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Effects of slow release gonadotropin releasing hormone analog on milt characteristics and plasma levels of gonadal steroids in greenback flounder, *Rhombosolea tapirina*

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INTRODUCTION

Gonadotropin releasing hormone analogs (GnRHa) have now been tested successfully a range of marine and freshwater species and been shown to be an effective strategy for improving milt quantity and quality (Mylonas and Zohar, 2001; Zohar and Mylonas, 2001; Lim et al., 2002). Greenback flounder (*Rhombosolea tapirina*) is currently under consideration as a potential culture species in south-eastern Australia. Treatment of male greenback flounder with injection of human chorionic GtH (hCG) or GnRHa increased milt volume, confirming the role of GtH in stimulating increases in milt volume. However, the effect of longer time exposure to GnRHa remains unknown. The present study was designed to examine the long-term effects of GnRHa on milt production, milt characteristics and plasma levels of androgens and 17,20-dihydroxy-4-pregnen-3-one (17,20 β P) in greenback flounder.

MATERIALS AND METHODS

Male flounder were randomly selected from stocks grown at the School of Aquaculture, University of Tasmania, Launceston, Tasmania. The first experiment was conducted over four weeks from February 21 to March 21, 2002, when cultured fish are typically in the early stages of spermiation. Each fish received either a blank cholesterol pellet (control) or pellets to give doses of either 50, 100, or 200 $\mu g.kg^{-1}$ body weight (BW) LHRHa . The second experiment was conducted for 6 weeks from May 7 to June 18, the period when most fish are spermiated in our culture system. Fish were implanted with either a blank pellet (control) or pellets containing 16.1 ± 0.6 μg LHRHa to give doses of 200 $\mu g.kg^{-1}$ BW. At 14 days a second pellet was implanted in

each fish to extend the period of GnRHa release. GnRHa pellets were produced following the recipe of Lee et al. (1986). Pellets were prepared individually according to the BW of each fish and implanted into the abdominal cavity of the fish through a small incision made with a scalpel blade. Plasma steroids were extracted with ethyl acetate, and concentrations of extracts measured by radioimmunoassay for testosterone (T), 11-ketotestosterone (11KT) and 17,20βP using the reagents and protocol given in Pankhurst and Kime (1991) and Pankhurst and Carragher (1992).

RESULTS

In both experiments, fish treated with GnRHa pellets consistently showed a significant increase in milt volume relative to controls, and the increase of milt volume was more rapid than the increase in numbers of spermatozoa. Spermatocrit (Sct) was significantly lower in GnRHa-treated fish than in controls. Plasma levels of T and 11KT were elevated at 7 and 14 days p.i. in fish treated with GnRHa and elevated plasma T and 11KT levels were accompanied by increased milt volume early in the spermiation period. In contrast, there was no significant difference in plasma T levels between GnRHa-treated and control fish in fish that were moderately spermiated at the time of implant. Treatment with GnRHa tended to result in lower plasma levels of 11KT and higher levels of 17,20βP relative to controls. There was no significant difference in sperm motility and pH and osmoloality of seminal plasma in both experiments. Conclusively, even though T and 11KT levels in plasma were slightly affected by GnRHa treatment early in the spermiation period, it is possible that there was no relationship between elevations of these androgens and increase in milt volume. The results suggest that the increase in milt volume is at least partially gonadotropin (GtH)-dependent and there is a positive relationship between the elevation of plasma 17,20βP and the increase in milt volume in response to implantation of GnRHa pellet. Slow release GnRHa administration had no effect on sperm activity or pH and osmolality of seminal plasma in either experiment.

REFERENCES

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