Refining continuous around the clock surveying of experimental fish by computer-vision and artificial intelligence – 3R annual meeting Nov. 6-7, 2023

REAL-TIME FISH

Real-time monitoring of fish welfare

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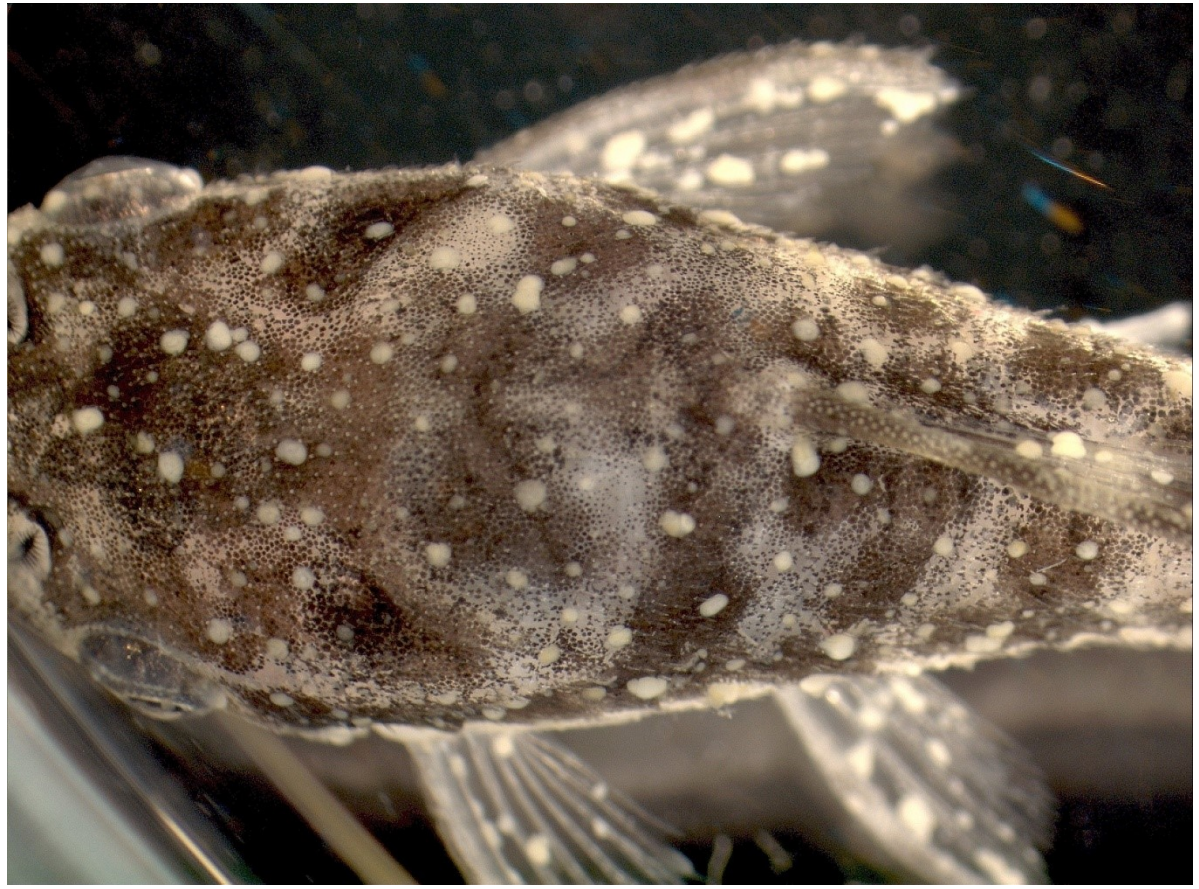
Aim and hypothesis of the 3R project

- Aim: We wish to develop a real-time monitoring system based on computer vision with artificial intelligence to automatically send an alarm to an observer when fish alter behaviour, or when clinical signs indicate that humane endpoints will soon be reached.
- Hypothesis: Clinical signs in fish including disturbances of equilibrium, skin colour, and visible epidermal parasites can be detected by video surveillance and computer vision that allow for a rapid removal of fish as soon as they reach the predefined humane endpoints.

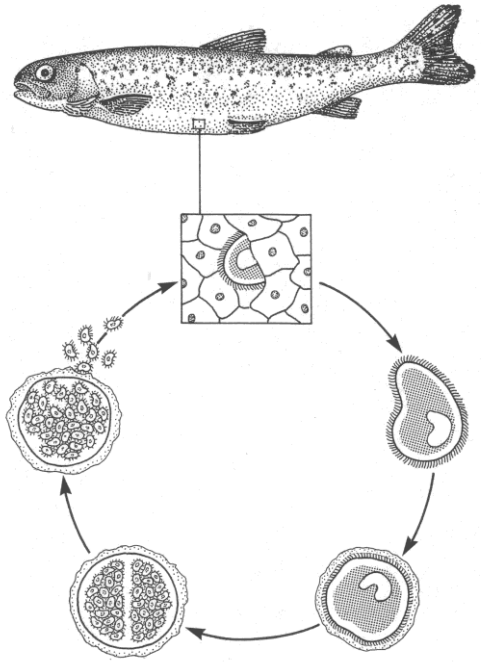
As it is today - when performing vaccination or breeding trials with rainbow trout - we are present in the challenge facility 24 h around the clock and observe the fish exposed to the disease agent continuously

- When a fish exhibit clinical signs we remove it from the fish tank and euthanize (Tricaine methane sulphonate immersion)
- Labor intensive
- Staff intensive
- 6 h shifts around the clock
- An automatic monitoring system based on video surveillance would be beneficial to all

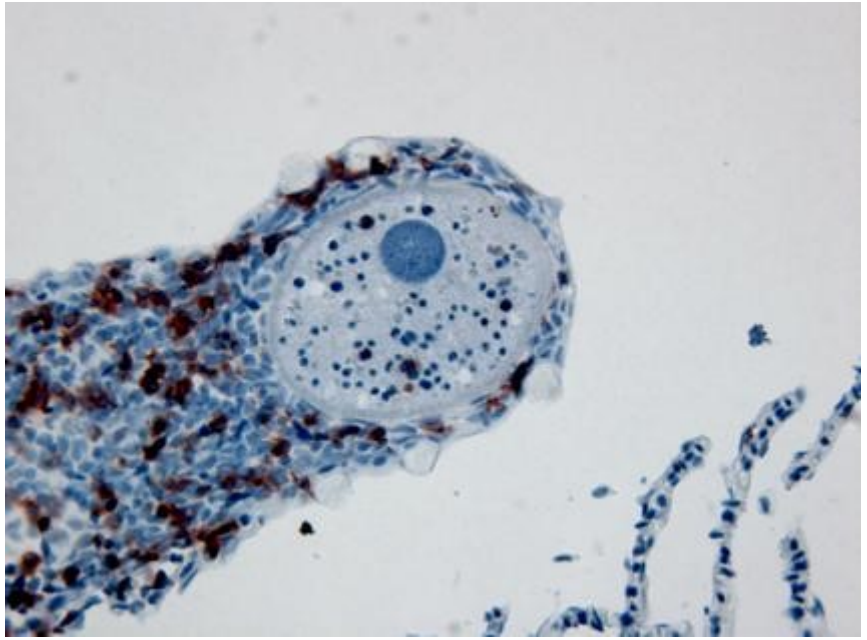
Our model: White spot disease - WSD - in fish caused by the ciliated protozoan *Ichthyophthirius multifiliis* invading the epidermis of freshwater fish



Life cycle for the WSD agent *Ichthyophthirius multifiliis*



Trophont in fish filament ready to burst out into the water



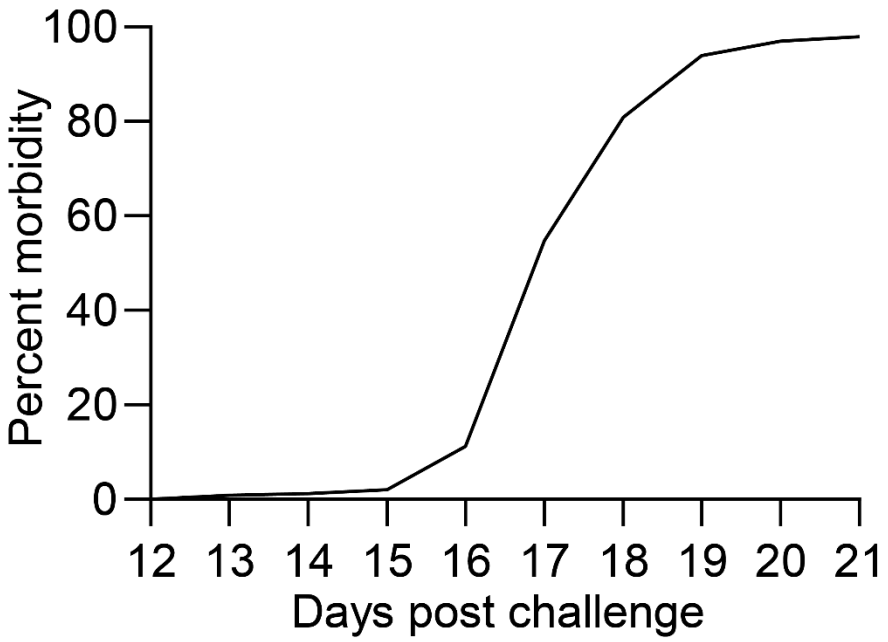
The trial conducted with 2X14 rainbow trout (body weight 10-13 g, body length 9-12 cm) in freshwater fish tanks in the Frederiksberg Campus infection facility May-June 2023. Temperature 19 °C. Recirculation by internal filters. Continuous oxygenation by aeration stones securing oxygen saturation. Enrichment by plastic plants. Video cameras located similarly for continuous recording over three weeks.

- **Control tank**
- Sham infection
- No adverse reaction
- No behavioural changes
- No disease
- No morbidity

- **Exposure tank**
- Cohabitation
- Clear reactions
- Marked behavioural changes
- Clear disease signs
- High morbidity

From the trial. The parasitic stage (trophont) can be seen as white spots. When they are fully developed they burst out of the skin and swim as white freely swimming dots (tomonts) in the water. This process challenges the osmoregulation and physiology of the fish and elicits clear behavioural changes.

The parasite *Ichthyophthirius* is highly pathogenic and if kept untreated the infection will lead to near 100 % mortality in a rainbow trout population. In experimental settings we need to define and monitor moribund fish – in order to remove and euthanize the fish before they die from the disease.



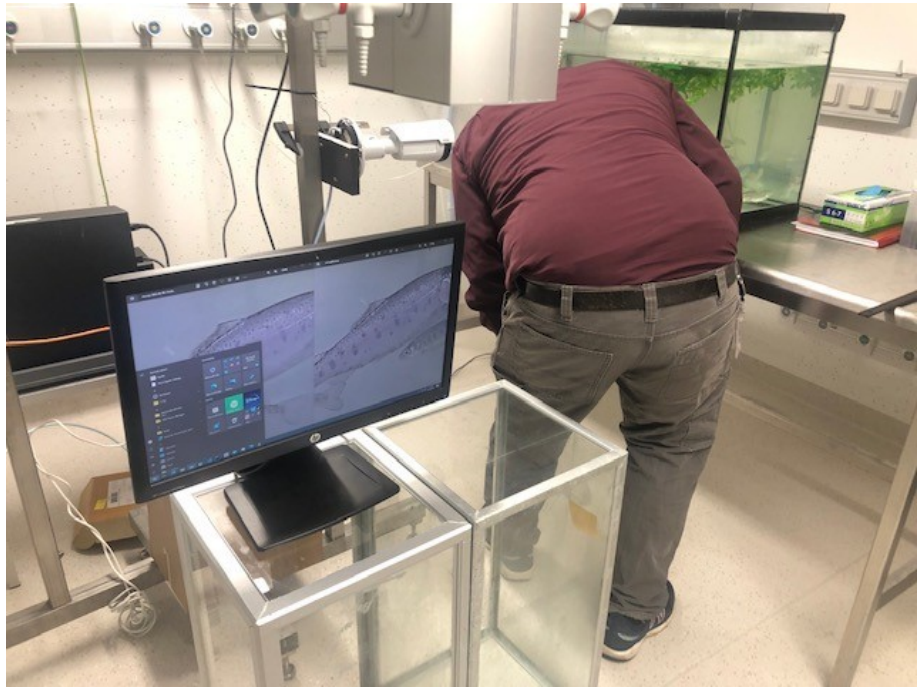
What to record and measure?

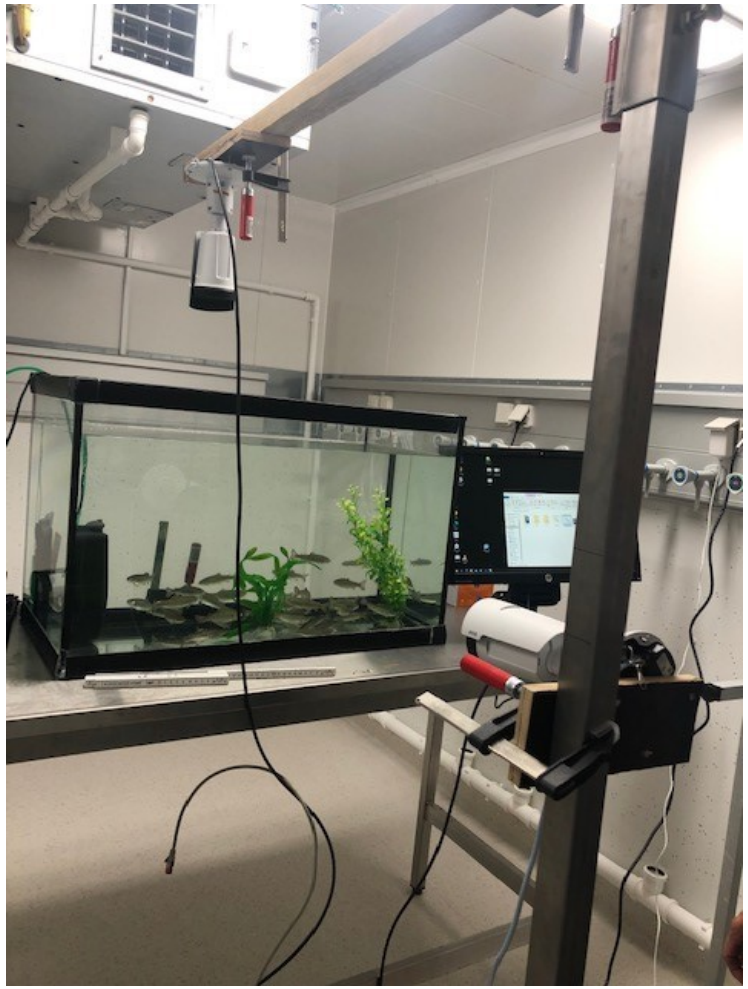
- Clinical signs to be recorded by the system
- The general clinical signs to be recorded in the new system comprise equilibrium disturbances (swimming upside-down, swimming on the side, lying on the fish tank bottom, surface seeking, increased ventilation rates, inactivity, lethargy). We try to combine these observations with colour changes (darkening of the skin) and the number of parasites in the fish. The specific disease signs to be recorded by the side-view computer-vision camera is the number and size of the parasites in the fish epidermis (white spots).

The set-up

- The parasite is visible as it develops white spots (up to 1 mm in diameter) in the epidermis of the fish (skin, fins) and we seek to monitor the infection by computer-vision combined with an algorithm based on artificial intelligence (one camera – side view into the glass fish tank or submerged camera in case of non-transparent fish tank walls). Another camera will have a top view and monitor clinical signs (aberrant behavior indicating impact of the pathogen on host physiology and welfare).

Setting up the video-equipment at the Frederiksberg Campus infection facility





The system in development:
First approach was to regard each fish as a rectangle

What could the video and the computer digest and not digest?

- **Aim:**
- Lethargy
- Anorexia
- Flashing
- Equilibrium disturbance
- White spot numbers on the individual fish

- **Obstacles:**
- Trophonts (large ciliates) escaping from the fish epidermis disturbes the monitoring.
- Scales on the fish epidermis reflects light and may trick the system.
- Trophonts released/escaped from the epidermis reflects light and may trick the system.

The next steps

- Identify the pixels in an individual fish
- Expand the analysis from square detection to pixel analysis
- Still a way to go before we have the functional turn-key alarm system calling-in the staff for care-taking

Thanks to the 3R Center for the support and thank you for your kind attention