



## THE NEUROENDOCRINE CONTROL OF PUBERTAL DEVELOPMENT IN ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*)

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### Introduction:

The bluefin tuna (BFT, *Thunnus thynnus*), a large pelagic fish with remarkably high commercial value, has become an endangered species due to overfishing [1, 2]. Recently, significant progress on spawning induction in captive BFT has been achieved providing the basis for the species' domestication [3]. To further promote the development of a self-sustained BFT aquaculture, we investigated first sexual maturity in BFT reared from an immature stage in captivity. Accordingly, our major objectives were to evaluate: (i) maturational status of the brain-pituitary-gonadal (BPG) axis, and (ii) responsiveness of the BPG to exogenous hormones. Special emphasis was given to characterize the KiSS/GPR54 system that constitutes the trigger of puberty onset, and the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH) that act as central regulators of gonadal development and gamete maturation.

### Methods:

*In vivo studies* - In a first trial (July, 2009), sexually immature BFT juveniles (n=20) and sexually mature individuals (n=19), were sampled during BFT natural spawning season within the Mediterranean Sea. In a second trial (June-July, 2010), sexually immature BFT juveniles were treated with poly [ethylene-vinyl acetate] (EVAc) implants containing KiSS-peptides (n=6). The remaining fish (n=14) were used as untreated controls. Three weeks post implantation all fish were sampled. Morphometric parameters were recorded and the relative gonadosomatic index values were calculated. The first spiniform ray of the dorsal fin was removed to determine the age of the fish, while brain, pituitary and gonad

tissues were removed and stored frozen until use for gene expression and hormonal measurements. Additionally, gonad slices were fixed in Bouin's fixative for histological analysis.

*In vitro studies* - Uniformly sized gonad fragments derived from treated- or untreated BFT juveniles (trial 2) were challenged (16 h) with graded doses of recombinant BFT LH or FSH (rLH and rFSH, respectively) previously produced at IOLR-NCM using the methylotrophic yeast *Pichia pastoris* expression system. At the end of the experiment, media and tissues were collected for further analyses. Gonadal sections were subjected to immunohistochemical staining using anti-proliferating cell nuclear antigen (PCNA). Quantification of germ cell proliferation was performed by measuring the surface of anti-PCNA positive germ cells using the QWIN image analysis software.

*Hormonal and gene expression analyses* - Sex steroid were extracted from gonadal tissue with diethyl ether. The dry extract was assayed for 11-ketotestosterone or estradiol levels using the specific ELISAs. The LH levels were measured in pituitary extract using an ELISA modified for tuna species [4]. The pituitary FSH levels were measured using our newly calibrated Immuno-Dot Blot assay. Expression levels of target genes were measured in relevant tissues employing the quantitative real time PCR technique.

### Results and Discussion:

The growth parameters recorded for the captive BFT juveniles are consistent with the length-weight relationship established for wild Mediterranean BFT stocks. The histological analyses of the gonads indicate advanced sexual maturation in BFT males compared to females, yet it is not yet clear whether this phenomenon



typifies wild stocks or is induced due to the culture conditions. The hormone measurements show expression and accumulation of both gonadotropins in the pituitaries of immature and mature BFT. The pituitary LH content increased concomitantly with the age of the fish, exhibiting sex dimorphic patterns (i.e. 3-fold higher levels in females) in adult but not in juvenile BFT. The pituitary FSH levels, however, were elevated in 2Y immature males and in fully mature adults. Comparable to mammals [5], the intra-pituitary FSH/LH ratio was found to be higher (>1) in sexually immature than in maturing or pubertal BFT. Nevertheless, in the 3Y BFT females, which were all immature, the onset of puberty appears to require some other prerequisites, such as a rise in the LH storage above a minimal threshold. Our *in vitro* trials further demonstrated the capacity of rFSH and to a lesser extent that of rLH to stimulate cell proliferation in the immature ovarian and testicular fragments. Both rFSH and rLH have failed to stimulate steroidogenesis, yet pre-treatment with KiSS containing EVAc implants (trial 2) appeared to potentiate FSH-stimulated steroidogenesis in the immature testes.

#### Conclusions:

Our results highlight the importance of the intra-pituitary FSH/LH ratio during the period of peripubertal/pubertal transition. Similarly to what was established for mammals, the fact that such ratio in sexually immature BFT is higher than in pubertal stages indicates that it may be used as an important endocrine clinical marker in fish as well. Our *in vivo* and *in vitro* studies further attest the growing notion that KiSS and

FSH are critical regulators of the initiation of pubertal development in general and of testis maturation in particular.

Future studies testing the effects of captivity and hormone-based treatments on precocious maturity at relatively small body size are expected to facilitate the handling in confined environments, and to greatly improve the cost-efficiency of BFT farming.

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