Thursday 14:30 Add to Calendar

Effects of GnRHa slow-release implants in the steroid profile of isolated couples of Arapaima gigas (Schinz, 1822)

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Introduction

Arapaima gigas is an Amazon osteoglossid species interesting to aquaculture development in Brazil, but lack of reproduction in captive has been limited industry expansion for decades. The reproductive dysfunction is difficult to be characterized due to the lack of sex identification tools and limited assessment of reproductive condition. This said, hormonal manipulations of reproduction and physiological investigations on isolated couples are necessary. The role of pheromones in the reproduction of *A. gigas* seems to be linked to a fluid secreted from cephalic canals, however its biochemistry has not yet been investigated. The aim of the study were 1) to induce spawning of isolated broodstock pairs with GnRHa slow-release implant technique (Zohar and Mylonas, 2001) and monitor steroid levels in blood plasma; and 2) characterise cephalic secretions through proteomic and steroid analyses during reproduction and parental care post spawning.

Materials and Methods

After sex identification, couples were isolated in 330 m^2 earthponds at day 0 (January 19th 2014) and implanted at day 62 (n=13 couples) with EVAc sGnRHa implants (85 $\mu g.kg^{-1}$♀; 45 $\mu g.kg^{-1}$ ♂). Blood and cephalic fluid were sampled at 119 days post hormonal induction. Levels of testosterone (T), 11-ketotestosterone (11-KT) and 17β-estradiol (E2) were assayed by RIA and ELISA following validation in the species. Groups of small (<39 kg) and large (>51 kg) broodstock were implanted and effects compared to a sham control (>45 kg) group (n=5 couples per treatment). Temporal variations in steroid levels were tested for groups with ANOVA (Holm-Sidak) and Spearman Rank was used to correlate steroid levels in blood plasma and cephalic fluid. Level of significance was set at $P \leq 0.05$.

Results

Steroid levels did not significantly change in control group along the study. Two weeks post hormonal induction, plasma levels of 11-KT increased by 2.2-fold (P=0.03) and 7.3-fold (P<0.001) in small and large male broodstocks, respectively (Fig. 1A). Within the same period, plasma levels of T increased by 2.4-fold (P=0.002) and 2.0-fold in small and large males, respectively. In larger females, plasma levels of T increased by 3.7-fold (P<0.001) two weeks post-induction (Fig. 1B), but levels of E_2 did not change significantly post hormonal induction (Fig. 1C).

A positive and significant correlation was found between levels of 11-KT (r = 0.828) and T (r = 0.373) in blood plasma vs. cephalic fluid (Fig. 2 A and B). Due to the low plasma E_2 levels assayed, no correlation was observed for E_2 (Fig. 2 C).

Discussion and Conclusion

Following induction with sGnRHa, no apparent spontaneous spawnings were observed although assessment was indirect (e.g. change in swimming and feeding behaviour) as it was not possible to confirm egg deposition. However, despite the lack of expected spawning, the hormonal induction resulted in an elevation of plasma T levels in both males and females, and also plasma 11-KT levels in males, thus indicating a positive hormonal stimulation on gonad steroidogenesis. A possible dopaminergic inhibition and/or suboptimal aromatase activity could explain the low plasma E_2 levels observed in females. Given the multiple GnRH forms identified in teleosts, sGnRH used in the present study may not be potent enough to regulate oocyte maturation in *A. gigas.* Further trials are required to test the effects of other GnRH forms (mGnRHa) in combination with dopamine antagonist on oogenesis and spawning induction of isolated *A. gigas* couples. The correlation found between plasma and cephalic levels of sex steroids suggest the use of the cephalic secretion in the release of metabolites such as potential pheromones. These initial results paves the way into future research on other pheromones present in the cephalic secretion, their olfactory sensitivity in the species and potential use in the captive reproduction of *A. gigas*. The proteomic study on the cephalic fluid will be presented in poster format.

References

Zohar, Y. & Mylonas, C. C. (2001). Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture (Amsterdam, Netherlands)* **197**, 99-136.

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