Accepted Manuscript

Influence of the lunar cycle on plasma melatonin, vitellogenin and sex steroids rhythms in Senegal sole, *Solea senegalensis*

C. Oliveira, N.J. Duncan, P. Pousão-Ferreira, E. Mañanós, F.J. Sánchez-Vázquez

PII: S0044-8486(10)00289-9
DOI: doi: 10.1016/j.aquaculture.2010.05.003
Reference: AQUA 629154

To appear in: *Aquaculture*

Received date: 5 January 2009
Revised date: 3 May 2010
Accepted date: 4 May 2010

Please cite this article as: Oliveira, C., Duncan, N.J., Pousão-Ferreira, P., Mañanós, E., Sánchez-Vázquez, F.J., Influence of the lunar cycle on plasma melatonin, vitellogenin and sex steroids rhythms in Senegal sole, *Solea senegalensis*, *Aquaculture* (2010), doi: 10.1016/j.aquaculture.2010.05.003

Influence of the lunar cycle on plasma melatonin, vitellogenin and sex steroids rhythms in Senegal sole, *Solea senegalensis*

C. Oliveira[1], N.J. Duncan[2], P. Pousão-Ferreira[3], E. Mañanós[4], F.J. Sánchez-Vázquez[1]*.

[1] Physiology Department, Faculty of Biology, Murcia University, Murcia, Spain

[2] IRTA, 43540 Sant Carles de la Rapita, Tarragona, Spain

[3] IPIMAR, Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal

[4] Institute of Aquaculture of Torre La Sal (IATS-CSIC), Castellón, Spain

* Corresponding Author:

Dr. F. Javier Sánchez Vázquez

Department of Physiology, Faculty of Biology

University of Murcia, Campus Espinardo

30 100 Murcia, SPAIN

javisan@um.es

Abstract

The effect of the moon light cycle on plasma melatonin rhythms was examined in Senegal sole (*Solea senegalensis*) exposed to natural outdoor or artificial indoor lighting conditions. Furthermore, in a second experiment, the effect of the lunar cycle on vitellogenin and sex steroids (Testosterone, T; Estradiol, E2; 11-ketotestosterone, 11kt) was studied using mature individuals during reproductive season. In the first experiment, during full moon, plasma melatonin peaked at night in covered tanks (deprived of night illumination) from both outdoor (133.2 ± 12.8 pg ml^{-1}) and indoor (190.6 ± 41.5 pg ml^{-1}) groups. However, for fish in the open tanks, exposed to approximately 0.3 lux of illumination, nocturnal plasma melatonin was significantly reduced ($p<0.05$), approaching to mid-light (ML) values, (79.6 ± 7.1 and 81.8 ± 14.0 pg ml^{-1}, for outdoor and indoor groups, respectively). During new moon a similar pattern was observed in outdoor group: fish in the covered tank showed higher melatonin values than those in the open tank, which were exposed to the near undetectable night illumination. In the second experiment, plasma sex steroid concentrations were significantly higher during the full moon compared to the new moon. In the case of females, E2 concentration reduced from 2.4 ± 0.6 to 0.4 ± 0.1 ng ml^{-1} between full and new moon samplings, while T decreased from 0.3 ± 0.0 to 0.2 ± 0.0 ng ml^{-1}. Vitellogenin, however, did not show such differences between moon phases. In males, 11kt exhibited a plasma concentration of 14.3 ± 2.1 ng ml^{-1} during full moon and 4.7 ± 0.7 ng ml^{-1} during new moon while T values were 2.6 ± 0.4 and 1.0 ± 0.1 ng ml^{-1} for full and new moon, respectively. In conclusion, these findings pointed out the high sensitivity to moon light of the Senegal sole, which could be using the melatonin signalling to synchronize their reproduction rhythms to the lunar cycle.

Key words: lunar rhythms, melatonin, sex steroids, vitellogenin, *Solea*

1. Introduction

Most environmental events occur cyclically, with different periodicities (annual, monthly and daily), influencing biological rhythms. The lunar cycles act through two different pathways: the tides and moonlight. This interacts with daily and seasonal environmental changes influencing several aspects of physiology and behaviour in mammals, birds and fish (Leatherland et al., 1992; Zimecki, 2006). For example, in the case of fish, the genus *Siganus* sp. shows evidence of a relationship between reproduction and environmental changes induced by the moon, with peaks of spawning and sex steroids, occurring once a month at a species-specific lunar phase (Takemura et al., 2004a).

Light is an important synchronizer of biological rhythms (Aschoff 1981). In vertebrates, photoperiod is transduced by the pineal organ into a melatonin rhythm. The duration of the nocturnal rise of melatonin is photoperiod-dependent, while the amplitude of elevation is lower in a dim illuminated (full moon) than in a dark night (new moon), thus providing the animal with daily, seasonal and lunar information (Reiter, 1993). In fish the pineal organ and its hormone melatonin are likely to be the mediators between environmental cycles and reproduction rhythms (Amano et al., 2000; Bayarri et al., 2004b; Bromage et al., 2001). Melatonin is thought to act on the hypothalamic-pituitary-gonad axis, controlling reproduction timing and the production of sex steroids in the gonads. However, the exact role of melatonin regulation is yet unknown (Falcón et al., 2007).

Senegal sole (*Solea senegalensis*) is a species with nocturnal rhythms of behaviour, melatonin and spawning (Bayarri et al., 2004a; Oliveira et al., 2009b). This species also showed seasonal periodicity in their melatonin rhythms, with water temperature modulating the amplitude of the rhythm (Vera et al., 2007), and

reproduction rhythms, with spawning, vitellogenin and sex steroids having a peak in spring (Anguis and Cañavate 2005; Gúzman et al., 2008). In addition, this species is very sensitive to artificial light at night, as a 1 hour light pulse of approximately 1 lux reduced melatonin titres to daytime values (Oliveira et al., 2007). Furthermore, the Senegal sole pineal is hypertrophied and shows a marked asymmetry in its position, reflecting an adaptation to the demersal life of this flatfish (Confente et al., 2008). This high sensitivity of Senegal sole to light can be useful for the detection of moonlight, possibly enabling synchronization to the lunar light cycle. Indeed, lunar rhythms of behaviour (Vinagre et al., 2006) and spawning (Oliveira et al., 2009b) have been observed in this species. The effect of natural outdoor moonlight on melatonin production, however, remains unknown.

The objective was to study light perception of the lunar cycle and its transduction into a melatonin signal by Senegal sole. To this end, we investigated the influence of nocturnal illumination in Senegal sole kept outdoor submitted to a natural moon light cycle, or kept indoor exposed to an artificial light cycle, mimicking the lunar phases (full moon vs. new moon). Furthermore, vitellogenin and sex steroids (estradiol, testosterone and 11keto-testosterone) were also determined in order to investigate the effects of the lunar cycle on the regulation of reproduction.

2. Material and Methods

2.1. Animals and facilities

The Senegal sole used for experiment 1 were spawned from wild broodstock (F1 generation) and reared in captivity in a research centre in the north of Spain (Instituto Español de Oceanografía - Planta de Cultivos EL BOCAL, Santander, Spain). Fish of three or more years of age were transferred to IRTA Sant Carles de la Rapita

(40º36.9'N0º36'E, Tarragona, Spain) in January 2006, and at the start of the experiment (November 2006) they were separated between two groups: one outdoorheld under natural conditions of photoperiod and temperature, and the other indoor, held under simulated natural conditions. All fish were taken from the same population that had a sex ratio of males to females of 1:0.7.

The outdoor group consisted of 32 fish with mean body weight of 733 ± 45 g, held in two 3000 L rectangular (5.5 x 0.8 x 0.8 m) fibreglass tanks (n=16 fish per tank), while the indoor group consisted of 24 Senegal sole with average weight of 883 ± 52 g held in two 2000 L circular (1.7 m diameter) fibreglass tanks (n=12 fish per tank). In each group, one of the tanks was covered with black shade net. All tanks were approximately 1 m deep and the water was pumped from the "Bahia Alfacs" in the Mediterranean Sea, and filtered to 50 µm with pressurised sand filters. Each week the fish were fed the following regimen over 6 days: Le-7 Elite (Skretting, Burgos, Spain) on three days, polychaetes (*Nereis virens*) (Seabait, Ashington, UK) on one day, cooked mussel (*Mytilus galloprovincialis*) (Frigorificos Berbes Fribesa, Vigo, Spain) on one day and chopped squid (*Loligo gahi*) (Rabade, de Rabade, Lugo, Spain) on one day. Dry diet was fed at 0.5 % biomass daily and wet diet was fed at 0.8 % biomass daily.

For experiment two, we used a group of 118 wild broodstock that had been kept for over 3 years in 18,000 L outdoor tanks with 1.2 m deep in the facilities of the Aquaculture Research Centre IPIMAR (37°1'59.72"N, 7°49'12.12"W, Olhão, Portugal). The 16 individuals used in this experiment had a mean body weight of 1742 ± 423 g and mean total length of 53 ± 4 cm. The sex ratio was 1:1. The lighting conditions were natural (photoperiod and lunar cycle) and food was provided daily in the form of polychaetes, mussels and squid.

In both facilities, the water in the tanks showed very low turbidity, and tanks were supplied with continuous aeration and water exchange in excess of 600% daily. Each fish was identified with an internal passive integrated transponder (PIT) tag (ID-100 TROVAN UNIQUE 12 mm tags, Zeus Euroinversiones, Madrid, Spain). Handling of the fish was in accordance with the European Union Directive (EEC, 1986) for the protection of animals used for experimental and other scientific purposes.

2.2. Experimental design

Experiment 1: Influence of the lunar cycle on melatonin rhythms

The two outdoor tanks (exposed to natural moon light) were placed away from artificial light contamination. One was totally open, with Senegal sole exposed to natural photoperiod and lunar cycle, and the other was covered with shade net, with fish exposed to an attenuated natural photoperiod, but not to a lunar light cycle. Water temperature naturally oscillated between 10 and 18ºC during the months of the experiments (November to March). Maximum light intensity reaching the water surface during the day was 4500 lux and 150 lux (Precision luxmeter MX-Elektronik Mini-Lux, OPTE-E-MA Engineering GmbH, Martinroda – Germany), respectively for the open and covered tank; during full moon it was 0.3 and 0.01 lux respectively for open and covered tank, while it was undetectable in both tanks during new moon. These values are the means obtained around the tanks during a cloudless day and night.

To artificially simulate a lunar cycle, the indoor group of Senegal sole was used. Fish were reared under natural photoperiod (produced with artificial light, and simulating dawn/dusk), and one was illuminated with a dim artificial light during the entire dark phase (0.3 lux), mimicking moonlight in the nights corresponding to full moon, and the other was covered with a light proof cover, to give complete darkness

during all nights. To avoid changing the spectral composition of light while reducing light intensity, the fluorescent tube (GRO-LUX, 40 W, Germany) was covered with aluminium foil in which holes were made until the desired light intensity was reached. The fluorescent tube was 1.25 m long, which approached the diameter of the tank and provided uniform illumination. Water temperature naturally oscillated between 11 and 18ºC and maximum light intensity reaching the water surface during the day was around 200 and 25 lux, respectively for the night illuminated and covered tank. During the nights when full moon was mimicked, the light intensity reaching the surface of the water had a mean value of 0.35 lux in the illuminated tank and 0.01 lux in the covered tank.

Sampling was performed over two complete lunar cycles. To avoid the effect of cloud cover, samplings during full moon were performed on cloudless nights. In each full / new moon, blood samples were taken in mid-dark (MD) and in mid-light (ML) from all tanks (n=8 and n=6 in each sampling, respectively for outdoor and indoor groups). Before sampling, each fish was anesthetized in 0.25 ppt 2-phenoxyethanol (Sigma, Spain), and then blood samples (~1 ml) were obtained with heparinized syringe, by caudal puncture. MD samplings were carried out under the moonlight, in full moon nights, while during new moon a dim red light, known not to be perceived by Senegal sole (Oliveira et al., 2007), was used and fish heads were covered with aluminium foil. For ML samplings the light was sunlight. Blood was transferred to heparinised eppendorf tubes on ice until plasma was separated by centrifugation at 4^{o}C. Plasma samples were stored at -80ºC until the melatonin levels were measured. Melatonin levels in plasma samples were measured by a Radioimunoassay commercial Kit (Melatonin Direct RIA, Biosource, Belgium), with a lower limit of quantification (LLOQ) of 2 pg/ml, as described by Oliveira et al., (2007).

Experiment 2: Influence of the lunar cycle on vitellogenin and sex steroids rhythms

To investigate the influence of the lunar cycle on the rhythms of vtg, E_2 and T in females, and 11kt and T in males, Senegal sole from the wild broodstock of Aquaculture Research Centre IPIMAR were used. Sampling was performed during the reproductive season, in spring. Light intensity at the surface of the water was 0. 2 and 0.01 lux during the full and new moon, respectively. During the day, the maximum light intensity reaching the water surface was 1500 lux. Fish were blood sampled during full moon (20 April) and new moon (5 May) nights, at MD, and the procedure was similar to that described above (n=8 for females and n=8 for males). Blood was transferred to heparinised eppendorf tubes with 10 µl of aprotinin prior to centrifugation, and plasma samples were stored at -20ºC until vtg and sex steroids analysis. In order to assess the plasma concentrations of vtg, T and E_2 in females, and T and 11kt in males, samples were analysed in the facilities of the Institute of Aquaculture of Torre la Sal (IATS) (Castellón, Spain), by enzyme-linked immunosorbent assay (ELISA) using protocols previously validated for Senegal sole plasma samples (Guzmán et al., 2005 , 2008).

2.3. Statistical Analysis

To determine the existence of differences between different groups, mean melatonin levels were subjected to one-way ANOVA, followed by Duncan's post hoc test (with a degree of significance of $p < 0.05$). Differences in vitellogenin and sex steroid levels between moon phases were compared using a Student's t test (with a degree of significance of $p < 0.01$).

3. Results

3.1. Experiment 1: Influence of the lunar cycle on melatonin rhythms

Daily rhythms of plasma melatonin during full moon

In the outdoor group, a similar pattern of plasma melatonin concentrations was observed during both full moon nights. Melatonin concentrations in the open tank had a mean value of 79.6 ± 7.1 pg ml^{-1} at MD, which was not statistically different from daytime levels (ML) in both tanks, 48.7 ± 4.3 and 54.8 ± 6.0 pg ml^{-1} (for opened and covered tank, respectively) (fig. 1). However, in the tank covered with shade netting the melatonin values at night (MD) were significantly higher (133.2 ± 12.8 pg ml^{-1}).

In indoor group, the pattern was similar (fig. 2). During both samplings, the animals in the night illuminated tank presented melatonin concentrations significantly lower than those in the covered tank (81.8 ± 14.0 vs 190.6 ± 41.5 pg ml^{-1}) and comparable with those in ML in both tanks (48.5 ± 7.2 pg ml^{-1} for the night illuminated tank and 43.6 ± 9.0 pg ml^{-1} in the covered tank). Between outdoor and indoor groups, significant statistical differences were found between MD sampling on both covered tanks.

Daily rhythms of plasma melatonin during new moon

During new moon nights in the outdoor group, nocturnal plasma melatonin concentrations were significantly lower in Senegal sole in the open tank than those in the covered tank (fig. 3). This response was repeated in both sampling months. At MD the values were 33.9 ± 5.2 and 126.8 ± 24.2 pg ml^{-1} and in ML 21.5 ± 3.1 and 27.7 ± 2.7 pg ml^{-1} respectively for open and covered tank. The ML values from both tanks were statistically comparable with the values observed in the open tank at MD.

3.2. Experiment 2: Influence of the lunar cycle on vitellogenin and sex steroids rhythms

When the influence of lunar cycle was tested in mature individuals of Senegal sole, sex steroids showed significantly higher levels during the full moon, when compared to a new moon (fig. 4). In the case of females, E_2 concentration reduced from 2.4 ± 0.6 to 0.4 ± 0.1 ng ml^{-1} between full and new moon samplings, while T descended from 0.3 ± 0.0 to 0.2 ± 0.0 ng ml^{-1}. However, vitellogenin concentrations exhibited no differences between moon phases, with similar values of 2.6 ± 0.7 mg ml^{-1} during full moon, and 2.7 ± 0.7 mg ml^{-1} during new moon. In males, the same pattern was observed for the concentrations of sex steroids: 11kt had a plasma concentration of 14.3 ± 2.1 ng ml^{-1} during full moon and 4.7 ± 0.7 ng ml^{-1} during new moon and T values were 2.6 ± 0.4 and 1.0 ± 0.1 ng ml^{-1} for full and new moon, respectively.

4. Discussion

A clear effect of moon light on plasma melatonin, and sex steroids was found in Senegal sole. In the first experiment, the animals in both outdoor and indoor groups, showed inhibited nocturnal melatonin production during full moon conditions. This result is in accordance with previous indoors observations that pointed out Senegal sole as a species with a melatonin rhythm very sensitive to one hour light pulses of dim artificial light, and the threshold of intensity capable of inhibiting nocturnal melatonin was approximately 1 lux (around full moon intensity; Oliveira et al., 2007). However, nothing was known on the effects of natural outdoor light from the moon. Thus, our results bring about new insights into the natural environment responses revealing that during the new moon, melatonin values rose in the covered tank, but were lower in the open tank, though the light intensity at the water surface was undetectable. This result

establishes that Senegal sole can detect very dim levels of scattered light contamination at night.

Lunar melatonin rhythms have been reported in tropical fish species, such as golden rabbitfish, *Siganus guttatus,* in which a similar pattern of inhibition was observed both *in vivo* and *in vitro* conditions (Takemura et al., 2004b; Takemura et al., 2006). When the fish were exposed to moonlight at midnight of both full and new moon, an immediate and significant decrease in the plasma melatonin levels was produced in both moon phases, when compared with values of fish placed in a covered tank (Takemura et al., 2004b). Furthermore, cultured pineal organs respond to environmental light cycles as in the whole fish (Takemura et al., 2006). The sea grass rabbitfish, *Siganus cnaliculatus,* presented a different lunar melatonin rhythm: during full moon, the concentration of plasma melatonin in fish exposed to natural conditions was significantly lower than those from fish maintained in dark experimental conditions, while in new moon the values were similar. Otherwise, when fish were subjected to artificial illumination during the night, melatonin production was suppressed (Rahman et al., 2004), like in Senegal sole. The different response to moon light in Senegal sole and the two rabbitfish species mentioned may be due to differences in light sensitivity of the pineal organ or a species-specific response to the lunar cycle.

Senegal sole are strictly nocturnal fish, as they display locomotor activity preferentially during the night (Bayarri et al., 2004a). The nocturnal lifestyle of Senegal sole may make them particularly sensitive to environmental light at night and prone to show lunar rhythmicity. In fact, a semi-lunar activity pattern in the use of the intertidal mudflats by Senegal sole has been reported (Vinagre et al., 2006), with a highest average density of Senegal sole occurring during full moon at dawn/dusk. During

quarter and full-moon nights its distribution extended over the lower and upper mudflat, but during the new moon, colonisation was restricted to the lower mudflat.

Sex steroids in Senegal sole appeared to exhibit a lunar rhythm in both females and males, although a possible effect of the natural seasonal oscillation cannot be ruled out. Values of T, E2 and 11KT were significantly higher during full moon than during new moon. As for plasma vtg, however, no differences were observed between full and new moon nights and concentrations were similar to those recorded in this species for the months the sampling took place (Guzmán et al., 2008). In male forktail rabbitfish, *S. argenteus*, and golden rabbitfish, *S. gutattus*, both T and 11KT appeared to have a clear rhythmic pattern synchronised to the lunar cycle, peaking during full and new moons, respectively. In the case of the golden rabbitfish, the sex steroids peak was followed by a dramatic fall that coincided with spawning during the first lunar quarter (Rahman et al., 2000b; 2003). A similar pattern can be observed in Senegal sole, since daily and lunar spawning rhythms have been recently observed in this species, with a higher incidence of spawning events during the early hours of the last quarter and new moon nights (Oliveira et al. 2009b). Therefore, it should not be surprising that this species presented also lunar rhythmicity in sex steroids. The synchronization of Senegal sole spawning and sex steroids rhythms to the lunar cycle could be related either to the moonlight cycle or to the gravitational effects, since these are the two different pathways of interaction between moon and earth, and the second one should not be discarded. These findings strongly suggest that Senegal sole is able to synchronise reproduction to the lunar cycle, thus spawning can be directed towards the darkest nights to avoid predators and ensure maximal survival of the offspring. Indeed, reproduction should not be considered an exclusively seasonal phenomenon, since

female Senegal sole exhibited daily rhythms of sex steroids, in addition to seasonal rhythmicity (Oliveira et al., 2009a).

Tropical fish live in a relative stable environment with little photoperiod and temperature changes throughout the year, so they are thought to use lunar cues to synchronize many biological and physiological processes (Rahman et al., 2000a; Takemura et al., 2004b). Our results, however, showed lunar rhythms in a teleost from temperate zones, so the influence of the lunar cycles should not be put aside in these regions. In fact, lunar spawning rhythms have been registered in other species such as gilthead sea bream, which preferred the full moon to spawn (Saavedra and Pousão-Ferreira, 2006).

5. Conclusion

This research provides new insights into the effects of the moon light in the phototransduction and reproductive hormones of Senegal sole. The natural light from the full moon inhibited the nocturnal production of melatonin, which was also affected by ambient light during new moon in the open tank, attributing to this species a capacity to detect very low levels of illumination. Furthermore, the lunar cycle also seems to influence sex steroids levels in males and females, which remained elevated during a full moon and significantly lower during a new moon. As for vitellogenin, no influence could be detected. This influence could be related to moonlight cycle or gravitational effects. Together these results illustrate the ability of Senegal sole to perceive moon light and produce hormonal signals to synchronize their reproduction rhythms. Such evidences provides a better understanding of the light response and reproductive physiology of Senegal sole, allowing aquaculturists to improve broodstock management and lighting protocols.

Acknowledgements

This work has been financed by the Spanish Ministry of Education and Science (MEC) through project *Reprosolea* AGL2004-07984-CO2-01/ACU to FJSV and project AGL2006-13777-C03-01 to EM. CO received an FPI fellowship from the same institution. Thanks also to Josep Luis Celades, Noelia Gras and Joaquín Canoura from IRTA for the maintenance of sole and to those who helped during nocturnal samplings: Caridad Meseguer from UMU, Ignacio Carazo from IRTA, Claudia Bandarra, Ana Marçalo from IPIMAR and Wilson Pinto from CCMAR.

References

Amano, M., Iigo, M., Ikuta, K., Kitamura, S., Yamada, H., Yamamori, K., 2000. Roles of melatonin in gonadal maturation of underyearling precocious male masu salmon. Gen. Comp. Endocrinol. 120, 190-197.

Anguis, V., Cañavate, J.P., 2005. Spawning of captive Senegal sole (*Solea senegalensis*) under a naturally fluctuating temperature regime. Aquaculture 243, 133-145.

Aschoff, J., 1981. Handbook of Behavioural Neurobiology, vol.4: Biological rhythms. Plenum, New York.

Bayarri, M.J., Muñoz-Cueto, J.A., Lòpez-Olmeda, J.F., Vera, L.M., Rol de Lama, M.A., Madrid, J.A., Sánchez-Vázquez, F.J., 2004a. Daily locomotor activity and melatonin rhythms in Senegal sole (*Solea senegalensis*). Physiol. Behav. 81, 577-583.

Bayarri, M.J., Rodríguez, L., Zanuy, S., Madrid, J.A., Sánchez-Vázquez, F.J., Kagawa, H., Okuzawa, K., Carrillo, M., 2004b. Effect of photoperiod manipulation on the daily rhythms of melatonin and reproductive hormones in caged European sea bass (*Dicentrarchus labrax*). Gen. Comp. Endocrinol. 136, 72-81.

Bromage, N., Porter, M., Randall, C., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role on photoperiod and melatonin. Aquaculture 197, 63-98.

Confente, F., El M'Rabet, A., Ouarour, A., Voisin, P., De Grip, W.J., Rendón, M.C., Muñoz-Cueto, J.A., 2008. The pineal complex of Senegalese sole (Solea senegalensis): Anatomical, histological and immunohistochemical study. Aquaculture 285, 207-215.

EEC, 1986. Council Directive 86/609 EEC for the protection of animals used for experimental and other scientific purposes. Official Journal L358 (18/12/1986), 1-28.

Falcón, J., Besseau, L., Sauzet, S., Bouef, G., 2007. Melatonin effects on the hypothalamus-pituitary axis in fish.Trends Endocrinol. Metabol. 18, 81-88.

Guzmán, J.M., Marull, E., Ramos, J., Mylonas, C.C., Olmos, V., Mañanós, E., 2005. Indución hormonal a la puesta del lenguado senegalés (*Solea senegalensis*) mediante la administración de sistemas de GnRHa de liberación sostenida. Actas X Congreso Nacional de Acuicultura. Universidad Politécnica de Valencia, Valencia, Spain, pp. 490-491.

Guzmán, J.M., Norberg, B., Ramos, J., Mylonas, C.C., Mañanós, E., 2008. Vitellogenin, steroid plasma levels and spawning performance of cultured female Senegalese sole (*Solea senegalensis*). Gen. Comp. Endocrinol. 156, 285-297.

Leatherland, J.F., Farbridge, K.J., Boujard, T., 1992. Lunar and semi-lunar rhythms in fishes, in: Ali, M.A. (Eds.), Rhythms in Fishes. Plenum Press, NYC, p83-107.

Oliveira, C., Ortega, A., López-Olmeda, J.F., Vera, L.M., Sánchez-Vázquez, F.J., 2007. Influence of constant light and darkness, light intensity and spectrum on plasma melatonin rhythms in Senegal sole. Chronobiol. Int. 24, 615-627.

Oliveira, C., Vera, L.M., López-Olmeda, J.F., Guzmán, J.M., Mañanós, E., Ramos, J., Sánchez-Vázquez, F.J., 2009a. Monthly day/night changes and seasonal daily rhythms of sexual steroids in Senegal sole (*Solea senegalensis*) under natural fluctuating or controlled environmental conditions. Comp. Biochem. Physiol. A 152, 168-175.

Oliveira, C., Dinis, M.T., Soares, F., Cabrita, E., Pousão-Ferreira, P., Sánchez-Vázquez, F. J., 2009b. Lunar and daily spawning rhythms of Senegal sole (Solea senagalensis). J. Fish Biol. 75, 61-74.

Rahman, M.S., Takemura, A., Takano, K., 2000a. Correlation between plasma steroid hormones and vitellogenin profiles and lunar periodicity in the female golden rabbitfish, *Siganus guttatus* (Bloch). Comp. Biochem. Physiol. 127B, 113-122.

Rahman, M.S., Takemura, A., Takano, K., 2000b. Lunar synchronization of testicular development and plasma steroid hormone profiles in the golden rabbitfish. J. Fish Biol. 57, 1065-1074.

Rahman, M.S., Takemura, A., Nakamura, S., Takano, K., 2003. Rhythmic changes in testicular activity with lunar cycle in he forktail rabbitfish. J. Fish Biol. 62, 495-499.

Rahman, M.S., Kim, B.-H., Takemura, A., Park, C.-B., Lee, Y.-D., 2004. Effects of moonlight exposure on plasma rhythms in the seagrass rabbitfish, *Siganus cnaliculatus*. J. Biol. Rhythms. 19, 325-334.

Reiter, R.J., 1993. The melatonin rhythm: both a clock and a calendar. Experientia. 49, 654-664.

Saavedra, M., Pousão-Ferreira, P., 2006. A preliminary study on the effect of lunar cycles on the spawning behaviour of the gilt-head sea bream, *Sparus aurata*. J. Mar. Biol. Ass. U.K. 86, 899-901.

Takemura, A., Rahman, Md.S., Nakamura, S., Ju Park, Y., Takano, K., 2004a. Lunar cycles and reproductive activity in reef fishes with particular attention to rabbitfishes. Fish Fish. 5, 317-328.

Takemura, A., Susilo, E.S., Rahman, Md., Morita, M., 2004b. Perception and possible utilization on moonlight intensity for reproductive activities in a lunar-synchronized spawner, the golden rabbitfish. J. Exp. Zool. 301, 844-851.

Takemura, A., Ueda S., Hiyakawa, N., Nikaido, Y., 2006. A direct influence of moonlight intensity on changes in melatonin production by cultured pineal glands of the golden rabbitfish *Siganus guttatus*. J. Pineal Res. 40, 236-241.

Vera, L.M., Oliveira, C., Lopez-Olmeda, J.F., Ramos, J., Mañanós, E., Madrid, J.A., Sánchez-Vázquez, F.J., 2007. Seasonal and daily plasma melatonin rhythms and reproduction in senegal sole kept under natural photoperiod and natural or controlled water temperature. J. Pineal Res. 43, 50-55.

Vinagre, C., França, S., Cabral, H.N., 2006. Diel and semi-lunar patterns in the use of intertidal mudflat by juveniles of Senegal sole, *Solea senegalensis*. Estuarine Coastal and Shelf Sci. 69, 246-254.

Zimecki, M., 2006. The lunar cycle: Effects on human and animal behaviour and physiology. Postepy Hig. Med. Dosw. 60, 1-7.

Figure Captions

Figure 1. Plasma melatonin concentrations in Senegal sole from “outdoors” group, during two full moon nights, in December (A) and in March (B) (values expressed as mean ± S.E.M.). White columns correspond to ML samplings, black columns to MD samplings in the covered tank, and grey columns to MD samplings in the opened tank. White circle symbolizes the full moon: opened in the open tank, and covered (dashed white circle) in the covered tank. The numbers in brackets indicate the number of fish sampled per point. Different letters indicate different groups with significant statistical differences (ANOVA, Duncan’s test, $p<0.05$).

Figure 2. Plasma melatonin concentrations in Senegal sole from “indoors” group, during two full moon nights, in December (A) and in March (B) (values expressed as mean ± S.E.M.). White columns correspond to ML samplings, black columns to MD samplings in the covered tank, and grey columns to MD samplings in the lightened tank. White circle indicates lightened tank and dashed white circle the covered tank. The numbers in brackets indicate the number of fish sampled per point. Different letters indicate different groups with significant statistical differences (ANOVA, Duncan’s test, $p<0.05$).

Figure 3. Plasma melatonin concentrations in Senegal sole from “outdoors” group, during two new moon nights, in December (A) and in February (B) (values expressed as mean ± S.E.M.). White columns correspond to ML samplings, black column to MD sampling in the covered tank, and grey columns to MD samplings in the opened tank. Black circle symbolizes the new moon: opened in the open tank and covered (dashed black circle) in the covered tank. The numbers in brackets indicate the number of fish

sampled per point. Different letters indicate different groups with significant statistical differences (ANOVA, Duncan's test, $p<0.05$).

Figure 4. Plasma sexual steroids from female (a) and male (b) Senegal sole during full and new moon nights (values expressed as mean ± S.E.M.). In (a) black columns represent testosterone values while grey columns, estradiol levels. In (b) black columns represent testosterone values and grey columns, 11-ketotestosterone (11kt) levels. The numbers in brackets indicate the number of fish sampled per point. ** indicates statistical differences with a significance of $p<0.01$ and *** with a significance of $p<0.001$ (student t test).