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Contents

1 Project execution

1.1 Overview of general objectives

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. **The main objectives of the WEALTH project were:**

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.
5. Based on the above objectives, the final goal of the WEALTH project was 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 Wealth projects consisted of 5 interlinked work packages (Figure 1).

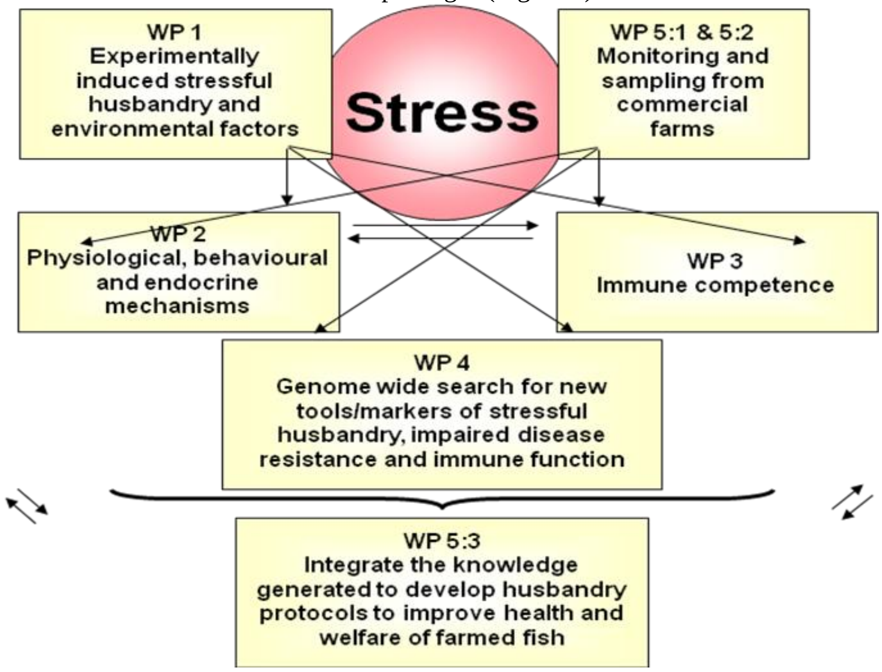

Figure 1. Structure of the Wealth project showing the link between WPs with monitoring and sampling on commercial fish farm, controlled experiments with potentially stressfull environmental and husbandry conditions, the impact on physiological, endocrine and behavioural mechanisms, as well as immune function and competence, search for new markers for stress and impaired immune function, and integration into recommendations on welfare indicators and husbandry protocols.

1.2 *Contractors involved*

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1.3 Summary of work performed and results

1.3.1 WP1.1 Husbandry and environmental factors – Atlantic salmon

Contractors involved: IMR, UGOT, NSVS, CEFAS.

A range of experiments have been conducted with Atlantic salmon; two in sea cages (1.1.1 and 1.1.2), 4 studies with postsmolts in seawater tanks (1.1.3-1.1.6) and one covering the transfer from freshwater to seawater (in tanks; 1.1.7).

Two sea cages studies (WP1.1.1 and 1.1.2) investigated how different aquaculture practises (feeding regime, placement of underwater lights and stocking density) affect the behaviour of the salmon, and the consequences on water quality, growth performance and possible health and welfare indicators. Initial analyses of the data show the variability of the actual density of the fish in the cages, and the variability of water quality parameters such as temperature, salinity and oxygen saturation. Both stocking density and light regime affected the behaviour of the fish (e.g. the swimming depth and the actual density) whereas less effect was found of the different feeding regimes. Initial analysis of growth performance and some welfare indicators suggest that problems arise under the given conditions when stocking density exceeded 26 kg/m^3. The observed density in these cages was often found to be much higher as the fish did not utilise the full volume of the cage in many cases.

Based on the results obtained in the sea cages, IMR has conducted 4 trials with variable hypoxia on salmon postsmolts in seawater tanks (Wp 1.1.3, 1.1.4, 1.1.5 & 1.1.6). Different levels of oxygen saturations were created by either increasing stocking density with fixed water flow – or reducing water flow (fixed or variable) or by combinations of reduced water flow and added oxygen in the inlet. The daily variability of oxygen was designed to resemble the range of observed values in commercial and experimental sea cages in WP5 and WP1, respectively. This way of creating lowered oxygen saturation also resulted in some build-up of carbon dioxide and ammonia in the water, but without exceeding assumed safe levels of these metabolites. All experiments were conducted in a set-up using 12 tanks (4 treatment groups in triplicate 7 m^3 tanks) with feed waste collection and continuous oxygen monitoring in the outlet. The experiments lasted for 40 to 60 days and the fish was monitored for a range of performance and welfare parameters. This included daily feed intake, feed conversion efficiency, growth, condition factor, fin damages, mortality and continuous monitoring of oxygen consumption in each tank.

Samples were collected for plasma and water cortisol and intestine permeability (analyzed in Wp2 by CEFAS and UGOT), and for analysis of expression of stress and hypoxia related genes by a 16K cDNA micro array in Wp4 (using head kidney tissue). At the end of each trial a proportion of the fish was challenged by treatments to simulate viral and bacterial infections (poly I:C, bacterial vaccine, DNA vaccine) and monitored for expression of cytokines and antiviral proteins by real-time quantitative PCR as a measure of the innate immune response. In Wp1.1.4 cells from head kidney were collected (macrophages), isolated and stimulated *in vitro* with poly I:C at NSVS before being analyzed for gene expression of cytokines and antiviral proteins after incubation.

The tank experiments revealed a clear correlation between oxygen saturation in the tanks and feed intake, with a gradual decrease in feed intake with lowered oxygen saturation in the range from 85 to 50%. Oxygen saturations fluctuating around 50% had a significant negative effect on growth rate and condition factor, but did not increase mortality. Constant low oxygen saturation (around 50%) was indicated to be more stressfull than repeated short (2h) or longer (5h) hypoxic episodes (dropping from 80 to 50% oxygen saturation), as judged by decreased feed intake and growth.

The continuous measurement of oxygen in the outlet of the tanks revealed that oxygen consumption is a very sensitive indicator of disturbances and stress such as changes in light or various sampling events. A clear effect was found on the haemoglobin composition in the blood of salmon exposed to the low oxygen levels, showing a shift to more hypoxia-resistant forms of haemoglobin.

The different ways of creating low oxygen/hypoxic episodes had different effects on cortisol in water and plasma (see WP2); a cortisol stress response was seen both in plasma and water in the low oxygen groups created by variable water flow, whereas no such cortisol response was seen in low oxygen groups created by constant low flow. This could indicate that strong variations in flow were experienced as more stressful to the salmon than low oxygen *per se*. On the other hand, in spite of no long term primary stressresponses, stress was indicated by a clear reduction in feed intake seen in all the low oxygen exposed groups. This may suggest prolonged secondary stress responses even when the stress hormonal levels have retained to normal and/or that other stress mechanisms were activated. Stressful conditions were also indicated by significant up- or down regulation of assumed hypoxia and stress related genes compared with controls as measured by a 16K cDNA salmon microarray following 10 or 58 days of exposure to around 50% oxygen saturation (see Wp4).

Examination of intestinal barrier functions using Ussing chambers (see Wp2) also indicated clear stress effects in all the low oxygen groups, where low oxygen as such resulted in impaired barrier functions, an effect that was reinforced by additional stressors like high temperature. The lowest oxygen saturation (fluctuating around 50%) was also indicated to compromise immune function compared with controls (at around 80% oxygen saturation) measured both after *in vivo* and *in vitro* (macrophages from head kidney) stimulation of the innate immune system. This was measured as differences in gene expression of a range of cytokines and antiviral proteins with real-time quantitative PCR shortly after treatments that simulate viral and bacterial infections (poly I:C and bacterial vaccine stimulation). These results together may imply that the low oxygen treated animals were more susceptible to infectious disease outbreaks than controls. However, there were large individual differences within each treatment group in the innate immune response following stimulation – preventing a clear-cut picture. Further trials are needed to verify these effects on innate immune responses, and also to demonstrate the assumed links between modified primary barriers, innate immune responses and the actual disease resistance.

Altogether, these studies clearly indicate that lowered oxygen saturation (to around 50% saturation) has negative effects on the welfare in salmon. Further, these well-controlled tank studies imply that the low oxygen levels that were detected episodically in salmon sea cages (Wp5 and Wp1) can have negative effects of fish welfare, and possibly affect disease resistance. This also corroborates the findings from the stocking density study that was conducted in salmon experimental sea cages (Wp 1.1), which suggested impaired welfare in terms of reduced growth and increased incidence of fin damages when stocking density exceeded approximately 25 kg/m^3. This was also associated with episodes of low oxygen levels in the cages. However, it should be noted that oxygen levels in sea cages are affected by a range of other factors apart from stocking density, making it difficult to draw precise conclusions on stocking density and welfare.

Combination of hyperoxygenation and low water flow in freshwater created chronic stress in salmon pre-smolts compared to controls under normal flow and oxygen levels (WP1.1.7), as evident by plasma and water cortisol levels analysed under WP2. This resulted in significantly higher mortality following IPNV challenge in postsmolts after seawater transfer (WP3). No mortality was seen in IPNV unchallenged fish in seawater either coming from the hyperoxygenated or the normoxic group. Tissue samples from this trial were analysed for intestinal primary barrier functions, cytokines and other immune and stress parameters under WP's 2, 3 and 4 to establish a better understanding on the link between chronic stress, impaired barrier functions, reduced immune function and increased susceptibility to IPNV induced mortality. However, large individual variations precluded firm conclusions.

1.3.2 WP1.2 Experimentally induced stressful husbandry and environmental factors for sea bass

Contractors involved: IFREMER, ICRAM, HCMR and WU.

WP1.2.1 Flow-through tank studies – sea bass

<u>WP1.2.1.1 Flow-through tank studies, IFREMER:</u>
An experiment was conducted from October 2004 to February 2005 to study effects of stocking density on fish behaviour, health and growth performances while maintaining water parameters at non-limiting levels. About 800kg of sea bass (75g individual mean weight) were transported by truck from a fish farm and distributed among 15 tanks of 1 m^3 each at densities of 10, 20, 40, 70 and 100 kg/m^3. Each density was run in triplicate tanks. Two different super-oxygenated seawaters (14 and 25 mg O2.l^{-1}) were mixed and supplied to the tanks to obtain a flow rate per tank ranging from 0.5 to 2.5 m^3/h depending on the stocking density. This prevented build up of wastes (gaseous, dissolved or particulate) in the rearing water. Fish were fed to satiation once a day and uneaten pellets were counted in the particle trap of each tank, in order to calculate the exact quantity of daily feed intake. Stocking density was maintained in a narrow range (+15 to 20%) by removing excess biomass at days 21, 42, 63, 84, 105 and 126. At days 1, 42 and 105, total tank biomasses were controlled and a sample of 40 fish per tank was measured and weighted individually. Fish swimming behaviour was monitored by acoustic telemetry. Mortality was recorded daily. Water samples were collected regularly to monitor temperature, salinity, oxygen, pH, ammonia, urea, nitrite, nitrate, phosphate. Cortisol was analysed in blood plasma and in rearing

water (WP2; ICRAM, CEFAS). At day 105, fish from treatment 10, 40, 70 and 100 kg/m^3 were randomly sampled for immune response monitoring through a virus challenge (WP3). Water parameters were maintained constants throughout the experiment at limiting levels: temperature = 21.2±0.6°C, salinity = 36.7±1.6 $g.L^{-1}$, oxygen>102% saturation, pH>7.1, ammonia<0.6 $mg.L^{-1}$ N-(NH_3+NH_4^+), urea <0.2 $mg.L^{-1}$N-Urea, nitrite < 0.08 $mg.L^{-1}$N-NO_2, nitrate <1 $mg.L^{-1}$ N-NO_3, phosphate <0.08 $mg.L^{-1}$ P-PO_4.

Differences in swimming behaviour were noted between the extreme treatments in this flow-trough study with stocking densities ranging from 10 to 100 kg/m^3 where water quality was maintained at non-limiting levels. A slight negative effect was seen on feed intake (-10%) and growth in the 100$kg.m^{-3}$ group compared with the other stocking densities. No noticeable incidence of wounds or fin damages was seen at the highest stocking densities. Whole blood analysis showed no significant difference in cortisol, sodium, potassium, chloride, pH, urea, glucose, hematocrit, hemoglobin and total protein between the 10, 40 and 100 kg/m^3 stocking density. The blood parameters PCO_2 and HCO_3 were higher in the 100 $kg.m^{-3}$ group. The study suggests only minor negative effects of high stocking density (up to 100$kg.m^{-3}$), provided that water quality parameters are kept on non-limiting levels.

During the period September 2005 to August 2007, 2 experiments were carried out at the IFREMER station in Palavas les Flots (WP1.2.1.1 and 1.2.1.2, see below). They were conducted in 1 m^3 tanks in triplicate under real "monoparametric" conditions: the water quality was kept identical in all the tanks and the only variable parameter was the oxygen concentration in the first experiment and the combination of oxygen and carbon dioxide concentration in the second experiment. All water quality parameters were monitored along the experiment (4 months) as well as mortality, daily feed intake, feed conversion efficiency, growth and fish condition. Samples were collected for plasma cortisol and analysed in WP2 by ICRAM (WP2.6). During that period, investigations were carried also out on (1) the proteinemia of the fish collected from the experiments on fish density in flow through and recirculation systems and the experiments on gas supersaturation and (2) the variation of the serum protein panel by proteomic analysis on fish from the experiment on fish density in flow through system (reported under WP3) 3) serum lysozyme analysed by ICRAM in WP3. At the end of the experiment, fish from all treatments were challenged.

<u>WP1.2.1.2 - Effects of hyperoxic water conditions under different total gas pressure in sea bass, IFREMER</u>
Investigation on the chronic effects of 2 levels (medium and high) of hyperoxia obtained by dissolution of oxygen under high pressure (resulting in a high total gas supersaturation) and 1 medium level of hyperoxia obtained by dissolving oxygen under low pressure (i.e. no total gas pressure supersaturation) on growth performances of sea bass. The fish were submitted in triplicate tanks during 84 days at 2 levels of hyperoxia (medium and high) obtained by dissolving oxygen under high pressure (respectively called T2 medium HP, and T3 high HP) and 1 medium level of hyperoxia obtained by dissolving oxygen under low pressure and called T4 medium LP. An additional treatment was the T1 control: T1 (control): [O_2] = 7.0 $mg.L^{-1}$, 99% sat, -150 TGP in mm Hg, T2 HP (medium): [O_2] = 7.9.9$mg.L^{-1}$, 139% sat, +29 TGP in mm Hg, T3 HP (high): [O_2] = 715.6 $mg.L^{-1}$, 220% sat, +167 TGP in mm Hg, T4 LP (medium): [O_2] = 135 $mg.L^{-1}$, 135% sat, -160 TGP in mm Hg. Physiological responses (cortisol and oxidative status analysed by ICRAM in WP2), morphology, physiology and immune competences in sea bass were also investigated (see Wp2.6).

Mortality was very low in control and T4 (medium LP) groups during all periods. A low mortality affected the fish in T2 (medium HP) treatment peaking at 1.3 and 1.4% for period 2 and period 4, respectively. T3 (high HP) treatment was the most affected with mortalities expressed as % of the fish per tank per period. The visual observation of the fish in the tanks did not showed a clear difference in swimming activity amongst the treatments in the first days of the experiment and after. The fish submitted to T3 (high HP), mainly during periods 1, 2 and 3, exhibited in water the same skin appearance in the 3 tanks: some fish (10 to 15% fish/tank) had a clearest body colour than control fish and had irregular grayish-white patches at the basis of the dorsal, caudal and pectoral fins. Some whitish patches appeared on caudal and pectoral fins. The dead fish showed tiny gas bubbles in the eyes and in the skin of fins when removed from water. The daily amount of ingested food (DAIF) per tank showed larges variations amongst the tanks due to external and internal factors and their complex combinations. From the first day of the experiment to the end, the control fish T1 had a significantly lower feed intake than the fish of the others treatments which exhibited a FI 7.5% higher. The global SGR was significantly affected in T3 where fish were subjected to high hyperoxia and high total gas pressure conditions and represented 92% of the growth observed in control fish. In T2 conditions, the fish exhibited a slightly better growth than the control fish (+5%). The FCR was significantly higher (+16%) in the treatment T4 high HP compared to the others. No statistical difference was found in Na, K, and pH amongst the treatments. TCO_2, PCO_2 and HCO_3 increased in relation with increasing hyperoxia.

WP1.2.1.3 Combined effects of hyperoxic and hypercapnic water conditions in sea bass, IFREMER:

Test of the chronic effects of 3 levels (control, medium and high) of hyperoxia associated to hypercapnia conditions (control around 7 mg/l of O_2 and CO_2, medium around 12 mg/l O_2 and 29 mg/l CO_2 and high 17 mg/l O_2 and 53 mg/l CO_2), on growth performances, physiological responses (cortisol, glucose, osmolality, ions and oxidative status analysed by ICRAM in WP2.6), morphology, physiology and immune competences (serum lysozyme and complement activities analysed by ICRAM in WP3.2) in sea bass.

No mortality was observed from d1 to d63 in all treatments. The visual observation of the fish in the tanks showed a clear difference in swimming activity between the treatments in the first days of the experiment. The fish submitted to T3 exhibited a lower swimming activity than control. The fish swam in slow motion, and in a group in the middle of the tank. When fed, the fish were indifferent towards the food and had a low feeding activity. Within a period of 30 days, the swimming and feeding behaviour of the fish of T3 became progressively similar to the control fish. At the end of the experiment, no visual differences in behaviour were observed amongst the treatments. The behaviour of the fish submitted to T2 was intermediate between Control fish and T3. The daily amount of ingested food (DAIF) per tank showed larges variations due to external and internal factors and their complex combinations leading most of the time to unclear graphs. When mobile means (3 following data) of DAIF were expressed in % of control fish and were plotted against time, more clear trends appeared over the experiment.

From the first day of the experiment to the end, the fish subjected to T3 had a significantly lower feed intake than control fish. The average feed intake in T3 is 84% of the control T1. The feed intake of T2 exhibited the same trend, but slightly and not significantly lower than controls (94%). The global SGR was deeply and significantly affected in T3 (see Table 2) where fish were subjected to high hyperoxia and hypercapnia conditions and represented 84.5% of the growth observed in control fish. In T2 conditions, fish exhibited an intermediary growth which was 93% of the control fish but not significantly different. The FCR was similar in the 3 treatments, as the predicted result of a reduced feed intake and a reduced growth in the same percentage. No statistical difference was found in Na, K, Glucose, and pH. TCO_2, PCO_2 and HCO_3 increased dramatically in relation with increasing hypercapnia and hyperoxia. Hematocrit and haemoglobin content decreased when increasing hyperoxia. The apparent oxygen uptake was significantly higher in T2 (120%) and T3 (132%) compared to the control fish T1 (100%).

WP1.2.1.4 Effects of cortisol implants in sea bass, IFREMER/UGOT/ICRAM

Investigation on short and long term effects of a stress-model created by intraperitoneal slow release implants of cortisol in sea bass. Growth performances, physiological responses, morphology and physiology of some primary barrier tissues, and immune competences in sea bass were investigated (see WP2 and WP3). The experimental design was based on the comparison of effects in fish of three different treatments in duplicate tanks:

- fish non injected (control or T1).
- fish injected with implant without cortisol (0 µg hydroxycortisone /g body weight) or T2.
- fish injected with cortisol implant (75 µg hydroxycortisone /g body weight) or T3.

Two groups of 135-140 fish each were subjected at random at the same treatment in 2 different tanks and were maintained in satisfactory rearing conditions (see above) during 42 days. The total number of experimental tanks was 6. No mortality was observed after injection from d1 to d18 in all treatments. At d19, 1 and 6 dead fish respectively were removed from the 2 tanks submitted at the cortisol treatment (T3), 5 hours after a failure in the lightning system. From d20 to the end of the experiment, no mortality was observed. The visual observation of the fish in the tanks showed a clear difference in swimming activity between the treatments in the first days of the experiment. The fish with cortisol implants (T3) exhibited a faster swimming activity and few of them seemed des-oriented, swimming fast in the opposite direction of the other fish. When fed, the fish under cortisol treatment went faster towards the contact point of the pellets with the surface seawater, but they ate less the pellets on the bottom of the tank as the fish of other treatments did. Within a period of 15 days, the swimming and feeding behaviour of the treated fish (T3) became progressively similar to the other fish. The feed intake per tank was significantly 21% lower in cortisol treated fish (T3 group) than in control (T1) and T2 conditions. The daily amount of ingested food (DAIF) per tank showed larges variations due to external and internal factors and their complex combinations leading to unclear graphs. When mobile means (3 following data) of DAI F were expressed in % of control fish and were plotted against time, clear trends appeared over the experiment. The fish injected without cortisol (T2) showed a feed intake 85% lower than control fish in the very first days of the experiment, but they recovered a similar feed level within 7 days. The fish injected with cortisol (T3) exhibited a DAIF corresponding to 70% of the control one during the 15 first days, then there was a progressive increase from d16 to d30 to recover a level closed to the control fish T1.

The global SGR is deeply and significantly affected in T3 where fish were injected with cortisol implants and represents only 61% of the growth observed in control fish. No difference was found between control fish and those which received an implant without cortisol. As a result of a lower feed intake and a reduced growth rate, the FCR of the fish in T3 was significantly higher by 143% than control and T2. The Coefficient of Variation of Weight and the Condition Index were not significantly different in any treatment, even if the mathematical values were slightly un-favourable in the T3 condition compared to control. No statistical differences were found between treatments in Na, K, TCO_2, glucose, hematocrit, pH, PCO_2, HCO_3 and haemoglobin. The apparent oxygen uptake was significantly different in T3 than in the other treatments, and was 115% higher than the control fish.

<u>WP1.2.1.5 Experimental land based flow-through tanks with sea bass, HCMR</u>

Experiment 1A Hypoxia in experimental land based flow-through tanks with sea bass
A flow-through study has been conducted in duplicate $1m^3$ seawater tanks at 4 different oxygen concentrations, ranging from 3.6 to 8.2ppm (temperature 26-28^oC). The stocking density was the same in all tanks and fish were fed to satiation. The experiment lasted 9 weeks and fish were sampled and weighed every 3 weeks after anaesthetization. The parameters measured included feed consumption, growth and feed utilization efficiency. Blood and tissue samples were taken to measure haematological and immunological parameters, and samples were collected for barrier tissue morphology with special emphasis on skin.

Hypoxia affected feed consumption to a small degree, while feed efficiency and fish growth were affected to a higher degree. Haematological values indicated an adaptation of the fish to the low environmental oxygen. Only neutral mucus cells could be seen on the skin of sea bass indicating that stress was mild for the fish. The number of mucus cells per skin area decreased with the oxygen level of the water. After 9 weeks of rearing the numbers of mucus cells were lower in all groups – all of them showing similar values – except the one at the lowest O_2 content having significantly higher numbers. Immunological parameters studied did not indicate significant differences among treatments.

Two new trials have been conducted using sea bass. One (Experiment 1B) involved rearing of sea bass under 3 different oxygen levels and the second (Experiment 2) use of different flow rates under reduced oxygen levels and comparison among them and with conditions of adequate flow rates and oxygen water content.

Experiment 1B. Effect of different oxygen levels on sea bass performance
This experiment was run to complement the results of experiment 1A, which was performed before, by extending the oxygen levels of water down to 2ppm. Three duplicate groups of sea bass of about 110g initial weight were used and the levels of oxygen in the tanks was kept at 2.2, 3.5 and 7.0ppm by providing oxygen in the tanks, along the water entrance, through diffusion stones connected to electronic valves controlled through a PC programme. Temperature was at 26.0 ± 2.0^OC and water flow rate at 30l/kg fish/h. The fish were fed by hand to visual satiation and non consumed feed was collected and counted. During the experiment, oxygen consumption was measured and three samplings were carried out on day 3, 10, 23, after the oxygen was lowered, for measurements of certain physiological (WP2.6, hematocrit, hemoglobin, number of red blood cells, lactate) and immunological (WP3.2, blood respiratory burst activity, lysozyme and antibacterial activity of complement) parameters. Samples of tissues were taken for histological analyses. Fish were also subjected to microbiological examination. Fish were individually weighed for calculating performance parameters. After the end of the experiment remaining fish were subjected to acute stress and mortalities as well as appearance of pathogens, previously present in the intestine, to internal organs (liver, spleen and kidney) monitored (WP2.8).

Experiment 2. Effect of different water renewal rates and oxygen levels on sea bass performance
Four triplicate groups of about 185g weight were used. The flow rates and oxygen level of the control group was 30l/kg fish/h and 8ppm O_2 in average. The oxygen level of the other treatments was kept at 3 to 4 ppm and flow rates ranged at 16, 23 and 30l/kg fish/h for treatments 3 to 4 respectively. The fish were fed by hand to visual satiation and uneaten feed was collected and counted. Fish were individually weighed at 50 and 80 days and samplings were performed at 25, 47 and 77, after the initiation of the experiment. Samplings involved collection of blood for measuring of certain physiological (WP2.6, cortisol, ROMs, AOP) and immunological parameters ACH50 analysed by ICRAM(WP3.2), skin collected for observing changes in morphology (WP 2.7), other tissues for histological observations and microbiological analyses. Fish were observed for the existence of parasites. Water samples were collected for measurements of total ammonia, nitrate levels and PH. After the end of the experiment remaining fish were subjected to acute stress and mortalities as well as appearance of pathogens, previously present in the intestine, to internal organs (liver, spleen and kidney) monitored (WP2.8).

WP1.2.2 Recycling systems studies – sea bass

WP1.2.2.1. Stocking density in experimental recirculation system tanks with sea bass, IFREMER:
A recirculation experiment was carried out from May to July 2005 to test effects of stocking densities on fish behaviour, health and growth performances, while maintaining water parameters at non-limiting levels. About 650kg of sea bass (135 g individual mean weight) were transported by truck from a fish farm and distributed among 12 tanks of $1m^3$ each at densities of 10, 40, 70 and 100 kg/m^3 in triplicate tanks. Two different recirculated seawaters (normal O_2 saturation and 29 mg $O2.l^{-1}$) were mixed and supplied to the tanks in order to obtain a flow rate per tank ranging from 0.5 to 2.5 m^3/h depending on the stocking density. This prevented build up of wastes (gaseous, dissolved or particulate) in the rearing tanks. The recirculated water was mechanically filtered, then passed through a degassing system, a biological filter, a UV disinfecting unit, and part of it in an oxygenation super-saturation system. The make up water quantity was maintained around $2m^3$ per kg feed fed to the fish. Fish were fed at satiation once a day and uneaten pellets were counted in the particle trap of each tank, to calculate the exact quantity of daily feed intake. Stocking density was maintained in a narrow range (+20%) by removing excess biomass at days 21, 42 and 63. Total tank biomasses were controlled and a sample of 40 fish per tank was measured and weighted individually. Fish swimming behaviour was monitored by acoustic telemetry and mortality was recorded daily. Water samples were collected regularly to monitor temperature, salinity, oxygen, pH, ammonia, urea, nitrite, nitrate, phosphate. Blood samples were collected for plasma cortisol analysed by ICRAM (WP2.6). At the end of the experiment, fish from treatment 10, 40 and 100 kg/m^3 were randomly sampled for immune response monitoring through a virus challenge. The water parameters were maintained constant throughout the experiment at non-limiting levels: temperature = 23.5±0.1°C, salinity = 37.2±0.5 $g.L^{-1}$, oxygen around 114 % saturation, pH around 7.1, ammonia<1,6 $mg.L^{-1}$ N-(NH_3+NH_4^+), urea <0.2 $mg.L^{-1}$N-Urea, nitrite < 1,6 $mg.L^{-1}$N-NO_2, nitrate around 12 $mg.L^{-1}$ N-NO_3, phosphate <1.5 $mg.L^{-1}$ P-PO_4.

The fish activity, based on visual control, showed large differences between the extreme treatments in the study from the recirculating system. Feed intake, growth rate and feed conversion ratio were not significantly different between the treatments 10, 40 and 70 kg/m^3. No noticeable incidence of wounds or fin damages was seen at the highest stocking densities.

WP1.2.2.2 Impact of different chronic stressors on performance, energy metabolism, stress responsiveness and behaviour in European sea bass kept in a recirculation system, WU:

One experiment was carried out in which performance, metabolic and welfare indicators were assessed under varying conditions of 2 fish stocking densities (7 and 35 kg/m^3) and 5 feeding levels ranging from maintenance to satiation levels. The experiment took place at the fish metabolic chambers at Wageningen University while monitoring O_2, CO_2, NH_4, NO_3^-, pH and urea. Respiratory, water quality and serologic data were analysed to assess metabolic consequences of different feeding levels at different densities. Video recordings allowed behavioural observations. Additionally, the sea bass was subjected to a 1h acute stress test following the different treatments. Data were analysed to verify stress responses and the presence of coping styles, as indicators of impaired welfare.

Results show a trend for increased maintenance requirements and lower feed intake in the high-density treatment. High stocking density increased cortisol plasma levels. Integration of behavioural and physiological data suggested the presence of coping styles.

At WU 3 further experiments were conducted (exp. 4-6) on the impact of different chronic stressors on performance, energy metabolism, stress responsiveness and behaviour in European sea bass kept in a recirculation system. Exp. 4 focused on the combined effect of stocking density and altered water quality (i.e., increased total bicarbonate and decreased dissolved oxygen levels with increasing stocking density) by applying 4 stocking densities (10, 30, 60, or 100 kg/m^3). Within exp 5 and 6 stocking density did not vary, and contrasts in various water quality parameters were assessed: a contrast in total bicarbonate by the addition of CO_2 to the inlet water (with or without CO_2 addition; exp 5) and 4 levels of dissolved oxygen (4.0, 5.5, 7.2 or 9.0 mg/L; exp 6). In exp 5 the effect of feeding level (satiation versus restricted feeding at maintenance) was also assessed. These experiments were carried out in the metabolic chambers of WU. Performance, energy metabolism, water quality were measured in relation to the studied chronic stress factors above. Video recordings and blood sampling at the end of these experiments were made to assess the impact on behaviour and stress responsiveness for WP2.

Results showed that increasing stocking density with coincided changes in water quality (decreased dissolved oxygen and increased bicarbonate and reduced pH) (Exp 4) reduced growth and feed intake. Energy metabolism measurements showed that reduced feed intake was partially compensated by a reduction in the energy requirements for maintenance. Results of exp 5, indicated that the reduction in growth, feed intake and maintenance requirement in exp 4 at the high stocking density were not due to the difference in total bicarbonate in the water. In Exp 4, no effect of CO_2 addition was present on performance and energy partitioning. Exp 6 showed that part of the reduction in feed intake, growth and maintenance requirements with increasing stocking density were related to the lower dissolved oxygen levels. Expressed as percentage of the gross energy intake, all studied chronic stress factors had no impact on the energy losses with faeces (i.e., digestibility of nutrients) and losses through branchia and urinary.

WP1.2.3 Acute and chronic exposure to high CO_2 levels

ICRAM:

The experiment on hypercapnia (four CO_2 levels: 2-5 mg/l control, 15-20 mg/l low, 30–35 mg/l medium, 50-55 mg/l high) was completed and reported in the mid report (Wp 1.2.3 p. 43-48; Wp 2.5. p. 55-67).

A study tested the effects of carbon dioxide (CO_2) in sea bass cultured in recycling tanks. Four test groups, in duplicate, were exposed for 45 days to water CO_2 concentration from ambient (2.8 ±2.0 mg/l^{-1}, control) through 16.9 ±3.3 mg/l^{-1} (low) and 32.7 ±4.3 mg/l^{-1} (medium) to 51.2 ±7.8 mg/l^{-1} (high), at 20±1 °C, 27 ppt salinity and 9.5 mg/l^{-1} dissolved oxygen (130% sat). Water pH was not buffered and corresponding pH values were 7.6±0.2, 6.8±0.1, 6.6±0.1 and 6.4±0.1. Fish sampling (n=890) and blood and tissues sampling (n=4000) were performed before CO_2 treatments and during acute (3, 6, 24, 48, 96 hours) and chronic exposure (8, 22, 45 days).

Samples (blood, serum and head kidney) were collected in order to investigate the effects of carbon dioxide on stress parameters and on innate and specific immunity in sea bass. Results are presented under Wp2.6 and WP3.2.2. Additional tissue samples (gill and kidney) were collected to investigate histopathological changes occurring in target organs under hypercapnic conditions (WP2.7).

Sea bass exhibited a good tolerance to chronic hypercapnia. After 45 days, no mortality was observed in the highest treatment group (51.2 mg/l^{-1}) and there were no significant effects of CO_2 on body weight, standard length and condition factor. However, the exposure to hypercapnic conditions induced significant changes in blood gas status and acid-base balance, according to water CO_2 concentrations. Difference in blood PCO_2, [CO_2], pH, PO_2, HCO_3^- and Cl^- contents were detected between hypercapnic and normocapnic groups during acute exposure and at day 45. The increase in blood HCO_3^- ions was related to a decrease in Cl^- concentration, suggesting a compensatory mechanism by means of HCO_3^-/ Cl^- ion exchange at the gill level. Acid-base imbalance appears to affect the highest hypercapnic groups, with a marked accumulation of blood HCO_3^- and plasma chloride loss. No differences were observed on osmolality, Na^+, K^+, glucose, complement activity and lysozyme levels and on the oxidative status of hypercapnic groups and controls.

This study represents a contribution to the identification of long-term sub-lethal effects of hypercapnia in sea bass in order to estimate carbon dioxide concentration tolerated by sea bass in recycling systems (Art. 12 and 21 of the “Recommendation on the welfare of farmed fish”, Council of Europe).

WP1.2.4 Effects of anaesthesia on stress response in sea bass

ICRAM:

Anesthetics are routinely used in aquaculture to minimize stress and to avoid injuries during husbandry practices, as well as to euthanize fish in an ethical way. This study analysed the efficacy of different anesthetics and their potential to reduce stress in sea bass. The experiments examine whether anesthesia procedures affect stress response and welfare in sea bass and whether they interfere with stress physiology measurements during blood sampling. Different anesthetics were tested: tricaine methanesulphonate MS222, 2-phenoxyethanol, clove oil (70-90% eugenol) and Aqui-S® (50% iso-eugenol) and a mixture of water and ice. Stunned fish were used as controls. Blood samples were collected at different time intervals (6 and 20 min) and analysed for plasma cortisol concentration. Data on water quality were monitored during the trial.

In sea bass, different anaesthetics showed different capacity to block the activation of HPI-axis and reduce cortisol stress response during handling and blood sampling procedure. The most effective was clove oil at 100 mgl^{-1}, even if a marked cortisol increase occurred during 20 min clove oil anaesthesia, suggesting that this chemical does not effectively inhibit the HPI-axis in sea bass. Clove oil at dose of 60 mgl-1 and Aqui-S® at 100 mgl^{-1} showed similar efficacy, due to their approximately equivalent concentration of eugenol and iso-eugenol, as active substances. MS222 and 2PHE induced a drastic increase of cortisol levels after 6 min. Anaesthesia in ice/water resulted the most appropriate method for performing blood sampling in sea bass, showing a good capacity to reduce stress response during both short and long exposure. Ice-water anaesthesia is also easy to perform, inexpensive and safe for environment and users.

1.3.3 WP2 Physiological, behavioural and endocrine mechanisms

Contractors involved: UGOT, IMR, CEFAS, WU, ICRAM, HCMR, IFREMER, NSVS

WP2.1 Development of new labelling techniques for pathogenic bacteria

UGOT, IMR and NSVS:

Work was done to try to construct and transform a plasmid containing the gene coding for GFP and ampicillin resistance into *Aeromonas salmonicida*. A plasmid vector containing a gene coding for green fluorescent protein (GFP) was constructed. However, due to a methylated site the restriction enzyme was unable to cut the DNA. Instead a PCR product of the GFP gene was constructed and ligated into the plasmid. This resulted in green colonies when grown on agar plates containing ampicillin. This successfully constructed plasmid vector should then be transformed into *A.s* using electrophoration. After transformation, *A.s* could be grown on agar plates containing ampicillin. However the colonies did not appear green indicating that the gene coding for GFP was not expressed in *A.s*. One possible explanation is that the promotor is from E. coli and it may not function in *A.s*. In order to solve this problem, actions will be taken to find a promotor that functions in *A.s* and then transform this into the bacteria.

A method to investigate translocation possibilities and rates of IPNV across the primary barrier of the intestine, using the *in vitro* Ussing chamber technique, is under development. We are under the progress to develop a DELFIA (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) assay in order to be able to quantify low concentrations of translocated virons. Concomitantly, a virus concentration method is under development to be used to concentrate translocated viral particles and further subject them to the DELFIA.

WP2.2 Transfer of non-invasive stress assay methodology to Atlantic salmon and sea bass, and development of normaliser for loading density

CEFAS:

Laboratory experiments were completed examining the effects of oxygenation of seawater and freeze storage of seawater samples on the stability of cortisol (the stress hormone) and melatonin (a potential normaliser) in seawater. Oxygenation of seawater and freeze storage of seawater samples did not reduce the recovery of cortisol. A significant correlation was demonstrated between cortisol release rate (an absolute measure of stress status calculated from water cortisol measurements) and plasma cortisol levels in Atlantic salmon (data from WP1.1.7) showing that water cortisol level does reflect the level of cortisol in the blood. Water cortisol and melatonin levels were measured in water samples collected in WP1.1.3, 1.1.4, 1.1.5, 1.1.6, and the water cortisol results largely concurred with blood sampling data. The transfer of the methodology to seawater Atlantic salmon was therefore validated and has been published. Cortisol release rates in seawater Atlantic salmon correspond well to rates previously observed in freshwater rainbow trout. However, the lower levels of water cortisol previously observed for sea bass are not due to oxygenation of the seawater or freeze storage of samples, and the reason remains unknown.

The non-invasive stress assay methodology was used to assess cortisol levels in fish exposed to bacterial and viral pathogens at various times pre- and post-challenge. Water cortisol levels were shown to become greatly elevated during acute viral (Viral Haemorrhagic Septicaemia and rainbow trout) and bacterial (*Aeromonas salmonicida* and freshwater Atlantic salmon) challenges 1-2 days before mortality became apparent, and remained elevated during the period of mortality. The potential causes and consequences of this elevation in cortisol level (supported by previously published plasma cortisol data) have been reviewed in a publication.

The laboratory experiments demonstrated that melatonin (a potential normaliser) is not broken down by oxygenated seawater, only slight losses occur during freeze storage, but that significant loss occurs during evaporation of solvent (used to recover the cortisol/melatonin from the concentration matrix) under air but not under nitrogen. The addition of an antioxidant (resveratrol) alleviated this latter problem. However, very low levels of melatonin have been recovered from fresh and frozen samples from WP1 experiments collected during the dark, despite addition of resveratrol. The methodology for measuring creatinine (a nitrogenous excretory product) in fresh and seawater as an alternative normaliser was successfully developed: creatinine is measured using a colorimetric plate assay after concentration from the water using appropriately sized cation exchange columns.

WP2.3 Experimental stress challenges on sea bass

See WP 2.5 below

WP2.4 Endocrine impact on physiology and immunology of Atlantic salmon

UGOT and NSVS:

In order to establish causal relationships between endocrine control, physiology and immunology, fish were implanted with sustained-release implants of growth hormone (GH; bovine growth hormone gel implant) and cortisol (F; slow release implant based on vegetable lipids). 2 persons from partner 6 stayed with partner 7 at one occasion (implantation) and 1 person from partner 6 stayed with Partner 7 for sampling at the end of the experimental period. The reminding fish from the experiment in WP 2.4 were transferred and continued within the experiment of WP 1.3.2. 6 fish from each tank (12 fish/group) were sampled for plasma for delivery to WP 2.6 and 2.9 for analyses of plasma for GH, IGF-1, cortisol and ions. Liver and kidney were also sampled for analysis for gene expression, delivery to WP 4.1. The reminding fish were transferred to WP 3.1.2.

WP2.5 Behavioural stress responses in sea bass

IFREMER:

In an attempt to characterize behavioural responses susceptible to indicate health and welfare status of fish under rearing conditions, we investigated swimming activity levels and rhythms as well as space occupation by sea bass, *Dicentrarchus labrax*, in relation to stocking density in flow through tanks using acoustic telemetry. Five individual sea bass were tagged per tank at low (10 kg m^{-3}), medium (40 kg m^{-3}) and high density (100 kg m^{-3}) and tracked for 48 hrs each (720 hrs in total), one fix every 2 s. Data obtained in this study showed that swimming levels significantly decreased with increasing density. At low density, a tendency to hyperactivity was also observed during the period preceding feeding and could be linked to a feeding anticipatory behaviour. In parallel, an inversion of the day/night rhythm was observed: when density increased, sea bass became predominantly diurnal whereas they were nocturnal at low and medium densities. Finally, stocking density also tended to modify sea bass preferential space use: fish more frequently occupied the tank centre with increasing density. This first study provided relevant information about significant fish swimming activity shifts in relation to stocking density and its use as operational welfare indicator remains under development

WU:

In experiments (exp 4-6 - WP1.2.2) done at Wageningen University, European sea bass were subjected to an acute stress test to measure the impact of the studied chronic stressors on stress responsiveness. This test comprised of netting fish for 1 h. Blood samples, taken from control fish and netted fish, were analysed for cortisol, glucose and lactate content. Video recording were analysed regarding behaviour of fish during the experimental period focussing on the swimming activity of sea bass. Additional in exp. 4, behavioural observations were made during a netting procedure which were related to blood stress parameters.

Behavioural observations showed that a strong relation with the measured energy requirements for maintenance (WP1.2.2.2). In Exp 4, increasing stocking density (coinciding with altered water quality) reduced the swimming speed of the sea bass. Similarly in Exp 6, the swimming speed was reduced at the low oxygen levels. Exp 5, showed that swimming speed was not affected by the created contrast in total bicarbonate level. Basal cortisol levels measured at the end of the experiments were not affected by any of the chronic stressors, except for feeding level (Exp 5), restricted fed fish had lower basal cortisol levels compared to satiation fed fish. Chronic stress affected the response of fish to an acute stressor (increased cortisol levels). In all experiments, the cortisol response to the acute stressor measured at the end of the experimental period was enhanced with increasing levels of chronic stress. Considerable variation in the behavioural response during the netting stress test was present. However, this variation in behaviour was not related to the variation in blood cortisol levels. Therefore, the existence of different copying strategies in sea bass can-not be confirmed, based on the data on the netting stress test.

WP2.6 Physiological stress responses in Atlantic salmon and sea bass

UGOT, IMR, ICRAM and HCMR:

Plasma and tissue samples for analyses of several physiological and immunological parameters were delivered from 5 experiments on Atlantic salmon and 10 experiments on sea bass according to the tables below.

ATLANTIC SALMON			
Exp.	**Experiments**	**Sampling**	**Analysed samples**
Exp. 1	WP 1.1.3	Plasma for analysis of cortisol, sodium, potassium, chloride, calcium concentrations and total osmolarity. Proximal and distal intestines in Ussing chambers; electrical parameters, permeability, bacterial translocation. Proximal and distal intestines in RNAlater, proximal and distal intestines fixed in formalin.	Plasma cortisol, Ussing chambers; electrical parameters, permeability, bacterial translocation as presented in WP 2.7.
Exp. 2	WP 1.1.4	Plasma for analysis of cortisol, sodium, potassium, calcium, chloride concentrations and total osmolarity. Proximal and distal intestines in RNAlater, proximal and distal intestines fixed in formalin	Plasma cortisol, sodium, potassium, calcium, chloride concentrations and total osmolarity.
Exp. 3	WP 1.1.5	Plasma for analysis of cortisol, sodium, potassium, calcium, chloride concentrations and total osmolarity. Proximal and distal intestines in Ussing chambers; electrical parameters, permeability. Proximal and distal intestines in RNAlater, proximal and distal intestines fixed in formalin	Plasma cortisol. Proximal and distal intestines in Ussing chambers; electrical parameters, permeability as presented in WP 2.7.
Exp. 4	WP 1.1.6	Plasma for analysis of cortisol, sodium, potassium, calcium, chloride concentrations and total osmolarity. Ussing chambers; electrical parameters, permeability. Proximal and distal intestines in RNAlater.	Plasma cortisol, Proximal and distal intestines in Ussing chambers; electrical parameters, permeability as presented in WP 2.7.
Exp. 5	WP 2.3	Plasma for analysis of cortisol, bone, liver, muscle, gills, proximal distal intestine in RNAlater, proximal and distal intestine and gills fixed in formalin.	Plasma cortisol Plasma GH and IGF-1 levels as presented in WP 2.9.
Exp 6	WP 1.2.4	Plasma for analysis of cortisol, Ussing chambers; electrical parameters, permeability.	Plasma for anlaysis of cortisol, Ussing chambers; electrical parameters, permeability as presented in WP 2.7.

SEA BASS			
Exp.	**Experiments**	**Sampling**	**Analysed samples**
Exp. 1	WP1.2.1.1 (Stocking density in flow through)	Blood sampling for stress response	Plasma samples (n=90) for cortisol analysis (in WP2.6) Plasma samples (n=90) for complement activity (CH50) (in WP3.2.)
Exp. 2	WP 1.2.1.1 (Stocking density in recycling)	Blood sampling for stress response	Plasma samples (n=90) for cortisol analysis (in WP2.6)
Exp. 3	WP 1.2.1.2 (Hyperoxia)	Blood sampling for cortisol stress response	Plasma samples (n=312) for cortisol and plasma samples (n=150) for reactive oxygen metabolites (ROMs) and total antioxidant levels (AOP) (in

			WP2.6)
Exp. 4	WP 1.2.1.3 (Hyperoxia & hypercapnia)	Blood sampling for stress and immunological analysis	Plasma samples (n=90) for cortisol, glucose, osmolality, Na^+, K^+, Cl^-, ROMs, AOP (in WP2.6) Plasma samples (n=90) for CH50 and lysozyme (Lys) (in WP3.2.)
Exp. 5	WP 1.2.1.4. (Cortisol implant)	Plasma for analysis of cortisol, Ussing chambers; electrical parameters, permeability. Blood and tissue sampling for stress response	Plasma for analysis of cortisol, Ussing chambers; electrical parameters, permeability as presented in WP 2.7. Plasma samples (n=126) for cortisol, glucose, osmolality, NEFA, triglycerides (in WP2.6) Plasma samples (n=372) for CH50, Lys, T and B cells (in WP3.2)
Exp. 6	WP 1.2.1.5 (Oxygen/water renewals)	Blood sampling for stress response	Plasma samples (n=268) for cortisol, plasma samples (n=140) for ROMs and AOP (in WP2.6) Plasma samples (n=204) for CH50 (in WP3.2)
Exp. 7	WP 1.2.3 (Hypercapnia)	Blood and tissue sampling for immunological and histological analysis	Blood and plasma samples (n= 2040) for CH50, Lys, NBT test, Anti Vibrio Ig, agglutination test, T and B cells in HK and PBL (in WP3.2) Samples of gill and kidney were analyzed as presented in WP2.7
Exp. 8	WP 1.2.4. (Anaesthesia)	Blood sampling for cortisol stress response	Plasma samples (n=180) for cortisol analysis (in WP2.6)
Exp. 9	WP 5.2.2. 1 (On farm - environment conditions	Blood sampling for stress response	Plasma samples (n=220) for ROMs and AOP (in WP2.6)
Exp. 10	WP 5.2.2.2 (On farm - stocking density)	Blood sampling for stress and immunological analysis	Plasma samples (n=180) for cortisol, glucose, osmolality, NEFA, ROMs, AOP (in WP2.6) Plasma samples (n=1515) for CH50, Lys, NBT test, Ig tot, Anti Vibrio Ig, agglutination test (in WP3.2)

The main and classical primary stress response measured in all experiments was plasma levels of cortisol. Further, partner 6 analysed plasma levels of ions and total osmolality as an indication of disturbed homeostasic ability. Low oxygen levels in the water and high fish densities, resulting in bad water quality, are without a doubt a sub optimal environment for the fish. This environment will challenge the homeostasis and the fish will need to adopt a change in physiological systems in order to adapt to the new environment. This physiological change can be defined as allostasis and the cost of cooping with the new environment, i.e maintaining homeostasis, can be defined as allostatic load. Elevated plasma cortisol levels were found mainly in the 30 kg/m^3 and 70 kg/m^3 group (experiment 1) and in the beginning of the experiment. After a period in these higher densities, the fish has been able to adapt to its environment and the plasma cortisol levels decline. However, coping with its environment has energy cost. Consequently, as more energy is needed to maintain homeostasis in the new environment, energy must be reallocated from other physiological systems like for example allocation to growth and/or the immune system. Although the fish is able to maintain the homeostasis, the cost for this may be lowered growth and increased disease susceptibility. Fish subjected to an altered environment such as chronically decreased oxygen levels will indeed have to change its physiology on order to be able to adapt to its new environment. Fixed O_2 levels as controlled by variable flow, was shown to significantly elevate in 60% and 50% DO compared to 80% and 70% DO groups (experiment 3). However, the cortisol levels declines slightly during the treatment and after 48 days no differences in plasma cortisol were observed. Also in experiment 4, the plasma cortisol levels tended to be higher in the groups subjected to a low water oxygen tension, independently of constant or periodically lower levels of oxygen. However, the experiment was terminated after 29 days, and therefore it can not be stated if the fish are able to reach a new allostatic state. A fixed water flow creating low and fluctuating O_2 levels (Experiment 2) did not create any differences in plasma cortisol after 10 days. It is clearly plausible that there was a transient increase in plasma cortisol that peaked before day 10 and then levelled out. This pattern of hypo-oxygenation seems to be a milder stressor and the fish had probably been able to adapt to this environment and a higher allostatic state and hence higher allostatic load already after 10 days. After 28 days the cortisol levels were higher in the 80% and 70% group. This was not expected and suggests that there might have been a disturbance in these groups at this sampling point. In the 50% DO group, the plasma cortisol levels tended to be lower compared to the other groups. It is important to bear in mind that plasma level of cortisol is dependent on both cortisol release and clearance rate and decreased levels of cortisol can be a result of an absolute increase in the biological activity of the corticosteroid system with both increased release rates and increased clearance rates. Thus, increased plasma levels of cortisol may not exclusively be a good stress marker. It appears to respond quite differently to different stressors, in most instances it is elevated – at least transiently, whereas in others it is un-affected or can even show a tendency towards lower plasma levels of cortisol in fish living in a sub optimal environment. This is well in line with previous research showing that cortisol is a good marker for acute stress while may not be as informative for chronic stress, if not consecutive sampling is performed.

<u>ICRAM:</u>
ICRAM investigated the effects of some environmental parameters (eg. oxygen, carbon dioxide) and husbandry practices (e.g. stocking density, water renewal) on physiological stress response in sea bass. A total of 1,662 sera samples were delivered from 10 experiments carried out in Wp 1.2 (IFREMER, NCMR), Wp 1.2.1.4 (cortisol implantation) and Wp 5.2 (ICRAM) and analysed for cortisol, glucose, non esterified fatty acids (NEFA), triglycerides, osmolality, Na+, K+, Cl- concentration (n=5576 analyses).

Two complementary assays for "in vivo" assessment of oxidative stress in sea bass were validated. The d-ROMs Test (Diacron International, Italy) for the measurement of reactive oxygen metabolites (ROMs) were validated by electronic spin resonance spectroscopy. The OXY-Adsorbent Test, for the measurement of the total plasmatic antioxidant capacity (AOP), was studied in comparison with other colorimetric methods. Analytical procedures for specific application of both tests to sea bass plasma samples were developed. Measurements of the ROMs and AOP levels were performed on 1,120 samples delivered from different experiments (Wp 1.2 and Wp 5.2.) and reference levels for sea bass were determined.

<u>Oxidative status of fish -</u> Two colorimetric assays (d-ROMs Test® and OXY-Adsorbent Test®, Diacron International, Italy) for the assessment of plasma oxidative status have been validated for sea bass. Both assays resulted suitable for the analysis of plasma oxidant and antioxidant status in sea bass. Risk of oxidative stress were observed in sea bass exposed to hyperoxia (250% O_2 sat), and hypercapnia (50-55 mg/l CO_2) & hyperoxia (230-250% O_2 sat). Under hypoxic conditions (3 mg/l O_2 concentration), an enhancement of antioxidant defences was observed. This response was already reported as an adaptive response to hypoxia, allowing sea bass to cope with potential oxidative stress arising from tissue re-oxygenation. Similar results were obtained under the same hypoxic conditions at different water renewal (16-23-30 lt/kg/h).

<u>Chronic stress model in sea bass -</u> Cortisol implantation (Wp1.2.1.4) induced a significant elevation of plasma cortisol levels in sea bass. Hormone-implanted fish (75 μg/g BW) showed significant higher plasma cortisol levels after 7 days, compared to control and vehicle implanted fish. Hypercortisolemia induced a significant increase of plasma glucose levels, osmolality and NEFAs concentration in implanted fish. Conversely, plasma triglycerides concentration significantly decreased. The metabolic effects produced by chronic hypercortisolemia

in implanted fish were consistent with the role of cortisol on the regulation of energy metabolism and osmoregulation mechanisms in fish.

Environmental conditions - Cortisol response seems to be not affected by long-term exposure to hypoxia (3 mg/l O_2 concentration), hyperoxia (up to 250% O_2 saturation) and to a combination of hyperoxia and hypercapnia (230-250 % O_2 saturation and 50-60 mg/l CO_2). Conversely, the exposure to extreme hypercapnia and to a combination of hyperoxia and hypercapnia significantly affected the ionic and osmotic regulation, suggesting a maladaptive response to blood acidosis, probably due to an impairment of gill function.

Overall results indicate that sea bass is a very tolerant species able to cope with different environmental conditions trough physiological adjustments. Threshold levels of 150 % O_2 saturation and 25 mg/l CO_2 concentration should be recommended for sea bass, in order to preserve its physiological status and good welfare conditions.

Stocking density – High stocking density, up to 100 kg/m^3 did not chronically affect plasma cortisol level of sea bass reared in experimental flow-through and recirculated tanks. Conversely, cortisol level measured on sea bass reared on commercial farm are affected by acute stress during sampling procedure, and resulted higher than cortisol measured in sea bass under laboratory conditions. This finding suggests that cortisol is not a suitable operational welfare indicator for sea bass on-farm. Differently, blood metabolites, as glucose and NEFA showed the existence of different energetic status in sea bass reared at low (10 kg/m^3) and high stocking densities (45 kg/m^3) in flow through tanks on farms. In conclusion, stocking density itself seems to not be a chronic stressor in sea bass and stocking density effects on energy metabolism and growth performances were probably due to related factors such as water quality, food availability and fish social behaviour.

WP2.7 Morphology of primary barrier tissues in Atlantic salmon and sea bass

IMR:

Samples of intestines and gills were delivered from WP1.1.4, 1.1.5 and 1.1.6 and analyzed using histological and ultrastructural analysis to techniques to verify the presence/absence of lesions resulted from exposure to hypoxia in Altantic salmon. In all experiments, 5 fish from each tank were randomly netted from each tank in one netting and anesthetized in methomidate (12.5 mg/ml) at subsequent occasions after start of treatment. At the sampling a lateral incision was made and the intestines dissected out. Sections from midgut (2-3 cm caudal to the last pyloric caeca) and hindgut (middle part of the hindgut) were immediately transferred to McDowell’s fixative and stored at 6°C until further processing. Gill tissues were also sampled from these fish and immediately transferred to the fixative as described above. Portions of intestinal segments and gill tissues from 5-10 fish from each dietary group were dissected into smaller pieces for transmission electron microscopy (TEM). Semithin (1.0 µm thick) ultramicrotome sections were stained with 2% toluidine blue and examined under light microscope. After fixation and dehydration some gill specimens were prepared for scanning electron microscopy (SEM).

Hypoxia treatment (WP 1.1.4 and 1.1.5) had no clear effect on the histological or ultrastructural (TEM) features in Atlantic salmon intestines. Likewise, hypoxia did not appear to have any major structural impacts on light microscopical features on gill tissues. However, salmon gill tissue have features that change significantly depending on where on the orientation on the microtome and where in the primary filaments samples originate. It was accordingly difficult to measure secondary filament height, width etc on these images. It was therefore decided to prepare and analyse subsets of the samples for SEM analysis. Studies at low magnification did not reveal any effects of hypoxia on the distance between primary or secondary filaments.

ICRAM:

Samples of kidney and gills were delivered from WP1.2.3 and analyzed using histological techniques to verify the presence/absence of lesions resulted from exposure to hypercapnic conditions in sea bass. Samples were collected from four fish for each CO_2 treatment (2-5 mg/l control, 15-20 mg/l low, 30–35 mg/l medium, 50-55 mg/l high) at different sampling time (4, 8, 22 and 45 days). Tissues were fixed in Bouin’s fluid and stored in alcohol 80% for further analysis. Tissues were embedded in paraffin, sectioned at 5µ and stained with haematoxylin/eosin (H/E) and Alcian blue. All sections were evaluated by three pathologists in a blinded fashion.

Sea bass exposed to hypercapnia did not show any significant histopathological changes in the caudal kidney at glomerular level. Conversely, atrophy and degeneration of the tubular epithelium and pyknosis of nuclei were detected in the kidneys of fish exposed to 50-55 mg/l CO2. At gill level, multifocal fusions of secondary lamellae, hypertrophy and hyperplasia of gill epithelium and hyperplasia of mucus cells were observed in sea bass exposed to hypercapnia. A marked dilatation of lamellae's apex, potentially caused by a lost of elasticity of endothelium of terminal gill vessels, was observed in fish expose to the highest CO_2 concentration after 45 days. This lesion is consistent with a chronic sufferance at gill level, and could be associated to the alteration of physiological respiratory mechanisms (see mid report, WP 2.5).

HCMR:
Skin samples of sea bass (WP1.2.1.5, exp.2) kept at normal conditions (flow rate 30 l $kg^{-1}h^{-1}$, oxygen level 7-9 ppm) and at inferior conditions (flow rate 16 l $kg^{-1}h^{-1}$, oxygen level 3-4ppm), taken after 77 days of rearing were analysed. Differences in the number of mucus cells were not significant, indicating that this factor is possibly not sensitive to the conditions applied.

WP2.8 Intestinal barrier functions and translocation of enteric pathogens in Atlantic salmon and sea bass

UGOT:
Plasma and tissue samples for analyses of several physiological and immunological parameters were delivered from 6 experiments according to the table above. Live intestines were delivered from WP1.2.4 (sea bass), WP 1.1.3, WP1.1.5 and WP1.1.6 (Atlantic salmon) and analysed with regards to barrier function, physiological transporting function as well as to translocation of enteric pathogen bacteria. All intestinal preparations were assessed for the electrical parameters; potential differences, electrical resistance and short circuit current. Likewise all preparations were assessed for paracellular permeability using the marker hydrophilic molecule ^{14}C-mannitol. In experiment 1, heat inactivated pathogen bacteria, *Aeromonas salmonicida* was used to assess the rate of bacterial translocation across the intestinal barrier, whereas in exp. 3 and 4 fluorescent microspheres of bacterial size were used to assess trans-cellular uptake pathways across the intestinal epithelium.

The electrical parameters assessed provide information on the viability and functionality of the intestinal epithelium as well as on the permeability *i.e.* the integrity of the epithelial barrier. High stocking densities (exp 1), with the subsequent decrease in water quality, negatively reduces both physiological functionality like active transport mechanisms and the barrier properties of the epithelium. Reduced oxygen tension as created by variable water flow and constant stocking density, both constant and using daily fluctuations, similarly decreased both active transport mechanisms and reduced the barrier integrity. Thus, the experiments assessed by the Ussing chamber methodology show that the intestinal epithelium is clearly affected by sub-optimal husbandry conditions. The magnitude of the detrimental response was also affected by temperature, so that an increased temperature worsened the effect considerably. The impairment occurs both at the physiological and physical level of the barrier, resulting in a decreased ability to transport both ions and nutrients as well as a in a destroyed intestinal barrier, leading to a lowered barrier function and possibilities for entry of harmful agents. In line with this bacterial and micro-sphere translocation are indeed affected by the different types of chronic stressors used resulting in increased translocation rate of pathogen bacteria or fluospheres of bacterial size.

The detrimental effects on the intestinal epithelium may in part be governed by high circulating levels of cortisol. Elevated plasma cortisol levels have been shown to coincide with increased paracellular permeability of proximal as well as distal intestine in the rainbow trout. These types of damages to the epithelium will create "holes" for particles to diffuse freely between the lumen and the blood, thus affecting electrical parameters and the diffusion rate of small molecules like mannitol. Thus, there seems to be a direct or indirect effect of increased circulating levels of cortisol on the intestinal primary barrier, leading to decreased integrity with a suggested increased bacterial translocation rate as a consequence. Increased epithelial permeability and bacterial translocation will make the underlying mucosa more exposed to foreign antigens from the gut lumen which may generate an inflammatory response. This in turn may attract neutrophiles and increase the amount of secreted cytokines, which in turn can disturb the barrier integrity even further.

To our knowledge, is these experiments the first attempts to assess *in vitro* intestinal barrier functions and intestinal electrical properties in sea bass intestine using the Ussing chamber system. In comparison with the better characterised salmonid intestines the sea bass show permeability and barrier characteristics similar to the anterior intestine of Atlantic salmon and rainbow trout. The TER was clearly affected by the treatment, in that both implanted groups showed increased TER in comparison to the non-treated control, whereas no differences in P_{app} were observed between groups.

Translocation of enteric pathogens in sea bass reared under different environmental conditions, HCMR:
The appearance of the different bacterial species in sea bass intestine was seasonal. Oxygen levels appeared to affect the microbial load of the intestine but this required a prolonged rearing time to become apparent (more than a month). Low water flow rates increased the bacterial load of the intestine, however did not affect the

qualitative picture of the intestinal flora. Application of handling stress allowed intestinal bacteria to pass in internal organs. More pathogenic species like *Vibrio alginolyticus* and *Vibrio harveyi* were isolated in higher frequencies from internal organs than less pathogenic species like *Vibro costicola* and *Pseudomonas spp*.

WP2.9 Endocrine profiles of the GH-IGF-I system in Atlantic salmon

UGOT:

Plasma delivered from WP 1.1.7 showed interesting results of IPNV-challenge on plasma IGF-1 levels. Plasma levels of GH were slightly increased after transfer of the fish to SW. No difference between IPNV infected and non-infected fish were apparent. IGF-1 on the other hand decreased drastically after infection with IPNV. The plasma levels of IGF-1 were the same before and after transfer to SW in the non-infected group, similarly no effect was seen in response to hyperoxygenation. Infection with IPN virus, on the other hand, decreased the IGF-1 levels to the same extent in both the hyperoxic and normoxic groups, an effect that persisted for approximately 12 days post infection.

Plasma samples were also delivered from WP 2.4 and WP 3.2.1. 6 fish per tank (duplicate) were sampled 48 days after hormone implantation and 1, 2, 7 and 21 days after IPNV challenge. Blood samples were obtained from the caudal vein using heparinised syringes, within 3 minutes from netting. Plasma was obtained through centrifugation and analyses of growth hormone (GH) was performed according to an established double-antibody salmon growth hormone radioimmunoassay protocol. Analyses of insulin like growth factor-1 (IGF-1) l were analyzed using a radioimmunoassay originally developed for coho salmon IGF-I.

As expected, 45 days after implantation, the groups receiving bovine GH showed decreased circulating levels of endogenous GH. This is in agreement with earlier studies and indicates a negative feedback on the endogenous pituitary release of GH. The two groups implanted with bovine GH, also showed increased circulating levels of IGF-I, which was equally expected as GH mediates IGF-1 release from the liver. There seems also to be an increase in the circulating levels of GH in cortisol (F)-implanted fish as indicated in both the F and GH+F implanted groups. IPNV challenge in the resent experiment showed a very low, if any, effect on the GH/IGF-I system. In the previous experiment within the project (WP 1.1.7) it was shown a clear decrease in plasma levels of IGF-1 after IPNV challenge, an effect that was restored at 14 days post infection. However, in the present study the effect seems to be smaller but reversed. Additional experiments are needed to fully elucidate the connections.

1.3.4 WP3 Immune competence and endocrine interaction – Atlantic salmon and sea bass

Contractors involved: NSVS, UGOT, IMR, IFREMER, ICRAM, UM, CISIC

WP3.1 Immune competence and endocrine interaction – Atlantic salmon

NSVS, UGOT, IMR:

The immune and endocrine systems of fish are intimately linked, and communication between the two systems appears essential for the maintenance of homeostatic function. The understanding is that stress alters immune system function both through acute and chronic responses. Studies have been carried out to elucidate both acute and chronic stress responses on the immunity and immunocompetence in Atlantic salmon. In these studies we have used an experimental approach of acute stress following hyperoxygenation, chronic stress following hypoxia and chronic stress through implantation and slow release of cortisol from the injection site. These treatments have been combined with infection with IPN virus.

Through acute stress studies (hyperoxia) we have seen that Atlantic salmon become more susceptible to IPN virus challenge and that the stressed fish died at significantly higher rate compared to controls. This hyperoxygenatic treatment in FW also resulted in a tendency towards increased P_{app} and translocation of *A. salmonicida* in the distal intestine and significantly elevated plasma cortisol levels. Subsequent IPNV challenge further increased P_{app} and the translocation rate of *A. salmonicida* in the proximal intestine. IPNV challenge also increased the number of Goblet cells and granulocytes in the *lamina propria* and enterocyte layer of the proximal intestine. In 2 out of 5 fish, massive necrosis was observed in the intestinal epithelia after IPNV infection with significant loss of cellular architecture. The IPNV challenge further elevated plasma cortisol levels. These results indicate that hyperoxygenation and reduced flow in the FW stage may serve as stressors with impact also during later stages of development. Fish with an early history of hyperoxygenation showed a higher stress response concomitant with a disturbed intestinal barrier function which may lead to increased disease susceptibility.

Chronic hypoxic stress was studied over a period of approximately 2 months and here an in vitro approach was taken. Macrophages were isolated from head kidney from stress and control fish and stimulated with poly I:C which will mimic a virus infection. The tendency is a down-regulation of immune related genes as assessed by real-time PCR methods in cells originating from stressed fish but the variability between fish give non-statistical difference for many of the cytokines/immune genes studied but the trend is clear for all candidate genes studied.

Chronic stress following implantation gave a down-regulation of Mx and acute-phase proteins in liver. There is a general trend towards down-regulation of immune-related genes in kidney, except for a marked (unexplained) up-regulation at 2 months post implantation (not shown). Fish were infected with IPNV virus and from culture F-implanted fish showed higher number of virus positive compared to the control group. Different immune genes/immune-related genes were also measured in kidney of F-implanted and IPNV challenged fish and there is a general trend towards down-regulation at early time points post challenge.

In summary, the studies carried out provide data in support of cortisol having immunosuppressive effects in Atlantic salmon and this result in higher prevalence of IPNV positive fish post challenge. We see that responses to IPNV infection combined with chronic stress are best studied by primary barrier function analyses as well as by profiling immune responses in the kidney and there are indications that the endocrine and immune systems are interconnected and have a bidirectional system of communication.

FRS:
Two IPNV challenge trials were conducted on salmon postsmolts under WP3.1. The first trial showed low mortality, and the trial was repeated. The second trial showed mortalities of 14.3 and 43.4% in intraperitoneally (IP) injected and cohabiting fish respectively. A peak was found in Mx protein gene expression using real time quantitative PCR. The Mx peak appeared to be independent of interferon 1 alpha (IFN) also measured with real time quantitative PCR. Moreover, the Mx peak seemed to be related to the photoperiod regime used to produce smolt, and may be related to stress and/or the cortisol peak normally seen during the parr-smolt transformation in salmon. Expression of IPNVSP (virus), Mx, GIFN and IP10 increased drastically in kidney at day 6 in the i.p. IPNV challenged postsmolts. No significant increase was detected in this tissue for IL1, TNF or IFN in the i.p. challenged fish. There was no induced expression of IL1 nor TNF in any of the tissues sampled in Exp. 1 from the cohabitant fish. No response in any of the genes assayed in the gill could be detected. MX, IFN, IP10 and GIFN appeared to be significantly induced but at a later stage between day 9 and 13. However, the kinetics does not show a clear peak of activation as was the case in the i.p. fish. The variability between individuals also appeared to be much higher in the cohab fish when compared to the i.p. fish. IL1 was not induced by the IPNV infection in any of the experiments. This indicates that (i) IL1 is not involved in immune responses to IPNV (ii) no opportunistic bacterial infection occurred in the challenges. Similarly there was no induced expression of TNF in contrast with previous results showing a drastic TNF induction after IHNV infection.

WP3.2 Immune competence and husbandry conditions – sea bass

IFREMER:
A challenge trial with nodavirus was conducted on sea bass at day 104 in the stocking density experiment under WP1.2.1. No significant differences were seen among the different stocking densities (ranging from 10 to 100 kg/m^3 in flow-trough tanks). A peak in mortality was seen between day 5 to 7 following challenge and total morbidity ranged from 66 to 84% on day 15 following virus challenge. No mortality was seen in the control groups.

Other challenge trials were systematically carried out at the end of all the experiments with sea bass, under WP1221 (stocking density in recirculation); WP1212 (effect of hyperoxia); WP1213 (combined effect of hyperoxia and hypercapnia) and WP1214 (cortisol implants), either using nodavirus and or bacteria (*Vibrio anguilarum*). No of the nodavirus challenge tests allowed to discriminate among the different treatments (stocking density or gas saturation levels or blood cortisol concentration): the mortality peak and average mortality level at the end of the experiment were in the normal range (70 to 90% of cumulative mortality) whereas the control shown no mortality. The bacterial tests are less relevant in most of the case because the fish are most of the time vaccinated against *Vibrio anguilarum* at an early stage by the farmers. It allowed to discriminate among the gas treatments (WP1213) with a statistically demonstrated difference between the highest hyperoxia with hypercapnia concentration 53 mg/l CO_2 and 17 mg/l O_2: 60% cumulative mortality compared to 30 to 40% for the other treatments.

ICRAM:

Innate and specific immune parameters were investigated in sea bass exposed to different environmental conditions (eg. temperature, hypoxia, hypercapnia, and combined hypercapnia/hyperoxia) and reared at different stocking densities and water renewals. An additional study was carried out to investigate the effects of chronic elevation of plasma cortisol on innate and specific immunity of sea bass implanted with exogenous cortisol at 75 µg/g BW dose for 5 weeks (Wp 1.2.1.4). Samples were delivered from 8 experiments carried out under laboratory conditions (Wp 1.2) and on farm (Wp 5.2).

A total of 4,730 analyses for innate immune parameters (plasma lysozyme, complement and the respiratory burst activity of phagocytes) and for specific immune parameters (percentage of B and T lymphocytes in head kidney and peripheral blood, their proliferation capability *in-vitro*; anti-*Vibrio anguillarum* immunoglobulins content and their agglutination capacity) were performed.

Environmental conditions - Both innate and specific immune responses in sea bass were affected by environmental conditions, mainly by water temperature, hypercapnia and hypoxia.

Seasonal temperature changes strongly influenced the humoral innate immune system of sea bass. Complement activity show a seasonal trend significantly correlated with water temperature, increasing during summer and decreasing in winter at low water temperature. Serum lysozyme levels displayed slight variations throughout the period.

Hypercapnia strongly affects innate and specific immune system in sea bass. Under chronic hypercapnic conditions (50-55 mg/l CO2), B and T lymphocytes decreased in both head kidney (HK) and blood. Lower percentages of T cells in the head kidney could be related to a chronic erythropoietic tissue stress, as observed in histological kidney sections (see WP2.7). The respiratory activity of blood phagocytes was reduced under hypercapnic conditions. The respiratory burst is oxygen-dependent and its reduction may be driven predominantly by the significant decrease in blood PO_2 observed in all hypercapnic groups (see Mid report, WP2.5, pp. 58). *In-vitro* proliferation of head kidney lymphocytes was significantly affected by chronic exposure (22 days) to high hypercapnic conditions, while short term exposure seemed to have no effect. This decreased capability may be due to a tissue stress in HK (see WP2.7), and/or by an apoptosis provoked by high plasma cortisol concentration. The serum content of *anti-Vibrio* immunoglobulins and their agglutination capacity were lower in fish exposed to hypercapnia after 45 days. Short and long term exposure to hypercapnia did not affect complement (CH50) and lysozyme (LYS) activities.

Similarly, long-term exposure to hyperoxia and hypercapnia, up to 230-250% O_2 saturation and 50-60 mg/l CO_2 did not affect CH50 and LYS in sea bass. Serum complement activity did not change in sea bass chronically exposed to hypoxia (3 mg/l O_2) compared to fish kept under normoxia (7-9 mg/l O_2). However, CH50 was found to be significantly higher in sea bass kept under hypoxic conditions and low water renewal, probably because microbial load was higher in rearing tanks supply at low water exchange.

Stocking density - Stocking density up to 30 kg/m^3 did not affect cellular and humoral immune response of adult sea bass. Conversely, juvenile sea bass seem to be more sensitive to high stocking density (40 kg/m^3) during growth-out phase, showing lower CH50 levels and lower response to vaccination against *V. anguillarum*. Under laboratory conditions, extreme stocking density (100 kg/m^3) affected serum lysozyme activity.

Chronic stress model in sea bass - The immune system is an important target for the glucocorticosteroids released by the hypothalamus-adrenal axis. Evidence of strong relationship between endocrine and immune system came out from the cortisol implantation study. Chronic elevation of plasma cortisol in implanted sea bass negatively affected both innate and specific immune system and induced immunosuppression. Serum complement, lysozyme activities resulted significantly decreased in cortisol implanted sea bass compared to vehicle-implanted fish and controls. Hormone implantation significantly reduced the percentage of circulating B lymphocytes, while B cells in the head kidney seem to be not sensitive to high cortisol concentrations. Further studies are needed to investigate whether B cells in the head kidney display cortisol active receptors or not. Our results indicate that both B and T sub-populations are sensitive to high circulating cortisol level in sea bass. Therefore, both acute and chronic stress would have a negative effect on resistance of sea bass to microbial infection as a result of the reduced percentage of antibodies producing cells in the blood of stressed fish.

UM:

UM has analyzed the expression of several pro-inflammatory, anti-inflammatory and chemokine genes identified by Partner 9 IBMC in the gene libraries obtained in this project. The results have identified a molecular signature of *V. anguillarum*-infected and -vaccinated fish. In addition, they also suggest that the virulence mechanisms of *V. anguillarum* in sea bass involve the inhibition of leukocyte respiratory burst and apoptosis, and thereby providing a safe haven for growth.

CSIC:
We carried out experiments both in sea bass (the susceptible species) and in sea bream, as a carrier of the disease to study the modulation of inflammatory cytokines, such as TNF-α, IL-1β and Mx protein and find possible explanations of the differential susceptibility to the disease. The results were published in Molecular Immunology (Poisa-Beiro et al., 2008) and suggested the early activation of TNF-α and IL-1β in kidney as a generalized response against nodavirus infection; a highly over-expression of TNF-α in the brain of sea bass possibly related to the neurodegenerative symptoms of the disease and the up-regulation of Mx protein as an antiviral mechanism in both species but much higher in sea bream, suggesting an explanation why is an asymptomatic carrier of the disease. Moreover, we analyzed sea bass tissue samples provided by IFREMER within WP 1.2 to study the effects of hyperoxia stress in combination with a nodavirus infection in sea bass. Altered patterns of expression of TNF, IL-1 and Mx by qPCR were observed suggesting that stresses fish could have a diminished capacity to respond to a nodavirus, and also indicating that good aquaculture practices are highly recommended to avoid economic loses.

1.3.5 WP4 Genome wide search for new tools/markers for stress – Atlantic salmon and sea bass

Contractors involved: IMBC, NSVS, UM, CSIC, IMR, HCMR-IMBC

IMR/NSVS:
Differentially expressed genes at short-term (11 days) in controls were compared to exposure to two different levels of oxygen, 80% and 55% oxygen saturation, respectively. Long-term exposure (58 days) to two different levels of hypoxia (80% or 55%) was compared to 11 days findings. Furthermore differential expression at 11 days and 58 days in the 80% and 55% hypoxia groups were compared. Exposure to short-term hypoxia (11 days), either to 80% or to 55% oxygen saturation, shows that the differentially regulated genes under these two conditions. The majority of these genes were annotated to metabolism, immune-related or unknown genes. While most metabolism genes were up-regulated following exposure to 80% oxygen, there was an even mixture between up- and down-regulated genes in the 55% group. For immune-related genes an up-regulation was seen in the 80% group, while for the 55% group, the general trend was a down-regulation (13 of 14 genes). Following long-term exposure in the 55% group, genes related to oxygen dependent processes were down-regulated including α and β globin, cytochrome-c oxidase (Cyt C), hemoglobin α chain, and biliverdin-IX beta reductase. Genes related to transcription/translation were up-regulated and included amminopeptidase, cathepsin D and S, whereas poly A binding protein was down- regulated. A range of genes related to metabolism was up-regulated, including ATP synthase, selenoprotein Pa, N-acetyltransferase and nucleoside disphosphate kinase.

This experiment shows that microarray is a suitable tool to detect genes associated with differential regulation following exposure to hypoxia, as the first step to identify new welfare related genes and processes. The changes in global gene expression patterns and a list of the most affected genes represent urokinase receptor, α and β globin, ferritin, cytochrome c oxidase, billiverdin reductase, ubiquitin and nephrosin. These are all candidate genes for detection of hypoxic stress in Atlantic salmon.

UM:
The mechanisms of the cellular immune response involved in the protection of fish against infection by the pathogenic bacterium *Vibrio anguillarum* have been studied. Sea bass specimens were injected with live or formalin-killed *V. anguillarum* and the respiratory burst of leukocytes was measured. The infection of fish resulted in a strong inhibition of the respiratory burst, in contrast with the slight increase in respiratory burst of leukocytes from fish injected with dead bacteria. In addition, it was found a concomitant down-regulation of p22phox and p40phox, two components of the NADPH oxidase, in the leukocytes from infected fish. To investigate whether these differences may be the result of a dysregulation of cytokines expression in infected fish, the expression of several sea bass cytokines identified by HCMR-IMBC, including interleukin-6 (IL-6), IL-8 and three CC chemokines, was studied by real-time PCR. Surprisingly, cytokine expression was fairly similar in leukocytes from both live and formalin-killed *V. anguillarum*-challenged fish, the response being even higher and longer lasting in infected fish. Furthermore, the expression of two key apoptotic caspases, caspase-3 and -9, was down-regulated in leukocytes from infected fish, but remained unaltered in fish injected with formalin-killed bacteria. The results have identified a molecular signature of *V. anguillarum*-infected and -vaccinated fish. In addition, they suggest that the virulence mechanisms of *V. anguillarum* in sea bass involve the inhibition of leukocyte respiratory burst and apoptosis, and thereby providing a safe haven for growth. The results have been published in Molecular Immunology.

CSIC:

CSIC produced a subtracted cDNA library from infected sea bass with nodavirus by SSH technique, in order to identify genes potentially involved in innate immunity (period 1). A complete analysis of SSH cDNA clones was carried out in period 2. 206 reliable EST sequences representing 71 singletons potentially up-regulated in infected kidneys were obtained. 18 out of 206 (8.7 %) were immune or stress related genes such as heat shock protein 90 beta, beta-2 microglobulin precursor, apcs-prov protein, beta-galactoside-binding lectin (galectin), mannose receptor C1, Cd209e antigen or serum lectin isoform 2. Among the ESTs potentially over-expressed we focused in three lectins (two C-type lectins and a galectin-1), given their role in pathogen recognition and neutralization. qPCRs were conducted for *in vivo* expression studies, showing a clear difference between infected and control samples for the three lectins. Also, a recombinant galectin-1 (rGalectin-1) was expressed, which functional and immune properties suggested that lectins could have an important defense role in response to a nodavirus infection.

HCMR-IMBC

Within WP 4.2 IMBC-HCMR have produced EST sequences and submitted them to bioinformatic analyses. Six cDNA libraries were created out of spleen, gills, intestine, liver, head kidney and peritoneal exudates tissues of sea bass infected with *Vibrio anguillarum.* Infection was done by partners 5 & 10 for 4h and for 24h. For the cDNA libraries total RNA of tissues infected for 4h and 24h were pooled. The libraries were tested for insert size by PCR and run on an agarose gel. The inserts range from 600 bp to >1.0 kb. In total 5111 EST sequences were obtained from all libraries and 1841 (36%) are unique sequences. Four cDNA libraries were also created out of spleen, brain, liver and Head kidney of sea bass infected with nodavirus. For the cDNA libraries total RNA of tissues infected with Nodavirus were extracted. In total 9573 sequences representing 3075 unique sequences (32%) were obtained and analysed. Among those sequences of several immune related proteins were identified for the first time in the order of Perciformes, as well as in Teleostei in general.

Simple Sequence Repeats (SSR) or Microsatellites are frequent in non-coding regions and are used as molecular marker. Detection of SSR within ESTs, exonic microsatellites or EST-SSRs presents a shortcut to obtain Microsatellite markers. Due to the fact that EST-SSRs are exonic they have two advantages over intergenic microsatellites. First it is expected that their flanking region are more conserved so that the primers can be used even in related species and second it is assumed that they are in strong linkage disequilibrium with functionally important sites. Therefore they are used in population genomics or gene mapping in order to map genes of economic significance. In silico mining for repeat motifs within the obtained sequences has been performed with the program Msatfinder (http://www.genomics.ceh.ac.uk/msatfinder/.) Among the unique sequences (3075) 371(12%) sequences were found containing Simple Sequence Repeats.

1.3.6 WP5.1 Monitoring and sampling from commercial farming of Atlantic salmon

Contractors involved: IMR and sub contractors CAC AS and Kobbevik og Furuholmen Fiskeoppdrett AS.

Effects of environmental variation on the swimming behaviour and experienced water quality parameters of caged Atlantic salmon at four commercial salmon farms in Western Norway

The water quality and fish behaviour were studied on four commercial salmon farms located at different sites in Western Norway; two on the outer coast and two in protected fjords. The stocking density in the cages was rather high (8-16 kg/m^3), but below the Norwegian legal limit of 25 kg/m^3. The study revealed large fluctuations in oxygen with time, and both within and between cages. Water flow was greatly affected by the cages themselves, and of large importance for the resultant oxygen levels in the cages. Swimming depth and actual fish density also showed large variation both between cages and with time, and the observed density was in many cases much higher than the theoretical stocking density, reflecting that the salmon often only utilise a limited part of the cage. The levels of ammonia and carbon dioxide were generally low, and below levels that are known to give problems for salmon. Thus, these sea cage trials suggest that oxygen was the main limiting water quality factor in the salmon cages.

Effects of environmental variation & crowding events the swimming behaviour and experienced water quality parameters of caged Atlantic salmon at CAC, Western Norway

The primary aim in this study was to observe the swimming behaviour of caged Atlantic salmon in commercial cages during late spring/early summer at a fjord site, when strong temperature gradients develop in the surface layers. Operational procedures, such as sorting or delousing by bath requires that the fish are crowded by reducing the cage volume and may force the salmon into non-preferred water qualities. The second aim in this study was to investigate if the swimming behaviour (schooling density, depth) and feed intake of Atlantic salmon was influenced by the crowding events.

The study was carried out from May to June on a commercial salmon farm (CAC, Western Norway) with twelve 30 m deep net cages (24*24*30m) with a volume of 17,280 m^3 each held up by a floating steel structure connected to a barge housing technical equipment and feed storage. Behavioural observation was carried out in the two cages closest to the barge. These cages held approximately 60 000 salmon each (Salmobreed strain) with a mean body weight of 2.4 kg, giving a total biomass of around 140 tons/cage and a stocking density of 7.4 kg/m^3 at the start of the study. The cages had been stocked in October the previous year as one year old smolts, and subjected to an artificial photoperiod (LL) from January by means of two 1000 W underwater lamps positioned in the center of each of the cages at 7 and 15 m depth. The fish was fed by an automatic feeding system in meals. Daily feed ration was calculated by a feeding program and adjusted to appetite by farm workers by the use of underwater cameras.

The swimming depth and fish density of the groups in each cage was continuously observed by a PC-based echo integration system (Lindem Data Acquisition, Oslo, Norway). Daily feed ration and appetite as % of biomass was calculated based on the estimated daily increase in biomass based on amount of feed delivered. Sea temperature, dissolved oxygen (mg/l) and water current speed was automatically recorded daily at 1, 7 and 20 m depth at a reference point at the far end of cage system by a stationary sensor system. On 3 occasions a more detailed vertical profiles (0-26 m) of temperature, salinity and oxygen was recorded for a 3 day period in the centre of the two cages and at a reference point between the cages and the barge using profiling CTD's connected to automatic winches. In addition a vertical profile of light intensity was recorded during midday and night on the 3 May.

A crowding event simulating the crowding procedure took place on April 27 in cage 2 using cage 1 as a control. The lifting of the net took about 40 min and the cage volume at the end was estimated to about 15 % of the full volume with an estimated fish density of 49 kg/ m^3. The fish was crowded for 0.5 h before the net was lowered and full volume retained after 10 m. On the June 1 the nets in cages 1 and 2 were lifted from 30 m to approximately 15 m depth, and subsequently. The fish in the cages was gradually crowded and pumped over a sorting grid in a well boat. Following sorting the full cage volume was restored.

In general environmental stratification is influenced by a complex interaction of factors like fresh water run offs, upwelling, wind and tidal driven currents, solar radiation and primary production. The regular recordings of temperature, oxygen and water current speed at different depths clearly demonstrated that the site had considerable temporal changes in vertical environmental stratification. Vertical stratification in salinity was only recorded on two occasions by the CTD, but indicated a typical fjord site with a halocline gradually increasing salinity with depth from around 20 at the surface to 35 ppm at the bottom of the cage. During the study period sea water temperature at 1 m depth increased from about 7 to 17 °C while temperature stayed below 10°C at 7m until mid June. At 20 m depth there was only a very slow increase from 7-8 °C.

There was a general decrease in DO at 1 m depth during the period consistent with the increase in sea temperature and general reduction in water current speed, but this seemed to be partly caused by instrument drift when comparing with the CTD-data. At 7 m the DO was stable, but with a marked decrease at the end of the period consistent with an increase in temperature. DO at 20 m showed a marked increase to supersaturated water at the end of the period possibly connected to inflow of nutrient rich deep water and increased phytoplankton production, as indicated by increased current speeds at 20m depth in June.

The swimming depth and schooling density of salmon was clearly influenced by the spatial and temporal variability in environmental factors. In general there was a pronounced diurnal variation in swimming depth whit the fish crowded at high densities in the warm surface layer during the day and dispersed towards the lights during the dark hours. During early May when the thermocline was relatively weak the fish were more evenly distributed, but when the surface water started to warm up in mid May, the salmon crowded at extreme high densities (> 200 kg/m^3) near the surface during daylight. High current speeds in the surface area in this period lead to sufficient supply of oxygen.

The crowding of the fish led to a sharp oxygen drop down to around 4.5 mg $O_2 l^{-1}$at one m depth in one of the two cages in the beginning of the crowding period. During the pumping and sorting period the oxygen saturation were between 5-8 mg O_2 l^{-1} (60-75% saturation. The fish showed clear change in behavior the next days after crowding, where the group of fish were more dispersed in the cages and did not concentrate in the warm surface layers as before. The appetite also dropped, even after several days without food, and needed several days to go back to normal.

Monitoring and sampling at Kobbevik and Furuhomen fiskeoppdrett AS (salmon farm)
A detailed environmental profiling and hydroacustic monitoring was also conducted at the subcontractor Kobbevik and Furuholmen Fiskeoppdrett AS, in Western Norway on February 8-22 and April 12-22 2006. The farm is located on a coastal site, and consists of eight 25 m deep 25x35m cages. In February 2006, the biomass ranged between 212 and 291 tonnes per cage, with 3-5 kg mean body weight (theoretical stocking density between 9.7-13.3 kg/m^3). In April 2006, the biomass ranged between 141 and 172 tonnes per cage (lower biomass due to harvest of part of the population; theoretical stocking density between 6.5-7.9 kg/m^3). Fish behaviour and environmental profiles were registered in four cages in each period. The swimming depth and fish density of the groups in each cage was continuously observed by a PC-based echo integration system. Detailed vertical profiles (0-20 m) of temperature, salinity and oxygen was recorded in the centre of the four cages and at a reference point between the cages and the barge using CTD's connected to automatic winches. Samples were collected from fish for potential analyses of stress and immune parameters in WP 3 and 4.

On the sampling dates February 8-22 2006, water temperature was higher in the lower parts of the cage, and at the end of the sampling period the cold surface water occupied the upper 15 m of the cage depth. The fish showed a clear daily pattern in swimming depth and were positioned deep in the cage at day time and closer to the surface at night time. The swimming depth tended to be deeper for the majority of the fish in the final part of the sampling period when the cold surface water occupied a larger part of the depth of the cages. The observed fish density was generally low with the highest values around10 kg/m^3. The highest densities were found in the surface layers at night time in the beginning of the sampling period while the highest density was in the deeper part of the cage late in the sampling period.

In the second sampling period, on April 12-22 2006, the highest temperature was found in the surface, with a gradual increase in temperature over time, also resulting in a more even temperature distribution at the end of the sampling period. Most of the fish was concentrated in the upper 10m during this sampling period apparently avoiding the cold deeper water, and possibly also seeking to the surface due to longer day length and higher feeding motivation. The observed fish density was higher in this sampling period compared to February 2006 in spite much lower stoking biomass due to harvesting from the cages between the two sampling periods. The fish in the surface layers swam at a density between 10-25 kg/m^3.

The results indicate that environmental factors such as water temperature stratification and light conditions have a major effect on the vertical distribution of the salmon in the cages and also in the resulting fish density in the cage at specific depths. The study verify previous studied showing that the salmon do not use the whole water column in the sea cages, but seems to prefers the highest available temperature as long as this is below optimal temperature. This behaviour is also modulated by the light-dark cycle and season (possibly affected by photoperiod). Although the observed fish density was higher than the theoretical stocking density also in this study do to the aggregation of fish specific depths, the observed fish densities were moderate and much lower than previously observed at other commercial salmon sites with stratified water. As we did not detect any specific environmental or production problems at this site during the production cycle, we did not analyse the samples further for molecular or morphological stress and welfare parameters.

1.3.7 WP5.2 Monitoring and sampling from commercial farming of sea bass

Contractors involved: IFREMER, ICRAM, HCMR

IFREMER:
Sea bass health status versus water quality was monitored in three very different environmental conditions: sea cages located close to the shore, sea cages in off shore conditions and tanks in recirculation in two private farms (Cannes Aquaculture and Méditerranée Aquaculture).

Sea bass health status versus water quality was monitored in three very different environmental conditions: sea cages located close to the shore, sea cages in off shore conditions and tanks in recirculation in two private farms: Cannes Aquaculture (cages) and Méditerranée Pisciculture (tanks in recirculation). For the different rearing systems, water quality (in the system, makeup water or surrounding water) and hydrodynamic was monitored in parallel to the fish performances and health status.

Concerning recirculation systems, sea bass growth was monitored over three years (years 2005 to 2007) in Mediterranean Pisciculture , keeping the water quality parameters in safe ranges. The system allowed to obtain 150 g fish on average after 11 months with a FCR ranging between 1 and 2,4. The experiment demonstrated that in all conditions, the blood immunoglobuline concentration is not a good welfare indicator. However, the level of parasitic infestation, gives an practical good idea on health status of the fish. In spite of Formalin treatments, the most common specific parasite of the bar "*Diplectanum aequans*" is very resistant and particularly touches the least robust individuals of the fish batches. The main conclusions are that, in a routine operation, the recirculation system gives rather good results in terms of fish survival and growth, with limited pathological events and quite optimal biologic condition for sea bass rearing. The analysis did not show any evidences of health or body integrity degradation. Welfare degradation is directly linked with punctual degradation of the water quality during crisis, mainly due to accidental mismanagement of the treatment loop.

Concerning cage culture, three different sites belonging to the company Cannes Aquaculture were monitored during three years. They represent regular 'close to the shore' and 'offshore' conditions. The effect of the cage net on the water currents was studied using a ADCP current meter ("Workhorse" of RD Instruments) moored on the bottom at the vertical of the cages during more than one month and positioned horizontally with four sliding plastic feet. The main results shown that in deep waters (around 30-35 m deep), when the seawater surface flow reaches the obstacle formed by the raft, it tries to shape it laterally. The seawater flow passing down to the floating cages and their nets increased its speed just down the sliding friction layer and reaches there its higher values. Upwards, in the subsurface layer, between the bottoms of the cage nets and the water surface, a flow goes up but decreases in speed and changes in direction (due to the Coriolis force). In very shallow waters (less than 15 m deep), closed to the shore line, with a gentle littoral drift of the inner area of a gulf, it is preferable to moor the raft perpendicular to the shore line, even if the farm is built with two parallel floating cages rafts.
Some behavioural investigations were also carried out in the cage system.

Swimming activity rhythms and levels as well as space occupation by sea bass were also examined in a sea cage (6.5 x 6.5 x 8 m, ca. 25000 fish of 400 g stocked at 30 kg m^{-3}) and were recorded using acoustic telemetry (9 fish, IBDT Sonotronics) and archival tags (11, DSTs STAR ODDI). Acoustic tracking was realized over a 13 days winter period and successful for 3 individuals (one fix every 2 min on average). For archival tags, only 4 tags were retrieved and 3 had been recording swimming depth and temperature for 9 months (Nov 2005-June 2006). Telemetry results showed an even day/night swimming activity levels in November with fish staying in close association with the bottom. Archival tags recordings revealed a preferential distribution in the water column between mid-water and bottom of the net cage in winter, over the whole water column in spring and near the surface zone in early-summer. In addition, on a 24 hrs period basis, sea bass presented a vertical movement rhythm: they swam closer to the surface during the day, especially around feeding events, and near the bottom at night. That vertical movement was pronounced during winter, faded during spring and almost disappeared in summer. In conclusion, we have seen that swimming activity monitoring in sea bass was able to greatly improve our knowledge on behavioural activities that were sensitive to husbandry and environmental conditions. Further data analysis coupling (*e.g.* behaviour and other parameters listed in the other WPs) should be conducted in order to calibrate such promising early welfare indicators.

ICRAM:
Intensive production of sea bass in Italy is achieved mainly on land-based farms located in coastal areas (4320 ton, 57% of total production). About 40% production of intensive land-based production is produced in farms utilizing geothermal waters at constant temperature (1673 ton out of 4320 from land-based systems). According to this peculiar condition, two farms were monitored in Italy: i) MARIBRIN, a land-based farm consists of forty flow-through tanks supplied with water pumped from underground geothermal wells at constant temperature (22-24°C) and 38 ‰ salinity; ii) SMEG, a land-based farm consists of 56 flow-through tanks supplied with seawater pumped from the sea at a variable temperature.

Farm MARIBRIN.
Two full-scale stocking density trials were carried out on sea bass reared in geothermal water with marine salinity and at constant temperature (24° C) at fixed water renewal, from August 2006 to May 2007. Two stocks of sea bass of different size/age classes were used: juveniles (10-150g) stocked at 15, 30 and 40 kg/m^3 final density and sub-adults (200-400g) stocked at 10, 20 and 30 kg/m^3 final density.

Farm SMEG.
The trial started in June 2004 and lasted 12 months. The objective was to record environmental and husbandry conditions during an annual rearing cycle of sea bass reared in a flow-through system under natural thermal regime.

Environmental and husbandry conditions, growth performance, mortality and the health status of fish were recorded during two production cycles and samples for stress physiology and immunity analyses were collected in both farms.

High fish biomass significantly affected water quality. Water ammonia (TAN-NH_4) was higher in tanks where fish were reared at the highest densities (30 kg/m^3 for adult sea bass and 40kg/m^3 for juveniles). Nitrite, phosphate and nitrate concentrations also increased in relation to stocking density. However, water flow at four water exchanges per day was able to maintain nitrite, nitrate, ammonia concentration, and the un-dissociated ammonia fraction, within limits that are non-harmful for sea bass.

Juvenile sea bass maintained for 12 months at three different stocking densities showed differences in growth performance. Mean total body weight, mean total length and specific growth rate (SGR) were lower in both juveniles and adults sea bass kept at high stocking density (40 kg/m^3 and 30 kg/m^3, respectively). Condition factor did not differ between low and high stocking density groups, although an increase in size heterogeneity was observed in both juveniles and sub-adults kept at low density. Similar results on size variation at low density were reported in salmonids, as a possible consequence of formation and maintenance of hierarchies.

Effects of stocking density on growth performances were not detected in sea bass kept at 10, 40 and 70 kg/m3 under laboratory conditions. Differences between studies may be due to water quality, which was maintained at non limiting levels for all water parameters under laboratory conditions (WP1.2.1) and was significantly reduced on farm, especially in rearing tanks stocked at the highest stocking density.

For monitoring health and welfare of sea bass stocked an autopsy-based method was developed. Results indicated that fin damage is the most common lesion in both juvenile and adult fish sea bass, whereas skin damage (scale loss, erosion, haemorrhages, ulcers) and vertebral column anomalies were less frequently observed. Kidney and gill damage increased in fish stocked at high densities, confirming a possible effect of poor water quality, as reported in salmonids. The occurrence of fin damage is higher in adult sea bass kept at low stocking densities, suggesting that social interaction and/or dominance hierarchies could be more pronounced at low density. The autopsy-based method proved to be a useful, inexpensive and routinely applicable tool to evaluate the welfare of farmed sea bass. In particular, fin damage may be considered an operational welfare indicator for sea bass, as also reported for salmonids.

HCMR:
Two farms was planned to be monitored one in sheltered area with low currents and high water temperatures during the summer (Farm Kalloni) and one with stronger currents in the surrounding area (Farm Aquaculture Argosaronic).

Farm Aquaculture Argosaronic. This farm was included in the project in order to run the experiment of different densities with sea bass. Three cages of 10m*10m and 5m depth, 500m^3 capacity were used in which 5000, 10000 and 15000 fish about 475g were transferred resulting in initial fish load of about 4.7, 9.5 and 14 kg/m^3, respectively. Rearing was performed from July to December and fish were fed about 1.6% of their weight during most of the rearing time. Oxygen was measured frequently inside and outside the cages and water currents monitored. Samplings were performed at the beginning as well as for 4 more times during the experiment. Fish were analysed for the existence of pathogens and histology of internal organs.

Fish were reared in this farm at three different densities approaching 11, 19 and 24kg/m^3 at the end of the rearing. Temperatures ranged from 27 to about 15^{o}C. The water current in the area were high from 10 to 15 cm sec^{-1} but close to the cages were lower, of the order of 2cm sec^{-1} since these were at a more protected area. Oxygen levels in the cages were lower than those from outside water by 0.8, 1.1 and 1.4ppm oxygen for the three loading densities used, but did not fall below 4.5ppm. The only negative effect on fish performance observed was a slightly higher incidence of the myxosporean parasite *Sphaerosphora* spp in bile with increasing fish density but the infestation was not severe.

Farm Kalloni. This farm was monitored from the beginning of the project. Monitoring and sampling continued during the second period of the project. Water currents in the area of the farm and oxygen levels inside the tanks were monitored in summer months of 2006. Samplings of fish were continued during 2006 and 2007. Fish were analysed for the existence of pathogens and histology of internal organs. Samples of blood were also taken from both farms for measurement of antibacterial activity of complement and lysozyme in serum. The last results are presented in WP3.

Measurement of currents in this farm indicated that these had a strong periodicity of the order of a day and that these were characterized by oscillations in parallel to the seaside. The transfer of new water in the gulf was weak and did not favour the water renewal since it was a closed environment This could explain the occurrence of low oxygen levels in all the area and not only in cages, when weather conditions favoured this. Microbiological and parasitological analysis of fish indicated the existence of pathogens in two samplings and infestation by parasites in one sampling. Histological examination of fish sampled during summer-autumn period indicated certain lesions in gill tissue, mild to moderate fatty infiltration of liver cells and hemorrhages in kidney. Samples taken in winter and spring indicated generally a better tissue appearance.

1.3.8 WP5.3 Integrate the knowledge generated to develop husbandry protocols to improve health and welfare of farmed fish

Contractors involved: All

In WP5.3 we have arranged two projects meetings (in Arcachon on October 7-8, 2006 and Sete, on April 19-21 2007) to integrate the knowledge generated in other WPs to further improve husbandry protocols, suggest minimum environmental requirements and best practises for improved fish health and welfare. The outcomes of the results and discussions are planned as a Wealth review paper. Moreover partners from the Wealth projects participated with a range of oral presentations and posters the Finfish health and special Fish welfare sessions at the WAS/EAS meeting in Florence in May 2006, at the EAS meeting in Istanbul in October 2007, and also at the EAS Aqua Nor forum, “Welfare as a driver for technological development in aquaculture”, held in Trondheim, Norway, August 15-16 2007. A summary from the EAS Aqua Nor forum is available at: http://www.easonline.org/files/Meetings/aqua_nor_forum_summary.pdf. The major results and conclusions of the Wealth project was published in a popular form in a 12 page flyer that was distributed at the EAS Aqua NOR forum in Trondheim in August 2007 (Annon. 2007: “WEALTH - Welfare and health in sustainable aquaculture”, available at http://wealth.imr.no/__data/page/6068/Wealth_leaflet_web.pdf). Based on the results and discussions at the project meetings in Arcachon and Sete, the Fish health and special Fish welfare session at the WAS/EAS meeting in Florence in May 2006, and the EAS forum during the Aqua Nor meeting in Trondheim August 2007, we have made a range of conclusions and recommendations listed in next chapter.

1.4 Summary of end results, conclusions and recommendations

1.4.1 Summary of end results and main achievements

The Wealth project has provided a large body of **baseline data from commercial farms** on environment and fish health (e.g. temperature, oxygen, carbon dioxide, behaviour, fish densities, damages/histopathological changes, status of immune parameters). We have identified some limiting factors and key hazards in various productions systems (e.g. low or very high O_2, high CO_2 or stocking density).

The project has provided a range of **new research tools and approaches** (e.g. methods to measure expression of immune or hypoxia related genes, micro arrays, in vivo and in vitro immune stimulation, intestine permeability, cortisol in water). The combined use of tank and sea cages studies, has provided the both control (experimental tanks and small experimental cages) and realism (a range of commercial tank and sea cages systems for salmon and sea bass). This approach has established links between environmental factors, stress physiology, immune responses and disease resistance.

The data provide basis for further development of validated **welfare indicators** for use in research and on commercial farms (i.e. operational welfare indicators). The suggested welfare indicators includes growth performance, feed intake, fin and skin damages, cortisol in water, intestine permeability, assays to profile genes related to chronic stress, hypoxia and immune function, new techniques for *in vitro* and *in vivo* immune challenges, and various disease tests.

The project has provided **recommendations** for thresholds for environmental factors, stocking density in cages and tanks. However these issues need further attention due to the complex interaction between different environmental factors, husbandry practises, different life-stages and various production systems. Recommendations of aquaculture protocols, environmental threshold and optimum values need to take into account this complexity. Generally there is limited precise information of relevance for welfare in salmon and sea bass, in particular are there only few studies that have clearly demonstrated clear links between environment, stress and immune function/disease resistance or other key welfare aspects.

1.4.2 Conclusions and recommendations

- Fish welfare can be affected by a range of hazards related to environmental factors and husbandry practises (see table 1). In some cases exposure to these hazards also leads to increased susceptibility to infectious diseases, which in turn is a major fish welfare issue by its own. The Wealth project has examined some of these factors, with particular focus on stress factors that can compromise immune function and potentially increase the risk of disease outbreaks.
- The key-limiting factors differ between production systems and life-stages; e.g. O_2 and temperature in sea cages, CO_2 and ammonia in recirculated tanks, and CO_2 and potentially high O_2 (super-saturation) in hyperoxygenated tanks.
- Sea cage environment is highly variable in time and space and needs close monitoring. There is a strong need for more precise knowledge about such environments, their impact on the fish, and new monitoring technology providing measurements that also can serve as operational welfare indicators.
- Stocking density effects depends on water quality, and is difficult to assess these factors separately (i.e. identify the effect of fish density per se independent of water quality). At high stocking densities, space-limitations can affect swimming behaviour, feed intake and increase the risk of physical damage (e.g. fin abrasions), either as a consequence of physical contact with the rearing unit or due to direct agonistic behaviour.
- There is a lack of validated welfare indicators – in particular those suitable for sea cage farming. A range of indicators needs to be applied to assess fish welfare (see table 2 for overview of some potential welfare indicators).
- Oxygen consumption is a sensitive immediate indicator of stress, and continuous oxygen monitoring may serve as one of several operational welfare indicators.
- Fish behaviour's (swimming, appetite etc) can also be useful operational welfare indicators, but interpretations can be difficult.
- The project has demonstrated links between stress and disease susceptibility – but not in all cases for various reasons (e.g. difficulties with disease challenge models such as nodavirus challenge). This needs to be further examined as "proof of concept", e.g. by using new in vivo and in vitro challenge/stimulation models.
- A range of new molecular and physiological tools have been developed/successfully tested on salmon and sea bass, e.g. cortisol in water, primary barrier tests, in vivo and in vitro immune challenges, as well as profiling of key immune and stress related genes after various challenges.
- These tools enable new studies designed to verify threshold and optimum levels for a range of biotic and abiotic factors and various husbandry practises in terms for fish welfare and disease resistance. These tools will also be essential in validating welfare indicators, both in providing basic knowledge in underlying mechanisms essential in understanding the link between environment and welfare, as well measuring coping costs when the individuals try to maintain their normal physiological and behavioural functions (figure 2).

1.4.3 Specific recommendations

1. Improved monitoring regimes should be implemented in salmon and sea bass farms to be able to have better overview of variation in the sea cage environment - and to take actions to avoid situations with high risk of impaired welfare. This was warranted by the findings of suboptimal and sometimes critical environmental conditions in commercial salmon and sea bass farming systems.

2. Hyperoxygenation of around 150% O_2 saturation combined with low specific water flow in freshwater gave a long-lasting stress response and increased susceptibility to IPN virus challenge after seawater transfer of salmon post-smolts. Hyperoxygenation is a common practice in salmon smolt farming, and the results suggest that this can increase the risk of disease outbreaks. Hyperoxygenation and high carbon dioxide was also found to increase mortality in sea bass after bacterial challenge. These results suggest that the combination of high O_2 and CO_2 levels have detrimental effects on disease susceptibility in both salmon and sea bass farming - and this common procedure should be revised. However, further studies are needed to clarify if this is due to CO_2, O_2 or a combination of these parameters.

3. Chronic hypercapnia (over 25-30 mg/l CO_2) affects both physiology and immune system of sea bass reared in recycling systems under experimental conditions. Results suggest elevated CO_2 concentrations should be avoided to preserve good welfare and health of fish. Further studies are needed on commercial sea bass farms to determine threshold CO_2 levels in relation to different rearing systems.

4. Variable hypoxia in the range of 50-70% oxygen saturation was found to reduce appetite, growth and feed conversion efficiency in salmon in seawater. Thus, improved growth performance can be expected by avoiding such low oxygen levels in commercial cages. Improved oxygen conditions can be achieved by lowering stocking density, by cleaning of cages, and by improving the design and orientation of sea cages.

5. Hypoxia around 55% of oxygen saturation was indicated to negatively affect the innate immune response following challenges that simulates virus and bacterial infection. This suggest that exposure of salmon to such oxygen levels may increase the susceptibility to infectious diseases. Maintaining oxygen levels at a higher level in sea cages may thus reduce the risk of infectious diseases outbreaks. However, further research is needed to corroborate these indications.

6. The results from the project suggest that the current limit of 25kg/m^3 in salmon cages in Norway is high. Approaching this limit can increase the risk of impaired salmon welfare. It could be considered to lower this limit to 20 kg/m^3 to reduce the risk of undesired oxygen conditions in the sea cages that in turn can impair welfare and potentially increase the likelihood of disease outbreaks.

7. Controlled tank experiments with sea bass indicated that stocking densities up to 70kg/m^3 had no negative effect on growth performance or other welfare indicators tested - provided that the water quality was maintained at non-limiting levels. However, these findings were contrasted with poor environmental conditions found in commercial sea bass tank and sea cage systems together with histopatological changes on the fish. Further studies are therefore needed on commercial sea bass farms to verify safe levels of environmental factors and stocking densities to secure fish welfare.

8. A range of new physiological and molecular tools have been developed – and partly tested – to study welfare of sea bass and salmon. This includes tools to measure expression of a range of genes related to immune function. These tools should be applied in well controlled experimental set-ups to explore underlying mechanisms of fish welfare and to determine safe limits for environmental conditions. This could include tests on how various husbandry protocols and farming conditions affect the immune response following various tests representing viral and bacterial challenges – to further increase our knowledge on impact of such protocols and conditions on fish health, and ultimately overall fish welfare.

9. The analysis of respiratory burst of leukocytes from blood, head kidney or peritoneal exudate is proposed as a quick, easy and reliable indicator to monitor sea bass health status. The inhibition of this response may be an indication of an infection with virulent bacteria.

10. The expressed recombinant galectin-1 (rGalectin-1), which functional and immune properties (cytokine level expression and respiratory burst activity**)** have been assessed, and may have a possible protective role administrated as a therapeutic agent.

Based on the scarcity of published results in the field of fish welfare, and the discovery of the complexity in providing threshold and optimum values, as well in assessing specific husbandry protocols on their impact on stress, health and ultimately the overall welfare, we have identified the **following knowledge gaps and need for further research**:

1. Validated welfare indicators;
    a. For use in research
    b. Operational indicators for use on farms
    c. More detailed monitoring on farms (including need for new monitoring technology)
2. Further insight in physiological links between environment, husbandry protocols, stress and health/welfare;
    a. Improved challenge tests (*in vivo* and *in vitro* models) and measuring parameters
    b. Understanding mechanism (based on new tools available)
3. Threshold values for limiting factors;
    a. Variation with species, life-stages and production systems
    b. Interaction effects (e.g. Additive or synergistic effects; “stress on stress”)
    c. Models (to provide input for risk-assessment)

Table 1. Some key fish welfare issues in farming associated with abiotic and biotic environmental factors, feed and feeding, and management. Issues in *italics* have been studied in the Wealth project with focus on the factors in the yellow boxes (***bold***). Fish requirements and tolerance differs between species and life-stages, and the limiting factors and hazards for fish welfare also differs between production systems such as flow trough tanks, hyperoxygenated tanks, recirculated tanks, fresh water cages and sea cages.

Abiotic	**Biotic**	**Feed & Feeding**	**Management**
Temperature	***Stocking density***	Balance major nutrients	*Sorting and handling*
Salinity	Aggression/agonistic behaviour	Vitamins, minerals and additives	Biomass monitoring
Oxygen	Predators	Pellet size/type	***Health monitoring***
Metabolites *(e.g. CO_2 and NH_3)*	***Pathogens***	*Feeding regime*	Disease/parasite treatments
Water current	Toxic algae	Feed amount	Vaccination
Waves	Jelly fish etc	Starvation periods	***Environment monitoring***
Light	Bio-fouling (affecting flow)	Alternative diets (e.g. Soya-based)	Net change and cleaning
Enclosure size/ Cage distortion	*Ability for schooling*	Medicines and vaccines in feed	Transport
Pressure		Dietary toxins (e.g. fungal toxins)	Emergency killing
Access to air (salmonids)			Removal of dead fish

Table 2. Suggested welfare indicators and their usefulness in fish farming (for research and on farms).

Welfare indicator	**Time aspect for detection (typical)**	**Comment**
Appetite/feed intake	Rapid (hours)	Decrease under unfavourable conditions such as hypoxia, but show often large natural/unexplained meal to meal variation
Growth rate	Slow to (weeks/months)	Lower growth rate is often a sensitive sign of unfavourable conditions, but high growth rate do not always guarantee high welfare (e.g. growth related skeletal disorders)
Condition factor	Slow (weeks/months)	Decreasing and/or very low condition factor can indicate unfavourable conditions, but show large natural/seasonal variation. Very high condition may also be indicative of problems.
Feed Conversion Rate	Slow (weeks/months)	Sensitive to increased coping costs, but show large variation and can be difficult to measure
Size variability	Slow (weeks/months)	Increased size variation can be indicative of antagonistic behaviour, under/uneven feeding, or other unfavourable conditions
Changes in oxygen consumption	Rapid (minutes/hours)	Sensitive indicator of stress and various disturbances, but increases also with general activity, e.g. related to feeding
Behaviour surveillance (displays, avoidance, panic reactions, preferences, swimming activity, crowding, schooling, feeding activity...)	Rapid (seconds, minutes)	Respond to various disturbances (e.g. environmental, social, predators etc), preferences can be indicative of optimum values/situations, some behaviours can be difficult to monitor in some systems, depends on the ability of the animal to perceive the hazard.
Fin damages	Slow (days/weeks/months)	Integrative over time, indicative of agonistic behaviours, physical damage inappropriate feeding regime and stocking density.
(Skeletal) Deformities	Slow (weeks/months)	Integrative over time, sensitive to various environmental disturbances, especially at early life-stages or related to broodstock management/gamete quality
Eye damages	Slow (days/weeks/months)	Integrative over time, indicative of agonistic behaviours, physical damages, inappropriate feeding regime and stocking density.
Cataracts	Slow (weeks/months)	May be indicative of some environmental disturbances and feed problems
Mortality	Variable (minutes to days)	Often unspecific and difficult to establish cause, increased mortality rate verify problems
Disease outbreaks	Variable (days to weeks)	Disease outbreaks is a big welfare problem in its own, but can also be indicative of unfavourable environment or husbandry conditions
Skin colour	Rapid or variable (minutes, hours, days)	Dark skin colouration can be indicative of stress, disease or parasite load in some species)
Stress hormones (in blood and water)	Rapid (minutes)	Indicative of physiological stress, difficult to sample in blood under farming conditions. Water samples can provide information on cortisol and stress level in tanks. Chronic stress (weeks) may not be reflected in sustained increased hormone levels.
Other stress parameters	Variable (hours to days)	Reliable stress indicators that require standardized blood sampling procedure and availability of

(plasma ions, osmolality, NEFA)		physiological basal levels. Sensitive indicators of physiological status of fish in relation to acute and chronic stressful conditions. Not routinely suitable on farm.
Immune parameters	Slow (days/weeks/months)	Integrative over time, indicative of welfare and health status in relation to chronic exposure of stressful environmental and husbandry conditions. Not routinely suitable on farm.
Histopathological changes (e.g. gill & intestinal damages)	Slow (days/weeks/months)	Indicative of diseases, tissue damage either caused by pathogens, vaccine damages or environment/social stress
Primary barriers and physiological changes (e.g. intestinal permeability)	Slow (days/weeks/months)	Sensitive to chronic physiological stress such as moderate hypoxia, can potentially be indicative of increased disease risk. Also sensitive to a prior history of stress, i.e. in an earlier life stage. Not routinely suitable on farm.
Immune parameters after stimulation/challenges	Variable (hours to days)	Compromised immune function is believed to be associate with severe physiological stress, but can be difficult to assess (i.e. due to lack of knowledge of mode of action)
Gene expression profiles (e.g. Micro arrays)	Variable (hours to days)	Stressed fish show an altered gene expression profile, which could affect the response of fish against infections. Can indicate affected mechanism and identify genes of interest. Signatures may be established that can indicate severe chronic stress or other situation with compromised welfare
Molecular markers	Variable (hours to days)	The functional characterization of several genes and expression studies by quantitative PCR ca provide clues about the mechanism of disease and stress resistance of fish. These candidate genes could be used for diagnostic and therapeutic purposes in aquaculture.
Disease challenge tests (mortality and pathogen persistence/prevalence after challenge)	Variable (hours to days)	Can show physiological cost of coping under severe/chronic stress condition, but can be difficult to conduct (high variability, lack of good disease models) and are ethically problematic
Acute stress test	Rapid (minutes, hours)	Acute stress test (e.g. cortisol measurements) after periods with chronic stress can be indicative of costs of coping and limitations in scope of further stress resistance.
Indirect assessment by environment monitoring of key parameters (e.g. oxygen, CO_2, ammonia, temperature, salinity, pH)	Rapid (minutes, hours)	It can often be easier to monitor the environment than the fish response, but interpretations should be based on knowledge on safe thresholds for different parameter at different life-stages and interaction between parameters. Some parameters can be difficult/costly to monitor continuously (e.g. CO_2, ammonia)

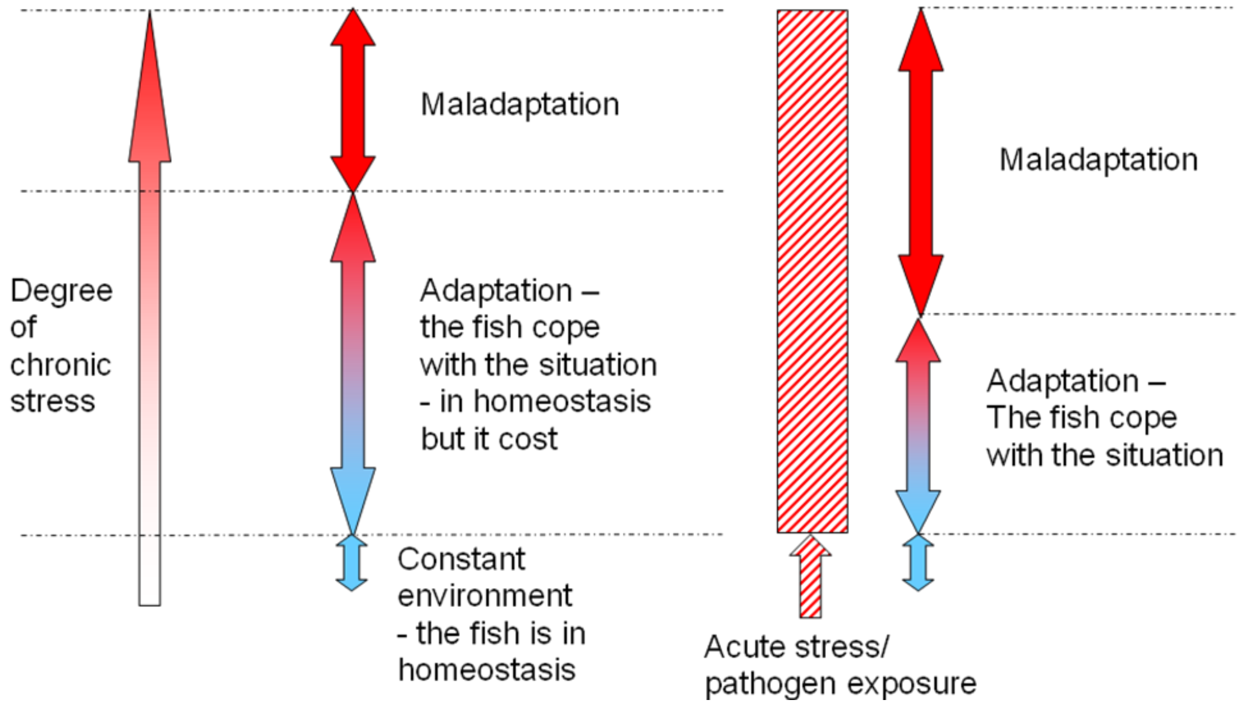

Figure 2. Chronic stress and coping abilities in fishes. After a period with chronic stress which the animal can cope with, the scope of adaption is reduced in order to cope with new acute or chronic stressors or pathogen exposure. Thus, when the scope of adaption is exceeded, stress become maladaptive, thereby increasing the likelihood of disease outbreaks and/or mortality.

1.5 Intentions for use and impact

Fish welfare comprises the maintenance of homeostasis and normal physiological and behavioral functions during all life-stages. The **Wealth** project has provided as range of new molecular and physiological tools and approaches that can serve the basis for new research projects targeting fish welfare in farmed fish.

This includes the identification of a range of key genes related to immune function in salmon and sea bass and development of real-time q PCR assays to monitor their expression. Use of cDNA microarray hybridisation in salmon has also indentified genes being affected by hypoxia and chronic stress, and monitoring their expression may potentially serve as welfare indicators in experimental set-ups.

A range of other physiological methods has been applied and validated to evaluate effects of potentially stressfull environmental conditions and husbandry practises, including measurement of cortisol in water and measurement of primary barrier integrity and permeability. Both these methods have been shown to respond well to chronic stress (over several weeks or months) such as hypoxia or poor water quality created by high stocking density.

Methodology for the measurement of fish oxidative status has been also validated and an increased risk of oxidative stress was detected in sea bass chronically exposed to stressful environmental conditions (e.g. hypoxia, hypercapnia and hyperoxia).

Some physiological parameters (ions, osmolality, non esterified fatty acids) have been found to be sensitive stress indicators and can be used to understand how fish cope with different environmental and husbandry conditions and to recognize detrimental effects on health and welfare.

Morphological indices such as various fin abrations/damages, skin mucus cell numbers and structure of gill lamella or has also been shown to respond to situations such as hypoxia and high stocking density. These morphological methods may also serve as operational welfare indicators in commercial farming.

Environmental monitoring in a number of commercial sea bass and salmon farms (both tanks and sea cages) has revealed large temporal and spatial (in cages) variation in water quality. Environmental monitoring with high resolution in time (and space in sea cages) serve as operational welfare indicators in two ways; first oxygen consumption has been shown to be very sensitive to stress and various disturbances, hence close monitoring of oxygen may be a rapid indicator of impending problems in fish farms. Secondly, continuous monitoring can help to ensure that environmental conditions are maintained within safe levels, and assist in the management on fish farms.

Actions to be taken will depend on the production system; in tanks both flow and water quality can be controlled in a more efficient way than in sea cages to ensure sufficient oxygen and removal of waste products such as ammonia and CO_2. However, we have also indications that the common practise of hyperoxygenation to maintain sufficient oxygen levels in tanks with limited water flow can be harmful to the fish, and increase susceptibility to infectious diseases. In sea cages, there is less possibility to control water flow and oxygen levels. The primary management solutions to maintain good water quality in cages is to keep stocking density at moderate levels, careful selection of sites with sufficient water currents, a set-up of cages that facilitate good water exchange and proper cleaning of nets. Use of deeper cages can provide the fish with better possibilities to select optimal environmental conditions, such as avoiding extreme temperatures or impact of biotic factors such as algal blooms or jelly fish strikes that are confined to specific depths. On the other hand, we have seen that salmon tend to aggregate at specific depths presumable due to such environmental preferences, hence, the resultant observed fish density is often much higher than the theoretical stoking density. Moreover, at some sites oxygen saturation can be low also in the surrounding waters, thus the above mentioned approaches will not be sufficient to provide sufficient oxygen levels in cages. Under such situations, artificial addition of oxygen can also be a possible solution.

The Wealth project has provided basis for evaluation of safe threshold levels for some water quality parameters such as CO_2 levels in sea bass on-growing and hypoxia in salmon postsmolts using a range of morphological, physiological and immunological parameters. However, we suggest that the issues of threshold values for both salmon and sea bass should be further explored with the new tools that has been developed, e.g. to study impact on immune related genes following immune stimulation. Also, the interaction between water quality parameters should be studied more in detail, e.g. the relative importance of high CO_2, pH and high oxygen pressure under hyperoxygenated situations.

Wealth has also provided new knowledge that can be the basis for development of operational welfare indicators both in salmon and sea bass. Some of these possibilities are implemented in an ongoing cooperation with the FASTFISH project. Some of the results can also serve as a basis for fish welfare legislation, e.g. related to threshold values, potential welfare indicators and changes in fish farming practises, e.g. stocking density in sea cages.

2 Plan for using and disseminating the knowledge

2.1 Section 1 - Exploitable knowledge and its Use

Overview table

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
Recommen-dations for fish farming		*Aquaculture industry and legislation*	*Available at project web site from April 2008*	*No - published on web*	*All*
Suggested welfare indicators		*Aquaculture industry and legislation*	*Available at project web site from April 2008*	*No - published on web*	*All*

2.2 *Section 2 – Dissemination of knowledge*

Overview table

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
September 2004	Juell, J. E., Oppedal, F., Johansson, D., Kelly, M., Stiansen, J.E., & Fosseidengen, J.E., 2004. Behavioural indicators of welfare in fish production cages. In: Book of Abstracts. AqEng "Engineering the future". Leuven, Belgium	Research, Industry	Scientific community worldwide		1
December 2004	Oppedal, F., Juell,. J-E., Johansson, D., 2004. Oppdrettsmiljø og fiskevelferd. Animal welfare in fisheries and aquaculture Workshop, Bergen	Research, Industry	Norway		1
September 2004	Juell, J. E., Oppedal, F., Johansson, D., Kelly, M., Stiansen, J.E., & Fosseidengen, J.E., 2004. Behavioural indicators of welfare in fish production cages. In: Book of Abstracts. AqEng 2004 "Engineering the future". Leuven, Belgium, 12-16 September 2004. pp. 594-595.	Research, Industry and Commission	Scientific community worldwide		1
October 20-23, 2004.	Evensen Ø, Glette J, Sundell K, Taranger GL, 2004. Welfare and health in sustainable aquaculture – Wealth. EAS Barcelona, Oral presentation.	Research, Industry and Commission	Scientific community worldwide		1, 6, 7
December 2004	Oppedal, F., Juell,. J-E., Johansson, D., 2004. Oppdrettsmiljø og fiskevelferd. Animal welfare in fisheries and aquaculture Workshop, Bergen Aquarium,	Public	Norway		1
2004	Johansson, D., Juell, J.E., Oppedal, F. Stiansen, J.E. og Fosseidengen, J. E., 2004. Oksygen i laksemerder: Har du tett luftfilter? *Norsk Fiskeoppdrett* 8, 52-55.	Public	Norway		1
2004	Johansson, D., Juell, J.-E., Oppedal, F., Stiansen J.E. and Fosseidengen, J.E., 2004. Merd-miljø og fiskevelferd i lakseproduksjon på kyst- og fjordlokaliteter på Vestlandet. *Fisken og Havet*, *Havforskningsinstituttet*, Bergen, Norway, No14-2006: 55 pp.	Public	Norway		1
April 2005	Juell JE, Oppedal F and Johansson D, 2005. Hva kan atferdstudier lære oss om fiskevelferd i oppdrettsmerder. Akvaveterinærenes Forening,	Research	Norway		1
May 2005	Juell, JE, 2005. The 3 R's and behavioural studies in aquaculture: Trade-off between possible animal suffering and relevance. International conference on the harmonization of use and care of fish in research. Gardermoen,	Research, Industry	Scientific community worldwide		1
June 2005	Kristiansen, T.S., Juell, J.E., Oppedal, F., Johansson, D., Fosseidengen, J.E. 2005. Overvåkning av fisk og miljø i merd. - Er dagens FoU teknologi morgendagens produksjonsteknologi? SINTEF- Nordisk Work Shop om Fiskevelferd og oppdrettsteknologi, Rica Nidelven Hotell, Trondheim, 21-22 juni 2005. (In	Research, Industry	Norway		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	Norwegian)				
August 2005	Santos, G.A., Lupatsch, I., van Anholt, R.D., Schrama J.W. and Verreth., J.A.J. 2005. The effect of stocking density on acute stress response in European sea bass (*Dicentrarchus labrax* L.). Aquaculture Europe, Trondheim, Norway.	Research, Industry	European countries		11
August 2005	T. Ellis, T., James, J.D. & Scott, A.P. (2005). Branchial release of free cortisol and melatonin by rainbow trout. J. Fish Biol. 67 535-540.	Research	Scientific community worldwide		3
April 2005	Juell, JE, 2005 Atferd hos oppdrettsfisk og konsekvenser for medisinforing. Næringsseminar om medisinforing, Schering Plough. Trondheim	Research, Industry	Norway		1
September 2005	Gadan K, Fridell F, Glette J and Evensen Ø, 2005. Effect of overcrowding on the susceptibility of Atlantic salmon (*Salmo salar* L.) to experimental challenge of IPN virus. EAFP conference Copenhagen	Research	Scientific community worldwide		1, 7
September 2005	Gadan K, Fridell F, Glette J and Evensen Ø, 2005. Effect of hyperoxygenation on the susceptibility of Atlantic salmon (*Salmo salar* L.) to experimental challenge of IPN virus. EAFP conference in Copenhagen, Denmark (poster).	Research	Scientific community worldwide		1, 7
September 2005	Juell JE, Behavioural studies and fish welfare in aquaculture: What questions are we asking? Scandinavian Society for Laboratory Animal Science. Mini symposium on Fish, Oslo,	Research	Scandinavian countries		1
November 2005	Conference: EAS Meeting, Trondheim (Poster presentation)	Research	European countries		11
2005	Juell, J.E and Kristiansen, T. 2005 Fiskevelferd - et positivt eller negativt perspektiv for næringen? In: Boxaspen K. et al. (eds.). Kyst og Havbruk 2005. Fisken og havet, Særnr. 2, 75-77.	Public	Norway		1
January 2006	Juell JE, 2006. Feeding behaviour of farmed fish. Workshop on oral medication, 11-12.01.2006, Inverness, Scotland.	Research	Scientific community worldwide		1
January 2006	Flyer on Welfare and Health in sustainable Aquaculture	Public	Worldwide		1, 6, 7
February 2006	Juell JE, Kristiansen, T, Fosseidengen, J.E, Johannson, D. & Oppedal, F., 2006. Utfordringer i merdmiljøet. Oppdrett av torsk-utfordringer for videre vekst! Nasjonalt Torskenettverksmøte	Research	Norway		1
March 2006	Gadan K, Fridell F, Taranger GL, Santi N, Mercy IS and Evensen Ø, 2006. Effekt av hypoxi på laksemakrofagers cytokinproduksjon etter Poly-I:C stimulering. HAVBRUK 2006, Bergen, 29-31 March 2006. Poster. (In Norwegian)	Research	Scientific community worldwide		1. 7
March 2006	Petochi, T. P. Di Marco, A. Priori, G. Marino. Chemical-clinical welfare indicators in farmed fish. (IZS Government Veterinary Institute, Legnaro, Italy. Oral	Research	Italy		12

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	presentation				
March 2006	Oppedal, F., Juell, J-E., Fosseidengen, J.E. og Johansson, D., 2006. Temperatur- og lysregulerende atferd hos laks i merd: konsekvenser for fiskevelferd? Programkonferanse Havbruk 2006, Sandsli, 29-31	Research	Norway		1
May 2006	Abstract and talk at international conference, Aqua 2006, Florence, Italy T. Ellis, J. James & A.P. Scott "A non-invasive cortisol assay for seawater Atlantic salmon."	Research	Scientific community worldwide		3
May 2006	Fridell F, Gadan K, Sundh H, Taranger GL, Glette J, Sundell K and Evensen Ø, 2006. Effect of hyper-oxygenation and low water flow on primary stress response and the susceptibility of Atlantic salmon *Salmo salar* L. to experimental challenge of IPN virus. WAS/EAS meeting, Aqua 2006, Firenze, Italy, 9-13 May 2006 (oral presentation).	Research	Scientific community worldwide		1, 6, 7
May 2006	Taranger GL, Sundell K, Evensen Ø, Blancheton JP, Ellis T, Alexis, M, Meseguer J, Ellis T, Kotoulas G, Novoa B, Verreth J, Marino G and Glette, J., 2006. WEALTH - Welfare and health in sustainable aquaculture", welfare session. WAS/EAS meeting, Aqua 2006, Firenze, Italy, 9-13 May 2006. Oral presentation.	Research	Scientific community worldwide		1, 6, 7
May 2006	Taranger GL, Fridell F, Oppedal F, Kvamme BO, Johansson D, Juell JE, Olsen RE, Sundell K, Gadan K, Evensen Ø and Glette J, 2006. Operational husbandry protocols to ensure welfare and health in farmed fish; Welfare and health in sustainable aquaculture – WEALTH. Finfish Health session, WAS/EAS meeting, Aqua 2006, Firenze, Italy, 9-13 May 2006. Oral presentation.	Research	Scientific community worldwide		1, 6, 7
May 2006	Oppedal, F., Johansson, D. og Juell,. J-E., 2006. Swimming depth and schooling density of caged Atlantic salmon in vertical temperature and light gradients. AQUA 2006 conference 9-13 May, Firenze, Italy.	Research	Scientific community worldwide		1, 6, 7
May 2006	Sundh H, Fridell F, Gadan K, Evensen Ø, Glette J, Taranger GL and Sundell K. Intensive husbandry conditions and IPN virus affects the intestinal primary barrier in Atlantic salmon (*Salmo salar*). Proceedings to: AQUA 2006, Florence, Italy.	Research	Scientific community worldwide		1, 6, 7
May 2006	Santos, G.A., Schrama, J.W., Verreth, J.A.J., 2006. The effect of stocking density on energy metabolism, behaviour and stress responses of European sea bass (*Dicentrarchus labrax* L.). AQUA 2006. 9-13 May, Florence, Italy.	Research	Scientific community worldwide		11
May 2006	Marino, G., P. Di Marco, A. Priori, T. Petochi, A. Massari, A. Mandich, M.G. Finoia, N. Romano. Effects of environmental hypercapnia in sea bass *Dicentrarchus labrax*: blood gas and acid-base status, stress and immune response to acute and chronic exposure. EAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and oral presentation	Research and Industry	Scientific community worldwide		12

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
May 2006	Di Marco, P., A. Priori, T. Petochi, A. Massari, A. Mandich, M.G. Finoia, G. Marino. Physiological and oxidative stress responses in sea bass *Dicentrarchus labrax*, exposed to environmental hypercapnia. EAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and poster presentation	Research and Industry	Scientific community worldwide		12
May 2006	Marino, G., P. Di Marco, T. Petochi, A. Priori, M.G. Finoia. Blood gas and acide-base status in sea bass exposed to environmental hypercapnia. EAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and poster presentation	Research and Industry	Scientific community worldwide		12
May 2006	Petochi, T., A. Priori, P. Di Marco, M.G. Finoia, G. Marino. Effects of long-term exposure to hypercapnia on innate immune response of sea bassEAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and poster presentation	Research and Industry	Scientific community worldwide		12
May 2006	E. Caccia, G. Marino, G. Scapigliati, L. Abelli, V. De Molfetta, L. Mastrolia, M. Mazzini and N. Romano. Effects of oxygen and carbon dioxide on the adaptive immune system of cultured sea bass *Dicentrarchus labrax*. EAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and poster presentation	Research and Industry	Scientific community worldwide		12
May 2006	Lemarié G., S. Sammouth, E. Gasset, G. Breuil, G. Marino and J. P. Blancheton. Effect of stocking density on sea bass *Dicentrarchus labrax* performance in a recirculating system. Oral presentation at AQUA 2006, WAS/ EAS European Aquaculture Society Congress. Florence, Italy.	Research and Industry	Scientific community worldwide		2
June, 2006	Poster presentation to the 8th Panhellenic symposium of Oceanography and Fisheries, to be held in Thessaloniki. Effect of different oxygen levels on sea bass growth. Vatsos I.N., Kalogiros H., Giagnisi M. and Alexis M.	Research and Industry	Greece		4
June 2006	Marino, G., P. Di Marco, T. Petochi, A. Priori, M.G. Finoia. Welfare indicators in farmed fish. SIBM Italian Society of Marine Biology. Grosseto, Italy. June 2006. Abstract and oral presentation.	Research	Italy		12
July 2006	Project web-site	Research, Industry, EU Commission and public	worldwide		All
July, 2006	Sepulcre MP, Dios S, Novoa B, Figueras A, Meseguer J, Mulero V, 2006. Identification of immune-relevant genes from sea bass (Dicentrarchus labrax) by Suppresion Subtractive Hybridization. 10th international congress of the internacional society for developmental and comparative immunology, Charleston (USA), 1-6 July 2006 (Poster).	Research	Scientific community worldwide		5
August 2006	Poster at international conference, 23rd conference of European Comparative Endocrinologists, Manchester UK	Research	Scientific community		3

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	A.P. Scott, M. Sebire, I. Katsiadaki, T. Ellis "Non-invasive measurement of fish steroids in water"		worldwide		
September 2006	Juell JE, Fosseidengen JE and Kristiansen TS, 2006. Utfôring tilpasset torskens begrensede vertikalvandring. AKVAFORSK-semiar "Foring av torsk" Molde, 07.09.2006. (In Norwegian)	Research	Norway		1
September 2006	Marino, G., P. Di Marco, T. Petochi, A. Priori, M.G. Finoia. Welfare indicators in farmed fish. (SIBM Italian Society of Marine Biology, Grosseto, Italy. June 2006).	Research	Italy		12
September 2006	Di Marco, P., A. Priori, T. Petochi, M.G. Finoia, G. Marino Effect of hypercapnia on total oxidant and antioxidant status in cultured sea bass *Dicentrarchus labrax*. UZI Unione Zoologica Italiana. Naples, Italy, September 2006. Abstract and oral presentation.	Research	Italy		12
October 2006	Abstract and talk at international conference: COST 867, Arcachon, France A.P. Scott & T. Ellis "Progress towards a 'dipstick' test for fish welfare"	Research	European scientific community		3
October 2006	Oppedal, F., Juell,. J-E. og Johansson, D., 2006. Oppdrettsmiljø og fiskevelferd - forsøksfisk i storskala oppdrettssystem. Forsøksdyrkurs, Haukeland universitetssykehus, 25 okt. 2006.	Research	Norway		1
October 2006	Juell JE, 2006. Fish welfare research at Institute of Marine Research, Norway. COST-867 "Fish welfare in European aquaculture" Archachon, France, 9-11 October 2006.	Research	European scientific community		1
October 2006	Kvamme BO, Taranger GL, Sundell K, Evensen Ø, Blancheton JP, Ellis T, Alexis M, Meseguer M, Ellis T, Kotoulas G, Novoa B, Verreth J, Marino G and Glette J, 2006. Welfare and Health in Sustainable Aquaculture – WEALTH. COST867, Arcachon, France, 9-11 October 2006.	Research	European scientific community		1-12
October 2006	Juell JE, 2006. Fish welfare research at Institute of Marine Research, Norway 2006. COST-867 "Fish welfare in Europeean aquaculture" Archachon, France 9-11 October.	Research	European scientific community		1
October 2006	Schrama, J.W., van de Nieuwegiessen, P.G., Santos, G.A., Martins, C.I.M. and Verreth J.A.J, 2006. Behaviour in relation to fish welfare: individual differences (coping styles) and effects of husbandry conditions. In Meeting of COST Action 867 "Welfare of fish in European aquaculture", October 9 – 11 2006, Arcachon, France.	Research	European scientific community		11
October 2006	Coeurdacier J. L. Alteration of total protein and proteome in serum of sea bass (*Dicentrarchus labrax*) reared at different stocking densities. Oral and poster presentation at « Congrès Société Française d'Electrophorèse et d'Analyse Protéomique (SFEAP) » St Malo, France. 16-19 October 2006	Research	France		2

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
October 2006	Gadan K, Santi N, Evensen Ø, 2006. Effect of stress on the balance between mixtures of recombinant IPNV isolates in persistently infected Atlantic salmon fry. 9th International Symposium on Double-Stranded RNA Viruses, South-Africa, 21-26 October 2006	Research	European scientific community		7
November 2006	Oppedal, F., Johansson, D. og Juell,. J-E., 2006. Kan enkeltindividers atferd lære oss mer effektiv produksjon? Produktivitetskonferansen 2006. MonAqua AS. Norway.	Research, industry	Norway		1
2006	Fridell F, 2006. Stress og mottakelegheit for sjukdom, hjå atlantisk laks. Eupharma seminar, Leknes, Norway. (In Norwegian)	Research, education	Norway		1
2006	Braastad, B., Juell, J.E & Damsgård, B. 2006. Animal behaviour – A new concept in aquaculture and fisheries In: Damsgård, B., Juell, J.E., & Braastad, B.O. 2006 (eds.). *Welfare in Farmed Fish. Report 5/2006 Fiskeriforskning*, Tromsø, Norway, pp 5-11.	Research, education	Norway		1
2006	Juell, JE., Johansson, D. & Oppedal, F., 2006 Effects of the cage environment and social interactions on the swimming behaviour and welfare of Atlantic salmon In: Damsgård,B., Juell, J.E., & Braastad, B.O. 2006 (eds.). Welfare in Farmed Fish. Report 5/2006 Fiskeriforskning, Tromsø, Norway, pp 91-98	Research, policy, education	Norway		1
2006	Oppedal, F., Johansson, D., Fosseidengen, J.E. og Juell, J.E., 2006. På hvilket dyp bør lyset plasseres? Norsk Fiskeoppdrett, 10: 58-59.	Public	Norway		1
2006	Oppedal, F., Johansson, D. & Juell, J.E., 2006. The cage environment laboratory: Behavioural studies in a realistic farm environment. In: Damsgård, B., Juell, J.E., & Braastad, B.O. 2006 (eds.). Welfare in Farmed Fish. Report 5/2006, Fiskeriforskning, Tromsø, Norway, pp 24-27	Research, policy, education	Norway		1
January 2007	Kvamme BO, Fridell F, Gadan K, Oppedal F, Evensen Ø and Taranger GL, 2007. Effektar av hypoksi på laksens immunsvar. Frisk Fisk meeting, Tromsø, 23.-25. januar 2007.	Research and Industry	Norway		1, 7
January 2007	Fridell F, Oppedal F, Gadan K, Sundh H, Taranger GL, 2007. Effekten av hypoksi på laksens ve og vel, kan ein måla fiskevelferd? Frisk Fisk meeting,Tromsø, Norway	Research and Industry	Norway		1, 6, 7
January 2007	Kvamme, B.O., Fridell, F., Gadan, F., Oppedal, F., Evensen Ø. and Taranger, G.L, 2007. Effektar av hypoksi på laksens immunsvar. Frisk Fisk meeting. Tromsø, Norway. 23-25 January 2007. (In Norwegian)	Research and Industry	Norway		1, 7
February 2007	Oppedal, F., 2007. Improved management of oxygen flow in net cages – OxFlow. CREATE day, Trondheim, Rica Nidelven hotel, 14-15	Research and Industry	Norway		1
February 2007	Oppedal, F., Bratland, S. and Olsen, R.E., 2007. Kortisol i vann – preliminære	Research and	Norway		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	resultat. EWOS – oppdrettsmøte. Matre, 22 February 2007	Industry			
March 2007	Petochi, T., P. Di Marco, A. Priori, M.G. Finoia, G. Marino. Innate immune response of reared European sea bass *Dicentrarchus labrax* to different environmental and husbandry conditions. SIICS - Italian Society of Developmental and Comparative Immunobiology. Naples, Italy. Abstract and oral presentation.	Research	Italy		12
March 2007	Romano, N., E. Caccia, T. Petochi, S. Meloni, L. Mastrolia, G. Scapigliati, L. Abelli and G. Marino. Effects of different carbon dioxide concentrations on the adaptive immune system of cultured sea bass (*Dicentrarchus labrax* SIICS - Italian Society of Developmental and Comparative Immunobiology. Naples, Italy. Abstract and oral presentation.	Research	Italy		12
March, 2007	Blancheton J.P., R. Piedrahita, A. Belaud, Y. Moutounet, S. Fivelstad, G. Lemarié (2007*b*). Gas control in land based aquaculture, physiological and technical aspects : Gaz totaux et oxygène. 15-16 March 2007. *Journées du SFAM 2007, Montpellier, France*	Research and producers	France		2
April 2007	Dios S, Poisa-Beiro L, Montes A, Aranguren R, Figueras A, Novoa B, 2007. Nodavirus increases the expression of Mx and inflammatory cytokines in fish brain. 7TH international symposium on viruses of lower vertebrates, Oslo, Norway, 22-25 April 2007	Research	Worldwide European scientific community		10
April 2007	Poisa-Beiro L, Dios S, Figueras A, Novoa B, 2007. New lectins characterized by Supression Subtractive Hybridization (SSH) in head kidney from sea bass infected with nodavirus. 7TH international symposium on viruses of lower vertebrates, Oslo, Norway, 22-25 April 2007(Poster).	Research	Worldwide European scientific community		10
April 2007	Gadan K, Mercy IS, Santi N and Evensen Ø, 2007. Stress, immunocompetence and IPN virus infections in Atlantic salmon. ISVLV symposium, Oslo, 22-25 April 2007.	Research	European scientific community		7
May 2007	Kristiansen, T.S., Begout, M-L., Toften, H., Villaroel, M. 2007. What is an operational welfare indicator? COST 867 conference, 2nd COST 867 Welfare of fish in European aquaculture, Work Shop, Varese, Italy.	Research	European scientific community		1, 2
May 2007	Talk at international conference: COST 867, Varese, Italy. T. Ellis "Review of cortisol and fish welfare"	Research	European scientific community		3
May 2007	Oppedal, F., Kristiansen, T., Bratland, S., Stien, L., Folkedal, O. and Juell, J.E., 2007. Salmon experiments in WP1: Pilot postsmolt study, Parr-presmolt-smolt study and postsmolt in cages, FASTFISH mid-term meeting, Crete, 21-23	Research	European scientific community		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
June 2007	Santos, G.A., Schrama, J.W., Rombout, J.H.W.M, Verreth, J.A.J., 2007. Studies on the effects of cronic stressors on energy metabolism, welfare and immunity of European sea bass (*Dicentrarchus labrax* L.). 2007. AQUA Twin Event 11 – 12 June, Patras, Greece.	Research	European scientific community		11
June 2007	Petochi, T., N. Romano, P. Di Marco, A. Priori, E. Caccia, G. Scapigliati, S. Meloni, L. Abelli, G. Marino. Innate and adaptive immunity of reared sea bass *Dicentrarchus labrax* under environmental hypercapnia. NOFFI, Nordic Society for Fish Immunology Congress. Stirling, Scotland, June, 2007. Abstract and poster presentation.	Research	Scientific community worldwide		12
June 2007	Faucher K., Millot S., Blancheton J.P., Lemarié G., Dutto G. & Bégout M.-L. 2007. Effect of stocking density on cultured fish swimming behaviour: First results on sea bass using acoustic telemetry in flow through tanks. *7th Conference on Fish Telemetry*, Silkebørg, Denmark. 17-21 June, 2007. Oral presentation	Research	European scientific community		2
June 2007	Faucher K., Millot S., Struski C. & Bégout M.-L., 2007. Assessment of sea bass swimming activity and preferential space use in sea cages using acoustic telemetry and archival tags. *7th Conference on Fish Telemetry*, Silkebørg, Denmark. 17-21 June, 2007. Poster.	Research	European scientific community		2
August 2007	Taranger GL, Fridell F, Kvamme BO, Oppedal F, Johansson D, Juell JE, Olsen RE, Sundth H, Gaddan K, Sundell K and Evensen Ø, 2007. Welfare biology in sea cage culture of salmon and sea bass. "My sea home" session. EAS Aqua NOR forum, "Welfare as a driver for technological development in aquaculture", Trondheim August 15-16, 2007. Oral presentation and panel member. See summary at: http://www.easonline.org/files/Meetings/aqua_nor_forum_summary.pdf	Research	Scientific community worldwide		1, 6, 7
August 2007	Welfare Fish welfare in land-based production systems from flow through to recirculation in relation to water quality and fish density. Jean-Paul Blancheton, Emmanuelle Roque d'orbcastel, Jeannine Person and Gilles Lemarié. "My sea home" session. EAS Aqua NOR forum, "Welfare as a driver for technological development in aquaculture", Trondheim August 15-16, 2007. Oral presentation and panel member. See summary at: http://www.easonline.org/files/Meetings/aqua_nor_forum_summary.pdf	Research	Scientific community worldwide		2
September 2007	Petochi T., Di Marco P., Priori A., Longobardi A. M.G. Finoia. Development of an autopsy-based method to assess on-farm welfare in sea bass. EAFP European Association of Fish Pathologists. Grado, Italy. Abstract and poster presentation.	Research	Scientific community worldwide		12
September 2007	K. Gadan, I. Singh Mercy, H. Sundh, K. Sundell and Ø. Evensen. Effects of Growth hormone and cortisol on the immune response after challenge with infectious pancreatic necrosis virus in Atlantic salmon (*Salmo salar*). EAFP. September 2007.	Research	Scientific community worldwide		1, 7

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
October 2007	Stien, L. H., Folkedal, O., Gytre, T., Kristiansen, T., Nilsson, J., Torgersen, T., 2007. Monitoring the welfare of farmed fish. The Research School SCOFDA - Sustainable Control of Fish Diseases in Aquaculture, Two day workshop on welfare and health of fish, Copenhagen, 30th to 31th October, 2007. (Invited lecturer).	Research	Scientific community worldwide		1
October 2007	Marino, G., T. Petochi, P. Di Marco, J.P. Blancheton, G. Lemariè, A. Priori, A. Priori, A. Massari, M.G. Finoia Developing operational welfare indicators for farmed sea bass *Dicentrarchus labrax*. EAS, Istanbul, Turkey. Abstract and poster presentation.	Research and Industry	Scientific community worldwide		12,2
October 2007	Effect of anaesthetics on stress physiology and welfare in sea bass *Dicentrarchus labrax* P. Di Marco, T. Petochi, A. Priori, M.G. Finoia, A. Longobardi, A. Massari, A. Mandich, G. Marino. EAS, Istanbul, Turkey. Abstract and poster presentation.	Research and Industry	Scientific community worldwide		12,2
October 2007	Long-term exposure to combined hyperoxia and hypercapnia on stress and innate immunity of sea bass *Dicentrarchus labrax*. P. Di Marco, A. Priori, T. Petochi, M.G. Finoia, J.P. Blancheton, G. Lemariè, A. Cevasco, G. Marino. EAS, Istanbul, Turkey. Abstract and poster presentation.	Research and Industry	Scientific community worldwide		12,2
October 2007	Effect of hyperoxia and hypercapnia on total oxidant and antioxidant status in cultured sea bass *Dicentrarchus labrax* P. Di Marco, A. Alberti, D. Macciantelli, A. Priori, Finoia, A., T. Petochi, J.P. Blancheton, G. Lemariè, G. Marino. EAS, Istanbul, Turkey. Abstract and poster presentation.	Research and Industry	Scientific community worldwide		12,2
October 2007	Blancheton J.P., J. Person-Le Ruyet, G. Lemarié Relation water quality fish requirements. *Aquaculture Europe 07 - Annual Meeting of the European Aquaculture Society, Istanbul, Turkey, Short course Principles of recirculation aquaculture systems (RAS) technology, Istanbul, Turkey*. Oral presentation.	Research and Industry	Scientific community worldwide		1
October 2007	Blancheton J.P., R. Piedrahita, A. Belaud, Y. Moutounet, S. Fivelstad, G. Lemarié (2007*a*). Gas control in land based aquaculture, physiological and technical aspects. *Aquaculture Europe 07 - Annual Meeting of the European Aquaculture Society, Istanbul, Turkey, Short course Principles of recirculation aquaculture systems (RAS) technology, Istanbul, Turkey*.	Research and Industry	Scientific community worldwide		1
October 2007	Oppedal, F., Bratland, S., Stien, L., Nilson, J. and Folkedal, O., 2007. Må vi tenke mer på dyrevelferd i norsk fiskeoppdrett? - Akutt stress hos settefisk, Microteket, EWOS seminar, Stjørdal, 31 October 2007. (In Norwegian)	Research and Industry	Norway		1
October 2007	Oppedal, F., Johansson, D. og Juell., J-E., 2007. Vanngjennomstrømning, oksygentilgang og faktorer som påvirker fiskens atferd i store merder. Akvaveterinærenes høstkurs 2007, Park Inn hotell, Stavanger, 23-24 October 2007. (In Norwegian)	Research and Industry	Norway		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
November 2007	Oppedal, F., Bratland, S. and Folkedal, O., 2007. Akutt stress hos laks - preliminære data. Produktivitets-konferansen 2007, Kristiansund, November 1 2007. (In Norwegian)	Research and Industry	Norway		1
November 2007	Oppedal, F., Bratland, S., Stien, L., Nilson, J. and Folkedal, O., 2007. Må vi tenke mer på dyrevelferd i norsk fiskeoppdrett? - Akutt stress hos settefisk, Microteket, EWOS seminar, Bergen, 7 Nov 2007. (In Norwegian)	Research and Industry	Norway		1
December 2007	Taranger GL, Sundell K and Evensen Ø, 2007. WEALTH: "Welfare and health in sustainable aquaculture". Working Group (WG) in the field of aquaculture. Research, Data Collection & Scientific Advice Unit of DG Fisheries & Maritime Affairs, EU Commission, Brussels, 3 - 5 December 2007. (Invited oral presentation).	Research	Scientific community worldwide		1, 6, 7io
December 2007	Oppedal, F., Dempster, T., Kristiansen., T., Korsøen, Ø., Folkedal., O, 2007. Nedsenking/ heving av merder – biologiske aspekter. CREATE-Submersible cage, prosjektmøte, Trondheim, 20 des. 2007	Research and Industry	Norway		1
2007	Annon. 2007. "WEALTH - Welfare and health in sustainable aquaculture". 12pp. (Wealth leaflet available at http://wealth.imr.no/__data/page/6068/Wealth_leaflet_web.pdf)	Research, Policy and Industry	Worldwide		1-12
2007	Fridell, F., Kvamme, B.O., Brix, O., Oppedal, F., Olsen, R.E., Taranger, G.L. 2007. Søket etter nye stress og velferdsmarkørar hjå Atlantisk laks. *Norsk Fiskeoppdrett*, 11:54-57	Public	Norway		1
2007	Fridell, F., Taranger, G.L, Kristiansen T.S. 2007. New research will help farmers manage fish health and welfare. *Fish farmer*, 30:12-13	Public	Norway		1
2007	Gytre, T., Stien, L. H., Oppedal, F., 2007. How are things in the sea-cage? *Marine Research News* NO. 18-2007	Public	worldwide		1
2007	Johansson, D. 2007. Production cage environment and salmon beaviour. *PhD Thesis*. University of Bergen, 2007.	Research, Public	Scientific community worldwide		1
2007	Juell, J.E, Nilsson, J., Olsen, R.E. Fridell,F., Kvamme, B.O., Oppedal, F., Humborstad, O.B., Mangor-Jensen, A. Stien, L.H and Kristiansen, T., 2007. Dyrevelferd i akvakultur og fiskeri – et nytt fagområde i rask vekst. In: Dahl, E., P.K. Haug, T. Karlsen, Ø (eds.). Kyst og Havbruk 2007. Fisken og Havet, særnr 2-2007, s. 141-148.	Public	Norway		1
2007	Kristiansen, T. Johansson, D. Oppedal, F. og Juell, J.E., 2007. Hvordan har oppdrettsfisken det i merdene? In: Dahl, E., P.K. Haug, T. Karlsen, Ø (eds.). Kyst og	Public	Norway		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	Havbruk 2007, Fisken og Havet, særnr 2-2007, pp. 151-155				
2007	Peer-review paper: Scott, A.P., Ellis, T. (2007). Measurement of fish steroids in water—a review. General and Comparative Endocrinology 153 392–400.	Research	Scientific community worldwide		3
2007	Peer-reviewed paper: Fridell F, Gadan K, Sundh H, Taranger GL, Glette J, Sundell K and Evensen Ø. Effect of hyperoxygenation on the susceptibility of Atlantic salmon (*Salmo salar* L.) to experimental challenge of IPN virus. Aquaculture 270, 23-35	Research	Scientific community worldwide		1, 6, 7
2007	Peer-reviewed paper: Ellis, T., James, J.D., Sundh, H., Fridell, F., Sundell, K., Scott, A.P. (2007). Non-invasive measurement of cortisol and melatonin in tanks stocked with seawater Atlantic salmon. Aquaculture, 272, 698-706	Research	Scientific community worldwide		1, 3, 6
2007	Peer-reviewed paper: Ellis, T., Bagwell, N., Pond, M., Baynes, S., Scott, A.P. (2007). Acute viral and bacterial infections elevate water cortisol concentrations in fish tanks. Aquaculture, 272, 707-716	Research	Scientific community worldwide		3
2007	Peer-reviewed paper: Johansson, D., Juell, J.-E., Oppedal, F., Stiansen J.E. and Ruohonen, K., 2007. The influence of the pycnocline and cage resistance on current flow, oxygen flux and swimming behaviour of Atlantic salmon (*Salmo salar* L.) in production cages. Aquaculture 265, 271-287.	Research	Scientific community worldwide		1
2007	Peer-reviewed paper: Johansson, D., Ruohonen., K., Kiessling, D., Oppedal, F., Stiansen, J.E., Kelly, M., and Juell, J-.E., 2006. Effect of environmental factors on swimming depth preferences of Atlantic salmon (*Salmo salar* L.) and temporal and spatial variations in oxygen levels in sea cages at a fjord site. Aquaculture 254, 594-605.	Research	Scientific community worldwide		1
2007	Peer-reviewed paper: Oppedal, F., Juell, J.-E. and Johansson, D., 2007. Thermo- and photoregulatory swimming behaviour of caged Atlantic salmon: Implications for photoperiod management and fish welfare. Aquaculture 265, 70-81	Research	Scientific community worldwide		1
2007	Peer-reviewed paper: Sepulcre MP, Sarropoulou E, Kotoulas G, Meseguer J, Mulero V. 2007. *Vibrio anguillarum* evades the immune response of the bony fish sea bass (*Dicentrarchus labrax* L.) through the inhibition of leukocyte respiratory burst and down-regulation of apoptotic caspases. Mol Immunol 44 (15): 3751-3757.	Research	Scientific community worldwide		5, 9
2007	Peer-reviewed paper: Rønneseth A, Wergeland HI, Devik M, Evensen Ø, Fausa Pettersen E, 2007. Mortality after IPNV challenge of Atlantic salmon (*Salmo salar* L.) differs based on developmental stage of fish or challenge route. Aquaculture 271 (1-4): 100-111.	Research	Scientific community worldwide		7
February 2008	Oppedal, F., Bratland, S., Stien, L., Nilson J., og Folkedal, O. 2008. Må vi tenke mer på dyrevelferd i norsk fiskeoppdrett? - Akutt stress hos settefisk, Fiskeridirektoratet,	Research, Industry	Norway		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	Bergen, 7 February 2008.				
2008	Kvamme B.O., Oppedal F., Torgersen T., Fridell F., Sundh H., Sundell K. Oksygennivået viktig for oppdrettsfiskens helse og velferd. Kyst og Havbruksrapporten 2008, Fisken og Havet, særnr 2-2008, pp. xxx-xxx	Public, research, education	Nordic countries		1, 6
2008	Kvamme BO, Fridell F, Oppedal F, Stien L, Torgersen T, Sundh H, Sundell K, Kristiansen T, Juell JE and Taranger GL, 2008. Hypoksi og Hyperoksi - Kva kan det føre til. ScanVacc Seminars, Trondheim, Norway, 2008. (In Norwegian)	Research	Norway		1, 6
2008	Kvamme BO, Fridell F, Oppedal F, Stien L, Torgersen T, Sundh H, Sundell K, Kristiansen T, Juell JE and Taranger GL, 2008. Hypoksi og Hyperoksi - Kva kan det føre til. ScanVacc Seminars, Stavanger, Norway, 2008. (In Norwegian)	Research	Norway		1, 6
2008	Kvamme BO, Fridell F, Oppedal F, Stien L, Torgersen T, Sundh H, Sundell K, Kristiansen T, Juell JE and Taranger GL, 2008. Hypoksi og Hyperoksi - Kva kan det føre til. ScanVacc Seminars, Bergen, Norway, 2008. (In Norwegian)	Research	Norway		1, 6
2008	Peer reviewed paper: Poisa-Beiro L, Dios S, Montes A, Aranguren R, Figueras A and Novoa B. 2008. Nodavirus increases the expression of Mx and inflammatory cytokines in fish brain. Mol Immunol 45 (1): 218-225	Research	Scientific community worldwide		10
2008	Peer-reviewed paper: Coeurdacier et al. Global serum protein as stress and health indicator for reared sea bass (*Dicentrarchus labrax* L., 1758). Submitted to Aquaculture	Research	Scientific community worldwide		2
2008	Peer reviewed paper: Sundh H, Olsen RE, Fridell F, Gadan K, Evensen Ø , Glette J, Taranger GL, Sundell K. The intestinal primary barrier in Atlantic salmon (*Salmo salar*): effect of hyperoxygenation and reduced flow in freshwater and subsequent IPN virus challenge in sea water. (Submitted)	Research	Scientific community worldwide		1, 6, 7
2008	Peer reviewed paper: Scott, A.P., Hirschenhauser, K., Bender, N., Oliveira, R., Earley, R.L., Sebire, M., Ellis, T., Pavlidis, M., Hubbard, P.C., Huertas, M., Canario, A.V. (submitted). Non-invasive measurement of steroids in fish-holding water: guidelines for practical application. Behaviour. (Submitted)	Research	Scientific community worldwide		3
2008	Peer reviewed paper: Fanouraki, E., Papandroulakis, N., Ellis, T., Mylonas, C., Scott, A., Pavlidis, M. (submitted). Is water cortisol concentration a reliable index for the assessment of stress in European sea bass, *Dicentrarchus labrax*? Behaviour (Submitted)	Research	Scientific community worldwide		3
2008	Peer reviewed paper: Sarropoulou E, Sepulcre P, Mulero V, Poisa-Beiro L, Figueras A, Novoa B, Terzoglou V, Reinhardt R, Kotoulas G., 2008. Profiling of immune specific mRNA transcripts of the European sea bass *Dicentrarchus labrax*. (In	Research	Scientific community worldwide		5, 9, 10

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	preparation).				
2008	Peer reviewed paper: Gadan K, Sandtrø A, Mercy IS, Santi N, Evensen Ø: Reversion to wild type of avirulent strains of infectious pancreatic necrosis virus in Atlantic salmon is associated with increased replication rate and induced by stress (manuscript) (In preparation).	Research	Scientific community worldwide		7
2008	Peer reviewed paper: Sandtrø A, Gadan K, Evensen Ø, Santi N: Experimental infection of Atlantic salmon fry with genotype mixes of infectious pancreatic necrosis virus results in a persistent infection consisting of quasi-species of the virus formed in vivo (manuscript in preparation)	Research	Scientific community worldwide		7
2008	Peer reviewed paper: Gadan K, Sundh H, Sundell K, Evensen Ø: Studies of immune parameters and virus persistence in Atlantic salmon parr injected with slow-release implants of cortisol, GH and cortisol/GH combinations (planned publication).	Research	Scientific community worldwide		6, 7
2008	Peer reviewed paper: Johansson D, Ruohonen K, Oppedal F, Juell JE. Swimming depth and thermal history of individual Atlantic salmon (*Salmo salar* L) in production cages under different ambient temperature conditions. (submitted).	Research	Scientific community worldwide		1
2008	Peer reviewed paper: Poisa-Beiro L, Dios S, Hadmed H, Vasta G, Figueras A, Novoa B. 2008. New lectins characterized by Supression Subtractive Hybridization (SSH) in head kidney from sea bass infected with nodavirus. (In preparation).	Research	Scientific community worldwide		10
2008	Peer reviewed paper: Kvamme, B.O., Fridell, F., Taranger, G.L. Effects of long term hypoxia on gene regulation after an immune stimuli by poly I:C examined by microarray analysis. (Planned publication).	Research	Scientific community worldwide		1
2008	Peer reviewed paper: Kvamme, B.O., Oppedal, F., Fridell, F., Sundh, H., Taranger, G.L. Performance of Atlantic salmon postsmolts at different stocking densities and water qualities (hypoxia). (Planned publication).	Research	Scientific community worldwide		1, 6
2008	Peer reviewed paper: Kvamme, B.O., Fridell, F., Gaddan K., Henrik Sundh H., Sundell K., Evensen Ø., Taranger, G.L. Effects of chronic hypoxia on the immune response of Atlantic salmon (*Salmo salar*, L) post-smolts stimulated by poly I:C. (Planned publication).	Research	Scientific community worldwide		1, 6, 7
2008	Peer reviewed paper: Fridell, F., Kvamme, B.O., Evensen, Ø., Taranger, G.L. Long term hypoxia on Atlantic salmon (*Salmo salar*, L) down regulates genes associated to metabolism and immunology, examined by microarray (planned publication).	Research	Scientific community worldwide		1, 7
2008	Peer reviewed paper: Fridell, F., Sundh, H. Oppedal, F., Kvamme, B.O., Ellis, T. Olsen, R.E., Juell, J.E., Brix, O., Sundell, K. and Taranger, G.L. Effect of different oxygen levels on potential welfare indicators in Atlantic salmon (*Salmo salar* L.)	Research	Scientific community worldwide		1, 6, 7

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	postsmolts. (planned publication).				
2008	Peer reviewed paper: Santos, G.A., R. Mamauag, N. Kamari, J.W. Schrama, J.H.W.M. Rombout and J.A.J. Verreth. Effect of stocking density on growth, feed utilization and energy metabolism in European Seabass (*Dicentrarchus labrax*). In Preparation.	Research	Scientific community worldwide		11
2008	Peer reviewed paper: Santos, G.A., J. Capelle, J.W. Schrama, J.H.W.M. Rombout and J.A.J. Verreth. Effect of hypercapnia on growth, feed utilization and energy metabolism in European seabass (*Dicentrarchus labrax*). In Preparation.	Research	Scientific community worldwide		11
2008	Peer reviewed paper: Santos, G.A., R. Ventura, J.W. Schrama, J.H.W.M. Rombout and J.A.J. Verreth. Effect of dissolved oxygen on growth, feed utilization and energy metabolism in European seabass (*Dicentrarchus labrax*). In Preparation.	Research	Scientific community worldwide		11
2008	Peer reviewed paper: Coeurdacier et al. Preliminary approach by 2D electrophoresis and MS-MS analysis of proteome alteration in serum of sea bass (Dicentrarchus labrax L., 1758) reared with different stocking densities. In Preparation.	Research	Scientific community worldwide		2
2008	Peer reviewed paper: Roque d'orbcastel et al. Effects of stocking density in sea bass in experimental flow-through systems. Aquaculture, in preparation.	Research	Scientific community worldwide		2
2008	Peer reviewed paper: Sammouth et al. Effects of stocking density in sea bass in experimental recirculating systems. Aquaculture, in preparation.	Research	Scientific community worldwide		2
2008	Peer reviewed paper: Combined effect of hyperoxia and hypercapnia on sea bass in flow through system	Research	Scientific community worldwide		2
2008	Peer reviewed paper: T. Petochi, P. Di Marco, A. Priori, M.G. Finoia and G. Marino. Stress response to acute and chronic exposure to carbon dioxide in European sea bass (*Dicentrarchus labrax*). In Preparation.	Research	Scientific community worldwide		12
2008	Peer reviewed paper: N. Romano, T. Petochi, E. Caccia, A. Priori, G. Scapigliati, P. Di Marco, M.G. Finoia and G. Marino. Immune response to acute and chronic exposure to carbon dioxide in European sea bass (*Dicentrarchus labrax*) In Preparation.	Research	Scientific community worldwide		12
2008	Peer reviewed paper: Total oxidant and antioxidant status in sea bass *Dicentrarchus labrax* under different environmental conditions. P. Di Marco, A. Alberti, D. Macciantelli, A. Priori, Finoia, A., T. Petochi, J.P. Blancheton, G. Lemariè, G. Marino. In Preparation.	Research	Scientific community worldwide		12,2

Planned/ actual Dates	**Type**	**Type of audience**	**Countries addressed**	**Size of audience**	**Partner responsible /involved**
2008-2009	Peer reviewed paper: Effect of anaesthetics on stress physiology and welfare in sea bass *Dicentrarchus labrax* P. Di Marco, T. Petochi, A. Priori, M.G. Finoia, A. Longobardi, A., Mandich, G. Marino. Planned publication.	Research	Scientific community worldwide		12

2.3 Section 3 - Publishable results

1. Conclusions and recommendation from the project are publicly available at the project web site: http://wealth.imr.no

2. Suggested fish welfare indicators are publicly available on at the project web site: http://wealth.imr.no

3. Public dissemination of research results: The following dissemination of results are publicly available by March 2008:

Actual dissemination from Wealth by April 2008

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
September 2004	Juell, J. E., Oppedal, F., Johansson, D., Kelly, M., Stiansen, J.E., & Fosseidengen, J.E., 2004. Behavioural indicators of welfare in fish production cages. In: Book of Abstracts. AqEng "Engineering the future". Leuven, Belgium	Research, Industry	Scientific community worldwide		1
December 2004	Oppedal, F., Juell,. J-E., Johansson, D., 2004. Oppdrettsmiljø og fiskevelferd. Animal welfare in fisheries and aquaculture Workshop, Bergen	Research, Industry	Norway		1
September 2004	Juell, J. E., Oppedal, F., Johansson, D., Kelly, M., Stiansen, J.E., & Fosseidengen, J.E., 2004. Behavioural indicators of welfare in fish production cages. In: Book of Abstracts. AqEng 2004 "Engineering the future". Leuven, Belgium, 12-16 September 2004. pp. 594-595.	Research, Industry and Commission	Scientific community worldwide		1
October 20-23, 2004.	Evensen Ø, Glette J, Sundell K, Taranger GL, 2004. Welfare and health in sustainable aquaculture – Wealth. EAS Barcelona, Oral presentation.	Research, Industry and Commission	Scientific community worldwide		1, 6, 7
December 2004	Oppedal, F., Juell,. J-E., Johansson, D., 2004. Oppdrettsmiljø og fiskevelferd. Animal welfare in fisheries and aquaculture Workshop, Bergen Aquarium,	Public	Norway		1
2004	Johansson, D., Juell, J.E., Oppedal, F. Stiansen, J.E. og Fosseidengen, J. E., 2004. Oksygen i laksemerder: Har du tett luftfilter? *Norsk Fiskeoppdrett* 8, 52-55.	Public	Norway		1
2004	Johansson, D., Juell, J.-E., Oppedal, F., Stiansen J.E. and Fosseidengen, J.E., 2004. Merd-miljø og fiskevelferd i lakseproduksjon på kyst- og fjordlokaliteter på Vestlandet. *Fisken og Havet, Havforskningsinstituttet,* Bergen, Norway, No14-2006: 55 pp.	Public	Norway		1
April 2005	Juell JE, Oppedal F and Johansson D, 2005. Hva kan atferdstudier lære oss om fiskevelferd i oppdrettsmerder. Akvaveterinærenes Forening,	Research	Norway		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
May 2005	Juell, JE, 2005. The 3 R's and behavioural studies in aquaculture: Trade-off between possible animal suffering and relevance. International conference on the harmonization of use and care of fish in research. Gardermoen,	Research, Industry	Scientific community worldwide		1
June 2005	Kristiansen, T.S., Juell, J.E., Oppedal, F., Johansson, D., Fosseidengen, J.E. 2005. Overvåkning av fisk og miljø i merd. - Er dagens FoU teknologi morgendagens produksjonsteknologi? SINTEF- Nordisk Work Shop om Fiskevelferd og oppdrettsteknologi, Rica Nidelven Hotell, Trondheim, 21-22 juni 2005. (In Norwegian)	Research, Industry	Norway		1
August 2005	Santos, G.A., Lupatsch, I., van Anholt, R.D., Schrama J.W. and Verreth., J.A.J. 2005. The effect of stocking density on acute stress response in European sea bass (*Dicentrarchus labrax* L.). Aquaculture Europe, Trondheim, Norway.	Research, Industry	European countries		11
August 2005	T. Ellis, T., James, J.D. & Scott, A.P. (2005). Branchial release of free cortisol and melatonin by rainbow trout. J. Fish Biol. 67 535-540.	Research	Scientific community worldwide		3
April 2005	Juell, JE, 2005 Atferd hos oppdrettsfisk og konsekvenser for medisinforing. Næringsseminar om medisinforing, Schering Plough. Trondheim	Research, Industry	Norway		1
September 2005	Gadan K, Fridell F, Glette J and Evensen Ø, 2005. Effect of overcrowding on the susceptibility of Atlantic salmon (*Salmo salar* L.) to experimental challenge of IPN virus. EAFP conference Copenhagen	Research	Scientific community worldwide		1, 7
September 2005	Gadan K, Fridell F, Glette J and Evensen Ø, 2005. Effect of hyperoxygenation on the susceptibility of Atlantic salmon (*Salmo salar* L.) to experimental challenge of IPN virus. EAFP conference in Copenhagen, Denmark (poster).	Research	Scientific community worldwide		1, 7
September 2005	Juell JE, Behavioural studies and fish welfare in aquaculture: What questions are we asking? Scandinavian Society for Laboratory Animal Science. Mini symposium on Fish, Oslo,	Research	Scandinavian countries		1
November 2005	Conference: EAS Meeting, Trondheim (Poster presentation)	Research	European countries		11
2005	Juell, J.E and Kristiansen, T. 2005 Fiskevelferd - et positivt eller negativt perspektiv for næringen? In: Boxaspen K. et al. (eds.). Kyst og Havbruk 2005. Fisken og havet, Særnr. 2, 75-77.	Public	Norway		1
January 2006	Juell JE, 2006. Feeding behaviour of farmed fish. Workshop on oral medication, 11-12.01.2006, Inverness, Scotland.	Research	Scientific community worldwide		1
January 2006	Flyer on Welfare and Health in sustainable Aquaculture	Public	Worldwide		1, 6, 7
February 2006	Juell JE, Kristiansen, T, Fosseidengen, J.E, Johannson, D. & Oppedal, F., 2006.	Research	Norway		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	Utfordringer i merdmiljøet. Oppdrett av torsk-utfordringer for videre vekst! Nasjonalt Torskenettverksmøte				
March 2006	Gadan K, Fridell F, Taranger GL, Santi N, Mercy IS and Evensen Ø, 2006. Effekt av hypoxi på laksemakrofagers cytokinproduksjon etter Poly-I:C stimulering. HAVBRUK 2006, Bergen, 29-31 March 2006. Poster. (In Norwegian)	Research	Scientific community worldwide		1. 7
March 2006	Petochi, T. P. Di Marco, A. Priori, G. Marino. Chemical-clinical welfare indicators in farmed fish. (IZS Government Veterinary Institute, Legnaro, Italy. Oral presentation	Research	Italy		12
March 2006	Oppedal, F., Juell, J-E., Fosseidengen, J.E. og Johansson, D., 2006. Temperatur- og lysregulerende atferd hos laks i merd: konsekvenser for fiskevelferd? Programkonferanse Havbruk 2006, Sandsli, 29-31	Research	Norway		1
May 2006	Abstract and talk at international conference, Aqua 2006, Florence, Italy T. Ellis, J. James & A.P. Scott “A non-invasive cortisol assay for seawater Atlantic salmon.”	Research	Scientific community worldwide		3
May 2006	Fridell F, Gadan K, Sundh H, Taranger GL, Glette J, Sundell K and Evensen Ø, 2006. Effect of hyper-oxygenation and low water flow on primary stress response and the susceptibility of Atlantic salmon *Salmo salar* L. to experimental challenge of IPN virus. WAS/EAS meeting, Aqua 2006, Firenze, Italy, 9-13 May 2006 (oral presentation).	Research	Scientific community worldwide		1, 6, 7
May 2006	Taranger GL, Sundell K, Evensen Ø, Blancheton JP, Ellis T, Alexis, M, Meseguer J, Ellis T, Kotoulas G, Novoa B, Verreth J, Marino G and Glette, J., 2006. WEALTH - Welfare and health in sustainable aquaculture”, welfare session. WAS/EAS meeting, Aqua 2006, Firenze, Italy, 9-13 May 2006. Oral presentation.	Research	Scientific community worldwide		1, 6, 7
May 2006	Taranger GL, Fridell F, Oppedal F, Kvamme BO, Johansson D, Juell JE, Olsen RE, Sundell K, Gadan K, Evensen Ø and Glette J, 2006. Operational husbandry protocols to ensure welfare and health in farmed fish; Welfare and health in sustainable aquaculture – WEALTH. Finfish Health session, WAS/EAS meeting, Aqua 2006, Firenze, Italy, 9-13 May 2006. Oral presentation.	Research	Scientific community worldwide		1, 6, 7
May 2006	Oppedal, F., Johansson, D. og Juell,. J-E., 2006. Swimming depth and schooling density of caged Atlantic salmon in vertical temperature and light gradients. AQUA 2006 conference 9-13 May, Firenze, Italy.	Research	Scientific community worldwide		1, 6, 7
May 2006	Sundh H, Fridell F, Gadan K, Evensen Ø, Glette J, Taranger GL and Sundell K. Intensive husbandry conditions and IPN virus affects the intestinal primary barrier in Atlantic salmon (*Salmo salar*). Proceedings to: AQUA 2006, Florence, Italy.	Research	Scientific community worldwide		1, 6, 7

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
May 2006	Santos, G.A., Schrama, J.W., Verreth, J.A.J., 2006. The effect of stocking density on energy metabolism, behaviour and stress responses of European sea bass (*Dicentrarchus labrax* L.). AQUA 2006. 9-13 May, Florence, Italy.	Research	Scientific community worldwide		11
May 2006	Marino, G., P. Di Marco, A. Priori, T. Petochi, A. Massari, A. Mandich, M.G. Finoia, N. Romano. Effects of environmental hypercapnia in sea bass *Dicentrarchus labrax*: blood gas and acid-base status, stress and immune response to acute and chronic exposure. EAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and oral presentation	Research and Industry	Scientific community worldwide		12
May 2006	Di Marco, P., A. Priori, T. Petochi, A. Massari, A. Mandich, M.G. Finoia, G. Marino. Physiological and oxidative stress responses in sea bass *Dicentrarchus labrax*, exposed to environmental hypercapnia. EAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and poster presentation	Research and Industry	Scientific community worldwide		12
May 2006	Marino, G., P. Di Marco, T. Petochi, A. Priori, M.G. Finoia. Blood gas and acide-base status in sea bass exposed to environmental hypercapnia. EAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and poster presentation	Research and Industry	Scientific community worldwide		12
May 2006	Petochi, T., A. Priori, P. Di Marco, M.G. Finoia, G. Marino. Effects of long-term exposure to hypercapnia on innate immune response of sea bassEAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and poster presentation	Research and Industry	Scientific community worldwide		12
May 2006	E. Caccia, G. Marino, G. Scapigliati, L. Abelli, V. De Molfetta, L. Mastrolia, M. Mazzini and N. Romano. Effects of oxygen and carbon dioxide on the adaptive immune system of cultured sea bass *Dicentrarchus labrax*. EAS, AQUA 2006. 9-13 May, Florence, Italy. Abstract and poster presentation	Research and Industry	Scientific community worldwide		12
May 2006	Lemarié G., S. Sammouth, E. Gasset, G. Breuil, G. Marino and J. P. Blancheton. Effect of stocking density on sea bass *Dicentrarchus labrax* performance in a recirculating system. Oral presentation at AQUA 2006, WAS/ EAS European Aquaculture Society Congress. Florence, Italy.	Research and Industry	Scientific community worldwide		2
June, 2006	Poster presentation to the 8th Panhellenic symposium of Oceanography and Fisheries, to be held in Thessaloniki. Effect of different oxygen levels on sea bass growth. Vatsos I.N., Kalogiros H., Giagnisi M. and Alexis M.	Research and Industry	Greece		4
June 2006	Marino, G., P. Di Marco, T. Petochi, A. Priori, M.G. Finoia. Welfare indicators in farmed fish. SIBM Italian Society of Marine Biology. Grosseto, Italy. June 2006. Abstract and oral presentation.	Research	Italy		12
July 2006	Project web-site	Research, Industry, EU Commission	worldwide		All

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
		and public			
July, 2006	Sepulcre MP, Dios S, Novoa B, Figueras A, Meseguer J, Mulero V, 2006. Identification of immune-relevant genes from sea bass (Dicentrarchus labrax) by Suppresion Subtractive Hybridization. 10th international congress of the internacional society for developmental and comparative immunology, Charleston (USA), 1-6 July 2006 (Poster).	Research	Scientific community worldwide		5
August 2006	Poster at international conference, 23rd conference of European Comparative Endocrinologists, Manchester UK A.P. Scott, M. Sebire, I. Katsiadaki, T. Ellis "Non-invasive measurement of fish steroids in water"	Research	Scientific community worldwide		3
September 2006	Juell JE, Fosseidengen JE and Kristiansen TS, 2006. Utfôring tilpasset torskens begrensede vertikalvandring. AKVAFORSK-semiar "Foring av torsk" Molde, 07.09.2006. (In Norwegian)	Research	Norway		1
September 2006	Marino, G., P. Di Marco, T. Petochi, A. Priori, M.G. Finoia. Welfare indicators in farmed fish. (SIBM Italian Society of Marine Biology, Grosseto, Italy. June 2006).	Research	Italy		12
September 2006	Di Marco, P., A. Priori, T. Petochi, M.G. Finoia, G. Marino Effect of hypercapnia on total oxidant and antioxidant status in cultured sea bass *Dicentrarchus labrax*. UZI Unione Zoologica Italiana. Naples, Italy, September 2006. Abstract and oral presentation.	Research	Italy		12
October 2006	Abstract and talk at international conference: COST 867, Arcachon, France A.P. Scott & T. Ellis "Progress towards a 'dipstick' test for fish welfare"	Research	European scientific community		3
October 2006	Oppedal, F., Juell,. J-E. og Johansson, D., 2006. Oppdrettsmiljø og fiskevelferd - forsøksfisk i storskala oppdrettssystem. Forsøksdyrkurs, Haukeland universitetssykehus, 25 okt. 2006.	Research	Norway		1
October 2006	Juell JE, 2006. Fish welfare research at Institute of Marine Research, Norway. COST-867 "Fish welfare in European aquaculture" Archachon, France, 9-11 October 2006.	Research	European scientific community		1
October 2006	Kvamme BO, Taranger GL, Sundell K, Evensen Ø, Blancheton JP, Ellis T, Alexis M, Meseguer M, Ellis T, Kotoulas G, Novoa B, Verreth J, Marino G and Glette J, 2006. Welfare and Health in Sustainable Aquaculture – WEALTH. COST867, Arcachon, France, 9-11 October 2006.	Research	European scientific community		1-12
October 2006	Juell JE, 2006. Fish welfare research at Institute of Marine Research, Norway 2006. COST-867 "Fish welfare in Europeean aquaculture" Archachon, France 9-11 October.	Research	European scientific community		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
October 2006	Schrama, J.W., van de Nieuwegiessen, P.G., Santos, G.A., Martins, C.I.M. and Verreth J.A.J, 2006. Behaviour in relation to fish welfare: individual differences (coping styles) and effects of husbandry conditions. In Meeting of COST Action 867 "Welfare of fish in European aquaculture", October 9 – 11 2006, Arcachon, France.	Research	European scientific community		11
October 2006	Coeurdacier J. L. Alteration of total protein and proteome in serum of sea bass (*Dicentrarchus labrax*) reared at different stocking densities. Oral and poster presentation at « Congrès Société Française d'Electrophorèse et d'Analyse Protéomique (SFEAP) » St Malo, France. 16-19 October 2006	Research	France		2
October 2006	Gadan K, Santi N, Evensen Ø, 2006. Effect of stress on the balance between mixtures of recombinant IPNV isolates in persistently infected Atlantic salmon fry. 9th International Symposium on Double-Stranded RNA Viruses, South-Africa, 21-26 October 2006	Research	European scientific community		7
November 2006	Oppedal, F., Johansson, D. og Juell,. J-E., 2006. Kan enkeltindividers atferd lære oss mer effektiv produksjon? Produktivitetskonferansen 2006. MonAqua AS. Norway.	Research, industry	Norway		1
2006	Fridell F, 2006. Stress og mottakelegheit for sjukdom, hjå atlantisk laks. Eupharma seminar, Leknes, Norway. (In Norwegian)	Research, education	Norway		1
2006	Braastad, B., Juell, J.E & Damsgård, B. 2006. Animal behaviour – A new concept in aquaculture and fisheries In: Damsgård, B., Juell, J.E., & Braastad, B.O. 2006 (eds.). *Welfare in Farmed Fish. Report 5/2006 Fiskeriforskning*, Tromsø, Norway, pp 5-11.	Research, education	Norway		1
2006	Juell, JE., Johansson, D. & Oppedal, F., 2006 Effects of the cage environment and social interactions on the swimming behaviour and welfare of Atlantic salmon In: Damsgård,B., Juell, J.E., & Braastad, B.O. 2006 (eds.). Welfare in Farmed Fish. Report 5/2006 Fiskeriforskning, Tromsø, Norway, pp 91-98	Research, policy, education	Norway		1
2006	Oppedal, F., Johansson, D., Fosseidengen, J.E. og Juell, J.E., 2006. På hvilket dyp bør lyset plasseres? Norsk Fiskeoppdrett, 10: 58-59.	Public	Norway		1
2006	Oppedal, F., Johansson, D. & Juell, J.E., 2006. The cage environment laboratory: Behavioural studies in a realistic farm environment. In: Damsgård, B., Juell, J.E., & Braastad, B.O. 2006 (eds.). Welfare in Farmed Fish. Report 5/2006, Fiskeriforskning, Tromsø, Norway, pp 24-27	Research, policy, education	Norway		1
January 2007	Kvamme BO, Fridell F, Gadan K, Oppedal F, Evensen Ø and Taranger GL, 2007. Effektar av hypoksi på laksens immunsvar. Frisk Fisk meeting, Tromsø, 23.-25. januar 2007.	Research and Industry	Norway		1, 7

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
January 2007	Fridell F, Oppedal F, Gadan K, Sundh H, Taranger GL, 2007. Effekten av hypoksi på laksens ve og vel, kan ein måla fiskevelferd? Frisk Fisk meeting,Tromsø, Norway	Research and Industry	Norway		1, 6, 7
January 2007	Kvamme, B.O., Fridell, F., Gadan, F., Oppedal, F., Evensen Ø. and Taranger, G.L, 2007. Effektar av hypoksi på laksens immunsvar. Frisk Fisk meeting. Tromsø, Norway. 23-25 January 2007. (In Norwegian)	Research and Industry	Norway		1, 7
February 2007	Oppedal, F., 2007. Improved management of oxygen flow in net cages – OxFlow. CREATE day, Trondheim, Rica Nidelven hotel, 14-15	Research and Industry	Norway		1
February 2007	Oppedal, F., Bratland, S. and Olsen, R.E., 2007. Kortisol i vann – preliminære resultat. EWOS – oppdrettsmøte. Matre, 22 February 2007	Research and Industry	Norway		1
March 2007	Petochi, T., P. Di Marco, A. Priori, M.G. Finoia, G. Marino. Innate immune response of reared European sea bass *Dicentrarchus labrax* to different environmental and husbandry conditions. SIICS - Italian Society of Developmental and Comparative Immunobiology. Naples, Italy. Abstract and oral presentation.	Research	Italy		12
March 2007	Romano, N., E. Caccia, T. Petochi, S. Meloni, L. Mastrolia, G. Scapigliati, L. Abelli and G. Marino. Effects of different carbon dioxide concentrations on the adaptive immune system of cultured sea bass (*Dicentrarchus labrax* SIICS - Italian Society of Developmental and Comparative Immunobiology. Naples, Italy. Abstract and oral presentation.	Research	Italy		12
March, 2007	Blancheton J.P., R. Piedrahita, A. Belaud, Y. Moutounet, S. Fivelstad, G. Lemarié (2007*b*). Gas control in land based aquaculture, physiological and technical aspects : Gaz totaux et oxygène. 15-16 March 2007. *Journées du SFAM 2007, Montpellier, France*	Research and producers	France		2
April 2007	Dios S, Poisa-Beiro L, Montes A, Aranguren R, Figueras A, Novoa B, 2007. Nodavirus increases the expression of Mx and inflammatory cytokines in fish brain. 7TH international symposium on viruses of lower vertebrates, Oslo, Norway, 22-25 April 2007	Research	Worldwide European scientific community		10
April 2007	Poisa-Beiro L, Dios S, Figueras A, Novoa B, 2007. New lectins characterized by Supression Subtractive Hybridization (SSH) in head kidney from sea bass infected with nodavirus. 7TH international symposium on viruses of lower vertebrates, Oslo, Norway, 22-25 April 2007(Poster).	Research	Worldwide European scientific community		10
April 2007	Gadan K, Mercy IS, Santi N and Evensen Ø, 2007. Stress, immunocompetence and IPN virus infections in Atlantic salmon. ISVLV symposium, Oslo, 22-25 April 2007.	Research	European scientific community		7
May 2007	Kristiansen, T.S., Begout, M-L., Toften, H., Villaroel, M. 2007. What is an	Research	European		1, 2

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	operational welfare indicator? COST 867 conference, 2nd COST 867 Welfare of fish in European aquaculture, Work Shop, Varese, Italy.		scientific community		
May 2007	Talk at international conference: COST 867, Varese, Italy. T. Ellis "Review of cortisol and fish welfare"	Research	European scientific community		3
May 2007	Oppedal, F., Kristiansen, T., Bratland, S., Stien, L., Folkedal, O. and Juell, J.E., 2007. Salmon experiments in WP1: Pilot postsmolt study, Parr-presmolt-smolt study and postsmolt in cages, FASTFISH mid-term meeting, Crete, 21-23	Research	European scientific community		1
June 2007	Santos, G.A., Schrama, J.W., Rombout, J.H.W.M, Verreth, J.A.J., 2007. Studies on the effects of cronic stressors on energy metabolism, welfare and immunity of European sea bass (*Dicentrarchus labrax* L.). 2007. AQUA Twin Event 11 – 12 June, Patras, Greece.	Research	European scientific community		11
June 2007	Petochi, T., N. Romano, P. Di Marco, A. Priori, E. Caccia, G. Scapigliati, S. Meloni, L. Abelli, G. Marino. Innate and adaptive immunity of reared sea bass *Dicentrarchus labrax* under environmental hypercapnia. NOFFI, Nordic Society for Fish Immunology Congress. Stirling, Scotland, June, 2007. Abstract and poster presentation.	Research	Scientific community worldwide		12
June 2007	Faucher K., Millot S., Blancheton J.P., Lemarié G., Dutto G. & Bégout M.-L. 2007. Effect of stocking density on cultured fish swimming behaviour: First results on sea bass using acoustic telemetry in flow through tanks. *7th Conference on Fish Telemetry*, Silkebørg, Denmark. 17-21 June, 2007. Oral presentation	Research	European scientific community		2
June 2007	Faucher K., Millot S., Struski C. & Bégout M.-L., 2007. Assessment of sea bass swimming activity and preferential space use in sea cages using acoustic telemetry and archival tags. *7th Conference on Fish Telemetry*, Silkebørg, Denmark. 17-21 June, 2007. Poster.	Research	European scientific community		2
August 2007	Taranger GL, Fridell F, Kvamme BO, Oppedal F, Johansson D, Juell JE, Olsen RE, Sundth H, Gaddan K, Sundell K and Evensen Ø, 2007. Welfare biology in sea cage culture of salmon and sea bass. "My sea home" session. EAS Aqua NOR forum, "Welfare as a driver for technological development in aquaculture", Trondheim August 15-16, 2007. Oral presentation and panel member. See summary at: http://www.easonline.org/files/Meetings/aqua_nor_forum_summary.pdf	Research	Scientific community worldwide		1, 6, 7
August 2007	Welfare Fish welfare in land-based production systems from flow through to recirculation in relation to water quality and fish density. Jean-Paul Blancheton, Emmanuelle Roque d'orbcastel, Jeannine Person and Gilles Lemarié. "My sea home" session. EAS Aqua NOR forum, "Welfare as a driver for	Research	Scientific community worldwide		2

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	technological development in aquaculture", Trondheim August 15-16, 2007. Oral presentation and panel member. See summary at: http://www.easonline.org/files/Meetings/aqua_nor_forum_summary.pdf				
September 2007	Petochi T., Di Marco P., Priori A., Longobardi A. M.G. Finoia. Development of an autopsy-based method to assess on-farm welfare in sea bass. EAFP European Association of Fish Pathologists. Grado, Italy. Abstract and poster presentation.	Research	Scientific community worldwide		12
September 2007	K. Gadan, I. Singh Mercy, H. Sundh, K. Sundell and Ø. Evensen. Effects of Growth hormone and cortisol on the immune response after challenge with infectious pancreatic necrosis virus in Atlantic salmon (*Salmo salar*). EAFP. September 2007.	Research	Scientific community worldwide		1, 7
October 2007	Stien, L. H., Folkedal, O., Gytre, T., Kristiansen, T., Nilsson, J., Torgersen, T., 2007. Monitoring the welfare of farmed fish. The Research School SCOFDA - Sustainable Control of Fish Diseases in Aquaculture, Two day workshop on welfare and health of fish, Copenhagen, 30th to 31th October, 2007. (Invited lecturer).	Research	Scientific community worldwide		1
October 2007	Marino, G., T. Petochi, P. Di Marco, J.P. Blancheton, G. Lemariè, A. Priori, A. Priori, A. Massari, M.G. Finoia Developing operational welfare indicators for farmed sea bass *Dicentrarchus labrax*. EAS, Istanbul, Turkey. Abstract and poster presentation.	Research and Industry	Scientific community worldwide		12,2
October 2007	Effect of anaesthetics on stress physiology and welfare in sea bass *Dicentrarchus labrax* P. Di Marco, T. Petochi, A. Priori, M.G. Finoia, A. Longobardi, A. Massari, A. Mandich, G. Marino. EAS, Istanbul, Turkey. Abstract and poster presentation.	Research and Industry	Scientific community worldwide		12,2
October 2007	Long-term exposure to combined hyperoxia and hypercapnia on stress and innate immunity of sea bass *Dicentrarchus labrax.* P. Di Marco, A. Priori, T. Petochi, M.G. Finoia, J.P. Blancheton, G. Lemariè, A. Cevasco, G. Marino. EAS, Istanbul, Turkey. Abstract and poster presentation.	Research and Industry	Scientific community worldwide		12,2
October 2007	Effect of hyperoxia and hypercapnia on total oxidant and antioxidant status in cultured sea bass *Dicentrarchus labrax* P. Di Marco, A. Alberti, D. Macciantelli, A. Priori, Finoia, A., T. Petochi, J.P. Blancheton, G. Lemariè, G. Marino. EAS, Istanbul, Turkey. Abstract and poster presentation.	Research and Industry	Scientific community worldwide		12,2
October 2007	Blancheton J.P., J. Person-Le Ruyet, G. Lemarié Relation water quality fish requirements. *Aquaculture Europe 07 - Annual Meeting of the European Aquaculture Society, Istanbul, Turkey, Short course Principles of recirculation aquaculture systems (RAS) technology, Istanbul, Turkey*. Oral presentation.	Research and Industry	Scientific community worldwide		1
October 2007	Blancheton J.P., R. Piedrahita, A. Belaud, Y. Moutounet, S. Fivelstad, G. Lemarié (2007*a*). Gas control in land based aquaculture, physiological and technical aspects. *Aquaculture Europe 07 - Annual Meeting of the European Aquaculture Society,*	Research and Industry	Scientific community worldwide		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	Istanbul, Turkey, Short course Principles of recirculation aquaculture systems (RAS) technology, Istanbul, Turkey.				
October 2007	Oppedal, F., Bratland, S., Stien, L., Nilson, J. and Folkedal, O., 2007. Må vi tenke mer på dyrevelferd i norsk fiskeoppdrett? - Akutt stress hos settefisk, Microteket, EWOS seminar, Stjørdal, 31 October 2007. (In Norwegian)	Research and Industry	Norway		1
October 2007	Oppedal, F., Johansson, D. og Juell., J-E., 2007. Vanngjennomstrømning, oksygentilgang og faktorer som påvirker fiskens atferd i store merder. Akvaveterinærenes høstkurs 2007, Park Inn hotell, Stavanger, 23-24 October 2007. (In Norwegian)	Research and Industry	Norway		1
November 2007	Oppedal, F., Bratland, S. and Folkedal, O., 2007. Akutt stress hos laks - preliminære data. Produktivitets-konferansen 2007, Kristiansund, November 1 2007. (In Norwegian)	Research and Industry	Norway		1
November 2007	Oppedal, F., Bratland, S., Stien, L., Nilson, J. and Folkedal, O., 2007. Må vi tenke mer på dyrevelferd i norsk fiskeoppdrett? - Akutt stress hos settefisk, Microteket, EWOS seminar, Bergen, 7 Nov 2007. (In Norwegian)	Research and Industry	Norway		1
December 2007	Taranger GL, Sundell K and Evensen Ø, 2007. WEALTH: "Welfare and health in sustainable aquaculture". Working Group (WG) in the field of aquaculture. Research, Data Collection & Scientific Advice Unit of DG Fisheries & Maritime Affairs, EU Commission, Brussels, 3 - 5 December 2007. (Invited oral presentation).	Research	Scientific community worldwide		1, 6, 7io
December 2007	Oppedal, F., Dempster, T., Kristiansen., T., Korsøen, Ø., Folkedal., O, 2007. Nedsenking/ heving av merder – biologiske aspekter. CREATE-Submersible cage, prosjektmøte, Trondheim, 20 des. 2007	Research and Industry	Norway		1
2007	Annon. 2007. "WEALTH - Welfare and health in sustainable aquaculture". 12pp. (Wealth leaflet available at http://wealth.imr.no/__data/page/6068/Wealth_leaflet_web.pdf)	Research, Policy and Industry	Worldwide		1-12
2007	Fridell, F., Kvamme, B.O., Brix, O., Oppedal, F., Olsen, R.E., Taranger, G.L. 2007. Søket etter nye stress og velferdsmarkørar hjå Atlantisk laks. *Norsk Fiskeoppdrett*, 11:54-57	Public	Norway		1
2007	Fridell, F., Taranger, G.L, Kristiansen T.S. 2007. New research will help farmers manage fish health and welfare. *Fish farmer*, 30:12-13	Public	Norway		1
2007	Gytre, T., Stien, L. H., Oppedal, F., 2007. How are things in the sea-cage? *Marine Research News* NO. 18-2007	Public	worldwide		1
2007	Johansson, D. 2007. Production cage environment and salmon beaviour. *PhD Thesis*.	Research,	Scientific		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	University of Bergen, 2007.	Public	community worldwide		
2007	Juell, J.E, Nilsson, J., Olsen, R.E. Fridell,F., Kvamme, B.O., Oppedal, F., Humborstad, O.B., Mangor-Jensen, A. Stien, L.H and Kristiansen, T., 2007. Dyrevelferd i akvakultur og fiskeri – et nytt fagområde i rask vekst. In: Dahl, E., P.K. Haug, T. Karlsen, Ø (eds.). Kyst og Havbruk 2007. Fisken og Havet, særnr 2-2007, s. 141-148.	Public	Norway		1
2007	Kristiansen, T. Johansson, D. Oppedal, F. og Juell, J.E., 2007. Hvordan har oppdrettsfisken det i merdene? In: Dahl, E., P.K. Haug, T. Karlsen, Ø (eds.). Kyst og Havbruk 2007, Fisken og Havet, særnr 2-2007, pp. 151-155	Public	Norway		1
2007	Peer-review paper: Scott, A.P., Ellis, T. (2007). Measurement of fish steroids in water—a review. General and Comparative Endocrinology 153 392–400.	Research	Scientific community worldwide		3
2007	Peer-reviewed paper: Fridell F, Gadan K, Sundh H, Taranger GL, Glette J, Sundell K and Evensen Ø. Effect of hyperoxygenation on the susceptibility of Atlantic salmon (*Salmo salar* L.) to experimental challenge of IPN virus. Aquaculture 270, 23-35	Research	Scientific community worldwide		1, 6, 7
2007	Peer-reviewed paper: Ellis, T., James, J.D., Sundh, H., Fridell, F., Sundell, K., Scott, A.P. (2007). Non-invasive measurement of cortisol and melatonin in tanks stocked with seawater Atlantic salmon. Aquaculture, 272, 698-706	Research	Scientific community worldwide		1, 3, 6
2007	Peer-reviewed paper: Ellis, T., Bagwell, N., Pond, M., Baynes, S., Scott, A.P. (2007). Acute viral and bacterial infections elevate water cortisol concentrations in fish tanks. Aquaculture, 272, 707-716	Research	Scientific community worldwide		3
2007	Peer-reviewed paper: Johansson, D., Juell, J.-E., Oppedal, F., Stiansen J.E. and Ruohonen, K., 2007. The influence of the pycnocline and cage resistance on current flow, oxygen flux and swimming behaviour of Atlantic salmon (*Salmo salar* L.) in production cages. Aquaculture 265, 271-287.	Research	Scientific community worldwide		1
2007	Peer-reviewed paper: Johansson, D., Ruohonen., K., Kiessling, D., Oppedal, F., Stiansen, J.E., Kelly, M., and Juell, J-.E., 2006. Effect of environmental factors on swimming depth preferences of Atlantic salmon (*Salmo salar* L.) and temporal and spatial variations in oxygen levels in sea cages at a fjord site. Aquaculture 254, 594-605.	Research	Scientific community worldwide		1
2007	Peer-reviewed paper: Oppedal, F., Juell, J.-E. and Johansson, D., 2007. Thermo- and photoregulatory swimming behaviour of caged Atlantic salmon: Implications for photoperiod management and fish welfare. Aquaculture 265, 70-81	Research	Scientific community worldwide		1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
2007	Peer-reviewed paper: Sepulcre MP, Sarropoulou E, Kotoulas G, Meseguer J, Mulero V. 2007. *Vibrio anguillarum* evades the immune response of the bony fish sea bass (*Dicentrarchus labrax* L.) through the inhibition of leukocyte respiratory burst and down-regulation of apoptotic caspases. Mol Immunol 44 (15): 3751-3757.	Research	Scientific community worldwide		5, 9
2007	Peer-reviewed paper: Rønneseth A, Wergeland HI, Devik M, Evensen Ø, Fausa Pettersen E, 2007. Mortality after IPNV challenge of Atlantic salmon (*Salmo salar* L.) differs based on developmental stage of fish or challenge route. Aquaculture 271 (1-4): 100-111.	Research	Scientific community worldwide		7
February 2008	Oppedal, F., Bratland, S., Stien, L., Nilson J., og Folkedal, O. 2008. Må vi tenke mer på dyrevelferd i norsk fiskeoppdrett? - Akutt stress hos settefisk, Fiskeridirektoratet, Bergen, 7 February 2008.	Research, Industry	Norway		1
2008	Kvamme B.O., Oppedal F., Torgersen T., Fridell F., Sundh H., Sundell K. Oksygennivået viktig for oppdrettsfiskens helse og velferd. Kyst og Havbruksrapporten 2008, Fisken og Havet, særnr 2-2008	Public, research, education	Nordic countries		1, 6
2008	Kvamme BO, Fridell F, Oppedal F, Stien L, Torgersen T, Sundh H, Sundell K, Kristiansen T, Juell JE and Taranger GL, 2008. Hypoksi og Hyperoksi - Kva kan det føre til. ScanVacc Seminars, Trondheim, Norway, 2008. (In Norwegian)	Research	Norway		1, 6
2008	Kvamme BO, Fridell F, Oppedal F, Stien L, Torgersen T, Sundh H, Sundell K, Kristiansen T, Juell JE and Taranger GL, 2008. Hypoksi og Hyperoksi - Kva kan det føre til. ScanVacc Seminars, Stavanger, Norway, 2008. (In Norwegian)	Research	Norway		1, 6
2008	Kvamme BO, Fridell F, Oppedal F, Stien L, Torgersen T, Sundh H, Sundell K, Kristiansen T, Juell JE and Taranger GL, 2008. Hypoksi og Hyperoksi - Kva kan det føre til. ScanVacc Seminars, Bergen, Norway, 2008. (In Norwegian)	Research	Norway		1, 6
2008	Peer reviewed paper: Poisa-Beiro L, Dios S, Montes A, Aranguren R, Figueras A and Novoa B. 2008. Nodavirus increases the expression of Mx and inflammatory cytokines in fish brain. Mol Immunol 45 (1): 218-225	Research	Scientific community worldwide		10

Bergen, 17 April 2008, Geir Lasse Taranger