Pain perception, aversion and fear in fish

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ABSTRACT: There is now compelling evidence that teleost fish possess similar nociceptive processing systems to those found in terrestrial vertebrates. Noxious stimulation of these nociceptors — specialised pain receptors — in the skin around the snout of fish generates neural activity that can be electrophysiologically recorded, and induces a number of behavioural and physiological changes. To determine whether changes in behaviour are more than simple responses to the noxious stimulation it is necessary to demonstrate that higher order cognitive processes such as mental state or 'affective state' are involved. However, quantifying the 'motivational affected state' of an animal — a concept encompassing not just pain but also fear, hunger, thirst and pleasure — is difficult owing to its subjectivity. Recent empirical work is beginning to test these concepts in fish, and we review a number of these studies and suggest how these general methodologies could be used to further our understanding of fish cognition and the capacity for fish to experience mental states such as fear or suffering.

KEY WORDS: Teleost fish · Nociception · Pain · Fear · Emotion · Suffering · Welfare

INTRODUCTION

The suggestion that fish can perceive pain and that they may have the capacity to suffer (i.e. experience negative emotional or mental states) has sparked a controversial and lively debate (Huntingford et al. 2006). Some argue that there is now good evidence that fish possess the same types of specialised receptors as birds and mammals that allow the detection of noxious stimuli (nociceptors), and that the nervous system and brain of several species of fish appear sufficiently complex to allow us to conclude that they have the capacity for fear and suffering (Sneddon et al. 2003a, Chandroo et al. 2004a, Braithwaite & Huntingford 2004, Braithwaite 2006). On the other hand, there are others who propose that fish are merely responsive, passively reacting to stimuli as they encounter them with little or no ability for cognition or self-awareness (Rose 2002, 2007, this issue). There is a growing interest in resolving this debate, and in determining whether our interactions with fish cause negative responses that generate suffering. Here, we provide an overview of the scientific evidence associated with pain perception and cognitive capacities in fish.

A number of recent in-depth reviews tackling issues pertinent to this debate already exist, for example, in relation to (1) fish welfare (Huntingford et al. 2006, Chandroo et al. 2004a), (2) fish cognition (Braithwaite 2006), and (3) questions relating to sentience and consciousness in fish (Chandroo et al. 2004a,b). These reviews cover a considerable breadth of research on pain, welfare and animal cognition and discuss how our interactions with this diverse group of vertebrates may affect their well-being (i.e. the balanced condition between biological, physical and cognitive states, Weed & Raber 2005). Our intention here, therefore, is to focus on what we view as the key elements of the debate over pain in fishes. We review evidence central to our understanding of the capacity of fish for pain perception, and we describe experiments that have investigated whether fish have emotions such as fear. Using these 2 lines of investigation we consider whether fish have the cognitive capacity to suffer from events or processes that we would consider to be painful and aversive in humans and other terrestrial vertebrates. In taking this approach, we identify areas that still lack understanding, and we suggest some approaches and methodologies that will be useful

to future work directed at understanding the consequences of noxious stimulation in fish.

NOCICEPTION AND THE EFFECTS OF NOXIOUS STIMULI IN TELEOST FISH

Many animals possess nociceptors i.e. specialised receptors associated with free nerve endings that detect potentially damaging, noxious stimuli. The nociceptive systems of birds and mammals are well understood and we have a good working knowledge of how noxious stimuli generate responses in these animals (e.g. Garry et al. 2004). In contrast, we have much less understanding of these processes in fish. For example, it is only recently that nociceptors have been described for teleost fish (Sneddon et al. 2003a). Nociceptors are linked to 2 classes of fibre that arise as free nerve endings in the dermal tissue. These 2 fibre types differ in diameter and convey information at different rates: (1) A-delta fibres are slow-conducting, small, myelinated fibres and are associated with 'first' pain (particularly in relation to noxious thermal stimulation), and (2) C fibres are smaller, unmyelinated, have an even slower conduction velocity and are associated with 'second' pain, the longer-lasting unpleasant sensation associated with tissue damage.

In 1971, Whitear described a range of nerve fibres within teleost skin, including free nerve endings associated with the outer epidermal surface. Some of these were linked with chemosensory cells in the skin, and other coarser fibres were presumed to be associated with mechanosensation. She further speculated that some of the free nerve endings may be pain receptors. Subsequent to these observations, however, little else was done to investigate the role of these free nerve endings in pain perception in fish. After Whitear's observations, there were a few investigations of the peripheral nervous systems in jawless fish (agnathans) and cartilaginous fish (elasmobranchs), which described the presence of A-delta fibres in some species of ray, such as the stingray *Dasyatis sabina* and longtailed ray *Himantura fai*, but the second type of fibres, C fibres, were either reported as absent or were found in very low numbers (Coggeshall et al. 1978, Leonard 1985, Snow et al. 1993, 1996).

The growing interest in fish welfare motivated the decision to look in more detail at the anatomy of the peripheral nervous system in rainbow trout *Oncorhynchus mykiss*. To formally study fish nociception, Sneddon et al. (2003a,b) followed 3 lines of investigation: (1) Sections of trigeminal nerve tissue (the main facial nerve) were studied for neuroanatomical evidence confirming the presence of the 2 specialised nociceptive fibres, A-delta and C fibres. (2) Electro-

physiological responses to noxious stimuli were quantified. (3) The effect that noxious stimulation had on fish behaviour was measured. In particular, the behavioural work was aimed at determining whether the responses seen in the fish were simple and reflexive in nature, or whether higher-order cognition was involved.

The trigeminal nerve consists of 3 branches (the ophthalmic, the maxilliary and the mandibular), and all 3 were carefully removed from terminally anaesthetised rainbow trout. The different branches were fixed, embedded in resin and then 1 μm thin sections were taken, stained and viewed under a light microscope (see Sneddon 2002). Using diameter size as the descriptor, A-delta and C fibres were identified. The diameter and distribution of these fibres were found to be fairly uniform across all 3 branches of the trigeminal nerve. Unmyelinated C fibres were found in small clusters and were often associated with a Schwann cell. The A-delta fibres were more abundant and made up approximately one third of all the fibre types. A-alpha and A-beta fibres were also present (Sneddon 2002). When the relative abundance of the different fibre types was quantified, there were relatively few C fibres — approximately 4% of all fibre types. This is in contrast to observations in mammals where up to 50% of the fibres may be C fibres. Why there are these differences between mammals and fish is unclear. It would be interesting to know whether this lower frequency of C fibres is consistent in other nervous tissue within the trout. Do other areas of the body, such as the flank or the areas around the fins, for example, contain more C fibres? Similarly, is the relatively low number of C fibres consistent across different fish species, or do some fish have more than others? And, are nociceptors generally distributed evenly across the skin of the fish, or are some areas more highly innervated than others, as is the case in birds and mammals?

Having confirmed the presence of A-delta and C fibres in the trigeminal nerve, Sneddon et al. (2003a) physiologically recorded action potentials from afferent cell bodies in the trigeminal ganglion of rainbow trout. Deeply anaesthetised fish, with water and anaesthetic flushed over the gills, were physically supported in a specially built cradle that permitted access to the brain from above. Following removal of the brain-case and skin, the fish were decerebrated (i.e. the olfactory lobes, optic lobes and the cerebellum were removed), and a neuromuscular blocker was administered to prevent muscle movement such as twitching. The trigeminal ganglion was then exposed, de-sheathed and covered with paraffin to allow it to remain moist. Glass electrodes were used to record activity from afferent cell bodies. Single cell activity in the trigeminal was recorded in response to noxious stimuli

applied to the head of the fish. Three types of noxious stimuli were used; mechanical (pressure), chemical and thermal. Receptor fields on the head of the fish were identified using a glass mechanical probe, and were then stimulated using von Frey filaments (fine hair-like probes of different thickness that enable thresholds of mechanical stimulation to be determined). The diameter of the receptor field was quantified and then reactivity to thermal and chemical stimuli was tested. Thermal sensitivity was tested by focusing a quartz light onto the skin, causing the receptor to be heated up to 58°C. Chemical sensitivity was assayed by applying a small drop of 1% acetic acid to the receptor. A drop of water applied to the receptive fields was used as a control stimulus to determine whether the physical action of putting a drop onto the receptor would affect it — the water never stimulated the receptors. Conduction velocities were measured by applying an electrical pulse directly to the receptor and then timing the latency for the action potential to reach the trigeminal ganglion.

Fifty-eight receptors were found on the head of the fish, just over half of which were described as pressure or touch receptors. However, 22 of the 58 receptors were classed as nociceptors. These responded with a slowly adapting firing pattern to the von Frey filament mechanical stimulation. Two nociceptor types were identified; 18 receptors were classed as polymodal nociceptors in that they responded to mechanical, thermal and chemical stimuli, and 4 were classed as mechanothermal nociceptors. Thus, electrophysiological evidence, together with the presence of the A-delta and C fibres, indicate that teleost fish such as trout possess the necessary neuroanatomy and neurophysiology to transduce and process information about stimuli that would be regarded as painful by humans.

The work described above demonstrates that fish have the basic neural systems for perceiving noxious stimuli; the next step is to determine how the detection of such stimuli affects the behaviour of the fish. Can the behavioural responses be described in terms of reflexive responses, or is the behaviour modulated at a higher cognitive level? To address this question, Sneddon et al. (2003a) designed an experiment to quantify changes in motivation to feed after application of a noxious stimulus. To infer that more complex cognition is involved in the behavioural responses we need to demonstrate that the fish's affective state has changed in response to the stimulus, for example has its attention or its motivation to perform a task changed in response to the stimulus? One way to address this, for example, is to consider the motivational state of the fish as an affective state — a state that reveals something about an animal's mental state or 'mood' (Paul et al. 2005). We might quantify this by determining whether an animal perceives a stimulus as positive or negative (i.e. is it willing to work towards obtaining the stimulus, or does it seek to avoid it?). In these terms, we could also consider emotion to be a form of affective state, and this is something we will return in the following section. Affective states are considered high-order cognitive processes because they are likely to involve considerable information processing in which multiple pieces of information need to be assessed and integrated to generate an appropriate decision or behavioural response.

To investigate how pain affects motivational state, 20 juvenile rainbow trout (30 to 100 g) were individually housed in tanks that provided a sheltered, darkened area in one half of the tank, and an open area that contained a surface food-ring attached to the wall on the opposite side of the tank. Prior to the trials, the fish were conditioned to approach the food-ring in response to a light cue. Once the conditioned response was learned, trout were randomly assigned to 1 of 4 treatments; 2 of these were control procedures, and 2 applied noxious stimulation. All fish were anaesthetised and handled. The first control group was then allowed to recover, fish in the second control group were given 0.1 ml of saline injected into the snout near the mouth, a third group was treated in the same way but they were injected with 0.1 ml of a 0.1% acetic acid solution, and a fourth was injected with 0.1 ml of bee venom (1 mg ml^{-1} saline). The 2 noxious stimuli were selected because of their known effects in mammals; acetic acid is an irritant known to stimulate mammalian nociceptors, and bee venom causes an inflammatory response. All fish were observed as they recovered, and their motivation to feed was gauged by their response to the light cue signalling food availability.

Trout treated with noxious stimuli (acetic acid or bee venom) showed prolonged suppression in their motivation to feed. It took these fish 170 min on average before they started to approach the food-ring in contrast to the 80 min it took for the control and saline injected fish to start feeding. The suppressed motivation to feed was observed when the fish were offered their normal diet of pellet-feed, but was also seen when preferred prey (live bloodworm) were offered. The precise mechanism underlying this observed decrease in feeding motivation is not yet known, but it should be noted that cortisol (a corticosteroid hormone involved in stress responses) can affect learned conditioned responses (Grillon et al. 2004).

Other affects observed in the noxiously stimulated fish included dramatically increased opercula beat rates compared to the control fish. Elevated opercula beat rate is a measure commonly used to infer a physiologically stressed state in fish (e.g. Lucas et al. 1993, Brown et al. 2004). Changes in opercula beat rate are a

sensitive response to disturbance, but it should be recognised that they do not always reflect the severity of the stimulus (Baretto & Volpato 2004). Additional observations included anomalous behaviours in the noxiously stimulated fish; fish in both groups were seen to rock from side to side on their pectoral fins whilst resting on the substrate. Similarly, fish injected with acetic acid were seen rubbing their snouts, where the noxious stimulus had been administered, on the walls and substrate of the tank. These anomalous behaviours were not observed in either of the control groups.

Taking these 3 levels of analysis together (i.e. anatomical, electrophysiological and behavioural), there is compelling evidence that teleost fish such as the rainbow trout possess nociceptors and that noxious stimulation adversely affects their behaviour. These behavioural changes not only reflect underlying changes in physiology, but also show that the experience of pain affects motivational state (i.e. motivation to feed).

EMOTION AND THE CAPACITY FOR FEAR AND AVERSION IN FISH

Emotions are thought of as psychological processes that enable animals to avoid danger and harm but allow them to work towards reward or desirable resources (Paul et al. 2005). As such, they can be seen as adaptive traits that enable an animal to fine-tune its behaviour to the environment or conditions in which it finds itself. In their recent review, Paul et al. (2005) elegantly highlight the problems involved in determining animal emotion. Unlike the study of human emotion, where subjects are able to verbally communicate their subjective experiences, to study emotion in animals we need to rely on behavioural techniques to quantify changes in behaviour and physiology. Such tools may reveal that emotion has been aroused, but to quantify the level and type of emotion is often difficult. Thus we must exercise some caution when we interpret behaviour in terms of fear, anxiety or pleasure. These are very much human-based terms, describing emotions that we feel and can recognise and empathise with in other humans. This is not to say that non-human animals may not have emotions, but it should be recognised that these will be qualitatively different to the emotions that we experience. Thus, it is important that we remember this difference and we must take care if we use the same words, or terms, to describe such mental and behavioural states in non-human animals.

In humans, fear is considered to be a negative emotion that arises in response to perceived danger and involves multiple components. For example, fear may not only encompass negative subjective feelings of dread, but may also be accompanied by perspiration, a rise in heart beat rate and a desire to move, or run away (Paul et al. 2005). This combination of responses underlies the emotion of fear, but need not necessarily be thought of as one complete process; indeed, it can be broken down into separate events or states (LeDoux 1996). We can therefore investigate fear by quantifying these separate processes even in animals where it is not possible to conclude that the animal is conscious of emotion (Tooby & Cosmoides 1990, Öhman 1999). It is also important to note that cognition can play a role in this component-view of emotion, because processes associated with information processing (i.e. cognition) change when there are changes associated with emotional state (Clore & Ortony 2000). Support for this comes from human studies which show that there are biases in cognitive processes associated with a subject's affective or emotional state (Mathews & MacLeod 1994). However, there is now evidence that similar cognitive biases arise in rodents and birds (Harding et al. 2004, Emery 2006).

There is some discussion in the literature as to whether emotional and cognitive processes can occur independently of each other (Paul et al. 2005). Panksepp (2003), for instance, considers that the affect system underlying emotion is processed in subcortical and basic areas of the brain and nervous system, whilst the cognitive system is associated with cortical activity. The fact that the development of the cortex is a relatively recent event in terms of evolutionary history can be seen to support the concept of independence in these 2 brain areas. Others, however, argue that emotion and cognition necessarily work together and cannot be seen as independent (Lazarus 1999, Forgas 2000). Given that cognitive processes do occur in fish (Braithwaite 2006), it seems reasonable to ask whether cognition is involved in the emotional and affective states exhibited in fish? In the next section, we consider 3 different experiments that have focused on 'fear' and avoidance behaviour in fish.

EMPIRICAL EVIDENCE OF AVOIDANCE BEHAVIOUR AND FEAR IN FISH

Trout typically show considerable avoidance of novel objects (neophobia), avoidance being quantified as the delay before the fish starts to approach the novel object. In an extension of the behavioural studies, Sneddon et al. (2003b) investigated rainbow trout attentional state by temporarily placing a novel object (a brightly coloured plastic column) into the home tank and then comparing the avoidance responses of control fish (anaesthetised, handled and treated with a saline injection in the snout as above), with test fish

(anaesthetised, handled and treated with 1ml of 0.1% acetic acid solution). Unlike control fish, the acetic acid group were found to spend more time closer to the object, suggesting that the avoidance response or attentional state was impaired in fish treated with a noxious stimulus (Sneddon et al. 2003b). If this impairment is due to the fish experiencing and being distracted by the noxious stimulation, it should be possible to reverse this impairment by administering some form of pain relief. The experiment was therefore repeated, but this time, in addition to injecting either saline (control) or acetic acid (noxious stimulus) into the snout, all fish were given an analgesic intramuscular injection (morphine sulphate). The control and acetic acid treated groups now showed similar levels of avoidance of the novel object. Thus, provision of the analgesic decreased the impairment of the avoidance response. These results support the earlier observations of Ehrensing et al. (1982), who found that intracranial application of morphine in goldfish decreased their response to electric shock. Subsequent application of opiate antagonists, however, reversed this effect. Although compelling, this approach by Ehrensing et al. (1982) was criticised because the application of morphine might be expected to reduce reactions to a stimulus regardless of whether it was associated with pain or not. The approach used by Sneddon et al. (2003b) however, counters this criticism because, here, the effect of the morphine was to revive the novel object avoidance behaviour in the fish.

It is important to note at this point that relatively little work has addressed analgesia in fish, and recent work clearly indicates that there are considerable differences between species. For example, the clearance of morphine from the bloodstream after intraperitoneal injection is much slower in 2 species of fish, the winter flounder *Pseudopleuronectes americanus* and seawater-acclimated rainbow trout, than has been found in mammals (Newby et al. 2006). Such findings emphasise the need for some careful, comparative studies on fish analgesia.

Other approaches investigating fear and avoidance have used associative learning conditioning paradigms to determine motivational affective states in fish. Yue et al. (2004) assessed fear responses in rainbow trout by conditioning them to associate an aversive chase by a plunging net with a light cue. After training, 65% of the fish learned to anticipate the presentation of the net when given the light stimulus and they responded by swimming away to another compartment to avoid the net prior to its deployment. This learned avoidance remained after 7 d, demonstrating that subjects had the capacity for long-term memory. The authors argue that by using such an approach they are able to quantify the motivation of the fish to avoid aversive stimuli,

and also to investigate the nature of the memory associated with the avoidance response. Startle responses have also been used as a behavioural indicator to characterise the emotion of fear in fish (Davis et al. 1976, Dunlop et al. 2006). The fact that the fish were able to learn and modulate their behaviour to avoid aversive stimuli such as electric shocks indicates that these responses in fish can be learned and are not just reflex responses. The motivations underpinning the aversion learning shown by Yue et al. (2004) are, of course, subjective (Dawkins 1993, Duncan 2002), and it is still necessary to address whether the observed aversion response equates to what may reasonably be considered as a negative affected state.

While the approach taken by Yue et al. (2004) is clearly useful, caution should be used in drawing inferences from this technique. For instance, it is vital in the design of conditioning experiments that the associative stimulus used is not also found to be aversive. Light, for example, is often found to act as an aversive stimulus for fish (Hoar et al. 1957), and it is possible that this stimulus may also produce reflexive responses in some species. Again, we need to recognise that not all fish species respond in the same way to the same stimulus, and this variation in response will need to be considered when working with other species. Furthermore, experiments using associative learning between an aversive stimulus and a signalling cue must be able to differentiate between behaviour driven by the animal's affective state (i.e. higher order cognitive processing) and that simply governed by the stimulus response processes of associative learning.

In a similar study, Dunlop et al. (2006) compared the avoidance learning behaviour of rainbow trout and goldfish *Carassius auratus*. They used 2 intensities of electric shock as aversive stimuli; these were given in specific regions of an open access tank to determine whether the fish could associate a location with the electric shocks. They also investigated whether the fish could modulate their behaviour in response to the intensity of the noxious stimulus. Finally, they investigated how the presence of a positive stimulus, visual access to a conspecific, modulated the avoidance response (Dunlop et al. 2006).

Trout and goldfish were stimulated by two 1 m lengths of insulated copper wire that were twisted around one another to create a light-weight lead. At one end the wires were connected to a power source, at the other the 2 wires were scraped free of insulation and were attached to the fish by gluing them onto the skin just behind the gill cover, or operculum. Fish were given open access to a $1.85 \times 30 \times 30$ cm tank. Pen marks drawn on the outside of the tank divided the tank into 4 equally sized sections. Two sections at one end were selected as the non-stimulating zones and

when the fish were in these 2 compartments no electrical stimulation was delivered. The next section was denoted the lower intensity stimulating zone, and the furthest end was the higher intensity stimulating zone. Both trout and goldfish responded to electrical stimulation either by briefly freezing followed by escape swimming behaviour, or by giving a single, powerful 'tail-flip' that propelled them away from the zones associated with electrical stimulation.

Dunlop et al. (2006) extended the approach taken by Yue et al. (2004) in that they investigated more than the learned association between the stimulus and the response. Unfortunately, there is a lack of independence in some of the data used to compare the time spent in the different areas of the tank, and thus we need to be careful in what we conclude from this part of the study. However, other data that were not compromised in this way showed that avoidance learning did occur, and that this involved learning to associate specific places with the noxious events. The 2 species also showed a number of differences in how they responded to the stimulus. Again, this highlights the importance of recognising that fish species vary in a number of ways, including physiological and behavioural responses. For example, Dunlop et al. (2006) found that the fish modulated their responses based on the intensity of the stimulus, and also changed their behaviour in the presence of a conspecific. Curiously, trout showed a strong motivation to stay close to a companion fish at the cost of continuous lower intensity electrical stimulation. In contrast, the goldfish moved out of the stimulating zone, but stayed in the adjacent compartment rather than move as far away as they could. The authors speculated that this was so that the goldfish could remain close to the conspecific, but was far enough away to avoid the electrical stimulation.

The experiments by Dunlop et al. (2006) and Yue et al. (2004) demonstrate that aversive behaviour in fish can be quantified, and that the aversion to noxious stimulation involves the integration of multiple pieces of information. Coordinating and integrating qualitatively different pieces of information, such as spatial learning, social context, and avoidance responses, makes considerable demands on the nervous system. And an ability to integrate these types of information coherently is considered to be a process requiring higher order cognition (Tononi et al. 1998).

DETERMINING NEURAL FUNCTION IN DIFFERENT AREAS OF THE FISH FOREBRAIN

Recent interest in the neural mechanisms underlying spatial and avoidance learning in fish has led to the striking discovery that different areas within the goldfish forebrain have functionally distinct properties, and that these are homologous to key mammalian brain structures. Teleost brains undergo a different developmental process to mammalian brains; where the mammalian neural tube folds in on itself, the teleost neural tube folds out (Rodríguez et al. 2006). This developmental difference means that, in comparison to a mammalian brain, the neural structures of the fish forebrain are reversed in order. Anatomical, hodological and lesion data now reveal that the lateral and medial pallial regions of the teleost forebrain are homologous to the mammalian hippocampus and amygdala. The amygdala in mammals is linked to processing information with an emotional content (e.g. aggression and fear), whereas the hippocampus is linked to timing and spatial learning and memory (Killross et al. 1997, Eichenbaum et al. 1992). Establishing that goldfish have these homologous structures represents an important advance in our understanding of the fish nervous system and the evolution of the vertebrate brain (Broglio et al. 2003).

The role of goldfish lateral and medial pallial forebrain regions was confirmed in a series of experiments that combined brain lesioning with behavioural observations (Portavella et al. 2002). The results from this work clearly demonstrate the ways in which these 2 different brain areas control different types of learning and memory task. Portavella et al. (2002) trained goldfish to avoid an area of a tank using the simultaneous presentation of a green light and an electric shock. Fish were then given different types of brain lesion surgery, and their ability to avoid being given an electric shock in response to the green light was measured. A lesion to the dorsomedial area of the forebrain impaired the avoidance response. However, fish given a sham (control) lesion, or a dorsolateral forebrain lesion showed no impairment in the avoidance response. In a second study, fish were given the light cue 5 s before a shock was administered. In this type of task, both avoidance and timing information is important. In this second experiment, the avoidance response was impaired in fish with either a dorsomedial or a dorsolateral lesion, but the sham (control) fish showed no deficit in their performance. Finally, goldfish were trained in a spatial task and then lesioned in the same ways as before. In this instance, only fish with dorsolateral lesions showed impaired performance. Taken together, these 3 experiments indicate that different types of task are processed in different areas of the forebrain. In particular, the dorsolateral and dorsomedial regions of the goldfish brain process different types of information. Also, the aversion behaviour observed by Portavella et al. (2002) represents a complex behaviour, with avoidance behaviour anticipating the delivery of noxious stimulation.

The work by Portavella et al. (2002) again demonstrates that the avoidance response to the electric shock is not solely dependent on simple reflexive responses. Rather, it would appear to involve complex behaviour, controlled by various parts of the brain that are reacting to anticipated aversive stimuli. The homology between goldfish dorsolateral and dorsomedial forebrain areas and the mammalian hippocampus and amygdala can be seen in the results of Portavella et al. (2002); simultaneous presentation of light and shock maximised emotional learning and memory (the light was associated with the noxious sensation of the electric shock) and when the amygdala homologue was ablated the fish had difficulty in avoiding the electric shock. However, tasks that required timing or spatial relationships to be remembered became impaired only when the dorsolateral areas, structures which are homologous with the mammalian hippocampus, were ablated.

Our increased understanding of teleost brain anatomy and the finding that fish have structures homologous to the hippocampus and amygdala open up the potential for some new and exciting advances. For example, it is now possible to use molecular techniques to determine brain areas where there are changes in activity. Using these techniques, we can treat a fish with a noxious stimulus and then visualise the active regions in the brain. Such approaches, combined with behavioural studies investigating the effects of anticipation of aversive stimuli, could provide a powerful new approach to the study of fish welfare.

CONCLUSION

We review 2 research areas that are central to the debate of whether fish can perceive pain and whether they have the emotional capacity to suffer. We suggest that research demonstrating the ability for nociception in teleosts is compelling. Teleosts possess specialised cutaneous pain receptors that are sensitive to damaging stimuli such as excessive heat and noxious chemicals and to mechanical pressure. The presence of a nociceptive system is clearly a necessary component for the perception of pain, but alone it does not provide evidence that the fish have an awareness of stimuli we would consider to be painful in ourselves or other terrestrial vertebrates such as birds and mammals. However, the fact that changes in the normal behavioural repertoire of the fish studied occurred after the administration of a noxious event does allow us to infer that the fish attended to this noxious stimulus in a way that was not just merely responsive.

Motivational affective states are becoming more prevalent in the evaluation of animal welfare (Broom 1998), and researchers are beginning to quantify such states in fish. Approaches like this are necessary to show that fish have the behavioural and physiological attributes that characterise conscious cognition or affected states. The successful application of such an approach will necessarily require the classification of behaviours associated with pain and fear in fish as they have in other species (Duncan 2002). Nevertheless, many of the responses fish have to aversive stimuli are similar to those found in mammals and birds and, given that fish brains have the capacity to remember and anticipate (Braithwaite 2006), such findings indicate that fish potentially have the capacity for long-term suffering.

LITERATURE CITED

Baretto RE, Volpato GL (2004) Caution for using ventilatory frequency as an indicator of stress in fish. Behav Process 66:43–51

Braithwaite VA (2006) Cognition in fish. Behav Physiol Fish 24:1–37

Braithwaite VA, Huntingford FA (2004) Fish and welfare: Do fish have the capacity for pain perception and suffering? Anim Welf 13:S87–S92

Broglio C (2005) Hallmarks of a common forebrain vertebrate plan: Specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. Brain Res Bull 66:277–281.

Broglio C, Rodríguez F, Salas C (2003) Spatial cognition and its neural basis in teleost fish. Fish Fish 4:247–255

Broglio et al. (2005)

Broom DM (1998) Welfare, stress and the evolution of feelings. Adv Stud Behav 27:371–403

Brown C, Gardner C, Braithwaite VA (2004) Population variation in lateralised eye use in the poeciliid Brachyraphis episcopi. Proc R Soc Biol Lett 271, S455 – S457. DOI 10.1098/rsbl.2004.0222

Chandroo KP, Duncan IJH, Moccia RD (2004a) Can fish suffer? Perspectives on sentience, pain, fear and suffering. Appl Anim Behav Sci 86:225–250

Chandroo KP, Yue S, Moccia RD (2004b) An evaluation of current perspectives on consciousness and pain in fishes. Fish Fish 5:1–15

Clore GL, Ortony A (2000) Cognition in emotion: always, sometimes or never? In: Lane RD, Nadel L (eds) Cognitive neuroscience of emotion. Oxford University Press, Oxford p 24–61

Coggeshall RE, Leonard RB, Applebaum ML, Willis WD (1978) Organization of peripheral nerves and spinal roots of the Atlantic stingray, *Dasyatis sabina*. J Neurophysiol 41:97–107

Davis RE, Kassel J, Schwagmeyer P (1976) Telencephalic lesions and behaviour in teleosts, *Marcopodus opercularis*: reproduction, startle reaction, and operant behaviour in the male. Behav Biol 18:165–177

Dawkins MS (1993) Through our eyes only? The search for animal consciousness. Spectrum/Freeman, Oxford

Duncan IJH (2002) Poultry welfare: Science or subjectivity? Br Poul Sci 43:643–652

Dunlop R, Millsopp S, 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

Eichenbaum H, Otto T, Cohen NJ (1992) The hippocampus — what does it do? Behav Neur Biol 57:2–36

Ehrensing RH, Michell GF, Kastin AJ (1982) Similar antagonism of morphine analgesia by MIF-1 and naloxone in *Carassius auratus.* Pharmacol Biochem Behav 17:757–761

Emery NJ (2006) Cognitive ornithology: the evolution of avian intelligence. Phil Trans R Soc B 361:23–43

Forgas JP (2000) Introduction: the role of affect in social cognition. In Forgas JP (ed) Feeling and thinking: the role of affect in social cognition. Cambridge University Press, Cambridge, p 1–28

Garry EM, Jones E, Fleetwood-Walker S (2004) Nociception in vertebrates: key receptors participating in the spinal mechanisms of chronic pain in animals. Brain Res Rev 46:216–224

Grillon C, Smith K, Haynos A, Nieman L (2004) Deficits in hippocampus-mediated Pavlovian conditioning in endogenous hypercortisolism. Biol Psychol 56:837–843

Harding EJ, Paul ES, Mendl M (2004) Cognitive bias and effective state. Nature 427:312

Hoar WS, Keenleyside MHA, Goodall RG (1957) Reaction of juvenile Pacific salmon to light. J Fish Res Board Can 14:815–830

Harding EJ, Paul ES, Mendl M (2004) Cognitive bias and effective state. Nature 427:312

Huntingford FA, Adams CE, Braithwaite VA, Kadri S, Pottinger TG, Sandoe P, Turnbull JF (2006) Current understanding on fish welfare: a broad overview. J Fish Biol 68:332–372

Killross S, Robbins TW, Everitt BJ (1997) Different types of fear conditioned behaviour mediated by separate nuclei within the amygdala. Nature 388:377–380

Lazarus RS (1999) The cognition-emotion debate: a bit of history. In: Dalgleish T, Power MJ (eds) Handbook of cognition and emotion. Wiley, Chichester p 3–19

LeDoux J (1996) The emotional brain. The mysterious underpinnings of emotional life. Simon & Schuster, New York

Leonard RB (1985) Primary afferent receptive field properties and neurotransmitter candidates in a vertebrate lacking unmyelinated fibers. Prog Clin Biol Res 176:135–45

Lucas M, Johnstone ADF, Priede IG (1993) Use of physiological telemetry as a method of estimating metabolism of fish in the natural environment. Trans Am Fish Soc 122:822–833

Mathews A, MacLeod C (1994) Cognitive approaches to emotion and emotional disorders. Annu Rev Psychol 45:25–50

Newby NC, Mendonça PC, Gamperl K, Stevens ED (2006) Pharmacokinetics of morphine in fish: winter flounder (*Pseudopleuronectes americanus*) and seawater-acclimated rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol 143:275–283

Öhman A 1999 Distinguishing unconscious from conscious emotional processes: methodological considerations and theoretical implications. In: Dalgleish T, Power MJ (eds) Handbook of cognition and emotion. Wiley, Chichester, p 321–352

Panksepp J (2003) At the interface of the affective, behavioral and cognitive neurosciences: decoding the emotional feelings of the brain. Brain Cogn 52:4–14

Paul ES, Harding EJ, Mendl M (2005) Measuring emotional processes in animals: the utility of a cognitive approach. Neurosci Biobehav Rev 29:469–491

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

Rodríguez F, Broglio C, Durán E, Gómez A, Salas C (2006) Neural mechanisms of learning in teleost fish. In: Brown C, Laland K, Krause J (eds) Fish cognition and behaviour. Blackwell, Oxford, p 243–277

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

Rose JD (2007) Anthropomorphism and 'mental welfare' of fishes. Dis Aquat Org 75:139–154

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, Gentle MJ (2003a) Do fish have nociceptors? Evidence for the evolution of a vertebrate sensory system. Proc R Soc Lond B 270:1115–1121

Sneddon LU, Braithwaite VA, Gentle MJ (2003b) Novel object test: examining pain and fear in the rainbow trout. J Pain 4:431–440

Snow PJ, Plenderleith MB, Wright LL (1993) Quantitative study of primary sensory neurone populations of 3 species of elasmobranch fish. J Comp Neurol 334:97–103

Snow PJ, Renshaw GMC, Hamlin KE (1996) Localization of enkephalin immunoreactivity in the spinal cord of the long-tailed ray *Himantura fai.* J Comp Neurol 367:264–273

Tononi G, Edleman GM, Sporns O (1998) Complexity and coherency: integrating information in the brain. Trends Cogn Sci 2:474–484

Tooby J, Cosmoides L (1990) The past explains the present: emotional adaptations and the structure of ancestral environment. Ethol Sociobiol 11:375–424

Weed JL, Raber JM (2005) Balancing animal research with animal well-being: establishment of goals and harmonization of approaches. Inst Lab Anim Res (ILAR) J 46:118–128

Whitear M (1971) The free nerve endings in fish epidermis. J Zool 163:231–236.

Yue S, Moccia RD, Duncan IJH (2004) Investigating fear in domestic rainbow trout, *Oncorhynchus mykiss*, using an avoidance learning task. Appl Anim Behav Sci 87:343–354