

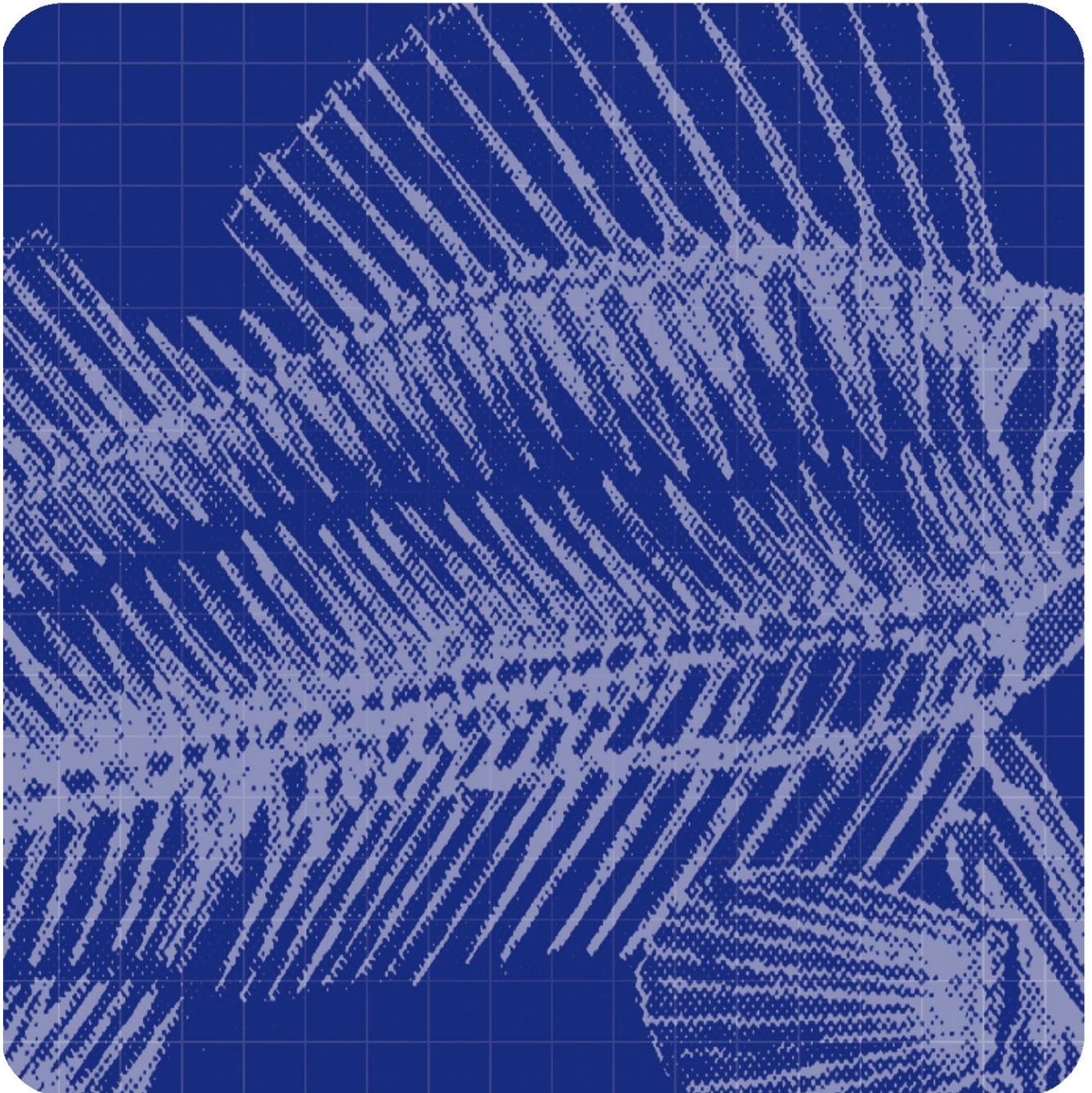


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Welfare in farmed fish

Børge Damsgård, Jon-Erik Juell and Bjarne O. Braastad





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<i>Summary:</i> <p>The interest of fish welfare is increasing, both in Norway and internationally, leading to an increasing need for scientific knowledge about basic and applied questions related to how farmed fish are produced. The strategic institute programme (SIP) "Welfare in farmed fish" was financed by the Research Council of Norway, for the five years period from 1 January 2001 to 31 December 2005. The core partners in the SIP were the Norwegian Institute of Fisheries and Aquaculture Research (Fiskeriforskning) in Tromsø, Institute of Marine Research (IMR) in Bergen and the Norwegian University of Life Sciences (UMB) at Ås.</p> <p>The general aim of the programme was to increase the basic competence about fish welfare by the integration of scientific fields such as fish behaviour, fish physiology and fish health as tools to understand welfare related topics in farmed fish, and to use these tools to evaluate fish welfare based on the animal's behavioural needs.</p> <p>This report aims at giving the reader an insight in the scientific work carried out in this strategic research programme and the progress made in this relatively new scientific field of aquaculture research. Chapter 3 gives a conceptual and theoretic background of animal welfare, and chapter 4 describes the methodological progress financed in part by the programme. Chapter 5-11 reports results and progress related to both basic and more applied fish welfare research which gives an illustration of the interdisciplinary nature of the subject.</p>	

TABLE OF CONTENTS

1	INTRODUCTION	1
2	ABOUT THE AUTHORS	3
3	ANIMAL WELFARE - A NEW CONCEPT IN AQUACULTURE AND FISHERIES	5
3.1	Animal welfare – what is it really?	5
3.2	Definition of animal welfare	8
3.3	Welfare in fish	9
3.4	Welfare indicators and monitoring in fish farming	10
3.5	References	11
4	DEVELOPMENT OF RESEARCH FACILITIES AND METHODS IN FISH WELFARE.....	15
4.1	Fish Behaviour Laboratory: Measuring individual traits under controlled conditions	15
4.2	The fish learning laboratory	20
4.3	The water quality laboratory: Controlled manipulation and assessment of environmental parameters	21
4.4	The cage environment laboratory: Behavioural studies in a realistic farm environment.....	24
4.5	Non-invasive methods for assessment of fish welfare	27
4.6	References	29
5	REPRODUCTIVE SUCCESS, SPAWNING BEHAVIOUR AND WELFARE IN BROOD-STOCKS.....	33
5.1	Introduction	33
5.2	Background on factors influencing reproductive success and behaviour	33
5.3	Reproductive behaviour and mating success in cod.....	34
5.4	Social behaviour and reproductive hormones in Arctic charr during a spawning season	40
5.5	References	43
6	PRENATAL STRESS IN FARMED SALMON; IMPACTS ON PHYSIOLOGICAL, MORPHOLOGICAL AND BEHAVIOURAL CHARACTERISTICS	47
6.1	Physiology and morphology.....	47
6.2	Stress proteins.....	50
6.3	Behaviour	51
6.4	References	53
7	LEARNING AND FISH WELFARE: LEARNING ABILITIES AND CONSTRAINTS IN COD AND HALIBUT.....	57
7.1	Introduction	57
7.2	Learning in cod.....	59
7.3	Classical conditioning of halibut.....	61
7.4	Differences in cognitive abilities in cod and halibut?	62
7.5	What does it mean that cod can be trace conditioned?.....	62
7.6	References	63

8	SOCIAL INTERACTIONS BETWEEN JUVENILE FISH AND INDIVIDUAL VARIATION IN BEHAVIOUR DURING THE ON-GROWTH PHASE.....	65
8.1	Introduction	65
8.2	Motivational background for agonistic interactions.....	65
8.3	The effects of winning contests on subsequent social interactions in Arctic charr.....	66
8.4	Effects of size differences and nutritional state on aggression and threat-sensitive behaviour in juvenile cod	68
8.5	References	72
9	WATER QUALITY PREFERENCE	75
9.1	Introduction	75
9.2	Temperature preference in Atlantic cod.....	77
9.3	Temperature preference studies with other species.....	79
9.4	Studies of tolerance and behavioural preference of ammonia	80
9.5	References	81
10	OPTIMISING INTENSIVE REARING CONDITIONS TO SECURE FISH WELFARE AND HEALTH.....	83
10.1	Introduction	83
10.2	Does intensive farming reduce the welfare status of salmon smolts?.....	83
10.3	Do high carbon dioxide concentrations and acid water affect the welfare of Atlantic salmon?.....	84
10.4	Do high oxygen and carbon dioxide levels affect the welfare status of salmon smolts?.....	85
10.5	References	87
11	EFFECTS OF THE CAGE ENVIRONMENT AND SOCIAL INTERACTIONS ON THE SWIMMING BEHAVIOUR AND WELFARE OF ATLANTIC SALMON.....	91
11.1	Microhabitats in big volumes	91
11.2	The pycnocline and the environmental quality of a site.....	92
11.3	Social environment, stocking density, and swimming behaviour	93
11.4	Swimming and feeding behaviours in a uniform physiochemical environment and a natural photoperiod.....	94
11.5	Effect of behavioural thermoregulation and phototactic behaviour on swimming depth and schooling density	95
11.6	Welfare of caged salmon.....	96
11.7	Practical implications	98
11.8	References	98

ANNEX. INTERNATIONAL PUBLICATIONS FROM THE PROGRAMME

1 INTRODUCTION

The strategic institute programme (SIP) ”*Welfare in farmed fish*” was financed by the Research Council of Norway, under contract 143213/I10, for the five years period from 1 January 2001 to 31 December 2005. The core partners in the SIP were the Norwegian Institute of Fisheries and Aquaculture Research (Fiskeriforskning) in Tromsø, Institute of Marine Research (IMR, Havforskningsinstituttet) in Bergen and the Norwegian University of Life Sciences (UMB, previously Agricultural University of Norway) at Ås. The programme has been coordinated by senior scientist Børge Damsgård (Fiskeriforskning), and has in total involved more than 20 scientists.

The programme includes five work packages, including:

1. **Social interactions and feeding hierarchies**
(coordinator: Børge Damsgård, Fiskeriforskning)
2. **Environmental preference and tolerance in fish**
(coordinator: Bjørn Steinar Sæther, Fiskeriforskning)
3. **Social interactions and environmental preferences of caged salmon**
(coordinator: Jon-Erik Juell, IMR)
4. **Learning in fish**
(coordinator: Tore S. Kristiansen, IMR)
5. **Welfare indicators**
(coordinator: Bjarne O. Braastad, UMB)

The general aim of the programme was to increase the basic competence about fish welfare by the integration of scientific fields such as fish behaviour, fish physiology and fish health as tools to understand welfare related topics in farmed fish, and to use these tools to evaluate fish welfare based on the animal’s behavioural needs.

The programme has been central in the development of fish welfare as a scientific topic in Norway, and several of the key scientists in the SIP were actively involved in the Norwegian White Paper on welfare published in 2003, the report on research needs in animal welfare in Norway published in 2005, and the development of a new Animal Welfare Act in Norway. In addition, the SIP has been a platform for the development of EU projects on fish welfare, and the core partners in the SIP are involved in several international projects and networks related to fish welfare, such as *SEAFOODplus*, *WEALTH*, *CONSENSUS*, *FASTFISH*, and the new COST Action 827 *Welfare of fish in European aquaculture*.

During the programme period, the core institutions have developed research laboratories aimed to work specifically with welfare related questions, such as the *Water Quality Laboratory* and *Fish Behaviour Laboratory* (Fiskeriforskning), and the *Fish Learning Laboratory* and *Cage Environment Laboratory* (IMR), described in chapter 5.

Educational aspects are important in strategic programmes, and three PhD-scholarships and one post-doc scholarship have been included in the work packages. The first two PhDs, Linda A. Hansen (Fiskeriforskning) and Marit Skog Eriksen (UMB) are expected to fulfil the PhD during 2006, while the third, Jonatan Nilsson (IMR), will be delayed to 2007 due to an unforeseen change of PhD candidate. More than 40 international publications are currently

planned published or already published, based on studies funded totally or partly from the SIP programme (Annex).

This report aims at giving the reader an insight in the scientific work carried out in this strategic research programme and the progress made in this relatively new scientific field of aquaculture research. Chapter 3 gives a conceptual and theoretic background of animal welfare, and chapter 4 describes the methodological progress financed in part by the programme. Chapter 5-11 reports results and progress related to both basic and more applied fish welfare research which gives an illustration of the interdisciplinary nature of the subject.

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3 ANIMAL WELFARE - A NEW CONCEPT IN AQUACULTURE AND FISHERIES

Bjarne O. Braastad, Børge Damsgård & Jon-Erik Juell

Animal welfare has been a subject of discussion ever since humans first kept pets and production animals. Fish, either captured or farmed, has until recently avoided this focus, perhaps partly since it was assumed that fish could not perceive pain. Philosophical discussions, conceptual development and biological research on animal welfare therefore mainly pertain to mammals and partly to birds. In this chapter we will present the conceptual understanding of animal welfare based on these animal classes, and then discuss how fish can fit in with this understanding.

3.1 Animal welfare – what is it really?

Animal welfare has mainly to do with the quality of life of animals. The concept has both an *ethical dimension* and a *factual dimension* (Tannenbaum 1991; Sandøe & Simonsen 1992; Fraser 1999; Report to Norwegian Parliament no. 12 2002/2003 on the keeping and welfare of animals). The ethical dimension is concerned with the values of animals in relation to questions like whether animals can claim a certain quality of life, when the quality of life is good enough, and what precisely is quality of life for animals. The factual dimension is concerned with scientific knowledge about how an animal is influenced by its environment, how it experiences its situation, and which social and physical factors contributing to poor or good conditions. There are important interactions between the ethical and factual dimensions. Values influence our choice of facts to consider, and factual information influences our values. Discussions on animal welfare have roots back to the English philosopher Jeremy Bentham who argued that animals must be treated well because they can suffer (Bentham 1789). Although biology and veterinary medicine are crucial sciences when animal welfare is discussed, disciplines like ethics, psychology, social sciences, economy and law contribute to the understanding of and consequences for how we can treat animals. The first country to implement such considerations into a law was England in 1822. Norway followed in 1935, while China probably has the most recent law put into force in 2005. Below the focus will be on the factual dimension of biological sciences.

Various factual approaches to animal welfare

The industrialization of agriculture after World War II led to increasing concerns about the welfare of production animals on farms. The book *Animal Machines* by Ruth Harrison (1964) was a bombshell that resulted in a report to the British Parliament on the state of animal welfare in agriculture (Brambell Committee 1965). The report concluded with certain criteria that persons responsible for animals should aim to fulfil in order to ensure adequate welfare, the so-called *five freedoms* (after later modifications by Farm Animal Welfare Council in 1992):

- Freedom from hunger and thirst – by ready access to fresh water and a diet to maintain full health and vigour,
- Freedom from discomfort – by providing an appropriate environment including shelter and a comfortable resting area,

- Freedom from pain, injury or disease – by prevention or rapid diagnosis and treatment,
- Freedom to express normal behaviour – by providing sufficient space, proper facilities and company of the animal’s own kind, and
- Freedom from fear and distress – by ensuring conditions and treatment, which avoid mental suffering.

The Brambell committee considered that, for terrestrial animals, the two first points were virtually fulfilled and that satisfaction of behavioural needs was most far from being fulfilled. These freedom requirements could be adapted also to aquatic animals, perhaps with modified wording. The report of the Brambell committee instigated a continuous scientific effort in Europe, and later other continents, to understand how animals perceive their various environmental conditions and how animals should be kept to improve their welfare. Unfortunately, during the first decades, research aimed at developing improvements of the environment before understanding exactly what the needs of the animals were. To some extent, applied research preceded basic knowledge. In aquaculture welfare research, this lesson should be learned encouraging the development of basic understanding that applied solutions could be based on.

The concept of animal welfare is approached from three different angles emphasizing different aspects of an animal’s quality of life; the animal’s biological functioning, the animal’s natural life, and the animal’s subjective experience of its situation (Duncan & Fraser 1997; Lund 2002). These approaches are visualized in Fig. 1, indicating also that they overlap to some extent. There is no solution as to which approach is ‘correct’. Each contributes with relevant facets of animal welfare and will therefore be presented briefly.

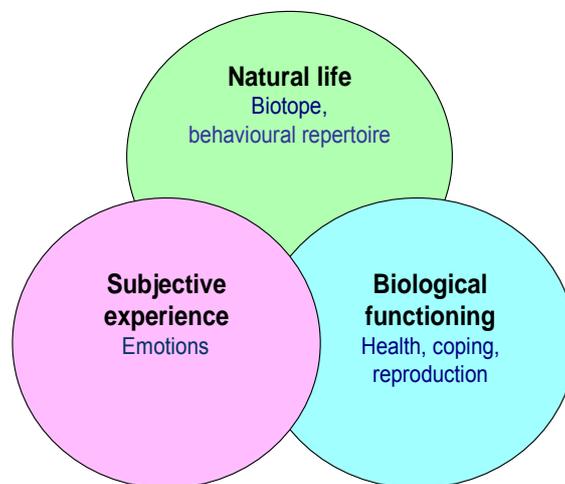


Figure 1 Three different approaches to the concept of animal welfare.

Biological functioning

A major comprehension of animal welfare is related to the biological functioning of an animal, meaning that an animal which is biologically well functioning with good health, low stress level, and adequate growth and reproduction, also has a good quality of life (Broom 1986; 1991). Broom’s well-known definition of animal welfare is in agreement with this

view; *an animal's state as regards its attempt to cope with its environment* (Broom 1986). According to Broom's definition, homeostasis is the ultimate goal. Coping comprises the biological mechanisms an animal utilizes to handle a significant threat to its stability or homeostasis and regain control (Benus *et al.* 1991). Immunological mechanisms, physiological mechanisms including stress biology, and behavioural strategies contribute to an animal's coping ability.

Natural life

A naturalistic understanding of animal welfare focuses on an animal's possibility to utilize its full behaviour repertoire according to its genetic preadaptation and live in an environment as close as possible to its natural biotope (Kiley-Worthington 1989; Rollin 1993). This also involves the principle of giving animals the benefit of doubt when scientific knowledge is insufficient. This approach is typical for organic farming (Lund 2002). A challenge with this view is that while freedom of movement and fulfilment of behavioural needs are well taken care of in natural life, predators, parasites, starvation, and extreme climatic conditions can sometimes compromise welfare, e.g. in reindeer and sheep on outfields.

Subjective experience

More lately increasing focus has been given to the significance of an individual's subjective experience of its situation when animal welfare is considered (Duncan 1996). A good quality of life requires that there is no mental suffering (Duncan 1993). Animal welfare research has mainly dealt with negative emotions related to stress, fear and pain (Dawkins 1980; Zayan & Duncan 1987). Later, some scientists have given more attention to the positive emotional state (Dawkins 1990; Fraser 1995). According to the definition of Berry Spruijt, animal welfare is *the balance between positive (reward, satisfaction) and negative (stress) experiences or states* (Spruijt *et al.* 2001). Satisfaction and joy is a temporary condition in which the animal has reduced the difference between a negative situation and what is expected or desired (Wiepkema 1985; Spruijt *et al.* 2001). Dissatisfaction or even frustration is the result of an inability to achieve this reduction. This is related to a homeostatic model for an animal's needs, in which a comparator mechanism checks whether there is a discrepancy between an ideal value ("Sollwert") and an actual value ("Istwert"; Wiepkema 1985). A discovered discrepancy gives rise to a need, which releases certain behaviour as part of a strategy to reduce the discrepancy and regain homeostasis. According to Spruijt's definition, this Sollwert might well be a positive condition related to positive emotions, and not only representing a need to increase welfare from a negative state to a neutral state.

It would be utopia to try to create an environment completely free from negative emotions. However, detrimental effects of negative emotions could be reduced by providing conditions for the animals that also stimulates positive emotions, reward and expectations of reward.

Animal welfare relates to the individual

The welfare level is a temporary characteristic of the individual animal. This follows directly from the main facets of welfare, regardless of welfare approach or choice of definition; emotions, pain, disease, injury, and behavioural repertoire are all aspects of the individual. Even if the average individual has a reasonably good welfare, individuals with a poor coping ability or which dominant individuals frequently attack might have poor welfare.

Environmental design and management of production animals should therefore take particular care of such individuals.

In large populations of animals, like large flocks of broilers or farmed fish, it is impossible to assess the welfare level for each individual. Welfare measurements must therefore be adapted e.g. in a way that samples of various types of individuals are collected.

Health and animal welfare

Health and animal welfare is not the same issue, but there is a certain overlap. Good health is certainly not enough to conclude on the welfare level. The choice of welfare approach determines whether the subjectively experienced health condition or a more objective health condition affects welfare. According to the subjective approach, good welfare requires good health to the extent that suffering is avoided and adequate physical functioning is ensured. The biological functioning approach requires also that health is objectively good, e.g. that there are no parasites or pre-pathological conditions even if the individual does not experience it as unpleasant.

3.2 Definition of animal welfare

In the report on the needs for animal welfare research in Norway (The Research Council of Norway 2005), the following definition of animal welfare was adopted:

Animal welfare is an individual's subjective experience of its mental and physical state as regards its attempt to cope with its environment

This definition expands Broom's definition (Broom 1986) to emphasize two important facets of welfare; that the welfare is a property of the individual, and that it deals with this individual's subjective experience of its state as the balance between positive and negative perceptions. Measures of health, behaviour, and physiology are important tools when assessing the welfare level. The *mental state* includes emotional and cognitive states that are affected by the present stimuli and memory of previous experience with similar stimuli, either unconditioned or conditioned. The *physical state* comprises physical and physiological states that influences, or potentially influences, mental states. The *environment* includes the social environment (conspecifics or humans in species that may form a social bond with humans), the physical environment (climatic factors, housing conditions, water quality), and other biotic factors (pathogens, parasites, predators, and stockpersons).

A consequence of this definition is that welfare could be measured on a scale from extremely poor to extremely good, with the neutral state as a zero point where no negative stimuli or frustrations are experienced, i.e. the homeostasis level in earlier models. Welfare could then be improved in two ways; (i) minimizing the distance from a point on the negative side to the homeostatic point, and (ii) maximizing the distance from the homeostatic point to a point on the positive side, e.g. by giving the animals unexpected positive stimuli, like titbits, or keeping them in a state of positive expectancy by positive conditioning.

3.3 Welfare in fish

The above welfare definition creates two main challenges when applied to fish. Firstly, we lack basic knowledge about emotional expressions in fish. We do not know to what extent emotions are found in fish, which factors could influence such emotions positively or negatively, or how fish might display various emotions. The consequence would be to ensure good biological functioning and good health as prerequisites for good welfare in farmed fish, also avoiding conditions that might inflict pain, and meanwhile exploring their emotions and cognitive functions for a future increased focus on subjective emotional experience. Secondly, farmed fish are held in large groups in an aquatic medium that makes it very difficult to observe the individual fish in its farm environment. To include welfare assessment in the day-to-day management of cultured fish there is a strong need to develop operational welfare measures.

Stress in fish

Fish have physiological stress responses similar to mammals (Wendelaar Bonga 1997; Iwama *et al.* 1997) and farmed fish may be exposed to both acute and chronic stress during their lifespan. The primary stress responses are involving release of stress hormones (e.g. adrenalin, cortisol), which lead to a secondary stress responses that stimulate oxygen uptake and transfer, mobilization of energy substrates, and reallocation of energy away from growth and reproduction. This mobilising of energy and attention to handle the stressor is named a “fight or flight” response, which in nature is a process that is essential for survival. The stress response is mainly an adaptive response, but repeated or chronic exposure to stressors will be maladaptive (tertiary stress responses) when the fish’s adaptive capacity is exceeded, and high costs are paid with respect to growth and health. If farmed fish are exposed to severe rearing condition over time, chronic stressors may have consequences for welfare, health and mortality. Long-term stress may lead to maladaptive stress responses such as behavioural changes, reduced appetite and growth; decrease general immunocompetence and reproductive changes - often indicators of unacceptable welfare.

Pain and suffering in fish

Pain perception and suffering in fish have been debated for some years and our understanding of these questions may have large impact on the way we handle and slaughter farmed fish. In this debate is important to separate between *pain* (nociception) and *pain perception*. Fish have a similar nerve system as mammals to communicate nociception from the body to the brain. The question about pain and brain activities in fish is, however, largely unexplored. Some scientists argue that fish, birds and some mammals, do not perceive pain because they lack a neocortex, which are important in pain perception in most mammals (Rose 2002). Other brain structures may, however, have the same functions as the neocortex (Braithwaite & Huntingford 2004) and there are studies indicating that fish have numerous pain receptors and show long term behavioural indicators when exposed to pain stressors (Sneddon *et al.* 2003).

The question of suffering in fish is more complex than the debate over pain perception. Reviews by Chandroo *et al.* (2004 a, b) focus on the animal’s consciousness about suffering, asking the question if fish have cognitive skills similar to mammals. This is tested in learning experiments, and Chandroo argue that several experiments indicate a highly advanced learning ability characteristic for consciousness in higher animal species. The question of suffering in fish is however still under debate.

Factors affecting welfare in fish

In practical fish farming, good fish welfare requires rearing conditions where the fish can maintain homeostasis and normal development and are protected against physical damages and chronic stress. Most aquatic animals, including fish, go through extensive physiological and morphological changes in the development from egg to mature individuals. Developing methods to measure welfare and factors within such variation requires studies underpinning basic biological mechanisms. Relatively minor factors during early life stages may have severe effects in later stages, and the interactions between the different environmental factors and the biology of the animal are complex. A high number of factors throughout the farming period may thus affect fish welfare, including for example the physical and chemical environment, feeding, social interaction, the occurrence of fish pathogens, parasites and predators, and the procedures during handling, transport and slaughtering.

3.4 Welfare indicators and monitoring in fish farming

The subjectively experienced mental and physical states of an animal cannot be measured directly. We need reliable indicators of these states. This requires research confirming both the *reliability* and *validity* of the suggested indicators. To be used in different environmental conditions or for different breeds of animals, the indicators might also have to be *calibrated* so relevant individual variation could be revealed.

The most obvious welfare indicators are related to growth, health, injuries, stress, and reproduction. Behaviours resulting in injuries to the individual or its conspecifics are also obvious indicators. Indicators of motivational and emotional states are much more difficult to find, even in mammals. Extensive research is needed to develop such indicators. Yet, some general experience from mammal and avian species can be useful. *Social stress* may be indicated from an elevated frequency of aggression higher and withdrawal from the social group. *Frustration* may lead to frequent shift in behaviour and result in stereotypic behaviour, i.e. repetitively performing a simple behaviour with no obvious function. *Fear* or *fearfulness* can be measured in a number of standard test situations, although their validity must be properly addressed. *Emotions* and *behavioural motivations* are indicated by communication signals or various types of conflict behaviour, i.e. behavioural elements related to conflicting or thwarted motivations. *Abnormal movements* could be related to a high population density or otherwise thwarted movements. *Apathetic behaviour*, i.e. lack of response to external stimuli, could be the result of a long-lasting condition with negative, frightening stimulation and a complete lack of rewarding stimuli. *Learned helplessness* is a similar condition in which the individual has experienced that any attempt of response to stimuli leads to “penalty”, resulting in an animal that avoids all behaviour. *Overflow activities* and *vacuum activities* are types of inadequate behavioural responses or responses to inadequate stimuli when the animal is highly motivated for something that appears to be inaccessible.

In farmed fish, behavioural indicators similar to those developed for terrestrial farm animals should be sought. Animal welfare is ideally an integration of a large number of factors related to the mental and physical state of an animal. In terrestrial production animals, particularly in pigs and cattle, several ways of producing a *welfare index* have been attempted, e.g. the Austrian Tiergerechtheitsindex (TGI, Bartussek 2001). These have mainly measured various aspects of the housing environment and management. One of the main problems with this approach is to assign quantitative weights to the various welfare factors. The relative importance of the different factors for the welfare of the animal would probably also show

interspecific and time-dependent variations. A feature of the welfare definition adopted in Chapter 3.2, and its interpretation, is that good welfare could more simply be recognized as a condition where there are more indicators on the positive side than on the negative side of the homeostasis levels. Constructing more complex welfare indexes than this is a challenge currently addressed by the scientific community e.g. in the EU projects: *Welfare Quality: Science and society improving animal welfare in the food quality chain* (FOOD-CT-2004-506508) on terrestrial animals and *Development of operational indicators of welfare in farmed fish* on fish.

Monitoring fish welfare

The European aquaculture industry is facing several challenges in the field of animal welfare, e.g. poor environmental quality, and high frequency of morphological deformities and high mortality rates. To improve animal welfare standards in fish farming standardised protocols to monitor and assess welfare is needed. A basic repeated surveillance of animal welfare of farmed stocks would be an important tool to discover deviation and intervene as early as possible. Such systems are already used in some terrestrial farm animals, and may become an important tool to document ethical qualities of farmed fish in the future (Damsgård 2005). Consumer studies have indicated that the interest for production and quality traits is increasing and has led to the development of traceability systems giving product information from the farm to the consumers.

A fish welfare surveillance system may aim to increase the general knowledge for the prevention of welfare related problems; including risk assessments and the identification of topics that need further scientific or legal considerations. Such surveillance must be based on scientifically acceptable and validated welfare indicators, based on both the behavioural, physiological and immunological traits of different fish species. A set of rapid, inexpensive and non-invasive screening methods may be used as welfare indicators. The indicators are not necessarily the most important welfare issues, but may give rapid information about the status of a specific population. Welfare is however individually based, and such indicators may only provide average changes in e.g. a sea cage, and not information of each single animal.

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4 DEVELOPMENT OF RESEARCH FACILITIES AND METHODS IN FISH WELFARE

4.1 Fish Behaviour Laboratory: Measuring individual traits under controlled conditions

Børge Damsgård & Bjørn-Steinar Sæther

Fish behaviour and behavioural needs of farmed fish has been identified as one of the key research needs in order to develop tools to evaluate fish welfare. It is a limited number of Norwegian research facilities dedicated to behavioural studies of individual fish, and Fiskeriforskning have developed an observational platform based on video signals, and the software *The Observer VideoPro* and *EthoVision* from Noldus (Wageningen, The Netherlands). The platform have been used over a range of experimental setups, and the key issue of the lab is to quantify behavioural events (e.g. aggression, feeding) and fish movements (e.g. swimming speed, habitat selection) and correlated these to for example physiological, immunological or genetic differences between individual fish. The platform consists of three units:

Tank units:	Observation tanks Preference chamber Water preference tank
Camera units:	CCD cameras Time coding Recording
Analyses units:	The Observer Video-Pro EthoVision Pro MatMan

Observation tanks

Size, shape, depth and other feature of an observation tank may affect the result in behavioural studies, and a scientific sound experimental setup must meet the compromise between an satisfactory environment for the fish, and the possibilities to study the behaviour. On one hand we need to mirror an “normal” rearing situation, and at the same time be able to study individual fish. In addition, such individual behaviour may often be related to various physiological parameters, and such measurements are difficult to synchronize with the behavioural sampling. We use several types of observation tanks, ranging from 15 × 28 cm (6 l) aquariums for juvenile fish, to large 5-m diameter tanks for large adults (Fig. 2). The tanks are filmed with underwater cameras, or from out of the tanks from the side, under the tank or from above the tank. It is however important to film the whole tank area, and that is often difficult with a side view. The problem with reflections from movements of the water surface is limited by filming from under the tank, but most commonly filming from above is used to monitor 2-dim movement and behavioural events. A side view will give more detailed behavioural data, but it is difficult to measure movements. Simultaneous side view and above water filming enables the calculation of 3-dim movement.



Figure 2 Tank units for observation of fish behaviour at Fiskeriforskning. a) Regular 100 l rearing tanks with video cameras above each tank, b) 1700 l preference tanks with PIT-tag antennas in the channel between each tank and video cameras above each tank, c) 6 l aquariums for juvenil fish with cameras on the side of the aquariums, and d) 5-m diameter large scale tank with underwater pole camera and zoom cameras above the tank. Photo: Fiskeriforskning.

The camera systems (Panasonic CCD cameras) are used with wide angle lenses to cover the whole tank, or remote controlled zoom lenses to track individual fish. In systems with several tanks, multiplexers, quad units and time lapse machines are used to reduce the amount of video samplings. The multiplexer select sequences from camera 1 to camera n, for example between 10 cameras with 10 seconds intervals, while the quad units separate the monitor screen in up to four parts without shifting between the cameras. The time lapse machine only record parts of the normal 24 frames per second, and e.g. by recording only 6 frames per second, the film last four times as long. In order to analyse the behavioural data the software need a time stamp, either made by a “time code generator” or by using the internal time code in digital films. Because of the time coding, the software often have problems with multiplexers and time lapse machines, while quad units may easily be used together with The Observer Video-Pro and EthoVision. In order to track fish with EthoVision, the video films must not necessarily be recorded, and a quad unit enable sampling from four tank units simultaneous.

All behavioural studies are based on individual behaviour, and individual fish must thus be recognisable on the film, either directly by EthoVision, or on the recorded film. EthoVision Pro may separate between individual animals using size, shape, contrast or colour (Fig. 3). If it is not possible to use size e.g. between two fish, the fish has to be marked in order to be recognised. Under the fish, filmed from under the tanks, the fish may be marked with Alcian Blue on the white belly, while the most common is dorsal tagging with either different shapes or colour tags.

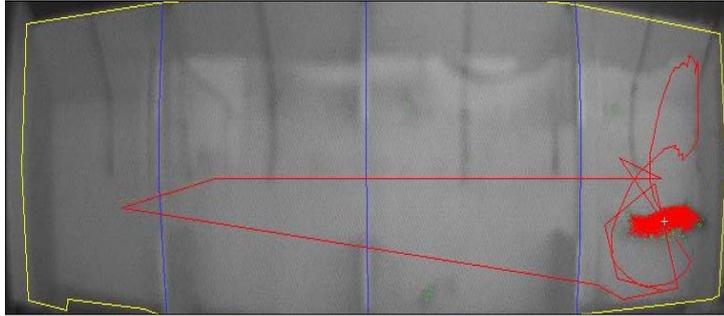


Figure 3 Tracking of fish using EthoVision. The yellow line represent the edge of the tank, the blue lines separate between the areas in the tank, and the red line represent the movement pattern of the fish.

Preference chamber

The preference chamber provides a gradient of the environmental factor in question, and leaves the fish with the opportunity to move freely within this gradient. No other learning than actually experiencing this gradient is involved. In principle any environmental factor that can be reproduced with a gradient can be studied in the system. The system was developed for studies of temperature preference, and is equipped to manipulate, control and record the water temperature. Fish, being a poikilotherm animal, tend to spend most of their time in a narrow range of temperatures, defined as their preferred temperatures. This active behaviour has been termed "behavioural thermoregulation" (Reynolds & Casterlin 1998), indicating that the fish actively regulate its body temperature by selecting an adequate environment. The preference chamber allows the fish to move freely in a temperature gradient, and a temperature preference can be decided by monitoring the temperature at which the fish spends its time. The chamber is constructed so that naïve fish should provide reliable data with minimal adoption or acclimation. Compared to shuttle box systems, there is no time lag, progressive heating or chilling involved, and the available temperature gradient is not controlled by the fish.

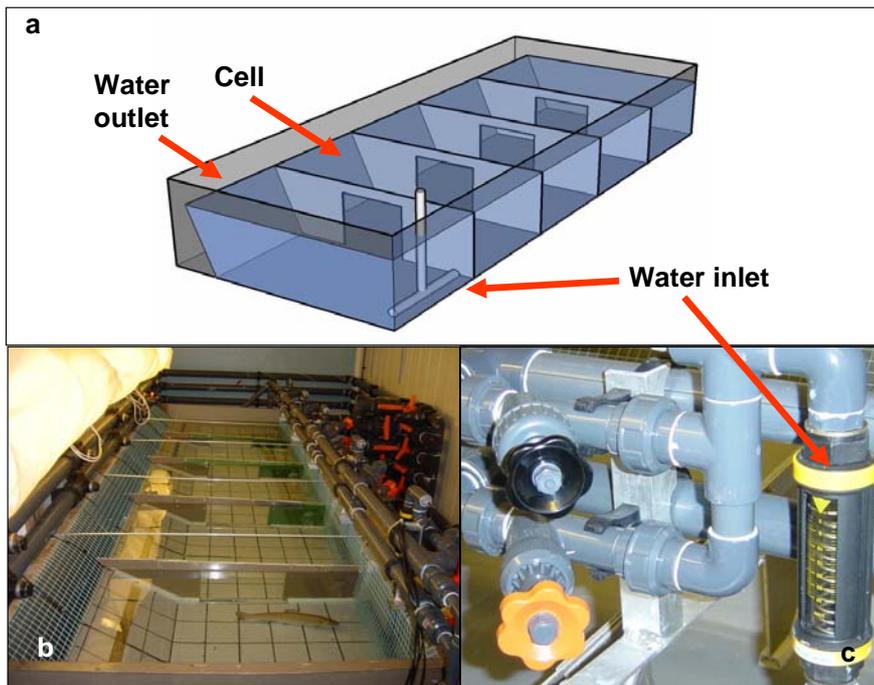


Figure 4 The preference chamber at the behavioural laboratory, Fiskeriforskning. a) A schematic drawing of the system, including 5 cells with different water qualities. The water mixing units and inlets are identical in each cell, b) Side view of the chamber with a fish in the nearest cell, and c) Water mixing unit with flow meter. Photo: Fiskeriforskning.

The chamber is divided into identically sized cells which the fish can move freely between via 30 cm wide openings in the cell walls. Separate water supply to each cell enable control of the temperature gradient by blending of water from two sources, either natural, heated or cooled (Fig. 4). A temperature gradient can be produced by gradually increasing the temperature of the supplied water between cells. The resulting temperatures are recorded at 9 places, in the middle of each cell as well as between them, and stored every 60 seconds (Grant Squirrel). This provides an accurate log of temperatures available to the fish at any given time. An example of a gradient is given in Fig. 5.

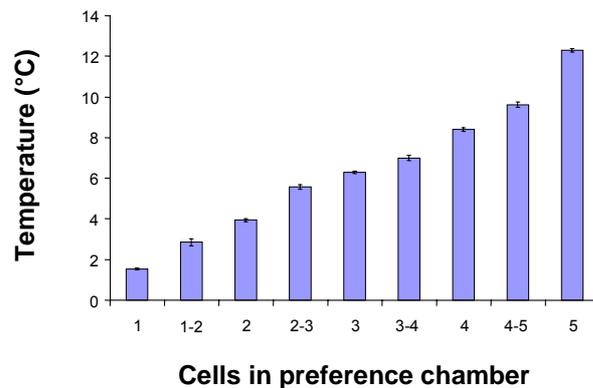


Figure 5 Typical temperature gradient produced in the preference chamber, Fiskeriforskning. Minimum temperature was 1.5 °C and maximum temperature was 12 °C. Variation is given as standard deviation.

The temperature at which the fish spends its time can be recorded using two different methods. 1. Data storage tags (Lotek DST 1200-10) attached to the first dorsal fin are programmed to record the temperature of surrounding waters at 60 second intervals, giving an accurate temperature track record. 2. The chamber was videotaped and all movements of the fish during trials recorded. Time spent in each cell based on video recordings could then be combined with the recorded temperature in each cell (Grant squirrel data) providing an alternative method to locate the fish in cases where tags can not be used. When environmental factors that cannot be monitored by DST's are studied, positioning based on video recording is the only useful method.

Trials are restricted to single fish to avoid confounding effects of social interactions. Each trial starts with the fish being anaesthetised and tagged with the DST between 0830 and 0900. After tagging and recording of weight, length and sexual maturation, the fish is transferred to the preference chamber to recover. The temperature gradient is adjusted to provide as wide range as possible. The DSTs start logging at 0900 and logs throughout the trial. After 24 hours the temperature gradient in the chamber is reversed, so that the cold end become the warm end and vice versa, and the trial continued for another 24 hours. By reversing the gradient the fish has to change position to confirm its choice by selecting again, thereby controlling for possible tank effects.

A measure of activity level can be achieved by combining the DST track record with the information on individual cell temperature. As the temperature in each cell is known, the whereabouts of the fish, and frequency of movements between cells, can be tracked.

Preference tanks

The preference tanks consist of three 1.7 m³ circular tanks (diameter 150 cm, height 120 cm; Fig. 2 b) interconnected by two black plastic pipes (45 cm long, 43 cm diameter). Each tank has separate water supply controlling water flow, salinity and water current direction and speed. A vertical perforated pipe in the centre of each tank drains the water from the whole water column, with water level controlled by a common water outlet giving identical water

level in each tank. To minimise exchange of water between tanks, water currents are adjusted so that the water flows past the pipes in the same direction at each side. The end tanks can optionally be supplied with water of other qualities, by addition of a small amount of water through the water inlet. This enables manipulation of the water quality in the end tanks without physical disturbance of the fish, and without changes in the water currents or flow. The pipes connecting the tanks are equipped with Trovan Passive Integrated Transponder-tag (PIT-tag) antennas that provide a radiofrequency field in the pipes. Passages of fish tagged with PIT-tags are registered and time of the incident is logged on a PC. This logging system keeps track of the fish in the system, providing data on the time spent in each tank. The frequency of movements also gives information about activity level. Each tank is also equipped with a video camera providing a top-down view of the activity in each tank. Combined with a time lapse video recorder, the videotapes provide information of the whereabouts of the fish. Due to confounding effects of social interactions, it is envisaged to use the system with single fish only.

4.2 The fish learning laboratory

Tore S. Kristiansen & Jan Erik Fosseidengen

Learning plays a pivotal role in the development of behaviour of all vertebrates and learning ability is an essential trait for survival in a different habitats and changing environments. A better understanding of the learning abilities and limitations of farmed fish is important for the understanding of how and why behaviours of farmed fish develop and how the fish cope with and handles environmental challenges in the aquaculture environment. Among the more than 20.000 fish species there are large variations in sensory and cognitive capacities, but even if learning abilities of fishes have got increasing scientific interest the last decades (Laland *et al.* 2001), this field is relatively unexplored. For the fish farmer the knowledge about fish learning abilities can also be used to manipulate (or educate) fish behaviour and develop less stressful and more efficient handling and feeding routines.

In this SIP program, the “Learning in farmed fish” project has studied associative learning abilities of cod, *Gadus morhua*, and halibut, *Hippoglossus hippoglossus* (see chapter 8) with focus on ability to associate events separated in time and space (Lieberman 2000). For these studies we have built a “learning laboratory” at IMR, Austevoll, consisting of four 7 m³ tanks (diameter 3 m) placed in a separate building (Fig. 6). The fish behaviour is monitored by video cameras placed right above each tank, and to avoid disturbance of the fishes the studio for monitoring of behaviour and control of feeding automates and light signals is located in a nearby building. The system is designed for running various combinations of appetitive classical conditioning experiments, where fish food is used as unconditioned stimuli (US) and flashing light from a transparent circular tube floating on the surface in the feeding area is used as conditioned stimuli (CS). The onset and offset of light signals and feeding automats are regulated by programmable electric relays, which makes it possible to design experiments with any combinations of interstimuli intervals (ISI: time from onset of CS to onset of US) and trace intervals (time from offset of CS to onset of US). Each camera is connected to a programmable DVD-HD recorder, and together this system can be pre-programmed to run the experiments fully automatic. The DVD-recordings give date and time of all recordings and the analyses of behaviour can be done on separate PCs using the Noldus – The Observer program (for DVD) or the DVD player on the PC.

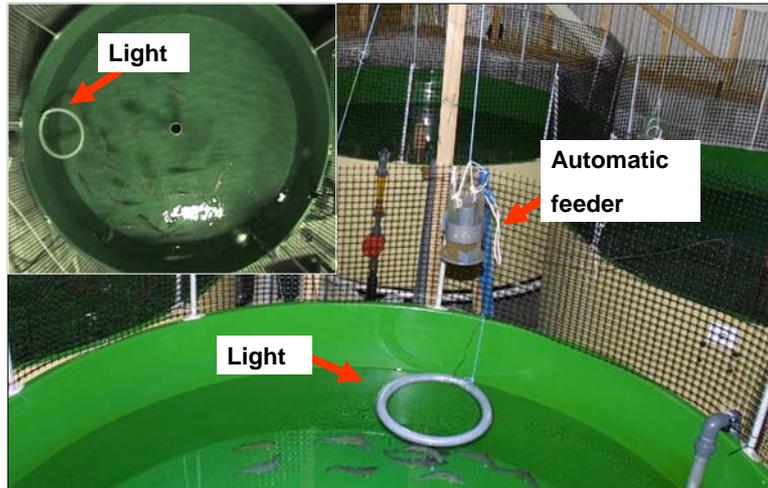


Figure 6 The fish tanks, feeder and signal system in the learning laboratory, Austevoll, IMR. Photo: IMR.

To study operant conditioning (the ability to associate own actions with outcome) and individual learning abilities we have developed a system based on Trovan passive transponder tags (PIT) and antennas. The antenna makes an electro magnetic field that induces an electric current in PIT-tags that enters the field (less than approximately 30 cm from the antenna). The tag will then transmit the tag number radio signals back to the antenna, which again sends the tag number back to the computer where it is stored together with registration date and time. The registration of all or any tag number can also be used to start an electric relay connected to a feeding automate. In this way the PIT-system can be used as a demand feeding system where the fish get a pre-programmed reward every time (or at a given probability) it approach the antenna. Since individual fish is registered we can study who and how fast the fish learns to operate the system and how much it is willing to work for the rewards. By using more than one antenna the fish can also be trained to choose between feeders with different food types (reward values) and various experiments related to ability to remember visual signs and locations can also be studied. The antenna can be placed outside the tank wall and visual signs can be taped to the inside of the tank wall.

4.3 The water quality laboratory: Controlled manipulation and assessment of environmental parameters

Hilde Toften

Land-based flow-through and recirculation systems are becoming more and more important in the intensive culture of fish. The advantages of these systems are better control over the biological and chemical quality of the inlet water, reduction in recipient pollution and independence from climatic conditions. Because these systems have high investment and energy costs it is necessary to lower the costs of water pumping and reduce water exchange in combination with oxygen supply.

Earlier, when the fish production was based on the natural content of oxygen in the inlet water, oxygen was the parameter that limited the carrying capacity, and subsequently, the flow rate had to be high. During the 90-ties, it has been increasingly common to supply the inlet water with pure oxygen, and thus, oxygen is no longer the limiting factor. When oxygen is added, the reduction in water flow is mainly limited by the accumulation of excretion products from the fish as ammonia (NH_3), urea, carbon dioxide (CO_2), suspended solids and induced low pH. Addition of oxygen also makes it possible to increase the biomass loading in the system resulting in high fish densities.

At Fiskeriforskning, we have focused on the welfare consequences of such poor water quality conditions following highly intensive husbandry practises. However, to conduct these types of studies, it is necessary to have adequate facilities. Until recently, there have been limited possibilities to carry out studies on many aspects of water quality in flow-through and recirculation systems. In 2002 we started to improve the existing facilities in Tromsø, and the experimental infrastructure at the Aquaculture Station is one of the best in Europe and enables us to do water quality experiments under highly controlled conditions.

A prerequisite for a sound interpretation of the results is that the quality of the raw water is good, and the Aquaculture Station in Tromsø has good raw water. This has recently been proven by the national water quality surveillance program, VK-2003, lead by Norwegian Institute of Water Research (NIVA). For example, the fresh water is not acidified, as many other Norwegian fresh water sources are, and there is low content of metals (like aluminium) in the water that could cause extra problems for the fish.

The “*Water quality laboratory*” in Tromsø consists of four main units: 1) Mixing unit; 2) Experimental unit; 3) Spider unit; 4) Water analysis unit (Fig. 7).

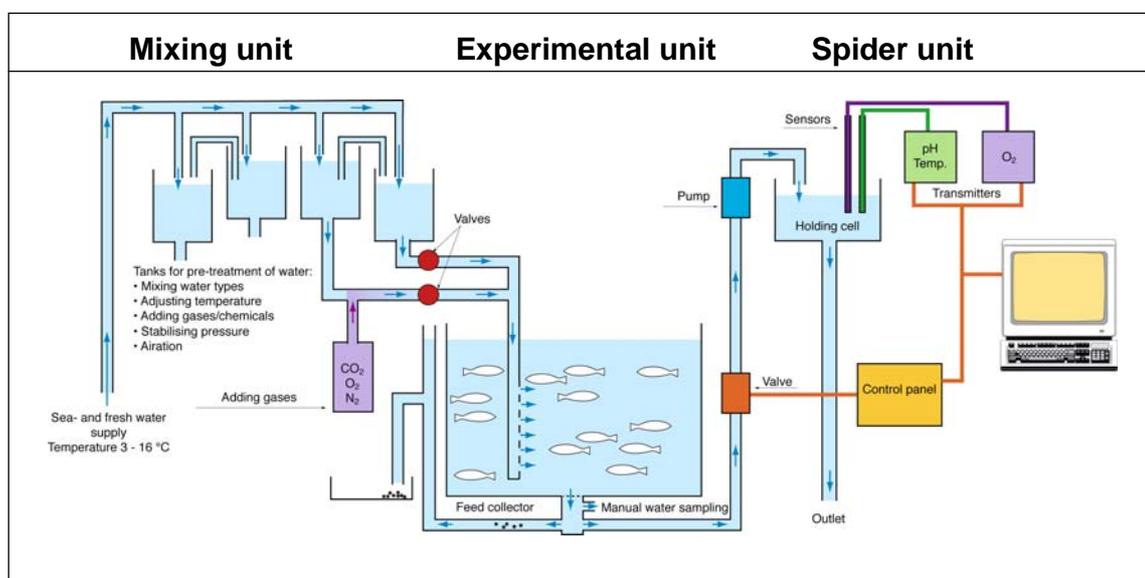


Figure 7 Schematic overview of the Water Quality Laboratory at Fiskeriforskning, including the mixing unit, the experimental unit and the Spider unit.

The Mixing unit

The mixing unit is used for premixing the water to obtain the specific water qualities needed in each experiment and is placed one floor above the fish tanks. It consists of a wet room with four pumps, several pipes, valves, digital flow meters and column degassers, two gas injectors (Aga AS), three large tanks (600 l), six smaller tanks (100 l) and equipment for monitoring oxygen, temperature and salinity.

The large tanks are used to mix the different water types available at the Aquaculture Station, for example unheated and heated fresh and sea water (up to 16 °C). Both the fresh and the sea water has been filtered and degassed in a packed column before entering the mixing unit. In addition, the sea water has been UV treated. The different water types are pumped from the Aquaculture Station pipeline system and degassed a second time before entering the large tanks. From each of the large tanks, the water can be directed through the gas injectors or to one or several of the six small tanks. The amount of water from both the large and the small tanks can be easily measured and controlled by digital flow meters. The water from the small tanks is then supplied to the fish tanks by gravity.

The experimental unit

The indoor experimental unit has 18 self-cleaning circular tanks (0.5 m³ each) allowing the tests of maximum 9 different treatment groups. Each tank is equipped with digital water flow meters, a vertical inlet pipe, a feed automate and a feed waste collector. Each tank has three outlets; one via the feed waste collector (Fig. 8), a second for manual water sampling and a third for automatic water sampling and recording (the Spider unit). Light intensity, photoperiod and artificial dawn and dusk can be controlled.

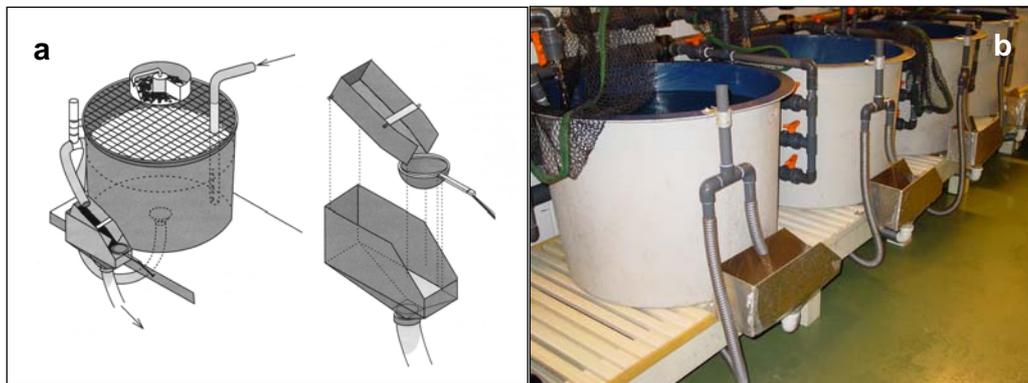


Figure 8 *Feed waste collector system used at the Water Quality Laboratory, Fiskeriforskning. a) Schematic drawing of a collector, and b) Picture of feed waste collectors on 500 l rearing tanks. Photo: Fiskeriforskning.*

The Spider unit

The Spider unit is an automatic system for continuous monitoring of temperature, oxygen and pH (Fig. 7). The water parameters are monitored by the use of pipes, solenoid valves and a measure chamber where an oxygen probe and a pH probe are connected to a data logger. Recording in the data logger, opening and closing of each solenoid valve are managed by an automatic controller device.

The water analysis unit

The water samples collected manually can be analysed further at the water analysis unit. This unit is equipped with a Carbon Dioxide Electrode (Orion model 95-02) for measurement of carbon dioxide concentrations, an Ammonium Electrode (Orion 95-12) for measurement of ammonia concentrations, a Thermo Orion Sure Flow Ross Combination Electrode (8172BN) for measurements of pH and a Orion 3 Star, Conductivity Portable Electrode (Orion 013005MD) for measurement of salinity. Parameters like oxygen saturation, red ox potential, conductivity and pH can be simultaneously measured and logged with a multi-parameter instrument (YSI 556 MPS, Yellow Springs Instruments, Ohio, USA) and the YSI 5566 TPG sensor is used to measure the total gas pressure (TGP). In addition, we collaborate with the Norwegian Institute for Water Research (NIVA) and Norwegian University of Life Sciences (UMB) for a more complete analysis of the water.

4.4 The cage environment laboratory: Behavioural studies in a realistic farm environment

Frode Oppedal, David Johansson & Jon-Erik Juell

About 95 % of the biomass in Norwegian aquaculture is produced in seawater cages. During this on-growing phase, more than 15 % of the fish is lost due to mortality and unknown causes. It is therefore surprising that little focus has been put on environmental variability and its effects on physiology, behaviour and welfare of caged salmon. To deepen our understanding we need more detailed studies of the environmental complexity and behavioural and physiological response of the fish in commercial settings. It can be argued that large-scale studies involving thousands of animals may compromise the need to refine and reduce the number of research animals possibly suffering. However, a crucial ethical aspect in this context is whether the experiment will result in relevant data for the research topic in question. Behavioural studies related to fish farming often aim at understanding the priorities of the animal – what they prefer and need – in order to establish production protocols that ensure acceptable standards of animal welfare (Dawkins 2003). When fish are taken out of the commercial setting, the environment, the behaviour, the management and the social interactions related to group size may be influenced in a way that questions can be raised regarding their relevance to commercial production.

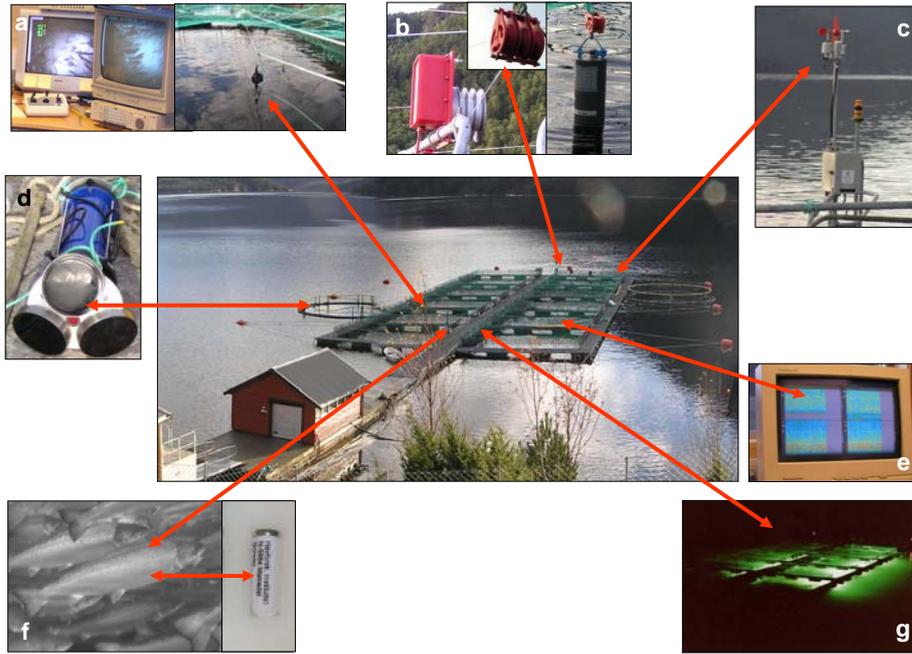


Figure 9 Pictures and sketch of the cage environment laboratory at IMR. a) Remote control cameras observe individual and group behaviour, b) CTD's attached to automatic winches records depth profiles of temperature, salinity, dissolved oxygen and light, c) A weather station measures wind and air temperature, d) Acoustic current profilers measures water current speed and direction around the cages while a velocimeter is used inside cages, e) A PC based echo sounder records the swimming depth and fish density of groups, f) Data Storage Tags records swimming depth and thermal history of individual fish, and g) Underwater lamps can be deployed at different depths. Photo: IMR.

The Cage Environment Laboratory

In order to study the environmental influences on fish behaviour, growth and welfare in a realistic setting while simultaneously maintaining scientific quality, a unique cage environmental laboratory was established at Institute of Marine Research (IMR) sea cage site at Solheim in Masfjorden (Fig. 9). The six middle cages in this 10 cage “steel farm” was equipped with systems monitoring the environment and fish behaviour with high resolution in time and space.

Environmental screening

The cage environment is monitored using CTDs with oxygen probes (YSI 6600; Yellow Springs Instruments, Ohio, USA or SD204; SAIV, Bergen, Norway or XR420; RBR, Ottawa, Canada) mounted on automatic winches (HF5000; Belitronics, Lunde, Sweden) inside 6 replicate cages. Additionally, a light probe (LI192 and LI193, Licor Biosciences, NE, USA) is mounted on a 7th reference CTD outside cages. Water current velocity and directions are measured using acoustical current profilers (Continental and Aquadopp by Nortek, Rud, Norway) in different positions around the farm and an acoustical velocimeter (ADV by Nortek) within the cages. Water samples for e.g. NH₃ and CO₂ analysis are taken with a Ruttner sampler. Fouling of the nets can be quantified visually with underwater cameras. Hence, high-resolution temporal and spatial screening of the environment within and around the farm is achieved with a sampling frequency of four depth profiles or more per hour. An example of the temperature fluctuations from August to December is shown in Fig. 10.

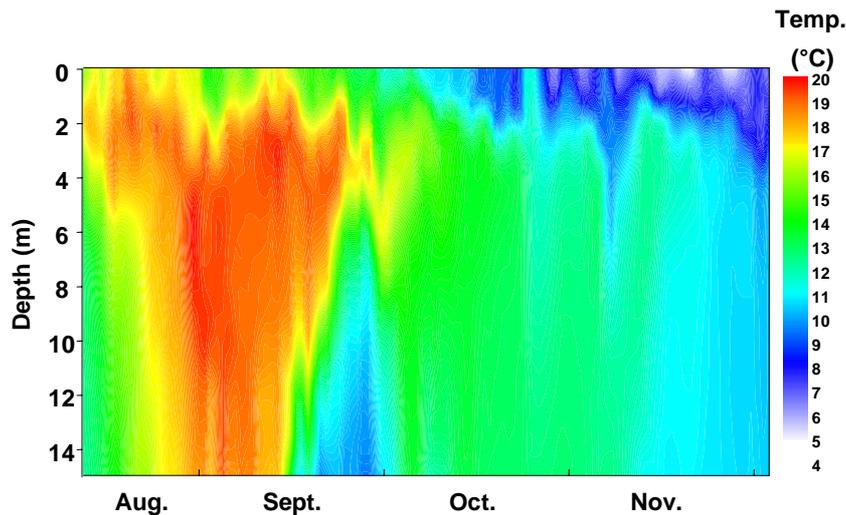


Figure 10 Spatial and temporal temperature fluctuations from summer to winter in the cage environment laboratory, IMR.

Behavioural observations

In each of the cages behavioural patterns such as group structure, horizontal distribution and swimming speed are observed by underwater cameras (Orbit, SM Remote systems, Frakkagjerd, Norway) connected to digital recorders. Each camera is remotely positioned at depth, distance from net wall and can be panned and tilted in 360 degrees. Infrared light sources can be used to observe behaviour at night within 1.2 m distance from camera. The swimming depth and schooling density of groups is observed simultaneously and continuously in the 6 replicate cages by a PC-based echo integration system (Lindem Data Acquisition, Oslo, Norway) described by Bjordal *et al.* (1993) connected to upward-facing transducers, with a 42° acoustic beam, positioned under the centre of each cage.

Individual behaviour is observed using the cameras, and also with tags (DST: data storage tags; Star-Oddi, Reykjavik, Iceland) inserted into the abdominal cavity of individual fish. The tags record swimming depth and body cavity temperature at pre-set time intervals. When the fish is retrieved the swimming depth and thermal history of the fish is downloaded from the tag.

Within the fish farming industry management protocols differ with regard to e.g. stocking density, photoperiod, feeding and net change operations. Understanding the mechanisms that control the environmental variation in cages and its interplay with management protocols to influence physiological and behavioural responses is a fruitful approach to how to ensure the welfare of caged fish. The Cage Environment Laboratory provides a platform to carry out challenge and preference tests in relation to motivational and environmental gradients in a realistic social and environmental setting.

Modelling the results

With a multi-factorial environment and several behavioural measures, analysis of the data must be improved through innovative statistical tools. A novel application of a regression tree method (Venables & Ripley 1999) has been introduced in the analysis in order to reveal relations and quantify the relative importance of the environmental factors in explaining variation in e.g. swimming depth. The importance and the interaction of different factors predicting the leaf values (e.g. fish density, oxygen levels) are revealed in the splits and the horizontal length of the branches in the regression tree (see chapter 11).

Development towards a commercial stress-monitoring tool

Behavioural shifts are often a primary response to stressful condition and may be used as indicators that can give an early warning to the farmer about sub-optimal conditions. By using the methods developed in the cage environment laboratory on field surveys at commercial fish farms, events of fish altering their behaviour and space use by crowding in certain water volumes has been recorded. Integrating environmental and behavioural indexes in the stress monitoring protocols may well prove useful. This approach is pursued in a current EU-project where behavioural observations in commercial conditions are linked to physiological and immune responses to acute and chronic stressors in controlled tank environments (EU-project WEALTH). This knowledge will be further used in an upcoming EU-project (FASTFISH) to develop tools for on-farm assessment and documentation of stress level and fish welfare.

4.5 Non-invasive methods for assessment of fish welfare

Øyvind Aas-Hansen & Børge Damsgård

Physiological telemetry enables monitoring of physiological parameters such as ventilation rate, heart rate, electric muscle activity (electromyogram, EMG) and others in unrestrained, free-swimming fish (Baras & Lagardere 1995). In conjunction with the integrated EU-funded project SEAFOODplus, Ethiqua (<http://www.seafoodplus.org>), we have developed a concept for the use of physiological telemetry as a method for the assessment of fish welfare in aquaculture systems. This concept involves the construction of an electronic tag termed “SmartTag” which is attached to a statistically appropriate number of fish and provides online measurements of changes in water pressure within the buccal cavity, thus providing detailed information of the fish’s ventilation (respiration rate and profile; including information relating to respiratory volumes). The tag itself is specially produced by the Norwegian technology company Thelma AS (<http://www.thelma.no>), and consists of a battery unit, a differential pressure sensor and an ultrasound (50 – 120 kHz) acoustic transmitter. The transmitted signals are received by a hydrophone, amplified and filtered, and displayed and stored on a computer. Thus, rather than monitoring properties of the rearing environment (such as water quality parameters or other potential sources of distress), the SmartTag enables measurements of how the rearing environment affects the physiology of individual, free-swimming fish (Fig.11).

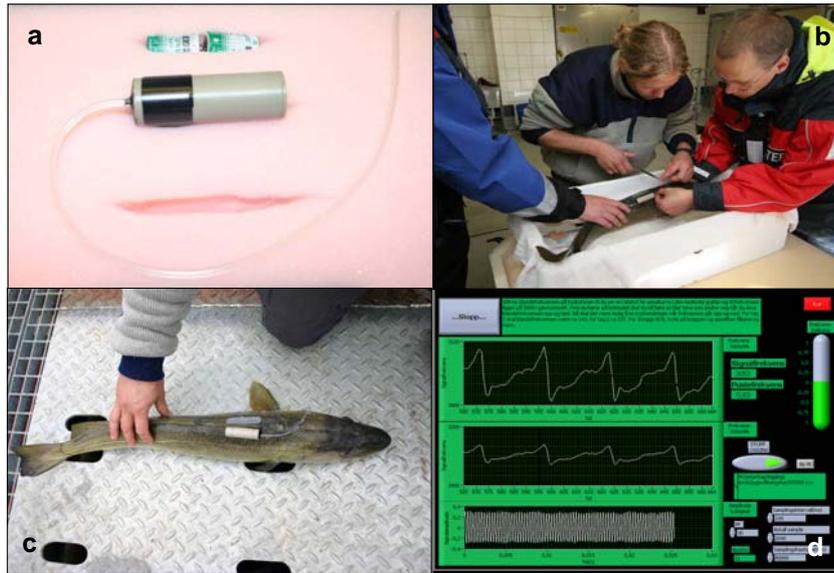


Figure 11 a) Smart Tag prototype, b) & c) Externally tagging of a cod during general anaesthesia, and d) Following restitution and release back into its net-pen, the cod's ventilation pattern is continuously monitored on a computer.

The underlying principle for the use of ventilation pattern or other candidate physiological parameters for assessment of welfare in free-swimming fish is that most factors which may cause distress or in some way represent a potential welfare concern also induce compensatory physiological responses. Such compensatory responses may or may not include induction of stress responses (Wendelaar Bonga 1997; Barton 2002), and represent controlled variables whose function is to regulate more vital parameters (such as blood oxygen level and pH, arterial blood pressure, energy substrate availability) at near-constant levels, thus ensuring life-important maintenance of homeostasis. Measurements of compensatory physiological responses therefore represent good candidate welfare indicators which, in theory, may provide sensitive and fine-graded quantification of the potential distress experienced by the animal. There is no single physiological parameter, however, with the qualities of a “universal welfare indicator” showing sensitive and linear responses to all factors which may cause a potential distress to the animal. Also, the number of physiological parameters suitable for telemetric measurements obviously is very limited. A number of laboratory studies suggest, however, that the parameter chosen in SmartTag namely quantitative measurements of ventilation represents the perhaps most promising indicator of a wide range of important welfare factors for use in physiological telemetry. We know, for example, that ventilation is significantly affected by factors such as hypoxia, hypercarbia and changes in water pH or metabolite levels (Smith & Jones 1982; Borch *et al.* 1993; Waller *et al.* 1997; Wasielesky *et al.* 1997; Reid *et al.* 2000), toxic or sub-toxic levels of metabolites and xenobiotics in feed or water (Williams *et al.* 1997; Handy *et al.* 1999; McKim *et al.* 1999; Valenzuela *et al.* 2003; Pane *et al.* 2004), anemia (Smith & Jones 1982) parasite infections (Laitinen *et al.* 1996; Finley & Forrester 2003), diseases (Byrne *et al.* 1991), as well as during the general stress response (e.g. Laitinen & Valtonen 1994) and factors which presumably also include psychological responses such as shelter, fear and pain (Fischer 2000; Sneddon 2003; Sneddon *et al.* 2003; Hawkins *et al.* 2004). In comparison, the data on for example heart rate measurements are less consistent (e.g. Borch *et al.* 1993; Thorarensen *et al.* 1996), partly because the use of heart rate as a physiological indicator is complicated by the fact that the regulated variable (blood pressure)

normally also is strongly influenced by changes in the heart's stroke volume and in the total peripheral resistance of the cardiovascular tree.

Ventilation is, however, strongly dependent also on metabolic factors such as temperature, exercise and feeding, as well as species, size, life-stage, and possible diel- an seasonal processes (e.g. Shelton 1970). It is therefore of important that the relationship between ventilation pattern and the above mentioned factors are carefully determined in well-designed calibration experiments (Baras & Lagardere 1995; Barreto & Volpato 2004). For the case of SmartTag, such calibration experiments include the use of swim tunnel respirometry, manipulative water quality treatments and comparison with more traditional, non-invasive and invasive measurements such as behavioural tests and blood sampling for assessment of stress responses.

SmartTag function as a very promising tool in fish welfare research. Potentially, further development of the SmartTag concept may justify its use also as a tool for monitoring and documenting fish welfare status in commercial aquaculture. In particular, this latter aspect may prove more important in the future, along with the development of larger fish production units with increasing number of fish, and the increased prevalence of submerged- and offshore aquaculture facilities where the opportunities for continued supervision of the fish is limited and the need for a remote early-warning system therefore is greater.

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5 REPRODUCTIVE SUCCESS, SPAWNING BEHAVIOUR AND WELFARE IN BROOD-STOCKS

Linda A. Hansen, Helge K. Johnsen & Børge Damsgård

5.1 Introduction

The reproduction of an individual depends on a variety of internal factors, such as health and social status, and external factors such as sex ratio and seasonal cues (Sargent & Gross 1993). If the physical or social environment changes, individual fish may respond through either phenotypic plasticity or by some genotypes adapting more successfully and thereby leaving relatively more offspring (Wootton 1990). In aquaculture and for fish incorporated in breeding programmes the physical environment may vary, but otherwise fish density, food availability and other external factors remain quite stable. There may none the less be large variations in a number of individual characteristics within a generation of fish, such as age and size at maturation, and reproductive success. Also the reproductive input with subsequent reductions in growth and survival after a spawning season may vary.

5.2 Background on factors influencing reproductive success and behaviour

Cost of reproduction

As reproduction is energetically costly both concerning physiological processes and behaviour, there may be a critical level of nutrient reserves involved in the decision whether or not to mature. Exercised female sockeye salmon displayed lower per cent body lipids than non-exercised females, and the exercised females displayed a delayed maturity, lower egg deposition rates, and were more likely to die prior to egg ovulation (Patterson *et al.* 2004). Male 3-spine sticklebacks, *Gasterosteus aculeatus*, experience on average a 91 % reduction in liver glycogen and 73 % reduction in liver lipid during the breeding season (Chellappa *et al.* 1989). Intensely displaying sand goby males have lower lipid content than less active males, indicating that this signalling increased energy expenditure or that low condition males increased their signalling effect (Svensson *et al.* 2004). The investment in reproduction may also vary between the sexes. Arctic charr (*Salvelinus alpinus*) females may lose about 80 % of their body lipid during spawning and over-wintering, while the males loose 50-55 % (Jobling *et al.* 1998). Reproduction in farmed fish may cause both economical losses to the farmer due to reductions in fish growth, as well as leave fish in a nutrient depleted condition, with physical injuries and as more susceptible to disease and parasites.

Aggressive behaviour as a tactic related to reproduction

Male fighting is often a successful tactic despite the cost involved in aggression (de Gaudemar & Beall 2003). During the reproductive season a large size may be important for male milt production and competitive advantage (Fleming & Gross 1994, Quinn & Foote 1994), female choice of males (Berejikian *et al.* 2000), female aggression towards males (Petersson & Järvi 1997), and female redd quality (Myers 1986) and egg number (Hutchings

1991). In theory a higher competitive success leads to more spawnings. Females may establish territories and males fight for temporary access to the female (Fleming 1998), or males can establish and defend territories around females and high quality nest sites (Foote 1990). In externally fertilising fish there may be an advantage of spawning close to a female, instead of as a subordinate male further away (Blanchfield & Ridgeway 1999) and mate guarding may produce more offspring (DeWoody & Avise 2001).

Mate choice

Polygamous mating among males and female mate choice mean that almost all females can find a mate but many males are unable to breed. When mate choice occur it can be based on either immediate or on delayed benefits from the genetic composition of the partner. Sexual selection is a more common behaviour in females than males. However, there are exceptions when the females are polygamous, the male's supply of sperm is not indefinite, and the males may exercise mate choice or parental care. The courtship structures are therefore an honest signal of energy reserves. Size, age and status can indicate viability of mates, which may be heritable, and a female may then select for good genes (Turner 1993).

Reproductive hormones

Hormones operate within the animal and pheromones between animals, serving to synchronise spawning interactions and reproductive behaviour with gamete maturation (Kobayashi *et al.* 2002). The act of reproduction must be perfectly timed with both internal and external cues, and the sum of endogenous and exogenous factors promotes or inhibits the reproductive behaviour of individual fish. Exogenous factors may be social stimuli that trigger or inhibit endocrine processes in the body. Males mating in favoured positions have higher levels of the sex steroid 11-KT (Pankhurst 1995), and high androgen levels are generally thought to correlate with increased sperm quality (Folstad & Skarstein 1997). In addition the plasma concentration of T and 11-KT are higher in aggressive, dominant salmonid males than in subordinate males (Liley & Kroon 1995; Cardwell *et al.* 1996) with similar tendencies in 17,20 β -P in Arctic charr (Elofsson *et al.* 2000).

The studies performed in this strategic research programme compared two species with different behavioural needs and environmental requirements in culture. Cod is a demersal spawner with similar preferences as other potential aquaculture species such as haddock and saithe. Arctic charr is a salmonid with reproductive mechanisms similar to salmon, rainbow trout and other species already common in aquaculture.

5.3 Reproductive behaviour and mating success in cod

In recent years cod has become an important species in aquaculture, increasing the need for more knowledge on the behaviour of cod. In spite of this few studies have been performed on its mating behaviour since Brawns' (1961) detailed description of cod reproductive behaviour in a small group of fish. Cod spawn in open waters and display no parental care, but knowledge is scarce on the connection between spawning behaviour and reproductive success in cod (Hutchings *et al.* 1999). The spawning period lasts for about two months with year to year variation (January-June) in timing correlated to water temperature (Hutchings & Myers 1994). Female fish may spawn up to 19 batches with a 2 to 6 days interval, and the egg quality varies with fish size, physiological stress level and egg batch (Kjesbu 1989). Males

mature earlier than females, and in culture fish that is not light treated may mature after two years (Hansen *et al.* 2001).

Behavioural patterns may improve the reproductive success of individual cod, such as swimming pattern, territoriality, aggression, courtship, mate choice, and use of pheromones and sounds (Brawn 1961; Hutchings *et al.* 1999). The physiological and behavioural mechanisms behind the release of eggs by a female cod are poorly known. Most likely it is caused by a combination of internal and external cues. The ventral mounts associated with courtship behaviour and spawning are mostly observed during night hours, but many of these are unsuccessful in eliciting the release of eggs from female cod (Hutchings *et al.* 1999). Multiple paternity occurs in many fish species (Martinez *et al.* 2000), and more than one male cod might spawn with a female fish. Although several male cod may contribute in a spawning the largest is expected to sire higher proportions of offspring (Bekkevolden *et al.* 2002). A large investment in size and activity can be an efficient strategy if the female cod practise mate choice. Choices can be made on the basis of qualities such as successful ventral mounts, larger sperm batches to ensure fertilisation and less chance of being interrupted during the courtship if the male is large and dominant (Hutchings *et al.* 1999). Brawn (1961) observed that only one male cod, the largest, was seen to spawn and all spawning occurred in his territory. Correspondingly, as larger repeat female spawners normally exhibit higher fecundity (Kjesbu 1994), one could expect more male attention directed towards larger females (Blanchfield & Ridgeway 1999).

An important question is what consequences farming conditions potentially have on the reproductive behaviour, reproductive success and general welfare of mature cod. Factors like fish density, distribution in space, and handling frequency may have an effect on the reproduction of individual fish. One potential consequence of confinement is irregular spawners who suffer a low fertilisation rate due to stress and a broken ovulatory rhythm (Kjesbu 1989; Patterson *et al.* 2004). Agonistic and courtship behaviours related to reproduction, such as those described by Hutchings *et al.* (1999) and Brawn (1961), may be adapted to or lost in the farming environment. Furthermore, the success of breeding program on a new species may depend on knowledge on the species in question, including its reproductive biology. The brood-stock fish used in a breeding program have to perform well in an aquaculture environment, feed well, invest surplus energy in a large gonad, take part in the seasonal spawning and produce a large quantity of offspring of high quality. With this study we aimed to describe behavioural mechanisms involved in cod reproduction, on a group and individual level. Individual qualities and the observed behaviour during the spawning season was compared to individual spawning success measured through parental testing, searching for characteristics of individuals with a high reproductive success in a farm environment.

The study was performed at the Aquaculture Research Station in Tromsø in February 2003, on 15 female and 15 male cod of the North East Arctic stock. The average initial weights and lengths of female and male fish were 6.1 kg and 5.9 kg, respectively. Individual identification of fish was done by varying numbers and placements of T-bar marks on the dorsal side of the fish. The fish were kept in a circular grey tank, 5-m in diameter and 1.8-m deep, with water of ambient temperature and at a natural photoperiod. Two cameras were mounted above the tank: a wide-angle camera capturing most of the tank and a pan/tilt- zoom camera. Group and individual behaviours were video recorded at irregular intervals during the daytime, depending on how active the fish were, amounting to 19 h of analysed recordings from the wide angle and from the zoom camera. Performers and receivers of ventral mounts and agonistic behaviours (chases, bites and nips) as described by Brawn (1961) and Hutchings *et*

al. (1999), and spawning (release of egg and milt) were registered, and for ventral mounts also the duration of the behaviour. The definition “ventral mount” is used rather than courtship in this study as this behaviour could be observed between males as well as towards females and therefore might be interpreted as other reproductive behaviour than courtship. Fertilised eggs were sampled in the morning, and estimations of spawning time were made from the developmental stage at that time related to water temperature. A maximum of 60 offspring from all spawnings was genetically tested for paternity by using an assay system including the five microsatellite loci *Gmo8*, *Gmo19*, *Gmo35*, *Gmo37* and *Tch11*. This system enables the identification of the offspring parents with high efficiency (99,9 %). For more detailed descriptions of the method see Delghandi *et al.* (2003). In total 311 egg from 42 egg batches were collected and analysed.

Reproductive behaviour during the spawning season

The number of male performers and female receivers of ventral mounts peaked in the middle of the spawning period, a period that lasted from 10 March to 28 April 2003 (Fig. 12). The number of male receivers of ventral mounts was quite stable, and towards the end of the spawning period a transition was apparent, from a similar share of male and female receivers to mostly male receivers. Social behaviour that could be interpreted as aggression and territoriality was not observed during the study period. The average ventral mount lasted 13.2 ± 0.6 seconds (\pm SE) (median 10, range 1- 74 s), and performers were always male fish. There were also occasional occurrences of two simultaneously performing fish towards the same receiver. Male fish that were frequent performers were also more likely to be frequently receivers. Female fish did not receive ventral mounts more frequently than did male fish. Frequent male performers of ventral mounts displayed a negative, but non-significant relationship to SGR ($0.5 > p > 0.25$) and final weight ($0.1 > p > 0.05$). Female fish had a more pronounced reduction in condition factor ($p = 0.017$) and also a significantly smaller SGR than male fish. When looking at the sexes separately ($n = 15$) there was no correlation between any size characteristics and received vertical mounts. In general there were large individual differences in frequency of performed and received vertical mounts.

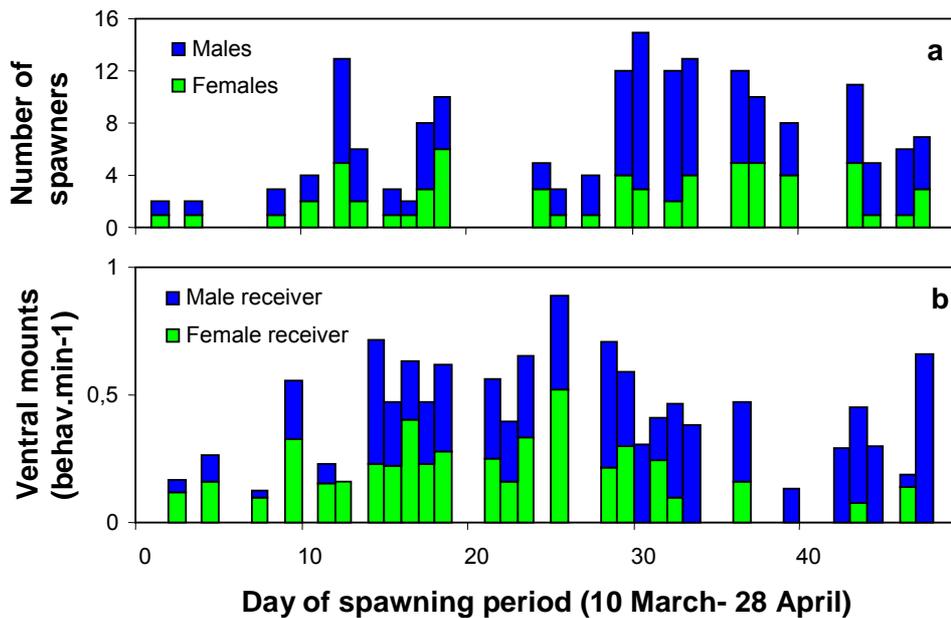


Figure 12 Reproductive behaviour in cod seen as: a) Daily variation in number of male (blue bars) and female (green bars) spawners during the spawning period, and b) Observed number of ventral mounts per minute received by male fish (blue bars) and female (green bars) from the first to the last day of the spawning period. The sum of male + female reception equals the number of ventral mounts performed by male fish.

Reproductive success measured by microsatellite analyses

Pedigree analyses revealed that significantly more male than female fish participated in spawning events ($p < 0.01$). As much as twelve out of fifteen male fish were distinguished by their DNA as participating one day, while the maximum found for the female fish on one single day was six individuals (Fig. 12). Female fish were found to spawn 4.3 ± 0.7 (1-10) times and male fish 7.4 ± 0.8 (2-14) times during a total spawning period of 51 days. Female fish and male fish had a spawning period of 20.6 ± 3.4 (1-38) and 25.8 ± 3.1 (1-46) days, respectively. The spawning interval of female fish was $6.1 \text{ days} \pm 0.8$ (1- 25) while male fish spawned every 4.0 ± 0.4 (1-20) day. Most spawnings were estimated to occur in the evening between 1600-2000 (16 spawnings) and in the early morning between 0400-0800 (10 spawnings). Also at night, between 2400-0400, there were some activity (8 spawnings). Between 2000-2400 and during the daytime, between 0800-1200 and 1200-1600, there were very few spawnings (2, 2 and 4 spawnings, respectively). There was no significant correlation between individual involvement in ventral mounts and actual spawning (Fig. 13). There were no indications of mate choice, as a majority of the offspring analysed came from combinations of one male and one female fish that spawned only once together. Of the male-female spawning combinations found three times or more, all were different from the most common male-female ventral mount combinations.

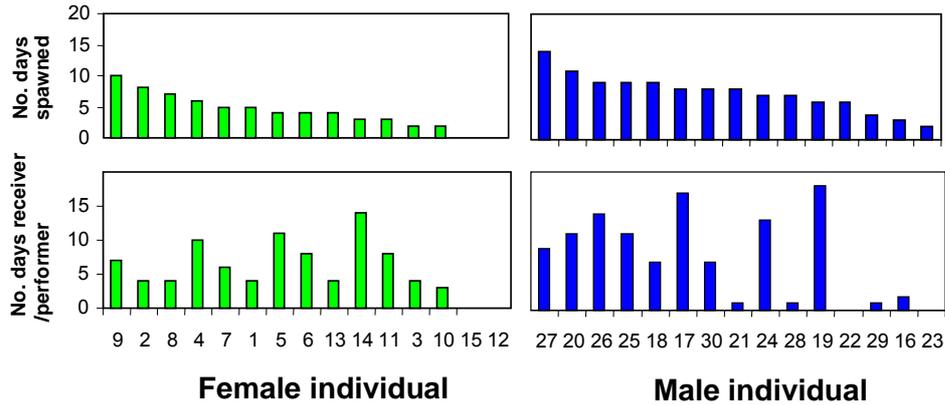


Figure 13 Individual participation in spawning and ventral mounts through a spawning season measured as total number of days the female (green bars) individuals 1-15 and male (blue bars) individuals 16-30 were found to spawn, and number of days the same females and males were observed to receive or perform ventral mounts, respectively. The individuals are consistently ranked from highest to lowest based on the number of days they were observed to spawn.

Comparing behaviour and reproductive success in one single spawning event

Two spawnings were recorded on film. Both followed ventral mount interactions between various individuals, and a large number of individuals were involved each time. The spawning activity was so intense it was impossible to visually distinguish the contribution of individual fish, and there were separations into several dynamic “spawning groups”. Microsatellite analysis performed on 60 sampled egg fertilised at 1630 on 11 April, one of two spawnings caught on film, illustrate the degree of multiple paternity and lack of correlation between ventral mounts and spawning activity. Four female and nine males participated. In the hour before this spawning event there were eight ventral mounts observed, but only once was a female involved and the rest were male-male interactions. Among the nine spawning males three had performed, three had received and one both performed and received ventral mounts, while two males were involved in neither. The recipient female was not among the spawning females that day.

In this study male cod displayed a similar spawning intensity curve as wild populations of cod, with a mean date of peak spawning on 1 April, but no similar peak was seen in female spawning. The estimated spawning times, with nocturnal activity dominating, are comparable to earlier studies (Brawn 1961; Kjesbu 1989), and the presence of a diel cycle of spawning in farmed cod is indicated by a higher frequency of spawnings in late afternoon and at night than in other parts of the day.

Both the male and the female cod experienced a significant reduction in weight and condition factor. As female fish generally invest more in gonad tissue than male fish (Turner 1993), it is not surprising that the individual weight reduction observed in this study was larger in the female than in the male fish. There were, contrary to previous findings (Brawn 1961; Hutching *et al.* 1999), no significant indications of a relationship between fish size and reproductive behaviour or spawning success. Similar findings were made by (Rakitin *et al.* 1999; 2001) where differences in male fertilisation success could not be explained by differences in body size, condition-factor or weight loss over the season. In general large males should have higher fertilisation success than smaller males if pair spawning is common

in a fish species. If however group spawning is more prevalent then relative reproductive success should depend more on quantity and quality of sperm produced by different males (Rakitin *et al.* 1999). Based on this theory and the lack of relationship between fish size and spawning success observed it would seem like there was group rather than pair spawning taking place in the present study. Furthermore, as most offspring had different parental background, the notion of mate choice in a farm environment with a high density of fish is not supported. However, it is possible that the observed irregularity of spawning in the present study prevented some of the female cod from performing actual mate choice. Furthermore, the confinement in a tank as opposed to natural conditions in the wild will affect the observed mating success in cod measured with pedigree analyses. The water in a tank is only replaced at a certain rate and the resulting retention of spawned egg and milt could mask possible mate choice or advantages of being close to the spawning female. The number of satellite males would be expected to increase under such circumstances and it would be difficult to draw any conclusions as to the presence of mate choice based on pedigree studies.

The large individual difference in activity level and spawning participation among the fish was similar between the present study and the two previous studies by Hutching *et al.* (1999) and Brawn (1961). But whereas the smaller male fish in the study by Brawn (1961) were intimidated by one dominant male and therefore showed little activity, this seemed not the case in the present study. Many male fish were active and the present study found no indications of visual aggression and territoriality being important mechanisms in captive cod reproductive behaviour. There were indications of an opportunistic spawning behaviour where the fish are influenced by the behaviour of others and spawn in several groups rather than in single pairs of one male and one female. In an evolutionary perspective this might be an advantage, in the same way that multiple paternity in Atlantic salmon, *Salmo salar*, may increase genetic variability and provide greater average fitness in salmon alternative life histories (Garcia-Vazquez *et al.* 2001). It is more difficult to explain in an evolutionary context the large amount of male-male ventral mounts that are not visibly aggressive, but more similar to courting. Perhaps the ventral mount interaction is a step in the male-male intimidation process, just practice on the courting movements or wrong choices made by the male?

Stress might have influenced the spawning participation and latency, causing a large individual variation. The physiology associated with maturation and spawning appears tightly coupled with stress physiology, and the response to stressors can be quite polymorphic, both within and between species (Schreck *et al.* 2001). As the male ventral mounts did not elicit any response in the female fish most of the time, it appears that the actual time of spawning could be under female control. Possibly the large individual variation in participation and success were brought on by differences in physiological responses to the stress a farming environment can impose farmed fish (Patterson *et al.* 2004). Kjesbu (1989) noted that seven out of 18 female cod were classified as stressed based on the behaviour, and that this was reflected in irregular spawning intervals and low fertilisation rate of the eggs. Stressed cod also initiate fewer courtships, and produce abnormal larvae more frequently (Morgan *et al.* 1999). One implication of this on cod domesticating and cultivating is an increasing need to study the behaviour and select individuals not only on a fast-growth basis, but also based on stress-tolerance and health maintenance in a farm environment. Cod mating systems in general needs to be studied further as to their potential implications for conservation biology on broadcast-spawning fish (Rowe *et al.* 2004). The physical and social environment affect individual life-history strategies, physiology and behaviour in varying degrees. This causes the large individual variation that is also apparent in the reproductive behaviour and spawning success of farmed cod.

5.4 Social behaviour and reproductive hormones in Arctic charr during a spawning season

Arctic charr have distinct seasonal variations in plasma concentrations of sex hormones, growth rate and condition (Tveiten *et al.* 1998). There is a peak in male testosterone (T) and 11-ketotestosterone (11-KT) plasma concentration in October, while female estradiol (E₂) peak in August-September, and a decline in levels during the spawning period for both sexes. Charr are in peak condition in August and September, just prior to the reproductive season (Tveiten *et al.* 1998). However, at this point food intake and growth starts to decline in maturing fish and there is a sharp increase in oocyte growth, gonado-somatic index (IG) and plasma sex hormones. The physiological decision to proceed with maturation may be condition dependent (Frantzen *et al.* 1997) and related to energy reserves accumulated during the summer feeding (Tveiten *et al.* 1996). Maturing and non-maturing fish exhibit large differences in feeding motivation, behaviour and physiological profiles. Non-maturing Arctic charr exhibit a low plasma sex hormone concentration throughout the year (Frantzen *et al.* 2004) and may not experience an anabolic effect of sex hormones early in the reproductive cycle (Tveiten *et al.* 1998).

The aim of the Arctic charr reproduction study was to investigate a possible association between reproductive behaviour and physiology. The dynamics of reproductive behaviour and temporal changes in sex hormone synthesis in salmonids in general and Arctic charr in particular is only partly described. We investigated the link between the sex hormones E₂, T and 11-KT and reproductive behaviour through observation of individual reproductive behaviour such as agonistic actions, courtship and redd digging, in combination with analyses of individual variation in plasma levels of reproductive hormones.

The study was carried out at the Aquaculture Research Station in a circular grey tank, 5-m in diameter and 1.2-m deep, at ambient water and light regime. Based on red pigmentation and differences in male and female head shape, 15 assumed females and 15 assumed males (660 to 1540 g) were selected among three-year-old Arctic charr of the Hammerfest strain on 9 August 2002. After the experiment the fish were dissected, and the final sex distribution was nine males and 21 females. Three of nine male fish and 18 of the 21 females matured during the fall. The fish were marked with 2 by 2 cm tags (1-30) at the base of the dorsal fin in order to identify the individual fish when video filming.

At the beginning of the experiment and subsequently once a month the fish were measured for weight and length. Video sampling of fish behaviour was done 9, 4 and 2 days prior to each monthly sampling, and observation data from these three days were pooled when presented together with monthly weight characteristics and plasma hormonal values. Group and individual reproductive and aggressive behaviour was filmed with a wide-angle and a pan/tilt-zoom camera. In total 23 hours of recordings from the wide angle and 23 hours from the zoom camera were analysed.

The reproductive behaviours analysed were divided into agonistic interactions (Peterson *et al.* 1999), male quivering (an intense body vibration close to a female) and female redd digging (a sharp, sideways movement of the body against the tank bottom). Male quivering and agonistic interactions perceived as chases, charges and bites were registered attempting to identify the performer and the receiver. Plasma concentrations of the sex steroid T (males and females), E₂ (females) and 11-KT (males) were assessed by means of radioimmunoassay (RIA) according to Schulz (1984; 1985).

Variation in behaviour through the spawning season

The frequency of performed reproductive behaviour varied through the spawning season. Aggressive behaviour was infrequent until the end of September, with less than one observation of aggression per minute performed by the 30 fish. From 24 September there was a marked increase in aggression, and between 27 September and 21 October the level of aggression was high, with a pooled group performance of approximately 6 aggressive acts per minute. By the end of October the level of aggression decreased to amounts similar to experiment initiation. The increase in aggressive acts coincided with an increase in male performance of reproductive behaviour. Male fish performed courtship quivers directed at females throughout most of the period of elevated aggression, but after 21 October the frequency of this behaviour decreased. The first observed display of redd digging was on 30 September and the last on 21 October. Females showed a sharp incline in redd-digging performance on 16 October with a low frequency of observations prior and after this date. The timing of male quivering and female digging was positively related. Mature females displayed aggression less frequently and for a shorter time period than the mature males (Fig. 14).

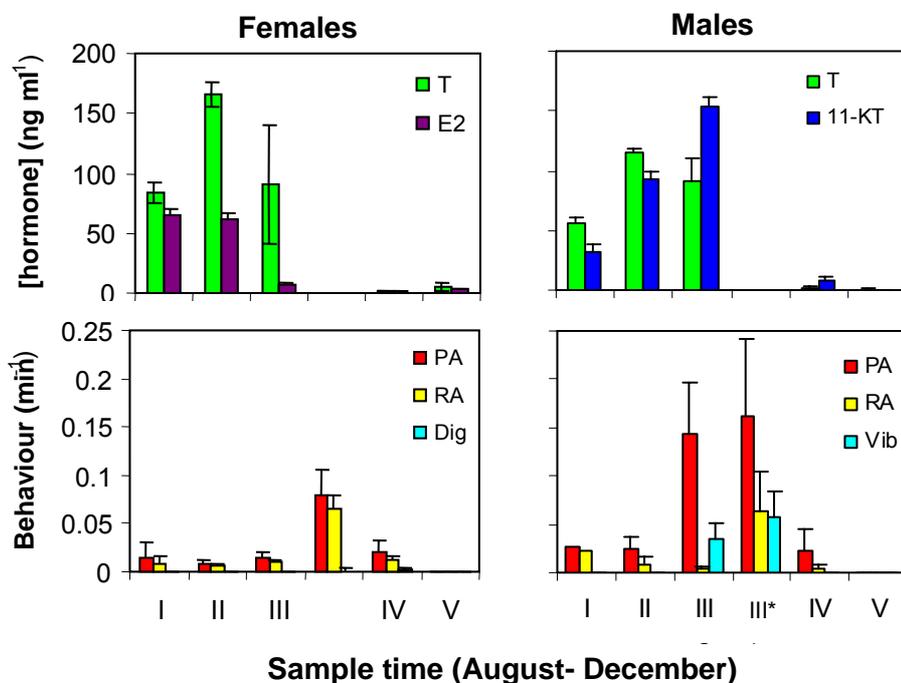


Figure 14 Variations in sex hormone levels and performance of reproductive behaviour in mature female and male Arctic charr during a spawning season. Top panel: Observations of monthly variation in plasma concentrations of female and male testosterone (T) (green bars), female estradiol (E2) (purple bars) and male 11-ketotestosterone (11-KT) (blue bars). Bottom panel: Variation among females and males in number of performed (PA) (red bars) and received (RA) (yellow bars) aggressive behaviours per minute observed, and frequency of redd-digging among the females (Dig) and courtship vibration performed by the males (Vib) (light blue bars).

Sex hormones and aggressive behaviour in male fish

Plasma sex hormone profiles were markedly different between the two groups, with a consistent low level in the immature male fish ($T < 10$ and $11\text{-KT} < 12.5$ ng ml⁻¹). The mature males displayed a top in plasma T concentrations in the early September sample and a top in plasma 11-KT concentrations in the early October sample, decreasing to low levels in November and December (Fig. 14). The immature fish were significantly less aggressive than

the mature males. The largest peak in both performed and received aggression among mature males corresponds well with the time of highest plasma concentrations of 11-KT but not of T. Furthermore, this peak was seen during the same time period as the frequency of female redd-digging behaviour was highest. Immature male fish gained weight during the autumn, while mature males experienced a negative SGR and were visually in a poorer condition concerning wounds and shell-loss.

Sex hormones and aggressive behaviour in female fish

The mature females displayed highest plasma T concentrations around the beginning of September, while E₂ peaked in the beginning of August. Plasma levels of E₂ were reduced to less than 10 ng ml⁻¹ by the beginning of October, similar to that displayed by immature fish the whole autumn. This drop in plasma hormone concentrations occurred prior to the short period in October with redd-digging behaviour and increase in female agonistic behaviour (Fig. 14). Immature females displayed very little aggressive behaviour although they were receivers of some aggression in the same time period. The six active nest digging mature females displayed more aggressive behaviour than the non-active mature females (48.3 ± 15.2 and 8.5 ± 2.2 acts individual⁻¹, respectively), but the individual frequency of performed agonistic behaviours was not related to the plasma levels of T or E₂. There were no significant differences in weight, condition factor or SGR between the mature and immature females.

Who performs and who receives agonistic behaviours?

The immature females displayed very little aggression, and only towards other immature females, while the mature females were most frequently aggressive towards other mature females. The immature males directed a majority of chases, charges and bites towards the immature females. The mature males performed a large amount of aggression with immature females as the receivers, although much aggression was also directed towards other mature males. Furthermore, there were large between-groups differences in how frequently fish were performers compared to receivers in agonistic interactions. The mature males performed aggression more frequently than they received aggression (82.3 ± 8.4 % performed aggressions) compared to the other groups of fish (immature males 52.9 ± 13.1; mature females 38.3 ± 6.3; immature females 4.8 ± 4.8 % performed aggressions).

When maturing fish display a large increase in agonistic behaviour not seen in non-maturing fish, this indicates that processes associated with reproduction rather than feeding influenced their behaviour. An increase in occurrences of mature male aggressive interactions in late September coincided with the onset of male courtship. These behaviours were observed when plasma T levels were decreasing, plasma levels of 11-KT increasing, the males were running-ripe and the first females had ovulated and initiated redd digging. The timing of this increase suggest that T and E₂ are either not involved or have an indirect effect on reproduction-related aggression. Male 11-KT may however be more closely linked with reproductive aggression and courtship behaviour, as this steroid show peak values in the same period as the mature males are most aggressive and perform courtship behaviour. This correspond with the relationship between 11-KT and aggression in sticklebacks, where the very high levels of 11-KT in the sexual phase stimulates both reproductive behaviour and development of male secondary characters (Mayer *et al.* 2004). In general the charr in the present study displayed plasma concentrations of sex steroids in agreement with other studies on salmonids (Mayer *et al.* 1992; Frantzen *et al.* 1997), and also variations in frequency of agonistic and courtship behaviour similar to that of the stickleback (Pall *et al.* 2002).

A small number of the mature females protected areas around them by chasing, charging towards and biting both neighbouring and passing mature females. This has previously been seen in other charr populations where females would defend prepared redds from other females (Johnston 2002). The redd-digging females were far more aggressive than the remaining mature females although not remarkable in regards to T or E₂ levels, size or condition factor. This suggest that under the study conditions other factors than those studied here were of importance in inducing behavioural differences among the mature females.

The concurrence of male courtship and female redd digging behaviours in the spawning season suggest a cyclic variation in behaviour that appear to be determined in part by the stage of the spawning period and by activities performed by the other sex. This is similar to findings in other studies, suggesting that internal androgens may affect male initial aggressive behaviour and quivering, while priming and releasing hormones produced by ovulated females induce further physiological and behavioural changes in males shortly before spawning (Kobayashi *et al.* 2002). In both female and male Arctic charr further studies are needed on the physiological “decision” to mature, and on the hormonal and behavioural mechanisms behind reproduction. Knowledge of these mechanisms is useful not only on an academic and ecological level, but also in aquaculture when for example the farm environment for brood-stocks is evaluated, or attempts are made to reduce the number of early maturing fish.

5.5 References

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6 PRENATAL STRESS IN FARMED SALMON; IMPACTS ON PHYSIOLOGICAL, MORPHOLOGICAL AND BEHAVIOURAL CHARACTERISTICS

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6.1 Physiology and morphology

Prenatal stress may be defined as stress experienced by a mature (fish) or pregnant (mammal) female, which can affect the development of her offspring. In mammals, confronting a female with a stressor elicits activation of the autonomic nervous system, firing in the hypothalamus-pituitary (HPA) axis, and subsequent enhanced concentration of glucocorticoids secreted by the adrenal cortex (Braastad 1998). These glucocorticoids can pass from the mother to the embryo, and the effects on the offspring, in turn, is suggested to be associated to the enhanced level of glucocorticoids experienced during the prenatal period. Prenatally stressed offspring are generally found to have increased mortality, lowered birth weight, reduced growth, deviant morphology and depressed immune function (Braastad 1998; Eriksen *et al.* 2003). Behaviour is also affected by prenatal stress, as prenatally stressed offspring show increased fearfulness and reduced stress coping in aversive situations, impaired resource-holding power (RHP), cognitive deficits, as well as infringed sexual and maternal behaviour (Braastad 1998).

Likewise, the teleost maternal endocrine state and that of her brood is strongly associated; hormones that regulate and facilitate embryonic growth and development come from the maternally derived yolk, where they are accumulated over the course of oogenesis (Schreck *et al.* 1991). There is thus the potential for environmental challenges to influence steroid levels in maturing females and modify developmental processes of offspring. In the intensive aquaculture industry, it is essential that common husbandry procedures ensure gamete and progeny quality for successful broodstock management. However, farmed fish are subjected to a wide range of temporary and chronic challenges during various phases in their life cycle, and the conditions to which fish farm broodstock are subjected during sexual maturation might hence be a significant factor in determining the nature of gametes produced and the succeeding offspring characteristics (Campbell *et al.* 1992; Eriksen *et al.* 2005 a, b).

Coping with various stressors is an omnipresent aspect of life for wild and reared fishes, and reproduction is one biological mechanism especially susceptible to its possible detriments (Schreck *et al.* 2001). Within the complex series of physiological alterations that comprise the teleost stress response, activation of the hypothalamic-pituitary-interrenal (HPI) axis and the sympathetic nervous system are of pivotal significance (Schreck *et al.* 2001). Common procedures in hatcheries, such as handling, transportation, cleaning and crowding, as well as problems with water quality, are stressors that may negatively influence reproductive parameters, and in a chain of a number of stressful incidents, discrete impacts of each event may contribute to an overall reply of the animal that compromises its entire functioning capability (Schreck 1990). In fishes, stress exerted upon mature females has generally been shown to result in reproductive suppression, reflected by reduced levels of sex steroids and vitellogenin in plasma, decreased fecundity and oocyte size, as well as altered timing of ovulation (Schreck *et al.* 2001). Beyond the abovementioned effects, some studies even demonstrate that exposing females to biotic and abiotic challenges during sexual maturation

also might impinge on the coming generation, for instance lowering offspring survival rates and diminish size at hatching (Weiner *et al.* 1986; Mount *et al.* 1988; Campbell *et al.* 1992, 1994; McCormick 1998; Eriksen *et al.* 2005 a).

Any organism is distinguished by regularity of its phenotype and numerous vital mechanisms ensure that ontogenetic processes are kept within specific confines. This capability to manage development under the current environmental conditions is called developmental stability (Møller & Swaddle 1997). However, ontogenetic disorders do occur, and is most often measured in terms of fluctuating asymmetry (FA), denoting small, random deviations from symmetry in normally bilateral symmetrical traits. Developmental imprecision can also be determined as the frequency of phenodeviants, referring to any relatively gross, conspicuous departure from the adaptive, phenotypic target of development (Møller & Swaddle 1997). Although phenodeviance usually is quite rare it can be more prevalent in certain situations, e.g. in intensive aquaculture (Barahona-Fernandes 1982; Divanach *et al.* 1996). Anomalous specimen generally have increased mortality rates, growth diminution and enhanced susceptibility to disease; malformations may thus consequently undermine the value of reared fish and also compromise animal welfare (Divanach *et al.* 1996). The aetiology of these syndromes is not fully understood, but environmental stressors are cited to initiate aberrations in development (Divanach *et al.* 1996; Møller & Swaddle 1997). With respect to possible teratogenes within aquaculture, the significance of the maternal environment has received little attention; the focus has primarily been on the young individual and its rearing environment (Divanach *et al.* 1996). However, in mammals it is shown that the endocrinological state of the female during pregnancy may affect morphological traits of the progeny (Braastad 1998). Thus, the aetiology concerning development of morphological anomalies in farmed fish might be better understood if prenatal factors are incorporated. We therefore designed a longitudinal experiment aiming at scrutinizing various impacts on offspring ontogeny attributable to increased levels of cortisol in mature farmed female salmon and a subsequent episode of hyperthermic exposure during incubation (Eriksen *et al.* 2005 a).

The experiment was conducted during a period from December 2002 to April 2003. The female salmon were farmed Atlantic salmon broodstock (*Salmo* Breed, mean weight 9.9 ± 1.3 kg) obtained from Bolaks, Eikelandsosen, Norway. These fish were held in groups of 200 individuals in 25 m³ fibreglass tanks supplied with aerated fresh water delivered at a rate of 500 l min⁻¹ at ~1 °C under natural photoperiod. Six days prior to the expected spawning date, thirty mature females were selected, tagged (PAT tags, Os Husdyrmerkefabrikk) and randomly divided into three groups, representing three implant treatments (Fig. 15). The fish in treatments 2 and 3 were injected intraperitoneally with coconut oil containing low and high levels of cortisol, respectively. In the peritoneal cavity, the implant solidifies and releases the hormone slowly over time. The two injection solutions were prepared by adding cortisol (No. H-0888, Sigma Aldrich, Sigma Chemical Co., St. Louis, MO, USA) to liquid coconut oil at 40 °C to yield a final concentration of 50 and 100 mg cortisol ml⁻¹ oil. In treatment 1, the females received sham injections containing only the vehicle and so served as controls. Prior to injections, the females were anaesthetised with metacaine (MS 222), weighed and then injected intraperitoneally with 1 ml of the emulsion per kg body weight, using a 18-gauge needle connected to a plastic syringe. This resulted in an implant of 50 and 100 mg cortisol per kg for treatment 2 and 3, respectively. Controls were injected with coconut oil, 1 ml kg⁻¹.

At stripping six days later, the treated fish were again caught, anaesthetised with metacaine, and a sample of eggs (0.25 l) was collected from each of the mature females in the three groups. Eggs were collected from seven, eight and nine females in treatment 1, 2 and 3, correspondingly. The eggs from each treatment were pooled and fertilized by a mixture of

milt obtained from three untreated male salmon. The same three males were used to fertilize all the eggs, hence avoiding male ID to be confounded with treatment. Further, the eggs were divided in two separate batches, which were kept at two different temperatures from fertilization to first feeding. One served as a control (8 ± 0.3 °C incubation temperature) and one was exposed to mild hyperthermia (10 ± 0.2 °C incubation temperature). The two manipulations (cortisol administration and increased temperature) gave rise to six different offspring groups, ranging from non-stressed (group 1) to highly stressed fish (group 6).

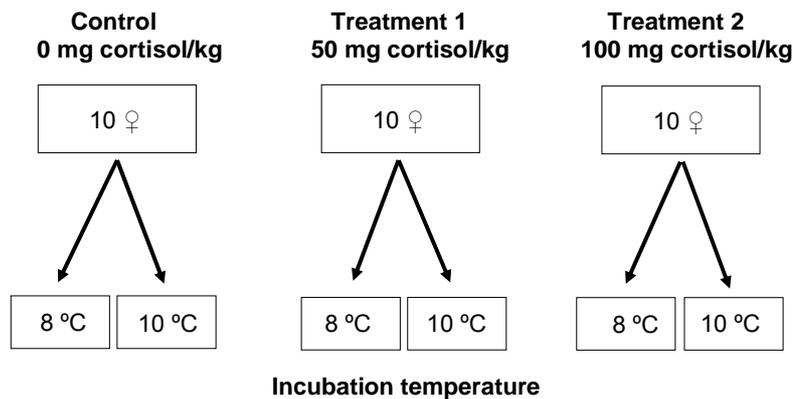


Figure 15 Experimental setup for the cortisol treatment and incubation temperature.

At hatch (510 daydegrees = dd), first feeding (810 dd), 1730 dd, 2842 dd and 10 months post hatch, some 60 offspring per group were randomly sampled and euthanized with an overdose of metacaine. Main parameters that were recorded include fork length, body weight, yolk sac size (at hatch), and frequency of deformities (Fig. 16) and also level of fluctuating asymmetry. Group mortalities were registered daily throughout the experimental period.

Augmented levels of maternal cortisol inflicted a range of progeny parameters at hatch (510 dd) and first feeding (810 dd), as reflected in increased mortality, decreased length and weight, diminished yolk sac volume, decelerated yolk sac utilization and enhanced prevalence of morphological deformities. In addition, the offspring originating from cortisol-implanted females had a decreased capacity to cope with the additional hyperthermic exposure. The most pronounced consequences on offspring performance were demonstrated in specimens exposed to both enhanced prenatal cortisol and a subsequent episode of hyperthermia.

Maternal cortisol increment also caused long lasting impacts on fry survival, growth and morphological development. At 1730 dd and 2842 dd, it was generally found that prenatal cortisol exposure reduced survival rates, increased growth and enhanced the frequency of deformities (Fig. 16). Offspring displaying anomalous morphology had reduced fork length and body weight compared to their normal counterparts (Eriksen *et al.* 2005 b).

Ten months post hatch, there were still some impacts attributable to the prenatal cortisol exposure, in that the offspring originating from the cortisol implanted females were longer, heavier and more asymmetric than offspring from the females not implanted with cortisol. The offspring displaying anomalous morphology were larger and had a higher condition factor than the normal ones. Again, the most prominent consequences were seen in progeny exposed to both increased levels of prenatal cortisol and hyperthermia exerted during incubation.

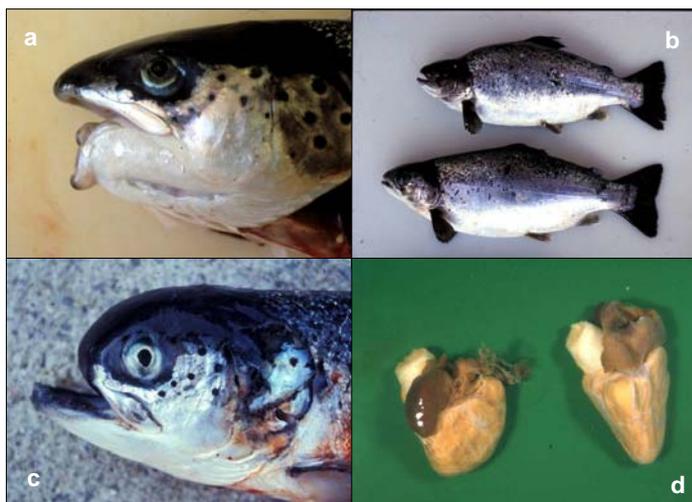


Figure 16 Morphological anomalies in farmed Atlantic salmon, similar to those observed in the current experiment. a) Malformed lower jaw, b) Anomalous vertebral column, c) Pug head and d) Anomalous cardiac shape from farmed salmon (left) and normal cardiac shape wild salmon (right). Photo: Trygve Poppe, The Norwegian School of Veterinary Science.

6.2 Stress proteins

All living organisms have developed a common defence mechanism against excess temperatures and other environmental biotic and abiotic stresses, collectively known as the Heat Shock Response, HSR (Edwards *et al.* 1997). The HSR is distinguished by rapid postponement of normal protein synthesis and concomitant production of genes encoding the heat shock proteins, aptly named stress proteins. Stress proteins belong to a highly conserved family of proteins that confers protection to cells following stress by preventing aggregation or improper folding of proteins and accelerating proteolysis of proteins that have been damaged beyond repair. In addition to their functions during stress, they participate in many cellular processes under normal conditions, acting as molecular chaperones, stabilizing the folding and assembly of newly synthesized proteins (Edwards *et al.* 1997). Stress proteins and glucocorticoids are central features of an organism's adaptive reply to adverse environments, and there is increasing evidence that steroid hormones may interact with the cellular stress response, as reflected in previous data supporting the existence of a functional relationship between the HSR and the HPA-axis in vertebrates (Blake *et al.* 1991). Although many aspects of the HSR in teleosts have been well described, relatively little is known about its prevalence in offspring originating from mothers exposed to stress during sexual maturation and oogenesis.

Cortisol has recently been shown to downregulate HSP70 levels, and HSP70 is documented to be a temperature sensitive stress indicator (Blake *et al.* 1991; Udelsman *et al.* 1993; Matic *et al.* 1995; Edwards *et al.* 1997; Takle *et al.* 2005). In the abovementioned experiment, it was therefore investigated whether increased cortisol levels in mature salmon females and subsequent hyperthermia affected the offsprings' HSP70 response at several selected stages during organogenesis.

At 102, 120, 160 and 200 dd, eggs were sampled for RNA isolation. Sampled eggs were frozen directly in liquid nitrogen and then transported on dry ice to a -80°C freezer for storage. Total RNA was extracted from five eggs per sample using TRIzolTM Reagent (Life Technologies). The total RNA concentrations were determined spectrophotometrically (by measuring the A_{260}/A_{280} ratio) following DNase treatment (Ambion) of 1 μg of total RNA. Thereafter, DNA-free RNA was reverse-transcribed by the use of the TaqMan[®] Gold RT-PCR Kit (Applied Biosystems). The concentrations and design of primers and MGB-TaqMan probes for real-time amplification of HSP70 and GAPDH cDNA (Table 2) were as previously described by Takle *et al.* (2005). Real-time semi-quantitative RT-PCR was performed by use of the ABI Prism 7700 sequence detection system (Applied Biosystems). The “comparative Ct method” (User Bulletin #2, ABI PRISM 7700 Sequence Detection System, Applied Biosystems, Foster City, USA), with optimizations (Takle *et al.* 2005), was used for relative quantification. In total, each pooled sample was run in triplicate. For calculation of the HSP70 mRNA expression, GAPDH was used as an endogenous control, and HSP70 expression in untreated embryos, 8°C and 0 mg cortisol at each investigated stage, was used as a calibrator.

It was predicted that increased levels of maternal cortisol would diminish the HSP70 response in the embryos. At 160 dd, the amounts of mRNA HSP70 were generally low in all the progeny, and magnitudes of HSP70 tended to be reduced as a consequence of prenatal cortisol exposure. However, maternal cortisol exposure was not found to affect HSP70 expression at 102 and 120 dd. Even so, maternal cortisol administration increased amounts of HSP70 at 200 dd in embryos that originated from mothers receiving the highest cortisol dose. In contrast, a different pattern was evidenced in offspring exposed to mild hyperthermia, where only embryos whose mothers had been implanted with the medium cortisol dose displayed increased HSP70 expression. Furthermore, hyperthermia was expected to initiate an increased HSP70 expression. A pronounced HSP70 mRNA up-regulation was exhibited at 120 dd in embryos exposed to 10°C . This observation was however not demonstrated in offspring originating from cortisol administered mothers. Temperature stress also seemed to initiate elevated amounts of HSP70 at 200 dd, but then again only in offspring originating from females receiving the intermediate quantity of cortisol. Finally, it was predicted that cortisol would decrease heat-initiated HSP70 transcription. However, the effects of maternal cortisol increments and hyperthermia on HSR responses appeared rather vague, and unequivocal patterns were not discovered.

Hence, increased maternal cortisol levels and a subsequent mild hyperthermic exposure were shown to affect HSP70 expression in salmon embryos. However, the results were rather ambiguous, and illustrate the necessity to further investigate the relation between pre-spawning stress and its possible impacts on progeny Heat Shock Response (HSR) along with its plausible linkage to teratogenic mechanisms in farmed fish.

6.3 Behaviour

Animals have evolved to cope with the environment, and stress is one mechanism that enables the animals to manage a changing environment or an environment that is not optimal to the individual. The adaptive value of stress will however apply mainly in the short term, and chronic stress due to continual suboptimal environment might cause severe impairment in animal welfare (Pickering 1998).

When an individual is exposed to one or more stressors the hypothalamus-pituitary-axis (HPA) is activated. In this process the stressor stimulates the secretion of corticotrophin releasing factor (CRF) and arginine vasopressin (AVP) from the hypothalamus that in turn stimulates the secretion of acetylcholinesterase (ACTH) from the pituitary. ACTH stimulates the release of corticosteroids from the adrenals (Braastad 1998). In teleosts the primary corticosteroid is cortisol. A mature or pregnant female that experience stress may thus produce cortisol that in turn may be transferred to the unborn offspring through placenta, or in case of fishes, to the eggs. Other effects of maternal stress on the offspring include impairment in egg size and survival of eggs and fry (Campbell *et al.* 1994; Eriksen *et al.* 2005 a) and morphology (Eriksen *et al.* 2005 a, b). Of behavioural disruptions the most recorded effects have been altered locomotor activity, motor development, learning ability, anxiety (see Braastad 1998), and altered social and reproductive behaviour (Braastad 1998). In some situations feeding behaviour has additionally shown to change due to prenatal stress (Weinstock 1997; Lesage *et al.* 2004). Also, maternal stress has shown to impair the offspring ability to cope with other stressors (Braastad 1998, Eriksen *et al.* 2005 a).

Previous studies have shown rapid changes in behaviour after exposure to stressors (Little *et al.* 1993; Peakall 1996; Smith & Logan 1997). Generally, a stressor that stimulates the HPA-axis will cause immediate alterations both in biochemical and behavioural biomarkers because of the rapid nervous response. Easy quantifiable behavioural biomarkers may thus serve as early indicators of impaired welfare. Behavioural biomarkers are defined as general biomarkers in that different stressors may cause the same behavioural alteration in an individual (Depledge 1994). Different stressors may be chemicals, and environmental stressors such as altered temperature impaired water quality and high stocking density.

The aim of this study was to investigate behavioural effects of prenatal stressed Atlantic salmon offspring of mature mothers injected with cortisol prior stripping, and where the eggs were incubated in two different water temperatures. Another aim was to study the effects of prenatal stress by using easily quantifiable behavioural traits as general biomarkers. As easily quantifiable traits different variables of activity were studied (swimming, angles of turns, bottom dwelling behaviour), in addition to feeding behaviour since these previously have been used as behavioural biomarkers.

In the experiment juvenile farmed Atlantic salmon (approximately 2 g) were used. They were offspring from mothers that prior stripping was injected with 0 (sham), 50, or 100 mg cortisol per kg fish. The eggs were placed in hatching batches and exposed to water temperatures of 8 or 10 °C. The offspring were thus divided into 5 groups; group 1 ($0_{\text{cort}}/8$ °C, control) = mothers exposed to 0 mg kg⁻¹ cortisol and eggs exposed to 8 °C; group 2 ($50_{\text{cort}}/8$ °C) = mothers exposed to 50 mg kg⁻¹ cortisol and eggs exposed to 8 °C; group 3 ($50_{\text{cort}}/10$ °C) = mothers exposed to 50 mg kg⁻¹ cortisol and eggs exposed to 10 °C; group 4 ($100_{\text{cort}}/8$ °C) = mothers exposed to 100 mg kg⁻¹ cortisol and eggs exposed to 8 °C; group 5 ($100_{\text{cort}}/10$ °C) = mothers exposed to 100 mg kg⁻¹ cortisol and eggs exposed to 10 °C.

The small-scale behavioural experiments were conducted in an aquarium that was divided into three compartments separated with two transparent removable walls (Fig. 17). The aim was to study the interaction between two individuals from different groups, placed in each of the outer compartments with an empty compartment in between. After 10 minutes with video filming of the fish behaviour, feed was introduced in the central compartment, the walls were removed and filming continued for another 5 minutes. Since there were no sign of social interaction between the fish the behaviour of each fish was recorded and grouped according to the exposure regime.

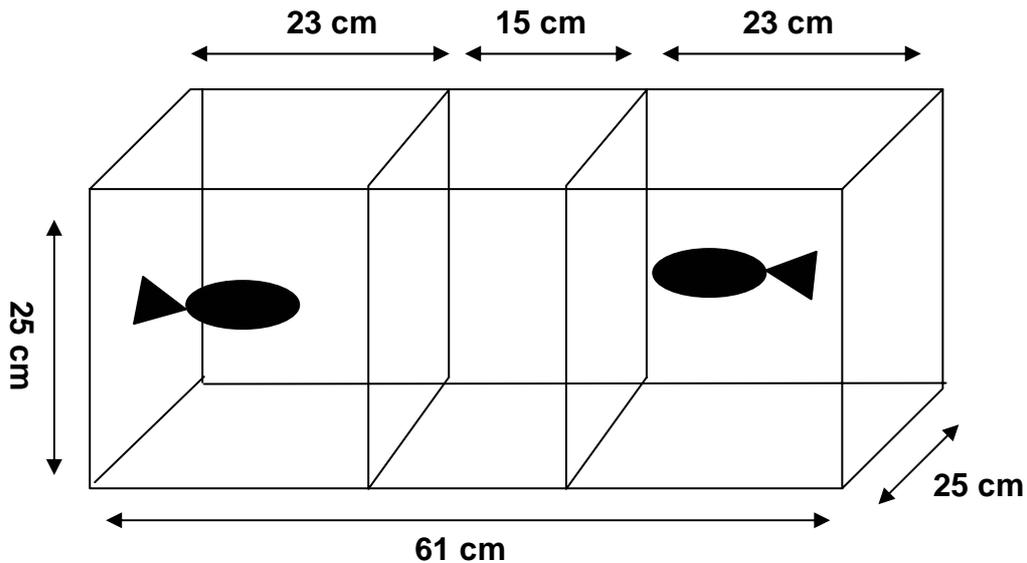


Figure 17 Illustration of the aquarium applied in the behavioural experiments.

No significant effects of cortisol or temperature alone were detected, however the fish exposed to a mixture of mild hyperthermia (10 °C) and cortisol (100 mg kg⁻¹) (group 5) were calmer than many of the other groups. This trend was most obvious in the variables “time spent on swimming” and “time spent at the bottom” where the 100_{cort}/10 °C fish were calmer than all the other groups except from the 50_{cort}/8 °C fish that also were calm. If this effect was a result of cortisol alone a similar effect in the 100_{cort}/8 °C fish would have been expected, but this was not the case. In the variable “angle of turns” the fish in group 4 (100_{cort}/8 °C) were more active than the other groups except from the 50_{cort}/10 °C fish that were as active. Latency time to feeding start was also investigated; no differences between the groups were recorded.

The results presented in this study show no clear tendencies, and one explanation may be that the time from the exposure of the mothers to cortisol to the behavioural experiments in the offspring was so long that the tertiary stress response, behaviour, was not affected enough. On the other hand, since there are signs of behavioural effects it is suggested that the behaviour is altered and that it is important to study more relevant variables in order to detect the behavioural effects of prenatal stress.

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7 LEARNING AND FISH WELFARE: LEARNING ABILITIES AND CONSTRAINTS IN COD AND HALIBUT

Jonatan Nilsson, Anders Fernö, Jan Erik Fosseidengen, Aase K. E. Marthinsen, Ruud van den Bos & Tore S. Kristiansen

7.1 Introduction

Although it has been known for decades that fish have relatively good memory and learning ability (Gleitman & Rozin 1971), fish are still viewed by most people as nonconscious, instinct driven animals with an infamous three-second memory (Laland *et al.* 2003). However, an increased research effort during the last decade has revealed that the cognitive capacities of fish are far more sophisticated and complex than earlier assumed (Laland *et al.* 2003), and we are now entering a new paradigm where fish receive animal welfare considerations and legislation similar to mammals.

The ability to learn is an essential tool to adjust behaviour in a variable and changing environment where the fish meets a multitude of sensorial impressions and situations in its everyday life. Since all impressions always are more or less different, the fish must have the ability to conceptualise (group) impressions into “same” or “similar” or “different” and categorize them into objects, concepts and situations of relevance (or not) for its survival and reproduction. To do this the fish must be able to associate sensorial impressions with each other and associate earlier experiences with new.

Which relevant objects the fish can perceive depend on the fish sensory apparatus. The ability to perceive relevant objects has been favoured by evolution, and different fish species have been equipped with specialized sensory organs to map the environment they live in, and in particular to sense relevant objects such as predators, prey, and mates. Which objects are relevant at a given time depend on internal and external states, and may vary considerably within and between individuals through ontogeny and habitat change. Rather than selecting for genetic codes for relevant and non-relevant objects, evolution has therefore favoured the ability to associate objects or events with stimuli that affect the chance for survival or reproduction, so that the object can be avoided or utilized at a later encounter. This type of learning is called associative learning, and is the basis for classical conditioning (Lieberman 2000). In classical conditioning a neutral stimulus (NS) precedes a stimulus that affects the survival or fitness of the animal (unconditioned stimulus, US), and when the response to the NS is similar to that of the US the NS has become a conditioned stimulus (CS). In *trace conditioning* there is an interval (trace) between the CS and the US, and successful trace conditioning requires awareness of the CS-US relationship, and is dependent on an intact hippocampus in mammals (Clark & Squire 1998). Fish lack the hippocampal structures found in mammals, but the lateral telencephalic pallium has been proposed to be homologous to hippocampus (Braford 1995; Butler 2000). Fish with intact lateral telencephalic pallium can successfully be trace conditioned, while fish with damaged lateral telencephalic pallium have reduced capacities for trace conditioning (Portavella *et al.* 2004), suggesting that fish have an anticipation of upcoming rewards and hence have a basic form of representation of their surroundings.

Fish have also the capacity to learn from their own actions, a type of learning called operant conditioning. In operant conditioning the probability for a response to reoccur is increased if the response is rewarded in some way. Operant conditioning differs from classical conditioning in that in operant conditioning animals detect a relationship between their own actions and a reward, while in classical conditioning they detect a relationship between an object or event (CS) and a rewarding or aversive stimulus (US). Through operant conditioning fish can improve behavioural skills such as foraging techniques and escape behaviour (Huntingford 1993). As classical conditioning often involves actions from the individuals, like approaching or escaping, classical conditioning experiments may involve elements of operant conditioning if the animals learn that their action response to the CS is followed by a reward. Likewise, classical conditioning of groups of fish may be influenced by social learning. Social learning is the use of information that is provided through observations or interactions with other individuals, like when a naïve fish experiences fear by the sight of a predator after observing the flight response in more experienced individuals, or copying other individuals to find food patches (e.g. Brown & Laland 2003). Training programmes of social learning of survival skills can be used to improve the survival rate of hatchery-reared restocking populations (Suboski & Templeton 1989; Brown & Laland 2001).

The environment of intensive aquaculture is very different from the natural habitats to which fish are evolutionarily adapted. Tanks and cages give no opportunity for migration, the stocking densities are very high, and water quality may change rapidly. A species' ability to adapt to an aquacultural environment depends partly on how broad its natural niche is. The rearing environment must be within the species' physiological range of tolerance. Species that are environmental generalists will have a better basis for adaptation to the aquacultural environment than those that are environmental specialists, and hence be more suited for farming. It is not only the physiological characteristics of a species that may limit its suitability for domestication. The fish must also manage to cognitively process the sensory information presented by the farming systems. The farmed fish must learn to deal with frequent social interactions, an environment with homogeneous structure and limited space, low variation of food, and highly stressing events such as handling. They may also meet sudden, fundamental changes of the environment, e.g. transfer from an indoor tank to a sea cage. If they are unable to adjust their behaviour to a changing environment, they will be maladapted and suffer from stress, reduced growth, and diseases. Cognitive capacities of a fish species will therefore influence its candidacy for domestication, or at least set limitations on the production systems used.

The cognitive capacities of fish may also influence the value we give fish in terms of ethical treatment, and it has been argued that the concept of welfare can only be applied to sentient species (Duncan 1996). Whether fish are conscious and sentient has been a subject for debate (e.g. Rose 2002; Sneddon 2003), but recent research suggests that they are more likely to be sentient than not (Chandroo *et al.* 2004).

In order to investigate basic cognitive and learning capacities of the relatively new aquaculture species cod and halibut, a number of classical conditioning experiments of these species have been conducted. As trace conditioning requires awareness of the CS-US relationship (Clark & Squire 1998), trace conditioning experiments with cod were conducted in order to investigate if cod can be aware of a CS-US relationship and have anticipations of an upcoming reward, i.e. if they have some form of consciousness.

7.2 Learning in cod

Classical conditioning by sound signals

A group of 9 cod, ranging from 160-290 g, was trained to associate a sound (CS) with food (US). Two squared 2 m wide tanks were connected by a 1 m duct through which the fish could swim freely. An underwater speaker was placed in the corner of one of the tanks, and a feeding automat hung above the speaker. The fish were fed sinking pellets twice a day, and every feeding session lasted for 2 minutes. A pulsating 150 Hz sound signal started one minute before each feeding session, and continued until the onset of feeding (delay conditioning). When the fish had detected the relationship between sound and food, at the onset of the CS they showed changes in behaviour such as an increased swimming speed and more swimming near the surface. They did, however, not approach the speaker and waited in the feeding area during the CS. Because of the reflecting walls, the properties and the direction of sounds in a tank are interrupted (Hawkins 1993) and it may be difficult for the fish to locate the sound source. Nonetheless, the experiment shows that cod can learn to associate a reward (food) with a signal, i.e. a sound.

Classical conditioning by light signals

Subsequently conditioning experiments (delay and trace conditioning) were conducted where the CS was a light signal instead of a sound signal, while the US was still food (sinking pellets). Two groups of 15 fish ranging from 200-500 g were held in circular tanks, 3 m diameter, filled with approximately 70 cm water of 7-9°C. A floating ring of 50 cm diameter containing a series of lights was placed under the feeding automat, so that food dropped down into the ring. The fish were fed every 4th hour, and every feeding session lasted for one minute. Each trial was recorded by a camera hanging above the tank and stored on DVD for later analyses. In the first experiment the light ring started to flash 12 seconds before each feeding session, and continued to 12 seconds after onset of feeding, so that the CS and the US overlapped (delay conditioning). After a few trials the fish started to approach the CS/US area at the onset of the CS, and after about 15 trials the response to the light signal was similar as to food. In parallel control experiments, in which the light signal was not paired with food (2 hours interval between light and feeding) the fish did not show any tendency to approach the CS. New groups of fish were trained to associate the light signal with food when there was an interval (trace) of 20, 60 or 120 seconds between the offset of the CS (5 flashes with a total duration of 12 seconds) and the onset of the US. In the 20 seconds trace experiment the fish started to approach the CS/US area at the onset of the CS after a few trials, similar to the delay experiment, and the fish stayed in the area during the trace waiting for the food to arrive (Fig. 18). Also in the 60 seconds trace experiment the fish started to approach the CS/US area at the onset of the CS after a few trials, but they did not stay in the area during the trace until after about 20 trials. In the last experiment when the trace was 120 seconds, the fish did not approach the CS/US area until after more than 20 trials, and even then the response to the CS was weaker than in the other experiments. The fish never waited in the area during the 120 seconds trace.

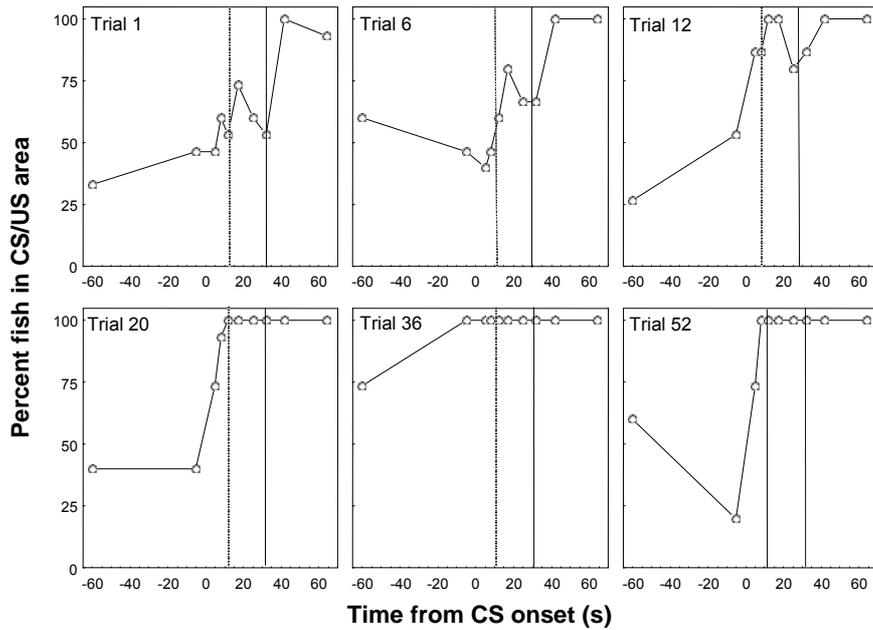


Figure 18 Percentage of a group of 15 cod that stayed in the quarter of the 3 m tank where the CS (light) and US (feed) were located during 6 trials in the 20 seconds trace experiment. The trace is marked out.

Memory test

In order to investigate if cod remembered the CS-US association after a long period without the CS, the fish groups of the delay and 20 seconds trace experiments were transferred to separate 1.5 m diameter tanks after the conditioning experiments, and kept there for 87 and 69 days, respectively. The fish were then transferred back to the 3 m tanks in the learning laboratory and acclimatized for 30 hours before the memory test. The test consisted of giving the fish the same CS of 5 light flashes from the light ring repeated 6 times with two hours' interval without reward. For both delay and trace groups the fish responded to the CS in a similar manner as at the end of the conditioning experiments, i.e. approached the light ring at the onset of the CS. Cod thus seem to have at least the capacity to remember associations that they have learned three months earlier.

Sign or goal tracking

When a classical conditioned animal associates the CS directly with the US the link between the two stimuli may be so strong that the animal is incapable of dissociating them, and the CS itself becomes a goal for the animal's action. This is referred to as sign tracking, as opposed to goal tracking where the US is the goal for the behavioural actions. When the CS and the US overlap in space, as in the experiments described above, and the animals respond to the CS by approaching the CS/US area, it is not obvious whether they approach the CS or the US. In order to investigate if cod are sign tracked or goal tracked, we separated the CS (light ring) and the US (food) in space, with the light ring and feeding automat on opposite sides of the 3 m tank, to see whether the fish approached the US area (marked out by a dummy ring floating under the feeding automat) when the CS came on, or if they approached the CS. One delay and one trace experiment were conducted. In the delay experiment the total duration of the CS was 60 seconds, of which 30 seconds overlapped with the US. In the trace experiment the

duration of the CS was 12 seconds (5 flashes) and the trace 20 seconds. The fish were fed every 4th hour, and each feeding session lasted for one minute. When the fish had detected the CS-US relationship, they always approached the light ring at the onset of the CS. However, before the delivery of food the fish left the CS area and moved to the US area (Fig. 19). This was the case in both the delay and trace experiments. Cod thus show features of both sign and goal tracking. As the fish moved towards the US area before the delivery of food they showed that they were aware of where and when the reward was delivered, but still they always approached the CS area before moving towards the US area. This may indicate that they have difficulties in dissociating the US from the CS. Alternatively, they may have learned a behavioural sequence due to operant conditioning: "if we approach the CS area at the onset of CS, food will be delivered in the US area", i.e. the fish "believe" that food is a reward for their action response to the CS.

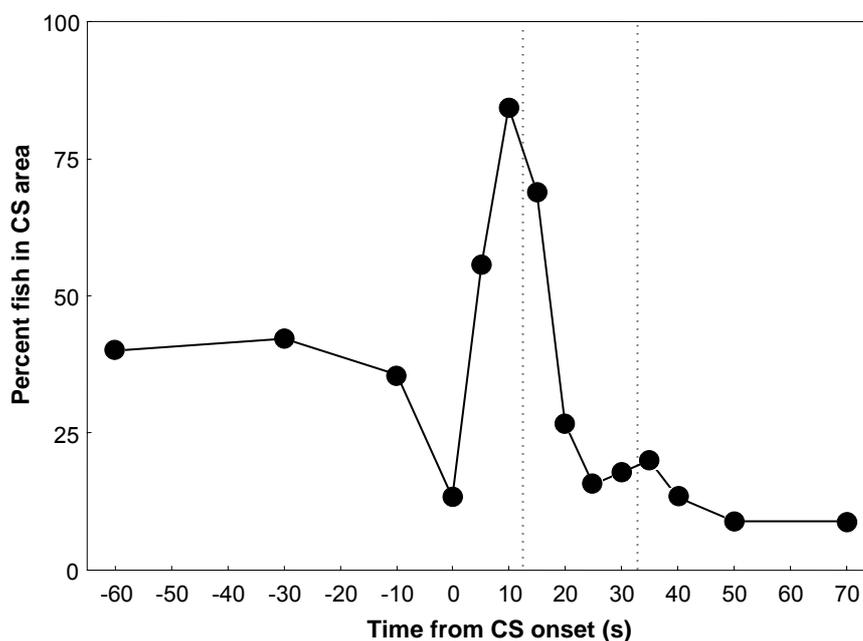


Figure 19 Percentage of a group of 15 cod in the CS area before, during and after CS in a trace conditioning experiment where CS (light) and US (feed) were located at opposite sides of the 3 m tank. Values are means of trial 30, 40 and 50. The tank was divided in 2 equal halves; CS and US area, and fish that are not in the CS area are thus in the US area. The 20 s trace is marked out. Onset of CS is at T_0 and onset of US at T_{32} . Feeding lasted for 1 minute.

7.3 Classical conditioning of halibut

A group of 10 halibut of 1800-2500 g were held in a 2 m tank and fed sinking pellets twice a day. A pulsating 143 Hz sound signal from an underwater speaker in the feeding area was given before each feeding session. The signal lasted for 40 seconds, and it started 20 seconds before onset of feeding, i.e. a 20 seconds overlap (delay conditioning). The fish showed no signs of associating the sound signal and food. Even after 60 trials, the fish did not respond to the sound, neither by approaching the feeding area nor by showing changes in their behaviour.

In a new experiment we attempted to train a group of halibut to associate a light signal (CS) and food (US). The fish were fed sinking pellets twice a day, and each feeding was paired with flashes from a light ring floating under the feeding automat, similar to that used in the

cod experiments described above. The light signal had a total duration of 30 seconds, and started 12 seconds before onset of feeding (delay conditioning). After 30 trials about 50 % of the fish responded to the CS by approaching the CS/US area, which was approximately the same percentage of fish that responded to the food. The experiment demonstrates that halibut can be conditioned to associate a light signal with a reward (food), while conditioning to a sound signal could not be demonstrated. The percentage of fish responding to the light signal was considerably lower in halibut than in cod. However, this was also the response to food. A relatively large proportion of the halibut showed little interest in food, and the reward value of the US was therefore low or lacking for those individuals. Hence, they had no interest in the CS either.

7.4 Differences in cognitive abilities in cod and halibut?

Cod could be conditioned to associate a sound signal as well as a light signal with food, while halibut could only be conditioned to a light signal. Further, the response to the light signal was stronger with a larger percentage of responding fish in the cod experiments than in the halibut experiments, and cod started to respond to the CS after fewer trials than halibut. Does this mean that cod have better cognitive abilities than halibut? Not necessarily. Species with different natural feeding strategies, such as rats and domestic cats, show different behavioural responses to a CS when the US is a food reward (van den Bos *et al.* 2003). There are a number of differences between cod and halibut that may explain the differences found in our experiments. Firstly, cod, but not halibut, possess swim bladder which functions as an amplifier, and cod have better hearing capacities than other flatfishes such as dab (Hawkins 1993), and probably better than halibut as well. Cod communicates with sound (Brawn 1961), and have a wide repertoire of foraging strategies of which many are dependent on hearing, and cod are likely to be more attentive to sounds in general than are halibut. This may explain why cod were more easily conditioned to a sound signal. Secondly, the response to food was relatively low in the halibut experiment where the CS was a light signal, and it came as no surprise that the response to the CS did not exceed the response to the US, as the US is the motivation to respond to the CS. Thirdly, cod is a more social species than halibut, and through social learning the cod may have learned by each other and hence reduced the number of trials needed before they started to respond to the CS.

7.5 What does it mean that cod can be trace conditioned?

Cod detected the CS-US relationship after a few trials at a trace duration of 60 seconds and waited near the feeding automat until the arrival of food, i.e. showing that they had an anticipation of the upcoming reward. That cod could be successfully trace conditioned, even at such long trace durations, indicates that they were aware of the CS-US relationship (Clark & Squire 1998). Although fish most likely do not experience consciousness in the way we do, our experiments demonstrate that cod have representations and anticipations of their surroundings. Cod, and probably other fish species as well, should therefore be considered as sentient animals, with the consequences this may have for application of the concept of animal welfare to fish (Duncan 1996).

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8 SOCIAL INTERACTIONS BETWEEN JUVENILE FISH AND INDIVIDUAL VARIATION IN BEHAVIOUR DURING THE ON-GROWTH PHASE

Linda A. Hansen, Katrine Skajaa & Børge Damsgård

8.1 Introduction

From the moment of fertilisation to the time of death, individuals compete for limited resources such as food, space or mates. Begon *et al.* (1990) defined competition as an interaction between individuals, caused by shared requirements for a resource in limited supply, and leading to a reduction in growth, survivorship or reproductive output for the individuals involved. Those who compete for resources with the highest success rate will be favoured by natural selection through a higher lifetime reproductive success (Sargent & Gross 1993). Behaviour is dynamic and depends upon the relative differences between individuals, particularly when there is competition for limited resources such as food and space. The exclusion of some individuals from resources is common in fish (Chellappa *et al.* 1999; Harwood *et al.* 2002; Kim *et al.* 2004). When resources are clumped rather than dispersed, such as in a farm environment, aggression may be an effective behavioural tactic in competitions (Grant & Guha 1993). As the amount of resources decreases or the number of individuals increase, competitive behaviours are expected to become more frequent. In fish farming on a commercial scale the fish is placed in an environment dissimilar from their natural habitats, and at higher densities than what is usually found in nature. When the general welfare of the fish is satisfactory, most individuals will manage to acquire sufficient food and maintain a good health. However, small changes in the physical or social environment may lead to increased competition and inter-individual variation in growth and health. Individual differences in traits like aggression and competitive ability will become more important during periods of high competition. The degree of interference an individual experiences may not only depend on the food abundance, but also on the competitive ability of its co-inhabitants. Asymmetric competition, where some individuals are affected far more than others, causes small initial differences to be transformed into large differences with time. As a result, the characterisation of populations by an arbitrary average can under specific circumstances be very misleading (Begon *et al.* 1990).

8.2 Motivational background for agonistic interactions

In a fish life growth and reproduction varies in relevance with age and season. The motivational basis for feeding and reproductive behaviour is quite different. During the growth phase the resources worth fighting for are usually connected to either food or territories where there is both food and adequate shelter. During reproduction food often becomes much less important while access to potential mates and breeding territories increases greatly in value. The outcome of competitions for limited resources depend on a number of factors, both internal and external. The behaviour of individuals will vary with for example size or age (Leiser *et al.* 2004), hormonal status (Johanssen & Björnsson 1994), and in some cases social status and experience (Leiser *et al.* 2004). The strategies and tactics displayed by a fish also depend on external factors, such as seasonal variation in the environment. There are internal, causal factors underlying the observed behaviour, and the

factors may interact. They may also be influenced by the appearance of external cues and by deprivations of such cues, and change over time, both during performance and over longer time periods (Colgan 1993).

8.3 The effects of winning contests on subsequent social interactions in Arctic charr

Within certain boundaries there is room for learning and for using prior experiences in subsequent adjustments of behaviour and in decision making. Preceding and following a contest between two fish there are complex behavioural interactions, underlying mechanisms, and dynamics of social status that affect both the winning and losing individual. The dominant and subordinate status connected with winner and loser effects are more than just the sum of aggression, and may possibly better be described as a patterning of interactions between individuals (Drews 1993) or a sum of an individual's resource holding potential (Dugatkin 1997). Prior territory possession, fighting ability and motivation as well as the effects of recent victory or defeat on behaviour affect social interactions (Dugatkin 1997). The loser effect might remain for quite some time after a loss and subordinate individuals may be characterised by suppressed aggressive behaviour, reduced feeding, and low locomotor activity (Øverli *et al.* 1998). Memory of prior victory and defeat can be stored and used to generalise a new situation, where prior winners thus behave as a winner and prior losers behave as a loser. Such changes in behaviour may be an important way to avoid unnecessary fighting or competition for resources (Harwood *et al.* 2001). The cause and effect mechanisms behind social interactions are still ambiguous. Losing animals show a tendency to be defeated at a subsequent contest with an otherwise equally matched individual (Chase *et al.* 1994; Mesterton-Gibbons & Dugatkin 1995).

Using the salmonid Arctic charr as a model species, the dynamics of contest outcomes in charr dyads was studied. Each individual was tested randomly and sequentially against several other fish, either against prior winners, prior losers or non-aggressive fish. This method makes it possible to investigate the dynamics of winner and loser effects across time, size, and various opponents. According to the hypothesis a dominant winner will tend to remain a winner, while a subordinate loser will continue to lose contests as long as it meet more motivated fish. Former experience, either winning or losing a contest should be reflected in the subsequent performance, independent of factors such as fish size or contest method.

The experiment was conducted at the Tromsø Aquaculture Research Station in July 2002. Forty one-year old Arctic charr of the Hammerfest strain were individually marked and measured for weight and length. The fish were distributed pair-wise in twenty 100 litre circular tanks, and kept at the natural light regime of the latitude, which in July means continuous illumination. Formulated feed was supplied in surplus by automatic disc feeders for six hours during the daytime. All twenty tanks were video filmed for three hours during the feeding period, and individual fish could be identified by observation from above. For each trial, a day 1 and a day 2 sub-sample of five minutes of each recorded hour were analysed. In total, 30 minutes from each trial was analysed using the software program The Observer Video Pro (Noldus Information Technology, Wageningen, The Netherlands). The number of aggressive behaviours (chases, attacks and bites) (Pettersson *et al.* 1999; Noakes, 1980) performed and received by each fish were assessed. The individuals in each pair initiating or receiving aggressive acts most frequently was designated as a dominant "winner"

or subordinate “loser”, respectively, with a win index from zero to nine describing the total number of wins by each individual in the nine trials.

After each two-day trial all dyad pairs were changed. Individuals met a new, randomly chosen contestant, in a new tank. The handling during pair change did not seem to have any effect on the aggressive behaviour of the fish, and aggression between two fish was even observed during transfer in a bucket. This procedure was repeated nine times. The experiment was ended after three weeks of observation by sedating the fish, followed by an overdose of sedative to kill the fish. All individuals were again measured for weight and length, and examined for potential fin and body abrasions. Fin wear, loss of scales and wounds were categorised from 1 to 3, where grade 1 indicate no or little damage, while grade 3 fish had several areas of scale loss and both tail and pelvic fins were reduced by approximately 50 % of normal size. As one fish was excluded from the experiment after trial number two due to a developing wound, the number of pairs was reduced to 19, giving a total number of 171 pairwise trials in the experiment. There was no difference in frequency of performed aggressive behaviours, percentage of active fish or amount of performed aggression between day one and day two of trial 2-9 when testing across all trials and days. The low number of aggressive behaviours on day one was probably due to the effects of handling and sedation at experiment initiation. The first trial was therefore only used to assess the social status of the fish, and was otherwise not included in the analyses.

After a prior win an individual charr became a winner of subsequent contests more often than fish having experienced a prior defeat or performed no aggression (Fig. 20). The outcome of a contest was also related to the opponents' prior experience of winning or defeat. An individual would more often win a contest if it met a prior loser than a prior winner or non-aggressive fish. Prior winners were more frequently aggressive than prior losers or non-aggressive fish, and prior losers displayed a tendency to be more aggressive towards other prior losers than towards prior winners. Prior winners meeting prior losers were more aggressive than prior losers meeting prior winners. They were also more aggressive than prior non-aggressive fish meeting prior winners. This suggests that the aggressive behaviour of individual fish was not independent of the opponent, but may vary according to the competitive threat an opponent represents. Number of contest wins was not related to initial body weight, length, or condition factor. There was no effect of percent size difference between opponents on neither number of wins nor amount of aggressive behaviours performed, despite large size differences (20 to 50 %) in many of the trials. There was a significant positive relationship between number of wins and individual specific growth rate during the experimental period, and fish winning more contests obtained less fin wear, loss of scales and wounds.

The competitive ability of Arctic charr appears to be affected by prior experiences in the same manner as has previously been shown in other fish species (Hsu & Wolf 2001; Dugatkin & Druen 2004) and thought modelling (Mesterton-Gibbons & Dugatkin 1995). A skewed distribution of win indexes between individuals indicates the presence a partial dominance hierarchy that is not comparable to the very definable hierarchy described by Dugatkin (1997) as results of a winner effect. Neither were there an active alpha and perhaps beta individual, and a large group of inactive fish, as when only a loser effect applies. Size differences in fish have normally been thought to determine the outcome of contests, unless the differences are small (Beaugrand *et al.* 1996; Damsgård *et al.* 1997; Adams *et al.* 1998). In the present study initial weight, length or condition factor were not reliable predictors of contest outcome or frequency of performed aggressive behaviours. Individual weight did not influence the competitive ability of individual fish regardless of some dyad weight differences being rather

large. Victory and defeat nonetheless influenced individual growth, with a higher SGR among frequently winning fish and a low SGR among frequently losing fish indicating an effect of contest outcome on feed intake and subsequent growth. This is comparable to other studies on feeding and aggression in Arctic charr. Subordinate fish tend to suppress their aggressive behaviour, reduce feeding and become less active (Øverli *et al.* 1998), and the effect of subordination may last for a surprisingly long time. The results from the present study indicate the presence of a winner and a loser effect in Arctic charr, and that these effects are active even with relatively large weight differences between the opponents. A consequence of winning dyad contests is larger weight increments and less body injuries in dominant individuals.

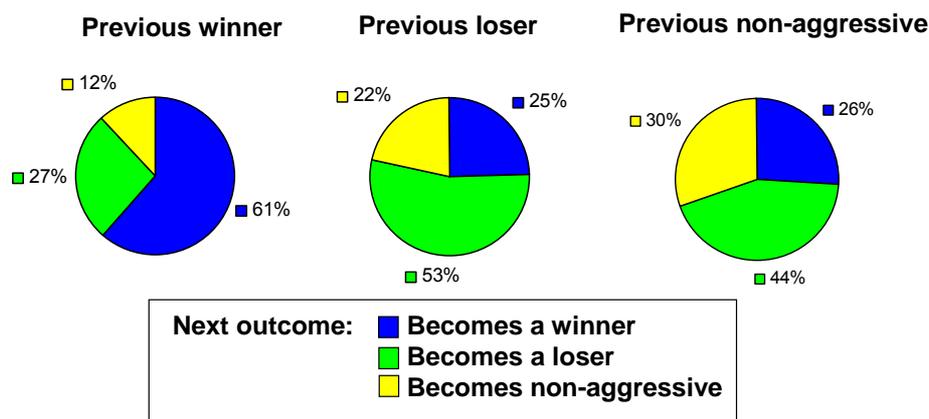


Figure 20 Winners effect in juvenile Arctic charr seen as success or failure in a pair-wise contest in relation to the outcome of the previous contest. The percentage of the 38 fish observed through eight subsequent trials that experience a win, loss or become non-aggressive are shown for previous winners, previous losers and previous non-aggressive fish.

8.4 Effects of size differences and nutritional state on aggression and threat-sensitive behaviour in juvenile cod

When size differences are conspicuous and perceived by the opponents, contests are settled without much agonistic investment or escalation. On the contrary, when opponents are matched in most aspects they rely more on displays and are more prone to escalate (Beaugrand & Goulet 2000). Size correlates naturally with strength, with the larger individual usually winning. An effect of individual size on position within a social or feeding hierarchy has been found in salmonids (Nakano 1995). With no prior residency involved or with relative size differences of 20-30 %, size has been shown to uniquely determine dominance outcome (Beaugrand *et al.* 1996). Ultimate large size in Atlantic salmon can be a consequence of dominance status. Salmon fry that start feeding earlier are dominant to their later feeding siblings, despite not being larger. The early feeders nonetheless soon establish and maintain a size advantage (Metcalf & Thorpe 1992).

Nutritional status is an important factor in determining competitive ability and motivation in fish. As internal energy levels change, so will the motivational basis for performing certain behaviours, thus linking social status and nutritional status. In juvenile rainbow trout fasted fish were found to be dominant over fed fish after 3 days, but after 6 and 9 days they

expressed similar competitive ability. After 12 days however there was a tendency for fed fish to be dominant over fasted fish (Johnsson *et al.* 1996). Variations in nutritional state may change the threat sensitivity of an individual. In coho salmon, food intake and specific growth rates increase after a period of starvation, and this compensatory growth result from a change in risk-taking behaviour (Damsgård & Dill 1998).

As large fish markedly reduces the growth of small fish through aggressive attacks and inhibition of activity (Dou *et al.* 2004), tactics and preferences dictated by social status, size or developmental status serve to reduce conflicts and predation risk. This implies that fish are capable of adjusting their relative use of contest and scramble competition depending on cost and benefits in relation to number of competitors and feeding opportunities (Syarifuddin & Kramer 1996). There will be a trade-off between the need for a resource and the cost of a competition, and subordinate fish may express a strategy of reduced involvement in aggression, possibly to avoid costs (Nicieza & Metcalfe 1999; Harwood *et al.* 2002). That aggression as a behavioural tactic involves an energetic cost is indicated through a negative correlation between growth rate and agonistic behaviour (Vollestad & Quinn 2003). Subordinate fish that minimise their energy use can obtain a net energy gain, whereas those that adopt a high-return/high-cost foraging strategy may actually lose more energy than they obtain (Metcalf 1986).

From foraging theory it is predicted that aggressive behaviour can be scaled to the degree of competitive threat (Dill 1983). According to this hungry cod should represent a greater competitive threat than fed cod, inducing more agonistic behaviours in the opponent. Furthermore, food-deprived individuals should defend a food resource more actively against an opponent than would satiate fish. It would also be expected that food-deprived cod should become less threat sensitive in competitions for food, as either its nutritional state changes or the relative threat of the opponent varies. Through quantifying aggressive and feeding behaviours, swimming behaviours, and positional interactions between two individual cod of varying size and nutritional status, this study aims to compare the relative strength of an external (size of opponent) and internal (nutritional status) stimuli on threat-sensitive behaviour in cod during the vulnerable juvenile stage.

The study was performed at the Tromsø Aquaculture Research Station. Two groups of juvenile Atlantic cod, large and small fish hatched with two months separation in time (0.7-1.7 g), were used in the experiment. Between the fish in the pair-wise trials there were a 57.1 ± 1.1 % difference in weight. Trials were initiated on 11 December, and by 23 December 2003, a total of 221 trials had been run, testing 442 fish. Eight pair-wise trials were performed simultaneously in small aquaria (15.3×28.7 cm, 6 l). Above the eight aquaria four cameras were mounted, each camera filming two aquaria. On one side a plastic tube was positioned, extending down to 0.5 cm above the bottom, and through this tube feed could be delivered during the trials. In most trials this was sufficient to keep the feed in a specific area defined as the food zone of the trial arena in the automatic tracking system EthoVision (Noldus Information Technology, Wageningen, The Netherlands). The food zone was about 1/3 of the total area (15.3×10 cm). Trials were run on four subsequent days allowing one or both of the contestants to be fed or food-deprived for one, two, three, or four days. Two fish, one larger and one smaller, were introduced into each aquarium, and the fish were filmed for 90 minutes. Food was supplied 60 minutes after initiation. Thirteen combinations of fish were tested, including control groups where big, fed (BF) fish were tested against small, fed (SF) fish. The other group combinations were: BF fish against small fish not fed for one to four days (S1 to S4), SF fish against big fish not fed for one to four days (B1 to B4), and B1 to B4 against S1

to S4 fish. The combination abbreviations were $S_F B_F$ (control), $S_x B_F$, $S_F B_x$ and $S_x B_x$, respectively, with x indicating days of food deprivation.

A fifteen-minute sub-sample of each trial was analysed, including the five minutes prior to supplying feed and the ten minutes after feeding. Individual aggressive and feeding behaviours were manually scored using the software program The Observer Video-Pro (Noldus Information Technology, Wageningen, The Netherlands). Feeding and aggressive behaviours were recorded, pooling the aggressive behaviours charge, chase and bite (Petersson 1999) when presenting the data. The performer and receiver of aggressive behaviours were recorded.

An effect of food deprivation on the nutritional status of the fish was indicated by the relative condition factor (RKF), which was higher in fed than in food-deprived fish. The small fish were in the majority of groups found to be less aggressive (Fig. 21, top panel), feeding less frequently (Fig. 21, mid panel), and their swimming speed was lower when compared to the bigger contestant. Aggressive behaviours were almost absent in the small fish, and the small fish reacted to attacks by the big fish by fleeing or hiding in 85 % of such interactions. The small fish was therefore termed the subordinate, and the big fish the dominant individual of the two contestants. Based on this it is assumed that the small, subordinate fish would be far more affected by the behaviour of the large, dominant fish than the other way around. The results are therefore presented with the subordinate fish as focal animal and the dominant fish as an external factor influencing the subordinates' behaviour to varying degrees according to relative differences in size and nutritional status.

Internal factors

Few effects of starvation on the behaviour of the subordinate contestant were evident despite the lower nutritional status in food-deprived fish. Subordinate aggressive behaviour was infrequent and not affected by food-deprivation. The frequency of feeding events were also generally low in the subordinate fish and food deprived fish did not feed more than the fish in the control group. The four-days food deprived subordinate fish did show more interest in food as it spent more time in the food zone than subordinate fish in the control group, with similar tendencies for two and three-days food-deprived fish (Fig. 21, bottom panel). There was no effect of food deprivation on swimming speed. The distance between two contestants was higher after four days of food-deprivation of subordinate fish compared to the distance between fish in the control group.

External factors

A reduced RKF in big, food-deprived fish compared to the fed control fish suggest that it could represent an increasing threat to the subordinate fish. A behavioural effect of food deprivation in the dominant fish could however only be detected in increasing feeding frequency by the large fish, and not in aggression or other studied behaviours. Dominant fish spent more time moving towards the subordinate fish as the relative size differences increased size, and the subordinate fish also spent less time within the food zone with increasing size difference between the two contestants. Increasing relative size differences and distance between contestants was positively related in the control group ($S_F B_F$), but this external effect was not evident when one or both fish were food deprived ($S_F B_x$, $S_x B_F$, $S_x B_x$). In all combinations except the control group there was a significant positive relationship between the frequency of the subordinate in the food zone and the approach speed of the dominant

contestant. This suggests that the approach speed of the dominant fish towards the subordinate could be an external factor changing in strength according to the behaviour of the subordinate fish

Interaction between internal and external factors

Four days of food-deprivation was seemingly not sufficient to significantly change the threat-sensitive behaviour of subordinate fish. However, with four days of food-deprivation the time spent in the food zone by the subordinate SxBx fish increased. It was visually comparable to the values in the internal factor (SxBF) combination and tended to be higher than in the control group, despite a food-deprived, larger contestant. This suggests that an intersection between food-deprivation of the subordinate as an internal effect and food-deprivation of the dominant opponent as an external effect may have been reached. A positive relationship between increasing size difference and distance between opponents was present only in the control group, suggesting an interaction between the external and internal effects that cancel out this response when one or both fish were food-deprived.

Juvenile cod kept in pairs displayed large size-dependent differences in behaviour in the present experimental study. The larger fish were more frequently aggressive than the smaller fish in all groups, and thus had a strong competitive advantage over smaller, subordinate individuals not even overruled by hunger in the subordinate opponent. Dominance due to a larger size is expected (Beaugrand *et al.* 1996; Harwood *et al.* 2002), particularly when relative size differences are as large as they were in this study. Exceptions do exist, for example when size differences are small or where other factors are more important than size. On a general basis a large individual will gain and maintain a dominant status through agonistic behaviours. The high level of aggression among large fish and low level among small fish in this study was stable through the fifteen minutes of observation, and independent of the presence of food. This is comparable to behaviour in Arctic charr, where dominant fish continue to attack even after a dominant-subordinate relationship has been established, and subordinate individuals have ceased feeding and reduced all other behaviours to a minimum (Noakes 1980). Feeding would for larger fish involve very little risk in the present study, as small fish rarely performed aggressive behaviours. Larger fish are also assumed to tolerate starvation better than smaller fish (Jonas & Wahl 1998). The lack of an expected increase in feed intake in the small food-deprived fish suggests that the cost of confronting the dominant opponent was too high compared to the benefit of obtaining food. The large fish were, regardless of nutritional status of either individual, very aggressive, whereas a strategy of reduced involvement in aggression among smaller fish may have served to reduce costs of confrontations (Nicieza & Metcalfe 1999). Regardless of the potential cost of confrontations the food-deprived small fish in the current study did spend more time in the food zone than its larger, fed opponent, and despite no increase in actual food intake an increased interest in food is indicated. The results indicate that the cost of feeding, in this case the cost of being exposed to a bigger, aggressive individual, overrules the certain risk of starvation and the lost growth opportunities if not obtaining food, for at least as long as four days of food deprivation. The results further emphasise the importance of showing awareness towards agonistic behaviour among cod in a culture environment. Individuals of varying size, often considerably larger size difference than experienced in the current experiment, are reared together in culture without any possibility for individual selection of habitats or refuges. Size-differences and monopolisation of food could compromise the welfare of smaller individuals and with time increase the initial variation in size.

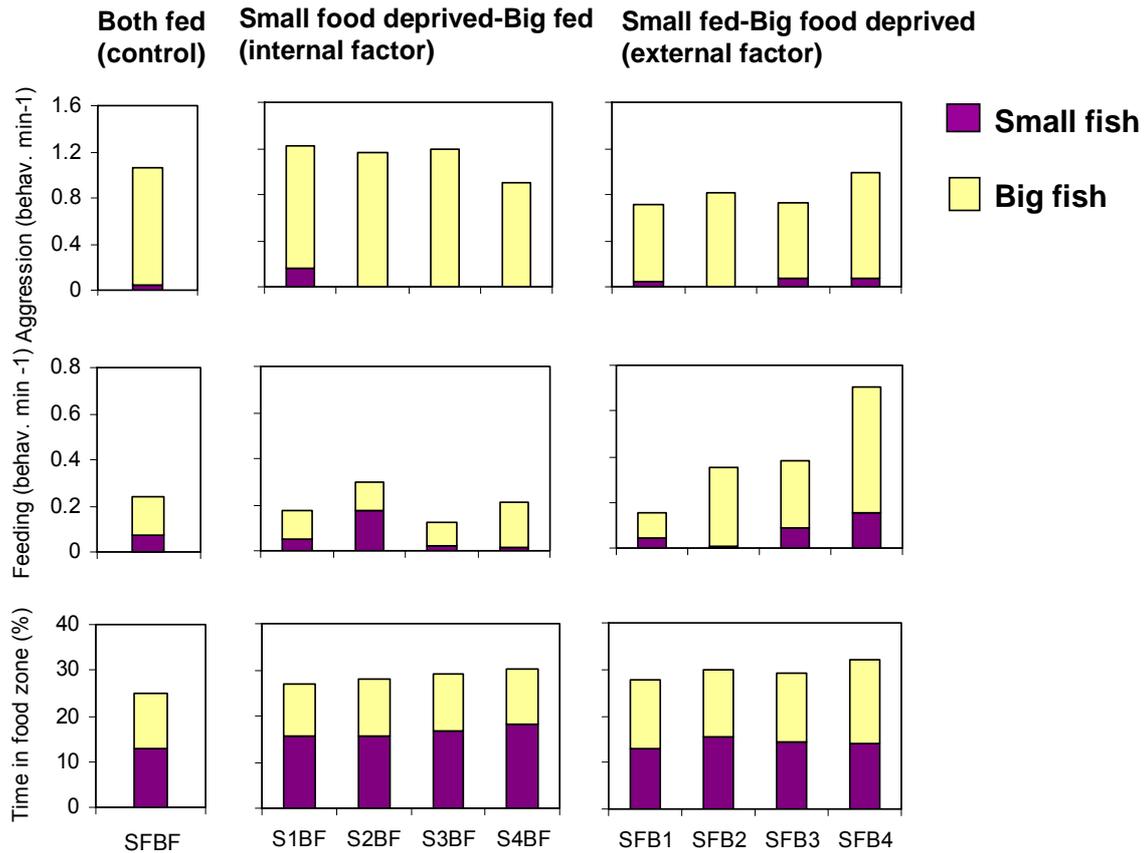


Figure 21 Frequency of aggression (behaviour min^{-1}), feeding (behaviour min^{-1}), and time spent in the food zone (% of total available time) in pairs of juvenile cod of different size and nutritional status. Mean value of behaviours are shown for small (dark bars) and large fish (white bars) in the control group where both fish were fed, for food-deprived small fish with fed large fish (S_xB_F), and for fed small fish together with food-deprived big fish (S_FB_x). Group letters denote S for small fish, B for big fish, F for fed and x for one to four days of food-deprivation.

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9 WATER QUALITY PREFERENCE

Bjørn-Steinar Sæther, Børge Damsgård & Arne Mikal Arnesen

9.1 Introduction

Fish are in many ways more dependent on its environment than land living animals. Being poikilotherms their body temperature is mainly decided by the surrounding water temperature although it may be a few degrees higher than the surrounding water. In addition, their internal chemical milieu are only separated from the surrounding waters by thin biological membranes (the skin, gills or the lining of the gastrointestinal tract), the chemical composition of the surrounding water may have a major influence on the fish. The composition of body fluids is controlled by the fish and kept within physiologically reasonable limits. These regulatory processes have an energetic cost and if severely challenged may cause stress to the fish. Thus, environmental conditions that significantly disturb the homeostasis of the fish should be avoided.

The tolerance range for different environmental factors vary with species, age/size as well as maturation or energetic status. The most studied environmental factor in aquatic animals is perhaps temperature, with early studies focusing on its regulatory effect on biological processes. The temperature range that a fish is capable of adapting to represents its thermal tolerance, and the tolerance limits may differ for different physiological processes such as growth and reproduction (Jobling 1994). Species able to cope with large temperature variability are known as eurythermic, as opposed to stenothermic animals which are adaptable only to slight temperature variations (Jobling 1994). Although biological processes can take place within a wide temperature range, the optimal temperature range may be very limited. For example, reproduction in fish may take place within a range of temperatures (indicative of tolerance), but high quality offspring is produced only when the broodstock is kept within narrow temperature ranges (Jobling *et al.* 1995; Tveiten *et al.*, 2001). Therefore, temperature also act permissive by suppressing development if the environmental conditions are beyond acceptable limits for reproduction, still well within acceptable limits for survival and growth (Jobling 1994).

Poikilotherm animals presented to thermal gradients spend most of their time in a narrow range of temperatures, defined as their preferred temperature (Fry 1947; Johnson & Kelsch 1998). This active behaviour of fish has been termed "behavioural thermoregulation" (Reynolds & Casterlin 1979). Preferred temperatures have been suggested to be correlated to physiological optimums of various functions (Brett 1971; Crawshaw & O'-Connor 1997) and good correlation between preferred temperatures and optimum temperatures for growth of fish has been demonstrated (McCauley & Casselman 1980; Jobling 1981). Hence, by an active selection of the optimum temperature for growth, fish should be able to maximise their growth rate. Accordingly, Jobling (1981) suggested that the preferred temperature could be used to predict the optimal temperature of growth in fish. However, later studies demonstrated correlations with other processes. For example; optimal temperature for growth is a few degrees Celsius higher than optimal temperature for feed conversion (Jobling 1994). When given a choice two salmonid species were found to prefer the temperature for optimal for feed conversion rather than growth (Larsson 2005).

It seems that optimal should be interpreted as the temperature that best supports the combined challenges put on the fish, remembering that the mechanisms has evolved in the wild. In other words, fish show behavioural temperature regulation that we can refer to as temperature preference, assuming that this preference integrate optimisation of several internal processes. If the temperature preference is indicative of what is optimal to the fish, this will add valuable information to the knowledge gained from traditional tolerance or level-effect studies. For aquaculture purposes, any discrepancy between holding and preference temperature may imply that we ignore the needs of the fish and has a potential to optimise holding conditions.

Fig. 22 illustrates that the response to temperature is relative to acclimation temperature (Jobling 1994). As preference temperature gradually become the new acclimation temperature, and the new acute preference temperature gradually close in on the final temperature preference zone, the acclimation temperature and acute preference will gravitate in the same area. The graph also illustrates that the temperature tolerance range (indicated by the zone marked with crosses) also is dependent on acclimation temperature. Further, the lowest and highest tolerance temperatures are related to acclimation temperature until ultimate upper incipient or ultimate lower incipient temperatures are met. The area of this polygon, given as degrees squared $[(^{\circ}\text{C})^2]$, quantifies the thermal tolerance of a species, and eurythermic animals have a larger $(^{\circ}\text{C})^2$ than stenothermic animals. Tolerance limits for growth, indicated by the shaded polygon, are narrower than that of survival indicating that the fish will cease to grow before survival is compromised. The maturation process is suggested to be the process with the lowest $(^{\circ}\text{C})^2$ and has optimal temperature below that of e.g. feeding and growth. Temperature preference in maturing fish is reduced, possibly because reproduction is a critical process with high priority that is weighted in the integrated response that preference represents.

Water quality parameters other than temperature may also affect the fish. Under intensive farming conditions metabolites are aggregated in the rearing water, and experimental exposure to ammonia reduces both appetite and growth in fish. Even if the exposure concentration is below lethal levels, and within the tolerance range, any adaptation implies use of energy to counteract the influence from external ammonia (Ip *et al.* 2003). Fish has a very well developed chemosensory system, and use this to evaluate habitats, seek for food and avoid predators (Sorensen & Caprio 1998). Accordingly, they can assess and select water quality, at least for some parameters, that does not compromise their internal environment.

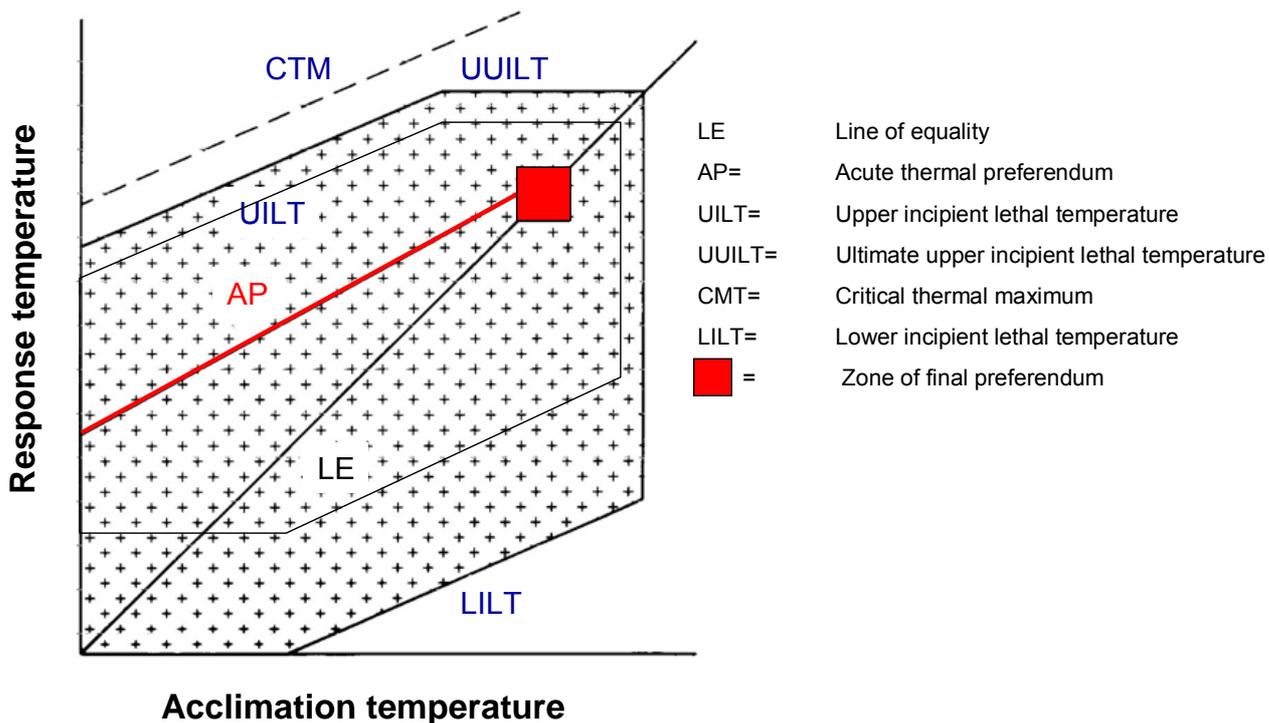


Figure 22 Temperature preference polygon describes how acute thermal preferendum (AP) relates to acclimation temperature. LILT and UILT represents the lower and upper incipient temperatures where, in case of lethal limits, survival is not compromised. Both are dependent on acclimation temperature until the ultimate limits are reached and these are constant whatever acclimation temperature. Line of equality is found when acclimation temperature and response temperature are the same. As long as final temperature preferendum is not reached, the acute thermal preferendum gradually becomes the new acclimation temperature, and the acute preferendum will then change. This leads to a gradual drift towards the final thermal preferendum, where acclimation temperature and acute thermal preferendum stays the same. Redrawn from Jobling 1994.

9.2 Temperature preference in Atlantic cod

In a series of experiments the thermal preference in Atlantic cod was investigated using the preference system described in chapter 4.1, where fish can swim freely in a controlled temperature gradient. Trials were done over a period of three years, involving building and testing of the preference system. The results presented concerns cod (300-800 g) from trials conducted in the period from 24 November to 4 February, thereby avoiding confounding effects of season which is known to influence temperature preference in cod. The fish were acclimated to natural temperatures for the time of year, declining from approximately 6.3 °C in November to 3.3 °C in January-February.

The trials were conducted over a 48 hour period, with the gradient reversed after 24 hours. Response temperature the last 4 hours in each diel was compared, and assumed to represent final thermal preferendum. The fish behaviour in the period after transfer to the preference system may be referred to as a learning period, as the fish were swimming back and forth surveying the whole tank. Typically, the activity level gradually declined with the fish spending more time in a smaller area and temperature range. This behaviour was normally repeated when the temperature gradient was reversed after 24 hours, but with a shorter "learning period". The response temperature on day 1 and day 2 is plotted in Fig. 23. If the

zone of final thermal preferendum is reached, the resulting temperature of choice should relate 1:1 to each other (match the line of equality). A fitted line to our result had an elevation of 1.08, giving a 1:1.08 relationship between day 1 and 2, suggesting that final thermal preferendum was met. The data show relatively high degree of variability, which may reflect the complexity of thermal preference itself. The fish were not fed during trials so the filling of the gastrointestinal system declined as the trials progressed. Both sexes were represented, some about to mature the following spring. However, these factors do not contribute significantly to explain the observed responses.

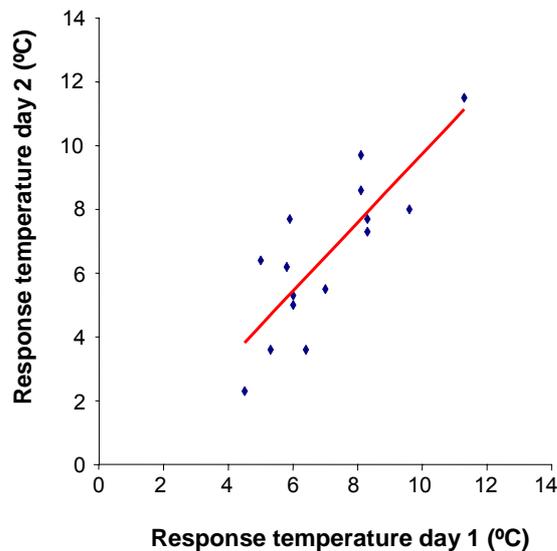


Figure 23 Response temperature in Atlantic cod over two consecutive days. The trials were conducted in the period 24 November to 4 February, with acclimation temperature between 6.3 and 3.3 °C.

The response temperature on day 2 significantly declined with increasing fish body weight (Fig. 24). The results match reasonably well with a previous study of preference during autumn/winter on smaller cod (Clark & Green 1991). However, preferred temperature is lower at any given size compared to the temperature that supports maximum growth (Jobling 1988; Björnsson *et al.* 2001; Björnsson & Steinarsson 2002). Studies of temperature effects on growth have shown that temperature for maximal growth declines with increasing body size (Jobling 1988; Björnsson *et al.* 2001; Björnsson & Steinarsson 2002), so does optimal temperature for growth (Jobling 1994).

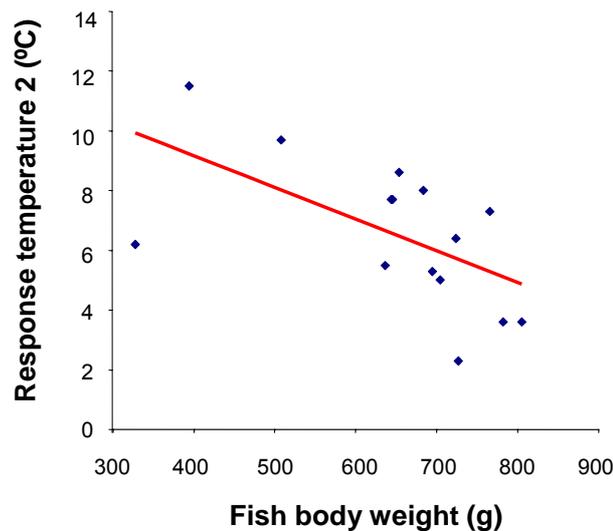


Figure 24 Response temperature in Atlantic cod of different size. The response is based on data following 48 hours in a preference system. The trials were conducted in the period 24 November to 4 February, with acclimation temperature between 6.3 and 3.3 °C.

The response temperature given by cod in the behavioural lab facility designed for assessment of preference temperature seems suitable to predict final temperature preferendum in Atlantic cod. It offers a reasonable easy and fast method to estimate this increasingly important parameter, although the physiological basis for interindividual variability needs further studies. Thorough knowledge of the fish's biology and physiological status are essential for interpretation of the results. The biological system that the fish represents is expected to optimise the conditions for all internal processes that is going on, and the importance of each of them are likely to be different

9.3 Temperature preference studies with other species

Preference studies have been conducted on other species than cod using the same laboratory facility. Results from Arctic charr show that the behaviour of this species probably makes them suitable for this kind of studies as they are active and explore the whole temperature range. Different to cod, they remain active passing through the whole temperature range throughout the trial. Results from only a few trials could be accepted, given the set of criteria given in chapter 5.1. The fish in 5 of these 6 trials showed a higher response temperature on day 2 as compared to day 1, indicating that they had not reached their final preferendum yet. These fish were held at natural ambient temperature before trials, which at that time were below 1 °C. Before new trials on charr are conducted, the protocol need to be considered. When plotting response temperatures between day 1 and 2 in these 6 trials, the curve should theoretically cross the line of equality at approximately 12 °C. Although this is a very limited set of data, they indicate a final thermal preferendum 2-3 °C below the temperature found to support maximal growth in Arctic charr (Jobling 1983). Published results on temperature preference in Arctic charr suggest that they prefer temperatures slightly above 9 °C (Peterson *et al.* 1979), or 10.8/11.8 °C dependent on strain and size (Larsson 2005).

A short series of trials on common wolffish (*Anarhicas lupus*) have been conducted. The wolffish is a more sluggish species than the two previous tested. They are bottom dwelling and lack swim bladder. Typically, they spend most of their time lying on the tank bottom

without moving around at all. This behaviour may explain why they generally needed longer time to respond to changes in temperature. They seem to prefer spending their time in the coldest end of the temperature gradient, but during our trials we were unable to determine the actual thermal preference due to lack of cooled water. We simply could not get the water cold enough to make the fish move from the end of the gradient, and results from these trials did not meet the criteria for validity of the results.

Attempts were made to study another bottom dwelling species, Atlantic halibut. These individuals showed no signs of behavioural thermoregulation in the experimental set-up, and the attempt was terminated after testing 3 individuals. None of them moved within the gradient, and took a change in temperature from 2 to 12 °C in 20 minutes without moving at all. The status or the biology of this fish may explain the lack of response, and new trials on other fish should be conducted before the halibut is written completely off.

In conclusion the temperature preference system established in the behavioural lab seems appropriate for thermal preference studies. The behavioural thermoregulation that is challenged here may be best studied in species that are not bottom dwelling.

9.4 Studies of tolerance and behavioural preference of ammonia

In a recent study of long term effects (96 days) of ammonia exposure on growth in Atlantic cod, a rather low tolerance level was indicated (Foss *et al.* 2004). Growth was compromised at 0.11 mg UIA-N l⁻¹ (unionised ammonia, NH₃) but not at 0.06 mg l⁻¹, indicating a threshold level somewhere in between. The cod did, however, compensate for increased ambient ammonia at 0.06 mg l⁻¹ by increasing urea production. Thus, based on this long term growth study and economic rationale, a recommended level of ambient UIA-N in the water should not exceed 0.06 mg UIA-N l⁻¹. However, the fish compensated for increased ambient ammonia at this level and may experience stress at lower levels than those compromising growth.

The behavioural response of cod to ammonia was studied in the preference tanks described in chapter 5.1. Water flow in each tank was adjusted to 20 l m⁻¹, with a water current of 3-5 cm sec⁻¹. Cod (660 g (SD 166), n=29) was anaesthetised and tagged (PIT-tag ID-100) at the base of the first anal fin the day prior to the trial, and introduced to the preference tanks to recover. The following day recording of behaviour started. This gave a baseline record of activity level, and whether the fish was appropriate or not to continue the trial was decided based on its track record between 0900 and 1000 hours. Fish that did not move between tanks was excluded (n=5), and a total of 24 fish completed the trials. At 1000 hours, ammonia of a known concentration was added to tank 1 for 1 hour. The added ammonia was from a stock solution that was added at a rate of 45 ml min⁻¹. At 1100 hours addition of ammonia was stopped, and the tanks were left to clear out remaining ammonia the following two hours. At 1300 hours the same concentration of ammonia was added to tank 3 for another hour, before the experiment was terminated at 1400. Five fish were tested sequentially for each concentration of ammonia, except for the highest concentration that due to previously described exclusions was reduced to four. The Trovan system kept track of the fish during the whole sequence of events, and the behavioural response of the fish was based on the last 30 minutes of each period with ammonia exposure.

Atlantic cod significantly avoided exposure to ambient ammonia at concentrations as low as 0.030 mg UIA-N l⁻¹, but not at 0.015 mg UIA-N l⁻¹. The avoidance response occurred at about half the concentration where compensatory urea production was observed and at approximately one third of the concentration that imposed reduced growth (Foss *et al.* 2004). Before results based on behavioural avoidance can be implemented in practical work and basis for protocols, further knowledge about the exact significance of the avoidance behaviour is needed. It seems likely though that the fish's sophisticated sensory system is very sensitive when assessing threshold levels of water components that elicit a response by the fish. Although this reveals levels that are unlikely to cause severe stress to the fish, it may still prove useful when evaluating acceptable water quality standards.

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10 OPTIMISING INTENSIVE REARING CONDITIONS TO SECURE FISH WELFARE AND HEALTH

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10.1 Introduction

Intensive rearing, including high fish densities, low specific water flow, and oxygen supplementation, seems to be the normal practice in most commercial land-based fish farms nowadays. Although the intensification of aquaculture practices have been profitable and enabled the farmers to increase their production within the existing facilities, they probably have had a diametrical opposite effect by reducing the water quality and thereby leading to increased chronic stress, growth impairment and health problems, all indications of reduced welfare status. To prevent poor water quality from acting as a limiting factor for optimal growth and welfare of the fish, it is necessary to optimise the production and identify those rearing conditions that secure the welfare of farmed fish.

We have conducted a series of experiments to study the welfare consequences of high fish density and different water qualities in Atlantic salmon under the parr-smolt transformation. The fish welfare status have been evaluated based on combinations of different biological measures such as changes in growth, blood physiology, susceptibility to IPN, immunology, and other health measures.

10.2 Does intensive farming reduce the welfare status of salmon smolts?

Reduced specific water flow combined with high fish loadings will inevitably result in an increased concentration of catabolic waste products, such as carbon dioxide (CO₂) and ammonia (NH₃), in the fish tanks (Lemarié & Toften 2003). In addition, the reduced water exchange will allow suspended solids, uneaten feed, and faeces to be accumulated in the tanks.

These changes in water quality may impose many negative effects on the fish. Fivelstad & Binde (1994) reported histological changes in the gills (swelling and hyperplasia) and kidneys (nephrocalcinosis), reduced growth and condition factor in a group of Atlantic salmon exposed to low water flow (0.16 - 0.33 l kg⁻¹ min⁻¹) and oxygen supplementation compared to the control (0.49 - 0.59 l kg⁻¹ min⁻¹). Similarly, Sommer *et al.* (2001) found suppressed growth rates and disease resistance in the treatment group of Atlantic salmon smolts exposed to low water flow (0.14 - 0.18 l kg⁻¹ min⁻¹) and oxygenation compared to the control (1.22 l kg⁻¹ min⁻¹). They also found higher mortality rates in treated fish after challenge with *Infectious Pancreatic Necrosis Virus* (IPNV), indicating a link between long-term exposure to reduced water quality and increased susceptibility to IPN. Also in sea bass, it has been found a negative correlation between specific water flow and growth (Lemarié & Toften 2003). In this experiment, it was also demonstrated that the reduction in growth rate was at least partly due to a reduction in feed intake. These studies indicate that the welfare status of the fish may be reduced by lowering the water exchange.

In our previous study with Atlantic salmon (Sommer *et al.* 2001), it was difficult to separate the effects of high density from the effects of poor water quality. In general, it is believed that there is a link between high fish densities and increased disease susceptibility in fish (Ellis *et al.* 2002), and a possible mechanism might be that high densities can suppress the immune defence (Wedemeyer 1996). However, this has not been sufficiently documented and there has been a dispute whether the cause of the observed effects of increasing density could be water deterioration (Ellis *et al.* 2002). Thus, we wanted to follow up our earlier study and try to identify which factor was the most important. In addition, we wanted to study the effects of high and fluctuating oxygen levels. This is a real problem in many commercial farms and may have damaging effects on the fish (Alabaster & Lloyd 1980; Wedemeyer 1996; Staurnes *et al.* 1998).

Thus, we have exposed groups of Atlantic salmon (initial weight of 40-50 g) to different treatment conditions (fish density, specific water flow and O₂) for 5 weeks during the parr-smolt transformation period in freshwater. Subsequently, the fish were transferred to seawater and some of the fish were challenged with IPN virus. Mortality, physiological and immunological performance of the fish was registered regularly, both in freshwater and seawater. Water quality parameters, such as water flow, water current, temperature, salinity, O₂, CO₂, NH₃, pH, total gas saturation and metals, were monitored frequently throughout the experiments.

The results confirmed our previous findings that fish exposed to low specific water flow, oxygenation, and high fish density showed reduced feed intake and growth in fresh water. In some of the groups, these negative effects continued after the exposure had ceased and the fish had been transferred to seawater. There was a clear tendency to increased mortalities after IPN challenge in groups subjected to the most intensive rearing conditions compared to the control groups, although some of these differences were non-significant. Low specific water flow, high levels of CO₂ and O₂, and high fish density were identified as risk factors. A combination of these factors gave the lowest growth and highest mortality rates.

10.3 Do high carbon dioxide concentrations and acid water affect the welfare of Atlantic salmon?

When oxygen is no longer the limiting factor, the reduction in water flow is mainly limited by the accumulation of excretion products from the fish as carbon dioxide (CO₂), ammonia (NH₃), urea and suspended solids (Colt & Watten 1988; Fivelstad & Binde 1994; Grøttum & Sigholt 1996; Person-le Ruyet & Boeuf 1998; Fivelstad *et al.* 1995; 1998; 1999 a, b). Among these factors, carbon dioxide seems to be the most critical water parameter because the increase in CO₂ will cause a reduction in pH, and therefore, the risk of high NH₃ levels is rather low. Thus, we wanted to examine the welfare consequences of high carbon dioxide concentrations and acid water.

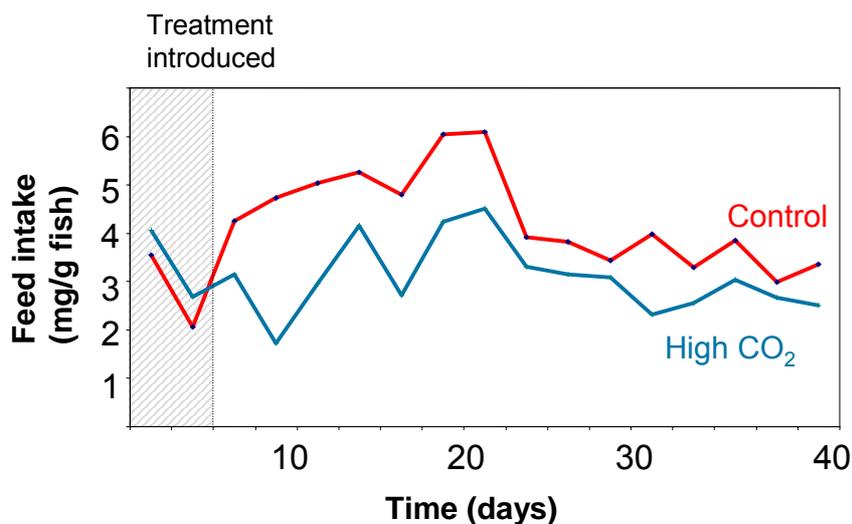


Figure 25 Feed intake in Atlantic salmon reared at two levels of CO₂ and pH (Control: 5.2 mg l⁻¹ CO₂ and pH 6.5; High CO₂: 34.7 mg l⁻¹ CO₂ and pH 5.6) for 40 days.

Seven groups of Atlantic salmon (initial weight of 40 - 50 g) were exposed to different water quality treatments: 1) Control (good water quality: 4.5 l mg⁻¹ CO₂ and pH 6.4); 2) Acidified water (pH 6.05); 3) Acidified water (pH 5.9); 4) Acidified water (pH 5.7); 5) 9 mg l⁻¹ CO₂ (pH 6.05); 6) 13 mg l⁻¹ CO₂ (pH 5.9); 7) 21 mg l⁻¹ CO₂ (pH 5.7). The treatments (two replicates) were given for 5 weeks during the parr-smolt transformation period in freshwater. The same fish density (25-30 kg m⁻³), specific water flow (1.3 l kg⁻¹ min⁻¹) and temperature (10 °C) were used in all experimental tanks. After the treatment in freshwater, the fish were transferred to seawater and some of the fish were challenged with IPN virus. Mortality, feed intake, growth, physiological and immunological parameters of the fish was registered regularly, both in freshwater and seawater. Water flow, water current, temperature, salinity, O₂, CO₂, pH, total gas saturation, and metals were monitored frequently throughout the experiment.

The results show that fish exposed to the highest CO₂ levels (and reduced pH) had reduced feed intake (Fig. 25) and growth during treatment in freshwater (Fig. 26). In some of the groups, these negative effects continued after the exposure had ceased and the fish had been transferred to seawater. There was a tendency to increased mortalities after IPN challenge in groups subjected to the highest CO₂ concentrations compared to the control groups; however, these differences were not significant (Fig. 27). Acid water alone affected neither feed intake, nor growth or mortality after IPN challenge.

10.4 Do high oxygen and carbon dioxide levels affect the welfare status of salmon smolts?

Addition of oxygen to levels above normal saturation seems to be a normal situation in intensive culture systems for fish. Fish seems to have developed a strategy for having a low partial pressure of oxygen in their arterial blood (Massabuau 2001), possibly to avoid the toxic effects of oxygen. An environment supersaturated with oxygen to levels above the

threshold for deregulation in the fish may lead to oxidative stress, reduced immunity, DNA damage (Staurnes *et al.* 1998), which eventually can increase disease outbreak, reduce growth, and possibly increase deformities and death (Toften *et al.* 2005).

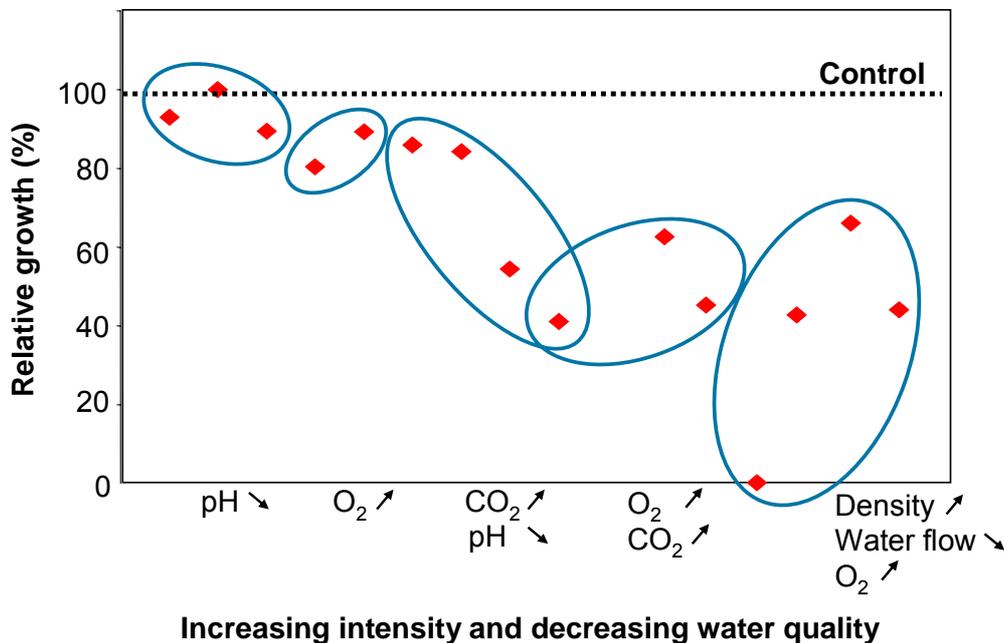


Figure 26 Relative growth (as percentage of specific growth rate of controls) in Atlantic salmon reared at different rearing conditions (acidified water, increased CO₂ and O₂, reduced specific water flow and increased fish density).

In intensive farming, the combination of high O₂ and CO₂ levels frequently occur, but we know very little how the fish responds to these conditions. Hyperoxia would increase the concentration of CO₂ and reduce the pH in the blood, and combined with high CO₂ levels in the water, this could aggravate the situation. On the other hand, the toxicity of NH₃ increases with decreasing oxygen levels (Alabaster *et al.* 1979; Thurston *et al.* 1981; Wajsbrodt *et al.* 1991) and the tolerance for NH₃ increases with increasing oxygen levels (Foss, 2002). This could also be the case for high O₂ and CO₂ levels.

Thus, we have exposed six groups of Atlantic salmon (average initial weight of 37 g) to different water quality treatments: 1) Control (85 % O₂ saturation; 5.2 mg l⁻¹ CO₂; pH 6.5); 2) MO₂/LCO₂ (130 % O₂; 5.0 mg l⁻¹ CO₂; pH 6.5); 3) HO₂/LHCO₂ (155 % O₂; 5.9 mg l⁻¹ CO₂; pH 6.5); 4) NO₂/HCO₂ (85 % O₂; 34.7 mg l⁻¹ CO₂; pH 5.6); 5) MO₂/HCO₂ (130 % O₂; 30.7 mg l⁻¹ CO₂; pH 5.6); 6) HO₂/HCO₂ (155 % O₂; 31.2 mg l⁻¹ CO₂; pH 5.6). The treatments (2 replicates) were given for 5 weeks during the parr-smolt transformation period in freshwater. The fish density (25-35 kg m⁻³), specific water flow (1.02 l kg⁻¹ min⁻¹) and temperature (10 °C) were approximately the same in all experimental tanks. After the treatment in freshwater, the fish were transferred to seawater and some of the fish were challenged with IPN virus. Mortality, feed intake, growth, physiological and immunological parameters of the fish was registered regularly, both in freshwater and seawater. Water flow, water current, temperature, salinity, O₂, CO₂, pH, total gas saturation, and metals, were monitored frequently throughout the experiment.

Preliminary results show that all treatment groups had lower growth than the control group during the treatment period in freshwater (Fig. 26). The three groups that were subjected to high carbon dioxide concentrations showed the poorest growth. It seems that a moderately increased oxygen level can counteract some of the negative effects of CO₂. There were no differences in growth between treatment groups after transfer to seawater. However, there was a tendency to increased susceptibility to IPN virus in all treatment groups compared to the control, with the highest mortality observed in groups where both the CO₂ and O₂ levels were high (Fig. 27). In some of the groups there were reduced chloride concentrations and increased partial pressures of CO₂ in the blood, and this reflected mainly the concentration of CO₂ in the water. Our results indicate that high levels of CO₂ and O₂ in the water may lead to reduced health and welfare in salmon smolts.

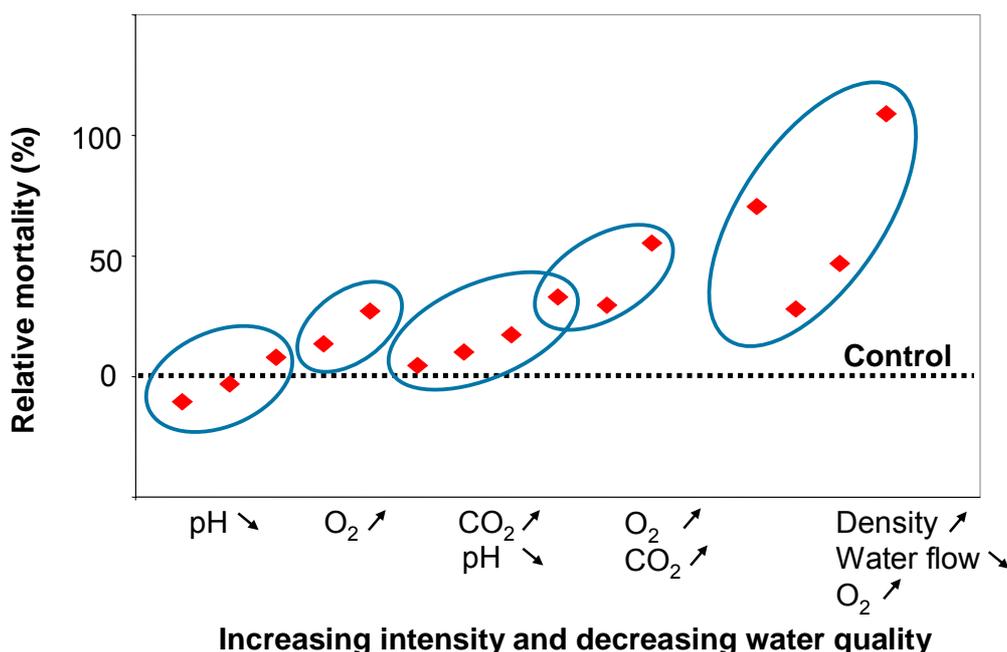


Figure 27 Relative mortality (as percentage of specific growth rate of controls) in Atlantic salmon reared at different rearing conditions (acidified water, increased CO₂ and O₂, reduced specific water flow and increased fish density) and challenged with IPN virus.

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11 EFFECTS OF THE CAGE ENVIRONMENT AND SOCIAL INTERACTIONS ON THE SWIMMING BEHAVIOUR AND WELFARE OF ATLANTIC SALMON

Jon-Erik Juell, David Johansson & Frode Oppedal

11.1 Microhabitats in big volumes

In order to improve production efficiency and secure fish welfare, the size of floating marine cages for Atlantic salmon production has increased considerably over the last decades. Early cages had a volume of 7-900 m³ and a depth of 5-7 m while a modern cage is typically around 12-1500 m³ and more than 20 m deep, and a further increase in size is anticipated. A state-of-the-art salmon farm may consist of 6-12 such cages configured in a compact steel structure (Fig. 28) or more dispersed individual circular cages. Each farm may hold several hundred thousands salmon and a biomass of more than 3000 metric tonnes. In view of the economical importance of this growing phase in salmon production, surprisingly few studies have been carried out to investigate the environmental conditions and behaviour of the fish in these systems. Furthermore, on-farm studies have recently been advocated as a highly useful approach in animal welfare studies (Dawkins 2004 a) and methods of behavioural observation of fish in cages have progressed for some time (Juell 1995, Johansson *et al.* 2005). A series of recent investigations based on the methods developed in the cage-environment laboratory (see chapter 4.4) has demonstrated that the cage volume normally offers a range of microhabitats with large temporal and spatial variation in physical and chemical factors that influence the physiology and behaviour of fish and ultimately may result in reduced welfare for caged salmon.



Figure 28 Modern cages enclose large volumes with a number of microhabitats with different environmental qualities. Photo: IMR.

11.2 The pycnocline and the environmental quality of a site

The fjords and archipelago of the Norwegian coastline provides a suitable sheltering for cage production of fish. In these sheltered areas the farms are often exposed to variable freshwater runoffs. Especially in fjords this produces a transition layer, the pycnocline, where there is a typical rapid change in water density with less dense brackish water above and more oceanic like water below. The pycnocline is often a distinct border between two highly different thermal and osmotic environments (Johansson *et al.* 2005 a, b, Fig. 29). In addition, the pycnocline may also have a strong effect on the background level of dissolved oxygen. The water layer above the pycnocline is oxygenated both through its contact with air and by algal production, but the pycnocline represents an effective obstacle to further mixing with the water layers below (Fig. 29). The oxygen content below the pycnocline is thus mainly dependent on algal production. In periods when photosynthetic production of oxygen is low (e.g. late autumn), this may induce hypoxic conditions (Johansson *et al.* 2005 a, b). The pycnocline may also influence the oxygen level through its influence on the water flow in different layers of the cage. Johansson *et al.* (2005 b) demonstrated that both the direction and the velocity of the water might differ between layers and influence the level of oxygen in different parts of the cage. The occurrence and strength of the pycnocline is consequently important factors for the environmental quality of a site.

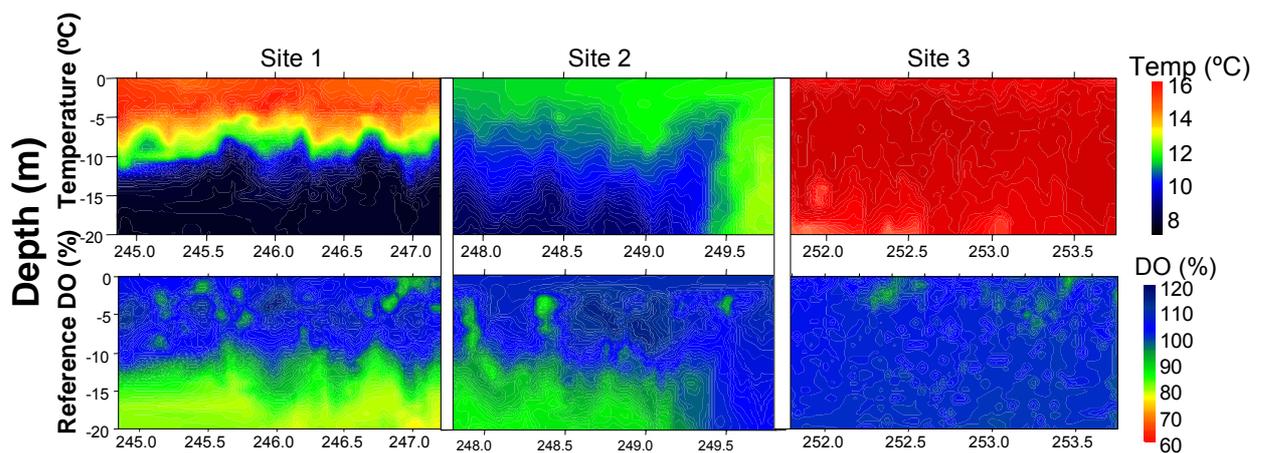


Figure 29 The pycnocline is an important factor for the quality of the cage environment. Isoplots of temperature and oxygen variation during a 50 h period at three commercial salmon sites.

In a 14-day survey of four commercial salmon farms on the west coast of Norway, it was clearly demonstrated that the salmon might be exposed to highly different physiochemical conditions within short distances in time and geographical position (Johansson *et al.* 2005 b). For example, in a farm located midway in a major fjord the pycnocline divided the cage into approximately equal layers of 16 °C at the top and 8 °C in the bottom part. In a second farm, located in a smaller fjord, with a smaller precipitation area and freshwater runoff, the pycnocline was closer to the surface and the brackish environment was maximum 10 % of the cage volume. At both sites, water flow and oxygen levels were related to tidal cycles. At a third site, located in the archipelago, the thermal and osmotic conditions were more uniform and not influenced by the tide. At a fourth site, located in the archipelago at the “mouth” of a large fjord, the pycnocline was broken down by changes in wind conditions, in effect changing the site from a “fjord-site” to a uniform “coastal site” in few hours. These highly variable environments at the different sites and with changes in time are likely to explain

some of the differences in performance (growth, feed conversion) and fish welfare observed in commercial salmon production through its influence on the behaviour and physiology of the fish (e.g. Claireaux *et al.* 1995; 2000).

11.3 Social environment, stocking density, and swimming behaviour

The social interactions of parr, presmolt and postsmolt Atlantic salmon are influenced by its social environment. It has been demonstrated that caged postsmolt salmon will initiate schooling behaviour (polarized swimming direction) at relatively low group sizes (Juell 1995). Caged salmon which are held in groups of tens or even hundreds of thousands individual fish, thus normally school in circular patterns during daytime (Sutterlin *et al.* 1979; Juell 1995; Oppedal *et al.* 2001). It is important to recognise that this is a fundamental shift in behavioural mode where the control of the behaviour is transferred from the individual to the group level (Toats 2004). As a consequence, aggressive interactions and the establishment of dominance hierarchies are highly unlikely and have not been reported in such groups. In contrast, aggression is easily induced between a pair of postsmolts held in a cage (Juell 1995). It has been suggested that caged salmon postsmolts keep up schooling throughout their seawater growth, contrary to its wild counterparts, as a behavioural adaptation to reduce the stress of the high density environment in commercial cages (Fernö *et al.* 1988; Juell 1995).

The stocking density in salmon cages is primarily a management and juridical measure used to regulate production and not based on considerations of fish welfare. For example, 25 kg per m³ has for several years been the upper legal limit in the Norwegian salmon industry, while 15 kg m³ is used in UK. Stocking density is simply calculated as the total biomass in the cage divided by the cage volume. However, stocking density bears little biological meaning in the cage context as it designates a situation where the fish is uniformly distributed in the cage volume (Fig. 30). Several studies have documented that the schooling behaviour of salmon induces considerably higher schooling densities as a response to e.g. natural (Huse & Holm 1993; Fernö *et al.* 1995) and artificial illumination (Oppedal *et al.* 2001; Juell *et al.* 2003; Juell & Fosseidengen 2004), motivational gradients (Juell *et al.* 1994; Fernö *et al.* 1995), and feeding regimes (Bjordal *et al.* 1993; Juell *et al.* 1994 b; Ang & Petrell 1998). Nonetheless, it is interesting to investigate if the stocking density *per se* influences the swimming behaviour and welfare.

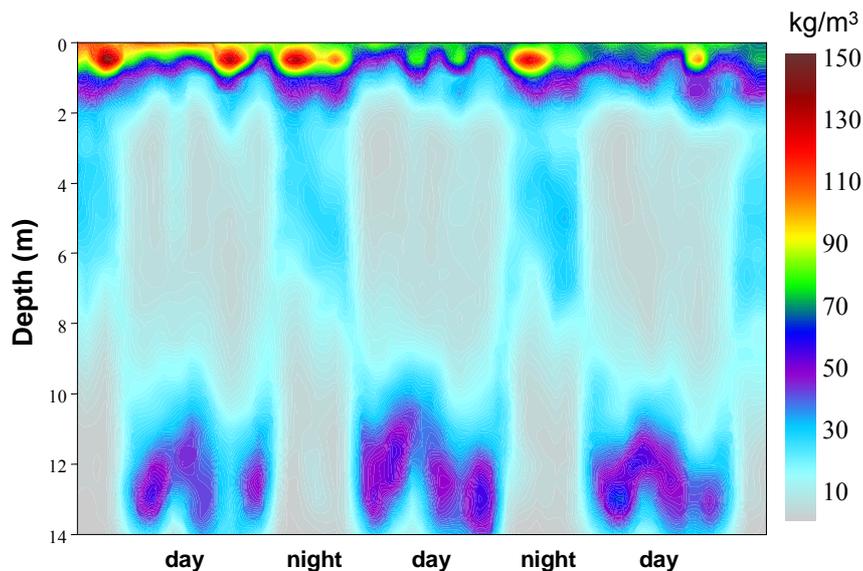


Figure 30 Stocking density is a poor predictor of the extent to which fish crowd. The mean observed fish density (kg m^{-3}) of triplicate cages with Atlantic salmon stocked at a density of 19 kg m^{-3} is shown over a three-day period in September.

In a study investigating swimming behaviour of caged salmon stocked at different densities in a stratified fjord environment, Johansson *et al.* (2005 a, b) used multivariate analysis (regression tree) to show that variation in swimming depth could be explained by temperature and light intensity preferences in connection with the pycnocline. The most preferred swimming depth was at temperatures in the range of 16-18 °C, and diurnal migrations related to shifts in light intensities were observed. As the preferred temperature was available only in a limited part of the cage, this induced a reinforcement of the effect of stocking density on schooling density. Competition for optimal thermal space crowded the fish resulting in both higher schooling densities and more fish exposed to suboptimal environmental conditions at high compared to normal density.

In conclusion, stocking density is a poor measure of schooling density of caged salmon and is therefore unlikely to be a reliable predictor of welfare effects normally associated with high animal densities in production systems (see also Ellis *et al.* 2002; Turnbull *et al.* 2005; Dawkins 2004 b).

11.4 Swimming and feeding behaviours in a uniform physiochemical environment and a natural photoperiod

The swimming behaviour of caged salmon is influenced both by motivational (e.g. hunger and stress level) and environmental factors. In a physiochemical uniform environment it has been suggested that the swimming depth and schooling density of groups of caged salmon can be explained as a trade-off between hunger level and surface avoidance (Juell *et al.* 1994; Fernö *et al.* 1995) with seasonal (Smith *et al.* 1993) and diurnal rhythms related to daylength, temperature and hunger level. Under such conditions and natural photoperiod, the salmon ascend to feed and descend away from the surface when hunger level is reduced. Feed availability (feed ration and feeding intensity) will therefore strongly influence the feeding and swimming behaviour while stressful husbandry operations, including e.g. reduction in

cage volume and depth, tend to reduce feeding motivation and can be observed as a reduced surface response (see Juell 1995 for a review). At dusk, the salmon ascend as a response to the fading light and reduces their swimming speed, while at dawn the school disperses with a subsequent descent and increase in swimming speed (Juell & Westerberg 1993; Huse & Holm 1993; Fernö *et al.* 1995).

11.5 Effect of behavioural thermoregulation and phototactic behaviour on swimming depth and schooling density

The use of photoperiod management by artificial lights to postpone the unwanted incidence of sexual maturation in sea water (Hansen *et al.* 1992; Porter *et al.* 1999; Hansen *et al.* 2000) (which cause problems both for the production efficiency and the welfare), overrides both the diurnal and seasonal patterns in swimming depth (Oppedal *et al.* 2001). Lamps mounted above water induce a strong vertical light intensity gradient and may induce crowding at the surface (Juell *et al.* 2003). Further, when the high number of fish in commercial cages is attracted towards the surface light this contributes to the attenuation of the light, and are likely to reinforce crowding. By the use of submersible lamps it is possible to create vertical light intensity gradients at predetermined depth intervals and actually control the swimming depth and schooling density in salmon cages (Juell & Fosseidengen 2004). This photopositive behaviour is interpreted as a behavioural adaptation to maintain schooling (which is primarily based on visual contact) when the daylight fades at dusk, instead of the fish relying on the slow process of dark adaptation in the salmon eye (Ali 1959; Guthrie 1986).

However, in thermally stratified waters as often found when there is a pycnocline (Fig. 29), the swimming depth and resulting schooling density are also strongly influenced by behavioural thermoregulation (Oppedal *et al.* 2001). Recent results from a study in the cage-environment laboratory at IMR, Matre demonstrated a behavioural trade-off between thermal preferences and light intensity levels sufficient to maintain schooling behaviour. When the vertical light intensity gradients were altered by positioning underwater lamps at different depths, the most frequently observed swimming depth at night were repeatedly modified by the temperature gradient. In daytime, swimming depth was strongly related to the depth of peak temperatures (Fig. 31). The strength of this behavioural thermoregulation was correlated to the range of available temperatures and resulted in a highly variable utilisation of the cage volume.

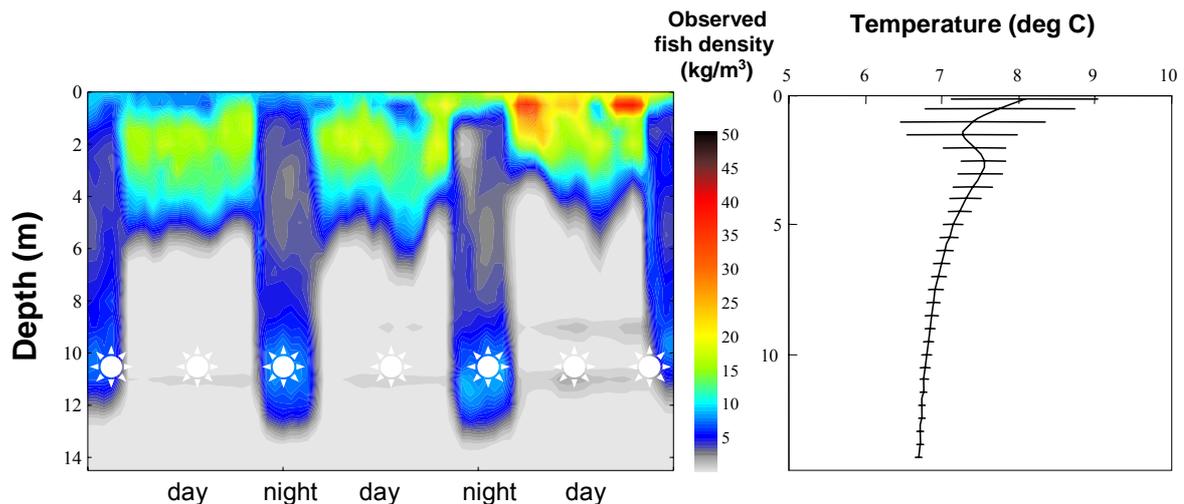


Figure 31 Variation in swimming depth and fish density of caged Atlantic salmon illustrates the behavioural trade-off between temperature preference and the need for sufficient schooling light. The cages were equipped with submersible lamps positioned at 10 m depth. The contours are the mean observed fish density (kg m^{-3}) for three days in triplicate cages stocked at 4 kg m^{-3} in late April and with the mean vertical temperature profile shown in the left panel.

11.6 Welfare of caged salmon

Salmon in floating cages is exposed to spatial and temporal variations in their environment. The farmer can partly influence the range of variability by site selection and choice of cage technology (volume, depth, and cage configuration). As salmon seems to rely on access to surface to inflate their swim bladder, submersible cages is not yet used to any extent in salmon farming, although technological developments to find solutions are underway. When site and technology have been chosen, the physiochemical cage environment is to a large extent subject to unpredictable natural variations. However, the farmer may influence the cage environment through management procedures either indirectly through stocking density, mesh size, and frequency of net change (oxygen saturation, see Johansson *et al.* 2005 a, b) or directly through feeding, photoperiod regimes (Juell *et al.* 2004), and active oxygenation (Bergheim *et al.* 2005).

This complex environment may influence the welfare of caged salmon in several ways. For example, temperature and oxygen conditions outside the tolerance range of salmon will induce both behavioural (avoidance and crowding) and physiological stress responses. The temporal aspects (frequency and duration) and severity (level) of such environmental stressors will determine if this results in short, acute stress responses with limited recovery costs or if it develops into a chronic state of stress with a series of negative consequences for the welfare of the animal (Bevelhimer & Bennet 2002), such as breakdown of primary barriers like skin, gills, and intestines (Olsen *et al.* 2005), as well as fin erosions, eye cataracts, and a compromised immune system.

Turnbull *et al.* (2005) constructed a multivariate welfare index based on a combination of physiological and morphological measures and found that the welfare of caged salmon was reduced above a breaking point of 22.1 kg m^{-3} . They could, however, not identify the mechanisms behind the observed reduction in welfare and suggested that limits on stocking density are probably not sufficient, or a particularly relevant measure, to secure welfare of

caged salmon. In a study performed in the CEL, where stocking densities of caged Atlantic salmon increased from 5.6 to 14.5 or from 15.7 to 32.1 kg m⁻³ from August to December we observed reduced performance recorded as a decrease in appetite, growth, condition, and increased feed conversion, eye cataracts (Fig. 32), fin erosion, body lesions, and mortality at stocking densities above 26.5 kg m⁻³. This study indicated that stocking density may contribute to reduced welfare, but again, the exact mechanisms behind remains unclear. For example, the prevalence of eye cataracts was within the levels observed on Irish and Scottish and Norwegian farms (Wall 1998; Ersdal *et al.* 2001). Such levels of eye cataract caused economic problems for growers due to impaired growth performance, blindness, secondary infectious diseases, and mortality (e.g. Wall 1998; Menzies *et al.* 2002). However, eye cataracts have been attributed to a number of farming conditions such as fluctuations in the water salinity, low temperatures (Hargis 1991), dissolved gas supersaturation, exposure to sunlight, temperature shocks, rapid fluctuations in water temperature (e.g. Bjerkaas *et al.* 2001), genetic factors (Wall & Richards 1992), nutritional factors (e.g. Waagbø *et al.* 2003; Breck 2004), and rapid growth and smoltification processes (Waagbø *et al.* 1996).

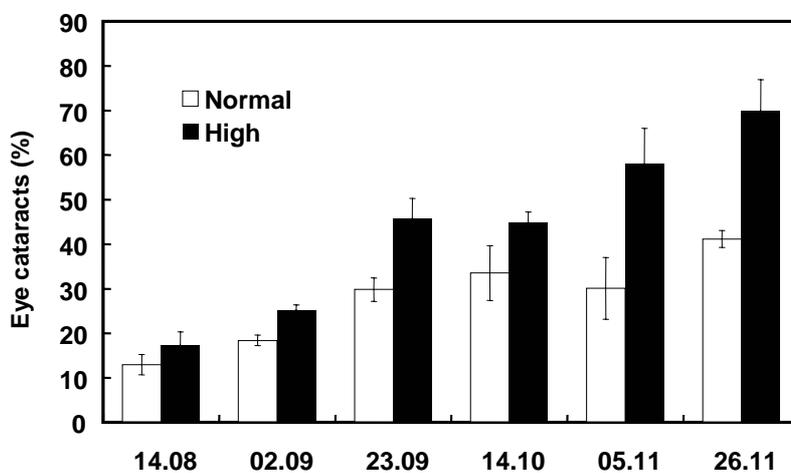


Figure 32 Increased levels of eye-cataracts in high-density cages may reflect competition for environmental resources.

In the present study the increased prevalence of cataracts at the higher stocking density may have been due to a combination of some of the above-mentioned causes, such as deteriorated water quality, fluctuations in water temperature, and possible forced swimming in the colder, less saline surface water. The gradual increase in the prevalence of cataracts in both groups in the early parts of the trial may be explained by hyperthermal conditions in August-September or exposure to high temperature variations in November-December when competition for thermal space was strong due to the high fish densities. This example serve to illustrate that both natural environmental variations and management practices as well as the behavioural responses may interact to compromise the welfare of caged salmon. With reference to the five freedoms of the Brambell committee (1965), one could argue that, at least for a large proportion of the group, (i) the freedom to express normal behaviour is violated by not allowing caged salmon to avoid an unsuitable environment by behavioural adaptations, (ii) the freedom from discomfort is violated due to the likely chronic stress effects in such environments, and (iii) the freedom from pain, injury and disease is violated due to the increase in prevalence of eye cataract, fin erosion, and mortality.

11.7 Practical implications

This new knowledge about the cage environment and behavioural responses of caged salmon should be implemented in future management practices and legislation framework for the salmon industry. For example, sufficient and reliable supply of oxygen to the fish is critical to efficient and sustainable aquaculture production. Oxygen fuels production and strongly influences growth, utilisation of feed, and fish welfare (Huguenin 1997; Chabot & Dutil 1999; Pichavant *et al.* 2001). Suboptimal levels of oxygen (hypoxia) will induce stress, which may lead to impaired welfare and, if severe, result in increased mortality. Protocols for oxygen management in cages, including site-specific assessment of production capacity and cage configuration, as well as oxygen monitoring, should be developed. Further, the knowledge about the behavioural thermoregulation and phototactic behaviour of caged Atlantic salmon should be utilised to avoid crowding and contribute to efficient light and feeding regimes. To achieve this there is a strong need to increase the environmental and behavioural monitoring in production cages and implement these data in management tools that can give early warning of deteriorating conditions compromising fish welfare.

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ANNEX

INTERNATIONAL PUBLICATIONS FROM THE PROGRAMME

- Aas-Hansen, Ø. & Damsgård, B. Ventilatory responses to water quality manipulation in cod (*Gadus morhua*). (MS unpubl.)
- Arnesen, K.R., Toften, H. & Killie, J.E. Effects of hypoxia on growth, health and risk of vibriosis in juvenile Atlantic cod. (MS unpubl.)
- Bjørklund, F., Toften, H., Johnsen, H.K., Damsgård, B. Effects of high density and low specific water flow on physiology and welfare of juvenile Atlantic cod. (MS unpubl.)
- Damsgård, B., Sørum, U., Uglestad, I., Eliassen, R. & Mortensen, A., 2004. Effects of feeding regime on susceptibility of Atlantic salmon (*Salmo salar* L.) against cold water vibriosis. *Aquaculture*, 239: 37-46.
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- Eriksen, M.S., Takle, H., Espmark, Å., Braastad, B.O., Salte, R. & Bakken, M. Pre-spawning stress in farmed Atlantic salmon (*Salmo salar*): Maternal cortisol exposure and hyperthermia during embryonic development influence offspring HSP70 expression. *Aquaculture* (MS submitted).
- Eriksen, M.S., Espmark, Å., Braastad, B.O., Salte, R. & Bakken, M. Long-term effects of maternal cortisol exposure and mild hyperthermia during embryogeny on survival, growth and morphological abnormalities in farmed Atlantic salmon (*Salmo salar*) offspring. *Aquaculture* (MS submitted)
- Eriksen, M.S., Espmark, Å., Poppe, T., Braastad, B.O., Salte, R. & Bakken, M. Developmental instability in farmed Atlantic salmon: Also a matter of the maternal environment? (MS unpubl.)
- Eriksen, M.S., Bolin, T., Damsgård, B., Braastad, B.O. & Bakken, M. Behavioural responses of Arctic charr (*Salvelinus alpinus*) subjected to multiple stressors. (MS unpubl.)
- Espmark, Å.M., Eriksen, M.S., Salte, R., Braastad, B.O. & Bakken, M. Impacts of prenatal stress on behaviour in juvenile farmed Atlantic salmon. (MS unpubl.)
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- Handeland, S., Stefansson, S.O., Kristiansen, T., Rosseland, B.O. & Toften, H. Effects of high density on freshwater and seawater performance of Atlantic salmon. (MS unpubl.)
- Hansen, L.A. & Damsgård, B. Dynamics of dominance: Winners effect in the salmonid Arctic charr. (MS unpubl.)

- Hansen, L.A., Johnsen, H.K. & Damsgård, B. Individual variation in social behaviour and reproductive hormones in mature and immature Arctic charr (*Salvelinus alpinus*) during a spawning season. (MS unpubl.)
- Hansen, L.A., Damsgård, B. & Delghandi, M. Reproductive behaviour and mating success in captive Atlantic cod. (MS unpubl.)
- Hansen, L.A., Damsgård, B. & Delghandi, M. Reproduction behaviour in mixed groups of migratory and non-migratory Atlantic cod. (MS unpubl.)
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- Siikavuopio, S.I. & Sæther, B.-S. Effects of chronic nitrite exposure on growth in juvenile Atlantic cod, *Gadus morhua*. Aquaculture. (In press)
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- Sæther, B.-S., Arnesen, A.M. & Damsgård, B. Temperature selection in Atlantic cod in an experimental preference system. (MS unpubl.)
- Sæther, B.-S., Damsgård, B., Bolin, T., Øverli, Ø. & Jobling, M. Aggression in Arctic charr fed at different rations: effects on plasma cortisol, feed intake and growth. (MS unpubl.)
- Sæther, B.-S., Siikavuopio, S.I. & Damsgård, B. Effects of ammonia exposure on feed intake and growth in Arctic charr. (MS unpubl.)
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- Skajaa, K., Mortensen, A. & Toften, H. Effects of supersaturated water on growth and survival of juvenile Atlantic cod (*Gadus morhua* L.). (MS unpubl.)
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