

# Aspects of fish welfare in aquaculture practices

JAC Roques<sup>1,2</sup>, W Abbink<sup>1</sup>, E Schram<sup>1</sup>, H van de Vis<sup>1</sup>, G Flik<sup>2</sup>

## Introduction, aim

- Interest in welfare of farmed animals, including fish, is increasing
- Aim: increase our knowledge on welfare of farmed fish through the study of acute and chronic welfare impairments:
  - > **acute**: pain stimulus e.g. by fin clipping, electric shock
  - > **chronic**: prolonged exposure to nitrogenous waste compounds
- Parameters investigated: (stress) physiology; behavior, growth
- Studies on: common carp, tilapia subsp., A. catfish, pike perch, zebrafish

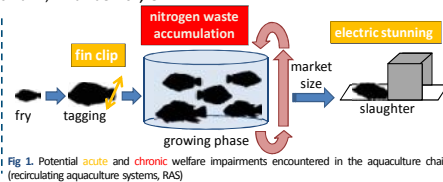


Fig 1. Potential acute and chronic welfare impairments encountered in the aquaculture chain (recirculating aquaculture systems, RAS)

## I) Acute welfare impairment: pain and nociception in fish

- Search for nerve fibers involved in nociception and pain perception
- Study of two standardized stimuli, potentially (mildly) painful in fish
- Physiological and behavioral responses investigated during 24 h
- For each time point, a control for handling stress was included
- Follow-up study: effect of tailfin clip on habituation in zebrafish, using the novel tank paradigm

### Results: Gill histology and physiology

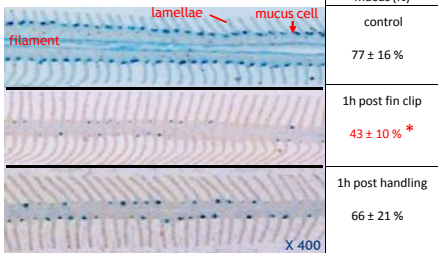


Fig 3. Quantification of branchial mucus cell frequency in Nile tilapia. Only the 1 h post-fin clip group showed significant a decrease of filled mucus cells (\*)

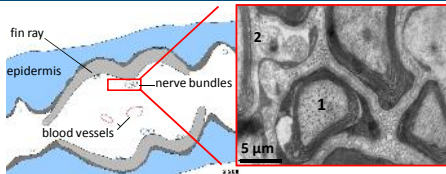


Fig 2. Nerve fibers in the tailfin of common carp. Presence of myelinated A-δ (mediating acute pain, 1) and unmyelinated C-fibers (mediating chronic pain, 2)

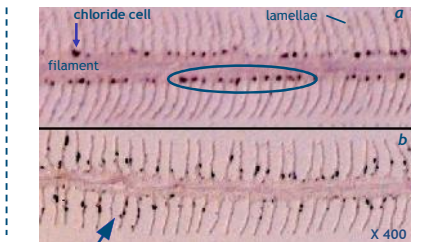


Fig 4. Branchial chloride cells, control situation (a). Lamellar migration (blue arrow) occurs in both fin clipped and handled groups from 6 h onwards (b)

### Physiological parameters

- Plasma cortisol, glucose, lactate, osmolality and branchial Na<sup>+</sup>/K<sup>+</sup>-ATPase activity were similarly affected by the painful stimulus and the handling procedure
- Tailfin clip and the electric shock resulted in differential responses regarding readout parameters and in time

### Behavior

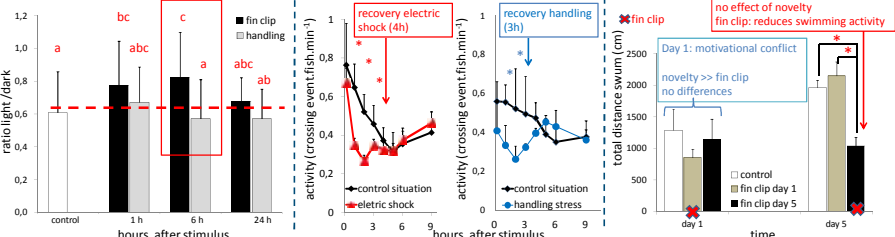


Fig 5. Scototaxis (dark/light preference) of Nile tilapia as function of treatment (fin clip vs. handling). Clipped fish are less present in the dark and more active. Fig 6. Activity of Mozambique tilapia, as function of time, for the handled group. Recovery is faster and more active. Fig 7. Distance swum by zebrafish in the novel tank test (6 min), at day 1 and day 5, for fish receiving no fin clip, fin clip on day 1 or on day 5. Presence of a motivational conflict on day 1, significant effect of fin clip on day 5 (\*)

### Conclusions and perspectives:

- Nociceptive A-δ and C-fibers in the tailfin together with behavioral and branchial responses show that both stimuli may be perceived as painful
- The results substantiate a differential response to a presumed painful stimulus compared to that of handling treatments
- The nature of the stimuli can explain the differences between them; fin clip: tissue-damage, similar to common injury encountered; electric shock: never encountered before, may elicit a deeper, longer lasting reaction
- Studies on brain activity *in vitro* (C-fos expression) and *in vivo* (fMRI) following a fin clip to show that the nociceptive evokes pain sensation

## II) Chronic welfare impairment: exposure to nitrogenous waste products

- All nitrogenous waste (NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>) are more or less toxic to fish
- Effects range from physiological disturbances → reduced growth → mortality
- Chronic exposure (4-6 weeks) of A. catfish and juvenile pikeperch to a concentration range of ammonia and nitrate

### Plasma physiology

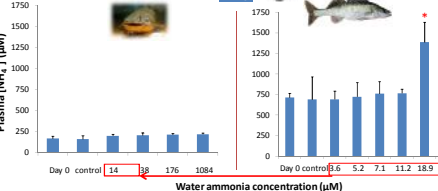


Fig 8. Plasma NH<sub>4</sub><sup>+</sup> of A. catfish (left) and juvenile pike perch (right) exposed to ammonia. Limited capacity to maintain low plasma [NH<sub>4</sub><sup>+</sup>]. On-set of ammonia defence mechanisms plasma [NH<sub>4</sub><sup>+</sup>]. No defense mechanisms

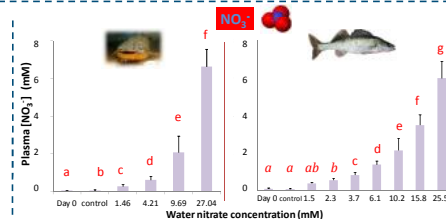


Fig 9. Plasma NO<sub>3</sub><sup>-</sup> of A. catfish (left) and juvenile pike perch (right) exposed to nitrate. Gradual increase of plasma [NO<sub>3</sub><sup>-</sup>] in both species. No on-set nitrate defence mechanisms; lower toxicity compared to ammonia

### Gill morphology

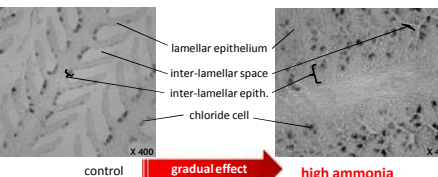


Fig 10. Gill epithelium of A. catfish exposed to 0 (control, left) and 1084 µM ammonia (right). Defense mechanism against ammonia entry: morphology drastically affected. A. catfish: air breather: gas exchange are not impaired; other fish would not survive

### Feed intake

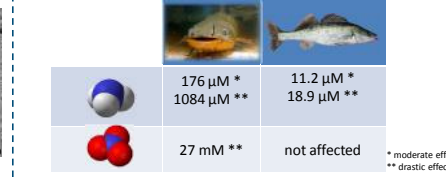


Fig 11. Cumulative feed intake of A. catfish and juvenile pike perch exposed to different ambient ammonia and nitrate concentrations. A. catfish is affected by very high ammonia and nitrate. Juvenile pike perch is already affected by very low ammonia but not by nitrate

### Conclusions and perspectives:

- Threshold determined for both species
- Tolerance to toxic Nitrogenous waste products is compound-specific and species-specific. Ammonia is a serious threat for A. catfish and pike perch, the latter being the more sensitive. With growth as readout, pike perch is rather tolerant to nitrate. The nitrate levels reached in RAS can affect growth performance of A. catfish
- Combining chronic welfare impairment with acute welfare impairment and assess the response

recommendations	A. catfish	juvenile pike perch
NH <sub>3</sub>	< 24 µM	< 3.4 µM
NO <sub>3</sub> <sup>-</sup>	< 10 mM	< 25 mM