

## WEALTH - Welfare and health in sustainable aquaculture

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Despite continuous improvements of diagnoses, sanitary controls and proactive treatments methods such as vaccinations, the health and welfare of farmed fish still remains a major problem for the European aquaculture industry. The causes are to be found in environmental conditions, husbandry practices, including the genetic make-up of the stocks. These impacting parameters make up a complex matrix determining the health and welfare of the fish. Although some important components have been identified and studied, the current knowledge is incomplete and fragmented, and a holistic view of how health and welfare of farmed fish can be maximized is still lacking.

### **Main objectives:**

1. To gain comprehensive knowledge on health and welfare in farmed fish by focusing on two of the major aquaculture species in Europe, Atlantic salmon and sea bass, not only to improve the farming situation for these fish, but to transfer the obtained knowledge to other important aquaculture species such as rainbow trout and sea bream.
2. To study a range of the most important environmental factors and husbandry practices in freshwater-, seawater- and recirculation-system aquaculture in order to identify how these may compromise welfare and health of farmed fish.
3. To gain an integrated understanding of the physiological and molecular mechanisms underlying the interactions of husbandry practices and environment on stress conditions affecting welfare and diseases resistance in farmed fish.
4. To identify innate and acquired immune parameters affected by environmental factors and husbandry practices resulting in compromised welfare and health, and to develop effective molecular tools to study and monitor the immune function, barrier functions and stress responses of farmed fish.

Based on the above objectives, it is the final goal of the WEALTH project to develop and validate operational husbandry protocols for improved welfare and health of farmed fish, including methods for early prediction and management of disease outbreaks and compromised welfare. The European aquaculture industry will be encouraged to adopt these protocols under the devise “welfare and health creates WEALTH!”. The project consists of five interlinked workpackages to achieve these objectives, summarised in Figure 1.

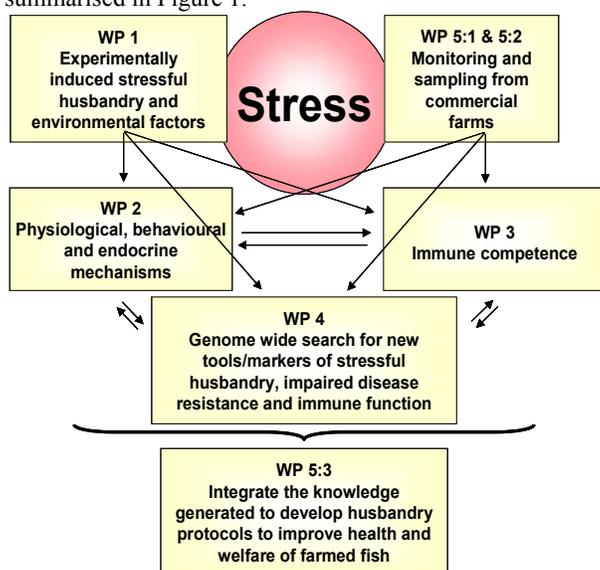


Figure 1. The relationship between the different work packages in WEALTH.

The lack of molecular markers of environmental and infectious stress has severely impaired the development of appropriate diagnostic tools and preventive and therapeutic agents in aquaculture. Therefore, the WEALTH project has applied a functional transcriptome approach in order to identify as much as possible stress molecular markers, rather than be limited to the study of a single gene cloned using a homology approach.

Analysis of expression of known immune related genes in various tissues in nodavirus infected sea bass revealed increases in interleukin 1, TNF- $\alpha$ , dicentracin and TGF. The expression increased after 1 and 3 days in brain, whereas opposite responses were seen in the kidney samples. Based on these observations Suppression Subtractive Hybridization (SSH) libraries were produced from brain and kidney in nodavirus exposed sea bass. SSH libraries were also produced from *Vibrio anguillarum* infected sea bass. In order to decide the samples for constructing the libraries, experimental infections were carried out to determine the expression of already known cytokines and phagocyte activation. This revealed that several genes were up-regulated 4h post-infection, and that the profiles differed between fish exposed to live or dead bacteria. Thus, one SSH library was constructed from control vs. 4h post-infection, and another from infected fish vs. fish injected with dead bacteria. Moreover, we have generated several normal cDNA libraries from different tissues of fish experimentally infected with the pathogenic bacteria *Vibrio anguillarum* and virus NNV.

Preliminary analysis of the SSH libraries led to the identification of 11 immune related genes that seems to be differentially expressed during infection. Strikingly, 5 out of 11 have been identified for the first time in fish. The uncovered genes can be classified into (i) Inflammatory genes: cc chemokine for leukocyte recruitment, pentraxin/C-reactive protein, which participates on innate immunity response in complement activation and opsonization, NALP is involved on IL-1 $\beta$  secretion, cathepsinG/Granzyme H related with target cell lysis in cell-mediated immune response and galectin-I, involved in neutrophils recruitment and activation; (ii) Leukocyte receptors: Ly6D, which is a component of lymphocyte antigen complex and IL-2R, which is involved on T-lymphocyte activation and proliferation, and beta-2-microglobulin, which is a subunit of MHC class I and is required for peptide presentation to CD8<sup>+</sup> T cells. Also, the analysis of normal cDNA libraries has displayed innate immune related genes such as lectins, hepcidin and members of the cathepsin or complement component family. The functional characterization of these genes will shed light into the mechanism of disease and stress resistance of fish and. They will be included in a stress/immune DNA micro array that will be an important tool for the diagnosis and monitoring of vaccine efficiency of cultured sea bass.

A range of RT real-time quantitative PCR assays for immune genes have been developed and optimised for Atlantic salmon, including Mx, type I IFN, type II IFN, TNF $\alpha$ , IL1 $\beta$ , and IP10. These assays have been applied to various experimental IPNV challenge and chronic stress experiments. Preparation has also been undertaken to apply micro arrays on samples from the WEALTH project, both for salmon and sea bass. We aim at sequencing 5,000 ESTs from sea bass and generate a cDNA micro array with stress/immune-related genes in cooperation with other projects. Thus, a partnership has been established with the project Marine Genomics to get access to a sea bass array that are being developed. Such micro array might be useful to monitor the efficiency of immunostimulatory and immunization treatments, and to assess consequences of sub-optimal rearing environments and husbandry procedures. In the case of Atlantic salmon a 16.000 cDNA micro array from the GRASP project has been tested, and is currently applied on head kidney samples from Atlantic salmon exposed to various chronic stress treatments.

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