Focus on Humane End Points in Fish experiments: Optimization by use of AI?

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DVO Annual Meeting - June 12th, 2025
Lundbeck Pharma
Valby

Work conducted together with

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- Jeppe Seidelin Dam
- Dorte Schrøder-Petersen

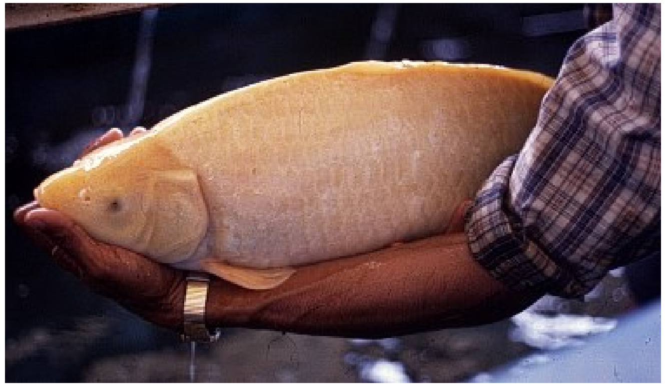
- Danish Technological Institute, Taastrup, Denmark

First of all:
Why do we conduct experiments involving fish?

- **Experimentation with fish, which are reared in aquacultural settings, are needed in order to find answers to basic questions related to growth, production and fish health. Here salmonids, cyprinids, percids, moronids and pleuronectids dominate the field.**

The background is the increasing importance of Aquaculture worldwide

- A climate friendly approach, which at present is supplying more than 50 % of fish consumed worldwide. Need to secure sustainability of the industry.



Fish are continuously exposed to various pathogens in the environment.
These comprise pathogenic virus, bacteria and parasites.

How do we prevent disease?

We have focus on vaccination and selective breeding by use of genetic markers

- **Vaccination**
- **Selective breeding of disease resistant fish**

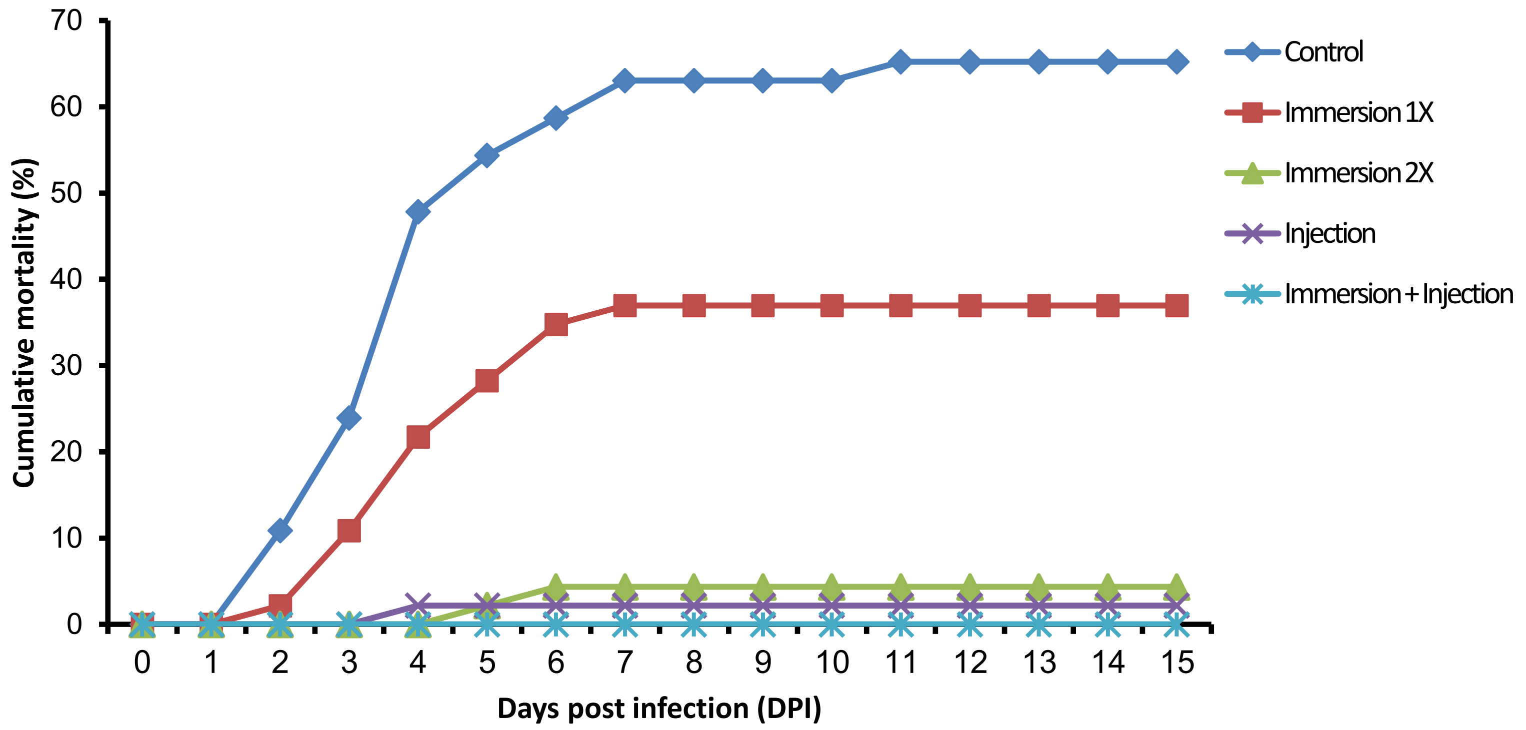
Vaccination by injection
Fish are always anaesthetized during the process

- High degree of protection

Even very small fry can achieve protection: vaccination by immersion

First step is to produce the fish: The fish are hatched and reared at the hatchery under pathogen-free conditions

When testing effects of vaccination or selective breeding we need to expose fish to live pathogens and follow the disease development. Here we see effect of vaccination.



For the selective breeding studies we also have to expose the disease free fish to one particular pathogen – and then follow the disease progression around the clock.

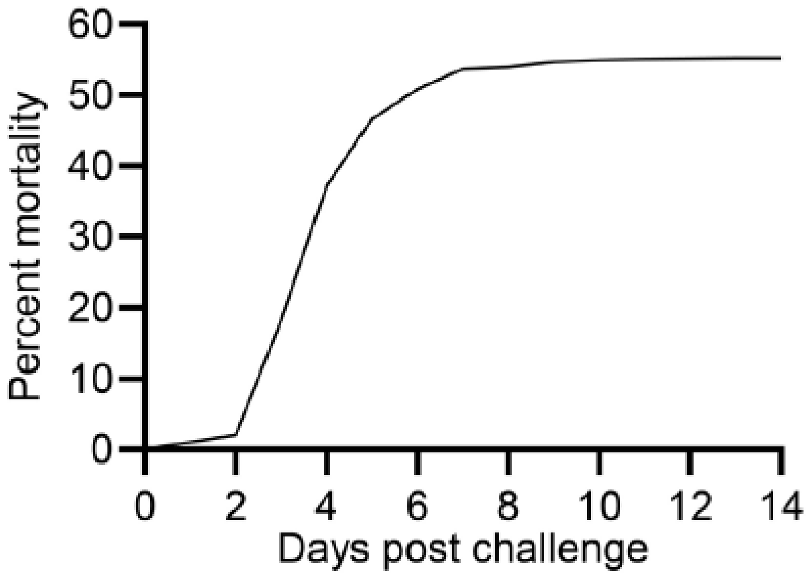
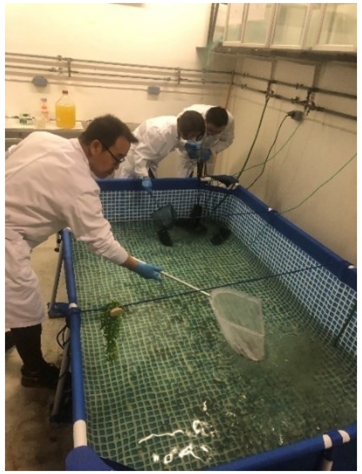
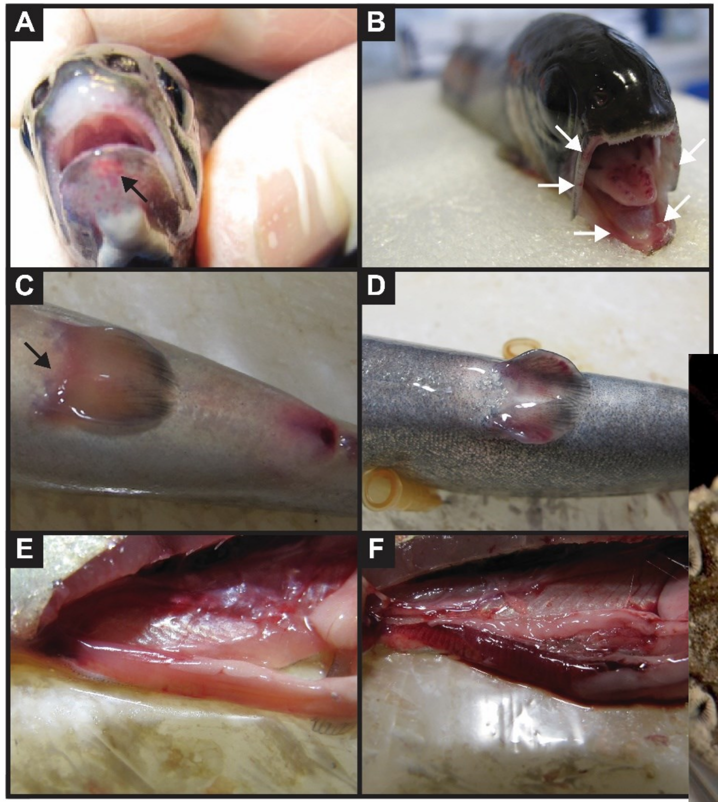


FIGURE 1 | Mortality curve for rainbow trout exposed to *V. anguillarum*.



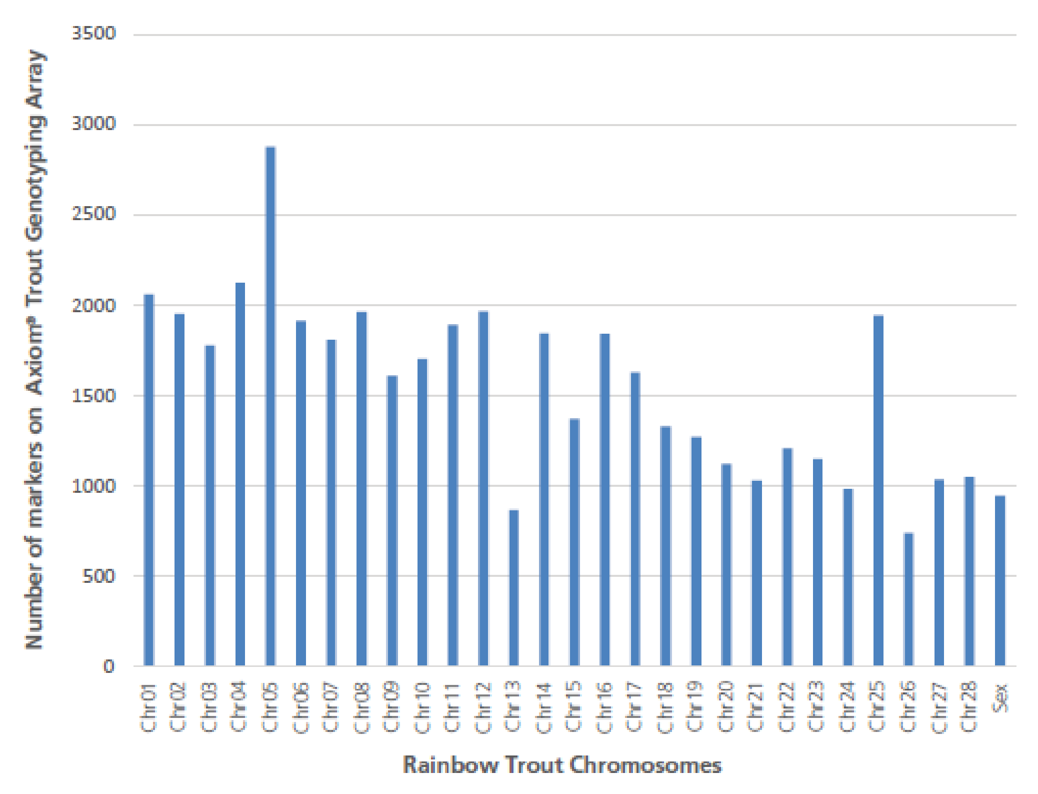
When ever a fish exhibits clinical signs it is taken out of the tank, euthanized, recorded as dead, and DNA typed – and any survivors (preferably 50 %) are DNA typed as well at the end of the experiment

How does it look like?
Here we see the pathology of Enteric Red Mouth Disease (left), White Spot Disease (middle) and furunculosis (right) in rainbow trout.



And then we genotype the sampled fish?
The tool we use for genotyping is an Affymetrix platform (Axiom® Trout). Describing presence or absence of 57,100 SNPs in the rainbow trout genome

Figure 1: Distribution of the SNPs on Axiom® Trout Genotyping Array across the rainbow trout chromosomes.



Then we can identify whether each individual fish carries the genes associated with resistance. Here we see vibriosis resistance associated with chromosome 21

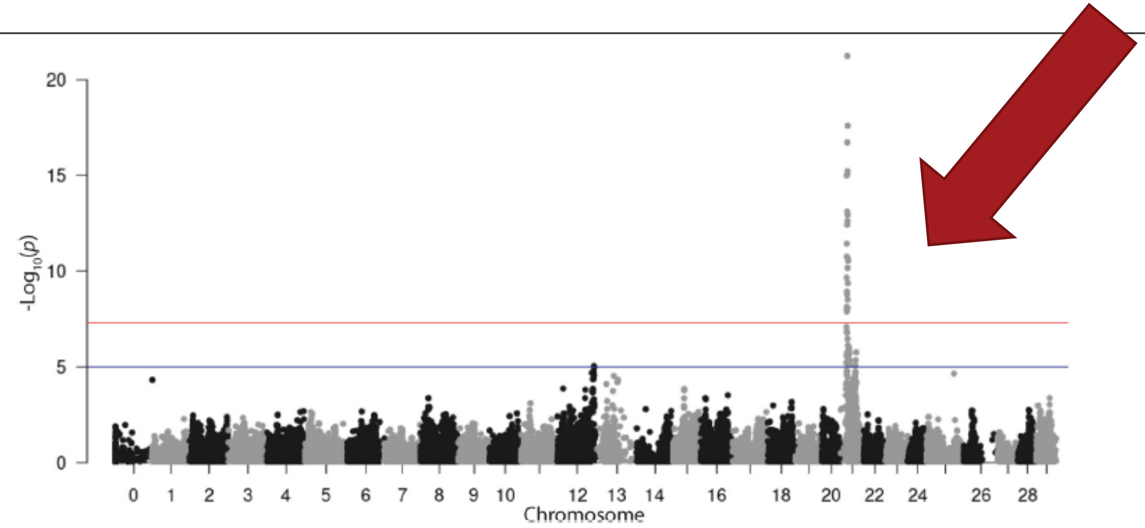
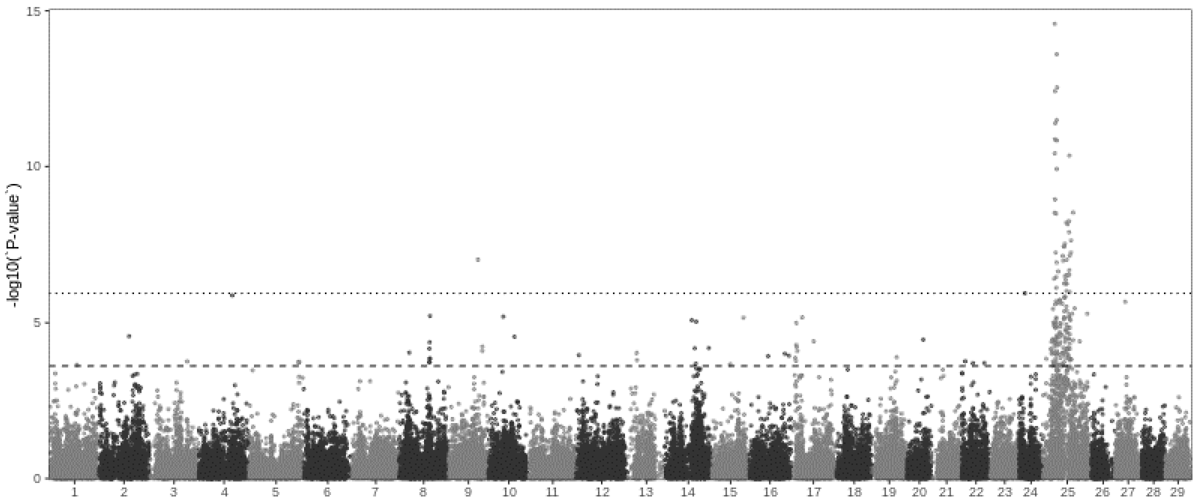
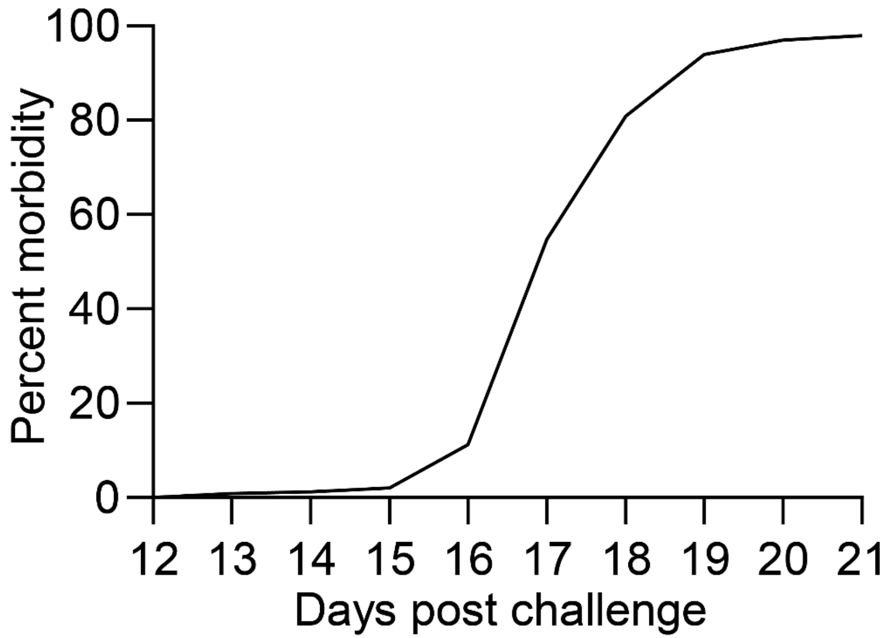


FIGURE 2 | Manhattan plot from an LMM-LOCO GWAS of time to death in rainbow trout after exposure to *V. anguillarum*.

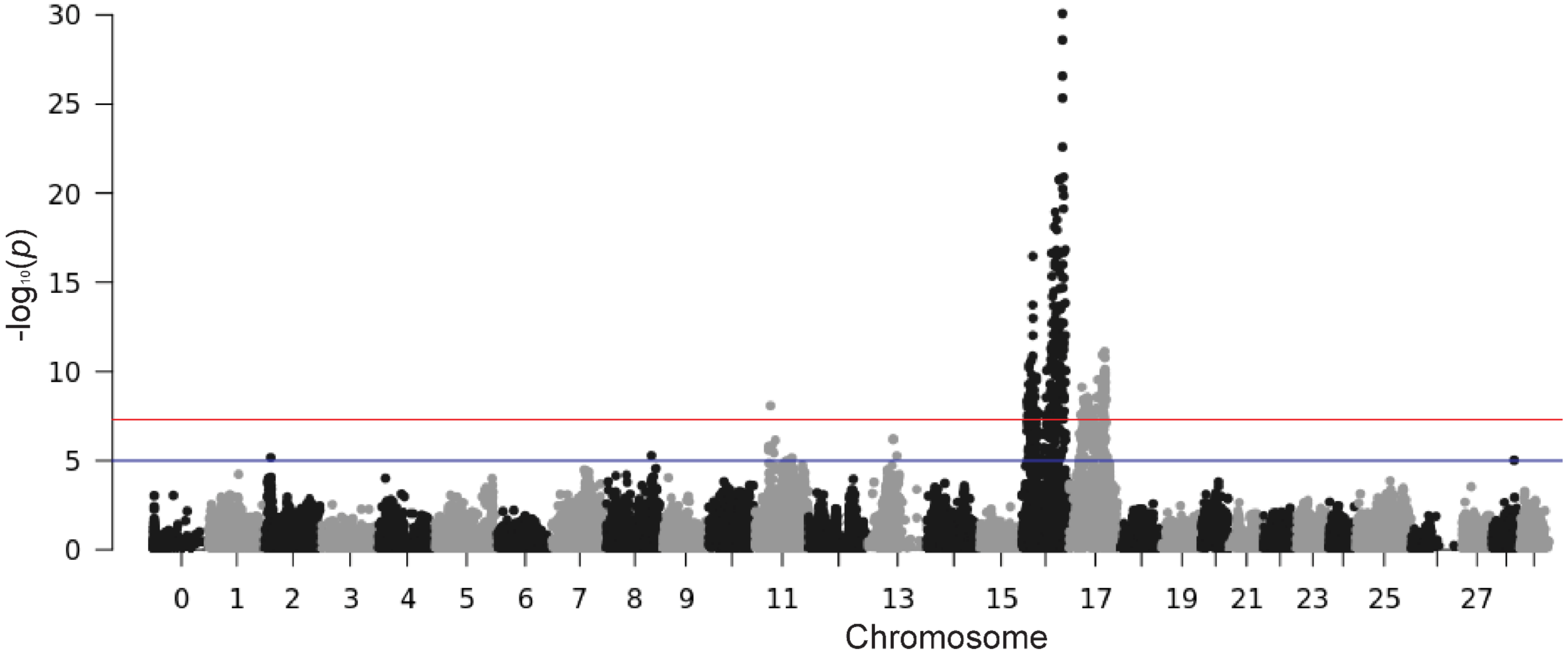
Chromosome 25 is carrying genes associated with resistance to RTFS/BCWD as inferred from our Manhattan plot



Another Example from our Danish rainbow trout selection study.
Here the infection with the parasite *Ichthyophthirius causing* White Spot Disease



SNP analysis shows that chromosomes 16 and 17 – nicely illustrated by this Manhattan plot - carry the genes associated with resistance towards the parasite *Ichthyophthirius*



But how to define humane end points in these challenge studies?

Humane end points in fish experiments

- Death is not applicable as humane end point
- Fish must be removed from the experiment and euthanized when clinical disease signs appear

- Humane endpoints:
- Equilibrium disturbances
- Visible haemorrhages
- Body colour (darkening)
- Anorexia
- Lethargy
- Surface gasping

Euthanasia

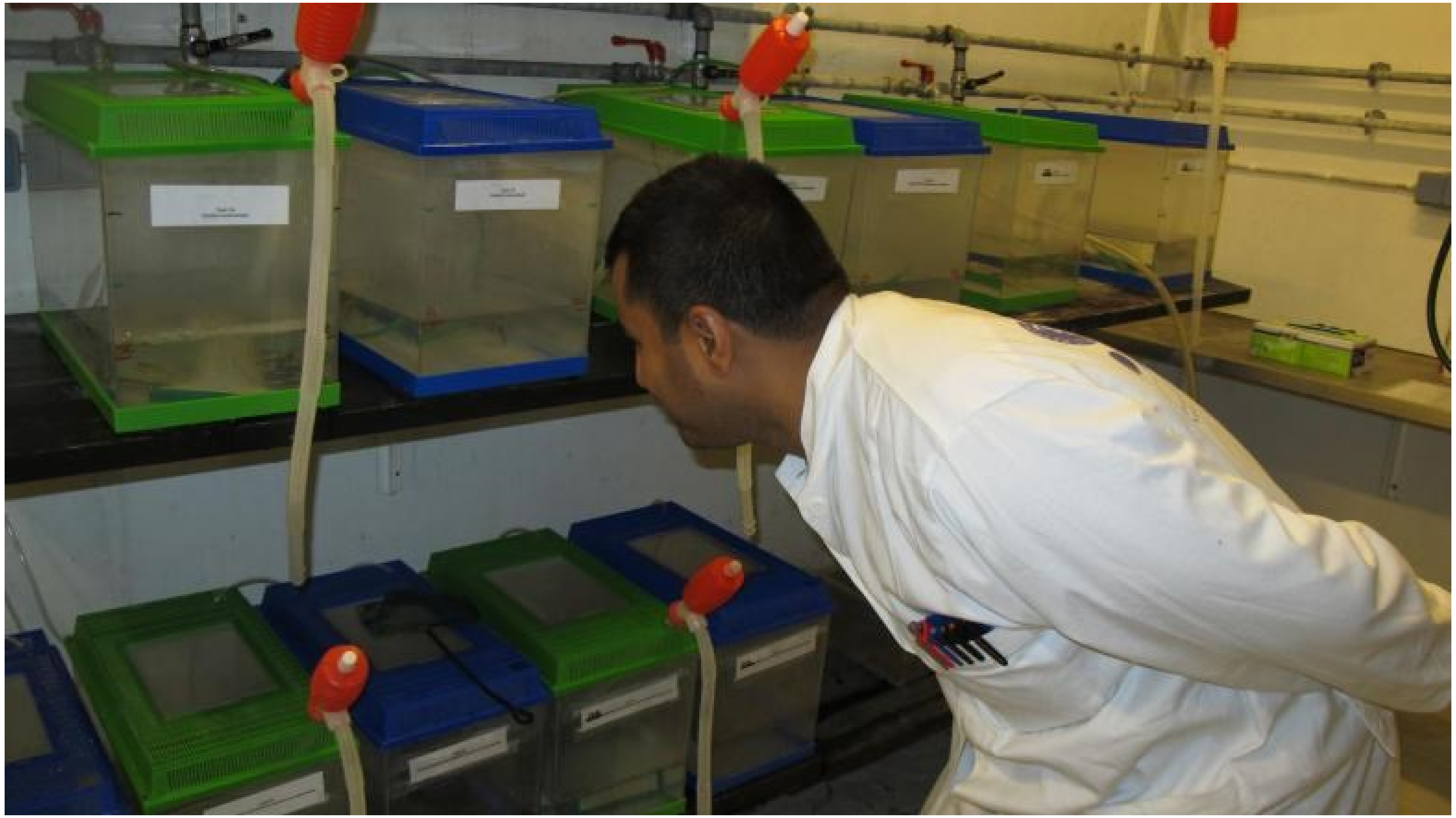
- Lethal dosage MS222 (200-300 mg/l)
- Insert scalpel in fish brain to re-assure death

Humane endpoint: Equilibrium disturbances

Humane endpoint: Ulcers

Counting the visible pathogens. Here the white spots of *Ichthyopthirius multifiliis*

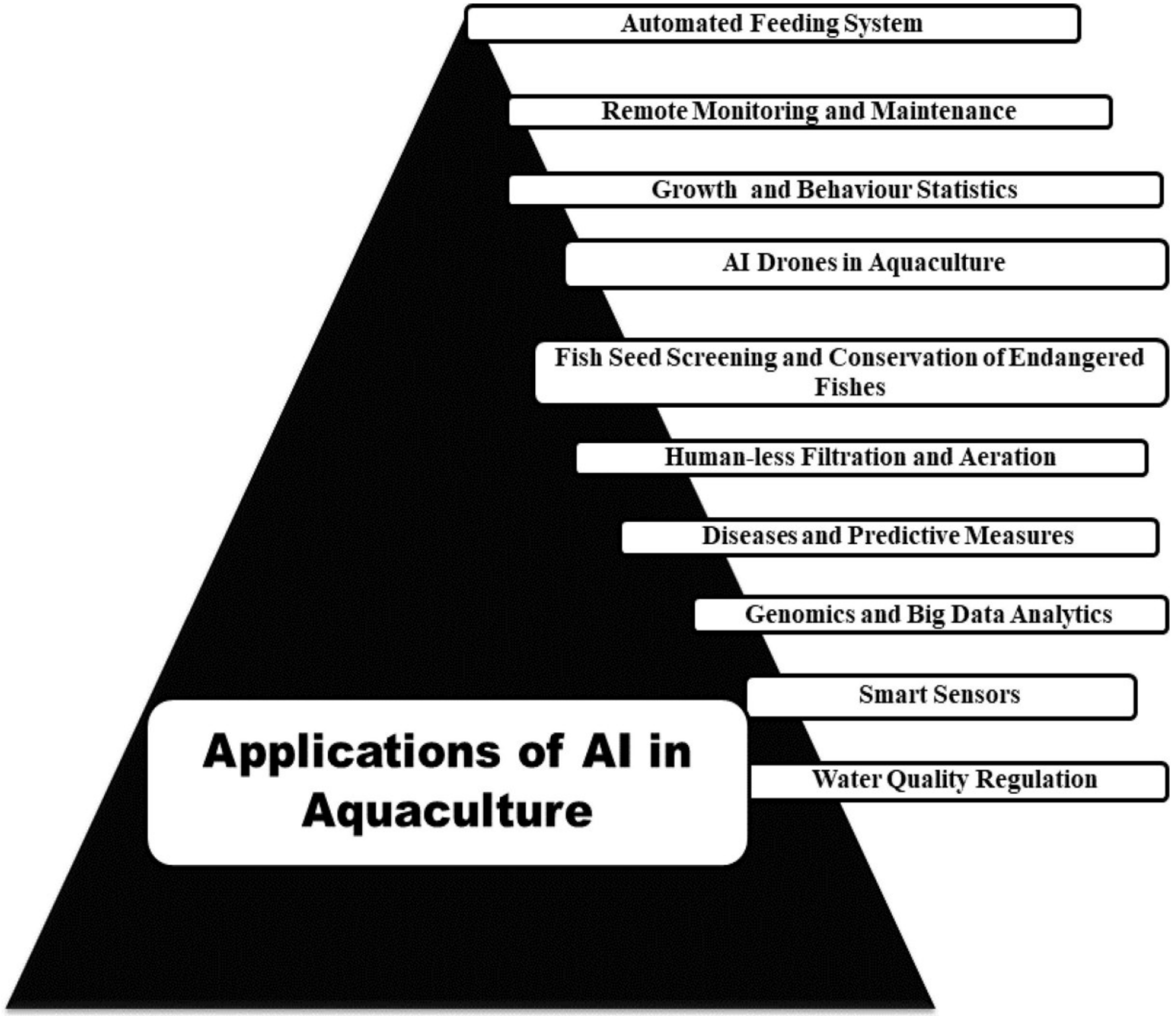
Infection in the laboratory: continuous checking of disease signs around the clock



Continuous monitoring of experimental fish is a must - but it is laborious to inspect fish 24 h around the clock for 21 d

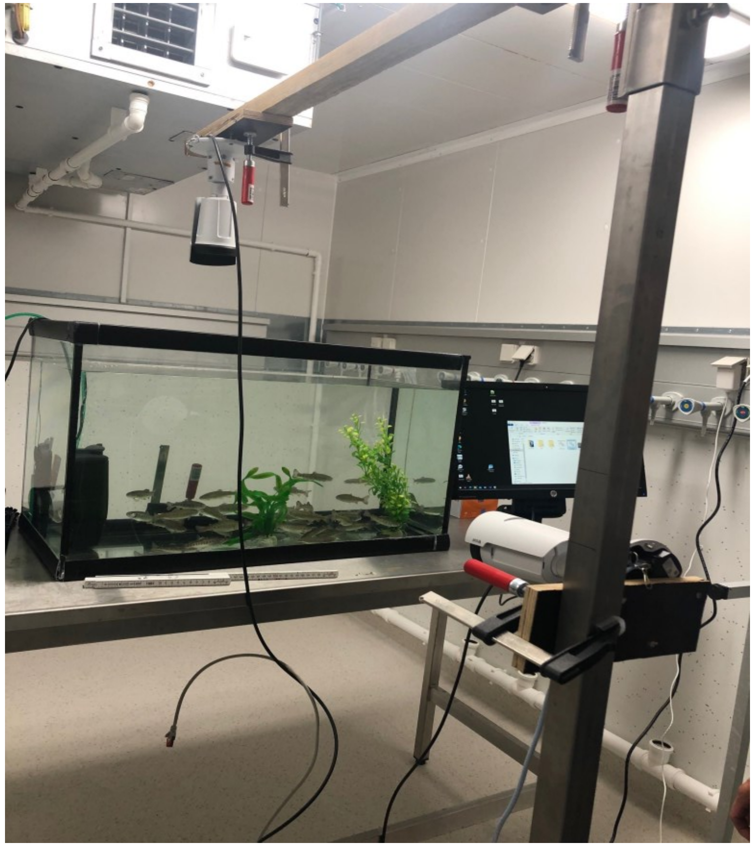
- Can we automatize the monitoring process?
- Can it be done by continuous video-recording?
- Can AI be applied to detect early clinical disease signs?
- Can the automatic detection be connected an alarm function calling staff to remove diseased fish from the on-going experiments?
- Can the continuous video-recording and AI analysis reveal new important humane end-points in fish experiments?

Artificial Intelligence AI is already used in for other purposes in fish production. So why not apply AI for Humane endpoints?



Rather et al. 2024. J. Food Chem.

We recently – together with the Technological Institute of Denmark - conducted a challenge study over 14 d using the rainbow trout/*Ichthyophthirius* infection model. Top and side view video-cameras were applied and recordings saved in computer



The cameras

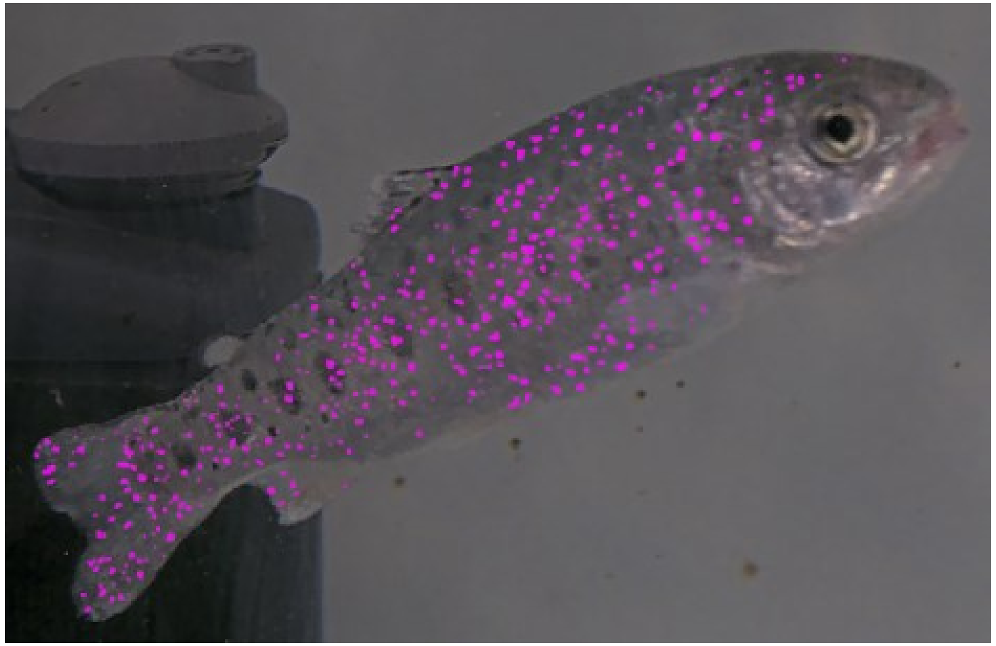
- Cameras were set to 30 frames per second with a resolution of 3840 × 2160 and set to the highest supported bitrate (80–100 Mbit/s).
- At the end of the experiment, videos were manually evaluated and analysed to note the occurrence of the behavioural patterns/symptoms

The algoritm

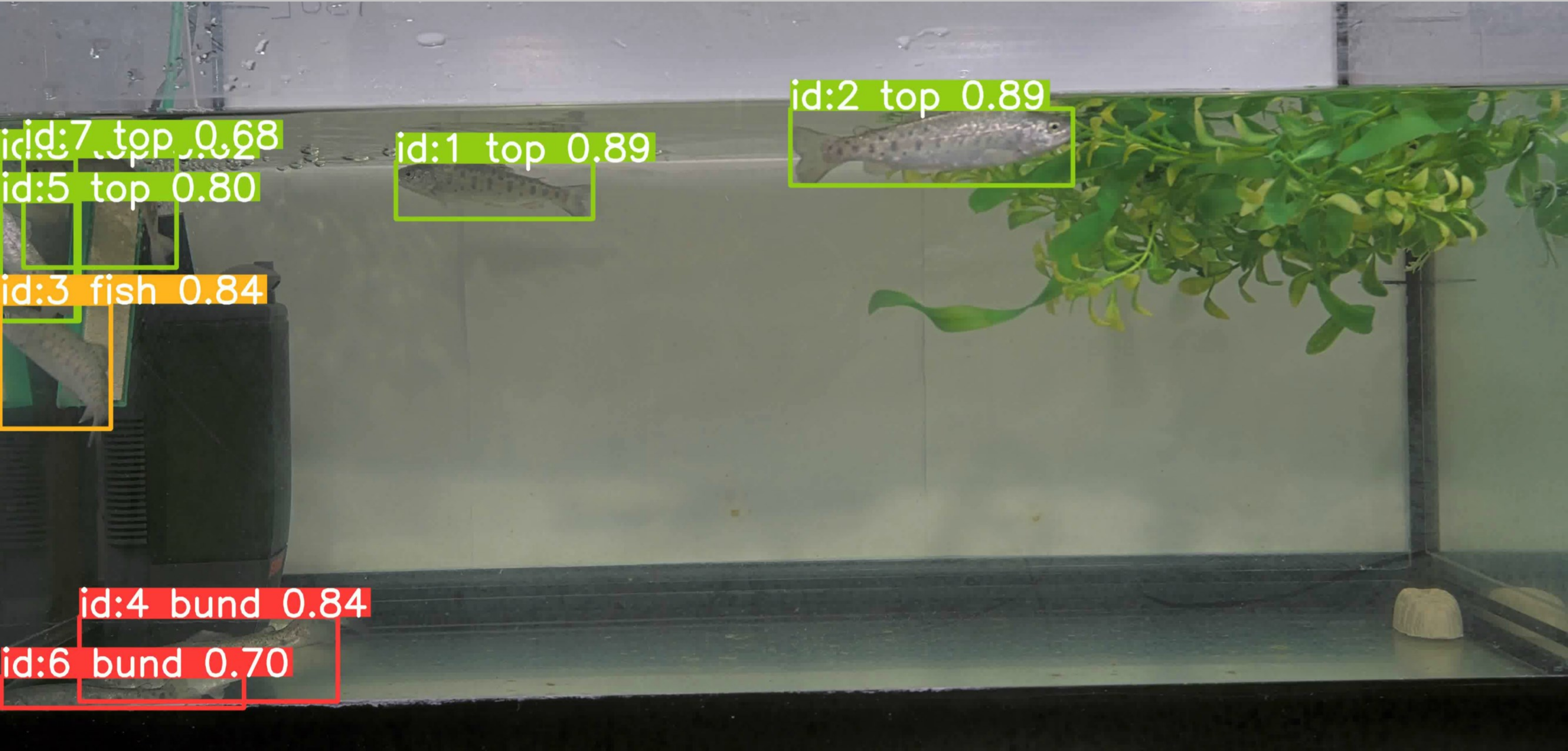
- **The YOLO-v8 method (Jocher, Chaurasia, and Qiu 2023) was trained for the object detection and position classification using the custom annotation, with a yolov8n architecture and an 80%/20% split between training and validation for 150 epochs and batch size of four, selecting the best minimal loss on the validations set. The rotation classifier was trained using YOLO-v8 Classification methods with a yolov8n_cls architecture and for five epochs and a batch size of 25, selecting the best minimal loss on the validations set.**

- **Random data argumentation using scaling, shear, translation, flip left and right, mosaic and mixup (Zhang et al. 2018) was used for the training of both models. Both the fish object detector and rotations classifier were used for inference on every frame of the video streams recorded for infected and baseline fish. The detections in each frame were combined with single animal tracking by performing multiple object tracking using the OCSort method (Cao et al. 2023) implemented in the BoxMOT library (Broström 2023).**

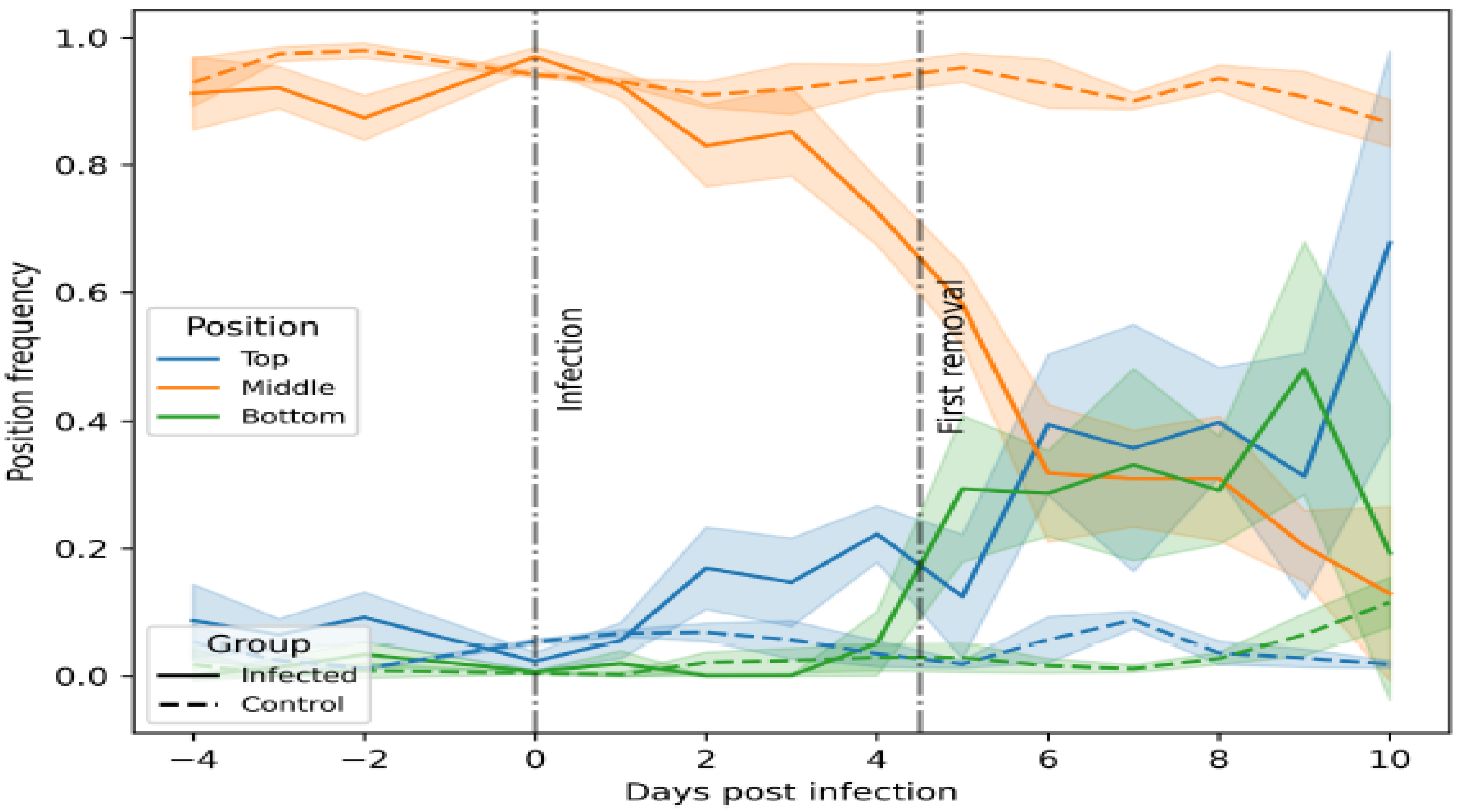
Humane endpoint: Visible pathogens



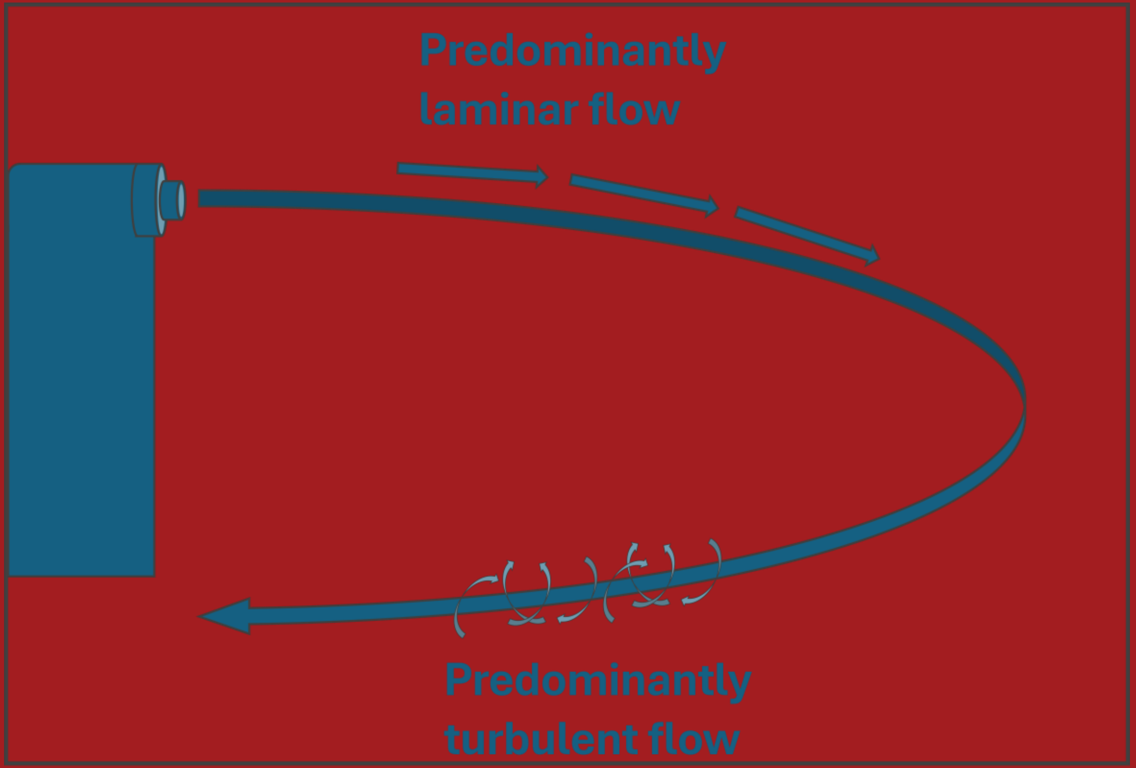
Detection of deviant behavior –top/bottom



Location of fish in the tank with and without infection



BUT WHY DID THE FISH LEAVE THE MIDDLE AND CENTRAL POSITION IN THE FISH TANK AND OCCUPY THE UPPER WATER LAYERS?



We hypothesize that fish with infected gills and thereby decreased oxygen uptake capability seek positions with high water flow, which will augment oxygen uptake

The behaviour of the fish in the earliest infection phase was found to be the best indicator of infection

- Classical humane end-points in ICH-infected fish appeared relatively late in the infection course

- Active relocation and placement of the fish in the tank was the earliest and best indicator

The AI application enabled us to detect the effect much more fast and easily compared to manual recording. We cannot exclude that AI can have a future role in humane end-point recording

Acknowledgments

Innovation Fund Denmark – TECHFISH and GREFIB

The Danish 3R center

Innovationsfonden

GUDP – EGYR

gudp

Ministeriet for Fødevarer, Landbrug og Fiskeri

Thanks to all our co-workers:

ORIGINALARTICLES

J Fish Dis: Quantitative trait loci (QTL) associated with resistance of rainbow trout *Oncorhynchus mykiss* against the parasitic ciliate *Ichthyophthirius multifiliis*

R Jaafar1 | J Ødegård2 | H Mathiessen1 | A M Karami1 | M H Marana1 L von Gersdorff Jørgensen1 | S Zuo1 | T Nielsen3 | P W Kania1 | K Buchmann1

- **Frontiers in Genetics: A Major QTL for Resistance to Vibrio**
- **anguillarum in Rainbow Trout**
- **Asma M. Karami1*, Jørgen Ødegård2, Moonika H. Marana1, Shaozhi Zuo1,**
- **Rzgar Jaafar1, Heidi Mathiessen1, Louise von Gersdorff Jørgensen1, Per W. Kania1,**
- **Inger Dalsgaard3, Torben Nielsen4 and Kurt Buchmann1**

Scientific Reports: Whole-genome association study searching for QTL for *Aeromonas salmonicida* resistance in rainbow trout
Moonika H. Marana1✉, Asma M. Karami1, Jørgen Ødegård2, Shaozhi Zuo1, Rzgar M. Jaafar1, Heidi Mathiessen1, Louise von Gersdorff

Fish & shellfish Immunology:

Full length article

Immune gene expression and genome-wide association analysis in rainbow trout with different resistance to *Yersinia ruckeri* infection

Shaozhi Zuo [a], Asma M. Karami [a,*], Jørgen Ødegård [d], Heidi Mathiessen [a], Moonika H. Marana [a], Rzgar M. Jaafar [a], Louise von Gersdorff Jørgensen [a], Mohamed Abdu [a], Per W. Kania [a], Inger Dalsgaard [b], Torben Nielsen [c], Kurt Buchmann [a]

Aquaculture Reports: Validation of two QTL associated with lower *Ichthyophthirius multifiliis* infection and delayed-time-to-death in rainbow trout Kurt Buchmann a,*, Torben Nielsen b, Heidi Mathiessen a, Moonika H. Marana a, Yajiao Duan a, Louise V.G. Jørgensen a, Shaozhi Zuo a, Asma M. Karami a, Per W. Kania a

Published in the Journal of Fish Diseases 2024

Journal of Fish Diseases

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RESEARCH ARTICLE OPEN ACCESS

AI-Driven Realtime Monitoring of Early Indicators for *Ichthyophthirius multifiliis* Infection of Rainbow Trout

Rikke Bonnichsen[1] | Glenn Gunner Brink Nielsen[1] | Jeppe Seidelin Dam[1] | Dorte Schrøder-Petersen[1] | Kurt Buchmann[2]

Thank you for your kind attention