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Letter to the Editor - Applied Animal Behaviour Science

*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)*

Newby and Stevens' (2008) paper "The effects of the acetic acid 'pain' test on feeding, swimming, and respiratory responses of rainbow trout (*Oncorhynchus mykiss*)" examines the effects of a noxious stimulus on the behaviour of trout in an attempt to replicate research conducted in my laboratory (Sneddon, 2003a; Sneddon, 2007; Sneddon et al., 2003a,b; Reilly et al., 2008). However, the authors used a different protocol to the one already published and I would like to respond to some of their points of discussion to provide scientific explanations for their results using data from my laboratory. The authors show that swimming behaviour and time to resume feeding are not impaired by administering 2% and 5% acetic acid subcutaneously but the fish do show the classic rise in respiration rate that has already been published. Recent electrophysiological investigations from my group have demonstrated that applying 2% acetic acid topically destroys nociceptor output and the neuron effectively dies (Ashley et al., 2006, 2007). Therefore, the lack of anomalous rubbing behaviours and resumption of feeding in the Newby and Stevens (2008) experiment can be attributed to them injecting such a high concentration of acid. If no nociceptive information is being conducted to the central nervous system then no behavioural changes will be elicited. This demonstrates the importance of following the experimental design of published studies to get comparable results (Sneddon, 2003a; Sneddon, 2007; Sneddon et al., 2003a,b; Reilly et al., 2008).

The authors also used a completely different housing design where fish were held in barren, cylindrical tanks rather than standard, rectangular tanks with gravel. This may preclude the ability to perform behaviours such as rocking, where the fish is situated on a gravel substrate and rocks to and fro on either pectoral fin (Sneddon et al., 2003a), and rubbing where the fish rubs the injection site into the gravel and sides of tanks (Sneddon, 2003a; Sneddon et al., 2003a; Reilly et al., 2008). If the tanks are cylindrical without flat surfaces or gravel, there is no substrate for the animals to perform these behaviours. Our research has also demonstrated that rainbow trout do not perform anomalous behaviours or exhibit such high physiological alterations in a barren environment as they do in an enriched environment. This data was presented at the International Fish Biology Conference (2006), the International Applied Animal Behaviour Conference (2006) and at the Society for Experimental Biology Meeting (2007; Sneddon, 2007). The authors, Newby and Stevens, were present during the 2006 spoken presentation at the International Fish Biology Conference in St. John's, Canada and discussed the results with us. Therefore, they are well aware that these behaviours are not performed in a barren tank set up. We have shown that barren conditions are more stressful for rainbow trout and result in higher levels of plasma cortisol. These stressful conditions result in increased preopiomelanocortin (POMC) in the brain of these noxiously stimulated trout but not in saline injected control fish (Sneddon et al., submitted for publication). POMC is the precursor to beta-endorphin and the enkephalins which act as natural painkillers within the nervous system of vertebrates in a mechanism called stress-induced analgesia (McNally, 1999). Therefore, 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 (Arends et al., 1999) as demonstrated by the loss of equilibrium in the noxiously stimulated fish. 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. Again, it is vital to follow established experimental protocol to obtain similar results (Sneddon, 2003a; Sneddon, 2007; Sneddon et al., 2003a,b; Reilly et al., 2008).

The Newby and Stevens' (2008) study raises a very serious ethical point about the treatment of animals in pain experimentation. In their protocol, they restrain trout without anaesthesia and insert a hypodermic needle into a very sensitive area of skin innervated by nociceptors (Sneddon, 2002, 2003b; Ashley et al., 2006) and administer a high concentration of damaging substance. I believe that this is not a humane way to administer such a noxious substance given that fish are aquatic animals and removing them from the water is effectively suffocating them causing significant stress (Arends et al., 1999). The ethical guidelines followed by this journal state that "Procedures with animals that may cause more than momentary or minimal pain or distress should be performed with appropriate sedation, analgesia, or anesthesia in accordance with accepted veterinary practice. Surgical or other painful procedures should not be performed on unanesthetized animals" (http://www.cioms.ch/frame_1985_texts_of_guidelines.htm). I believe that these guidelines should be adopted in future studies concerning pain in aquatic animals and that appropriate anaesthesia should be used when administering noxious stimuli.

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