FSH PLASMA LEVELS DURING TESTICULAR RECRUDESCENCE OF PRECOCIOUS AND NON PRECOCIOUS MALE EUROPEAN SEA BASS USING A NEWLY DEVELOPED SPECIES SPECIFIC ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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Introduction:

Sea bass (*Dicentrarchus labrax*) is an important cultured marine fish in Europe in which puberty is intensively studied. In this species large males attain puberty earlier than small ones [1]. Therefore the high rate of growth achieved under intensive culture, results in a high proportion of precocious males before attaining marketable size. At puberty the individual becomes able to reproduce for the first time implying the functional competence of the brain–pituitary–gonad (BPG) axis [2]. Gonadotropins, the follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are components of this axis and are known to be fundamental in the regulation of steroideogenesis and gametogenesis. Although the duality of gonadotropins in fish is well established, the distinct role of piscine FSH and LH is still not clear for most species studied so far mainly due to the scarcity of assays to measure FSH in plasma. By comparing FSH and LH plasma levels in male sea bass that decide or not to mature, we aimed to gain knowledge on the functional discrimination of sea bass gonadotropins in the regulation of the onset of puberty. These data was generated using a newly developed species specific ELISA for FSH.

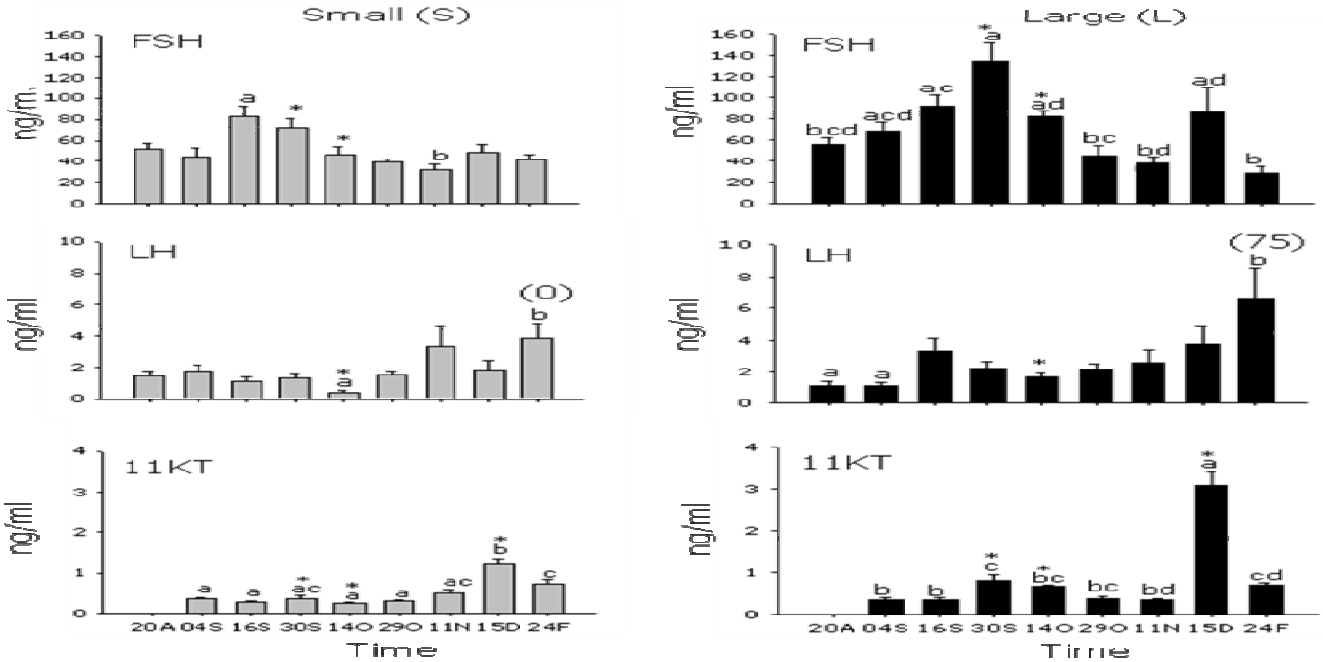
Methods:

Animals and experimental conditions: In early March six month old juvenile male sea bass were placed under simulated natural photoperiod (NP, 40º NL). At sampling fish were size sorted into two groups, one consisting of small (S) and the other of large fish (L). Fish were sampled from August to February, blood collected and plasma used for FSH, LH [3] and 11ketotestosterone (11KT) [4] analysis. Gonads were dissected, processed and testicular growth stages classified according to [1]. *FSH Assay:* The ELISA developed for sea bass FSH used polyclonal antibodies raised against recombinant FSHβ produced in yeast (*Pichia pastoris)* and recombinant FSH dimer produced in the baculovirus system as standard curve and coating.

Results and Discussion:

Small fish displayed a remarkable delay in the progression of testicular development that prevented them from achieving spermiation in February. Large fish entered in spermatogonial mitosis towards meiosis in

Fig.1. Plasma profiles of FSH, LH and 11KT of S and L yearling male sea bass during the first reproductive cycle. Different letters indicate differences among sampling points. Asterisks denote differences between groups at the same sampling time. In brackets percentage of spermiating males in February.



September, almost 1.5 month earlier than S group attaining full spermiogenesis in February. The highest FSH plasma levels were observed in L fish which almost doubled the FSH values of S fish at this time ($p<0,05$). These high levels of FSH were linked to spermatogonial proliferation and associated with the onset of gametogénesis, as in other teleosts, [5]. A transient FSH peak ($p<0.05$) was observed at mid December in L fish coincident with increasing levels of LH suggesting a possible collaborative role of both gonadotropins in eliciting the 11KT surge observed at this time that in turn would drive active spermatogenesis forward and meiosis of germinal cells [2, 4,]. Finally, in L fish LH increased from mid October onwards peaking in February coinciding with full spermiation, revealing its prominent role at late gametogenesis. In S fish levels of LH were lower, started to increase one month later and peaked in February but with about half of the value shown by L fish at this date. In both groups 11-KT exhibited the highest levels by middle of December although values of L doubled those of S ($p<0.05$).

In L fish a transient but significant elevation of 11KT was observed by the end of September coincident with the highest levels of FSH observed at this date, confirming the steroidogenic role of FSH in sea bass [6] and its mediation in stimulating early stages of spermatogenesis as previously suggested in other fish [5]. Though small fish had similar endocrine profiles as L fish, their amplitude were much lower being most likely the reason why functional competence of the BPG axis in former fish did not occur. Apparently fish size is a permissive condition to guaranty full effectiveness of the hormonal actions. The ELISA for sea bass FSH showed a high degree of parallelism between the standard curve and serially diluted plasma and pituitary samples of this species. The assay has a high sensitivity and reliability.

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