

Biomarkørar frá táknu svabum

- ikki deyðiligt amboð til forsøgn av fiska heilsuni -

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Vitan til varandi aling 2021

The goal of this project is to develop a routine monitoring tool for fish health, as a service to the salmon farming industry

Based on the scientific findings in this project

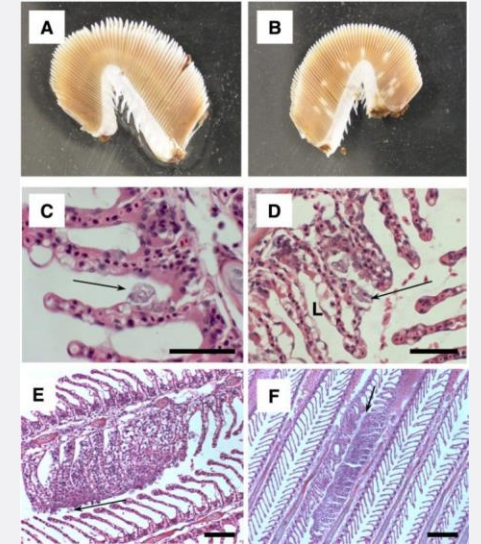


Current monitoring tool for gill health

- Histology (slow, expensive and time-consuming)
- PCR - Present/absent of infection agent
- Geared toward late-stage inflammation and damage

- We will develop an early warning tool
- Biomarkers from salmon epithelia cells – first line of defence
 - Quantitative/objective tools directly monitor immune function/fish health

- Sampling should be Non-lethal
 - The 3 Rs – science are guiding principle for more ethical use of animals in testing



Gills AGD-affected salmon

Chosen biomarkers

- Mucins
 - Glycosylated proteins produced by epithelial cells
 - Humans: biomarkers for several diseases including cancer and airways disease
- Immune related response:
 - RIG family (receptors are key players sensors of virus infection, recognize diff. viruses)
 - Interferons (signal molecules in response to virus infection)
 - Cytokines (broad group of signal proteins – stimulate the immune system)
- Scientific validated biomarkers in salmon (controlled experimental set-up)
- Tested their expression in different stressors in salmon
 - Handling stress (3h and 24h)
 - Exposed to AGD
 - Exposed to different viruses (IPNV, ISAV, SAV)
- Salmon tissue:
 - Gill tissue
 - Cell lines (head kidney leukocytes)

Description of biomarkers

Description	Gene name	Tissue	Note
Mucins	<i>muc2.1</i>	Gills	- Secreted mucin, increase after acute handling stress, up-regulated in 3h and 24h
	<i>mucin 2</i>	Gills	- Mucin genes showed most variability when exposed to AGD ^b and linked to disease severity
	<i>mucin 5</i>	Gills	
	<i>mucin 18</i>	Gills	
Immune genes:			
RIG family	<i>RIG-I</i>	TO cells	- Genes were up-regulated upon virus infection in general
	<i>LGP2</i>	TO cells	
Interferons	<i>IFNα</i>	TO cells	- Up-regulated upon IPNV ^c and ISAV ^d infection
	<i>TNF-α1</i>	TO cells	
	<i>IFNβ</i>	TO cells	- Up-regulated upon SAV ^e infection
Cytokines	<i>IL-12</i>	TO cells	- Genes showed most variability when exposed to AGD and linked to disease severity
	<i>interleukin 4/13a</i>	Gills	
	<i>interleukin 4/13b2</i>	Gills	

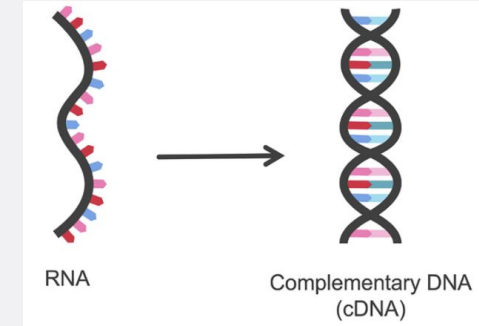
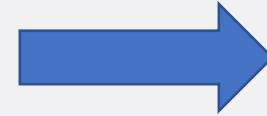
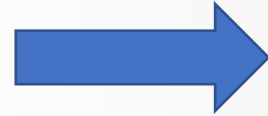
Description of the chosen biomarkers. ^aTO cells are derived from Atlantic salmon head kidney leucocytes, ^bAmoebic Gill Disease (AGD), ^cInfectious Pancreatic Necrosis Virus (IPNV), ^dInfectious Salmon Anaemia Virus (ISAV) and ^eSalmonid Alphavirus (SAV).

References:

1. Marcos-López, M. *et al.* Gene expression analysis of Atlantic salmon gills reveals mucin 5 and interleukin 4/13 as key molecules during amoebic gill disease. *Sci. Rep.* **8**, 1–15 (2018).
2. Sveen, L. R., Grammes, F. T., Ytteborg, E., Takle, H. & Jørgensen, S. M. Genome-wide analysis of atlantic salmon (*Salmo salar*) mucin genes and their role as biomarkers. *PLoS One* **12**, 1–15 (2017).
3. Nerbøvik, I. K. G. *et al.* Molecular cloning of MDA5, phylogenetic analysis of RIG-I-like receptors (RLRs) and differential gene expression of RLRs, interferons and proinflammatory cytokines after in vitro challenge with IPNV, ISAV and SAV in the salmonid cell line TO. *J. Fish Dis.* **40**, 1529–1544 (2017).

Sampling/method

Non-lethal sampling

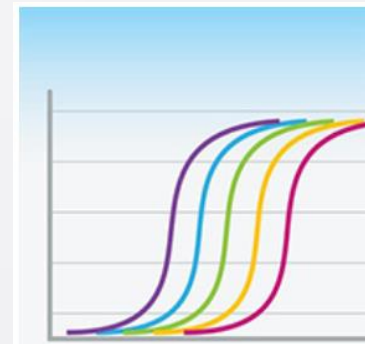


- Gill swab during sea lice counting
- DNA/RNA Shield medium

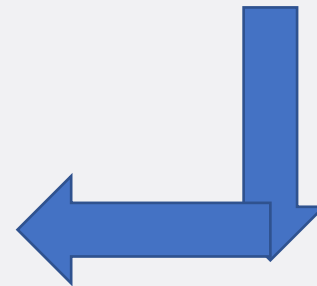
Extract RNA

- Convert RNA to cDNA
- Required input: 500 ng total RNA

RT-PCR



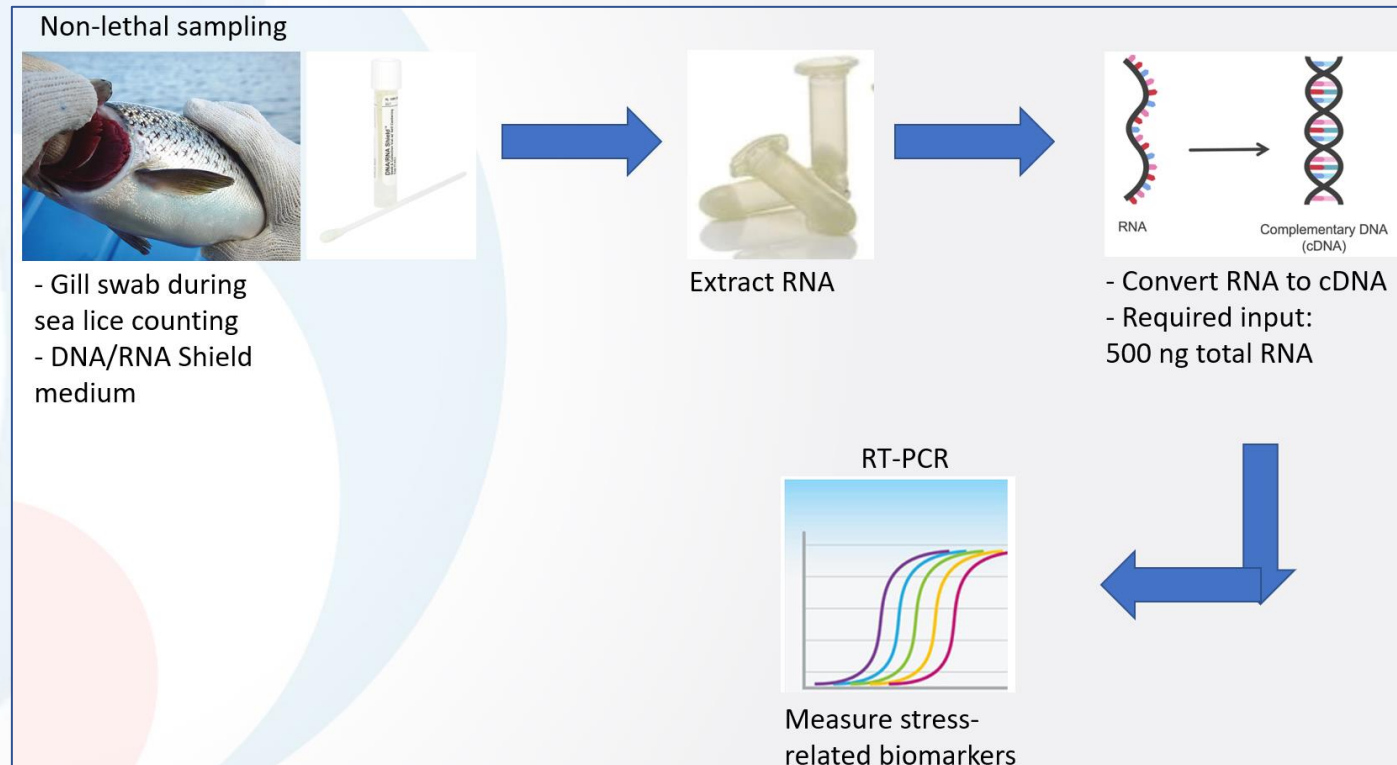
Measure stress-related biomarkers



Preliminary results – Pilot study

Questions to address in this pilot study:

- 1) Do we get enough material from gill swabs (RNA)?
- 2) Is the quality sufficient for gene expression analysis (PCR)?
- 3) Can we detect the chosen biomarkers (genes) via PCR?



Preliminary results – Pilot study

- 1) Do we get enough material from gill swabs (RNA)?
 - The biomarkers has been validated in gill tissue and cell lines from salmon
 - 500 ng total required for cDNA synthesis

Results:

Qubit fluorometer: RNA quantity = 16 – 32 μg (sufficient for cDNA synthesis)

Preliminary results – Pilot study

2) Is the quality sufficient for gene expression analysis (PCR)?

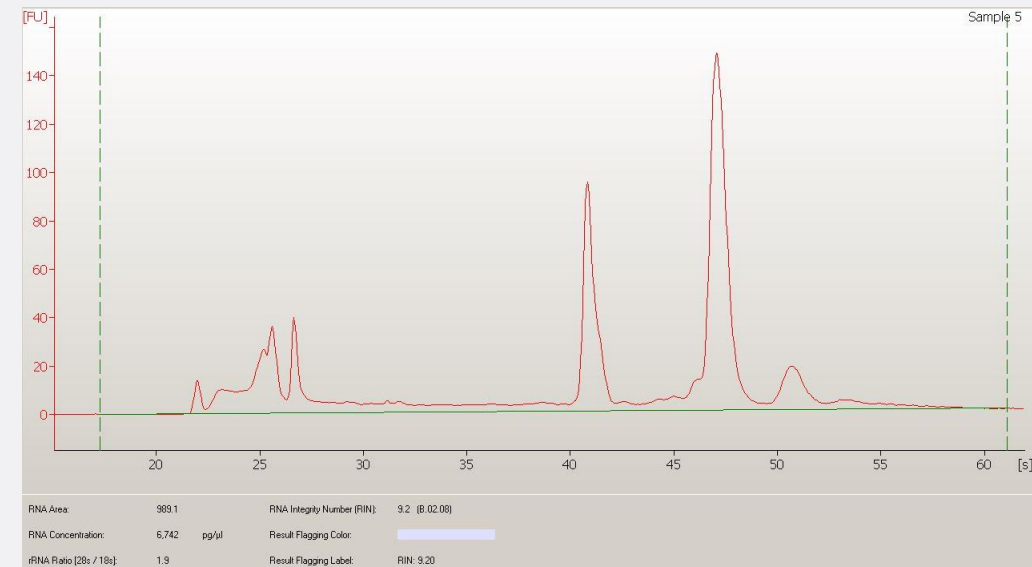
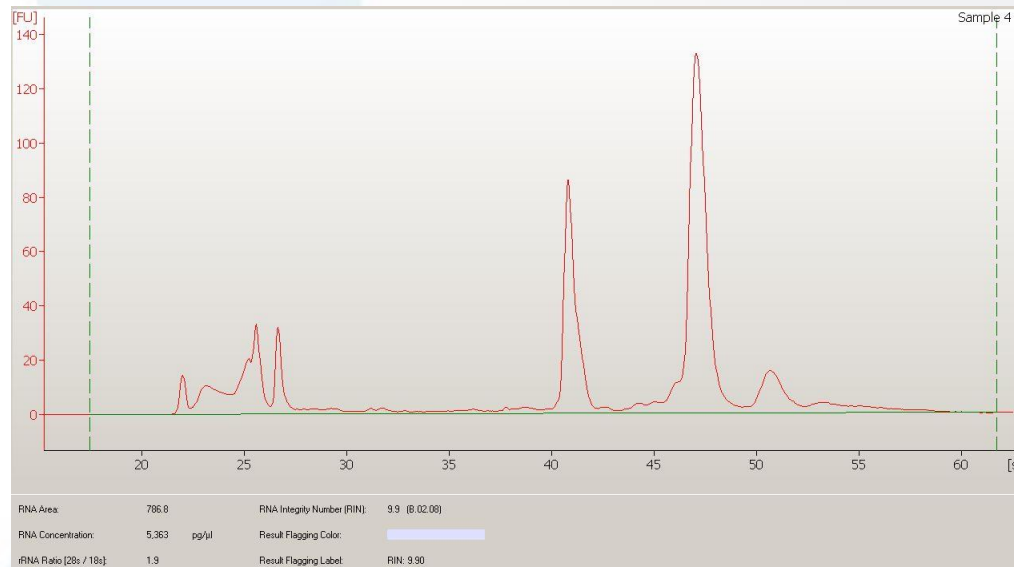
- High quality RNA is important for gene expression analysis

Results:

RIN number: 9.2 – 9.9 (highly intact RNA from gill swabs)



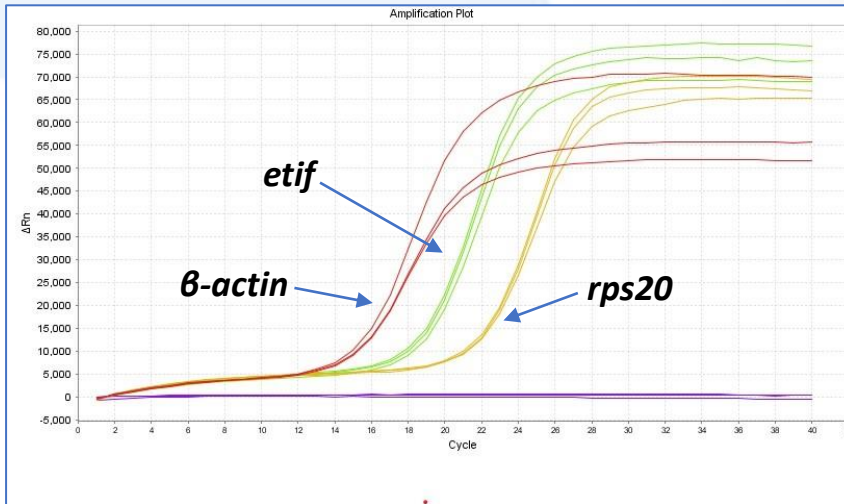
2100Bioanalyzer



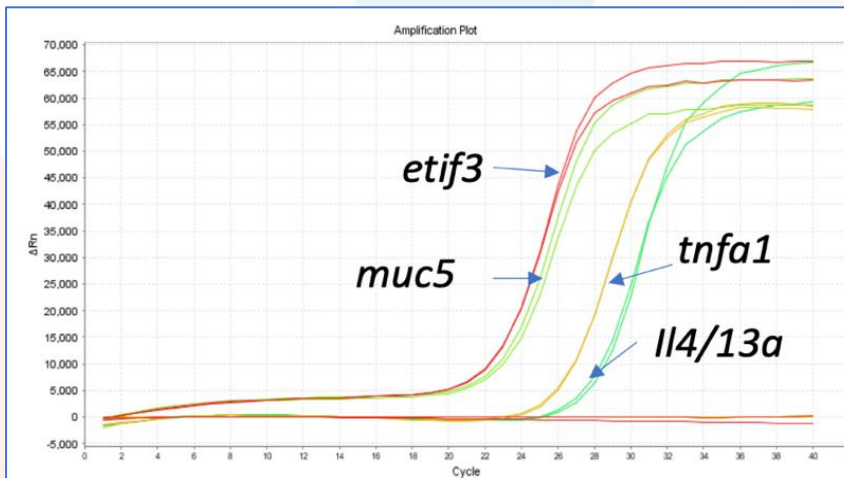
Preliminary results – Pilot study

3) Can we detect the chosen biomarkers (genes) via PCR?

Housekeeping genes/controls



Biomarkers



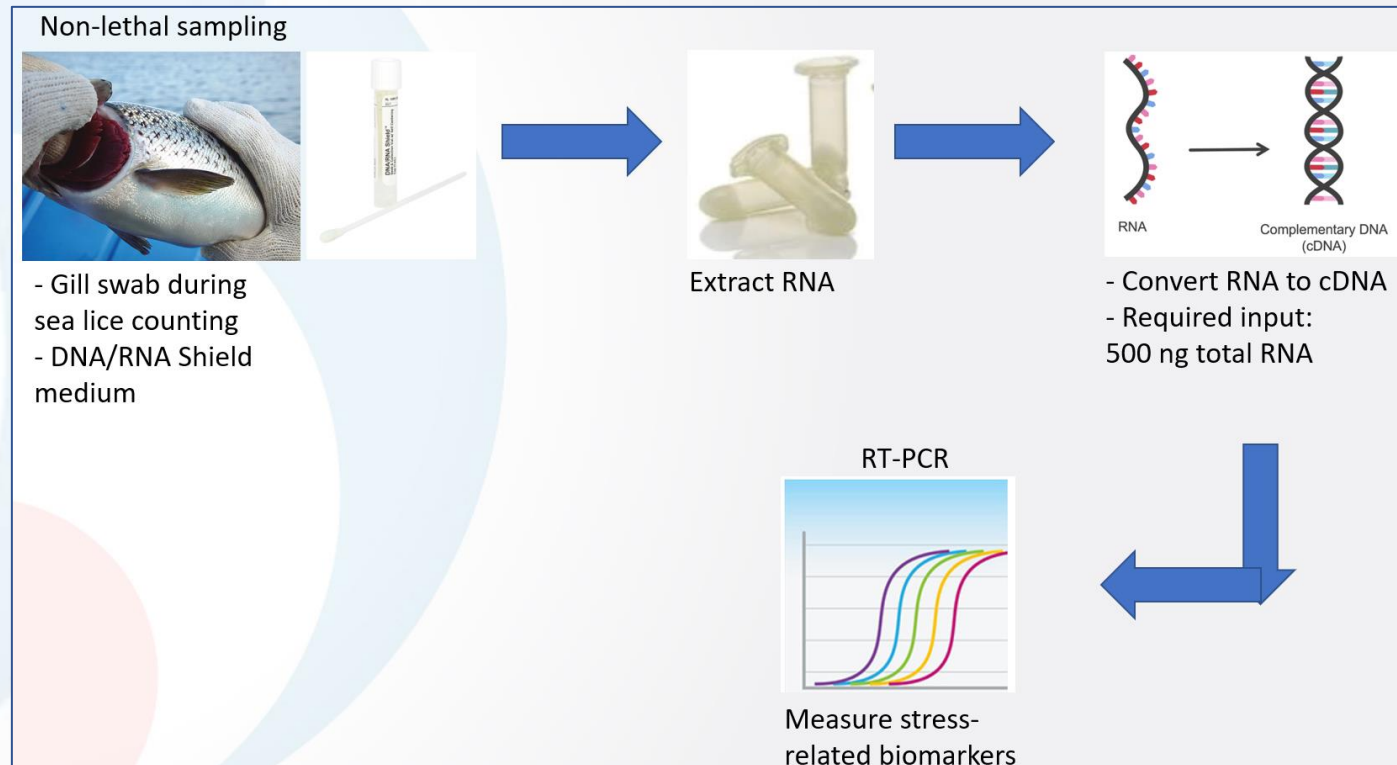
Results:

- Housekeeping and target genes are detectable and amplify well
- This pilot study also demonstrate the feasibility in gill swabs

Preliminary results – Pilot study

Questions to address in this pilot study:

- 1) Do we get enough material from gill swabs (RNA)? - yes
- 2) Is the quality sufficient for gene expression analysis (PCR)? - yes
- 3) Can we detect the chosen biomarkers (genes) via PCR? - yes



Project plan

- WP1
 - Validate gill swabs vs gill tissue
 - Correlate molecular and histology signs of gill inflammation
- WP2
 - Validate the biomarker panel during an at-sea production cycle

Sea Transfer



Slaughter



Sampling once at any point during at-sea production



Gill Dissection (60 fish)



Gill swab (RNA)



Gill tissue (RNA)



Gill tissue (Formalin)

Compare gill swabs to gill tissue and histology



Validation

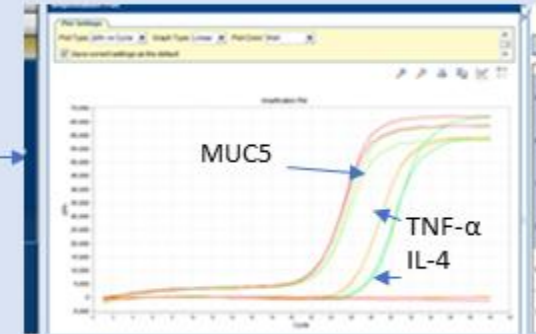
RNA from Gill tissue

RNA from Gill swabs

Histology

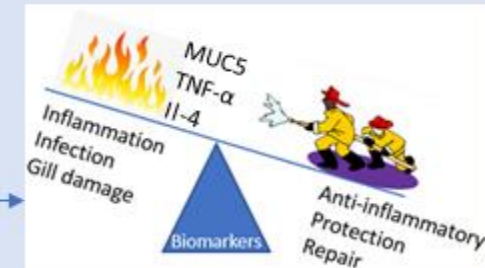


Histological Gill Score



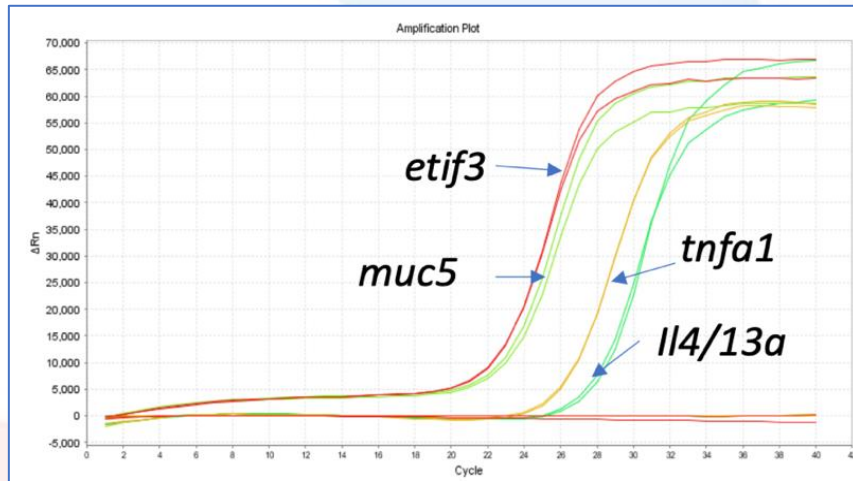
Compare

Histology to gene expression



Expected results/Perspectives

Biomarker expression from gill swabs
pilot study



Animal-based welfare indicators

Parameter	Reporting
Sea lice load	Sea lice number: Chalimus stages (<i>L. salmonis</i> and <i>C. elongatus</i>) - <i>L. salmonis</i> (pre-adult- and adult stages) - <i>C. elongatus</i> (adult stage)
AGD gill score	0 to 5
Other gill abnormalities	0 to 5
Condition factor (K)	Weight and length of the fish
Scale loss/skin damage	0 to 3

Table 2. Direct and animal-based welfare indicators describing the appearance of the individual fish. Condition factor is calculated from the weight and length of the fish.

Environmental data	Operational data
- water temperature	- mortality
- salinity	- growth rate
- water current	- feed up-take
- meteorology	- presence of pathogens
- dissolved oxygen	- historical data on delousing
- harmful microalgae blooms	- vaccination history

- Develop a routine monitoring tool for fish health
 - Reports to the industry
- Increase knowledge – immune system in fish

Thank you for your attention

Others participants in project:

- **Hiddenfjord/Luna**
- **Alf Seljenes Dalum, Histopathologist at PatoGen, Norway**