VARIATIONS OF STEROID HORMONES AND FSH LEVELS IN ATLANTIC BLUEFIN TUNA CAUGHT IN THE MEDITERRANEAN BASIN DURING SPAWNING AND POST-SPAWNING SEASON.

Carnevali, O. (1), Maradonna, F. (1), Sagrati, A., (1), Bovari, G. (1), Lombardo, F. (1), Pignalosa P. (2), Bonfanti, E. (1,3) Nocillado, J. (3), Palma, P. (3), Gioacchini, G. (1), Elizur A.(3)

(1) Department of Life and Environmental Sciences, Polytechnic University of Marche, 60131 Ancona, Italy. Email o.carnevali@univpm.it

(2) OCEANIS Srl, Ercolano (NA), Italy

(3) Genecology Research Centre, Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Qld, AUSTRALIA

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Introduction

The Atlantic bluefin tuna, *Thunnus thynnus*, is a highly valued commercial species around the world. In the last decades, interest in the basic biology of this species significantly increased. Recent studies shed light on the correlation between molecular markers related to growth with canonical morphological indexes, advancing the knowledge on the metabolic system of the species and offering new useful information for the aquaculture industry (Api et al., 2018). A complex interplay occurs between growth factors and steroid hormones in the hypothalamus, representing a key factor in the neuroendocrine control of its reproductive function. In teleosts, three sex steroid hormones, 17β-estradiol (E_2), 11-ketotestosterone (11-KT) and 17 α20 β- dihydroxy-4-pregnen-3-one (DHP) are produced in gonadal tissues under the control of pituitary gonadotropins (GTH) and these are essential for gametogenesis.

Methods

Pools of plasma from three animals (500 ul) were extracted twice with diethyl ether. Concentrations of Estradiol-17b (E_2) and Testosterone (T) and progesterone (P) were measured by commercially available competitive enzyme-linked immunosorbent assays (ELISA) (Cayman Chemicals, Ann Arbor, MI, USA) which measured the total amount of steroids in the plasma.

Circulating FSH levels were determined using a heterologous ELISA developed using a *Seriola lalandi* recombinant FSH, produced in the yeast *Pichia* expression system and validated for tuna. Vitellogenin (VTG) concentration in the plasma was assayed using an ELISA method previously validated and described by Rahman et al. 2000. Gonad samples were fixed in Bouin's solution and prepared for histological examination using standard biological procedures. The fixed tissues were embedded in paraffin and sectioned (4 μm) with a microtome (Leitz 1512). Each gonad was fully

sectioned and processed for Mayer hematoxylin-eosin staining and observed under a Zeiss Axioskop microscope. Microphotographs were captured using a high resolution digital camera (Canon EOS 6D).

Results and Discussion

The results revealed that changes in plasma levels of steroid hormones, (E_2) and (T) closely correlated with gonadal development and reproductive season. In both male and female fish, E_2 levels significantly varied between spawning (summer) and post- spawning (autumn/winter) seasons, with the highest levels observed in the summer. T levels remained almost constant in males in both seasons. In contrast, a significant decrease was observed in post-spawning females. Concerning progesterone (P) levels, no significant variation was observed in the two sampling periods. The follicle-stimulating hormone (FSH) levels significantly increased at post-spawning season and were associated with significantly lower gonadosomatic index (GSI) values in both sexes. During the spawning season, FSH levels decreased concomitant with increase in GSI. At spawning, vitellogenin levels resulted significant higher in female than in male. In post spawning fish, levels resulted lower than the detection limits. Results of hormonal assays agreed with histological observations showing in summer late vitellogenic/reproductive stage ovaries and spermiating males. In autumn, few vitellogenic oocytes could be observed within the ovaries while males were still fluent.

Conclusion

The results obtained add to the basic knowledge of the reproductive biology of Atlantic blue fin tuna and could be used to enhance the development of artificial breeding technology for this high value species.

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