ANNUAL VARIATIONS OF MATURATION INDUCING STEROID IN TWO CULTURED GENERATIONS OF SENEGALESE SOLE, *SOLEA SENEGALENSIS*.

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Introduction:

The Senegalese sole is a highly valuable species in Southern Europe. But, the development of sole aquaculture is limited by its dependence on captures from the wild to constitute broodstock of wild fish, since F1 soles (hatched in captivity) are unable to produce fertilized spawning in captivity. In a previous study, an F2 generation was obtained by hormonal treatment of F1 breeders [1]. These F2 fish were grown to puberty and, spawning performance and plasma profiles of sexual steroids (testosterone (T), estradiol (E2) and 11-ketotestosterone (11-KT)) and vitellogenin (VTG) followed through three consecutive years [2], and compared with previous data on F1 [3] and wild [4] sole breeders. The present study was aimed to add further information by analyzing plasma profiles of the maturation inducing steroid (MIS; 17alfa,20beta-dihidroxi-4-pregnen-3-one) in F1 and F2 broodstock, in an attempt to get comparative data between all three generations (wild, F1, F2) and potentially detect endocrine differences that could be associated with failed reproduction in cultured generations.

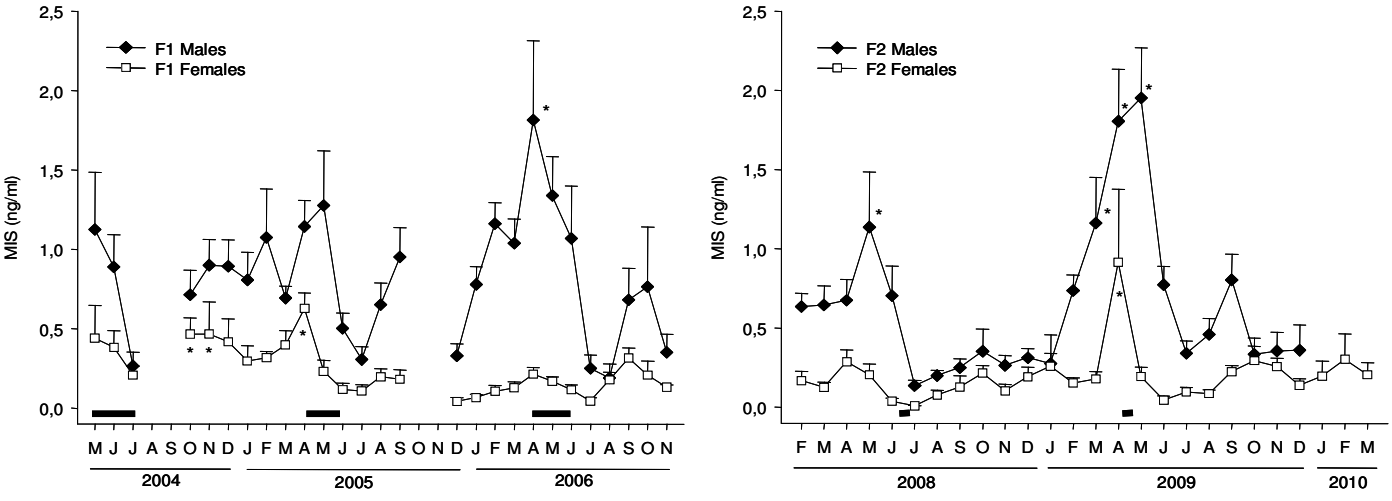
Methods:

Fish were reared at our facilities (40ºN, 0ºE) under natural photoperiod and temperature. Females and males were sampled monthly from puberty (3 years old) for weight, length and blood through three consecutive years. Plasma levels of MIS were analyzed by using a newly developed ELISA method. Specific antibodies were produced in rabbits (Agrisera AB, Sweeden) against conjugated MIS-CMO, kindly provided by Dr. A. Scott (CEFAS, UK); a commercial acetylcholinesterase tracer (Cayman, USA) was used. The ELISA showed a sensitivity (Bo-2SD) of 40 pg/ml, and intra- and inter-assay coefficients of variation of 2.49 % (n=11) and 9.91 % (n=69), respectively. One wayANOVA ($p<0.05$) was performed for statistical analysis.

Results and Discussion:

Plasma levels of the MIS are shown in figure 1. Two annual peaks of the MIS were detected in F1 females, in spring (major spawning season) and autumn (secondary spawning season). Significant differences from basal levels (minimum detected in December 2005) were only found in October-November 2004 and April 2005, on 4+ year-old fish. Profile and plasma levels in F2 females were similar to those of F1's, with significant peak levels in spring and minor elevations in autumn. Similarly, wild broodstock have also shown maximum MIS levels in January-February, coinciding with the beginning of their spawning period [4]. Previously described annual profiles of T, E2 and VTG in F1 and F2 soles also showed annual peak spring levels, correlated with highest maturational degree of the gonad and beginning

Fig. 1. Plasma levels of MIS in F1 (left) and F2 (right) generations of Senegalese sole during three consecutive years after puberty, when fish were 3, 4 and 5 years old. Significant differences respect minimum values for each sex and generation are represented by *. Spawning periods are indicated by horizontal black bars. Data given as mean ± SEM (n=5-8).



of spawning [2,3]. The spawning period seemed to be shorter in F2 than in F1 broodstock (Fig. 1).

In F1 males, annual profiles of MIS were similar than those observed in F1females, with highest levels in spring and lower elevations in autumn. Peak levels of MIS were observed in April-May of each year, being significantly different from basal levels (August 2006) only during the spring of 2006 (5 years old). F2 males showed a similar profile, with significant peak levels of MIS in May 2008 (3 years old) and March-April-May 2009. The annual profiles of T and 11-KT analysed in previous studies [2, 3] were similar to those obtained for MIS, as occurred in females. In general, levels of MIS were higher in males than in females of both generations, similarly to what has been previously described for wild breeders [4].

Conclusion:

The levels and annual profiles of MIS found in F1 and F2 sole were similar, with major peaks during the main spawning season (spring), and lower elevations during the secondary spawning period (autumn). These profiles were similar to those of T, 11-KT, E2 and VTG previously described for these cultured generations. No fertilized spawning were obtained from F1 or F2 breeders. Previous works by others [4] showed similar hormonal profiles in wild broodstock, which do reproduce normally in captivity. Thus, no differences have been observed between the hormonal profiles of wild, F1 and F2 sole breeders that could be associated to the observed differences in the reproductive performance between wild and cultured sole generations.

Research funded by the Spanish Ministry of Science and Innovation (MICIIN; AGL2006-13777-C03-01) and JACUMAR (2009, III National plan for the cultivation of sole).

References:

[1]GUZMÁN JM, RAMOS J, MYLONAS CC, MAÑANÓS E. 2011. Comparative effects of human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone agonist (GnRHa) treatments on the stimulation of male Senegalese sole (*Solea senegalensis*) reproduction. Aquaculture, 316: 121-128.

[2]BAYARRI MJ, PIQUER V, GUZMÁN JM, RAMOS J, MAÑANÓS E. 2010. F2 generation of Senegalese sole: biometrical, hormonal and spawning monitoring. In: Proceedings of Aquaculture Europe 2010. Porto, Portugal.

[3]GUZMÁN JM, NORBERG B, RAMOS J, MYLONAS CC, MAÑANÓS E. 2008. Vitellogenin, steroid plasma levels and spawning performance of cultured female Senegalese sole (*Solea senegalensis*). Gen.Comp. Endocrinol., 156: 285-297.

[4]GARCÍA-LÓPEZ A, ANGUIS V, COUTO E, CANARIO AVM, CAÑABATE JP, SARASQUETE C, MARTÍNEZ-RODRÍGUEZ G. 2006. Non-invasive assessment of reproductive status and cycle of sex steroid levels in a captive wild broodstock of Senegalese sole *Solea senegalensis* (Kaup). Aquaculture, 254: 583-593.