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Influence of the photoperiod and light intensity on growth performance, Melatonin and Insulin-like growth factors gene expression on *Acipenser persicus* during the embryonic stage

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Abstract

The effects of different light regimes (24L: 0D, 150 lux, 12L: 12D, 300lux, 0L: 24D, 0 lux, and control treatment) on growth, melatonin concentration, and Insulin-like growth factors gene expression of Persian sturgeon (*Acipenser persicus)* embryos were evaluated. Four groups of Persian sturgeon eggs (450 g) were incubated in Youshchenkov incubators with three replications for each treatment. The experiment was conducted during an 8- day period of incubation. Growth rate, plasma melatonin levels, and IGF-growth factors were investigated at 1, 4, and 8 days post fertilization. Results showed that higher mean weights growth were observed in fertilized eggs exposed to a 24L: 0D photoperiod with 150lux light intensity (p<0.05). The larval body length and hatching rate of fertilized eggs were significantly higher in embryos incubated at 12L: 12D with 300lux. The melatonin hormone level between sampling days and treatments were significantly different and it would be concluded that it may be related to the species. During the first-day post fertilization, the IGF-I and IGF-IR gene expression were significantly higher than other days. However, IGF-I and IGF-IR gene expression at different treatments showed no significant difference at day 4 and 8 post fertilization. According to the results, the stimulating role of IGF-I and IGF-IR gene expression in growth at the one-day post fertilization under different light regimes can be concluded. These findings suggest that the 12L:12D photoperiod with 300lux light intensity is the appropriate light regime for Persian sturgeon eggs incubation. The results of the present study would provide fundamental information for further use of light regimes during the early development of sturgeon larvae to optimize rearing protocols in sturgeon hatcheries.

Keywords: *Acipenser persicus*, Growth, Photoperiod and light intensity, Melatonin, Insulin-like growth factors, Gene expression

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Introduction

Persian sturgeon (*Acipenser persicus*) is one of the Acipenseriformes fish which has been existing for over the past 200 million years (Williot *et al*., 2002). Persian sturgeon is endemic to the southern Caspian Sea and like to other sturgeon species in this Sea, its stock has declined dramatically due to anthropogenic interferences such as overfishing, loss of natural habitats for reproduction and pollutions like to other sturgeon species in this Sea (Chebanov and Billard, 2001). The Persian sturgeon has been listed as critically endangered species in CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) (IUCN, 2010). In order to save this species efficiently and also develop aquaculture industry and minimize pressure on wild populations, artificial propagation has been attempted during the last decades in Iran (Kazemi *et al*., 2012). This task is rather difficult because the most mortality happens during larval development and active feeding mainly as a result of small size, the ability of swimming, and sensitivity to environmental changes (temperature and light) (Rice *et al*., 1987; Miller *et al*., 1988).

To improve growth performance of sturgeon during early life rearing, different strategies such as manipulation of food ingredients (Abedian Kennari *et al*., 2007; Hafezieh *et al*., 2010), addition of growth stimulants (Zare *et al*., 2017) , and environmental conditions manipulation (Lara-Flores *et al*., 2003; Zolfaghari *et al*., 2011; Kazemi *et al*., 2012; Poursaeid *et al*., 2012) have been used. In teleosts, light has great influences on the life cycle from the embryonic developmental stages to sexual maturation in adults (Downing and Litvak, 2002; Migaud *et al*., 2010).

The light is one of the factors would effect on growth. Alteration in photoperiod has had great effects on growth in some species (Boeuf and Le Bail, 1999). The light regimes might have different effects on various developmental stages. It has been reported that photoperiod and light intensity manipulation, improved growth during fish rearing in several aquaculture species. For example, rainbow trout subjected to different photoperiod, exhibited positive and significant growth rate (Taylor *et al*., 2005). The light may also effect on embryonic development. According to the previous studies, it has been suggested that the light has an effective impact on growth and respect to species, are the most often diverse. Different light regimes have been reported to influence on embryos and hatching rate for a number of aquaculture species, such as Atlantic halibut (Forsell *et al*., 2001), *Epinephelus strariatus* (Ellis *et al*., 1997), Haddock *Melanogrammus aeglefinus* (Downing and Litvak, 2002), Summer flounder *Paralichthys dentatus* (Watanabe and Feeley, 2004), Asian catfish *Clarias macro* and African catfish *C. gariepinus* (Mino *et al*., 2008), *Lutignus guttatus* (Duncan *et al*., 2008), *A. persicus* (Kazemi *et al*., 2012; Poursaeid *et al*., 2012).

The secretion of melatonin is closely related to the light-dark cycle and its amount depends on the light presence in the environment. During the light-dark cycle, high levels of melatonin are observed in the dark, while the amount of concentration of this hormone in the light period is very low and even stopped

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(Kalamarz *et al*., 2009). This template exists in all vertebrates, including fish. Studies have shown that melatonin can result in the development of larvae of fish, the proliferation of fetal cells and the rapid development of fish (Kalamarz *et al*., 2009), and morphological and physiological changes of freshly hatched larvae to young fish (Kvetnoy *et al*., 2002).

In teleost, growth is affected by endocrine control of GH (growth hormone) (Duan, 1998) – IGF-I (insulin-like growth factor I) axis (Duan *et al*., 1993; Wood *et al*., 2005). External (Photoperiod, light intensity and temperature) and internal (hormonal factors and nutritional state) cues, directly or indirectly stimulate growth-related hormones production (Björnsson *et al*., 2002). Growth hormone acts directly on target tissues by stimulating mitosis and energy metabolisms, and indirectly by regulating the production and release of IGF-I in the liver and other tissues (Fox *et al*., 2010). IGF-I has been shown to promote cell differentiation and proliferation, and ultimately growth in teleost (Chen *et al*., 2000; Degger *et al*., 2000). Considering the role of photoperiod on the level of plasma GH and growth, it would be suggested that levels of the liver IGF-I mRNA are indirectly regulated by the light. For these reasons, IGF-I suggested as the most appropriate growth biomarker candidate (Picha *et al*., 2008).

In the present study, we studied the effects of light on IGF-I mRNA expression during embryos development in Persian sturgeon. We also evaluated the possibility of using IGF-I mRNA expression as a growth indicator in Persian sturgeon embryos in response to different photoperiod and light intensity.

Materials and methods

Egg collection and general experimental conditions

Persian sturgeon brood stocks were caught from the Caspian Sea on January 2017 and held at the Shahid Beheshti Sturgeon Propagation and Rearing Center (Rasht, Iran), under the ambient conditions. During February, six adult individuals (four females and two males) were induced to spawn using LHRH-A2 hormone (3 g kg^{-1} body mass) intramuscular injection (Mohammadi *et al*., 2015). After fertilization, Eggs adhesiveness was removed by a 45 min treatment with clay – water suspension. Fertilized eggs (approximately 40% fertilization rate) were incubated in Youshchenkov incubators (Williot *et al*., 1997). An experimental incubation unit consists of 450 g or 14400 eggs. During the incubation period, water temperature and dissolved oxygen were 14 °C and 7.5 mg L^{-1}, respectively. Fertilized eggs were hatched 7 days past fertilization. For the embryonic developmental stages, four treatments with 3 replicates were used for each treatment.

Light treatments

To determine the influence of photoperiod on development and hatching of Persian sturgeon eggs, Youshchenkov incubators were subjected to the one of four light treatments: (T1) 24:0 h light/dark (24L: 0D), 150lux; (T2) 12:12 h light/dark (12L: 12D), 300lux; (T3) 0:24 h light/dark (0L: 24D), 0lux; (T4) ambient condition (as control treatment). There were three

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replicates of each treatment. Yellow bulbs provided light to the surface of incubators from 80 cm distance. Incubators were placed in the dark rooms to protect them from external light sources. Light intensities were adjusted by an electric dimmer (40-600 W, 230 V, 50 HZ, Part Iranian Co., Iran). Light intensity at the incubator surfaces was measured in lux using a light meter equipped with a photometric sensor (TES-1336A, 20-20000 lx, Tes Electronic Corp., Taiwan).

Biometric samples

During the experiment in 1, 4 and 8 days post fertilization (dpf), 10 eggs have been sampled from each repeat/treatment, individually. The weight of eggs and fresh hatched larval were calculated with digital scales (GF-300 model with an accuracy of 0.001 g, Japan A & D Company). The diameter and total length of the eggs after photographed eggs were assessed using the Images software program and by the equipped loop with the DS-L2 digital eye (Nikon, Japan), with a precision of one-hundred of a millimeter. For gene expression analysis, egg samples for each treatment were rapidly moved to cryo-tubes, snap frozen on liquid nitrogen, and then transferred to -80 ^{o}C freezers until analysis.

The duration of the present experiment was 8 days and the mean temperature, dissolved oxygen, oxygen saturation and pH was 15.9 ° C, 1.8 mg L^{-1}, 82.5% and 6.5, respectively.

Melatonin analysis

The levels of melatonin hormone in embryonic samples have been measured by ELISA (Biotek Elx 800, USA) using the Melatonin hormone kit (East Biopharm, Hangzhou, China, Cat.No: CK-E90865) according to the manufacturer's protocol.

Gene expression analysis

Total RNA from the pooled eggs (5-6 eggs) was purified using BIOZOL (Bioer-Bioflux, China) according to the manufacturer's instructions. RNA quality and quantity were verified using a Nanodrop spectrophotometer (Nanodrop Technology, Wilmington, DE, USA) via examination of absorbance ratios at OD 260/280 (range 1.98-2.06) and OD 260/230 (range 1.96-2.07) and by visual inspection of the integrity of both the 18S and 28S ribosomal RNA bands on a 1.5 % agarose gel. Samples were adjusted to a final concentration of 200 ng µl and treated with DNase I, RNase-free (Fermentas, France) followed by an ammonium acetate precipitation. First strand cDNA synthesis was performed on 5 µg of DNase treated total RNA using a M-MuLV Reverse Transcriptase kit (Fermentas, France) with 2.5 µM oligo (dT) 20 (Resuehr 2003). Species-specific qPCR primers were designed for Persian sturgeon IGF-I and IGF-IR sequences available from Genebank (GenBank no. GU325629.1 and JF732901.1) with the Primer 3 software (http://frodo.wi.mit.edu/primer3/) (Table 1). RT-qPCR reactions were performed in a final reaction volume of 12 µl using 1 x SYBR Bio Easy SYBR Green I Real-Time PCR Kit (Bioer, China), 2.5 µM of ROX reference dye, 0.2 µM of each primer with 2 ng of cDNA template. All reactions were performed on a CFX96 (Bio-Rad, USA) and utilizing CFX-manager software.

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Each reaction was amplified in triplicate and consisted of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing, and extension for 30 s at primer-specific temperatures (Table 1). RPL6 was used as the housekeeping gene for data normalization due to its constant expression through different developmental stages(Akbarzadeh et al., 2011). The 2–ΔΔCt method for relative gene expression analysis described by Livak and Schmittgen (2001) was used to calculate the gene expression values.

Table 1: Name, sequence, and annealing temperature (T) of primers used in the present study to quantify gene transcripts abundance through real-time PCR.

Primer name	Primer sequence (5'-3')	Annealing T (^{o}C)	Amplicon size (bp)	Application
IGF-I-qPCR-F	CAG TTT GTG TGT GGG GAG AG	65	183	Real-time PCR
IGF-I-qPCR-R	GGC ACG TAC AGA GCG TGA G	65		
IGF-IR-qPCR-F	AGG AGG CCG CTG AGA TGG GGA AAG	65	155	Real-time PCR
IGF-IR-qPCR-R	GTG GCC GGG AGC ATC AAT GAT GGT	65		
RPL6-qPCR-F	GTGGTCAAACTCCGCAAGA	65	149	Real-time PCR
RPL6-qPCR-R	GCCAGTAAGGAGGATGAGGA	65		

Data collection

For determination eggs size, quality, and hatching rate, five aliquots of at least 10 eggs were sampled from each incubator for each treatment i 1, 4, and 8 days post fertilization (Fig. 1). The samples were fixed in 4% buffered formalin for descriptive purposes. Ten additional eggs from the above mentioned time points were also sampled and immediately deep freeze in liquid nitrogen and stored at -80 °C until gene expression analysis.



Figure 1: Images of fertilized eggs of Persian sturgeon at different days. a) one-day post fertilization, b) 4 days post fertilization, c) 8 days post fertilization (hatching).

Statistical analysis

All data passed Levene's test for homogeneity of variance before one-way ANOVA. Post hoc multiple comparisons of means were used Tukey test. Whenever appropriate, comparison of two means was also performed using T-test. All statistical analysis was undertaken using IBM SPSS 20.0 (www.ibm.com). Differences were considered statistically significant at $p<0.05$.

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Results

Eggs weight

Eggs weight was not significantly diverse between different treatments at 1 and 4 days post fertilization (dpf). However, at 8 days post fertilization, the egg weight at different photoperiod and light intensity regimes compared with 1dpf and 4dpf significantly decreased (p<0.05, ANOVA). The results showed that the eggs at T1group (treatment) had higher mean weights compared to other experimental groups (T2, T3, and C groups) (Fig. 2).

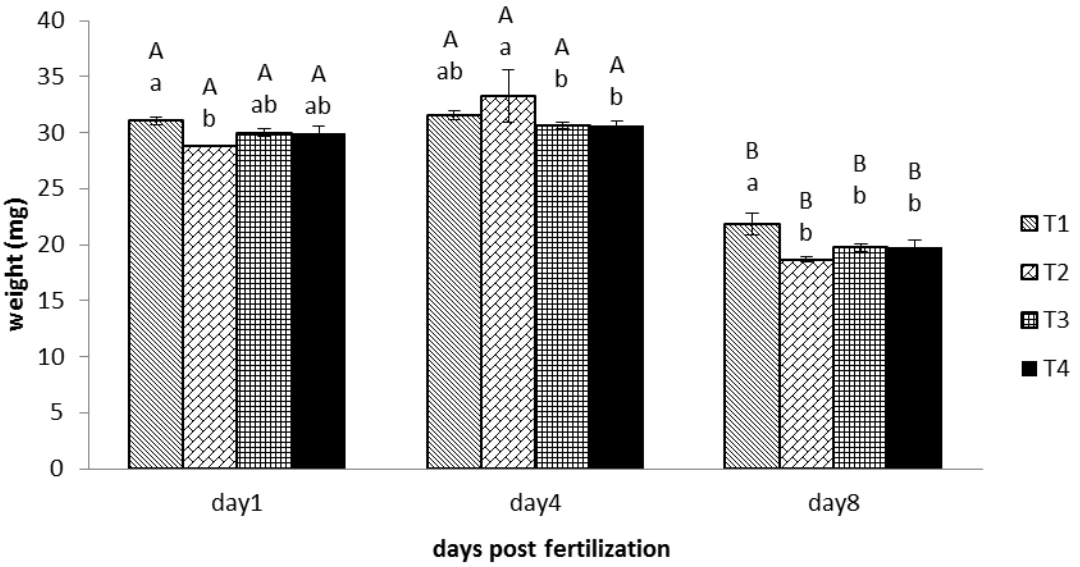


Figure 2: Mean±S.E. weight (mg) of fertilized eggs of Persian sturgeon at different experimental treatments. Different letters in each column represents significant differences (p<0.05).

[T1 (24L: 0D, 150 lux), T2 (, 12L: 12D, 300lux), T3 (0L: 24D, 0 lux) and T4 (control treatment)].Capital letters indicate a significant differences between eggs weight or larvae of different treatments in different days of the experiment, while lower case letters indicate a significant difference between eggs weight or larvae of different treatments in a given day of the experiment. Non-similar alphabets in each of the two groups showed a significant statistical difference (p<0.05).

Eggs size

The results showed that at 1 day and 4 days post-fertilization, eggs diameters at different light regimes were not significantly different. However, eggs diameter at 8 days post fertilization (hatching day) increased significantly. This could be due to the size of hatching larvae. The presented results showed that different photoperiods influenced larval body length (mm). The embryos incubated under 12L: 12D, 300lux (T2), were significantly larger in length than embryos incubated at T1, T3 and natural conditions (T4) in larval body length (Fig. 3).

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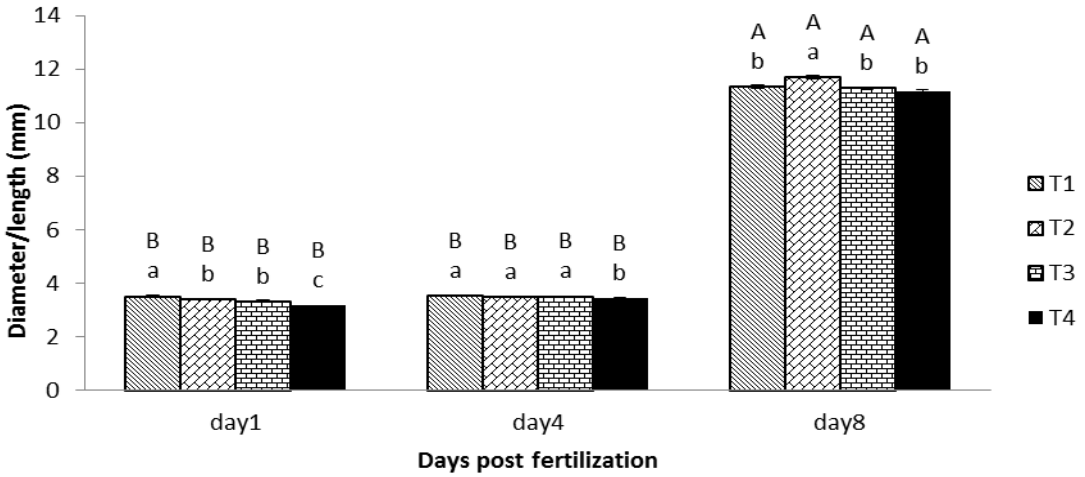


Figure 3: Mean±S.E. Diameter/length (mm) of fertilized eggs of Persian sturgeon at different experimental treatments.

Different letter in each column represents significant differences (p<0.05). [T1 (24L: 0D, 150 lux), T2 (12L: 12D, 300lux), T3 (0L: 24D, 0 lux) and T4 (control treatment)]. The upper case letters indicate a significant difference between the eggs diameter or length of larvae of different treatments in different days of the experiment, while the lower case letters indicate a significant difference between the eggs diameter and the larval length of the different treatments in a given day of the experiment. Non-similar alphabets in each of the two groups showed a significant statistical difference (p<0.05).

Hatching rate

Differences in hatching rate were identified for different photoperiod treatments. The hatching rate of fertilized eggs was ranging from 44.41% to 75.56%. The highest hatching rate was observed in treatment 2 (12L: 12D, 300lux). However, the eggs incubated under natural photoperiod (control group) and 3 treatments, complete darkness (24D, 0lux) displayed low hatching rate from 44.41 – 50.99% (Fig. 4).



Figure 4: Hatching percentage of fertilized eggs of Persian sturgeon at different experimental treatments. [T1 (24L: 0D, 150 lux), T2 (, 12L: 12D, 300lux), T3 (0L: 24D, 0 lux) and T4 (control)].

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Melatonin

Melatonin hormone level in fertilized eggs extraction at the end of the incubation stage affected by different photoperiod treatments individually and its average concentration in different treatments ranged between 93±1.55 to 142± 1.09 ng L^{-1} (p<0.05). The results of melatonin hormone level showed that the highest concentration of this hormone in fertilized eggs extract of control treatment (natural photoperiod) at one-day post fertilization (day 1) and the lowest concentration was observed in hatching eggs extracts of the larvae with 12L:12D, 300 lux (T2) at 8 days post fertilization (day 8, hatching day). There was a significant difference between days of sampling and treatments at the one-day post fertilization (day 1) were significantly higher than other days of sampling (p<0.05) (Fig. 5).

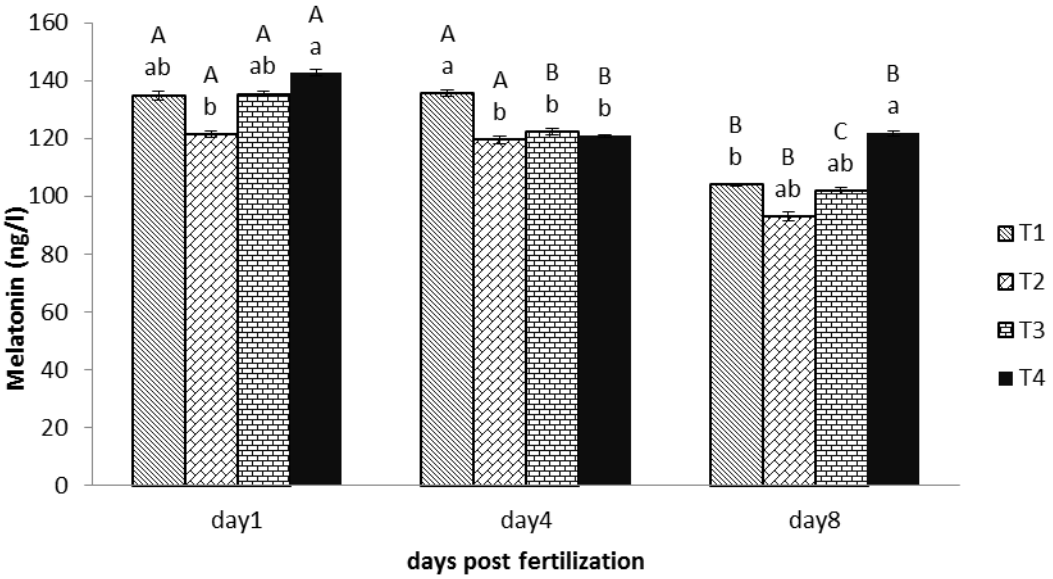


Figure 5: Melatonin hormone level in fertilized eggs extraction of Persian sturgeon at different experimental treatments. [T1 (24L: 0D, 150 lux), T2 (12L: 12D, 300lux), T3 (0L: 24D, 0 lux) and T4 (control treatment)]. The upper case letters indicate a significant difference between the concentration of egg melatonin or larvae of different treatments in different days of the experiment, while the lower case letters indicate a significant difference between the concentration of egg melatonin or the larva of different treatments in a given day of the experiment. Non-similar alphabets in each of the two groups showed a significant statistical difference (p<0.05).

IGF-I mRNA expression at different treatments

Mean relative IGF-I mRNA expression in fertilized eggs at different treatments showed a significant difference between the four photoperiod and light intensity treatments at the four and eight days post fertilization (day 4 and day 8). However, the IGF-I gene expression was significantly higher in the 12L: 12D, 300 lux treatment (T2) at day 1 (1-day post fertilization) (p<0.05) (Fig. 6).

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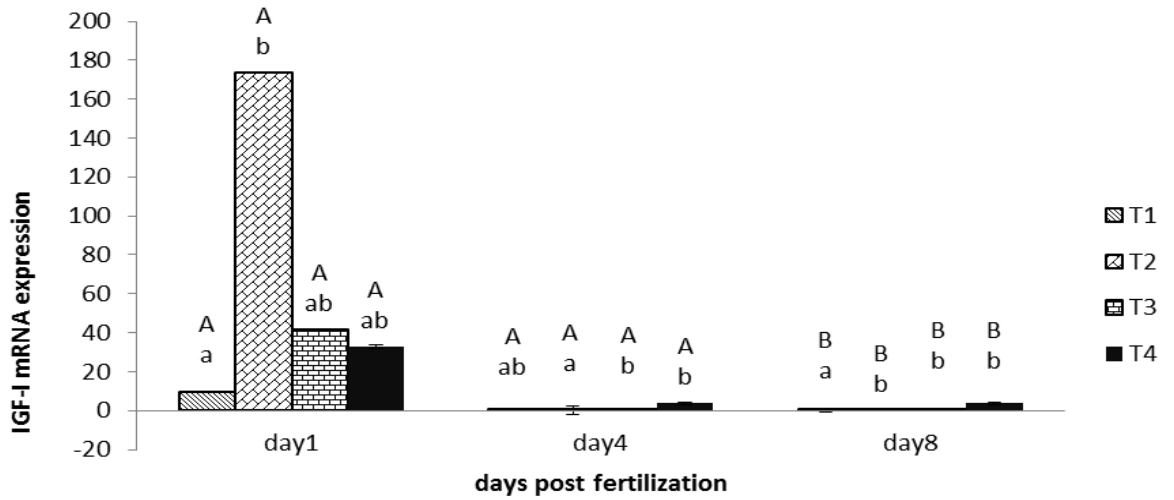


Figure 6: Mean relative IGF-I mRNA expression in fertilized eggs extraction of Persian sturgeon at different experimental treatments. [T1 (24L: 0D, 150 lux), T2 (, 12L: 12D, 300lux), T3 (0L: 24D, 0 lux) and T4 (control treatment)]. The upper case letters indicate a significant difference between the amount of expression of the IGF-I gene of the egg or the larvae of different treatments in different days of the experiment, while the lower case letters indicate a significant difference between the amount of expression of the IGF-I gene of the egg or the larva of different treatments in a given day of the experiment Is. Non-similar alphabets in each of the two groups showed a significant statistical difference ($p<0.05$).

IGF-IR mRNA expression at different treatments

No significant difference was observed between different experimental treatments, for fertilized eggs IGF-I receptor mRNA expression at 4 and 8 days post fertilization (Fig. 7). At day 1, IGF-I receptor mRNA expression in eggs incubated under natural light condition was significantly higher compared to other treatments ($p<0.05$).

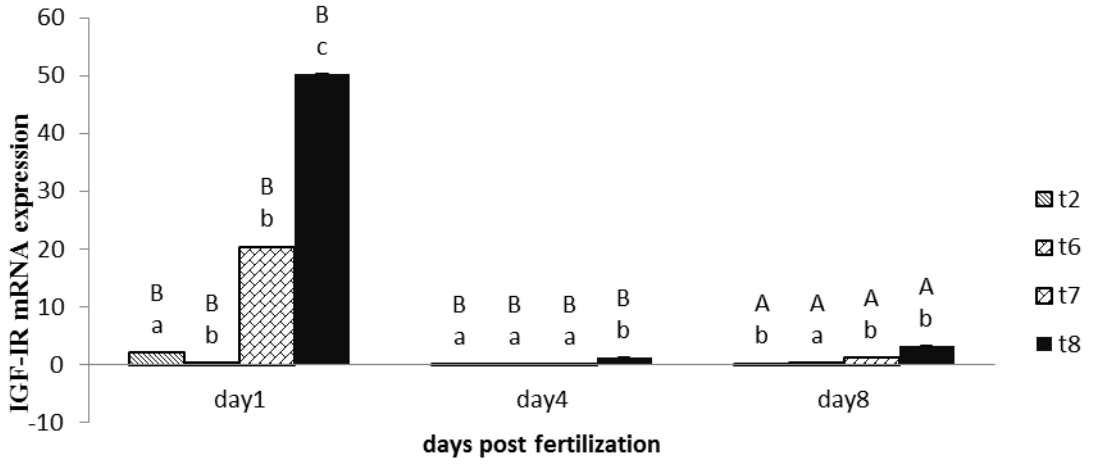


Figure 7: Mean relative IGF-IR mRNA expression in fertilized eggs extraction of Persian sturgeon at different experimental treatments. [T1 (24L: 0D, 150 lux), T2 (, 12L: 12D, 300lux), T3 (0L: 24D, 0 lux) and T4 (control)].

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Discussion

It has been suggested that the key role of the light and changes in it would have significant effects on growth in some farmed fishes (Picha *et al*., 2014). In aquaculture, fish subjected to different light regimes for different purposes including growth increase, maturation inhibition, changing in smoltification (introduction into seawater) (Boeuf and Le Bail, 1999). Previous work results suggested the GH-IGF axis is biomarkers for growth at different photoperiods (Komourdjian *et al*., 1976; Marchant and Peter, 1986). Thus, in the present study, we investigated the effects of different photoperiod and light intensity on growth and IGFs transcription at embryonic stages in Persian sturgeon. In this study, it was found that the mean weight and total length of hatching eggs of Persian sturgeon were influenced by different light treatments, so that, the maximum weight and length in larvae were observed in the T2 light treatment (12L:12D, 300lux). The results also showed, despite the same eggs fertilization rate in all treatments (93%), the percentage of hatching was different and affected by the light. At the end of incubation stage, the eggs incubated in the T2 treatment (12L:12D, 300lux) exhibited the highest percentage of hatching (75%), while eggs incubated in control treatment had the lowest hatching rate (44%). Our results showed that photoperiod with continuous lighting, 24L:0D with 150 lux light intensity, T1 treatment, or continuous darkness, 0L:24D, 0 lux, (T3 treatment), as well as natural light condition (control treatment, T4) during incubation, were not suitable conditions for incubation of eggs in this species in order to increase the percentage of hatching and production of larvae. Studies on different species and even one species at a certain stage have resulted in different results. The results of the present study on Persian sturgeon fertilized eggs during incubation stage showed that there were no significant differences in hatching percentage between different light treatments. However, in a study on Persian sturgeon, the continuous dark treatment (0L:24D, 0lux) showed the higher hatching rate, survival, weight and number of larvae (Kazemi *et al*., 2012). Another study on Persian sturgeon in different photoperiod treatments and the same light intensity for all treatments showed that the photoperiod regimes 16L:8D and L00:24D had the maximum hatching efficiency, minimum mortality and maximum size in larvae (Poursaeid *et al*., 2012). It can be concluded that the effect of the light period and intensity is different according to age and growth stage among species (Loew and Sillman, 1998; Ruchin, 2007).

On the other hand, ecological differences in different regions can have different results in hatching and morphological development of produced larvae (Giorgi, 1981). The hatching rate for Ling cod eggs (*Ophiodon elongatus*) differed in two various natural locations across the Pacific Ocean. It seems that the ecological difference between two regions with different natural light periods could affect the percentage of hatching rate and the developmental time of larvae after hatching. On the other hand, it was found that the percentage of hatching eggs of Ling cod was not affected by different photoperiods, while low light intensity (at low and one lux) could significantly

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increase hatching percentage and produce more healthy larvae than high light intensity (563lux) and also 0lux (Cook and Rust, 2002). Because the eggs of Ling cod are demersal, so, they need low light intensity for hatching. The light intensity on the hatching rate percentage of Flounder (*Paralichthys dentatus)* eggs in low rang was resulted in optimal growth of larvae in subsequent stages (Watanabe and Feeley, 2004). It seems that the adaptation of these species to the low light intensity conditions for hatching eggs in the natural environment is the most important factor for achieving the highest percentage of hatching and optimal growth rate of larvae at low light intensity. The present study results and previous studies showed that different environmental conditions and egg structure indicate the control and diffusion of light on incubation or hatching time in some species of fish. Therefore, the proper amount of light intensity for optimal hatching eggs is important in different fish species (Cook and Rust, 2002).

Various environmental conditions and egg structure indicate the different control and diffusion of light on the period of incubation or hatching time in some species of fish. Therefore, the amount of light intensity suitable for hatching optimal eggs is important in fish (Cook and Rust, 2002). The eggs of Haddock (*Melanogrammus aeglefinus)* exposed to continuous light were hatched more rapidly and exposed to continuous darkness were significantly hatched later than other treatments, and the size of the body of the larvae produced in the light and dark period was shorter and longer than other periods of light, respectively (Downing and Litvak, 2002). Our results showed that the appropriate light period for the stage of incubation of Persian sturgeon egg in terms of growth and hatching percentage was 12L:12D. Present results were contrary to previous studies on this species, which suggested the continuous darkness or 16L:08D photoperiod treatment for Persian sturgeon egg incubation (Kazemi *et al*., 2012; Poursaeid *et al*., 2012). Our results were in agreement with the results in other species including Haddock. Because, as other research results in different species showed, the 12L:12D photoperiod largely is coincided with the living conditions of Iranian sturgeon in the natural environment

In the present study, the highest concentration of melatonin hormone was observed on the first day after hatching in all photoperiod treatments. Based on our results, during incubation stage, the concentration of the melatonin hormone was enhancement by increasing the duration of the light (24L:0D), as well as in the natural photoperiod (control treatment). Some studies have shown that pineal gland vision receptors, secrets serotonin hormone or precursor of melatonin hormone and other molecules involved in the transmission of light before hatching, which may thus affect the hatching time of eggs. Because the pineal gland can receive and analyze the light data before hatching eggs, therefore, it has an effect on the hatching time of the eggs (Powers and Raymond, 1990), However, optic receptors of the pineal gland may be differentiated before hatching and earlier than the cells in the retina (Ekstrzm and Meissl, 1997), which are very suitable for changing light conditions. Therefore, in

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response to the light period, light intensity stimulates the pineal gland in the hypothalamus and then, with the help of each other, controls the growth, metabolism and daily regulation of the activities. But in continuous dark and light periods, this physiological process may be interrupted and damaged and lose its function (Tuckey and Smith, 2001). On the other hand, the primary distinction of pineal neurons is important in establishing neural patterns of the brain (Wilson and Easter, 1991), because the primary neural pathway may be involved with an environmental stimulus to hatch adjustment (Downing and Litvak, 2002). Against to present study results, in halibut, light cause hatching eggs to stop (Helvik and Walther, 1992). However, in some species of fish such as *Danio rerio* and *Orizias latipes* (Schoots *et al*., 1983) and Atlantic salmon, *Salmo salar*, light-dark conditions are known as an effective hatching factor. Therefore, the major hatching of the eggs of these species is similar to that of Persian sturgeon, which is used in this study, occurred in the light-dark cycle.

Few studies on the effects of light and dark periods have shown that the production and the plasma level of melatonin hormone increase rapidly with the onset of the darkening period (Porter *et al*., 2001), and decrease with the length of the light period (Prayogo, 2012). By increasing or decreasing the intensity of light step by step, even in the dark phase, the amount of blood melatonin in the rainbow trout (*Onchorhynchus mykiss*) decreased and increased, respectively (Taylor *et al*., 2006). It was also reported that the concentration of melatonin hormone in the Atlantic salmon blood plasma also decreased significantly with the addition of artificial light in dark time and decreased with increasing light period (Porter *et al*., 1999). Also, increased larvae activity after hatching is associated with a decrease in the plasma melatonin concentration of blood plasma that reaches its maximum in the presence of light to the base and in the dark (Ekstrzm and Meissl, 1997), which is consistent with the results obtained in this study. Some studies have shown that, in dark conditions, the level of the melatonin hormone increases and reaches the highest level, maintaining a high level of production of melatonin in the first days of life and in a completely dark condition (Bolliet *et al*., 1996), can be justified a higher survival rate (Campagnolo and Nuñer, 2008), weight gain (Reiter, 1986), organogenesis and larval development (Kvetnoy *et al*., 2002; Kalamarz *et al*., 2009) on early days After hatching. The results of the present study confirmed that the light periods can have a different effect on the amount of melatonin production.

In teleost, energy metabolism and growth are affected by an endocrine system involving a variety of hormones. The GH-IGF-I axis control growth by metabolic and growth-promoting function (Duan, 1998). In the present study, the IGF-I and IGF-IR gene expression have been observed during embryonic developmental stages. However, the highest IGF-I and IGF-IR mRNA expression have been observed on the first day after hatching in all light treatments. Similar to our results, a study on Persian sturgeon IGF-I gene expression revealed that IGF-I transcription level was very low

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during embryonic stage (2 days before hatch and hatching day) compared to larval developmental stages (Miandare *et al*., 2013). The presence of a significant difference in IGF-I and IGF-I-R during first-day post-fertilization is in agreement with a significant difference in the eggs weight increase at the one-day post fertilization under different light regimes. Considering the present results, the stimulating role of IGF-I and IGF-IR in growth can be concluded. To our knowledge, there is no report on the association of GH-IGF-I axis gene expression and different photoperiod regimes with early developmental stages of any fish species. However, several studies reported the relationship between IGF-I and growth under different photoperiods. The positive association between growth and hepatic IGF-I mRNA expression was observed in different teleost (Beckman *et al*., 1998; Shimizu *et al*., 1999; Cruz *et al*., 2006), salmon (Beckman *et al*., 1998) and gilthead sea bream (Mingarro *et al*., 2002) in different photoperiods. The present study investigated the effects of the light on different performances in Persian sturgeon eggs. The present results suggest that the light effects on the physiological process in Persian sturgeon eggs during incubation and embryo development. However, the short-term duration of the study (only 8 days) may not be allowed the significant effects of different photoperiod and light intensity regimes on physiological aspects of eggs. Considering the importance of incubation stage during embryonic developmental at fish life, the effects of these slight changes would be observed in subsequent stages.

In conclusion, different photoperiods and light intensity have some effects on growth (egg weight), hatching rate, melatonin concentration, IGF-I and IGF-I-R mRNA expression in Persian sturgeon eggs incubated. The results suggesting that the appropriate light regime for Persian sturgeon eggs incubation is the 12L: 12D photoperiod with 300lux light intensity. In general, the hatched larvae can be obtained with a higher survival percentage in these light conditions. Also, these findings clearly showed that photoperiods with light intensities used to optimize light conditions during the incubation stage for the growth and survival of eggs were in accordance with the concentration of physiological indices. The results of the present study would provide a fundamental for further use of the light regimes to induce growth in sturgeon aquaculture.

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