Project no. **022720**

Project acronym: **FASTFISH**

Project full title:

On farm assessment of stress level in fish

FASTFISH

Instrument: Scientific Support to Policy (SSP)

Area 1.3 – Modernisation and sustainability of fisheries, including aquaculture-based production systems

PUBLISHABLE FINAL ACTIVITY REPORT

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Project coordinator name: Tore S Kristiansen

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1. PROJECT EXECUTION

1.1 Overview of general objectives

The main objective of the SSP priority is to provide a scientific basis for the development of modern, sustainable and competitive aquaculture sector in Europe. A pre-requisite to achieve this objective is to design aquaculture systems as an integrated part of a healthy ecosystem. A major step in this direction is to develop operational protocols that secure acceptable standards of animal welfare. This will reduce the risk of catastrophically events due to sub-lethal and lethal environmental conditions, which impose high stress-levels on fish and increase the risk of disease outbreaks. To this end it is important to develop protocols for monitoring of pre-critical stress levels in populations of farmed fish.

Main Objectives

Using Atlantic salmon (*Salmo salar*) and European sea bass (*Dicentrarchus labrax*) as model organisms the project has the following objectives:

WP1 FAST-INDICATORS

- Identify non-invasive and reliable behavioural indicators that can be used to monitor and quantify acute and chronic stress levels in farmed fish.
- Validate and quantify these indicators as proxy measures for stress using immunological and physiological methods.

Figure 1: Schematic overview of the FastFish project.

WP2 FAST-TOOL

- Develop databases and protocols for on farm monitoring of environmental data and the validated stress level indicators.
- Develop an expert system for assessment and management of stress levels in aquaculture production systems based on analysis of monitored parameters.
- Identify the prerequisites and market mechanisms for a successful implementation of FAST-TOOL in the aquaculture industry, and estimate the costs and benefits of implementation for the fish farmer.

WP3 FAST-TEST

- Implement, test and refine the database, protocols and expert system (FAST-TOOL) in commercial aquaculture in periods with potentially high and low stress levels.

1.2 Contractors involved

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Project logo: FASTFISH

1.3 Summary of worked performed and results

1.3.1 WP1.1 Identification and validation of behavioral stress level indicators in European sea bass

Contractors involved: HCMR, UOC, IFREMER, IMR

1.3.1.1 BEHAVIOURAL ONTOGENY OF SEA BASS

Contractors involved: UoC, HCMR

The study of behavioral activities and their ontogeny can reveal the associated relationships between the animal and their surrounding environment which can in turn contribute to the selection of the most suitable conditions for the cultivated fish "welfare". The present study focused on the behavioral ontogeny of European sea bass swimming and feeding behavior in captivity in two different rearing technologies: intensive (Papandroulakis et al., 2002) and mesocosm rearing system (Divanach and Kentouri, 2000).

It was demonstrated that during ontogenesis, individuals go through some clear behavioral changes that are strongly related with their morphological ontogeny and also with the rearing technique. In particular, as far as the **swimming activity** is concerned (sustained i.e. normal swimming), our results showed that from pre-larval to larval stages, swimming activity shifted from an initial spasmodic, intermediated swimming where larvae spent more time resting than acting, to a more continuous, coordinated and energetic swimming under both rearing conditions. Under intensive rearing conditions, "sustained" swimming velocity (so-called "routine speed"), from just after larvae entered the heterotrophic life, showed an increasing trend between the stages studied with an important increment between the phase from Flexion (urostyle torsion) until Beginning of metamorphosis (formation of dorsal and anal fin). After that stage, and until the formation of all fins, swimming velocity reduced again, but remained higher than the mean velocity found at the stage from First feeding until Flexion. In mesocosm rearing, although this increment in swimming velocities was observed during developmental time, there was no obvious or intense increment between stages studied as may be expected in the stage between Flexion and Beginning of metamorphosis as observed in intensive rearing. This difference could be explained by taking into account the different conditions present in each rearing system. Fish in mesocosm undergo the same morphological changes at the stage from Flexion to Beginning of metamorphosis as do fish of the intensive rearing system and in conformity with other marine species but this important morphological change does not seem to have a big impact on swimming activity probably because fish have more "space" than fish in intensive rearing (almost ten times more water volume per individual). Also, fish in mesocosm rearing system, are subjected to lesser competition for food which in combination with the larger space availability potentially enhanced the fish welfare. Nevertheless, in all cases the "routine speeds" estimated in the present study are within the range of those reported for Sparidae species (Kentouri, 1985).

The activities that follow the transition from pre-larval to larval stage can be easily explained from the consecutive modifications of the morphological features. It is obvious that the formation of fin rays, the increment of body dimensions and the opening of mouth modify the swimming abilities and the feeding behavior. Also, the efficiency of behavior related to **prey searching and hunting** is strongly related to the full development of the swim bladder occurring at 10 dph (days post hatching) for larvae from intensive rearing system and at 5 dph for larvae from the mesocosm rearing system. This is consistent with data that have been reported by Chatain (1986) and Georgalas et al. (2007), concerning the swim bladder development in sea bass. Those authors report similar changes in larval swimming behavior that could be related to the complete swim bladder development.

During cofeeding phase (progressive elimination of live prey in the food) larvae continued to show strong preference to live feed, albeit dry food was inserted a few days before, giving the larvae enough time to recognize the new prey. This underlined the need for vivid stimulants (encounters/shocks in the water column from displacements) for the modulation of their hunting response. The cofeeding phase could not occur earlier than 31 and 32 dph for intensive and mesocosm rearing respectively demonstrating an adaptation and learning period of larvae for the new food (dry granules) and though displaying an ontogenetic evolution of the behavior.

Larvae of both rearing systems showed a clear **directionality** (fish tended to swim parallel to the tank walls) at the developmental stages of Beginning of metamorphosis and Formation of all fins (early juveniles). This is a first sign of schooling behavior which in natural habitats occurs mostly in juveniles and happens with counter-current positioning (ontogeny of rheotactism). Behavioral variation was also observed concerning the **distribution and dispersion** of the larvae in the tank between populations originating from the intensive or mesocosm systems. Larvae from the intensive rearing system seemed to occupy the whole tank the whole duration of the autotrophic phase without any particular patterns while larvae from the mesocosm rearing showed a sequence of repositioning in the water column with developmental time. In particular larvae at the beginning of the autotrophic stage (0 dph) were found in swarms right under the water surface and from 2 dph started to migrate to deeper zones still in the first half of the tank. They progressively moved to deeper zones (3 dph occupy up to 2/3 of the water column) and by the end of the autotrophic stage (4 dph) larvae were distributed in the whole tank. While the larvae of the intensive rearing showed a random orientation towards the whole tank, the larvae of the mesocosm rearing at the end of the autotrophic phase (4 dph) showed a beginning of positive reaction to natural light (positive phototropism). During heterotrophic phase, larvae of both populations were distributed in the whole tank except for feeding times when individuals moved to upper zones where food was more abundant.

Conclusions – discussion

Our aim was to compare the ontogeny of the behavior of sea bass larvae reared at two different rearing techniques and our results have presented some similarities but also some differences in behavior between the two populations. The most marked ones involved differences in the swimming performance and speed but also some divergence in feeding behavior. Based on the above, one can conclude that intensive rearing fish present a behavioral delay when compared to mesocosm reared fish and this can be justified by the fact that mesocosm conditions are approaching those found in the field whereas the intensive ones comprise a more stressful environment for cultivated fish. This stressful environment possibly has an impact on the behavioral evolution of the fish.

Moreover, little is known on the behavior of sea bass larvae and therefore comparisons are difficult to make. Understanding the behavioral variation and capabilities of these early-life history stages of sea bass is however, essential to intelligent management, since larval ethology could be regarded as a tool for the assessment of fish welfare, which could be combined with other tools used as indicators of larvae quality.

1.3.1.2 CORTISOL ONTOGENY IN EUROPEAN SEA BASS AND THE STRESS RESPONSE DURING EARLY LARVAL AND JUVENILE STAGES.

Contractors involved: UoC, HCMR

Although the stress response in adult fish exposed to various acute or chronic stressors has been studied in several aquaculture species, the ontogeny of the stress response in Teleosts remain poorly understood. The aim of the task was to determine basal cortisol levels and to investigate the cortisol stress response in larval and early juvenile stages of the European sea bass, *Dicentrarchus labrax*. A main objective was to validate and quantify behavioral indicators as proxy measures for stress using classical physiological indexes (plasma and whole body concentrations of cortisol) as well as non-invasive novel methods such as the determination of cortisol levels released into the water.

Basal whole body concentrations of cortisol were low in embryos, at hatching and in pre-larvae, higher in the preceding developmental stages and maximum at the full formation of scales. Basal whole body cortisol concentrations during early ontogeny in sea bass where much higher than those observed in the respective developmental stages in other marine species, like the gilt-head sea bream. There were no significant differences in whole body cortisol concentrations between the two rearing methods used (semi-intensive - mesocosm and intensive rearing systems) in control fish, but the magnitude of the cortisol stress response was much higher in intensively reared fish compared to fish reared in mesocosm and exposed to the same acute stressor.

Acute stress incidents (light, air, transport and handling) at various developmental stages (mouth opening, flexion, post-flexion, formation of all fins, and full formation of melanophores) resulted in increased whole body cortisol concentrations in intensive but not in fish from mesocosm rearing system. The magnitude of the stress response, in both rearing systems was lower at mouth opening in comparison with the melanophores developmental stage. Stress during transfer to weaning (*i.e.* after melanophores covered the whole body; Total Length ~20 mm), resulted also in high whole body cortisol concentrations and high cortisol release rates into the bucket water. Transport simulation of juvenile fish in two stocking densities (20 *vs.* 50 kg m^{-3}, mean body weight of 30 g) or acute stress (1 min air exposure, mean body weight of 1.8 g) resulted in high cortisol release rate within the first 4 hours of the experiment.

High basal plasma concentrations were found in adult sea bass, in accordance with previous results obtained by other research groups in the same species. Further experiments showed that cortisol release rate into the water were affected by the size of fish, with large fish tending to release more cortisol than smaller sea bass. However, it has to be noticed that sea bass releases lower amounts of cortisol into the water than those expected based on the high whole body and plasma cortisol concentrations.

Conclusions – discussion

1. The ontogeny of the stress response was described for the first time in sea bass reared under two different larvae rearing systems (semi-intensive/mesocosm & intensive).
2. The ontogenetic pattern of basal cortisol was similar to that observed in other marine Teleosts. However, the magnitude of the response is much higher than that reported in other warm-water Teleosts.
3. The presence of cortisol in fertilized eggs is very probably the result of maternal transfer during ontogenesis. The onset of cortisol production occurred near the transition from endogenous to exogenous feeding, indicating that cortisol biosynthesis is important for adapting to different nutritional sources for energetic, growth and osmoregulatory purposes.
4. Application of the same stressor during early ontogeny did not elicit a significant increase in whole body cortisol content in sea bass pre-larvae (mouth opening) but gave maximum response at the melanophores' stage. Lack of the stress response at mouth opening may be due either to the lack of maturation of the Hypothalamus-Pituitary-Interrenal (HPI) axis or to the suppression of a fully developed HPI axis for protective purposes.
5. Cortisol release rates into the water were determined for the first time for sea bass and, in general, for warm-water marine species. This method can be applied to evaluate the stress response in juveniles, especially in closed water systems. Thus, transport simulation of juvenile fish (mean body weight of 30 g) in two stocking densities (20 *vs.* 50 kg m-3) or acute stress (1 min air exposure) (mean body weight of1.8 g) resulted in high cortisol release rate within the first 4 hours of the experiment.
6. There was a strong positive correlation between plasma cortisol concentration and release rate in adults. Cortisol release rate into the water were affected by the size of fish, with large fish tending to release more cortisol than smaller sea bass.

Future priorities:
1. to investigate the adaptive role of high basal whole body or plasma concentrations of cortisol in larvae and adult sea bass,
2. to determine the mechanism of cortisol production and the elements of a fully developed HPI axis during early ontogeny,
3. to investigate possible role of cortisol in the transition to exogenous feeding;
4. to develop a more proper unit to express cortisol release rate into the water by taking into account differences in size, gill morphology and general activity of the fish.

1.3.1.3 SEA BASS BEHAVIOURAL STRESS LEVEL INDICATORS IN INTENSIVE AND MESOCOSM LARVAL REARING SYSTEMS

Contractors involved: HCMR, UoC

The stress response in fish exposed to various acute or chronic stressors has been studied in several aquaculture species, but there is still lack of indicators that could be easily applied in farms. The aim of the work performed was to identify non-invasive indicators easily applicable in industrial scale. The work covered the two most critical phases of the production, i.e. during larval rearing and during on growing both in cages and in tanks. Sea bass is relatively robust to stress and recover fast from acute stress in larvae but can also be very sensitive in earlier stages (pre-larval) due to intense stressful conditions. Sea bass larvae exhibited responses that were similar to previously established behavioral patterns of the Sparidae family (Kentouri and Divanach 1983) and concerned the levels of behavioral expressions (behavioral modes) identified as:

• Development of supernormal behaviors: a state of fallacious good or sensible health, where the reduction of the thresholds of specific resistance can lead to husbandry risk;

• Expression of behavior of anticipation or transfer and development of subsequent organic and eventually behavioral compensatory or substitutive perturbations: it corresponds to a state of bad health, with a certainty of low performances that comprise mortality risks.

• Possible evolution of necrotic lesions and development of pre-lethal behaviors, generally regressive. Acquisition of lethal-like and expression of pre-lethal behaviors can be observed and are generally spasmodic: it can constitute states where epidemic or chronic (differed or instant) mortality can occur with a certainty of total loss of the live stock.

In order to determine a precise ethogram of sea bass larvae and to challenge fish with stressors, larval rearing was performed using two rearing systems with assumed low and high stress level: large mesocosm tanks (40 m^3) and small intensive tanks (0.5 m^3). During the early rearing phase, collection and analysis of behavioral data was performed by monitoring (i) the velocity of the individuals (value and direction) and (ii) the vertical distribution in the tanks. The populations were exposed to relevant acute stressors (changes in photoperiod, high aeration in the tanks (*i.e.* high currents), inadequate feeding, handling, etc…). Monitoring of stressed and unstressed populations was performed for fish originating from both rearing systems and at various developmental stages (tail flexion, fin development, metamorphosis, juveniles). For video recordings analysis dedicated software was developed.

Results indicated that during larval rearing the growth performances of the larvae was different between the two rearing systems with mesocosm larvae growing almost 20% faster than the intensively reared ones. Regarding larval behavioral indicators, such as velocity and direction, these were changing during the development and their distribution in the water column differed according to rearing systems (*cf.* table below). The differences in growth and also in quality may be attributed to several reasons such as environment of rearing and also food availability but all these parameters apparently are related to the general welfare of the individuals. Therefore it can be stated that indeed Mesocosm system has a significant higher welfare index when compared with the classical intensive rearing system.

Conclusions – discussion

In conclusion, it appeared that the individuals reared with the mesocosm system were responding more intensively during stress application. This is expressed either with higher velocities or more rapid changes in moving direction, or by preferring lower layers in the water column of the tank and staying closer to the bottom. This response can be considered as more "natural", or more close to wild population responses.

1. The behavior of the sea bass larvae reared under two different larvae rearing methods (mesocosm -semi-intensive & intensive) was described at different developmental stages and under different conditions.
2. There were different responses according to the rearing system. The differences were varying also according to the developmental stages of the larvae.
3. The velocity and the distribution of the larvae are behavioral indices that can be used to describe their condition.
4. The individuals reared with the mesocosm system are responding more intensively during stress by changing their velocity and their position in the tank.

Table 1. Sea bass behavioural ontogeny of swimming speed, direction, and vertical distribution in tanks

Stages	Conditions	Velocity ($BL.s^{-1}$)		Direction		Distribution per layers	
		Mesocosm	Intensive	Mesocosm	Intensive	Mesocosm	Intensive
Tail Flexion	Calm / feeding	2	2	Permanent	Permanent	Overall	Lower
	Stress / feeding	3	3	Changes	Changes	Lower	Middle
	Stress	3	3	Changes	Changes	Lower	Middle
Fins Dev.	Calm / feeding	3	3	Permanent	Permanent	Middle	Middle-upper
	Stress / feeding	3.5	3	Rapid changes	Changes	Lower	Middle
	Stress	4	3	Rapid changes	Changes	Lower	Overall
Meta-morphosis	Calm / feeding	3.5	2	Permanent	Permanent	Lower	Lower
	Stress / feeding	4.5	2	Permanent	Permanent	Middle-lower	Middle-lower
	Stress	4.5	2	Permanent	Permanent	Overall	Middle-lower
Juveniles	Calm / feeding	2	3	Permanent	Permanent	Overall	Overall
	Stress / feeding	3	3	Permanent	Permanent	Lower	Middle- lower
	Stress	3	3	Changes	Permanent	Middle-lower	Lower

1.3.1.4 SEA BASS BEHAVIOURAL STRESS LEVEL INDICATORS IN SEA CAGES

Contractors involved: HCMR, IMR

The monitoring of populations initially reared with the mesocosm and the standard intensive methods was further continued after their transfer to the sea cages for ongrowing. The ongrowing took place in two different sites, at the pilot scale facility of HCMR and the commercial facility of Nireus SA. Behavioral monitoring of the populations was performed by observation, video recording and the use of a sonar system. Monitoring was performed while the fish were under calm conditions, during feeding and also when a stress event occurred (presence of persons either in the water or on the cage platform, presence of boats, presence of persons during night-time etc).

First, during ongrowing, populations of mesocosm system origin grew without differences reaching 400 g after 18 months. Populations from intensive hatchery presented differences in growth from 350 to 400 g mean weight during the experiment duration.

Behavioral analysis was based mostly on echo sounder data and showed that fish responded to the different conditions by changing their position as expected and that this position is different according to the various conditions tested. During feeding and stress, populations have the tendency to move deeper. The displacements (i.e. the movements) that the populations were presenting were larger during feeding and stress. During feeding, populations originating from the two initial rearing systems responded differently with the populations coming from mesocosm presenting systematically greater movement responses. The space occupied by the population was also presenting a systematic difference during stress when populations originating from intensive larval rearing system occupied less space. Results obtained were reproducible in time and location presenting no significant differences for the studied variables (displacement and spatial distribution) and when populations of similar size but reared in different sites were compared.

Conclusions – discussion

1. The behavior of the sea bass during ongrowing from juvenile to commercial size was described under different conditions.
2. The behavior can be described using three variables: the mean position, the individual displacement and the spatial distribution.
3. During on growing, individuals from mesocosm origin presented more intense reactions than fish originating from intensive rearing system.

1.3.1.5 SEA BASS BEHAVIOURAL STRESS LEVEL INDICATORS IN ONGROWING TANKS

Contractors involved: IFREMER, UoC

Different experimental sea bass strains were available during the course of FASTFISH project. We therefore used that opportunity to strengthen our investigations in ongrowing tanks by working with fish of known genetic origin. In total, six populations were tested issued from four strains differing according to their level of domestication or selection (Vandeputte et al., 2009). The 6 populations were used in 3 set of experiments with either acute or chronic stress applied.

Self feeding, swimming and acute stress: Two experimental studies in ongrowing tanks of feeding motivation and swimming behavior were realized. In both cases, individual and group feeding demand were continuously evaluated (using self-feeders coupled with Pit-tag detection) allowing the identification of meal duration and rhythm, amount distributed, and ID of the fish triggering the rod. Uneaten food was measured to estimate group feed intake. The effects of a standardized acute stress applied 2 times over the experiments were monitored. Swimming behavior was investigated for the group and particular high triggering individuals using video cameras before or after stress were applied. The objectives of the study were to determine the i) effects of acute stress on feeding and swimming behaviour; and ii) whether domestication and genetic selection influenced those behaviours. The acute stressor applied was a rapid draining of the tank and 30 s of air exposure after which the fish were removed from the tank, anaesthetized, identified and measured for wet mass and fork length. In the first experiment (Exp. 2a), at the end of the experiment, fish were sub sampled for assessment of physiological status (blood glucose, liver glycogen, brain serotonin). Skin samples were collected in the dorsal or ventral zone of the fish for melanin dosing by partner 2. In the second experiment (Exp. 2b), physiological variables analysed were plasmatic lactate and glucose levels before and after stress were applied in order to confirm stress treatments and to link behavioural and physiological indicators.

Results on feeding behaviour highlighted that acute stress strongly altered all measured variables: fish self-feeding behavior, growth and physiological status. In Exp. 2a, after the first acute stress, both populations increased their feed demand and intake, modified their feeding rhythm and growth rate. On the opposite, the second acute stress had no effect on the feed demand or intake, while the feeding rhythm was modified and the growth rate depressed especially for selected fish. At the end of Exp 2a, fish from both populations but especially selected ones, showed physiological values (*i.e.* low hepatic glycogen, high plasmatic glucose and brain serotonin levels) associated to high stress levels. In conclusion of Exp2a, wild-origin and selected fish clearly presented differential adaptive behavioural and physiological capacities but selected fish seemed less adapted to acute stress and characterised by a lower welfare potential than their wild counterparts. Melanin dosing realized by partner 2 did not present any significant difference between populations nor zone (ventral vs. dorsal zones). Results of Exp 2b showed the same trends: an increase in feed intake was observed associated with a decrease in feed efficiency ratio (FER) for selected fish whereas a decrease in feed intake and an increase in FER was seen for domesticated fish. Feeding rhythms were clearly modified with a reinforcement of feeding activity around 12:00. SGR largely varied in relation to strain and period (before or after stress events). Fulton index was higher for domesticated fish before stress but the trend reversed after the first acute stress. Plasmatic lactate increased significantly after each stress event hereby confirming the efficacy of the chosen stressor and the potential use of the chosen indicators as proxy measures of stress in sea bass. Plasmatic glucose levels evolution suggested the existence of compensatory mechanisms (an increase was only observed after the first stress) but such mechanisms are likely to have a maximum threshold.

Swimming behavior studies focused on space use in the tank displayed by the high-triggering fish *vs*. group space use, and on swimming speed of the high-triggering fish in relation to stressors and time. Final objectives of the study were i) to validate a behavioral indicator based on swimming activity and ii) to determine whether domestication or genetic selection for growth elicited differential responses.

Results showed, on one hand, that the first acute stress has a higher effect on all measured variables (space use, swimming speed) whatever the population hereby underlining fast adaptation and compensation capacities. On the other hand, space use of the high-triggering fish directs that of the other fish in the group. Concerning swimming speed of the high-triggering fish, it appears to be a secondary indicator of stress effects on motivation to feed. That study has also put forward that various components of swimming behavior are differentially modified by domestication and selection which in turn encourage us to follow the careful examination of such welfare indicators of cultivated fish.

Exp. 3. Self feeding and chronic stress: A study on feeding motivation with chronic stressors was conducted. Group feeding demand was continuously evaluated using self-feeders allowing the calculation of meal duration and rhythm, and amount distributed. Uneaten food was measured to estimate group feed intake. Four populations of sea bass were tested in 1 m^3 tanks in triplicate. Experimental periods were defined as the period between two measuring day (D): P1 from D1 to D14; P2 from D15 to D35; P3 from D36 to D63 and P4 from D64 to D91. After a first control period, the fish were submitted from D35 and during 56 days to a chronic stress treatment including frequent and random application of 4 acute stressors (either as single or associated stressor: pursuing fish with a net during 1 min, switching off the light for 2s during the day or, conversely, switching on the light for 2 s during the night, and over flying a bird predator silhouette above the tank during 30 s). Our aim was to compare feeding behavior related variables (i.e. indicators) between the period before stress and the two periods during stress application. At the end of the experiment, fish were sub sampled for assessment of physiological status and skin samples were taken for melanin dosing by partner 2.

Results showed that the two variables that were measured, i.e.: fish self-feeding behavior and growth were both altered by chronic stress. During the first chronic stress period (P3), all strains increased seriously their feed demand and intake (+27 %) but only slightly modified their feeding rhythm while their growth rate decreased (fish issued from domesticated or selected parents) or remained stable (fish issued from wild parents). The second chronic stress period (P4) showed again an increased in the feed demand and intake (+16 %) and led to a strong feeding rhythm modification; conversely to what happened during P3, here, these modifications were associated with an improvement of the growth rate of all groups (+53 %). For all physiological variables (blood chemistry, glucose, liver glycogen and brain serotonin) measured at the end of the experiment, values were within the normal ranges evaluated by different authors for sea bass (references in Millot 2008, Millot et al. 2008) which indicated a good health status of fish and no inter population differences could be found. Melanin dosing realized by partner 2 presented significant differences between the four populations tested (with higher values for one highly selected population) and zones (values for ventral < values for dorsal zones) but no significant interactions could be found.

Conclusions - Discussion

Overall the study on acute stress effects in sea bass highlighted that feeding and swimming behaviour were differentially modulated by the level of domestication and selection. The promising results encourage us to follow research on cultivated fish welfare monitoring using such indicators knowing that feeding activity is a primary modulated variable when swimming activity modification appears as a secondary indicator of feeding motivation alteration. Fish appeared capable to cope with repeated acute stress presented as a chronic stress situation and to some degree capable of adaptation and habituation as reflected by feeding behaviour plasticity and growth performance at the end of the experiment. According to this study, fish issued from all strains presented the same adaptation abilities and thus the same welfare potential under our constrained environment.

The main objectives 1 & 2 in WP1.1 were investigated thoroughly and for both feeding motivation and swimming behavior patterns, some responses to stressors could be identified and therefore considered as potential indicators of fish responses to stressors. However, juvenile sea bass populations tested appeared rather robust and weakly sensitive to the stressors retained in the study. Physiological results, blood lactate and glucose in particular, were used to validate behavioral indicators to acute stress response as proxy measures of stress in sea bass production.

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1.3.2 WP1.2 IDENTIFICATION AND VALIDATION OF BEHAVIORAL STRESS LEVEL INDICATORS IN ATLANTIC SALMON

The overall objective was to identify non-invasive and reliable behavioural indicators that can be used to monitor and quantify acute and chronic stress levels in farmed Atlantic salmon. Further these should be validated and quantified using physiological or immunological methods. These relations were studied in Atlantic salmon during the three experimental series, two in tanks (7m^3, 600-1250 fish in each) and one in sea cages (2160m^3) IMR, Matre research station. The sub-goals of the experiments were to identify behavioural indicators of acute stress (temperature rise or drop, hypoxia or hyperoxia, light on/off, handling and vaccination) and measure the latency of the stress behaviour and reduced appetite after the acute stressor had been applied. In the tank experiments we used excretion of cortisol into the water and hyper consumption of oxygen to verify stress levels.

In order to verify if the behavioural indicators were dependent on development stage, swimming and anticipatory behaviour in parr, smolt, and post-smolt stages of Atlantic salmon were studied. Anticipatory behaviour is, to our knowledge, in this series of experiments for the first time tested and used as an indicator of feeding motivation in fish. Motivation is a key concept in behavioural biology that describes the emotional or affective states that make an animal perform certain behavioural needs and regulates the strength of the behavioural response. Emotional or affective states may be indirectly studied by behavioural patterns reflecting the presence of internal organising signals (Spruijt *et al*., 2001). The appropriate time to "read out" the sensitivity of reward systems is the moment at which the animal is expecting a relevant stimulus (rewarding or aversive). Thus the activity of this system should be assessed by measuring anticipatory behaviour, which can be elicited by a classical (Pavlovian) conditioning schedule (Lieberman, 2000; Spruijt *et al*., 2001). Both salmon presmolt and postsmolt was after few days conditioned to associate a blinking light in the feeding area with arrival of food 30 seconds after the onset of the signal, and responded by crowding in the feeding area. We interpret strong anticipatory behaviour as high motivation to feed (positive state), and have thus used reduction in and recovery time to normal anticipatory behaviour as an assessment of stress levels.

All tanks were monitored with both under water and surface video cameras. A computer based video image analysis procedure for assessing vertical fish distribution in aquaculture tanks was developed based on the under-water recordings (Stien et al., 2007). This method was used for measuring the response of anticipatory behaviour in postsmolt reared in tanks, while a similar analysis of video from cameras above the tanks was developed for describing the anticipatory behaviour in presmolt.

1.3.2.1 BEHAVIOURAL STRESS LEVEL INDICATORS IN ATLANTIC SALMON PARR AND SMOLTS IN FRESH WATER TANKS

Contractors involved: IMR

Atlantic salmon parr in 12 circular indoor tanks (5.3m^3, 1250 individuals per tank) were acclimated and then conditioned to a blinking light (CS) prior to every meal (US). The experiment was then divided into the parr, presmolt and smolt stage and exposed to acute stressors within each stage.

The main aims of the experiments were to identify behavioural indicators of acute stress (temperature rise or drop of 6.5 °C, hyperoxia up to 380 percent oxygen saturation, light on/off, chasing, handling and vaccination) and measure the latency of the stress behaviour and reduced appetite after an acute stressor had been applied.

The fish responded immediately to the stressors by hyper consumption of oxygen, followed by rapid recovery to baseline consumption levels when the stressors were terminated. Elevated levels of cortisol released into the water were detected up to two hours after finished stressors. Both the amplitude of oxygen hyper consumption and cortisol release rate from baseline reflected the strength of the different stressors, and confirmed that for example the chasing stressor we applied was a much stronger stressor than the relative mild stressor of temperature increase.

The strength of physiological responses between the different stressors were reflected by duration of reduced anticipatory behaviour (only analysed at the presmolt stage) and feed intake. After the mild stressor of temperature increase at the presmolt stage the cortisol release rates were only slightly elevated above the control levels concurrently with reduced anticipatory behaviour and feed intake, where the fish had recovered normal anticipatory behaviour accompanied by compensatory feed intake at the second meal three hours later. Interestingly and contrary to after the temperature stressor, the hyperoxia stressed presmolt had a significant elevation in cortisol release and a clear reduction in anticipatory behaviour, but feed intake close to normal levels. The more severe stressor of chasing resulted in a highly elevated cortisol release rate after finished stressor, reduced anticipatory behaviour before all three meals of the day (up to 6 h after stress), reduced feed intake in the two first meals after stress, and also reduced total daily feed intake for the four next days that feed intake was measured.

These results shows that anticipatory behaviour is a sensitive stress measure and time to recovery to normal levels after stress exposure were much longer than the latency of elevated cortisol release rates and oxygen hyper consumption. The relationship between stress level and the physiological indicators can be measured as the strength of the acute response, i.e. strong stressors give immediate high cortisol release rates and oxygen consumption, but latency of the response is short for both weak and strong stressors. However, the latency of reduced anticipatory behaviour seems to be correlated with stress level, and can therefore be a behavioural indicator of the latency of the psychological effects after exposure to acute stressors.

The relationship between feeding motivation and feed intake seem not be straight forward, indicating that physiological stress responses and the subjective stress experience of the fish should be treated as different systems, where the relationship between them is dependent on the form and strength of the given stressor. The assessment of feeding motivation must however be considered to be an all over good welfare indicator, taking into account that we interpret a positive conditioned response as an indicator of well-being.

Anticipatory behaviour at the parr stage to different stressors was not measurable with the methods developed. The cortisol response was low after temperature change, significant after switching light off and largely significant after chasing. In total, the fish responded by lowered appetite on the sudden light off and chasing while no effects was seen on temperature stress. The cortisol response was low after temperature change, significant after switching light off and highly significant after chasing. The parr responded by reduced appetite on the sudden light off and chasing.

At the smolt stage, the combined data of feed intake and cortisol release rate display correlations with high cortisol release and low feed intake being observed at the severe stressors of chasing and seawater transfer. Following the mild stressors of hyperoxia (<240%) and temperature reductions no effects were seen. The data collected from seawater transfer are not directly comparable with the other stressors applied as the fish were not brought back to its previous conditions. It is however interesting to see that there was a similar strong peak in cortisol release both after chasing and seawater transfer which reached baseline levels within

4 hours while reduced appetite lasted for days in the seawater transferred fish, but only for a few meals after chasing. This observation stress the importance of looking at several measures at the same time and that cortisol release on its own is not sufficient.

1.3.2.2 BEHAVIOURAL STRESS LEVEL INDICATORS IN ATLANTIC SALMON POST SMOLT IN SEA WATER TANKS AND CAGES

Contractors involved: IMR

In the tank experiments of acute stress in salmon post smolt the fish were divided into groups with two different feed rations (65% ration=high hunger, and 90% ration=low hunger) to evaluate the effect of metabolic state on stress responses. Three different stressors were given; 6ºC temperature increase over four hours, hypoxia by reducing the water flow (35% saturation at outlet for two hours), and darkness, sudden lights off (2h), and subsequently on again. The difference in metabolic state between the two groups was apparent by higher baseline oxygen consumption in the low hunger (higher feed intake) groups. Both groups had however the same amplitude of hyper consumption of oxygen during the stressors, and about the same levels of elevation in cortisol release, which indicate similar coping efforts, but oxygen consumption closer toward the maximum capacity in the low hunger group.

The effect of feed ration on the conditioned response was evident as the high hunger groups had a faster recovery rate of anticipatory behaviour after the stressors, and also an all over stronger and more consistent anticipatory behaviour before feeding compared to the low hunger groups. In comparison; the high hunger groups had recovered normal anticipatory behaviour one and two hours after temperature stress, and one hour after terminated darkness, while the low hunger groups recovered normal anticipatory behaviour 3 hours after temperature stress an showed a neutral to negative response to the conditioning signal for up to the last meal of the day 6 hours after terminated darkness stress. No systematic behavioural responses or increased cortisol release were found after the hyperoxia stressor.

The temperature and darkness stressor gave both weak cortisol responses, even if the darkness stressed fish showed very strong behavioural reactions and dived immediately towards the bottom, had a hyper consumption of oxygen and did not respond to the light blinks or the first feeding. There was a systematic difference in conditioned response between controls and temperature stressed fish; the stressed groups showed an immediate negative response by moving away at the first light blink, indicating psychological stress. Also manual analyses of individual swimming behaviour showed that the temperature stressed fish deviated significantly from the controls. Behaviours interpreted as indications of exhaustion, such as backing on current, and escaping by moving in either current direction, were highly represented in the stressed fish. Behavioural indications of exhaustion was however stronger in the high feed intake fish, in line with their higher oxygen demand and prolonged suppression of feeding motivation after stress compared to the high hunger fish.

Anticipatory behaviour measured with the developed video image analysis method (Stien et al. 2007) show that psychological stress in groups of fish can be assessed at a relative detailed level, and that the psychological component of stress may last longer than the apparent physiological restitution of cortisol and oxygen consumption.

The conditioning procedure in sea cages did not give observable response, probably due to too weak conditioning signal. The salmon responded behaviourally and reduced the feed intake the first feeding after stress (light on off during night). Evident behavioural responses of burst type swimming at high speed and short time changes in vertical positioning was observed after a stressor of alternating light and darkness, and a systematic response of dense schooling in a tornado shaped structure after a stressor of forcing the fish to crowd at surface.

As chronic stressor in cages we used hindered the access to the surface and air filling of swim bladder. The submerged fish swam an average of 1.6 times faster (0.88 BL s-1) and in more structured schools than control fish (0.55 BL s-1), to compensate for the reduced buoyancy. There were no significant effects on feed intake and growth, and relatively short time submergence (21 days) given the prevailing circumstances (spring and underwater lights) did not act as a strong chronic stressor. However, later studies have shown that longer-term submergence to deeper depths and during darkness may act as a chronic stressor for salmon, giving strong behavioural changes. The results also show the potential for submergence of salmon in sea-cages for short periods to avoid negative surface events (Dempster et al., 2009).

1.3.1.3 OXYGEN CONSUMPTION AND CORTISOL RELEASE RATES AS PHYSIOLOGICAL STRESS LEVEL INDICATORS

Contractors involved: IMR

From the studies in WP 1.2 including acute stressors in the life stages from parr, via presmolt and smolt to postsmolt we found consistency between the levels of physiological responses to different stressors, taken into account differences in cortisol production and oxygen consumption between the life stages. This demonstrates that the physiological stress measures of cortisol and oxygen consumption is relevant for the different life stages of farmed salmon that we investigated.

Cortisol released to water was found useful as a non invasive method for verification of physiological stress, but is relative expensive and cumbersome method. Hyper consumption of oxygen can along with cortisol, or even unaccompanied by other physiological measurements be used as a precise assessment of acute stress. Oxygen consumption has the advantages that it can be measured in continuous time series with high precision and be assessed online by electronic DO sensors in inflow and outflow water. The consumption can at any time be compared with an expected rate, i.e. if the expected increase in oxygen consumption during feeding is not seen, this will indicate low food intake caused by some stressful event. Lasting oxygen consumption below expected level given the prevailing conditions may indicate chronic stress caused by suboptimal environmental conditions or disease.

1.3.1.4 EFECTS OF CHRONIC STRESS ON IMMUNE COMPETENCE IN ATLANTIC SALMON SMOLT AND POSTSMOLT

Contractors involved: NSVS, IMR

The main aim has been to study the effects of chronic stress on development of immune competence. In these studies we have used an experimental approach of chronic stress following hypoxia, hyper oxygenation, and the combination of hyper oxygenation and hypercapnia. As there are good indications that cortisol (corticosteroids) will influence T helper type 1 (Th1) immune responses to a greater extent that Th2 responses, antigen formulations/constructs biased towards one or the other immune mechanisms have been made. These vaccines where: PVK - a recombinant fusion protein, consisting of the detoxified PE subunit of Pseudomonas aeruginosa known to stimulate humoral immune responses in mice and zebra fish, and the viral capsid protein VP2 of a high virulent IPNV strain (NVI 015). The vaccine is assumed to give bias towards humoral immune responses (Th2). A DNA vaccine encoding the G-protein of Viral Haemorrhagic Septicaemia Virus (VHSV), as there is good documentation that DNA vaccination will induce cell mediated immune responses (Th1). Finally; a water based commercial vaccine towards *Vibrio anguillarum* O1, O2a and O2b, Alpha Marine Vibrio (PHARMAQ). These vaccines were used in Exp. 5 and 6. In Exp. 7 the fish were immunised with a commercial oil-based vaccine, Norvax® Compact 6 (Intervet Norbio).

Briefly we have addressed the effect of chronic stress on the ability of fish to mount an antibody response to vaccines, to produce regulatory cytokines and other immune relevant genes assessed by quantitative real-time PCR. The role of chronic stress on the development of immunocompetence has also be studied in fish as ability to survive viral challenge.

Exp		Chronic stressor	Duration of experiment	Fish stage	Water quality
WP1.2	Exp.5	Hypoxia (50% O2)	21 Days post vaccination	Post-smolt	Sea water
	Exp.6	Hyperoxia (~170 % O2)	45 Days post vaccination	Pre-smolt/smolt	Freshwater
WP3.2	Exp.7	Hyperoxia and hypercapnia	44 Days post vaccination	Pre-smolt/smolt	Freshwater

Exp. 5: This experiment was run to observe the effect of hypoxia (50% saturation) on the development of immune competence in Atlantic salmon post-smolt (mean weight ± s.d.: 156±50 g) reared in seawater. The fish had previously been immunised with a commercial oil-based vaccine without the IPNV component, but unfortunately experienced an IPN outbreak prior to the experiment. The experiment started when the mortality had stopped and the appetite had returned to normal. One month following conditioning the fish (mean weight 144g) were vaccinated and the chronic stress phase was commenced. Fish were injected 0.1ml vaccine intraperitoneally (IPNV and Vibrio), while 0.1ml were injected intramuscularly for the DNA vaccine. Each vaccine was used for all fish in two tanks, one kept at a normoxic environment, while fish in the other tank were submitted to chronic low oxygen level (50% saturation). Low O_2 levels were established by low water flow, immediately after vaccination.

Analysis of IPNV specific antibodies by ELISA revealed high titres in all groups, both prior to and post immunisation. The titres were higher post immunisation, and significantly higher in the stressed group ($p<0.05$). The distribution of antibody titres in the control fish shows that approximately half of the fish had high titres and half of the fish had low titres or were negative. 22 days post immunisation the fish in the non-stressed group had a similar distribution as the controls, albeit at slightly higher levels. In the stressed group there were no fish having low or negative titres. As the fish experienced and IPN outbreak prior to the experiment, the results suggest that stress caused by low O_2 levels in the tanks reduced the ability of the fish to avoid infection, but not the ability to mount an antibody production. Analysis of Vibrio specific antibodies revealed high titres in all groups, both prior to and post immunisation. There were no statistical differences between the control group and the two vaccinated groups. The fish had previously been vaccinated with a commercial oil-based vaccine, and clearly the Vibrio anguillarum antibody titre was still high and that revaccination with the water based Vibrio vaccine was not able to increase these high titres further.

Exp. 6: This experiment was run to observe the effect of hyper oxygenation (>150% saturation in outlet water (~170%) on the development of immune competence in Atlantic salmon pre-smolt (mean weight 44g) reared in freshwater. One week into chronic stress, fish were immunised following the same regime as described for the Exp. 5. Each vaccine was used for all fish in two tanks, one kept at a normoxic environment, while fish in the other tank were submitted to hyperoxia. 45 days post vaccination the fish immunized against IPNV were transported to the Institute of Marine Research (Bergen) for a challenge trial. This trial was performed in four replicate tanks with 20 fish (mean weight 211g) in each group. The three groups were as follow: Vaccinated fish kept in either a hyperoxic environment (stressed fish) or normoxic environment (non-stressed) and a third non-vaccinated control group. The tanks were supplied with seawater with a mean temperature of 11 ºC. Fish were challenged by injecting 12 extra fish (non- vaccinated) fish with recombinant IPNV (rNVI15R) 0.25 ml of a solution of 3.5 x107 $TCID_{50}$ /ml). The shedders were marked by the elastomar-colour method to separate them from the cohabitants. Mortality was recorded on a daily basis, and the experiment was terminated 39 days post challenge.

Analysis of IPNV specific antibodies by ELISA revealed low titres in all groups, both prior to and post immunisation. At day 21 post vaccination the stressed fish had significantly higher antibody level compared to the non-stressed fish, but there were no differences at day 45. The PVK vaccine had low antibody generating capacity in general. There was no clear effect of chronic stress on the development of the IPNV specific antibody titres. Analysis of Vibrio specific antibodies revealed increasing titres following vaccination with the water based commercial Vibrio, but there was no effect of hyperoxic water (chronic stress) on the development of specific antibodies. The titres were low compared to Exp.5 where an oil-based multivalent vaccine was used.

During the challenge trial low mortalities in all groups were detected between 3 to 9 days, but between ten to fourteen days massive mortality was observed in all groups. 39 days post challenge cumulative mortality had reached 100% for the unvaccinated groups, 92% for the vaccinated groups kept in a normoxic environment, while being 97% for the vaccinated group kept in hyperoxic environment. The IPNV immunised fish kept in a hyperoxic environment were not statistically significant from the non-immunised challenged controls (Chi-square = 2.13). The IPNV immunised fish kept in a normoxic environment were statistically different from the non-immunised controls (Chi-square = 6.31, $p \leq 0.025$). The relative percent survival (RPS) was however low for both immunised groups, being 2.6 and 7.6 % for the stressed and non-stressed groups respectively.

Exp. 7: This experiment was run to observe the effect of hyper oxygenation and hypercapnia on the development of immune competence in Atlantic salmon pre-smolt (mean weight 180g) reared in freshwater. The fish were immunised with a commercial oil-based vaccine, Norvax® Compact 6 (Intervet Norbio). Three of the groups were held at 16 ºC (production of O+ smolt), while 1 group were kept at a low water temperature, 6 ºC and in normoxic water (production of 1+ smolt). The three groups at 16 ºC were kept at the following conditions with regard to O_2 and CO_2 levels. The control group were held in normoxic water (>90% O_2 and normal water flow (60 l/min). Two of the groups were subjected to chronic stressors. One group to chronic hyperoxia (170% O_2 saturation/ normal flow through 60 l/min). The high intensive hypercapnia group was in the beginning of the experiment given both low water flow and hyperoxia, but to get a low enough water flow to raise the CO_2 level we had to reduce the supply of oxygenated water. With only 10 l/min water supply in these groups this also led to much higher content of feed waste particles and faeces in the water giving turbid water. To get out the waste feed the tanks had to be flushed, which gave extra stress to these groups.

Analysis of Vibrio specific antibodies by ELISA revealed high titres after vaccination in all groups kept at 16 ºC. The use of a commercial oil-based multivalent vaccine caused equal titres as observed in Exp.5 ($\log_2$ titre ~ 8). We did not observe statistical significant differences between the fish held in normoxic water compared to the groups of fish subjected to either hyperoxia or hypercapnia. However the effect of water temperature were obvious, as vaccinated fish kept at 6 ºC were not statistically different from the non-vaccinated controls at day 44 post vaccination. The mean growth rates of the hypercapnia and hyperoxia groups were reduced to 62% and 88%, respectively, of mean growth rate in the control groups, showing significant negative effects of the low water flow (hypercapnia, t-test $p<0.001$) and hyperoxia (t-test, $p<0,031$) compared to control groups (Pooled groups, since no significant difference within groups). The condition factor of the hyperoxia and hypercapnia groups were also significantly lower than control.

Gene expression results (from Exp. 5 and 6): As two of the vaccines used in Exp. 5 & 6 were injected intraperitoneally, being distributed throughout the abdominal cavity, the injection site reactions measured at gene expression level were difficult to assess. Studies of regulatory cytokines, immune relevant genes and immune cell markers have therefore been studied in the head kidney. The head kidney is an important hematopoietic organ, and shows morphological similarities with the bone marrow in higher vertebrates. But it also serves as a secondary lymphoid organ - lymph node analogue important in the induction and elaboration of immune responses.

The most responsive genes were as follow; Interleukin 1-β (IL1-β), Complement factor D, IL-10 and Mx. For IL-1β, an important mediator of the inflammatory response involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis a marked up-regulation at day 2 were observed in both studies. This up-regulation was observed for both the PVK and the Vibrio vaccine, while the DNA vaccine did not induce IL-1β. The trend suggested strongest induction for the Vibrio vaccine in both Exp. 5 and 6. However, in Exp. 6 the PVK vaccinated stressed fish, showed significantly higher IL-1β levels than the non-stressed parallel group of fish. At day 8 however, the stressed fish showed significantly lower IL-1β than the normoxy group. The level of IL-1β induction was strikingly different in the two experiments, being around 30-fold and 3-4 fold in Exp. 5 and 6 respectively. The reason for this is not clear, but might be due to the fish physiological status, pre-smolt versus post-smolt. However a clear trend suggesting an impact from either hypoxia (Exp.5) or hyperoxia (Exp.6) on IL-1β in head kidney were not observed, a common trend observed for all genes throughout the material.

The immunoregulatory cytokines, IL-10 and IL-12 are believed to play opposite roles in regulating cell mediated immunity (Th1) and antibody responses (Th2). The IL-10 cytokine has pleiotropic effects in immunoregulation and inflammation. It down-regulates the expression of Th1 cytokines, and also enhances B cell survival, proliferation, and antibody production. On the contrary IL-12 is involved in the differentiation of naive T cells into Th1 cells, and is therefore known as a T cell stimulating factor, which can stimulate the growth and function of T cells. In both Exp. 5 and 6 the Vibrio vaccine induced IL-10 at day 2 post vaccination, while IL-10 was only found upregulated following immunisation with the PVK vaccine in Exp. 6. The DNA vaccine did not induce IL-10 expression. This makes sense as there is good documentation that DNA vaccination will induce responses biased towards Th1. The effect of vaccination on IL-12 production were not as clear as IL-10, and it remains elusive whether this was due to sampling at wrong time points for this cytokine, or efflux of IL-12β producing cells from the head kidney. In Exp. 6 (day 8), the DNA vaccine showed significant higher IL-12β production than the two other vaccines, but at the same level as the non-vaccinated controls.

Mx and IFN-α are mainly involved in the innate immune response against viral infection. In Exp. 5 only the DNA vaccine induced Mx. It is known that DNA vaccines in fish induce an early upregulation and expression of the antiviral protein Mx. In Exp. 5 the Mx genes peaked at day 8, and were also induced at day 21. In Exp. 6 the Mx levels for the non-vaccinated controls were high, compared to vaccinated fish. A putative explanation for high Mx in the non-vaccinated controls remains elusive, but at day 8 we observed the same picture as seen in Exp. 5. However there were no significant effect of the stressors on Mx expression in Exp. 5 and 6. For IFN-α the results revealed no clear peaks, differences between the vaccines or effect from stressors. Complement factor D is a protein involved in the alternative complement pathway, cleaving factor B. The alternative pathway can operate without antibody production, and is a part of the innate immune system, or the natural defence against infections. Complement factor D were up-regulated by the PVK vaccine at day 8. This up-regulation were observed in both experiments, and not affected by the stressors. In Exp.6, complement factor D were slightly higher in the vaccinated fish and at all time-points than for the non-vaccinated controls.

We also studied the expression of cell markers in the head kidney following vaccination. Any increase or decrease could be due to an actual change in expression. But it is also apparent that some changes probably were due to efflux of cells from head kidney to the site of vaccine injection. CD3 (T-cell marker), CD4 (T-helper cells, T-regulatory cells, monocytes, macrophages), CD8 (Cytotoxic T-cells, Natural killer cells), T-cell receptor, MHC I (most nucleated cells) and MHC II (Professional antigen presenting cells - APC) were all studied in Exp 5 and 6. In Exp. 6 there was a general drop in expression of all these markers between day 2 and 8. This drop was most profound for the PVK vaccine, slightly less for the Vibrio vaccine and least/or absent for the DNA vaccine. The reason for the PVK vaccine causing most prominent reduction of these markers is not known, but it might be speculated if this was due to the use of oil-adjuvants. The oil-based adjuvants allow a prolonged delivery of antigens because they help form a depot and this action might increase the number of cells “drained” from the head kidney. The expression of CD8 in DNA vaccinated fish were the only cell marker being statistically higher than the non-vaccinated controls (day 21 and 45), which makes sense for a vaccine believed to yield good T-cell responses. Furthermore there is reason to believe that expression of pro-inflammatory cytokines, immune regulatory cytokines and antimicrobial cytokines were influenced by the efflux of cells from head kidney, as many of these are produced by macrophages and lymphocytes. In Exp. 5 there was no apparent drop in cell markers following vaccination, as observed in Exp. 6. The outbreak of IPN just before start up might serve as an explanation for this difference. By real-time PCR, IPNV were detected in 61 % of the fish used for gene expression data. An on-going infection, or persistent infection, might have caused drainage of cells from head kidney before immunisation. Interpretation of data from Exp. 5 should be done with care due to concurrent IPNV. There were however no clear effects observed from the stressors hypoxia or hyperoxia in these cell markers in Exp. 5 and 6.

Furthermore, we have tested a set of genes whose expression might be altered during chronic stress. With regard to oxygen-induced stress, this is likely to be seen in all metabolically active tissues including liver cells, as the liver is a major detoxifying organ. Hydrogen peroxide and superoxide is harmful by-products of many normal metabolic processes. To prevent damage, these must be quickly converted into other, less dangerous substances. Catalase functions to catalyse the decomposition of hydrogen peroxide to water and oxygen. While the enzyme superoxide dismutase (SOD), catalyses the conversion of superoxide into oxygen and hydrogen peroxide. Finally metallothionein (MT) and heat shock proteins (HSP) are considered as general stress proteins, and shown to be affected by oxidative stress. The glucocorticoid receptor (GR) is a ligand-activated transcription factor that binds with high affinity to cortisol and other glucocorticoids, while steroidogenic acute regulatory protein (STAR) is a transport protein that regulates cholesterol transfer within the mitochondria, which is the rate-limiting step in the production of steroid hormones. The general trends observed for these genes in Exp 6 were as follow: Highest gene expression levels were found in the non-vaccinated controls. Following vaccination, the expression levels dropped significantly for all genes studied. The non-vaccinated stressed controls that had been exposed to hyperoxic water for 8 days were not different from the non-stressed and non-vaccinated controls for any of the genes studied. This trend was also observed after vaccination. For some of the genes (Catalase, SOD, HSP90) minor but statistically significant differences between different vaccine types were observed. The biological relevance of these is not known.

Conclusions

In summary neither hypoxia, hyperoxia nor hypercapnia affected the development of specific antibody levels following immunisation of Atlantic salmon. This was best seen in Exp. 7 were strong specific antibody responses were achieved following vaccination with an oil-based multivalent vaccine, but not affected by the stressors. The antibody titres were however markedly affected by water temperature. Especially the “hypercapnia” groups were reared under very poor environmental conditions with low water flow, variable temperature, relatively high CO_2, fluctuating dissolved oxygen levels, and high turbidity (POM). The large reduction in growth rate, reduced appetite and stronger effects and longer latency of acute stress, clearly indicated suboptimal rearing conditions and we should assume relative high chronic stress level. However, our assumptions of reduced immune response in chronically stressed fish was not fulfilled in this experiment, nor in the WP1.2 experiments, and it seems that reduction in immune response is not a good indicator of chronic stress and cannot be used to validate chronic stress level in Atlantic salmon.

The PVK vaccine had low antibody generating capacity, and was in Exp. 5 dominated by the seroconversion caused by natural infection prior to the experiment. An interesting finding though that the stress caused by low O_2 levels in the tanks seemed to reduce the ability of the fish to avoid infection, but not the ability to mount antibody production. The water based commercial Vibrio vaccine yielded rather weak responses, and these were not significantly affected by stress. Assessment of immunocompetence by an IPNV challenge test in fish vaccinated with the PVK vaccine showed that the relative percent survival (RPS) was low for both immunised groups, being 2.6 and 7.6 % for the stressed and non-stressed groups respectively. This difference was not statistically significant. Furthermore, the gene expression data did not reveal a clear impact from the stressors hypoxia (Exp. 5) and hyperoxia (Exp. 6) on the genes studied, and the set of possible stress markers in the liver were not affected by hyperoxia, although some were affected by vaccine type. The importance of maintaining immunological competence might be a prioritised task for Atlantic salmon during chronic stress periods. However, it might be speculated that Atlantic salmon is highly capable to habituate to both high and low O2 levels in the water, at least when these are kept constant.

1.3.3 WP2.1 Development of internet based database and expert system (FAST-TOOL) for monitoring, documentation and assessment of fish stress level and welfare in fish farms

Contractors involved: IMR, HCMR

1.3.3.1 DEVELOPMENT OF DATABASE AND INTERNET BASED USER INTERFACE

Work on a web application and database system for day-to-day registration of environmental data, stress level indicators, husbandry data for salmon and sea bass hatcheries and ongrowing farms (sea cages) started spring 2006. The web application was named FAST-TOOL and the prototype is available at http://www.imr.no/fasttool. FastTool is based on the latest in Internet technology (Asynchronous JavaScript and Java Server Pages) to provide a seamless interaction between the application and the database. The database is implemented in the open source database management system PostgreSQL and follows relational database system principles (Halpin 1999). A key part of the work has been to make the database as flexible as possible so that it will be suitable for both complex users (e. g. researcher using the database to store research data) and for fish farmers dealing with a more limited amount of data.

The first prototype of the FAST-TOOL application provided one input dialog where the user could enter the daily data input to FAST-TOOL. Testing this prototype of FAST-TOOL on fish farmers and technical personal working at IMR's own rearing facilities revealed that they soon became overwhelmed by the number of parameters asked for by the dialog and lost interest. It was therefore decided to make a new prototype where the data input was done step-by-step.

When entering this new prototype of the FAST-TOOL-application the first webpage which opens is the 'Summary page' (Figure 1.3.3.1-1). The upper part of the summary page contains eight action links. Each of these links opens a dialog where the user can enter data about:

1. The fish farmers' subjective assessment of fish welfare (welfare index).
2. Type of pen, depth, volume, algae growth and sight depth.
3. Fish species, life stage, biomass, number of fish, average length, number of dead fish.
4. Daily food and daily waste food.
5. Feeding behaviour.
6. Water temperature, dissolved oxygen and salinity.
7. Fish behaviour.
8. Fish appearance.

Each of the action links are marked with a status box. A red box stating 'hurry' means that these data are overdue, i.e. they should already have been entered into the database via the web application. A yellow box stating 'enter' means that the data is scheduled to be entered today and a green box stating 'ok' means that there is no need to update these data today or that they already have been updated.

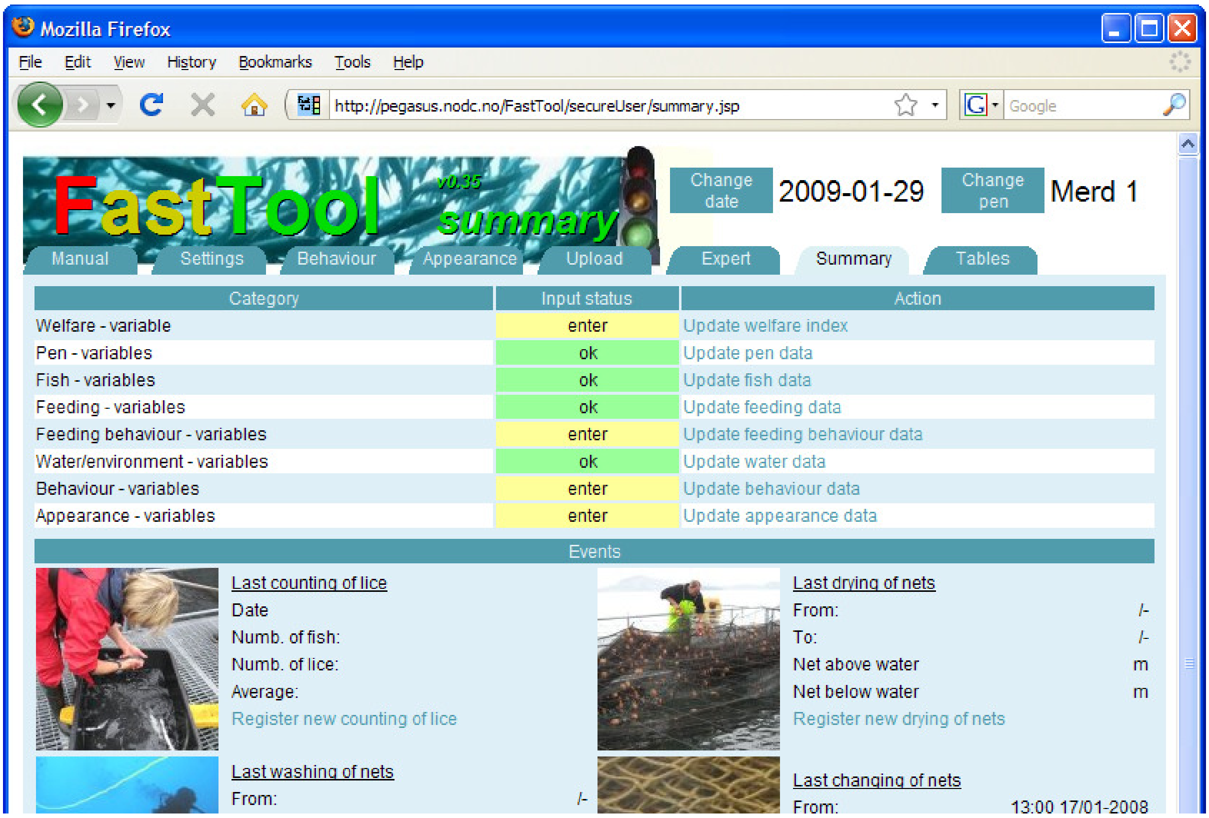

Figure 1.3.3.1-1: The main page / summary page of the FastTool web application. This page gives an overview of which data categories that are to be entered by the fish farmer today (upper part of page) and a range of different events that the fish farmer can report (lower part of page).

In the case shown in Figure 1.3.3.1-1 the ', the 'Update feeding behaviour data', the 'Update behaviour data' and the 'Update appearance data' status boxes are yellow stating 'enter'. These data should therefore be entered as soon as possible.

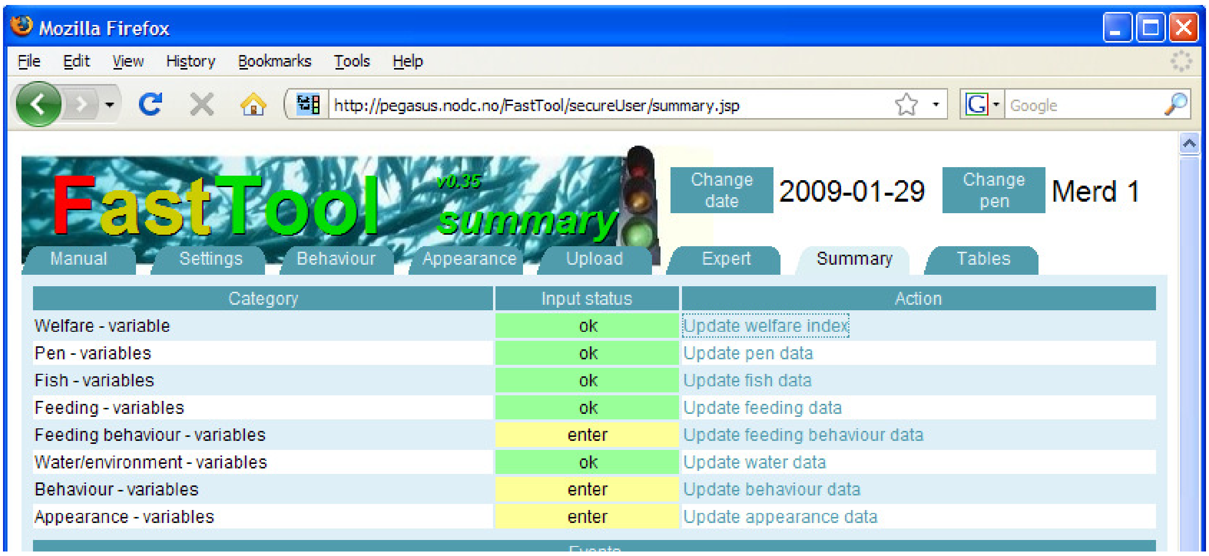

Figure 1.3.3.1-2: The main page / summary page of the FAST-TOOL web application. The user has now entered (compared to in Figure 1.3.3.1-1) entered the welfare index data.

Starting at the top, clicking the 'Update welfare index'-action opens a dialog asking for the farmers subjective assessment of the welfare of his fish into one of the categories 'Excellent', 'Very good', 'Good', 'Ok', 'Sub optimal', 'Bad' and 'Terrible'. The user inputs his or hers subjective assessment of fish welfare by clicking on the respective welfare category and then on the 'OK'-button. The dialog then closes and the input status for the welfare index in the summary page becomes green and stating 'ok' (Figure 1.3.3.1-2). This illustrates the step-by –step approach of the input. The users continuous for the rest of the categories until all status-boxes have become green.

One of the main inputs to FAST-TOOL is fish behaviour. The fish behaviour dialog (Figure 1.3.3.1-3) asks the fish farmer to indicate relative fish density in the upper, middle and lower part of the water column, fish density in the centre, intermediate and outer part of the pen and to rate if he sees certain behaviours on a scale from False to Unsure to True. These behaviours are: jumping, shoaling, fish at surface, looser fish, fish standing against current, swimming activity, schooling, wall syndrome and high aggression. On holding the mouse on top of each of the sliding bars an image and a text describing the behaviour appears in the 'Information'-section of the dialog.

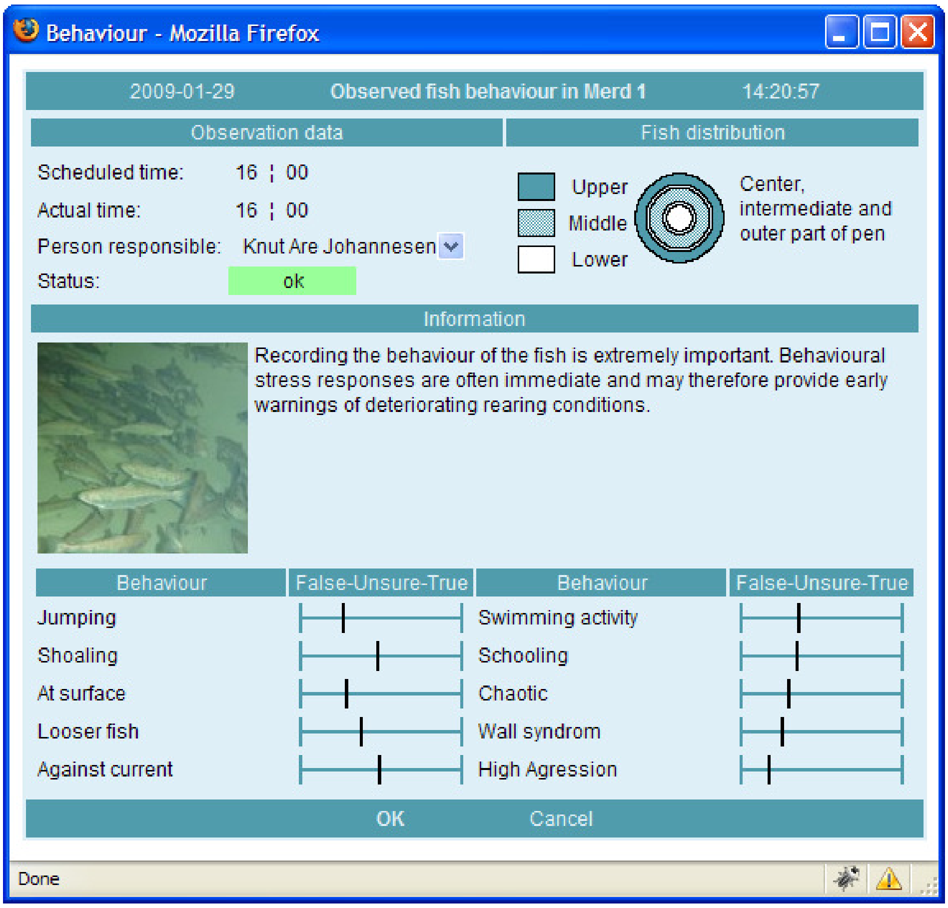

Figure 1.3.3.1-3: Dialog for registering the behaviour of the fish. The upper part of the dialog describes the distribution of the fish, both vertically and horizontally. The lower part of the page is a set of sliding bars where the user can rate the degree of different behaviours. The middle part of the dialog gives information according to where the mouse is placed .If the mouse is placed on tom of the 'Jumping'-sliding bar an image of jumping fish and description of how to grade this behaviour appears.

In order to get standardised registration of behaviour a behaviour ethogram was constructed. The user can at any time watch this ethogram by accessing FAST-TOOLs behaviour ethogram—webpage. The behaviour webpage contains name of the different behaviours, short descriptions of the behaviours, images and movies. A similar page has also been created for fish appearance.

In addition to manual input (described above) it is also possible for the fishfarmer to upload data into the FAST-TOOL database. These data include data from CTD's(SAIV MINI STD/CTD – model SD204, SAIV AS, Norway) with additional sensors for oxygen, turbidity and fluorescence, data from a echo sounder system (Lindem Data Acquisition, Norway) and data from a feeding program (FishTalk, AKVA group, Norway). Uploading of data is done in the 'Upload'-page (Figure 1.3.3.1-4).

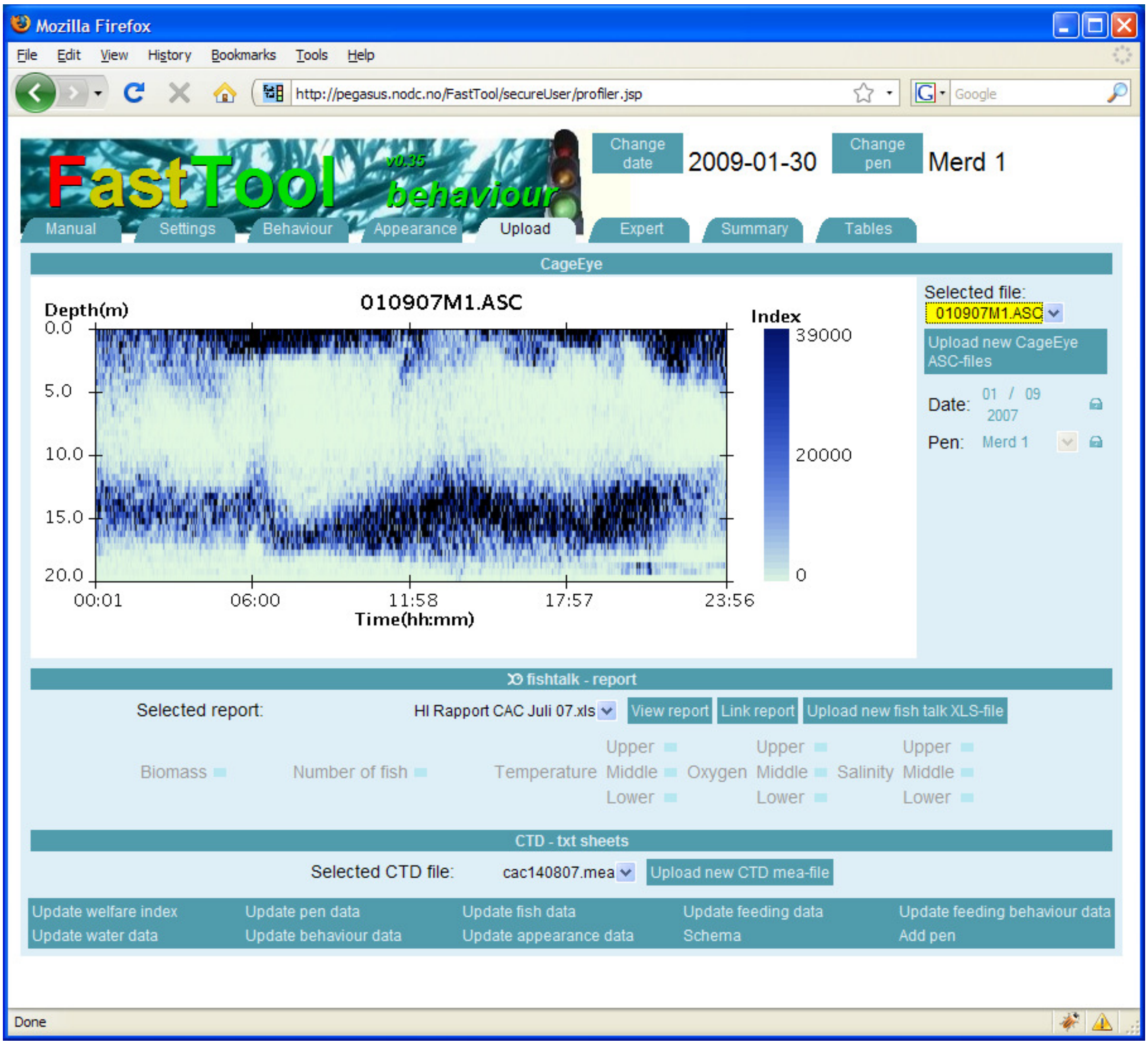

Figure 1.3.3.1-4: Upload page. This page allows the fishfarmer to upload echo sounder data, data from the FishTalk feeding program and CTD-data.

1.3.3.2 FROM FAST-TOOL TO WELFAREMETER EXPERT SYSTEM

Although tests of the second prototype of FAST-TOOL showed far better results in terms of user- friendliness and hence compliance , long term trials at commercial fish farms revealed that it still was difficult to get day to day compliance from the farmers. It was therefore concluded that FAST-TOOL had to become more automatic (less dependent on manual input of data) and more attractive to the farmer (the farmer must everyday be rewarded by new information when entering FAST-TOOL). The FASTFISH project did therefore in cooperation with Tendo Tech AS (Norway), SAIV AS (Norway) and Morten Hammersland programvare (Norway) develop a prototype of a measurement system that provides a profile of the environmental conditions in a sea cage from top to bottom at regular intervals (typical every two hours) and automatically transmits the data via the mobile phone network (GPRS) to the FAST-TOOL database. This cooperation was made possible through additional funding from BTO (Bergen Technology Transfer, Norway).

The FAST-TOOL v2 - Welfaremeter prototype consists of a profiling probe (CTD), a control unit, a database, an expert software program and an internet web application (Figure 1.2.3.2-1Figure). The probe measures temperature, oxygen, salinity, fluorescence and turbidity for each half meter downwards in the cage. The control unit determines how often the probe profiles the water column and sends the measurement data via the mobile phone network (GPRS) to a database at The Norwegian Marine Data Centre (NMD), Institute of Marine Research. These data are then analysed by the expert software which gives an evaluation of the environmental conditions in the cage and calculates a welfare index from 0 (terrible welfare) to 100 (excellent welfare). The results are then presented on the FAST-TOOL v2 – Welfaremeter internet web application.

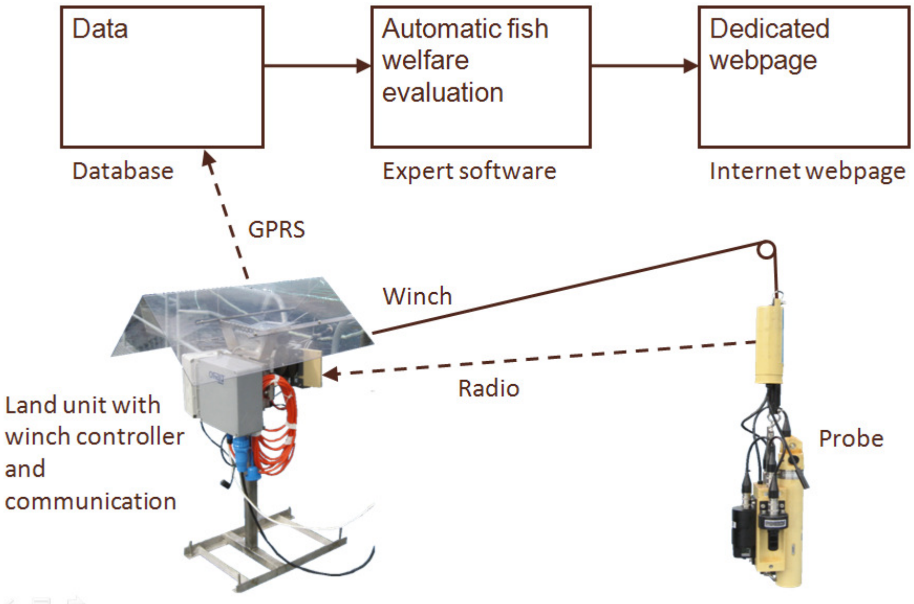

Figure 1.3.3.2-1: The Welfaremeter prototype.

The expert software of the FAST-TOOL v2 - Welfaremeter prototype is based on knowledge about how Atlantic salmon react to different environmental conditions and mathematical modelling of salmon metabolism given the measured environmental conditions (metabolism is the sum of all chemical reactions in an organism that lead to continued life, growth and reproduction). For instance the chemical processes in the cells decelerate at low water temperatures, while too high temperatures may lead to collapse of the metabolism (Pennell and Barton 1996). Satisfactory temperatures for salmon lies in the range between about 7 and about 17 °C, and for sea bass between about 8 and 28 °C. Further, low oxygen saturation will lead to higher metabolic costs of oxygen uptake and lower maximum uptake rates. Changes in environmental conditions may lead to stress-induced increase in oxygen consumption and oxygen consuming physiological acclimation processes. Over time these responses will habituate and diminish.

When new data arrive in the database, it is immediately analysed by the expert software and classified as good, fair or potentially harmful to fish. The expert software calculates a welfare index from 0 (terrible welfare) to 100 (excellent welfare). This index is based on modelling of metabolic scope (the salmon's capacity to extract oxygen beyond its basic needs from the water under the current environmental conditions) and factorial scope (the maximum continuous oxygen uptake divided by the standard oxygen consumption, reflecting the salmon's robustness for stress and environmental perturbations under the current environmental conditions). The modelling behind these two parameters has been developed in the software package Stella (Isee systems, USA) and is based on the ecophysiological growth model Ecophys.fish (Neill et al. 2004). A first version of this model is included in the prototype expert software, calculating metabolic scope and factorial scope. A preliminary presentation of the model is given in Torgersen 2009 and a peer reviewed paper is soon to be submitted (Torgersen, in prep.).

The FAST-TOOL v2 - Welfarmeter internet web application is available at www.imr.no/welfaremeter and displays live data from an ongoing trial. The main visible features of the webpage are three graphs and a speedometer (Figure 1.3.3.1-6). The speedometer gives the welfare index. The top graph on the page illustrates the environmental conditions in the cage from top to bottom. The measurements by the probe are indicated by points, the x-axis gives the scale of the selected environmental parameter and the y-axis gives the depth. The background of the graph is divided into red, yellow and green zones. If a measurement is in a red zone, it means that the conditions at this depth are potentially harmful to the fish. Yellow zone indicates less good conditions and green zone indicates good conditions. The user can easily choose between the different environmental parameters (including metabolic scope and factorial scope) by clicking on a menu at the side of the graph. The other two graphs are based on similar principles, but specify the development over time.

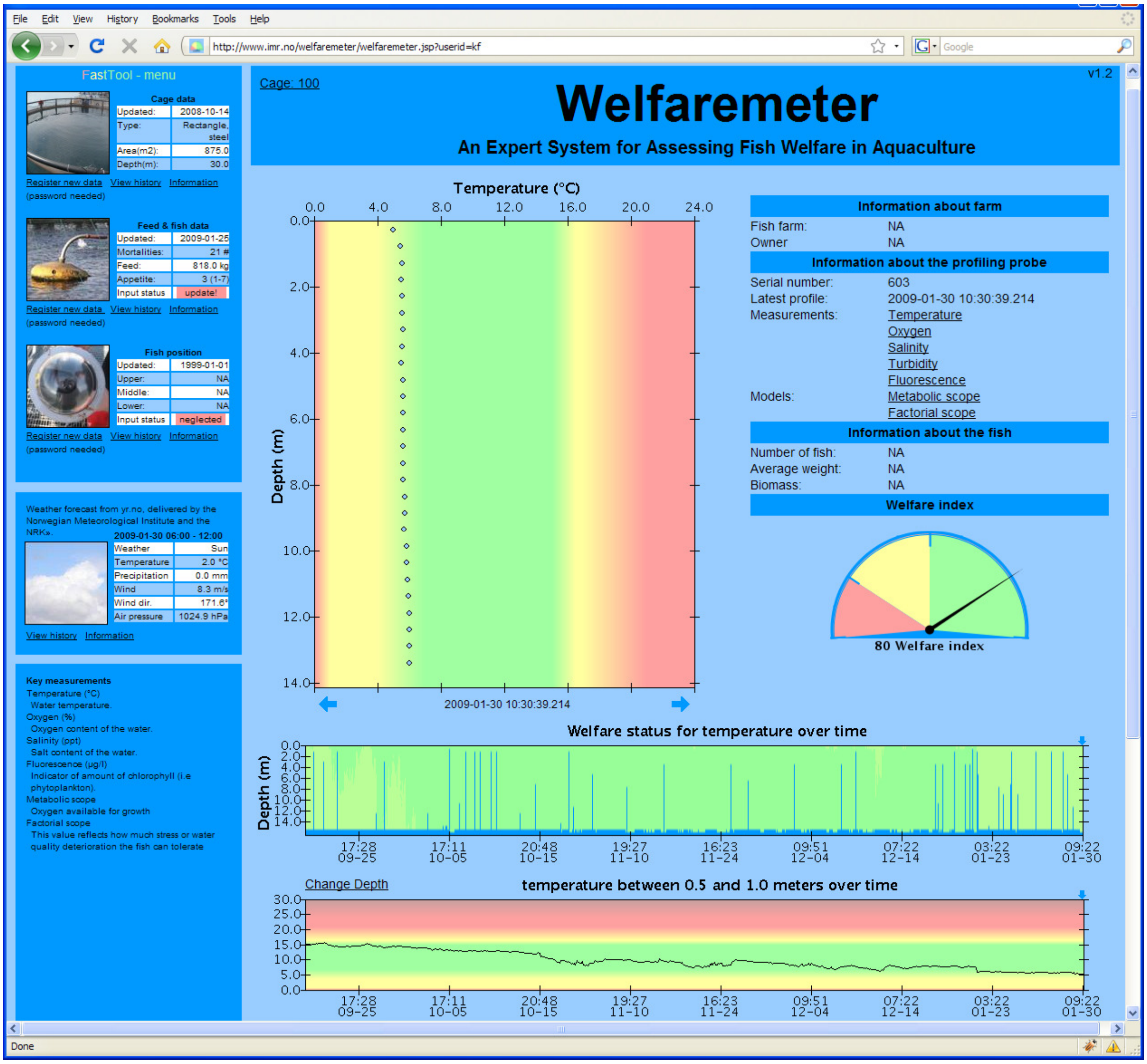

Figure 1.3.3.2-2: Screenshot of the FAST-TOOL v2 Welfaremeter web application. The main graph shows the result from the last CTD profiling of the sea cage from top to bottom. By clicking on the links to the right of the graph the fishfarmer can change between temperature, oxygen, salinity, turbidity, fluorescence, metabolic scope and factorial scope. The welfare index is shown as a dial to the right. The two lower graphs show the development of the selected parameter over time.

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1.3.4 WP2.2 Costs and benefits of implementation of fish welfare monitoring systems in the aquaculture industry

Contractors involved: LEI-WUR, IMR, HCMR

According to the specific role and aim of stakeholders with FAST-TOOL a segmentation was made based on desk research in farmers, suppliers, retailers/food services and other chain partners, NGOs, government, research, code-of-conduct organizations (CCOs). An abundance of information was available: publications, brochures, research reports, strategic plans, articles, books, contracts, and websites of both CCOs and interest groups that deal with aquaculture. The subsequent data collection process included two stages. First, a survey was performed about the intentional use of FAST-TOOL of fish farm managers by the attitude – behavior model. A questionnaire was developed based on literature and discussion with researchers and tested with an operational manager of a fish farm as well as a manager of several fish farms in Norway and Greece in 2008. Second, an initial implementation plan for FAST-TOOL was designed drawing on the governance literature, the survey and discussions with researchers. Finally, experts were asked to comment on the initial findings from desk research and the survey, and gave their opinions on strategic issues faced by the specific organization.

Adoption of FAST-TOOL can be achieved if fish farmers are legally obliged to apply the tool in their farm systems. A faster and probably more successful approach is to enable market forces to stimulate adoption through governance (governments collaborating with private actors). The aim was to develop an implementation approach for FAST-TOOL. In the current complex society an increasing number of organizations in society are concerned about aquaculture and fish welfare. All these stake-holders at some point will play a role in the implementation of FAST-TOOL. Stakeholders can be grouped in farmers, suppliers, retailers/food services and other chain partners, NGOs, government, research, code-of-conduct organizations (CCOs) who formulate criteria for fish welfare and sustainability.

FAST-TOOL requires the involvement and acceptance of this idea by chain parties, special interest groups, consumer organizations and government. Adoption of a technological innovation requires adaptation in culture (value and norms) and structure (routines, organizations, issuing etc.). In that case, implementation of a fish monitoring tool is a strategic process towards sustainable aquaculture development. Transfer of technology used to be a common approach to establish implementation. However, the increasing complexity of agricultural issues such as animal welfare and environmental conservation require a broad context of other stakeholders then fish farmers to have the new system contribute to sustainable aquaculture development. The implementation should acknowledge the role of stakeholders that have a financial and/or social impact on the system. Furthermore, it acknowledges the value of stakeholders sharing ideas and information among themselves rather than relying on direction or advice from government agencies or other professionals. Therefore, it will encourage ‘ownership’ both of problems and solutions and working together on further development.

Conclusion and discussion

FAST-TOOL is set up as a participatory approach that needs self-assessment of the fish behavior by fish farm managers. The challenge for conceptualizing FAST-TOOL is to find a way to incorporate different views of stakeholders while keeping the aim of improving welfare by monitoring. The products that can stem from FAST-TOOL are separated in four categories that are shown in the Table 1.3.4.1 The Welfare Network Levels. The whereto depends on the intentions, the major challenge will be the incorporation of multiple stakeholders. The structure shows the activities of the system: measuring welfare objectively (level 1), developing aquaculture (level 2), criteria formulating (level 3) and consumer marketing (level 4). The question of where to in levels 1-4 serves directly from the question who uses the information and who learns who sets the rules.

Table 1.3.4.1 Welfare Network Levels

	Level 1	**Level 2**	**Level 3**	**Level 4**
Objectives FAST-TOOL	Objective assessment of welfare	Education and human development	Certification and Bench marking	Marketing information and creating awareness for consumers
Data	Raw data 0-1000	Parameters 0-100	Criteria 0-10	Classes 0-1 (,2,3)
Stakeholders	Fish farmers Research	Fish farmers Extension Suppliers Government	Fish farmers NGO CCO Retailer Other chain	Fish farmers NGO Retail CCO
Approach	Assessment	Judgement in study group Extension	Defining values and criteria Creating incentive for competition	Overall welfare parameter Promotion
Key points *Results*	Efficiency of assessment Information	Impact of advice Improved technical results	Conflict resolution Improvement and/or retention of reputation	Product market combination Marketing with added value

Implementation of FAST-TOOL is a strategic process where monitoring of fish behavior and their welfare is also about politics, choices and directions, a social process. When welfare is important to all stakeholders there should be intention to incorporate multiple perspectives. This implies the formulation of common goals between stakeholders that establish an institutional arrangement that uses the information from the monitoring to develop certain products, facilitated by a platform.

Monitoring welfare is embedded in negotiations and collaborative decision making by groups of stakeholders, each using specific information from FAST-TOOL. The platform roles can be extended to deal with the adoption of new insights, monitor the progress and achievements about learning and verification, and find ways how farms with low welfare can be given temporary access and opportunities to market their fish. The platform needs to be supervised by a body to remain trust in the system, facilitate stakeholder processes and concur the conflicts between stakeholders. In addition, the body's role can be extended to deal with the adoption of new insights, monitor the progress and achievements about learning and verification, and marketing of welfare friendly fish.

Adoption of the tool can be achieved if farmers are legally obliged to apply the tool in their farm systems. A faster and probably more successful approach is to enable market forces to stimulate adoption through governance. Sustainable aquaculture development by the approach of a monitoring system will be a result of the dynamics and the interaction between entrepreneurs, supply chain organizations, societal organizations and the consumers. The involved stakeholders share the opinion that market parties need to be the initiators for improving welfare. In cooperation at the European level with the EWAC initiative a national daughter body can aim to fulfill the specific local needs, for example the extent towards government is represented in the body and is involved in the decision making.

The scientific validated information forms the base for the negotiations between stakeholders about the indicators. The state-of-the-art on understanding of welfare of fish is currently developing and new available technology will increase the understanding about the relationship between stressors and fish behavior. The information is to be extended with environmental data because most stakeholders regard the impact not solely of welfare but also the environment.

Scientific validated information does not automatically imply validation of the information by other stakeholders. Interpretation of the indicators will become important processes when developing products from FAST-TOOL in groups of stakeholders that hold different views on welfare and sustainability development. Market parties will departure from the standpoint for criteria formulating of consumer segments aimed at higher welfare part of a broader reconfiguration of quality both in response to consumer concerns and retailers and food services own commitment to quality and welfare and sustainability including these in their sourcing standards. Whereas, special interest groups will depart what is best for the fish in the trade-off between costs of welfare investments and to what extent these raise the level of welfare. Scenario analysis is a facilitating instrument that outline's the different paths of development and could provide the insight of the process underlying the path ways.

The development and implementation of a fish welfare assessment is also about sophistication of the technical aspects of the monitoring system. Managers of fish farms need to develop the habit to work with the system. Self-assessment puts large requirements on the managers' capabilities to perform monitoring and data entry on a daily basis. Farm managers' involvement can be enhanced by a participatory approach and providing education and human development and creating the awareness of fish welfare in society and the relationship with farm performance.

Building capacity and experience by all stakeholders about the approach followed by monitoring welfare needs priority at the first stage of development. Aimed first at the mantle of pioneers who function as a benchmark and to start communication about welfare with the aquaculture sector. Implementation is best be performed in a lee environment initially, giving the potential conflicting material at hand and the possibility of losing the required trust in the approach necessary for the intention to operate transparent. Gradual implementation is therefore preferred, also because of the effort put in the development of FAST-TOOL so far.

1.3.5 WP3.1 FAST-TEST Implementation, testing and evaluation of behavioral stress level indicators and use of FAST-TOOL in sea bass cage farms

Contractors involved: HCMR, IMR

A summary of the activities

1.3.5.1 MONITORING OF SEA BASS SEA CAGES

The evaluation of the FAST-TOOL application was performed during the on-growing of 4 populations; two in a pilot scale farm (Farm 1) and two in a commercial sea bass farm (Farm 2). The pilot scale rearing experiment were performed at the Hellenic Centre for Marine Research (HCMR) experimental sea farm located in the bay of Souda on Crete, Greece (Figure 1.3.5.1-1, Farm 1). The commercial sea bass farm (Figure 1.3.5.1.-2, Farm 2) was located at Fokida, mainland Greece and is owned by the NIREUS SA.

Figure 1.3.5.1-1: Sea bass cages at HCMR's experimental sea farm (Farm 1).

Figure 1.3.5.1-2: Commercial sea bass farm (Farm 2)

Farm 1: The testing of FAST-TOOL at Farm 1 started 05.10.2007 and ended 22.11.2007. During this time data about rearing practices, water environment, fish behaviour and fish appearance were entered into the application. Data was recorded for two sea cages of sea bass (Dicentrarchus labrax). The first population (11,800 individuals, 300g mean weight) was originated from mesocosm rearing, while the second population (11,700 individuals; 275g mean weight) was originating from intensive hatchery. The two populations were located in rectangular cages of 6x6 in perimeter and of 6m in depth, i.e. an approximate water volume of 216 m3. Fish density in the mesocosm cage was 16.3 kg/ m^3 and for the intensive sea cage 15.0 kg/ m^3. The two populations were offered commercial diets by hand feeding twice daily throughout the rearing period.

Farm 2: The testing of FAST-TOOL at Farm 2 started 26.09.2007 and ended 26.11.200. During this time data about rearing practices, water environment, fish behaviour and fish appearance were collected into the FAST-TOOL application. Data was recorded for two populations of sea bass. The first one was originated from HCMR, reared in mesocoms during the larvae stage, and the other from an industrial intensively producing hatchery. Both sea cages had depth of 10 m and a total water volume of 1350 m3. At the start of the experimental period the sea cage of mesocosm sea bass contained 47,865 fish (24g mean weight), while the sea cage of the intensive sea bass had almost similar conditions with 44,650 fish (27g mean weight). Fish density in the mesocosm sea cage was 0.8 kg/ m^3 and for the intensive one 0.9 kg/ m^3.

Water environment: The collected data about water environment presented variations in terms of temperature and oxygen but not in salinity. Temperature conditions at Farm 1 varied between a minimum of 23.0 °C and a maximum of 25.2 °C. Temperatures above 24 °C are considered as potentially stressful for the fish by the FAST-TOOL application. Ideal temperature for sea bass is around 19 °C. It is also worth noting that the temperature was slightly lower in the upper part of the water column compared to the lower. Temperature conditions at Farm 2 varied between a minimum of 22.0 °C and a maximum of 23.5 °C. At this farm no stratification between the upper, middle and lower part of the water column of the sea cages were noted. Oxygen conditions at Farm 1 varied between a minimum of 67 % and a maximum of 97 % saturation. Oxygen conditions of less than 80 % saturation are considered to be potentially stressful to the fish by the FAST-TOOL application. The low oxygen concentrations are related to high temperature conditions in Farm 1 and not to general bad water conditions as outside the cage oxygen saturation was at normal values for the period. A second parameter that affects further the measured values is the biomass in the cage (more than 15 kg/m3) which may be consider as high for cage farming. Oxygen conditions at Farm 2 varied between a minimum of 60 % and a maximum of 86 %. Both farms reported little or no stratification in oxygen saturation between the upper, middle and lower part of the water column. Both Farms report stable salinity throughout the experiment period. Farm 1 had a stable salinity of 38 ppt and Farm 2 a stable salinity of 39 ppt. Both these salinities are within the appropriate zone for sea bass set by the FAST-TOOL application.

Direct measurement of fish welfare: Data collected related to the direct measurement of fish welfare were the manual welfare index, and the number of dead fishes. The welfare index is a subjective rating by the fish farmer of the welfare of the fish. The index has a scale from 0 (terrible welfare) to 100 (excellent welfare). The manual welfare index at Farm 1 was constant set at a score of 60 for both mesocosm and intensive populations. For Farm 2 the score varied between 80 and 100. The mesocosm sea bass at Farm 2 had a mean rating of 96 ±2 (s.e) and the intensive sea bass a mean rating of 90 ±2 (s.e)($p=0.031$, paired t-test). At Farm 1 the dead fish was collected and counted on a daily basis, while they on Farm 2 were collected at irregular intervals. Since data was not put into the FAST-TOOL application on a daily basis the mortality data became difficult to interpret.

Feeding behaviour: All the fish were reported to display high anticipatory behaviour before feedings, high feeding activity and high surface activity during feedings. These behaviours are associated with good appetite and low stress level. One interesting observation was that the sea bass in Farm 2 which had been reared under intensive conditions as larvae repeatedly displayed lower feeding activity and surface activity during feeding compared to the mesocosm fish. This was supported by the feeding data: There were little difference in the amount of daily feed between the two populations at Farm 1, but the mesocosm sea bass was on average fed slightly more than the intensive (29.1 kg ±0.7 (s.e) vs. 27.8 kg ±0.9 (s.e) , $p=0.001$ paired t-test). Although this difference was small at Farm 1 it was distinct at Farm 2, where the mesocosm population was fed on average 38.6 kg ± 1.1 (s.e) while the intensive population was fed on average only 30.2 kg ± 0.3 (s.e) ($p<0.001$, paired t-test).

Fish distribution: Fish distribution was monitored by the farmer to indicate if the bulk of the fish were situated in the upper, middle or lower part of the sea cages. In general at both farms the fish were positioned in the upper and middle part of the sea cages. Near the end of the period part of the population in both pens at Farm 2 moved near the bottom. At this time the oxygen conditions were also higher (96% saturation) in the lower part of the cage compared to the middle and upper part of the sea cage (91%

saturation). When the oxygen conditions again become equal at the end of the period the fish return to the upper and middle part of the sea cages.

Fish behaviour: Several specific behaviours were also monitored by the farmers by rating the amount of fast swimming fish , slow swimming fish, fish standing still against current, shoaling fish, fish schooling in a circular pattern, fish schooling in a tornado patter (tight circle),fish standing against bottom, fish standing against surface, fish swimming in a random fashion, fish swimming with a clear direction, looser fish (fish standing by themselves outside the main body of fish), wall syndrome (fish swimming slowly very close to the net wall), fish standing in corners and aggressive fish. The objective was to correlate any of these behaviours with high or low welfare. The data showed little difference in the behaviour adult mesocosm and intensive fish at Farm 1, but there were clear differences in the behaviour between the juvenile mesocosm and intensive fish at Farm 1. This is as expected since juvenile fish are likely to be more influenced by what they experienced as larvae compared to more adult fish. All in all, this confirms that fish behaviour is indicative of fish welfare and stress-level.

Fish appearance: Data on fish appearances included the amount of looser fish (thin stick like fish), deformed fish, fish with splitting fins, fish with heavily worn fins, fish with missing eyes, fish with small wounds, fish with large wounds and the amount of fish with other clear symptoms of disease or damage. Of these appearances only occurrence of looser fish and deformed fish were recorded during the FAST-TOOL test. Results showed that Farm 1 had occurrence of both looser fish and deformed fish, while no such fish were observed at Farm 2. Both these appearances are linked to poor welfare by the FAST-TOOL system.

Biomass increase: The cumulative biomass increase, as percent of initial biomass, presented clear differences between the farms. As mentioned before, this is mostly related to the differences in the initial mean weight of the individuals at the two sites. In farm 1 the mean weight of the individuals was near to 300 g while the populations in Farm 2 had a mean weight of 25 g. This difference has a significant effect in the growth performance and also in feed assimilation. Younger individuals perform better and therefore any direct comparison between the farms is not possible. However, there are also differences between the populations within each farm. At Farm1 the mesocosm and the intensive populations had a total cumulative growth of 7.2 and 8.9 % respectively. A more clear difference was presented in Farm 2, as the mesocosm and the intensive populations had a total cumulative growth of 48.3 and 39.4 % respectively. Feeding behaviour indicated to the FAST-TOOL system that the mesocosm sea bass at Farm 2 were doing better than the intensive sea bass. This is also supported by the cumulative biomass increase. It is 9% higher for the mesocosm sea bass at Farm 2 compared to the intensive sea bass.

The general evaluation of the applicability of the FAST-TOOL in the sea bass farms differed according the evaluation site.

Evaluation of FAST-TOOL at Farm 1: In Farm 1, the observations of rearing practices, water environment, fish behaviour and fish appearance was performed by a highly skilled technician. Even though the technician had the competence to implement his task, as there was no internet available at the farm the online character of the application was lost. An Excel spreadsheet, easy to print, was constructed for data entry purposes available both in English and Greek. The situation is expected to change in the near future with available internet at Greek fish farms.

Farm 1 was well equipped to support FAST-TOOL as there were available underwater cameras and an echo sounder system, being thus possible for the technician to observe the behaviour and appearance of the fish. There were also equipment for measuring water temperature, oxygen saturation and salinity.

Regarding data collection, the person responsible for this was also involved in other activities and therefore it was not possible the daily involvement with the system. This resulted in a relative low compliance as almost 50% of the working days data was collected and entered into FAST-TOOL. This underlines the need for a clearer dedication from the farm in order to appoint the responsible for entering data into FAST-TOOL. Otherwise data may be missing due to practical reasons.

Evaluation of FAST-TOOL at Farm 2: In Farm 2, the observations required for FAST-TOOL was performed by one of the technicians working at the farm. This person was trained by the technician doing the FAST-TOOL registrations at Farm 1. As in Farm 1, there was no Internet connection available at Farm 2, so the same Excel spreadsheet prepared for Farm 1 was used. Although a potential source or error was the unfamiliarity of the technician performing the data collection with computers, due to the general data collection system followed in Farm 2 this technician was indeed familiar in collecting data in sheets manually. Following data collection the paper sheets were faxed to Farm 1 for data entry in the online FAST-TOOL system. It is clear that if FAST-TOOL is going to be a success in the future internet must be available on farms and the users must be familiar with computers.

Farm 2 was well equipped to support FAST-TOOL as there were available underwater cameras and an echo sounder system, being thus possible for the technician to observe the behaviour and appearance of the fish. There were also equipment for measuring water temperature, oxygen saturation and salinity.

Data collection was almost similar as in Farm 1 as again data were collected 50 % of the working days. Several are the reasons for this, the most important being that FAST-TOOL was no in the daily routine of the farm and therefore data collection was performed when possible. This relative low compliance again underlines the need for higher commitment from the farm management and the involvement of a several individuals for entering data into FAST-TOOL.

Conclusions: The test showed that it is possible to register data about fish behaviour and fish appearance not only in a pilot scale experimental farm but also in a commercial one. In the future, when internet connection is available, not only data entry will be easier but also the response of the system will be available engaging further the farmer in the use of the tool. It will also be advantageous to limit the number of inputs to FAST-TOOL. This is likely to increase compliance as the work will be less. The tests were therefore on a whole positive for future use of FAST-TOOL.

1.3.5.2 BEHAVIOUR AND PERFORMANCE OF INTENSIVELT AND MESOCOSM REARED JUVENILES DURING ONGROWING IN SEA CAGES

Intensively (500l tanks, at start 100 eggs*l^{-1}) and *Mesocosm* (40m^3 tanks, at start 8-10 eggs*l^{-1}) reared sea bass juveniles were monitored for their behavior and performance until marketable size was reached. Monitoring started from hatchery stage, during the initial pre-growing phase and continued during ongrowing in cages. The first stage of monitoring in hatchery was implemented in the HCMR facility, while the subsequent monitoring in cages was implemented in two different farms located at Souda, Crete and Nafpaktos, continental Greece. The Souda farm was a pilot scale experimental farm, while the Nafpaktos one was an industrial one. The populations monitored in Souda were originated from the intensive and Mesocosm hatcheries of HCMR while the populations in Nafpaktos were originated from a commercial intensive hatchery and from the Mesocosm HCMR hatchery. The populations were evaluated during different developmental stages and under different conditions. During the hatchery phase the behavior of the populations were monitored using video recordings and estimation of velocity and distribution in the tanks water column. Additionally samples for hormonal estimations were also taken. During the cage rearing echo sounders were used and the parameters controlled were the mean position of the biomass, the exhibited displacements of the population and the space occupied in the cage.

The first evaluation was implemented **during the transport** from the larval rearing tanks to the pre-growing facility. The results of this experiment showed a clear difference in the response of the two populations. In terms of their velocity individuals from *Intensive* rearing have at the first half of the observation period an almost constant velocity of 4 BL s^{-1} that was increased to 6 BL s^{-1} at the second half of the observation period before starting decreasing again. During the first period individuals show disoriented with changing directions, while the second half they present a constant direction in their motion. On the contrary individuals from *Mesocosm* origin exhibited a constant decrease in their velocity from 4 to 2 BL s^{-1} during the observation period. The *Mesocosm* individuals showed also continual changes in their direction showing a higher disorientation and lose motion. Regarding their distribution in depth, fish from *Mesocosm* had a more wide and normal distribution in the water column while individuals from *Intensive* rearing occupy mostly the upper layers of tank. This is probably because of the significant increase in light intensity. Regarding the cortisol levels in the tissue of the individuals no significant different was noticed between the two rearing systems.

During pre-growing populations, originated from *Mesocosm* and *Intensive* hatchery, were monitored during calm and stressed conditions. They presented differences depending on their origin during calm conditions with the individuals from *Intensive* conditions to have a higher velocity than the *Mesocosm* ones. When stressed, individuals from *Mesocosm* increase their velocity and lose their direction, while the intensively reared maintain their velocity. Following the stressing period individuals from both systems recover within approximately one hour to exhibiting similar behaviour as during calm conditions. Regarding their distribution pattern juveniles during feeding, independent on the rearing method, create a main group near the bottom where they stay throughout the period. When stressed, individuals from both rearing systems, stay at the lower layers of the water column. During periods of high current (mechanical stress) individuals from *Mesocosm* are more likely to create patches in the middle of the tank. The populations under observation at the hatchery stage that were exposed to different stressors did not present any difference in terms of growth from others reared with similar conditions. Significant growth difference was present between *Mesocosm* and *Intensive* system during the hatchery stage with the Mesocosm individuals having a much better performance

During ongrowing the evaluation of populations was focused on periods of potential high risk such as the reproductive period of the sea bass in February, the high temperature periods in August September, and the periods of the first rains in November when potentially polluted freshwater runoffs may affect the farms.

<u>During the period of reproduction </u>in February there was almost no significant difference between the populations reared in Souda while some differences in the mean position were presented in the Nafpaktos reared populations. There are differences in the response of the populations when compared with periods outside the reproductive season. The Souda populations during the reported period had a mean position closer to the surface in all tested conditions and independent from their origin. The Mesocosm originated individuals were showing smaller movements while on the contrary the ones from intensive hatchery were presenting bigger movements. This pattern was similar for calm conditions and during feeding. No difference was observed on the response of the individuals during stress. No difference was observed related to the occupied space independent from conditions or origin.

<u>In the high temperature period</u> during August/ September in Souda during August the temperature reached 27 ^{o}C while in Nafpaktos during September temperature reached 24 ^{o}C. The populations from different origin in Souda presented significant differences in their mean position and occupied space but not in displacement for all tested conditions. For the populations reared in Nafpaktos there are differences only in mean position in depth for all tested conditions. When the responses are compared with ones presented outside the considered period then the mean position of intensively reared populations in August was closer to the surface during calm periods while the Mesocosm originated populations presented the same

difference when stressed. The displacements of the populations, under all conditions, were smaller during the warm period and this was more obvious for the mesocosm population. Regarding the occupied space, it was smaller, in all tested conditions, again during the warm period. These differences show that the populations during warm period are less active and that the Mesocosm originated individuals are more sensitive to these "extreme" conditions.

During the high risk periods due to freshwater runoffs in November, the comparison of populations from different origin show differences regarding the position and the occupied space for the individuals reared in Souda. For the populations reared in Nafpaktos almost all parameters are different during the calm period and when stressed. Comparing the response of the populations reared in Souda with the ones outside the reported period then differences are noted regarding the mean position of the populations that is bigger for the Mesocosm populations during stress conditions and for the intensive population during the calm period.

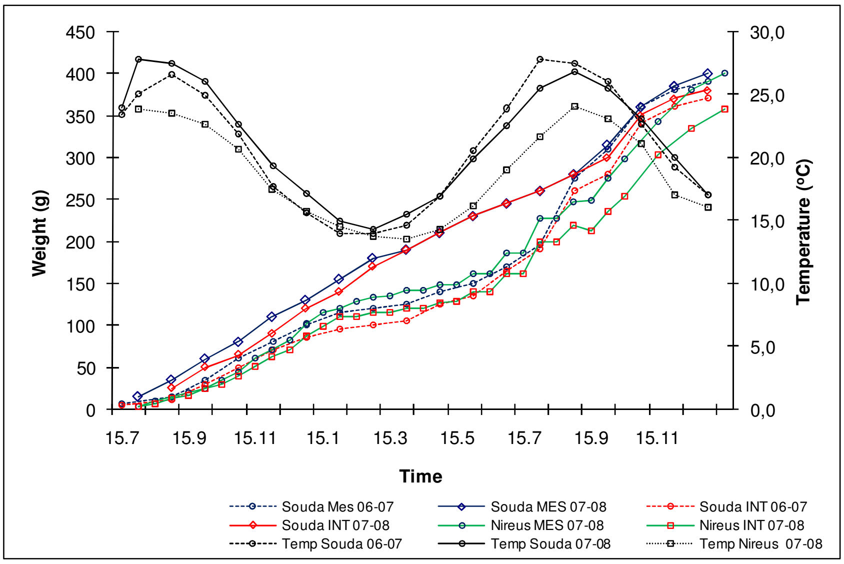

Figure 13.5.2-1. Growth performance of the populations reared at HCMR and Nireus facilities. The monthly temperature variations in the two locations are also plotted.

Regarding the **growth performance**, there were no differences that could be related to the acute stressors applied as all population presented the expected growth. The major difference in growth was observed in the industrial scale farm in Nafpaktos between the populations from Mesocosm and from the commercial intensive hatchery, where the Mesocosm fish grew better. In the Souda farm the intensive fish were slightly smaller than the mesocosm groups, but there were no significant differences in growth between the intensive and mesocosm fish.

Discussion - Conclusions

There are observed differences in the behavioral pattern of fish coming from different hatcheries. The most prominent being the ones at the hatchery stage when the individuals exhibit differences both in their behavior but also in their growth performance.

During ongrowing the differences are apparent during the "extreme" warm period and during the reproductive season. These differences were expressed mostly in the reaction (displacement) that the population presented and also their position in the cage.

The difference in growth performance between the populations reared in the industrial farm is significant, but in this case could this not be assigned only to larval rearing method. Since the two populations were originated from different brood stocks and there were no parallel cages, also genetically and random effects could be involved in addition to larval rearing method. However, long experience with mesocosm reared fish sold to fish farmers have shown low degree of deformities, higher ratio of females and better growth in cages.

1.3.6 WP3.2 FAST-TEST Implementation, testing and evaluation of behavioral stress level indicators and use of FAST-TOOL in Atlantic salmon cage farms and research facilities

Contractors involved: IMR, NSVS

The main objectives in WP3.2 were to develop and validate operational protocols for stress screening in different life-stages of salmon farming, and test and evaluate the practical use of FAST-TOOL and the behavioural stress level indicators developed in WP1.2, both in a new research station experiments and in commercial cage farms. To validate if suppressed immune response could be used as a validation method for chronic stress, a new experiment was set up with assumed higher stress level than in the WP2.1 experiments. At last we wanted to test evaluate the prediction power of FAST-TOOL in commercial salmon farming and semi commercial experiments

1.3.6.1 TESTING AND EVALUATION IN SEA WATER TANKS AT RESEARCH STATION TORE

Based on the experiences from WP1.2 a new experiment was designed to test and evaluate effects of production intensity on behavioural indicators and immune response in production scale experiments with good environmental control. In this experiment we wanted to evaluate the practical use of FAST-TOOL by the research technicians and the registration protocols used. Salmon originating from the same egg group was exposed to conditions simulating different intensity of production (5m^3 tanks, 450 fish in each, triplicate groups, 16°C in A-C):

- **A** (0^+ smolt, control): high water flow (60l/min, DO at outlet aprox. 90%,)
- **B** (0^+ smolt, hyperoxia), high water flow (60l/min, DO around 170% saturation)
- **C** (0^+ smolt, hypercapnia): low water flow (10l/min, 5-20 mg/l CO_2, DO aprox. 90%,)
- **D** (1^+smolt, low temperature): high water flow (60l/min, DO at outlet aprox 90%), and low temperature (6°C)

A protocol for behavioural screening using FAST-TOOL was set up at regular intervals for one tank from each of the four treatment groups. All tanks were monitored with surface and under water video cameras. All feedings were video recorded, starting 5 minutes before feeding. Temperature and oxygen in outflow water was registered automatically every 30 min by sensors placed in the outflow water, and stored in a separate database. To study behavioural and appetite effects of acute stress the fish were given acute stress events during the experiment (chasing with brush for 20 min, 30 min before first feeding, on three occasions).

Fish performance

The mean growth rates of the hypercapnia and hyperoxia groups were reduced to 62% and 88%, respectively, of mean growth rate in the control groups, showing significant negative effects of the low water flow and hyperoxia compared to control groups. Because of problems with the adjustment of DO and temperature, some of effects was probably also caused by the variable conditions in oxygen and temperature. As expected the cold water 1^+group (6°C) grew only 15% compared to control. The condition factor of the hyperoxia and hypercapnia groups was also significantly lower. There were few observations of severe fin injuries and no difference between groups, and almost no mortalities in any group.

Fish behaviour

The horizontal group positioning and individual swimming behaviours asked for in FAST-TOOL were found to be useful behavioural indicators of both acute and chronic stress levels. The hypercapnia fish performed differently than the control also in behavioural terms, were found to have a horizontal distribution closer to the tank wall than the control and the hyperoxia fish, which may be explained by a preference for positioning in the inlet water with low CO_2 content that was distributed parallel to the tank wall. The swimming behaviour was variable between days, but the hypercapnia and to a lesser degree the hyperoxia fish were found to be more active compared to the control as they had an all over higher frequency of position shifts, defined as a behaviour of active swimming to reposition. We interpret this behaviour as fish seeking away from the aversive conditions by moving, and consider it as good candidate for assessment of stress levels in groups of fish.

Acute stress

There were evident and long time responses to the chasing stressors in form of altered swimming behaviour and reduced feed intake. The majority of the fish had baseline behaviour of swimming against current (stationary position), in a donut shaped school close to the tank centre in the control groups, and in a dense group towards the tank wall in the hypercapnia groups (Fig 1.3.6). The school structure dispersed during the stressor as the fish started swimming in an erratic manner close to the tank bottom and towards the tank wall. The individual swimming behaviour the hour after the stressor was characterized by a high degree of position shifts, variable swimming direction, and high swimming activity compared to before stress. Over the next hours the fish returned gradually to less “chaotic” behaviours as they mainly kept a stationary position towards current, but remained swimming with greater horizontal spread compared to the baseline schooling behaviour. All the tanks had regained their baseline behaviour at the measurement at 8.25 hours after the stressor. The control groups recovered faster than the chronic stressed groups. The feed intake in all groups was significantly reduced on the stress day, and was also reduced the day after stress compared to the day before stress, in line with our earlier results of chasing in salmon presmolt in WP1.2.

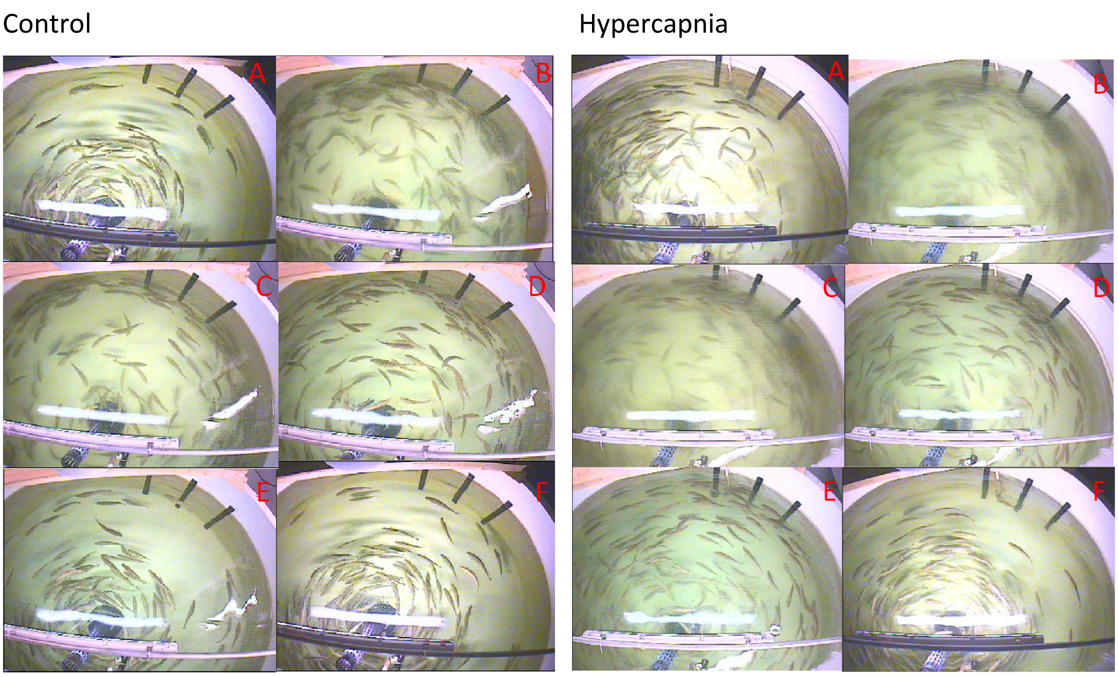

Figure 1.3.6 - 1 Swimming behaviour in salmon pre-smolt before and after a stressor of chasing for 20 min in control group (left section) and hypercapnia group (right section). Time after finished stressor (indicated in picture): A=Baseline, B=2 min after stress, C=15 min after, D=2.25 h, E=4.25 h, F=8.25 h.

Immune response

Analysis of Vibrio specific antibodies by ELISA revealed high titres after vaccination in all groups kept at 16 ºC. The use of a commercial oil-based multivalent vaccine caused equal titres as observed in WP1.2 Exp.5. However we did not observe statistical significant differences between the fish held in normoxic water compared to the groups of fish subjected to either hyperoxia or hypercapnia. However the effect of water temperature was obvious, as vaccinated fish kept at 6 ºC were not statistically different from the non-vaccinated controls at day 44 post vaccination.

Evaluation of the use of FAST_TOOL

The WP3.2 experiment presented here was the first test of the FastTool web application after it had been improved after the first pilot tests. In this test data were recorded nearly every day by the staff at the station, and we concluded that the changes prompted by the first test were a success. The main negative feedbacks of the users were:

- too many behavioural categories, some categories were seldom observed and seemed irrelevant
- better definition of the categories and training of the users in how to judge and observe the behaviours were necessary,
- too little feedback of results made it little rewarding to enter data

The last comment was not surprising, since we at this moment had focused on the input part of the database and the expert system was not yet developed. We also came aware of that we had a problem with two conflicting strategies. In this version we wanted the user to judge different behaviours as objectively as possible without saying anything of what was good or bad. We then could evaluate if the high stress treatments gave different results from the control groups, and then later use the behaviours that was most different as stress level indicators. In a more developed version the expert system should be based on validated behavioural indicators that indicate high or low the stress level. The user would then get immediate feedback on stress level in the tanks, but to reach this level we had to be able to judge and verify the different behaviours in relation to stress level.

The observation method where the observer classified the presence of defined behaviours by operating a slide-bar was found useful, but have potential for refinement. The scale of grading on the slide-bar system was interpreted differently between test persons. Grading by approximate percent points was found to give the most consistent results between days and also between observers, and should therefore be considered as the most rigid standard of observation. Different approaches did also bias the all over assessments of activity level and grade of chaotic swimming. These important measures may instead of a direct assessment of the observer be automatically translated in the registration tool by combining scores for the less subjective assessments of fish swimming stationary on current, drifting with current and fish shifting position, and thereby give all over scores that are easier to interpret for the user. A reduction in behavioural categories, combined with better feedback will hopefully increase the motivation for using the system as well.

Conclusion

There were clear differences in growth and behaviour between groups with different chronic stress level, and in feed intake and behaviour before and after acute stress in the same groups. There were no significant differences in growth or behaviour between groups within the same treatment. The behavioural indicators implemented in FAST-TOOL were able to detect differences between caused by chronic and acute stress. The user interface of the database for monitoring of behaviour was evaluated as easy to use and understand, but better training and consensus on how to interpret the indicators should be given before start of the registrations. Better and fast feedback of the interpretations of the input should be given.

There were found no effects of hypercapnia or hyperoxia (chronic stress level) on immune response towards a *Vibrio* vaccine, indicating that the salmon reduce growth before immune response. However, low temperature (6°C) reduced the immune response completely.

1.3.6.2 TESTING AND EVALUATION OF FAST-TOOL IN SEA CAGES AT COMMERSIAL SALMON FARMS

In order to evaluate FAST-TOOL in commercial production sites of salmon, the FAST-TOOL application was tried throughout two complete production cycles of Salmon at two different salmon farms along the Norwegian coast.

The first farm consists of twelve sea cages and a barge with feed hall, workshop and offices. The offices are equipped with several computers with internet connection. It should therefore be easy for the fish farmers to access the FAST-TOOL internet application.

A production cycle of a new generation of salmon started at this farm 9th of June 2007. The goal was to use this production to test FAST-TOOL and further develop FAST-TOOL, i.e. based on input and feedback from the fish farmers at the site to improve and further develop FAST-TOOL. During this experiment data from four sea cages were entered into the FAST-TOOL-application. This data included information about rearing practises, feeding, water environment, fish behaviour, fish appearance and disease. In addition to the manual input, data from a profiling CTD (SAIV MINI STD/CTD – model SD204, SAIV AS, Norway), an echo sounder system (Lindem Data Acquisition, Norway) and software used at the farm (FishTalk, Akva group) were uploaded to FAST-TOOL.

The first prototype of FAST-TOOL was presented to the fish farmers at the farm in June 2007, and one of the fish farmers at the site was assigned the task to enter data into FAST-TOOL. The fishfarmer was also going to suggest improvements to the FAST-TOOL-application.

Although the fish farmer displayed enthusiasm about the project, in reality the compliance for entering data into FAST-TOOL on a daily basis was poor. In retrospect the first versions of FAST-TOOL were too detailed and complicated. The fishfarmer was asked to enter data about large number of different variables about the fish, water environment, fish behaviour and fish appearance on a daily basis. At the same time FAST-TOOL gave little back in the form of stress level assessment, and it was too few reports. Also; some of the other software used at the farm asked for some of the same parameters as FAST-TOOL. Having to enter data twice (both in FAST-TOOL and the other software) was reported as being discouraging and irksome.

In response to the feedback from the fishfarmer FAST-TOOL were continuously being updated. This inevitably meant that there on occasions were bugs, lowering the fish farmers' confidence in the software. Especially grievous were bugs that meant that after entering data into a dialog the data was not stored. Finally there were problems with the firewalls at both the local net at the farm (the farm was connected to the internet through Marine Harvest's company net) and the firewall at the Institute of Marine Research were FAST-TOOL is located. . These problems were not consistent, some days they appeared while other days they were completely absent. This made finding the source of the problem difficult. However, in the end it was revealed that the problem was a combination of the settings of the local firewall and the settings of server at the Norwegian Marine Data Centre (NMD) where the FAST-TOOL web application and database were located. As soon as we found this out we were able to change the setting of the server at NMD, and since then these problems have not re-emerged.

In short, the lessons learned from the test at Farm 1 about how to construct the next version of FAST-TOOL were:

1. There should be as little manual input as possible
2. If possible all uploading of data should be done automatically, independent of man.
3. The user must get immediate and useful feedback from the system.
4. The system should be as independent of software and internet services at the farm as possible.
5. The website should be as simple and intuitive as possible.
6. The website must be automatically updated at least daily; otherwise it is not interesting for the fish farmer to open the website.

7. Historic data and analysis of these should be easily available.

Based on these lessons it was decided to make a completely new version of FAST-TOOL. This new version has been, and continues, to be tested at another salmon farm. The technological development of the new necessary equipment was made possible through additional financial support from Norwegian research funds and run as parallel project (details not reported here).

This second farm consists of eight 35x25 steel cages and a barge with office and workshop. The office is equipped with several computers with internet connection. It should therefore be easy for the fish farmers to access the FAST-TOOL v2-internet-application, called WELFAREMETER. This application is open for anyone to look at (www.imr.no/welfaremeter, to enter data it is necessary with username and password). A production cycle of a new generation of salmon started at the farm 29'Th of October 2007 and is expected to last until June 2009. The FAST –TOOL v2 – Welfaremeter system has been running on the farms since day one of the production.

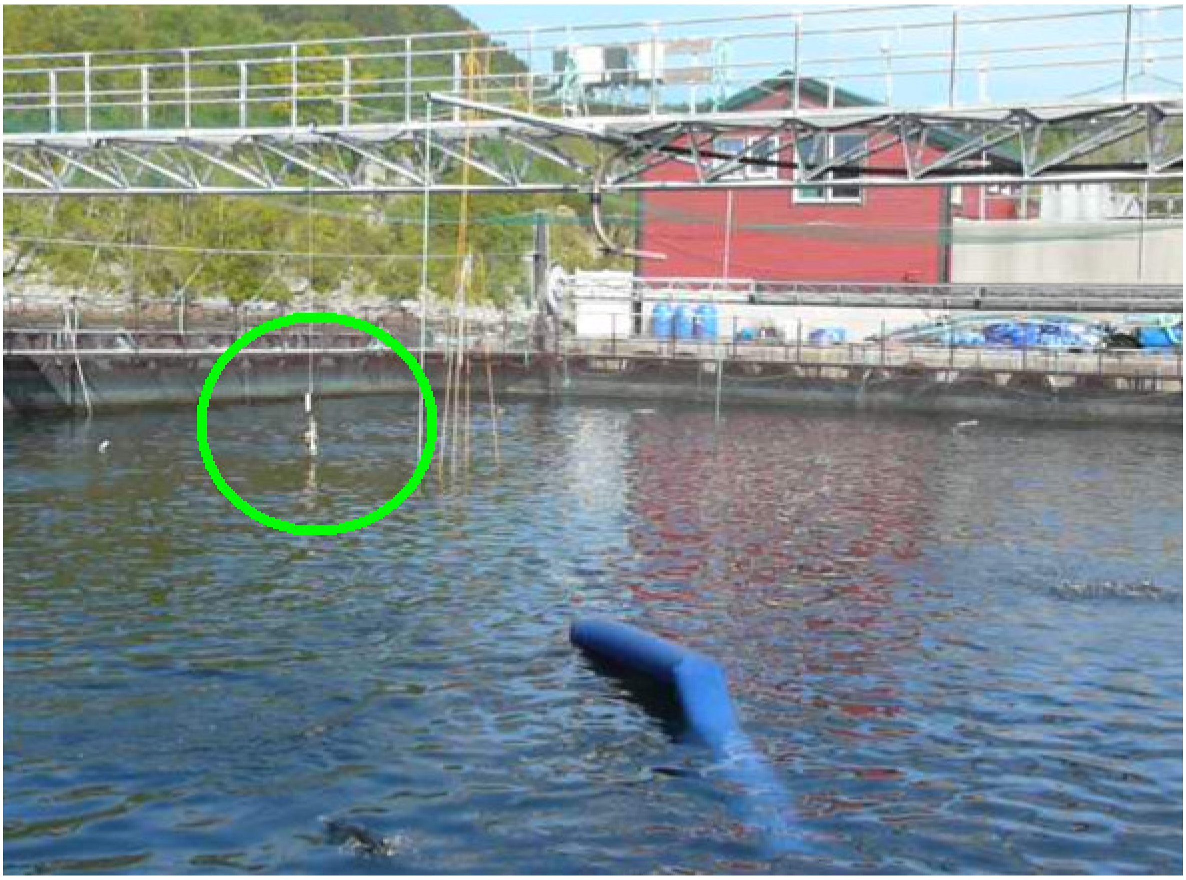

Figure 1.3.6.2-1: The sea cage at the Brattavika salmon farm which is equipped with the Welfaremeter system. The CTD-probe is encircled with a green circle. The winch and control unit stands on the bridge above the sea cage.

In short the Welfaremeter system consists of a profiling probe (CTD, 1.3.6.2-1), a control unit, a database, an expert software program and an internet webpage. The probe measures temperature, oxygen, salinity, fluorescence and turbidity for each half meter downwards in the cage. The control unit directs how often the probe performs a profiling and sends the measurement data via the mobile phone network (GPRS) to a database at The Norwegian Marine Data Centre, Institute of Marine Research. These data are then analysed by the expert software which gives an evaluation of the environmental conditions in the cage as either very good, good or potentially harmful for the fish, base on published knowledge about salmon preferred environment and tolerance areas. In this test the probe is set to do a profiling every two hours.

At any time the fishfarmer can go to the FAST-TOOL v2 –Welfaremeter website and see the temperature (1.3.6.2-2), oxygen, salinity, turbidity and fluorescence conditions in the sea cage. In this way it is always interesting to enter the website; it contains updated data.

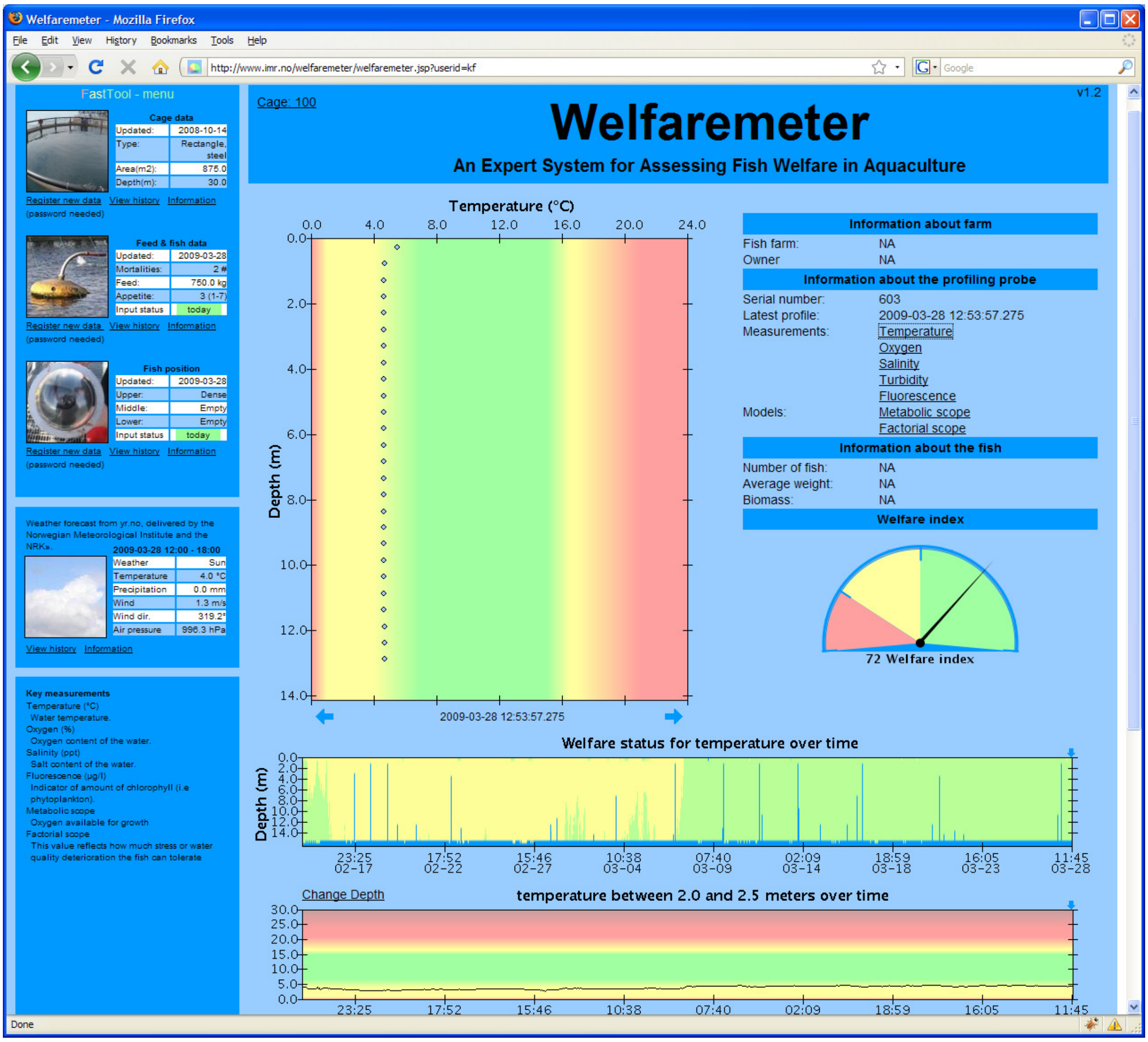

Figure 1.3.6.2-2.: The Welfaremeter internet page (http://www.imr.no/welfaremeter). The user has selected to view temperature (March 28, 2009). The main graph shows the current temperature from top to bottom of the sea cage and the two other graphs show welfare and temperature the last 43 days. By pointing at any part of this history plot you get a vertical plot from this day and time. Also by pointing at the dots on the vertical plot a sign with the exact values will show up.

The response from the fish farmer has been very positive. According to his own statements he is looking at the FAST-TOOL v2 – Welfaremeter webpage several times a day. Encouraged by this we have started to add improved dialogs from FAST-TOOL v1 onto the FAST-TOOL v2 – Welfaremeter webpage. These dialogs can be accessed through the FAST-TOOL-menu positioned on the left side of the page (Figure 1.3.6.2-2**Feil! Fant ikke referansekilden.**). Currently, there are dialogs for adding basic data about the sea cage (type, area and depth), basic daily data about the fish (number of dead fish, amount of feed and a subjective appetite assessment), and data about fish position before and during feedings. To encourage this input the last input is always visible and a status box indicates whether the input is updated or not. This approach has so far yielded good results. The fishfarmer has so far been entering data into the system several times a week, some weeks every day since the dialogs were made available in the beginning of September 2008. From the first version of FAST-TOOL we feel we have got significant progress, and if we look back on the demands from the first test we now have the following improvements:

1. Amount of manual input is reduced and made simpler. Improved protocols and instructions for registration of fish behaviour and behavioural stress level indicators are needed.
2. Environmental data are logged by a profiling probe measuring salinity, temperature, dissolved oxygen, turbidity and fluorescence. The data are uploaded automatically to the FAST-TOOL database every 2 hour, and has given stable data input for more than a year. Automatic uploading of data from other farm control software and echo sounders are still lacking.
3. The user gets immediate and useful feedback from the system. Improved overall welfare assessment needed.
4. The profiling probe is independent of software and internet services at the farm using only mobile phone network. User interface available everywhere as long as connected to internet.
5. The website is tried to be as simple and intuitive as possible, but still need improvement.
6. The website is updated as soon as new data are entered from loggers or manually. Input data are also immediately analysed before presentation; fish farmer visits web site every day.
7. Historic data and analysis of these are easily available and presented. Generation of an overall welfare assessment analysis and documentation report is needed.

The FASTFISH project has contributed a lot to the development of a web based database and expert system for assessment of environmental conditions, fish behaviour and performance, but there is still away to go to get a functional system that will be implemented by the industry. During the project we have learned that it is of no use to try to implement a premature system, which will be judged negatively and not used by the industry. We still need a period with further development of the monitoring technology, find ways to automatically integrate all available farm data, further develop models and analysis methods, and increase the user's interest and knowledge about observation of fish behaviour and performance, and data collection in general. During 2009 and 2010 we will try to solve many of the remaining challenges in a follow up project financed by the Norwegian research council and The Norwegian fisheries and aquaculture industry research fund, and will be carried out by IMR, NOFIMA Marin, University of Oslo, and industry partners.

1.4 Summary of end results, conclusions and recommendations

1.4.1 Summary of end results and main achievements

The FASTFISH – on Farm Assessment of STress level in FISH - project has been a combination of basic and applied research, also including small study of societal issues related to implementation of monitoring and documentation tool. The main goal of WP1 was to study behavioural changes related to acute and chronic stressors in different developmental stages of both Atlantic salmon and European sea bass, and search for behavioural indicators that can be used as operational indicators of stress level. An important part was also to study at physiological and immunological responses to the stressor to be able to compare behavioural and physiological responses. In WP2 the goal was to develop an internet based database and expert system for on farm monitoring of rearing environment, potential stressful events, fish behaviour and behavioural stress level indicators, and fish performance. In WP3 the implementation of the tool was tested and evaluated both in “production scale” research experiments and in commercial farms.

BEHAVIOURAL INDICATORS IN SEA BASS

Several larval rearing experiments were performed in two different rearing systems: Intensive “high stress” (high stocking density, small tanks) and Mesocosm “low stress” (low stocking density, large tanks with more natural environment). Large amounts of video footage have been recorded by the project. New innovative video image analysis methods have been developed both for sea bass and salmon to study behavioural changes related to stress, including swimming speed and direction, and vertical and horizontal distribution.

In sea bass, the behavioural ontogeny from hatching to juvenile stage was described for larvae reared by the two different methods. There were marked differences in growth rate, swimming performance and speed, and feeding behavior, between fish reared with different methods. Intensive reared fish had a delayed behavioral ontogeny and growth compared to mesocosm reared fish.

In tanks the velocity, swimming pattern and group structure, and the vertical distribution of the larvae were behavioral indices that can be used to describe their stress level. The fish reared in mesocosm were responding more intensively during stress by more rapid change of their velocity and position in the tanks. In cages the mean position of the shoal, the displacement of mean position and the spatial distribution during and after stress and, measured by echo sounder, are behavioural indices that can be used to assess stress level and latency of stress reactions. Also here fish originating from mesocosm larval rearing presented more intense reactions than fish originating from intensive rearing system. However, sea bass of intensive and mesocosm origin had similar growth and mortality rates during ongrowing in cages.

Use of electronic self feeders to measure feeding motivation, showed that sea bass during ongrowing recovered fast from acute stress (lowering of water level, air exposure). Overall the study highlighted that feeding and swimming behaviour were differentially modulated by the level of domestication and selection. Feeding activity is a primary modulated variable when swimming activity modification appears as a secondary indicator of feeding motivation alteration. Fish appeared capable to cope with repeated acute stress presented as a chronic stress situation and to some degree capable of adaptation and habituation as reflected by feeding behaviour plasticity and growth performance at the end of the experiment. According to this study, fish issued from different strains presented the same adaptation abilities and thus the same welfare potential under our constrained environment. Physiological results, blood lactate and glucose in particular, were used to validate behavioral indicators to acute stress response as proxy measures of stress in sea bass production.

PHYSIOLOGICAL STRESS RESPONSES IN SEA BASS

In sea bass, the ontogeny of the physiological stress response (cortisol) was described for the first time for fish reared under two different larvae rearing systems. The ontogenetic pattern of basal cortisol was similar to that observed in other marine teleost fish. However, the magnitude of the response was much higher than that reported in other warm-water teleosts. The onset of cortisol production occurred near the transition from endogenous to exogenous feeding, indicating that cortisol biosynthesis is important for adapting to different nutritional sources for energetic, growth and osmoregulatory purposes.

Cortisol release rates into the water were determined for the first time for sea bass and, in general, for warm-water marine species. This method can be applied to evaluate the stress response in sea bass juveniles reared in tanks. There was a strong positive correlation between plasma cortisol concentration and release rate in adults. Cortisol release rate into the water were affected by the size of fish, with large fish tending to release more cortisol than smaller sea bass.

BEHAVIORAL STRESS LEVEL INDICATORS IN SALMON

The overall objective was to identify non-invasive and reliable behavioural indicators that can be used to monitor and quantify acute and chronic stress levels in farmed Atlantic salmon. Further these should be validated and quantified using physiological or immunological methods. These relations were studied in parr, presmolt, and postsmolt during four experimental series, three in tanks (7m^3, 450-1250 fish in each) and one in sea cages (2000m^3, 3300-4000 fish in each) IMR, Matre research station. The sub goals of the experiments were to identify behavioural indicators of acute stress (moderate stressors: temperature rise or drop, hypoxia or hyperoxia; strong stressors: light on/off, chasing, handling and vaccination) and measure the behavioural changes compared to non stressed control tanks, and the latency of the stress behaviour after the acute stressor had been applied.

All tanks were monitored with both under water and surface video cameras. Computer based video analysis procedures for assessing vertical fish distribution in aquaculture tanks was developed based on the under-water recordings. To assess feeding motivation we used conditioned response to light blinks (CS) associated with a food reward. Reduced anticipatory behaviour after onset of CS was found to be a good and sensitive behavioural indicator of stress levels that could be measured by automatic video analyses of both vertical and horizontal fish distribution.

Positive (no/low stress) behavioural indicators relevant as operational indicators were:

- Polarised slow swimming (on current) with few position shifts (all stages)
- Circular school/shoal, holding position in the tank (parr and presmolt)
- Strong anticipatory response to conditioned signal - crowing in feeding area before feeding
- Strong response to feed - feed competition -most fish in feeding area (all stages)

Negative (moderate/high stress) behavioural indicators relevant as operational indicators were:

- Low vertical distribution in tanks - most fish near tank bottom (all stages)
- Nervous swimming with many position shifts (all stages)
- Weak anticipatory response to conditioned signal - no crowing in feeding area before feeding
- Increased sensitivity to stressors - startle response to conditioned signal (light flash)
- Low feeding response and feed intake (all stages)
- Fast chaotic swimming, near tank bottom (all stages, response to sudden light on or chasing)
- Fast vertical and horizontal swimming in sea cages resulting in self organising "tornado" schools or other emergent rapid changing school formations (post smolt/ongrowing salmon in cages)
- Positioning near tank wall/water inlet (fresh water stages, low water quality or after acute stress)
- Looser fish or stick fish - fish with low condition factor and apathetic behaviour (disease or poorly adapted to rearing conditions, long time chronic stress)
- High gill cover movement frequency - low oxygen or gill damage.

Physiological stress level indicators

In the tank experiments we analysed excreted cortisol into the water and hyper consumption of oxygen to verify stress levels. Weak stressors like temperature increase and hyper- or hypoxia gave low and short lasting increase in cortisol release rates, almost near the detection range of the analysis method, while strong stressors like chasing, and handling/vaccination gave significantly increased cortisol release rates lasting for 2-4 h after removal of stressor. Increased oxygen consumption was also a sensitive indicator of both negative stress and food consumption, which is possible to measure in real time and relatively cheap and easy to integrate in online monitoring systems, as long as it is possible to measure oxygen concentration in inflow and outflow water. In general were the physiological stress level indicators more short lasting than the behavioural/psychological indicators like conditioned anticipatory behaviour and swimming behaviour, and also reduction in feed intake. No or small compensation of lost feed intake caused by acute stress were observed at later feedings.

Immune response as indicator of chronic stress

The main aim has been to study the effects of chronic stress on development of immune competence. In these studies we have used an experimental approach of long time chronic stress following hypoxia, hyper oxygenation, and the combination of hyper oxygenation and hypercapnia. Briefly we have addressed the effect of chronic stress on the ability of fish to mount an antibody response to vaccines, to produce regulatory cytokines and other immune relevant genes assessed by quantitative real-time PCR. The role of chronic stress on the development of immunocompetence has also been studied in fish as ability to survive viral challenge.

In summary neither of the chronic stressors affected the development of specific antibody levels following immunisation of Atlantic salmon, not even in groups reared under very poor environmental conditions with low water flow, variable temperature, relatively high CO_2, fluctuating dissolved oxygen levels, and high turbidity from faeces and waste feed, which gave almost 40% reduction in growth rates. The antibody titres were however markedly affected by water temperature. Our hypothesis of reduced immune response in chronically stressed salmon was not fulfilled in these experiments, and it seems that reduction in immune response is not a good indicator of chronic stress and cannot be used to validate chronic stress level in Atlantic salmon. The importance of maintaining immunological competence might be a prioritised task for Atlantic salmon during chronic stress periods. However, it might be speculated that Atlantic salmon is highly capable to habituate to both high and low O_2 levels in the water, at least when these are kept constant.

DEVELOPMENT OF INTERNET BASED DATABASE AND EXPERT SYSTEM (FASTTOOL) FOR MONITORING, DOCUMENTATION AND ASSESSMENT OF FISH STRESS LEVEL AND WELFARE IN FISH FARMS

A web application and database system (FastTool) for day-to-day registration of environmental data, stress level indicators, husbandry data for salmon and sea bass hatcheries and ongrowing farms (sea cages) has been developed. The web application is available at http://www.imr.no/fasttool. FastTool is based on the latest in Internet technology to provide a seamless interaction between the application and the database. The database is implemented in the open source database management system PostgreSQL and follows relational database system principles. During the project period several updates have been done based on user evaluations. The main summary page contains eight action links where the user can enter data about:

1. The fish farmers' subjective assessment of fish welfare (welfare index).
2. Type of pen, depth, volume, algae growth and sight depth.
3. Fish species, life stage, biomass, number of fish, average length, number of dead fish.
4. Daily food and daily waste food.
5. Feeding behaviour.
6. Water temperature, dissolved oxygen and salinity.
7. Fish behaviour.
8. Fish appearance.

In order to get standardised registration of behaviour a behaviour ethogram was constructed. The user can at any time access FAST-TOOLs behaviour ethogram—webpage. The behaviour webpage contains name of the different behaviours, short descriptions of the behaviours, images and movies. A similar page has also been created for fish appearance. In separate folder in the web page contains pages for input of farm and fish data, and protocol and setup of schedule for the data registration. It is also possible for the fish farmer to upload data into the FAST-TOOL database. These data include data from CTD's(SAIV MINI STD/CTD – model SD204, SAIV AS, Norway) with additional sensors for oxygen, turbidity and fluorescence, data from a echo sounder system (Lindem Data Acquisition, Norway) and data from a feeding program (FishTalk, AKVA group, Norway).

Although new tests showed far better results in terms of user- friendliness and hence compliance, long term trials at commercial salmon and sea bass fish farms revealed that it still was difficult to get day to day compliance and good quality data from the farmers. Based on feedback and observations of the users we got the following conclusions:

- There should be as little manual input as possible
- If possible all uploading of data should be done automatically, independent of man.
- The user must get immediate and useful feedback from the system.
- The system should be as independent of software and internet services at the farm as possible.
- The website should be as simple and intuitive as possible.
- The website must be automatically updated at least daily with new rewarding information
- Historic data and analysis of these should be easily available.

To achieve this FAST-TOOL v2 - Welfaremeter prototype was developed, supported by additional funding. It consists of a profiling probe (measuring DO, T, S, turbidity, fluorescence) a control unit, a database, an expert software program and an internet web application. The control unit determines how often the probe profiles the water column and sends the measurement data via the mobile phone network (GPRS) to a database at The Norwegian Marine Data Centre at IMR. These data are immediately analysed by the expert software which gives an evaluation of the environmental conditions in the Welfaremeter internet web application (http://www.imr.no/welfaremeter). The expert software is based on knowledge about how Atlantic salmon tolerance to different environmental conditions and mathematical modelling of salmon metabolism given the measured environmental conditions. In this way the fish farmer can be updated about the current environmental conditions in the farm and also look at how the rearing conditions have been throughout the production. The last development has been to integrate the two systems into one, and make V2 a two way system where the farmer can enter farm data. During the FASTFISH project we have gone a long way towards developing a functional monitoring system and through FASTTOOL v2 we have the several improvements, but also remaining needs for further development:

- Amount of manual input is reduced and made simpler. Improved protocols and instructions/training for registration of fish behaviour and behavioural stress level indicators are needed.
- Environmental data are logged by a profiling probe several times a day. The data are uploaded automatically to the FAST-TOOL database and has given stable data input for more than a year. Automatic uploading of data from other farm control software and echo sounders are still lacking.
- The user gets immediate and useful and easy to understand feedback from the system. Improved overall welfare assessment integrating and weighting all information is needed.
- The profiling probe is independent of software and internet services at the farm using only mobile phone network. User interface available everywhere as long as users are connected to internet.
- The website is tried to be as simple and intuitive as possible. Further development of analysis methods and models are needed.

- Historic data and analysis of these are easily available and presented. Generation of an overall welfare assessment analysis and documentation report is needed.

The FASTFISH project has contributed a lot to the development of a web based database and expert system for assessment of environmental conditions, fish behaviour and performance, but there is still away to go to get a functional system that will be implemented by the industry. During the project we have learned that it is of no use to try to implement a premature system, which will be judged negatively and not used by the industry. We still need a period with further development of the monitoring technology, find ways to automatically integrate all available farm data, further develop models and analysis methods, and increase the user's interest and knowledge about observation of fish behaviour and performance, and data collection in general. During 2009 and 2010 we will try to solve many of the remaining challenges in a follow up project, financed by the Norwegian research council and The Norwegian fisheries and aquaculture industry research fund, that will be carried out by IMR, NOFIMA Marin (fish breathing frequency tags), University of Oslo(echo sounders). The "Welfaremeter" profiling probe buoy and the FAST-TOOL database and expert system will be developed to an industrial product during the next few years.

COSTS AND BENEFITS OF IMPLEMENTATION OF FISH WELFARE MONITORING SYSTEMS IN THE AQUACULTURE INDUSTRY

A survey among farm managers in Norway and Greece did show positive attitudes towards the FastTool idea and monitoring of fish welfare in general. Adoption of FAST-TOOL can be achieved if fish farmers are legally obliged to apply the tool in their farm systems. A faster and probably more successful approach is to enable market forces to stimulate adoption through governance (governments collaborating with private actors). The aim was to develop an implementation approach for FAST-TOOL. In the current complex society an increasing number of organizations in society are concerned about aquaculture and fish welfare. All these stake-holders at some point will play a role in the implementation of FAST-TOOL. Stakeholders can be grouped in farmers, suppliers, retailers/food services and other chain partners, NGOs, government, research, code-of-conduct organizations (CCOs) who formulate criteria for fish welfare and sustainability.

FAST-TOOL requires the involvement and acceptance of this idea by chain parties, special interest groups, consumer organizations and government. Adoption of a technological innovation requires adaptation in culture (value and norms) and structure (routines, organizations, issuing etc.). In that case, implementation of a fish monitoring tool is a strategic process towards sustainable aquaculture development. Transfer of technology used to be a common approach to establish implementation. However, the increasing complexity of agricultural issues such as animal welfare and environmental conservation require a broad context of other stakeholders then fish farmers to have the new system contribute to sustainable aquaculture development. The implementation should acknowledge the role of stakeholders that have a financial and/or social impact on the system. Furthermore, it acknowledges the value of stakeholders sharing ideas and information among themselves rather than relying on direction or advice from government agencies or other professionals. Therefore, it will encourage 'ownership' both of problems and solutions and working together on further development.

1.4.2. CONCLUSIONS AND RECOMMENDATIONS

- Behavioural indicators can be used to assess stress level in all stages salmon and sea bass, and relatively simple and easy to observe indicators like vertical distribution, group structure, swimming speed and orientation, can be used to measure deviations from normal conditions. However, self assessment of fish behaviour demands better training and motivation of the fish farmers, and availability of the necessary tools and technology. New innovative technology like hydro acoustics and video image analysis should be further developed to develop monitoring tools independent of man. We also believe that stronger focus on behavioural indicators will increase the attention to fish welfare and coping ability and improve the stockman ship.

- Behavioural stress level indicators seemed more sensitive to stress and were observable for a longer period than the latency of physiological effects as cortisol excretion and oxygen consumption. Oxygen consumption has been shown to be a potentially easy to measure stress level indicator that can be used to measure both deviations in food consumption and active metabolism.

- Acute stress led to reduced food intake, and time to recovery were dependent of the severity of the stress. The fish did not seem to compensate fully for lost feed intake, indicating that exposure to any acute stressor will lead to reduced growth rates and potential loss for the fish farmer. All unnecessary handling should therefore be avoided and more careful handling methods should be developed.

- The links between chronic stress and immune response was not evident. In spite of detailed studies of immune responses in salmon, no significant effects of chronic stress on disease resistance (vaccine response) were found. The importance of maintaining immunological competence might be a prioritized task for Atlantic salmon during chronic stress periods. However, this must be the fact only within limits, and we expect this to be an exponential relationship where small changes in stress dose or exposure time can have large impact when the fish approaches its tolerance limits. More studies are needed to find the tolerance limits for chronic stress.

- The FASTFISH project has contributed a lot to the development of a web based database and expert system for assessment of environmental conditions, fish behaviour and performance, but there is still away to go to get a functional system that will be implemented by the industry.

- By using modern data and telecommunication technology multiple information from both electronic sensors, feeding systems and events observed by man, have been integrated in a common database. Interpretation and analysis of data are done in real time and presented on the internet to licensed users. Further development will be done to integrate information from hydro acoustic transducers (echo sounders), farm management, and feeding software. Developments of industrial products are started.

1.4.3 SPECIFIC RECOMMENDATIONS

The monitoring of rearing conditions, fish behaviour and stress and welfare conditions in fish farm are still at a low level. Especially in cage culture there exists few datasets with integrated detailed information about environmental data, fish behaviour and performance, and other farm data, like feeding and husbandry stress. In general there exists no consensus for assessment of stress level or fish welfare.

Farmed fish are protected by the same animal welfare legislation as other farmed animals in many European countries, and these laws and regulations demand the fish farmers to have sufficient education, technology and equipment to secure the animals welfare. However, since there exists no consensus or accepted methods to assess or document fish welfare it is impossible for the fish farmer to comply with these regulations, and also impossible for the food authorities to enforce them.

Animal welfare is an archetypical concept, in the same way as for example happiness and it is not possible to give an exact definition or measurement, but must be rated on a gradient from terrible/bad to good/excellent. It is also a multidimensional concept and it is necessary to integrate many different aspects and parameters related to the overall assessment of welfare. By this we mean a systematic attempt to assess the welfare status of animals in relation to their rearing environment and how they are handled, based on observations of the animals, their biological and physical environments, and scientific knowledge. These methods should be diagnostic to identify the causes of poor (or good) welfare and should be the basis for advice on how to improve their welfare. Such assessment methods must be feasible and valid for specific species, developmental stages and production systems.

The welfare status of animals depends on their physiological and psychological state and on the way animals assess their individual needs. These systems involve behavioural and physiological responses that guide the animal to fulfill their proximate and ultimate goals, which are survival, growth and reproduction. It seems legitimate to assume that the welfare of an animal depends on if and how their needs are fulfilled, and that indicators correlated with the fulfillment of their needs may be good welfare indicators.

Overall assessment of fish welfare involves development of theories, models and methods to assess and score welfare and the problem of weighting the scores against each other. Another problem is weighting the welfare of low performing individuals against welfare of the whole group. The task also involves ethical considerations, and it should be addressed by a multidisciplinary group of scientists, also involving ethicists, and building on similar studies from terrestrial animals. To build trust in the developed methods it is also important to involve stakeholders, and develop good dissemination and education plans.

1.5 Intentions for use and impact

Fish behaviour is a sensitive and operational indicator of stress level. Sustainable aquaculture development by the approach of a monitoring system will be a result of the dynamics and the interaction between entrepreneurs, supply chain organizations, societal organizations and the consumers., Technology and software for automatic monitoring of fish behaviour should be developed and integrated in farm management, but demands trained and motivated observers and time to do the observations.. We believe a stronger focus on behavioural stress level indicators will have large impact on improvement of stockman ship and fish welfare.

The FASTFISH project have made the first approach to develop the first database and expert system for real time overall assessment of rearing conditions, fish behavior and performance, and husbandry events. The software has gone through a testing and evaluation in commercial systems, and strength and weakness have been detected and new actions have been initiated for further improvements of monitoring and communications technology and software and models included in the expert system. Before this can be implemented on a large scale in the industry and start to collect detailed datasets a well tested and fully developed system must be made and a business model must be implemented.

It was foreseen that the results obtained in the project can aid in establishing a general tool that can serve as an on-farm recording and warning system to identify early signals of high stress level or periods of chronic stress that eventually may result in increased risk of disease outbreaks. Although the FASTFISH have focused on sea bass and salmon the results obtained will apply to closely related fish species. Nevertheless, FAST-TOOL will provide a general framework for stress assessment in all species, but will need to be customized to the various species and farming systems. The dynamic nature of FAST-TOOL should make it ideal as a general stress assessment tool for a rapidly changing aquaculture industry.

Adoption of the tool can be achieved if farmers are legally obliged to apply the tool in their farm systems. A faster and probably more successful approach is to enable market forces to stimulate adoption through governance. Stakeholders share the opinion that market parties need to be the initiators for improving welfare. In cooperation at the European level with the EWAC initiative a national daughter body can aim to fulfill the specific needs, for example the extent towards government is represented in the body and is involved in the decision making.

The technical scientific validated information forms the base for the negotiations between stakeholders about the indicators. The state-of-the-art on understanding of welfare of fish is currently developing and new available technology will increase the understanding about the relationship between stressors and fish behavior. The information is to be extended with environmental data because most stakeholders regard the impact not solely of welfare but also the environment.

Scientific validated information does not automatically imply validation of the information by other stakeholders. Interpretation of the indicators will become important processes when developing products from FAST-TOOL in groups of stakeholders that hold different views on welfare and sustainability development. Market parties will departure from the standpoint for criteria formulating of consumer segments aimed at higher welfare part of a broader reconfiguration of quality both in response to consumer concerns and retailers and food services own commitment to quality and welfare and sustainability including these in their sourcing standards. Whereas, special interest groups will depart what is best for the fish in the trade-off between costs of welfare investments and to what extent these raise the level of welfare. Scenario analysis is a facilitating instrument that outline's the different paths of development and could provide the insight of the process underlying the path ways.

The development and implementation of a fish welfare assessment is also about sophistication of the technical aspects of the monitoring system. Managers of fish farms need to develop the habit to work with the system. Self-assessment puts large requirements on the managers' capabilities to perform monitoring and data entry on a daily basis. Farm managers' involvement can be enhanced by a participatory approach and providing education and human development and creating the awareness of fish welfare in society and the relationship with farm performance. Through using the FastTool expert system the fish farmer will also be given training in how to observe behaviour and be updated on new scientific knowledge.

Building capacity and experience by all stakeholders about the approach followed by monitoring welfare needs priority at the first stage of development aimed first at the mantle of pioneers who function as a benchmark and to start communication about welfare with the aquaculture sector. Implementation is best be performed in a lee environment initially, giving the potential conflicting material at hand and the possibility of losing the required trust in the approach necessary for the intention to operate transparent. Gradual implementation is therefore preferred, also because of the effort put in the development of FAST-TOOL so far.

2. PLAN FOR USING AND DISSEMINATING OF 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 2009*	*No - published on web*	*All*
Suggested welfare indicators		*Aquaculture industry and legislation*	*Available at project web site from April 2009*	*No - published on web*	*All*
Database and expert system for fish farm monitoring	New monitoring technology. Database and expert system service	*Aquaculture industry and legislation*	*Still under development. Link to prototype web pages on project web site*	*Actual*	*IMR, Industry partners*

2.2 Dissemination of knowledge

Planned/ actual Dates	Reference - Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Mar 06	*FASTFISH Flyer, web presentation* http://ec.europa.eu/research/fp6/ssp/fastfish_en.htm	*Public*	*International*	*Unknown*	*All, + EU-commision*
Mar 06	*FASTFISH Technical leaflet. Profet policy.* http://www.profetpolicy.info/index.php?option=com_docman&task=doc_download&gid=21	*Public*	International	*Unknown*	*All, + EU-commision*
May 06	*Kristiansen, T.S. On farm assessment of stress level in farmed fish. Presentation World Aquaculture/ EAS conference, Firenze 10 May 06. Oral presentation and extended abstract*	*Conference audience*	International	*Ca. 200*	*1,all*
Jun 06	*FASTFISH Project web page* http://fastfish/imr.no	*Public*	International	*Unknown*	*1, all*
Sep 06	*Flyer, web presentation* http://www.imr.no/english/__data/page/6335/Can_fish_behaviour_be_used_to_monitor_stress_level_fishwelfare.pdf	*Public*	International	*Unknown*	*1, all*
Sep 06	*Web presentation IMR News* http://www.imr.no/english/news/news_2006/monitoring_of_stress_and_fish_welfare	*Public*	International	*Unknown*	*1, all*
Sep 06	*Stien, L.H., Austevoll, I., and Kristiansen, T.S., 2006. Description of fish behaviour by video and computer vision NOBIM konferansen 2006. 7-8 September 2006. Oslo. Oral presentation.*	*Conference audience*	International	*Ca 100*	*1*
Oct 06	*Kristiansen, T.S. 2006. On farm assessment of stress level in fish. 1st COST 867 Welfare of fish in European aquaculture, Work Shop. Archachon, France. 9-11, October 2006. Oral Presentation.*	*Conference audience*	International	*Ca 100*	*1, all*
Oct 06	*Juell, J.E. 2006. Fish welfare research at Institute of Marine Research, Norway. . 1st COST 867 Welfare of fish in European aquaculture, Work Shop. Archachon, France. 9-11, October 2006. Oral Presentation*	*Research*	*European scient. comm.*	*Ca 100*	*1*
Nov 06	*Kristiansen, T.S. 2006. Overvåking av fiskevelferd i kommersielle oppdrettsanlegg (Monitoring of fish welfare in commercial fish farms). West- Norway Fish-farming organization. Yearly meeting, Bergen, Norway. Oral Presentation.*	*Conference audience*	*National Norway*	*Ca 50.*	*1*
Jan 07	*Fish farmers magazine 30, No 1 2007:12-13 Popular scientific paper*	*Public*	*International*	*Unknown*	*1, all*
Feb 07	*FASTFISH Web page presentation Norwegian School of Veterinary Science web page (www.veths.no)* *http://www.veths.no/templates/Project.aspx?id=8757*	*Public*	*International*	*Unknown*	*4,1*
Feb 07	*Fridell, F., Taranger, G.L, Kristiansen T.S. 2007. New research will help farmers manage fish health and welfare. Fish farmer 30:12-13 Fish Farming International, February 2007: p16. Popular scientific publication*	Public	*International.*	*Unknown*	*1*

Planned/ actual Dates	Reference - Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	(Interview).				
Feb 07	*Kristiansen, T.S. 2007. On farm assessment of stress level and fish welfare. CREATE day, Trondheim, Rica Nidelven hotel, 14-15 February 2007. Oral presentation.*	*Conference audience*	*Norway, USA*	*Ca 50*	*1*
Feb 07	*Oppedal, F., Bratland, S. og Olsen, R.E., 2007. Kortisol i vann – preliminære resultat (Cortisol in water – preliminary results). EWOS – oppdrettsmøte Matre 22 februar 2007. Oral presentation*	*Fish farmers*	*Norway*	*18*	*1*
Mar 07	*Juell, J.E., Nilsson, J., Olsen, R.E., Fridell, F., Kvamme, B.O., Oppedal, F., Humborstad, O.B., Mangor-Jensen A, Stien, L.H., Kristiansen, T.S. 2007. Dyrevelferd i akvakultur og fiskeri – et nytt fagområde i rask vekst. (Animal welfare in aquaculture and fisheries - a new growing research area) Fisken og havet, særnummer 3, 2007. Popular scientific publication.*	Public	*National/International*	*Unknown*	*1*
Mar 07	*Kristiansen, T.S., Johansson, D., Oppedal., F., Juell, J-E. 2007. Hvordan har oppdrettsfisken det i merdene. (How are the fish in the sea cages) Kyst og Havbruk 2007, Fisken og Havet, Særnummer 2. 2007:151-154. Popular scientific publication*	*Public*	*National/International*	*Unknown*	*1*
May 07	*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. 14-16 May 2007. Oral presentation*	*Conference audience*	*International*	*Ca.100*	*1,all*
Jul 07	*Stien, L. H., Bratland, S., Austevoll, I., Oppedal, F., Kristiansen, T. S., 2007. A video analysis procedure for assessing vertical fish distribution in aquaculture tanks. Aquac. Eng, 37, 115-124. Peer- reviewed scientific publication*	*Public*	*International*	*Unknown*	*1*
Aug 07	*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*	*Conference audience*	*International*	*Ca 200*	*1*
Sep 07	*Kristiansen, T.S. 2007. FASTFISH - On farm assessment of stress level in fish. Seminar with General-Director of DG Research, EU Commision, Vil Vite Senteret, Bergen, 7 September 2007. Oral presentation*	*Invited audience*	*NationalNorway*	*Ca 75*	*1*

Planned/ actual Dates	Reference - Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Sep 07	*Fanouraki, E., Papandroulakis, N., Ellis, T., Mylonas, C.C., Scott, A.P. and Pavlidis, M. Is water cortisol concentration a reliable index of stress in European sea bass, DICENTRARCHUS LABRAX? Bioactive Water Borne Chemicals: Pheromones and Welfare Indicators in Fish, Faro, 17-19 November 2007, pp. 38.*	*Conference audience*	*International*	*Ca. 100*	*5*
Oct 07	*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) Oral Presentation*	*Conference audience*	*Norway*	*76*	*1*
Oct 07	*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. Oral Presentation (Invited lecturer).*	*Conference audience*	*International*	*Ca 50?*	*1*
Oct 07	*Stien, L.H., Nortvedt, R., Roth, B, 2007. Welfare of farmed fish from harvest to killing – meeting the future challenges. Invited speaker. The research school SCOFDA – Sustainable Control of Fish Diseases in Aquaculture. Two-day workshop on welfare and health of fish. 30-31 Oct. 2007, Copenhagen, Denmark Oral Presentation*	*Conference audience*	*International*	*Ca 50*	*1*
Nov 07	*Oppedal, F., Bratland, S. and Folkedal, O., 2007. Akutt stress hos laks - preliminære data. Produktivitets-konferansen 2007, Kristiansund, November 1 2007. (In Norwegian) Oral Presentation*	*Public, fish farmers*	*Norway*	*135*	*1*
Nov 07	*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) Oral Presentation*	*Public*	*Norway*	*92*	*1*
Dec 07	*Kristiansen, T.S. 2007. FASTFISH "On farm assessment of stress level in fish". 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 speaker) Oral presentation*	*Invited audience*	*Europe*	*20*	*1, all*
Jan 08	*Oppedal, F., 2008. Welfare and behaviour in research fish - research fish in large-scale systems. Research animal course, Haukeland universitetssykehus, 10 January 2008. Oral presentation*	*Scientists*	*Norway*	*36*	
Mar 08	*Kvamme, B.O., Oppedal, F., Torgersen, T., Fridell, F., Sundh, H., og Sundell, K.S. 2008. Fiskevelferd – oksygenet viktig for oppdrettsfisken sin helse og velferd. I: Boxaspen, K., Dahl, E., Gjøsæter, J., Sunnset, B.*	*Public*	*Norway*	*Unknown*	*1*

Planned/ actual Dates	Reference - Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	Kyst og Havbruk 2008, Fisken og Havet, særnummer 2-2008, 134-137. Popular scientific publication				
Mar 08	*Stien, L.H., Gytre, T., Torgersen, T., Fosseidenegen, J., E., Kristiansen, T., 2008. Velferdsmeter. I: Boxaspen, K., Dahl, E., Gjøsæter, J., Sunnset, B. Kyst og Havbruk 2008. Havforskningsinstituttet, Norge, pp 113-115. Popular scientific publication*	*Public*	*Norway*	*Unknown*	*1*
Mar 08	*Bégout M-L, Millot S, Vandeputte M, Chatain B, 2008. Evaluation de la tolérance au stress de différentes lignées génétiques de bar (Dicentrarchus Labrax) : exemples d'indicateurs comportementaux discriminants. Réunion du Syndicat Français de l'Aquaculture Marine et Nouvelle (SFAMN), mars 2008, Montpellier. Oral presentation*	*SFAMN & Public*	*National*	*Ca. 50*	*3*
Apr 08	*Folkedal, O., Stien, L.H., Oppedal, F., Kristiansen, T.S, 2008. Atferdsindikatorer for akutt stress hos kondisjonert postsmolt. Foredrag Havbruk 2008, Norges forskningsråd, Havbruksprogrammet, konferansen SAS hotellet, Tromsø. 7-9 april. 2008. Oral presentation*	*Conference audience*	*Norway*	*Ca 200*	*1*
April 08	*Edouard Launet, Popular scientific paper: Interview M.L. Bégout. Libération, Cahiers scientifiques, April 1st Popular scientific paper (Interview).*	*Public*	*National*	*Unknown*	*3*
Apr 08	*Gytre, T., Kristiansen, T.S., Torgersen, T., Stien, L.H. 2008. Velferdsmeter. Havbruk 2008, Norges forskningsråd, Havbruksprogrammet, konferanse SAS hotellet, Tromsø 7-9 mai. 2008. Poster*	*Conference audience*	*Norway*	*Ca 500*	*1*
Apr 08	*Kristiansen, T.S. 2008. Kan man måle fiskens velferd. Innledningsforedrag, invitert foredragsholder. Havbruk 2008, Norges forskningsråd, Havbruksprogrammet, konferansen SAS hotellet, Tromsø 7-9 april. 2008 Oral presentation (inviteted speaker) Oral presentation*	*Conference audience*	*Norway*	*Ca 200*	*1*
Apr 08	*Stien, L.H., Kristiansen, T.S., Folkedal, O., Oppedal, F., Torgersen, T., Gytre, T. 2008. Nye systemer for overvåking av oppdrettsmiljø og fiskevelferd. Foredrag Havbruk 2008, Norges forskningsråd, Havbruksprogrammet, konferanse SAS hotellet, Tromsø 7-9 april 2008. Oral presentation*	*Conference audience*	*Norway*	*Ca 200*	*1*
Apr 08	*Stien, L.H., Gytre, T., Kristiansen, T.S., Torgersen, T. 2008. Experiences in creating two systems for online assessment of fish welfare. Annual Wellfish (COST 867) meeting 15-17 April 2008, Cracow, Poland. Oral presentation*	*Conference audience*	*International*	*Ca 50*	*1,2*
Apr 08	*Torgersen, T., Neill, W. H., Vabø, R., Huse, G., Folkedal, O., Stien, L. H., Kvamme, B. O., Kristiansen, T. S., and J. E. Juell. Dynamic models as fish welfare tools. Oral presentation Annual Welfish (COST 867) meeting, Krakow, Poland. 15-17 April 2008. Oral presentation*	*Conference audience*	*International*	*Ca 50*	*1,2*

Planned/ actual Dates	Reference - Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Apr 08	*Stien, L. H., Torset, A., Kristiansen, T., Torgersen, T., Sagen, H., and T. Gytre. Welfaremeter. COST 867 meeting, Krakow, 14-17 April 2008, Poland. Poster*	*Conference audience*	*International*	*Ca 150*	*1,2*
May 08	*Oppedal, F. og Kristiansen, T., 2008. Fiskevelferd og friskere fisk. Foredrag på workshop om 'Hvordan oppnå en forvaltning som sikrer en bærekraftig oppdrettsnæring som ikke er en trussel for villfisk stammene i Norge' arrangert av Mattilsynet, Clarion hotell, Bergen airport, 29 mai 2008. Oral presentation*	*Food authorities*	*National*	*Ca 25*	
Sep 08	*Gytre, T., Stien, L. H., Torgersen, T., Sagen, H., Kristiansen, T., 2008. A system for online assessment of fish welfare in aquaculture. Presentation ICES Annual meeting, Halifax. Canada 22-25 Sept. 2008. Oral presentation*	*Conference audience*	*International*	*Ca 100*	*1*
2008	*Fanouraki, E., Papandroulakis, N., Ellis, T., Mylonas, C.C., Scott, A.P., Pavlidis, M. , 2008. Water cortisol is a reliable indicator of stress in European sea bass, Dicentrarchus labrax. Behaviour 145, 1267-1281. Peer-reviewed scientific publication*	Public	*International*	*Unknown*	*5,2*
Sep 08	*Stien, L. H., Gytre, T., Torgersen, T., Sagen, H., Kristiansen, T., 2008. A system for online assessment of fish welfare in aquaculture. ICES CM 2008/R:18. Paper ICES Annual Meeting, Halifax, Canada. Popular scientific publication*	Public	*International*	*Unknown*	*1,2*
Sep 08	*Oppedal, F. 2008 Fagruppe dyrevelferd sine aktiviteter på Matre - et pittelite utdrag. Workshop Länstyrelsen Sverige, Havforskningsinstituttet Matre, 3 september 2008 Oral presentation*	*Conference audience*	*Norway*		
Sep 08	*Kristiansen, T.S. 2008. Overall welfare assessment (OWA) of Atlantic salmon. FASTFISH session, Work Shop "On farm assessment of fish welfare". EAS Aquaculture Europe 2008, Krakow, Poland 16-18 September. Oral presentation + Chair*	*Conference audience*	*International*	*Ca 50*	*1,all*
Sep 08	*Oppedal, F., Olsen, R.E., Torgersen, T., Folkedal, O., Stien, L., Bratland, S. and Kristiansen, T., 2008. Behavioural and physiological responses to environmental stress and handling stress in Atlantic salmon. FASTFISH session, EAS Aquaculture Europe 2008, Krakow, Poland 16-18 September. Oral presentation*	*Conference audience*	*International*	*Ca 50*	*1,4*
Sep 08	*Stien, L.H., Gytre, T., Torgersen, T Kristiansen, T.S. 2008 New technology and methods for on farm assessment of environmental conditions and welfare in salmon farms. FASTFISH session, EAS Aquaculture Europe 2008, Krakow, Poland 16-18 September. Oral presentation*	*Conference audience*	*International*	*Ca 50*	*1,2*
Sep 08	*Folkedal, O., Stien, L.H., Oppedal, F., Kristiansen, T.S MOTIVATIONAL STATUS AND STRESS COPING AS OPERATIONAL WELFARE INDICATORS IN FARMED SALMON Salmo salar L. EAS Aquaculture Europe 08, Krakow, Poland, 15-18 September 2008 .Oral Presentation + extended abstract*	*Conference audience*	*International*	*Ca 100*	*1*

Planned/ actual Dates	Reference - Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Sep 08	*Kristiansen, T.S, Begout, M-L., Evensen, Ø., Immink, V., Oppedal, F., Papandroulakis, N., Pavlidis, M., Stien, L.H. 2008. On farm assessment of welfare in Atlantic salmon (Salmo salar L.) and sea bass (Dicentrarchus labrax L.) aquaculture – Results from the EU project FASTFISH. EAS Aquaculture Europe 08, Krakow, Poland, 15-18 September 2008. Oral presentation + extended abstract*	*Conference audience*	*International*	*Ca 100*	*1,all*
Sep 08	*Neofytou, M., Divanach P. & Kentouri M, 2008. Behavioral ontogeny of larvae and early juveniles of the European sea bass (Dicentrarchus labrax L.) (Pisces: Moronidae) in intensive rearing. Poster, EAS Aquaculture Europe 08, Krakow, Poland, 15-18 September 2008.*	*Conference audience*	*International*	*Ca 500*	*5,2*
Sep 08	*Papandroulakis N., Pavlidis M., Lika K., Fanouraki E., Papadakis G., Asderis M., Anastasiadis P., Stefanakis S., Vardanis G. and P. Divanach. Assessment of stress level and welfare in sea bass. FASTFISH session, EAS Aquaculture Europe 2008, Krakow, Poland 16.10.09. Oral presentation*	*Conference audience*	*International*	*Ca 50*	*1,all*
Sep 08	*Torgersen, T. Ole Folkedal, Bjørn Olav Kvamme, Mette Remen, Tore S. Kristiansen, Tom Hansen, William H. Neill. Quantifying the response and acclimation/habituation to environmental stress in Atlantic salmon. EAS Aquaculture Europe 08, Krakow, Poland, 15-18 September 2008. Oral presentation + extended abstract*	*Conference audience*	*International*	*Ca 100*	*1,all*
Oct 08	*Kristiansen, T.S. 2008. Kunnskap om dyrevelferd – "happy fish" gir mindre svinn. Invitert foredragsholder. Produktivitetskonferansen, Kristiansund 2008. 29-30.10.2008 Oral presentation*	*Conference audience*	*Norway*	*Ca 200*	*1,all*
Oct. 08	*Millot S., 2008. Domestication, sélection et comportement du bar : Variabilité des aptitudes comportementales et de la tolérance au stress de groupes génétiquement distincts de bar,* Dicentrarchus labrax. *PhD dissertation thesis, Université de La Rochelle, France, 186 pp. PhD dissertation thesis*	*Public*	*International*	*Ca 50*	*3*
Dec 08	Folkedal, O., MOTIVATIONAL STATUS AND STRESS COPING AS OPERATIONAL WELFARE INDICATORS IN FARMED SALMON Fastfish work shop II, Augustin hotel, Bergen 03.12.08 *Oral presentation*	*Invited fish farmers*	*Norway*	*Ca 20*	*1*
Dec 08	*Oppedal, F. Olsen, R.E., Torgersen, T., Folkedal, O., Stien, L., Bratland, S. og Kristiansen. Adferd og fysiologiske responser på miljø- og håndteringsstress hos laks. FASTFISH workshop II - On Farm Assessment of STress level in farmed FISH, Augustin hotel, Bergen, 3 desember 2008. Oral presentation*	*Invited fish farmers*	*Norway*	*Ca 20*	*1*
Dec 08	*Torgersen, T. "Oksygen tilgjengelig for vekst"? (Oxygen available for growth). Fastfish work shop II, Augustin hotel, Bergen 03.12.08. Oral presentation*	*Invited fish farmers*	*Norway*	*Ca 20*	*1*

Planned/ actual Dates	Reference - Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Mar. 09	*Vandeputte M, Chatain B, Millot S, Bégout M-L 2009. Effet d'une génération de sélection ou de domestication du bar sur les performances de croissance, de qualité et de comportement alimentaire et testage de souches. 20ième Journée technique piscicole SYSAAF, Paris.* Oral presentation	*SYSAAF & Public*	*National*	*50*	*3*
Mar. 09	*Péan S., Millot S., Bégout M.-L., Chatain B. Effets des stress chroniques et aigus sur le comportement alimentaire et les performances de croissance de différentes souches de bar européen (Dicentrarchus labrax). Quatrièmes Rencontres de l'Icthyologie Française, Paris. Oral presentation*	*RIF & Public*	*National*	*Ca 100*	*3*
2009 *In press*	*Dempster, T. Korsøen, Ø., Folkedal, O. Juell, J-E., Oppedal, F. 2009. Submergence of Atlantic salmon (Salmo salar L.) in commercial scale sea cages: a potential short-term solution to poor surface conditions. Accepted for publication in Aquaculture 2 December 2008. Peer reviewed scientific publication*	Public	*International*	*Unknown*	*1*
2009 *In rev*	*Bratland, S., Stien. L.H., Braithwaite, V., Juell, J-E., Oppedal, F., Kristiansen, T.S. Subm. From fear to anticipation. Using aversive light stimuli to train reward conditioning in Atlantic salmon (Salmo salar L.). Aquaculture Peer reviewed scientific publication*	Public	*International*	*Unknown*	*1*
May 2009	*Neofytou M., Divanach P. & Kentouri M.2009. Behavioral ontogeny of the European sea bass (Dicentrarchus labrax L.) (Pisces: Moronidae) in intensive rearing. Accepted for oral presentation in 9th Panhellenic Symposium for Oceanography and Fisheries 13-16 May 2009, Patras, Greece .* Oral presentation	*Conference audience*	*International*	*Ca 100?*	*5*
May 2009	*Fanouraki E., Papandroulakis N., Pavlidis M., 2009. NON-INVASIVE STRESS INDICATORS IN MEDITERRANEAN MARICULTURE: EFFECT OF FISH SIZE ON WATER CORTISOL RELEASE RATE. 9th Panhellenic Symposium of Oceanography & Fisheries. 13-16 May 2009, Patras, Greece.* Oral presentation	*Conference audience*	*International*	*Ca 100?*	*5*
	Planned peer reviewed scientific publications after end of project				
2009	*Folkedal, O., Stien, L. H., Torgersen. T., Olsen, R. E., Oppedal, F., Kristiansen, T. S.. Effects of acute temperature increase on cortisol response, feeding motivation and swimming behaviour in farmed Atlantic salmon. Aquaculture. Planned submitted April 2009*	Public	*International*	*Unknown*	*1*
2009	*Folkedal, O., Stien, L. H., Torgersen. T., Olsen, R. E., Oppedal, F., Fernö, A., Kristiansen, T. S. Behavioural indicators of stress in salmon parr. Aquaculture Planned submitted April 2009.*	Public	*International*	*Unknown*	*1*
2009	*Haugland, Ø., et al. Effects of hypoxia, hyperoxia and hypercapnia on vaccine responses of Atlantic salmon (Salmo salar L.) - Fish and Shellfish immunology*	Public	*International*	*Unknown*	*4*
2009	*Karantzali E., S. Kolias, N. Papandroulakis and M. Pavlidis, Developmental expression of glucocorticoid receptor during early ontogeny in gilt-head sea bream, Sparus aurata, and European sea bass, Dicentrarhus*	Public	*International*	*Unknown*	*5*

Planned/ actual Dates	Reference - Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	labrax. Accepted for an oral presentation and thus qualifies for preparation of a full paper for publication in the special "larvi'09" volume of Aquaculture.				
2009	*Millot S., Bégout M.-L., Péan S., Leguay D., Vernier A., Chatain B. Chronic stress effects on the self-feeding behavior and growth performance of four sea bass strains. Planned submitted April 2009*	Public	*International*	*Unknown*	*3*
2009	*Millot S., Bégout M.-L., Di Poï C., Chatain B. Acute stress effects on the self-feeding behavior, growth performance and physiological status of different sea bass strains. Planned submitted May 2009*	Public	*International*	*Unknown*	*3*
2009	*Millot S., Bégout M.-L., Péan S., Durand E., Chatain B. Acute stress effects on the individual and group swimming and space use behavior of different sea bass strains. Planned submitted June 2009.*	Public	*International*	*Unknown*	*3*
2009	*Neophtou, M. In prep. STUDY OF THE BEHAVIORAL ONTOGENY OF LARVAE AND EARLY JUVENILES OF THE EUROPEAN SEA BASS (Dicentrarchus labrax L.) (Pisces: Moronidae) IN TWO DIFFERENT REARING SYSTEMS (INTENSIVE AND MESOCOSM TECHNIQUE). PhD Thesis University of Crete, including 3 manuscripts of peer reviewed papers.*	Public	*International*	*Unknown*	*5*
2009	*Fanouraki, E. in prep. Development of welfare indicators in marine aquaculture fishes (working title), PhD Thesis, University of Crete, incl 3 peer reviewed papers*	Public	*International*	*Unknown*	*5*
2009	*Fanouraki, E., N. Papandroulakis, C. C. Mylonas and M. Pavlidis. Species-specificity of stress response in eight marine fish species exposed to acute stressors under farmed conditions. In preparation*	Public	*International*	*Unknown*	*5*
2010	*Folkedal, O. STRESS COPING IN FARMED SALMON, Ph.D Thesis, including 3 papers. University of Bergen*	Public	*International*	*Unknown*	*1*