# CtrlAQUA SFI

## Centre for Closed-Containment Aquaculture

Centre leader Dr. Åsa Espmark Nofima
(from 1. Oct 2017)

Senter for forskningsdrevet innovasjon
Norges forskningsråd

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# Restructuring Norway

- Research-based innovation will be extremely important for Norway in the years to come
- The word «innovasjon» mentioned 464 times in the governments Industry Report to parliament
- «The centres for research-based innovation (SFI) shall develop knowledge *in* and *for* the industries. These centres are pivotal in the long-term efforts to restructure Norwegian industries»

# CtrlAQUA SFI  2015-2023

- Financed 50/50 by RCN and partners

-Total budget:199 mill NOK (= 31 mill CAD)

## Objective

- Develop technological and biological innovations to make closed-containment aquaculture systems a reliable and economically viable technology, for use in strategic parts of the Atlantic salmon production cycle;

➡ contributing to solving the challenges limiting the envisioned growth in aquaculture

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# 23 types of floating closed systems in sea in Norway in 2017

- ✓9 prototypes have been built
  - ✓Agrimarine
  - ✓Akvadesign
  - ✓AquaDome
  - ✓Ecomerden
  - ✓FishGlobe
  - ✓Flexibag/Botngaard
  - ✓Neptun, Aquafarm Equipment
  - ✓Preline
  - ✓SalmonHome no. 1
- ✓+14 others at various prototype stages


Foto: MSC Aqua, AquaDome


Foto: SalmoHome no 1, Finn Håkon Eines


Foto: Akvadeisgn

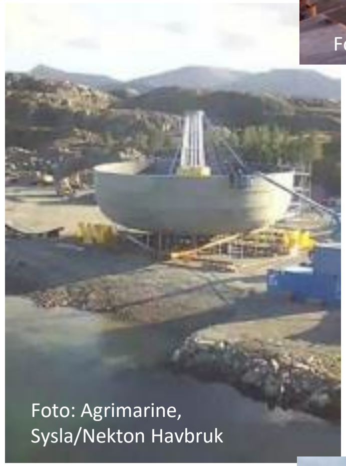
Foto: Agrimarine, Sysla/Nekton Havbruk


Foto: : Ecomerden


Foto: Lerøy, Preline


Foto: FishGlobe


Foto: Flexibag Nekton Havbruk


Foto: Aquafarm Equipment

# 21 CtrlAQUA SFI partners

## Host institution:

- Nofima

## R&D partners:

- UNI Research
- University of Bergen
- Norwegian University of Science and Technology (NTNU)
- The Freshwater Institute, WV, U.S.
- University of Gothenburg
- University College of Southeast Norway

## User partners:

### Technology suppliers:

- Krüger Kaldnes
- Storvik Aqua
- Aquafarm Equipment
- Oslofjord Ressurspark
- FishGLOBE
- Botngaard

### Farming companies:

- Marine Harvest
- Cermaq
- Grieg SeaFood
- Lerøy SeaFood Group
- Bremnes Seashore
- Smøla Klekkeri & Settefisk

### Biotechnology companies:

- Pharmaq
- Pharmaq Analytiq

# Dept of Training & Recruitment

**Led by Prof. Sigurd Stefansson, Univ of Bergen**

- Major goal of CtrlAQUA, to educate 15 PhD’s
- Status 2017:
  - 7 PhD’s
  - 2 post-docs
  - 10 MSc’s

NTNU
Kunnskap for en bedre verden

HSN



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# Dept. Fish Production & Welfare

Dept. leader Prof. Lars Ebbesson uni Research

**Objective: Contribute to innovations to improve survival, welfare, performance and secure profitability**

- **Identify** physiological and environmental requirements for post-smolts in CCS
- **Optimize** environmental parameters
- **Innovate** new welfare and robustness indicators
- **Maximize** output from each CCS platform without compromising welfare and robustness

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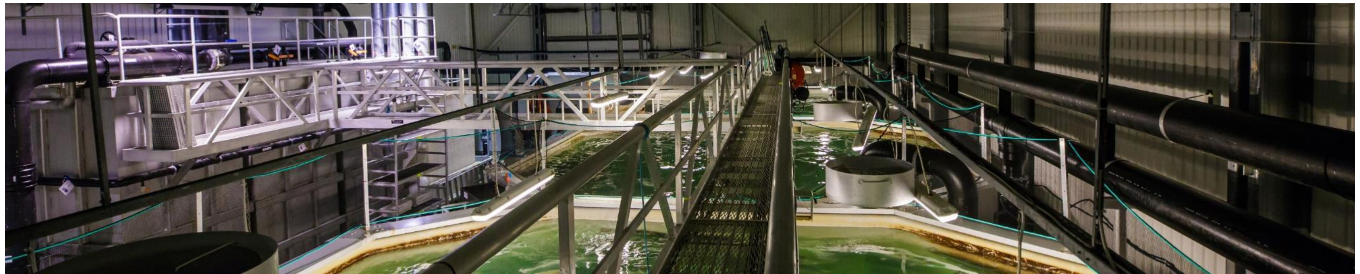
# Dept. Technology & Environment

Dept. leader Jelena Kolarevic Nofima

**Ojective facilitate innovation of CCS technology, water treatment processes, and sensors, to achieve a high level of production control.**

- **Sensing** the environment: to run the technology efficiently and to ensure fish welfare and performance
- **Limits** to the environment: to ensure fish welfare and performance, to dimension and design CCS technology correctly, and to reduce production costs
- **Controlling** the environment: to reduce risk, ensure fish health and reduce production costs



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# Dept. Preventive Fish Health

Dept. leader Lill-Heidi Johansen Nofima

**Objective: Contribute to inventions to prevent, detect and control disease in closed containment systems**

- **Strengthen fish robustness and disease resistance** with focus on barrier functions and cardiovascular capacity.
- **Strengthen pathogen control** and handling of disease outbreaks in CCS.
- **Develop new or refined vaccines** and protocols for pathogens representing a special threat in CCS.

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# The rationale behind each of the CtrlAqua projects:

- To establish the knowledge-base necessary for achieving biological and technological innovations in closed systems used in the production chain

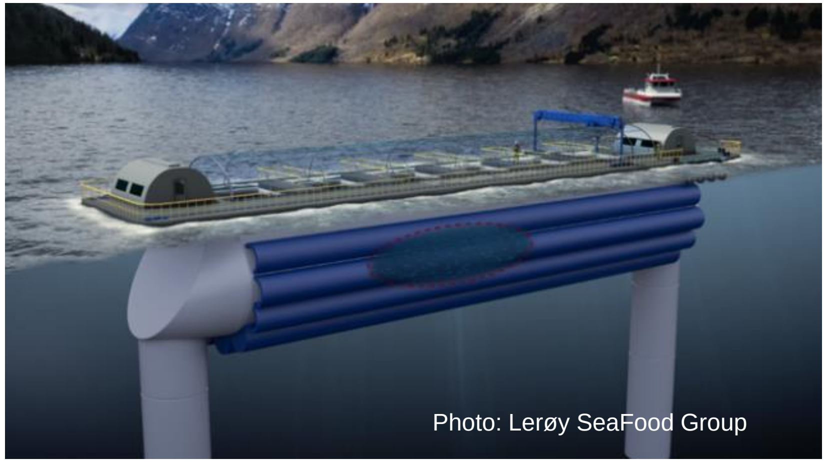
Photo: Lerøy SeaFood Group


Photo: Marine Harvest

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# 14 Projects in CtrlAqua 2017

## Technology & Environment

SENSOR
CO2RAS
HYDRO
BIOMASS
INTAKE
EXPO

## Fish Production & Welfare

ROBUST
OPTIMIZE
RIGID S-CCS
(PRELINE, NEPTUN,
CONCRETE)
BENCHMARK

## Preventive Fish Health

BARRIER
MICROPARASITES
PREVENTIVE
(POCNAD, CARDIO)

**+ADM (all)**

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# Cont. CtrlAqua projects

- **PHOTO and BENCHMARK:**
  - What photoperiod and salinity conditions are optimal in RAS?

- **PARTICLE:**
  - To assess fish on RAS tolerance to particles: Can we keep high particle levels in water and reduce production cost?

- **ROBUST and BARRIER:**
  - Effect of stressors on brain predictive markers, understand the effect of stressors on mucosal tissue (skin):

    -predict and improve tolerance to chronic and acute stress as high fish density, temperature variations etc

    - Understand mechanisms that enhance fish robustness

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# Cont. CtrlAqua projects:

- **RIGID S-CCS (CONCRETE, PRELINE, NEPTUN) and INTAKE:**
  - Evaluation of performance, welfare and health in large scale CCS
  - Study feasibility of water filtration to improve biosecurity:
    - Test and improve how salmon respond to large scale closed systems during production

- **TRANSFER:**
  - To determine effects of transfer temperature gradients on post-smolt physiology and welfare:
    - What happens afterwards? Little knowledge about optimal conditions and procedures for transfer of post-smolts from closed systems to cages

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# Cont. CtrlAqua projects

- **CO2RAS:**
  - To improve RAS dimensioning: The $CO_2$-tolerance of salmon in RAS must be known

- **SENSOR, PREVENTIVE, MICROPARASITES:**
  - New sensors; knowledge about the water quality and pathogen presence - get accurate sensor data

- **HYDRO:**
  - Develop models, validate in real systems and use models for experiments:
  - Predict the hydrodynamic environment in huge new systems to make tools for optimizing conditions for the fish and the system

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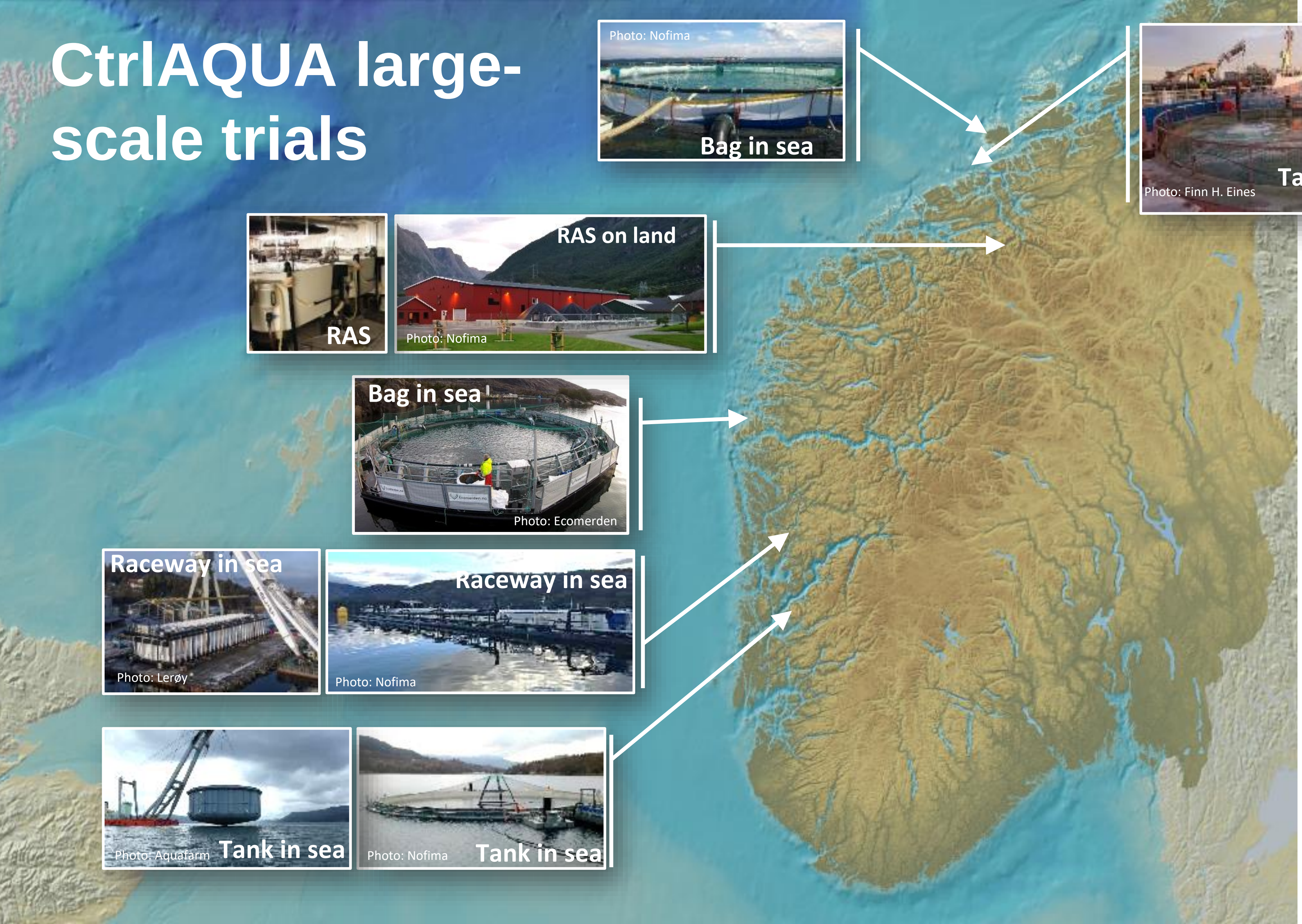
# CtrlAQUA large-scale trials

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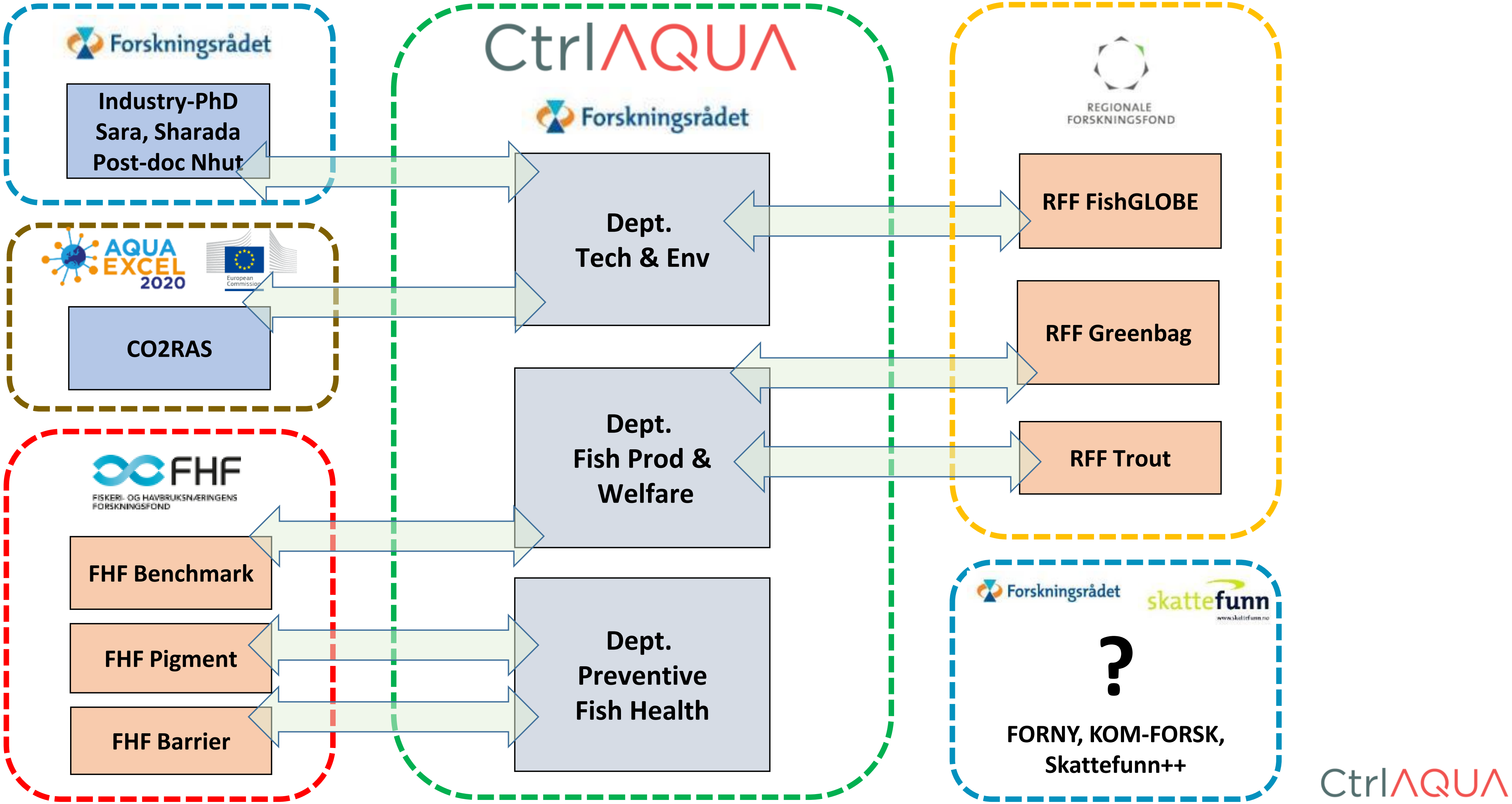
# Associated projects, to dig deeper than SFI's and/or to commercialize



# Some research highlights

- None, or very little sea lice in CCS
- High survival during post-smolt stage
- New findings on salmon environmental requirements in CCS
- We can identify good fish welfare, not only bad
- Skin behaves almost as a living sensor
- Screening has identified biosecurity challenges
- Nano-compounds promising for sensor protection
- Flow models for huge tanks developed


Photo: Terje Aamodt


Photo: Nofima

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# Dept. Preventive Fish Health



**Lill-Heidi Johansen**
**Dept leader**
**Researcher, Nofima Dept Fish Health**

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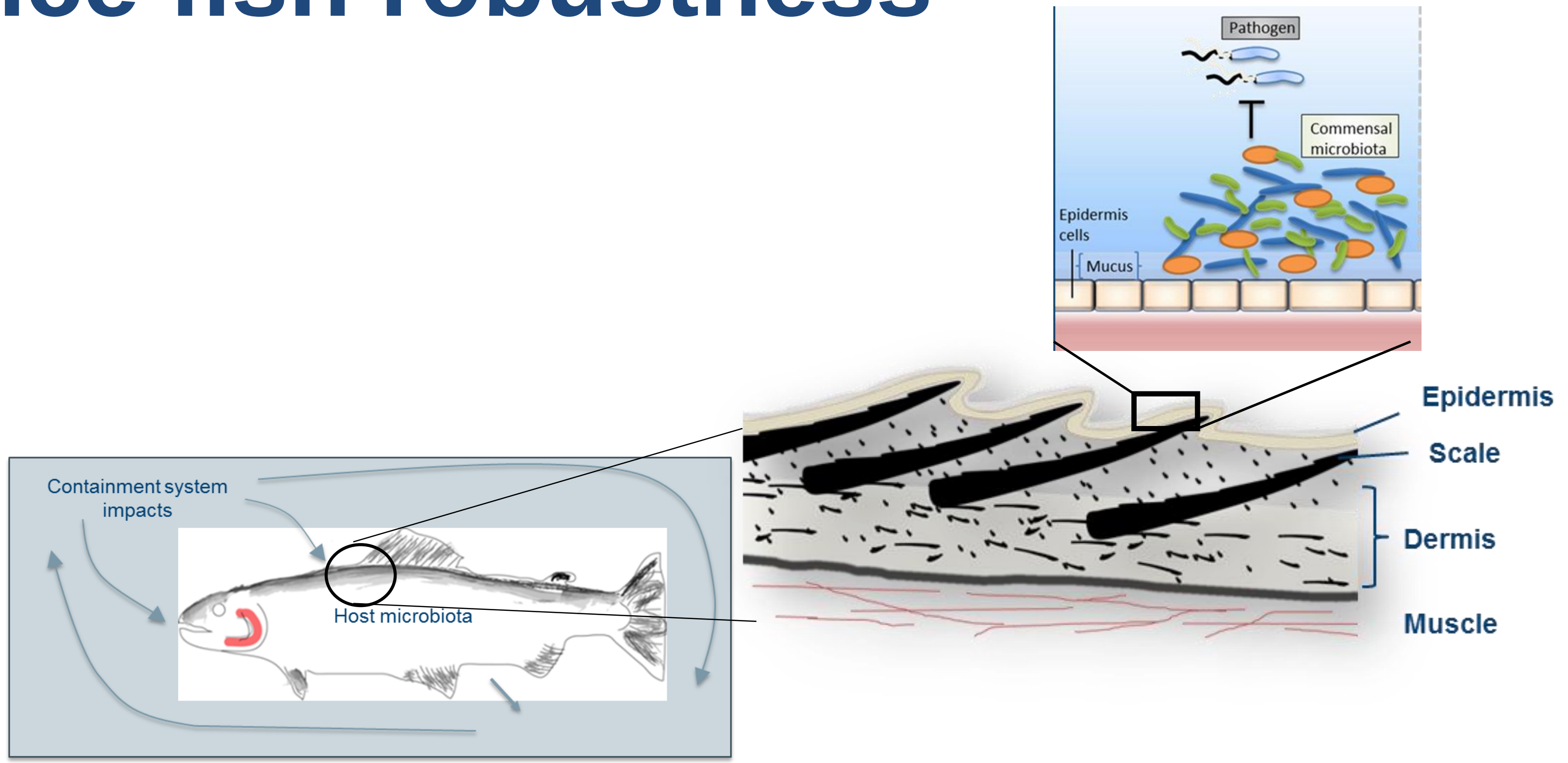
# BARRIER - understand mechanisms that enhance fish robustness

- Mucus barrier
- Skin integrity
- Gene regulation
- Immune activity
- Microbial interactions





Nofima
UNIVERSITY OF GOTHENBURG
uni Research

Christian Karlsen (PL), Elisabeth Ytteborg, Aleksei Krasnov, Gerrit Timmerhaus, Ida Rud, Jelena Kolarevic,

Kristina Sundell, Henrik Sundh,

Tom Ole Nilsen, Sigurd Handeland

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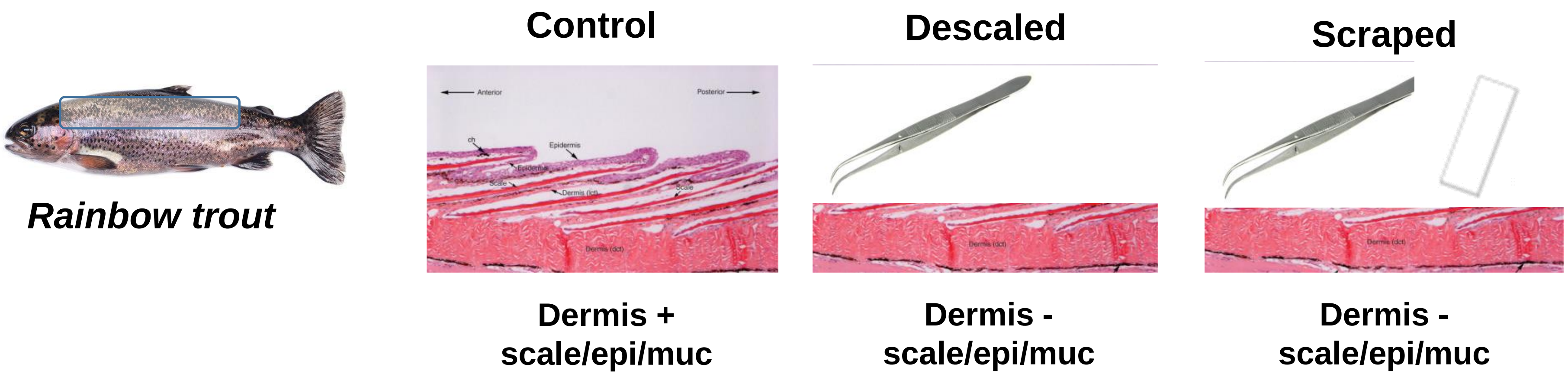
# BARRIER: Studies of scale loss and skin barrier function

***Treatments***

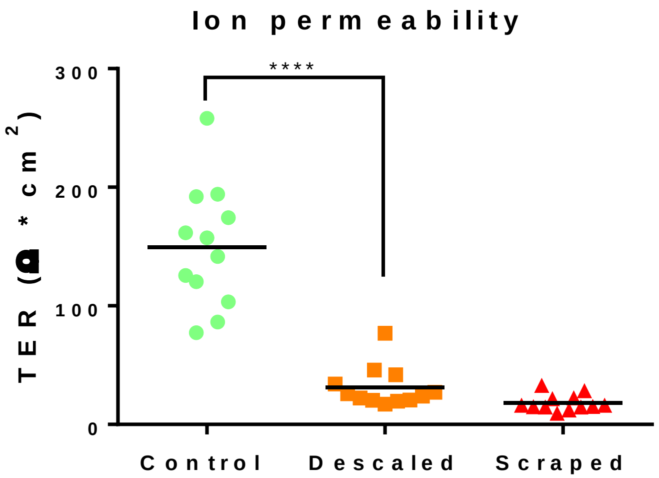


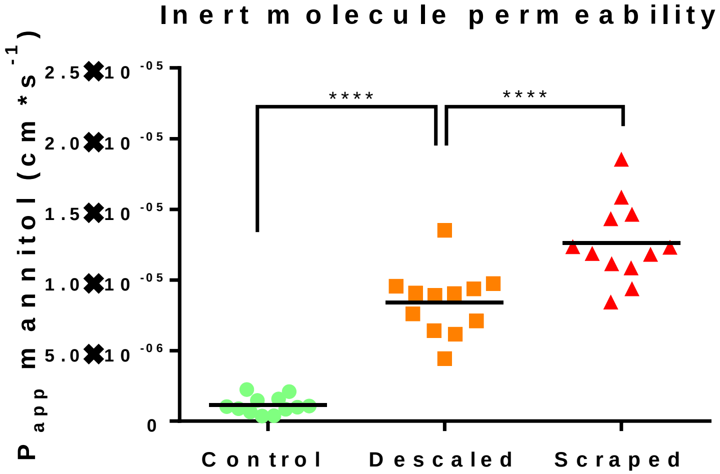

**-Main barrier consists of:**

-Scales, epidermis and mucus

-Scale loss is sign of impaired skin barrier function

-Loss of main barrier is likely to increase susceptibility to pathogens

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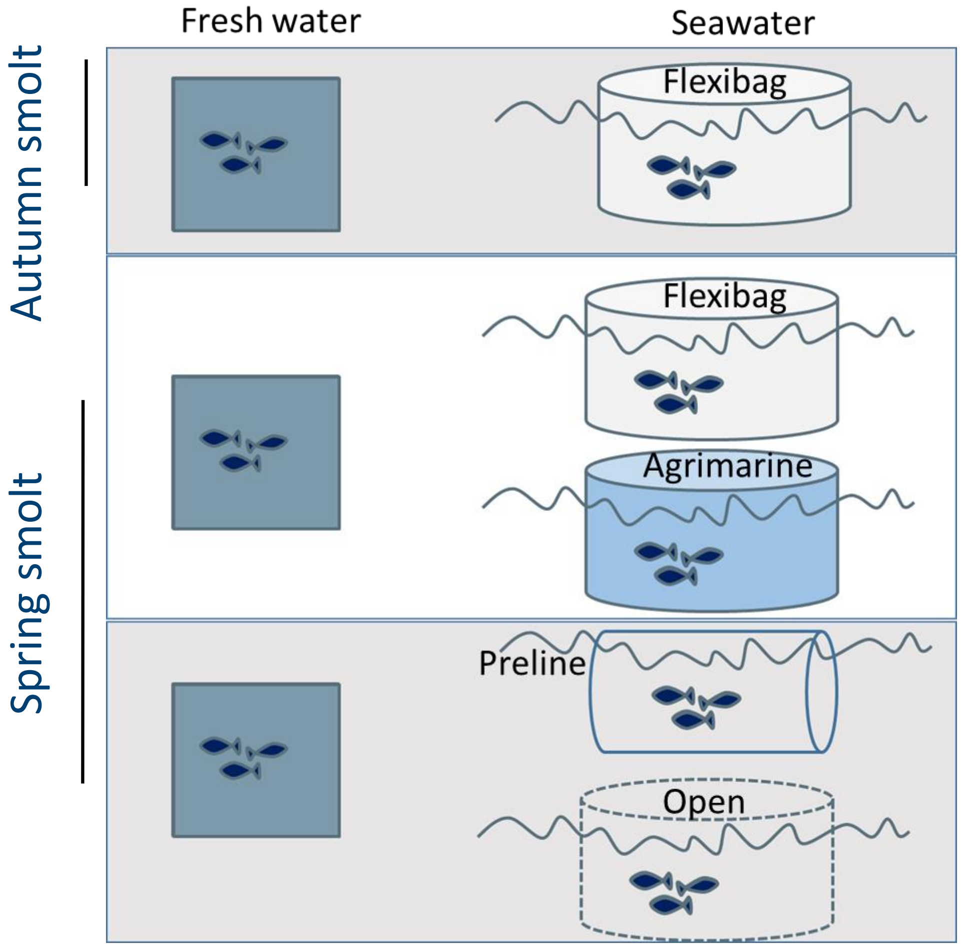
# BARRIER; Skin characteristics of salmon reared in S-CCS and open systems

- 3 smolt groups in FW distributed to different facilities in SWT at different time of year
- Sampling **1 and 4 months** after transfer




## Overall results:

- Greater difference *between time-points* than between the systems
- First time-points in seawater systems were close to smolt results
- Gene expression: increased cutaneous secretion and gradually enforced protection against pathogens.
- Histology: development of epidermis thickness, number of mucus cells increased with time after seawater transfer

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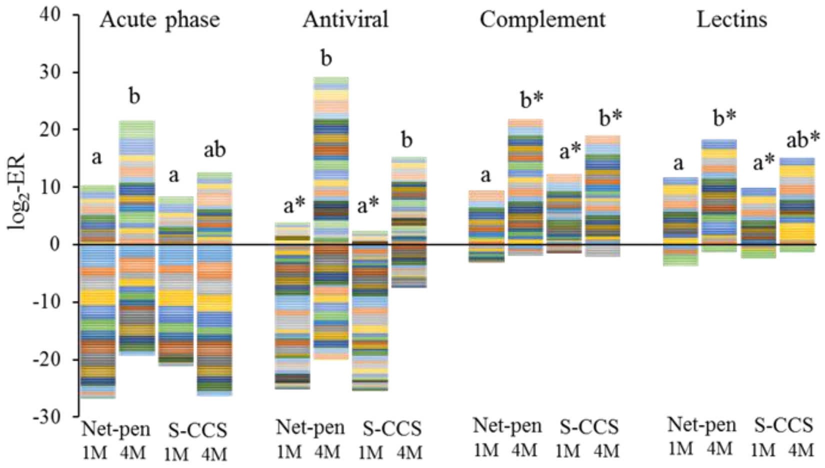
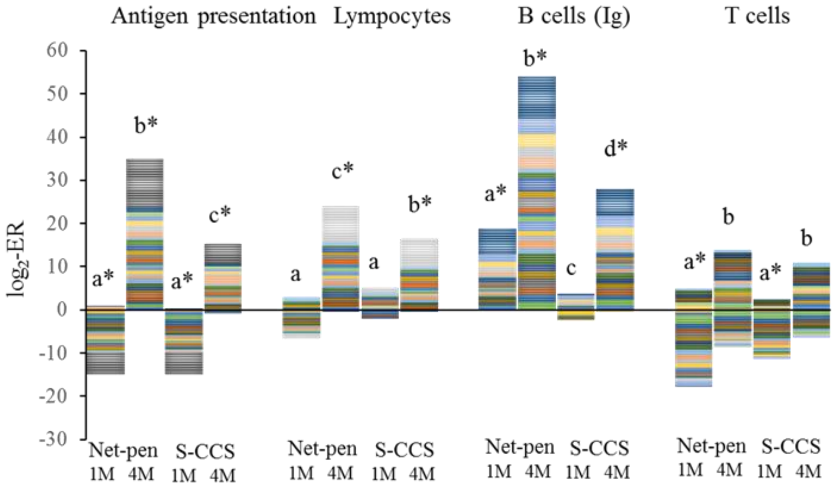
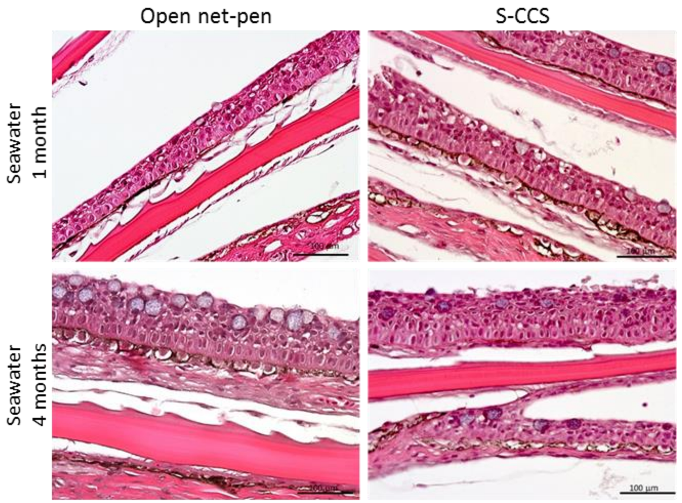
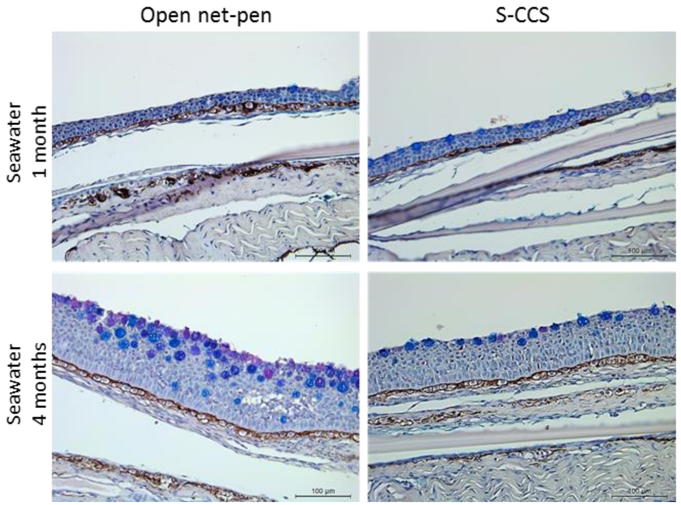
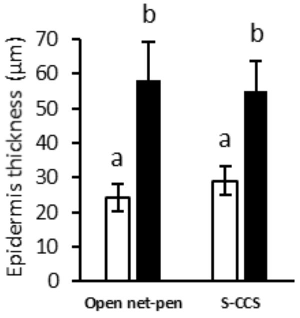
# BARRIER: Development of immune competence and structural integrity of postsmolt skin

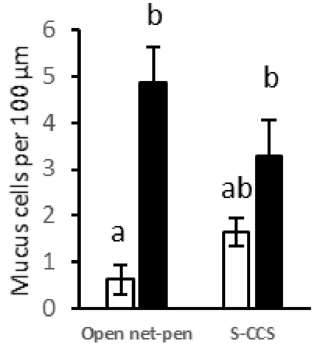


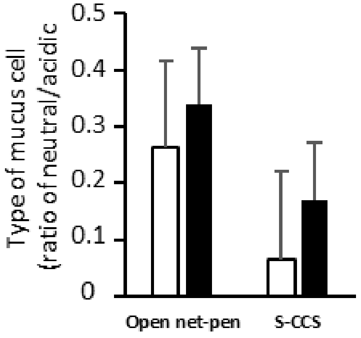

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# BARRIER: Development of immune competence and structural integrity of post-smolt skin after SW transfer

-Gained better understanding of the increased susceptibility to pathogens associated with welfare problems and losses of post-smolts.

-Rearing facilities with environmental controls/barriers that restrict fish pathogens, especially during immunosuppressed periods, may be key to enhance animal welfare and overall production performance.

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# MICROPARASITES in semi-closed containment systems (S-CCS)

Project leader Are Nylund, Univ of Bergen

-Screening of RAS and S-CCS production systems:

- -Preline (Lerøy seafood group),
- -Flexi-bag (Smøla Klekkeri og Settefisk),
- -Neptun (Marine Harvest)

**Objective:**

To identify and characterize the most important known and emerging microparasites in CCS and S-CCS:

- -mapping of diversity, prevalence and load
- -mapping of transmission routes (introduction into CCS and S-CCS)
- -identification & characterization of emerging pathogens

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# Microparasites

## -Real time RT PCR assays developed

| | |
|---|---|
| Infectious salmon anaemia virus, | ISAV |
| Salmonid alphavirus, | SAV |
| Infectious pancreatic necrosis virus, | IPNV |
| Piscine orthoreovirus, | PRV (*S. salar*) |
| Piscine myocarditis virus, | PMCV |
| Atlantic salmon calicivirus, | ASCV |
| Salmonid gill poxvirus, | SGPV |
| *Moritella viscosa* | MoV |
| *Tenacibaculum* spp | Ten |
| Tenacibaculum finnmarkense | TeF |
| *Yersinia ruckeri* | *YR* |
| *Candidatus* Branchiomonas cysticola | CaBC |
| *Candidatus* Syngnamydia salmonis | CaSS |
| *Candidatus* Clavichlamydia salmonicola | CaCS |
| *Candidatus* Piscichlamydia salmonis | CaPS |
| *Parvicapsula pseudobranchicola* | Parvi |
| *Paramoeba perurans* (Syn. Neoparamoeba perurans) | PP |
| Paramoeba spp. | Parsp |
| *Ichthyobodo necator* (Costia) | Costia-N |
| *Ichthyobodo salmonis* (Costia) | Costia-S |
| *Paranucleospora theridion* | PT |

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# S-CCS

- Four productions in Preline, one in Neptun, and two at Smøla followed since start of project.

- **Results so far:**
  - Production in S-CCS so far do not result in higher mortalities compared to open systems.

  - Diversity and prevalence of microparasites vary with time of year (summer-autumn production vs winter-spring production).

  - Relatively large number of microparasites ( f. ex. ISAV, IPNV, PRV, SGPV) are introduced into the S-CCS with the smolt.

  - Salmon lice enter existing prototype S-CCS; densities detected so far are within the set limit for such systems

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# Emergence of a New virus: *Cyclopterus lumpus* Flaviviridae virus (CLFV)

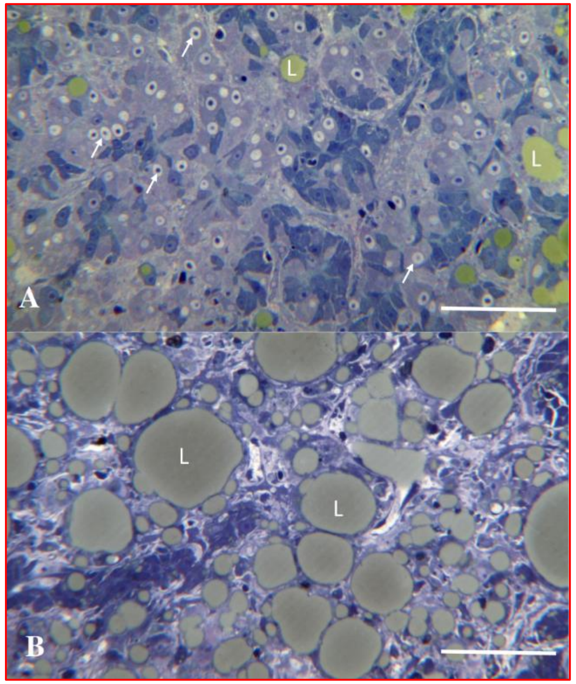
-Detected in lumpfish used as cleanerfish in 2016

-Associated with high mortalities in production of lumpfish

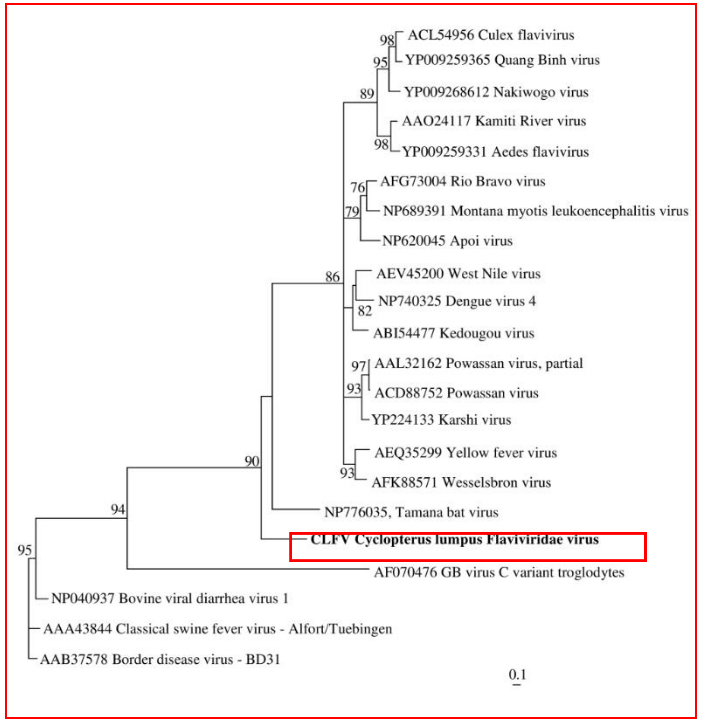
-Found in both south and north Norway

-Real time RT PCR assays developed for detection

-Screening of lumpfish and farmed salmon suggest that the virus will not infect salmon




Liver pathology




Phylogenetic position of CLFV

Skoge, R. H., Brattespe, J., Økland, A., Plarre, H., Nylund, A. (2017). New virus in family Flaviviridae detected in lumpfish (*Cyclopterus lumpus*). Arch. of Virology. https://doi.org/10.1007/s00705-017-3643-3

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# MICROPARASITES

## Testing real time RT PCR on water and biofilm from RAS and S-CCS:

-Indications that all studies of biofilms in S-CCS and RAS facilities using real time RT PCR will have to be optimized for each facility.

- Probably necessary to optimize method for each sampling time point since we don`t know how changes in the biofilms during the year will influence the quality of RNA extraction and PCR reaction.

- Raises a serious question about the applicability of real time RT PCR screening of biofilms

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# PREVENTIVE: Improved disease prevention, pathogen detection and immune prophylaxis in CCS

Project lead by Nofima (Lill-Heidi Johansen PL)

Partners: Univ of Bergen, UniResearch, ORP, Pharmaq, Pharmaq analytic, Lerøy, Cermaq, Marine Harvest, Grieg seafood

## Task CARDIO

Task leader Gerrit Timmerhaus Nofima

- **Objective:**
- Finding optimal water velocity for post-smolts to promote (cardiac-) health

# CARDIO: Benchmarking at user partner

## Big scale smolt trial 2016-2017

Increased water speeds tested in big scale at smolt production site (Marine Harvest)

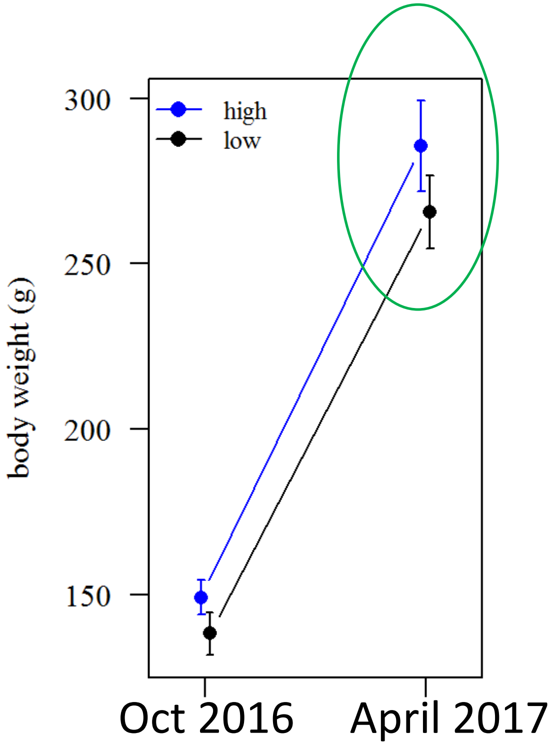
Water speeds:

-Low: ~0.6 body length per second

-High: ~1.0 body length per second

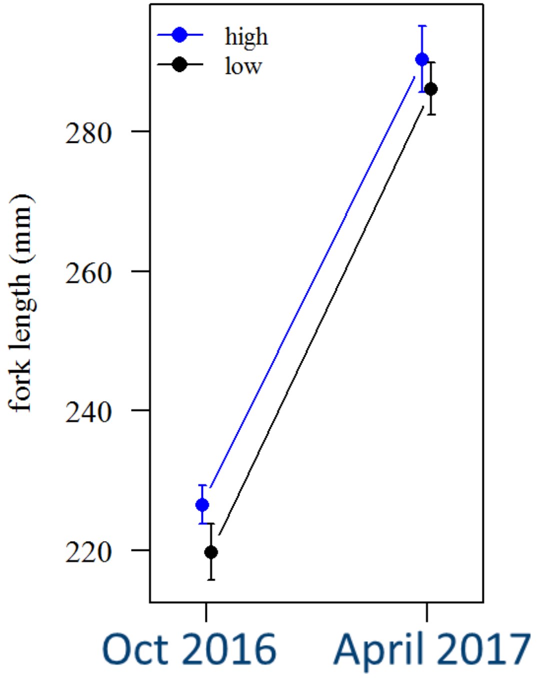
Monitoring and sampling Oct-16 and April-17:

- Body size, cardio somatic index, gill and heart samples



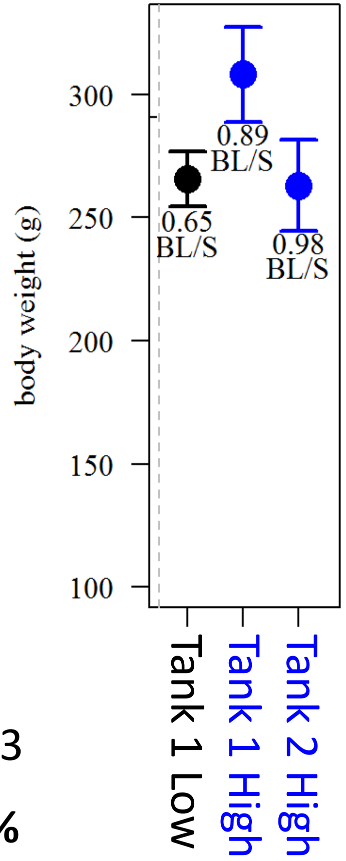

Two factor Anova: 0,0270

**Weight «High» gr: +7.74%**




Two factor Anova: 0,0253

**Length «High» gr: +2.29%**



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# Cont. CARDIO Benchmarking at user partner

## Conclusions:

- Higher water speeds have the potential to increase the growth rate of smolts
- Differences in replicates showed that
  - finding the optimal speed is challenging
  - higher water speeds may not have positive effects
- More research is needed for to confirm the results and optimize the water speed

# CARDIO: Optimum water velocities for post-smolts

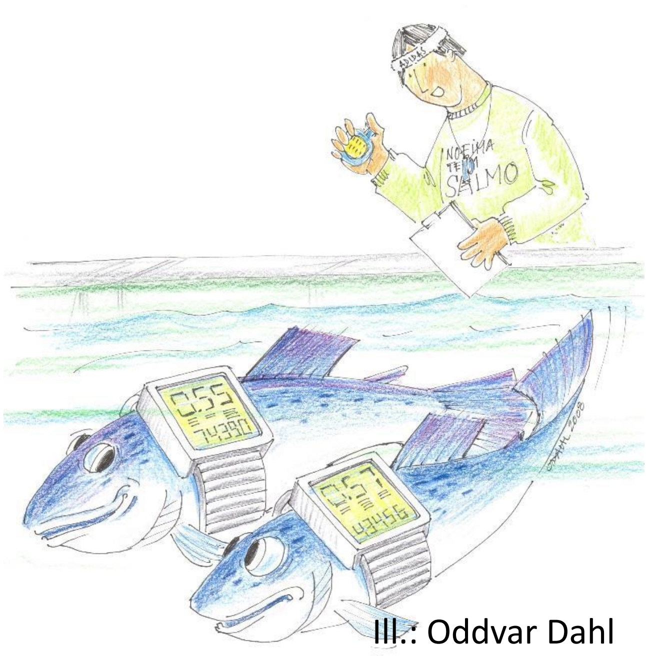
Previous trials: positive effects due to medium (1 BL/s) and high (1.5 to 2 BL/s) intensities were found for young fish until smolt stage:

## Main objective:

- **identify optimum water velocities for post-smolts prior to sea transfer in RAS**
  - Identify related positive effects on growth and welfare:
    - Growth rate, feed conversion rate
    - Welfare scores

## Other objectives:

- Training effects on cardio somatic index and muscle cellularity
- Training effects on mucosal health (gill, skin) and blood parameters
- Evaluation of stress response



Ill.: Oddvar Dahl

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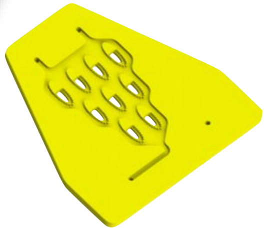
# Task POCNAD

## Automated analyses of gene activity - on line pathogen monitoring and warning sensor

Steven Hughes, Bård Haug, Frank Karlsen, Nhut Tran, Oslofjord Ressurs Park (ORP)
Gerrit Timmerhaus, Lill-Heidi Johansen, Nofima

## 2 patented technologies:

**Particle:**
**Refining**
**Separation**




**Gene analysis:**
**Point of care**
**Lab on a chip**

**SepRef**

**PocLoc**

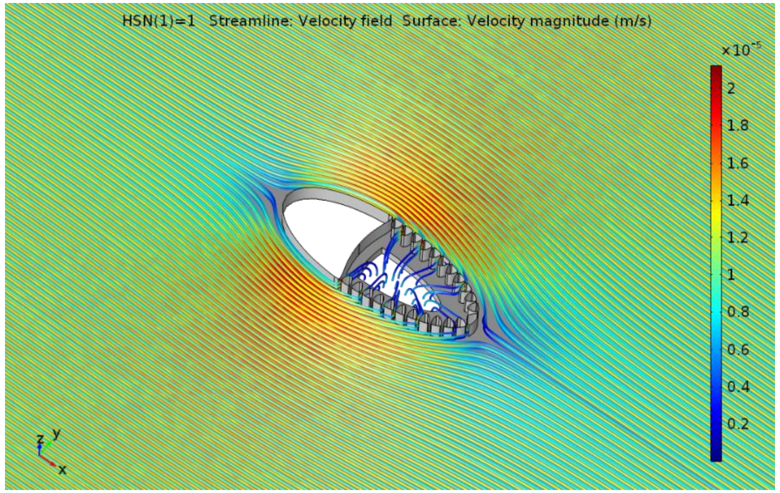
**NASBA technology:** Primers/probes in one temp. reactions:
Increased sensitivity, less false positives or begatives

O/R/P Oslofjord Resource Park CtrlAQUA

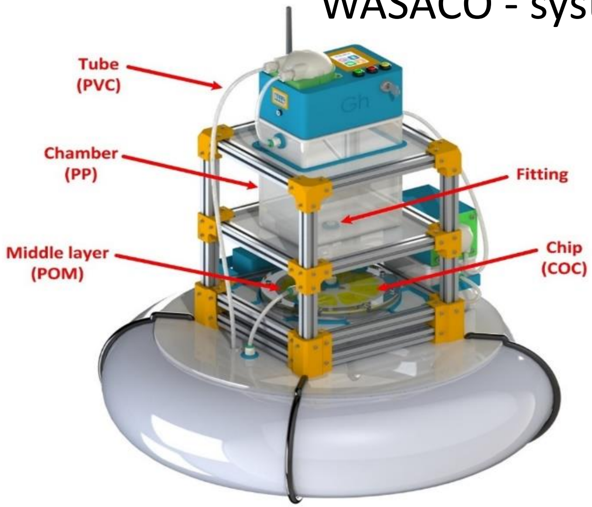
# SepRef technology

- Separation of particles smaller or larger than 180 micron are possible using SepRef with both single and multiple chips (WASACO)

- Possible to concentrate and separate from even very large volumes of complex water.



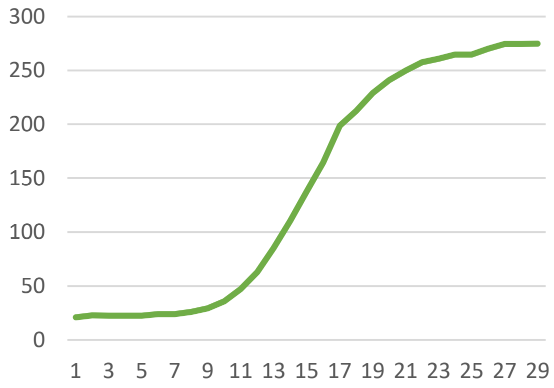

WASACO - system



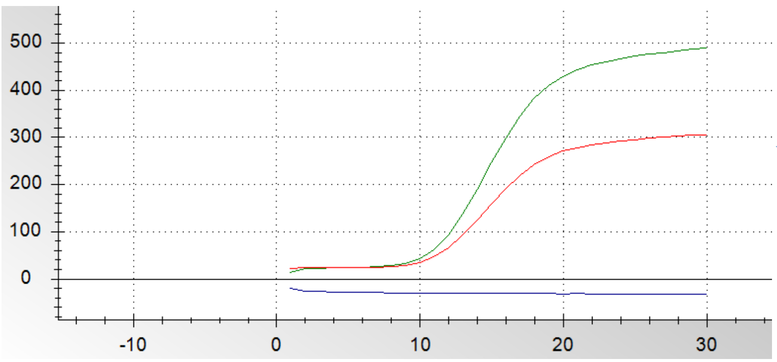

Nofima

# The PocLoc and POCNAD technology – Real-time NASBA detection of RNA

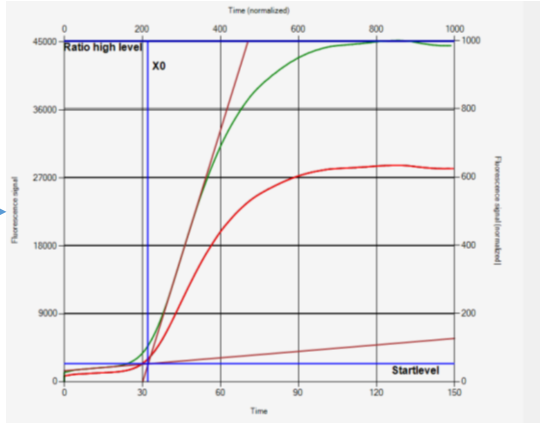
Detection of 16S RNA Gram-neg bacteria
E-coli K12



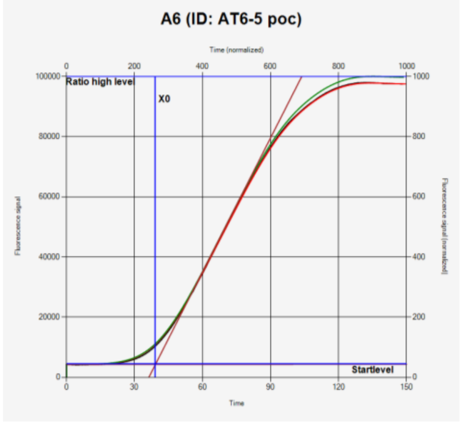

Detection of human mRNA




Detection of human mRNA

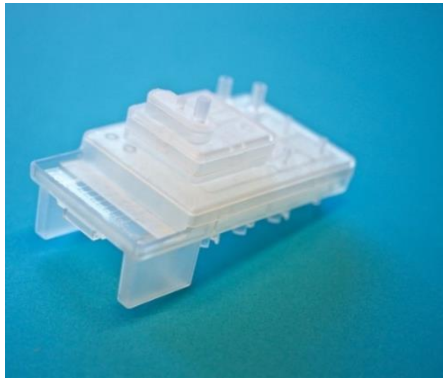



Detection of 16S RNA Gram-neg bacteria
E-coli K12





The POCNAD cartridge



**Conclusion:**

**The POCLOC reaction chamber support the same analytical sensitivity as conventional NASBA**

Conventional real-time NASBA reader


O/R/P Oslofjord Resource Park

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# POCNAD

- **Nofima's role:**

  Support the development and test the device

- **Tasks:**
  - NASBA primer/probe design for salmonid alpha virus (SAV)
  - Test NASBA assays
  - Evaluate the functionality with "real-life" samples

- **Results (preliminary):**
  - NASBA SAV primers/probes designed and tested
  - Assay developed for SAV