

## Evidence for pain in decapod crustaceans

RW Elwood

School of Biological Sciences, Queen's University, Belfast BT9 7BL, UK; email: r.elwood@qub.ac.uk

### Abstract

Vast numbers of decapods are used in human food and currently subject to extreme treatments and there is concern that they might experience pain. If pain is indicated then a positive change in the care afforded to this group has the potential to produce a major advance in animal welfare. However, it is difficult to determine pain in animals. The vast majority of animal phyla have a nociceptive ability that enables them to detect potential or actual tissue damage and move away by a reflex response. In these cases there is no need to assume an unpleasant feeling that we call pain. However, various criteria have been proposed that might indicate pain rather than simple nociception. Here, with respect to decapod crustaceans, four such criteria are discussed: avoidance learning, physiological responses, protective motor reactions and motivational trade-offs. The evidence from various experiments indicates that all four criteria are fulfilled and the data are thus consistent with the idea of pain. The responses cannot be explained by nociception alone but, it is still difficult to state categorically that pain is experienced by decapods. However, the evidence is as strong for this group as it is for fish but the idea that fish experience pain has broader acceptance than does the idea of decapod pain. A taxonomic bias is evident in the evaluation of experimental data.

**Keywords:** animal welfare, avoidance learning, decapod, motivational trade-off, nociception, protective motor reaction

### Introduction

The number of crustaceans captured or farmed for human consumption is vast. For example, the number of tiger prawns (*Penaeus monodon*) used in 2008 is estimated at 214 billion (ie 214 million, million) animals. As this species comprises about 12% of the number of crustaceans used per year the total number is over 1,600 billion animals, a number that vastly exceeds the combined numbers of chickens, pigs, sheep and cattle killed. The following is an attempt to put the numbers into perspective. A person who eats a 200 g portion of beef every day will consume 25 animals over a lifetime of 80 years. If that person has a prawn cocktail as a starter for just one meal, more than 25 animals could be consumed in one day.

Live crustaceans are often transported for days, with claws either bound or mutilated so they are non-functional. Some crab fisheries involve the claws being pulled off and retained whilst the animal is thrown back to the sea alive but unable to feed (Patterson *et al* 2009). When whole animals are landed the method of killing is usually by placing in boiling water. Despite these treatments, our understanding of the potential for suffering within this group has been largely neglected (Kellert 1993; Sherwin 2001). Here, by applying specific criteria (Bateson 1991; Sherwin 2001;

Elwood 2011), coupled with an experimental approach (Patterson *et al* 2007; Barr *et al* 2008; Appel & Elwood 2009a,b; Elwood & Appel 2009; Barr & Elwood 2011) we ask if these animals experience pain. The substantial numbers of them used in the food industry and the extreme treatments to which they are exposed should indicate the potential for improved welfare if evidence of pain is found.

### Pain or nociception?

Within the United Kingdom, all vertebrates have been protected in scientific research since 1986 (Animal Scientific Procedures Act), despite considerable disagreement about which vertebrate taxa might experience pain (Rose 2002; Braithwaite 2010; Carere *et al* 2011; Mason 2011). Recent work, however, has been influential in the greater acceptance that pain occurs in fish (reviewed in Sneddon 2009; Braithwaite 2010). Invertebrates are generally excluded from such protection (apart from *Octopus vulgaris*) because their protective reactions are viewed as nociceptive reflexes (see below) rather than involving pain. Legislation in the UK has been proposed to widen protection to some other species of cephalopods, but not decapod crustaceans. To understand why some animals have been protected whereas others are excluded we need to be clear about the definition of pain.

Pain in humans is described as:

an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (IASP 1979).

The problem with studying pain in animals is that they cannot describe their emotional experiences and thus other definitions have been proposed. One commonly used definition is:

an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance and may modify species specific behaviours, including social behaviour (Zimmerman 1986).

A shorter definition that excludes the criteria by which pain may be assessed is “an aversive sensation and feeling associated with actual or potential tissue damage” (Broom 2001). Thus, pain is a centrally organised emotional interpretation of the effects of a noxious stimulus and is a strong motivation to get away from or otherwise stop the effects of the noxious stimulus.

As a first step in understanding pain we have to be clear about the mechanisms by which the noxious stimulus is initially perceived. These sensory systems are termed nociceptors (Sherrington 1906) and nociception is defined as “the neural processes of encoding and processing noxious stimuli” (Loeser & Treede 2008) or “the capability of animals to detect and react to stimuli that may compromise their integrity” (Besson & Chaouch 1986). There is no suggestion that nociception involves an unpleasant feeling and there is no implication that central processing and decision-making are involved in responses. Rather, nociception involves a reflex response that helps minimise tissue damage. In some taxa, however, the input from nociceptors is experienced as pain but that does not mean that nociception leads to pain in all animal taxa.

Nociception has a clear function in that it protects the animal from continuing tissue damage and should thus enhance fitness. Nociception is thus widespread and virtually universal amongst animal phyla (Elwood 2011). If animals typically have this nociceptive protection we may ask what further fitness enhancement is provided by the emotional unpleasant feeling of pain? Presumably, pain provides a mechanism of long-term awareness of the noxious stimulus and tissue damage and a strong motivation to avoid that stimulus in the future (Bateson 1991; Elwood 2011). Thus, nociception provides immediate action whereas pain enables long-term protection.

Elwood (2011) lists eight criteria suggested by various authors (Bateson 1991; Sherwin 2001; Broom 2007) that might be used to indicate pain. That paper suggests that four commonly used criteria, specifically: 1) the presence of nociceptors; 2) a ‘suitable’ central nervous system; 3) decreased responses with analgesics or opioids; and 4) high level of cognitive ability, allow only limited insights into whether or not an animal might experience pain. However, it concludes that greater insights might be gained from studies on the remaining four, specifically: 5) avoidance

learning; 6) physiological changes; 7) protective motor reactions; and 8) trade-offs between pain responses and other motivational requirements. Here, recent experiments are concentrated on these latter four criteria and the extent to which the data are consistent with the notion pain is indicated. I also suggest other approaches that might help to determine if pain is likely in decapod crustaceans.

### Avoidance learning

As noted above, the function of pain seems to be that the high motivation to avoid that experience enhances avoidance learning and protects the animal from future damage. Thus, we expect that taxa that experience pain should show rapid avoidance learning and there have been several experiments on this topic in decapods. Denti *et al* (1988) used an inhibitory avoidance technique on the crab, *Chasmagnathus granulatus*. Each subject was placed in a dark compartment and allowed to walk to a light area and some then received a shock. When the crabs were tested again the latency to enter the light compartment was greater for those that received a shock than for the control group. The association from a single trial between the shock and the light chamber persisted for up to 3 h. Crayfish (*Procamarus clarki*) were able to associate a light signal with a shock and learned to avoid the shock by walking forward to the other end of a shuttle box but would not learn to tail flick when the light was presented if facing away from the other end of the shuttle box (Kawai *et al* 2004).

A recent study used the shore crab (*Carcinus maenas*) which typically avoid light and seek dark spaces under rocks or weed (Magee & Elwood, unpublished). In the laboratory, we allowed crabs to select one of two dark shelters within an otherwise light area. Crabs quickly and reliably entered a shelter to avoid the light. Some crabs were randomly selected to receive a shock when they entered the shelter and received further shock if they remained in that shelter. Other crabs were not shocked in the first chosen shelter. After 2 min, the crabs were returned to the central start point in the light and allowed to select a shelter again. For this and subsequent trials those that received a shock in the first shelter would receive a shock in that shelter again but not in the alternative shelter and the reverse would occur for those that did not receive a shock in the first shelter. We found that most crabs went back to the shelter selected in their original trial and whether or not they had received a shock did not alter that choice. However, a change in response to the shock subsequently became evident because those that received a shock in the second trial were more likely to switch shelters in the third trial compared to those that had not received a shock in the second trial. Thus, the crabs quickly learned to discriminate between the two shelters and avoided the shock shelter.

Crabs will also learn to move a single appendage. This was shown in *Ocypode ceratophthalma* by applying a shock to a single leg whenever the crab lowered that leg into water. The animal learned to hold the leg above the water and thus avoid shock (Hoyle 1976) and similar leg avoidance movement was described in *Carcinus maenas* (Barnes &

Dunn 1981a,b). Using intact mud crabs (*Eurypanopeus depressus*), Punzo (1983) showed that leg avoidance learning was accompanied by increases in RNA and protein synthesis in the brain and that disruption of the protein synthesis also disrupted learning. This study identified specific areas of the protocerebrum within the brain as being involved in the learning of this avoidance. Curiously, 'debrained' crabs also show the ability to learn to lift their leg to avoid shock, suggesting that the thoracic ganglia of these animals play a role (Hoyle 1976; Barnes & Dunn 1981b).

Another approach was employed with hermit crabs (*Pagurus bernhardus*), some of which received a shock to the abdomen within the shell (Appel & Elwood 2009b; Elwood & Appel 2009) and all were later offered a new shell. Shocked crabs approached the new shell more quickly and showed a much quicker investigation of the new shell before moving into it. Quick investigation is typical of crabs that are in shells of very poor quality (Elwood & Stewart 1985) suggesting that the shocked crabs perceived their shells as being of particularly low quality. They showed a high motivation to move out of the shell even 24 h after the shock (Appel & Elwood 2009b). These studies indicate rapid avoidance learning and persistent memory of a noxious stimulus.

### Physiological changes

Corticosteroids are elevated after acute noxious stimuli are applied to vertebrates and there is a range of other physiological responses to potential pain. These include changes in heart rate, pupil dilation and blood gases (Short 1998; Sneddon *et al* 2003a). Decapods also have a stress hormone called the Crustacean Hyperglycaemic Hormone (CHH) (as well as specific biogenic amines) that function in a way similar to corticosteroids in vertebrates in that glycogen is converted to glucose and also causes elevated lactate (eg Lüschen 1993). In the edible crab (*Cancer pagurus*), forceable removal of a claw (a practice used in some fisheries) in a way that causes tissue damage induced a rapid rise in glucose and lactate, whereas those induced to autotomise their claw did not show these effects (Patterson *et al* 2007). That is, tissue damage results in a marked physiological stress response.

### Protective motor reactions

Vertebrates show prolonged licking, grooming or rubbing of an area of the body subject to a noxious stimulus (Weary *et al* 2006). For example, a rat or mouse injected with acetic acid or formalin on the forelimb licks and grooms that area and this is one of a number of standard 'pain tests' employed to test the effectiveness of analgesics (Shibata *et al* 1989). Trout injected in the lip with acetic acid also rub the lip in the gravel of a tank (Sneddon *et al* 2003a). This approach was taken with the glass prawn (*Palaemon elegans*) by brushing acetic acid (or sodium hydroxide) onto one antenna when the animal was out of water and then replaced in the water. This caused a marked increase in grooming, and also rubbing the antenna against the wall of the tank compared to control animals brushed with water

(Barr *et al* 2008). This grooming was directed at the treated antenna rather than the untreated antenna and there was no overall increase in activity that could explain the response. It has been suggested that the response could be just a grooming response rather than a nociceptive response (Puri & Faulkes 2010) presumably simply to clean the area. Of course, the same could be said of similar observations on rodents but those observations are regarded as valid evidence of pain (Sherwin 2001). We have also noted prolonged abdominal grooming at the site of a shock in hermit crabs that evacuate their shells (Appel & Elwood 2009a,b), which we have not seen when crabs are cracked out of their shell by a bench vice or removed from their shells during a shell fight (Dowds & Elwood 1985). Prolonged grooming and rubbing indicates an awareness of the specific site of the noxious stimulus and is not easily explained as a reflex.

### Trade-offs between pain responses and other motivational requirements

A key feature of pain is that it is a strong motivational factor to terminate and avoid stimuli that give rise to the pain (Elwood 2011). Pain is thus not all or nothing but, in humans at least, can be graded. If pain is part of a motivational system then we would expect that pain responses would be traded-off against other motivational requirements. Thus, fish subject to shock whilst feeding are less likely to give up feeding to avoid the shock if they have been deprived of food (Millsopp & Laming 2008). In that case the response to the shock cannot be a reflex because the response depends on other motivational requirements. This general approach allows for a number of inferences. First, some form of central processing must be involved so that the different motivational requirements can be assessed and a decision made. Second, we can use the giving up of a resource as a measure of the noxiousness or unpleasantness of the potential pain-inducing stimulus. We might expect mild pain not to stop a hungry animal from feeding whereas severe pain would cause the animal to move away from the food. Third, a minor shock might not cause an animal to give up a desired resource but it might give up a less desired resource.

Taking this approach we have tested hermit crabs by shocking them within their shells and shocked crabs might evacuate their shells. Hermit crabs show strong preferences for particular species of shell as determined by shell choice experiments (Elwood *et al* 1979) and the tendency to fight for new shells (Dowds & Elwood 1983). To test for motivational trade-offs, crabs were shocked repeatedly within the shells starting with a low voltage and increasing the voltage with each subsequent shock. There was no effect of shell species on the voltage that caused the crab to first respond in terms of a brief jerking movement but those in the less preferred species got out of their shell at a lower voltage than did those in the preferred species (Appel & Elwood 2009b). A subsequent experiment used a single shock at a voltage, judged to be just below that which would cause crabs to evacuate (Elwood & Appel 2009). Nevertheless, some crabs did evacuate but more did so from the less

preferred species. A third experiment used a single species of shell but some crabs were tested while the odour of a predator was present and these evacuated at a higher voltage than did those in the absence of an odour (Magee & Elwood, unpublished). Thus, it is clear that hermit crabs trade-off competing demands in their responses to electric shock in a way that cannot be explained by a nociceptive reflex response.

Shocked hermit crabs show two curious responses apart from just evacuating shells or grooming their abdomen. For example, a number of crabs that evacuated the shell then felt deep into the shell in a way that is consistent with them searching for the source of the noxious stimulus (Appel & Elwood 2009a,b). Further, although the shell is an essential resource and these are typically in very short supply in the natural habitat, some crabs walked away from the shell after getting out and some appeared to attempt to climb the wall of the observation chamber. Note we also found that shore crabs will leave a dark shelter if shocked even though a dark shelter is an important resource for these animals. These observations of hermit crabs abandoning shells or shore crabs leaving dark shelters are consistent with giving up a highly valuable resource in order to escape the noxious stimulus.

### Animal welfare implications and conclusion

The studies noted above are consistent with the concept of pain and demonstrate that the responses to noxious, potentially tissue damaging stimuli go beyond that predicted by nociceptive reflex. However, there is still a resistance to the idea of pain in decapods (Brathwaite 2010) although others are more open to the idea (Carere *et al* 2011; Mason 2011). It is worth noting that the evidence for pain in decapods and fish is rather similar. However, there is considerably more acceptance for pain in fish, a clear case of the argument by analogy not being applied equally among taxa (Sherwin 2001). Both fish and decapods show swift avoidance learning (Denti *et al* 2004; Dunlop *et al* 2006), specific stress responses (Chandross *et al* 2004; Patterson *et al* 2007), motivational trade-offs (Millsopp & Laming 2008; Elwood & Appel 2009) and prolonged rubbing or grooming (Sneddon *et al* 2003a; Barr *et al* 2008). Based on the evidence, fish and decapods would seem to have a similar likelihood of pain experience (Elwood 2011). Whilst the data for both are consistent with the idea of pain that is not the same as stating that they definitely do experience pain, however, I am prepared to argue that there is a strong possibility. As such, both taxa should be treated as though they are able to experience the negative affective state of pain.

There is a case for further research to attempt to resolve the issue of pain in decapods. I have argued that showing analgesic effects of morphine and effects of local anaesthetics add little to the concept of pain because both interfere with nociceptive input (Elwood 2011). However, much more persuasive are studies in vertebrates that demonstrate that subjects develop a preference for food or water containing analgesics when noxious stimuli are applied (eg Danbury *et al* 2000). I am not aware of such approach in any invertebrate, but a demonstration of a shift

in preference for analgesics would be strong evidence for a negative affective state. Second, a demonstration of CHH release and heart-rate change after shock would support the idea of pain in crustaceans. Further, it would be interesting to examine such physiological change to conditioned stimuli that were subsequently applied in the absence of unconditioned noxious stimuli. If such changes were demonstrated it would suggest anticipation of the noxious stimulus in a way predicted if the animal could experience pain. Third, examples in which the animal was distracted from attending to the noxious stimulus and then showing a reduced response would be consistent with the idea of pain. Also, studies in which 'pain' distracts from other important stimuli, as shown by a decrease in neophobia in fish (Sneddon *et al* 2003b), should be applied to decapods. Depending on the findings, these approaches have the potential to weaken or strengthen the case for pain in decapods but, regardless, the arguments by analogy should be applied equally to vertebrates and invertebrates if welfare science is to make progress.

### Acknowledgements

I am grateful to Lisa Collins for gathering data on weights of crustaceans captured or reared and used in human food that enabled estimates of numbers used.

### References

- Appel M and Elwood RW** 2009a Gender differences, responsiveness and memory of a potentially painful event in hermit crabs. *Animal Behaviour* 78: 1373-1379. <http://dx.doi.org/10.1016/j.anbehav.2009.09.008>
- Appel M and Elwood RW** 2009b Motivational trade-offs and the potential for pain experience in hermit crabs. *Applied Animal Behaviour Science* 119: 120-124. <http://dx.doi.org/10.1016/j.applanim.2009.03.013>
- Barnes WJP and Dunn PDC** 1981a Neural correlates of leg learning in the shore crab, *Carcinus maenas*. *Marine Behaviour and Physiology* 8: 83-97. <http://dx.doi.org/10.1080/10236248109387005>
- Barnes WJP and Dunn PDC** 1981b Learning of leg position in the shore crab, *Carcinus maenas*. *Marine Behaviour and Physiology* 8: 67-82. <http://dx.doi.org/10.1080/10236248109387004>
- Barr S and Elwood RW** 2011 No evidence of morphine analgesia to noxious shock in the shore crab, *Carcinus maenas*. *Behavioural Processes* 86: 340-344. <http://dx.doi.org/10.1016/j.beproc.2011.02.002>
- Barr S, Laming PR, Dick JTA and Elwood RW** 2008 Nociception or pain in a decapod crustacean? *Animal Behaviour* 75: 745-751. <http://dx.doi.org/10.1016/j.anbehav.2007.07.004>
- Bateson P** 1991 Assessment of pain in animals. *Animal Behaviour* 42: 827-839. [http://dx.doi.org/10.1016/S0003-3472\(05\)80127-7](http://dx.doi.org/10.1016/S0003-3472(05)80127-7)
- Besson JM and Chaouch P** 1986. Peripheral and spinal mechanisms of nociception. *Physiological Reviews* 67: 88-186
- Braithwaite V** 2010 *Do Fish Feel Pain?* Oxford University Press: Oxford UK
- Broom DM** 2001 Evolution of pain. In: Soulsby EJJ and Morton D (eds) *Pain: Its Nature and Management in Man and Animals*. Royal Society of Medicine International Congress Symposium Series, Volume 246 pp 17-25. Royal Society of Medicine: London UK

- Broom DM** 2007 Cognitive ability and sentience: which aquatic animals should be protected? *Diseases of Aquatic Organisms* 75: 99-108. <http://dx.doi.org/10.3354/dao075099>
- Carere C, Wood JB and Mather J** 2011 Species differences in captivity: where are the invertebrates? *Trends in Ecology and Evolution* 26: 211. <http://dx.doi.org/10.1016/j.tree.2011.01.003>
- Chandroo KP, Duncan IJH and Moccia RD** 2004 Can fish suffer? Perspectives on sentience, pain, fear and stress. *Applied Animal Behaviour Science* 86: 225-250. <http://dx.doi.org/10.1016/j.applanim.2004.02.004>
- Danbury TC, Weeks CA, Waterman-Pearson AE, Kestin SC and Chambers JP** 2000 Self-selection of the analgesic drug carprofen by lame broiler chickens. *Veterinary Record* 146: 307-311. <http://dx.doi.org/10.1136/vr.146.11.307>
- Denti A, Dimant B and Maldonado H** 1988 Passive avoidance learning in the crab *Chasmagnathus granulatus*. *Physiology and Behavior* 43: 317-320. [http://dx.doi.org/10.1016/0031-9384\(88\)90194-1](http://dx.doi.org/10.1016/0031-9384(88)90194-1)
- Dowds BM and Elwood RW** 1983 Shell wars: assessment strategies and the timing of decisions in hermit crab fights. *Behaviour* 85: 1-24. <http://dx.doi.org/10.1163/156853983X00011>
- Dowds BM and Elwood RW** 1985 Shell wars 2: the influence of relative size on decisions made during hermit crab shell fights. *Animal Behaviour* 33: 649-656. [http://dx.doi.org/10.1016/S0003-3472\(85\)80088-9](http://dx.doi.org/10.1016/S0003-3472(85)80088-9)
- Dunlop R, Millsopp S and Laming P** 2006 Avoidance learning in goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*) and implications for pain perception. *Applied Animal Behaviour Science* 97: 255-271. <http://dx.doi.org/10.1016/j.applanim.2005.06.018>
- Elwood RW** 2011 Pain and suffering in invertebrates. *ILAR Journal* 52: 175-184.
- Elwood RW and Appel M** 2009 Pain in hermit crabs? *Animal Behaviour* 77: 1243-1246. <http://dx.doi.org/10.1016/j.anbehav.2009.01.028>
- Elwood RW and Stewart A** 1985 The timing of decisions during shell investigation by the hermit crab, *Pagurus bernhardus*. *Animal Behaviour* 33: 620-627. [http://dx.doi.org/10.1016/S0003-3472\(85\)80086-5](http://dx.doi.org/10.1016/S0003-3472(85)80086-5)
- Elwood RW, Barr S and Patterson L** 2009 Pain and stress in crustaceans? *Applied Animal Behaviour Science* 118: 128-136. <http://dx.doi.org/10.1016/j.applanim.2009.02.018>
- Elwood RW, McClean A and Webb L** 1979 The development of shell preferences by the hermit crab, *Pagurus bernhardus*. *Animal Behaviour* 27: 940-946. [http://dx.doi.org/10.1016/0003-3472\(79\)90032-0](http://dx.doi.org/10.1016/0003-3472(79)90032-0)
- Hoyle G** 1976 Learning of leg position by the ghost crab *Ocypode ceratophthalma*. *Behavioral Biology* 18: 147-163. [http://dx.doi.org/10.1016/S0091-6773\(76\)92038-1](http://dx.doi.org/10.1016/S0091-6773(76)92038-1)
- IASP (International Association for the Study of Pain)** 1979 Pain terms: a list with definitions and notes on usage. *Pain* 6: 249-252
- Kawai N, Kono R and Sugimoto S** 2004 Avoidance learning in the crayfish (*Procambarus clarkia*) depends on the predatory imminence of the unconditioned stimulus: a behavior systems approach to learning in invertebrates. *Behavioural Brain Research* 150: 229-237
- Kellert RS** 1993 Values and perceptions of invertebrates. *Conservation Biology* 7: 845-855
- Loeser JD and Treede RD** 2008 The Kyoto protocol of IASP basic pain terminology. *Pain* 137: 473-477
- Lüschen W, Willig A, Peter P and Jaros PP** 1993 The role of biogenic amines in the control of blood glucose level in the decapod crustacean, *Carcinus maenas* L. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* 105: 291-296
- Mason GJ** 2011 Invertebrate welfare: where is the real evidence for conscious affective states? *Trends in Ecology and Evolution* 26: 212-213. <http://dx.doi.org/10.1016/j.tree.2011.02.009>
- Millsopp S and Laming P** 2008 Trade-offs between feeding and shock avoidance in goldfish (*Carassius auratus*). *Applied Animal Behaviour Science* 113: 247-254. <http://dx.doi.org/10.1016/j.applanim.2007.11.004>
- Patterson L, Dick JTA and Elwood RW** 2007 Physiological stress responses in the edible crab *Cancer pagurus* to the fishery practice of de-clawing. *Marine Biology* 152: 265-272. <http://dx.doi.org/10.1007/s00227-007-0681-5>
- Patterson L, Dick JTA and Elwood RW** 2009 Claw loss and feeding ability in the edible crab, *Cancer pagurus*: implications of fishery practice. *Applied Animal Behaviour Science* 116: 302-305. <http://dx.doi.org/10.1016/j.applanim.2008.08.007>
- Punzo F** 1983 Localization of brain function and neurochemical correlates of learning in the mud crab, *Eurypanopeus depressus* (Decapoda). *Comparative Biochemistry and Physiology Part A: Physiology* 75: 299-305. [http://dx.doi.org/10.1016/0300-9629\(83\)90085-3](http://dx.doi.org/10.1016/0300-9629(83)90085-3)
- Puri S and Faulkes Z** 2010 Do decapod crustaceans have nociceptors for extreme pH? *PLoS One* 5: e10244. <http://dx.doi.org/10.1371/journal.pone.0010244>
- Rose D** 2002 The neurobehavioral nature of fishes and the question of awareness and pain. *Reviews in Fisheries Science* 10: 1-38. <http://dx.doi.org/10.1080/20026491051668>
- Sherrington C** 1906 *The Integrative Action of the Nervous System*. Oxford University Press: Oxford, UK
- Sherwin CM** 2001 Can invertebrates suffer? Or how robust is argument by analogy? *Animal Welfare* 10: S103-S118
- Shibata M, Ohkubo T, Takahashi H and Inoki R** 1989 Modified formalin test: characteristic biphasic pain response. *Pain* 38: 347-352. [http://dx.doi.org/10.1016/0304-3959\(89\)90222-4](http://dx.doi.org/10.1016/0304-3959(89)90222-4)
- Short CE** 1998 Fundamentals of pain perception in animals. *Applied Animal Behaviour Science* 59: 125-133. [http://dx.doi.org/10.1016/S0168-1591\(98\)00127-0](http://dx.doi.org/10.1016/S0168-1591(98)00127-0)
- Sneddon LU** 2009 Pain perception in fish: Indicators and endpoints. *ILAR Journal* 50: 338-342.
- Sneddon LU, Braithwaite VA and Gentle MJ** 2003a Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proceedings of the Royal Society of London B270*: 1115-1121. <http://dx.doi.org/10.1098/rspb.2003.2349>
- Sneddon LU, Braithwaite VA and Gentle MJ** 2003b Novel object test: examining nociception and fear in the rainbow trout. *Journal of Pain* 4: 431-440. [http://dx.doi.org/10.1067/S1526-5900\(03\)00717-X](http://dx.doi.org/10.1067/S1526-5900(03)00717-X)
- Weary DM, Neil L, Flower FC and Fraser D** 2006 Identifying and preventing pain in animals. *Applied Animal Behaviour Science* 100: 64-76. <http://dx.doi.org/10.1016/j.applanim.2006.04.013>
- Zimmerman M** 1986 Physiological mechanisms of pain and its treatment. *Klinische Anaesthesiologie Intensivtherapie* 32: 1-19