

REPRODUCTION, OLFACTION AND DOMINANCE BEHAVIOUR IN SENEGALESE SOLE (*Solea senegalensis*) (E. Fatsini)

Summary

Senegalese sole (*Solea senegalensis*) is a flatfish species with increasing importance for the aquaculture industry due to its good performance (survival and growth) in captivity and high market price. However, one of the principal bottlenecks to the expansion of the species culture is the reproductive behavioural dysfunction in cultured males (born and reared in captivity), which complicates establishing a closed cycle in captivity. The reproductive behavioural dysfunction is exhibited by the cultured males that do not participate in the courtship and consequently most eggs obtained from cultured broodstocks are unfertilised. Therefore, Senegalese sole production relies on wild males, which is unsustainable in the long term. With the aim to understand and look for solutions to this bottleneck, the present thesis focuses on different aspects of behaviour related to reproduction and the olfactory system that may control the reproductive behaviour.

The effect of the presence of spawning wild Senegalese sole breeders on the reproductive behaviour and reproductive success of cohabiting cultured breeders was examined. Two groups formed with wild and cultured animals and one control group formed with only cultured sole were compared during three consecutive spawning seasons. Fertilised spawns were not obtained from the control group. However, fertilised spawns were obtained from the mixed-origin groups, principally from the wild fish. In the experimental group cultured males were observed to participate in the courtship in “Follow” behaviours, which are behaviours associated with competition among the fish (principally males) chasing each other. One cultured male fertilised spawns in 2014. Over the three years the participation of culture males (and females) increased, which suggested a learning process in this species for behaviours associated to reproduction.

To establish if culture males and females had the olfactory structure for chemical communication the structure of the olfactory rosettes upper (UOR) and lower (LOR) of wild ($n = 10$) and cultured ($n = 10$) Senegalese sole juveniles were compared. This report was the first description of the olfactory rosettes of Senegalese sole and the structure was similar to other fish species above all flatfish. No significant differences in tissue structure, cell types and cellular distribution pattern (olfactory sensory neurons) were observed between cultured and wild specimens, however, differences were found between UOR and LOR in number of lamellae and amount of goblet cells in the ridge region of the lamella, which were more frequent in LOR.

To determine if cultured males and females had the olfactory functionality to communicate chemically, transcriptomic profiles using RNA-seq of the UOR in cultured ($n = 3$) and wild ($n = 3$) Senegalese sole mature males were characterized and compared. A total of 2,387 transcripts were differentially expressed between cultured and wild

mature males. Transcripts of some olfactory receptors (OR, TAAR and V2R-like) and other transcripts associated with the control of reproduction (brain aromatase and Tachykinin) demonstrated clear differences in functionalities between origins. Furthermore, cultured males presented higher expression of genes related to goblet cells and mucin production that controls inherent and adaptive immune responses. Many of these changes could be explained by different nutritional status and diet preference.

To establish the form of chemical communication, the olfactory sensitivity of cultured Senegalese sole, juveniles ($n = 12$) and adults ($n = 12$) to urine and ovarian fluid from mature conspecific (wild and cultured) was evaluated using electro-olfactogram (EOG). Urine was confirmed to be a potent olfactory stimulus for both conspecific stages, juvenile and adult, inducing large-amplitude, concentration-dependent EOG responses, with thresholds of detection at a dilution of $1:10^6$. Significant differences in the amplitude of perception to urine in relation to sex and maturity of both the donor and the receiver indicated that urine may play a role in reproduction by communicating sex and maturity status. In addition to this, urine from mature females evoked a small, but significant increase in plasmas levels of luteinizing hormone (LH) in mature males, further demonstrating that urine-released odorants play a role in reproduction in the Senegalese sole. However, perhaps contrary to expectations, the olfactory potency of urine from wild females was significantly lower than urine from cultured females.

To examine dominance in Senegalese sole, two different groups of Senegalese sole juveniles (early; $n = 74$ and late; $n = 34$) were used to conduct dyadic dominance tests (feeding response and place preference test) and group tests (4 groups; $n = 6$). In addition, transcripts related to dominance in other species were tested to differentiate between dominant and subordinate individuals. This was the first study related to dominance behaviour in this species, which could be very relevant to the low participation in the parental contribution during the spawning season. Dyadic tests found that dominance existed in relation to feeding and space (restricted area with sand) and observed that dominant animals exhibited a higher frequency than subordinate animals of the behaviours resting the head on an individual, approaching and swimming above another individual. Additionally, dominant sole dominated the sand at the end of the test and occupied the sand area at the end of the test. Moreover, these behaviours (resting the head, approaching and swimming above another) that were associated with dominance were corroborated in group-test, where two index (rest the head Index and position before feeding) determined the two dominance categories in the same group. Two transcripts related to neurogenesis (*nrd2*) and neuroplasticity (*c-fos*) were differentially expressed between dominant and subordinate sole juveniles demonstrating the different transcriptomic activity between dominant and subordinate sole.

In some species SCS have been related to reproductive exit and gene expression. In the present study the stress coping styles (SCS) (proactive, intermediate and reactive) in Senegalese sole juveniles ($n = 30$) was determined in relation to several genes associated with coping styles in other species were tested to find a marker. There were four transcripts which linked behavioural SCS categories with brain gene expression, *gapdh-2* (metabolism), *pparβ* (lipid metabolism and feeding behaviour), *igh-Ia* (growth and feeding behaviour) and *per1* (circadian rhythm and feeding behaviour).

The present thesis has demonstrated that the presence of spawning wild sole increased the participation of cultured cohabitating sole in courtship behaviour and spawning, which suggested a learning aspect to reproductive behaviours. The olfactory system appears to have importance in these behaviours and courtship with the capacity to increase LH plasma levels and differentiate between sex and maturity. In addition, similar behaviours (to courtship) were identified to be related to dominance in feeding and space. Together these advances strengthen the importance of these research lines as areas that can give a solution to the reproductive dysfunction that can enable the aquaculture industry to close the species life cycle in captivity to make Senegalese sole culture sustainable.