First Field Evidence of Probable Aerosol-Mediated Transmission of an Atlantic Salmon Pathogen in RAS

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The project is funded by the European Union's Horizon 2020 research and innovation programme under the Marie Sktodowska-Curie grant agreement No 956481

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Introduction

Modes of pathogen transmission, in general, are vertical and horizontal.

Horizontal transmission is well-documented in fish (most common).

The horizontal transmission is close contact mediated (most fish pathogens involved in spiking studies)

Vertical transmission is well-documented for a few pathogens; a classic example is IPNV.

ISA virus is transmitted horizontally, with no evidence to suggest vertical transmission.

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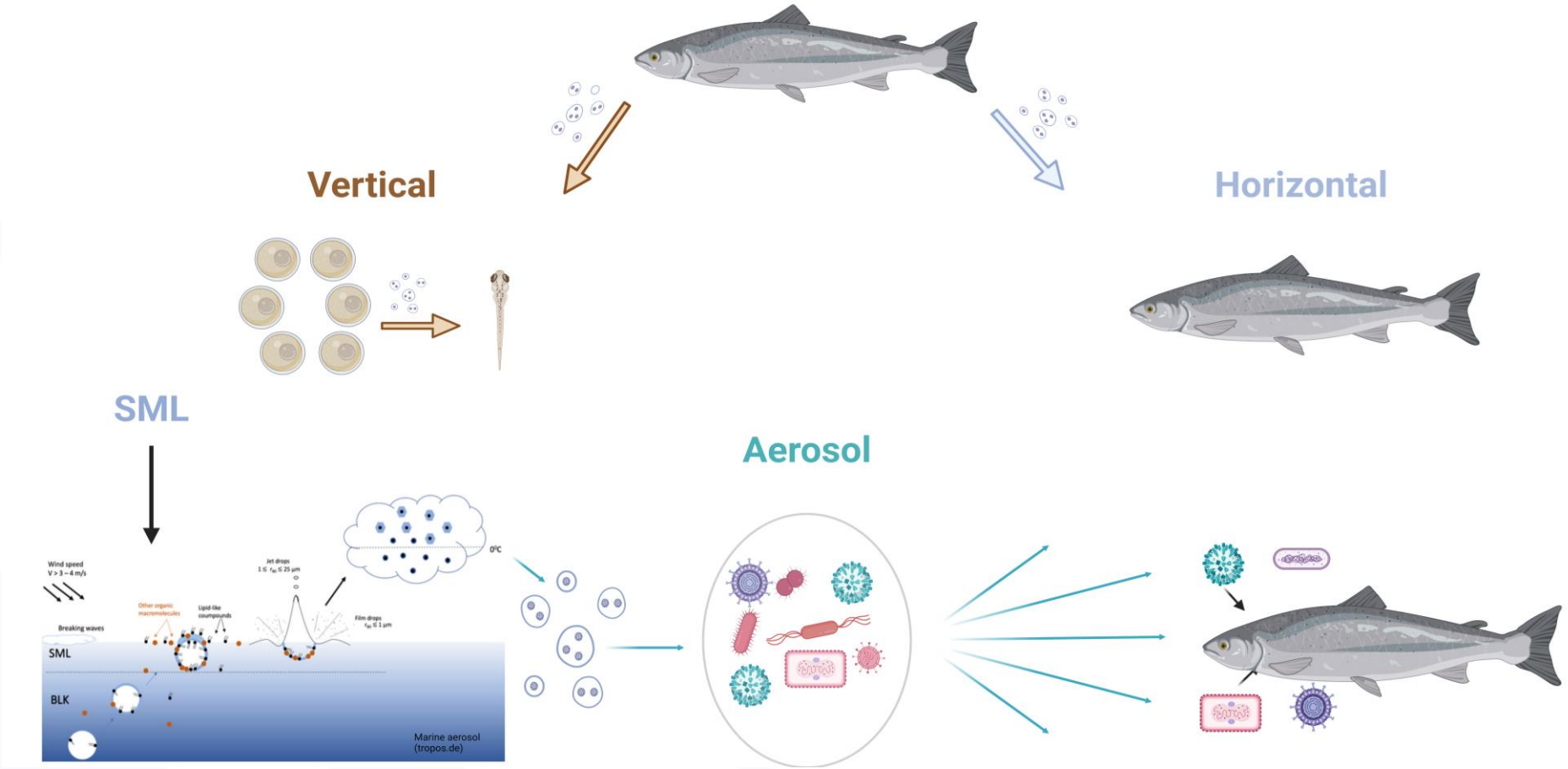

Previous studies have shown that the Sea Surface Microlayer can generate aerosols that transmit viruses and bacteria over long distances.

RAS, an intensive system, should produce aerosols since it uses large quantities of air for degassing ?

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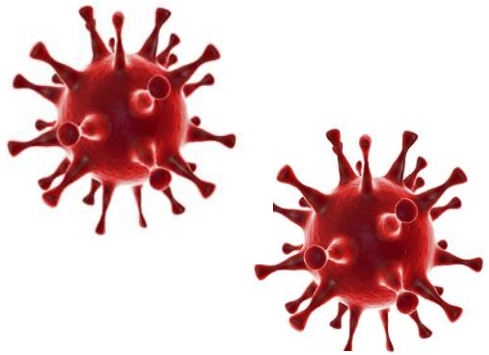
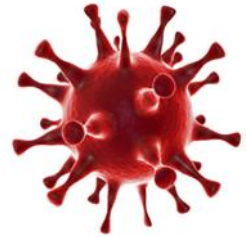
Aerosol mediated transmission



Aerosol transmission is a type of horizontal transmission.

Based on the theory that anything in a system that can “move” can probably be aerosolised.



This mode is well-documented in terrestrial animals and humans.

It has been documented for a few fish pathogens like *Aeromonas salmonicida* and *A. hydrophila* (Bishop et al., 2003; Gołaś et al., 2022)

Ichthyophthirius multifiliis and *Amyloodinium ocellatum* (Wooster and Bowser, 1996; Roberts-Thomson et al., 2006)

What about fish viruses? And in RAS?

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Methodology

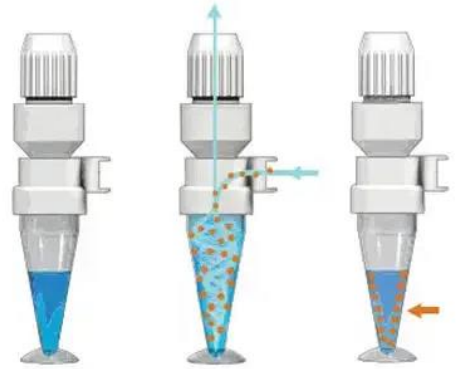
Two commercially validated aerosol samplers, the Coriolis Micro and Coriolis Compact (Bertin Technologies, France), were used.

Centrifugal concentrators that concentrate into a liquid or adsorb the particles on the sampling cone surface

For the first sampling, fish and water samples were also collected with aerosol samples.

Coriolis Micro

Coriolis Compact



Unidirectional airflow

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Methodology : Sampling

Fish: Gill and kidney swabs

Water: 1L

1st sampling was conducted to identify the pathogen dynamics in RAS (2 different RAS systems)

2nd sampling followed the 2nd fish group with additional aerosol sampling (1 RAS system)

Aerosol: Coriolis Compact and Coriolis Micro 1h each; 3 positions

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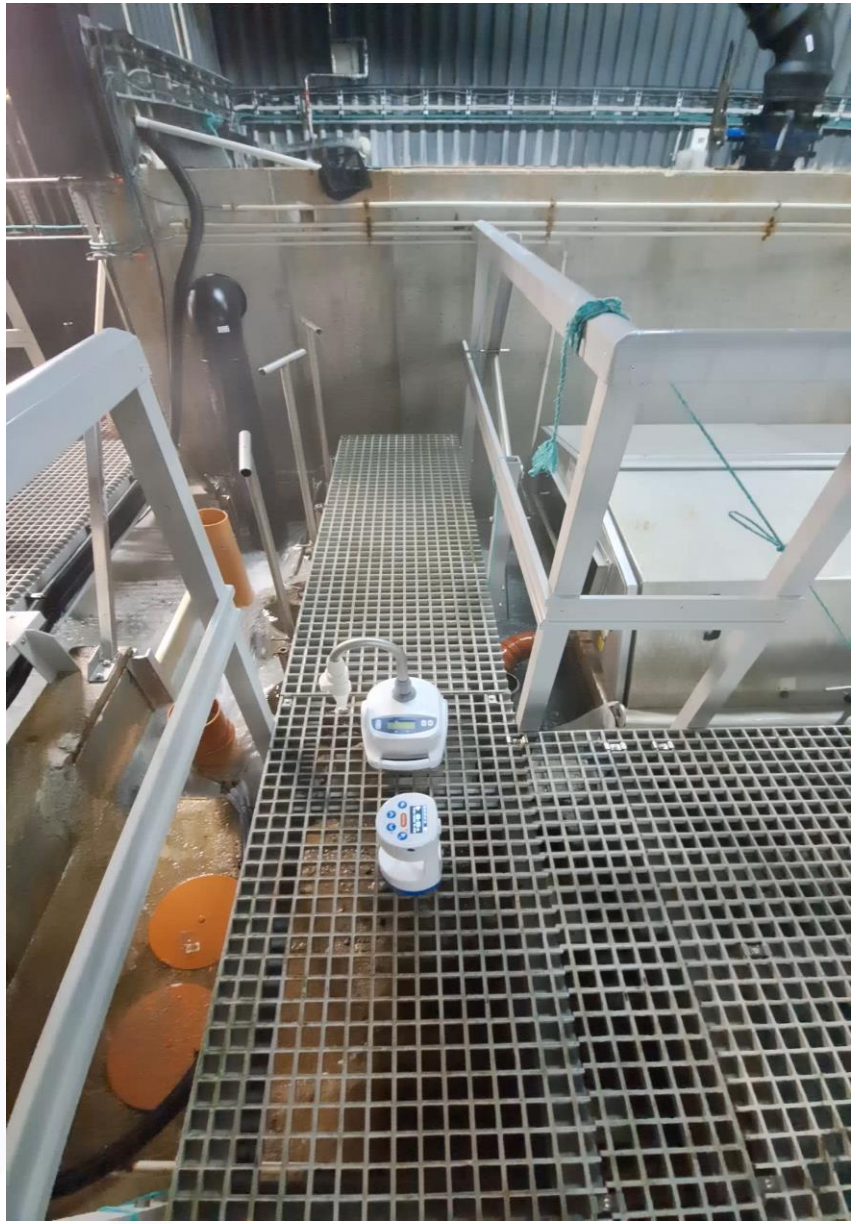

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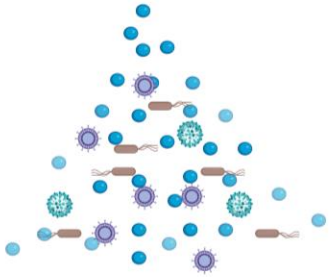
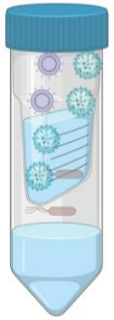
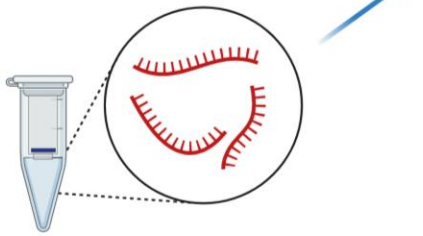
Sample collection



Coriolis Micro

Coriolis Compact

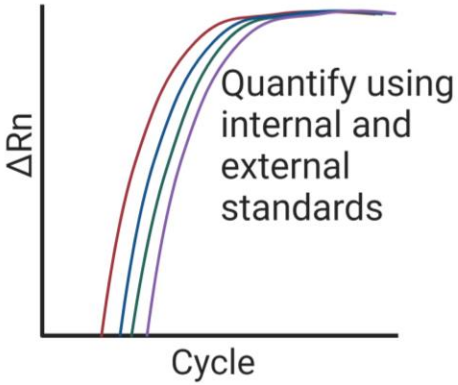




Extracted DNA/RNA

Extraction procedure similar to water/fish samples

RT-qPCR



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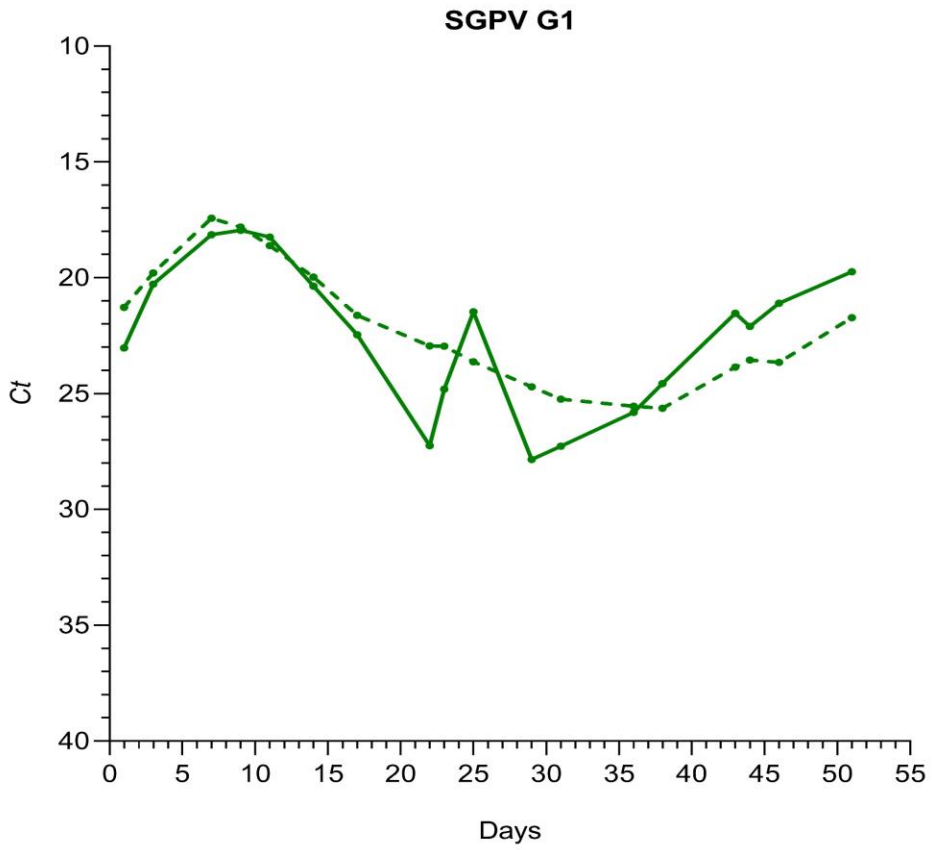
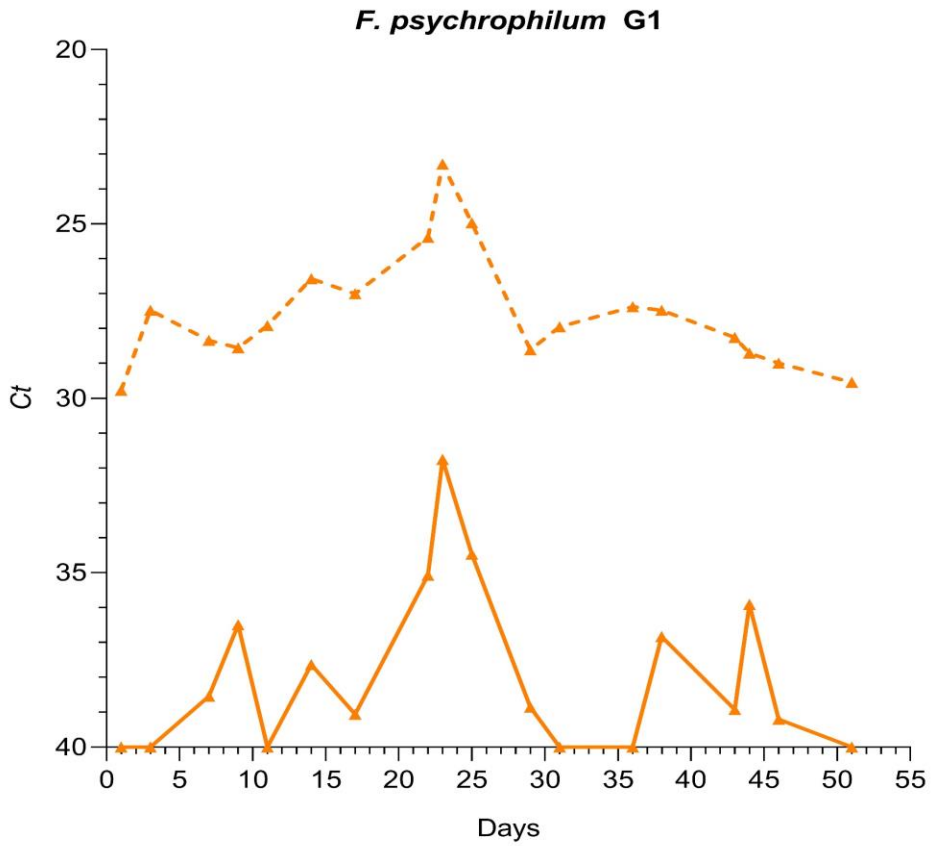
Results: Infection dynamics of various pathogens





/ Fish

/ Water

Ct > 40; negative results

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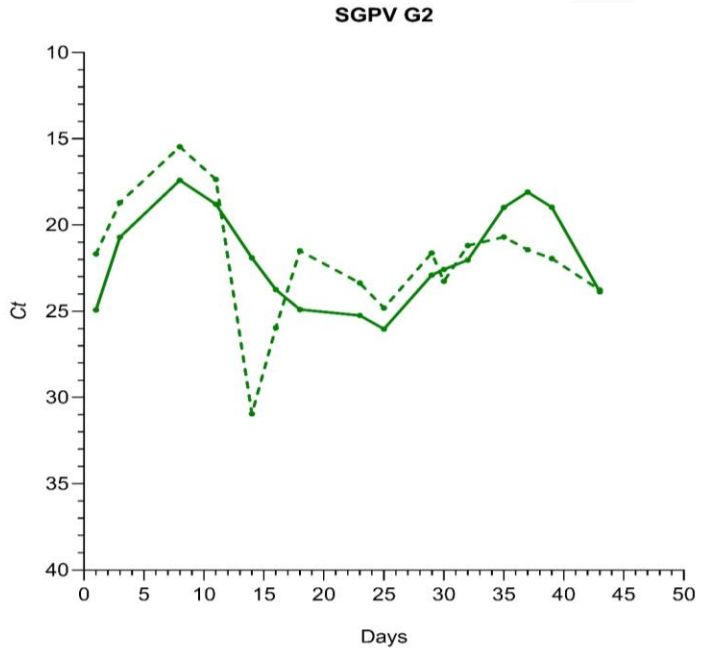

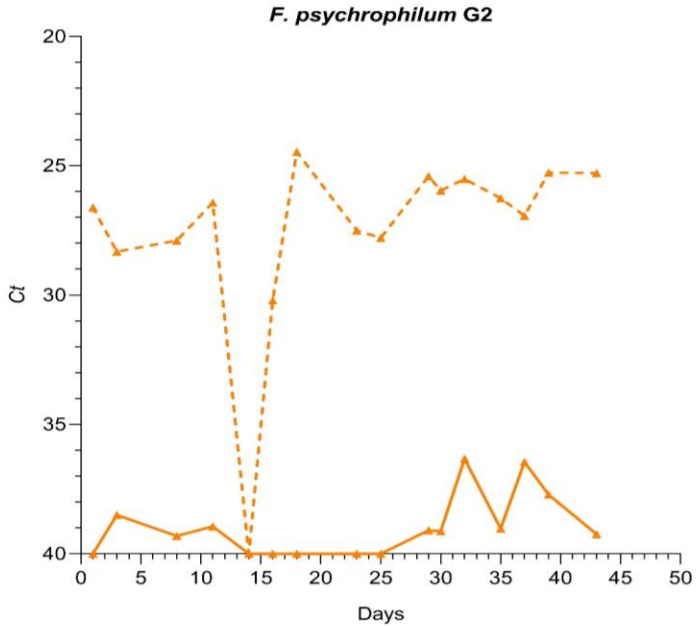

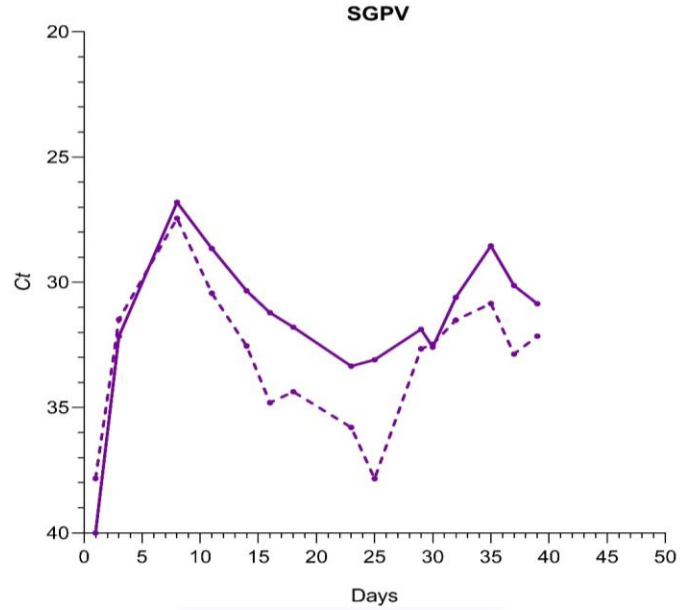

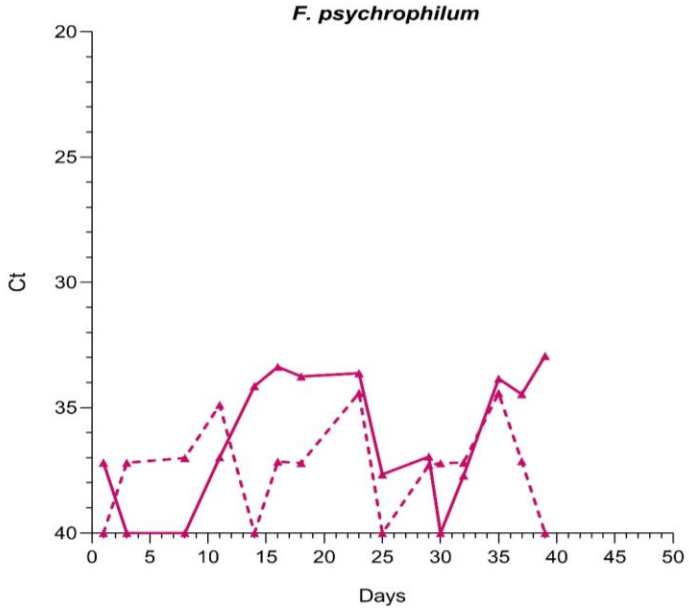

Biofilter

Ct > 40; negative results

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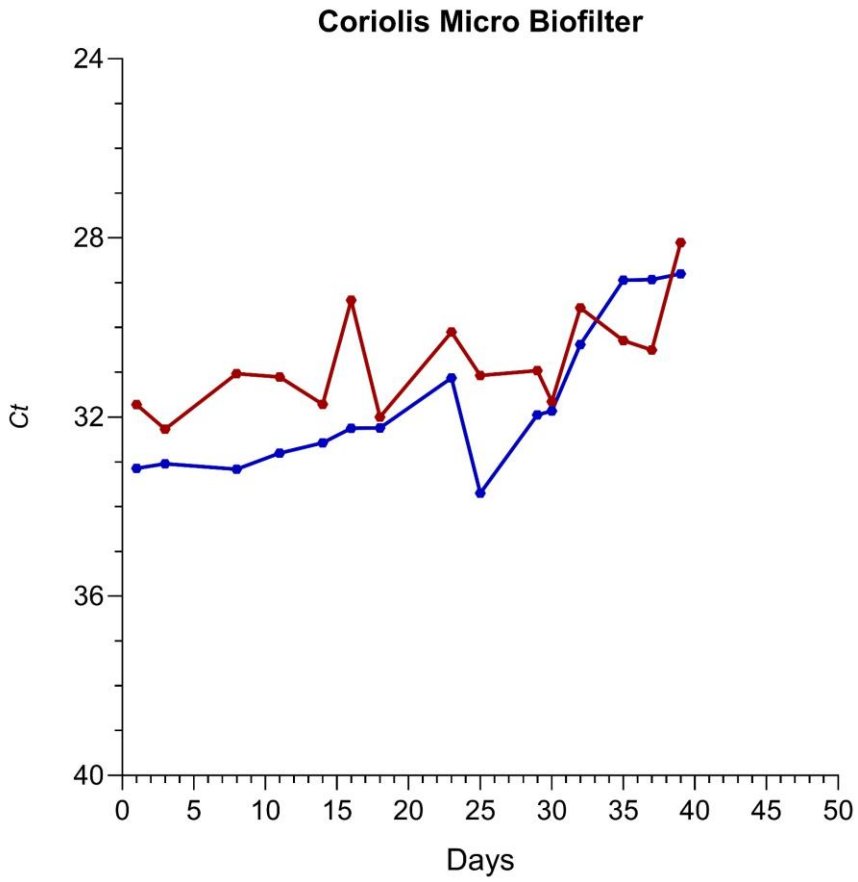
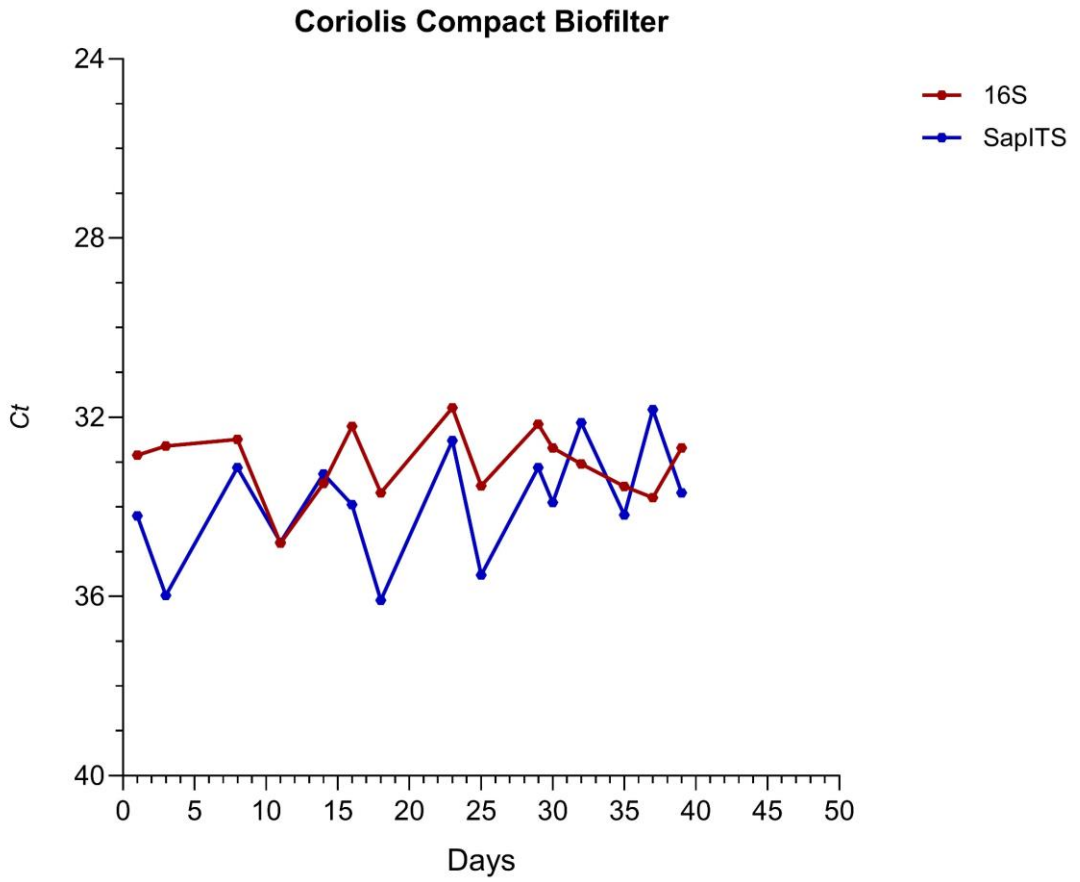
Benchmarking of aerosol samples





Ct > 40; negative results

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Does it answer our research question? Not really!

- Although we see the pathogens in the air, is it live?
- Or is it only eDNA/eRNA
- Failure to isolate IPNV in cell line (initially)
- However, we were able to grow bacteria on culture plates

- 16S sequencing of these bacterial isolates revealed environmental bacteria such as *Rhodococcus spp.*, *Microbacterium spp.*, *Staphylococcus equorum* etc.
- So partial success!

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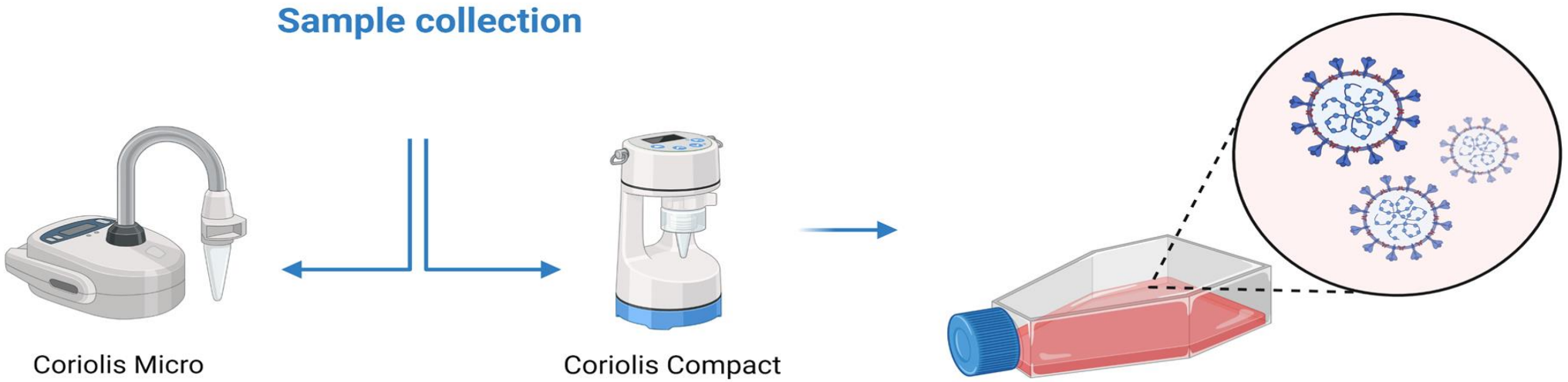
What was done next?

- Targeted sampling during an outbreak event of IPNV
- Samples were collected using the two samplers
- The sampling area was the biofilter room
- 2 cell lines BF-2 and EPC, 2 passages



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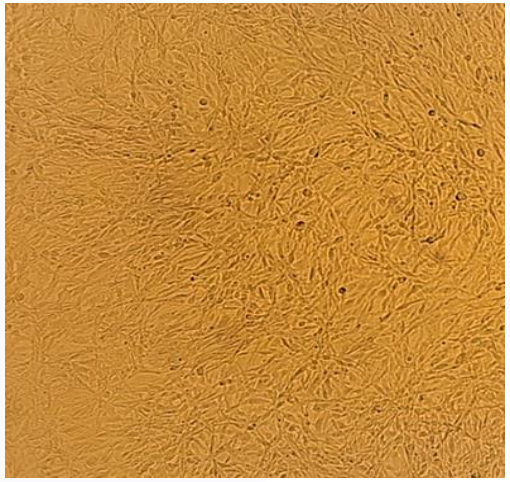
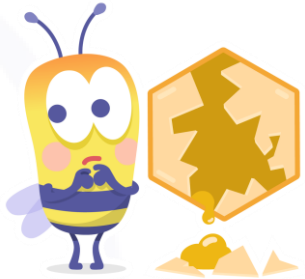
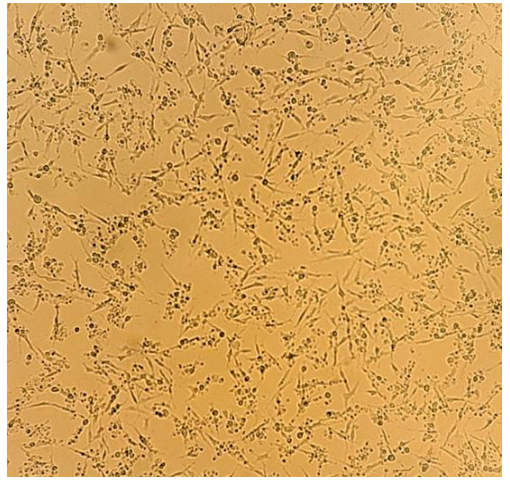
Is it just eRNA(again), or did we find live virus?

BF-2



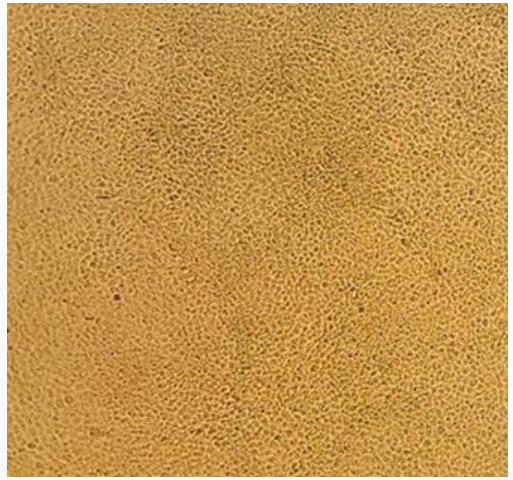
Healthy cells

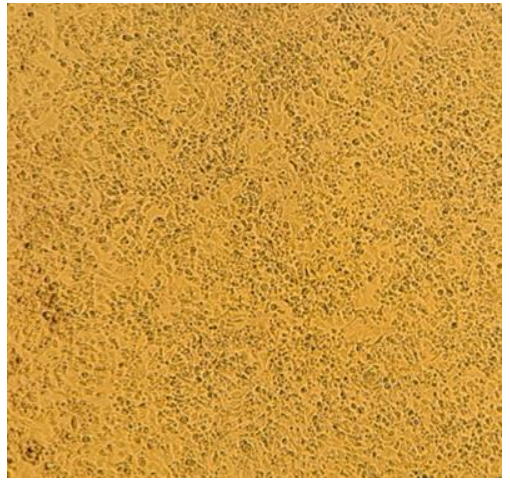


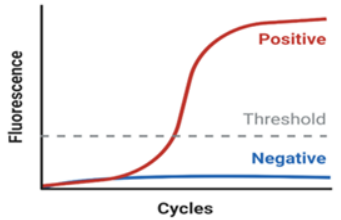


Not so healthy cells

EPC







Confirmation with RT-qPCR

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Is our research question answered now?

- Yes!
- We have proof that live fish pathogens (viruses) are aerosolised
- Transmission: Some previous studies have shown that there is tank-to-tank transfer in experimental studies
- More work is needed to consolidate the concept of aerosol-mediated transmission (experimental setup)

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Implementation

RASOPTA — Safeguarding Future Production of Fish in Aquaculture Systems with Water Recirculation

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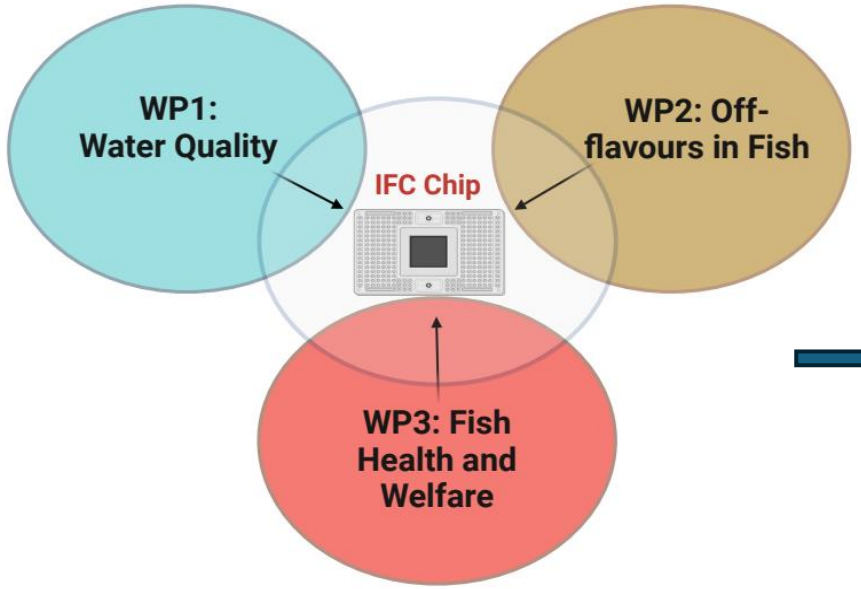
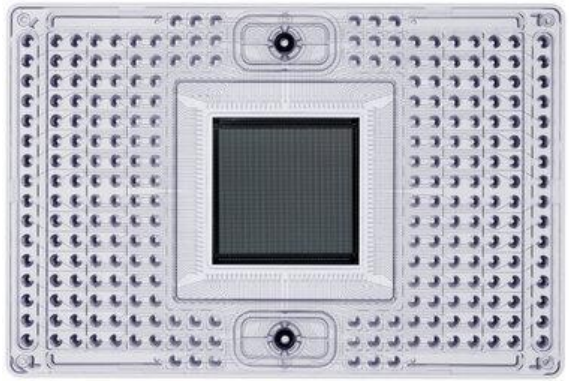
Work package 1: Water quality
Contributors: Fernando Fernando, Sara Sousa e Brito, Sujan Khadka

Work package 2: Off-flavours
Contributors: Julia Södergren, Pedro Martínez Noguera, Mariana Rodriques da Silva, Matteo Egiddi

Work package 3: Fish health and welfare
Contributors: Manuel Thibaud Blonc, Dhiraj Krishna, Cyril Henard, Hazim Sajiri, Hanxi Li





48 samples

48 different test/assay

One single output

Fast, efficient and high throughput (as opposed to conventional methods)

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Takk fyri!

Thanks to Debes, Petra and Maria

The PATO team

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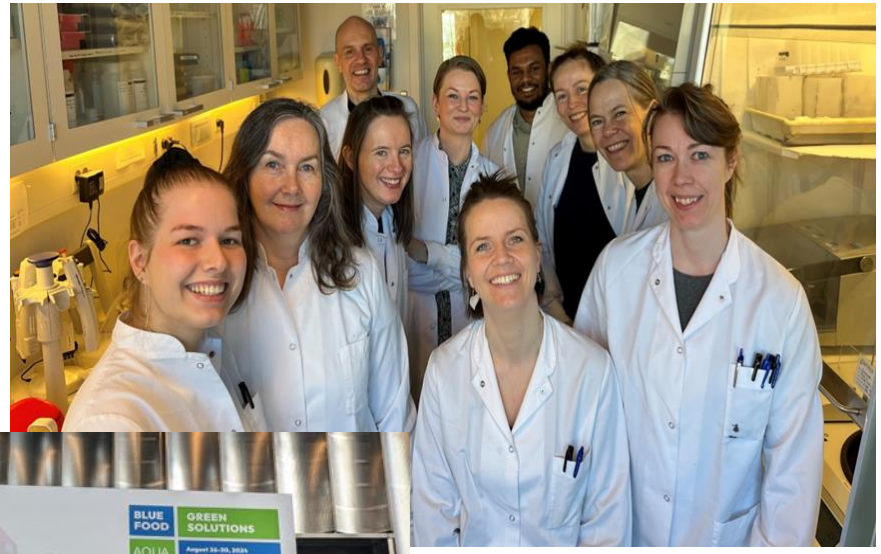
EU Horizon 2020



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