Pain perception in fish: Why critics cannot accept the scientific evidence for fish pain?

Response to Rose et al. 2012 Can fish really feel pain? Fish and Fisheries, in press.

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Rose wrote a review 10 years ago at the request of recreational fishing societies in the USA to address the issue of pain perception by fish (Rose 2002). His review concluded that anglers need not be concerned about the possibility that fishes experience pain during angling procedures because these 'senseless' creatures do not have an identical brain to humans so cannot experience pain consciously. Rose has no track record in investigating animal pain and has not published one empirical study on the topic. Shortly after his review was published I was the first scientist to prove fish had nociceptors, receptors to detect tissue damaging stimuli that would give rise to the sensation of pain in mammals and humans (Sneddon 2002; 2003a). I also demonstrated that the fish's physiology and behaviour were adversely affected over 3 hours after the insult, performing anomalous behaviours not reported previously and that fish stopped feeding until they recovered (Sneddon 2003b). All of the negative changes in behaviour and physiology were dramatically reduced by the use of a painkiller, morphine (Sneddon 2003b). These findings were a very important contribution toward then nascent interest in fish welfare and attracted substantial media and public interest. If these findings had been derived from studies on a mammal, they would be accepted as evidence of pain perception. In Rose and colleagues' latest review (Rose et al. 2012) the same argument is used with no new perspectives: if fish do not have an identical brain to humans then they are not consciously aware and cannot "feel" pain. Accepting this opinion means that no animals (except possibly primates) can experience pain. Rose's premise is that if the pain that an animal experiences is not the same as human pain and they do not possess a human brain then they cannot experience the negative affective component associated with injury. This biased and highly anthropomorphic view of pain provided by Rose and colleagues does give license for the angling and fisheries industries to treat fish without consideration for the welfare of the animal. None of these authors have produced any scientific data to disprove the existence of pain in fish but instead write reviews that only cite favourable references. They have also included a number of incorrect facts in their current review about my published studies. Their review is based upon their personal opinion and is far from exhaustive. It is clear from their public statements this is really the agenda from these authors. There is published scientific evidence for fish and indeed animal pain, although

their pain is not going to be identical to the human experience. However, as humane, ethical, educated beings we must minimise any negative situation into which animals may be placed and seek to reduce any damage that is likely to lead to some sensation of a negative welfare state. Fish and other animals may have a more primitive pain experience, which concurs with the laws of evolution; pain must have arisen in other animal groups, but as humans we have the most highly developed experience of pain. It could be argued that Rose and colleagues have adopted a creationist view of pain by suggesting that pain and consciousness has suddenly arisen only in humans, contradicting the laws of evolution. I address the short-comings and opinions expressed in the Rose et al. (2012) review below.

1. Defining pain in animals

Pain is an important sensory system that alerts an animal or human to potential damage and motivates it to avoid that injurious stimulus or protect itself from further damage. Therefore, it would adaptive to evolve such a system and many "simple" animals possess specific receptors, nociceptors, that detect damaging stimuli e.g. the sea slug *Aplysia*. Pain is not just the perception of the injury but also the negative affective state that accompanies the injury. It is impossible to know how someone feels internally when they are in pain unless they communicate it to you. You empathise as you may have felt the type of pain that they report. This illustrates how difficult it is to measure pain in humans that cannot speak (e.g. neonates) or animals that do not share our language. What we must do is make robust scientific measures of changes in behaviour and physiology after a potentially painful event compared with animals treated in the same way but experiencing no painful treatment (controls) and if these are adversely affected and reduced by administering a painkiller, then one can judge that the experiment was painful to the animal. Rose's argument is that since you cannot measure the subjective state or internal feelings of an animal then they cannot "feel" pain. One cannot directly prove what an animal feels, but equally Rose has ignored the fact you cannot then disprove it and it would be unscientific to do so. Rose and colleagues state that 'pain is a subjective experience that cannot be directly observed' but then goes on to seemingly demand this observation as the only one that will satisfy them.

2. Brain structures and pain

Rose and colleagues claim that only humans and possibly primates can experience pain as only they possess the highly developed neocortical structures necessary for consciousness. This definition supports their stance as no other animals possess such a structure, however, humans with an intact neocortex and thalamocortical connections who are completely conscious cannot feel pain if they have congenital insensitivity to pain with anhidrosis due to mutations in the NTRK1 gene (Lee et al., 2009). Therefore, this gene would seem to be the key to experiencing pain, and indeed defines whether humans perceive pain, and many studies have identified this gene in fish (Zfin, 2010; Catania et al., 2007; Germana et al., 2002; 2004; Vecino et al., 1998). Rose then refers to a number of human "studies" but most of the papers cited are actually reviews and the studies on decerebrate or vegetative humans are based on incredibly small sample sizes which is something Rose suggests is a problem in animal studies. Of course it would unethical to experiment on humans sending them into a vegetative state so all of the studies that actually report on human cases are due to accidental damage and as such are very rare and based on imprecise and uncontrolled damage occurring in patients. Therefore, caution does need to be applied to these studies. However, recent research using brain imaging has demonstrated that vegetative patients (now termed unresponsive wakefulness syndrome) who were previously considered not to feel pain do show equivalent brain activity in the sensory discriminative pain network and the affective pain network (feelings of pain) to normal, healthy humans (Markl et al. 2013). Rose et al. (2012) also states that feelings and emotions are not the same things, and appears to be happy to apply emotions to fish but not feelings. I personally have never used the term "feelings" when discussing fish pain as it is such an ambiguous term given that "feel" can mean a sensory stimulation is perceived or indeed an internal feeling of pain, fear or stress. Alternatively, this can also mean positive emotions such as hunger or thirst that motivate the animal to seek food or drink. Pain can also be thought of an important motivator to protect animals and ensure their survival which is of course adaptive. One of the key factors in attributing consciousness to an animal is being able to recognise itself in a mirror test. Among fish the species tested often react to their own image by attacking it, seeing the reflection as an intruder or competitor. However, we must consider the evolution and ecology of fish – when would they come into contact with their own mirror image? Terrestrial animals would come to water bodies to drink and would see their own reflection but this is precluded by living under water. This difference in ecology would influence whether mirror self recognition would work and explains why fish have not evolved to recognise themselves in this way (Lev-Yadun & Katzir 2012). However, fish can recognise themselves through smell

and considering how fish live in a world where light is filtered out at depth, a reliance on other forms of communication are especially important. Cichlid fish can recognise their own odour distinct from others but also distinct from closely related kin (Thunken et al. 2009). Therefore, this is evidence for self recognition and the ability to discriminate one owns smell from others.

3. The criteria for animal pain

Rose criticises studies published suggesting pain is not clearly defined. In fact, I have written extensively on this subject stating that definitions of human pain are not precise enough and that as scientists we need something that we can measure experimentally. I have updated Bateson's criteria (1992) which provided a number of useful tick boxes to which I have added suspension of normal behaviour over a prolonged period (Sneddon 2004; 2011). These criteria are considered in isolation in Rose's review which is misleading – all of these criteria must be met for an animal to considered capable of pain perception, not just one. Therefore, fish must have nociceptors, processing of potentially painful information in higher brain areas and not just reflex centres in the spinal cord or hindbrain; have neural pathways from the nociceptors on the body and head to these higher brain areas; possess opioid receptors and endogenous opioid substances within the nervous system; painkilling or analgesic drugs should reduce any adverse responses to a potentially painful event; the animal should learn to avoid a potentially painful stimulus and this should be so important to the animal it should occur within one to a few trials of training; and finally that normal behaviour should be adversely affected and that this should not be an instantaneous withdrawal response - in fish (depending upon the potentially painful event) the behaviour can be negatively affected for up to 6 hours. Fish comply with all of these criteria (Sneddon 2011). None of these variables have been measured in isolation as Rose and colleagues mistakenly suggest. Together the results from robust scientific studies provide reliable evidence that fish fulfil the collective requirements for animal pain.

4. Errors in the Rose et al. review

Rose's critique of studies on pain in fish does not provide the full details but rather he and his colleagues only report isolated parts and some are, alarmingly, incorrect. For example, they state fish injected with acetic acid in the frontal lips, a standard pain test in

mammals and humans, ate within 3 hours – in fact they took on average three hours, whereas fish which were just handled and those injected with saline (non-painful) resume within 80 minutes. It takes humans approximately 3 hours to recover and stop feeling the pain of the acetic acid test (Sneddon et al. 2003). The authors then suggest that if there was a substantial stress response the fish would not have fed at all, however, this confirms that the fish were responding to the pain test and it was not just a reaction to stress. These fish resumed feeding when their physiology and behaviour returned to normal.

Rose and colleagues do not convey the established fact that different types of pain cause different reactions via their different mode of activating nociceptors. For example, if one gets vinegar (acetic acid) in a cut this feels like an acute sharp pain, however, capsaicin from hot chillis feels like a burning sensation. Both elicit different sensory experiences, so it is hardly surprising that when trout were given venom, an agent that causes inflammation and itch, they behaved differently to acid stimulation, an irritant which directly excites nociceptive nerve endings. Rose et al. (2012) suggests that the acid and venom have a toxic effect on the gills and produce the increased ventilation rate which is much greater than that of a stress response or of that seen in the control groups. The flaw in this argument is that if it was a toxic effect painkillers such as morphine (Sneddon 2003b), lidocaine and carprofen (Mettam et al. 2011) would not reduce this adverse change in physiology. It is clear that these drugs reduce the impact of the potentially painful stimulation and one sees a reduction in ventilation rate, fish are quicker to resume feeding and the suspension in normal swimming activity is ameliorated.

Rose and colleagues criticise one study which uses a range of doses of drugs with the aim of finding the most effective and fail to see that lower doses may have a reduced efficacy in improving recovery which is a baffling criticism. Rose et al. (2012) then compares one study to a repeat attempt by Newby and Stevens (2008) and states that conditions were identical including tank design – this is completely incorrect. I provided a critique on the Newby and Stevens study (Sneddon 2009) which in fact used cylindrical flow through tanks with no bottom for the fish to rest upon and no gravel substrate - how could the fish therefore perform the “rocking” on the bottom of the tank or rub the lips against the sides of the tanks or gravel substrate as was demonstrated by my laboratory’s results? Newby and Stevens did not use anaesthesia when injecting their fish with acid which would be an illegal experimental procedure in many countries. The stress of being injected with a noxious

stimulus using forcible restraint coupled with being held in a barren environment in the Newby and Stevens (2008) study would lead to high cortisol levels as demonstrated by the loss of equilibrium in the noxiously stimulated fish in their study. This most likely led to stress-induced analgesia since high cortisol results in the release of beta endorphin in fish (van den Burg et al., 2005) and, therefore, no suspension in feeding or performance of pain-related behaviours was observed as pain would be reduced centrally by endorphins which act as painkillers within the mammalian nervous system. Newby and Stevens also used a very high concentration of acid (above 5%) and electrophysiological studies within my laboratory demonstrate that in trout, concentrations above 2% do indeed destroy nociceptor activity so no information is conveyed to the brain which may explain some of their results. Rose and colleagues extrapolate from a salmonid, the rainbow trout, where 2% is the maximum acetic acid concentration as evidenced by extensive electrophysiological studies (Sneddon 2002; 2003a; Ashley et al. 2007; 2008; Mettam et al. 2012) to cyprinid fish, zebrafish and common carp – I have never conducted electrophysiology on either of these species and so do not know what concentrations of 2% and above do, however, our behavioural studies have shown that the cyprinids are more robust and possibly have a higher pain threshold. Pain thresholds differ between species of mammals as well as responses to pain and indeed pain thresholds differ even within species and certainly between humans.

These authors also claim that anaesthesia affects behaviour, yet it's clearly laid out in previous studies that sham handled animals that receive no injection show identical behavioural and physiological responses to fish injected with saline (Sneddon et al. 2003). Thus, they should be aware there is no difference between handled and anaesthestised fish and those fish that also receive a saline injection. From an ethical perspective, we must implement the ethos of replacement, reduction and refinement (the 3Rs) to reduce the numbers of animals we use in experiments, therefore, why repeat sham handled animals when we have proved that they are identical to saline injected fish? The authors criticise the anomalous behaviours of rocking and rubbing, yet are surprised they have not been included in a recent study published which tested analgesic drugs in trout. Perhaps they have missed the point of this study, which was to use robust measures of potential pain for the animal carer or researcher to use rather than subtle behaviours they may not be familiar with. Swimming, feeding and opercular (gill) beat rate are easily identifiable and simple to record. Overall, the studies these authors draw attention to would be perfectly acceptable as evidence for sensory pain perception in mammals and given their errors in critiquing these, they appear

to be massaging the details to suit their opinion. It is interesting to note that one of the authors, Stevens, has published on analgesia in fish and indeed one of his studies states "morphine acted as an analgesic when administered via the water as demonstrated by significantly decreased rubbing behaviour in response to the presence of a noxious stimulus (subcutaneous injection of 0.7% acetic acid)", yet this finding is inexplicably not discussed in this review (Newby et al. 2009; Correia et al. 2011).

5. Differences between animals and humans

The authors of this review then seek to criticise studies from several laboratories who have employed well established, published neurobiological techniques and shown that potentially painful stimuli result in electrical activity in the higher brain areas in fish. These techniques have been employed in mammalian studies without criticism. Neuroanatomical studies do show that both A-delta and C fibres are present in rainbow trout (Sneddon 2002; 2003a) and common carp (Roques et al. 2010), structures that act as nociceptive neurons in mammals. Rose states that Sneddon (2002; 2003; 2004) does not address the differences between mammals and fish in terms of percentage fibre composition. Fish have fewer C fibres than mammals and C fibres are proposed to transmit longer term nociceptive signals after damage and are important in sensory pain. However, after careful reading, they would note that suggest evolutionary reasons for the disparity are provided. Fish live in an aqueous world, therefore, there will be a difference in how damage occurs to fish compared with terrestrial animals. Buoyancy of fish in water means less damage due to gravity (falling), noxious chemicals may be more diluted in aquatic water bodies and changes in temperature are less dramatic compared with terrestrial environments thus pain from gravity, extremes of temperature and noxious chemicals may be experienced to a greater degree by terrestrial animals. This is just a hypothesis, but irrespective of this my electrophysiological studies show clearly that trout A-delta fibres act in the same way as mammalian C fibres reacting to a variety of noxious stimulus. The authors' argument is anthropomorphic again that the fish nociceptive system should be identical to the human system, however, A-delta fibres conduct at a faster speed so perhaps the fish system is faster and more efficient. Rather than taking Rose's anthropocentric view it is incredibly important to avoid placing human needs onto an animal that has a completely different life history, experiences different environmental demands, and has been subject to entirely distinct evolutionary pressures that has shaped its nervous system. We cannot expect animals to be completely similar to us, and indeed studies

on the brain of birds, for example, have shown that animals evolve quite different neural structures to perform the same function (Jarvis et al. 2005). Animals have gone down a different evolutionary path and fish are one of the most successful animal groups. Given Rose and colleagues appear to hold creationist views it is likely they will not accept evolution as a viable or valid explanation for fish evolving a nociceptive system that is not identical to that seen in humans, however, these empirical studies actually demonstrate that it is very similar.

6. The use of fish

Rose and colleagues proceed to discuss contexts where fish may sustain injury and again highlight information from studies that support their views but overlook many that show the converse. For example, they mention that tagging has no impact on behaviour and physiology but the measurements are taken 3 weeks after the tagging event so the fish have most likely healed and recovered (Wagner & Stevens 2000). In the case of catch and release angling, they rely heavily on unpublished data from one of the authors that has not been subject to peer review and this author works for a fishing company that catches, tags and releases fish so these data have not been obtained from a wholly independent source. Indeed the authors' agenda becomes clear when they suggest that improving the way fish are treated could affect fish industries such that enhancing welfare will have a detrimental economic impact. There are also studies not cited that demonstrate behaviour and physiology are adversely affected by fishing (review in Cooke & Sneddon 2007; Norwegian Food Safety Agency (2010) *Risk Assessment of Catch and Release*) but these are surprisingly omitted. Rose has publicly stated that anglers have been "stigmatised" but to my knowledge animal welfare scientists who believe we have the right to use animals but should do so humanely have actually promoted improved fishing practices. Indeed I have produced a set of recommendations with one of the Rose's co-authors, Cooke, who in 2007 agreed that there was compelling evidence that fish perceive pain and it that it is important to improve their welfare in catch and release fishing (Cooke & Sneddon 2007). I worked with other eminent authors on guidelines to improve this fishing practice for the Norwegian Food Safety Agency (2010) to improve the welfare of fish.

There are many examples of farmed animals where consumers are willing to pay more for better welfare, for example, eggs from free range chickens. This also applies to fish where consumers are now demanding to know where the fish are caught from and the method

used. Regulations are based upon published scientific studies that do provide convincing evidence that fish experience pain, fear and stress and as responsible, moral beings we have to ensure the wellbeing of the animals we use. Lack of consideration for fish has led to unsustainable fishing practices and populations crashes of important species (Sneddon & Wolfenden 2012) as well as death of non-target animals such as birds, cetaceans, turtles and so on in fishing apparatus. Surely it is better to be proactive and ensure the wellbeing of fish for improved economic return in aquaculture, fisheries and ornamental fish trade. More importantly, these authors suggest that we should have free reign on experimental procedures without any thought for the impact on the animal. Not only is the idea completely irresponsible, but studies have clearly shown that when welfare of experimental animals is considered and humane treatment is applied, there is less variation in the scientific data making the results more robust, valid and reliable. It is not surprising these authors take an archaic view of fish welfare since in a recent review they misguidedly suggest that it is acceptable to treat wild fish in any way and have little or no regard for their wellbeing as we should consider ourselves as predators (Diggles et al. 2011). However, natural predators only kill to satiate their hunger and stop once satisfied. They do not kill many other non-target animals in the process of killing the fish that they consume and they do not massively disrupt the environment when doing so. To deliberately cause injury and suffering is unethical and as moral beings we have a duty of care to animals that we place in the completely unnatural environment of fishing equipment. Finally, Rose et al. (2012) concludes that fish welfare is important. This could be considered hypocritical since they have failed to understand the basic premise of welfare that animals must suffer from negative states in order to have poor welfare.

7. Conclusion

I do applaud the authors for highlighting an important area of science that funding bodies should make a real priority of. More science needs to be funded to investigate pain in fish so that government and public regulations can be informed by rigorous science rather than opinionated reviews. I do hope that some of the scientific community will continue to formulate hypotheses after the results are known (HARKing) since many of our major scientific discoveries have been made in this way. For example, cloning of Dolly the sheep, discovery of major drugs such as penicillin and Viagra and the utility of X-rays.

I am rather surprised that I was not given the opportunity to review this publication by Rose et al (2012) before it was published. I could have pointed out the many errors and

omission of important references to provide a more balanced text. As scientists, many of us are funded by public money and it is important that we take an unbiased view presenting our results and allowing the public to make up their own minds rather than trying to force through a personal agenda. I did request that I be allowed to write a response but the editorial board have refused, suggesting that the best thing I can do is to continue my research and promote improvements in the way we treat fish.

References

Ashley, P.J., Sneddon, L.U. and McCrohan, C.R. (2006) Properties of corneal receptors in a teleost fish. Neuroscience Letters 41, 165–168.

Ashley, P.J., Sneddon, L.U. and McCrohan, C.R. (2007) Nociception in fish: stimulus-response properties of receptors on the head of trout *Oncorhynchus mykiss*. Brain Research 116, 47–54.

Catania, S., Germana, A., Cabo, R., Ochoa-Erena, F.J., Guerrera, M.C., Hannestad, J., Represa, J. & Vega, J.A. (2007) Neurotrophin and trk neurotrophin receptors in the inner ear of *Salmo salar* and *Salmo trutta*, Journal of Anatomy, 210, 78–88.

Cooke, S.J. and Sneddon, L.U. (2007) Animal welfare perspectives on recreational angling. Applied Animal Behaviour Science 104, 176–198.

Correia, A.D., Cunha, S.R., Scholze, M. and Stevens, E.D. (2011) A novel behavioral fish model of nociception for testing analgesics. Pharmaceuticals 2011, 665-680.

Diggles, B.K., Cooke, S.J., Rose, J.D. and Sawynok, W. (2011) Ecology and welfare of aquatic animals in wild capture fisheries. Reviews in Fish Biology and Fisheries 21, 739–765.

Germana, A., Catania, S., Cavallaro, M., Gonzalez-Martinez, T., Ciriaco, E., Hannestad, J. & Vega, J.A. (2002) Immunohistochemical localization of bdnf-, trkb- and trka-like proteins in the teleost lateral line system, Journal of Anatomy, 200, pp. 477–485.

Jarvis, E.B. et al. (2005) Avian brains and a new understanding of vertebrate brain evolution. Nature Reviews Neuroscience 6, 151-159.

Lee, S.T., Lee, J., Lee, M., Kim, J.W. &Ki, C.S. (2009) Clinical and genetic analysis of Korean patients with congenital insensitivity to pain with anhidrosis, Muscle & Nerve, 40, 855–859.

Lev-Yadun S. & Katzir G., (2012) Mirror images: Fish versus terrestrial animals. J. Theoret. Biol. 182-184.

Markl A, Yu T., Vogel D., Muller F., Kotchoubey B. & Lang S. (2013) Brain processing of pain in patients with unresponsive wakefulness syndrome. Brain and Behaviour, online DOI: 10.1002/brb3.110

Mettam, J.J., Oulton, L.J., McCrohan, C.R. and Sneddon, L.U. (2011) The efficacy of three types of analgesic drugs in reducing pain in the rainbow trout, *Oncorhynchus mykiss*. Applied Animal Behaviour Science 133, 265–274.

Mettam, J.J., McCrohan, C.R. & Sneddon, L.U. (2012) Characterisation of chemosensory trigeminal receptors in the rainbow trout, *Oncorhynchus mykiss*: responses to chemical irritants and carbon dioxide. Journal of Experimental Biology, 215, 685-693.

Newby, N.C. and Stevens, E.D. (2008) The effects of the acetic acid "pain" test on feeding, swimming and respiratory responses of rainbow trout (*Oncorhynchus mykiss*). Applied Animal Behavior Science 114, 260–269.

Newby, N.C., Wilkie, M.P. and Stevens, E.D. (2009) Morphine uptake, disposition and analgesic efficacy in the common goldfish (*Carassius auratus*). Canadian Journal of Zoology 87, 388–399.

Norwegian Food Safety Agency (2010) http://english.vkm.no/dav/42f495efaf.pdf

Roques, J.A.C., Abbink, W., Geurds, F., van de Vis, H. and Flik, G. (2010) Tailfin clipping, a painful procedure: studies on Nile tilapia and common carp. Physiology and Behavior 101, 533–540.

Rose, J.D. (2002) The neurobehavioral nature of fishes and the question of awareness and pain. Reviews in Fisheries Science 10, 1–38.

Rose, J.D, Arlinghaus, R., Cooke, S.J., Diggles,B.K., Sawynok, W., Stevens, E.D and Wynne C.D.L. (2012) Can fish really feel pain? Fish and Fisheries, in press. DOI: 10.1111/faf.12010

Sneddon, L.U. (2002) Anatomical electrophysiological analysis of the trigeminal nerve of the rainbow trout, *Oncorhynchus mykiss*. Neuroscience Letters 312, 167–171.

Sneddon, L.U. (2003a) Trigeminal somatosensory innervations of the head of a teleost fish with particular reference to nociception. Brain Research 972, 44–52.

Sneddon, L.U. (2003b) The evidence for pain in fish. Use of morphine as an anaesthetic. Applied Animal Behaviour Science 83, 153–162.

Sneddon, L.U. (2004) Evolution of nociception in vertebrates: comparative analysis of lower vertebrates. Brain Research Reviews 46, 123–130.

Sneddon, L.U. (2009) The effects of the acetic acid "pain" test on feeding, swimming, and respiratory responses of rainbow trout (Oncorhynchus mykiss): a critique on Newby and Stevens (2008). Applied Animal Behaviour Science 116, 96–99.

Sneddon, L.U. (2011) Pain perception in fish. Evidence and implications for the use of fish. Journal of Consciousness Studies 18, 209–229.

Sneddon, L.U., Braithwaite, V.A. and Gentle, M.J. (2003) Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. Proceedings of the Royal Society of London B Biological Sciences 270, 1115–1121.

Sneddon L.U. & Wolfenden D.C.C. (2012) How do large-scale fisheries affect fish: Pain perception in fish? In Sea The Truth: Essays on Overfishing, Climate Change and Pollution, Nicolaas G. Pierson Foundation.

Thunken, T., Waltschyk, N., Bakker, T. C. M. & Kullmann, H. (2009) Olfactory selfrecognition in a cichlid fish. Animal Cognition, 12, 717-724.

van den Burg EH, Peeters RR, Verhoye M, Meek J, Flik G, Van der Linden A (2005) Brain Responses to Ambient Temperature Fluctuations in Fish: Reduction of Blood Volume and Initiation of a Whole-Body Stress Response. J Neurophysiol 93:2849-2855.

Vecino, E., Caminos, E., Becker, E., Rudkin, B.B., Evan, G.I. & Martin-Zanca, D. (1998) Increased levels of trka in the regenerating retinal ganglion cells of fish, NeuroReport, 9, 3409–3413.

Wagner, G.N. and Stevens, E.D. (2000) Effects of different surgical techniques: suture material and location of incision site on the behaviour of rainbow trout. Marine and Freshwater Behaviour and Physiology 33, 103–114.

Zfin (2010) [Online], http://zfin.org/action/marker/view/ZDB-GENE-980526-118

[28 August 2012].