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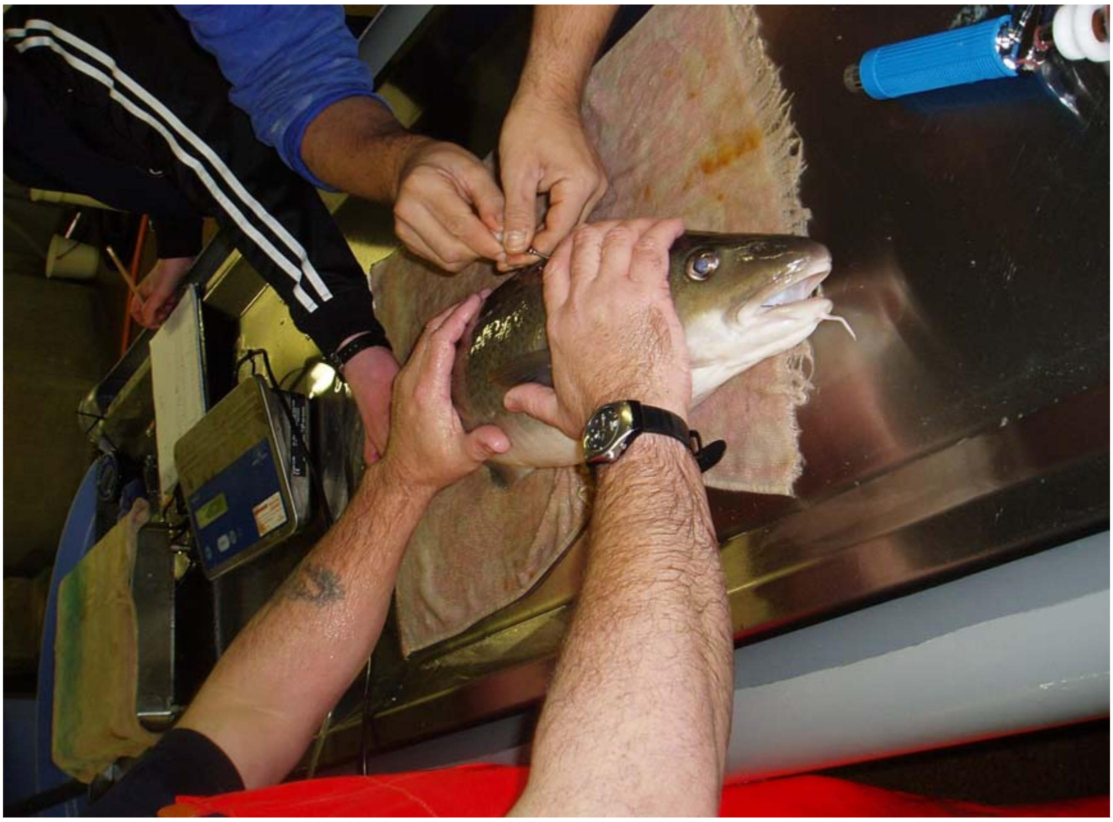
"Farmed fish and welfare"



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Foreword:

This report presents a general introduction and overview of aquaculture welfare issues, involved bodies, legislation and related EU funded research projects

A short overview over institutions working on issues related to welfare in farmed fish:

The World Organisation for Animal Welfare (OIE) (166 member countries). Main priority of the OIE involvement in animal welfare is the work on issues concerning animals used in agriculture and aquaculture for production, breeding and working purposes, focusing on transportation, humane slaughter and killing for disease control, and later on housing and management.

An *ad hoc* group dealing with aquaculture is now being established and will start working autumn 2004.

The Council of Europe. The Standing Committee for the protection of animals kept for farming purposes of the Council of Europe is currently working on a recommendation concerning farmed fish. Main issues which are intended to be covered by the recommendation are stockmanship and inspection, enclosures, buildings and equipment, management and emergency killing.

The European Food Safety Authority (EFSA). One of the scientific panels within EFSA is the Panel on Animal Health and Welfare (AHAW), and its work is co-ordinated by EFSA´s Scientific Committee. AHAW published two documents on welfare issues which do also concern farmed fish:

1. Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to the welfare of animals during transport, adopted on 30 March 2004
2. Scientific report on “welfare aspects of animal stunning and killing methods”, adopted on 15 June 2004

DG Health and Consumer Protection (Sanco) is responsible for food safety issues covering the whole production chain, including animal health and welfare.

DG Fisheries is the Directorate-General responsible for the Common Fisheries Policy (CFP), which covers all fishing activities, the farming of living aquatic resources, and their processing and marketing. The DG is also responsible for running a scientific research programme for fisheries as part of the Community's framework research programme. Together with DG Research, DG Fisheries is funding research projects on welfare related topics which should provide a scientific basis for policy making.

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Societal and political background

Traditionally, the welfare of fish compared to welfare of other, land farmed animals has not been an important topic to consumers, producers and legislators, an attitude which is reflected in past research projects and legislation directed towards welfare, which hardly ever took fish into consideration. So why was and is fish, compared to other animals, not a hot topic regarding welfare concerns? First, there is a lack of tradition in perceiving fish as sentient beings as fish do not evoke compassion and concern in humans in the same way other, warm-blooded animals do; second, there is still no consensus among scientists wether fish are able to perceive pain and to suffer; third, large scale, industrialised aquaculture is a relatively recent farming method.

Nevertheless, an increased concern for the welfare of fish in general and especially in aquaculture can be noticed in recent year, stimulated by research results suggesting awareness of pain and suffering, and reports on farming conditions detrimental to health and welfare.
A Norwegian study done by the National Institute for Consumer Research in 2002 reveal that only 49% of the consumers considered welfare of aquacultured fish to be acceptable. About 40% pinpointed that they avoided buying certain fish types because they are sceptical regarding what the flesh might contain of medicines and feed additives.

Welfare – general introduction

Traditional definitions of welfare often have either a feeling-, nature- or function-based approach or seek to combine those. Agreement on how to correctly and best define welfare has shown to be difficult. This difficulty has its origin in a disagreement regarding which the essential and most important parameters for good welfare are.

The function-based approach analyses the animals coping with the environment, or, as Broom quotes, "the welfare of an animal is its state as regards its attempts to cope with its environment". This definition is based on the assumption that the animal tries to maintain homeostasis, equilibrium, in its physiological system. The function-based definition assumes that a healthy body is an indicator for good mental health, and important welfare indicators are physiological parameters like stresshormones, health situation, reproduction and growth.

The feeling-based approach has the subjective, mental state of the animal in mind, and feelings like fear, pain and suffering play a major role for good welfare. Physiological and behavioural parameters are used when there is no possibility to assess the emotional components directly.

The nature-based definition of good welfare requires that the animal is given the possibility to perform normal and natural behaviour. The animal is viewed in a holistic way, and this welfare approach is important in for example ecological farming.

In order to secure welfare, the Brambell Committee Report postulated in 1965 the "five freedoms":

1. Freedom from thirst, hunger and malnutrition by ready access to fresh, clean water and adequate, nutritious food to maintain full health and vigor. (applied to fish, this means that fish should be provided with an adequate and nutritionally complete diet according to the species and age specific demands. Food deprivation before slaughter and transport should be a short as possible and adapted to the species and age)
2. Freedom from discomfort by providing an environment suitable to their species, including adequate shelter and a comfortable resting area. (applied for fish this means species adequate water quality parameters, flow rates and temperatures, appropriate light intensities and other needs)
3. Freedom from pain, injury and disease by prevention, rapid diagnosis and treatment. (applied to fish this means to prevent injuries through careful and gentle procedures, prevent infections and diseases through good sanitary conditions and eventually vaccination, avoid malformations)
4. Freedom to express normal behaviour by the providing sufficient space, proper facilities and company of the animal's own kind.
5. Freedom from fear and distress by ensuring living conditions which avoid mental suffering (applied to fish this means ensuring gentle and adequate handling procedures, stunning procedures before slaughter which render immediate insensitivity to the animals, human slaughter methods)

The five freedoms, originally postulated for land farmed animals, can and should be applied to aquaculture fish.

Pain and discomfort in fish

The question of pain awareness is of huge significance for the public concern about animal welfare. There is still no scientific agreement regarding the question whether fish are, or not, capable of perceiving pain.

Pain in humans is defined by the International Association for the Study of Pain (1979) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage", and further " activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state". Several researchers state that fish are unable to feel pain in a similar way to pain experienced by humans, arguing that there is no awareness of pain without consciousness, and the capacity of consciousness depends on functions of the neocortex. The neocortex is absent in fish.

Because of the difficulty in assessing emotions in animals, emotions and consciousness do not feature in the definition of pain in animals and the assessment of pain is strongly attached to behavioural and physiological parameters.

After reviewing the central and peripheral nervous system, neurotransmitters, as well as physiological and behavioural responses of fish, several new publications do conclude that it is highly probable that fish have the necessary requirements in order to perceive pain and to suffer. This position is also adopted by the European Food Safety Authority, which in its scientific report regarding stunning and slaughtering (2004) points out that there is enough evidence indicating that fish is capable of feeling pain and suffering, and this should be taken into consideration when talking about fish welfare.

Welfare indicators

The choice of indicators is dependant on the level and type of information required.

Physiological indicators:
Many husbandry activities in aquaculture, like handling of fish, transport and vaccination, lead to stress responses in the organism which, if chronic or intense, eventually can lead to welfare impairment. Stress responses can be divided into primary, secondary and tertiary responses. Primary responses to stressors are the release of catecholamines and corticosteroids. Secondary responses are characterised as the immediate reactions of blood and tissues to those hormones. Finally, the tertiary responses are determined as being long term responses influencing growth, reproduction and immune responses.
Hormone and glucose level in the blood can be measured and used as indicators for the animal´s stresslevel.

Behavioural indicators:
Impaired welfare as well as good welfare and environmental changes reflect in the behaviour of fish and can among others cause visible changes in colour, breathing frequency, social and swimming behaviour and feeding. Behavioural measures are therefore sensitive indicators of the biochemical and physiological changes occurring in response to stress. As altered behaviour is an easily observed response, giving immediate information without the need to use invasive methods, specific behavioural responses can be used as indicators for stressful and/or welfare-threatening situations.

Other indicators:
Further indicators for welfare are general condition of the fish (scale loss, damaged fins, etc), growth, reproduction, and health.

Welfare issues

There are several aquaculture conditions which may have an influence on animal welfare, including:

Fish densities in farming

Increasing stocking densities compared to the natural densities of fish species means that the number and the type of fish interactions increase. The naturally evolved behaviours are adaptive and reflect the most adequate way to react in order to cope with the environment in an efficient way, which implies that increased stocking densities normally affect this balance. The biology of the cultivated fish species is complex, to identify adequate stocking densities is therefore difficult and must take age, size, environmental conditions and biological characteristics of the species into consideration.

Environmental parameters

Fish are in constant interaction with their environment through the surface of the gills and the skin, therefore water quality (oxygen, ammonia, pH) and the level of present contaminants are crucial for welfare. Water quality should be constantly and carefully monitored and reflect the needs of each species and developmental stage.

Malnutrition

It is important to notice, that feed formulations can have both negative and positive effects on health and welfare; diseases originated from missing micronutrients being an example for a negative effect, the improvement of the immune system through the oral administration of glucan a positive one.

Most feed formulations contain high proportions of marine fish meal and oil in order to match the fatty acid and amino acid needs of the cultured fish. The main reasons are the high demand for protein, and secondly the high demand of polyunsaturated (PUFA) fatty acids. Inadequate levels of fatty acids have a negative impact on the immune function and can lead to a decrease in antibody production.

The major challenges regarding the vegetable alternatives are the different content and composition of FA and AA, and the presence of so-called anti-nutrients. Anti-nutrients represent a welfare problem in themselves, as they can lead not only to reduced absorption and digestion, but also to diseases in the intestinal systeme. In addition, the oil content of the vegetable alternatives is lower and the carbohydrate content poorly utilised by fish.

Food deprivation

Food deprivation is used in order to adapt production to the market demand, before transport to reduce metabolic rate and oxygen consumption and production of waste products, and before slaughter aiming to eliminate gut contents and reduce contamination risks.

Important to notice is here that the natural behaviour of reduced feeding is temperature-, age-, species and season dependent, implicating that depriving fish from food during non-natural periods might lead to reduced welfare.

Cataracts

The incidence of cataract (“grey star”) in intensive culture of Atlantic salmon has increased significantly, and also sea bass and sea bream have been shown to be affected by cataract. Behavioural changes and reduced growth due to difficulties in feeding are direct consequences of cataract.

Several factors are known to be related to cataract formation. Among these are environmental conditions (ex.: temperature), nutrition (ex.: deficiency in histidin, methionin, zinc), toxins, genetic predisposition and parasites.

Deformities (skeletal and tissue deformities)

Skeletal deformities represent not only an ethical and welfare issue but have a negative impact on economy and quality as well, due to growth impairment in the affected fish, elevated mortality, reduced fillet quality and smolt which cannot be sold. Skeletal deformities include spinal cord, head, mouth and opercular deformations, and affect most of the farmed species.

Factors responsible for the induction of malformations are genetic disposition, malnutrition (especially during fast-growth periods) and environmental parameters (temperature, oxygen content etc.).

During the last years, an increasing number of apparently healthy, large fish showed problems in coping with normal aquaculture procedures like transport, handling and grading due to tissue deformities like abnormally shaped hearts. A combination of sedentary lifestyle and breeding programs that do not take organ functioning into account could be the origin for the increasing amount of anomalies.

Vaccination – side effects

Vaccination programs have contributed significantly to reduce the losses due to disease outbreaks in fish farming, and represent in addition the major factor for the enormous reduction in antibiotic used in aquaculture. Vaccination, and especially intraperitonial vaccination with oil adjuvant vaccines, may be hampered by severe side-effects. Beside the loss of appetite, more severe side effects of oil-adjuvant vaccines are tissue adhesions of variable degree around the injection site, pigmentation and granulomata. Severe lesions can disturb and interfere with the normal function of the affected organs, resulting in reduced growth and impairing animal welfare.

Transport

Farming fish often includes transport of living fish, normally at high densities, with loading, capture, netting and pumping procedures involved. During transport inadequate water exchange depleting the oxygen content and accumulating carbon dioxid and ammonia may further contribute to stress reactions and impaired welfare.

Stunning/sedation

From an animal welfare point of view any method of slaughter should incorporate a stunning procedure that renders the animal immediately insensitive and unconscious until death. Many of the currently used slaughter methods are inadequate from a welfare perspective.

Table 1: Stunning (stun) and killing (kill) methods that are used for slaughter in the most common European farmed fish species. Not determined (n.d.) and not appropriated (n.a.) methods indicated as well (after Roth 2003).

	Stunning and killing method							
Fish species	Asphyxia	Thermal shock	Salt	Aqueous solution	Exsanguination	CO2	Electric	Percussion
Northern Europe								
Atlantic Salmon (*Salmo salar*)	Kill	Stun	n.a.	n.a.	Kill	Stun	Stun	Kill
Rainbow trout (*Oncorhynchus mykiss*)	Kill	Stun	n.a.	n.a.	Kill	Stun	Stun	Kill
Halibut (*Hippoglossus hipoglossus*)	n.d.	Stun	n.a.	n.a.	Kill	Stun	n.d.	n.d.
Eel (*Anguilla anguilla*)	n.a.	Stun	Kill	Kill	n.a.	n.a.	Kill	n.a.
Southern Europe								
Gilthead Seabream (*Sparus aurata*)	Kill	Stun/Kill	n.a.	n.a.	n.a.	n.a.	Stun	n.d.
Seabass (*Dicentrarchus labrax*)	Kill	Kill	n.a.	n.a.	n.a.	n.a.	Stun	n.d.
Commom carp (*Cyprinus carpio*)	Kill	n.d.	n.a.	n.a.	Kill	n.a.	Stun	Kill
Turbot (*Scophtalmus maximus*)	Kill	Stun/Kill	n.a.	n.a.	Kill	n.a.	Kill	n.d.

Past and current EC research projects on welfare issues

Research towards animal welfare has been funded and supported by the European Commission since 1994, with the start of the Fourth Framework Program (FP4). In

FP4, research on welfare was part of the Agriculture and Fisheries (FAIR) Programme. In FP5, under Key action 5 (Sustainable agriculture, fisheries and forestry) of the Quality of Life programme, funding of welfare related research projects continued. Under FP6, animal welfare is addressed mainly within Priority Area 5, Food Quality and Safety, and within the Policy oriented research. Research projects have been addressing relevant issues like cataracts, organic production, slaughter methods, stress, nutrition and breeding.

Report

1 Societal and political background

Traditionally, the welfare of fish compared to welfare of other, land farmed animals has not been an important topic to consumers, producers and legislators, an attitude which is reflected in past research projects and legislation directed towards welfare, which hardly ever took fish into consideration. Despite this fact, as will be shown later in the report, there are plenty of conditions in modern aquaculture which can and do impair welfare. So why was and is fish, compared to other animals, not a hot topic regarding welfare concerns?

One major reason is that fish do not evoke compassion and concern in humans in the same way other, warm-blooded animals do. Compared to calves and lambs for example, fish do not give loud when they are anxious or in pain, they have no facial expression, live in an aquatic environment which is unfamiliar to humans, and they rarely show behavioural reactions that are similar to our own. Fish are, in other words, seldom perceived as sentient individuals.

In addition, there is still no consensus achieved among scientists whether fish are able to perceive pain, or not, as the fish brain does not have those regions which are responsible for pain perception in human beings and other mammals (a short review on pain perception in fish is given later in this report). A third reason might be that large scale, industrialised aquaculture is a relatively recent farming method. Nevertheless, an increased concern for the welfare of fish in general and especially in aquaculture can be noticed in recent year, as will be illustrated below, stimulated by research results suggesting awareness of pain and suffering, and reports on farming conditions detrimental to health and welfare.

The Treaty of Amsterdam, adopted in 1997, classifies animals as sentient beings, and states that “in formulating and implementing the Community´s agriculture, transport, internal market and research policies, the Community and the Member States shall pay full regard to welfare requirements of animals…”.

Eight years later the new Constitution, which was adopted in June 2004, contains a re-worded article on the protection and welfare of animals. The word “fisheries” is here added, the text now being “in formulating and implementing the Union´s agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall pay full regard to the welfare requirements of animals, as sentient beings…”.

The recent re-rewording of the welfare article, now including fisheries, illustrates the increased focus on welfare issues in fisheries by European legislation.

A study finalised in 2001 on consumer concerns about animal welfare and the impact on food choice (EU FAIR CT98-3678), shows that consumers use animal welfare as an indicator of other products attributes, like food safety, quality and healthiness, equating thereby good welfare standards with good food standards. Although this study was only performed for land farmed animals, a Norwegian study done by the National Institute for Consumer Research in 2002, which also includes fish, shows similar results.
The Norwegian study revealed that only 49% of the consumers considered welfare of aquacultured fish to be acceptable. In comparison, the welfare of cattle and pigs was considered acceptable by respectively 73% and 79% of the consumers. Compared to fish, only poultry was considered by more consumers (73%) to be farmed under non-acceptable welfare conditions. About 40% pinpointed that they avoided buying certain fish types because they are sceptical regarding what the flesh might contain of medicines and feed additives. At the same time, when presented with the question if 2, 20 or 40% of farmed salmon was treated with antibiotics, only 17% of the consumers knew the correct answer (2%), showing that mal-information of the public can inflict with the perception of welfare.

2 Welfare – general introduction

Traditional definitions of welfare often have either a feeling-, nature- or function-based approach (FSBI 2000, Fraser 2004, Duncan 2004), or seek to combine those. Agreement on how to correctly and best define welfare has shown to be difficult. This difficulty has its origin in a disagreement regarding which the essential and most important parameters for good welfare are. Human perception of welfare and ethics play influence welfare definitions.

The function-based approach analyses the animals coping with the environment, or, as Broom (1986) quotes, “the welfare of an animal is its state as regards its attempts to cope with its environment”. This definition is based on the assumption that the animal tries to maintain homeostasis, equilibrium, in its physiological system. As an example, the animal can have the need to increase the uptake of liquid, or to get away from a predator, and can, through behavioural and physiological responses, react accordingly. Feelings (hunger, fear) and natural behaviour can be seen as methods or strategies to secure homeostasis, developed through evolution. The function-based definition assumes that a healthy body is an indicator for good mental health, and important welfare indicators are physiological parameters like stresshormones, health situation, reproduction and growth. Regarding reproduction and growth one considers especially bad reproduction and reduced growth as indicators for impaired welfare, as reproduction and growth can be maintained at a high level even if the animal is suffering under impaired welfare (ex.: chicken can grow fast even if they are not able to growth because of bone problems).

The feeling-based approach has the subjective, mental state of the animal in mind, and feelings like fear, pain and suffering play a major role for good welfare. Physiological and behavioural parameters are used when there is no possibility to assess the emotional components directly.

The nature-based definition of good welfare requires that the animal is given the possibility to perform normal and natural behaviour. The animal is viewed in a holistic way, and this welfare approach is important in for example ecological farming.

Summarising: the definition of welfare is complex and should comprise the animals behavioural and physiological needs, its physical health, production, reproduction and emotions. Dawkins (1988) claims that both presence of a negative stimulus, but also absence of an essential stimulus can reduce welfare, and recently increased there has been increased focus on the characterisation and application of positive welfare indicators. The absence of negative indicators does not give sufficient on the animals welfare status. With other words, good fish welfare is much more than only secure health and good waterquality.

Dawkins (2004) summarises different aspects of welfare by putting through the proposal that one should ask two questions in order to describe the welfare of an animal: “is the animal healthy”? and “does the animal get what it wants?”. These two questions comprise both the physical/functional and the mental component of welfare.

Even if one should be aware of the fact that ethical considerations influence welfare research, there is a difference between an objective scientific description of positive and negative welfare parameters, and the ethical evaluation of what is acceptable welfare. The ethical evaluation varies between people, societies and in a historical context. And it is also dependent on how the animal is perceived: does it feel pain? Is it the animal able to suffer? If the animal is not able to perceive pain and to suffer, why should it then be important to secure good welfare? Those thoughts, combined with a traditional lack of perceiving fish as sentient beings, have lead to the fact that welfare in aquaculture has received little attention when compared to other farmed animals.

In order to secure welfare, the Brambell Committee Report postulated in 1965 the "five freedoms":

1. Freedom from thirst, hunger and malnutrition by ready access to fresh, clean water and adequate, nutritious food to maintain full health and vigor.
   (applied to fish, this means that fish should be provided with an adequate and nutritionally complete diet according to the species and age specific demands. Food deprivation before slaughter and transport should be a short as possible and adapted to the species and age)
2. Freedom from discomfort by providing an environment suitable to their species, including adequate shelter and a comfortable resting area.
   (applied for fish this means species adequate water quality parameters, flow rates and temperatures, appropriate light intensities and other needs)
3. Freedom from pain, injury and disease by prevention, rapid diagnosis and treatment.
   (applied to fish this means to prevent injuries through careful and gentle procedures, prevent infections and diseases through good sanitary conditions and eventually vaccination, avoid malformations)
4. Freedom to express normal behaviour by the providing sufficient space, proper facilities and company of the animal's own kind.

5. Freedom from fear and distress by ensuring living conditions which avoid mental suffering
   (applied to fish this means ensuring gentle and adequate handling procedures, stunning procedures before slaughter which render immediate insensitivity to the animals, human slaughter methods)

The five freedoms, originally postulated for land farmed animals, can and should be applied to aquaculture fish.

3 Pain and discomfort in fish

The question of pain awareness is of huge significance for the public concern about animal welfare. There is still no scientific agreement regarding the question whether fish are, or not, capable of perceiving pain. A short summary of the state of the art regarding scientific knowledge on pain perception in fish follows.

In order to investigate if an animal is capable of perceiving pain, one has to show that the animal senses the potentially painful stimulus and that it reacts physiologically and behaviourally adversely to the stimulus. When discussing pain, one has to emphasise on the difference between nociception and pain, nociception being the activity induced in a nociceptor and the following nociceptive pathways. One crucial issue is to differentiate between a behavioural reflex (a defensive action for which no perception of pain is necessary) and a more complex action resulting from a stimulus perceived as painful. Nociceptive responses might be merely reflectoric reactions, and vice versa, reflectoric reactions do not necessarily implicate the presence of pain.

Pain in humans is defined by the International Association for the Study of Pain (1979) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage", and further " activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state". Rose (2002) states that fish are unable to feel pain in a similar way to pain experienced by humans, arguing that there is no awareness of pain without consciousness, and the capacity of consciousness depends on functions of the neocortex. The neocortex is absent in fish and no other, analogous brain structures is in Rose's opinion identified. Rose claims that pain is not possible if the neural activity associated with nociception does not reach the level of consciousness.

Because of the difficulty in assessing emotions in animals, emotions and consciousness do not feature in the definition of pain in animals and the assessment of pain is strongly attached to behavioural and physiological parameters (Bateson 1991; Morton & Griffiths 1985).

After reviewing the central and peripheral nervous system, neurotransmitters, as well as physiological and behavioural responses of fish, several new publications do conclude that it is highly probable that fish have the necessary requirements in order to perceive pain and to suffer (Braithwaite and Huntingford 2004, Chandroo et al. 2004, Gregory 1999, Klausewitz 2003, Sohlberg et al. 2004).

Anatomical requirements:
Nociceptors have been found for a variety of fish species, for example Sneddon et al. (2003) found several A delta and C nociceptors in trout, receptors which react exclusively to potentially painful stimuli, as pressure, temperature and chemical substances. These findings where accompanied by experiments in which behaviour after administration of a noxious substance was observed. The administration led to both strong physiological- and complex, non-stereotype behavioural reactions. In addition it was found that the administration of morphin (a substance which in humans reduces pain perception) reduced the adverse reactions.
The absence of complex, neocortex-like brain structures does not imply that the animal is unable to experience discomfort. Several authors claim that the, in fish highly developed, telencephalon might well have developed analogous functions to the mammalian neocortex (Verheijen & Flight, 1997). Furthermore, Børresen (2000) claims that the neocortex does not self "construct" feelings, but only modulates, moderates or enhances feelings arising in the more ancient parts of the brain.

Physiological requirements:
Neuropeptides and neurotransmitters known from other vertebrates are also present in fish, and these are of importance for the transmission of nociceptive signals. Among others, substance P, adrenalin, achetylcholin and dopamin have been found in fish. Other neuropeptides and endorphin, in mammalians responsible for painmodulation, have been found.

Behavioural requirements:
Teleost fish show complex behaviour and various experiments indicate that several fish species are in capable of learning and memory. This indicates a primary consciousness and a potential capacity to perform conscious, cognitive actions.

Summarising, there is much evidence suggesting that fish are capable of perceiving pain. This position is also adopted by the European Food Safety Authority, which in its scientific report regarding stunning and slaughtering (2004) points out that there is enough evidence indicating that fish is capable of feeling pain and suffering, and this should be taken into consideration when talking about fish welfare.

4 Welfare indicators

The characterisation of adequate welfare indicators is crucial in order to assess and evaluate the welfare of fish in experiments and "on farm". The choice of indicators is dependant on the level and type of information required, some indicators might be suitable for small-scale laboratory experiments, but there is a special need to describe operational on-farm indicators which are ease to observe and can give immediate information regarding the welfare of the animals.

4.1 Physiological indicators

Many husbandry activities in aquaculture, like handling of fish, transport and vaccination, lead to stress responses in the organism which, if chronic or intense, eventually can lead to welfare impairment. Stress can be defined as a state in which the equilibrium of an animal, the so-called homeostasis is either threatened or disturbed by an internal or external factor, the stressor. As a consequence of the exposal to a stressor, physiological and eventually behavioural responses are initiated, aiming to re-establish the equilibrium or the resting state. In this sense, a stress

response is a state during which the animal copes with a stressor by readjusting its biological activities (Bonga, 1997). Important stressors are animal interactions, aquaculture practices (handling, crowding, transport etc.) and environmental factors (temperature, water quality, pH, chemicals etc.).
According to this definition, stress is not necessarily to be considered as detrimental, but is merely a form of adaptive response. However, stress may loose its original adaptive value and turn detrimental and dysfunctional when becoming chronic or continuous (Bonga, 1997; Barton 1997). It is therefore important to differentiate between "eustress", which may even be considered as beneficial and stimulating, and "distress", a response to more threatening challenges which may lead to a pathological state (Bonga, 1997).
It is important to have in mind that the magnitude and the type of response can vary between species, and even on a strain and individual level (Pickering and Pottinger, 1997), and do also depend on the general condition and developmental state of the fish. As an example, it is known that salmon is especially sensitive to stressors during the smoltification period – this period is often also a period where divers handling procedures occur, transport and transfer to sea water, exposal to a new environment etc.

Stress responses can be divided into primary, secondary and tertiary responses. Primary responses to stressors are the release of catecholamines and corticosteroids. Secondary responses are characterised as the immediate reactions of blood and tissues to those hormones. Finally, the tertiary responses are determined as being long term responses influencing growth, reproduction and immune responses.

Hormone and glucose level in the blood can be measured and used as indicators for the animal's stress level. The disadvantage of using these indicators is that blood sampling itself stresses the animal and may influence and mask the results, in addition the method is labour intensive and rather expensive. Large variation between individuals and strains can make the interpretation of the results difficult. Alternative and indirect ways like to measure for example cortisol content in the water are possible, but also these methods are more suitable for small-scale experiments and not for on-farm measurement of welfare.

A short review of the main stress responses in fish:
The hypothalamic-sympathetic-chromaffin cell axis regulates the catecholamine level in the organism. Catecholamine (epinephrine and norepinephrine) levels normally increase within a couple of minutes as a response to a severe stressor, being mainly released from the chromafin cells in the head kidneys.
As a result of the elevated level of catecholamines, the oxygen uptake rate through the gills increases and the affinity of haemoglobin for oxygen is increased resulting in improved oxygen transport properties. In addition, catecholamine enhances the release of glucose from the liver, inducing a stress-related hyperglycemia in the blood and reducing the amount of glycogen in the liver.
Furthermore, the stress response itself may be impaired through stressors. As an example, the CA release or production maybe affected by pollutions/toxic elements. Chronic stress may desensitize the CA regulatory and target cells through downregulating their CA receptors (Bonga, 1997; Gilmour et al. 1994), which means that chronically stressed fish may have problems in reacting adequately to a sudden and acute threat.

The hypothalamic-pituitary-interrenal axis is in fish, as in general for vertebrates, responsible for the regulation of cortisol. Cortisol is the end product of this axis, its production occurs in the interrenal cells of the head kidney and is induced by the adrenocorticotropic hormone (ACTH) and corticotropin-releasing hormone (CRH). A 10-100 fold increase of cortisol can usually be detected after a stressful experience within a couple of minutes.
The main target organs of cortisol are liver, intestine and gills, and the major influence of cortisol is on the mineral balance and energetic metabolism. Cortisol plays an important role for the regulation of the uptake/extrusion of Na+ and Cl-, and is therefore essential to unable fish to live in a hypo- or hyperosmotic environment. It is known that cortisol stimulates the gluconeogenesis, a fact which possibly contributes to the weight loss occurring during chronic stress.
When using cortisol to measure stress responses it is important to note that natural variants of cortisol levels occur through the day, season and life cycle of the fishes; a high base line of cortisol must therefore not necessarily indicate chronic stress.

Apart from catecholamine and cortisol, neurotransmitters, neuroendocrine peptides and several hormones are involved in the stress response.
A result of a stress response is often the reallocation of energy resources from growth and reproduction to activities necessary to restore homeostasis, like respiration and locomotion, resulting in reduced growth, lower food conversion rates etc.

4.2 Behavioural indicators

Impaired welfare as well as good welfare and environmental changes reflect in the behaviour of fish and can among others cause visible changes in colour, breathing frequency, social and swimming behaviour and feeding. Behavioural measures are therefore sensitive indicators of the biochemical and physiological changes occurring in response to stress. As altered behaviour is an easily observed response, giving immediate information without the need to use invasive methods, specific behavioural responses can be used as indicators for stressful and/or welfare-threatening situations. Fish in aquaculture can be observed on an individual level (using transmitters) or on a group level.
Another important aspect of behavioural observation is the recent focus on not only negative but also positive welfare indicators. Using choice tests to allow fish to choose and thereby express their preference (for example allowing them to choose between water of different temperatures) is a commonly used approach for the investigation of positive welfare indicators.
Summarising, knowledge of the behaviour of each species and use of specific behavioural welfare indicators are important tools for the monitoring and improvement of aquacultural conditions.

In the following, some examples of behavioural indicators will be given.
An important indicator for the welfare of fish is behaviour during feeding. Feeding behaviour is varies according to the stresslevel and healthsituation of fish. It has been observed that behaviour of salmon during feeding consists of complex mechanisms involving threat, agressive biting, chasing and submission of fish. Behaviour during feeding and the amount of food taken up varies tremendously, and food intake seizes for example completely up to several days after vaccination or under bad environmental conditions. It is also known that juvenile Pacific salmon can develop

aggressive behaviour and hierarchies if feed from a point source. Changing feeding sources or spreading the feed is an easy and unexpensive way to improve welfare. Feeding behaviour shows therefore to be a sensible indicator for health and condition of fish.

Swimming patterns and behaviour or other potential welfare indicators. It has for example been observed that Halibut shows increasing swimming activity when the density in the cages is increased. Increased swimming was in the experiments correlated with a reduction and increased variability of growth, indicating impaired welfare (Kristiansen et al. 2004).
Indications of stress can be abnormal behaviour like spontaneous migrations towards one part of the cage or increased breathing activity (Aabel JP et al. 1991).

Good knowledge of the behaviour of each species can lead to aquaculture procedures which are more adapted to the animals natural behaviour, thereby improving health and welfare.

4.3 Other indicators

Further indicators for welfare are general condition of the fish (scale loss, damaged fins, etc), growth, reproduction, and health. The relationship between health, disease and welfare is complex, but without doubt good health is a necessary requirement for good welfare. Signs of disease normally indicate impaired welfare, and diseases may in addition result as a consequence of bad husbandry or stressful situations and indicate an underlying problem. But, infectious diseases can also naturally occur under good welfare conditions and are therefore not necessarily indicators for impaired welfare.
On the other side, absence of disease cannot unconditionally be interpreted as good welfare, as some diseases can be avoided through vaccination or treated with antibiotics or other therapy forms, thereby possibly “masking” poor welfare conditions. In addition, healthy fish may still suffer from reduced welfare due to other factors, like for example an environment which deprives from their natural behaviour.

5 Welfare issues

This chapter aims to give a short overview over several aquacultural conditions which may have an influence on animal welfare.

5.1 Fish densities in farming

Increasing stocking densities compared to the natural densities of fish species means that the number and the type of fish interactions increase. The naturally evolved behaviours are adaptive and reflect the most adequate way to react in order to cope with the environment in an efficient way, which implies that increased stocking densities normally affect this balance. The biology of the cultivated fish species is complex, to identify adequate stocking densities is therefore difficult and must take age, size, environmental conditions and biological characteristics of the species into consideration.
The biology of salmon illustrates the degree of complexity involved in establishing adequate densities, as salmon, when stocked under a certain threshold level develops an aggressive and territorial behaviour, on the other side, when the stocking densities are increased, changes towards a less aggressive schooling behaviour. Aggression has

evolved in order to establish and secure territories, feeding sources and reproductive success. If the number of aggressive actions increases due to an overcrowded environment, this results not only in a stressful situation for the fish defending its territory but can as well result in injuries in both aggressor and victim. As a result, damaged fins, scale loss in addition to chronic stress and other injuries can be common. Further on, hierarquic structures with dominant individuals can lead to a bimodal size distribution, which makes more frequent grading necessary. This again increases the required amount of handling procedures leading to more stressful conditions. This example from salmon aquaculture illustrates that low stocking density does not necessarily improve welfare, and that care must be taken in order to find optimal density levels. Another example is that, for some species like coho salmon and rainbout trout, high stocking densities have a negative effect on the feeding rates, while an opposite trend is observed in Arctic charr (Alanära 1996).

A secondary effect of frequent interactions between animals is the potential increase of horizontal disease transfer, either from fish to fish or through the water. Viruses, some protozoan parasites and some bacteria are capable of surviving in the water column or the sediment for some time after being shed by an infected host even it they are obligate pathogens (meaning that they need a living host in order to grow and reproduce).
Both facultative and obligate pathogens can be controlled through thorough disinfection procedures in closed systems and hatcheries, but in open seawater cultures and ponds a complete control is not possible. Interactions between fish and microbials which can be harmless under natural conditions, can result in disease outbreaks in farming environment due to added stress factors.
It is therefore necessary to provide fish with a low-stress environment which does not reduce the immunosuppressive functions, making the animals less succeptible to present pathogens.

5.2 Environmental parameters

Fish are in constant interaction with their environment through the surface of the gills and the skin, therefore water quality (oxygen, ammonia, pH) and the level of present contaminants are crucial for welfare. Water quality should be constantly and carefully monitored and reflect the needs of each species and developmental stage.

5.3 Malnutrition

During the last years, much has been invested into research of adequate feeding formulations, but there still remain a couple of open questions and problems to be solved. It is important to notice, that feed formulations can have both negative and positive effects on health and welfare; diseases originated from missing micronutrients being an example for a negative effect, the improvement of the immune system through the oral administration of glucan and other micronutrients a positive one.
As regarding salmonid diets, most of them contain high proportions of marine fish meal and oil in order to match the fatty acid and amino acid needs of the cultured fish. The main reasons are the high demand for protein, and secondly the high demand of polyunsaturated (PUFA) fatty acids. Some of those, like the fatty acids EPA and DHA cannot be synthesized by marine fish and are therefore essential, the content in the food being reflected by the content in the fish. Inadequate levels of fatty acids have a

negative impact on the immune function and can lead to a decrease in antibody production.
As production from intensive fish-farming is increasing, marine fish products for the feed industry are becoming limited and in addition the public is claiming alternative raw materials due to ethical reasons. Focus has been on the possibility of using plant raw materials, e.g. soja bean, as an alternative. The major challenges regarding the vegetable alternatives are the different content and composition of FA and AA, and the presence of so-called anti-nutrients. Anti-nutrients represent a welfare problem in themselves, as they can lead not only to reduced absorption and digestion, but also to diseases in the intestinal systeme. In addition, the oil content of the vegetable alternatives is lower and the carbohydrate content poorly utilised by fish.
There has been shown that a non sufficient provision with one or several nutrients can lead to severe disease and disfunction (cataracts, malformations, etc...), impairing animal welfare.

On the other side, health might be improved by feed ingredients. Glucan is already used as a general immunostimulant and currently effort is done to find a set of bacteria which could act as probiotics in fish. These bacteria should be auxiliary in improving the microflora of the fish and prevent pathogen proliferation.

5.4 Food deprivation

Food deprivation is used in order to adapt production to the market demand, before transport to reduce metabolic rate and oxygen consumption and production of waste products, and before slaughter aiming to eliminate gut contents and reduce contamination risks.
Fish are ectothermic, meaning that their body temperature varies and does not need to be maintained at a fixed level. Therefore periods with less or without food are less detrimental than for endothermic animals (FSBI 2000). In addition, wild fish shows natural changes in appetite, having periods (for example juvenile salmon in winter) during which eating is strongly reduced without necessarily impairing welfare. Important to notice is here that this natural behaviour of reduced feeding is temperature-, age-, species and season dependent, implicating that depriving fish from food during non-natural periods might lead to reduced welfare.

The welfare consequences of food deprivation for up to several weeks as a market-regulating mechanism, used in order to delay growth and slaughter during periods in which marked price is low, should be carefully examined.
The fasting period demanded in order to completely empty the gut before transport and slaughter should be adapted to species, fish size and ambient temperature, and be not longer then necessary.

5.5 Cataracts

The incidence of cataract (“grey star”) in intensive culture of Atlantic salmon has increased significantly, and also sea bass and sea bream have been shown to be affected by cataract (Berkaas et al. 2000, Ersdal et al. 2001). During the last years it has been reported that up to 90% of the salmon in a net pen can be affected with cataract of varying degree of intensity, leading to reduced feeding and other signs of discomfort (Bjerkås et al. 2000). An epidemiological study done in Norway in 1998 showed that most of the surveyed sites where affected, and that 80% of the examined fishes exhibited lens opacities of varying degree, 30% of them serious enough to affect vision. Apart from the health and welfare issues related to the high incidence of cataracts, the economic impact is considerable. Menzies et al. (2002) estimated the direct costs of cataracts in Norwegian farming due to mortality and weight loss to be between 747.000 Euros and 54.983.000 Euros in years with a low/high incidence of cataract.

The lens is mainly constituted by crystallins (proteins), and when affected by cataract, the normally transparent lens turns partially or completely opacious, scattering a certain proportion of the incoming light. Lens transparency is dependent on the regular arrangement of lens fibers and the structural properties of the crystallins (Breck 2004). The physiological mechanisms involved in cataract formation are both of cellular, intercellular and capsular nature and vary in accordance to the different causative factors. Behavioural changes and reduced growth due to difficulties in feeding are direct consequences of cataract.

Several factors are known to be related to cataract formation. Among these are environmental conditions (ex.: temperature), nutrition (ex.: deficiency in histidin, methionin, zinc), toxins, genetic predisposition and parasites.

5.6 Deformities

5.6.1 Skeletal deformities

Skeletal deformities represent not only an ethical and welfare issue but have a negative impact on economy and quality as well, due to growth impairment in the affected fish, elevated mortality, reduced fillet quality and smolt which cannot be sold. Deformities in salmon can appear during the egg stage, freshwater phase or even after transfer to the sea. In Hordaland (Norway), a study in order to access quality degradation in slaughtered salmon due to spinal deformities was performed in 1994, indicating that an average of 3% of the fish had to be degraded, variations between groups ranging from 0 to 20%. In farmed cod, up to 80% of some fingerling groups showed deformities in the neck region (Totland et al. 2004), resulting from the pressure of an unnormally large air bladder.
Other skeletal deformities include head, mouth and opercular deformations, and they affect not only salmon but most of aquacultured species. In sea bream, sea bass and milkfish spinal malformations, bending opercule and jaw malformations especially in hatchery reared larvae are quite common (Cahu et al. 2003).

Temperature is one of the major factors inducing deformities: for salmon eggs, temperature over 8°C during embryogenesis may lead to malformations, while the optimum incubation temperature for rainbow trout is supposed to be around 10°C ((Baeverfjord and Lein 2004). Not only during incubation, but also in the early freshwater stages temperature seems to play a role in inducing malformations, the incidence and severity of malformations in salmon increasing with increasing rearing water temperature (Baeverfjord and Wibe, 2004).
It has been shown that deformities due to elevated temperature during rearing in freshwater, which where moderate or hardly noticeable, can develop to more protuberant and serious deformities during the following rearing in seawater (Baeverfjord and Wibe, 2004).
For sea bass and sea bream, lordosis has been related to hydrodynamics in tanks as well as to inadequate light intensity and salinity (Cahu et al. 2003.) Cahu et al. (2003)

reviewed the effects of some nutritional components on the skeletal development of fish larvae. A deficiency in phospholipids, some highly unsaturated fatty acids, amino acids and peptides can increase the occurrence of malformations in larvae. In Japanese flounder, a high vitamin A level resulted in a higher incidence of bone deformities.
In summary, factors responsible for the induction of malformations are genetic disposition, malnutrition (especially during fast-growth periods) and environmental parameters (Helland et al., 2004, Roberts et al, 2001).

5.6.2 Tissue deformities

During the last years, an increasing number of apparently healthy, large fish showed problems in coping with normal aquaculture procedures like transport, handling and grading. Fish with small and abnormally shaped hearts seem to be overrepresented in this group, and reports have been made on several heart anomalies that might result in restricted cardiac function.
Poppe et al. (2003) compared ventricle morphology in farmed and wild Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) in order to describe potential morphological changes due to domestication and environment. The hearts from the farmed fish showed to be less pointed/triangular and exhibited conspicuous amounts of epicardial fat compared to the wild animals. The authors speculate that a combination of sedentary lifestyle and breeding programs that do not take organ functioning into account could be the origin for the increasing amount of anomalies.

5.7 Vaccination – side effects

Vaccination programs have contributed significantly to reduce the losses due to disease outbreaks in fish farming, and represent in addition the major factor for the enormous reduction in antibiotic used in aquaculture. In 1987 around 50.000kg of antibiotics where used in Norway, compared to 1000kg in 2002 (Grave et al. 1996, Håstein et al. 2004). The major break-through regarding vaccination in aquaculture was the introduction of oil-adjuvant injection vaccines, their effectiveness attributed to the depot effect of the oil which only slowly releases the antigens, resulting in a prolonged stimulation of the immune system (Midtlyng et al. 1996). Other application forms of vaccines are oral, bath or dip, but non is as effective as injection vaccines. For a recent review on vaccines against viral and bacterial diseases see Biering et al. (2004) and Håstein et al. (2004).
Vaccination, and especially intraperitonial vaccination with oil adjuvant vaccines, may be hampered by severe side-effects. Beside the loss of appetite, more severe side effects of oil-adjuvant vaccines are tissue adhesions of variable degree around the injection site, pigmentation and granulomata. Severe lesions can disturb and interfere with the normal function of the affected organs, resulting in reduced growth and impairing animal welfare (Poppe and Breck, 1997), and may lead to reduced harvest weight (Midtlyng 1997). Tissue lesions and adherence after vaccination with oil adjuvant vaccines are observed not only in salmon, but also in cod (Mikkelsen et al. 2004). The severity of the side effects is apparently dependent not only on vaccine formulation, but as well influenced by vaccination time, general condition of the fish, water temperature etc.
Sørum and Damsgård (2004) studied the effect of anaesthetisation and vaccination of the feed intake and growth of Atlantic salmon, considering that feeding behaviour is supposed to be a reliable criteria for evaluating health and welfare. The results showed that anaesthetisation only had an insignificant effect on feed intake and

growth, while vaccination with an oil adjuvant vaccine resulted in a significant decrease in feed intake for about 12 days.

A certain degree of side effects has been accepted, due to the positive welfare aspects gained through the protection given by the vaccines, but definitely further emphasize and research must be oriented towards formulations and vaccination programs which minimise the adverse effects.

5.8 Genetics

For salmonid fish, large genetic variation has been associated with important economic traits, which makes it possible to select and breed for those traits. Most of the breeding experiments focus on increased growth, age at sexual maturation, disease resistance and meat quality (Gjedrem 2000).
It is known from land farmed animals, that genetic selection for high productivity has created serious welfare problems. As an example, broiler chickens which where selected for fast growth and high body weight show bone and cartilage disorders, and cows selected for high milk production have an increased frequency of mastitis. One has therefore to be aware of non-desired side effects of selection.
Selective breeding for improved welfare of farmed fish, for example by selecting for increased stress resistance, might be a possibility to diminish welfare problems.

5.9 Transport

Farming fish often includes transport of living fish, normally at high densities, with loading, capture, netting and pumping procedures involved. During transport inadequate water exchange depleting the oxygen content and accumulating carbon dioxid and ammonia may further contribute to stress reactions and impaired welfare (Eriksen et al. 1997).
Eriksen et al. (1997) studied the effect of handling stress and water quality during live transportation (well-boat) and slaughter of Atlantic salmon on the anaerobic white muscle activity by measuring pH and high-energy phosphate contents in the muscle. They did not find any dramatic effects, concluding that transport and slaughtering does not necessarily have an adverse effect on flesh quality and welfare if good water quality is maintained and gentle and efficient handling procedures assured.
Other studies show that after a 2 hours transport plasma glucose levels did not return to basal level even after a 48 hour recovery period, indicating that under unfavourable conditions even longer resting periods are necessary.

The Health and Consumer protection Directorate General from the European Commission adopted a report (March 2002) on the welfare of animals during transport. This report considers horses, pigs, sheep and cattle, but does not consider fish. The Scientific Panel on Animal Health and Animal Welfare from the European Food Safety Authority published an opinion on the report, especially taken into consideration those animals not referred to in the original report, among them fish.
The recommendations for transport of fish emphasize on
- sufficient oxygen availability
- avoiding exposure to air during the loading and unloading procedures
- food deprivation time prior to transport should be adjusted according to species, size and temperature
- optimal design of transport vehicle and equipment ensuring minimal contact with the

animals
- adequate monitoring of water quality and fish condition

5.10 Pre-slaughter handling

Pre-slaughter handling procedures include transport, crowding, removing the fish from water. Handling before slaughter might not only result in impaired welfare, but it does as well have a strong impact on quality. Scale loss, gaping and blood spotting occurring after handling procedures account for 50% or more of the reasons for downgrading (Robb and Whittington 2004).
Crowding takes place in order to enable access to fish in a harvest cage and might lead to adverse reactions like escape attempts and burrowing, which can result in damage of fins and snouts and physical stress reactions (Southgate and Wall 2001). Water quality and oxygen content is also often depleted during crowding conditions if not carefully monitored. Subclinical or carrier diseases may become a clinical condition due to unfavorauble environmental conditions, and those can be difficult to treat due to imposed withdrawal times of chemicals (Wall, ….).
In an attempt to assess the influence of pre-slaughter handling on flesh quality, Robb et al (2000) used electro-stimulation of a carcass (Rainbow trout) immediately after death as a model for high levels of muscle activity prior to death. They found that electro-stimulation resulted in shorter times to rigor and flesh colour was lighter and less red. The onset of rigor is dependent on the ATP content, which decreases with muscle activity. According to the authors the colour changes might result from a higher level of insoluble proteins in the flesh, caused by the rapid drop in pH post mortem of exercised fish as it has been observed in red meat animals.
The effect of live-chilling and crowding on stress responses in muscle in Atlantic salmon prior to slaughter was studied by Skjervold et al. (2001), in addition, post-slaughter fillet quality was also analysed. Live chilling for one hour at 1C is applied as a method to cool the fish throughout slaughtering and increase the rigor onset preserving flesh quality. It could be shown that both crowding and chilling resulted in pre-slaughter stress, indicated by elevated cortisol, glucose and lactate levels compared to the control group. Post slaughter, the live-chilled fish had a delay in time to onset of rigor mortis, while crowded fish had a faster onset of rigor compared to control. It seems thereby that live-chilling can prevent some of the negative effects on quality caused by crowding, but the effect of chilling on welfare and welbeeing of fish has to be taken into consideration. It is important to note that live-chilling may in many cases prolong the time necessary for the fish to reach unconsciousness after stunning.

5.11 Stunning/sedation

As stated by Southgate and Wall (2001), from an animal welfare point of view any method of slaughter should incorporate a stunning procedure that renders the animal immediately insensitive and unconscious until death. The Council Directive n 93/119 from 1993 points out that “Animals shall be spared any avoidable excitement, pain or suffering during movement, lairaging, restraint, stunning, slaughter or killing”.

Roth (2003) gives an overview of the most commonly used stunning and killing methods for the different fish species (table 1).

Table 1: Stunning (stun) and killing (kill) methods that are used for slaughter in the most common European farmed fish species. Not determined (n.d.) and not appropriated (n.a.) methods indicated as well (after Roth 2003).

	Stunning and killing method							
Fish species	Asphyxia	Thermal shock	Salt	Aqueous solution	Exsanguination	CO2	Electric	Percussion
Northern Europe								
Atlantic Salmon (*Salmo salar*)	Kill	Stun	n.a.	n.a.	Kill	Stun	Stun	Kill
Rainbow trout (*Oncorhynchus mykiss*)	Kill	Stun	n.a.	n.a.	Kill	Stun	Stun	Kill
Halibut (*Hippoglossus hipoglossus*)	n.d.	Stun	n.a.	n.a.	Kill	Stun	n.d.	n.d.
Eel (*Anguilla anguilla*)	n.a.	Stun	Kill	Kill	n.a.	n.a.	Kill	n.a.
Southern Europe								
Gilthead Seabream (*Sparus aurata*)	Kill	Stun/Kill	n.a.	n.a.	n.a.	n.a.	Stun	n.d.
Seabass (*Dicentrarchus labrax*)	Kill	Kill	n.a.	n.a.	n.a.	n.a.	Stun	n.d.
Commom carp (*Cyprinus carpio*)	Kill	n.d.	n.a.	n.a.	Kill	n.a.	Stun	Kill
Turbot (*Scophtalmus maximus*)	Kill	Stun/Kill	n.a.	n.a.	Kill	n.a.	Kill	n.d.

Brief description of the main killing and stunning procedures:

Asphyxia

When removed from water and exposed to the air the gills collapse and the resulting minimal oxygen exchange results in anoxia. Time of death is temperature dependent and can take up to several minutes.

Thermal shock

Commonly used for the slaughter of small trout, sea bass and sea bream, the fish are placed into an ice and water slurry. The fish may remain alive in the slurry for several hours.

CO2

A bath in carbon dioxide saturated water results in a fall of blood pH leading to a central nervous system disruption, followed by immobility and eventual insensibility. Narcosis is known to be associated with strong aversive movements (Robb et al. 2000). The method is widely used in large scale salmon and trout farming, but is considered to be aversive not providing immediate insensivity, in addition reducing the flesh quality through lowering muscle pH. Aversive reactions, like rapid swimming and escape attempts can last for up to several minutes in trout and salmon, and up to more than one hour in eels (Marx et al. 1997). In Atlantic halibut carbon dioxide narcosis prior to exsanguinations resulted in rapid onset of rigor mortis and high hardness values compared to killing by Eugenol or blow on the head (Akse L and Midling K, 2000).

Electric stunning

Used on small trout and recently also being introduced for large scale salmon farming. The method has the potential to kill large numbers of fish humanely without exposing them to long periods of stress and without removing them from water, but incorrect current can cause fractures and haemorrhages due to strong muscle stimulation (Lines et al. 2001). It has been shown that electrical stunning can improve flesh quality by delaying rigor mortis and providing a firmer texture compared to fish stunned by CO2 (Roth et al. 2002), on the other side, the use of non-appropriated currents can cause carcass damage and result in lower quality of the product.

Percussion

Percussive stunning is commonly used on salmon and large trout. The method consists of a blow on the head, disrupting the normal brain functions by the pressure

changes. Provided that the blow is of sufficient accuracy, percussive stunning renders immediately unconsciousness. In semiautomatic stunning devices the fish are pushed with their head into a guide and touch a trigger which activates a hammer delivering a blow in the head rendering unconsciousness, the procedure is followed by immediate exsanguination.
This method is considered to be an efficient and humane way to slaughter fish, in addition results slower post mortem muscle activity and slower onset of rigor mortis compared to other methods. The increase in flesh quality is probably due to reduced physical activity before slaughter, thereby reducing the exposure to stress.
Spiking (iki jime)
Applied in for example tuna and large salmon, a spike is inserted into the brain and rotated causing the localised destruction of parts of the brain. As in percussive stunning, if applied correctly and accurate, spiking leads to unconsciousness immediately and fish show reduced physical activity at slaughter and consequently increased flesh quality.

Comparing different slaughter methods applied to Atlantic salmon by determination of the onset of brain failure, Robb et al. (2000) found that there was a significant difference in time for onset of brain failure between fish stunned by percussive blow or spike, and those stunned by CO_2 narcosis or gill cut. Stunned by the two latter methods it took up to several minutes until no brain activity could be recorded, while blow and spike resulted in brain failure after 15-30 seconds if correctly applied. A further study by Robb and Roth (2003) on brain activity following electrical stunning show that, when applied with the correct strength and pulse duration, electrical stunning can cause immediate loss of consciousness.
Comparing carbon dioxide, manual and electrical stunning on carp, eel and trout, Marx et al (1997) found that fish where anaestetized almost immediately with both stunning methods, while it took 3.2 min (trout), 9.2 min (carp) and 109.7 min (eel) to anaestetize the fish with CO_2. In addition, CO_2 stunning resulted in the lowest pH values and water holding capacities after slaughter and storage.
Traditionally, CO_2 narcosis has been the most common method of sedation used in salmon slaughter. During the last years percussive stunning and electrocution has been introduced as new methods for the slaughter of large amounts of fish. As an example, the Norwegian government authorised in 2003 both methods for sedation in addition to the previously authorised CO_2 narcosis and chilling (Garseth 2003).

Eel
The eel is a very robust and difficult to manipulate species, stunning and slaughtering therefore being difficult tasks to be performed in a human way. Eels have been traditionally killed by decapitation when sold in small numbers to consumers or through brining when they are to be smoked. After decapitation, heads can show activity and survive for about 30 minutes when stored in water and for about several hours in air (Verheijen and Flight 1997).
Brining generally consists in treating living eels with dry salt through witch after some minutes water is added before slaughtering by evisceration, this procedure is supposed to de-slime the fish making it suitable for further processing. Verheijen and Flight (1997) showed that eel, after a phase of escape movements followed by immobility, when taken out of the salt solution and washed, where not dead as often assumed but recovered even though they eventually died as a result of the

deteriorating procedure. When the brains where destroyed prior to the brining procedure the movement stopped after short time and no recovery was possible, the release of mucus seemed to be unaffected.
A further evaluated slaughter method (Lambooij et al. 2002a) is placing the eel in cold (-18C) brine after stunning through chilling them in ice water until the body temperature was <5C. EEG measures showed that after the stunning procedure some eels still exhibited responses to putative painful stimuli and the animals exhibited adversive behaviour. During the subsequent cold brining extreme depolarisation of membrane potentials only started after 27 sec.
Stunning has been used on different animal species including fish (trout, salmon) as a stunning method rendering immediate insensitivity if applied with sufficient force and accuracy. Lambooij et al. (2002b) evaluated the use of needle stunning for eel by observing behaviour before, during and after stunning, and measuring EEG and ECG. The method was considered suitable for human slaughter of eels, even though no commercial stunning method is yet available.
Electrocution can be an acceptable stunning method, if correct current and intensity is applied (Kuhlmann et al. 2001, Robb et al. 2002).

5.12 Organic production

During the last years, there has been an increasing demand from consumers for the so-called organically produced agricultural products, and according to the Soil Association (UK) the sales of organic salmon having increased by 33% in 2003. EU production of organic aquacultural farming in 2000 was about 4500 tons, most of it salmon. The general idea of organic production is to manage natural resources in a manner that avoids environmental damage, provide farming environments which attend to the natural needs and welfare of the species, base the production on renewable resources and minimise pollution. Organic production is often considered to improve and secure animal welfare through taking into account the natural physiological and behavioural needs of the farmed species in a larger degree than conventional farming.

Several institutions have recently developed guidelines and standards for organic aquaculture, applying to major farmed fish species, shrimp and shell.
Some of the basic requirements in organic fish farming are (extracted from the Norwegian Debio standards and the COABC´s standards):

- No triploid or genetically modified organisms
- Feed shall consist of organic certified feed and synthetic (colouring additives, antioxidants, amino acids etc) additives as well as ingredients that where produced using genetic engineering are not permitted.
- Use of drugs should be avoided and is only permitted when no other measures can be applied, vaccination is, with some restrictions, permitted. When synthetic drugs are used the withdrawal period is longer then for conventional farming. Biological disease combating (e.g. wrasse for delousing) is encouraged.
- Transport has usually to be limited to a maximum of 6 hours. The application of synthetic stimuli or tranquillisers during transport is not permitted.
- Food deprivation of fish prior to slaughter must not exceed 100 day degrees.
- Fish must be sedated prior to slaughtering via head trauma (CO_2 permitted in Norway, not in UK).

Despite the positive image organic farming has regarding health and welfare, it is important to notice that sometimes health and welfare in fact can be impaired through organic culture. Some of the major problems encountered in land based organic farming are feed formulations which do not meet the animals needs, low reproduction rates and reduced health due to limitations imposed on medical treatment. In addition, organically produced products are often of greater zoonotic risk for humans and may contain higher amounts of potentially harmful microorganisms compared to conventional products, this being due to the lower amounts of antibiotics and pesticides used and the use of faeces as fertilizers.
Organic aquaculture is a relatively new field and many questions and problems remain unsolved. Little is known on the consequences of the requirements on fish health and welfare, and further research is needed in order to secure both consumer and fish health.

6 Past and current EC research projects on welfare issues

Research towards animal welfare has been funded and supported by the European Commission since 1994, with the start of the Fourth Framework Program (FP4). In FP4, research on welfare was part of the Agriculture and Fisheries (FAIR) Programme. In FP5, under Key action 5 (Sustainable agriculture, fisheries and forestry) of the Quality of Life programme, funding of welfare related research projects continued. Under FP6, animal welfare is addressed mainly within Priority Area 5, Food Quality and Safety, and with the Policy oriented research. A short overview over past and current research projects related to welfare issues in aquaculture is given below.

FAIR-CT98-3372, Organic salmon production and consumption: ethics, consumer perceptions and regulation (Orcsal)
Total costs EUR 720 000; EU contribution EUR 400 000
Duration 24 months
Co-ordinator Dr James A. Young, University of Stirling

Primary objectives:

1. to evaluate definitions of organic salmon and aquaculture production from both industry and consumer perspectives
2. to explore consumers ethical perceptions of organic salmon in the major EU markets
3. to critically appraise the technical, animal welfare and environmental aspects implicit in organic salmon production in terms of ethical, social, economic and sustainability considerations
4. to explore critical issues in the regulatory and legal framework at the national and EU level, thus providing input to regulatory bodies developing standards for organic fish farming at the EU level.

FAIR-CT97-3127: Harvest procedures of farmed fish
Total costs EUR 1 585 085; EU contribution EUR 1 014 640
Duration 36 months, starting date 1.9.1997
Co-ordinator Dr Hans Van der Vies

Primary objectives:

1. optimisation of slaughter processes of cultivated fish with respect to fish quality and welfare
2. automation and application of optimum procedures on a pilot scale

FAIR-CT95-0152: Selective breeding for stress tolerance
Total costs EUR 1 285 310; EU contribution EUR 1 049 310
Duration 48 months, starting date 1.12.1995
Co-ordinator Dr T G Pottinger

Primary objective: to assess the feasibility of increasing the tolerance of finfish to stress selective breeding

FAIR-CT96-1840: Investigation and quantification of the stress associated with accumulation of carbon dioxide in eel farms with recirculating water
Total costs EUR 760 000; EU contribution EUR 760 000
Duration 36 months, starting date 1.12.1996
Co-ordinator Prof Carla Giuliana Bolis

Primary objective: to identify and quantify the physiological and oxidative stress associated with exposure to chronic hypercapnia in eels grown under intensive conditions in closed-cycle fish farms. To study the effect this stress has on performance in culture.

FAIR-CT97-3963: Cataracts in farmed fish – A multidisciplinary initiative for scientific progress
Total costs EUR 190 000, EC contribution 190 000
Duration 36 months, starting date 1.1.1998
Co-ordinator Dr Paul J Midtlyng, VESO, Norway

Primary objectives:

1. dissemination of scientific knowledge and state of the art in cataract research, facilitation of the exchange of study specimens and methodology
2. initiation of epidemiological studies to provide data on the occurrence and economy of the disease
3. stimulation of new research on the physiology of the fish eye and the pathogenesis of lens cataracts

FAIR-CT98-4217: FISCI: A new bio-index for the assessment of stress condition in aquacultured marine fish
Total costs EUR 895 565, EC contribution 670 000
Duration 36 months, starting date 4.1.1999
Co-ordinator Dr Maria Alexis, Nacional Centre for Marine Research

Primary objectives:

1. to detect, analyse and quantify stress effects in sea bream and sea bass, using changes in skin, mucus layer and gill epithelia as parameters (tissue morphology, enzymatic activity, stress hormones)

2. on the basis of analytical methods and stress parameters a "fish stress condition index (FISCI)" will be established, which can be used as a non-invasive method of stress detection in aquacultured fish

QLK2-1999-00799: Intraperitoneal immunopathological reactions following vaccination of farmed fish – studies of basic immune mechanisms
Duration 39 months, starting date 1.1.2000
Co-ordinator Øystein Evensen, Norwegian School of Veterinary Science

Primary objectives:
1. to elucidate the effect of single and combined bacterial components and vaccine formulations for the initiation, development and maintenance of intraperitoneal immune granulomas
2. to investigate the key cellular mechanisms related to the development of vaccine granulomas both with regard to cell profile and cytokine profile

Expected results:
1. to identify the contribution of the individual vaccine components regarding incution and maintenance of immune granulomas at the injection site
2. to obtain and overview of the kinetics of the inflammatory responses at the injection site and to elucidate the underlying immune mechanisms

   The results will be of importance for designing oil-adjuvanted vaccines optimising the side-effect profiles.

Q5RS-2000-30058: Researching alternatives to fish oil in aquaculture
Duration 48 months, starting date 1.1.2001
Co-ordinator Gordon Bell, University of Stirling

Primary objectives:
To research and develop vegetable oil alternatives to fish oils in aquafeeds without comprising health and welfare. This will also include further understanding of how the basic metabolism of the fish responds and adapts to changes in the dietary lipid composition.

Expected results:
Recommendations and guidelines to the aquaculture industry for substituting fish oils in aquafeeds, undermined by advanced understanding of the nutritional biochemistry and physiology of lipids in fish.

Q5RS-2000-30068: Perspectives of plant protein use in aquaculture (PEPPA)
Duration 36 months, starting date 1.12.2000
Co-ordinator J Kaushik, INRA-Ifremer

Primary objectives:
1. to define the ideal amino acid profile for maximum muscle protein growth and to evaluate the interrelationships between AA supply and protein, carbohydrate and lipid metabolism
2. to assess effects of AA and of plant protein on digestive and metabolic processes affecting muscle protein growth and adiposity

3. to develop fishmeal-free or low fishmeal diets and to assess the effects of those diets on fish performance and health

Expected results:

1. identification of the most suitable AA profile that can promote maximum protein growth and in-depth knowledge on nutritional, metabolic and endocrine factors that govern protein growth and their molecular basis
2. development of low/fishmeal free diets
3. social and economic analysis of a shift from fishmeal to plant protein based feeds in European aquaculture

Q5RS-2000-31656: Gastrointestinal functions and food intake regulation in salmonids: impact of dietary vegetable lipids (GUTINTEGRITY)
Duration 36 months, starting date 1.1.2001
Co-ordinator Björn Thrandur Björnsson, University of Gothenburg

Primary objectives:
Main objective is to provide a strong basis for the quantitative and qualitative incorporation of vegetable lipids into feed for salmonids, without compromising growth, health and welfare of the animals.

Expected results:

1. understanding of how vegetable lipids affect the gastrointestinal tract, especially the absorption processes, nutrient uptake mechanisms, barrier function, indigenous microbiota and disease resistance.
2. understanding of how vegetable lipids affect food intake, how this is mediated by gut peptides and hormones, and how fish are able to discern between lipid sources
3. understanding how the health and welfare of the fish is affected by disturbance of the intestinal integrity

Q5RS-2000-30271: Feed for aquatic animals that contains cultivated marine micro-organisms as alternatives to fish oil (PUFAfeed)
Duration 36 months, starting date 1.12.2000
Co-ordinator Lolke Sijtsma, Agrotechnological Research Institute

Primary objectives:

1. to develop alternative feed resources to fishmeal employing heterotrophic, autotrophic and mixotrophic micro-organisms
2. to elucidate fundamental aspects of lipid accumulation in algae and to develop the technology to produce microbial biomass and novel feeds

Expected results:

1. an efficient process for the production of microbial biomass containing long chain polyunsaturated fatty acids
2. production of feeds for aquaculture that are based on untreated or processed microbial biomass and determination of the performance in starter feeds
3. based on the results obtained in feeding trials with respect to growth, survival and stress tolerance of fish larvae as well as on economic data of the

production costs, the economic evaluation of the whole integrated process will be established

Q5RS-2001-02211: A functional genomic approach to measuring stress in fish aquaculture (STRESSGENES)
Duration 36 months, starting date 1.11.2001
Co-ordinator Patrick Prunet, INRA

Primary objectives:

1. to determine the gene expression profiles of selected tissues in fish exposed to standardised stressors
2. to identify genes whose expression is linked to adaptation response in stress
3. the difference in gene expression profiles between trout which display a divergent trait will be used to identify potential candidate markers of genotypic variation in stress responsiveness

Expected results:

1. construction of suppression subtractive hybridisation cDNA libraries derived from tissues collected from rainbow trout exposed to several stressors
2. preparation in microarrays, implementation of a bioinformatics management system, and the isolation of cDNA encoding for stress-regulation genes
3. use of selected families of rainbow trout to identify stress-regulated genes and to allow discrimination between stress-sensitive and stress-resistant individuals

Q5RS-2001-01233: Optimisation of rearing conditions in sea bass for eliminated lordosis and improved musculoskeletal growth (ORCIS)
Duration 36 months, starting date 1.11.2001
Co-ordinator Neil Stickland, University of London

Primary objectives:

1. to assess the influence of rearing temperature, nutrition (mainly vitamins C, E and selenium) and current velocities and their interactions on the incidence of lordosis
2. to assess for the same factors the influence on musculoskeletal development and growth at tissue, cell , protein and molecular level
3. to provide a tool for standardised assessment of quality taking into account the presence and absence of skeletal deformities and the effects of their different expression intensities on biological performance and product image

Expected results:
Further understanding of the reasons for vertebral deformities and indication for the optimum rearing conditions for its elimination and for improved musculoskeletal growth.

Q5RS-2001-01465: Calcium, the backbone of fish culture: importance in skeletal formation, reproduction and normal physiology (FISCAL)
Duration 42 months, starting date 1.12.2001
Co-ordinator Adelino V. M. Canário, University of Algarve

Primary objectives:

1. to identify the relative importance of dietary, endogenous and environmental sources of calcium during critical phases of development, growth and reproduction of sea bream
2. to determine the roles of the parathyroid hormone related protein (PTHrP) in whole animal calcium homeostasis
3. to study the molecular mechanisms that underpin bone formation in fish and the way PTHrP regulates this process

Expected results:
The project will provide new information on calcium physiology and allow the identification of causal factors in abnormal bone development in larvae, the mechanism by which calcium is incorporated into vitellogening, the general requirements of calcium for normally growing fish and the function of PTHrP in these processes.

FP6, 501984: Welfare and health in sustainable aquaculture (WEALTH)
Co-ordinator Johan Glette, Institute of marine research, Norway

Primary objectives:

1. to gain comprehensive knowledge on health and welfare in farmed fish focusing on Atlantic salmon and sea bass
2. to study important environmental factors and husbandry practices in freshwater-, seawater- and recirculation-system aquaculture in order to identify how these may compromise welfare and health of farmed fish
3. to understand the physiological and molecular mechanisms underlying the interactions of husbandry practices and environment on stress conditions affecting welfare and disease resistance in farmed fish
4. to identify innate and acquired immune parameters affected by environmental factors and husbandry practices resulting in compromised welfare and health, and to develop effective molecular tools to study and monitor the immune function of the farmed fish

Expected results:
To develop and validate operational husbandry protocols for improved welfare and health of farmed fish, including mechanisms for early prediction and management of disease outbreaks.

Health promoting, safe seafood of high eating quality in a consumer driven fork-to-farm concept (SEAFOODplus)

Subproject 5.2: Ethical quality traits in farmed fish: the role of husbandry practices and aquaculture production systems (ETHIQUAL)
Primary objectives:

1. to identify ethical quality based on behavioural and physiological traits
2. to develop smart tagging and monitoring technology for individual-based screening to collect records of parameters related to physiological activities such as lactate levels, EMG or blood flow rate, or behavioural activities

3. to monitor ethical indicators under experimental conditions for tagged fish exposed to specific challenges, indicators may include food demand, aggression, stress indicators, fin condition etc
4. to balance ethical and flesh quality in farmed fish
5. to identify the genetic basis for individual-specific responses to challenges
6. effects of transport on physiology, behaviour and fish flesh
7. to optimise pre-slaughter with respect to physiology and fish flesh. The optimised pre-slaughter conditions will be tested at two SMEs.

CRAFT 512991 STUNFISHFIRST "Development of prototype equipment for humane slaughter of farmed fish in industry"

Primary objectives:
1. to develop prototype equipment for humane slaughter of the selected farmed fish species, namely eel (*Anguilla anguilla*), tilapia (*Oreochromis niloticus*), sea bass(*Dicentrarchus labrax*) and turbot (*Psetta maxima*). Humane slaughter consists of stunning (rendering unconscious without avoidable stress prior to killing)
2. model electrical stunning so that the required parameters for effective stunning with high quality standards can be predicted for the selected fish species and other species
3. establish requirements for stunning of the species with respect to product quality and welfare
4. design, build four prototypes (one for each species) for electrical stunning¿
5. study power saving techniques to facilitate implementation of electrical stunning at small SMEs
6. test the prototypes at SMEs with respect to product quality, welfare and operational characteristics

7 Welfare – Economic value and product quality

Among the most important factors influencing the quality and value of flesh are fat content, fat distribution, colour and texture (Gjedrem 1997). Apart from genetics and nutrition, husbandry procedures and the resulting stress and exercise may have great influence on quality parameters (Pottinger 2000).
During exercise resulting from stressful procedures, the highly energetic adenosine triphosphate (ATP) is transformed into adenosine diphosphate (ADP), liberating energy originated from the phosphate bonds and generating free phosphate. When the ATP stores are depleted, in the predominantly anaerobic metabolism of the white fish muscle new ATP is generated by the catabolism from glucose to lactic acid, the main source for glucose being glycogen. As a result, intense exercise leads to the depletion of glycogen and the accumulation of lactic acid, resulting in a decline of the pH that reduces flesh quality.
Jerrett et al (1996) analysed the tensile strength of Chinook salmon white muscle after storage, comparing rested to exhausted muscle, finding that the strength of the muscle of rested animals was 2.7 times that of the exhausted. These findings illustrate the importance of reducing pre-harvest exercise in the production of high quality fish muscle.

On the basis of the physiological reactions described above, one can conclude that securing welfare and assuring good husbandry procedures are not only of ethical and moral relevance, but they do as well have a positive impact on product quality.

In their article "an economic perspective on fish welfare" Peddie and Scott (2003) describe ways to evaluate the costs of improving welfare and how to construct a model exploring the relationship of productivity with welfare. They emphasize on the different welfare concepts, one being the scientific approach, based on the physiological and behavioural needs of the animals, the other one being the economists approach which is consumer centred and influenced by science, culture, education, personal ethics and income. This makes the consumer centred approach on animal welfare more vulnerable to actual trends in marked and society, this being the reason for the authors to choose the term perceived welfare instead of absolute welfare. In their economic model, the authors assume that increasing productivity reduces welfare, and that aquaculture operates within a people centred range of desired welfare which has a lower limit at which no further welfare reduction is accepted even if production thereby can be increased. Attention is drawn to the fact that minimum welfare standards normally are established on a national basis, while free-trade is international, this resulting in a tendency to concentrate or move production to countries with lower welfare standards.
It is though important to keep in mind that productivity is not synonymous to quality, a fact not taken into account by the authors, and there are numerous examples where improved and good welfare leads to improvement of product quality. This is especially true for good practices in pre-slaughtering procedures, which reduce stress reactions in fish and result in improved flesh quality, but also other welfare issues like deformities and vaccination side-effects can result in reduced product quality and thereby have a negative economic impact.

8 Legislation

Definitions (www.euabc.com)
Resolution: A resolution is a non-binding statement, which defines objectives and makes political declarations. The European Council's resolutions set out the direction of future policy initiatives. Resolutions may be used by the EU Court to interpret laws. They may be referred to as a form of "soft law".

Recommendation: A non-binding decision, which only urges Member States to comply. A Member State cannot be fined for the breach of recommendations.

Directive: Directives are to be transferred into national law through the member states' parliaments and governments within 18 months. Through the years, the EU Court has proclaimed many directives to be directly applicable and even declared that countries are liable to pay compensation if they have not implemented a directive in time.

Directives are normally transformed into national laws by the national parliaments or most often by the governments through delegated acts.

Decision: An EU decision is binding on the persons, companies or Member States mentioned in the decision. It is not generally binding, as is the case with a regulation.

Regulation: An EU decision that directly binds all Member States and citizens in the whole of the EU. Whereas directives need to be "transformed" into national law, regulations are directly applicable. It is therefore forbidden to change EU regulations when putting them into national laws.

European Convention for the protection of animals kept for farming purposes Official Journal L 323 , 17/11/1978 p. 0014 - 0022

78/923/EEC: Council Decision of 19 June 1978 concerning the conclusion of the European Convention for the protection of animals kept for farming purposes Official Journal L 323 , 17/11/1978 p. 0012 - 0013

Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes Official Journal L 221 , 08/08/1998 p. 0023 – 0027

2000/50/EC: Commission Decision of 17 December 1999 concerning minimum requirements for the inspection of holdings on which animals are kept for farming purposes (notified under document number C(1999) 4534) (Text with EEA relevance) Official Journal L 019 , 25/01/2000 p. 0051 – 0053

Council Directive 91/628/EEC of 19 November 1991 on the protection of animals during transport and amending Directives 90/425/EEC and 91/496/EEC Official Journal L 340 , 11/12/1991 p. 0017 - 0027

Council Directive 95/29/EC of 29 June 1995 amending Directive 91/628/EEC concerning the protection of animals during transport Official Journal L 148 , 30/06/1995 p. 0052 – 0063

Council Resolution of 19 June 2001 on the protection of animals during transport, Official Journal C 273 , 28/09/2001 P. 0001 – 0001

Council Regulation (EC) No 1040/2003 of 11 June 2003 amending Regulation (EC) No 1255/97 as regards the use of staging points

Welfare of animals during transport (details for horses, pigs, sheep and cattle). Report from the Scientific Committee on Animal Health and Animal Welfare, adopted on 11 March 2002. http://europa.eu.int/comm/food/fs/sc/scah/out71_en.pdf

Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to the welfare of animals during transport. Adopted 30 March 2004.
http://www.efsa.eu.int/science/ahaw/ahaw_opinions/424/opinion_ahaw_01_atrans_ej44_en1.pdf

Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. (Question N° EFSA-Q-2003-093). Adopted by the AHAW Panel on the 15th of June 2004.
http://www.efsa.eu.int/science/ahaw/ahaw_opinions/495_en.html

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