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Welfare concerns in regards to catch and release fishery: Is this procedure a painful event?

Bachelorprojekt i Husdyrvidenskab

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Abstract

This project addresses the issue brought forward by The Ethical Animal Counsel in Denmark about the welfare of fish used in catch-and-release fishery. The focus area being whether those fish experiences negative welfare, in form of pain, when hooked. The term “welfare” embraces different aspects, and therefore no specific definition does yet exists. *The Five Freedoms* nevertheless provide good guidelines to judge the welfare for the animals that are in our care and control. It is clear that to be free from pain is a crucial factor. It has therefore been appraised whether fish have the necessary nervous system to sense pain, are sentient beings, and whether they react on supposable painful stimuli. Pain and nociception are not synonyms. Nociception is the detection of stimulus and pain is a psychological state. Fish are capable of nociception and do possess many of the necessary structures for pain, but lack maybe the most essential structure of pain perception, the neocortex. Nonetheless many suggest that the dorsal pallia may be similar in function, but this is still inconclusive. Plenty of data supports cognitive skills, together with avoidance behavior in fish when exposed to noxious stimulus. Moreover reduction of normal behavior when handled with morphine is documented. However these data are not evident enough to conclude with certainty that experiencing pain is possible in fish. Thus pain perception is claimed to be mistaken for simple nociceptive responses. Consequently it cannot definitively be concluded that fish are able to feel the pain when hooked through the mouth. Nonetheless this should not hinder actions to be taken in regards to catch and release fishery as the condition of the fish is severe impaired after this procedure, and may affect survival. Moreover we should provide fish with the benefits of the doubt as pain perception is still not proven impossible, and a number of data supports this theory.

Resumé

Dette projekt sætter fokus på den problematik der i 2013 blev belyst af Det Dyreetiske Råd, omhandlende fiskevelfærd med henblik på catch and release fiskeri. Hovedspørgsmålet er sågar om denne gruppe af dyr er i stand til at føle smerte ved fangst agten og efterfølgende. Udtrykket ”velfærd” inkluderer forskellige aspekter, og der findes derfor ingen klar definition på hvad velfærd er. *The Five Freedoms* er dog accepterede bud på hvordan dyrevelfærd kan vurderes i henhold til de dyr der er under vores indflydelse. Heriblandt er frihed for smerte en essentiel faktor. Det er derfor nødvendigt at finde svar på om fisk har et nerve system som er i stand til at registrer eventuelle smertefulde stimuli, og kan de reagere på disse stimuli som tegn på at disse opfattes som ubehag i fisken. Nociception og smerte er ikke entydige begreber. Nociception er den proces hvori der registreres et noxious stimulus af nervereceptorer i huden, hvorefter nerveimpulsen sendes videre op til hjernen, hvor den i mennesker kan registreres som en smerte følelse. Smerte er altså ikke en fysiologisk proces men et psykologisk stadie. Nociception er muligt i fisk og de besidder tilmed mange af de nødvendige strukturer med hensyn til smerte sansning. Der hersker dog stadigvæk en del tvivl om en sammenlignelig struktur til neocortex findes. En hel del data understøtter kognitive færdigheder i fisk. Ydermere ses der tydeligt en undvigende adfærd når de udsættes for skadeligt stimulus. Desuden ses en reduktion af normal adfærd, når de håndteres med morfin.

Disse samlede resultater er dog ikke entydige nok til at konkludere smerteopfattelse i fisk, da den formodentlige smerteopfattelse kan hævdes at være forvekslende med simple nociceptive reaktioner.

Ikke desto mindre bør dette ikke forhindre foranstaltninger i forhold catch and release fiskeri. Fiskene er som konsekvens i vært forringet tilstand efter denne procedure, og dette kan påvirke overlevelse. Ydermere da smerteopfattelse er stadig ikke bevist umulig, og en række data understøtter den teorien herom, bør vi lade tvivlen komme fisk til gode, og følge Det Dyreetiske Råds råd forslag om forbud mod catch and release fiskeri i fremtiden.

Introduction

The Ethical Animal Counsel in Denmark (Det Dyreetiske Råd, 2013) has issued a report regarding their concerns about sport fishery. The Report concluded that activities such as catch and release fishing, that allows first the catching of a fish by hook and then releasing of it into the wild again, should be reevaluated, as they may have a negative effect on welfare.

Fish represent the largest taxon of the vertebrate animals, with the number of species estimated to 35.000 (Roques et al., 2010). A huge portion of these species are used by humans in a range of different purposes, where they may function as either display or companion animals, as protein source, or are used for commercial or sport fishing. Despite the many interaction fields between this group of animals and humans, they do not seem to be involved in our ethical consideration on the same level as for example mammals do. This being despite the huge impact we have on their quality of life. Attention needs therefore to be drawn to activities which potentially have a harmful influence on the welfare of fish, such as when fish are hooked (Huntingford et al., 2006).

Why is it, that fish for so long have been ignored in regards to welfare? Firstly the answer might be due to the fact that fish do not vocalize, nor do they have recognizable facial expressions, which are primary cues or human empathy. This may also be one of the reasons that humans find it so hard to empathize with them. Secondly, fish have been, and still are, considered as "lower" organisms (together with the amphibians), in comparison to mammals being the "higher" organisms. This complexity is characterized by a more complex brain circuit. A fish brain and a human brain do in general have the same basic structure, but fish do not possess the telencephalic structure known as the neocortex.

Emotional states and experiences of these are based on neural activity in parts of the neocortex defined as the limbic forebrain, i.e. the prefrontal, insular and cingulate cortex, the hippocampus, the amygdala and the septal nuclei. Amygdale activity has in particular been correlated to negative emotions (Boissy et al., 2007). As fish lack these structures, scientists consequently disagree, arguing whether fish are capable of feeling pain. This leaves both scientists and the public in doubt about welfare actions for this group. In order to address these issues, different parameters must be considered to assess whether fish feel pain. Demonstration of sensory capacity to detect supposable painful stimuli, and the performance of adverse behavioral responses to such stimuli must show to be more than just reflexive, and consciousness are necessary (Sneddon et al., 2003a). Fish cannot tell if or how they feel and evidence for these capabilities must rely on what can be observed and measured rather than subjective states (Sneddon, 2009).

Motivation

Humans tend to discriminate between groups of animals when it comes to decide which species are "worthy" to be considered for welfare. We apparently find it quite all right to hook a fish through the palate in the procedure of catch and release fishing, whilst this would be considered as an unacceptable way of treating mammalian species. It became evident that such sort of discrimination between species, may be due to a lack of knowledge on how non-human species function, and whether we can expect them to feel and react in the same way as humans. This is properly one of the explanations of why welfare concerns of fish have not been considered in same degree as the mammalian species. If these animals are not able to separate good experiences from bad, why then care how to treat them?

The reason for diving into this issue was therefore, for me, to get a better understanding about the welfare of fish, and whether our different ways of using them, might compromise their welfare in term of painful events, especially in regards to sport fishery.

Objective

The main purpose of this assignment is to evaluate the welfare of fish in regards to catch and release fishing, as the procedure may impair the welfare of these species. It will therefore be necessary to consider the possibility for fish to perceive pain, to assess proper welfare strategies in the future. It is consequently the purpose to put forward different evidence for the capability of fish to feel pain. This will be addressed by appraising whether fish have the necessary nervous system, are sentient beings and whether they react on supposable painful stimuli. The effort manifested in this project is thus, the joint of different views, evidences, and debates on the subject. To establish whether fish can separate between different states of welfare, and whether their welfare could be impaired by different activities, it is essential to define what underlies the concept of good and poor welfare respectively. Moreover, the nervous system of the fish has to sense noxious stimuli, and perceive these. To assess whether this is possible, the neuroanatomy of the fish will be analyzed in comparison to the human nervous system. It will be relevant to look at the evidence for cognition and possible consciousness in fish, as consciousness is paramount in order to experiencing emotional states (feel). An organism must therefore acquire consciousness to be capable of pain perception. Therefore, different literature on whether fish are able to learn and remember will be put forward. Whilst mastering of different learning tasks could be considered as evidence for cognition. Physiological and behavioral responses, which occur in fish when exposed to suggested noxious stimuli, will provide an insight in what the fish are experiencing subjectively. Considering all these aspect together provides a better knowledge of what we can expect this group of animals to be capable of, and whether it includes different emotional states and feeling of them.

Different results and opinions will be discussed and an overall conclusion will be made of whether pain perception is possible in fish and if so whether this should result in changes of procedure in regards to catch and release fishery. At last suggestions to further studies in this area will be presented.

Hypothesis

My hypothesis is that fish are able to feel pain, as I assume this skill to be a natural defense mechanism present in all vertebrates in order to survive. Thus catch and release fishery, need to face some changes of procedure, as pain perception in fish mean that this activity certainly have a negative effect on welfare.

Methodology

This project is a literature study, based on knowledge and results from published scientific empirical works. This includes reviews and first hand articles. Moreover reports, text-books, and few web pages relevant for the subject have been used. The online literature search has been performed on the databases: Rex – Det kongelige bibliotek, Google Scholar, Science Direct, PubMed together with WEB OF SCIENCE. The literature used is confined to including only English and Danish.

The content will be descriptive, explanatory, analyzing and include different views on the topic. My hypothesis consists of two elements, and their relation to each other. One assessing whether pain perception is possible in fish, and the other enlightening the welfare aspect of catch and release fishery in relation to the conclusion of the pain question.

Defining of the project

This project will be limited to include only fish species belonging to the class of bony fish (*Osteichthyes*). The research of fish is very scarce and therefore only a small amount of literature is available. The best provided information is of the bony fish species, because they may have been easier to handle in scientific matter**.** Because of the huge diversity between different species of fish, and lacking research also among bony fish, it will not be possible to make generalized statements about bony fish, but rather provide different data and examples from separate species, which may at the end lead to an overall conclusion.

The neuroanatomy is as mentioned an essential factor to include in the debate, but only a brief summary of the different parts of the fish brain (focus point being the telenchephalon), together with the most relevant aspects in accordance to pain perception will be reviewed. Furthermore the noxious stimuli detection and nociceptive pathway to the neocortex in humans will also be explained, but this will not include a deeper explanation of the chemical aspects of either the detection process or the pathway. Different activities of fish use, which may have potential to impair the welfare of fish, will shortly be presented but only catch and release fishery will function as the main problematic in this project. No detailed description of the catch fishery will be brought forward.

When considering the term “emotions” there will be distinguished between (I) the functional aspects of an emotion; an emotional state, which includes behavioral, physiological and cognitive processes and (II) the conscious experience of an emotion, which refers to feeling an emotion.

1. Welfare

The following will have the purpose to establish the different terms in association with welfare. This is done in order to, recognize signs of both poor and good welfare and judge the situation with fish in regards to catch and release fishery.

1.1 What is welfare?

Many might sustain that fish are repeatedly overlooked in concerns of welfare. In order to make such statement, or for that matter any kind of statement about welfare, it must be made clear what one defines as good or poor welfare respectively.

Welfare is a complex concept, and no clear consensus on how to judge or define welfare exists (Vis et al., 2012). Three different ideas of the definition of good welfare are nevertheless commonly used and broadly accepted by scientists. These concepts are; (I) *Feeling-based definition,* (II) *Function based definitions* and (III) *Nature based definitions*.

Feeling-based definitions have the fundamental assumption that good welfare is measured in the balance of negative and positive experiences. It is therefore the emotional state of the animal which is important, and the life of an animal should be free from negative experiences, such as fear and pain. *Function based definitions* are set in terms of an animal's ability to adapt to its present environment. The welfare here is correlated with the biological state (function) of the animal, which requires good heath in a physiological aspect. Thereby, maintenance of homeostasis, and the animal's ability to cope with stressors, both infectious and non-infectious ones. *Nature based definitions* come from the view that behavior is species specific. In regard to this view, it is essential that the animal is able to express its arts specific behavior and live a natural life, in accordance with lives of its free conspecifics (Huntingford et al., 2006). Another recognized definition of what good welfare is, was brought forward in 1979 by the Farm Animal Welfare Council, in order to ensure welfare for farm animals, and sounds as follows:

1 Freedom from hunger and thirst
2 Freedom from discomfort
3 Freedom from pain, injury and disease
4 Freedom to express normal behavior
5 Freedom from fear and distress

Although one must recognize that these does not really apply to free living animals as in nature, animals are almost constantly exposed to one or more of these stressors described above. At the same time captivity does rarely meet all of these conditions. One might exclude the other. Moreover the different emphasizes brought upon welfare statements, often reflect the interest of the one speaking. This meaning that the aspect in focus if asking a veterinary or farmer may be disease, injury and low growth rate. Consumers of organic products may weigh the natural living aspect the highest, while the public on the other hand may be focusing on whether the animal is free from unpleasant feelings (Appleby et al., 2011).

1.2 How to assess whether a fish's welfare may be compromised

Changes in behavior often provide good welfare indicators. Behavioral markers can only be applied in assessing either good or poor welfare, when it is understood that normal behavior is species specific. It is therefore essential to understand the species-specific biology before drawing any conclusions in relation to welfare. Disadvantages of behavioral indicators might be that large individual differences in behavior within animal groups. Thus specific indicators could be difficult to quantify. The observers therefore need the sufficient skills to observe the animals 'body language'. What also should be noted is that behavioral responses under stressful conditions are dependent not only on the type of stressor, but also on the coping style that characterizes an individual (Martins et al., 2012).

Behavioral changes which could be observed amongst fish in respond to supposable poor welfare are for example failure of fish to reproduce, enhanced opercular beat rate, reduced feed intake and changes in swimming frequency. How well these signs work in any given case will, as mentioned, depend on the species concerned. Physical changes such as inhibit growth, or change in skin or eye color, and injury together with high mortality rates have also been used to reach knowledge of the welfare state of fish (Huntingford et al., 2006). The advantages of such physical parameters are that they are easily recognized and measured. Stress parameters, such as elevated cortisol level, on the other hand, are not as straightforward recognizable (Vis et al., 2013). However, what must be noted is that stress responses such as elevated blood pressure and heart rate do not require consciousness but only nociception. Therefore interpretation of these responses is confounded by stress responses to tissue damage (Appleby et al., 2011).

1.3 When fish are left in hands of humans

The behavioral indicators mentioned above present some of the consequences which may occur due to the different ways we use or handle fish. Table 1.1 puts forward some activities which might have a negatively impact on fish, according to different situations. Considering catch release fishery, especially two aspects may be problematic in regards to welfare. The first is the release of reared fish inappropriately equipped for survival in the wild. The second is the pain, which could be experienced through and after impaling of the mouth, if fish really can feel pain this will completely violate the basic assumption of what good welfare includes. (Det Dyreetiske Råd, 2013).

1.3 Partial conclusion

Different aspects of welfare have been reviewed and it seems that three main ideas can be represented as the framework of good welfare and includes the following: a physiological healthy state, to have more positive experiences compared to bad ones (experiencing good emotions), and the possibility to lead a natural life is also essential. These definitions represent welfare requirements in general, whereas *The Five Freedoms* present guidelines for good animal welfare when in control or affected by humans. The third point states that animals must be free of pain, injury, and disease. It may not be possible to live a whole life free from these factors, but these freedoms must be seen as guidance for humans to avoid applying such states to the animals in our control. Of course it is therefore necessary to estimate whether some activities can cause pain. Next section will discuss what pain is, and whether nociception can function as a parallel to pain.

Table 1.1 Examples of potential situations in which fish welfare may be impaired. Adapted from Huntingford et al., (2006)**.**

Activity	**Potential effects on welfare**
Aquaculture	- High densities in simple and constraining conditions, both in normal rearing conditions and for husbandry. - Poor water quality. - Food deprivation (*e.g*. during disease treatment and before harvest). - Handling and removal from water during routine husbandry procedures - Unnatural light-dark regimes, to control breeding. - Handling, constraint and, sometimes, low oxygen levels during transportation. - Increased exposure to predators, attracted to fish farms or used to grade out smaller fish. - Transmission of disease between wild and farmed stocks.' - Crowding, handling, removal from water and pain during slaughter
Commercial and sport fisheries	- Injury during trawling - Tagging/fin clipping during stock assessment - In both, tissue damage, physical exhaustion and severe oxygen deficit during capture. - In both, pain and stress during slaughter. - In angling, pain and stress in tethered fish when live bait is used. - In angling, stocked fish introduced to lakes may be denied the opportunity to migrate
Keeping ornamental fish and display fish in public aquaria	- For ornamental fish, capture by sub lethal poisoning. - For ornamental fish, permanent adverse physical states due to selective breeding. - For ornamental fish, release or escape of exotic species. - Inappropriate temperatures, poor water quality and physical constraint during transport. - Confined space and poor water quality once housed. - Inappropriate social conditions, with shoaling fish at low densities and predators with prey.
Scientific research	- Genetic-modification induced for scientific research may have detrimental effects on welfare. - Fish used in the laboratory for experimental purposes are often confined and may be exposed to a range of deliberately-imposed adverse physical, physiological and behavioral states. - Fisheries research often involves electrofishing, tagging, fin clipping or otherwise marking fish, which potentially cause pain and injury. - In both cases, handling during research procedures may cause injury

2. Nociception and pain - What is the relationship between the two?

A lot of literature with the purpose to describe how humans sense and perceive pain fails to distinguish between the feeling of pain and nociception. When a stimulus leading to pain is detected in humans, it happens through special cutaneous receptors. These receptors are free nerve cell endings of primary afferent neurons. They are often referred to with concepts such as "pain receptors" or "the pain pathway", but should instead be defined as nociception and nociceptive pathways respectively. Nociceptors detect potential or actual harmful stimuli. This mechanism ought to make us react rapidly and hinder further tissue damage. When a nociceptor is stimulated it triggers an action potential and the information is carried into the back of the spinal cord, the dorsal horn (site for sensory fibres). Neurotransmitters such as substance P, will then be released from the nociceptor and this will lead to the activation of second order neurons that conduct the impulses via the spinothalamic tract and to the thalamus. From the thalamus third order neurons will relay the impulses into the somatosensory cortex (Basbaum et al., 2009).

Two types of nerve cells (fibers) are said to be associated with pain detection, *A-delta fibers* and *C-fibers*. They are excited either by, mechanical, chemical or thermal stimuli which will or have the potential to cause cell damage. *A-delta-fibres* are thick myelinated nerve fibers which conduct at about 20 m/s (fast conducting), whereas the *C fibre*s are unmyelinated axons, which conduct at velocities generally less than 2 m/s, the ache is experiences later, but the pain tend to persist for longer. A-delta-fibers are responsible for signaling first pain, which results in immediately withdraw (Weber III, 2011a). These fibres respond to mechanical or thermal stimuli. C-fibers are the most abundant, and stimulation of these fibers leads to second pain, and function in respond to a more intense kind of stimuli, which is usually more difficult to localize. C fibres are polymodal, and the stimulus activating these fibres is chemical, mechanical or thermal stimulus (Basbaum et al., 2009).

Pain on the other hand does not occur unconditionally to the detection of the above described situations. The definition of pain according to *The International Association for the Study of* Pain (IASP, 2012) states the following: (I) Pain is an unpleasant sensory and emotional experience that is associated with actual or potential tissue damage or described in such terms (II) pain is always subjective. Pain is always a psychological state, and as result cannot be directly associated with nociception.

2. 1 Partial conclusion

The above underlines the importance of recognizing the difference between neuron activity and pain. It cannot be assumed that pain is felt in an organism, even though neuron activity can be detected when exposed to noxious stimuli. A closer look at the neuroanatomy of the fish will provide knowledge of how this functions, and what the main differences may be between bony fish and mammals.

3. Neuroanatomy of the fish

The nervous system is the essential structure making humans able to react on different stimuli, and the feeling of pain is suggested to rely on complex brain structures unique to mammals (Rose 2002). Comparable studies of the brain of different vertebras will therefore provide good evidence of whether pain can be sensed in fish. For long time, the accepted notion has been that all vertebrates have evolved through a linear progression, going from inferior to superior forms. This being characterized by increasing complexity of the nervous system. This scale leaves fish belonging to the lower group of vertebrates. Together with the amphibians, fish are considered being the least evolved. This is due to them possessing a less complex brain circuits. The central nervous system of fish as in mammals, contain the spinal cord, medulla oblongata and the brain. However, the brain of fish is small compared to body size (Bshary et al., 2014) and the forebrain (telencephalon) of the fish is a relatively simple structure lacking a neocortex and only having limited differentiations (Flood et al., 1976).

In humans the neocortex forms the neurological substrate for cognitive and emotive mental processes. Due to this, humans are considered as the “highest” organisms, being capable of having sophisticated cognitive abilities and intelligent behavior, whereas the lower group of organisms are believed to have only simple forms of behavior such as instinctive responses or reflexes (Broglio et al., 2011a). Nonetheless not everyone share this view, and Flood et. al. (1976) present the view from Nelson (1969) that the structure of the telencephalon of fishes cannot be interpreted as being primitive. It is believed that teleost fish are far from their Devonian progenitors, and have themselves advanced in evolutionary terms.

3.1 A brief overview of the fish brain

The brain structure of fish varies dramatically across species, but similar to other vertebrates the brain can in general be divided into the forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). Figure 3.1 illustrates the brain of the ray finned fish the Longnose gar (*Lepisosteus osseus*), e.g. bony fish, with its different structures and components. The forebrain is divided into the diencephalon and the telencephalon. The midbrain consists of the more rostral parts of the brain stem and a roof region (tectum). The hindbrain contains the caudal part of the brain stem medulla and cerebellum (Butler, 2000b).

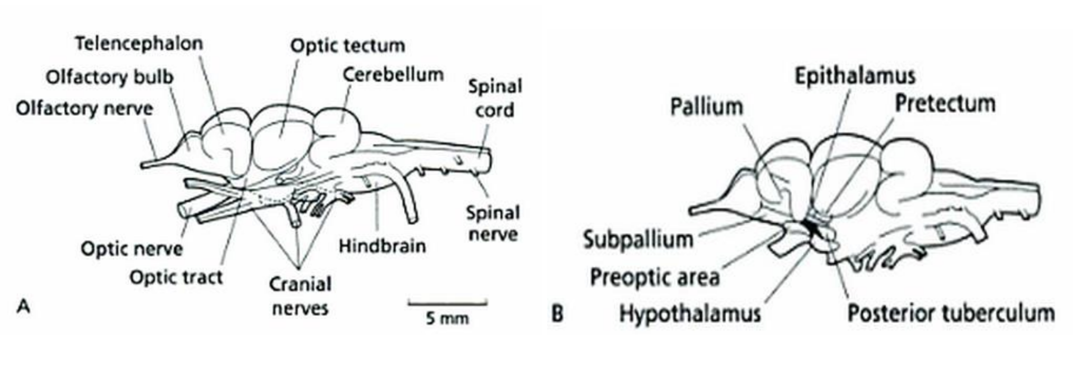

Figure 3.1 Drawing of the brain of a ray finned fish. Obtained from *The Laboratory Fish* (Butler, 2000b).

3.1.1 Rombencephalon:

The hindbrain of ray-finned fish (*actinopterygians*) contains most of the motor and sensory nerve nuclei together with the reticular formation. They do also have corpus cerebella that receives inputs from the electrosensory system and mechanosensory lateral line as well as octaval inputs and descending inputs from telencephalon. A unique structure in teleosts is the valvula, which is an extension of the corpus cerebella that in some species covers both the tectal roof and the pallial hemispheres (Northmore 2010).

3.1.2 Mesencephalon:

The optic tectum of the mesencephalon forms the roof of the midbrain and mainly receives visual inputs. The tectum is a sheet of neural tissue forming a twin-lobe structure that inflates over a fluid-filled ventricle. The two lobes are connected at the midline by the tectal commissure. The tectum controls eye movements, approach, and avoidance movements through its connections to premotor centers. In teleosts the optic tectum is a highly developed neural processor, indispensible for the sensory discrimination and rapid decisions required for behavioral reactions necessary for survival and reproduction (Northmore 2010). In teleost fish, the midbrain roof has an associative structure, the torus simicucircularis and torus longitudinalis, which are dominated by auditory and lateral line inputs (Butler, 2000a).

3.1.3 Prosencephalon:

The forebrain consists of diencephalon and telencephalon. Diencephalon includes the epithalamus, the dorsal and ventral thalamic nuclei, hypothalamus, peoptic area and the pretecum and posterior tuberculum (latter structures represent the caudal parts of diencephalon). The pretecum receives retinal projections. The epithalamus comprises the pinal gland and the habenular nucleus, which receives pinal afferent projections together with inputs from the subpallia of telencephalon. The hypothalamus encloses a number of nuclei, which for example in teleosts fish are characterized by evaginated inferior lobes (Butler, 2000b). These hypothalamic enlargements receive inputs from telencephalon and the brain stem (the gustatory nucleus) (Butler, 2011). The preoptic area is a rostral extension of hypothalamus, containing both small- and large celled nuclei and also with a suprachiasmatic nucleus. Along with hypothalamus it gives rise to reticular formation sites, which sends information to the spinal cord. Descending pathways also rise here, and these do affect visceral motor centers of the brain stem.

In ray-finned fish, the telencephalon develops through a unique process of outward folding of the prosencephalic alar plates called eversion (in contrast to evagination which occurs in other vertebras)(Salas et al., 2003). This process makes up two massive hemispheres separated by a single ventricular cavity and results in a medial to lateral reversal of the pallial areas (Broglio et al., 2003). The telencephalon has a dorsal (pallial) upper located area and a ventral (subpallial) lower area, and comprises the olfactory bulbs. The pallium is the general term used to describe the grey matter that covers the telencephalon(Chandroo et al., 2004). The ventral part does only contain nuclei, whereas the dorsal region has in some of the ray-finned fishes elaborated into a cortical-like region. A general difference between pallium and subpallium is that pallium projection neurons are glutamatic whereas the subpallium is GABAergic (Butler, 2011). The dorsal area can be divided into, pars centralis (Dc);

pars dorsalis (Dd); pars medialis (Dm); parslateralis (Dl); and pars posterior (Dp). These parts may be subdivided further. Similar is true in regards to the ventral area (Ito and Yamamoto, 2009).

3.2 Partial conclusion

A lot is still to be discovered about the brain of bony fish. What for now could be hypothesized is that the eversion of telencephalon has resulted in same brain structure as mammals only differently located.

4. Do fish have the necessary structure to sense and perceive pain?

The nociceptive pathway is known as the first step in pain registration. Nociceptors in mammals including humans are of two fibre types A-delta and C fibres (Chandroo et al., 2004). Both types have been found in the trigeminal nerve of the rainbow trout (*Oncorhynchus mykiss*) (Sneddon, 2002), and later by a study of Sneddon et al., (2003a) it was confirmed that some of the fibres could be characterized as nociceptors as they were activated in response to supposable noxious stimulus.

In bony fish (*Osteichthyes*) both types of fibers have been detected, with the A-delta fibers being the most abundant ones. This is in contrast to both amphibians, birds and mammals, where the C fibres usually compose between 50-65% of the total fibre number. In chrondrichthyes (elasmobranches) only A-delta fibres have been discovered while agnatha (hagfish and lamprey) do primary posses C fibres (Dunlop and Laming, 2005).

When a nociceptive stimulus is detected by nociceptors in fish, the signal travels to the spinal cord and is relayed to the thalamus. Here from, the impulse travels further to the telencephalon via the gray matter pallium, through which, thalamus and telencephalon are connected (Weber III, 2011b). To have opioid, receptors have been considered as a crucial factor in whether fish are capable of nociception. Buatti and Pasternak (1981), found opiate receptors in goldfish, and furthermore enkephalin like substances has been found in various brain areas. Enkephalin-like substances have also been discovered in other fish species for example catfish, African lungfish and rainbow trout. Furthermore the distribution of immune activity to components such as substance P and serotonin is much alike that seen in mammals (Sneddon, 2004).

4.1 Comparable structures and function in the vertebra brain

It is known from humans, that the major tracts involved in nociceptive processing and relaying information to the brain, are the trigeminal tract conveying information from the head and the spinothalamic tract conveying information from the rest of the body (Sneddon, 2004). Both have shown presence in fish. Nonetheless, no activity had earlier been detected in telencephalon due to noxious stimuli, and impulses confined to the dorsal root ganglion is interpreted as purely reflexive (Dunlop and Laming, 2005). Rose (2002) sustains that innate responses, like limb withdrawal, facial displays, and vocalization, to noxious stimuli are generated in subcortical levels primary in the spinal cord brain stem.

This argument has often been presented in the discussion about whether fish can experience emotions, as no recordings of forebrain activity have earlier been detected in fish during supposable noxious stimuli. Moreover, the forebrain of fishes has for long been thought to be only olfactory. Recently the olfactory projections have been traced to a limited circumscribed area in the pallium. Additionally, other ascending sensory inputs to other regions have been discovered in this area (Butler, 2011).

Dunlop and Laming, (2005), wanted to challenge the opinion that noxious stimuli would not be detected in the telencephalon of fish. In their experiment including goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*) they were able to record electrical activity during noxious stimulation in the telencephalon of both fish. At the same time the teleost forebrain receive a pattern of projections from the thalamus, likewise those of higher vertebras (Vis et al., 2013). The structures of the fish brain resembling the ones of the mammalian regions, in question of pain perception, are summed up in table 4.1. Rose (2002) uphold that the neocortex is the essential brain level for conscious awareness of pain, and it does not exist in fish, which according to Rose (2002) make them incapable of pain perception. Additionally, he states that the existence of complex capacities in humans, such as language and self-awareness, do not necessarily exist in other species. Different species do also have different evolutionary paths resulting in very different neurobehavioral adaptations. The failure of humans to recognize this, is caused by an anthropomorphically approach to science, which he claims hinders the true understanding of all non-human animals.

It has been suggested that the main subdivisions in the dorsal pallium in ray finned fish could be homologous to the neocortex, amygdale and hippocampus (Broglio et al., 2011a; Mueller, 2012). The dorsal pallium may correspond to the neocortex (Mueller, 2012). Hippocampus and amygdale are structures contained in the limicsystem of mammals, and these regions are responsible for memory and emotional assessment in humans respectively (Bshary et al., 2014). The medial pallium is the one thought to be homologous to the amygdala of land vertebrates. The similarities between amygdala and the medial pallium lies on the basis of developmental evidence and similarities in the pattern of gene expression, neurochemical distribution and neuroanatomical comparative evidence as well as behavioral data (Broglio et al., 2011b). Hippocampus involved in spatial, contextual, or relational memory (Portavella et al., 2002), is suggested to be parallel in function to the lateral pallium in fish. Most of the earlier studies confirming this hypothesis have included entire telencephalon ablation. However, the resulting effects could be considered a sum of the impairments of several specialized telencephalon-based behavioral systems instead of unspecific effects of the lesion on an undifferentiated brain-system. In 2005, Portavella et al. provides more specific data on this area. They performed a study in which different parts of telencephalon was lessoned. Goldfish that received either lateral pallium ablation (n=8), medial pallium ablation (n=8) and then they had a control group (n=8), all were trained in both avoidance and spatial procedures. The results showed that dorsal medial lesions of goldfish impaired acquisition and retention of conditioned avoidance response. The same effect has been recorded in land vertebrates, in case of amygdale lesions (Portavella and Vargas, 2005). Dorsal lateral lesions impaired spatial learning ability which was concluded by the authors resembling the effects of hippocampal lesions. The data therefore supports the hypothesis of brain region similarities. Yet other brain areas of fish and mammals have shown comparable in function. Both in mammals and fish, the lateral habenula (a mesencephalic nucleus) is activated in respond to aversive stimuli, which in turn affects motor and cognitive behaviors by inhibiting the activity of mesencephalic dopaminergic and serotoneric neurons (Bshary et al., 2014). Furthermore the development and function of the cerebellum is preserved between the bony fish and mammals (Bshary et al., 2014).

Table 4.1. Comparison of the fish and mammalian brain in accordance with pain sensation and perception.

Mammalian Brain	Fish Brain	Functions (humans) include
Hippocampus location: cerebral cortex as part of limbic system	**Lateral pallium of telencephalon**	Memory formation
Amygdala location: cerebral cortex as part of limbic system	**Medial pallium of telencephalon**	subjective feelings associated with negative emotions such as fear
Neocortex location: outer surface of cerebral cortex	**Dorsal pallial division**	conscious thought and perception of pain
Habenula Location: mesenchephalic nucleus	**Habenula**	inhibits the activity of mesencephalic dopaminergic and serotoneric neurons
The trigeminal- and spinothalamic tract Location: In the head and spinal cord respectively	**The trigeminal- and spinothalamic tract**	Sensory pathways conveying impulses from the head and the rest of the body respectively

4.2 Partial conclusion

The litterateur provides quite a lot confirmation for both morphological and functional similarities between the nervous system of fish and mammals. Yet some remarkable differences persist, such as the difference in distribution in the number of, respectively, a delta fiber and C fibers, and the size of the brain compared to body size. Even more research on this matter may in time result in a concrete picture of the whole nervous and brain circuits of fish. The following part will address the issue of consciousness in fish. Since it is still inconclusive whether the dorsal pallial division, function as the neocortex of humans, this theme is also under huge debate.

5. Cognition and consciousness

A lot of studies have suggested that fish are experiencing negative welfare, such as fear, stress and pain (Roques et al., 2010; Sneddon 2003; Sneddon et al., 2003a,b; Ehrensing et al., 1982). It is widely agreed that pain is a conscious experience (Brown, 2014). However it is been fiercely debated whether or not fish are consciously aware of different emotional states, such as pain, due to their lack a neocortex (Rose, 2002). The purpose of this section is to establish whether fish have cognitive skills, in order to possibly link these abilities to some sort of consciousness. Cognition is a subcategory of consciousness that incorporate recognition, thinking, and the ability to learn from and use ones knowledge (Den store danske, 2012). Learning and memory creating have frequently been viewed as signs of cognition in animals (Brown, 2014), and conscious may be inferred by examine of cognitive abilities (Chandoroo et al., 2004).

The concept of consciousness is broadly described as ones awareness of external and internal stimuli, and having the ability of excluding oneself from others (self awareness). A more detailed distinction is often made between (I) primary consciousness, which consist of raw sensory feelings, such as hunger, cold, hot and so on, (II) secondary consciousness, which is the ability to incorporate/combine observed events with memory to create awareness of the present and immediate past of the world around them and (III) tertiary consciousness, which is self-awareness, including thinking about one's actions and one's ability to speculate/think. Those which are not willing to give a consciousness to animals, may not distinguish between the different forms of consciousness, and may in fact be referring to the tertiary consciousness, which is quite certain to be unique to humans (Panksepp, 2005; Rose et al., 2014). In Figure 5.1 different areas of the human brain is highlighted, in respect to regions that are important for; emotion state (blue), feeling of emotion (red) and level of consciousness (green), and the regions for consciousness in humans are obviously confined to the cortex (Tsuchiya and Adolphs, 2007).

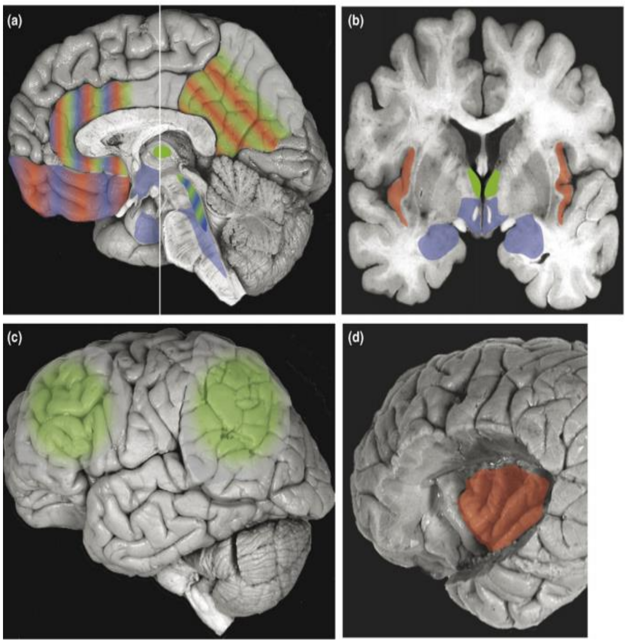

Figure 5.1: **(a)** Hypothalamus, amygdala, brainstem nuclei, including periaquedactal gray and parabrachial nuclei, orbitofrontal cortex and anterior cingulate cortex which are important for the expression of emotions are marked with blue. Anterior and posterior cingulate, including precuneus, and orbitofrontal cortex are important for the experience of emotion (red). **(b)** Coronal slice at the level of the white line in (a). The thalamus, hypothalamus and amygdala are also shown here. Insular cortex (red) is an important structure for the experience of emotion - also shown in (d). **(c)** Bilateral prefrontal and parietal cortices are broadly important for the level of consciousness (green). **(d)** The surface of the prefrontal cortex has been removed to reveal the insular cortex (red) (Tsuchiya and Adolphs, 2007).

Since it is not possible to measure mental states or feelings of animals, and whether the brain regions shown above, are present in fish is questionable, it is broadly agreed that behavioral responses are the best clues implying consciousness of animals (Chandroo et al., 2004). This issue is often approached experimentally (Brown, 2014).

Self-recognition is considered one of the key criteria for consciousness (Sneddon, 2011). The most applied test used in this context is the mirror self-recognition test. One classic example is a chimp having a sticky dot attached to its forehead, and then is put in front of a mirror. If the chimp recognize the mirror image as being its own reflection and tries to remove the dot, it would suggest self-recognition. Fish seldom pass this kind of test, and it is argued that self recognition does not necessarily happen only through vision, but that other factors such as chemicals cues play an essential role in aquatic ecosystems. This suspicion is supported by situations where male cichlids (*Pelvicachromis taeniatus*) have shown to prefer their own odor to those from brothers or unrelated males (Thuenken et al., 2009).

5.1 Are fish able to learn and store memories?

As mentioned capacity to learn and create memories of different events are viewed as strong evidence for cognitive abilities. Different learning tasks, such as spatial and reversal learning and classical- and operant conditioning are used for comparative cognition (Brown, 2014), and different indicators for learning skills in fish will be considered below.

Brown (2001) documented one trail learning in crimson spotted rainbow fish (*Melanotaenia duboulayi*). In the experiment the fish were exposed to a novel trawl device over a period of five runs (year 1997). Eleven months later, the experiment was then repeated (year 1998). In 1997 when the fish were first represented with the trawl approach the fish showed what could be defined as panic escape, but this response seemed to improve during repeated trials (five runs), and the fish performed a more rapid escape. Eleven months later the latencies were still low, as the fish seemed to recall the escape route. Moreover, fish that were familiar with the experimental tank (fish which were held in the tank for 3 weeks previous to the test) showed significantly lower escape latencies. The fact that the subjects did perceive their escape response, showed proof of the ability to learn and memorize (Brown 2001). Additionally intertidal rock-pool fish were shown to remember the location of, and learn to return safe tide pools after displacement. This task requires both spatial learning and memory skills. In the present context the development and evolution of cognitive abilities that enables individuals to remember cues in their environment and by them figure out how to refine their key resources (White and Brown, 2013). The tide-pool-dwelling goby (*Bathygobius soporator*) remembered the location of neighbor pools and where thereby able to jump directly into them at times with low tide. This was done even though it was not possible for the fish to locate the location visually (Aronson, 1971).

Territorial behavior is often seen in the animal kingdom. Being the dominant one in such hierarchies clearly has its advantages. Dominant individuals usually have prior access to life supporting resources, such as food, favorable sites, and mates. Dominance-subordinate relationships are often assessed by the relative fighting abilities of the opponents. Rainbow trout are able to judge its own likelihood of winning a future fight and change in aggression according to its opponent when it has had access to observational information of the individual from a previous contest. When a trout observe a trial between two conspecifics, that will establishes a dominance-subordinate relationship and the trout thereby alters its fighting strategy according to which of the previous opponents it is set up against (Johnson and Åkerman, 1998) the trout show observational learning (McGregor et al., 2001) learning is also seen in male Siamese fighting fish (*Betta splendens*), that were slower in assessing a previous winning competitor than a losing one. The winner was identified as the male displaying for the longest period of time, which refers to the one having erect gill covers while within one fish length of the side of the aquarium closest to the opponent. A second hypothesis was illustrated here, in that, when a previous winner was approached the subject the fight escalated to a higher level (measured by the proportion of subjects that attempted to bite the intruder) than with the losers. Assuming the fish recognized the winner as a stronger opponent and fought more furiously (Oliveira et al., 1998). Similar results were reported by McGregor et al., (2001), that found that the respond of the subject depended on whether the opponent was a winner or a loser, using same definitions of winners and behavior of the subject as described in latter experiment. The subject spent more time near and displaying together with more attempted bites and tail beats. The latencies in respond to the winner opponent, was nevertheless shorter than towards losers. These results support that fish do posses some form of cognition in that they show acquirement, processing, storing of information, and are able to act on information gathered from the environment (Shettleworth, 2001).

It has been argued that stable social groups have increased cognitive capability, by the out smarting of other group members (Brown, 2014). Such cases are also seen among fish. Male-male competition or female choice have been considered the reasons for sexual traits, such as mating displays and bright colors which for long have fascinated biologists. It seems though that these choices may not always happen independently, but it has been documented that females might "copy" the mate choice of another female (Dugatkin and Godin, 1992). Copying could serve to reduce the cost of mate assessment or increase the accuracy of choice (Gibson and Höglund, 1992). In two different preference tests, female guppies were set to choose freely between two males, and it was shown that the females obtained social cues and switched their initial mate preference, after observing another female choose differently. Social clues did influence on their choice (Dugatkin and Godin, 1992).

5.2 Are fish intelligent? - Evidence of manipulation and tool use in fish

Tool uses are also a factor associated with intelligence, hence a good indicator for cognition in fish (Brown, 2014). In fish it is difficult to speak of the same kind of "tool use" that can be observed in primates, due to their lack of grasping appendages/limbs. Furthermore their surroundings provide an ecological constrain in this aspect because water is more viscous than air and material is more buoyant in water than in air. Therefore, the term "tool use" might seem refined to primates, and another phrase suggested by Brown (2012) may be more fitted to the fish species; Active manipulation of an external object in the attainment of a goal. Even though not a lot of information of this kind of behavior has been registered, a few examples stand out and provide clear evidence about such kind of properties in fish.

A black-spotted tuskfish (*Choerodon schoenleinii*) has been observed to crack open a bivalve on an anvil. The fish grasp the bivalve in its jaws and take it to what seems to be a feeding station. The fish deliver alternate, well-placed blows to the left and right by rapidly rotating its body, eventually breaking the shell open. A similar approach is made by Choerodon anchorago, but these fish tend to release the grasp just a little before the anvils, and thereby throwing the bivalve towards the avils (Brown, 2012). Triggerfish (*Pseudobalistes fuscus*, family Balistidae) feed on sea urchins and shellfish by manipulating the environment by removing obstacles to reach hidden but visible) prey, and experimental evidence suggests that these fish use a well-developed spatial intelligence while trying to get access to their prey. This species also tries to blow water streams to turn sea urchins over. Furthermore some individuals of *Balistapus undulates* (also family: Balistidae) cut the urchins' spines so that they can lift prey up. Wrasses (Coris angulata, Cheilinus fasciatus, C. lunulatus) take sea urchins in their mouth to crush them by swimming against corals to get access to the meat (Bshary et al., 2002).

Use of objects is also seen in fish when protecting their eggs. Many fish species are known to place their eggs on various objects. The eggs are sticky and will therefore hang on to the site where they are left. The objects are often in the size and form that make the fish capable to carry it away in case of emergency. The South American cichlids (Cichlidae) provides for such an example. It lays its eggs on leaves or small rocks which can then be used as 'tablets' to transport around in its territory (Brown, 2012).

5.3 Partial conclusion

The data above provides supporting evidence that fish possess cognitive capacity, and master some form of tool use which former has been viewed as being a limited primate skill (Brown, 2014). Interestingly these manipulative skills seem to be confined to a limited number of fish taxa (e.g. the wrasse) (Brown, 2012). More to it several of data demonstrate proof of memory and learning in fish and this altogether could be seen as pretty advanced skills to master, so maybe pain perception, which is a natural defense mechanism in humans will also show present in fish. In search for an answer it will be relevant to look at how fish behave or change their behavior if exposed to events which would be perceived as painful in humans. Do the responses show parallel to ours?

6. Suspension of behavior response to noxious stimuli

The nervous system of fish does not provide strong enough evidence to conclude pain perception in fish. A method to get closer to an answer might be to expose fish to what humans would consider as a painful situation, and see how they react. A couple of scientists have applied different approach to address this issue.

Much of the argumentation for and against the evidence of pain perception in fish comes from Lynne U. Sneddon and James D. Rose, who seem to deeply disagree on this subject. It is therefore their opinions that will dominate this section.

In an experiment performed by (Sneddon, 2003a) the acute effects of administering a noxious chemical to the lips of rainbow trout were examined. The aim was to prove the capability of fish to perceive pain, by demonstrating that the behavior of animals are adversely affected by a potentially painful event and is not simply due to a reflex response. Twenty-five rainbow trout were assigned to four treatment groups. The trout had either, (i) sterile saline, (ii). acetic acid, (iii) acetic or (iiii) acid and morphine sulfate, injected into their lips. In addition one group got only morphine and the last group used for control was handled but did not receive any injection. The reason for using acetic acid is that this has been proved to give a nociceptive response in both animals and humans, and is typically used in pain tests (Sneddon, 2003a). For the acid injected fish it took almost double as long to resume feeding (approximately 170 minutes) compared with the other groups. In both acid and acid–morphine treatment groups an abnormal "rocking" behavior was reported (the fish were observed to move side to side on either pectoral fin). Additionally the author states that this type of behavior might fall into category with the stereotype behavior which often is observed in zoo animals. This type of behavior is seen as an indicator of poor welfare. Moreover both groups of fish were observed to rub their lips against the gravel and the side of the tank. Larger doses of morphine, properly would have dismissed the rubbing behavior in the acid-morphine group too. No difference in amount of swimming was observed in either group. Increased opercular beat rate was reported in the acid group, but not in the others. The different behaviors reported were interpreted by the author as being due to higher brain processing. The morphine did act as analgesic agent in the reducing of pain. The author sustain in regards to the rubbing behavior that this was not caused by simple reflexes but could be likewise the behavior, which would occur in higher vertebras as a pain-related response. Consequently Sneddon (2003) concludes that fish are capable of pain perception.

Rose et al., (2014) oppose to this view and argue that the elevated opercular beat rate reported in the latter experiment, might rather be due to gill irritation, than pain. It is proposed that this would have happen because of leakage from the injection site. The suggestion that the mouth rubbing should be an indicator for pain, owe to the fact that it was concluded that morphine reduced such behavior, is also refused by Rose et al., (2014). They claim that it might have occurred entirely through nociception. In addition they note that the morphine doses used were quite high, and the trout's response to the morphine must indeed be very different compared with mammals, where they state such doses would exceed lethal doses. Rose et al., (2014) utter in their report that many of the papers in which evidence for pain in fish have been found, are defined as a response that is 'more than a simple reflex'. Such a definition of a supposable pain response is according to Rose et al. (2014) to vague and ambiguous and therefore not a valid explanation for a painful event (Rose et al., 2014).

A similar experiment also obtained by (Sneddon et al., 2003a), was done in order to assess whether fish have cutaneous nociceptors which can detect noxious stimuli. They then establish whether the behavior of the fish was adversely affected by the noxious stimulus. The trout were assigned into different treatment groups also receiving different injections in their lips: (i) sterile saline; (ii) bee venom; (iii) acetic acid; and (iiii) control - fish handled but received no injection. In all groups increases in opercular beat rate were measured. Although, the acid and venom group showed significant bigger change in beat rate, before and after treatment. These two groups did also take longer in proceeding to eat than the saline and control group did, and were observed to perform a ‘rocking’ behavior, after handling. Though only the fish which had got the acid injection was seen rubbing their lips into the gravel, no such behavior was noted in the venom group. Either of the treatments seemed to have influence on the fish activity level, and none of the fish searched into the sheltered areas. All these behavioral changes were clear evidence according to the authors that trout not only are capable of detecting noxious stimuli but also that the fish were physiologic negatively affected by them, such as humans would be in the case of feeling pain.

In their paper *Can fish really feel pain*, Rose et al. (2014), express a great deal of skepticism towards this conclusion, and point out that such relatively large amount of venom or acid, would cause severe pain to a human, and that the trout seemed to be remarkably little affected by it. Furthermore, they question the fact that rubbing of lips into the gravel was only observed in the acid group and not in the fish injected with venom. They argue that if this behavior was really due to the feeling of pain, it should be reflected in both groups. This statement would nevertheless not be accepted by Reilly et al., (2008). They found very different behavior in response to potentially painful stimuli, in common carp, zebrafish (*Danio rerio)* and rainbow trout respectively and state that one should be aware of stimulus-specific behavior.

Avoidance learning is also considered a good indicator for an animal to be experiencing a negative state. Heath avoidance by goldfish has been documented by Nordgreen et al., (2009). Adverse stimuli avoidance in fish to electric shock has been reported in the papers of Sneddon, (2003a) and Sneddon et al., (2003a). The study in referred to in those reports, was done by Rudolph H. Ehrensing (1982). Here goldfish were exposed to electric-shock to test the effect of Prolyl-leucyl-glycinamide (MIF-1) and naloxone and their ability to reduce the effects of morphine. An agitated swimming response was used as an indicator of pain (Ehrensing et al., 1982). Dunlop et al., (2006) exposed trout and goldfish to electric shock in effort to show the avoidance of these fish to such noxious stimulus. When stimulated by high intensity shock it resulted in a freezing respond or escape from the area. During and after the stimulus presentations the fish spend more time in the non-stimulated zones compared with pre-stimulating period (Goldfish: $F_{(2,26)}$ = 36.098 and P = 0.0001, trout: $F_{(2,20)}$ = 12, 32 and P < 0.001). This shows significance in avoidance of the explosive zone in both groups. These results provide evidence that pain avoidance is not purely a reflex action. This interpretation is supported by Braithwaite and Boulcott (2007) that state:

> “*The fact that the fish were able to learn and modulate their behavior to avoid aversive stimuli such as electric shocks indicates that these responses in fish can be learned and are not just reflex responses”*.

Another experimental arrangement is hook avoidance tests. Askey, et al. 2006, did a research in order to determine whether fish are able to learn hook avoidance after being caught in catch-and-release procedure. This hypothesis was tested on rainbow trout. They found the catch rates to drop rapidly within seven to ten days, which indicates a change of behavior in previously captured fish. The fish were assumed to lower their own catchability. This experiment used fly-fishing gear, meaning that no life bait was used. Beukemaj, (1970) did in contrast use both natural bait and artificial bait, in a similar hook-avoidance experiment with pike (*Esox Lucius L*.). Just as in the latter experiment, catchability was decreased to very low levels after previous experience with the hook by almost half of the population. Such a result was only obtained in the case with the artificial bait (spinner fishing). Neither of these experiments were aimed specifically to prove any kind of emotional processing nor nociceptive responses. It could be argued though that the avoidance response was caused by previous negative experiences (earlier encounters with the hook), and eventually due to the feeling of pain. Yet another experiment performed fin clip of Nile tilapia (*O. niloticus*) and it was concluded that this kind of stimulus is rather painful for the fish (Roques et al., 2010).

Ashley et al. (2009) showed that dominant fish became less aggressive when suffering from supposable pain; acetic acid had been injected into the treatment groups, including both shy and bold fish. Shy fish showed timid behavior. Boldness defined as taking more risks, are more active, spend more time in open water, learn conditioned test faster and show more aggressiveness and dominant behavior towards shy fish. Moreover when exposed to a predator cue treatment groups decreased their use of activity and cover use, whereas control fish behaved with normal antipredator response, by increasing their use of cover but also became more active. This shows suspension of behavior due to negative stimulus. A similar response was documented by Sneddon et al., (2003b) in rainbow trout which seemed to lose their fear of novel objects, when acetic acid has been injected into their lips. The pain appears to distract from performing other tasks (Brown 2014)

For an overview of different research experiments that document painful event in fish, see the appendix table A.1, where the above papers will be presented.

6.1 Partial conclusion

It was stated by Sneddon (2003a) and Sneddon et al., (2003a), that electric-shock avoidance had been documented by Rudolph H. Ehrensing. Sneddon et al. (2003a) and Sneddon (2003a) have misinterpreted this study as only a physical reaction, such as an agitated swimming response (ASR) was observed and used as an indicator for pain. No avoidance behavior has been documented in this paper. Nonetheless avoidance testing demonstrates the importance of aversive stimuli to an animal. If one quickly learns to avoid a suggested averse stimulus, the stimulus presumably has a negative effect on welfare.

Discussion

In chapter one different definitions of welfare have been reviewed, and whether an animal suffer from impaired welfare may strongly depend on which of the aspects is being considered. The Five Freedoms bring forward a framework on how to analyze the welfare of animals. Noteworthy is, that the lack of scientific agreement about the existence of sentience in fish may hinder contributes to develop new strategies for the different ways of keeping fish. Nevertheless some of the cases provide strong evidence of impaired welfare. Activities such as catch-and-release fishery, has been documented to have a negative effect on the condition of the fish, and leaves a stress respond in the fish (Thorstad et al., 2003; Det Dyreetiske Råd, 2013). Especially variables such as impaling the mouth, bleeding from the hook wound and air exposure during these sections were found to negatively affect condition at release in such tasks. Moreover, the handling time was noted to have a harmful influence, but this result did not show statistical significance (Thorstad et al., 2003). Chances of surviving after being caught and set back differ according to the condition of the fish (D et Dyreetiske Råd 2013). Even though these examples should provide enough evidence to set in new strategies, when it comes to catch and release fishery, effort is hardly made to do so, because of the deep rooted thought of the incapability of fish to consciously experiencing pain.

This assignment presents the different opinions about whether fish are capable of nociception, being sentient, and if their response to noxious stimuli can be interpret as a sign of pain, which prevails among scientists. Chapter four concentrates on identifying the necessary nervous structures for fish to feel pain. Nociception is clearly possible in fish, and this happens through the same type of nociceptors as have been concluded to be responsible for carrying the nociceptive impulses, that lead to pain perception in humans. A lesser certainty is whether these nociceptive stimuli lead to the same awareness in fish as in humans. Fish do not possess a neocortex, and the lacking of such a complex network of neurons raise doubts about whether fish can feel pain. Nonetheless telencephalic neuron activity has been shown in response to noxious stimuli, and when Rose (2002) sustains that reflexive responses occur due to subcortical activity, telencephalon activity could be a sign of not pure reflexes. Yet Rose (2002) remains convinced in regards to neocortex being the only brain structure able to be responsible for pain perception. Firstly, this opinion could be argued to contradict an earlier declaration, by Rose (2002), which states that anthropomorphism is not valid to support scientific research, when at the same time claiming that only a neocortex structure as the one in humans, will prove evidence of pain perception. Secondly, it clearly ignores the fact that the central region of the bird telencephalon, called the dorsal ventricular ridge (DVR), has found to be similar in function and structure to the neocortex of humans. Reptiles too do not have a neocortex, but similarities of function between the mammalian neocortex and the cortical area in reptiles has also strongly been suggested (Dugas-Ford et al., 2012). This leaves the possibility open for the same affirming of neuro function of fish.

An anthropomorphic approach may to some instance hinder the understanding of other species. Still the importance and value of such comparable studies based on formerly information have provided fundamental knowledge about both similarities and differences in many species. This approach has for instance resulted in a lot of success when it comes to medicine research, where animals such as pigs have functioned as models for humans. This again underlines the possibility for fish to be able to perceive some sort of pain, when quite many similarities are found in their nervous system compared with that of other mammals. What must be strongly emphasized is that the literature only concludes these similarities of brain areas to be homologue with that of mammals, and not humans'. This again leads to inconclusiveness as no psychological state can for sure be measured in other animals but humans.

The evidence for learning and storing of memory, together with tool use, of fish has been reviewed. This was done to prove cognition in fish, intelligence and eventually consciousness. Even though fish seem not to be able to recognize oneself by visual cues, several data show that fish are able judge the performance of others and alter their own behavior in respect to what they have previously observed. They are able to learn and remember information for a period of time, and use that knowledge in the future (White and Brown, 2013). Proof of such ability in fish is overwhelming seen in the light of what previously has been thought about this animal group. Additionally, the fact that fish show evidence for being cognitive individuals could reflect some form of consciousness too. This may further suggest that fish would also be conscious in relation to painful events. Consciousness can stand alone as an animal can be conscious but nonetheless not possess cognition, although the ability to be conscious when showing cognitive abilities might be pretty strong. At the same time fish are clearly capable of acting in such ways that it improves their success in life. This is being exemplified by for instance the capability of fish to actively manipulate an external objective in the attainment of a goal. This skill may also imply some form of intelligence, and at least primary consciousness may seem to be paramount in order to survive. The literature represented in part 5, indeed also indicate secondary consciousness.

Different experiments done to prove pain feeling in fish are fiercely debated by Lynne U. Sneddon and James D. Rose. Rose (2014) provides several of conflicting statements about whether the different experiments and opinions brought forward by Sneddon (2003a) and Sneddon et al., (2003a) can be accepted as valid scientific evidence. Rose (2002) claims that leakage of the injection site would have caused the increased opercular beat rate due to gill irritation (documented in a research by Sneddon, (2003a)), and that mouth rubbing observed in trout, may be only a nociceptive reflex. This correspondence is also purely speculative, and none of which have been proved to be the true reasons for the responses. Additionally I argue that gill irritation means that the trout still are capable of feeling, and any irritation, is also an unpleasant experience, and should be not avoided. Rose (2014) at the same time strongly criticizes the vague response in the trout to such high doses of acids (0,1 ml) acetic acid (Sneddon et al., 2003a). This apparently little responsive effect could be owed to the fact that different species develop different protective mechanisms dependent on what kind of danger could occur in their environment. Applying this hypothesis on latter results, means that a plausible reason for the comparable little response, may lie with the small risk for encountering with acid substances, when living in water. The answer could also be found in the number of C fibres in bony fish being very small (see chapter 4). These are the fibres responsible for conducting chemical stimuli, this including acid impact. Thus as only very few nociceptors are present to carry this kind of stimuli, the perception of the emotional state may also be reduced compared with other types of stimuli.

In both hook avoidance test presented in this paper, the subjects show ability learning to elude hooks. One noteworthy thing was that fish previously fished with life bait, did not show significantly hook avoidance. This may raise the question if the fish did only learn to differentiate between the artificial bait (the spinners) and the life bait, and not learned to avoid an adverse feeling, as first assumed.

In general the number of animals used in most of the research is fairly low, n≤8 (Oliveira et al., 1998; Sneddon, 2003a; Dunlop et al., 2006; Portavella & Vargas, 2005), which may reflect ethical consideration. A conclusion is always stronger if more data is available.

Pain capability is very much an attitude topic, and the underlying interest of the ones debating this, has the tendency to shine through. The reason for addressing this subject was my worry when it came to the welfare of non-human animals and particularly the ones which I feel are being ignored in this respect to this matter. Fish leave an excellent example in that an activity, such as catch and release fishery would never seen acceptable in the animals, which we keep as pets. For most times we are trying to protect for example farm animals from suffering during culling. Nonetheless impale a fish through its palate seems not of much concern to us. It seems to me that three factors properly are the reason for this discrimination. Firstly, is the “cute factor”. Animals possessing the “cute factor”, are often protected to a much higher extend than for example insects and fish, which may not speak to our innate protector instinct in the same way as furry animals. Secondly is the lack of knowledge about the non-human animals. Thirdly, I think that the animals, which we interact with on daily basis, are at enhanced chances to receive better welfare.

Congenital insensitivity to pain with anhidrosis (CIPA) is an illness seen in a few hundred people worldwide. People suffering from this disease are in incapable of pain perception, together with sweat secretion and many dies within an age of three years. Pain function as an alarm clock, warning us about that something is not right, and in physiological aspect may cause tissue damage. Patients lacking this sort of alarming signaling, can have damages such as broken bones and server life threatening diseases to take place unnoticed and therefore do not receive proper treatment. Interestingly this proves a very good point as if fish were not able to experience the feeling of pain, how come that this species has survived up till now? What needs to be recognized is that pain is an essential factor when it comes to survival mechanisms of both humans and non-human animals. It functions as a guidance to keep us away from different dangerous stimuli (Illustreret videnskab, 2007).

The debate of pain perception in fish may persist long into the future, but we still need to take a stand in consideration to the welfare. Catch and release fishery, is truly problematic in that this is an activity only due to entertainment. The experience and excitement in fishing does not provide a good enough reason for potentially harming a living individual, and is therefore unacceptable. The Ethical Animal Counsel in Denmark (Det Dyreetiske Råd, 2013) consequently advises a prohibition against this type of fishing. The procedure of catch and release fishery would certainly be a painful event in humans. This will be the only thing to be certain of, and one must assume that the same is true in the case of other organisms, until other has been proven. Even though we do not know for sure whether fish are experiencing pain, it must be recognized that pain is a subjective experience, and subjective experiences of a fish may be very different from humans, but may still be an unpleasant experience.

Conclusion

The term "welfare" embraces different aspects, and therefore no specific definition exists. *The Five Freedoms* nevertheless provide good guidelines to judge the welfare for the animals that are in our care and control. Freedom from pain is a clear criterion for good welfare. When considering the literature regarding pain perception in fish, a lot of the studies conclude that such an event is possible (see appendix table 3). Fish are capable of nociception and do have many of the necessary structures for pain. Plenty of data supports cognitive skills, together with avoidance behavior in fish when exposed to noxious stimuli. Moreover reduction of normal behavior when handled with morphine is documented. Nonetheless these conclusions are easily dismissed by for instance the claims of behavioral responses from presumable noxious stimuli, being due to nociceptive events and therefore purely reflexive. Whilst strong proof of cognition may not mean that these animals are actually conscious beings and this remain inconclusive, as the mind of others cannot be measured. It therefore seems that signs for pain perception can always be excused by other explanations, so it cannot be concluded that fish can feel pain. Hereby my hypothesis cannot be confirmed, as the evidence for pain perception in fish, are not conclusive.

With the above statements it might for some be reasonable that the current treatment of fish does not need to change in any direction. As this does not prove that pain is experienced in a fish when caught during fishing. Nevertheless we should still give animals the benefit of the doubt, and try to avoid treatment of them which would be painful to us. It is therefore reasonable to consider the proposal uttered by the Ethical Animal Counsel in Denmark, about restraint catch and release fishery.

Further study

The assessment of pain in fish is still a fairly new approach. An effort ought to be made, is the discovery of, or a definite conclusion of fish having a neocortex-like structure. This will mean that they can equally be accepted, in welfare considerations in same way as mammals and birds. The structures in telencephalon showing response to noxious stimuli therefore need to be further studied to establish whether the neurons in these areas may be similar to that of the neocortex.

References

Appleby, MC, Mench, JA, Olsson, I, Anna, S. 2011. Animal welfare. UK, CAB International.

Aronson, LR, 1971. Furter Studies on Orientation and Jumping Behavior in the Gobid Fish, Bathygobius Soporator. Ann. NY. Acad. Sci. 188: 378-392.

Ashley, PJ, Ringrose S, Edwards KL, Wallington E, McCrohan CR, and Sneddon LU. 2009. Effect of noxious stimulation upon antipredator responses and dominance status in rainbow trout. Anim. Behav. 77: 403-410.

Basbaum, A I, Bautista, DM, Scherrer G, and Julius D. 2009. Cellular and molecular mechanisms of pain. Cell. 139: 267-284.

Beukemaj, J J. 1970. Acquired hook-avoidance in the pike esox lucius L. fished with artificial and natural baits. J. Fish Biol. 2: 155-160.

Boissy, A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling, LJ et al. 2007. Assessment of positive emotions in animals to improve their welfare. Physiol. Behav. 92: 375-397.

Braithwaite, VA, and Boulcott P. 2007. Pain perception, aversion and fear in fish. Dis. Aquat. Organ. 75: 131-138.

Broglio, C., Gómez A, Durán E, Salas, C, and Rodríguez F. 2011a. Brain and Cognition in Teleost Fish. Pages 325-358 in Fish Cognition and Behavior. Wiley-Blackwell,

Broglio, C, Rodríguez F, and Salas, C, 2003. Spatial cognition and its neural basis in teleost fishes. Fish Fish. 4: 247-255.

Brown, C. 2014. Fish intelligence, sentience and ethics. Anim. Cogn. : 1-17.

Brown, C. 2001. Familiarity with the test environment improves escape responses in the crimson spotted rainbowfish, melanotaenia duboulayi. Anim. Cogn. 4: 109-113.

Brown, C. 2012. Tool use in fishes. Fish Fish. 13: 105-115.

Bshary, R, Wickler, W and Fricke, H. 2002. Fish cognition: A primate's eye view. Anim. Cogn. 5: 1-13.

Bshary, R, Gingins S, and Vail, AL. 2014. Social cognition in fishes. Trends Cogn. Sci. 18: 465-471.

Buatti, MC., and Pasternak, GW. 1981. Multiple opiate receptors: Phylogenetic differences. Brain Res. 218: 400-405.

Butler, AB. 2011. Brain and Nervous System | Functional Morphology of the Brains of Ray-Finned Fishes. Pages 37-45 in Encyclopedia of Fish Physiology. Farrell, AP, ed. Academic Press, San Diego.

Butler, AB. 2000a. Chapter 20 - Nervous System. Pages 331-355 in The Laboratory Fish. Ostrander, G. K., ed. Academic Press, London.

Butler, AB. 2000b. Chapter 8 - Nervous System. Pages 129-149 in The Laboratory Fish. Ostrander, G. K., ed. Academic Press, London.

Chandroo, KP, Duncan IJH, and Moccia RD. 2004. Can fish suffer?: Perspectives on sentience, pain, fear and stress. Appl. Anim. Behav. Sci. 86: 225-250.

Den store danske. 2012: Kognition [online]. Gyldendals åbne encyklopædi, 21. marts 2012, København. [citeret 17. september 2014]. tilgængelig på internet: http://www.denstoredanske.dk/Krop,_psyke_og_sundhed/Psykologi/Psykologiske_termer/kognition

Det Dyreetiske Råd. 2013: Udtalelse om lystfiskeri [online]. Ministeriet for Fødevarer, Landbrug og Fiskeri, september 2013, København.[citeret 27. oktober 2014]. Tilgængelig på internet: http://fvm.dk/fileadmin/user_upload/FVM.dk/Dokumenter/Fiskeri/Indsatser/Lyst-_og_fritidsfiskeri/Udtalelse_om_lystfiskeri__DOK578351_.pdf

Dugas-Ford, J, Rowell, JJ, and Ragsdale, CW. 2012. Cell-type homologies and the origins of the neocortex. Proc. Natl. Acad. Sci. U. S. A. 109: 16974-16979.

Dugatkin, LA, and Godin, JJ. 1992. Reversal of female mate choice by copying in the guppy (poecilia reticulata). Proceedings: Biological Sciences. 249: 179-184.

Dunlop, R, and Laming, P. 2005. Mechanoreceptive and nociceptive responses in the central nervous system of goldfish (carassius auratus) and trout (oncorhynchus mykiss). The Journal of Pain. 6: 561-568.

Dunlop, R, Millsopp S, and Laming, P. 2006. Avoidance learning in goldfish (carassius auratus) and trout (oncorhynchus mykiss) and implications for pain perception. Appl. Anim. Behav. Sci. 97: 255-271.

Ehrensing, R H, Michell, GF, and Kastin, AJ. 1982. Similar antagonism of morphine analgesia by MIF-1 and naloxone in carassius auratus. Pharmacology Biochemistry and Behavior. 17: 757-761.

Flood, NC, Overmier JB, and Savage, GE. 1976. Teleost telencephalon and learning: An interpretive review of data and hypotheses. Physiol. Behav. 16: 783-798.

Gibson, RM, and Höglund, J. 1992. Copying and sexual selection. Trends in Ecology & Evolution. 7: 229-232.

Huntingford, FA, Adams C, Braithwaite, VA, Kadri S, Pottinger,TG, Sandøe, P, et al. 2006. Current issues in fish welfare. J. Fish Biol. 68: 332-372.

Illustreret videnskab (2007): Findes der mennesker der ikke kan føle smerte?[online]. illustreret videnskab IV nr. 2, 2002. [citeret 30. oktober 2014]. tilgængelig på internet: http://illvid.dk/mennesket/findes-der-mennesker-der-ikke-kan-foele-smerte

International Association for the Study of Pain (2012): IASP Taxonomy [online] International Association for the Study of Pain,Washington, D.C., 6. oktober 2014 [citeret 25. oktober 2014]. tilgængelig på internet:http://www.iasp-pain.org/Taxonomy?navItemNumber=576

Ito, H, and Yamamoto N. 2009. Non-laminar cerebral cortex in teleost fishes? Biol. Lett. 5: 117-121.

Johnsson, JI, and Åkerman, A. 1998. Watch and learn: Preview of the fighting ability of opponents alters contest behaviour in rainbow trout. Anim. Behav. 56: 771-776.

Martins, CI, Galhardo, L, Noble, C, Damsgård, B, Spedicato, MT, Zupa, W, et al. 2012. Behavioural indicators of welfare in farmed fish. Fish Physiol. Biochem. 38: 17-41.

McGregor, PK, Peake, TM, and Lampe, HM. 2001. Fighting fish betta splendens extract relative information from apparent interactions: What happens when what you see is not what you get. Anim. Behav. 62: 1059-1065.

Mueller, T. 2012. What is the thalamus in zebrafish? Front. Neurosci. 6: 64.

Nordgreen, J,Garner, JP, Janczak, AM, Ranheim, B, Muir WM, and Horsberg, TE. 2009. Thermonociception in fish: Effects of two different doses of morphine on thermal threshold and post-test behaviour in goldfish (carassius auratus). Appl. Anim. Behav. Sci. 119: 101-107.

Oliveira, R F, McGregor, PK, and Latruffe, C. 1998. Know thine enemy: Fighting fish gather information from observing conspecific interactions. Proceedings of the Royal Society of London.Series B: Biological Sciences. 265: 1045-1049.

Panksepp, J. 2005. Affective consciousness: Core emotional feelings in animals and humans. Conscious. Cogn. 14: 30-80.

Portavella, M, Vargas, JP, Torres, B and Salas, C. 2002. The effects of telencephalic pallial lesions on spatial, temporal, and emotional learning in goldfish. Brain Res. Bull. 57: 397-399.

Portavella, M, and Vargas, JP. 2005. Emotional and spatial learning in goldfish is dependent on different telencephalic pallial systems. Eur. J. Neurosci. 21: 2800-2806.

Reilly, SC, Quinn, JP, Cossins AR, and Sneddon, LU. 2008. Behavioural analysis of a nociceptive event in fish: Comparisons between three species demonstrate specific responses. Appl. Anim. Behav. Sci. 114: 248-259.

Roques, J A, Abbink, W, Geurds, F, Vis, H, and Flik, G. 2010. Tailfin clipping, a painful procedure: Studies on nile tilapia and common carp. Physiol. Behav. 101: 533-540.

Rose, JD, Arlinghaus, R, Cooke, SJ, Diggles, BK, Sawynok, W, Stevens, ED, et al. 2014. Can fish really feel pain? Fish Fish. 15: 97-133.

Rose, JD. 2002. The neurobehavioral nature of fishes and the question of awareness and pain. Rev. Fish. Sci. 10: 1-38.

Salas, C, Broglio, C and Rodriguez, F. 2003. Evolution of forebrain and spatial cognition in vertebrates: Conservation across diversity. Brain Behav. Evol. 62: 72-82.

Shettleworth, S J. 2001. Animal cognition and animal behaviour. Anim. Behav. 61: 277-286.

Sneddon, LU. 2009. Pain perception in fish: Indicators and endpoints. ILAR J. 50: 338-342.

Sneddon, LU. 2011. Cognition and Welfare. Pages 405-434 in Fish Cognition and Behavior. Wiley-Blackwell,

Sneddon, LU. 2004. Evolution of nociception in vertebrates: Comparative analysis of lower vertebrates. Brain Res. Rev. 46: 123-130.

Sneddon, L U. 2003a. The evidence for pain in fish: The use of morphine as an analgesic. Appl. Anim. Behav. Sci. 83: 153-162.

Sneddon, LU. 2003b. Trigeminal somatosensory innervation of the head of a teleost fish with particular reference to nociception. Brain Res. 972: 44-52.

Sneddon, LU. 2002. Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, oncorhynchus mykiss. Neurosci. Lett. 319: 167-171.

Sneddon, LU, Braithwaite,VA and Gentle, MJ. 2003a. Do fishes have nociceptors? evidence for the evolution of a vertebrate sensory system. Proceedings: Biological Sciences. 270: 1115-1121.

Sneddon, LU, Braithwaite, VA and Gentle, MJ. 2003b. Novel object test: Examining nociception and fear in the rainbow trout. The Journal of Pain. 4: 431-440.

Thorstad, EB, Næsje, TF, Fiske, P and Finstad, B. 2003. Effects of hook and release on atlantic salmon in the river alta, northern norway. Fisheries Research. 60: 293-307.

Thuenken, T, Waltschyk, N, Bakker, TCM and Kullmann, H. 2009. Olfactory self-recognition in a cichlid fish. Anim. Cogn. 12: 717-724.

Tsuchiya, N, and Adolphs, R. 2007. Emotion and consciousness. Trends Cogn. Sci. 11: 158-167.

Vis, H, Kiessling, A, Flik, G and Mackenzie, S. 2013. Welfare of farmed fish in present and future production systems. Dordrecht: Springer.

Weber III, E S. 2011a. Fish analgesia: Pain, stress, fear aversion, or nociception? Veterinary Clinics of North America: Exotic Animal Practice. 14: 21-32.

Weber III, E S. 2011b. Fish analgesia: Pain, stress, fear aversion, or nociception? Veterinary Clinics of North America: Exotic Animal Practice. 14: 21-32.

White, G, and Brown, C. 2013. Site fidelity and homing behaviour in intertidal fishes. Mar. Biol. 160: 1365-1372.

Appendix

Table A.1 Presentation of different researches that conclude pain in fish. Adapted from Rose (2014).

Measures used to infer pain	**Stimulus**	**Inference concerning measure(s**	**Species**	**References**
Voltage necessary to produce agitated swimming response	Electric shock and opioid system manipulations	Threshold level of pain	Goldfish (*Carassius auratus*)	Ehrensing et al. (1982)
Escape response to heat applied to trunk Elevation of heat escape threshold by morphine Hovering in lower half of home tank after testing	Heat applied to trunk	Goldfish perceived heat as noxious	Goldfish (Carassius auratus)	Nordgreen et al. (2009a)
Shock avoidance, 'not purely a reflex action	Electric shock	Electric shock 'might lead to an increase in fear', 'if fear is considered an emotion…the possibility of fish perceiving pain must be considered.'	Goldfish (*Carassius auratus*) Rainbow trout (*Oncorhynchus mykiss*)	Dunlop et al., (2006)
Behavior that is 'more than a simple reflex' Respiratory rate 'Rubbing' 'Rocking' Response to novel object	Acetic acid injections in jaws, morphine injection	Behaviors reflect pain or fear	Rainbow trout (*Oncorhynchus mykiss*)	Sneddon et al. (2003b)
Behavior that is 'more than a simple reflex'? Respiratory rate 'Rubbing' 'Rocking' Latency to feed	Acetic acid injections in jaws, morphine injection and Acetic acid or bee venom injections in jaws	Behaviors reflect pain, morphine reduced 'pain-related behaviors' Behavior reflects pain	Rainbow trout (*Oncorhynchus mykiss*)	Sneddon(2003a) and Sneddon et al. (2003a)
Swimming Preference for darker part of tank (Tilapia only)	Caudal fin clips	Differential response to fin clip shows this is a 'painful procedure'	Common carp (Cyprinus carpio) Nile tilapia (Oreochromis niloticus)	Roques et al. (2010)
Exploration of novel environment Use of 'cover' Response to alarm pheromone	Acetic acid injections into jaws	Reactivity to a 'painful stimulus' modified use of cover and response to 'predator cue' providing evidence for central processing of pain rather than a 'nociceptive reflex'	Rainbow trout (*Oncorhynchus mykiss*)	Ashley et al. (2009)
Swimming Preference for darker part of tank (Tilapia only)	Caudal fin clip	Differential response to fin clip shows this is a 'painful procedure'	Common carp (*Cyprinus carpio*) Nile tilapia (*Oreochromis niloticus*)	Roques et al. (2010)