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TESTING INTRA-VITAM DIAGNOSTICS FOR GILL DISEASE IN COMMON CARP – A CASE STUDY FOR CARP EDEMA VIRUS INFECTION AND EPITHELIAL OR ENVIRONMENTAL SAMPLING BASED METHODS.

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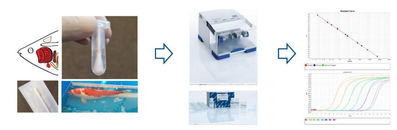
Cure4Aqua
Curing EU aquaculture by co-creating health and welfare innovations

Introduction

Recently, non-lethal or environmental sampling has been recognised as a tool for monitoring the presence of pathogens in aquaculture. Measurement of immune responses in the epithelium and the presence of pathogens in the epithelium and water appear to be particularly suitable for the detection of mucosal pathogens in fish. Gill and skin diseases are often multi-pathogenic, including co-infections with viruses, bacteria and parasites, and can induce a plethora of different immune responses. For example, carp edema virus (CEV) infection is indicative of immunosuppression of adaptive responses and often occurs with co-infections with ectoparasites such as *Ichthyobodo necator* and the bacterium *Flavobacterium branchiophilum*, which drive the proinflammatory responses and pathology, making diagnosis and treatment difficult. As carp cannot always be sacrificed for sampling during the production cycle, we tested the applicability and robustness of epithelial immune response monitoring methods and environmental DNA-based methods for the detection of pathogens associated with KDS.

Materials and Methods

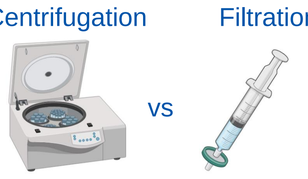
To test the selected methods for rapid detection of KDS, water samples, gill swabs and gill biopsies were collected during disease outbreaks and experimental infections and stored frozen at -20°C. Several centrifugation speeds and different pore size filters were used to select the best method for concentrating pathogens from water. Detection of carp edema virus, *Ichthyobodo necator* and *Flavobacterium sp*. was performed by qPCR after DNA extraction using a Qiagen DNA mini kit. Immune responses were measured using a Fluidigm array and correlated with pathogen load and pathological changes.

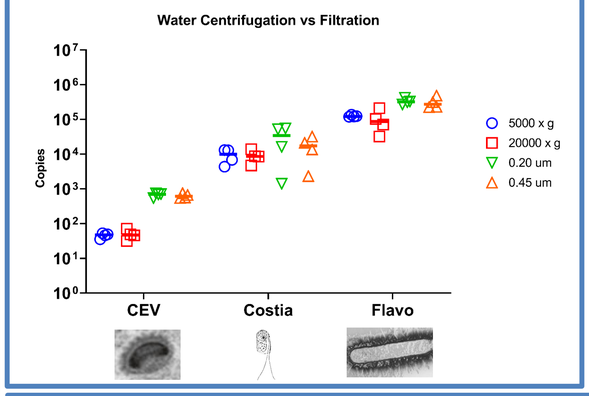


Results

Concentration procedure comparison

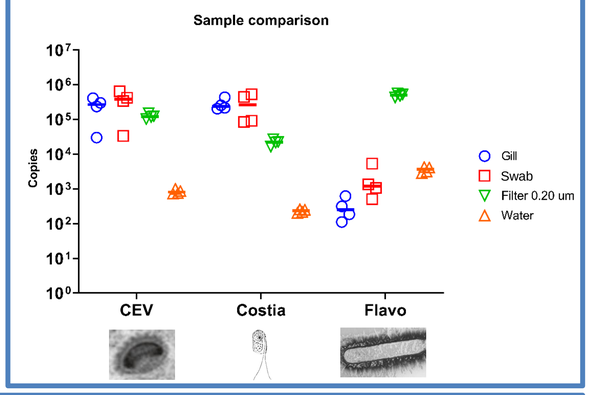
Water Centrifugation vs Water Filtration





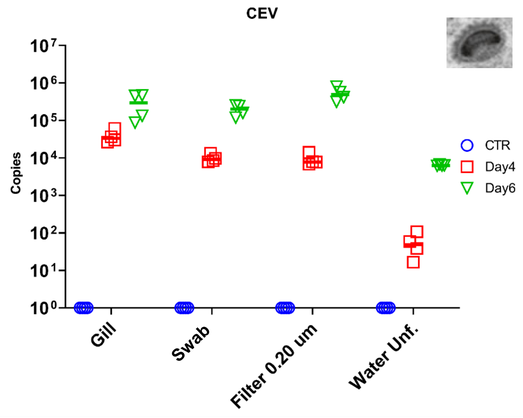
Sample type comparison during KSD outbreak

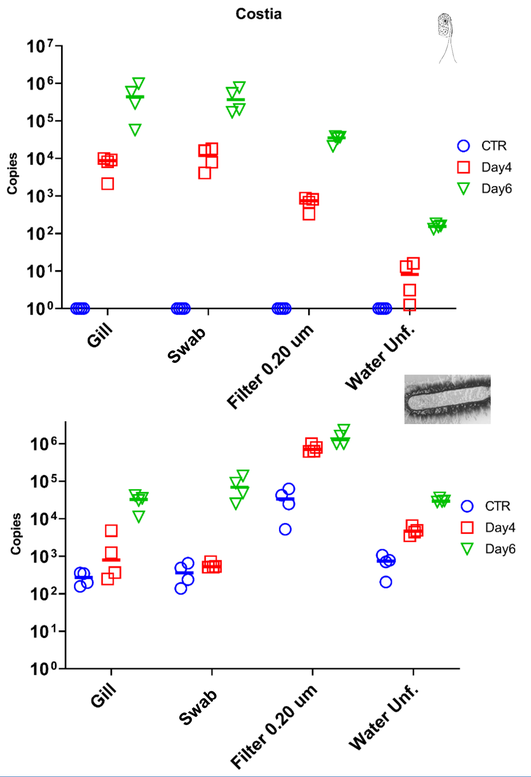
Gill biopsy vs Gill swab vs Water Filtration vs Water



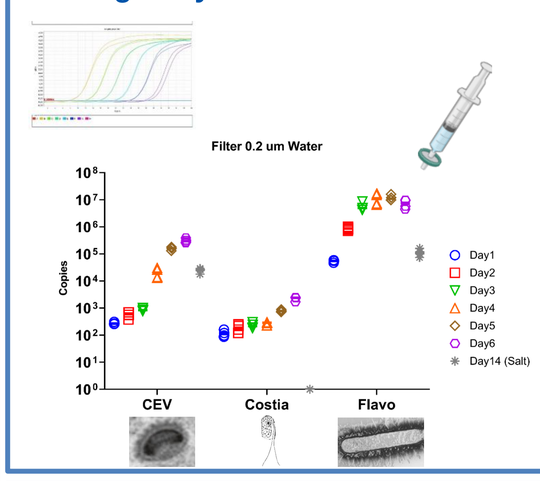
Sample type comparison vs infection dynamics

Gill biopsy vs Gill swab vs Water Filtration vs Water

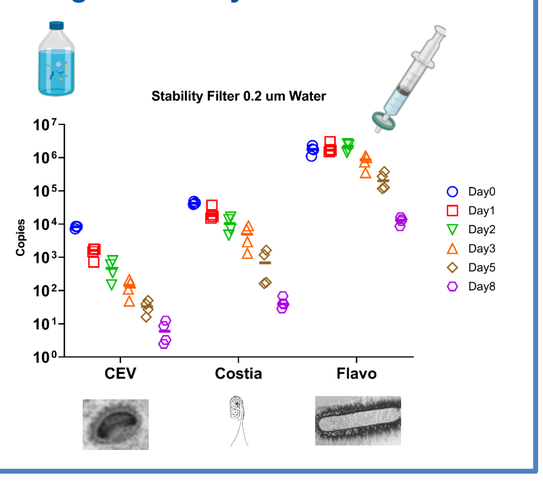




Pathogen dynamics in water



Pathogen stability in water



Main observations

- Filtration (0.20 µm and 0.45 µm) appeared to be the most reliable method for concentrating the pathogens associated with KDS outbreaks
- The detection of CEV, *I. necator* and *Flavobacterium* sp. was possible at very early stages of infection
- The CEV concentration increased rapidly on day 4 onwards when the first clinical signs were visible.
- Furthermore, the DNA of all pathogens could be detected in the water for at least 8 days after removal of infected fish.
- Gill biopsies and swabs allowed the detection of immune responses previously measured during CEV infections: increased antiviral and proinflammatory responses and decreased levels of adaptive immunity markers.

Detection of immune responses in gill biopsies and swabs vs gill samples

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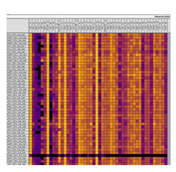
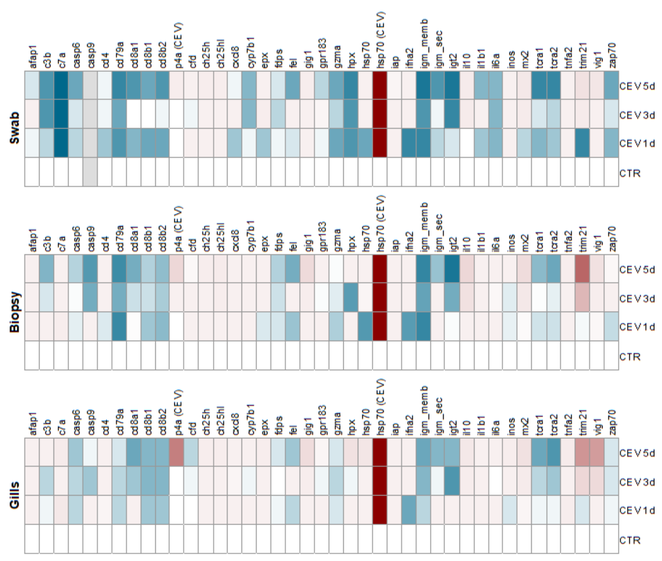


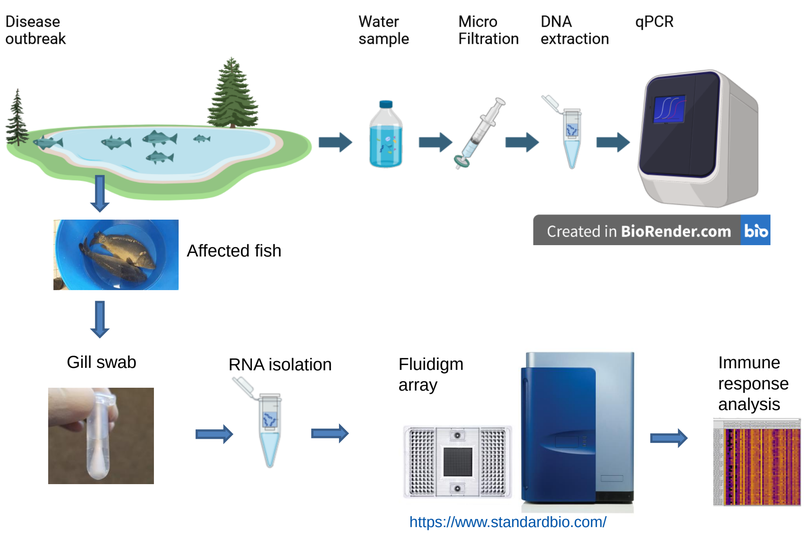
Gill swab

Gill biopsy

Gill sample (collected during necropsy)



Proposed diagnostic procedures



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Environment sampling – detection of pathogens

Minimal invasive fish gill sampling – detection how fish are doing

Conclusions

Concentration of all pathogens involved in multi-pathogen gill disease associated with carp edema virus infection was possible with a single water filtration procedure using e.g. a 0.20 µm syringe filter. eDNA-based diagnosis could therefore be a very efficient method for detecting outbreaks of KSD, flavobacteriosis and ichthyobodiasis, at least in relatively small water bodies such as small ponds or tanks. Gill swabs appeared to be as reliable as gill biopsies or post-mortem samples for detecting immune responses characteristic of KDS. Additional immune markers might need to be evaluated to better distinguish between other types of infection.

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