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RESEARCH ARTICLE

Selected coelomic fluid parameters of sterlet, *Acipenser ruthenus* L.: effects of light color and photoperiod

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Abstract. Light and color have effect physiological aspects of fish such as growth, the neuro-hormonal system, and reproduction. In the present study, the effects of light color and photoperiod on the levels of selected parameters of coelomic fluid (glucose, total protein, cholesterol, calcium, magnesium) of female sterlet (*Acipenser ruthenus*) were investigated. Thirty-six broodstock were kept in 12 experimental tanks (500 l) for six months. The fish were reared under four different light regimes (two photoperiods of 18L:6D and 6L:18D and two colors – red and blue) at an intensity of 150 Lx (three replicates). Fish reared under the red-long photoperiod had the highest concentration of the selected parameters of the coelomic fluid. Moreover, significant differences were noted in the all the parameters in fish reared under the red-long photoperiod in comparison with fish from the other treatments ($P < 0.05$). The results indicate that light (color and photoperiod) influences egg quality during the final stage of *A. ruthenus* reproduction.

Keywords: light color, photoperiod, coelomic fluid, sterlet

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Introduction

Sturgeon stocks have declined because of overfishing for meat and caviar production, habitat destruction, and environmental pollution (Billard and Lecointre 2001). Therefore, most species of acipenserids are listed as endangered by IUCN (2012). Sterlet, *Acipenser ruthenus* L., is the smallest sturgeon and is considered to be the most suitable species for biological, nutritional, and genetic studies because of higher early growth rates and earlier maturation compared with other sturgeon species (Sokolov and Vasiliev 1989). Light rays of different wavelengths pass through water to different depths depending on light absorption, diffusion, and the water content of admixtures and small organisms. Most species of fish have well-developed color sight, and are therefore very sensitive to colored light (Ruchin 2004). Photoperiod is classified as a directive and abiotic factor that can affect the growth performance of aquatic organisms by influencing the endocrine system. Photoperiod manipulation has been used successfully to improve the growth and reproduction of a number of teleost fishes. Additionally, photoperiod affects the hypothalamus-pituitary-internal axis (HPI) and is one of the main environmental cues for reproduction timing. Since illumination can affect the reproductive performance of fish, it is probable that light regime changes during the final

reproduction stages and near the time of spawning could affect broodfish breeding and spawning (Craig 2000, Borg 1994, Fontaine et al. 2006, Falahatkar et al. 2012).

The effects of colored light and photoperiod on various aspects of fish physiology have been investigated, including growth, survival, the neuro-hormonal system, reproduction, behavior, and the stress response (Volpato and Barreto 2001, Bayarri et al. 2002, Naor et al. 2003, Ruchin 2004, Karakatsouli et al. 2007, Bani et al. 2009, Banan et al. 2011, Pourhosein Sarameh et al. 2012). However, data regarding the effect of colored light and photoperiod on *A. ruthenus* broodstocks in the final stage of reproduction are scarce. Therefore, the aim of this study was to examine *A. ruthenus* coelomic fluid parameters to detect changes in glucose, total protein cholesterol, and ionic content (Ca^{2+} and Mg^{2+}) in broodstocks that were subjected to photomanipulation during the final stage of reproduction.

Materials and methods

Fish and experimental condition

This study was performed during a six-month period from November 2011 to April 2012 in at the International Sturgeon Research Institute in Rasht, Guilan, Iran. The fish (n = 36) were held in 12 square (100×100×42 cm) fiberglass tanks (rearing volume – 500 l) during an acclimatization period of five days. Data on initial fish length and weight are presented in Table 1. The fish were fed manually twice daily with a commercial diet (ALLER AQUA, Denmark) during the adaptation period and throughout the experiment. The dry matter content of the diet was 87.8% and comprised 40.1% crude protein, 19.6% crude fat and 5.62% crude ash. All tanks received equal amounts of food at each feeding time. The experimental tanks were cleaned (siphoned) every morning before the first feeding. Water temperature, oxygen content, and pH were 16-18°C, >5.3 mg l^{-1}, and 7.1-7.5, respectively, during the experiments.

Table 1
Sterlet (*A. ruthenus*) female sizes and weights in the test tanks under different colored light and photoperiod treatments

Treatment	n	Body weight (g)	Total length (cm)
6L:18D: red	9	557.6 ± 144	50.55 ± 2.18
18L:6D: red	9	730.4 ± 29.61	54 ± 1.8
6L:18D: blue	9	678.2 ± 58.45	52.44 ± 3.24
18L:6D: blue	9	701.4 ± 8.77	51.61 ± 1.11

Experimental design

The 12 tanks were assigned randomly to one of the four experimental treatments (three replicates per treatment) to assess the effects of the various photoperiods studied. All the tanks were separated by black plastic sheets and were illuminated with energy-saving tube lamps (Novin Light Co., Karaj, Iran). Four different photoperiods were established: two light colors (red and blue) and two light regimes (18L:6D and 6L:18D). The light was controlled by a light meter (LI-COR, model Li-1776, Lincoln, NE, USA). The light intensity at the surface of each tank in all treatments was adjusted to 150 lx.

Sampling protocol and measurements

To estimate the stage of germinal vesicle (GV) migration before spawning, 20-30 oocytes were collected, boiled for 5 min, and bisected from the vegetable pole (VP) to the animal pole (AP) to measure the distances from the VP to the AP and from the AP to the nucleus under a dissecting microscope.

Fish were sampled after being anesthetized in a buffered solution of tricaine methane sulphonate (MS 222), which induced the cessation of body and opercular movements within 4-8 minutes after exposure. Coelomic fluid and ovulated oocytes were obtained from females and deposited onto meshed textile. The coelomic fluid was then withdrawn with a syringe and transferred into micro tubes. Samples were frozen immediately and stored at –20°C until

further processing. The concentrations of magnesium and calcium were measured with the colorimetric procedure in an Autoanalyser Technican (RA 1000, Technican, Swords, Dublin, Ireland). The coelomic fluid biochemical constituents were analyzed using an auto analyzer (Eurolyser, Detect 1970) and commercial clinical investigation kits (Pars Azmoon kit, Tehran, Iran). Biochemical measurements were performed for total protein, glucose, and cholesterol. Ionic and organic compositions were measured three times per sample.

Statistical analyses

Statistical analysis was conducted using SPSS (Version11.5, SPSS Chicago, IL, USA), and differences of $P < 0.05$ were considered to be statistically significant. The data were analyzed with two-way analysis of variance (ANOVA). Duncan's test was used to identify significant differences among the various means. The values are presented as means ± SD.

Results and discussion

The results of the different color and light regimes on the mean values of coelomic fluid parameters are presented in Fig. 1a-e. The fish reared under a red-long photoperiod had the highest concentration of glucose, total protein, cholesterol, calcium, and magnesium. In addition, there were significant differences in the all the selected parameters of fish reared under a red-long photoperiod in comparison to the other treatments ($P < 0.05$). In sturgeon, as well as in other teleosts, an increase in glucose level is considered a secondary indicator of the stress response (Barton et al. 2000, Belanger et al. 2001). The other parameters measured in our study were also likely to change in response to stress or environmental variations. Changes in day length and light regime were involved in secondary stress responses in sterlet. Furthermore, ion concentrations were affected by changing light regimes. Our results are in good agreement with previous observations of Askarian et al. (2008) in beluga, *Huso huso* (L.). The results of another study by Volpato and Barreto (2001) reinforce the idea that colored light affects the HPI axis and, consequently, it may influence various biological systems. It should be emphasized that assessing fish welfare would also require measuring cortisol and glucose, which are the main end products of the HPI axis and a secondary indicator of stress response, respectively (Barcellos et al. 2009). It seems that female sterlet brooders are sensitive to light and finally react to constant light with an increased glucose level as a stress response. Because stress responses might change under different photoperiods, light regimes may cause higher or lower sensitivity (Biswas et al. 2006). Additionally, the exposure time of the stressor can influence the magnitude of physiological responses and the sensitivity of the physiological response depends on stressor type (Acerete et al. 2004). Thus, our results suggest that female sterlet are sensitive to long day light in the final stages of reproduction. The effect of photoperiod on egg quality is difficult to study, because manipulation of photoperiod often results in the modification of other parameters throughout oogenesis, including those during late oogenesis (Bobe and Labbe 2010). Moreover, available data indicate that photoperiod-induced manipulation of spawning data can impact egg quality. The importance of this effect on egg quality seems highly dependent on the type of photoperiod regime used. To conclude, the sensitivity of coelomic fluid parameters of sterlet brooders to constant photoperiods in this reproductive stage is indicated here, and the effects of the intensity, duration, and time of applying stressors in different reproductive stages on physiological responses are taken into account. More research is necessary to foster a complete understanding of these effects and sensitivities. The results obtained here along with those of future studies might help to develop a suitable photoperiod regime or to change photoperiods in different stages of maturity until the spawning of sterlet. Furthermore, it is necessary to conduct studies on the effects of photoperiod on altering the timing of maturation and various

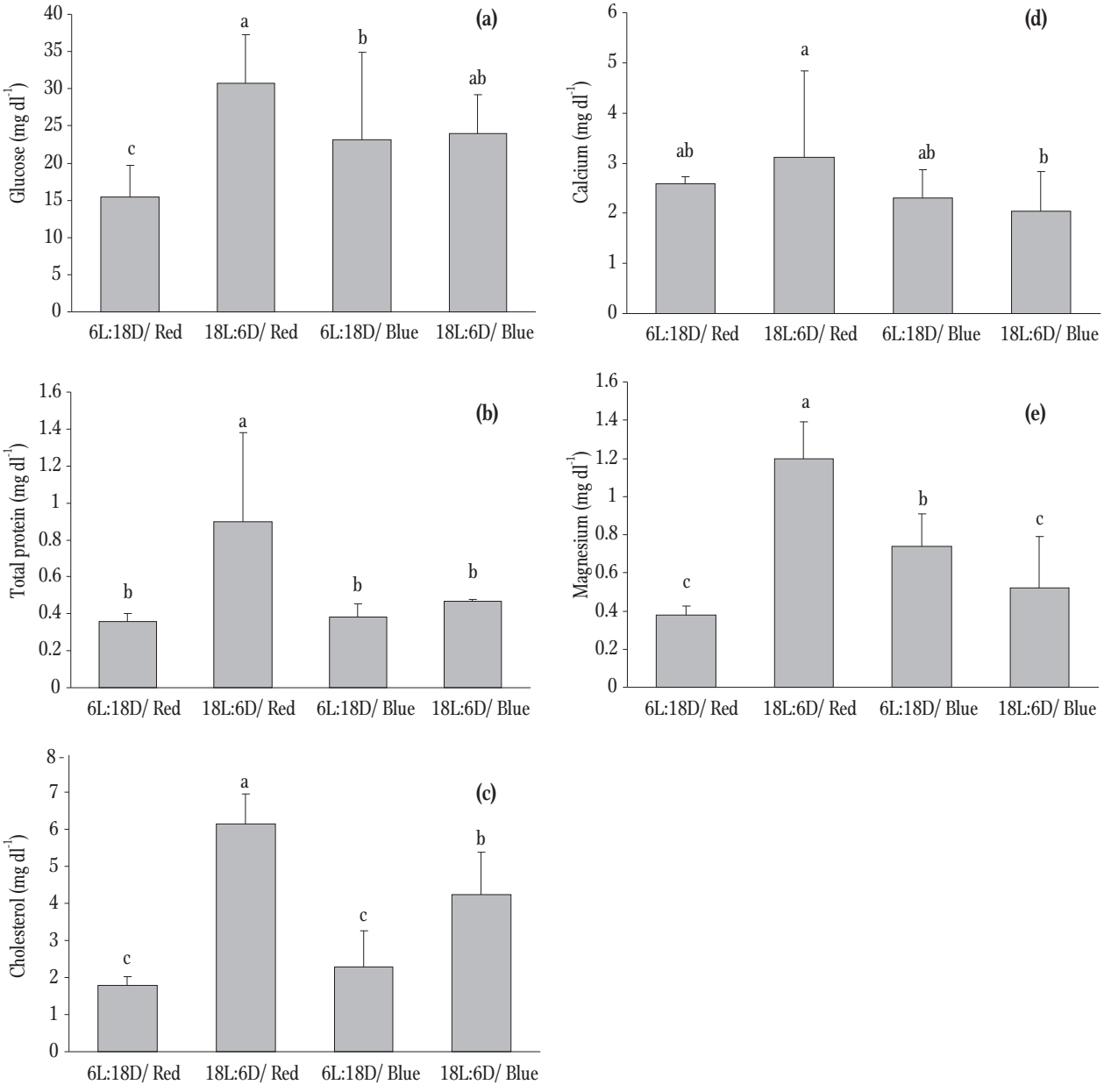

Figure 1. Mean values (± SD) of selected coelomic fluid parameters: glucose (a), total protein (b), cholesterol (c), calcium (d), and magnesium (e) of female starlet (*A. ruthenus*) broodstocks reared under different colored light and photoperiod treatments.

maturational processes, and the effects of light intensity, duration, and different photoperiod applications during different reproductive stages of sterlet on serum parameters, the immune system, the stress response, and reproductive performance.

Author contributions. H.A. and F.N. designed and performed the experiment; H.A. analyzed the data, M.S.A. wrote the manuscript.

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