

## MALE SEX REVERSAL IS NUTRITIONALLY AND GENETICALLY REGULATED IN SEA BREAM: INSIGHTS IN WELFARE AND REPRODUCTIVE RESILIENCE

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### Abstract

Gilthead sea bream is a highly cultured marine fish in all the Mediterranean area, but new and strict criteria of welfare are needed to assure that the intensification of production has no negative effects in animal farming. Most welfare indicators are specific of a given phase of the production cycle, but others such as the timing of puberty and/or sex reversal are of retrospective value. This is of particular relevance in the protandrous gilthead sea bream, in which the sex ratio is highly regulated at the nutritional level. Social and environmental factors (e.g. contaminant loads) also alter sex ratio, but the contribution of genetic component remains unclear. To assess this complex issue, five sea bream families representative of slow/intermediate/fast growth were grown out with control or a plant-based diet in a common garden system from early life to completion of their sexual maturity in three-year old fish. The plant-based diet enhanced the male-to-female sex reversal by more than 50% in average. This occurred in parallel with the progressive impairment of growth performance, which was indicative of changes in nutrient requirements as the result of the different energy demand for growth and reproduction through development. The effect of a different nutritional and genetic background on the reproductive performance was also assessed by measurements of circulating levels of sex steroids during two consecutive spawning seasons, varying plasma levels of 17 $\beta$ -estradiol (E2) and 11-ketotestosterone (11-KT) with age, gender, diet and genetic background. Principal component analysis of three-year-old fish displayed a gradual increase of the E2/11-KT ratio from males to females with the improvement of nutritional/genetic background. Altogether, these results support the use of a reproductive tract scoring system for leading farmed fish towards their optimum welfare condition, contributing to improve the productivity of the current gilthead sea bream livestock.

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## NEW ROLE OF AMH AS A STEROIDOGENESIS PROMOTING HORMONE IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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### Abstract

In higher vertebrates, Anti-Müllerian Hormone (AMH) is required for involution of the Müllerian ducts during male sexual differentiation and for negatively regulating gonadal development in both sexes. AMH signals through a transmembrane AMH type II receptor (AMHR2) with serine-threonine kinase activity. Despite the absence of Müllerian ducts in teleosts, orthologues of mammalian AMH have been described in fish, and a role of this hormone in sex determination and gonad differentiation has been demonstrated. In adult teleost gonads, Amh exerts a role at early stages of germ cell development in both males and females, however, the exact mechanism of Amh signaling and its implication in gonad development are poorly investigated. Recently, in sea bass, we have demonstrated that Amh signals through Amhr2, and that Amh is localized in Sertoli cells surrounding early germ-cell generations. In addition, information available of amh and amhr2 expression during sea bass ovarian development suggest a role of Amh during vitellogenesis. However, still knowledge is lacking about the function and the specific mechanisms of Amh signaling during gametogenesis. As tool for studying the mechanisms of Amh action, we have produced specific recombinant mature forms of this hormone, and have used them in *in vitro* transactivation experiments to study the intracellular signaling pathways of sea bass Amhr2. In addition, we have performed *in vitro* tissue cultures of ovaries and testis treated with recombinant Amh, and *in vivo* experiments through Amh injection in fish, to study the impact of this hormone in steroidogenesis, and its interaction with Fsh, both by analyzing steroid production and gene expression in gonads. We finally concluded that Amh promotes steroid production in immature testis and early vitellogenic ovaries, showing a clear involvement of sea bass Amh in gametogenesis.

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