

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





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Head of Biotechnology department

# 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

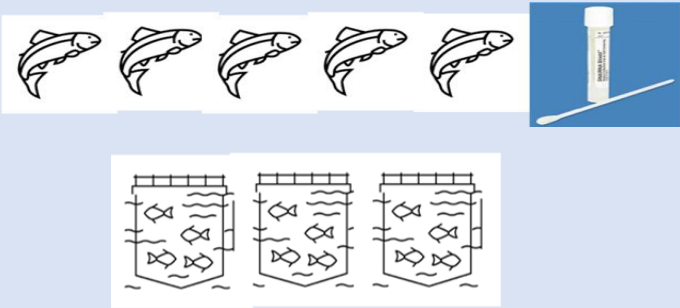
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# Sampling strategy based on routine operations

## 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

## Environmental risk



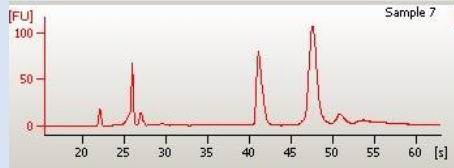
- Phytoplankton at 5m



Performed in collaboration

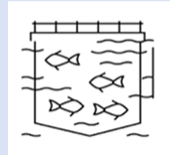
# Mucus gene expression by ddPCR

## RNA extraction



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

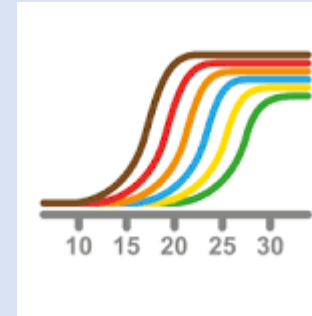
## cDNA



= 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  $\beta$ -actin and *etif3*

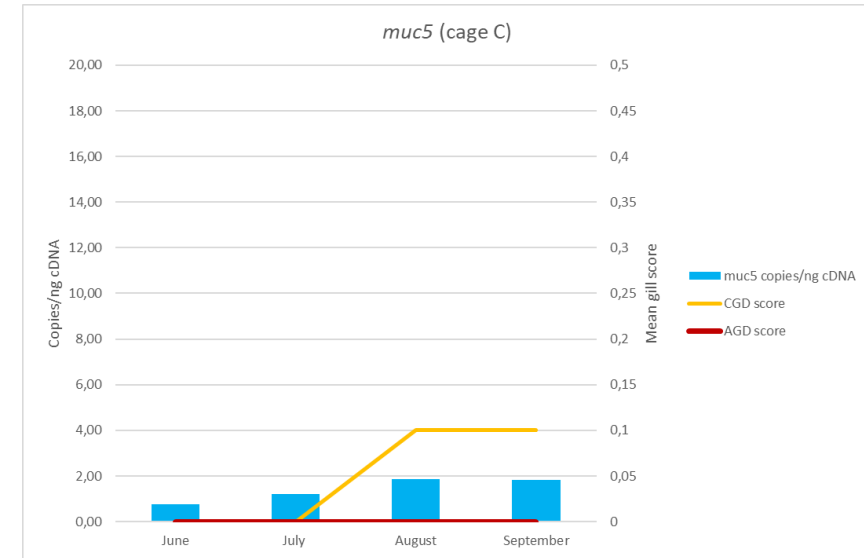
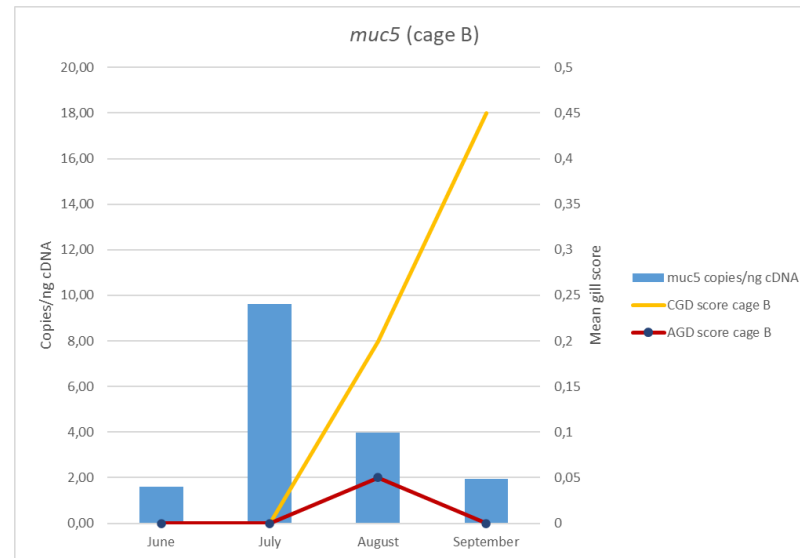
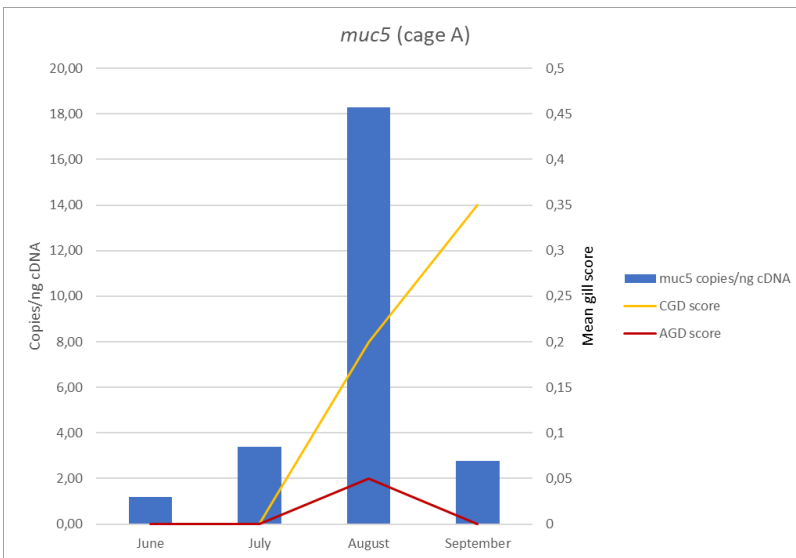
## ddPCR



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

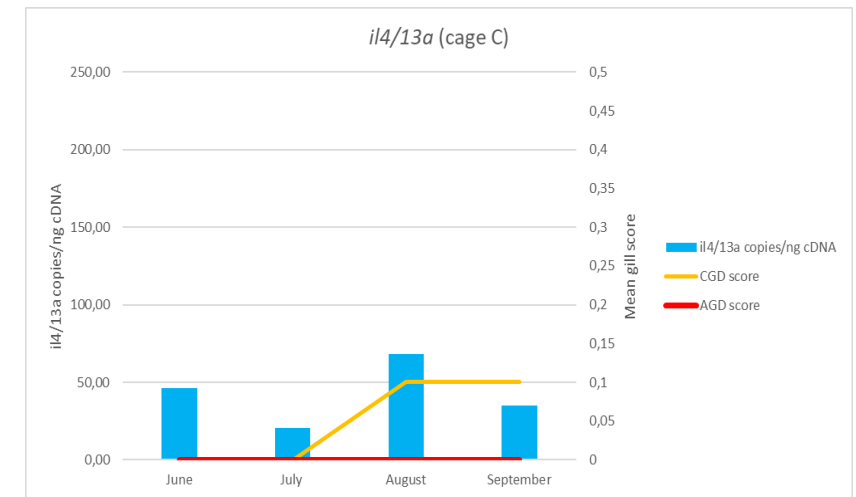
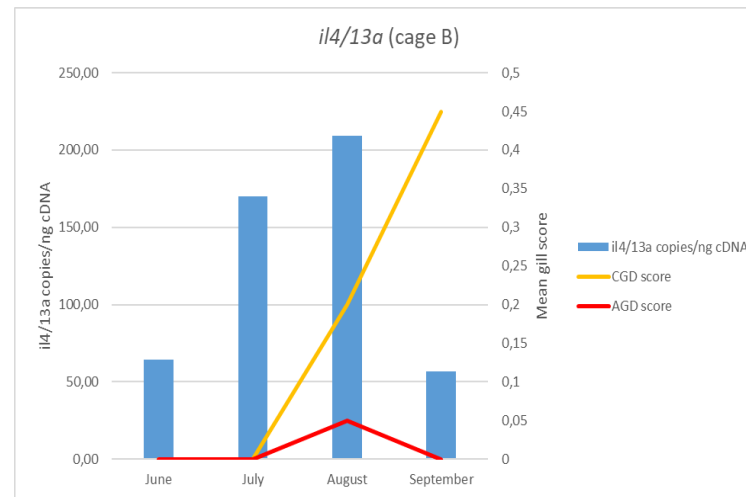
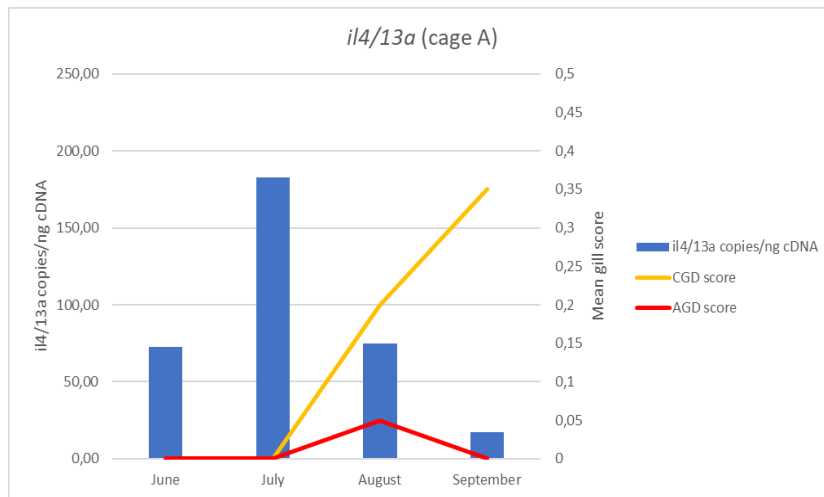
# ddPCR: *muc5* expression in mucus increased with the onset of gill issues and signs of AGD

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

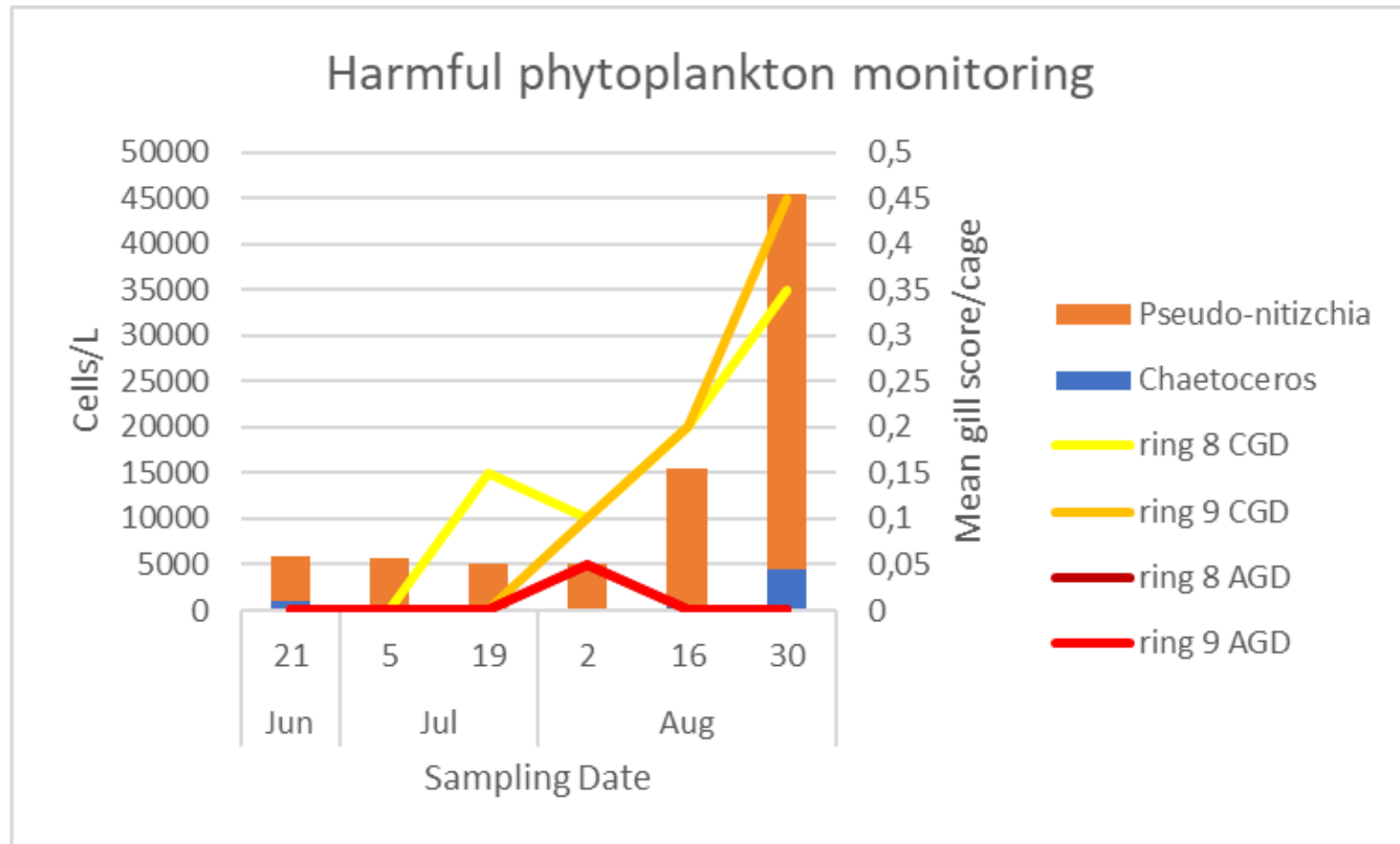
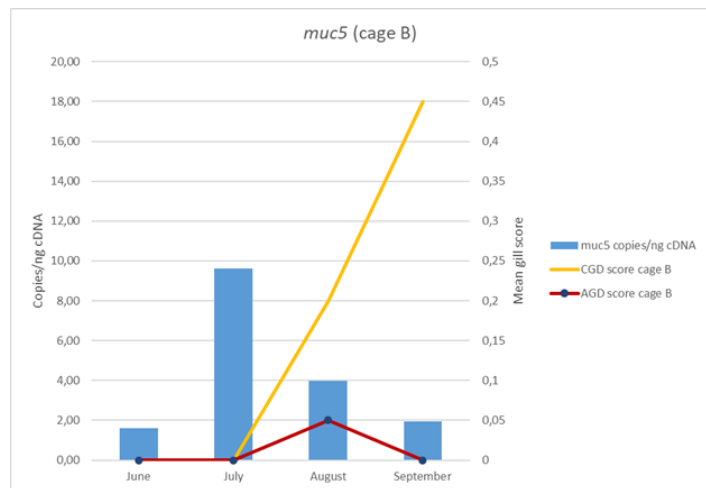
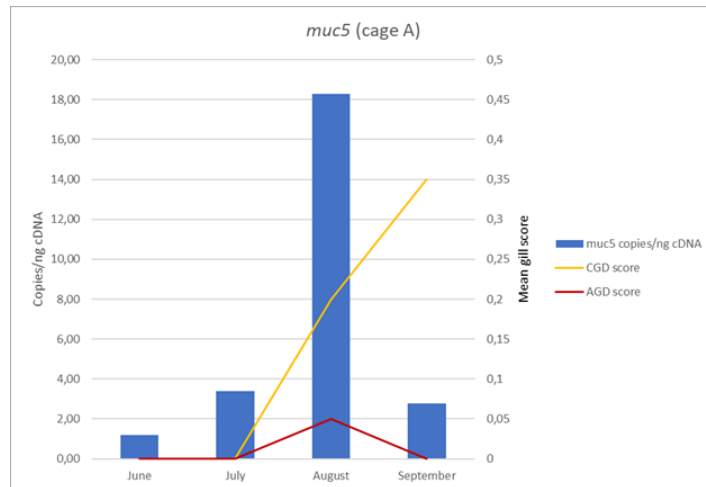
# ddPCR: *il4/13a* expression in mucus increased with the onset of gill issues and signs of AGD



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

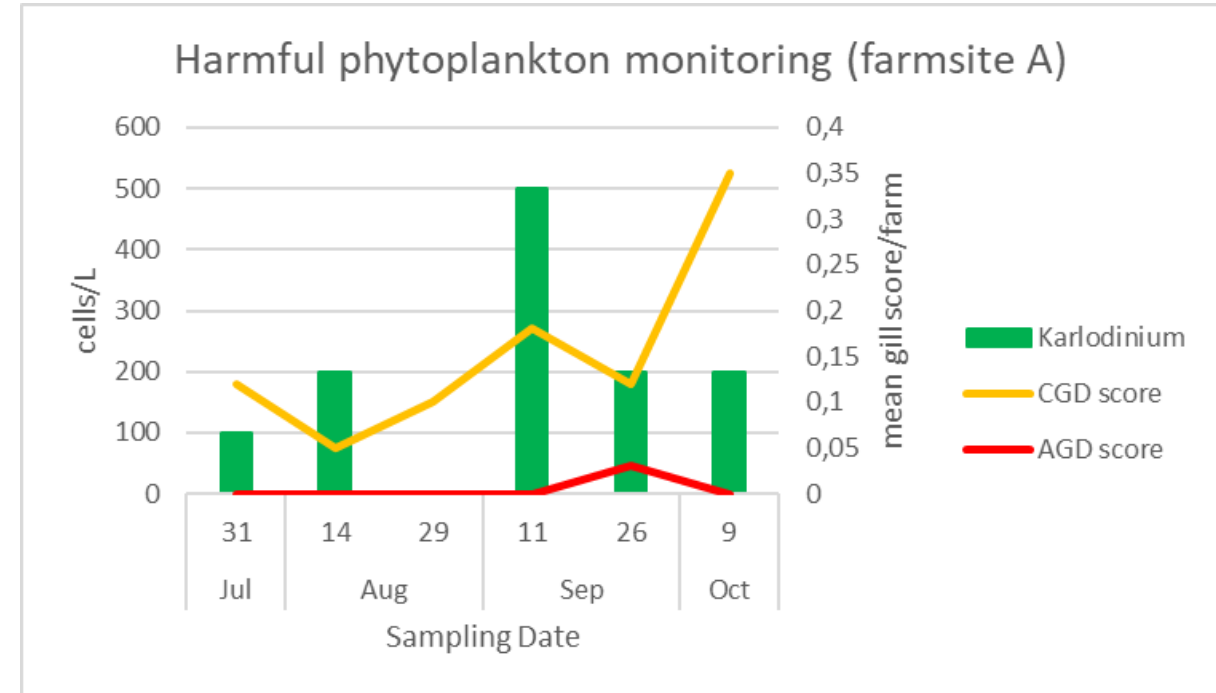
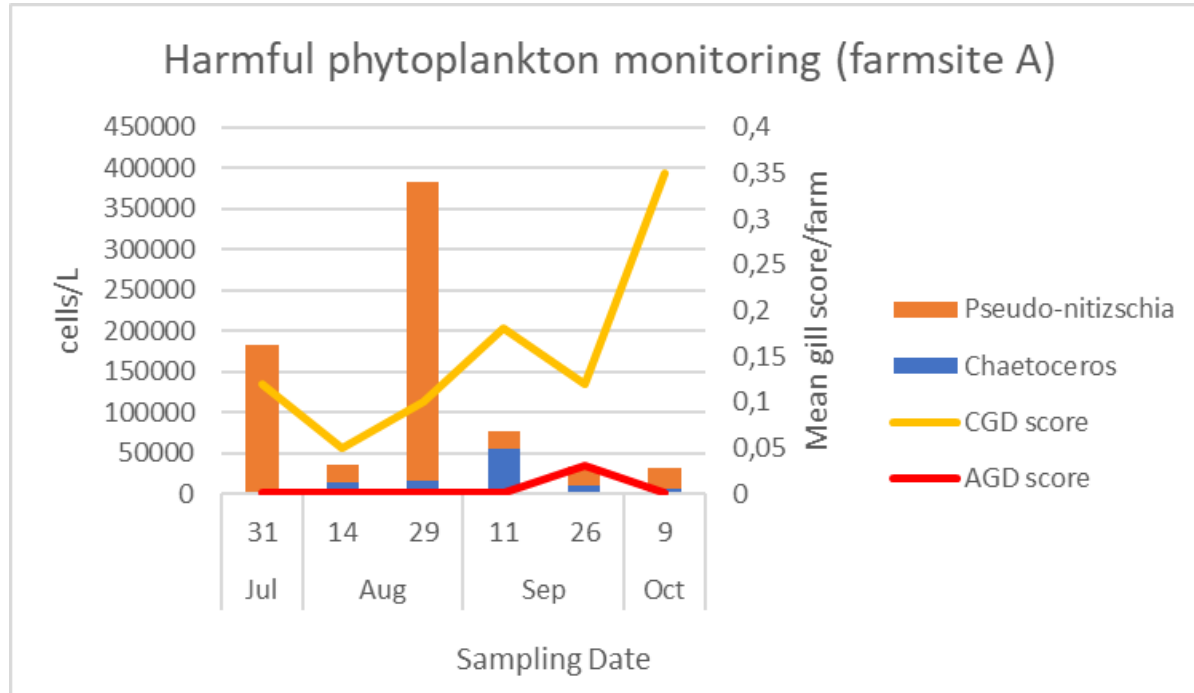
# Harmful phytoplankton increases with gill scores

muc5 increase is prior to the increase in phytoplankton



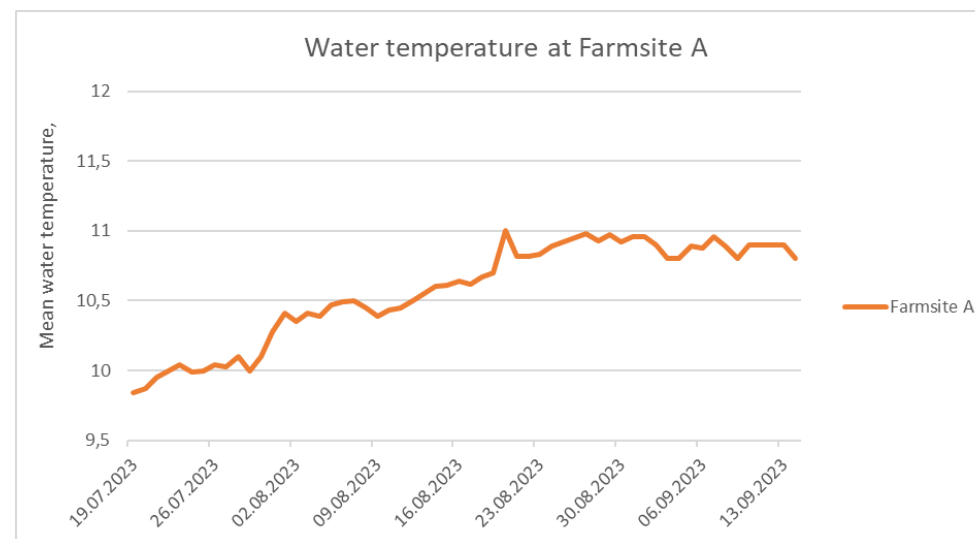
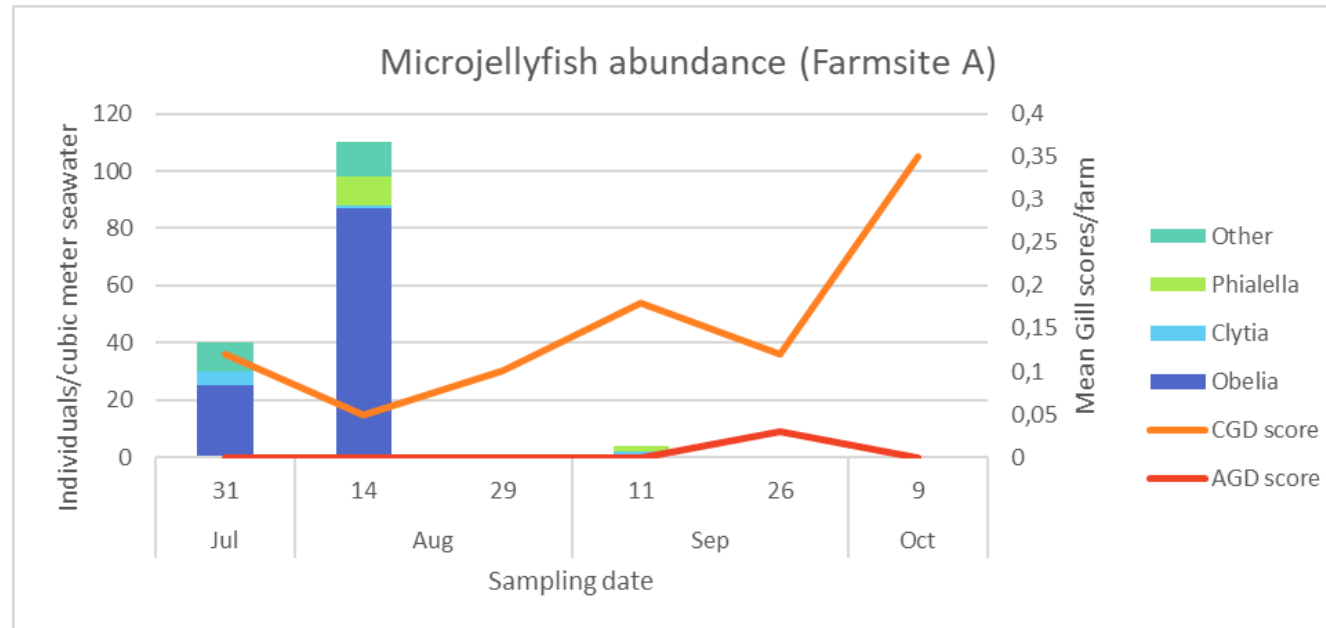
Phytoplankton sampled for the pilot project, not service data

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



Data provided by Bakkafrøst

# Jellyfish monitoring pilot project started in August 2023

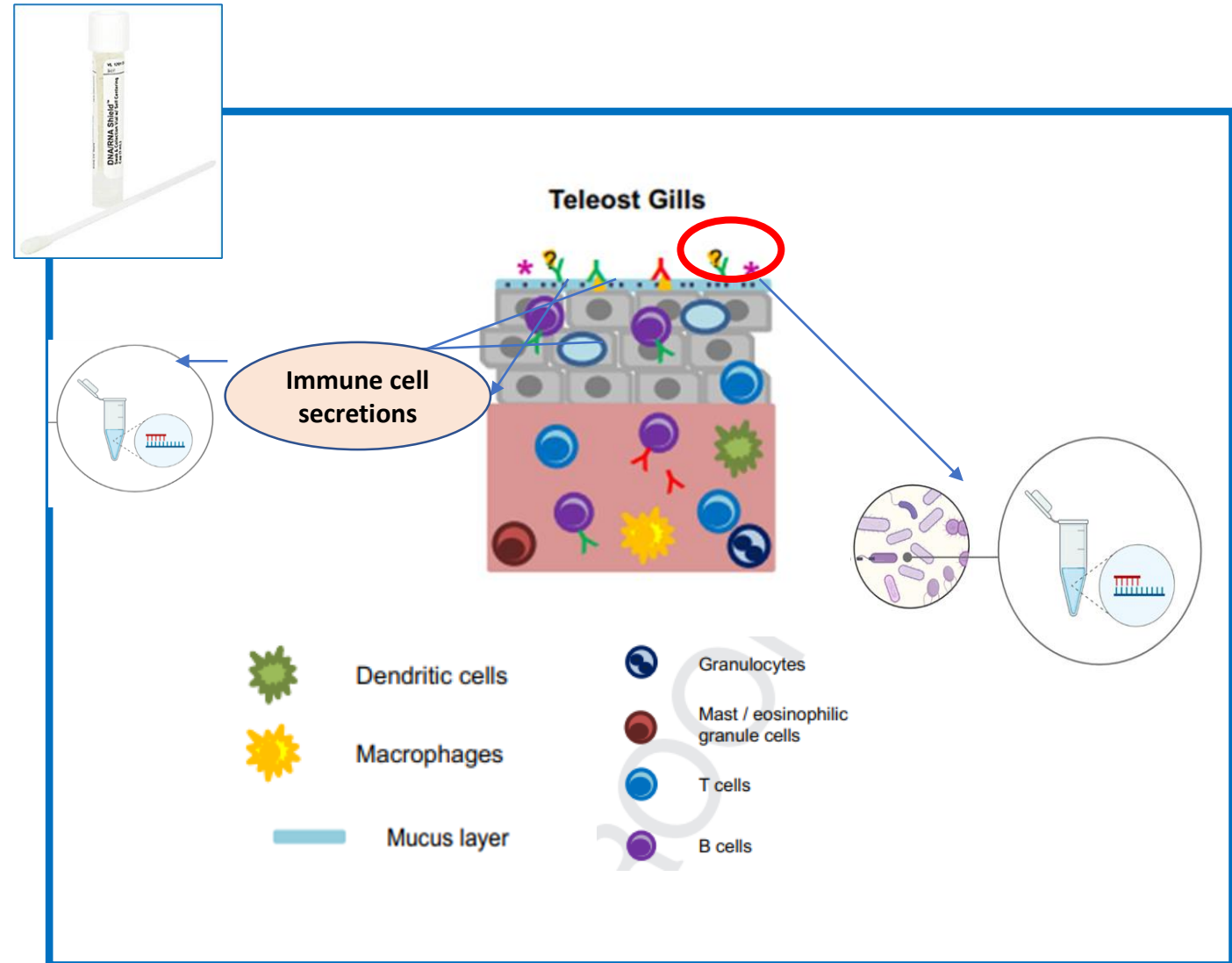


# Conclusion

- 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



# Acknowledgements

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- Phytoplankton (Ása Jacobsen, Elin Jacobsen, Eiríkur Danielsen)
- Jellyfish (Eiríkur Danielsen)
- Mucus gene expression (Kim Bergkvist)

- **Bakkafrøst**

- Phytoplankton and Jellyfish sampling (Jógvan Johansen)

- **University of the Faroe Islands**

- ddPCR collaboration (Anni Djuurhus)

- **Hydrozoan Scotland**

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Phytoplankton service data provided by Bakkafrøst

