PUBERTIMING - Photoperiod control of puberty in farmed fish: Development of new techniques and research into underlying physiological mechanisms

G.L. Taranger[1], S.O. Stefansson[2], N.R.Bromage[3] H. Migaud[3], O.Kah[4], J.J.Lareyre[5], M. Carillo[6] and R.W.Schulz[7]

[1]Institute of Marine Research, PO Box 1870 Nordnes, 5817 Bergen, Norway. geirt@imr.no. [2]University of Bergen, PO Box 7800, 5020 Bergen, Norway. [3]Institute of Aquaculture, University of Stirling, Stirling FK94LA; Scotland. [4]Centre National de la Recherche Scientifique, PO Box CS 74205, 35042 Rennes, France. [5]Institut National de la Recherche Agronomique, 35042 Rennes, France. [6]Instituto de Acuicultura de Torre de la Sal, Ribera de Cabanes, 12595, Torre de la Sal, Spain. [7]University of Utrecht, PO box 80058, 3508 TB Utrecht, The Netherlands.

Background

A major constraint in farming of fish is the timing of the first sexual maturation or puberty. Sexual maturation results in reduced growth, regressive changes in harvest quality, increased susceptibility to diseases, and reduced feed conversion efficiency, and ultimately increased production costs. The term puberty describes developmental processes that constitute the transition from an immature juvenile to a mature adult state of activity of the reproductive system. The two core components of the reproductive system are the gonads that produce germ cells as well as hormones, and the endocrine system that regulates the gonadal functions, the brain-pituitary-gonad (BPG) axis. Apart from the putative permissive factors related to body size, growth and/or adiposity, the question on how the quiescent state is of the BPG axis in juveniles is overcome has not yet been fully explained in mammals, and is rather unclear in fish. Photoperiod alteration has proved a simple and rapid way to delay puberty in farmed Atlantic salmon. Promising results have also been obtained in other European farmed fish such as rainbow trout, Atlantic cod, Atlantic halibut and sea bass.

Overall Objective of the project

Although photoperiod is wildly used in farm fish to control puberty, the efficiency of this method remains unreliable depending on the species but also on the production systems (in door/out-door, scale). It is also well established that other environmental factors (temperature) and genetic background modulate puberty onset. This project aims to better understand the modulation of key-factors responsible for initiating puberty in farmed fish and how environmental (nutrition) and genetic factors interfere with that process.

Three complementary research actions are conducted:

Aim 1: Improved knowledge on the mechanisms of activation of the brain-pituitary-gonad (BPG) axis during puberty in fish, including development of new tools to study the BPG axis in salmon, rainbow trout and sea bass.

Aim 2: Improved understanding of the importance of differences in light intensity and spectral quality in affecting the BPG axis and the initiation/postponement of puberty, by assessment of pineal melatonin production *in vitro* and *in vivo* in salmon and sea bass.

Aim 3: Improved understanding of the interactions between photoperiod protocols, genetic background and adiposity in arresting/promoting puberty in salmon and sea bass.

Technical content related to functional genomics approach

Orthologous genes regulating puberty in vertebrates are being identified in salmon, sea bass, and/or trout using methods based on protein sequence homologies, *in silico* analysis of known fish genome, and molecular phylogenetic analysis. Several candidate genes such as gonadotropin subunits (GTH-α, FSH-β, LH-β), gonadotropin-releasing hormone receptors (GnRHR's), estrogen receptors (ER's), androgen receptors (AR's) and gonadotrophin receptors (GtHR's; FSHR and LHR) have been cloned or provided by biological resources centres.

Partial or full sequences have been obtained for a range of genes of relevance in onset of puberty including FSHR, LHR, ARα and ARβ in salmon, GnRHRI in trout, GnRHRII in sea bass, and ERβ1 and ERβ2 . The FSH-β subunit proximal promoter (1.8 kb) has been cloned and sequenced in sea bass. Partial pharmacological characterization has been done with the salmon FSH-R and LHR, indicating that FSHR binds both FSH and LH, whereas LH-R appears to only bind LH. AR's and different ER's are also being characterized in functional assays.

The brain distribution of three different estrogen receptors (ERα, ERβ1 and ERβ2) has been characterized in the sea bass by *in situ* hybridisation, and the estrogenic dependence of the sea bass pituitary FSH and LH cells has been characterized by double *in situ* hybridisation. Real-time quantitative RT-PCR methods have been established for GTH-α, FSH-β, LH-β, FSH-R, LH-R and AR's in Atlantic salmon, and ERα, ERβ1 and ERβ2 (partial), GnRH's and GnRHR's in sea bass.

The biological significance of new identified genes in fish is currently investigated by combining expressional and functional approaches. The biopotency of physiologic ligands to activate the newly identified nuclear or membrane bound receptors are studied in transient transfection assays of heterologous cells. The spatio-temporal expression patterns of these genes are studied all over the reproductive cycle using quantitative real-time PCR and *in situ* hybridisation in appropriate tissues belonging to the brain pituitary gonadal axis.

Changes in mRNA or protein expression of the newly identified genes along the BPG will be determine between normal and continuous light treated animals to better understand the mechanisms underlying the inhibitory effect of a continuous light treatment on spermatogenesis onset. Information gained from these newly identified genes will be used as "landmarks" to validate large scale gene expression profiling using cDNA arrays.

Acknowledgements

The PUBERTIMING project is funded by EU FP5 Contract Q5RS-2002-01801.