Using gill mucus to monitor immune gene expression of farmed Atlantic salmon

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Head of Biotechnology department

Fiskaaling

knowledge for sustainable aquaculture

Focus on the inflammatory reactions to multi-factor and chronic stress

- ✓ Seasonal time-course of gill disease
- ✓ Commercial scale/natural outbreak of gill disease
- ✓ qPCR primers for mucus and immune-response genes

Gene expression analysis of Atlantic salmon gills reveals mucin 5 and interleukin 4/13 as key molecules during amoebic gill disease

Mar Marcos-López, Josep A. Calduch-Giner, Luca Mirimin, Eugene MacCarthy, Hamish D. Rodger, Ian O'Connor, Ariadna Sitjà-Bobadilla, Jaume Pérez-Sánchez & M. Carla Piazzon

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RESEARCH ARTICLE

Genome-wide analysis of Atlantic salmon (*Salmo salar*) mucin genes and their role as biomarkers

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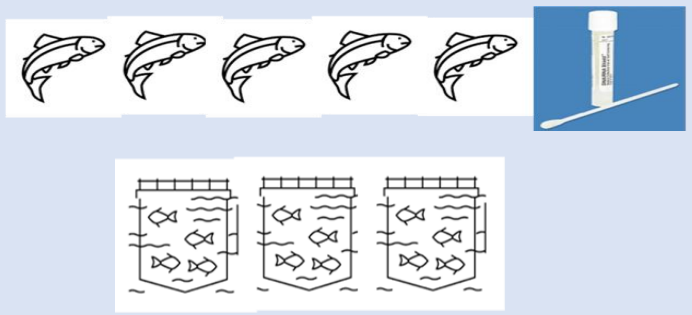
Sampling strategy based on routine operations

Mucus swabs



- Routine gill checks
- First 5 fish scored/cage, 3 cages
- Blinded to gill score
- Zymo DNA/RNA shield

Time-series of gill health

- Pilot study
- Sampling every other week (n=8)
- Plan: Capture seasonal increase in complex gills issues

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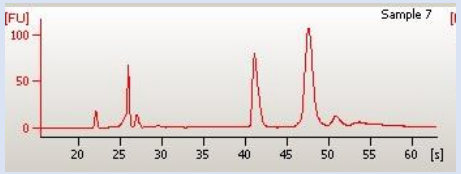
Performed in collaboration

Environmental risk

- Phytoplankton at 5m

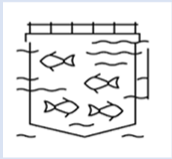
Mucus gene expression by ddPCR

RNA extraction



- Performed on individual gill swabs
- Zymo kit with bead-beating
- 400 ul supernatant input
- Mean of 48 ng/ul

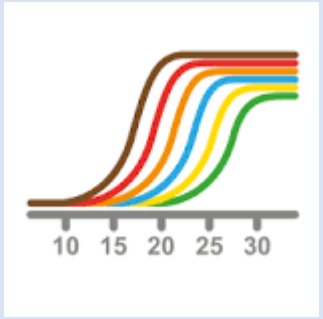
cDNA



= 1 cDNA

- Pooled 200ng RNA/swab
- Zymo clean and concentrate to 14 ul
- RT Bio-Rad iScript
- 1µg cDNA

qPCR



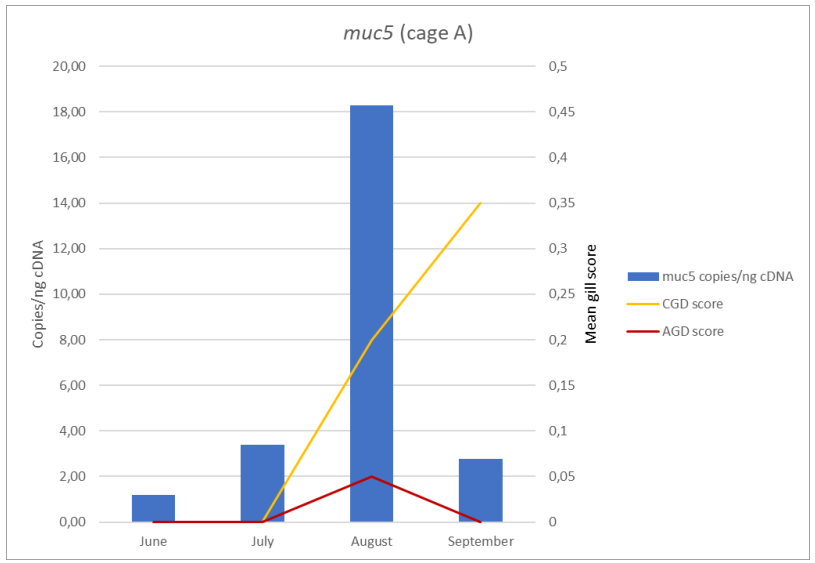
- SybrGreen
- Initial screening of gene expression
- *muc5* and *il4/13a* normalized to *β-actin* and *etif3*

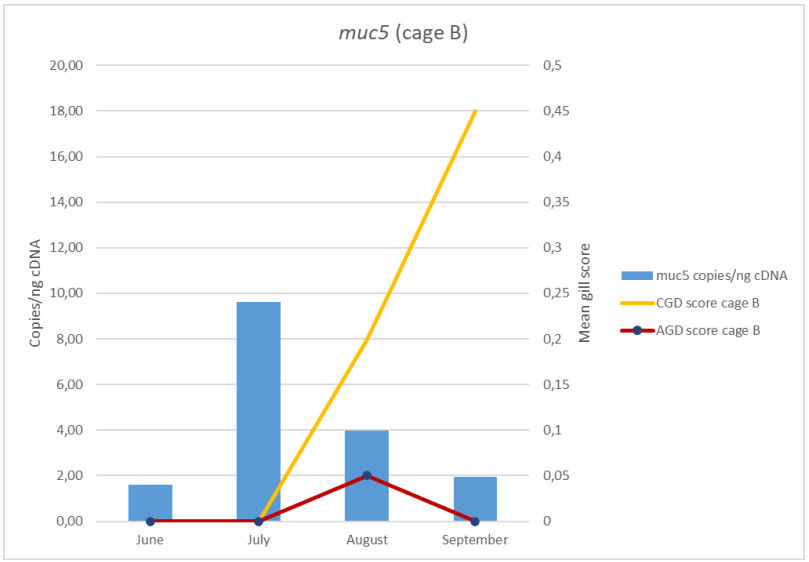
ddPCR

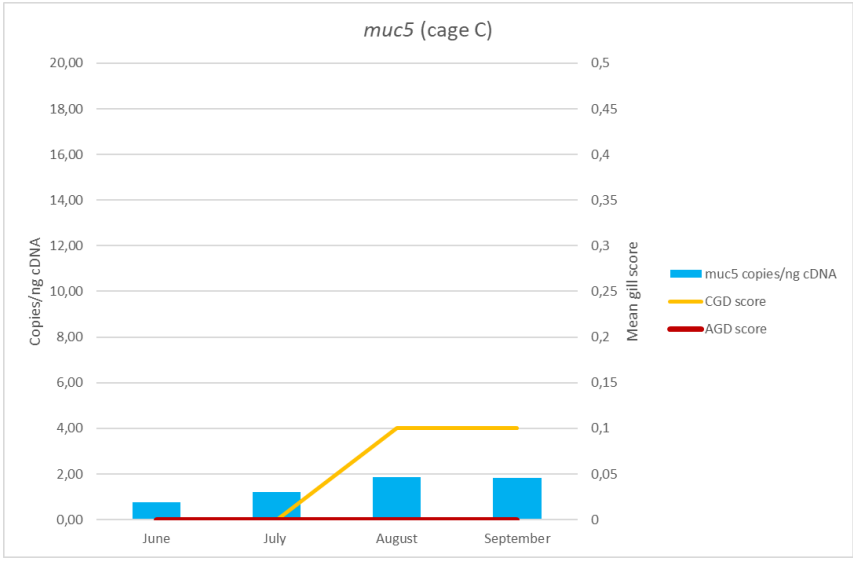
- Quantitative
- Performs with lowly expressed targets
- Less sensitive to PCR inhibitors

ddPCR: *muc5* expression in mucus increased with the onset of gill issues and signs of AGD

Trend of increase with severity of complex gill scores…but very general

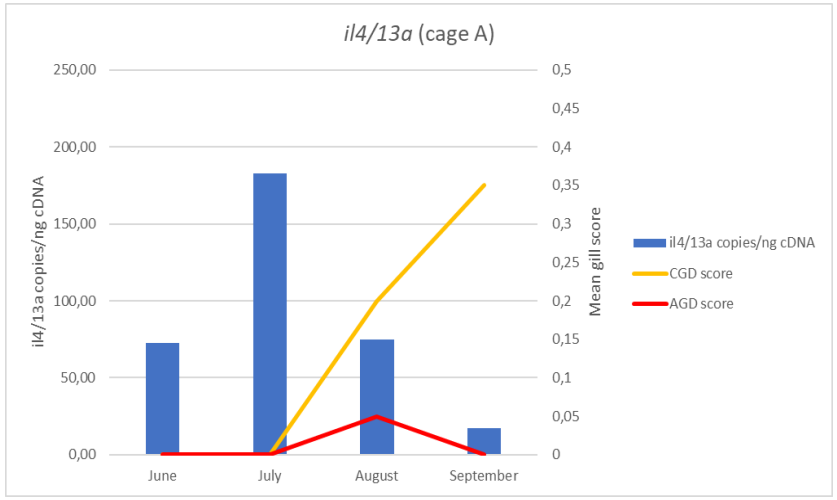


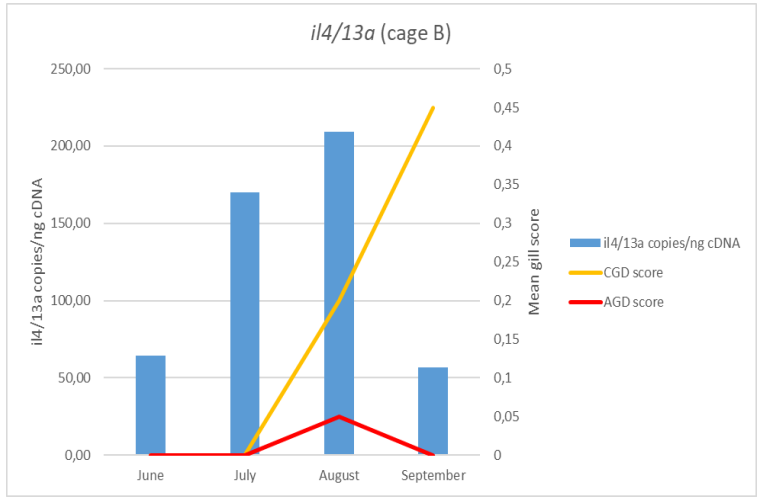


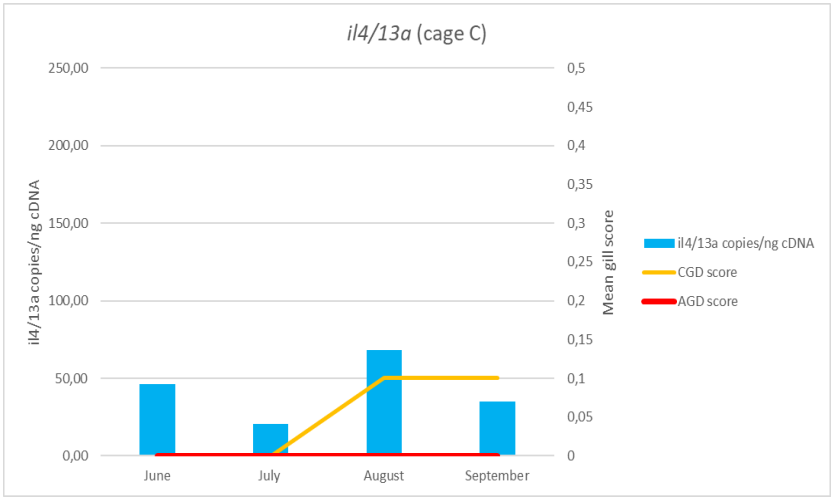


Possible reasons for the lack of correlation in August/September:
Other candidate genes are better targets for CGD disease management in mucus
Variability in scoring

ddPCR: *il4/13a* expression in mucus increased with the onset of gill issues and signs of AGD



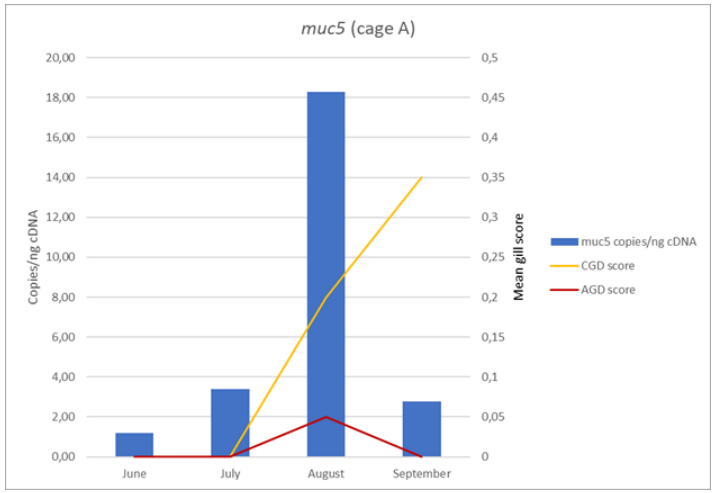


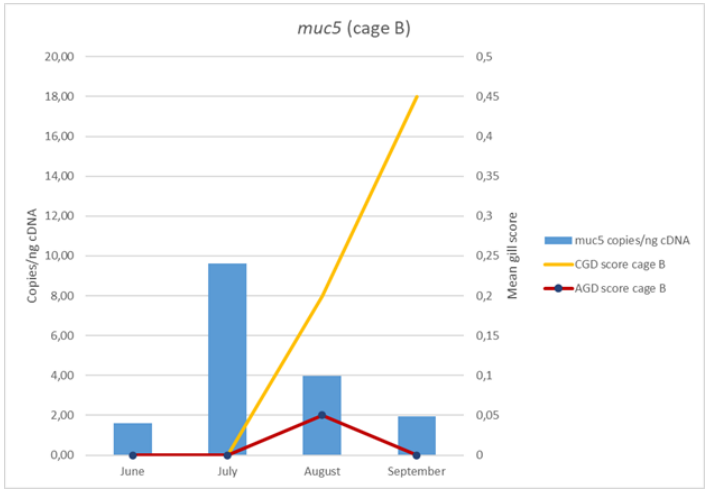


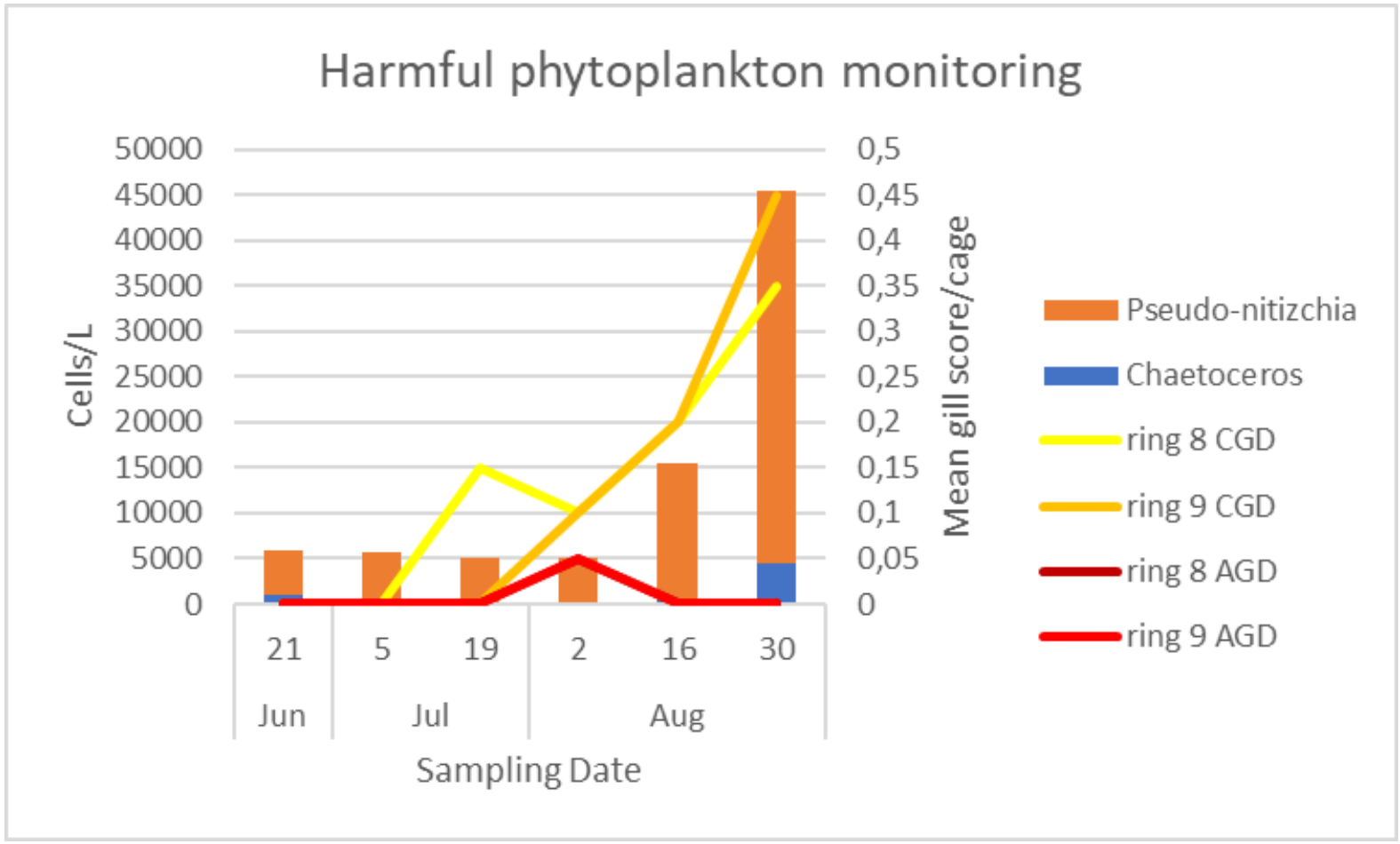
Possible reasons for the lack of correlation in August/September:
Other candidate genes are better targets for CGD disease management in mucus
Variability in scoring

Harmful phytoplankton increases with gill scores

muc5 increase is prior to the increase in phytoplankton

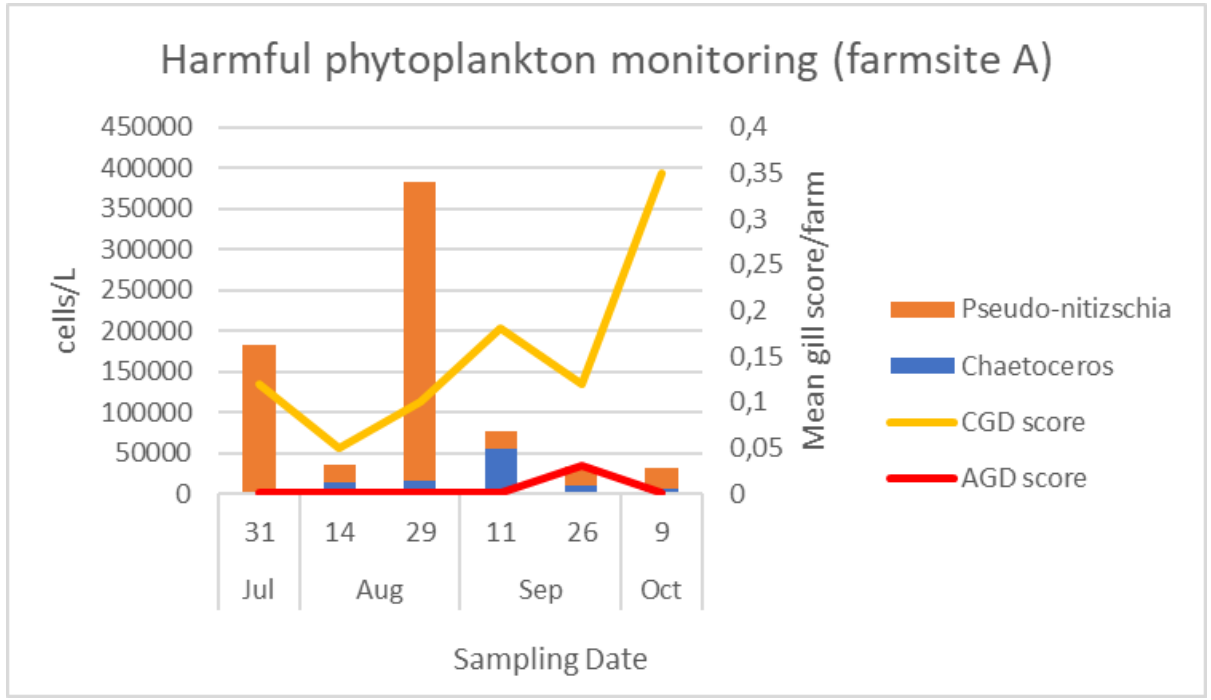


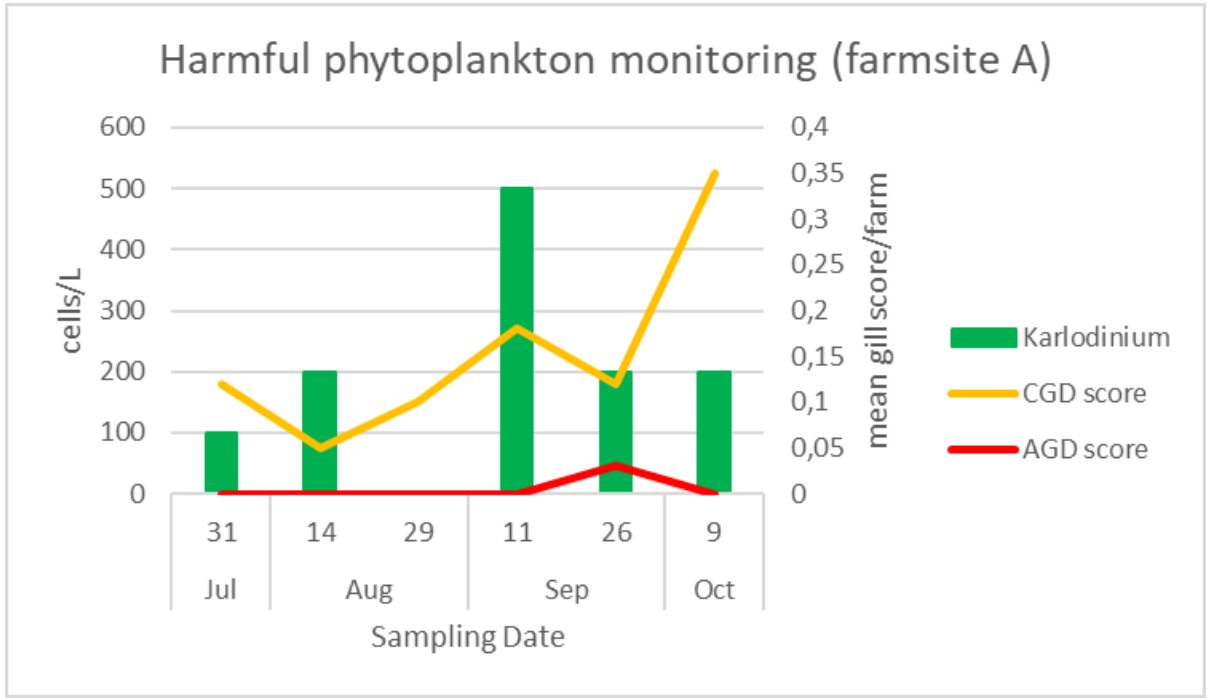




Phytoplankton sampled for the pilot project, not service data

2023 data: Phytoplankton monitoring as a service for the Faroese aquaculture industry





Data provided by Bakkafrost

Jellyfish monitoring pilot project started in August 2023

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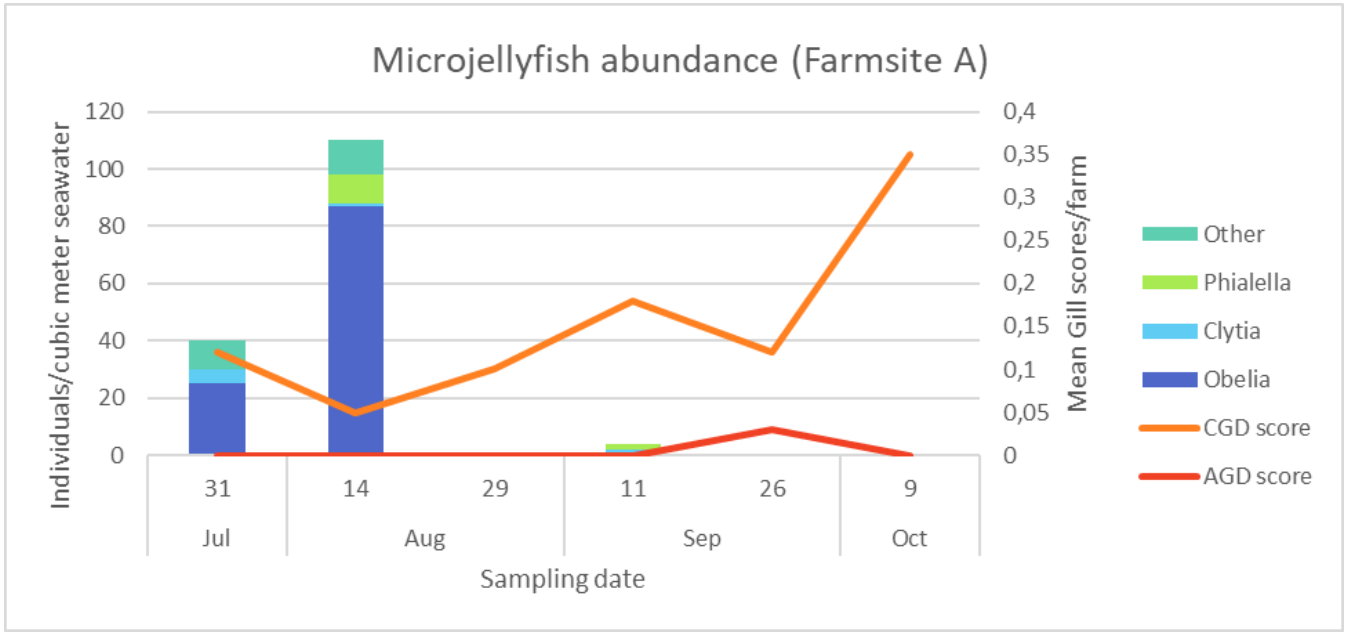
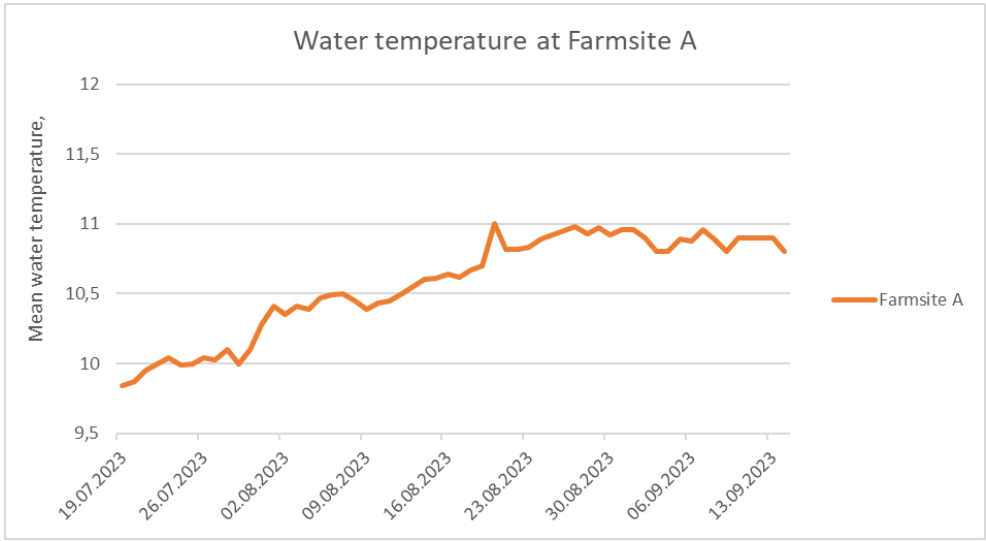
Performed in collaboration





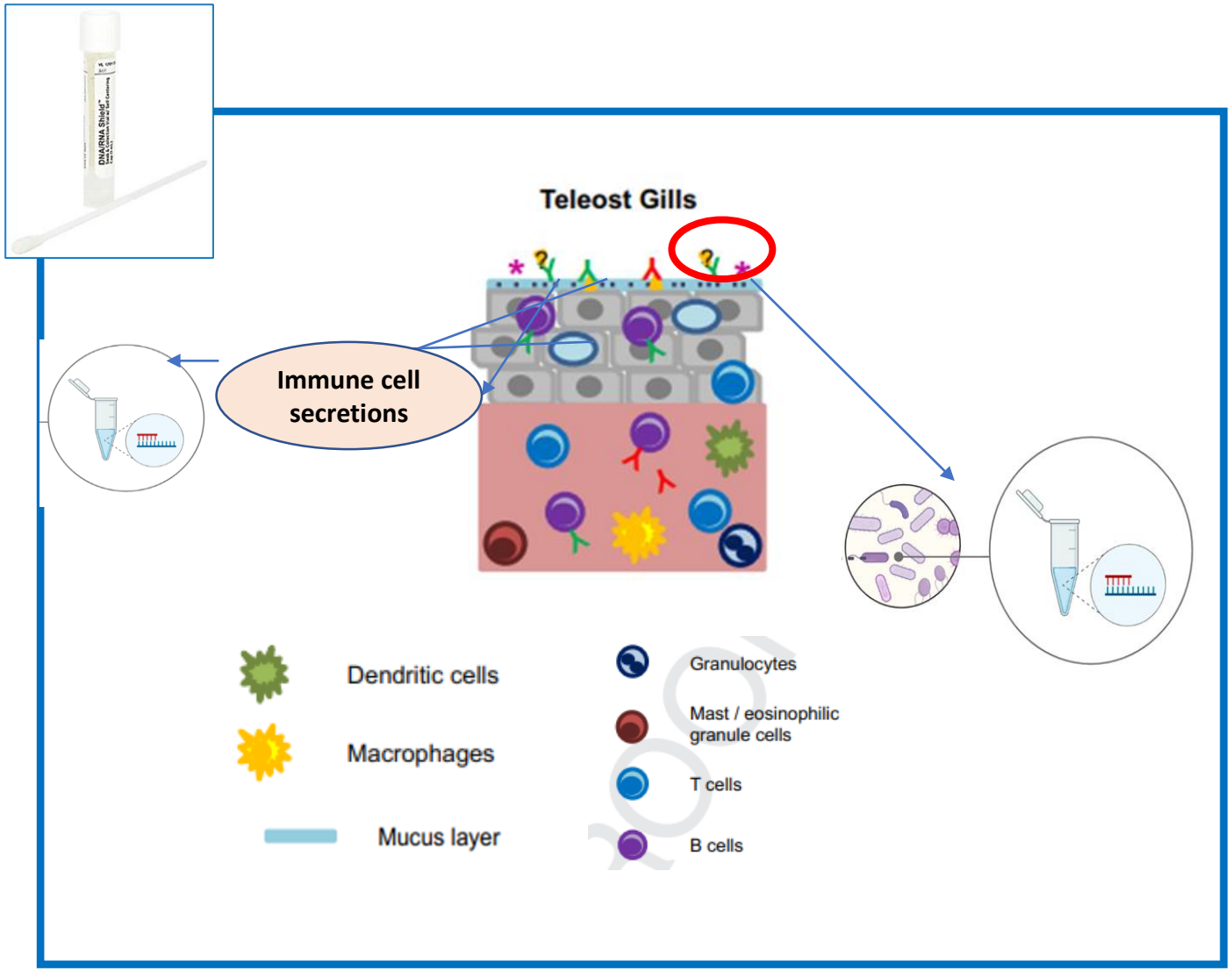
Conclusion

- Introduction to Faroese research on gill health
  - Phytoplankton
  - Jellyfish

- Field confirmation of other published studies
  - Mucin and immune response genes are regulated during gill disease
  - Identifiable in the mucus
  - Results are variable in complex gill disease

Future Directions: Early stages and triggers of complex gill disease

- Immune response and loss of microbial tolerance
- Phytoplankton and Jellyfish monitoring
  - Regional and seasonal differences in population dynamics



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  - Phytoplankton and Jellyfish sampling (Jógvan Johansen)
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