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The LIFECYCLE project: greater basic knowledge on fish biology will help find long-term solutions to aquaculture production bottlenecks

Early development and important life-stage transitions are important LIFECYCLE research areas. Sea bream larvae, 35 days post-hatch, stained for visualizing skeletal development.

The LIFECYCLE project (www.lifecycle-fp7.eu) is a major need-driven research effort on the biology of important aquaculture fish species within the 7th EU Framework Programme. The "need" for this project is of a fundamental and an applied character. In the context of modern animal production industry, fish rearing is still in its infancy, a branch where we're still largely dealing with raising animals in early stages of domestication. Our current understanding of many basic biological processes and their regulation throughout the lifecycle is still quite limited and thus, as cited from the call text, a major project goal is to "***clarify the mechanisms of essential biological functions related to the most relevant stages of aquaculture fish life-history (larval development, growth, maturation, reproduction) at both a physiological and molecular level***". Over the last two decades, the aquaculture industry has grown rapidly, intensified and diversified, and while a number of hurdles have been cleared, all parts of the production cycle have clearly identifiable problems. Although varying among the species, these concern sex differentiation, hatching, larval rearing, metamorphosis/smoltification, juvenile/adult ongrowth, puberty and sexual maturation. Therefore, the project is also dedicated to "***help to overcome bottlenecks in the production cycle of commercially important species***".

To this end, the research scope of the project was defined to cover four major areas of fish biology, organized in workpackages (WPs): **WP1 - Development and growth, WP2 - Adaptation, WP3 – Immunity, and WP4 - Sex differentiation and puberty**. To address all the detailed questions raised within the overall workplan (the project has these specific areas, turn, these focus areas decided the final composition of the LIFECYCLE consortium, creating a 14-partner consortium from 9 European countries. The work is mainly being carried out on Atlantic salmon, rainbow trout, sea bass and sea bream, but Atlantic halibut and Atlantic cod are also studied in specific research tasks.

A major topic which exemplifies well how basic science and aquaculture go hand in hand is that of **development** and **growth**, as addressed in WP1 Stable production of vigorous, healthy juveniles by hatcheries is in itself prerequisite to successful on-growth production, but during the on-growth phase itself, several questions remain concerning muscle and skeletal growth, as well as the processes which regulate the growth processes. Recent research shows strong influences of early life experience on the subsequent growth performance of fish in aquaculture right up until harvest. Understanding the mechanisms and consequences of this developmental plasticity is a key goal for WP1. The LIFECYCLE research will focus on determinants of egg and larval quality, including what information, in addition to the classically inherited parental genomic information, the broodstock female may transfer to the egg. Skeletal muscle and bone are heterogeneous tissue composed of several cell types that interact to affect growth patterns. The research focuses on the regulatory interactions between cell types and between tissues (cross-talk) in larvae and juveniles, and how it influences growth and malformations. New knowledge on flatfish metamorphosis will also be obtained, as this may not only help improve production and predictability in

flatfish larval production, but also give broad insights into development/production of marine fish larvae. An important aspect of growth and muscle quality as a quality trait is the regulation of appetite, metabolism and fat deposition, and LIFECYCLE will address this along with questions on how specific hormones exert their effects. WP1 will also contribute to the expanding genomic resources available for commercially important fish species providing powerful new tools to study fundamental biological processes and for translational research into aquaculture bottlenecks.

In order to develop, grow and mature normally, fish need to maintain stable internal conditions, irrespective of environmental fluctuations in factors like temperature and salinity. This ability to adapt to different environmental conditions, called **homeostasis**, is the main focus of **WP2**. The organs that make up the boundary between the environment and the body are the skin, gills and intestine. Gill and intestinal epithelia have vital functions in the transport of nutrients, gases, ions and water but they also act as barriers against harmful substances. During developmental and environmental changes these multifunctional tissues must be able to adapt to new situations.

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To address critical bottlenecks in aquaculture we're focusing on three important life-stage transitions which include changes in the culture environment: larval development, smoltification and sexual maturation. During these physiologically demanding periods, correct function of the transporting epithelia is of utmost importance. The role of the epithelia is to provide the fish with sufficient amounts of nutrients and energy, without disturbing homeostasis, so that they can perform remodelling and maturation of the physiological functions. The research focuses on salmonids, cod and halibut, but zebrafish and medaka will also be used for in-depth studies of adaptation mechanisms.

In LIFECYCLE, the development of ion- and water regulation will be examined, from primitive ion-transporting cells in the integument of larvae, to specialized cells containing specific transport proteins, in the gills and intestine. The complex patchwork of genes and factors controlling this cellular differentiation, as well as the responses of these cells and proteins to different salinities, will also be elucidated.

Rearing fish in brackish water usually results in higher growth rates and better food conversion, and the molecular, cellular and organismal mechanisms behind these observations will be investigated. At the level of the intestinal epithelium, there is a "competition" between nutrient- and ion-transport. Important question to be answered are if the higher growth rates are due to reduced osmoregulatory costs, or if salinity changes lead to changes in feed intake, digestibility and nutrient uptake? The hormonal control of these events will also be elucidated.

In Atlantic salmon, sexual maturation is accompanied by a change in environment and thus a change in the osmoregulatory work of the transporting epithelia. LFECYCLE will also study whether sex steroids regulate ion transport by binding to transporting cells in the gills, the mode of action of sex steroids in epithelial transport processes, and interactions with endocrine axes controlling osmoregulation and energy allocation will also be investigated.

Fish health and disease resistance is of central importance to sustainable aquaculture. Although much previous work has greatly expanded our knowledge of fish defences and how the immune system of fish responds to different pathogens and vaccines, there is still limited information on how the immune system changes during development and during key physiological bottlenecks in production. Thus the immunity workpackage (WP3) is examining stages of the lifecycle where the immune system may be challenged or compromised. These are divided into early life stages from egg to first feeding, and during major physiological changes (parr-smolt transformation) and during sexual maturation.

To study the defence mechanisms acting at the early stages of development, the presence of maternal transcripts encoding defence molecules in ova will be determined which may provide innate protection. During later embryonic, post-hatch and first-feeding stages, immune challenges with characterised bacterial and viral pathogens will establish how transcription of immune genes respond during ontogeny. This is an area of great importance for the understanding of how these early stages can repel pathogens, as the immune system is not fully developed and relies on innate protection. The establishment of specific immunity during the development is also unclear as vaccination requires a functional specific immune system. The capacity of fish B and T lymphocytes to recognize a diversity of pathogens will be tested at different stages.

Little is known about the interaction of hormones and immune response in fish. The immune response will be examined during two of the stages, controlled by major hormonal changes, both the parr smolt transformation

Onset of puberty and its regulation is a major LIFECYCLE research focus. Adult salmon being sampled at the Matre aquaculture research station, Norway.

and sexual maturation. During these stages the fish have high levels of stress, reproductive and peptide hormones each of which are likely to modulate the immune response. To examine the interactions in greater detail specific leucocyte cell types (such as neutrophils, B cells and macrophages) will be isolated and their response examined when stimulated with both immunostimulants and hormones. This will allow the interaction between hormones and the defence system to be investigated. The physiological interactions between cytokines and primary barriers will be examined in the intestine to determine modulation of permeability. Together this research will define protection against pathogens at the most vulnerable stages and how health management can be improved in the future.

WP4 addresses two key aquaculture bottlenecks in fish, **sex differentiation** and **precocious puberty**, both of which affect growth and therefore production. The overall aim is to understand how hormones and the environment regulate sex differentiation and then later in the lifecycle, pubertal maturation. In addition, for the research into puberty, we will study how external parameters (e.g. photoperiod, food availability, social behaviour) modulate the signaling by hormones. In the future, this basic knowledge will contribute to the development of new and increasingly sustainable options to modulate sex ratios and to reduce the incidence of early puberty. Moreover, controlling fertility in aquaculture will help to reduce the risks linked to the escape of farmed individuals.

The investigations will cover bony fish species at different positions in the phylogenic tree (Atlantic salmon, rainbow trout, sea bass, medaka and zebrafish), taking benefit from specific advantages of each species to study particular questions and providing the comparative basis required to develop generic approaches to lifecycle control in novel aquaculture species.

In LIFECYCLE, new basic knowledge will be produced on the mechanisms of sex differentiation. It will investigate the gonad and the brain of rainbow trout and sea bass, as these species have fundamentally different mechanisms of sex determination. In trout sex is purely genetically determined, whereas in sea bass sex is more labile with temperature being able to modify a basic genetic blueprint. A particular focus of these studies is placed on the female sex hormones, the estrogens, and investigations will clarify regulatory mechanisms affecting their production as well as the action of estrogens on gene expression and cellular function.

Salmon, trout and sea bass are used to study how physiological conditions (growth, adiposity) and external signals (photoperiod, behaviour) impact on the hormonal signaling that trigger the onset of puberty. As early puberty is a problem that is particularly relevant in males, testis maturation receives a particular focus compared to ovarian maturation. New basic knowledge is expected on how the hormone system integrates signals from the environment with internal feedback signals to regulate pubertal maturation.

Further, the molecular mechanisms with which the testis responds to maturation-promoting hormones (FSH and androgens playing important roles) are investigated, using trout and zebrafish as experimental models. Moreover, gene expression patterns receive particular attention in testes of salmon that were exposed to environmental conditions promoting or inhibiting puberty.

As exemplified above, the LIFECYCLE project therefore represents a major EU-supported effort to obtain fundamental information on processes which are of importance to raising fish in captivity and thereby helping find ways to improve the competitiveness and sustainability of European aquaculture. In this context, the impact of the project is highly dependent on the successful transfer of knowledge to the industry. Hopefully, members of the LIFECYCLE consortium will be active in presenting data at aquaculture conferences – only recently, 17 members of the consortium attended the Aquaculture Europe 2010 conference held in Porto. We also hope that the project website www.lifecycle-fp7.eu will be an effective way of distributing information to the aquaculture sector, and all suggestions from the industry on the content of the website as well as other ways of disseminating data are highly appreciated.

On behalf of the LIFECYCLE consortium,

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Visit the LIFECYCLE website at www.lifecycle-fp7.eu for updates on the project progress.

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