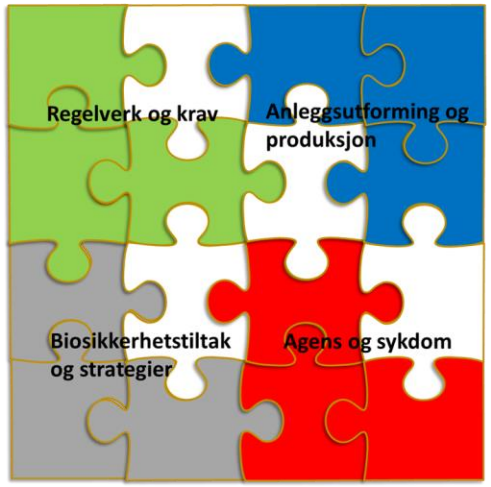


Sonal Patel/Aquaculture health

Effect of biosecurity measures on pathogen persistence in RAS

Norwegian
Veterinary Institute

Experiences from industry





Larsen, Sondre Veberg., Tørud, Brit., Hess-Erga, Ole-Kristian., Furseth, Kamilla., Patel, Sonal. (2024).
Praktisk biosikkerhetsarbeid i resirkulerende akvakultursystemer (RAS).
Akvaplan-niva rapport nr. 2024 64276.01 og VI rapportserie 2024_5. ISBN 97-82-449-0074-4.

FHF - prosjektdatabasen

Biosecurity measures (BM):

1. Fish out of system
2. BM1 + wash & disinfect tanks
3. BM1 &2+ partial wash and/or disinfection of water treatment system
4. BM1-3, Sanitization of whole system including bioreactor

Additional: varying period of **Fallowing**

Norwegian Veterinary Institute

Pathogens and disease history at the facilities

Facility	Last 5 Years	At Interview	Comment
1	ILAV-HPR0, PRV, HSMB	PRV	Working on PRV
2	ILAV-HPR0, Saprolegnia	ILAV-HPR0	
3	IPN	IPN	Acquired new IPNV strain
4	IPN	None	Introduced fry with IPNV only in one tank in 2021
5	PRV, HSMB, ILAV-HPR0, *Pseudomonas fluorescens*, Spironucleus salmonicida	PRV, HSMB	Screening of house strain, microbiome with Pharmaq Analytiq's NGS
6	ILAV-HPR0	ILAV-HPR0	
7	Yersinia, ILAV-HPR0, Costia, Flavobacterium, Tenacibaculum	*Tenacibaculum*, *Flavobacterium*, ILAV-HPR0	No active disease challenges except tail rot found with Tenacibaculum and Flavobacterium
8	ILAV-HPR0, part of ILA, *Vibrio* spp., *Ps. fluorescens*, *A. hydrophila*	Ps. fluorescens	*Ps. fluorescens* typically 1-2 weeks after moving to smolt section (fry)

- *Ca*. Branchiomonas cysticola
- *Yersinia ruckeri*
- *Pseudomonas* spp.
- Saprolegnia
- +++++

Typical biosecurity measures

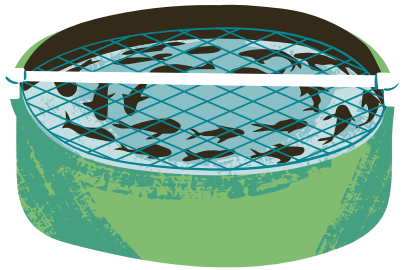
- Mechanical wash + with and without disinfection of tanks
- Treatment with lye pH > 12 and acid pH < 4
- Pipe wash + water treatment + anaesthesia in bioreactor. Chemicals with chlorine/PAA
- Dry biofilter occasionally/ drains water in FBBR
- Circulates O_3 throughout the water treatment
- LOZ (sodium hypochlorite)
- «Trial and error» and in several rounds

Identify practices and test in trial

Test effect of different biosecurity measures in use in the Norwegian Industry

- Fallowing
- Cleaning and water exchange
- Ozonation



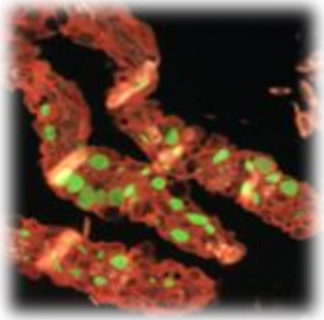


- **Survey of industry biosecurity**
- **Experimental trials**
- **Commercial RAS facilities**

Pathogens for experimental trial

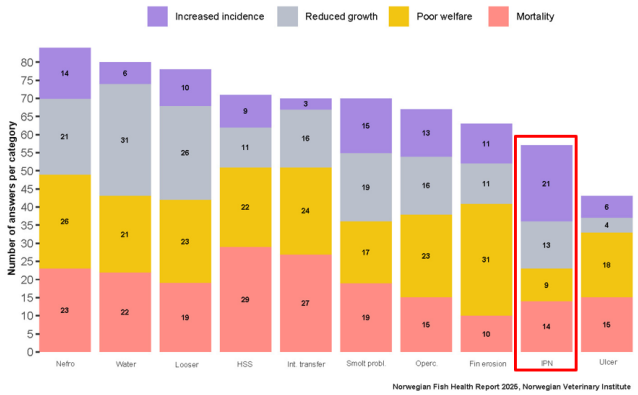
- Infectious Salmon Anaemia Virus (ISAV) HPRdel (virulent)

- *Ca*. Branchiomonas cysticola
  - Infectious pancreatic necrosis virus (IPNV)
  - Piscine Orthoreovirus (PRV)



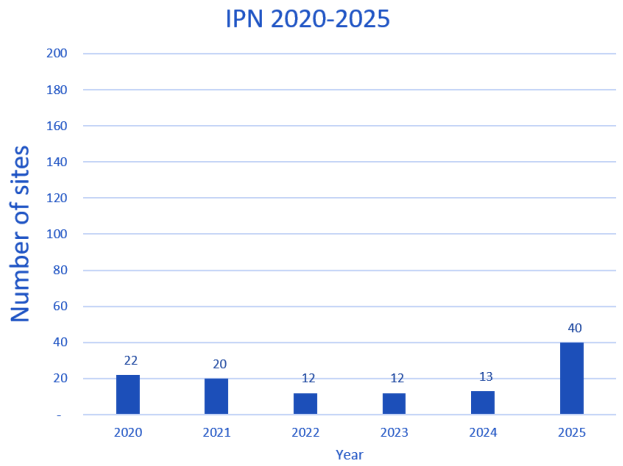
Fish health report – status 2025

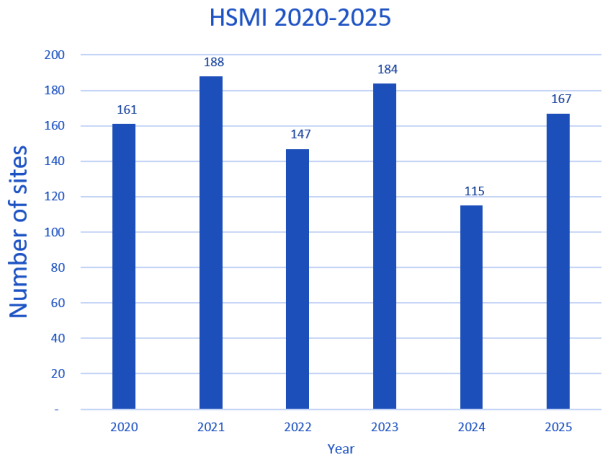
Smolt phase



Grow-out phase

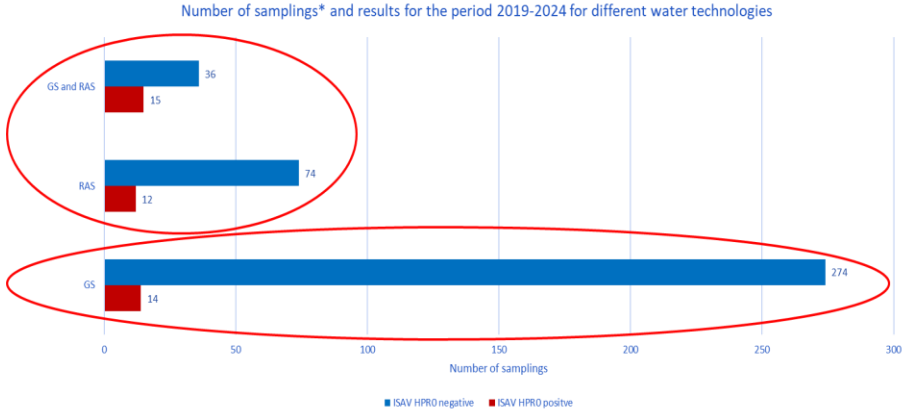






Figures: Victor Oliveira, Hege Løkslett, Torfinn Moldal, NVI

HPR0



Two Isavirus variants

Connection between ILAV HPR0 in smolt phase to ISAV HPRdel/ISA in Grow-out phase

Two trials





1. ISAV



IP challenge:

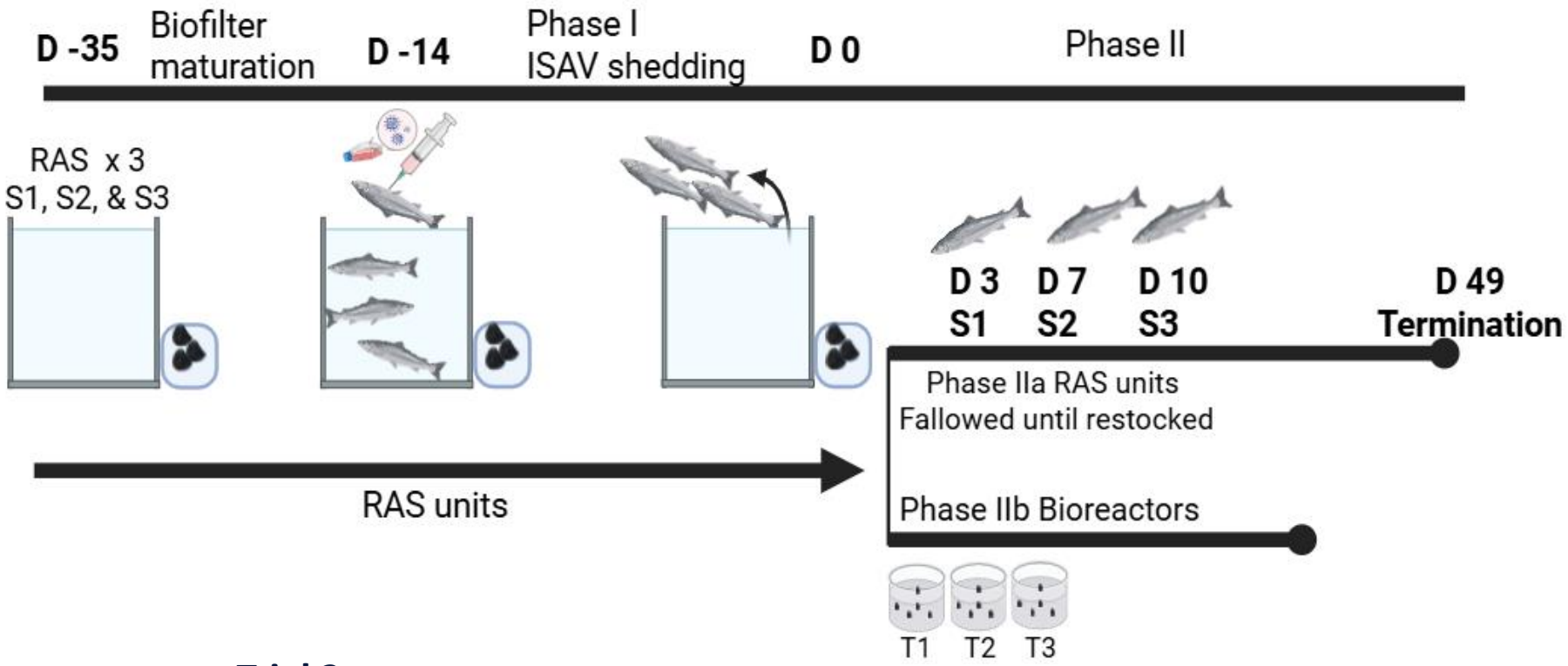
smolt 50 g

2. Ca. Branchiomonas cysticola+IPNV+PRV



Transport of infected smolt from commercial farm - ~78 g

A. Experiment set-up



Trial 2:

Shedding period 3 weeks

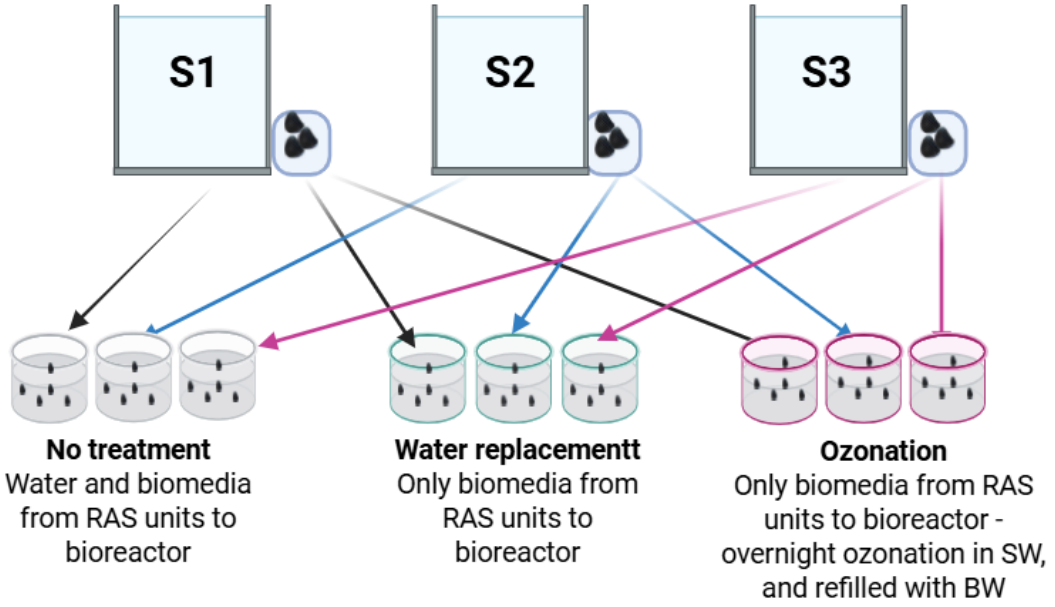
S1 - 5 hrs

S2 - 2 days

S3 - 4 days

Norweg Veterin

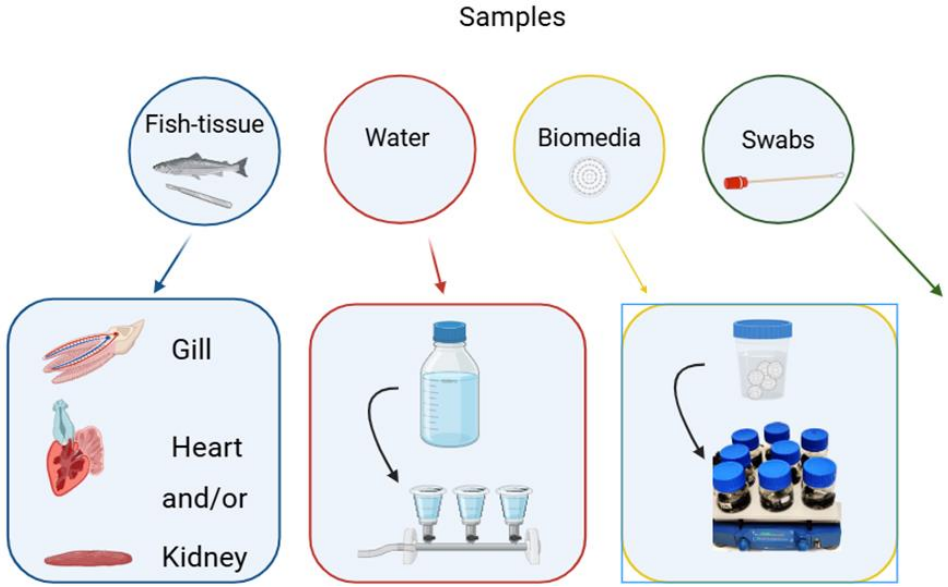
Phase II: small scale bioreactors





Sampling, processing and analyses







Infected "shedders"/ Nâive fish at termination

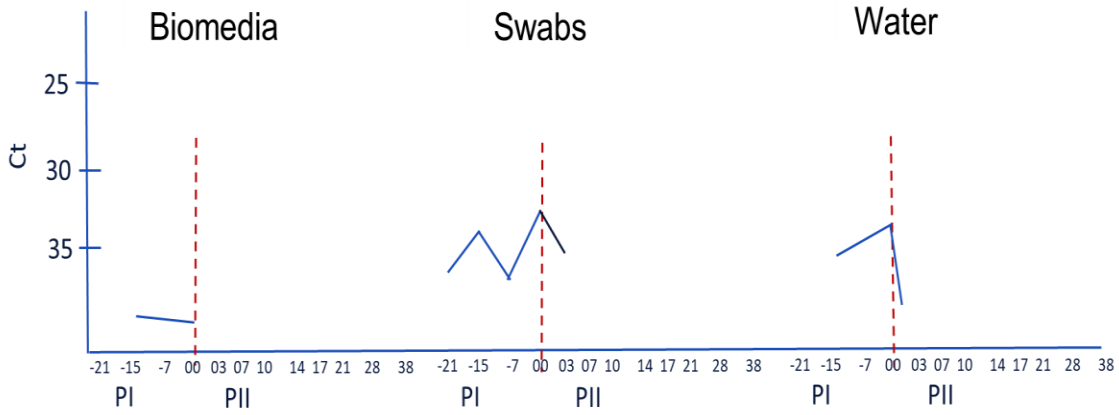
- qPCR
- Histology
- Immunohistochemistry

Analysis

- qPCR
- IFAT staining

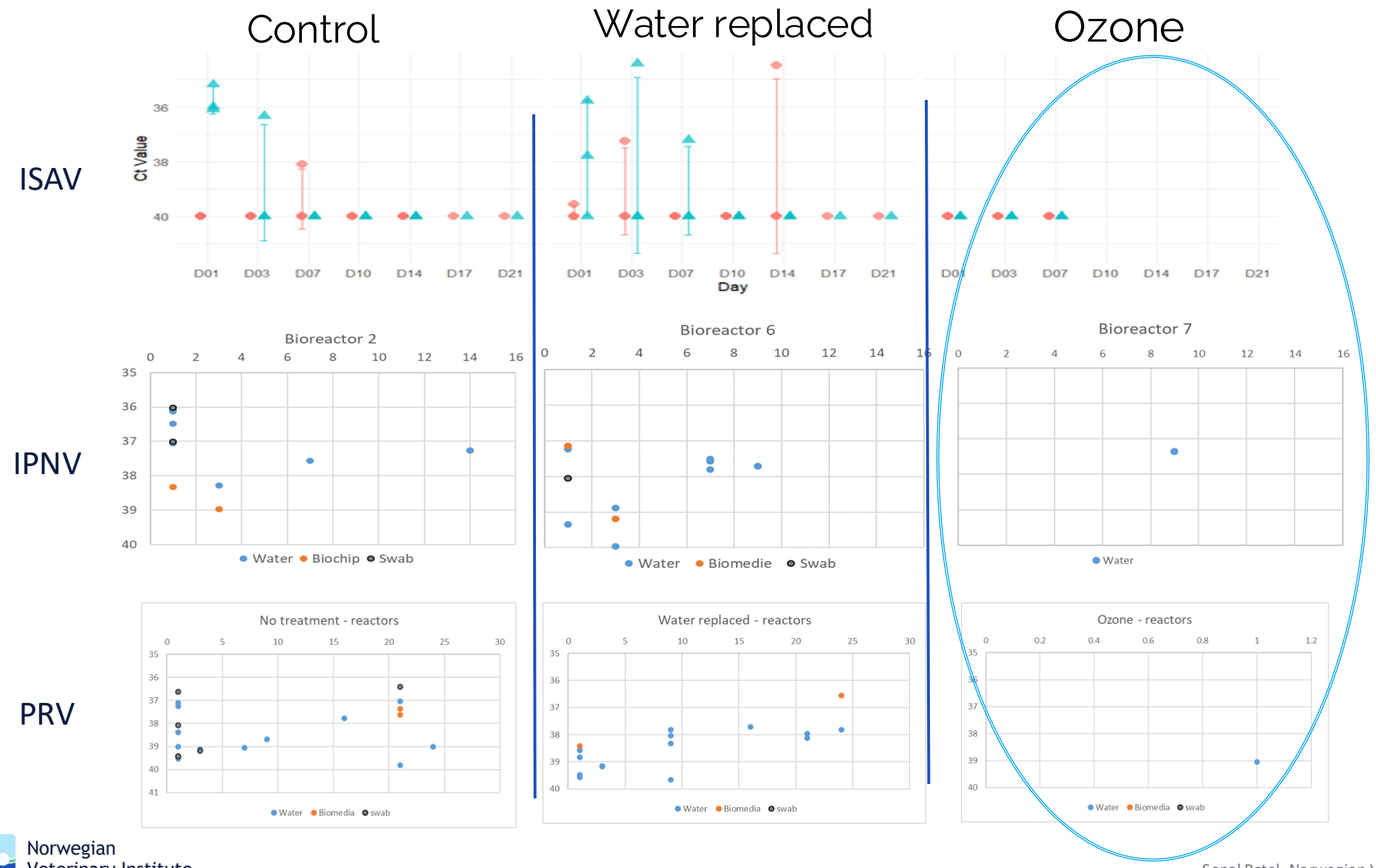
Persistence- Substantial pathogen dependent variation

Ca. B. cysticola



Bioreactors

Ca. B. cysticola: D1 water (No treatment); swab (ozone): Ct 38 - 39



Water
Biomedia
Swab

Summary – lab trials


BRAS
Vitenskapet

Spredning, overlevelse og inaktivering av fiskepatogener i labskala-RAS

Hvor lenge kan fiskepatogener overleve i et RAS uten fisk? For å få svar på dette spørsmålet har det blitt gjennomført kontrollerte laboratorieforsøk. Vi undersøkte overlevelsen av fiskepatogener i labskala-RAS, nærmere bestemt i vannet, på biologemer og på overflater under ulike forhold. Resultatene indikerer at brakklegging og ozonering kan ha stor effekt på overlevelsen av smittestoffer, men behandlingene kan påvirke overlevelse av bakterier (Ca. B. cysticola) og virus (IPNV og ILAV HPRdel) på ulik måte.

Fiskepatogener og biosikkerhetstiltak i RAS

Kontrollerte forsøk

Treatments

Fallowing :

Effective: *Ca.* Branchiomonas and ISAV HPRdel (virus degradation over time?)

- Pathogen load too low to sustain infection?
- Short persistence following removal of fish

Not effective: IPNV, PRV - näive fish positive at termination- so short-term (4 days) fallowing ineffective

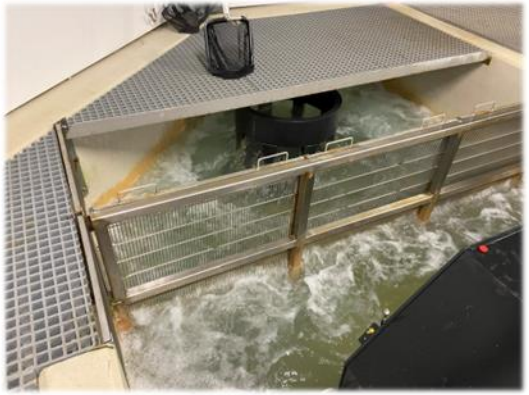
Water exchange/replacement: NO real effect

Ozonation: effective for elimination of ISAV HPRdel & *Ca.* Branchiomonas, but not completely for IPNV and PRV?

- Effect on biofilter flora? Effect on water quality
- Microbiome studies now being analysed…..STAY TUNED!

Commercial RAS facilities - 5 cases













Farm 2, Case 3: HPR0 and PRV

Day: -3 | 0 | 5 | 13 | 14 | 25 | 37 | 48 | 61

Confirmation of agent

Positive fish out

Naive fish in

~ 1.5 weeks after naive fish

Only swabs

~7 weeks after naive fish

~9 weeks after naive fish

Biosecurity measures: Wash, reuse water | Fallowing

Day sampling: -3 | 5 | 13 | 25 | 37 | 48 | 61

	HPR0	PRV	HPR0	PRV	HPR0	PRV	HPR0	PRV	HPR0	PRV	HPR0	PRV	HPR0	PRV
	90	N	40	N	30	N	10	N	N	30	80	N	90	N
	100	N	N	N	N	N	N	N	NA	NA	NA	NA	100	N
	100	100	N	N	N	N	N	N	NA	NA	100	N	100	N
	63	8	NA	NA	NA	NA	N	N	NA	NA	100	N	100	N

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Farm 4, Case 5a: Ca. Branchiomonas cysticola

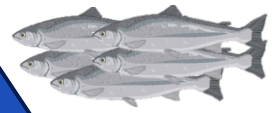
Day: -1 0 24 52 77

Confirmation of agent | **Positive fish out** | **Naive fish in** | **3.5 W after** naive fish introduced, Same day vaccination started, **Fish moved to RAS4** | **7 W after** naive fish, 3 W post vaccination

Biosecurity measures: Wash, disinfect tank | Fallowing

Day sampling:	-1	6	24	52	77
	33	N	N	N	N
	NA	NA	NA	NA	NA
	80	NA	N	N	NA
	90	NA	NA	N	NA

D0 & D4: 33% pos.
Vaccination machine



Fish moved to new department

Farm 4, Case 5b: Ca. Branchiomonas cysticola

Day: 0 (day of vaccination) 25-28 63 142 184

Fish from RAS3 after sampling of RAS4 | **~ 4 W** Post vaccination | **~ 9 W** Post vaccination

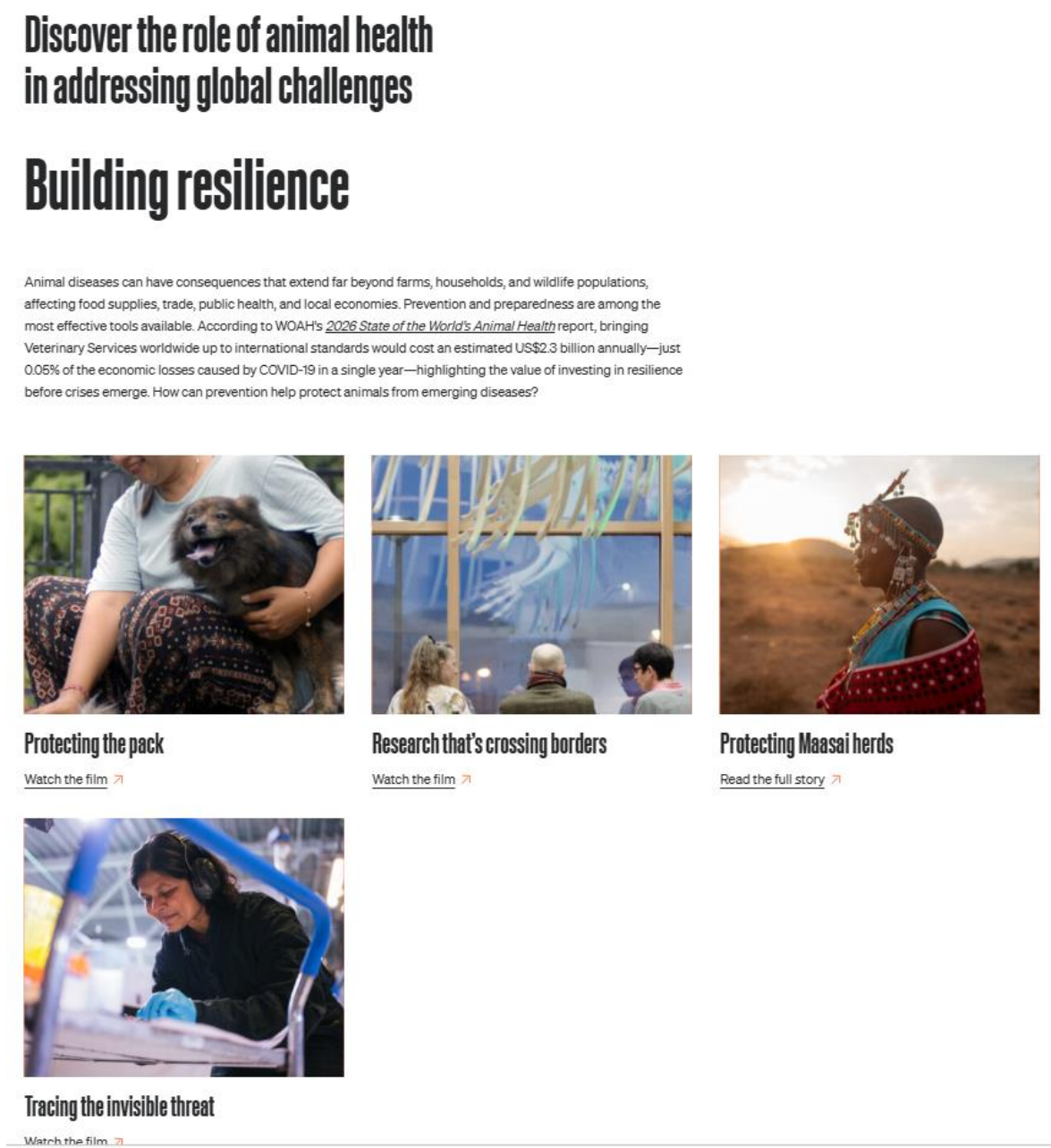
Day sampling:	0	25-28	63	142	184
	N	20	27	N	N
	N	NA	NA	NA	NA
	N	N	N	NA	N
	NA	N	N	NA	N

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[Animals & Us - WOAH - World Organisation for Animal Health]

[European Partnership for Animal Health and Welfare - YouTube]

[eupahw yout tube - Google Search]

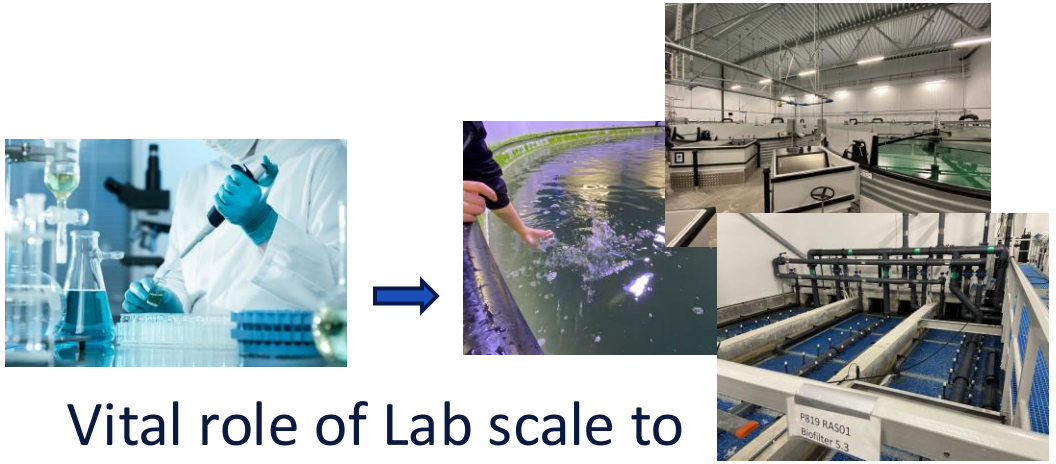


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Biosecurity measures

5 cases:

- *Ca.* Branchiomonas cysticola – 2 sites
- PRV – 3 sites
- IPNV – 1 site + historical case
- HPR0 – 1 site



Vital role of Lab scale to commercial scale testing

Thanks to Industrial partners:

Osland Settefisk	Lerøy Aurora
Salmar Settefisk	Salangfisk
Erko Settefisk	Mowi ASA
Helgeland Smolt	Hardingsmolt AS
Grieg Seafood ASA	Ænes Inkubator AS

Thanks to!

Sonal Patel, **Duncan Colquhoun**, Simon Weli, Merete Sture Gåsvær, Miriam Furne, Saima Mohammad, Henriette Kvalvik

Ole Kristian Hess-Erga, Paulo Fernandes

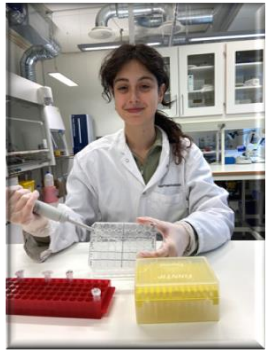
Sondre Veberg Larsen

Elisabeth Midtbø Viik

Reidar Handegård, Melanie Andrews, Mai-Linn J. Aga

Håkon Dale

Erik Sterud



Marta Moutinho
Master student
University of Porto

Finance

FHF - 091792

SOA15 A2-1

«Co-funded by the European Union under project 101136346 EUPAHW. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.»

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