MODIFICATION OF PHOTOPERIOD REDUCES EARLY SEXUAL MATURATION IN JUVENILE SEA BASS (*Dicentrarchus labrax*) (Hormonal aspects).

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Abstract

The effect of short-term exposure to continuous light (LL) to reduce early male sexual maturation was investigated in the sea bass. Four LL light regimes (1 or 2-month duration) were established during the pregametogenesis, whereas a simulated natural photoperiod (SNP) and a long-term exposure group (12-month duration) to LL were kept as controls according to previous trial in this species. Exposure to different LL treatments induced a differential response on the sex-steroid levels. Particularly, significant differences (ANOVA, n=20, $P<0.05$) in the levels of 11-KT were observed during the peak of reproductive period (February) in groups 4 and 5 (control) in comparison with groups 1, 2, 3, 6. These results suggested the role of this androgen as a putative threshold determining early puberty in the sea bass as previously reported. Profiles of T were similar during whole experimental period. E_2 levels at the beginning of pregametogenesis (September) presented significant differences between SNP groups (5 and 2) and LL treatments groups (1, 3, 4, 6). Thus, the identification of a short-term exposure to LL approach can be of interest for sea bass aquaculture, since it could reduce the presence of early sexual maturing males under farming conditions.

Key words: Photoperiod, continuous light, precocity, spermatogenesis

1. Introduction

The importance of European sea bass (Dicentrarchus Labrax L.) on the albanian aquaculture and the whole Mediterranean basin is pushing the researchers to find different forms to maximize the production. Just thanks to scientific research output for this species only from aquaculture in 2005 was 80 161 tones, leaving behind fishing with 11 481 tones [8].

As a result of this production increase, in conditions of intensive aquaculture, there are observed a number of aspects which hinder the further growth of production. One of these main problems is the high percentage of sea bass males that goes from 70 - 99% [4, 2, 5, 12, 22] and moreover males have lower body weight than females. In addition, 20 - 30% of these males mature sexually in the first year of life (in fact the time of achieving sexual maturity is the second year of life). These males are called precocious males or males with early maturity [4, 31]. Immediately after reaching sexual maturity these individuals invest part of the energy provided for development of gonads and reproductive processes. Therefore the object of work for research groups in many countries is reducing the number of these premature puberty individuals (precocious individuals).

To reduce or curb this phenomenon should observe how the process of puberty occurs in fish and the factors causing it.

1.1 Puberty in teleosts

Puberty is the process in which an animal is able to reproduce for the first time [20, 29]. Today is still not known precisely why the individual in a certain moment "starts" the process of puberty. It is thought that are external factors (photoperiod, temperature, pheromones), which interfere with the start of the process of puberty [20, 24, 29]. Bass is a species in which the change of light (photoperiod) affects more

than other factors in the onset of puberty and all reproductive processes. As a result of external stimuli (in our case the light) the signal passes the first gate of the body (the eyes) and goes to the pineal gland. This gland produces the hormone Melatonin [11], which is not well known how activates brain structures, but it is seen that after production melatonin a group of neurons in the lower structures of the brain (neurons Kiss), produce a peptide (peptide Kiss), which operates on hypothalamic to activate latter the system of GnRH (Gonadotropin Releasing Hormone) [10]. Also has thoughts that the GnRH system is activated by one hormone produced by adipose tissue of the body and precisely Leptine (Carrillo et al., unpublished data) (Fig. 1).

As a result of activation of the GnRH system, this system acts on the pituitary. This gland produces Gonadotropin hormones (GtHs). These hormones are Folikulo-stimulating hormone (FSH) and Luteinizing hormone (LH). These hormones act in gonads to produce steroids.

There are three steroids responsible for puberty: 11-Ketotestosterone (11-KT), Testosterone (T) and 17β-estradiol (E2). 11-Ketotestosterone (11-KT) is the most important steroid hormone in males and responsible for spermatogenesis [25, 15, 3, 24]. Estradioli-17β (E2), known as a female hormone, actually is an increasingly protagonist in male gonadal development. It is now confirmed that the E2 is very important during the pre-gametogenesis in male fish [14].

The study in question is limited to only measuring the values of steroid hormones in plasma and not measuring all hormones responsible for puberty in Dicentrarchus labrax. Profiles of steroid hormones can give accurate information regarding the percentage of individuals who have begun the process of puberty in male sea bass in the first year of life. At the same time the value of steroids may also give an answer on the effectiveness of different combinations of photoperiod regimes with constant light (LL), to reduce precocity in the first year for bass.

2. Materials and methods

The study was conducted at the Institute of Aquaculture Torre de la Sal (Castellón, Spain, 40 ^{0} N and 0 ^{0} E). Fish were provided by the company Aquanord (Gravelines, France).

- Duration of the experiment in August 2007 - April
- 3000 fish aged 5 months
- Initial density 2.5 kg/m3
- Feeding: 3 times/daily through automatic feeders
- 4 eksperimentales groups (groups 1, 2, 3 and 4) + 2 controls (group 5 and group 6)
- 12 tanks with 2000 l or 2 tanks for each experimental group (210 fish/tank)
- Light intensity of 650-700 lux

2.1 Experimental project

Fish were divided into 6 groups (4 experimental groups and two controls) as follows (Fig. 2).

2.2 Hormonal analysis

Blood sampling was done in the vein through a caudal puncture. This process was realized with the help of different size needles (according to size of fish) and syringes which were previously treated with heparin. Then, were extracted plasma samples (centrifuge 3000G, 4 ^{o}C), subject to analysis of steroid hormone levels of 11-KT, T and E2.

For the three hormonal tests was used the method EIA (Enzyme Immunoassay). EIA is a biochemical test that measures the concentration of a substance that is found in a sample, in our case plasma. This method works on the basis of antibody – antigen reaction (Fig. 3).

2.3 Statistical analysis

The data were analysed using a Two-Way ANOVA test to compare the effects of six photoperiodic regimes on growth and reproductive

performances including biometric parameters, body indexes, hormonal analyses of T, 11-KT and E_2 in plasma. Statistical differences at different sampling times are indicated in the present study. When necessary, normality was ensured by using the Kolmogorov-Smirnoff test after logarithmic transformation of data. Barlett´s test was used to establish homogeneity of variances. A multiple range test of Tukey HSD was used to examine significant differences between means. All data were expressed as mean ± SEM. Differences were accepted as significant when $P < 0.05$ [27].

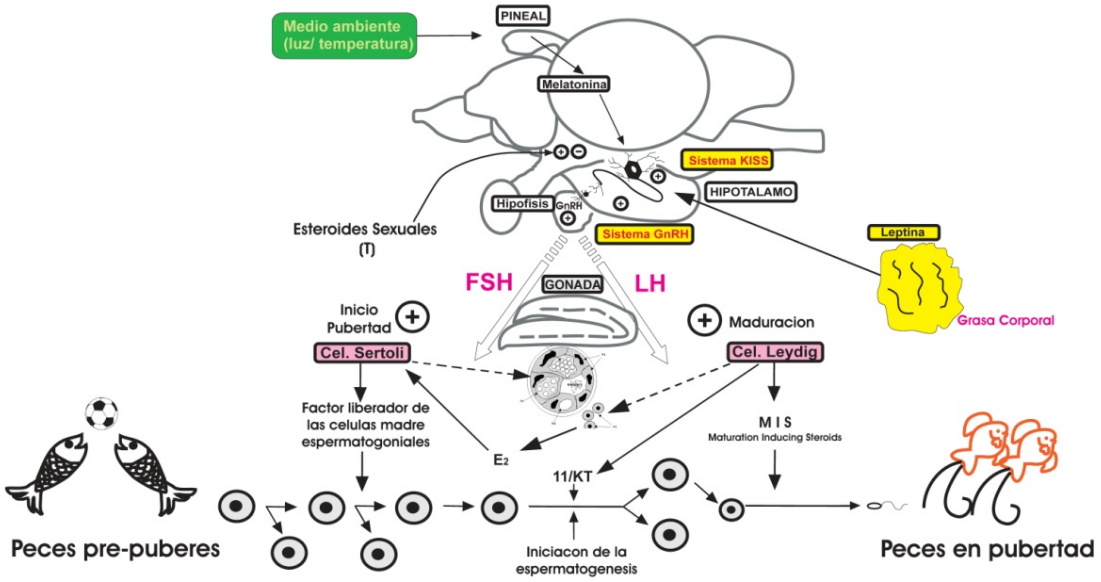

Figure 1. Scheme of the initiation of puberty in male fish (modified M. Carrillo)

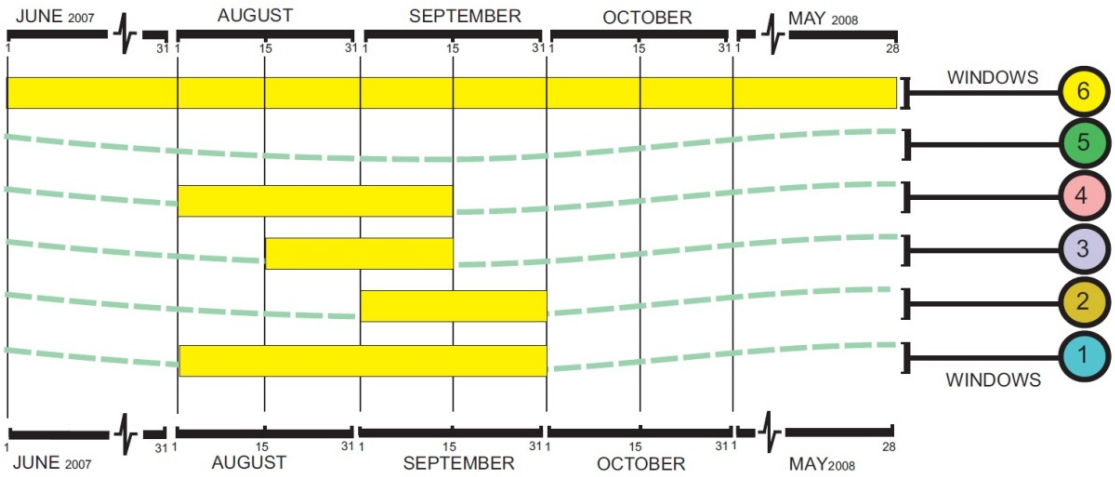

Figure 2. Experimental project of 6 different photoperiodic regimes applied on juvenile males of Dicentrarchus labrax in the first year of life. Groups 5 and 6 are controls, while 1, 2, 3 and 4 are experimental groups.

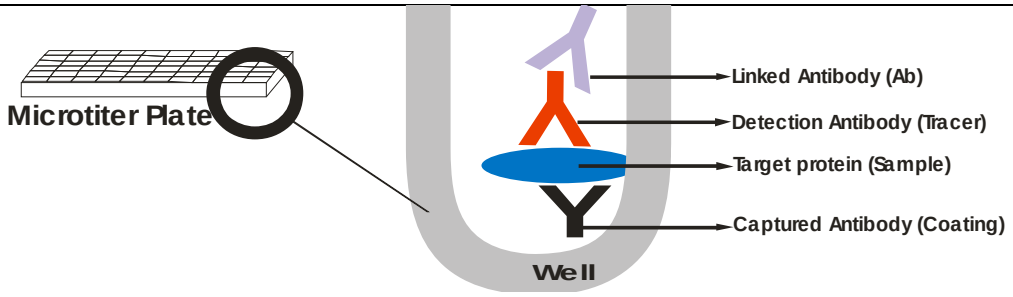

Figure 3. Scheme of the EIA method

3. **Results and Discussion**

This study demonstrated that short-term exposure to continuous light of one-month or two-month duration given at the pre-gametogenesis stage, between August and October, to the juvenile male sea bass might be equally effective as that of a long-term exposure to LL in reducing sexual maturation. Exposures to LL during 12 consecutive months have been previously demonstrated to be the most effective treatment to inhibit later stages of testicular development in male sea bass during their first year of life [9]. Although our results corroborated this statement, the application of critical LL treatments, if a photo-labile period defined, is critical for reducing the onset of puberty in this specie, thus facilitating lighting and farm operating costs in aquaculture. Our data demonstrated that fish under LL regimes through pre-gametogenesis (Window 1, 1 Aug-1 Oct; Window 2, 3 Sept-3 Oct and Window 3, 14 Aug- 14 Sept) exhibited immature gonads, except for Window 4 (LL, 1 Aug- 31 Aug). The ineffectiveness of this light regime may be due to that the exposure to LL is necessary during the whole month of September for maintaining the inhibition of gonadal development which already started during August. In addition, the short-term exposures to continuous light in this study, mainly the Window 1, 2 and 3, evidenced alterations in the reproductive wave in comparison to Window 5 (SNP group), affecting the progression of germ cells to more advanced stages of testicular development. Particularly, a reduction of the stage II of germ cell development was observed from November to January in these three shorter LL window regimes.

As previously suggested [9], light may have a direct effect on the arrest of meiotic divisions of the germinal cells and thus, on the gonadogenesis. Because stage II is considered the previous stage after which meiosis of germ cells occurs in the testis of the sea bass, the low rate of germ cells at stage II may limit stages of germ cell development to a more advanced gonadal maturation. Thus, due to light regimes including Window 1, 2 and 3 coincided with the time when spermatogonial proliferation and/or the beginning of meiosis of germ cells take place in the testis of the sea bass may account for improved photoperiod protocols for delaying first sexual maturation (puberty) in commercially farmed fish as the sea bass. Body weight and fork length presented similar pattern of growth variation in all experimental groups although some significant differences was observed among them. Mean weight of all fish under experimentation gradually increased from 14.4 ± 0.1 g at 6 months (July 2007) to 135.5 ± 0.5 g at 15.5 months of age (February 2008) (Fig. 4), whereas length increased from 10.0 ± 0.4 to 21.1 ± 0.02 cm for the same period of time (Fig. 5). CF varied from 1.3 to 1.5 in the SNP group, whereas in the remaining groups CF declined to 1.2 at 7.4 months of age (end of august), reaching similar values to the SNP group in January at 15.5 months of age (Fig. 6). The present data evidence that growth performance was not altered by photoperiod as recently observed in this species [9], although somatic growth may be enhanced after LL treatments as previously described in other fish species [13, 6]. These differences in growth can be explained by different photoperiod regime applied and reproductive investment in each species.

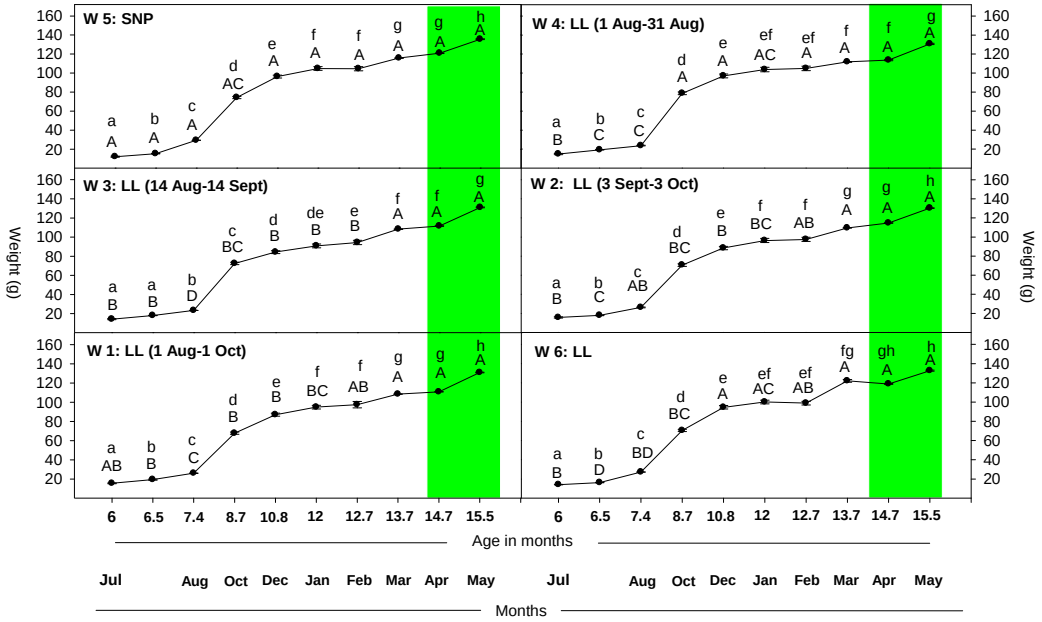

Figure 4. Body weight of juvenile sea bass males during their first annual cycle of life, from 6 to 15.5 months of age. Data as mean ± SEM from two replicates groups in each light regime. Each replicate was stocked with 210 fish (n = 420 fish per photoperiod regime; see additional information in Figure 2). Different capital letters above the symbols indicate significant differences between groups for a same date. Different lower case letters indicate significant differences throughout the period of experimentation (months) within each photoperiod regime.

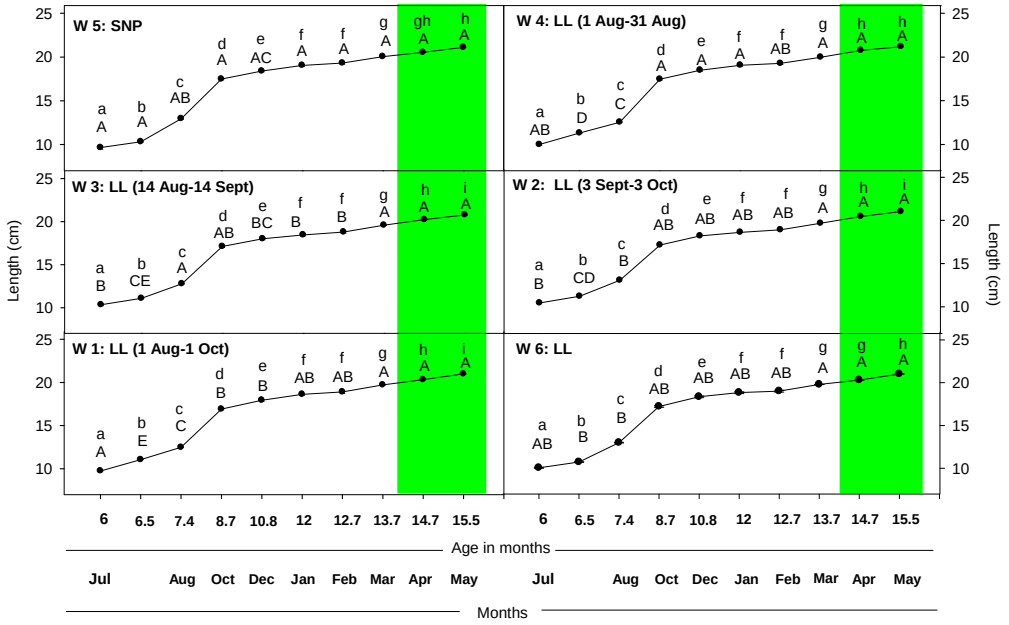

Figure 5. Fork length of juvenile sea bass males during their first annual cycle of life, from 6 to 15.5 months of age. Data as mean ± SEM from two replicates groups in each light regime. Each replicate was stocked with 210 fish (n = 420 fish per photoperiod regime; see additional information in Figure 2). Capital letters and lower case letters as in Figure 4.

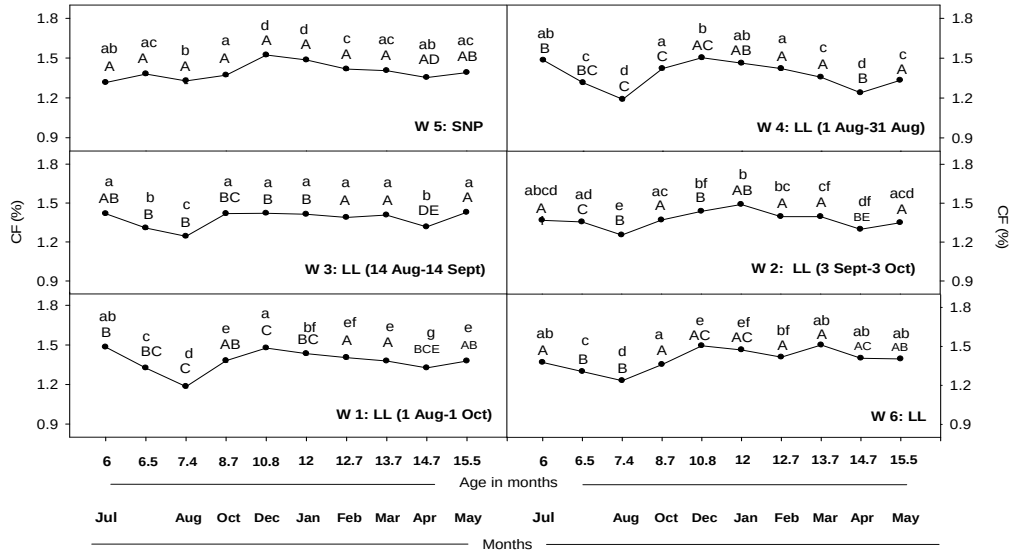

Figure 6. Condition factor of juvenile sea bass males during their first annual cycle of life, from 6 to 15.5 months of age. Data as mean ± SEM from two replicates groups in each light regime. Each replicate was stocked with 210 fish (n = 420 fish per photoperiod regime; see additional information in Figure 2). Capital letters and lower case letters as in Figure 4.

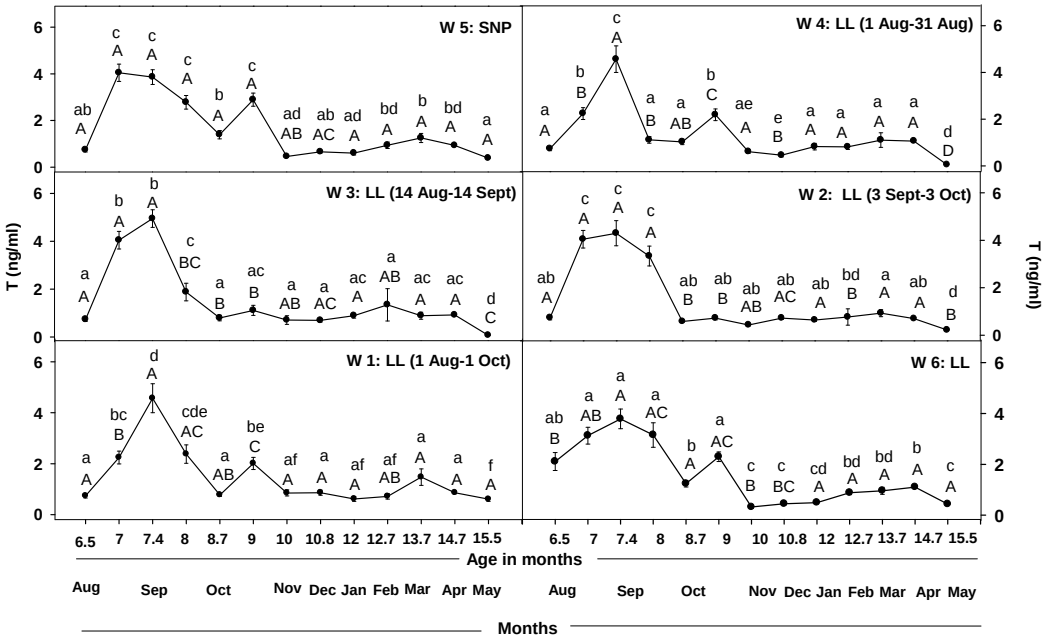

Figure 7. Seasonal profile of plasma testosterone (T) levels during the first annual cycle of life of sea bass males, from 6.5 months of age in August to 15.5 months of age in February, kept at different photoperiodic regimes (see additional information in Figure 2 for photoperiod treatments). Different capital letters above the symbols indicate significant differences between groups for a same date. Different lower case letters indicate significant differences throughout the period of experimentation (months) within each photoperiod regime.

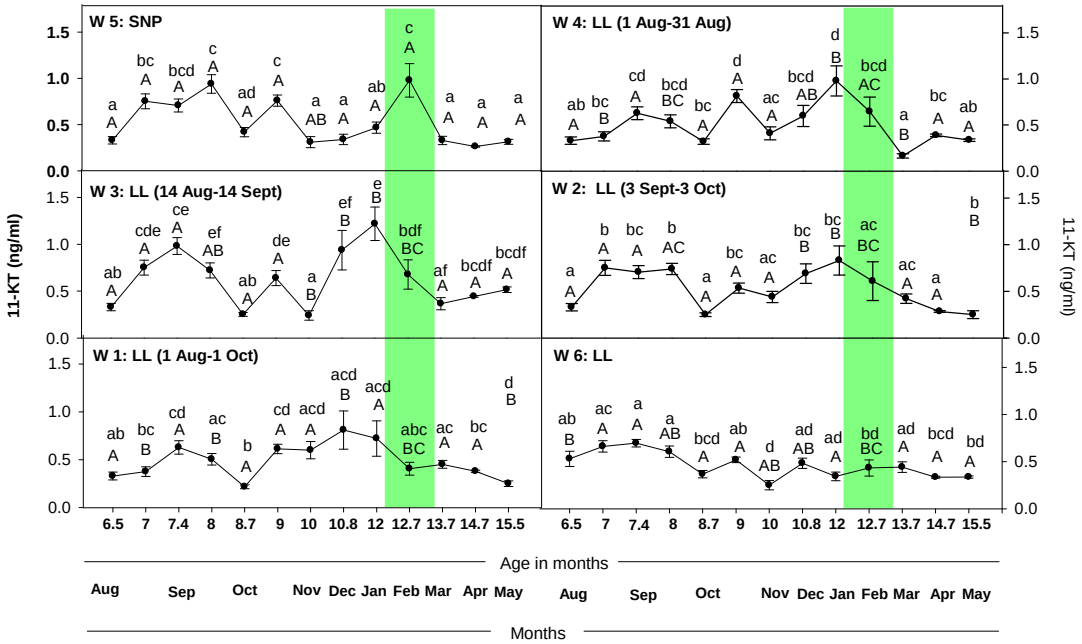

Figure 8. Seasonal profile of plasma 11-ketotestosterone (11-KT) levels during the first annual cycle of life of sea bass males, from 6.5 months of age in August to 15.5 months of age in February, kept at different photoperiodic regimes (see additional information in Figure 2 for photoperiod treatments). Capital letters and lower case letters as in Figure 7. The initiation of the spermiation period in fish under experimentation is highlighted as a shaded area.

Plasma T (Fig. 7), 11-KT (Fig. 8) and E_2 (Fig. 9) were affected by photoperiod treatments. Plasma T levels in the SNP group (*W*5) increased from August until they reached maximal levels between the mid-August (4.5 ± 0.37 ng ml^{-1}) and the beginning of September (3.86 ± 0.32 ng ml^{-1}). In addition, similar values were observed under those light regimes including groups *W*2 and *W*3, although fish maintained under light regimes such us groups *W*1, *W*4 and *W*6 displayed a slower increasing of T level from August to September, peaking one month later than the control. Subsequently, values of T decreased in all treatments, resulting lower than 0.97 ± 0.10 ng ml^{-1} around the beginning of October (8.5 months of age). A second and minor peak of plasma T was observed in mid-October (9 months of age), where the SNP group reached 2.89 ± 0.28 ng ml^{-1}. The remaining groups attained lower levels compared to SNP group, which were considerably reduced in those groups under light regimes including *W*1 (2.01 ± 0.24 ng ml^{-1}), *W*2 (0.72 ± 0.07 ng ml^{-1}), *W*3 (1.11 ± 0.21 ng ml^{-1}) and *W*4 (2.19 ± 0.25 ng ml^{-1}).

The levels attained by the LL group (*W*6; 2.3 ± 0.19 ng ml^{-1}) were not significantly different from SNP group at this point while the rest of LL groups yes. Plasma T levels decreased in all groups in November and remained low in February (0.91 ± 0.24 ng ml^{-1}) (Fig. 7). The comparative analyses of sex steroids levels in plasma between SNP and all LL regimes proved to be important to provide the progression into reproductive maturation condition. Thus, the seasonal profiles of plasmatic levels of T evidenced that the high levels of this hormone at the beginning of September, around 7-7.4 months of age, coincided with the sexual differentiation period in the sea bass, as previously reported in fish gonadal differentiation [17, 7]. After this time, the levels dropped and seasonally fluctuated until they reached similar levels in the peak of reproduction (13.7 months of age).

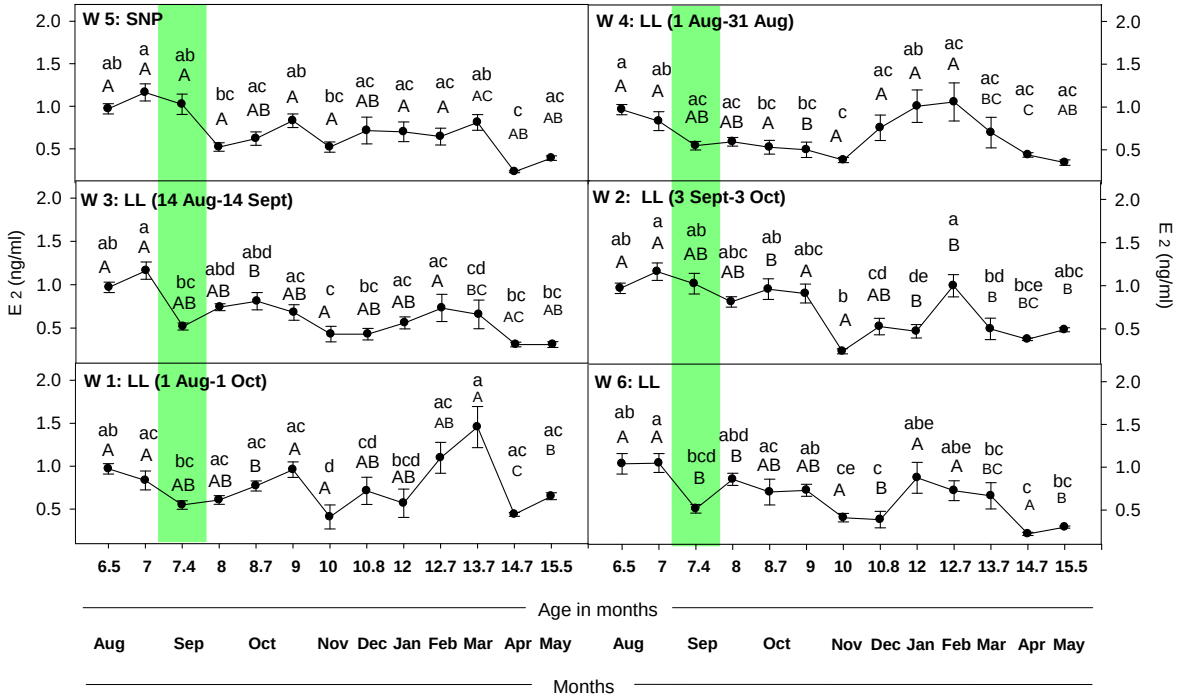

Figure 9. Seasonal profile of plasma 17β-estradiol (E_2) levels during the first annual cycle of life of sea bass males, from 6.5 months of age in August to 15.5 months of age in February, kept at different photoperiodic regimes (see additional information in Figure 2 for photoperiod treatments). Shaded area shows a critical time on which earlier events of gamentogenesis are taking place with significant changes of E_2 levels between controls and LL exposure fish.

11-KT plasma levels showed a similar pattern of evolution in all groups during the year. However, in February only the control group (*W*5) showed a significant rise of 11 KT during February. The rest of the groups did not show this 11KT plasma elevation during this month. More specifically, The SNP group (*W*5) reached 0.98 ± 0.18 ng ml^{-1}, whereas *W*1, *W*2, *W*3, *W*4 and *W*6 attained only 0.41 ± 0.07, 0.61 ± 0.20, 0.68 ± 0.16, 0.65 ± 0.16 and 0.43 ± 0.08 ng ml^{-1}, respectively (Fig. 8). The levels of plasma 11-KT were not as higher as T during gonadal differentiation. Later, 11-KT levels showed a moderate incrementing from January to February. This androgen is the most important steroid in fishes [3, 15, 24, 25] directly related with spermiogenesis [3, 15]. According to the differences in the levels of 11-KT observed in February between the SNP group and the LL groups, a second part of this study is in progress to corroborate the inhibitory effect of the LL treatments and elucidate a photo-labile period in this species for reducing gonadal development. Moreover, previous work in sea bass has suggested the role of 11-KT as a putative threshold determining early puberty in the sea bass and their unbalanced production might be limiting the stimulation of germ cell proliferation at the testicular level in this species [9, 23].

Differences in plasma E_2 pattern were detected around 7-7.5 months of age (early September), when the E_2 levels in the SNP group (*W*5) remained around 1.02 ± 0.12 ng ml^{-1}, whereas fish under LL regimes including groups *W*1, *W*3, *W*4 and *W*6 showed low T values (< 0.55 ± 0.05 ng ml^{-1}) at the same date. Fish under LL regimes including *W*2 showed comparable values to those observed in the SNP group at this time. Subsequently, values of E_2 varied until they reached an average value of 0.67 ± 0.12 ng ml^{-1} in January (Fig. 9).

Although E_2 is an important estrogen in females, several studies have reported that this hormone plays an important role in testicular growth during the early phases of development [19] and its receptor is present in testis of many teleost fishes [26, 30]. Steriodogenic enzymes, especially cytocrome P450-aromatase, convert T in E_2 [17, 18]. In this study, the maximum level detected was round 1 ng ml^{-1} coinciding with the accomplishment of the sexual differentiation process and early stages of gametogenesis (August–September). These results are in agreement with those reported by Papadaki et al. [21] in the sea bass with plasma levels around 0.8 ng ml^{-1} at similar dates which likely are regulated by FSH [16].

Additionally, high levels of E_2 (7 ng ml^{-1}) might be necessary to begin the renovation of SgA cells [1]. It is noticeably that in those fish exposed to LL that did not proceed into further stages of gonadal development, a dramatic decrease of plasma E_2 (about half of the values attained by controls) was displayed during September a period coinciding with testicular proliferation of stem cells. Thus, it supports the idea about the role of E_2 at early stages of gonadal development in males. However, a better understanding of the role of sex steroids as regulators of early stages of spermatogenesis seems to be crucial to clarify these statements [28].

4. Conclusions

- A long-term exposure to continuous light (LL), 12-month duration, during the whole sexual cycle including both pregametogenesis and gametogenesis is the most effective treatment to inhibit later stages of testicular development in male sea bass during their first year of life.
- The application of continuous light from 14 Aug-14 Sep (Window 3) was less effective in comparison to Window 2 (LL, 3 Sep-3 Oct) and Window 1 (1 Aug-1 Oct). The ineffectiveness of Window 4 (LL, 1 Aug-31 Aug) might be due to that the exposure to continuous light is necessary during the whole month of September for maintaining the inhibition of gonadal development which already started during August.
- Exposures to continuous light according to the application of Windows 1, 2, 3, and 6, provoked a significative decrease of plasma 11-KT levels during the peak of reproductive activity (February) while Window 4 showed similar plasma 11-Kt levels to those of the control group (Window 5, SNP). This indicates the importance of 11-KT during gametogenesis, particularly in advanced gonadal stages. This explains that the sexual maturation process might be controlled by photoperiod.
- High plasma E_2 levels observed in the control group (SNP) during the early stages of gametogenesis (August – September), showed the importance of this hormone during the process of testicular development. However, fish maintained under continuous light regimes showed a reduction of plasma E_2 levels in comparison to those in the control, suggesting that the administration of LL might affect the proliferation of testicular germ cells thus decreasing the production of E_2.

5. References

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