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Tailfin clipping, a painful procedure: Studies on Nile tilapia and common carp

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ABSTRACT

The fish welfare debate is intensifying. Consequently, more research is carried out to further our knowledge on fish welfare in aquaculture. We define here a series of key parameters to substantiate an acute response to a supposedly painful stimulus: a standardized tailfin clip.
Ultrastructural analysis of common carp (*Cyprinus carpio*) tailfin indicates the presence of A-δ and C-type axons, which are typical for transmitting nociceptive signals in (higher) vertebrates. In Nile tilapia (*Oreochromis niloticus*), responses to a tailfin clip were studied and the unavoidable acute stress associated with the handling required for this procedure. A series of key parameters for further studies was defined. The responses seen in 'classical' stress parameters (e.g., changes in plasma cortisol, glucose and lactate levels) did not allow discrimination between the clipping procedure and the handling stress. However, three parameters indicated a differential, stronger response to the clip stimulus itself: first, swimming activity increased more and clipped fish spent more time in the light (in a tank where half the volume is covered by dark material); second, the gill's mucus cells released their content as observed 1 h after the clip, and this response is transient (no longer observed at 6 h post clipping). Third, branchial Na^+/K^+-ATPase activity assayed *in vitro* was not affected by the procedures, but a remarkable migration of Na^+/K^+-ATPase immunoreactive (chloride) cells into the lamellar epithelium was observed as of 6 h post clipping. We conclude that the differential response to clipping supports that this is a painful procedure that evokes a transient specific physiological status.

1. Introduction

In humans, awareness of pain, fear and stress depends on functions controlled and executed by the highly developed hippocampus, amygdala, and cerebral frontal lobes and neocortex [1]. In fish, the telencephalon, which will evolve to these cerebral structures in higher vertebrates, is far less complex and anatomically and fundamentally different, which has led many to conclude that fish cannot experience pain, fear or stress [2,3]. One of the endeavours in research on fish welfare is the assessment of consciousness which is at the basis of pain and fear experience. There is ample evidence to conclude that fish experience stress and successfully mount behavioural and neuroendocrinological responses to cope with stress [4].

Reviews by Braithwaite and Huntingford [5] and Chandroo et al., [6] present convincing evidence that fish, despite their less developed telencephalon, have learning abilities at a level that implies cognitive abilities. For some species (rainbow trout *Oncorhynchus mykiss*, Atlantic cod *Gadus morhua*, common goldfish *Carassius auratus,* and Atlantic salmon *Salmo salar*), the first evidence has been advanced that fish may have the capacity to perceive painful stimuli and have a nervous substrate to experience fear and to suffer [7–9]. However, it has to be emphasized that it is unlikely that fish, as well as other animals, except maybe higher primates, have the capacity to experience suffering as human do [5]. Nociception, the detection of potentially harmful stimuli, is at the very basis of experiencing pain, i.e., interpreting the nociceptive stimulus. Pain perception thus involves both the nociceptive sensory machinery and the actual translation of harmful stimuli to the feeling of pain. Fish should possess then both a nociceptive system and some cognitive capacities to experience pain in a human sense. Indeed, a limited, yet firm, literature supports that fish detect harmful stimuli, respond to nociceptive stimuli and may conceptualize pain [5–7,10–12].

Next to the feeling of pain, fear and stress are motivational affective states that are relevant to fish welfare. In their seminal reviews Braithwaite and Huntingford [5], and Chandroo et al., [6] conclude that these affective states may well be attributed to fish. Recently, Nilsson et al., [8] demonstrated explicit memory in Atlantic cod and, therefore, it is reasonable to hypothesize that fish indeed have capacities to have some form of consciousness and be aware of pain.

Studies that deal with the welfare of fish are limited to only a few out of an estimated total of 35,000 species; indeed, the knowledge on fish can only be called fragmentary. Beyond natural variation, human influences on fish, e.g., through prolonged farming and domestication,

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may impinge on welfare-related aspects such as aquaculture-related stress physiology [13]. Clearly, big gaps in the knowledge on fish welfare exist. Nevertheless, the current literature suggests that fish deserve a better moral consideration than they have received so far [14].

The international association for the study of pain (IASP) defined pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [15]. Although pain has a subjective component that is difficult to convey without words, a non-verbal individual can still experience pain and benefit from pain-relieving treatment. In humans, methods to assess and quantify pain focus on cognitive abilities and subjective feelings. In studies on other mammals, emphasis is put on physiological parameters and behavioural activity, with little interest in the cognitive abilities and subjective feelings as is done for humans. However, few of these methods have been applied to demonstrate or quantify painful stimuli in fishes. A complicating factor in pain research is that the application of painful stimuli goes with an inherent stress response, for instance to handling (e.g., when blood is sampled) that interferes with the response to the fin clip. It is difficult to distinguish between stress responses and mild pain responses as these responses share a larger part of the stress physiology.

In this study, behavioural and stress-endocrine responses of the Nile tilapia (*Oreochromis niloticus)* to a presumed pain stimulus (tailfin clip) were investigated. In common carp (*Cyprinus carpio*), the clipped tissue was investigated at the ultrastructural level to identify nerve fibres classified in mammals and rainbow trout as pain fibres. Swimming activity was monitored and the fish's preference to reside in the lightened or darkened section of a compartmented aquarium. The stress parameters plasma cortisol, glucose and lactate, were measured. Parameters for osmoregulatory performance including Na^+/K^+-ATPase enzymic activity and chloride cell abundance and position in gills and plasma concentrations of Na^+, K^+ and Ca^{2+} were determined. In addition, mucus content of mucus cells in the gills was quantified.

This study was designed to discriminate the acute stress response inherent to the application of a fin clip as presumed pain stimulus from the fin clip proper through inclusion of the appropriate controls, and to select key parameters for future studies into this field of research.

Peripheral nerve fibres are categorized according to their diameter, conduction velocity and degree of myelinisation as A-α, A-β, A-γ, A-δ B- and C-fibres [16]. The A-fibres are myelinated for fast conduction of action potentials. The A-δ fibres are involved in the transmission of well-localized acute pain, while C-fibres lack a myelin sheet (are very simply isolated by glia) and therefore slowly conduct action potentials and involved in poorly localized unpleasant slow dull pain [7,13,17]. Fibres conducting in the velocity range of A-δ and C-fibres were identified in the trigeminal nerve of the rainbow trout and characterized as nociceptive fibres by Sneddon [7]. A-δ fibres (25%) were predominant over C-fibres (4%), displaying a different pattern compared with other vertebrates, where C-fibres can comprise from 50% (cat, human) up to 65% (frog) of the total fibre type [18].This difference in fibres composition is attributed to the water-to-land transition in vertebrate evolution [7].

A tailfin clip was chosen as pain stimulus; all the handling around the clipping procedure, but omitting the clip, served as control procedure to quantify the handling stress. Fins are vulnerable body parts that are easily damaged as a result of aggressive behavior between fishes or of aquaculture practices, such as sorting and transport.

2. Materials and methods

2.1. Ultrastructural analysis of common carp (Cyprinus carpio) tailfin

2.1.1. Nerve bundles

Tailfin clips of common carp were immersed in glutaraldehyde (2.5% v/v), $K_2Cr_2O_7$ (1% w/v) and OsO_4 (1% w/v) in 0.15 M cacodylic acid (pH 7.5) and embedded in Spurr's resin. Ultrathin sections (70–90 nm) were cut with an ultratome and mounted on square mesh nickel grids. On-grid sections were post-stained for 2 min with uranyl acetate and then lead citrate for 2 min and rinsed thrice with doubly distilled water. Nerve fibre types in cross sections were categorized based on diameter and the presence of myelin to distinguish Aα-, Aβ-, A-δ and C-fibres [7,17] (Table 1).

2.2. Responses of Nile tilapia (O. niloticus) to a tailfin clip

2.2.1. Fish

Female Nile tilapia, weighing around 200 g, were obtained from a local fish farm (Fishion Aquaculture BV, Mortel, The Netherlands) and after transport to the laboratory acclimatized for 2 weeks to the aquarium facilities of the Radboud University Nijmegen. The fish were kept in 140 l flow-through tanks with nine fishes per tank; the fish received pellet feed at 2% of the total body weight daily (Trouvit, Trouw, The Netherlands). The water quality was monitored for nitrogenous waste products weekly ($NO_2^- = 0.5$ mg/l; $NO_3 = 12.5$ mg/l; $NH_4^+ = 0.5$ mg/l; $O_2 = 7.0$ mg/l). Water pH (7.5 ± 0.2) and water temperature (25 ± 0.2 °C) were continuously monitored; the light regime was 12 h light: 12 h dark. The study was approved beforehand by the Animal Experimental Committee of Lelystad (Protocol: 2008139).

2.2.2. Fin clipping

Fish were caught with a net and restrained manually by one experimentator, while another clipped the caudoventral corner of the tailfin with a sharp, sterile pair of dissection scissors; next, the fish were returned to their original tank. In the control for handling stress treatment, fishes were handled the same way but not given the clip (instead gentle pressure was applied at the area the fin clip was provided to the other group).

2.2.3. Experimental set

Eight groups of nine fish were used (Table 2). Two control groups were sampled one day prior the treatments of the six experimental groups. The results of the two control groups were pooled, since no differences were found between these fish. Clipped and control for handling stress groups were sacrificed at 1, 6 and 24 h after the clip procedure. Fish were not fed 24 h before sampling.

2.2.4. Sampling

The fish were rapidly netted and deeply anaesthetized with 2-phenoxyethanol (1 ml/l; Sigma-Aldrich, St Louis, USA); this procedure took less than 2 min. Blood sampled by puncture of the caudal vessels with a heparinized syringe fitted with a 25 Gauge needle was immediately centrifuged at 4 °C and 13,000 rpm for 10 min to separate plasma and cells; plasma was snap-frozen and stored at −20 °C.

Two gill arches were excised and stored in SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole; pH 7.4) for later determination of Na^+/K^+-ATPase enzymatic activity or fixed in Bouin's fixative (15 volumes saturated picric acid: 5 volumes formaldehyde: 1 volume glacial acetic acid) for mucus cell and chloride cell histology.

2.2.5. Dark-light preference and swimming activity

Tanks were covered with black plastic to make 50% of the volume dark and 50% illuminated. The preference to reside in the light or dark and general swimming activity of the fish was determined by snapshots through undisturbed camera-viewing of the tanks in the week before the experiment (control) and after administration of the fin clips, prior to sampling. The fish were scored for presence in the dark or light part of the tank. Data are expressed as ratio of fish present in (as a group) in the light versus the dark. A score of 1.0 indicated that the fish were equally divided over the light and dark part of the tank. Control situation was assessed 1 h for 3 days prior the