# SCIENTIFIC OPINION

# General approach to fish welfare and to the concept of sentience in fish[1]
## Scientific Opinion of the Panel on Animal Health and Welfare

## (Question No EFSA-Q-2008-708)
## Adopted on 29 January 2009

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**PANEL MEMBERS**

Bo Algers, Harry J. Blokhuis, Anette Bøtner, Donald M. Broom, Patrizia Costa, Mariano Domingo, Mathias Greiner, Jörg Hartung, Frank Koenen, Christine Müller-Graf, David B. Morton, Albert Osterhaus, Dirk U. Pfeiffer, Mohan Raj, Ronald Roberts, Moez Sanaa, Mo Salman, J. Michael Sharp, Philippe Vannier and Martin Wierup.

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[2] The acknowledgments have been corrected in order to include all Working Group experts" individual names. No further changes have been introduced in the opinion. To avoid confusion the original version of the opinion has been removed from the website, but is available on request as is a version showing all the changes made.

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SUMMARY

Following a request from the European Commission, the AHAW Panel was asked to deliver a Scientific Opinion on the animal welfare aspects of husbandry systems for farmed fish. In addition to the already adopted scientific opinions regarding six different species of farmed fish, a scientific opinion on the general approach to fish welfare and to the concept of sentience in fish was adopted on 29 January 2009.

The scientific opinion focused on the neurobiology and special sense organs in fish, and their capacity to experience pain, fear and distress, expressions of sentience from a fish welfare point of view, taking into consideration the available scientific information. Only a small number of the circa 20,000 teleost fish species, from diverse groups, generally those of economic importance for fisheries or aquaculture, have been studied in any scientific detail. It is therefore important that generalisations across the range of species should not be used without qualification.

It was concluded that the concept of welfare is the same for all the animals, i.e. mammals, birds and fish, used for human food and given protection under the Treaty of Amsterdam. However, fish welfare has not been studied to the same extent as mammals and birds. Whilst similar measures of welfare developed for other animals are often relevant to fish, clearly defined protocols for fish welfare evaluation are lacking.

Due to the complex relationships among the various needs/requirements of farmed fish and their behavioural and physiological consequences, as for all animals it is impossible to find one single measurement or welfare indicator that will cover all possible husbandry systems, farmed species and situations. A range of welfare indicators should be considered when welfare is being evaluated. Indicators of fish welfare should be species-specific, validated, reliable, feasible and auditable.

Different species of fish have evolved highly sophisticated sensory organs to survive in changing and varied environmental conditions. There is scientific evidence to support the assumption that some fish species have brain structures potentially capable of experiencing pain and fear. The balance of evidence indicates that some fish species have the capacity to experience pain. However research and developments in the area of cognition and brain imaging techniques should be carried out in fish to further our knowledge and understanding of pain perception.

Defence and escape behaviours are dependent on cognitive and learning abilities related to fear. Responses of fish, of some species and under certain situations, suggest that they are able to experience fear. Fish possess a suite of adaptive behavioural and physiological responses that have evolved to cope with stressors. Many of these are homologous with those of other vertebrates. Fish show short term adaptive responses which may be important to the maintenance of homeostasis but these do not necessarily imply any harmful consequences. Prolonged exposure to stressors generally leads to maladaptive effects or chronic stress. Chronic stress responses indicative of poor welfare include reduction in immune function, disease resistance, growth and reproduction, eventually death. Cumulative stress responses occurring at different life stages have not been studied.

From studies of sensory systems, brain structure and functionality, pain, fear and distress there is some evidence for the neural components of sentience in some species of fish. Our knowledge and understanding of manifestations of sentience in fish, however, are limited.

**Key words:** welfare of fish, welfare assessment, sensory organs, brain structure, pain, fear, stress, sentience.

## BACKGROUND

Council Directive 98/58/EC concerning the protection of animals kept for farming purposes lays down minimum standards for the protection of animals bred or kept for farming purposes, including fish.

In recent years growing scientific evidence has accumulated on the sentience of fish and the Council of Europe has in 2005 issued a recommendation on the welfare of farmed fish[3]. Upon requests from the Commission, EFSA has already issued scientific opinions which consider the transport[4] and stunning-killing[5] of farmed fish.

## TERMS OF REFERENCE

In view of this and in order to receive an overview of the latest scientific developments in this area the Commission requests EFSA to issue a scientific opinion on the animal welfare aspects of husbandry systems for farmed fish. Where relevant, animal health and food safety aspects should also be taken into account. This scientific opinion should consider the main fish species farmed in the EU, including Atlantic salmon, gilthead sea bream, sea bass, rainbow trout, carp and European eel and aspects of husbandry systems such as water quality, stocking density, feeding, environmental structure and social behaviour.

In addition to the already adopted scientific opinions regarding six different species of farmed fish, a scientific opinion on the general approach to fish welfare and specifically on the concept of sentience in fish was requested

## ACKNOWLEDGEMENTS

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[3] Recommendation concerning farmed fish adopted by the Standing Committee of the European Convention for the protection of animals kept for farming purposes on 5 December 2005

[4] Opinion adopted by the AHAW Panel related to the welfare of animals during transport -30 March 2004

[5] Opinion of the AHAW Panel related to welfare aspects of the main systems of stunning and killing the main commercial species of animals- 15 June 2004

## INTRODUCTION

The Treaty of Amsterdam, which came into force in May 1999, reflects the concerns of the public about the welfare of sentient animals, including fish, farmed for food and hence there is a requirement for a scientific basis for the evaluation of animal welfare. On an international level, the OIE (World Organisation for Animal Health) is also concerned with fish welfare and aims to harmonise standards throughout its 172 member countries. Animal welfare has been defined and this definition adopted by a formal OIE General Session Resolution as presented below, which may be applicable to all the species of animals farmed for food.

The OIE Resolution (Article 7.1.1 of the Terrestrial Code) reads: "Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour and it is not suffering from unpleasant states such as pain, fear and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter / killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment."

For some farmed animals there is a significant body of scientific literature that can be drawn upon to assess or measure (directly or indirectly) the impact of various husbandry systems on the welfare of the animals concerned. However, for fish there are few such data owing to the fact that the welfare of intensively farmed fish has not been investigated to the same extent as that of terrestrial farm animals. Nevertheless, it is emphasised that absence of sound scientific evidence at present should not be seen as evidence of absence of suffering in farmed fish, and this fundamental principle is entrenched in the Treaty.

It is important to mention that the term "fish" covers a wide and very disparate group of vertebrates. The three major groups of fish are: Agnatha (hagfish, lampreys), Chondrichthyes (sharks, rays, sturgeons) and Actinopterygii (bony fish with teleost being the most prevalent). Most aquaculture finfish species are teleostean fish (Evans et al., 2005). There are more than twenty thousand living species of teleosts that have been evolving over 500 million years. They represent every aquatic environment and a vast range of physiological and behavioural traits. Only a small number of species, from diverse groups, generally those of economic importance for fisheries or aquaculture, have been studied in any scientific detail. It is therefore important that generalisations across the range of species should not be used without qualification.

The welfare of different species of farmed fish and the various farming systems used in Europe have been described in recent EFSA reports (EFSA 2008 a, b, c, d, e).


## 1.   Scope and objectives

The scope of this opinion is limited to exploring, based on available scientific literature, the neurobiology and special sense organs in fish, some of which are unique to fish and some occurring in other vertebrates, and their capacity to experience pain, fear and distress, expressions of sentience from a fish welfare point of view.

Officially recognised nomenclatures of brain regions and neuronal groups have been harmonised, to a certain extent, between mammals and birds but not between mammals or birds and fish. However, comparative brain structures between mammals and fish are provided to facilitate our understanding of the commonality and to stimulate further studies.

The concept of welfare is the same for all farm animals, i.e. mammals, birds and fish, used for human food and given protection under the Treaty of Amsterdam. Fish welfare however has not been studied to the same extent as terrestrial farm mammals and birds, neither welfare concepts nor welfare needs have been clearly understood for the various species of farmed fish. In the absence of studies and understanding of the needs of farmed fish some argue that the species specific welfare needs of individuals could be deduced from our understanding of the wild fish species. Whereas others argue that the requirements for farmed fish are different. Welfare of an animal refers to its physical, physiological and mental states. It is acknowledged that in a population when the welfare needs of individuals are met then welfare in the population could be considered good.

Where welfare is referred to as good within the scope of EFSA assessments, it implies a state that is positive for an individual and by assumption for the population as a whole. Where welfare is referred to as poor this indicates that individuals have negative states.

## 2. Welfare concepts

Animal welfare concepts include physical, physiological and mental states of individual animals. Various animal welfare concepts have been presented in the scientific literature and some are listed below. It is worth noting that scientists do not have tools to measure directly the mental states of an animal, unlike physical and physiological measures such as blood constituents, including the state of consciousness. However, an improved knowledge of neuroanatomy, physiology and species specific behaviours of animals can contribute to a better understanding.

Terrestrial farm animal welfare concepts that would ensure positive physical, physiological and mental states are encompassed in what is known as the Five Freedoms, as proposed in 1979 by the Farm Animal Welfare Council (FAWC) in the UK: i) Freedom from Hunger and Thirst - by ready access to fresh water and a diet to maintain full health and vigour; ii) Freedom from Discomfort - by providing an appropriate environment including shelter and a comfortable resting area; iii) Freedom from Pain, Injury or Disease - by prevention or rapid diagnosis and treatment; iv) Freedom to Express Normal Behaviour - by providing sufficient space, proper facilities and company of the animal's own kind; v) Freedom from Fear and Distress - by ensuring conditions and treatment which avoid mental suffering.

Fraser (Fraser, 1999) discussed various animal welfare concepts and divided them into animal function, animal feelings and how natural the husbandry system or conditions were. Feelings or experiences cannot be measured directly but may be inferred from measurements of physiology and behaviour and are a component of coping systems (Cabanac, 1979; Broom, 1998; Panksepp, 1998). Observations on animals in the wild are not involved in welfare assessment, but give a guide as to animals" likely functioning when removed from the environment in which they have evolved.

Welfare is a characteristic of an individual animal and is concerned with the effects of all aspects of its genotype and environment on the individual (Duncan, 1981). Broom (Broom, 1986) defined welfare as follows: "the welfare of an animal is its state as regards its attempts to cope with its environment". According to this definition, an animal"s welfare depends on the ease or difficulty of coping and also the extent of any failure to cope, which may lead to disease and injury. Furthermore, welfare also includes pleasurable mental states as well as unpleasant states such as pain, fear and frustration (Duncan, 1996; Fraser and Duncan, 1998).

MacIntyre et al. (MacIntyre et al., 2008) suggested that animal health is a central tenet of animal welfare. But a potential problem with this approach is that good animal health is only one facet of animal welfare concepts, for example, according to the five freedoms as proposed by the FAWC. The problem is further confounded by the fact that animals in good health could have poor welfare in some other way, e.g. feel deprivation due to an inability to perform species specific behaviours and *vice versa*. Animal health is also one of the welfare constituents in the recently adopted OIE resolution. The concept of animal welfare „needs" is a novel approach to defining species specific welfare requirements. This concept has been developed as an alternative to five freedoms proposed for terrestrial farm animals (FAWC, 1979). Such welfare needs are not clearly defined for fish; however, they can be directly related to basic physiological requirements that need to be constantly satisfied. Some of these are essential for life (vital needs) and include food and environmental factors such as water of good quality. Other welfare requirements include the ability to perform species specific behaviours which may vary according to the stage of development and situations (Jensen and Toates, 1993), for example, sows and hens need materials for and the opportunity to build nests (Van de Weerd et al., 2006). Such experiments have rarely been conducted in fish to reveal species specific behavioural requirements (Kiessling et al., 2006). In the absence of strong scientific evidence however it is reasonable to infer that a failure to meet such basic needs in some way may contribute to poor welfare. The concept of welfare needs/requirements of fish is important and developing.

Welfare refers to the state of an individual, when welfare needs of individuals in a population are met then welfare of the population can be considered to be good.

## CONCLUSION

The concept of welfare is the same for all the animals, i.e. mammals, birds and fish, used for human food and given protection under the Treaty of Amsterdam. However, fish welfare has not been studied to the same extent as mammals and birds.

## 3.    Welfare assessment

Welfare assessment should be based upon a thorough understanding of the biology of the species and the related needs and requirements of a farmed species. For example welfare can be assessed by measuring the non-fulfilment of these needs, or it may be assessed on the basis of deviation from normality. Poor welfare can be assessed by how far an individual animal has deviated from what is normal for animals in that environment and by comparing it with other kinds of animals kept in the same or a similar environment (Morton and Griffiths, 1985). To understand, compare and develop actions to improve fish welfare, defined protocols of welfare measures or indicators are needed. The scientific assessment of farmed fish welfare has been specifically addressed by (Huntingford et al., 2006). Some welfare research in terrestrial farm animals involves measuring direct indicators of welfare while other research evaluates what is important to animals by studies demonstrating positive preferences and motivation (Dawkins, 1990). Along with these one can also measure aversion i.e. negative preferences and how hard an animal will work to avoid, as opposed to access, an environmental variable. A limited amount of work on preferences and motivation has been conducted with fish (Yue et al., 2004; Yue et al., 2008). It has been recently demonstrated that significant differences in behavioural responses to a nociceptive event exist between different species of teleosts (Reilly et al., 2008).

Measures of physiological functioning, productivity, health and pathology and behaviour all form the basis of welfare assessment currently being used in field conditions. As an example, measuring disease resistance or the functioning of the immune system may offer one way of estimating the welfare "cost" of certain intensive fish farming conditions. Compromised immune performance can lead to disease outbreaks with associated direct negative welfare consequences. Moreover, lowered disease resistance is generally a consequence of maladaptive physiological stress, and disease challenge testing may therefore also be an indirect measure of such stress conditions.

Fish in a natural habitat display complex swimming, feeding, anti-predator and reproductive behaviours. Such behavioural traits are linked to genetic differences between species and individual animals, and are modified by the environment and learning through experience. Fish behaviour studies and analysis of behavioural responses exhibited by fish exposed to stressors are mostly devoted to fish in their natural environment (Schreck et al., 1997). Fewer studies have looked at fish behaviour in production systems, e.g. feeding behaviour (Volkoff and Peter, 2006), social interaction and hierarchies (Gilmour et al., 2005), which are considered to be important for fish welfare.

Due to the complex causal relationships among the various needs of farmed fish and their behavioural and physiological consequences, it is impossible to find one single measurement or welfare indicator that will cover all possible rearing systems, farmed species and potential situations. When the welfare of fish or other animals is assessed, sets of measures, which might be physiological (Oliveira R. F. et al., 1999; Ellis et al., 2004), behavioural or pathological (Huntingford et al., 2006) may be used alone or in combinations. Whilst a single measure could indicate poor welfare, a range of measures will usually provide a more accurate assessment of welfare because of the variety of coping mechanisms used by the animals (Koolhaas et al., 1999; Huntingford and Adams, 2005) and the various effects of the environment on individual species of fish.

Production variables might have a place in welfare assessment and a failure of fish to feed and grow often indicates poor welfare. However, high performance levels (e.g. high feed intake and good growth) do not necessarily indicate good welfare. Once again, provision of a nutritionally balanced feed is only one facet of welfare concepts or needs/requirements. In regard to mortality, at a population level, incidence of mortality may be a useful non-specific indicator of extremes of poor welfare.

Welfare indicators at the individual level refer to measurements on individual fish in a system, either by non-invasive monitoring in free-swimming fish, or with targeted sub-sampling of fish. Examples of individual measures include fin condition and parasite load. Representative sub-samplings are difficult in large farming systems, but can work well in smaller systems. The individual indicators commonly relate to the ability of the fish concerned to maintain a species specific normal physiological and behavioural state, including the ability to mount effective immune responses.

The fishes comprise the largest and most diverse vertebrate group. Therefore scientific evidence in one species does not necessarily apply to other species unless they are closely related.

**CONCLUSIONS**

Whilst similar measures of welfare developed for other animals are often relevant to fish, clearly defined protocols for fish welfare evaluation are lacking.

Due to the complex relationships among the various needs/requirements of farmed fish and their behavioural and physiological consequences, as for all animals it is impossible to find one single measurement or welfare indicator that will cover all possible husbandry systems, farmed species and situations.

## RECOMMENDATION

A range of welfare indicators should be considered when welfare is being evaluated. Indicators of fish welfare should be species-specific, validated, reliable, feasible and auditable.

## 4. Neurobiology and special sense organs

### 4.1. Sensory systems in fish

Fish perceive optical, positional, chemical, tactile, mechanosensory and electrosensory (lateral line), acoustic, and magnetic stimuli by receptors innervated by particular brain regions (Hodos W. and Butler A.B., 1997). Basic patterns of sensory innervations are common to all vertebrates for the relay of sensory inputs from all the different sensory receptor systems up to the brain and for the relay of motor output from the brain to motor cells. The specific sensory systems are discussed below.

The optical characteristics of water affect illumination intensity and spectral quality. This has led to the evolution of the fish eye to cope with these challenges and is thus rather different from other vertebrate eyes (Warrant and Locket, 2004). Light focused by the lens is transformed into neural signals in a similar manner to all other vertebrates via the specialised protein opsin in combination with the chromophore. The photic information is transformed by a complex retinal circuit to form receptive fields that begin to analyse the image. The information is conveyed to the optic tectum of the midbrain via the optic nerves where a retinotopic map of visual space is preserved across the tectum. They do not have eyelids or nictitating membranes and the large choroidal complexes are subject to pressure changes and to gaseous embolism. Thus the fish eye is particularly vulnerable to a variety of environmental effects which may happen in husbandry systems leading to poor welfare (Roberts, 2001).

Sound and vibrations travel well in water and fish are highly responsive and potentially sensitive but it is not known if it implies conscious or unconscious process. The ear of bony fish comprises three semi-circular canals, a utricle and a sacculae and lagena. The auditory receptors comprise a variable set of sensory organs that perceive sound from the environment. The ascending auditory pathways in mammals and fish are similar. The vestibular system of vertebrates detects position and motion of the head and is important for equilibrium or balance and coordination of head, eye and body movements. The semicircular canals and the otolith organs (utricle and sacculae) in fish are also used as the vestibular organs. Hair cells in the semi-circular canals detect rotational and angular acceleration and the information is conveyed to the brain via the vestibular nerve. The otolith organs detect the position of the head in relation to gravity and linear acceleration. In the brain of all vertebrates, vestibular information is conveyed to the cerebellum and the vestibular nuclei in the brain stem.

Fish have a highly elaborate chemosensory detection of information from the environment. The structural organisation of the peripheral olfactory organ is variable throughout fish species, although the ultra structural organisation of the olfactory sensory epithelium is extremely consistent (Hara, 1994). The olfactory receptor cells are bipolar neurones directly exposed to

the environment and signals are sent to the brain by its own axon (Cranial Nerve I). Four major classes of chemicals have been identified as specific olfactory stimuli and their stimulatory effectiveness characterized: amino acids, sex steroids, bile acids/salts and prostaglandins (Hara, 1994). Olfactory signals such as those involved in reproduction and feeding may be processed independently through two distinct subsystems (Laberge and Hara, 2001; Nikonov et al., 2005). The neuronal components are similar to the olfactory systems of mammals except that there is no connection between respiratory structures and the olfactory system in fish. As in terrestrial vertebrates the taste buds of fish are the receptors of the gustatory or taste organ. Taste buds are present in the oropharyngeal cavity and on the whole of the body surface (Hara, 1994). Chemical information detected by gustatory cells, is transmitted to the brain via the cranial nerves VII (facial), IX (glossopharyngeal), and X (vagal). In the brain, the facial and vagal lobes receive input from these cranial nerves and this is conveyed to the pons in the hindbrain. The information then ascends to the midbrain (hypothalamus) and to the telencephalon in the forebrain. In mammals, the gustatory input is via the same cranial nerves but fish lack the brainstem gustatory nucleus present in the mammalian hindbrain. Fish gustatory receptors detect amino acids, various organic acids, nucleotides and bile salts (Hara, 1994).

The lateral line system detects movement and vibration and in some cases electrical information or impulses and is found in all fishes and some amphibians but has been lost in reptiles, birds and mammals. The sensory organ consists of hair cells called neuromasts located in the lateral line canals or on the head and body. The lateral line system allows fishes to respond to water movements and other movements relatively close to the fish. This system alerts fish to prey, predators, school neighbours, water flow from environmental obstacles and in salmon reproductive vibrations (Satou M. et al., 1994) that facilitate orientation behaviour (Montgomery et al., 1997).

Some fish species have developed electroreception, as part of adaptation to living in turbid environments. Weakly electric fish produce electric signals with a specialised organ in their tail (Von der Emde, 1999). They are also electrosensitive and can perceive their self-generated signals for electrolocation and signals from other electric fishes. Many fish species are capable of detection but not production of electrical signals using electroreceptors. Electrosensitive marine fish might sense the geomagnetic field through electromagnetic induction to allow navigation during migration, although definitive evidence that such fish actually do so has not yet been obtained (Lohmann and Johnsen, 2000). The mode of transduction for the magnetic sense remains unknown. However, magnetite particles embedded in specific cells in the basal lamina within the olfactory lamellae of rainbow trout, *O. mykiss*, have been identified (Walker et al., 1997).

## CONCLUSION

Different species of fish have evolved highly sophisticated sensory organs to survive in changing and varied environmental conditions. Some of these sensory organs are absent in mammals, for example electroreceptors and the lateral line system.

## 4.2.  Brain structure and functional similarities

As vertebrates, fish, birds and mammals share a similar general brain structure. The fish brain consists of the forebrain (i.e. telencephalon and diencephalon), the midbrain (mesencephalon), and the hindbrain (rhombencephalon). Thus, the general anatomy of the teleost (bony) fish brain is similar to that of other vertebrate brains. However, the fish brain is smaller relative to

body size and less complex in structure than that of higher vertebrates (Kotrschal et al., 1998). Fish do not have the extensive cerebral cortex that mammals have, this being smaller relative to body size and without the characteristic folded (convoluted) and layered appearance of the mammalian cortex. The pallium (the grey matter that covers the telencephalon) has thickened to various extents in different classes of vertebrates, and in mammals it consists of a laminated structure, the cerebral cortex (Striedter, 1997). The possibility cannot be excluded, however, that parts of the brain other than the cerebral cortex have evolved the capacity for generating emotional states in fish (Huntingford et al., 2006).

The fish brain grows continuously, throughout life. The fish neurons are constantly replaced and the number of cells can increase if specific behavioural stimulations are given (Dunlap et al., 2006; Kihslinger et al., 2006; Kihslinger and Nevitt, 2006; Lema and Nevitt, 2006; Remage-Healey and Bass, 2007). Among fish there is a marked inter species variation in brain anatomy, often reflecting sensory specialization, fundamental differences in embryonic development, or the degree of cell migration and proliferation (Butler, 2000).

In vertebrates, specific brain structures have been identified as being associated with emotions and motivated behaviour. It is now indicated that the same function can be served by different brain structures in different groups of animals, e.g. cognitive functions in birds and mammals, (Jarvis et al., 2005). Comparative anatomical studies have shed some light on the potential functional role of fish brain structures in relation to motivational and affective states (Portavella et al., 2002). Sensory processing is carried out in different regions of the brain according to adaptations of the particular group of fishes (Rose, 2002; Vogt et al., 2003).

At the level of the telencephalon, since fish lack the higher cortical centres it has been argued that they may not experience pain as mammals (Rose, 2002). Neuronal pathways that connect to various forebrain structures are of fundamental importance to consciousness and the perception of pain and fear in mammals (Willis and Westlund, 1997), extensive interconnections exist between the telencephalon, diencephalon and mesencephalon in fish (Rink and Wullimann, 2004). In the majority of fish species, the pallium is unlaminated (Vogt et al., 2003), however there is evidence to suggest it has developed into a highly differentiated structure with respect to the processing of sensory information (Bradford M. R., 1995; Butler, 2000). The telencephalon in fish contains several brain structures that may be functionally homologous to those associated with pain and fear in higher vertebrates (Bradford M. R., 1995; Chandroo et al., 2004; Portavella et al., 2004), and known to be active after a noxious stimulus such as pin-prick stimuli in rainbow trout or goldfish (Dunlop and Laming, 2005). Information about noxious stimuli in fish may be processed in a functionally homologous area, not yet fully characterised, to that involved in pain processing in mammals. In mammals, the hippocampus, a telencephalic structure, is involved in memory and learning of spatial relationships whereas the amygdala, a structure which is also telencephalic, has long been known to be important in arousal and emotions, particularly fear responses (Carter, 1996; Maren, 2001). Recent studies have identified structures in the teleost telencephalon that appear to be homologous to the mammalian amygdala and hippocampus with alterations in fear, spatial learning and memory retrieval when these areas are lesioned (i.e. destroyed or made dysfunctional) (Portavella et al., 2002). Another important structure in the fish brain, the hypothalamus, is thought to perform functions similar to those of the hypothalamus in other vertebrates. The hypothalamus is involved in various functions, including sexual and other social behaviour, and is also responsible for the integration of both internal and external signals including those originating from those telencephalic areas that have been implicated in fear responses (Fox et al., 1997; Portavella et al., 2002; Chandroo et al., 2004).

The dorsomedial (Dm) telencephalon in fish has been implicated in emotional learning and is thought to be homologous to the amygdala in mammals (Bradford M. R., 1995; Butler, 2000; Portavella et al., 2004). In mammals the hippocampus is involved in memory and learning of spatial relationships and it is the dorsolateral (Dl) telencephalon in fish that is thought to be functionally homologous to the hippocampus. Dm lesions impaired acquisition of an avoidance response but had no effect on performance in a spatial learning task, while Dl lesions affected spatial learning but did not impair the acquisition of the avoidance response (Portavella et al., 2002). Therefore Dm and Dl areas of the fish telencephalon share functional similarities with the amygdala and hippocampus, respectively, in mammals.

Officially recognised nomenclatures of brain regions and neuronal groups have been harmonised between mammals and birds (Reiner et al., 2004) but not between mammals and fish.

**CONCLUSION**

There is scientific evidence to support the assumption that some fish species have brain structures potentially capable of experiencing pain and fear.

## 5. Pain

The concept of pain in vertebrates revolves around the perceived noxiousness of certain stimuli, which may have been conserved through evolution as a protective strategy.

Pain is defined as an aversive sensation and feeling associated with actual or potential tissue damage. The International Association for the Study of Pain (IASP, 1979) definition also highlights that pain is both a sensory and emotional experience in humans. It could therefore be argued whether animals, especially fish, have the conscious emotional states needed to experience pain in a similar manner to humans.

It has been suggested that this difficulty could be overcome if one is prepared to accept scientific reasoning that consciousness evolved gradually, and that different species of animals would therefore have different degrees or qualities of consciousness (Duncan, 1996; Århem and Liljenström, 1997). It has been stated that „Experiencing emotional states is not dependent on higher level consciousness (as found in humans and possibly some animal species such as the great apes), but on more basic forms of consciousness. Possession of these forms of consciousness is thought necessary for certain types of ability and behaviours, and it is therefore possible to determine which species of animals possess these characteristics" (Flecknell, 2008). Understandably, based on this principle, scientists have been evaluating and developing anaesthetics, analgesics and euthanasia agents for various fish species (http://www.nal.usda.gov/awic/pubs/Fishwelfare/anes.htm).

As pain is thought to have evolved as a protective mechanism to avoid tissue injury and is widely conserved in vertebrates, it has to be capable of generating an avoidance/withdrawal response if it is to have that function.

A number of criteria have been defined to provide a guide as to whether an animal, including fish, might be capable of experiencing pain (Bateson, 1991; Broom, 2001a; Broom, 2001b; Sneddon, 2004) these include:

i) the existence of functional nociceptors,

ii) the presence and action of endogenous opioids and opioid receptors,

iii) the activation of brain structures involved in pain processing,

iv) the existence of pathways leading to higher brain structures,

v) the action of analgesics in reducing nociceptive responses,

vi) the occurrence of avoidance learning,

vii) the suspension of normal behaviour associated with a noxious stimulus.

Nociception is the detection of a noxious stimulus and is usually accompanied by a reflex withdrawal response away from that stimulus immediately upon detection. Noxious stimuli are those that can or potentially could cause tissue damage so stimuli such as high mechanical pressure, extremes of temperature and chemicals, such as acids, venoms, prostaglandins and so on, excite nociceptive nerve fibres. (Martin and Wickelgren, 1971) and (Matthews and Wickelgren, 1978) identified sensory neurones in the skin and mouth of a lamprey (*Petromyzon marinus*) during heavy pressure, puncture, pinching or burning, and found that the output was like that which would be recorded in a mammalian nociceptor when responding to a painful stimuli. Studies of nerve responses, nerve and other tissue regeneration, behavioural responses and effects of analgesics indicate nociceptive function in the fins of salmonid and other fish (Becerra et al., 1983; Turnbull et al., 1996; Chervova, 1997). Studies of the rainbow trout *Oncoryhnchus mykiss* have shown that nociceptors are present on the trout face and are innervated by the trigeminal nerve (Sneddon, 2002; Sneddon et al., 2003a). These studies on nociceptor anatomy and physiology strongly support the hypothesis that the rainbow trout has the sensory equipment for detecting potentially painful stimuli. Fish have the necessary brain areas for nociceptive processing to occur (e.g. pons, medulla, thalamus (Sneddon, 2004).

Some argue that fish lack the neocortex, which plays a key role in the subjective experience of pain in humans (FSBI, 2002; Rose, 2002) whereas, others have suggested that it is possible that parts of the brain other than the neocortex have evolved the capacity of generating emotional states, including pain, in fish (Huntingford et al., 2006). In teleosts, the rudimentary cortex or the cerebral hemispheres possess complex projections to the diencephalon and midbrain (Northcutt, 1981). Recently, electrophysiological recordings have measured electrical activity in the forebrain of rainbow trout, and goldfish, (*Carassius auratus*) during noxious stimulation that differed from neutral stimulation (Dunlop and Laming, 2005). These can be seen as evidence to support nociception in various fish species.

In fish, as in other vertebrates, nociceptive information is relayed to the brain from the periphery via two major tracts. The trigeminal tract conveys information from the head while the spinothalamic tract conveys information from the rest of the body. In fish the trigeminal has been shown to project to the thalamus as it does in other vertebrates. The elasmobranch (Ebbesson and Hodde, 1981) and teleost groups (Goehler and Finger, 1996; Finger, 2000), both have the same basic components of ascending spinal projections as higher vertebrates.

The possession of opioid receptors, endogenous opioids and enkephalins is one of the requirements to determine whether nociception can occur in an animal (Bateson, 1991; Broom, 2001a; Broom, 2001b). These substances are involved in analgesia in the central nervous system of vertebrates and are produced in the brain in order to reduce pain. Met-enkephalin and leu-enkephalin are present in all vertebrates which have been tested and there are at least six opioid receptors described for teleost fish (Dores and Joss, 1988; Dores et al., 1989; Dores and Gorbman, 1990; McDonald and Dores, 1991). Opioids elicit anti nociception or analgesia through three distinct types of receptors in mammals (Newman et al., 2000) and these have been identified in the zebrafish, *Danio rerio* (Stevens, 2004). When goldfish are subjected to stressful conditions, there is an elevation of pro-opiomelanocortin, the precursor of the enkephalins and endorphins, just as there would be in humans (Denzer and Laudien, 1987).

Goldfish which are given electric shock show agitated swimming but the threshold for this response is increased if morphine is injected. It was also demonstrated that naloxone (opioid antagonist) blocks the morphine effect (Jansen and Greene, 1970). Work by Ehrensing et al., (1982) showed that the endogenous opioid antagonist MIFI down-regulates sensitivity to opioids in goldfish. Opiate receptors and enkephalin like substances have also been found in various brain areas of goldfish, *Carassius auratus* (Finger, 1981; Schulman et al., 1981) and rainbow trout, *O. mykiss* (Vecino et al., 1992). The distribution of enkephalins in the fish brain shows a similar pattern to that seen in higher vertebrates (Simantov et al., 1977; Vecino et al., 1992). Together, these studies indicate that the brain opioid structures are similar between fish and other vertebrates.

A simple reflex response to a noxious stimulus can indicate nociceptive function, however, adverse effects on an animal"s normal behaviour beyond a simple reflex may indicate a psychological component that is indicative of suffering, and suggests that the animal may be perceiving pain. Reflex responses occur instantaneously and within a few seconds but some of the responses of fish may be prolonged to 3 to 6 hours (Sneddon, 2006). A study investigated the behavioural response of rainbow trout which had been given subcutaneous injections of acetic acid and bee venom (algesics) to the lips (Sneddon et al., 2003a). These fish showed an enhanced respiration rate for approximately 3 hours, did not feed within this period, and showed anomalous behaviours such as rubbing of the affected area on the aquarium substratum and glass and rocking from side to side on either pectoral fin (Sneddon et al., 2003a; Sneddon et al., 2003b). These, therefore, appear to represent changes in behaviour over a prolonged period as a result of nociception.

The ability of analgesics to modulate nociception is also indicative of pain perception since they selectively act on this system. The adverse behavioural responses seen in the rainbow trout, *O. mykiss*, were quantified and when morphine was administered to fish injected with acid, there was a dose-dependent reduction in this rubbing behaviour as well as rocking behaviour and the enhanced respiration rate was also ameliorated (Sneddon et al., 2003a; Sneddon et al., 2003b). Further to this, acid injected fish did not show an appropriate fear response to a novel challenge supporting the idea that this painful stimulus dominates the fish attention (Sneddon et al., 2003b). Studies have shown that goldfish are able to learn to avoid noxious stimuli such as electric shock (Portavella et al., 2002; Portavella et al., 2004). Learned avoidance of a stimulus associated with a noxious experience has also been observed in other fish species (Overmier and Hollis, 1983; Overmier and Hollis, 1990) including common carp, and pike, avoiding hooks in angling trials (Beukema, 1970b; Beukema, 1970a).

There are strong debates on the question of pain in fish with opposing views (Rose, 2002; Sneddon, 2004; Sneddon, 2006). They also suggest that there is an important difference between knowledge about sensation and sentience (Derbyshire, 2008). All fish which have been investigated, including the Agnatha, and teleosts, have nociceptors and these look like and have a similar response profile to those of birds and mammals. Elasmobranchs and teleosts also possess the neurotransmitters –characteristically found in pain systems and opioids, including those that serve an analgesic function in humans and appear to act in the same way in fish. Behavioural and neurophysiological studies of several teleost species show that responses, behavioural avoidance and learning from experience of tissue damage occur in a similar way in fish and mammals. It appears that fish fulfil the criteria for animal pain and there is the potential for pain perception in fish. However, we do not have sound scientific evidence to ascertain the exact stage of development or life form at which the neuronal capacity to perceive nociception is adequate or fully developed in various species of fish.

## CONCLUSION

The balance of evidence indicates that some fish species have the capacity to experience pain

## RECOMMENDATION FOR RESEARCH

Research and developments in the area of cognition and brain imaging techniques should be carried out in fish to further our knowledge and understanding of pain perception.

## 6. Fear

Fear serves a function that is fundamental to survival and is the activation of a defensive behavioural system that protects animals against potentially dangerous environmental threats. In higher vertebrates fear involves mainly the amygdaloid and hippocampal regions of the brain although other areas are also implicated. Studies in fish have shown that these responses also appear to be dependent upon cognitive mechanisms and homologous limbic brain regions in the telencephalon.

Studies on fear conditioning in mammals measure levels of freezing and startle behaviour (Fendt and Fanselow, 1999). In fish, a number of different behavioural responses to potentially threatening stimuli have been described, depending upon the species, and include escape responses such as fast starts (Domenici and Blake, 1997; Chandroo et al., 2004; Yue et al., 2004) or erratic movement (Cantalupo et al., 1995; Bisazza et al., 1998), as well as freezing and sinking in the water (Berejikian et al., 1999; Berejikian et al., 2003). Such behaviours may serve to protect the individual species from the threat and a number of studies have illustrated that these behaviours can be shown in response to conditioning. Many fish species also release chemical alarm substances when injured. These are thought to act as warning signals, as conspecifics show a behavioural fright response to these chemicals (Smith, 1992; Lebedeva and Golovkina, 1994; Brown and Smith, 1997; Berejikian et al., 1999). These alarm behaviours include dashing movements, vigorous movements in the aquarium substratum, and fast swimming towards hiding places, remaining there for an extended period. These behaviours are thought to be associated with predator evasion (Hamdani et al., 2000). Learned avoidance studies not only show that a consistent range of behaviours are produced in response to fearful stimuli in fish but they also provide evidence that the displayed behaviour is not merely a reflex response. Studies in rainbow trout have shown that they learn to avoid an aversive stimulus indicating the existence of a cognitive process to be able to experience fear (Yue et al., 2004; Yue et al., 2008).

Learning is thought to be mediated in part by receptors in the brain that are activated by N-methyl-D-aspartic acid (NMDA). Administration of selective antagonists of NMDA receptors impair learning mechanisms such as associative learning and conditioned fear in mammals (Miserendino et al., 1990; Kim et al., 1991; Sanger and Joly, 1991; Maren, 2001). Experiments with goldfish have shown that intracranial administration of MK-801, an NMDA receptor antagonist, blocks specific aspects of Pavlovian fear conditioning in fish (Xu and Davis, 1992).

## CONCLUSION

Defence and escape behaviours are dependent on cognitive and learning abilities related to fear. Responses of fish, of some species and under certain situations, suggest that they are able to experience fear.

## 7.  Stress response

The response to stress is considered to be an adaptive mechanism that allows the fish to cope with stressors in order to maintain homeostasis, which is beneficial to the survival of the species. If the impact of the stressors is severe or long lasting, disturbances of homeostasis can become detrimental to the fish‟s welfare leading to a state associated with the term „distress‟ (Wendelaar Bonga, 1997; Barton, 2002; FSBI, 2002), reducing fitness (Broom and Johnson, 1993) and eventually death. The state of distress and reduced physiological fitness are indicative of poor welfare. During the last 20 years, there has been extensive research devoted to improving our knowledge and understanding of the biology of stress in fish. Physiological and behavioural responses to a large variety of physical, chemical and biological stressors including those seen in aquaculture have been measured (for review see (Iwama et al., 1997; Wendelaar Bonga, 1997; Barton, 2002; FSBI, 2002; Conte, 2004; Ashley, 2007). The first response to any stressor is the Exogenous Chaperone or Heat Shock Protein (HSP) response. This rapid synthesis of a suite of chaperonin proteins known as HSP‟s are generated by the host in response to exposure to any environmental or other stressor occupying a central role in the management of stress at the cellular, organism and population level (Srivastava, 2008). They are highly conserved and have been described for many aquatic species including salmonids and cyprinids (Heikkila et al., 1986; Mitani, 1989). The genes responsible for stress proteins in rainbow trout have been identified and sequenced and shown to be similar to those of mammals (Zafarullah et al., 1992). Since it is a consistent, early and readily measurable effect, it has been suggested that HSP levels can, with care, be used as indicators of individual stress levels in aquatic animals (Sanders, 1993). Hypothalamic-pituitary-interrenal (HPI) responses (which are homologous to mammalian Hypothalamic-Pituitary-Adrenal (HPA) responses) are generally considered as an adaptive strategy to cope with a disturbance to homeostasis. For example, poor water quality elicits a stress response in fish (Conte, 2004). Although fish are able to tolerate poor water quality conditions for a short period, depending upon the species, when the conditions become too challenging or prolonged, fish cannot maintain homeostasis and experience chronic stress which in the long term can impair immune function, growth and reproductive function. Furthermore, chemicals may have toxic effects at the level of cell and tissue but, in addition, elicit an integrated stress response which may be specific to the toxicant (Wendelaar Bonga, 1997).

The stress physiology of fish is directly comparable to that of higher vertebrates and in fish the physiological responses are manifested as primary, secondary and eventually tertiary stress responses (Wedemeyer et al., 1990; Wendelaar Bonga, 1997; FSBI, 2002; Ashley, 2007). The primary stress response to potentially harmful situations involves amongst other things the release of catecholamines (adrenaline and noradrenaline) from the chromaffin cells into the circulating system. Simultaneously, activation of the hypothalamic-pituitary-inter-renal (HPI) axis is observed, corticotrophin releasing factor (CRF) is released from the hypothalamus and acts on the pituitary resulting in the synthesis and release of adrenocorticotrophic hormone (ACTH) which in turn stimulates the synthesis and mobilisation of glucocorticoid hormones (e.g. cortisol) from the inter-renal cells. Released catecholamines and cortisol will result in an

activation of various physiological and behavioural mechanisms that constitute the secondary and possibly tertiary stress responses. The secondary changes include alteration of secretion of other pituitary hormones and thyroid hormones, changes in turn-over of brain neurotransmitters, mobilisation of energy by breakdown of carbohydrate and lipid reserve and by oxidation of muscle protein, improvement of respiratory capacity via increased heart stroke volume and increase blood flow to gills. As a consequence of this last effect, disruption of the hydromineral or osmoregulatory balance can occur. Tertiary stress responses also include reduction in immune function and disease resistance (Pickering, 1992; Balm, 1997), in growth (Barton et al., 1987; Pickering, 1992), and in reproduction (Pankhurst and Kraak, 1997; McCormick, 1998; Schreck et al., 2001). Some of these stress responses have been used to study welfare in certain fish species.

Behavioural responses can be seen as the first line of defence against adverse environmental changes, often triggered by the same stimuli that initiate the primary physiological stress responses. The exact behavioural response depends on the stressor concerned. For example, the response to an approaching potential predator might be flight, whereas the response to an approaching competitor might be attack. The behavioural response to abiotic environmental stressors, such as water temperature, oxygen levels or water current, includes a range of responses in movement pattern, spatial choice and social interactions, but these responses are poorly studied and/or described in most fish species. In addition, individuals of the same species may differ in the nature and magnitude of their behavioural responses to various stressors. Such behavioural differences, together with the physiological variation with which they are associated, are referred to as coping strategies. Some individuals adopt what is called a proactive coping strategy, showing adrenaline-based fright and flight responses, while others adopt a reactive coping strategy, showing cortisol based "freeze" and hide responses (Korte et al., 2005). These differences are correlated with variation in brain serotoninergic activity (Schjolden and Winberg, 2007) and are also affected by the extent of exposure to stressors.

Chronic stress is a major factor affecting the health of fish. As in mammals, the best link between stress and immune status arises through effects of cortisol which can suppress many aspects of the immune system (Wendelaar Bonga, 1997). However, the relationship between stress and immune function goes in two directions since components of the immune system can influence stress responses through modification of the secretion of hormones (Ottaviani and Franceschi, 1996; Balm, 1997). While disease is not always connected to poor environmental conditions (Huntingford et al., 2006), aquaculture practice presents many situations where stress and physical injury can strongly increase susceptibility to naturally occurring pathogens (Ashley, 2007). For example, diseases associated with low temperatures over winter period have been described in a number of different species (Tort, 1998). Fin erosion, also an important problem in aquaculture often occurs as results of aggressive interactions, leading to chronic stress and may increase susceptibility to infections (Turnbull et al., 1996). Fin erosion involves damage to live tissue containing blood vessels, nerves and nociceptors, which induces an inflammatory response and in some cases may result in secondary infections (St-Hilaire et al., 2006). An example of the strong interaction between environmental stress and a serious infectious disease is furunculosis. Many fish may carry the causative pathogen but clinical outbreaks occur normally after stressful events such as grading or transportation of fish. So predictable is the response that a predictive test for identifying carrier populations is the „furunculosis stress test‟ where samples of healthy fish are injected with cortisone to identify those populations in which some individuals might develop clinical disease if stressed (Hiney et al., 1994).

As in terrestrial vertebrates measurements of the levels of glucose and lactate in the plasma have been used as biomarkers of stress in fish (Arends et al., 1999; Acerete et al., 2004).

Measures of the expression of stress related genes might also provide useful markers (Gornati et al., 2004). Chronic stress has been also studied and exerts a strong effect on haematology (Montero et al., 2001), metabolism (Mommsen et al., 1999), neuroendocrine function (DiBattista et al., 2005), and osmoregulation (Wendelaar Bonga, 1997). However, reliable indicators of chronic stress are still under investigation and will probably rely on a range of measurements.

Avoidance of the maladaptative consequences of prolonged stress is a central concern in aquaculture and assessments of potential methods to reduce stress responses is an active area of research (Ashley, 2007). Thus, fish have been selectively bred for a reduced emergency responses: High responding (HR) and low responding (Kause et al., 2005) lines of rainbow trout have been generated by selection for consistently high or low cortisol response to a standard confinement test (Pottinger and Carrick, 1999). In addition, these two strains of rainbow trout also show a divergence in sympathetic reactivity as a response to confinement (Schjolden and Winberg, 2007). However, all testing was conducted under controlled laboratory conditions and the welfare and productivity of LR strains have not yet been compared under commercial conditions. Selection for reduced stress response does not however lead to reduced sensitivity to other stressors. Furthermore, such genetic selection may increase resistance but it does not eliminate the adverse effect of stressors. Manipulation of fish diet has been also shown to play an important role in inter-renal sensitivity. For example, vitamin E added in the diet has been shown in sea bream to slow down elevation of plasma cortisol levels in response to a stressor and to increase survival rate (Montero et al., 2001). In African catfish (Clarias gariepinus), vitamin C fed during early development induced lower inter-renal sensitivity (Merchie et al., 1997).

Although much research has been devoted to stress biology in fish, major questions concern the development of new techniques for non-lethal and non-invasive sampling of physiology and behaviour of fishes which would allow measurement of stress outside a controlled laboratory environment. Cumulative stress responses occurring at different life stages have not been much studied.

## CONCLUSION

Fish possess a suite of adaptive behavioural and physiological responses that have evolved to cope with stressors. Many of these are homologous with those of other vertebrates.

Fish show short term adaptive responses which may be important to the maintenance of homeostasis but these do not necessarily imply any harmful consequences. Prolonged exposure to stressors generally leads to maladaptive effects or chronic stress. Chronic stress responses indicative of poor welfare include reduction in immune function, disease resistance, growth and reproduction, eventually death. Cumulative stress responses occurring at different life stages have not been studied.

## 8. Sentience

In the context of welfare of farmed fish the physiological, biochemical and behavioural reactions of fish are considered to be part of the experience of pain, fear and distress. Which emotional states they may experience is unknown. It is assumed that it may happen on a more basic form of consciousness (Flecknell, 2008).

As in other vertebrates, there is also considerable variation among fish species in the behavioural responses to pain and fear (Reilly et al., 2008).

We do not know the extent to which feelings of pleasure (positive mental states) exist in fish, but the hormone oxytocin, associated with pleasure in humans and other mammals, occurs in fish, or is replaced by its analogue isotocin (Bentley, 1998). Scientists do not have a tool to objectively measure various mental states in animals, including fish. It could be argued that a level of awareness is a requisite to experience pain, fear and distress. An individual is able to exhibit cognitive awareness if it is able to assess and deduce the significance of a situation in relation to itself over a short time span (for examples see review (Chandroo et al., 2004)). A fish might respond to a predator that poses an immediate threat but the predator is not yet directly attacking. In this context, fish may also have some mental representations of their environment because of their ability to navigate and recognise social companions and form mental maps (Rodriguez et al., 1994; Swaney et al., 2001; Odling-Smee and Braithwaite, 2003). Fish can avoid, for some months or years, places where they had aversive experiences (Beukema, 1970b; Czanyi and Doka, 1993).

Animals acquire a cognitive ability at a certain stage of development. Hence it is relevant to consider at what time, during the life of a fish, their perceptual and cognitive abilities develop. It is likely that fish develop some cognitive ability only when they are able to perceive external stimuli. While little is known about the development of cognitive ability, we have some evidence concerning the stage of life at which the development of responsiveness to external stimuli starts (EFSA, 2005). In zebra-fish the embryos are already motile at the end of the first day of development, showing spontaneous twitching of the body axis. During the second day of development, the zebra-fish embryo starts to respond to touch stimuli. This response is coordinated by relatively few differentiated neurones and a simple scaffold of pioneering axons that relay touch inputs to muscle cells flanking the neural tube and elicit the characteristic startle response for escape (Louis Saint-Amant, 1998). Data concerning the development of cognitive abilities in different life stages in other fish species is lacking.

## CONCLUSION

From studies of sensory systems, brain structure and functionality, pain, fear and distress there is some evidence for the neural components of sentience in some species of fish.

Our knowledge and understanding of manifestations of sentience in fish, however, are limited.

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