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THE INVESTIGATE OF PHOTOPERIOD AND LIGHT INTENSITY EFFECTS ON MELATONIN RHYTHMS IN MALE AND FEMALE WHITESPOTTED RABBITFISH *(SIGANUS SUTOR)* OF PERSIAN GULF

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Abstract:

Annual variation of photoperiod is the most regular phenomenon that has strong predictive value in the temporal organization of seasonal activities in biological world, especially reproduction. The pineal organ, has the most important role in melatonin release under the different light condition. Melatonin is a much conserved feature in vertebrates that plays a central role in the entrainment of daily and annual physiological rhythms. The present study carried out in order to underestand different light intensity and photoperiod effects on melatonin release in whitespotted rabbitfish (siganus sutor) brood stock. For this purpose, 9 treatments, under artificial lighting conditions with 3 levels of photoperiod and light intensity include (16L/8D), (12L/12D), (6L/16D) and 1000, 2000, 3000 Lux, with a control treatment (natural light) on a period of 60 days studied. At the end of period analyzing the blood showed that the rate of melatonin in all of the male and female treatments in treatment of higher light intensity and photoperiod was lower than control treatment with a significant difference ($P < 0.05$).

Key words: *melatonin - photoperiod- light intensity- siganus sutor- Persian gulf.*

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INTRODUCTION:

Virtually all organisms have adapted their behaviors and functions to the daily and annual variations of the external cues. The alternation of light (L) and darkness (D), the 24 h LD cycle, is the most prominent and reliable of these cues [1]. The fish pineal organ acts as a photo transducer, conveying photoperiod information to the brain via neural pathways and via the release of indoleamines, primarily melatonin [2]. Primary research demonstrated in fish who their pineal removed, wasn't show rhythm of plasma melatonin, So pineal is the main source production plasma melatonin [3]. Although melatonin is synthesized in several tissues [4], its rhythmic synthesis is primarily localised in the pineal organ and the retina [5]. Melatonin rhythms provide the animal with information about the time of day and also the time of year, synthesizing and releasing high levels into the bloodstream during the dark period [6],[7] shown that artificial lights prevented the secretion of melatonin in European sea bass (*Dicentrarchus labrax*). As well as turned out in (DD) secretion of melatonin will be intensification [8]. The plasma melatonin rhythms in sea grass rabbit fish (Siganus canaliculatus) showed that in the period of natural light (12L / 12D) with a steady rhythm during the night higher than day [9]. However constant light (LL) prevented the secretion of important hormones such as melatonin and LH [10], Nevertheless melatonin level in cod fish in night lower than day considerably [11] (Wagner et al. 2001). Since rabbitfish, depend to tide extremely, The aim of the present study has been to dependence to sunlight and the relation between different light regimes and secretion of melatonin.

MATERIALS AND METHODS:

Animals:

Fish were caught during cruise on Lavan Island north beach situated Persian gulf in November and December 2011. Live fish moved to Persian Gulf and Sea Ecological Research Institute in Bandarabbas and up to project start were kept in 5-tonne fiberglass tanks. The animals were fed ''ad libitum'' using a commercial diet (The final concentrate shrimp) twice a day by hand, as well as crab meat sliced twice a week.

Experimental procedure:

Fish were exposed in 9 module that were separated by thick, black plastic cover, and each module had a 300-liter fiberglass tank with 5 siganus sutor. We used 9 light rejimes included light intensity (1000 – 2000 – 3000) Lux and photoperiod (16L/8D) ، (12L/12D) and (8L/16D). In addition, one tank was in natural lighting conditions as a control. According to this regime, Exposure to the fish began on January the ninth of 2012 for 60 days.

Measurement of plasma melatonin:

At the end of the period, the fish were completely unconscious. In order to measure the plasma melatonin, Of each fish at the rate of 2-4 ml of blood samples was by spinal cord vein puncture and 18 percent were cast into the pipe. Blood was centrifuged for 10 min at 1500 - 3000 rpm and 4 ^{0}C, and plasma was frozen at 80 ^{0}C. Plasma melatonin levels of each group were determined. For this purpose, a commercial ELISA kit (IBL, Hamburg, Germany) was used, as described by Bayarri et al. (2002, 2003 and 2004), Wagner et al. 2001[78,11,12].

Data analysis:

Data are expressed as means ±SEM values. The statistical differences between groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's test, with $P < 0:05$ taken as the statistically significant threshold.

RESULTS:

Melatonin levels in treatments that were under artificial lighting with the control treatment using ANOVA (one way anova) comparison and found that the secretion of these hormones in males and females of all treatments, a significant difference was less than the control treatment ($P < 0.05$). The amounts of melatonin in the two sexes were compared by t-test and the results showed that the value in male were significantly higher than females ($P < 0.05$).

Compare melatonin hormone in males and females based on the intensity of light:

In females, the highest rates were observed with optical evaluate the secretion of melatonin in the light intensity of 2000 lux, respectively. The light intensity of 1000 and 3000 had the luxury of a similar order. The hormone melatonin levels in males was observed in all three light intensity levels of the hormone correlated, so that in the same light intensity with decreasing photoperiod according to a certain order to the D16: L8 decreased melatonin levels . 3000 lux light intensity in the optical D8: L16 and D12: L12 had the highest amount of release (Figure 1).

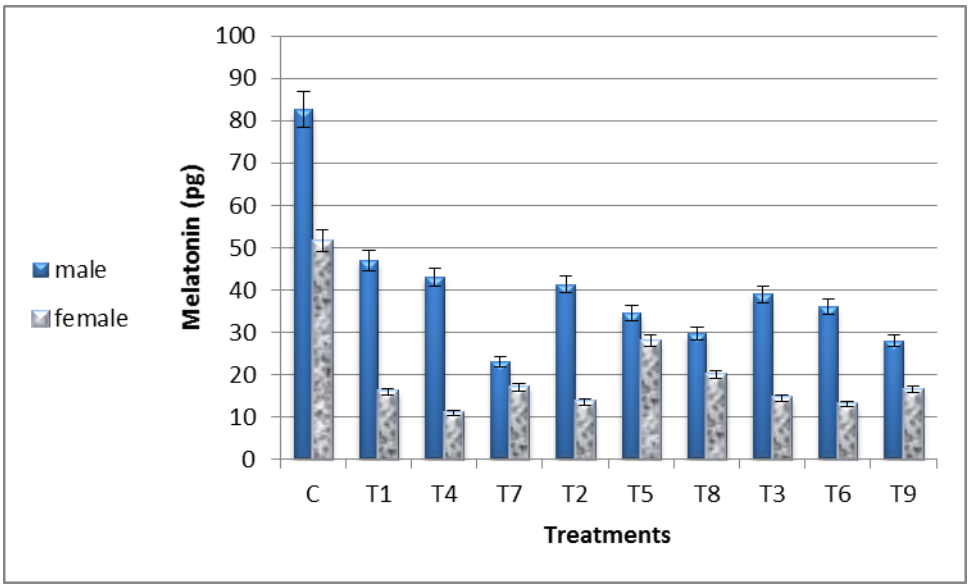

Fig 1: Comparison of male and female hormone melatonin according to light intensity (±SE)

Compare melatonin hormone in males and females based on the photoperiod:

By comparing the amount of melatonin females on different optical period proved to be the longest photoperiod (D8: L16) Melatonin three treatments with similar values, lower than other courses were light. The highest melatonin (With the exception of group 5 that which the females were limited samples), in the lowest of photoperiod treatments (D16: L8), respectively. But in the photoperiod (D12: L12) and (D16: L8) and a similar Process was observed, Figure 2 demonstrates that it is. The comparison of different photoperiod male, it was found that the highest levels of melatonin for a maximum photoperiod treatments D8: L16, In the event that the lowest rates of secretion was in the treatment of 7 with D16: L8 (Figure 2).

Finally, Duncan test showed that in females with changes in light intensity and photoperiod, difference is not significant in melatonin secration between different treatments with artificial light ($P>0.05$). As well as changes in the light intensity and photoperiod in males, significant differences between the controls (C) with all treatments at least photoperiod D16: L8 found; In the event that in another photoperiods this difference in light intensity was observed in 1000 and 2000 lux. In fact, the only treatments 1 and 4 had no significant difference with control.

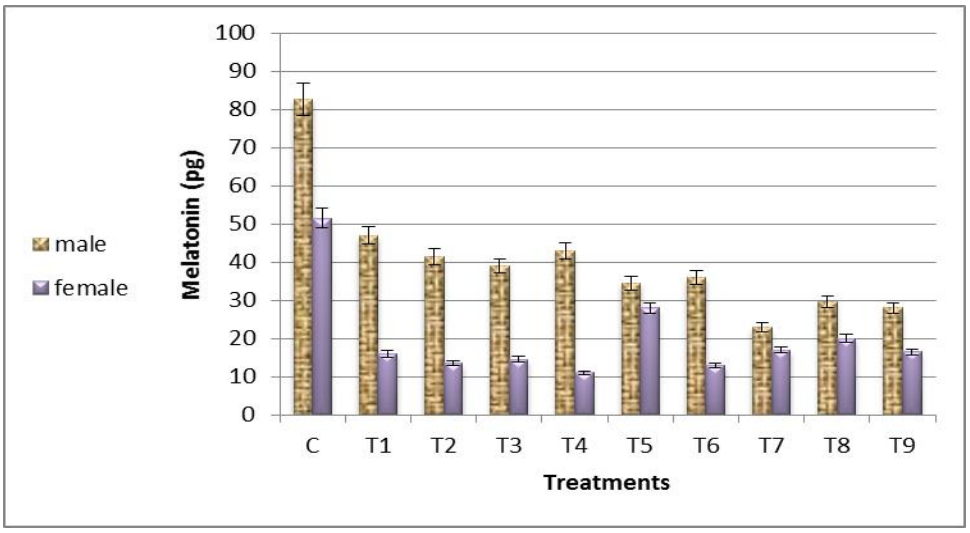

Fig 2: Comparison of male and female hormone melatonin according to photoperiod (±SE)

DISCUSSION:

This study is considered as early information about the impact of fluctuations in light intensity and photoperiod on melatonin rhytms in Whitespotted rabbitfish (*Siganus sotur*). According to the results of the measurement of plasma melatonin, Were observed in all treatment levels of this hormone in females, with a significant difference was less than male ($P < 0.05$).

Discussion:

The current study is considered as the initial information about the impact of duration fluctuations and light intensity on the amount of secretion of melatonin on the filtering of white spot fish (Siganus sotur) .

According to the results of plasma's melatonin measurement, was observed that in all treatments , the rate of this hormone in female sex is significantly less than male sex. ($P<0.05$)

Melatoninrate in the filtering of harvested fishes rom depths of 1.5 to 5 m in Persian gulf, was measured in the initial phase of period and equals to 50-154.

In the conditions which Wagner et al 2002 in the survey o melatonin rate in pineal of deep water fish (1500-4800 m) the amount of secretion of this hormone in *Synaphobranchus kaupi was estimated 2-70 pg* and 4-92 pg in *Coryphaenoides armatus.*

The reason of these differences can be due to the difference in the biological depth of fish. Deep-fishes are exposed to the less light meanwhile only some of the photo-spectrums are able to penetrate in the water depths.

This is true when in the upper levels of water , fish have the high quality of light (intensity , duration and photo-spectrum) that will have an effective impact on the melatonin secretion . In addition, the aforementioned differences can be led by venesection hours rom fish, Bayarri et al 2003 stated that melatonin amount in ***Dicentrachus labrax*** **at** 19 equals to 85 pg and at 13 is reached to less than 10 pg.

Also, Rahman et al 2004 [9] in the survey of melatonin rate in *Siganus canaliculatus* under the photoperiod of 12L: 12D have found that these levels significantly in 24 h have been rose with a pick and close to the 6 h of morning was decreased rapidly.

Although , another factor as the latitudes , optical spectrums and various seasons of year that the experiment was performed within it , can have a significant impact on the melatonin value of fish in the natural terms , but it seems that the most effective factor in the secretion amount of this hormone , is the physiological terms of the considered genus.

Generally , the artificial lights causes the prevention of releasing the melatonin levels of plasma (Bayarri et al) . in this research also observed that artificial lightning in format of intensity and various photoperiods , causes the melatonin amounts to be decreased in both male and female sexes compared to the control treatment .

Although, the increase or decrease of exposure, have the different impacts on the rate of melatonin in the various genera of fishes, generally the increase of intensity or photoperiod is with the decrease of melatonin rate.

In the investigation of the melatonin hormone amount in the filtering of white spot fish , due to the physiological differences existent in the male and female genus , the hormone levels completely separate in the male and female genus were compared . as mentioned above , the results coming from the increase of male genus ' melatonin to the female genus were significant . Meanwhile in each treatment, the male and female fish were kept in the same terms and in a tank.

Moreover, the venesection terms also for all fishes is similar and in the certain hour's o day was performed. According to the frequent studies that previously around the melatonin effect on the reproductive processes of various species o fish were done, the preventive impact of this hormone on the gonadal growth and development is undeniable.

Although the rate of melatonin hormone on the sexual maturation process of filtering the white spot fishes is not countered as one of the aims of this research and requires extensive discussion , but use of some witness observations , helps to the analysis of the differences of melatonin hormone between the male and female sexes .

Extracting the sperm from male's genital pore without applying any pressure to the abdominal zone during the venesection rom fish, indicates the full growth of gonads during the 60-days period. in the terms which the female fishes have not achieved the complete sexual maturation and gonadal growth and development process was continued too.

This issue is in accordance with findings of Lam and Soh in 1975. They stated that the sexual maturity of

Siganus canaliculatus is occurred sooner in captivity conditions than wild conditions and sooner in males than females.

During the studies of foroughi et al in 1381, also was proved that the growth and development of gonads in male sex of S.sotur was very remarkable such that sometimes occupied the whole abdominal cavity, provided that ovaries in female sex had not a good growth in the female sex and occupied a little size of abdominal cavity.

They also stated that the male fish have reached in May to the 4th step of maturation such that a mild pressure to the abdominal zone leads to the sperm secretion from these fish. In terms which the sexual maturation in female S.sutor compared to the male fish having the delay ability in a way that the 4th step of sexual maturation in female was observed in late of May and early of June .

The recent studies showed that the Pituitary hormonal regulation and gonads in fish, are most complex than previously identified.

The studies that principally were performed based on the fish, indicating the specific role of GtHs on the controlling of growth of Gonads.

The controlling the release of GtHs by Hypothalamus in bony fishes under the effect o seasonal reproduction cycle or gonadal maturation cycle.

Usually , it is believed that GtH-I is important for initial development of the gonads and vitellogenesis , meanwhile GtH-II irritates some events which cause to the final maturity of Oocyte and maturity of females as well as , sperms maturity in the males.

The results of experimental studies using pituitary cells cultivation broadly indicated that the release of GtH-II directly aroused by GnRH, Norepinephrine as well as serotonin.

When the sexual maturity of gonads occurred in its duration and vitellogenesis is occurring in females as well as when the maturation in the males is done, the response of GtH-I decreases compared to GtH-II. In salmom , during the period before the maturation, the release sensitivity of GtH-I in reaction to the GnRH for increasing the sensitivity of GtH-II will be increased by the sexual maturation.
Its amount in the first phases of Gametogenesis is low and in the period before than the maturation reaches to its maximum value. Meanwhile, the changes o secretion of GtH-I and GtH-II in the duration of sexual gonadal maturation, probably is associated with differences related to the production capacity o these two hormones.

The mentioned details can be an appropriate justification for high levels of sex steroids in the filtering of white spot female fish.

Because as is said , the filtering the female fishes in the pre-maturation step and gonadal development and growth process , cause to the raising the sexual steroids and since the melatonin secretion as a preventive factor for gonadal maturation is in contrast to the sexual steroids , therefor the melatonin level is kept low. Meanwhile as mentioned above , serotonin that is counted as the precursor of melatonin is one of the actors of GtH-II secretion.

In addition , the investigation of annual approach of reproductive activities of filtering the fish has identified that in the genus of *Siganus argenteus* , the values of 17-beta Estradiol as the most important sex steroids in the female fish , ater the fluctuations that in the April -June , had an increasing trend in the first part of July and a pick in the half od moth simultaneously with the maximum GSI and then decreased severely .(Graph 3)

As well as the amounts of 17-beta stradiol , Testosterone and DHP as well as during the year have been faced with several increasing and declining trends [13]. (Graph 4)

In addition , the investigation of annual trend of flat spawning activitioes revealed that in the species of Siganus Argenteus , the values of 17 b-steradiol as the most important sex stroid in the female fish after the fluctuation that were observed in the month from April to June m at the beginning of July had an increasing trend and in the medtime of it changed to a pick and then was decreased severely (graph 3). While , the values of 17 b-stradiol , testestron and DHP during the year had the several increasing and decreasing trend[13]. (graph 4)

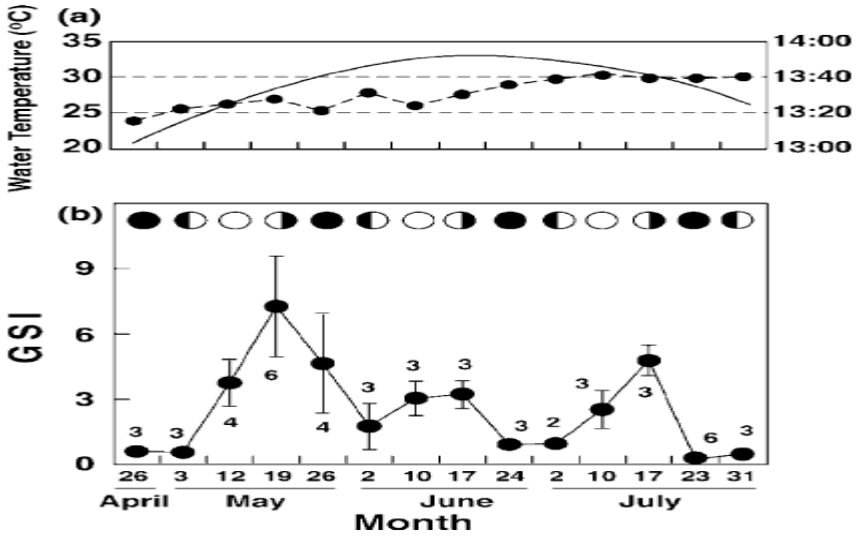

Fig 3 (a) : the daily changes in water temperature and photoperiod in Japan; and (b) GSI in females of Siganus argenteus during the breeding season . GSI changes were shown by the mean of SEM. [13,14]

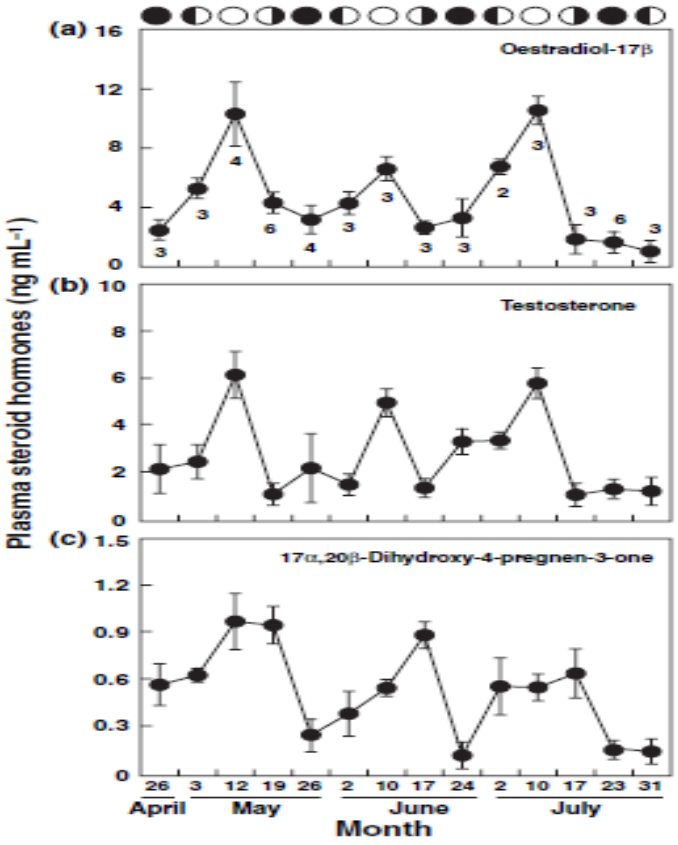

Fig 4: Weekly changes in : (a) 17- beta estradiol (b) of testosterone and (c) DHP in plasma Siganus argenteus during the annual reproductive cycle.[13,14]

In relation to the male secretion, GtH, 11KT and DHP influenced the evolution of sperm and spermatogenesis (Nagahama 1994)[14]. Rahman et al (2003)[15] also reported that

Filtering in male fish of *Siganus argenteus* (Siganus argenteus), testosterone levels and 11KT and DHP present in plasma occurs in the range of the full moon spermatogenesis is occurred as well as in the last week of month when the evolution of sperm is observed , just were increased as the peak.

Therefore , by survey of studies about the annual reproductive process of filtering the fishes , it can be stated that in the male sex of S.sutor also probable the sexual steroids rate including the testosterone that is the most important available androgen in the circulatory system of male fish , due to the evolutionary process of gonadal maturity , after the increasing trend as a pick , have fallen again. Hence, the decrease of sexual steroids, permit to the increase o melatonin hormone as a pick.

In comparison of the effect of artificial lights on the filtering of female white spot fish , it is observed that the amount of melatonin under the long light duration 16L:8D to the treatment having the minimum light duration of 8L:16D placed in the lower level.

This issue will approve the first hypothesis of research as well as the results were in line with Bayarri et al (2003) [7]. They proved the melatonin rate was less than the light duration of 12 L: 12D under the long light duration of 18L: 6D in European bass fish.

Zachman et al 1992 [16], stated that under the effect of instant photoperiod of 16 L: 8D, the melatonin rate in Salvelinus Fontinalis during the night is longer. Also, placing the Oncorhynchus mykiss during the long days or shorter days respectively cause the melatonin rate to be decreased or increased.

In this fish, Pineal gland translates the night duration to the increasing pattern to the melatonin secretion that probably will be used daily to the various forms. (Bromage et al 1998) . also, long photoperiod of 18L:6D in comparison with the normal photoperiod of 12 L :12D causes the maturation to be delayed in Siganus canaliculatus .(Soh and Lam 2003) based on the studies of Ekstrom (1997) , this issue probably due to the preventive effect of melatonin on the sexual maturation and its prevention from increasing the gonads size.

.In addition to this , the secreted melatonin in the photoperiod of 8L:16D with the exception of treatment No. 5 , was more than the photoperiod of 12L:12D. since that the treatment No.5 in comparison to the other treatments was faced with lack of female sex, probably this increase is for the experiment error or lack of sufficient space.

Generally, we can state that by decreasing the photoperiod, the amount of melatonin have been increased in plasma in the filtering of white spot female fish. Such that in the photoperiod o 16L: 8D, melatonin in each photo-intensity , approximately is equal , therefore the long photoperiod have destroyed the various photo-intensity impacts.

The similar studies on *Dicentrarchus labrax* , proves that the use of continuous light causes to the prevention of the key hormones production including melatonin and LH [10] (Bayarri et al 2009) , generally it must be considered that every species of fish in the various ways will react to the photoperiod consistencies , therefore the beginning of sexual gonads evolution and spawning occur in some periods of year that is specific to that species.

In relation to different light intensities apparently they could not change or regular fluctuations in fish filter material to create white spots.

In a way that photo-intensity of 1000 and 3000 lux caused the melatonin secretion rather equal meanwhile in the photoperiods of 8L: 16D and 12L: 12D, the photo-intensity have the maximum amount of melatonin.

Therefore, the final resulting approves that in the female sex of such fish filtering; dependency to the photoperiod is more than the photo-intensity. Although, Dunken test showed us a significant difference between the treatments under the different photo-diets) .P>0.05)

In association with male sex of S.Sutor , by analysis of data a new results and relatively different with previous studies on the other genera as well as filtering the female white spot fish.

Unlike the hypothesis of research, in comparison with rate of melatonin based on the photoperiod, was observed that in the longer photoperiods more melatonin was secreted. Such that by decrease of photoperiod rom 16L: 8D to 8L: 16D, the rate of melatonin regularly was decreased and the minimum melatonin was observed in the 7th treatment with the shortest photoperiod.

In a survey of melatonin of male sex based on the basis of different lighting intensities was proved that the amounts of these hormones are correlated between different treatments such that the highest melatonin value in light intensity of 3000 lux .

During the investigation of Zachmann et al (1992) [16] also was proved that Melatonin levels in Salvelinus fontinalis increased with increasing the light intensity. Apparently in the filtering of males, increasing the duration and optical severity has been increased the secretion of melatonin.

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