 8 weeks[1]

L:D cycle[2]	RBC[3]	WBC[4]	PCV[5]	Hb[6]	Glu[7]
24L	1.84[a]	29467	43	7.9[a]	82.00
18L:6D	1.71[a]	31067	42.66	6.5[ab]	85.00
12L:12D	1.59[ab]	24333	41.83	6.83[ab]	78.33
6L:18D	1.79[ab]	28800	40.5	6.86[ab]	80.00
24D	1.48[b]	25067	42.63	5.63[b]	76.00
Pooled SEM[8]	0.05	1411.58	1.12	0.33	1.64

[1]Values in same column with different superscript are significantly different at $P < 0.05$.
[2]24 h Light, 24 L; 18 h Light & 6 h Dark, 18L:6D; 12 h Light & 12 h Dark, 12L:12D; 6 h Light & 18 h Dark, 6L:18D; 24 h Dark, 24D.
[3]RBC ($\times 10^6$): Red blood cell.
[4]WBC (/mm^3): White blood cell.
[5]PCV (%): Hematocrit.
[6]Hb (g/dl): Hemoglobin.
[7]Glu (mg/dl): Glucose.
[8]Pooled standard error of mean: SD/√n.

24D photoperiod ($P < 0.05$). However, there was no significant differences in RBC among fish exposed to 24L, 18L:6D, 12L:12D and 6L:18D photoperiods, or among those reared under 24D, 12L:12D and 6L:18D photoperiods ($P > 0.05$). Hb of fish exposed to 24L was significantly higher than those of fish exposed under 24D photoperiod ($P < 0.05$). However, there was no significant differences in Hb among fish under 24L, 18L:6D, 12L:12D and 6L:18D photoperiods, or among those under 24D, 18L:6D, 12L:12D and 6L:18D photoperiods ($P > 0.05$). The PCV, WBC and plasma glucose of fish showed no significant differences among the different treatments ($P > 0.05$).

Discussion

During the 8 weeks of rearing, survival ranged between 93% and 96% and averaged 94.9%. The results of the present study indicated that juvenile Caspian roach were exposed to 24L and 18L:6D photoperiods had significantly better growth, WG and SGR than those exposed to 12L:12D, 6L:18D and 24D photoperiods. Similar results have been reported that growth performance could be enhanced by continuous light or extending the light photoperiod in Atlantic salmon juvenile, *Salmo salar* (Saunders and Harmon, 1990), red seabream juveniles, *Pagrus major* (Biswas et al., 2005, 2006). Also, the growth of sole, *Solea solea* (Fuchs, 1978), European seabass, *Dicentrarchus labrax* (Barahona-Fernandes, 1979), barramundi, *Lates calcarifer* (Barlow et al., 1995), olive flounder, *Paralichthys olivaceus* (Dou et al., 2003), black porgy, *Mylio macrocephalus* (Kiyono and Hirano, 1981), gilthead seabream, *Sparus aurata* (Tandler and Helps, 1985), rabbit fish, *Siganus guttatus* (Duray and Kohno, 1988) and snapper, *Pagrus auratus* (Fielder et al., 2002) larvae were better at 18 and 24 h light periods than at 12:12 h or shorter light periods.

Photoperiod is commonly believed to be one of the directive factors that could control fish growth during their early life stage through its influence on endogenous feeding rhythms and efficiency or food availability (Björnsson, 1997; Boeuf and Le Bail, 1999; Taylor et al., 2006). The finding that higher growth accompanied with both higher feed intake and feed efficiency under long and continuous photoperiods parallels the findings in other species, such as haddock, *Melanogrammus aeglefinus* (Trippel and Neil, 2003) and gilthead seabream, *Sparus aurata* (Kissil et al., 2001; Ginés et al., 2004).

It is well known that hematological parameters regarded as reliable indicators for assessment of health status in fish, but they can vary with season, temperature and nutritional status (Bond, 1979). In this study, the results demonstrated that photoperiod manipulation cause significant differences in the levels of hematological parameters such as RBC and Hb; but, did not cause significant in the PCV and WBC. The PCV has been shown to increase under stressful conditions (Pierson et al., 2004) and this could be attributed to the swelling of red blood cells (Biswas et al., 2006). The PCV has also been shown to decrease under chronic stress (Barcellos et al., 2004).

In addition, there was no significant difference in plasma glucose among the treatments in this study. Plasma glucose concentrations have long been used as indicators of stress in fish (Hattingh, 1976; Donaldson, 1981; Wedemeyer and McLeay, 1981). Yet, in many studies (Adams et al., 1985; Brown et al., 1986; Goss and Wood, 1988; Pottinger et al., 2002), under stress plasma glucose either remained unchanged or took a longer duration of stress to show the change. The results demonstrated that photoperiod manipulation did not cause a significant acute stress response in juvenile caspian roach as the levels of different stress indicators in fish exposed to different photoperiods. It has been demonstrated that chronic stress generally results in a higher elevation of cortisol and glucose concentrations in fish (Leonardi and Klempau, 2003). In this study, plasma cortisol was not determined; however, plasma glucose concentration in the blood indicates that photoperiod manipulation did not appear to cause significant chronic stress response in the fish. Similarly, the findings for juvenile red sea bream, *Pagrus major*, also demonstrated that photoperiod manipulation did not cause significant plasma glucose concentration in fish exposed to different photoperiods (Biswas et al., 2006).

In conclusion, the results suggested that the growth performance of juvenile caspian roach can be stimulated remarkably by the manipulated photoperiods used in 24L and 18L:6D photoperiods. The higher growth performance under manipulated photoperiods may be attributed to the feeding time as well as to improved appetite, greater feed intake and higher feed conversion efficiency. In addition, the photoperiod manipulation used in this study did not cause a significant stress response, and can therefore be used to stimulate the growth performance of juvenile Caspian roach, *Rutilus rutilus caspicus*.

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