



Can fish really feel pain?

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Abstract

We review studies claiming that fish feel pain and find deficiencies in the methods used for pain identification, particularly for distinguishing unconscious detection of injurious stimuli (nociception) from conscious pain. Results were also frequently misinterpreted and not replicable, so claims that fish feel pain remain unsubstantiated. Comparable problems exist in studies of invertebrates. In contrast, an extensive literature involving surgeries with fishes shows normal feeding and activity immediately or soon after surgery. C fiber nociceptors, the most prevalent type in mammals and responsible for excruciating pain in humans, are rare in teleosts and absent in elasmobranchs studied to date. A-delta nociceptors, not yet found in elasmobranchs, but relatively common in teleosts, likely serve rapid, less noxious injury signaling, triggering escape and avoidance responses. Clearly, fishes have survived well without the full range of nociception typical of humans or other mammals, a circumstance according well with the absence of the specialized cortical regions necessary for pain in humans. We evaluate recent claims for consciousness in fishes, but find these claims lack adequate supporting evidence, neurological feasibility, or the likelihood that consciousness would be adaptive. Even if fishes were conscious, it is unwarranted to assume that they possess a human-like capacity for pain. Overall, the behavioral and neurobiological evidence reviewed shows fish responses to nociceptive stimuli are limited and fishes are unlikely to experience pain.

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Perhaps nowhere is the truism 'structure defines function' more appropriate than for the brain. The architecture of different brain regions determines the kinds of computations that can be carried out, and may dictate whether a particular region can support subjective awareness. Buzsaki (2007)

Introduction

In the past decade, research addressing fish welfare has focused increasingly on the possibility that mental welfare is a legitimate concern, particularly the question of whether fishes feel pain and suffer (Huntingford *et al.* 2006; Braithwaite 2010). In our view, much of this research seems mission oriented and differs, accordingly, from the more detached tradition expected of basic science. Given the unquestioned societal importance of fish welfare, it is essential that welfare policies and practices be based on sound science. In an article addressing this important issue, Browman and Skiftesvik (2011) have concluded that 'Much of the literature on aquatic animal welfare is flawed by four non-mutually exclusive (and often inter-related) biases: under-reporting/ignoring of negative results, faith-based research and/or interpretations, hypothesizing after the results are known (HARKing), and inflating the science boundary. These biases have an insidious impact on the credibility of the "science" surrounding aquatic animal welfare.'

A critical evaluation of research literature pertaining to aquatic animal welfare is clearly

needed, particularly literature dealing with the issue of fish mental welfare. Here, we critically examine recent research on which claims for fish pain, suffering, and awareness are based and address the following issues:

1. proper conduct of pain research with fishes, including matters of experimentally assessing pain with valid measures;
2. technical and interpretational problems that undermine studies purporting to have demonstrated a capacity for pain awareness in fishes;
3. evidence from a wide variety of experimental and field studies that were not necessarily designed to study pain but offer insights into the possibility of pain experience by fishes;
4. claims for conscious awareness in fishes; and
5. costs to humans and fishes of invalid definitions and mistaken beliefs concerning fish pain and suffering.

Pain research with fishes – problems with definition and measurement

Pain research with human subjects has been productive on many fronts, particularly in the use of brain imaging methods, like positron emission tomography and functional magnetic resonance imaging to advance our understanding of the higher brain processes that underlie pain (Derbyshire 2004; Bushnell and Apkarian 2006). Imaging methods have been useful in delineating the brain areas specific to pain experience in humans because they can be obtained concurrently with

verbal reports of pain. In contrast, imaging techniques are of lesser value in non-humans because of difficulties in verifying that images of brain activation are actually accompanied in real time by feelings of pain. In this section, we illustrate the difficulties in attempting to identify pain in animals in general and examine the validity of recent claims for proof of pain in fishes (e.g. Huntingford *et al.* 2006; Braithwaite 2010; Sneddon 2011) and invertebrates (reviewed by Mason 2011).

The nature of pain in humans and implications for animal research on pain

Pain is a private experience. As such, it cannot be directly observed, verified, or measured. Many dependent variables in research are not directly observable, dissolved oxygen in water for example, but there exist standardized and validated instruments that can be used for this measurement purpose. A fundamental difficulty in research on pain is that there are no simple, unequivocal ways to measure it aside from verbal communication with human subjects and even that method is subject to error.

A valid working definition of pain is vital for efforts to explain its underlying mechanisms. To this end, the key features of the definition of pain by the International Association for the Study of Pain (IASP) are that pain is (i) an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage; (ii) pain is always subjective; and (iii) pain is sometimes reported in the absence of tissue damage and the definition of pain should avoid tying pain to an external eliciting stimulus (Wall 1999; IASP 2011). One of the most critical conceptual advances in the understanding of pain is the distinction between nociception and pain. As Wall (1999) emphasized, '...activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state.' This seemingly simple statement is actually fundamental to understanding what pain is and what it is not. Wall deliberately used the term nociceptor rather than 'pain receptor' and nociceptive pathways rather than 'pain pathways' because he understood that pain is not felt at the level of a sensory receptor, peripheral nerve, or pathway within the spinal cord or brain. Thus, there are no 'pain receptors.' Correspondingly, as Wall admonished, there are no 'pain pathways' in the nervous sys-

tem, just nociceptive pathways that also transmit non-nociceptive activity to some degree.

Tissue damaging stimuli excite nociceptors and this activity is conducted through peripheral nerves and across multiple synapses through the spinal cord, subcortical brain structures and then to the cerebral cortex (reviewed by Derbyshire 1999; Rose 2002). If a person is conscious when nociception-related activity arrives in the cortex, further processing by extensive cortical regions may but need not result in pain (Price 1999; Treede *et al.* 1999).

The activity in nociceptors and subcortical nociceptive pathways is processed unconsciously and is not directly accessible to conscious perception (Laureys *et al.* 2002). For example, carpal tunnel surgery is sometimes performed in awake patients following axillary local anesthetic injection, which blocks conduction in axons passing from receptors in the hand and arm to the spinal cord. Consequently, the patient can watch the surgery but feel nothing, in spite of intense nociceptor activation.

In distinction from nociception, pain is a result of specific patterns of activity in certain well-studied regions of the cerebral cortex and is quite separable from the activation of nociceptors or pathways conducting nociceptive activity to the cortex (Derbyshire 1999; Laureys *et al.* 2002; Laureys 2005). Whereas nociceptive neurons are widespread but not universal among animals (Smith and Lewin 2009), the higher brain structures known to be essential to conscious pain, specifically regions of neocortex and mesocortex, are found only in mammals (Rose 2002). This view of nociceptors is not different in principle from the conceptualization that rods and cones in the eye are correctly called photoreceptors but not vision receptors because their activation may result in unconscious visual processing but need not lead to consciously experienced vision. Visual images generated in the cerebral cortex can also be experienced in the absence of photoreceptor stimulation. Recent pain research has seen an increasing effort to clarify the nociception–pain dichotomy and to distinguish experimental procedures that measure nociception but not pain from those that have the potential for assessing pain (Vierck 2006; Rose and Woodbury 2008).

The nociception–pain dichotomy is not just a matter of academic terminology, but is essential to understanding the nature of pain. Pain is not felt at any subcortical level of the nervous system. It is clear that a reflex limb withdrawal response in a

human with a high spinal transection is a nociceptive reaction, that is, a nocifensive response, and not pain, because the person cannot feel any stimulus applied to body parts below the transection. Similarly, grimacing, vocalization, and organized avoidance reactions made in response to a nociceptive stimulus by an unconscious human, such as a decorticate individual, a person in a persistent vegetative state, or a lightly anesthetized person are nocifensive reactions alone because such people are incapable of consciousness, the essential condition for the experience of pain. Thus, purely nocifensive behaviors can be simple or relatively complex and exhibited by humans or other vertebrates (see below) with critical parts of their central nervous system damaged.

The separateness of pain and nociception is seen commonly in humans. Pain often, but not always, accompanies nociception; pain sometimes occurs without nociception; and the degree of pain is often poorly associated with severity of injury. First, nociceptor activation does not always lead to pain. People can sustain severe injuries in warfare, sports, or everyday life and either not report pain or report it differently than the extent of an injury would suggest (Beecher 1959; Wall 1979; Melzack *et al.* 1982). Second, people with 'functional' pain syndromes experience chronic pain without any tissue damage or pathology that would activate nociceptors. Third, pain can be greatly reduced or increased by 'psychological' manipulations such as a visual illusion (Ramachandran and Rogers-Ramachandran 1996) and created or reduced by hypnotic suggestion (Faymonville *et al.* 2003; Derbyshire *et al.* 2004) in spite of the fact that nociceptor activation is unmodified. Fourth, pain has a strong social learning component and depends greatly on one's prior experience with it, beliefs about it, and interpersonal interactions that accompany this experience (Flor and Turk 2006) rather than the extent of nociceptor activation *per se*. For example, a child's pain response depends greatly on behavior of caregivers (Kozłowska 2009). Fifth, pain can be faked or disguised as seen frequently in portrayals by actors. On the other hand, inhibition of pain-related behaviors in the face of extreme nociception is frequently cultivated as in the piercing rituals of the sun dance still practiced in traditional Plains Native American cultures (Mails 1998).

The dissociation between nociceptive stimulation and behavior is seen in animals as well. Injury-

related behaviors are frequently not expressed during violent, male–male conflicts (e.g. elephant seals, bull elk) or predator–prey interactions, where defense and escape are priorities. In contrast, ground nesting birds like the killdeer may display stereotyped, species-typical behaviors that seem to feign injury. Collectively, these facts about the relationship between nociception and nocifensive/nociception-related behaviors and pain should drive home the point that this relationship is highly variable, often unpredictable and that pain is clearly a separate process from nociception. As Wall (1979) put it concisely, '...pain has only a weak connection to injury...' This fact should make investigators of pain highly cautious in their interpretations of the relationship between nociceptive/nocifensive behaviors and the subjective experience of pain. Even where a verbal report of pain is available from humans, it is frequently difficult to interpret due to the importance of personality factors (Flor and Turk 2006). Unfortunately, as we will show, the nociception–pain distinction is commonly misinterpreted or totally disregarded in welfare biology and non-human studies of 'pain', and this is particularly the case in fish studies.

How pain is defined in scientific work and why it matters

The definition of pain is not merely a 'semantic' or 'academic' issue, but a matter of utmost importance for the practical world and the ethics of human–animal relations. There are differing types of definitions that are used in studies of nociception and pain: theoretical/explanatory definitions and operational definitions. The former definition, exemplified by the IASP definition mentioned above, is aimed at explaining what pain is. The IASP definition is commonly used if any definition is offered at all in experimental studies of fish 'pain' (e.g. Sneddon *et al.* 2003a; Nordgreen *et al.* 2009a; Roques *et al.* 2010). The operational definition, in contrast, explains how pain is measured in a particular experiment. For instance, the presence of shock avoidance learning has often been operationally (although incorrectly) defined as an indication of pain. In the case of operational definitions, the label (pain) used to describe the dependent variable in question (avoidance learning) may not have been validated from a methodological point of view and therefore lacks construct validity (Rose 2007). The labels used to describe

dependent variables may be chosen for convenience rather than because they have been proven to validly represent what is suggested by the label. Thus, in the absence of any validation, it is critical not to treat the dependent variable as a validated measure of its label, here pain. For example, in no case is a behavioral response to a noxious stimulus pain because pain is a subjective experience that cannot be directly observed. When an animal model is being used to investigate some aspect of pain, it is vital to know that the model system is actually valid for the purpose.

Nociception is not pain and emotions are not feelings

As shown above, clinical neurology provides human examples of the pain–nociception distinction, but clear examples have been in the animal literature for many years. Responses to noxious stimuli have been studied in several mammalian species following decerebration, in which all of the brain above the midbrain including the diencephalon, cortex and subcortical forebrain is removed. Although there is not universal agreement that rats are capable of consciousness, it is widely assumed that removal of the cortex alone, because of the well-known dependence of human consciousness on the neocortex (discussed below), would render such animals unconscious if they possessed consciousness when their brains were intact (reviewed in Rose 2002). Chronically decerebrate rats, which have the entire brain above the midbrain removed (e.g. Woods 1964; Rose and Flynn 1993; Berridge and Winkielman 2003), still react strongly to the insertion of a feeding tube, struggling, pushing at it with the forepaws, and vocalizing. When receiving an injection, these rats react indistinguishably from a normal rat: vocalizing, attempting to bite the syringe or experimenter's hand, and licking the injection site. These reactions are nocifensive, unconscious and are far more complex than 'simple reflexes' (in the language of Sneddon *et al.* 2003a and Braithwaite 2010). They are even ostensibly purposive, a fact that makes behavioral distinction between nociception and pain very difficult. In fact, many assumptions about indications of pain have been mistakenly based on behaviors that are sustained, organized, or directed to the site of nociceptive stimulation (Bateson 1992; Sneddon *et al.* 2003a), responses fully within the capacity of decerebrate rats.

The same types of evidence and logic that distinguish unconscious, nocifensive behaviors from conscious, pain-mediated behaviors also apply to the differences between emotions and feelings. The forging description of full-blown emotional behavior in decerebrate rats is just one of many lines of evidence, including research on humans, demonstrating a relationship between emotions and feelings comparable to that between nociception and pain (see Rose 2002; Berridge and Winkielman 2003; Damasio 2005; Rose 2007; LeDoux 2012 for a more detailed explanation). In this way of understanding affective responses, emotions are the fundamental unconscious, subcortically generated visceral, behavioral, hormonal, and neural responses to positive or aversive stimuli or situations, including learned reactions to these stimuli. Emotions are autonomous and functional in their own right, yet they also provide the pre-conscious raw material for the experience of conscious feelings, which arise through further processing by higher cortical regions (Berridge and Winkielman 2003; Damasio 2005; LeDoux 2012). These cortical regions are essentially the ones that underlie the conscious experience of suffering in pain (Rose 2002). This distinction between the terms 'emotion' and 'feeling' has not become as well established in the literature as that between nociception and pain; however, understanding the nociception–pain and emotion–feeling distinction is fundamental to understanding the difference between fishes and humans in their capacities for experiencing pain or conscious suffering.

Although human verbal reports of pain or feelings are not invariably reliably interpretable, validated rating scales or other psychometric tools adopted from cognitive psychology can provide adequately reliable means of measuring pain and other latent constructs in cooperative humans (Price 1999). Correspondingly, there is a long history in experimental psychology of using non-verbal behavioral methods to assess the internal 'psychological' state of an animal (Kringelbach and Berridge 2009). It is quite possible to assess the noxiousness (consciousness not implied) of a stimulus in terms of whether the animal will learn to avoid it, escape from it, or perform some behavior to escape that reflects the aversiveness of a nociceptive stimulus. An example of the last case is that a rat will leave a dark chamber and enter a brightly illuminated chamber (normally aversive to a rat) to escape a hot plate or electric shock

(Vierck 2006). Learned avoidance, or conditioned emotional responses to nociceptive stimuli, however, do not prove the existence of conscious pain or feelings, because associative learning of Pavlovian or instrumental types is well within the capacity of decorticate (Bloch and Lagarriguea 1968; Oakley 1979; Yeo *et al.* 1984; Terry *et al.* 1989), decerebrate (Bloedel *et al.* 1991; Whelan 1996; Kotanai *et al.* 2003), and even spinally transected mammals (Grau *et al.* 2006), as well as fishes with the forebrain removed (Overmier and Hollis 1983). The fundamental message here is that avoidance learning or conditioned emotional responses can be acquired in animals with central nervous system truncations that would make pain or conscious emotional feelings impossible.

Construct validation, an essential requirement for the identification of pain

A critical, but often overlooked, criterion for an animal model of pain is construct validity; that the model should actually be an indicator of pain and distinguish between pain and nociception as opposed to assessing nociception alone (Vierck 2006; Rose 2007; Rose and Woodbury 2008). In short, the animal model should be validated for assessing the process or variable that it is thought to assess. Many tests involving nocifensive behaviors in mammals like limb withdrawal, licking, vocalizing, writhing, or guarding have been used to assess pain because they have face validity. That is, they appear to reflect states comparable to those that would be associated with pain in humans. However, in the past few years, investigators in the pain science field have become increasingly aware that most of the standard animal tests for pain reflect nociception and nocifensive responses rather than pain. Critiques of the limitations in these models have been presented by Le Bars *et al.* (2001), Blackburn-Munro (2004), Vierck (2006), and Rose and Woodbury (2008). In the most recent edition of the *Textbook of Pain*, Vierck (2006) concluded that responses examined in the most frequently used tests, like those cited above, could be entirely mediated by spinal reflexes or brainstem/spinal motor programs, thus constituting unconscious nocifensive responses. Some higher brain influence probably contributes to these behaviors in an intact, awake animal, but the presence and nature of that influence is hard to separate from subcortical processes and it is also

likely to be unconsciously mediated. Consequently, none of these tests can be legitimately viewed as tests of pain, because the target behaviors can be expressed without consciousness. In some cases, investigators are aware of this constraint and strictly adhere to the term nociception rather than pain in interpreting their results (Vierck 2006). Unfortunately, this is far from a universal practice, and erroneous language and inference are common. Frequently, 'pain processing' or 'pain transmission' is used to describe what is clearly nociceptive processing at the receptor, spinal, or subcortical level (Rose and Woodbury 2008).

Development of well-validated models for pain, as opposed to nociception, is one of the most significant challenges in pain research, regardless of the animal model. To this end, some investigators have recently utilized more innovative paradigms based on the dependence of the suffering dimension of human pain on cortical functioning, especially the cingulate gyrus, insula and prefrontal cortex (Price 1999; Treede *et al.* 1999). On the assumption that similar cortical regions, where present, work in at least approximately similar ways across mammalian species, it would be possible to provide a preliminary validation of a putative animal model for pain by showing that behaviors allegedly reflecting pain depend on the functional integrity of these cortical zones known to mediate conscious pain in humans. There would still be a chance of confusing nocifensive behaviors with pain-dependent behaviors, but by placing at least part of the control of the response measure at the same cortical regions known to be essential to pain experience in humans, the potential for examining common mechanisms would be greatly facilitated. Unfortunately, for the question of pain in fishes, this approach cannot be used because the fish brain does not contain these highly differentiated, pain-mediating cortical regions, or true cortex, for that matter (see Rose 2002 for a more detailed discussion of fish brain structure), a fact that has led to the conclusion that pain experience meaningfully like humans is probably impossible for fishes (Rose 2002, 2007).

It has been argued that teleosts have forebrain structures homologous to some of those involved in human pain (Braithwaite 2010), but homology only means only that a structure is believed to have been present in a common ancestor of different species, in this case fishes and mammals (Butler and Hodos 1996). No functional equiva-

lency is established by neuroanatomical homology. Furthermore, the argument from homology essentially assumes a similar mode of functioning between fish and human brain. Consequently, the homologous structures in question, like the amygdala, would have to be operating in concert with cortical structures that are present in humans (and other mammals) but not in fishes, in order to enable it to generate 'fear' or any other consciously experienced feelings. This is because it is not the amygdala or any other limbic structure operating by itself, but rather a limbic-neocortical system that appears to generate emotional feelings (Damasio 1999; Derbyshire 1999; Amting *et al.* 2010).

'More than a simple reflex' – an inadequate definition

Recently, despite the methodological issues inherent in non-human mammalian pain research, a number of studies have been published purporting to evaluate the existence of pain experience in teleost fishes (reviewed in Braithwaite 2010; Sneddon 2011). The most common conclusion of these reports has been that evidence for pain was found. As this and the following sections will show, however, the studies in question have failed to adequately distinguish between response measures indicative of pain and those that could have been due purely to nociception.

Ideally, a research paper in this field should provide a clear operational definition of pain that explains the behaviors or other dependent variables that were observed as indicators of pain. Of course, the interpretations and conclusions of the study should hinge on and clearly state whether the operationally defined measures have been validated or whether they should be regarded as tentative. This restraint in interpretation is particularly important where independent variables are indirect measures of constructs that are inferred but not directly observable, like alleged internal states such as fear, pain, hunger, or consciousness. In many of the reports in which evidence for pain was allegedly found in fishes and even invertebrates, 'pain' was defined as a response that was 'more than a simple reflex,' or something similar [in the language used by Sneddon (2003a,b), Sneddon *et al.* (2003a,b), Dunlop *et al.* (2006), Barr *et al.* (2008), Appel and Elwood (2009), Ashley *et al.* (2009) and Elwood and Appel (2009)]. For reasons described below, we regard this definition as too vague and ambiguous.

DNA is more than a simple molecule, but not all more complex molecules are DNA. In the 'more than a simple reflex' definition, there is no explanation of what constitutes a 'reflex'. In addition, there is no explanation of how a simple reflex would differ from a complex reflex. It is implied, but not stated, that a 'complex' reflex would constitute evidence of pain, but no validating evidence of this assumption is ever offered. The term reflex is normally used to describe a very temporally limited, anatomically circumscribed response to a specific trigger stimulus. A 'simple reflex' would be exemplified by the patellar tendon (knee jerk) reflex, involving just one central synaptic relay, or a limb withdrawal reflex to a nociceptive stimulus involving at minimum, two spinal synaptic relays. Among the more complex reflexes would be vomiting or righting reflexes, which require coordinated action of numerous muscle groups, through the operation of multiple sensory and motor nerves and nuclei in the brainstem or the brainstem and spinal cord, respectively. Using the 'more than a simple reflex' criterion, virtually any sustained, whole animal behavior that seemed to result from a nociceptive stimulus would necessarily be considered evidence of pain. This practice constitutes the logical fallacy of false duality, discussed further subsequently.

As explained below, the existence of diverse, complex unconscious behaviors in animals and humans invalidates the assumption that a behavior that is 'more than a simple reflex' should be taken as evidence of consciousness or pain. An additional liability, from a perspective of good scientific practice, is that the vagueness and open-endedness of this 'definition' allow investigators to use their imaginations in hindsight rather than previously validated criteria to decide which of the behaviors seemingly evoked by a nociceptive stimulus should be taken as evidence of pain.

Unfortunately, most of the experimental literature on the subject of pain in fishes is flawed regarding the forgoing considerations of definition and interpretation. The following significant errors are evident in these studies: (i) invalid operational definitions where a dependent variable is insufficient to distinguish indications of nociception from pain; (ii) invalid levels of measurement, such as a purely anatomical or electrophysiological variable that may not have been specific to nociception or pain or was recorded in a context like anesthesia, where pain could not be present; (iii) no attempt

to provide an operational definition at all, but conclusions made regarding pain; or (iv) errors of interpretation concerning the relationship between an experimental manipulation and its relevance for pain. The last case typically involved administration of opiate analgesics and the drawing of conclusions regarding pain when the opiate's action at lower levels of the nervous system (i.e. an effect on nociception) may have been responsible for the drug's effect on behavior. Studies in which these kinds of errors were committed are presented in the following section.

Research related to the question of pain in fishes

A critical evaluation of behavioral studies claiming evidence for fish pain

Table 1 summarizes recent studies aiming to investigate pain in fishes but where, in fact, the measures or operational definitions (if any) would not validly distinguish pain from nociception. Perhaps, the most publicized of studies claiming to have demonstrated fish pain was by Sneddon *et al.* (2003a). This study, which examined behavioral effects of injections of large volumes of acetic acid or bee venom into the jaws of rainbow trout *Oncorhynchus mykiss* (Salmonidae), presented a more explicit and detailed description of theoretical criteria for the identification of pain than have most papers involving fishes or invertebrates. As such, it provides a useful case study to show how, like many studies of alleged pain in non-mammals, the authors have not properly distinguished nociception from pain or have used other invalid assumptions about pain. Many of the problems of technique and interpretation present in Sneddon *et al.* (2003a) have been evident in subsequent studies.

In their study, Sneddon *et al.* (2003a) claimed that various 'anomalous' behaviors produced by acid or venom injections satisfy criteria for 'animal pain' as put forward by Bateson (1992). Although Bateson's criteria have been popular in animal welfare research, they are based on invalid and outdated conceptions of pain and its neural basis. Sneddon *et al.*'s criteria, a subset of Bateson's were (i) to show that the animal has the same apparatus to detect a noxious stimulus that humans have; (ii) to demonstrate that a noxious event has adverse behavioral and physiological effects; (iii)

the animal should learn to avoid this noxious stimulus, and (iv) the behavioral impairments during a noxious event should not be simple reflexes.' These criteria fail to distinguish pain from unconscious nociceptive responses for the following reasons.

The first of Bateson's criteria requires the presence of nociceptors, which, as previously explained, are neither necessary nor sufficient for experiencing pain. Furthermore, the mere presence of a relatively primitive telencephalon is not sufficient for pain experience either. The conscious experience of pain most likely requires highly developed and regionally specialized forebrain neocortex (and associated limbic cortex), which fishes do not have (Northcutt and Kaas 1995; Striedter 2005).

The second criterion is invalid because, as explained earlier, physiological and behavioral responses to noxious stimuli are fully possible and (even in humans) regularly executed without consciousness, which is an essential requirement for pain (Derbyshire 1999; Laureys *et al.* 2002). Thus, these behaviors are not evidence of pain.

Criterion three is invalid because avoidance learning can involve only unconscious associative conditioning and, thus, fails to prove the existence of consciousness (explained above). This fact also negates claims for the demonstration of conscious fear in rainbow trout where associative conditioning was used as the behavioral response (Sneddon *et al.* 2003b; Yue *et al.* 2004).

The fourth criterion is also unacceptable for several reasons. A 'simple reflex' has not been defined or distinguished from a complex reflex or other behaviors. Furthermore, evidence from decorticate humans, such as the well-known case of Theresa Schiavo (Thogmartin 2005) as well as humans with sleep disturbances, demonstrates that we are fully capable of highly complex, seemingly goal-directed behavior while unconscious. Binge eating, climbing, driving, sexual assaults, homicides, and other complex behaviors can occur during states of unconsciousness in humans (Plazzi *et al.* 2005; Ebrahim 2006). Consequently, it is clear that very complex behaviors that are more than 'simple reflexes' can be performed unconsciously. The invalidity of the 'more than a simple reflex' criterion for pain has been explained in detail by Rose (2003, 2007), yet in a recent paper, Sneddon (2011) said 'Opinions against fish perceiving pain have stated that these responses are merely

Table 1 Measures used to infer pain in studies claiming evidence for fish pain.

Measures used to infer pain	Noxious experimental manipulation	Inference concerning measure(s)	Species	References
Voltage necessary to produce agitated swimming response	Electric shock and opioid system manipulations	Threshold level of pain	Goldfish (<i>Carassius auratus</i>)	Ehrensing et al. (1982)
Tail flick response to electric shock of caudal fin	Electric shock of caudal fin	Painful stimulus	Common carp (<i>Cyprinus carpio</i>)	Chervova and Lapshin (2011)
Behavior that is 'more than a simple reflex' Respiratory rate 'Rubbing' 'Rocking' Latency to feed	Acetic acid or bee venom injections in jaws	Behavior reflects pain	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Sneddon et al. (2003a)
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 (<i>Oncorhynchus mykiss</i>)	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	Behaviors reflect pain, morphine reduced 'pain-related behaviors'	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Sneddon (2003b)
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 (<i>Carassius auratus</i>) Rainbow trout (<i>Oncorhynchus mykiss</i>)	Dunlop et al. (2006)
Number of feeding attempts and time spent in the feeding/shock zone vs. shock intensity and vs. food deprivation	Electric shock	If a fish is willing to change this reflex response to a noxious stimulus, as shown here, it is possible that there is some sort of conscious decision making taking place.	Goldfish (<i>Carassius auratus</i>)	Millsopp and Laming (2008)
Ventilation rate Swim rate 'Rocking' 'Rubbing' 'Use of cover' Term nociception used	Acetic acid injections into jaws	Response to potentially painful stimulation	Common carp (<i>Cyprinus carpio</i>) Zebrafish (<i>Danio rerio</i>) Rainbow trout (<i>Oncorhynchus mykiss</i>)	Reilly et al. (2008a)
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 (<i>Oncorhynchus mykiss</i>)	Ashley et al. (2009)

Table 1 (Continued).

Measures used to infer pain	Noxious experimental manipulation	Inference concerning measure(s)	Species	References
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 (<i>Carassius auratus</i>)	Nordgreen <i>et al.</i> (2009a)
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 (<i>Cyprinus carpio</i>) Nile tilapia (<i>Oreochromis niloticus</i>)	Roques <i>et al.</i> (2010)
Ventilation rate Activity change Resumption of feeding	Acetic acid injections into jaws Injection of lidocaine or analgesic drugs	Behaviors reflect pain	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Mettam <i>et al.</i> (2011)

nociceptive reflexes... (Rose 2002; Iwama 2007).' This statement misrepresents the position expressed in detail by Rose (2002, 2003, 2007), wrongly reducing it to adherence to the false dichotomy type of interpretation that was condemned by Rose (2007).

The Sneddon *et al.* (2003a) study was also beset with contradictory data interpretation and failure to consider alternative explanations for their data (Rose 2003). In spite of the large injections of venom or acid, manipulations that would cause severe pain to a human, the trout actually showed remarkably little effect. Their activity level was not changed, they did not hide under a shelter in the tank and they fed spontaneously in <3 h. Furthermore, fish that received no injection at all or fish that received a saline injection did not feed, on average, for an hour and 20 min. Thus, a large saline injection (which would have been very painful to a human) produced no more effect than just handling. This is a significant point in view of the repeated assertion that hooking a fish in recreational angling would cause it pain (e.g. Braithwaite 2010; Mettam *et al.* 2011; Sneddon 2011).

The foregoing outcomes actually contradict claims that the trout were in pain. First, sustained pain should have triggered an endocrine stress response, initiated by brain release of corticotrophin releasing factor (crf), which typically causes locomotor activation in vertebrates, including

increased swimming in salmonids (Lowry and Moore 2006). No change in swimming occurred, however. Second, suppression of feeding is considered a reliable effect of stressful or noxious stimuli in fishes (Iwama *et al.* 1997; Huntingford *et al.* 2006) and also results from stress-related crf release (Bernier 2006). The comparatively rapid initiation of feeding (relative to uninjected or vehicle injected trout) is inconsistent with a presumption that the trout were suffering from pain, particularly if the effect of the acid persists for 3 h (Sneddon *et al.* 2003b). Third, while acid and venom-injected fish showed an infrequent rocking behavior (about once every 2–3 min), there is no reason to believe that it was more than an unconscious effect on balance, rather than a monkey-like (Sneddon *et al.* 2003a) indication of 'pain.' Reilly *et al.* (2008a) have recently implied that recovery from prior benzocaine anesthesia was likely to have impaired balance causing rocking behavior. Rocking was not observed when the experiment was conducted without anesthesia by Newby and Stevens (2008). Fourth, it was reported that acid-injected fish sometimes 'rubbed' their mouths against the gravel, but the venom-injected fish did not. The authors concluded that 'mouth rubbing' was due to pain. If so, why did the venom-injected fish that were supposed to also be in pain not perform this behavior? If 'mouth rubbing' was really due to pain or even nociception,

any method for producing nociception should produce 'mouth rubbing.' Furthermore, Sneddon *et al.* (2003a) interpreted the longer time to resume feeding by the venom or acid-injected trout as representing avoidance of mouth stimulation. If so, why did the trout 'rub' their mouths on gravel to reduce pain? These interpretations are clearly contradictory, non-validated and the behaviors have not proven to be repeatable (Newby and Stevens 2008). The use of the words 'mouth rubbing' is also unfortunately unobjective inasmuch as it implies intent on the part of the trout and is purely speculative. In short, the 'mouth rubbing,' feeding suppression, and 'rocking' put forth as behavioral assays for pain lack essential validation or even logically consistent interpretation and constitute an example of Hypothesizing After the Results are Known, what Browman and Skiftesvik 2011, label 'HARKing.'

In a study of the neurobehavioral effects of whirling disease, Rose *et al.* (2000; discussed in Rose 2007) observed infected rainbow trout frequently swimming with their mouths in contact with the bottom of the aquarium. This behavior could have been described as 'mouth rubbing' but such an interpretation would have been entirely speculative and this example shows how interpretation of this behavior is not as simple as it might seem. Irrespective of whether 'rubbing' is in response to nociception, irritation, or unknown factors, there is no justification for concluding that the behavior was consciously mediated, because seemingly purposive behaviors in response to noxious stimuli are commonly expressed by decerebrate animals (Woods 1964; Berridge and Winkielman 2003; Vierck 2006).

One of the few effects consistently produced by the acid or venom injections was an elevated opercular beat rate (Sneddon *et al.* 2003a; Newby and Stevens 2008). This response could have resulted from various effects of the acid or venom, particularly gill irritation due to leakage from the injection site. It should be recognized that oral venom and acid injections are poorly controlled manipulations in fishes, due to the certainty of leakage and circulation to the mouth and gills, thus constituting a broad, non-specific irritation, also involving gustatory and olfactory receptors rather than a specific activation of nociceptors. This issue should be addressed by the addition of a fluorescent dye to the injection solution to resolve the leakage question. But, even if increased opercular

beat rate was due to nociceptive stimulation of the mouth, this non-consciously controlled movement proves nothing about conscious pain. Given the fact that acid and venom injections would likely have produced a large scale and sustained nociceptor activation (Sneddon *et al.* 2003a,b), it is remarkable that the injections had so little behavioral effect. Instead of proving a capacity for pain, these results suggest remarkably high resilience to oral trauma by the trout, a trait later attributed to carp *Cyprinus carpio* (Cyprinidae) by Reilly *et al.* (2008a).

In another paper on rainbow trout, Sneddon *et al.* (2003b) reported that 'mouth rubbing' behavior after an oral acid injection was reduced by morphine and concluded this to be proof that mouth rubbing was an indication of pain. However, this morphine effect could have occurred entirely through actions on nociception (or other kinds unconscious neurobehavioral functioning) and constitutes no evidence that the trout were feeling pain. Also, opiates have diverse effects on the nervous system in addition to reducing nociceptive signaling (Strand 1999), so there is no certainty that the morphine effect was even specific to nociception. In addition, the morphine dosage used by Sneddon *et al.* (2003b) was exceedingly high (Newby *et al.* 2008; Newby and Stevens 2009). It should also be noted that following tritiated morphine injection in Atlantic salmon *Salmo salar* (Salmonidae), Nordgreen *et al.* (2009b) failed to find much of the isotope in central nervous system, most being in the head kidney. Sneddon (2003b) states 'morphine sulfate (0.3 g/1 mL sterile saline) was injected intramuscularly (0.1 mL/10 g fish weight).' This is equivalent to 10 mL kg⁻¹ or 3000 mg of morphine kg⁻¹. The typical dose for a small mammal is 2 mg kg⁻¹, and the dose for a large mammal or a human is much less. This huge dose, which did not alter the swimming behavior of the trout, was 10 times the lethal dose for any bird or mammal that has ever been studied. This result alone indicates that the response of trout to morphine is different from that of mammals, including humans. In a later communication on this point, Sneddon stated that the published dose was an error and the actual dose was 300 mg kg⁻¹ (Newby and Stevens 2009), still a huge dose that exceeds the lethal dose in mammals (Votava and Horakova 1952).

Subsequently, Newby and Stevens (2008) examined the reliability of the effects of oral acid

injections on the behavior of rainbow trout. They repeated the Sneddon *et al.* 2003a study with acetic acid injections, keeping experimental parameters as similar as possible (including testing the same species, temperature, tank size, fish size, food, and noxious stimulus). There were, however, some methodological differences, the most important being that Sneddon *et al.* gave the acid injections to anesthetized fish, whereas Newby and Stevens (2008) restrained unanesthetized fish during the procedure. Newby and Stevens reported an acute response to the noxious stimulus in that some fish lost equilibrium, whereas this response, immediate and transient, would have been masked in the Sneddon *et al.* study because the fish were anesthetized. In addition, the noxious stimulus did not change feeding behavior and the fish ate at the first opportunity, 15 min after injections. Further, the trout in the Newby and Stevens study did not exhibit 'rocking' or 'rubbing' behavior as reported by Sneddon *et al.* The fish in both studies responded to the noxious stimulus with a similar increase in rate of opercular movements. Newby and Stevens were not able to provide a definitive explanation for the differences in results between their study and those of Sneddon *et al.* but some possible reasons were considered in subsequent discussion (Newby and Stevens 2009; Sneddon 2009). The most significant difference between the two studies was the use of anesthesia for injections and its likely confounding effects on the results of Sneddon *et al.* (2003a). In a reply to the Newby and Stevens paper, Sneddon (2009) said that her Sneddon *et al.* (2003a) study employed 0.1% acid injections and that the 5% injections used by Newby and Stevens would have destroyed nociceptive afferents. This counterargument seems to be contradicted by the fact that in the study by Sneddon *et al.* (2003b) 2% acetic acid was used because the authors said it had more sustained behavioral effects than the 0.1% concentration, and Reilly *et al.* (2008a) used 5 and 10% acetic acid injections with carp and 5% injections with zebrafish *Danio rerio* (Cyprinidae).

With one exception, several studies by Newby and co-workers that examined effects of mouth or cheek injections of acetic acid never produced any 'rubbing' behavior. Newby *et al.* (2009) studied the effect of 0.7% acetic acid injection into the cheek muscle in goldfish *Carassius auratus* (Cyprinidae). In contrast to the other studies performed with rainbow trout (Newby and Stevens 2008)

and winter flounder *Pseudopleuronectes americanus* (Pleuronectidae; Newby *et al.* 2007b), 0.7% acetic acid injection into the cheek muscle of goldfish caused immediate locomotion at the time of the injection in fish treated with acetic acid but not in fish treated with saline (Newby *et al.* 2009). Every fish showed some 'rubbing' behavior, consisting of brief contact with the aquarium wall, immediately after the introduction of the noxious stimulus, which continued for more than an hour. The amount of 'rubbing' was significantly altered by the amount of morphine in the water. There was no significant difference in respiratory frequency between the control group and the fish injected with saline, suggesting that the injection itself was not more noxious than handling alone.

The result is in contrast to that of Reilly *et al.* (2008a) where injection of 5 or 10% acetic acid produced very little 'rubbing' or rocking in common carp. These authors admitted that 'rocking' behaviors observed in carp after the injection of acetic acid was like being off balance, swimming lopsided, and rocking from side to side. In two of five carp, 'rubbing' against the walls of the tank was observed. These authors concluded that 'common carp are incredibly robust fish,' because the carp did not behave so as to indicate that they were in 'pain.' Again, all fish were anesthetized by Reilly *et al.* (2008a) prior to being injected with acetic acid; hence, recovery from anesthesia potentially influenced the behavioral outcomes of their experiments. It is notable that the more recent published research using acid injections (Mettam *et al.* 2011) make no mention of 'anomalous behaviors' at all.

In the study by Mettam *et al.* (2011), the oral acid injection procedure with rainbow trout was employed to assess effects of an opioid, buprenorphine, a non-steroidal anti-inflammatory drug, carprofen, and a local anesthetic, lidocaine, on 'pain.' The study entailed a large number of manipulations, including dose-response variations. The results were a mixture of statistical outcomes that were both consistent and inconsistent or simply odd with respect to the possibility that these drugs could beneficially influence effects of acid injections. For example, low lidocaine doses were associated with longer times to resume feeding than in control fish. Acid injection by itself produced a remarkably small (although reportedly significant) effect, delaying feeding, relative to controls in only two of five fish tested. Plasma cortisol

concentrations were not elevated by acid treatment, an outcome attributed to the small sample size, but small sample size was argued to be justified by 'ethical considerations.' This is an unacceptable argument given the policy implications that these authors attach to their findings, which should demand adequate sample sizes to ensure results are robust. Overall, the sheer number of statistical comparisons between treatment and control conditions that appeared not to have been appropriately compensated by adjustments in *P* values made the conclusions of the investigators (including assertions that lidocaine spray should be used during hook removal in catch-and-release angling) unconvincing.

In a variation of the oral chemoirritant injection test, Ashley *et al.* (2009) conducted an investigation of whether a hypothetical 'painful stimulus' might modify use by 'bold' and 'shy' rainbow trout of cover and response to a 'predator cue' in the form of a rainbow trout skin extract. The criterion for the evidence of pain was, once again, behavior that was more than a 'nociceptive reflex.' The second part of the study intended to examine the effects of acid injection on 'dominant' and 'subordinate' trout placed in familiar or unfamiliar social groups. The results were very complex. In some instances, acid injections altered behavior and cortisol relative to saline-injected controls and in other instances they did not. The authors concluded 'Our findings provide new evidence that fish are considerably affected by pain and that the perception is not just a simple nociceptive reflex. In light of this, there are welfare implications that need to be reconsidered with regard to the treatment of fish.' As we have explained in detail, the oral acid injection procedure has not been validated as a legitimate test for pain. This particular study has not provided any further validation of this procedure either, simply restating the false duality that pain is demonstrated by behavior that is 'more than a nociceptive reflex.' While it is a possibility that some of the behavioral outcomes reported in this study may have stemmed from nociception, we reiterate our previously stated concerns with the potential multiple effects of chemoirritant injection. In their interpretation of the results, the authors did not consider that probable acid leakage from injection sites close to the nares could alter olfactory processing of the 'predator cue' or that acid leakage could stimulate opercular beat rate, confounding interpretations

with either measure. Of greatest importance is the fact that no conclusions regarding pain are warranted.

A report by investigators at the University of Newcastle, Australia, appears to shed light on the behavioral effects, or lack thereof, of acid injections. It was reported that in response to severe, chronic wounds, fish may appear to behave relatively normally and can continue activities such as feeding. In contrast, acute stimuli, such as exposure to a strongly acidic environment, may cause the animal to increase opercular movements, 'attempt to jump from the water,' and show abnormal swimming behavior (University of Newcastle 1993). The observations by this group lead to the interpretation that acid leakage from the injection site(s) could elicit patterns of abnormal behavior as a consequence of irritation rather than nociception and that specifically nociceptive stimuli, like wounds, have none of these effects, as the subsequent evidence also shows.

Chervova and Lapshin (2011) examined tail flick responses to electric shock of the caudal fin in common carp during treatment with three opiate agonist drugs. They concluded that the observed raised thresholds for 'deflections of the caudal peduncle' were evidence of raised threshold of pain perception. Clearly, this study fails to provide evidence for pain in that it is subject to the same constraint as that by Sneddon *et al.* (2003b) that the drug effects could have been occurring at lower levels of the nervous system and, in addition, the behavior, a threshold response to electrical stimulation, was not clearly shown to be nociceptive in nature, much less a consequence of conscious pain.

Studies involving surgery, wounding, or electronic tagging

In addition to studies supposedly designed to examine nociception or pain, there are many other relevant studies that potentially speak to the question of pain in fishes. These studies involved natural, presumably nociception-causing events, like surgery and wounding, as opposed to chemoirritant (acid) injections that would have diverse, non-nociceptive tissue effects, or electric shocks that would have non-specifically activated nociceptive and non-nociceptive afferents alike.

Several studies have examined behavioral effects of surgery in fishes. Wagner and Stevens (2000)

tested effects on spontaneous swimming behavior in rainbow trout of surgical implantation of dummy transmitters into the peritoneal cavity and incision closure by sutures. Swimming behavior appeared normal immediately after recovery from anesthesia. Three weeks after surgery, three measures of behavior, C-turns, sprints, and distance travelled, were not affected either by the amount of inflammation at the incision site or by the amount of inflammation at the entry and exit points of the sutures. The surgical techniques tested had little effect on fish behavior post-surgery, regardless of inflammation levels, including a range of conditions in which the incision might have been partially or completely open. The relative absence of C fibers in rainbow trout (discussed below) would likely eliminate the usual mechanism for inflammation-caused nociception (Price 1999).

Newby *et al.* (2007a) injected 12-mm glass-coated passive integrated transponders (PIT tags) into the peritoneal cavities of juvenile rainbow trout. These trout fed within 5 min of food presentation after a 30-min recovery from anesthesia. There were no significant differences between the PIT-tagged fish and anesthetized but untagged controls on latency to feed or amount eaten on the surgery day or the following day. Relative to previously tagged and recovered controls, PIT tag implantation did not affect time to fatigue in a fixed velocity swim test. 'Rubbing' behavior or 'rocking' behavior was not seen.

Narnaware and Peter (2001) anesthetized goldfish and performed cranial surgery involving opening of a bone flap for intracerebral injections. In tests initiated 15 min after surgery, the fish resumed feeding immediately and ate at the same rate as unhandled fish for a 30-min test period. In addition, intracerebral injection of neuropeptide Y receptor agonists substantially enhanced feeding. A similar result and lack of any effect of surgery on feeding in goldfish was reported in a prior study where intracerebral NPY infusions enhanced feeding immediately post-surgery (Narnaware *et al.* 2000).

In a study of post-operative analgesics after ventral midline incision to expose internal organs, followed by wound closure in koi *Cyprinus carpio* (Cyprinidae), Harms *et al.* (2005) found that surgery without analgesia or with ketoprofen analgesia resulted in reduced activity, lower position in the water column, and decreased feeding intensity

at multiple time points after surgery in untreated and treated fish. These effects were not seen in butorphanol treated fish. Post-surgical 'rubbing' or rocking was not seen in any koi and respiratory rate did not increase in any group at any time point. In post-surgical feeding tests, feeding intensity was said to be reduced, but relatively few fish showed feeding suppression, with 75 and 83% of the fish feeding at 30 min and 1 h after surgery, respectively. There were no differences detectable in feeding between fish treated or untreated with analgesics. These authors were interested in the issue of pain and analgesia in fishes but took a restrained view of its interpretation, stating: '...in laboratory animal settings, the debate over whether or how fish perceive pain may be less relevant than the questions of what physiological and behavioral effects are produced by noxious stimuli (such as surgery) that could impact research results and whether those effects can be ameliorated by therapeutic intervention.'

There is a variety of evidence arising from electronic tagging studies, suggesting that fishes are minimally affected by physical insults that would be painful and debilitating to humans. Most electronic tagging studies involve the intracoelomic surgical implantation of tags. The premise of electronic tagging field studies is that tagged fishes exhibit similar behavior and fates relative to untagged conspecifics (Brown *et al.* 2011). To that end, there have been over 100 studies that have examined the effects of intracoelomic tag implantation on fishes (reviewed in Jepsen *et al.* 2002; Bridger and Booth 2003; Wagner and Cooke 2005; Cooke *et al.* 2011). Although anesthesia is typically used to immobilize fish for surgery, analgesics are rarely used, and in some larger fish species, no anesthesia is used at all, rather the fish are simply inverted and surgery is successfully performed, while the fish are upside down in a state of tonic immobility (e.g. Meyer *et al.* 2007). Indeed, surgery under tonic immobility can result in higher post-operative survival rates than if the fish were anesthetised (Semmens *et al.* 2010).

The wide range of tagging studies has revealed that although there are a number of factors that can influence the behavior and survival of fishes post-surgery, these alterations are short-lived and often disappear before the incisions have healed. Certainly, there are often some detectable immediate effects of tagging (e.g., lethargy for a period of minutes to hours; Cooke *et al.* 2011), but it is

difficult to tease out the relative impacts of the anesthesia, surgery, and presence of the tag. Interestingly, most studies that use shams (surgery without actual placement of electronic tag) have documented similar outcomes relative to non-surgery controls, suggesting that the presence of the tag, especially if too big, is one of the primary drivers of alteration in fish behavior (e.g. Jadot *et al.* 2005) rather than surgery, *per se*. If fishes are provided with appropriate pre-operative and post-operative care, recovery is even more rapid.

Provided that the tag burden is minimal and the surgery is conducted by someone with appropriate training, fishes typically do not have behavioral impairments that last for more than several hours (Wagner and Cooke 2005). Indeed, many fish species resume 'normal' behavior immediately post-tagging. For example, Cooke *et al.* (2002) implanted radio transmitters in nest-guarding adult smallmouth bass and noted that they resumed parental care behavior within minutes post-release. Thoreau and Baras (1997) used motion-sensitive transmitters in an aquaculture tank to evaluate the activity of four tilapias during the recovery from anesthesia and surgical procedures. The authors reported that all four fish exhibited normal diurnal activity rhythm patterns (compared with control fish) throughout the study. However, activity levels were low during the first 12- to 24-h post-surgery. Several studies have also evaluated the effects of tagging on various aspects of social behavior (e.g. Swanberg and Geist 1997; Connors *et al.* 2002) and failed to document any differences relative to untagged fish. Others have implanted tags in migratory fishes and noted immediate resumption of migration behavior (Hockersmith *et al.* 2003; Moser *et al.* 2005). In the Columbia River Basin of the United States, tens of thousands of downstream-migrating smolts are implanted with tags annually to quantify loss associated with hydropower facilities (Jepsen *et al.* 2002; Brown *et al.* 2011). These fish are typically released within hours of tagging and those data are used, often in a legal context, to determine the level of compensation necessary. The compensation for hydropower mortality in fishes is based on the premise that tagged fish are behaving normally (Brown *et al.* 2011). If these fish were experiencing pain, we would infer that they would exhibit severely altered behavior and these studies would have not stood up to the rigors of legal proceedings. From an anthropomorphic

perspective, imagine having a tag, the size of a soft drink can top or, in early studies, the drink can itself (Bridger and Booth 2003), implanted in your peritoneal cavity and then, after a brief recovery period, being placed into an environment as challenging and dangerous as ones normally occupied by free-ranging fishes. If fishes were debilitated by pain or discomfort to the degree that would surely occur in a human, or other mammals, the kind of functioning exhibited by tag-bearing fishes would be surprising rather than the norm.

Neurological studies

A number of studies have examined various types of neurological evidence for mechanisms subserving nociception and possibly pain in fishes, sometimes with the aim of satisfying one or more of Bateson's criteria for animal pain. As pointed out previously (Rose 2002), the first evidence for nociceptive afferents, in this case, epidermal-free nerve endings in fishes, was published by Whitear (1971). In the absence of physiological verification, the actual sensory function of these afferents was unknown. More recent studies have been electrophysiological demonstrations of nociceptive afferents in the head (Sneddon 2002, 2003a; Sneddon *et al.* 2003a) including the cornea (Ashley *et al.* 2006, 2007) of rainbow trout.

In an electrophysiological study of goldfish and rainbow trout, Dunlop and Laming (2005) claimed to have identified neuronal responses in the spinal cord, brainstem, and telencephalon that were specific to nociceptive stimuli (a 'pin prod'). The authors' description of recording procedures and signal analysis methods suggests that their results may not be interpretable at all. Among numerous other problems, the state of anesthesia during recording was unclear, electrode placement, especially in spinal cord, was poorly controlled (electrodes were manually inserted and not histologically verified), and a lack of recording from well-isolated single neurons makes inferences about nociceptive response specificity questionable. The authors also claimed to have identified A-delta and C fiber type nociceptive afferents at multiple levels of the central nervous system, but this is technically impossible because (i) they were not recording directly from first-order afferents and the designation of A-delta or C fibers is properly restricted to first-order afferents; (ii) the use of a solenoid driven stimulus is of inadequate precision

for latency measurements of conduction velocity, and (iii) the number and durations of synaptic delays along conduction pathways in the spinal cord and brain were unknown and not considered (see Rose *et al.* 1993; Schlag 1978 for discussion of relevant technical issues).

In another electrophysiological study recording evoked potential responses to electrical skin stimuli in Atlantic salmon, Nordgreen *et al.* (2007) claimed to have demonstrated a pathway to the telencephalon of potential significance for pain. As with the previously described study, technical issues undermine interpretations of these results. The use of monopolar recording of field potentials from a stationary, low-impedance electrode does not conclusively show that the generator for the evoked potentials was in the telencephalon because this method cannot distinguish near-field from far-field potentials (see Dong 1981). Further, increased response amplitude with increased stimulus intensity prevents interpretations regarding selective activation of nociceptive afferents because raising stimulus intensity would recruit additional large-diameter, non-nociceptive fibers as well as small diameter, possibly nociceptive, afferents. Although a somatosensory pathway to the telencephalon in teleost fishes is likely on the basis of previously reported neuroanatomical information (Finger 2000; Xue *et al.* 2006a,b), no scientifically defensible conclusions regarding nociceptive or non-nociceptive somatosensory transmission to the telencephalon may be drawn from this study.

An anatomical study by Roques *et al.* (2010) reported that the average number of histologically identified A-delta and C fibers in tail nerves from common carp was 38.7% of the fiber size spectrum, a value comparable to the 36% reported for rainbow trout trigeminal nerve (Sneddon 2002). However, only 5% of these were C fibers, slightly more than the 4% C fibers reported in the rainbow trout trigeminal nerve by Sneddon (2002). Of course, not all A-delta or C fibers are nociceptors and the actual subset that is nociceptors can only be determined electrophysiologically, not histologically. Sneddon (2004) acknowledged that this proportion of small C fibers that would include nociceptors is far below numbers seen in mammals (or even amphibians), but did not convey the significance of this point for nociception and pain. It is known in humans that A-delta nociceptive afferents mediate first or bright pain. This sensation is rapid, brief, well-localized perceptually and not

particularly unpleasant, like a needle prick or the initial sensation after a more intense stimulus. In contrast, C fiber nociceptors, unmyelinated fibers that conduct very slowly, mediate the more slowly developing, sustained and diffuse, second pain experience, also called burning or dull pain (Price 1999). The suffering that we associate with burns, toothaches, or crushing injury is caused by C fiber activity, not A-delta fibers (Price 1999). If a person hits their thumb with a hammer, the immediate, well-localized stinging sensation is due to relatively fast signaling by A-delta nociceptors. The subsequent, slower wave of intense, more diffuse, and more agonizing pain is due to C fiber activity. If the C fiber activity is eliminated by an appropriate type of nerve block (Makenzie *et al.* 1975), the second pain experience is also eliminated. The distinct perceptual and affective differences between first and second pain are apparently due to the fact that A-delta fiber activation, and first pain is associated with activity in the S1 and S2 somatic sensory cortical regions, known for processing the physical properties such as the spatial location, temporal parameters, and intensity attributes of a nociceptive stimulus. In contrast, C fiber activation and second pain are associated with S2 somatosensory cortex activation, but also anterior cingulate gyrus cortex activation, the latter region playing a primary role in the generation of the suffering component of pain (Ploner *et al.* 2002; Derbyshire 2004). Second pain becomes intensified due to the spinal effects of repeated C fiber activation, and tonic C fiber activity is critical for causing hyperalgesic states like inflammatory and neuropathic pain (Price 1999).

The human sural nerve, as an example of a cutaneous nerve, innervates the skin of the calf and foot. It has 83% C fibers (Guo *et al.* 2004). Similarly, the cutaneous branch of the sural nerve in rats is 82% C fibers (Schmalbruch 2005). In humans, a rare condition called congenital insensitivity to pain (more correctly, insensitivity to nociception) results when there are fewer numbers of nociceptive afferents in peripheral nerves. Such individuals have diffuse congenital insensitivity to pain with anhidrosis, in which C fibers, only 24–28% of the sensory axon population but the A-delta afferent population is in the normal range (Roseberg *et al.* 1994; Guo *et al.* 2004). Given the extent of the pain insensitivity with even this many C fibers present, it is reasonable to ask of

what functional significance the extremely small number of C fibers might be in fishes. It appears most logical to assume that in teleosts, at least those species that have been studied, A-delta afferents serve to signal potentially injurious events rapidly, thereby triggering escape and avoidance responses, but that the paucity of C fibers that mediate slow, agonizing, second pain and pathological pain states (in organisms capable of consciousness) is not a functional domain of nociception in fishes. The likelihood that teleosts, compared with terrestrial vertebrate taxa, actually have a relatively limited capacity for nociception, makes the absence of unmyelinated nociceptors in elasmobranchs (Coggeshall *et al.* 1978; Leonard 1985; Snow *et al.* 1993; Smith and Lewin 2009) seem less of a conspicuous functional difference between cartilaginous and bony fishes.

Anatomical and electrophysiological studies of somatic sensory nerves and their spinal connections have been conducted in several species of elasmobranchs. The most striking finding was a near absence of unmyelinated axons in dorsal roots of Atlantic stingray (*Dasyatis Sabina* Dasyatidae), spotted eagle ray (*Aetobatus narinari* Myliobatidae), cow-nose ray (*Rhinoptera bonasus* Myliobatidae; Coggeshall *et al.* 1978), long-tailed ray (*Himantura uarnak* Dasyatidae), and shovelnose ray (*Rhinobatus battillum* Rhinobatidae; Snow *et al.* 1993). Small specimens of shovelnose ray and black-tip shark (*Carcharhinus melanopterus* Carcharhinidae) had larger numbers of fibers that were classified as unmyelinated but these axons had an unusual 1:1 association with Schwann cells suggestive of developing myelinated fibers (Snow *et al.* 1993). These anatomical results indicate that these elasmobranchs essentially lack C fiber-type nociceptors, an interpretation consistent with Leonard's (1985) failure to find in the Atlantic stingray any neurophysiological evidence of either A-delta or C fiber types of nociceptors. He found no polymodal nociceptors (the most common type, comprising 90% in humans (Price 1999) or nociceptors responsive to acidic or intense thermal stimuli. Leonard did observe some mechanoreceptive afferents that responded to high intensity stimuli. Consistent with this apparent paucity of peripheral nociceptors in elasmobranchs is the absence of Rexed's lamina I in the spinal cord dorsal horn in three species: brown stingray (*Dasyatis fluviorum*; Dasyatidae), eagle ray (*Aetobatis narinari*; Myliobatidae), and black-tip shark examined by

Cameron *et al.* (1990). Lamina I in mammals is a principal synaptic input zone for nociceptive activity and the neurons there comprise a major source for the transmission of nociceptive activity to the brain (Dostrovsky and Craig 2006; Todd and Koerber 2006). Consequently, its absence, together with the near total absence of unmyelinated afferents or nociceptive peripheral neurons, is a conspicuous indication that these elasmobranchs are ill-equipped to process nociceptive stimuli.

In two studies employing gene microarray analyses, Reilly *et al.* claimed to have identified expression of nociception-related candidate genes in the brains of rainbow trout (Reilly *et al.* 2008b) and common carp (Reilly *et al.* 2008b, 2009). In the first study, rainbow trout and carp were anesthetized and injected in the jaws with 5% acetic acid solution and killed 1.5, 3, or 6 h later. The control fish were injected with saline vehicle. Brains were processed with gene microarray technique, and it was reported that a large number of genes were differentially expressed between the acid-injected and saline-injected fish, a relatively small number of which were claimed to have a possible functional connection to nociception. Microarray technique has been used previously in an effort to identify gene expression potentially related to mechanisms of nociception or even pain, but a causal functional connection between gene expression and nociception or pain is exceedingly difficult to establish (Rose and Woodbury 2008). In addition, the two studies by Reilly *et al.* (2008b, 2009) employed an insufficient number of control conditions to make any interpretations related to nociception. As explained earlier, acetic acid is a chemoirritant that could produce gustatory and tissue irritating effects apart from nociceptor activation. At the very least, a control condition is necessary to show that the pattern of gene activation observed is specific to nociceptor activation and would not have resulted from any strong, non-nociceptive sensory stimulus. Control issues like this are obligatory in gene expression studies of the brain, as exemplified by the more commonly used early oncogene methods like *c-fos* (Alexander *et al.* 2001). Additional problems existed in the claim (Reilly *et al.* 2008b) that 5% acetic acid injection was a validated nociceptive stimulus with behavioral effects because in the same year, a paper by Reilly *et al.* (2008a) stated that 10% acetic acid injections were used in carp in addition to 5% injections because the carp were said to be

'tolerant' of 5% injections. The authors concluded (Reilly *et al.* 2008a) that 'In the carp, a nociceptive stimulus did not elicit an alteration in normal behavior or ventilation.' Consequently, the alleged nociceptive stimulus employed in the two gene expression studies with carp (Reilly *et al.* 2008b, 2009) had not been shown to be an effective nociceptive stimulus, thereby invalidating claims that any gene expression associated with 5% acid injections were a consequence of a truly nociceptive stimulus. In summary, neither of these studies provided any valid information concerning gene expression specific to nociception.

In addition to the forgoing problems of interpretation, Reilly *et al.* (2008b) asserted that Rose (2002) had stated that fish's response to noxious stimulation is 'limited to a reflex response at the level of the spinal cord and hindbrain' and that the brain was not active during noxious stimulation. This assertion is untrue and misrepresents the more mechanistically explicit position on the matter of fish pain that Rose (2002, 2007) has repeatedly expressed including the opinion that somatic sensory activity, including nociceptive activity is likely to reach the forebrain in fishes (see Rose 2007; fig. 2). Furthermore, for reasons thoroughly articulated in the present paper, Rose (2003, 2007) has never found the use of the term reflex to be acceptable as an explanation for the complex behaviors of fishes, including many of those elicited by nociceptive stimuli.

Feeding habits of fishes

Fishes commonly consume foods that would be very painful for us to eat, such as urchins, crabs, coral, barnacles, hard shellfish, stingrays, and a great many fish species with spiny, rigid, or venomous fin rays. Although species-specific types of oral handling by predatory fishes may reduce injury and predators show preferences for prey that are more easily ingested (Helfman *et al.* 1997), these fishes still frequently eat injurious prey. Predatory fishes are commonly found with numerous spines from marine catfish, urchins, or stingrays embedded in their mouths and throats (Smith 1953; Heemstra and Heemstra 2004). For example, specimens of the great hammerhead *Sphyrna mokarran* (Sphyrnidae), which preys on stingrays, have been found with as many as 96 stingray barbs embedded in the mouth, throat, and tongue (Helfman *et al.* 1997).

Herbivorous and omnivorous fishes swallow inedible materials like stones and sand and expel them from the mouth or through gill rakers or ingest and excrete them. These eating habits of fishes are difficult to reconcile with claims that they are troubled by pain or respond to nociceptive stimuli as we or other mammals would.

Insights from catch-and-release fishing

Studies of catch-and-release recreational fishing also provide useful insights into how fishes respond to injuries that would elicit pain in humans. Indeed, a fish cannot be angled without at least one hook being driven into tissue such as the jaw, roof of mouth, or other areas. Thus, injury is implicit in recreational fishing (Cooke and Sneddon 2006). However, capturing a fish by angling may or may not elicit a stress response associated with anaerobic exercise and handling (Cooke and Suski 2005). A study of diverse serological indications of stress in angling-captured salmonids by Wedemeyer and Wydoski (2008) is of particular interest. Stress-sensitive indicators, including blood glucose, chloride, osmolality, and hemoglobin were measured immediately after capture in wild brook trout *Salvelinus fontinalis* (Salmonidae), brown trout *Salmo trutta* (Salmonidae), cutthroat trout *Oncorhynchus clarkii* (Salmonidae), and Arctic grayling *Thymallus arcticus* (Salmonidae) that had been hooked and played for 1–5 min. The osmoregulatory and metabolic disturbances associated with capture by angling were minimal and judged to be well within normal physiological tolerance limits. In contrast, fish of the same species that were played for 5 min and then released into net-pens where they were held for up to 72 h showed blood chemistry alterations that appeared to be related to stress from confinement, showing that prerelease air exposure and handling cause more physiological stress than either hooking *per se* or playing time. Similar results have been obtained for other species, including snapper *Pagrus auratus* (Sparidae) and mao mao *Scorpius violaceus* (Kyphosidae) (Pankhurst and Sharples 1992; Pankhurst *et al.* 1992; Lowe and Wells 1996). This is not surprising given the results of Sneddon *et al.* (2003a) who found no significant differences in behavior of fish injected with saline compared with uninjected control fish. Embedding a fish hook is comparable with the mechanical tissue damage caused by embedding a

needle of similar size, but without the saline injection. This indicates that hooking is a less noxious stimulus than the supposedly effective acid or venom injections, an observation that is supported by empirical evidence from not only Wedemeyer and Wydoski (2008), but numerous other observations of fishes exhibiting normal behavior (such as recommencement of feeding) almost immediately after capture and release angling (Schill *et al.* 1986; Arlinghaus *et al.* 2008 and see below). Nevertheless, Sneddon *et al.* (2003a) concluded that their results were of relevance to angling, a statement that was simply not supported by their data.

Arlinghaus *et al.* (2008) examined the post-release behavior of northern pike *Esox lucius* (Esocidae) that were released with a lure in their mouth. Compared with controls, no differences in behavior (e.g., swimming activity levels in the wild) were noted after 24 h, although fish with lures exhibited some level of hyperactivity for the first hours post-release. Behavioral measures constitute sensitive indicators of the complex biochemical and physiological changes that occur in response to stress (Schreck *et al.* 1997) and may be indicative of altered or impaired capability of a fish in sensing and responding to its environment. From a practical perspective, outcomes of catch-and-release studies leave no major amount of unexplained variation in survival aside from identifiable physical or physiological factors that have been well studied and synthesized such as injury with bleeding, adverse water temperature, or prolonged air exposure (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Cooke and Suski 2005; Arlinghaus *et al.* 2007a). If 'psychological' distress or physiological stress caused by pain and suffering due to being caught was a significant factor in their well-being, post-release mortality of angled fishes would not be so fully explained by 'non-psychological' variables. The typical physiological recovery profile of hooked fishes is similar to those simply chased to exhaustion (Kieffer 2000; Cooke and Suski 2005). Vulnerability to predation or injury from environmental hazards such as strong currents would be particularly important threats to survival if a fish's neurobehavioral functioning was compromised by psychological distress due to being caught and handled (Cooke and Philipp 2004).

Apart from the neurological evidence, another way to evaluate whether being hooked causes pain to a fish is to examine whether fishes in a

natural situation behave in a way that indicates they are capable of pain and suffering. Some studies have attempted to identify behavioral effects, being caught on the probability of recapture. In experimental studies, some fish apparently learned to avoid certain lures or baits more readily than others (Beukema 1970a,b; Hackney and Linkous 1978; Raat 1985; Burkett *et al.* 1986). In other studies, fish were recaptured repeatedly, as many as 26 times (Britton *et al.* 2007), even in a short time frame. Although authors of these studies have typically assumed that a fish with a decreased probability of recapture had learned to avoid hooks, there is no direct evidence that this is the case or that learning was based on pain. Even in the studies where differential effects were produced by angling with various types of lures or baits, what these fish had learned is unknown. The repeated capture of angled fishes also is consistent with the notion that a catch-and-release fishing event does not induce a state that is similar to pain in humans. For example, a study of cutthroat trout revealed that in a reach of the Yellowstone River, fish were captured an average of 9.7 times in a single season, equivalent to once every 5 days as well as multiple recaptures (2–4) in a single day (Schill *et al.* 1986). Multiple recaptures have been documented in a number of species (e.g., Beukema 1970a,b; Hackney and Linkous 1978; Raat 1985; Burkett *et al.* 1986; Hayes 1997; Tsuboi and Morita 2004; Britton *et al.* 2007). Some of the best evidence of repeated captures on short timescales comes from a large, unpublished tagging data set from Australia (Sawynok, Infofish Australia, www.info-fish.net, unpublished data) that involved tagging of 619 279 fish with 39 034 angler recaptures. This ongoing study has revealed that 380 fish of 37 species have been recaptured the same day as tagged. Moreover, three fish of two species, goldspotted rockcod *Epinephelus coioides* (Serranidae) and barramundi *Lates calcarifer* (Latidae) were tagged then recaptured twice more on the same day (i.e. caught three times in 1 day). A total of 2141 fish were recaptured within a week. Interestingly, more fish were caught on the same day and the next day than the following days. A total of 245 (64.5%) fish caught on the same day were caught within hours by the person who tagged the fish (or a person they were fishing with) as part of the same trip. Two fish were caught on the next cast, 15 (30%) within 10 min and 31 (62%) within an hour. Clearly these recaptures over

short time periods are inconsistent with the notion that fish experience significant pain. Even in cases where artificial lures lost effectiveness but natural baits did not, the fish may have associated being caught with removal from the water and handling rather than hooking, *per se*.

What is the significance of a limited capacity for nociception in fishes?

Pain is taken not as a simple sensory experience signaling the existence of damaged tissue. The presence and intensity of pain is too poorly related to the degree of damage to be considered such a messenger. Pain is a poor protector against injury as it occurs far too late in the case of sudden injury or of very slow damage to provide a useful preventive measure. Instead it is proposed that pain signals the existence of a body state where recovery and recuperation should be initiated. (Wall 1979)

The relatively few teleost and elasmobranch species that have been studied have provided consistent results, indicating that these diverse taxa, compared with humans and other mammals, have a very limited capacity for nociception, particularly C fiber nociception that leads to agonizing, emotion-provoking pain in humans. Anthropomorphic thinking, a bias that obstructs objectivity about biological questions (Kennedy 1992; Wynne 2004; Rose 2007), would lead to expectations that fishes should be more like humans in their capacity for nociception, even pain (e.g. Chandroo *et al.* 2004). But, it is clear for all fishes that have been studied that this is not so, a fact demonstrating that these fishes have evolved and survived perfectly well without human/mammal like nociception, much less a brain capable of mediating human-like pain experience.

The weight of the literature described above, which shows that teleosts commonly display limited and relatively brief or even no behavioral disturbances due to injury, is entirely consistent with their limited capacity for nociception. In the case of elasmobranchs, sharks have long been known to be behaviorally unaffected by severe wounds, even seen to continue vigorous feeding after evisceration (Smith 1953; Goadby 1959 cited by Snow *et al.* 1993).

Valuable insights into the utility of the human capacity for first pain, second pain, and pain-related suffering were put forth by Wall (1979),

where he summarized evidence, more fully documented later (Melzack *et al.* 1982), that injury is often initially dissociated from pain, but connected with avoidance or escape from the source of injury, whereas minutes, hours, or days later, severe pain is experienced and is associated with behaviors compatible with healing and recovery, particularly inactivity. Seen in this light, the capacity for nociception in fishes is understandable rather than puzzling. A capacity for rapid detection (A-delta nociceptor) of potentially injurious stimuli that would trigger escape and possibly mediate rapidly learned (unconscious) avoidance could be beneficial to fishes that possess it. On the other hand, succumbing to behavior impairing, intense and sustained (C fiber nociceptor) signaling from injuries could greatly increase vulnerability to predation, reducing capacity to function and would therefore be selected against in a perpetually threatening and often highly turbulent environment. As the preceding sections of this article have shown, the response of fishes to injury, when evident, is normally transient, diminishing in minutes or hours, the opposite of the pattern described by Wall for humans and other mammals, where debilitating nociception and pain increase in hours and days after injury (Wall 1979).

None of this should be taken to suggest that injury is not detrimental to fishes or that teleosts, at least, do not quickly respond to such stimuli. However, rapid signaling by nociceptors is best viewed as just one of many modalities, like lateral line sensations or visual detection of looming predators, which mediate rapid escape and protective functions without leading to conscious suffering or fear. Among these protective functions are endocrine and other physiological adaptations to stress. A particularly instructive example is that in elasmobranchs, substance P and met-enkephalin, neuropeptide transmitters, which in mammals are closely linked with nociception and anti-nociception, respectively, are found in sensory neurons innervating the spinal dorsal horn (Cameron *et al.* 1990). This is the case even though the sensory axons involved and the dorsal horn structure of elasmobranchs are unlikely to mediate nociceptive function. Neuropeptides tend to serve multiple functions, however, such as promoting vasodilation and healing (Strand 1999), which would be valuable to organisms unresponsive to injury.

Claims for pain in invertebrates

The discussion of putative fish pain has been contemporaneous with a similar discussion and claims for the existence of pain in some invertebrates. Although we will not undertake a thorough consideration of this issue, or of the immense diversity of invertebrates (including allegedly special cases like cephalopods), many of the same issues regarding standards of evidence and interpretation that we have discussed regarding claims for fish pain are also present here. Invertebrate nervous systems are extremely diverse, but tend to be organized in a much more decentralized manner than are those of vertebrates. Invertebrate behavioral control functions are commonly mediated by spatially separate ganglia, rather than a highly centralized brain (for more details see Barnes *et al.* 2001 and Meinertzhagen 2010). This fact makes a capacity for the highly integrated and unified processing of information known to be essential to what we know as consciousness highly improbable. The term 'brain' is commonly used to refer to a collection of head ganglia present many taxa of invertebrates, such as insects, but the structural and functional organization of such 'brains' is highly variable across diverse invertebrates and decidedly unlike vertebrate brain organization, hox genes notwithstanding (Bullock and Horridge 1965; Alleman 1999).

Examples of recent studies with bees, crabs, shrimp, crayfish, and prawns illustrate that the same problems present in the 'fish pain' literature have been evident in attempts to identify possible nociceptive processes or pain in these invertebrates. In honeybees, stinging responses to electric shocks were measured as indicators of nociception (Núñez *et al.* 1983, 1998). Here, the behavior selected might be a legitimate indication of nociception, but electric shocks are not specific activators of nociceptive neurons. In the crab, *Chasmagnathus granulatus* (Decapoda, Grapsidae), spreading of the chelae and elevation of the carapace on flexed walking legs in response to electric shock was construed as a nociceptive response (Lozada *et al.* 1988). The issue of non-specificity of electric shocks applies here also, but morphine was found to attenuate the response. In prawns, *Palaemon elegans* (Crustacea, Decapoda), tail flicking and antennal grooming was elicited by pinching with a forceps, or application of solution containing acetic acid or sodium hydroxide to the distal antenna (Barr *et al.*

2008). Sustained antenna grooming, which was attenuated by a local anesthetic, was taken as evidence of pain experience rather than nociception because, according to these authors, the behavior was more than a reflex response and was an attempt by the crab to ameliorate the pain. In effort to replicate the results of the study by Barr *et al.* Puri and Faulkes (2010) applied similar acetic acid or sodium hydroxide solutions to the antennae of crayfish *Procambarus clarkia* (Decapoda, Astacidea) and to species of shrimp, *Litopenaeus setiferus* (Crustacea decapoda) and *Palaemonetes* sp. (Crustacea Decapod Natantia). They failed to evoke antenna grooming in any of the species and also found no electrophysiological evidence for nociceptive responses from antennae, concluding that the existence of nociceptive neurons should be demonstrated before concluding that putatively noxious stimuli were eliciting nociception-dependent responses.

In a study of hermit crabs (Crustacea, Decapoda) by Elwood and Appel (2009), electric shocks were used to stimulate evacuation from a shell. Surprisingly, crabs that had been shocked were more likely to enter a new shell offered 20 s later and they entered more quickly. This behavior was interpreted as indicative of pain because it was not reflexive, the experience was remembered and the behavior, according to the authors, was traded off against other motivational requirements. A second study with hermit crabs by Appel and Elwood (2009) produced a peculiar combination of results. Most of the shocked crabs moved back into the same shells they had evacuated but did not differ from unshocked crabs in the tendency to enter a new shell. Nonetheless, these authors concluded that their results proved that the crabs felt pain, again citing nonreflexive behavior, memory of an aversive event, and changes in motivation to obtain a new shell. None of the forgoing studies in which pain was supposedly studied were actually measuring behaviors that could not have been unconsciously mediated and, as discussed previously, the 'more than a reflex' criterion for pain is invalid for many reasons.

In reviewing studies claiming to have demonstrated pain or consciousness in invertebrates, Mason (2011) condemned the use of many criteria such as problem solving, stimulus recognition, avoidance conditioning, or physiological stress responses. She argued instead that '...evidence for conscious affective states should come specifically

from responses to stimuli that elicit approach and positive reinforcement, or avoidance and negative reinforcement, because in humans and perhaps other homeotherms, these give rise to positive and negative feelings.' We argue that these criteria are inadequate as well since, as we have shown above, these types of responses are fully within the capacity of animals (and humans) that are incapable of consciousness due to brain damage. Similarly, Mason's belief, that awareness is implied by behaviors supposedly indicative of 'motivational trade-offs,' is questionable. Phrases like 'motivational trade-offs' are loaded and constitute interpretations of the animal's alleged thinking and intent, when the situations described could just as well reflect unconscious 'if-then' contingencies.

It is clear from this brief review that research on invertebrate pain and awareness is beset with the same shortcomings that have undermined research with fishes: invalid criteria and measures for these states, as well as inflated interpretations, faith-based interpretations and HARKing (Browman and Skiftesvik 2011).

Arguments made for consciousness in fishes

It is considered axiomatic that pain depends on consciousness, so a demonstration that fishes can feel pain depends on showing that they are also conscious. With the realization that pain is a conscious experience and impossible in the absence of consciousness, investigators advocating the belief that fishes can feel pain have attempted to promote the idea that behaviors elicited by nociceptive forms of stimulation reflect conscious pain. There are several questions to be addressed regarding claims that fishes have conscious awareness: (i) what is consciousness and how is it identified?; (ii) how plausible is it that fishes could be conscious?; (iii) what is the evidence for consciousness in fishes?; (iv) of what value would consciousness be to fishes and at what cost?; and (v) if fishes were conscious, could we comprehend what that consciousness was like?

Any rigorous consideration of the existence of consciousness should be predicated on a clear definition of the properties that this consciousness is assumed to have, but unlike the IASP definition of pain, there are no well agreed upon benchmarks for defining the nature of consciousness in animals. Consequently, the absence of a clear

statement of the proposed nature of fish consciousness renders the construct conceptually amorphous, meaning different things to different individuals. This fact makes discussions of various hypothetical aspects of consciousness in fishes or other animals problematic at best (Allen 2011). We prefer to address this question from a less controversial and more empirically sound perspective that of the fundamental properties and neurobiological basis of consciousness in humans.

Although the exact terminology has varied from writer to writer, two principal manifestations of consciousness have long been recognized to exist in humans: (i) primary consciousness, the moment-to-moment awareness of sensory experiences and some internal states such as feelings and (ii) higher-order consciousness also called access consciousness or self-awareness (Macphail 1998; Damasio 1999; Edelman and Tononi 2000; Cohen and Dennett 2011; De Graaf *et al.* 2012; Vanhaudenhuyse *et al.* 2012). Higher-order consciousness includes awareness of one's self as an entity that exists separately from other entities; an autobiographical dimension, including memory of past life events; an awareness of facts, such as one's language vocabulary; and a capacity for planning and anticipation of the future. Differing components of neocortex and associated cingulate gyrus mesocortex have recently been shown to mediate these two forms of consciousness (Vanhaudenhuyse *et al.* 2012). Additional categories and subdivisions of consciousness have been proposed as well (e.g. medical awareness, De Graaf *et al.* 2012) but additional definitions and categorizations of consciousness remain a source of controversy (Baars and Laureys 2005; Overgaard *et al.* 2008).

There have been long accepted criteria for demonstrating the presence of consciousness in humans. Minimal criteria for identifying primary conscious applied by a clinical neurologist are as follows: (i) sustained awareness of the environment in a way that is appropriate and meaningful, (ii) ability to follow commands to perform novel actions, and (iii) verbal or nonverbal communication indicating awareness of the ongoing interaction (Collins 1997; Young *et al.* 1998). Recently, however, intensive study of vegetative states has shown that additional clinical categorizations such as 'minimally conscious states' may exist, but that even highly limited capacity for interaction and demonstration of awareness still requires extensive

functional integrity of frontoparietal 'association' neocortex (Baars and Laureys 2005).

Obviously, assessment of consciousness in an individual, like pain, is introspective and depends heavily on verbal interaction or comprehension. The clinical criteria are of great practical importance, sometimes with life-or-death consequences, as in decisions about brain death or termination of life support. Wakefulness is not evidence of consciousness because it can exist in situations where consciousness is absent (Laureys 2005). This point is not widely appreciated among those working with animals. For example, recovery of wakefulness following anesthesia is often inaccurately referred to as consciousness in species where consciousness has not been validly demonstrated to exist.

The capacity for verbal expression as a means for exhibiting conscious awareness is not necessarily a limiting factor because humans incapable of verbal expression or comprehension have still been able to show full awareness. In the neurological condition called 'locked in syndrome,' in which lower brainstem damage renders a person paralyzed except for voluntary eye movements, the victims are able to demonstrate awareness through arbitrary eye movement patterns that would not be evident in an unconscious person (Bruno *et al.* 2011). Even in the absence of a capacity for verbal expression or comprehension, humans can demonstrate consciousness if they possess it. Helen Keller, deaf and blind since infancy, was able to use arbitrary gestures to establish communication before she learned to sign (Donald 2001). In another well-documented case, a man with focal brain seizures that eliminated all forms of verbal expression or comprehension was able, during a sustained seizure, to travel, check into a hotel, and order food from a restaurant by inventing diverse, novel gestures (Donald 1991). These means of communication, of course, require that the communicator be aware of those with whom they are trying to communicate and possess a normal human 'theory of mind' with which they can anticipate the psychological traits of another human. Theoretically, trans-species communication in which a fish could convey its awareness to a human would be possible if the fish had the capacity for flexible and novel voluntary behavior as well as the inclination. The features of novelty, spontaneity, and flexibility are critical here.

Because of the dependency on verbal or some other flexible, deliberate form of communication for direct assessment of pain and consciousness, a requirement impossible to satisfy with fishes, a different approach, involving consideration of the neurological plausibility of consciousness and pain in fishes, was used to shed light on these issues (Rose 2002). Consider, for example, that there was no opportunity to behaviorally assess whether a species of fish had color vision. If an examination of the pigments in retinal photoreceptors revealed only one type of photopigment rather than multiple types, it would be quite implausible that this fish had color vision. Consciousness is like any other nervous system function in that it depends on specific and identified neural structures.

The neural basis of consciousness was reviewed and applied to the problem of fish pain by Rose (2002). Here, it is important to emphasize that although the specific neural processes that generated consciousness remain unknown, there is much solid evidence regarding the necessary neural structures and systems, including some of the neurophysiological processes that enable it, a very different matter. Subsequent research has further substantiated and refined the fundamental principles identified earlier, that, the existence of all the previously described forms of consciousness depends on neocortex, particularly frontoparietal 'association' cortex in distinction from primary or secondary sensory or motor cortex (Laureys and Boly 2007; Amting *et al.* 2010; Vanhaudenhuyse *et al.* 2012). Primary consciousness also requires supporting operation of subcortical systems including (i) the brainstem reticular formation to enable a working condition of the cortex and (ii) interactions between the cortex and thalamus as well as cortex and basal ganglia structures (Edelman and Tononi 2000; Laureys *et al.* 1999, 2000a,b,c). Higher-order consciousness depends on the concurrent presence of primary consciousness and its cortical substrate, but in addition, higher-order consciousness also requires functioning of broader regions of the neocortex (Edelman and Tononi 2000; Koch and Crick 2000; Iacoboni 2000; Vanhaudenhuyse *et al.* 2012). Human neocortex, the six-layered cortex that is unique to mammals, has specialized functional regions of sensory and motor processing, but activity confined to these regions is insufficient for consciousness (Koch and Crick 2000; Lamme and Roelfsma 2000; Laureys *et al.*

2000a,b; Rees *et al.* 2000). Although neocortex is usually identified as the critical substrate for consciousness, a critical role for some regions of mesocortex, particularly the cingulate gyrus, is well established. Mesocortical structures have fewer than six layers, but like neocortex, are unique to mammalian brains and highly interconnected with neocortex. The cingulate gyrus, in concert with neocortex, is particularly important for conscious awareness of the emotional aspect of pain (Vogt *et al.* 2003), other dimensions of emotional feelings (Amting *et al.* 2010) and self-awareness (Vanhaudenhuyse *et al.* 2012).

Brain structure dictates function, always. So if a fish has consciousness, it will be a product of the fish brain and different from ours accordingly. To the extent that human brains and fish brains differ, particularly the great differences between human neocortex and limbic mesocortex, vs. pallial structure in fishes, the properties of putative consciousness in humans and fishes would differ as well. Furthermore, with the tens of thousands of species of fishes, there are extremely wide variations in brain structure that are associated with variations in behavior and ecological adaptations. The mental life (whatever that might be) of these highly differing brains would have to be deciphered on a case-by-case basis. For example, megamouth sharks are filter feeders with body weights in excess of 1000 kg but brains weighing about 20 g, compared with a predatory hammerhead shark having a 60-g brain at one-fifth the megamouth's body weight, that is, 15 times heavier, when corrected for body weight (Striedter 2005). These dramatic differences in brain size in cartilaginous fishes may, in the case of small-brained species, be an example of symmorphosis, where elaborate body parts are no longer necessary in species with less demanding behavioral repertoires (Striedter 2005).

Some who argue for pain in fishes seem to realize the necessity of finding a plausible neural mechanism for it. Consequently, there have been the previously described studies devoted to identifying nociceptors and describing their properties as well as studies attempting (unfortunately with inadequate techniques, as described above) to show ascending nociceptive activity, ultimately reaching the pallium. Given that pain is a process dependent on concurrent consciousness, it is necessary to show that fish are conscious and that they have a neural system that could mediate

both nociception and pain, which, as described previously, are separate processes. But, if it is assumed that fish brains function according to a common vertebrate plan, which seems to be the supposition (Braithwaite 2010), then the mechanisms essential to both pain and consciousness must be identified and characterized in the pallium, a very challenging undertaking that remains to be attempted. As was explained in detail previously (Rose 2002), it is mechanistically unfeasible that the fish pallium (claims of homologies notwithstanding) could be found to function like a human or other mammalian cortex for purposes of pain or consciousness. Compared with human neocortex and mesocortex, the pallium in fishes is much smaller, unlaminated, and vastly simpler in types, numbers of neurons, and regional differentiation. In mammals, ascending systems are expanded at successively higher levels of the brain, such that the cortical representation of a given sensory system is typically mapped and remapped to become very spatially extensive and functionally diversified (Nieuwenhuys *et al.* 1998; Striedter 2005). Fish brains, in contrast, are examples of diminishing systems, in which ascending systems typically decrease in the size as they ascend to the pallium (Nieuwenhuys *et al.* 1998). Anyone proposing that a fish pallium could function like a human or mammalian neocortex or that there might be a substitute system in fish brains for generating consciousness, must provide convincing, empirical evidence that such a proposal is worth consideration. This has not been done.

Furthermore, and this is a point seldom considered, a great deal of human behavior is actually unconsciously mediated (discussed in Rose 2002, 2007) and our survival depends on that fact. The study of unconscious mental processes is a very active and expanding research field. In his book, *Strangers to Ourselves*, Timothy Wilson (2004) said: 'The mind operates most efficiently by relegating a good deal of high-level, sophisticated thinking to the unconscious, just as a modern jetliner is able to fly on automatic pilot with little or no input from the human 'conscious' pilot. The adaptive unconscious does an excellent job of sizing up the world, warning people of danger, setting goals and initiating action in a sophisticated and efficient manner.' When the lives and behaviors of fishes are examined, even the most complex of their social behavior, an adaptive unconscious is likely to suffice nicely. Thus, the scientific principle

of parsimony applies here: why propose the existence of a more complex process (consciousness) when a less complex one accounts for the data, not to mention the lack of a plausible mechanism for the more complex process?

Arguments have been put forward to support the contention that fishes experience adverse experiential states that 'humans associate with pain and emotional distress' (Huntingford *et al.* 2006). Huntingford *et al.* (2006) consider pertinent to this assertion claims that fishes are long-lived; that their behavior is complicated and not stereotyped, including that some species live in groups and can recognize individuals; that they remember locations of negative experiences; that they learn complex spatial relationships; and that neurobiological evidence shows that information processed in different areas of the brain can be integrated to produce avoidance responses. Similar arguments have been made by Braithwaite (2010). There is nothing inherent in these points (even if they were all valid) to necessitate a conclusion that fishes are conscious. Longevity is hardly a trait of all fishes and trees live much longer. Complexity has never been an acceptable proof of consciousness. As we show below, avoidance learning, spatial learning, and complex social behaviors do not depend selectively on pallial function in fishes. Individual recognition is another type of claim that depends greatly on how supporting data are interpreted. Regarding brain integration and avoidance learning, the same type of centralized brain processing is evident in brain functions generally, including unconscious ones, and hardly warrants attributions of consciousness.

Sneddon *et al.* (2003a) argued that the fact that behavioral responses in trout to two types of noxious stimuli extended 'over a prolonged period of time, suggested discomfort.' Huntingford *et al.* (2006; see also Braithwaite 2010; Braithwaite and Boulcott 2007) have claimed that behaviors 'that require more complex processes than associative learning' have been observed in fishes and are 'evidence of fish species [in which] the experience of suffering must be a real possibility.' These claims for the significance of complex learning must be taken as tentative at best. In the research cited, much depends on assumptions of what the learning task requires by the fish and subsequent interpretation of the data. The past century of experimental behavioral research has repeatedly demonstrated that the more extravagant claims

(like language learning by great apes) have usually failed to withstand critical scrutiny or more extensive subsequent investigation (Wynne 2008). We consider that it is very premature to conclude that consciousness has been demonstrated in fishes on the basis of such limited study.

Braithwaite (2010), Braithwaite and Boulcott (2007) and Huntingford *et al.* (2006) provide no evidence for their belief that reflexive and associate behaviors are unconscious, while more complex learned behaviors require consciousness. The evidence cited above of the complex and integrated behavioral responses that can be evoked in somnambulists, decorticate humans, and even decerebrate mammal species clearly contradicts arguments that behavioral complexity is evidence for conscious experience. Furthermore, the evidence detailed below of learning by fishes with the forebrain removed also argues against this point.

Braithwaite (2010) has also claimed that mutualistic feeding between moray eels and a species of grouper is evidence of fish consciousness, even self-awareness. Mutualisms are numerous and diverse, existing between virtually all classes of species, plants, animals, and even bacteria (e.g. between gut bacteria and ruminant herbivores). They are typically regarded by biologists as products of natural selection and evolution, rather than a result of insightful behavior on the parts of the participants (Leigh 2010), and the example given by Braithwaite is best viewed in this way. Learning may play a role in refining mutualism between fishes (Helfman *et al.* 1997), but inferences about consciousness, much less self-awareness, are unwarranted. If mutualisms by fishes were products of conscious insight, they should be more common, spontaneously occurring, and not follow such species-specific patterns.

Contradicting claims about consciousness is the extensive evidence that in fishes most aspects of neurobehavioral function are retained after cerebral hemisphere removal. This was shown many years ago by experiments in which the cerebral hemispheres were removed, leaving only the diencephalon, brainstem, and spinal cord intact in diverse fish species (Overmier and Hollis 1983). The behavior of these fishes was substantially preserved. They still found and consumed food, showed basic capabilities for sensory discrimination and many aspects of social behavior, including schooling, spawning, and intra-species aggression.

An exception was the loss of the sense of smell, which is processed entirely in the forebrain. Some species differences exist, but courtship, nest building, and parental care often persisted after forebrain removal. Classical conditioning and instrumental learning are intact in the absence of the forebrain. Avoidance conditioning, a type of instrumental learning, seems to be much more difficult, but nonetheless possible, for fishes with the cerebral hemispheres removed (Overmier and Papini 1986). This difficulty with avoidance learning is not due to reduced responsiveness to noxious stimuli because the startle and locomotor, including escape responses, to such stimuli are quite normal in fish without cerebral hemispheres. The general conclusion derived from many studies is that the basic patterns of fish behavior are controlled by lower brain structures, mainly the diencephalon, brainstem, and spinal cord. The cerebral hemispheres serve mainly to 'modulate' behavior, that is, to regulate its intensity or frequency and to refine its expression (Overmier and Hollis 1983).

In recent years, there have been many studies of more diverse forms of learning by fishes, especially goldfish. Of most relevance to the present discussion are those in which brain lesions were used to investigate the potential dependency of a form of learning, most frequently spatial learning, on telencephalic structures. A common outcome following various types of localized telencephalic lesions was that an effective lesion impaired but did not eliminate the type of learning in question and that specific spatial tasks were differentially affected by lesion location (López *et al.* 2000; Rodríguez *et al.* 2002; Portavella and Vargas 2005; Saito and Watanabe 2006; Vargas *et al.* 2006). It is especially noteworthy that in studies where total telencephalon ablation was performed, the result was either a deficit, with preserved capacity to learn one type of spatial task, while the ability to learn another was unimpaired (López *et al.* 2000; Rodríguez *et al.* 2002) or, in contrast, no deficit at all (Durán *et al.* 2008). In distinction to these results, either dorsomedial pallial lesions or total telencephalic ablation prevented learning of a taste aversion by goldfish (Martín *et al.* 2011). Similar to the results of older studies with classical conditioning paradigms, heart rate conditioning in goldfish was not affected by telencephalic ablation (Martín *et al.* 2011). It is clear from these studies that the forebrain definitely assists various types of learning, making the

learning faster and better, but localized telencephalic damage may not eliminate these forms of learning. The research we summarize here does not include cases of all forms of learning by fishes but these examples of more recent research employing brain lesions do not make a strong case that that learning by fishes depends on forebrain function to a degree that would suggest a consciousness dependency of that learning.

A final point regarding arguments related to fish learning as evidence for consciousness is that increasing evidence shows that even declarative forms of memory in humans, previously regarded as consciously mediated, can be encoded and retrieved unconsciously (Henke 2010).

If fishes were conscious, what would it be like?

There seems to be an assumption made by those advocating the belief that fishes are conscious that if this assumption were true, it would automatically be justifiable to assume that their consciousness would be human-like enough to conclude that fishes experienced human-like pain and suffering. There is no basis for this belief other than pure speculation. If fishes have consciousness, their consciousness must be so different from ours, as deduced from their brains and their behavior, that we have no idea what it would be like. We really only know the consciousness of our own species and that is hard enough to describe. Furthermore, fishes are highly diverse organisms and there are tens of thousands of species of them. Would the consciousness of a basking shark be like that of a barracuda?

Then, there is the question of the utility of consciousness or pain to a fish. Most fishes fail to reach adulthood and predation is the greatest reason for this. Those that do survive must react to attacks by predators within milliseconds (Helfman *et al.* 1997). Rapid reactions are best performed unconsciously, even in humans. Adding additional processing time with consciousness would likely prove fatal. The same constraints apply to predators, which must react to prey capture opportunities faster than the prey can escape. Furthermore, many predators are simultaneously prey, especially as juveniles, so escape and attack behaviors must be instantaneously ready at all times. Where is the value of consciousness here?

The same consideration applies to pain. The fossil record demonstrates that sharks and rays have

survived longer than teleosts have existed (Long 1995), apparently without a capacity for nociception, much less pain. Agonizing pain, due to C fiber activity, is a typical consequence of serious injury in humans. The lives of humans with pathological insensitivity to pain (really a lack of nociception) is revealing in this regard. Such people do not avoid injurious circumstances and neglect fractures, burns or diseases like appendicitis (Nagasako *et al.* 2003). Injuries, like tongue mutilation, occur and may become ulcerated and unwittingly traumatized further (Butler *et al.* 2006). The soft tissues and complex appendages (e.g. hands) of humans are clearly injury prone and costs of injuries are potentially great. Pain in humans initiates a variety of sophisticated behaviors that serve to protect injuries and promote healing.

A rapid response to potentially injurious stimuli, mediated by A-delta nociceptors and (unconscious) learning to avoid injurious situations where noxious stimuli occurred, would likely be selectively advantageous to fishes. However, preoccupation with conscious suffering, especially when little can be done to minister to injuries, would be selected against as it would be unlikely to benefit fishes, which must survive in an environment where they can ill afford to be debilitated by conscious suffering.

Even those scientists who would attribute some form of consciousness, such as primary consciousness, to fairly diverse species of vertebrates typically do not believe that fishes could have self-awareness (Donald 2001; Tulving 2005). The debate about that capacity has mostly been centered on whether it is unique to great apes or just humans (Macphail 1998; Donald 2001; Povinelli 2004; Wynne 2004; Terrace and Metcalfe 2005). This point is pivotal because one of the most critical determinants of suffering from pain is the personal awareness and ownership of the pain (Price 1999). This is why dissociation techniques, in which a person can use mental imagery to separate themselves from pain, are effective for reducing suffering (Price 1999). In contrast, without awareness of self, the pain is no one's problem. It is simply there, something to be reduced or avoided if possible, but not a 'personal' problem. The known importance of self-awareness for pain contradicts, Sneddon's (2011) claim that an absence of self-awareness in fishes would make their 'pain' worse.

Costs of invalid definitions and mistaken views of fish pain and suffering

There are many potentially damaging consequences of ongoing misrepresentations of what is known, or more accurately, not known, concerning the fish pain and suffering issue. Policies stemming from these misconceptions could undermine the health and welfare of fishes and humans alike and, if unchecked by more scientifically sound information, their impacts will likely become more widespread and damaging. A thorough consideration of these consequences will not be undertaken here, but we see five categories of human–fish relations that could be adversely affected by such misconceptions: (i) accurate understanding of the nature and welfare needs of fishes; (ii) scientific research with fishes; (iii) aquaculture and commercial fishing; (iv) direct contact between humans and fishes through 'recreational' fishing or captive fish ownership, and (v) fisheries management.

Increasing regulation of human conduct toward fishes, particularly in Europe (see Berg and Rösch 1998; Arlinghaus *et al.* 2007b, 2009, 2012; Ashley 2007; Meinelt *et al.* 2008; Arlinghaus and Schwab 2011), has been implemented to reduce alleged fish pain and suffering, but the analysis we have presented here shows that such regulations have been implemented without valid scientific justification. Predicating welfare policy on unsubstantiated and likely mistaken concerns about fish pain and suffering has the potential to undermine the scientific basis of fish welfare, an argument that Dawkins (2012) has recently raised concerning the credibility of welfare research more broadly. A justification for restrictive welfare policies is exemplified by the 'benefit of the doubt' dogma. This brand of logic peculiar to welfare biology is, in effect, an admission that the fish pain issue is not resolved (hence the doubt), but the consequence is to mandate policy as if the matter actually was resolved in favor of fish pain interpretations. This is a social–political maneuver that effectively exempts valid science from policy. The 'benefit of the doubt' dogma is not benign nor does it best protect fish welfare (Arlinghaus *et al.* 2009).

A disconcerting and costly irony of oppressive regulations of experimental protocols for the use of fishes is that some of the most decisive research that could be carried out to resolve the contentious issue of fish pain and suffering is usually prohibited across most of Europe. A key example is

the different results of studies involving acetic acid injections into the jaws of rainbow trout by Sneddon *et al.* (2003a,b) as opposed to findings of Newby and Stevens (2008). The likely difference underlying the opposing outcomes between the two studies (acknowledging the hazards of interpreting negative results) was that Newby and Stevens did not anesthetize the trout when giving acid injections. Anesthetization was considered by Sneddon *et al.* (2003a) to be mandatory from a 'humane' perspective, but had the most conservative of 'humane' criteria been allowed to rule here, this critical test of the reliability of the Sneddon *et al.* study might not have been attempted or published. Further elaboration on the possible consequences of mistaken assumptions that fish do feel pain can be found in a series of papers by Arlinghaus *et al.* (2007b, 2009, 2012).

Fish welfare without conjecture

As we stated at the start of this article, in questioning the evidence for pain awareness and suffering in fishes, we are not diminishing the importance of fish welfare concerns. However, we believe that in fostering fish welfare, implementation of legislation must be intelligently considered to ensure that it would not adversely impact humans socially and economically without necessarily benefiting fishes.

In recent papers regarding free-living fishes and aquatic invertebrates, Diggles *et al.* (2011a,b) argued for the superiority of function and nature-based approaches over a feelings-based approach to the welfare of fishes and aquatic invertebrates because the former two definitions do not contradict reality, do not invite use of double standards, and do not contravene basic scientific principles. Similarly, Arlinghaus *et al.* (2007b, 2009) argued for a pragmatic over a feelings-based approach to fish welfare, based on science and logic that acknowledge that there are human impacts on fish but that similarly acknowledge that humans depend on the uses of fishes. We reassert these views here. Function-based welfare does not depend upon assumptions of awareness or resolution of the scientific debate about whether fishes and aquatic invertebrates experience pain, suffering and emotional feelings. Function-based welfare can be measured and assessed within a factual and logical framework that can be supported by empirical science. We believe that using objective

information based on clearly validated indices of fish well-being, like reproduction, stress responses, growth, disease resistance, or detrimentally disturbed behavior, without anthropomorphic speculations about what a fish is allegedly feeling, will readily identify environmental or experiential conditions detrimental to welfare (Iwama *et al.* 1997; Erickson 2003; Nickum *et al.* 2004; Arlinghaus *et al.* 2007b, 2009; Iwama 2007). Furthermore, an objective, non-anthropomorphic examination of the normal behavior and adaptations of diverse species of fish will provide the best guide to species-specific welfare (Arlinghaus *et al.* 2007b; Turnbull and Kadri 2007).

Summary and conclusions

We have discussed the nature of pain and identified critical standards for the conduct of legitimate research in this area, especially the necessity of using definitions and measures that validly distinguish between nociception, the unconscious sensory detection of injurious stimuli and conscious pain. Our examination of the research literature revealed that these requirements have not been met in research leading to claims for fish pain.

Definitions, of pain such as 'more than a simple reflex,' are too vague and at odds with the existence of complex unconscious, nocifensive (nociception-evoked) behaviors. In addition, this definition has fostered the use of a false dichotomy that invalidly biases interpretations in favor of conclusions that fishes feel pain. Consequently, the research literature that alleges to show pain in fishes has failed to do so.

One of the most conspicuous shortcomings in discussions of scientific evidence for fish pain has been the selective consideration of evidence. There is a wealth of experimental and field research that speaks to the issue of fish pain and nociception in a very realistic way because this research examines the effects of actual injury or natural injury-producing stimuli rather than the more confounded manipulations like chemoirritant injections or electric shocks. Feeding, activity levels, and forced swimming have been examined after various types of surgeries in fishes. Typical results have been resumption of feeding and normal activity immediately or within minutes of recovery from anesthesia. Likewise, studies involving biotelemetry have consistently documented rapid recovery of normal behavior following transmitter implantation as well

as long-term survival and normal behavior. Studies of catch-and-release angling have consistently demonstrated resumption of normal activity immediately or within hours of release, with many instances of a fish being recaptured within minutes or hours of release and showing good long-term survival. In contrast to this information are a relatively small number of highly publicized studies, in which fishes showed minimal and almost trivial responses to seemingly noxious experimental procedures, yet have been the basis for claims of fish pain. Prominent among these were experiments employing injections of an acid solution into the jaws of rainbow trout. It was claimed that short-term suppression of feeding and 'anomalous' behaviors, including mouth 'rubbing' and 'rocking,' constitute evidence of pain. We have questioned the validity of these interpretations and cited evidence from several other studies involving similar acid injections as well as many other studies involving surgery in which no instances of such 'anomalous' behaviors were obtained.

Anatomical and physiological studies have presented evidence for A-delta and C fiber afferents in the trigeminal nerve in rainbow trout and carp tail nerves. While A-delta fibers, the type of nociceptive afferent responsible for triggering rapidly sensed, well-localized 'first pain' in humans, were fairly numerous, only an extremely small number of C fibers, the nociceptive afferent that is the most abundant fiber type in mammalian nerves and responsible for the more intense, suffering-producing 'second pain,' were found. Studies with elasmobranchs have consistently shown that sharks and rays lack unmyelinated (C type) fibers. Shark and ray species have also been found to lack a key region of the spinal dorsal horn known in mammals to be critical for transmitting nociceptive activity to the brain. These results bring into question the feasibility of pain-induced suffering or even intense, prolonged nociception in teleost as well as elasmobranch fishes. Our consideration of the available evidence leads us to conclude that fishes for which behavioral, physiological, and neurobiological evidence is available, are unlikely to have a capacity for the full range of nociception, especially the C fiber-mediated nociception that can cause agonizing pain in conscious humans. The behavior of teleost fishes subjected to natural injury or injurious stimuli is consistent, instead, with the interpretation that these fishes are likely able to detect acute nociceptive stimuli

so as to escape and (unconsciously) learn to avoid situations leading to such stimuli, but that prolonged consequences of nociceptive stimulation and injury, especially conscious pain, are highly unlikely. Elasmobranchs, notably sharks, appear to be even less responsive to nociception.

A source of confusion in the literature advocating fish pain is the claim for a capacity for conscious emotional feelings in fishes. The contemporary neurobiological literature has shown that there is a dichotomy of unconscious emotional responses and conscious feelings that is comparable to the nociception–pain dichotomy. Fishes are neurologically equipped for unconscious nociception and emotional responses, but not conscious pain and feelings.

In view of the necessity of consciousness as a precondition for pain experience claims have also been made for the existence of consciousness in fishes. Our assessment of these claims leads us to conclude that neither their rationale nor their supporting evidence is compelling, much less neurologically feasible.

The arguments we have presented support function and nature-based welfare standards that are predicated on objective indicators of fish well-being rather than a feelings-based standard that is highly speculative and scientifically unsubstantiated.

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