



European Union Reference Laboratory for Fish and Crustacean Diseases

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

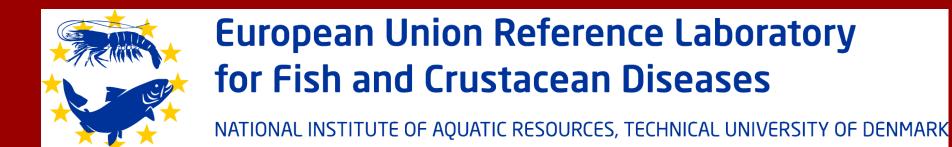
29th Annual Workshop of the National Reference Laboratories for Fish Diseases 26th -27th May 2025

Technical Presentations



Niccolò Vendramin and Thomas Weise 29th AW of the National Reference Laboratories for Fish Diseases, May 26th 2025

Survey & Diagnosis of fish diseases in 2024





Survey & Diagnosis of listed fish diseases in the European Community 2024

An Annual questionnaire

- General data: Number of farms and health status for Cat. C disease / presence of control programs for Cat. E disease
- 2. Epidemiological data: Number of outbreaks and increase/decrease in number of infected farms/severity
- 3. Laboratory data, NRL and regional laboratories: total number samples tested and samples tested positive for each disease

4. Reports from the individual European countries: general information on aquaculture production, fish health status, disease challenging production.





Report

- The report was collated in May, and will be sent to all of you for validation.
- Please check if the information given is correct!



Report on Survey and Diagnosis of Fish Diseases in Europe 2024



DISCLAIMER The EURL for Fish and Crustacean Diseases and the EU commission have no liability for the accuracy of the information and cannot be held liable for any third-party claims or losses of any damages related to this report.



General production data taken from:

FEAP Data refer to 2023





Additional relevant sources



National reports

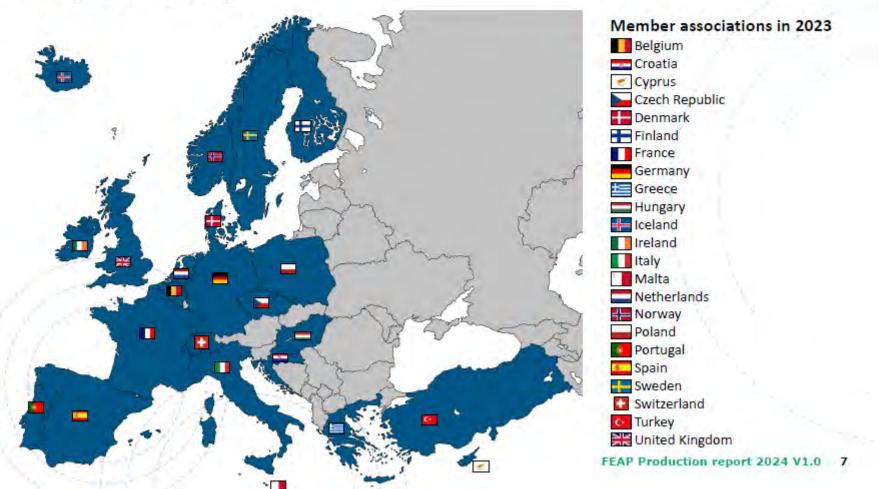


Fish feed of the future? A soldier fly larva, magnified 80 times. Photo: Jannicke Wilk-Nielsen, Norwegian Veterinary Institute



Fish Farming Production by country



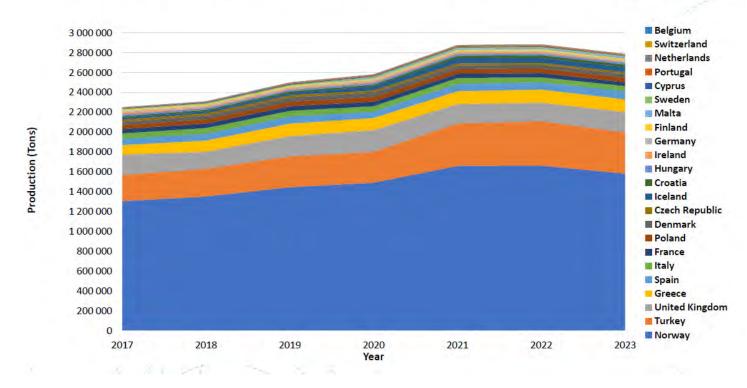




Fish Farming Production in Europe

Fish Farming Production in Europe



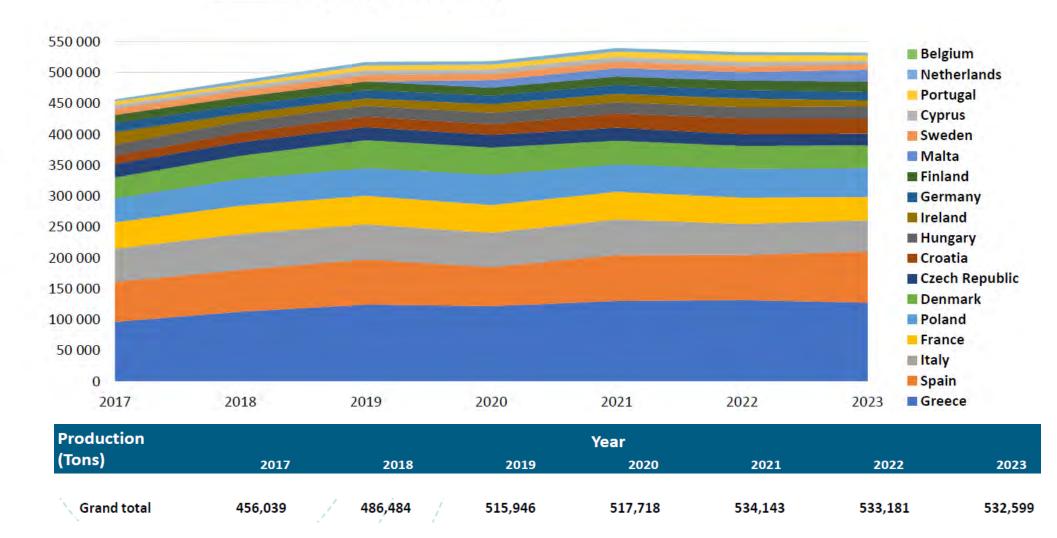


Grand Total (tonns) From report 2023 2,251,195 2,311,299 2,500,713 2,581,443 2,872,722 2,885,231 2,789,559		2017	2018	2019	2020	2021	2022	2023
	(tonns)	2,251,195	2,311,299	2,500,713	2,581,443	2,872,722	2,885,231	2,789,559



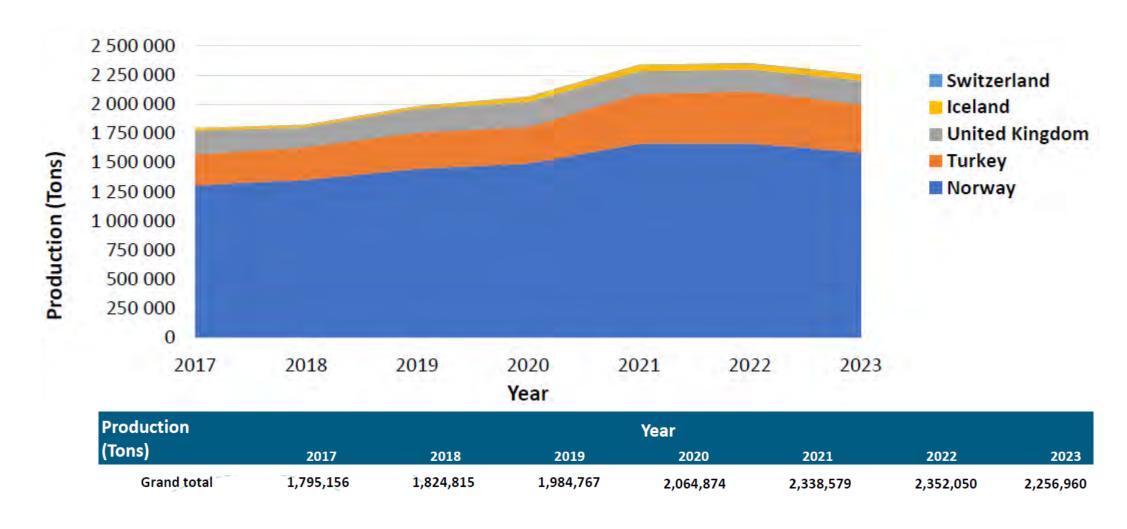
Production (Tons)

Fish Farming Production in EU countries





Fish Farming Production in non-EU countries





Farmed species of relevance in European aquaculture



Species 2023 AS 1,823,376





Species	2023		
RT table size	247,330		
RT large size	154,149		





Species	2023
Carp	52,357



Species	2023
Sea bass	236,332
Sea bream	244,186



DTU



Other species of relevance in European aquaculture

- Salmonids (Artcic char **7,274** tonns in 2023) was 12.000 Tonns in 2022
- Sturgeon (2,258 tonns produced in 2023 approx 226 T of caviar)
- Halibut (**2,380** tonns in 2023)
- Turbot (**13,030** tonns in 2023)
- African Catfish (12,276 tonns in 2023)
- Meagre (**13,927** tonns in 2023)
- Bluefin Tuna (32,200 tonns in 2023, 59% production Malta)
- Eel (**4,192** tonns in 2023)

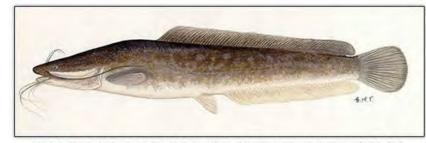


Figure 1. Clarias gariepinus (courtesy of South African Institute for Aquatic Biodiversity Artist: E.M.Tarr)

 Cleanerfish for sea lice mitigation (33 million of total cleanerfish deployed at sea in Norway (fiskedirektorat) in 2023 -33 in 2022 – 45 in 2021)









	Year		
2020	2021	2022	2023
11,790	13,549	14,000	18,910
	8,277	8,500	6,000
	- Y-1		4,000
	3,270	3,200	3,252
			38
	25,096	25,700	32,200
		2020 2021 11,790 13,549 8,277 3,270	2020 2021 2022 11,790 13,549 14,000 8,277 8,500 3,270 3,200

Farming of bluefin Tuna is sea ranching – catching wild juveniles transfer them in sea cages and feed them with fish for fattening

In Spain IEO achieved for the first time worldwide the reproduction of bluefin tuna in land-based facilities

A company in Cape Verde established first hatchery for Tuna https://www.nortuna.com/tasteful-seafood/atlantic-bluefin-tuna





European Aquaculture production

Total number of authorised/licenced fish farms in Europe is estimated to be about 37103











DTU

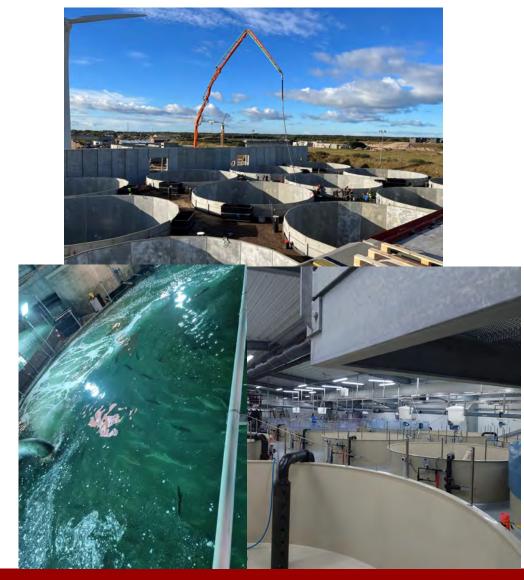


At least 15 countries reports RAS operations



Model 3 – biofilter- Raibow trout production Denmark

Fully recirculated Aquaculture facility
Indoor facilities
High value species or warmwater species





Production of species in RAS



Clarias gariepinus



Stizostedion Lucioperca



Tilapia niloticus



Pangasianodon hypophthalmus







With the implementation of the AHL we passed from 5 health categories to 4 health status

2006/88

Health Category	Health status	Intro from	Dispatch to
I	Disease free	ı	I-V
II	Surveillance programme	I	III+V
III	Undetermined: "Not known to be infected but not subject to eradication programme for achieving disease free status"	1, 11 & 111	III+V
IV	Eradication programme	I	V
V	Infected	I-V	v

Applies to infection with VHS, IHN, HPR-del ISAV, WSSV and other present or emerging diseases

2020/689	
Disease free	
Eradication program	6 years max- with possibility for additional 6 years
Farm under surveillance but not in eradication program (will not achieve	
Notified but Not in program (both infected and non infected)	Possibility for national program



One bias of the survey this year

• In the survey performed in 2024 (refer to data from NRL in 2023)

1.1 Total Number of fish farms authorized or active in 2023 in the country. Only include farms/sites that were active in 2023 or part of 2023.

Total 36838

In the survey performed in 2025 (refer to data from NRL in 2024)

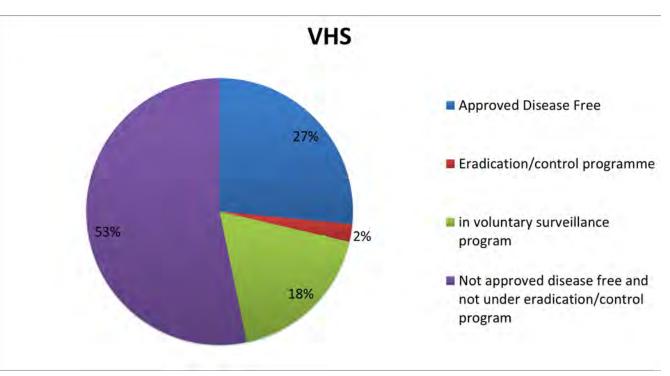
1.1 Total Number of fish farms authorized or active in 2024 in the country. Only include farms/sites that were active in 2024 or part of 2024.

Total

26881



Distribution of farms in zones and compartments according health status for VHS 8426 farms (*about 14000 farms categorized in 2023)



Health status for VHS:

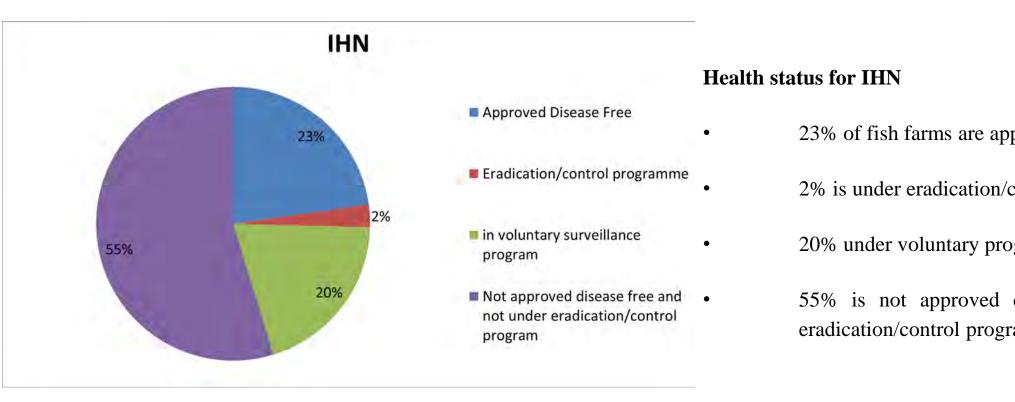
- 27% of fish farms are approved disease free
- 2% is under eradication/control program
- 18% under voluntary program
- 53% is not approved disease free and not under eradication/control program

19

DTU Title Date



Distribution of farms in zones and compartments according to health status for IHN including 8186 farms (*about 14000 farms categorized in 2023)

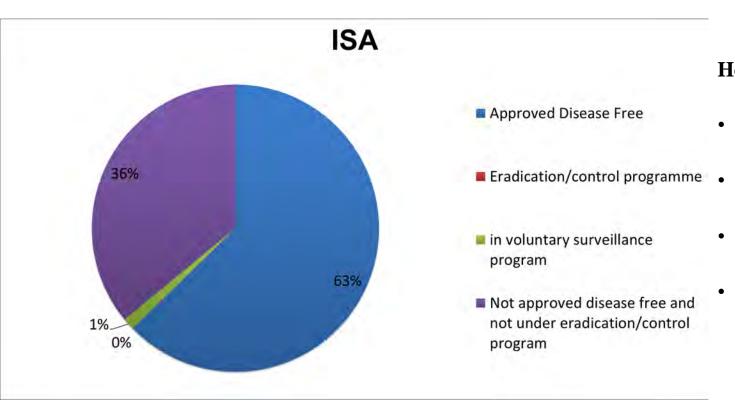


- 23% of fish farms are approved disease free
- 2% is under eradication/control program
- 20% under voluntary program
 - 55% is not approved disease free and not under eradication/control program

DTU Date Title 20



Distribution of farms in zones and compartments according to health status for ISA including 5074 farms with susceptible species



Health status for ISA (Infection with HPRΔ ISAV)

- 63% of fish farms are approved disease free
 - 0% is under eradication/control program
- 1% under voluntary program
 - 36% is not approved disease free and not under eradication/control program



KHVD is now category E disease - means a listed disease for which there is a need for surveillance within the Union

Are there control programs for the Cat. E disease KHVD? (Y/N)

If yes, please specify how the program is implemented



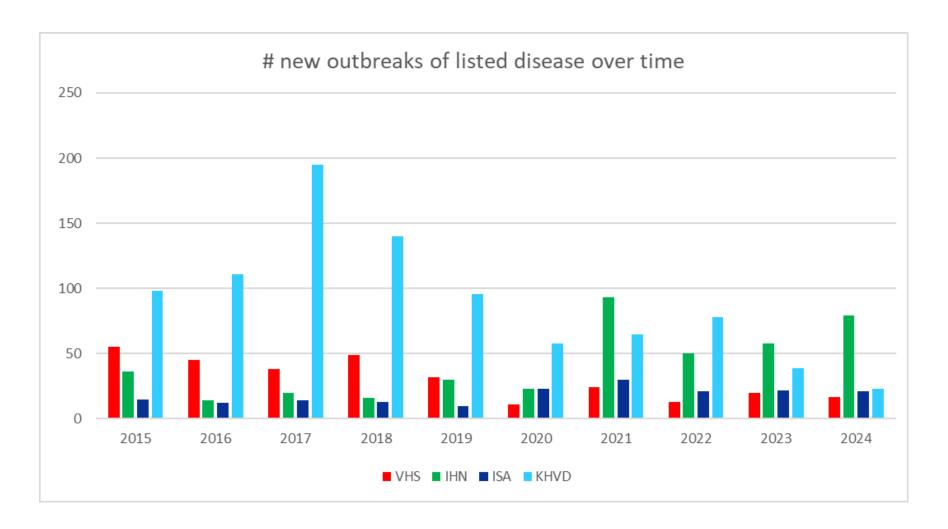
KHVD is category E disease - means a listed disease for which there is a need for surveillance within the Union

- 9 countries reported an active control program for KHVD
- This largely imply targeted surveillance of farm with susceptible species
- One MS has status of Free from KHVD
- Presence of compartments with freedom status (Italy sud Tirol, Germany voluntary Lower saxony)
- National surveillance programs in Bulgaria, Latvia, Republic of North Macedonia





Reported outbreaks of listed disease





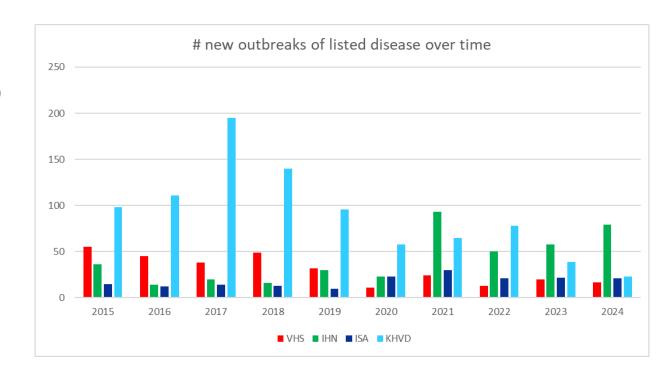


Disease outbreaks - VHS

• 17 confirmed cases in 2024 (20 reported in 2023)

- 2 in Austria
- 2 in Czech Republic
- 1 in France (detection during surveillance)
- 6 in Germany
- 5 in Poland
- 1 in Switzerland (decrease)





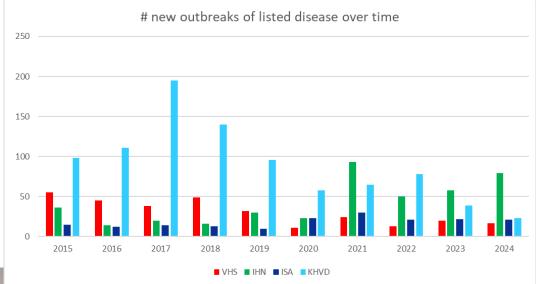


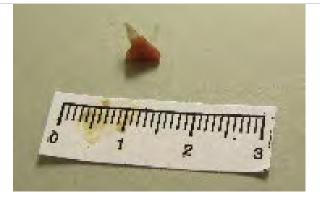
Disease outbreaks - IHN

- 79 outbreaks reported in 2024 (58 reported in 2023)
 - 11 in Belgium (increase in case number durinv voluntaey scresurveillance)
 - 1 in Czech Republic
 - 30 in Germany
 - 1 in Slovenia
 - 36 in Rep. of Macedonia



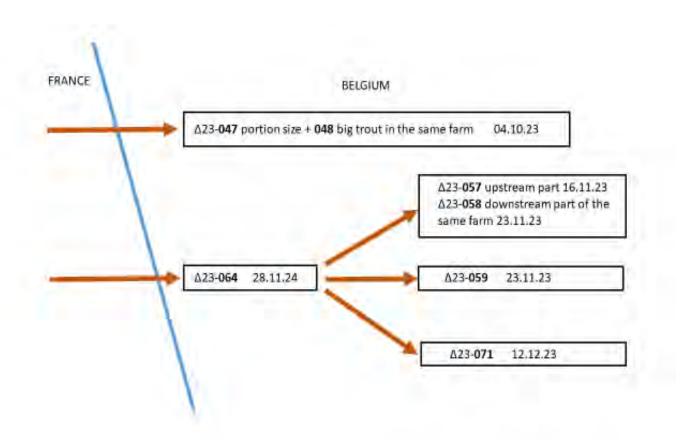








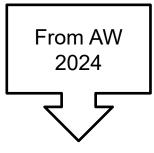
IHN outbreak in Belgium FROM last AW



F.Lieffrig – NRL belgium

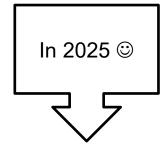


IHN in Belgium



Planned shipment of isolate from Belgium to **EURL**

Initiative on NGS for molecular tracing with higher resolution!



Full genome sequence obtained – analysis on going

More isolates from voluntary program to be sequenced

Dialogue with NRLs in Belgium and France on how to proceed





Disease outbreaks - ISA

- 21 outbreaks of ISA reported
- 20 in Norway similar to previous years (number of infected farms, case of ISAV HPRDel detection with no clinical signs)
- 1 in Faroe Islands
- Follow up presentations later in the workshop







Disease outbreaks - KHV

- 23 outbreaks reported
- Category E disease .
- Decrease of KHV of number of outbreaks in Hungary



Pics from CEFAS Gov.uk



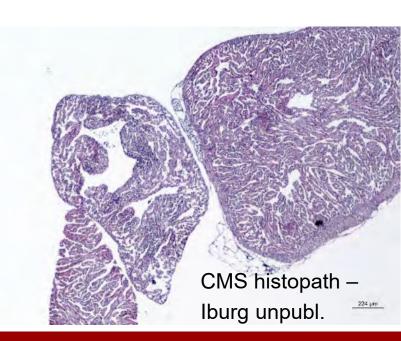


Other fish diseases problems in Europe Atlantic salmon

- PD reduced impact extensive vaccination campaign with DNA-vaccine
- Piscirickettsia salmonis SRS
- BKD
- Cardiomyopathies (CMS HSMI)
- IPN outbreak due to variants escaping QTL
- Skin ulcers







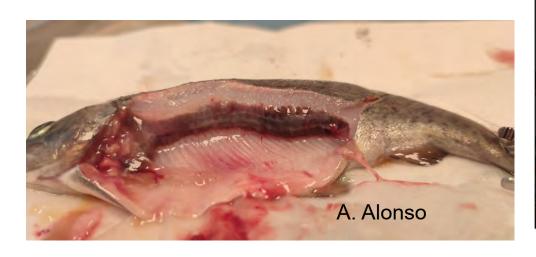


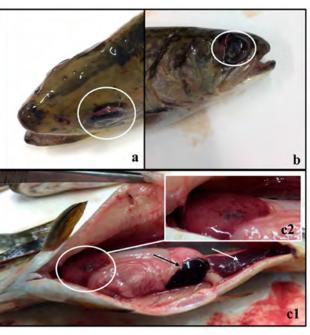
Other fish diseases problems in Europe Rainbow trout



Rainbow trout (Source: Seafish image)

- Lactoccosis (France, Portugal, Slovenia, Italy, Bulgaria)
- RTFS
- BKD





Pastorino et al., 2019

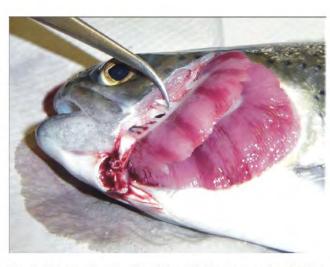


Fig. 1. Advanced stage of nodular gill disease grossly manifested in rainbow trout *Oncorhynchus mykiss* as swelling of gills with multiple pale nodules. Natural size.

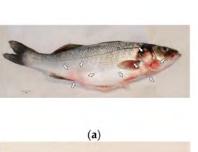
Peckova et al., 2010

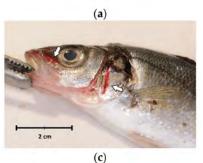
Other fish diseases problems in Europe seabass and seabream



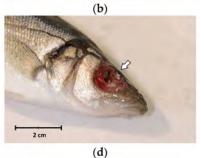


- In seabass and seabream :
- Diminished reports of VNN/VER
- Outbreaks of Aeromonas Veronii in sea cages
- Sparicotyle sp. in Gilthead sea bream
- Vibrio harveyi infection in Sea bass reported as relevant in Italy and Croatia
- Lactoccus garviae (unexpected summer mortality)

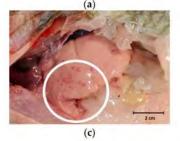




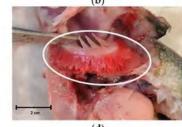












Salogni et al.,2024

SOA 15 – task 1.1. mapping biosecurity knowledge gaps in farmed finfish in EUrope

- Species working groups (sea bass and sea bream, Rainbow trout, Carp, A.salmon)
- Live questionnaire (freesurveyonline) at WS or remotely and discussion with participants
- Cross-species GAPS highlighted
 - Documentation of disinfection efficacy (UV, Ozone, etc) under field condition
 - Assessment of risk posed by wild fauna in maintaining infection
 - Viability of pathogen in the environment
 - Vertical /vertical associated transmission of pathogens
 - Lack of documentation systems for safe trade of live fish
 - Interpretation of eNA result testing







Conclusion on S&D 2024

- Stable production in EUROPE initiative for increase production from association of producers
- More RAS projects developing
- IHN slight increase, VHS stable
- ISA outbreaks in Norway and Faroe Island.
- Emergence of bacterial pathogens *L.garviae* (global warming ?)



Thank you for all the significant work, efforts and time used for compiling these data!!

And please use the report!

Thank you for your attention!

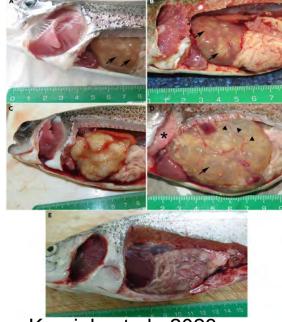




New disease observed in RAS farmed RT



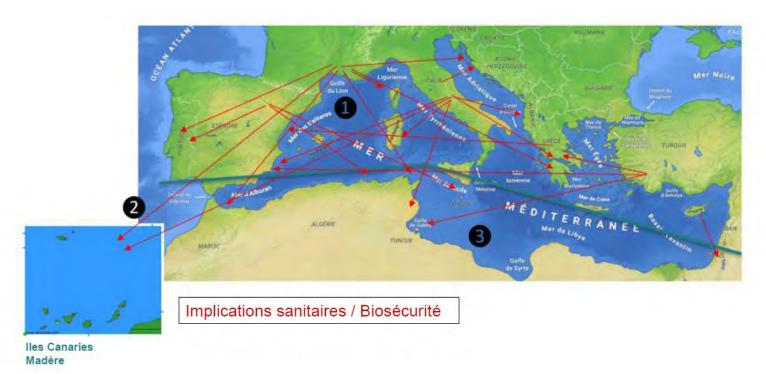
- Large size RT farmed in RAS
- Presence of granuloma in liver
- Increased mortality
- Extensive laboratory investigation including extensive list of pathogens have not identified a putative causative agent
- Preliminary testing for nidovirus –ve
- Further investigation by NGS ongoing

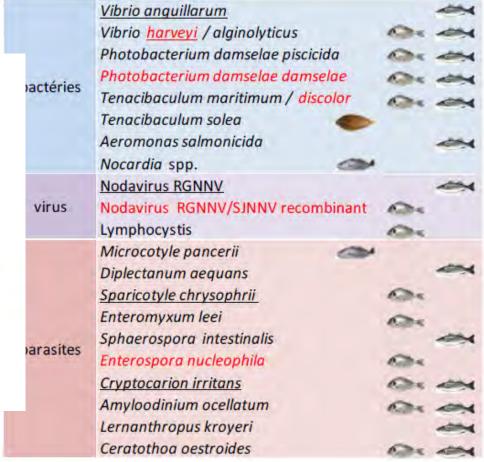


Karniely et al., 2023



From Alain le Breton – Selarl Veteau







IHN is key issue of the workshop and will be extensively adressed

3 Presentation today 2 on the ongoing situation in Denmark and Finland, 1 on Atlantic salmon susceptibility

1 (from the EURL) tomorrow

Belgium reported sudden occurrence of IHN in Rainbow trout also large size >1.5 kg

Two new farms detected in Republic of North Macedonia

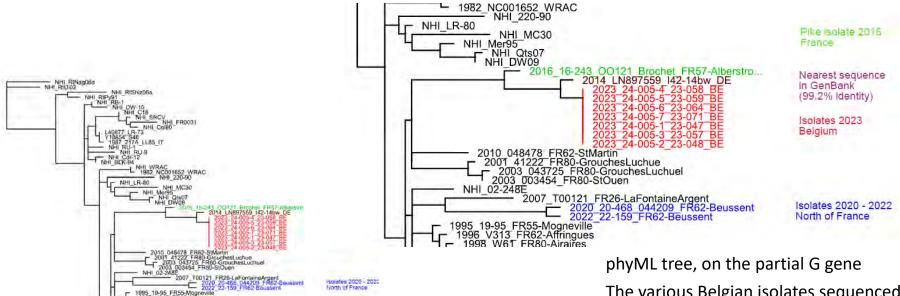
Number of infected farm decreased in Denmark (further details later on)





0.02

IHN outbreak in Belgium



The various Belgian isolates sequenced by the French NRL had epidemiological links, notably regarding their provider, who is known to work mainly with French fish farmers. The 7 isolates displayed 100% identity (on 651 nucleotides) and belonged to the genotype E.

The detected strains exhibited 98% identity (13 SNPs) with a strain isolated on northern pike in 2016 (in green in the tree) and 93.6 to 94% id (39 to 42 SNPs) with 2020 and 2022 isolates from department 62 (North of France, blue in the tree), and 99.2% identity to a german sequence in GenBank from 2014

Thanks a lot to French NRL (Marine Baud and Lenaig Louboutin) and Belgian NRL (Francois lieffrig) for sharing the results

Overview of the disease situation in Norway

EURL-workshop, 26th of May 2025

Torfinn Moldal, Scientific coordinator for fish health/veterinarian, PhD



The Norwegian Fish Health Report



Published annually since 2003
Separate chapter on farmed cod
Separate report on wild fish
Calculations of mortality risk also
for cleaner fish and farmed
Atlantic cod in sea



Photo: Jannicke Wiik-Nielsen

The statistical basis for the report

Access to data at site level from private laboratories since 2020

Survey to fish health personnel as well as advisors and inspectors in the Norwegian Food Safety Authority

Official registers on active sites, biomass, harvest and mortality

Categorization of death causes















Some key official production data

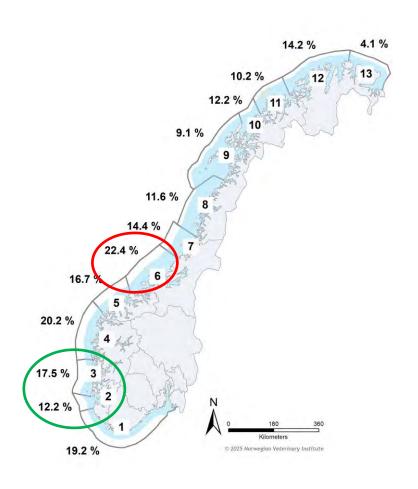
	2020	2021	2022	2023	2024
Salmonid juvenile production (active)	157	155	153	148	150
Salmonid ongrowing sea sites (active)	907	895	900	893	908
Number of fish transferred to sea (millions)					
Atlantic salmon	289	304	341	411	387
Rainbow trout	17,5	13,0	18,0	25,5	27
Harvested (tons)					
Atlantic salmon	1 393 129	1 557 739	1 539 375	1 520 348	1 545 853
Rainbow trout	92 864	84 077	76 653	81 251	95 152



Mortality, delousing and slaughter quality



Mortality Risk for Atlantic salmon in sea in 2024



57.8 million salmon and 2.4 million rainbow dead in the sea phase

Nationwide: 15,4 percent for salmon and 15,0 per cent for rainbow trout

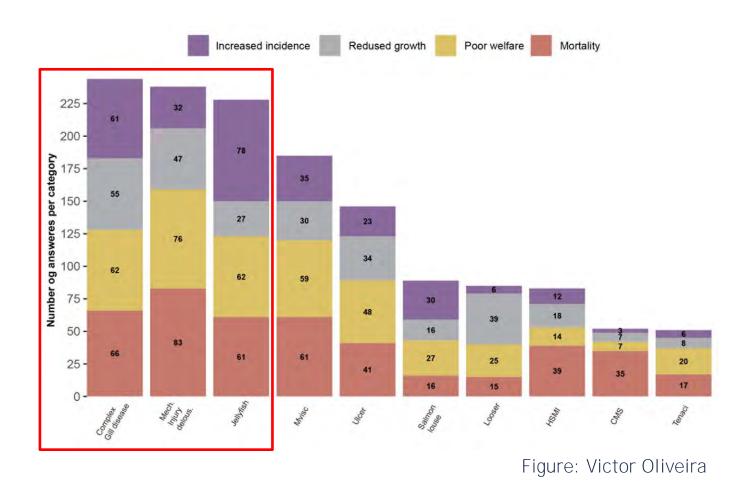
Lower risk for Atlantic salmon in 2024 than in 2023 nationwide

- Lower in PA2, PA3 og PA8-PA10
- Higher in PA4, PA6, PA7 og PA12



Map: Attila Tarpai

Ranking of health problems for salmon in sea

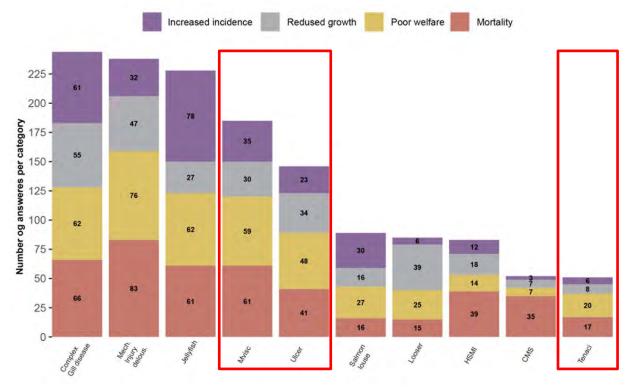




Wounds are likely the biggest challenge for salmon



Photo: Geir Bornø







Categorization of death causes for salmon in sea



A Infectious disease
A 01 Viral disease
A 01.170 Cardiomyopathy
syndrom



D Physiological causes



B Environmental impact



E Other causes



C Injuries or trauma



F Unknown

Categorization of death causes in salmon in sea

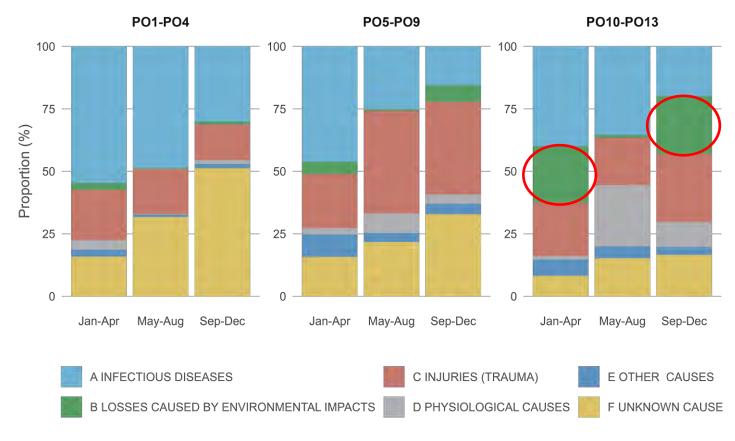
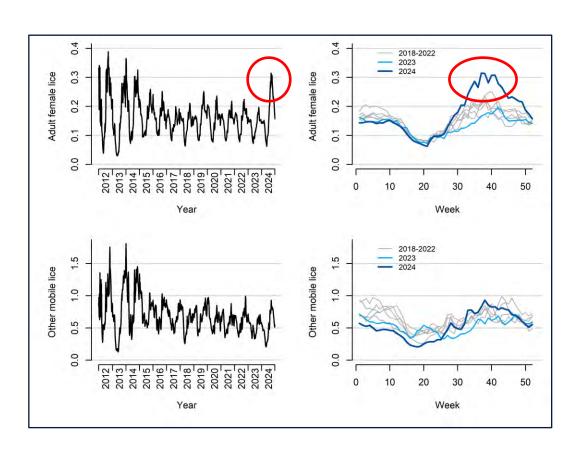
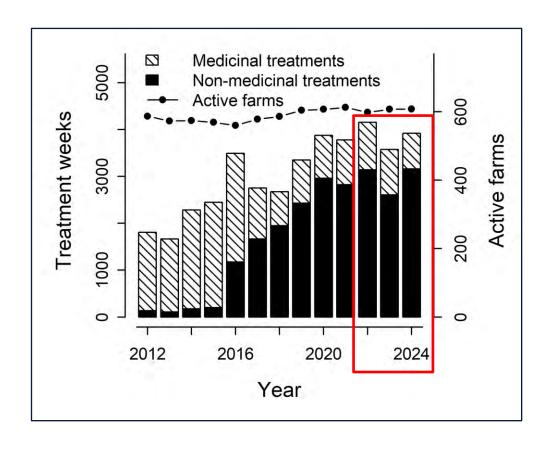




Figure: Annika Krutto and Victor Oliveira

Number of lice and lice treatments







Seasonal variation for slaughter quality

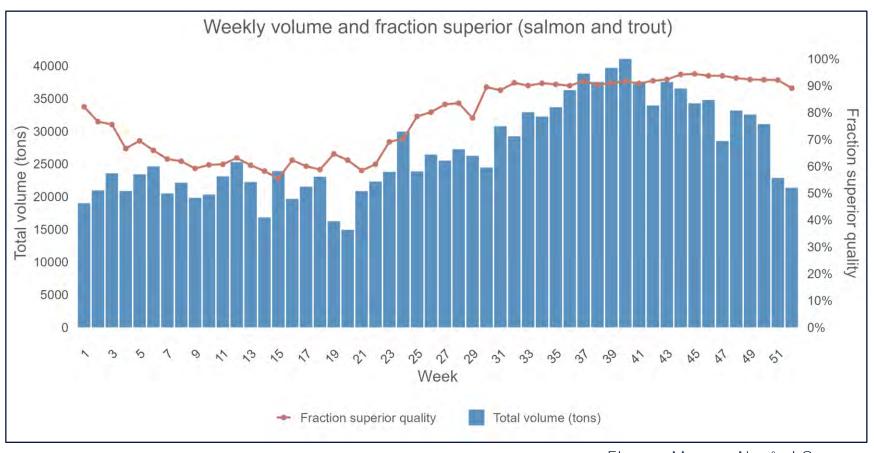
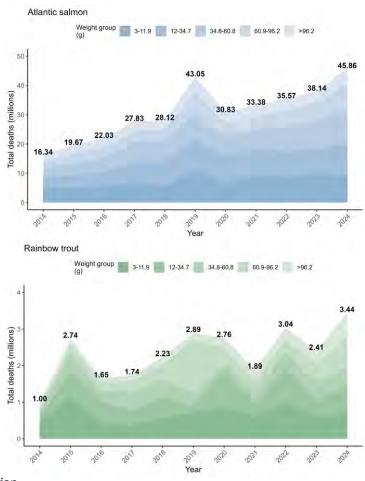
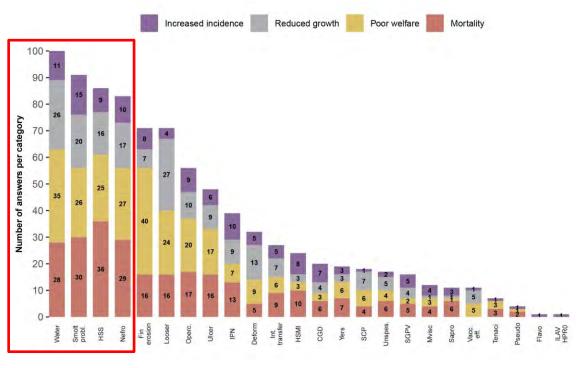




Figure: Magnus Nygård Osnes

Mortality in hatcheries (salmon and rainbow trout)









Vaccination and selected diseases



Sold vaccine doses per category

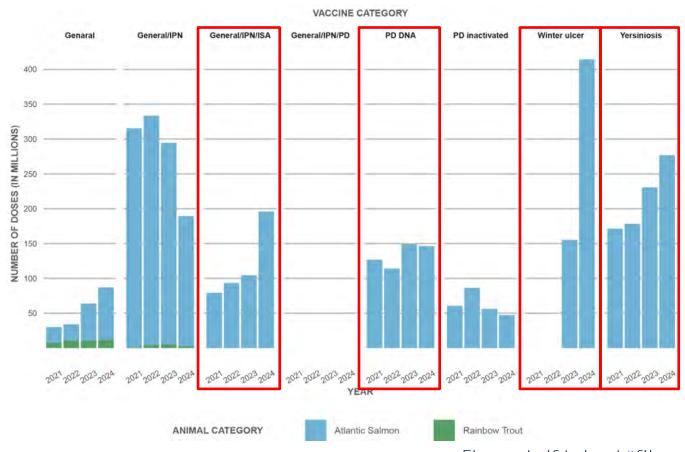
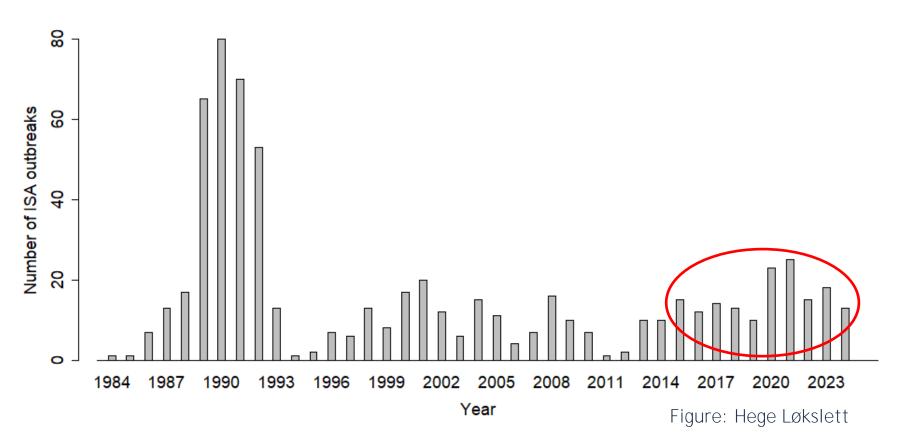




Figure: Leif Lukas Löfling

Number of confirmed ISA outbreaks per year





ISA outbreaks and suspicions in 2024



Outbreaks at 13 sites and suspicions at nine sites

Substantial portion in Mid-Norway

Several virus variants

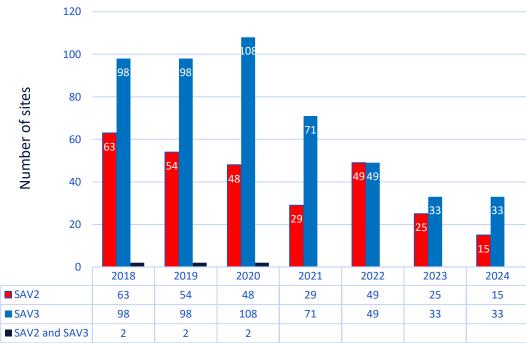
Outbreak at one site with vaccinated fish



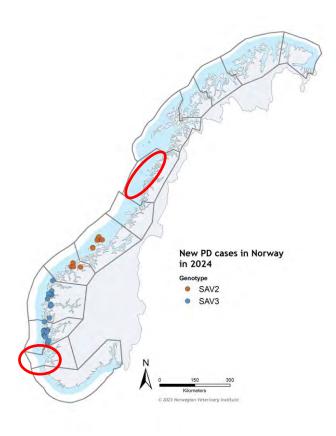
Map: Attila Tarpai

Pancreas disease (PD)





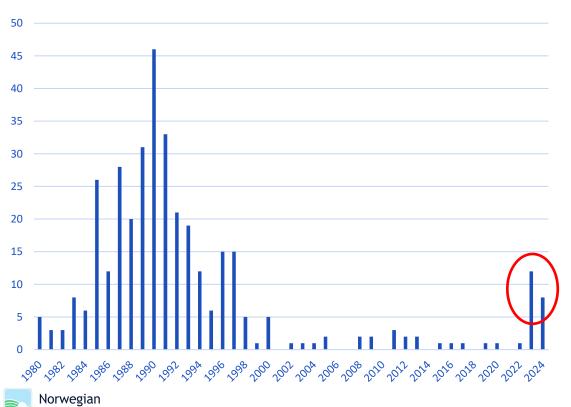




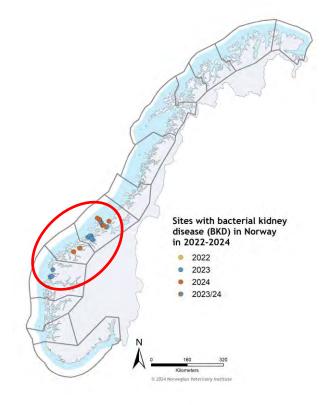
Map: Attila Tarpai

Bacterial Kidney Disease (BKD)



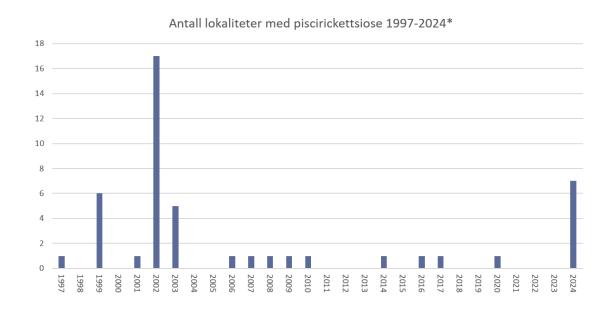


Veterinary Institute





Piscirickettsiosis



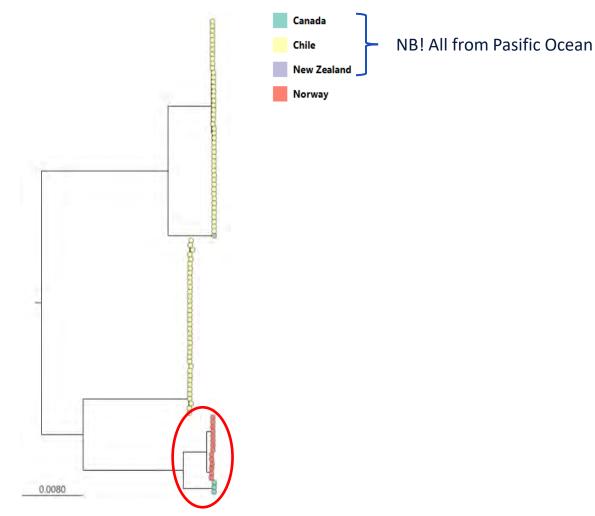
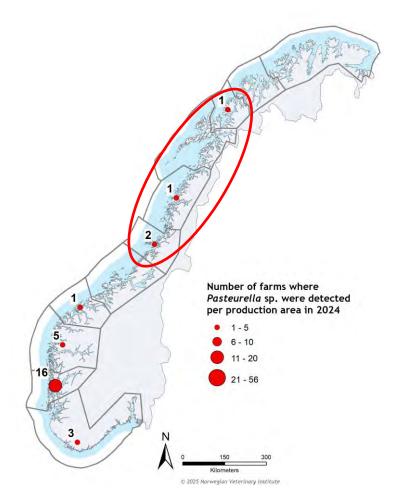




Figure: Snorre Gulla

Pasteurellosis



Map: Attila Tarpai



RESEARCH ARTICLE OPENACCESS

Associations Between Delousing Practices and Pasteurellosis in Farmed Atlantic Salmon

Leif Christian Stige D | Duncan J. Colquhoun D | Victor H. S. Oliveira D |

Norwegian Veterinary Institute, As, Norway

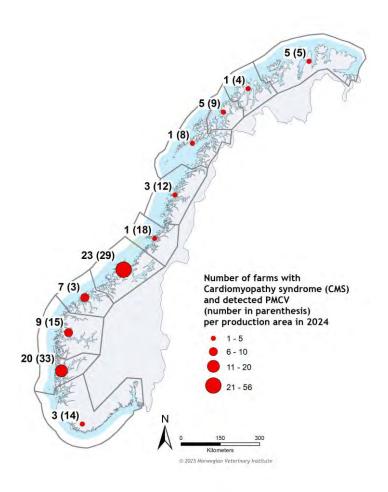
Correspondence: Leif Christian Stige (leif.christian.stige@vetinst.no)

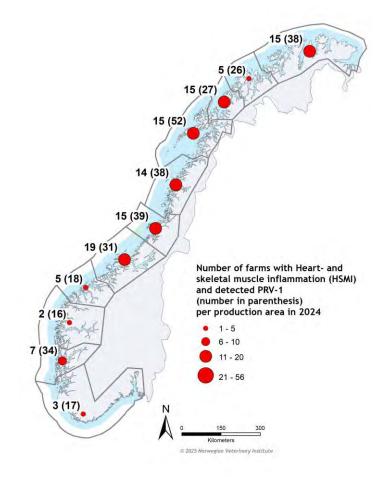
Received: 11 November 2024 | Revised: 8 January 2025 | Accepted: 17 January 2025

Funding: This study was funded by the Norwegian Seafood Research Fund through the project 'Pasteurellosis in Norwegian salmon' (project no. 901680).

Keywords: Atlantic salmon (Salmo salar) | Lepeophtheirus salmonis | Pasteurella | sea louse treatment

Viral myopathies







Infectious pancreas necrosis (IPN)

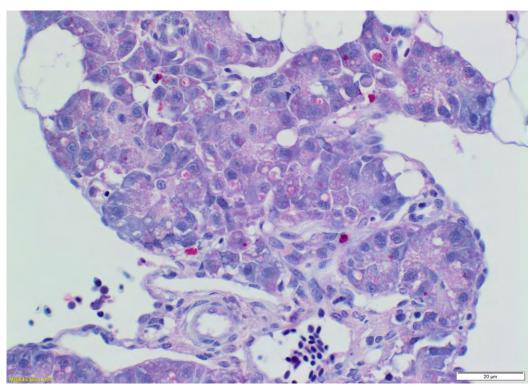
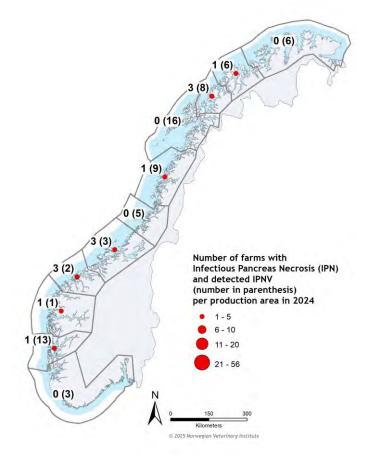


Photo: Lisa Furnesvik





Map: Attila Tarpai

Amoebic gill disease (AGD)



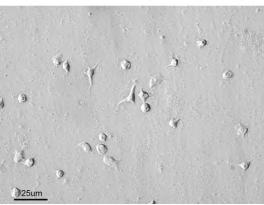
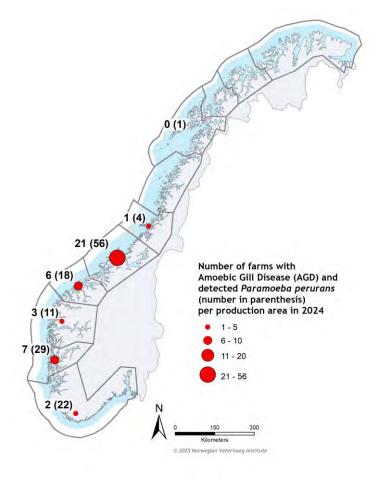


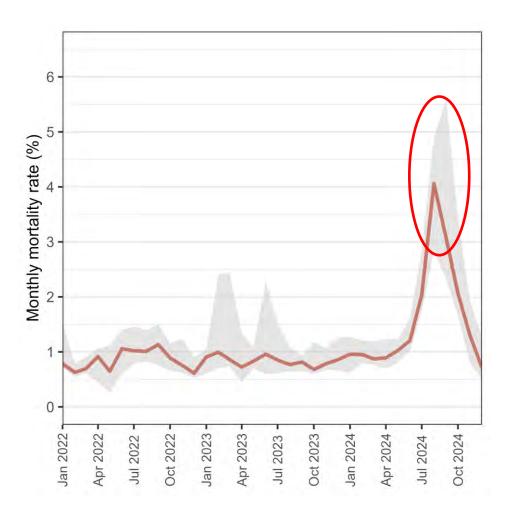


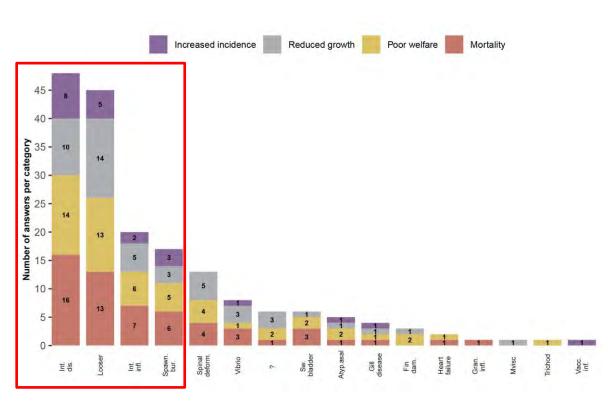
Photo: Jannicke Wiik-Nielsen





Health and welfare in farmed Atlantic cod







Health and welfare in farmed Atlantic cod

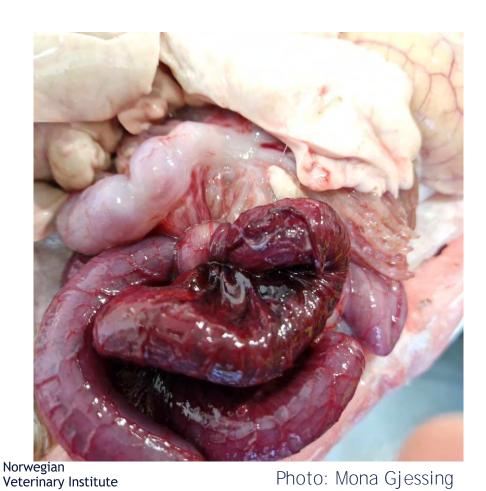




Photo: Anne Berit Olsen

Summary

Salmon lice, complex gill disease and wounds are still challenging for farmed Atlantic salmon

Jelly fish has led to poor welfare

Several bacterial diseases are on rise – effect of climate change?







Scientifically ambitious, forward-looking and cooperative – for One Health





Fish diseases in the Mediterranean Sea: state of the art and future challenges

Marsella A.1*, Toffan A.1

¹ Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory for Fish, Molluscs and Crustaceans Diseases, Legnaro, Italy

The Mediterranean Sea

- Semi-closed basin with limited water exchange
- Biodiversity hotspot
- 22 countries



- Global warming
- Invasive alien species
- Introduction and/or spread of diseases
- Pollution
- Overfishing
- Space competition





Surveillance for fish diseases in the Mediterranean Sea



Diseases affecting farmed fish species are not listed according to EU or WOAH



No surveillance programme are in place and thus no reporting is mandatory



Few informations about epidemiological situation are available



What is happening in the Mediterranean Sea?



Fish diseases in the Mediterranean Sea 2010-2024: settings



Gather information on infectious diseases affecting fish species in the Mediterranean Sea





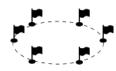
Farmed and wild finfish

Finfish infectious diseases in the Mediterranean basin: A systematic review with insights on vaccination possibilities

Anna Toffan a,e, o, Andrea Marsella a,e, Vasco Menconi a,e, Michela Bertola b,e

- 3 National Reference Centre for Fish, Shellfish and Molluscs Diseases, Legnaro, 35020, Padova, Italy
- National Reference Laboratory for Honey Bee Health, Legnaro, 35020, Padova, Italy Stituto Zooprofilattico Sperimentale delle Venezie, Legnaro, 35020, Padova, Italy

https://doi.org/10.1016/j.fsi.2025.110189



Mediterranean Sea only (brackish and saltwater)



From 2010 to 2024



- ✓ Peer-reviewed full-text
- ✓ English or French
- Disease or mortality reported



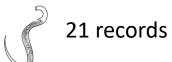
Fish diseases in the Mediterranean Sea 2010-2024: results

Abstract Full-text Review

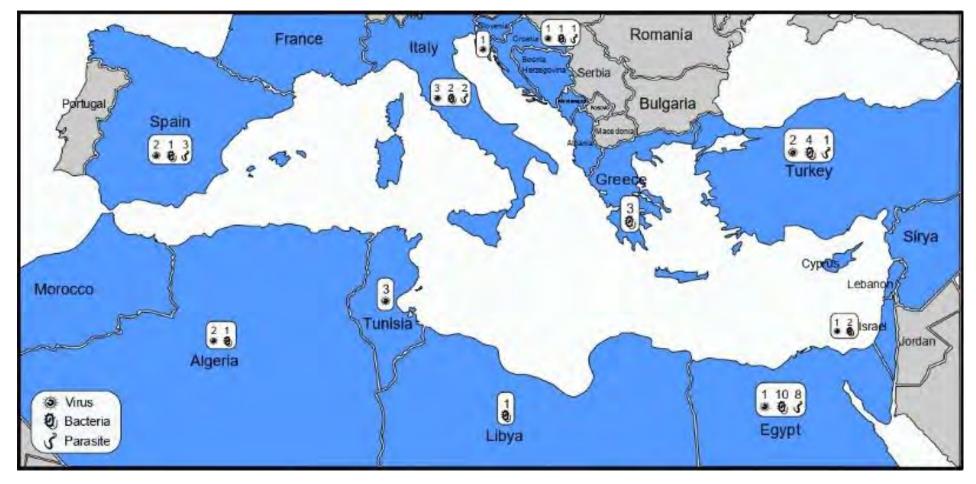
9021 papers screened 412 papers eligible 44 papers selected







Some co-infections were reported!





Fish diseases in the Mediterranean Sea 2010-2024: results



Viruses

Betanodavirus (11)

Megalocytivirus (2)

LCDV (2)

LCDV-Polyomavirus-Papillomavirus (1) HVA-IPN (1)

Totivirus infection (1)



arasites		
arasites		

Amiloodinium ocellatum (5)

Lernanthropus kroyeri

(3)

Cryptocarion irritans

(2)

Enterospora

nucleophila (2)

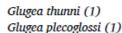
Ergasilus sieboldi (2)

Livoneca redmanii (2) Caligus clemensi (1)

Caligus enlogatus (1)

Caligus mugilis (1) Ceratothoa ostreoides

(1)



Myxobolus tilapiae

(1)

Miamiensis avidus (1)

Furnestinia echeneis

(1)

Diclidophora merlangi

(1)

Luxuroides spp. (1)

Diplectanum aequans

(1)

Ktariella polyorchis

(1)

Philometra obladae

(1)

Trichodina truttae (1)



Bacteria

Vibrio alginolyticus (10)

Mycobacterium marinum (6)

Vibrio harveyi (5)

Aeromonas hydrophila (4)

Photobacterium damselae piscicida (4)

Vibrio vulnificus (4)

Vibrio parahaemolyticus (3)

Photobacterium damselae

damselae (3)

Streptococcus agalactiae (3)

Pseudomonas fluorescens (3)

Vibrio sp. (2)

Mycobacterium pseudoshottsii

(2)

Vibrio anguillarum (2)

Tenacibaculum maritimum

Nocardia sp. (2)

Rickettsia-like (1)

Vibrio splendidus (1)

Aeromonas sobria (1)

Photobacterium malacitanum
(1)

Photobacterium andalusiense

Ichthyocystis hellenicus (1) Ichthyocystis sparus (1) Vibrio fluviatilis (1)

Lactococcus garvieae (1)

Vibrio gigantis (1)

Aeromonas sp. (1)

Pseudomonas sp. (1)

Mycobacterium avium (1)

Aeromonas veronii BIOVAR

veronii (1)

Pseudomonas aeruginosa (1)

Pseudomonas putida (1)

Pseudomonas alcaligenes (1)

Aeromonas veronii BIOVAR

sobria (1)

Aeromonas caviae (1)

Aeromonas salmonicida (1)

Edwarsiella tarda (1)

Vibrio pomeroyi (1)

Vibrio crassostreae (1)

Pseudomonas anguilliseptica

(1)

Staphylococcus homini (1)

Ichthyocystis sp. (1)

Chlamydia sp. (1)

Piscirickettsia salmonis (1)

Pasteurella multocida (1)

Proteus vulgaris (1)

Proteus mirabilis (1)

Grimontia hollisae (1)

Lacinutrix venerupis (1)





Fish diseases in the Mediterranean Sea 2010-2024: results



Quality assessment of the included papers referring to viruses.

Reference	water temperature	Reported mortality	Viral isolation	IHC	ID Molecular	Sequence	Exclusion of other causes of mortality	Final quality score
[114]	1	1	1	0	1	0	1	5
[10]	1	1	0	0	1	1	0	4
[10]	1	1	1	0	1	1	0	5
[11]	1	1	1	0	1	1	0	5
[14]	1	1	1	0	1	1	0	5
[12]	1	0	0	0	1	1	1	4
[18]	1	1	1	1	1	1	1	7
[37]	1	0	0	0	1	1	0	3
[13]	0	1	0	1	1	1	0	4
[17]	1	0	1	1	1	1	0	5
[115]	1	1	1	0	1	1	1	6
[20]	1	1	0	0	1	1	0	4
[30]	0	0	1	0	1	0	0	2
[16]	1	1	0	0	1	1	0	4
[31]	1	0	0	0	1	0	1	3
[36]	1	0	0	0	1	1	0	3
[15]	1	0	1	0	1	1	0	4
[116]	1	0	1	0	1	1	1	5
Total	16/18	10/18	9/18	3/18	18/18	15/18	6/18	4.3

Note: 1—the paper meets the quality criteria; 0—the paper is deficient in terms of the quality criteria.



Authors	Water temperature	Reported mortality	ID species level	ID Morphology	ID Molecular	Exclusion of other causes of mortality	Final quality assessment
[111]	0	1	1	1	1	0	4
[135]	1	0	1	0	1	1	4
[89]	1	1	1	1	1	0	5
[113]	0	0	1	1	1	0	3
[96]	1	1	1	1	1	0	5
[136]	1	1	1	1	0	0	4
[99]	1	1	1	0	0	0	3
[98]	1	1	1	1	0	0	4
[137]	0	0	1	1	1	0	3
[104]	1	1	1	1	0	0	4
[97]	1	1	1	1	1	0	5
[109]	0	0	1	1	1	0	3
[54]	1	1	1	1	1	1	6
[105]	1	0	1	1	0	0	3
[138]	1	1	1	1	1	0	5
[108]	1	1	1	1	1	0	5
[139]	0	1	1	1	1	0	4
[53]	1	1	1	0	1	1	5
[140]	0	1	1	1	0	0	3
[141]	0	0	1	0	1	0	2
Total	13/20	14/20	20/20	16/20	14/20	3/20	4.0

Note: 1—the paper meets the quality criteria; 0—the paper is deficient in terms of the quality criteria.



Reference	e Water temperature	Reported mortality	Identification (genus and species level)	Isolation	ID Molecular	MALDI- TOF	Sequence	Exclusion of other causes of mortality	Final quality score
[46]	1	1	1	1	0	0	0	0	4
[49]	1	0	1	1	1	0	0	0	4
[117]	1	1	0	1	1	0	0	0	4
[61]	0	0	1	1	1	0	1	0	4
[47]	0	0	1	1	1	0	0	0	3
[75]	0	0	1	1	1	0	1	0	4
[80]	0	0	1	1	1	0	1	1	5
[62]	0	0	1	1	1	0	1	0	4
[48]	1	1	1	1	1	0	0	0	5
[118]	1	1	1	1	1	0	1	0	6
[119]	0	1	1	1	1	1	0	1	6
[120]	0	1	1	0	1	0	1	0	4
[76]	0	0	1	1	1	0	0	0	3
[63]	0	0	1	1	1	0	1	0	4
[121]	1	0	1	1	1	0	1	0	5
[122]	1	1	1	1	1	0	1	1	7
[123]	0	0	0	0	1	0	1	0	2
[124]	0	1	1	1	1	1	1	0	6
[69]	1	1	2	1	1	0	1	0	7
[50]	1	0	1	1	0	0	0	0	3
[125]	0	1	1	1	1	0	1	1	6
[64]	1	0	1	1	1	1	0	0	5
[52]	0	1	1	1	1	0	0	0	4
[84]	0	1	1	1	1	0	1	0	5
[126]	0	0	1	1	1	0	1	1	5
[86]	0	1	1	1	1	0	1	0	5
[65]	0	0	1	1	1	0	1	1	5
[73]	1	1	1	1	1	0	1	0	6
[127]	1	0	1	1	1	0	1	0	5
[128]	1	1	1	0	1	1	1	1	7
[66]	0	0	1	0	1	0	1	0	3
[70]	1	1	1	1	1	0	1	0	6
[51]	0	0	1	1	1	0	0	0	3
[54]	1	1	1	1	1	0	1	1	7
[129]	1	1	0	0	1	0	1	0	4
[77]	1	1	1	1	1	0	1	0	6
[71]	1	1	1	1	1	0	1	1	7
[53]	1	1	1	1	1	0	1	1	7
[130]	0	0	0	1	0	0	0	0	1
[122]	1	0	1	1	1	0	1	0	5
[131]	0	0	1	1	1	0	1 0	0	4
[132]				1	1			1	3
[78]	0	1 0	1	0	0	0	0	0	2
[55]	1		1	1	1	0			4
[133]	1	1 0	1	1	1	1 0	1	1	8 5
[134] Total	1 23/46	23/46	1 41/46	1 40/46	1 42/46	5/46	1 31/46	12/46	4.7
Iotai	23/40	23/40	41/40	40/40	42/40	3/40	31/40	12/40	4./

Note: 1—the paper meets the quality criteria; 0—the paper is deficient in terms of the quality criteria.



Fish diseases in the Mediterranean Sea 2010-2024: conclusions

- Reports of diseases in the literature are sparse in time and geographical distribution
- Only 0.88% of papers examined addressed the question «Which infectious diseases relevant to finfish are being reported in the Mediterranean Basin?»
- Armonization of informations provided by papers should be achieved
- Armonization of the diagnostic workflow and procedures is paramount

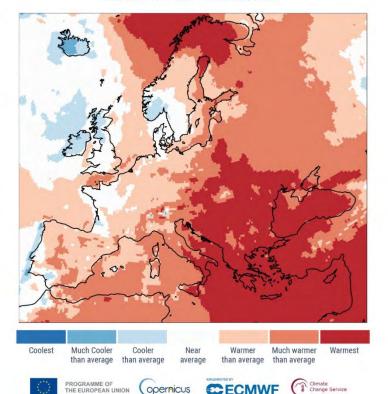
Contribution from a proper peer-reviewing process



Future challenges in the Mediterranean Sea

Anomalies and extremes in surface air temperature for June-August 2024

Data: ERA5 1979-2024 • Credit: C3S/ECMWF



Anomalies and extremes in surface air temperature for June–August 2024. Colour categories refer to the percentiles of the temperature distributions for the 1991–2020 reference period. The extreme ("coolest" and "warmest") categories are based on rankings for the period 1979–2024. Data source: ERA5. Credit: Copernicus Climate Change Service/ECMWF.

Seasonal average temperatures were predominantly above average over almost all of the continent, with record-high temperatures in southeastern Europe and northern Fennoscandia. In stark contrast, some regions in the western part of the continent, including Iceland, Ireland, northern and western parts of the United Kingdom (which overall experienced its coolest summer since 2015), and southern Norway were cooler than average.

The seasonal averages largely reflect the temperature distribution observed in July and August, while in June, a comparably larger area of western Europe experienced colder-than-average temperatures.

Parts of Europe saw substantial heatwaves during the summer. For example, in June, southern Italy, southeastern Europe and Türkiye were impacted by a heatwave, with temperatures reaching 40°C in many locations, and local temperature records broken. In July, heatwaves affected Italy, Greece, and Bulgaria, while in August, much of southeastern Europe, including Italy, Portugal, and Spain, experienced similar conditions. For more information on monthly variations in temperature anomalies, see the monthly bulletins of June, July and August.

The effect of the heatwaves is reflected in an above-average number of 'warm daytimes' over much of Europe throughout summer.

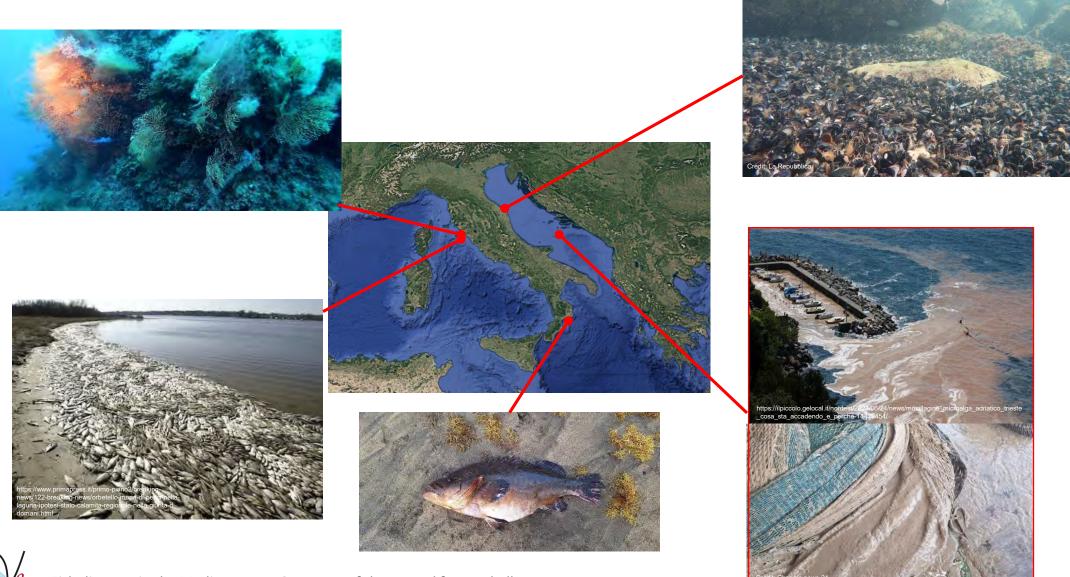


Water temperature in the Mediterranean Sea

- ❖ In 2024 an extremely severe heatwave hit the Mediterranean Sea
- the daily median temperature across the Mediterranean climbed to 28.9°C
- The increase of temperature, due to the length of the heatwave, affected the surface but also water at depths down to 50 m (and even more)



Relevant events in Italy during summer 2024





Summer 2024: Mucilage

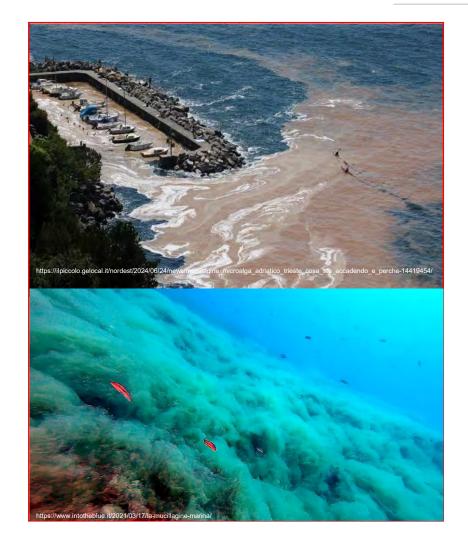
- Normal event except 2024
- Large patches on the surface deposited at the bottom

Mortality of bottom-dwelling organisms (reduced light penetration, anoxic area)

Displacement of fish population

Damage of fishing gears







Summer 2024: M. galloprovincialis mortalities

- ❖ 33 abnormal mortality events
- In 10 cases an infectious pathogen was detected
- All were linked to environmental parameters
- ❖ In some areas, almost 100% of the population have been lost



Summer 2024: Grouper mortalities caused by NNV

Numbers refer variably to analyzed or to affected fish → armonization of reports needed when possible

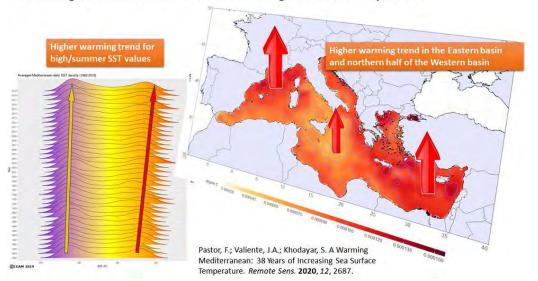




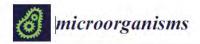


Global warming and NNV

A Warming Mediterranean: 38 Years of Increasing Sea Surface Temperature



- VNN has been considered since its discovery a «summer disease»
- A positive trend between increasing temperature of the water and increasing viral virulence has been suggested





Article

Impact of Global Warming on the Severity of Viral Diseases: A Potentially Alarming Threat to Sustainable Aquaculture Worldwide

Marine Combe 1,*,†, Miriam Reverter 1,2,*,†, Domenico Caruso 1, Elodie Pepey 1,3,0 and Rodolphe Elie Gozlan 1

- ISEM, Université de Montpellier, CNRS, IRD, 34095 Montpellier, France
- Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, University of Plymouth, Plymouth PL4 8AA, UK
- CIRAD, UMR ISEM, 34398 Montpellier, France
- * Correspondence: marine.combe@ird.fr (M.C.); miriam.reverter@plymouth.ac.uk (M.R.); Tel.: +33-7-62-25-68-08 (M.C.)
- † These authors contributed equally to this work.



NNV in new species



Mycteroperca rubra red grouper



Dactylopterus volitans flying gurnard



Balistes capriscus grey triggerfish



Fish diseases in the Mediterranean Sea: future challenges

- ❖ Aquatic environment is highly vulnerable to climate changes
- Aquaculture and fisheries will need adaptation plan against climate change
- Climate is affecting diseases epidemiology
- Research on climate change effects on pathogen-host-environment interaction will be helpful
- Surveillance and complete diagnosis of outbreaks will be helpful in mitigating diseases impact



Thank you for your attention

My warmest thanks to:

- Pantelis Katharios (HCRM Greece)
- Antonio Terlizzi (UniTS Italy)
- Francesc Padros Bover (UAB Spain)
- Carmelo Iaria (University of Messina –Italy)
- Fabio di Nocera (IZSM- Italy)
- Alessia Vetri (IZSVe)
- Karim Mezali (Université de Mostaganem-Algerie)







29th Annual Workshop of the National Reference Laboratories for Fish Diseases –May 2025

The Map as a tool on outbreaks and epidemiology

Britt Bang Jensen,
Caitlin Yoo
Section for Fish and Shellfish Diseases
DTU Aqua

DTU Aqua



As an NRL.....

- We recieve samples, with information on the farm and the samples
- Often, we don't know the farm history, or its connections to other farms
- This affects our ability to give advice and sometimes to select the right test



Al-generated image, Microsoft Copilot



The need:

- >Know where the farm is
- ➤ Know if neighboring farms are infected with disease
- ➤ Know the history of the farm



DTU Aqua



The approach:



- Use data that is freely awailable
- Use software that is freely available
- Simple, but highly adaptive tool



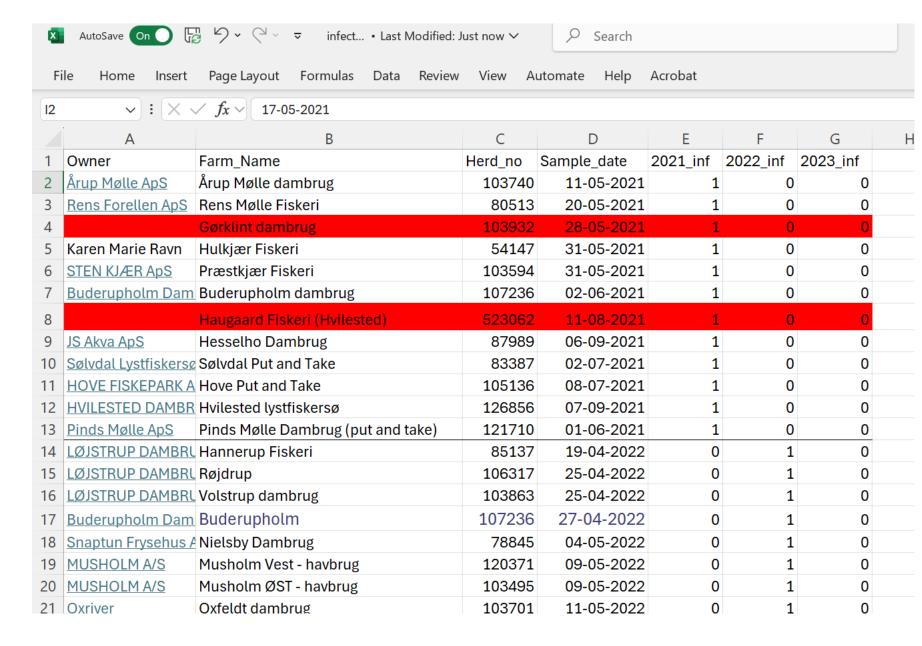
Data on farm location:



Dataset on farms



Data on IHN:







- "Shiny" package: Makes apps
 - <u>https://shiny.posit.co/</u>
- "Leaflet" package: Makes maps
 - <u>https://r-charts.com/spatial/interactive-maps-leaflet/</u>





Acknowledgments:

Caitlin Yoo, PhD-student





DTU Aqua



Experimental infection of rainbow trout (Oncorhynchus mykiss) and European perch (Perca fluviatilis) with Epizootic haematopoietic necrosis virus (EHNV).

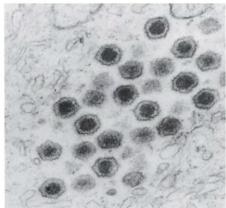
Anna Luiza Farias Alencar DVM, MSc, PhD Postdoc DTU Aqua



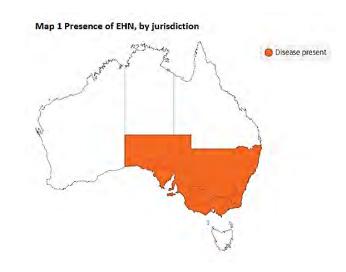


Epizootic haematopoietic necrosis virus (EHNV)

- Ranavirus (Iridoviridae family)
 - Same family as other fish viruses:
 - ECV, ESV, Cod iridovirus, Turbot iridovirus
- Listed disease by WOAH
- Outbreaks in wild European perch (Perca fluviatilis)
- Less severe in farmed rainbow trout (Oncorhynchus mykiss)
 - Endemic in southeast Australia
- Necrosis of haematopoietic tissue
 - Head kidney
 - Liver
 - Spleen



Langdon et al., 1986





Epizootic haematopoietic necrosis virus (EHNV)

Outbreaks in wild European perch

- Associated with high temperatures (summer)
- High mortality rates up to 95% juveniles mostly affected



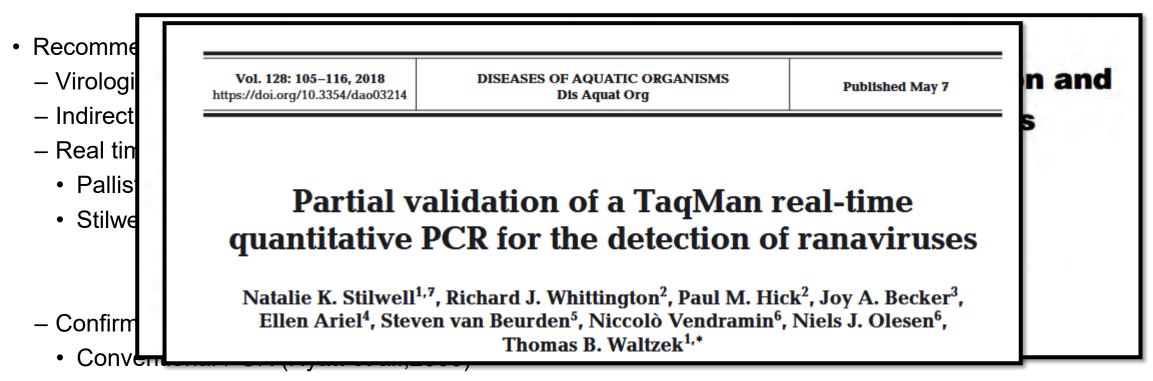
Outbreaks in rainbow trout

- Associated with stress (high stocking density, sudden changes in temperature, water quality etc)
- Low mortality can go unnoticed





Epizootic haematopoietic necrosis virus (EHNV)



- DNA sequencing of MCP gene discriminate EHNV from other ranaviruses
- No direct real time PCR for detection of EHNV currently available.



Background – Pilot study



DTU Aqua





Australian Center for Disease Preparedness - AFDL

Development of a new EHNV real time PCR assay

In progress...



Pilot study - aims

- 1. Comparison of virulence of 2 different EHNV isolates:
- A

- European perch (EP)
- Rainbow trout (RT)

High morbidity/mortality in control group

No development of disease in RT

Slow development of disease in EP

- 2. Follow pathogen kinetics (development of disease) in RT and EP
 - Weekly sampling
- 3. Generate panel of samples to be used in new assay validation



Material and methods – Virus isolates

Virus isolates

- EHNV 86/8774 (from RT) "old" isolate
- EHNV 24-00087 (from EP) "new" isolate
 - Received from CSIRO, Australia
 - Propagated in BF-2 cells

Inoculum	EHNV 87/8774	EHNV 24-00087
Titer (TCID ₅₀ /mL)	1,9 x 10 ⁵	1,3 x 10 ⁵
Ct (Stilwell et al., 2018)	17.48	18.71

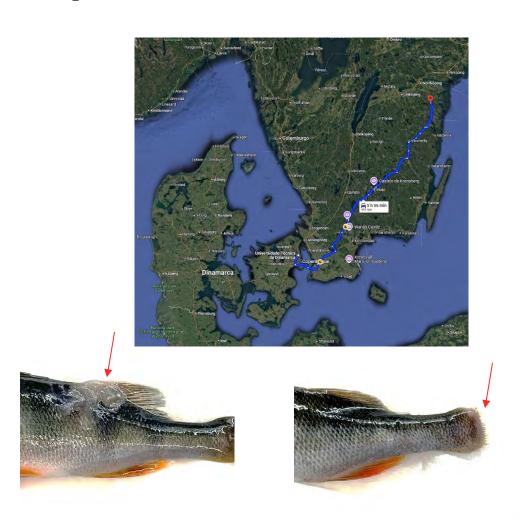
100uL per fish = 10^4 TCID50/fish



Material and methods – Experimental fish

European perch

- Commercial farm in Sweden (about 500km)
- Initially ~ 400 fish
- Losses during transport and quarantine (1 mo)
- Thin and weak
- Tested virology/bacteriology
 - Virology no CPE in BF2/EPC
 - Bacteriology growth in BA
- Avg weight: 17g
- Rainbow trout SPF from DTU facility
 - Avg weight: 34g





Material and methods - Cohabitation model

Cohabitation model

- Shedders x Cohabitants
- 6 x 180L tanks
- Salinity 20ppt
- Temperature 18°C

Species	Isolate	Total fish	Injected fish
European perch	Control (MEM)	38	10
Rainbow trout	Control (MEM)	80	30
European perch	EHNV 86/8774	60	30
Rainbow trout	EHNV 86/8774	80	30
European perch	EHNV 24-00087	60	30
Rainbow trout	EHNV 24-00087	80	30

- Fish were acclimatized 1 week in the experimental tanks
- Infection trial was conducted Oct-Nov 24
 - 28 days
 - Visit from CSIRO Dr. David Cummins and Dr. Lachlan Coff



Material and methods – Cohabitation model

Shedders:

- Clipping of adipose fin RT
- Clipping of upper lobe of caudal fin EP





Tail rot in EP – difficult to determine shedders in some fish



Material and methods – Sampling

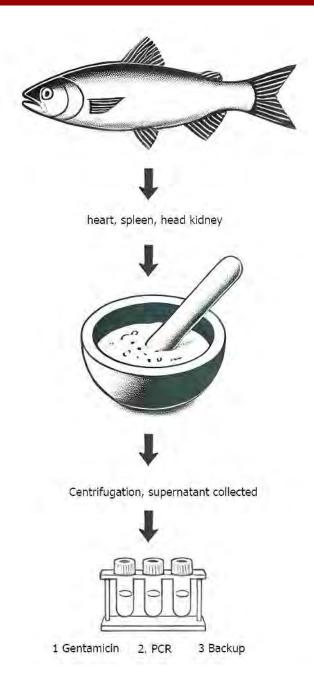
	EHNV 86/8774			EHNV 24-00087				Control (MEM)		
	R	RT		EP		T	E	Р	R	T
DPI	Sh	Со	Sh	Со	Sh	Со	Sh	Со	Sh	Со
7	3	10	3	0	3	10	3	0	3	10
14	5	5	5	5	5	5	6	5	5	5
21	5	5	5	5	5	5	5	6	5	5
28*	5	5	5	5	5	5	5	5	5	5



Material and methods – Sampling

- All samples (tissue homogenate)
 - Added to BF-2 cells
 - Subcultivated after 7 days in fresh BF-2 layer
 - Assessed for development of CPE

 Real time PCR: Ranavirus (Stilwell et al., 2018) and EHNV specific assay (not shown here) done in duplicates





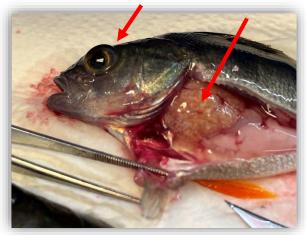
Results

- No development of disease in RT at any time during the trial
 - No clinical signs

- Development of disease in EP (shedders only)
 - Darkening of skin
 - Lethargy
 - Tail rot (fin rot) seen in cohabs too
 - Exophtalmia
 - Enlarged spleen
 - Liver lesions/degeneration









Conclusions

- Rainbow trout did not develop disease;
 - Able to detect some positive RT through qPCR and isolation in cell culture
- EP cohabitants showed increased survival when compared to shedders;
 - EP shedders developed disease with ~55% survival
- No significant difference in virulence among both EHNV isolates***;
- Generation of a panel of samples:
 - 2 species
 - 2 methods of infection
 - 2 isolates
 - Negatives/positives on cell culture x negatives/positives on qPCR
 - To be used in the validation of the new diagnostic assay



PBS (DFO/MPO)

Kyle Garver Laura Hawley

Thank you!

CSIRO

Peter Mohr Nick Moody David Cummins Lachlan Coff

DTU Aqua – Section for fish and shellfish diseases







Molecular tracing of *Renibacterium salmoninarum* in the North-East Atlantic through whole genome sequencing analysis

Alejandra Villamil Alonso, PhD Student Moldal Torfinn (Norwegian Veterinary Institute) Karin Lagesen (Norwegian Veterinary Institute)

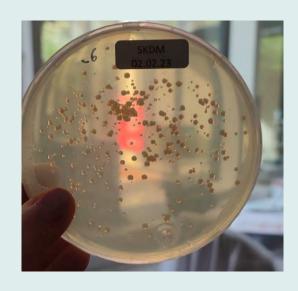
PhD Supervision team: Argelia Cuenca, Niccolò Vendramin, Lone Madsen

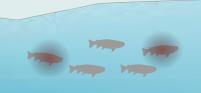
29th EURL Annual Workshop May 26th, 2025

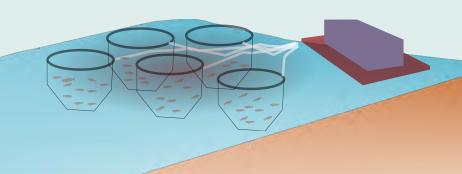


Renibacterium salmoninarum

- Gram-positive, intracellular
- Bacterial kidney disease in salmonids
- Diagnosis: ELISA, PCR, bacterial culture > very slow growth
- ~3.15 Mbp genome, Micrococcaceae family



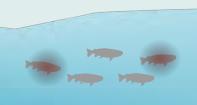


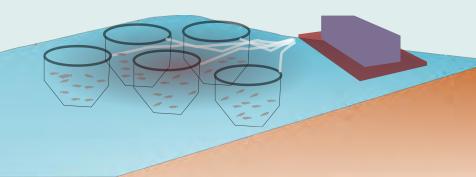




Renibacterium salmoninarum is responsible for bacterial kidney disease (BKD) and has been isolated from northern European salmonid farms since the 1980s

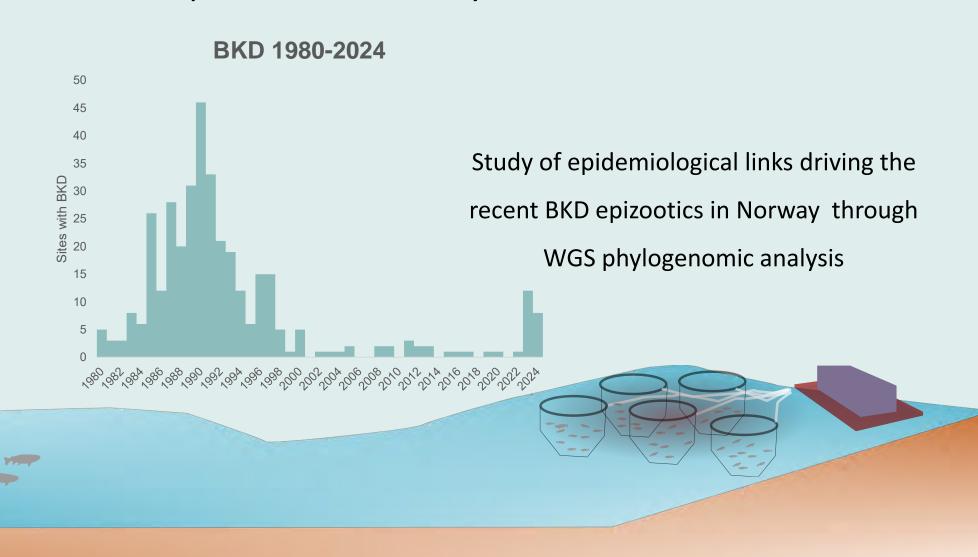
- Horizontal and vertical transmission
- Chronic infection
- **Persistent** pathogen
 - Subclinical infections
 - Environmental persistence

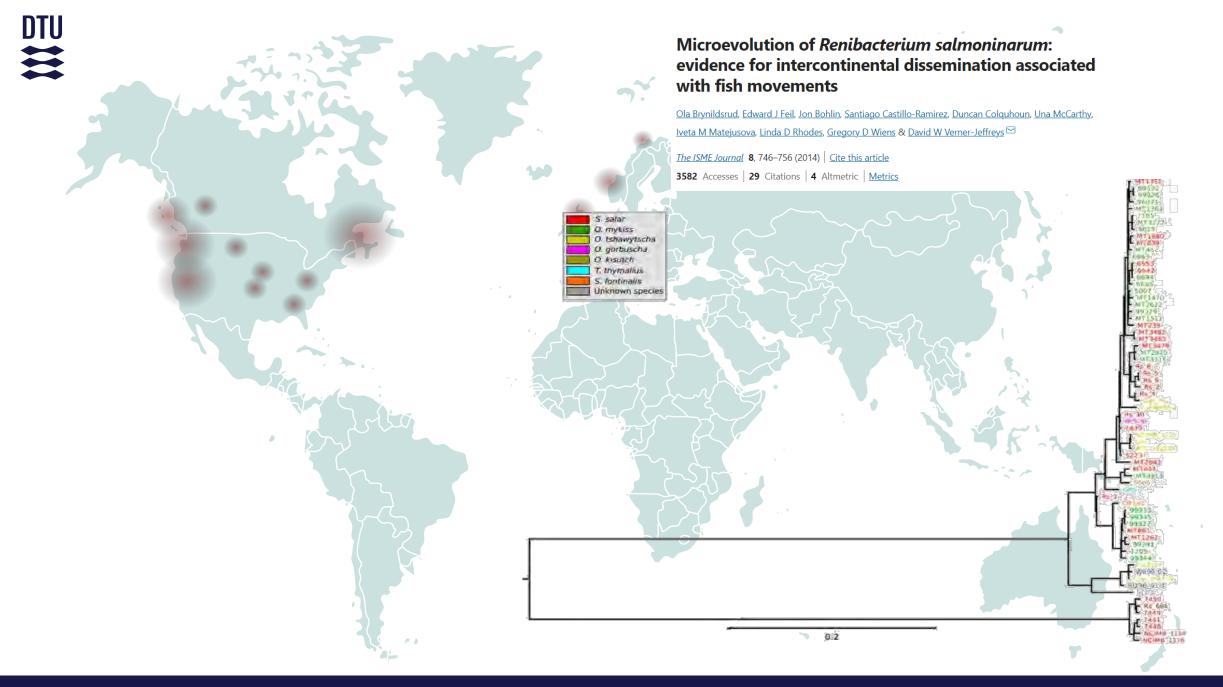


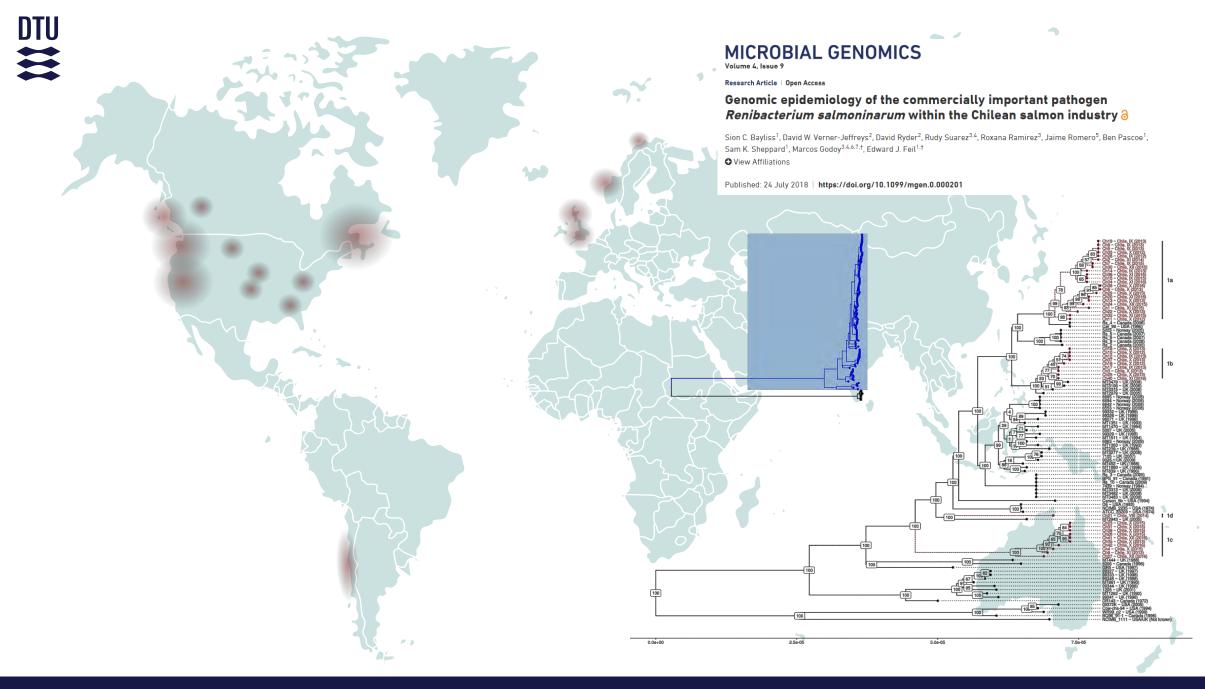




The 2022-24 BKD epizootics in Norway









Molecular tracing of *Renibacterium salmoninarum* in the North-East Atlantic

DTU (DK) - NVI (NO) collaboration (2023)

Keldur (IC) (2024)

Summer 2024: last isolates added

92 isolates

- → 67 Norway (35 cases since 2022)
- → 12 Denmark
- → 1 Faroe Islands
- → 12 Iceland

1980s to 2024

Atlantic salmon, rainbow trout, Artic char

Technical University of Denmark



Molecular tracing of *Renibacterium salmoninarum* in the North-East Atlantic

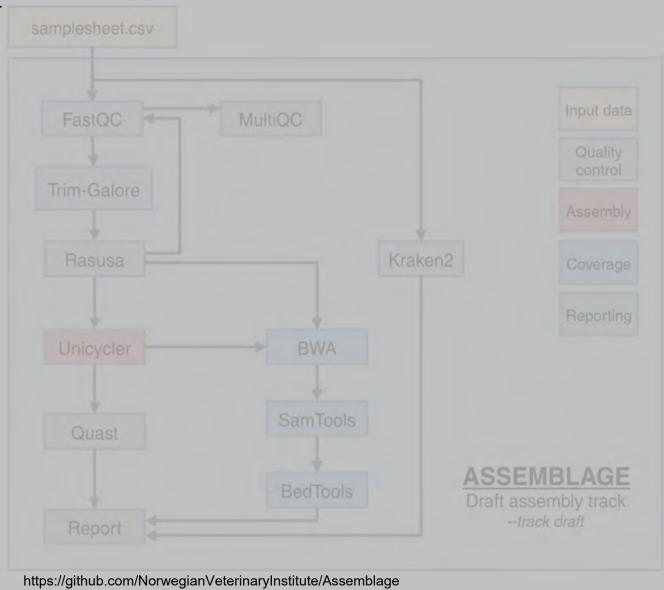
AIM

To study the epidemiological links driving the recent BKD epizootics in Norway, and to create an overview of the movements of the

bacterium within the North-East Atlantic region.

Technical University of Denmark





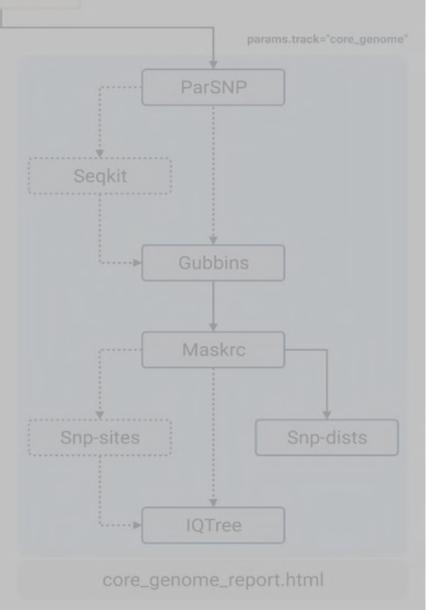
Methods

Collection of isolates, bacterial growth, and DNA extraction

Whole genome sequencing, assembly, and annotation



Assemblies



Methods

Collection of isolates, bacterial growth, and DNA extraction

Whole genome sequencing, assembly, and annotation

Phylogenomic analysis with ALPPACA pipelines (fastANI, core_genome)

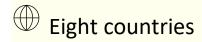
10

Kaspersen, & Fiskebeck. (2022). ALPPACA - A tooL for Prokaryotic Phylogeny And Clustering Analysis. Journal of Open Source Software, 7 (79), 4677.

Technical University of Denmark



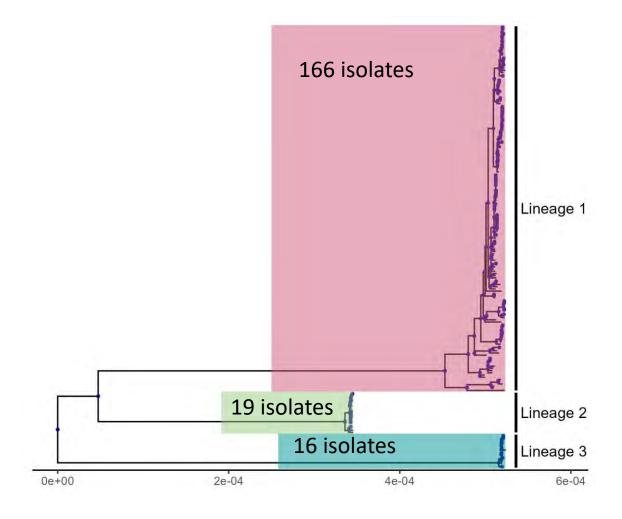
1. Past and recent movements of *R. salmoninarum* within the North-East Atlantic region



Eight fish species

From the 60s to present

Limited genomic intraspecies variation ANI>99%

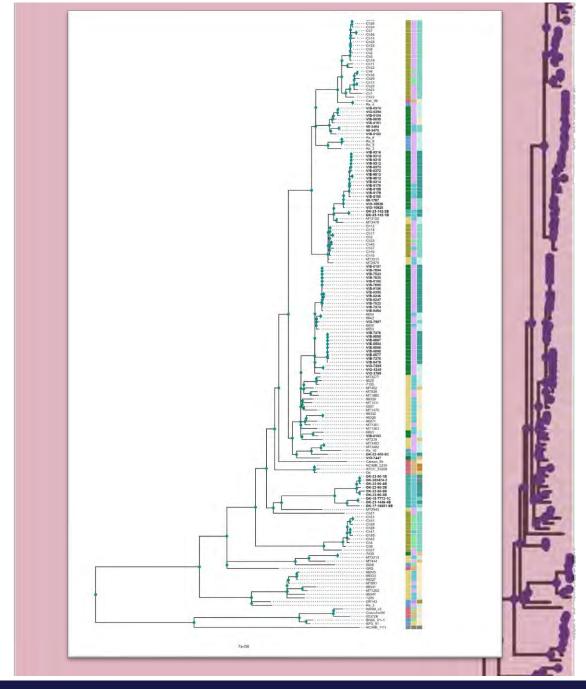


11



- All Danish isolates (2017-2022)
- 47 Norwegian isolates

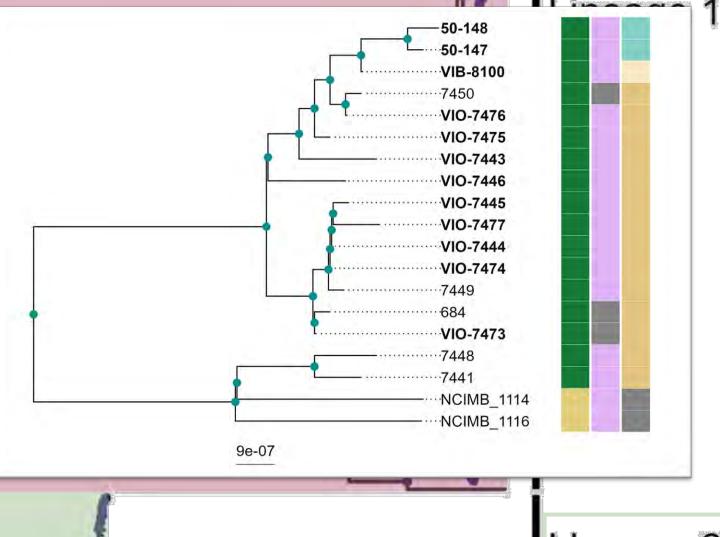
Intercontinental spread Aquaculture-associated Deeply sampled



Lineage 1

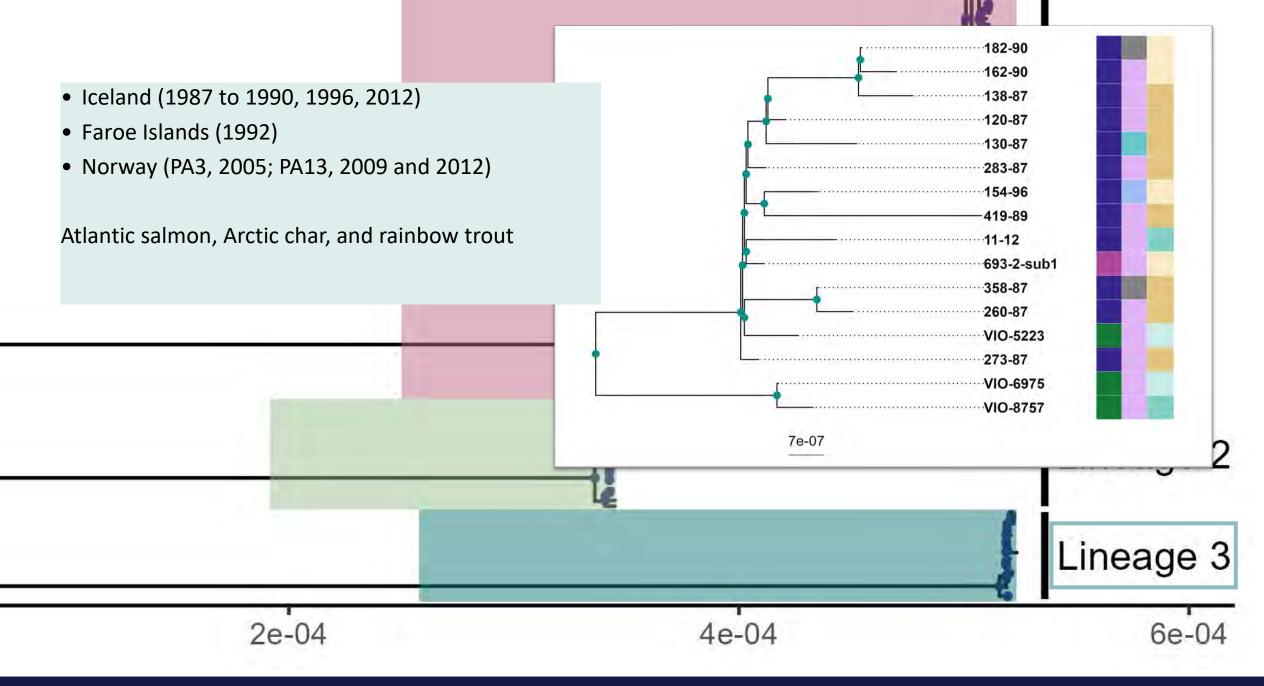


- River Dee, Aberdeen (1962)
- Norway PA3-7 (1985-1987, 1998, 2012)
- Atlantic salmon



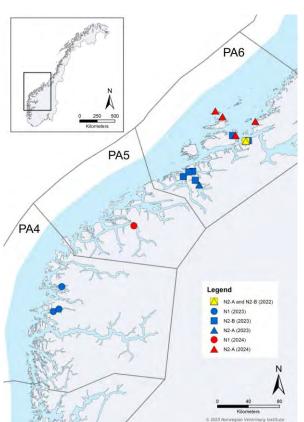
Lineage 2

alvalo@aqua.dtu.dk 13



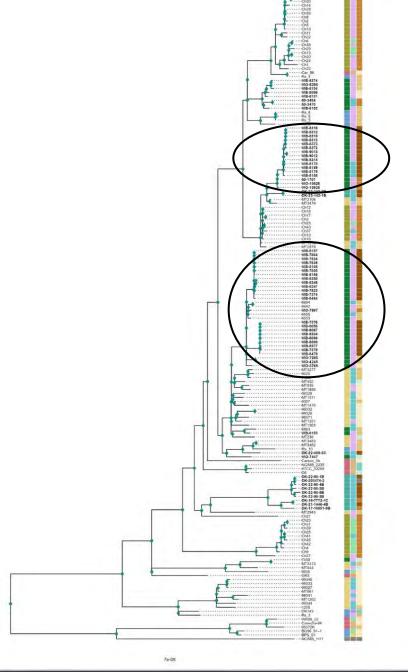
Technical University of Denmark

2. The 2022-24 BKD epizootics in Norway



Map: Attila Tarpai, Norwegian Veterinary Institute

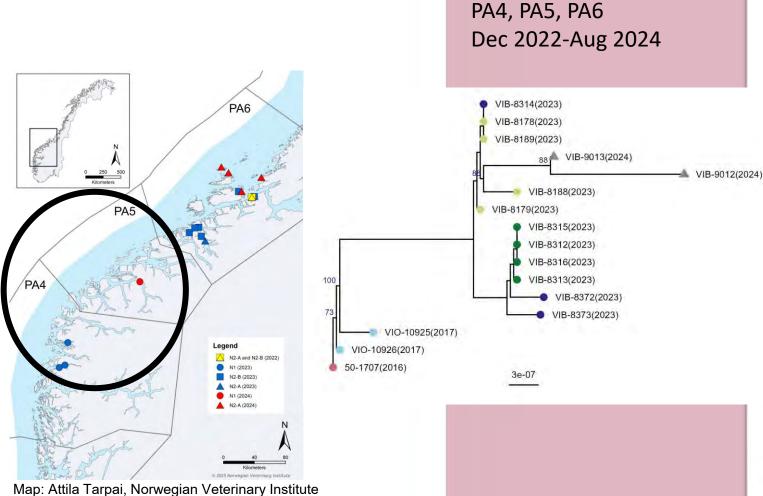
35 R. salmoninarum PA4, PA5, PA6 Dec 2022-Aug 2024

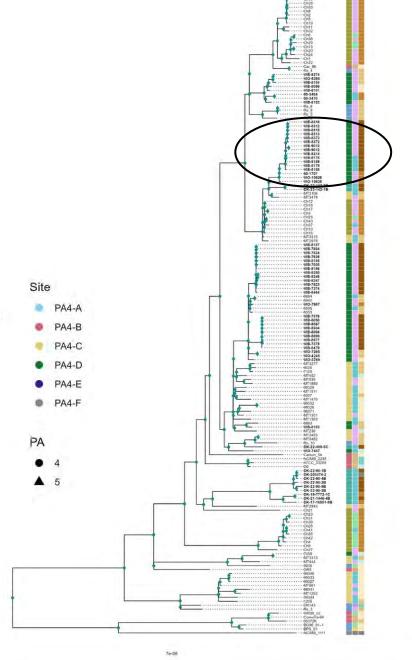


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2. The 2022-24 BKD epizootics in Norway

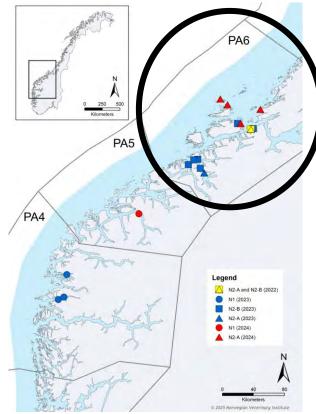
35 R. salmoninarum



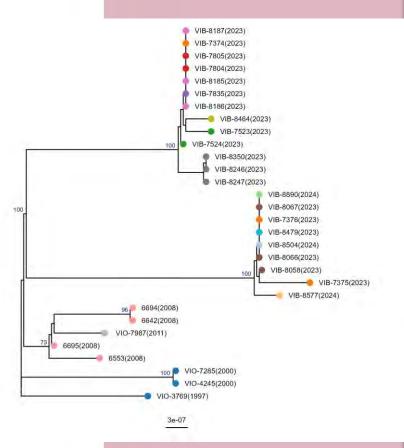


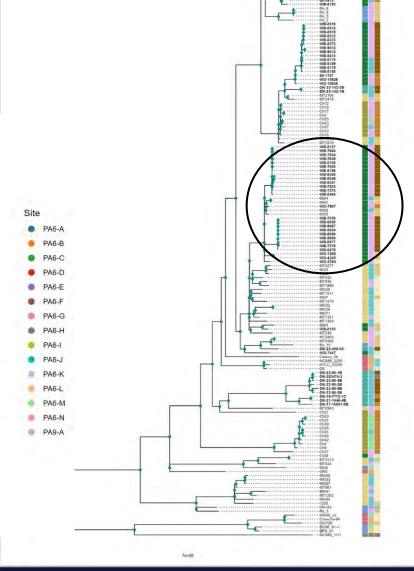
2. The 2022-24 BKD epizootics in Norway

35 R. salmoninarum PA4, PA5, PA6 Dec 2022-Aug 2024



Map: Attila Tarpai, Norwegian Veterinary Institute

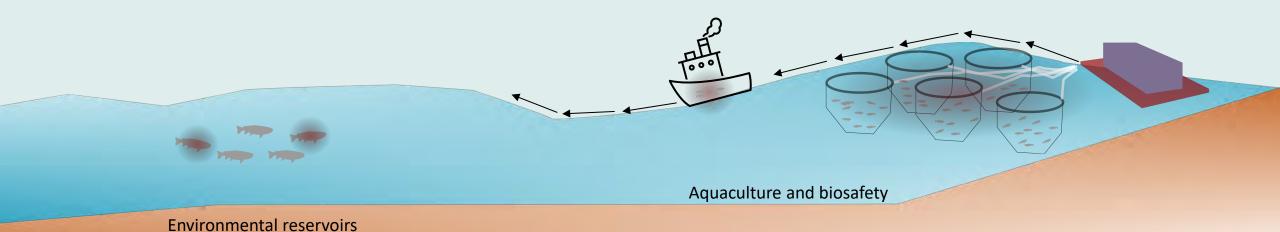






Main findings

- 1. Three Lineages of *R. salmoninarum*!
- 2. Two active hotspots of BKD drive the current epizootics in Norwegian farms



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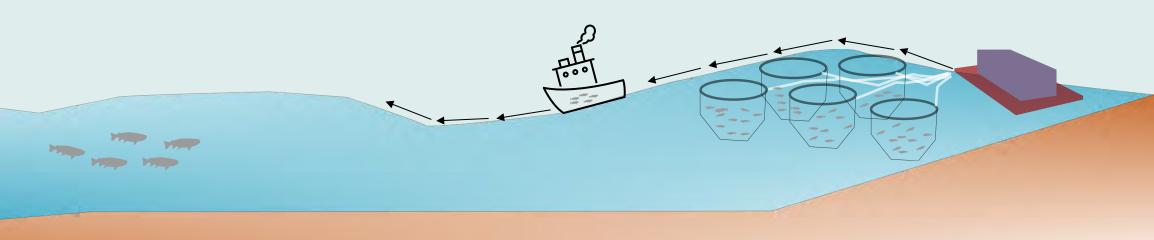
Renibacterium salmoninarum is responsible for bacterial kidney disease (BKD) and has been isolated from northern European salmonid farms since the 1980s, and it is still there!

To prevent the spread of *R. salmoninarum*

Active surveillance!!

Standardized diagnostic methods

Revision of disinfection procedures





Molecular tracing of *Renibacterium salmoninarum* in the North-East Atlantic through whole genome sequencing analysis

Technical University of Denmark

Argelia Cuenca

Niccolò Vendramin

Lone Madsen

Teena Vendel Klinge

Kári Karbech Mouritsen

The Institute for Experimental Pathology at Keldur, University of Iceland

Birkir Þór Bragason

Norwegian Veterinary Institute

Karin Lagesen

Moldal Torfinn

Cathrine Arnason

Snore Gulla

Duncan Colquhoun

Anne Berit Olsen

Hanne Nilsen

Åse Helen Garseth





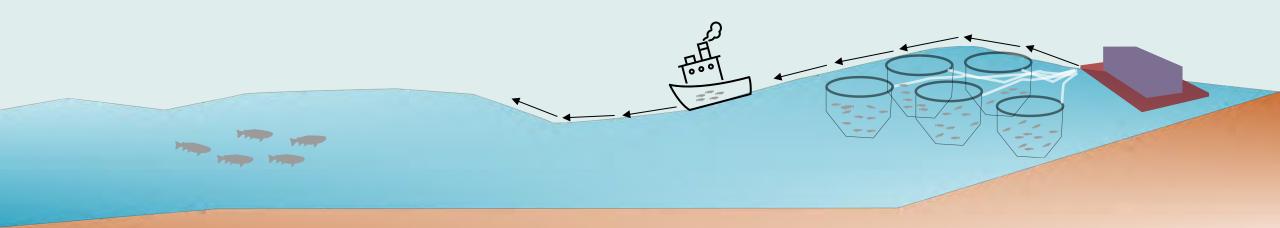




European Union Reference Laboratory for Fish and Crustacean Diseases NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK



THANK YOU FOR YOUR ATTENTION ©







Mediterranean Marine Lactococcosis

Luana Cortinovis

Istituto Zooprofilattico Sperimentale delle Venezie

29 th Annual Workshop of the National Reference Laboratories for Fish Diseases Lyngby - May 27th 2025

Italian NRL for fish, mollusc and crustacean disease



Part of the Istituto Zooprofilattico Sperimentale delle Venezie (Legnaro, Italy)

Four departments:

- Fish bacteriology and parasitology; crustacean and mollusc diseases
- Fish virology
- Aquatic epidemiology
- Experimental aquarium

More than 30 people among veterinarians, biologists, biotechnologists and technicians involved in the diagnosis and research of aquatic species diseases



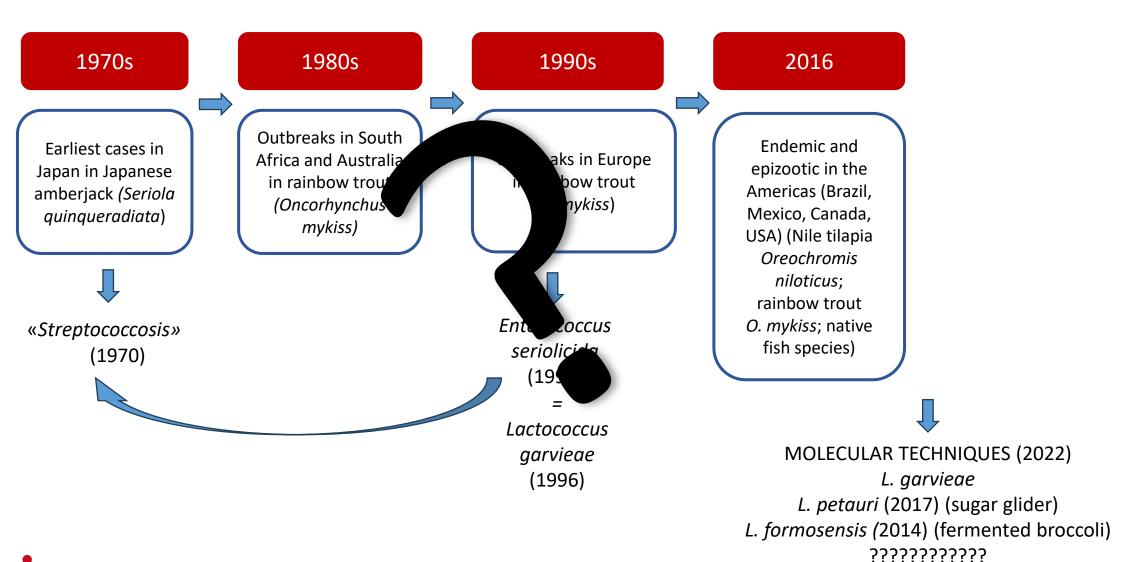


Outline

- 1) History of piscine Lactococcosis
- 2) Italian marine outbreaks
- 3) Outbreaks in Mediterranean Atlantic bluefin tuna
- 4) Characteristics of the disease
- 5) Diagnostic methods
- 6) Available literature
- 7) Italian monitoring plan



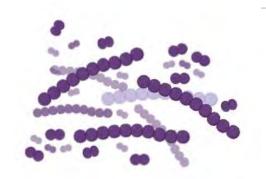
Piscine Lactococcosis





Lactococcus genus

23 different species belonging to the *Lactococcus* genus



Three species related to fish mortalities

L. garvieae

L. petauri

L. formosensis

Very similar species in terms of phenotypic characteristics and 16S rRNA sequence.

Different geographical distribution?

Two species considered as commensal in intestinal microbiota

- L. lactis
- L. piscium



Piscine Lactococcosis

1970s Earliest cases in Japan in Japanese amberjack (Seriola quinqueradiata) «Streptococcosis» (1970)

1980s

Outbreaks in South Africa and Australia in rainbow trout (Onchorhyncus mykiss) 1990s

Outbreaks in Europe in rainbow trout (O. mykiss)



Enterococcus seriolicida (1991)

Lactococcus garvieae (1996) 2016

Endemic and epizootic in the Americas (Brazil, Mexico, Canada, USA) (Nile tilapia Oreochromis niloticus; rainbow trout O. mykiss; native fish species)

2023

Severe outbreaks in the Mediterranean bassin and Canary islands in European sea bass (Dicentrarchus labrax) and Gilthead Sea Bream (Sparus aurata). Outbreaks in Atlantic bluefin tuna (Thunnus thynnus) in eastern Mediterranean sea

MOLECULAR TECHNIQUES (2022)

L. petauri (2017) (sugar glider)
L. formosensis (2014) (fermented broccoli)
??????????



Mediterranean bassin: Italian outbreaks

Late summer/early fall of 2023

Gulf of Follonica (western Italian coast) – 4 off-shore and 1 in-shore farms

First outbreaks in European sea bass (Salogni et al., 2024) and Gilthead sea bream (Esposito et al., 2025)

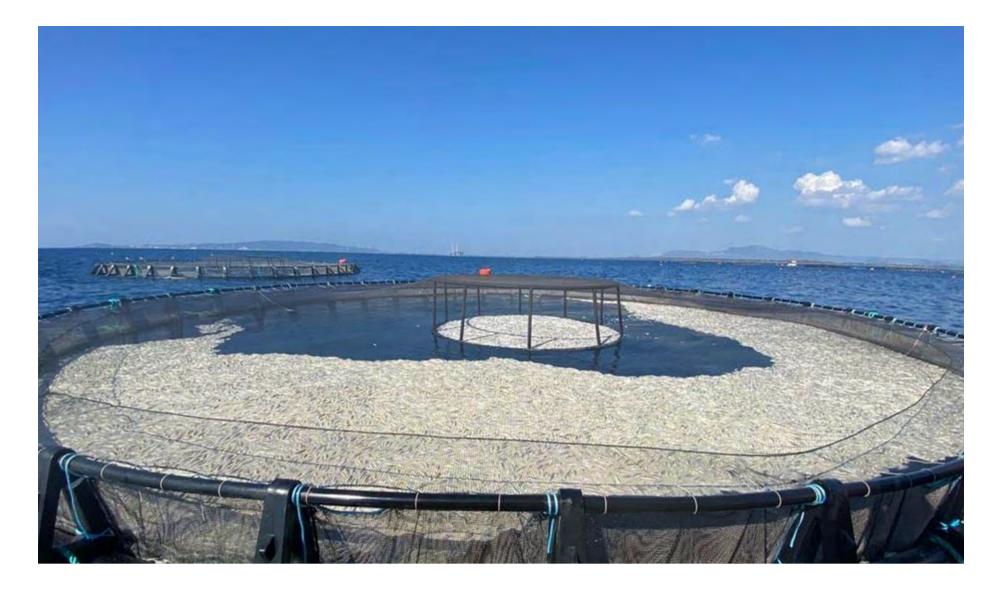
(Relatively) low mortality, unexpected diagnosis

But...





May-late October 2024





More than 2000 tons lost

11,8 million of euro lost

About 15% of italian European sea bass and Gilthead sea bream **production lost**

National production (API 2023): Gilthead Sea bream: 10.500 ton; European sea bass: 6.000 ton





Gulf of Follonica

Gulf of Orbetello

Gaeta



Mediterranean bassin: Italian outbreaks

According to the Italian NRL, from october 2023 to march 2025 (PASSIVE SAMPLING):

8 farms with recurrent episodes

103 analyzed cases: 73 in European sea bass, 28 in Gilthead sea bream 2 wild specimens (*Boops boops, Trachurus trachurus*)

ONLY

LACTOCOCCUS

GARVIEAE

WAS IDENTIFIED

TO DATE

IN ITALIAN

MARINE OUTBREAKS

Among them

68 cases in which Lactococcus garvieae was the only detected pathogen

35 coinfections

Vibrio harveyi
Aeromonas veronii biovar sobria
Photobacterium damselae subsp piscicida
Mycobacterium spp.
Sparycotyle chrysophrii, Diplectanum aequans...



Challenges

- Recurrent outbreaks are common and thought to be associated with bacterial persistence in carrier fish, avian piscivores, environmental biofilms and poor biosecurity measures
- Extreme mortality rates (tons and tons of dead fish) not supported by an efficient and timely removal and disposal system
- Control of active outbreaks with medicated feed (i.e. Erythromycin, Oxytetracycline)
 often fails, due to the acute nature of the disease, population inappetence, or
 antimicrobial resistance
- Autogenous or commercial vaccines are available in some regions but have shown mixed success in limiting disease
- Several coinfections recorded (with two, three or even more identified pathogens)



Atlantic bluefin tuna (*Thunnus thynnus*)

In September 2023 the IZSVe was contacted by an Atlantic bluefin tuna grow out facility located in central eastern Mediterranean for a mortality event.







Anamnesis on September 28th

- 1 out of 18 net-pen involved in the episode. Only few specimens involved. (net-pen size: 135K m³; depth: 35 m; density: 5 Kg/m³; avg size: 200 Kg)
- High seasonal water temperature (26 27°C)
- Behaviour: Moribund fish swim lethargically, sometimes unbalanced erratic swimming
- Feed (about 30 tonnes/day per net-pen): frozen herring, mackerel and sardine
- Appetite: Normal, but stomachs of dead fish found to be empty or filled with white fluid

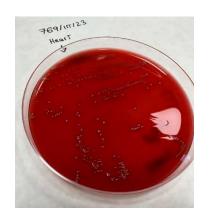




Bacterial isolation from RNAlater fixed samples

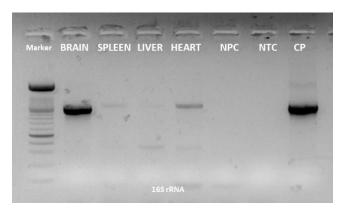
Organs fixed in RNAlater for 7 days before the isolation!!!!

Heart and brain were streaked on Blood Agar and incubated at 22°C and 37°C for 24h









Bacterial identification carried out through amplification and Sanger sequencing of:

- 16S rRNA gene (Davidovich et al., 2022)
- gyrB gene (Egger et al., 2023)
- 16S-23S rRNA Internal Transcribed Spacer Region (ITS) (Stoppani et al., 2023)

One week later

 In the first affected cage, the mortality increased from few specimens per day up to 107 dead tuna on October 3rd

- The outbreak extended to nearby cages:
- All of the dead and moribund fish and even the healthy-looking harvested fish, had white opaque eyes.
- The moribund fish presented white faeces.
- None of the fish had any external lesion, except from the eyes.
- Possible sources of infection were investigated:
- Frozen baits
- European sea bass and Gilthead sea bream farmed at about 5 Km from tuna net-pens
- Samples of water from inland wastewater

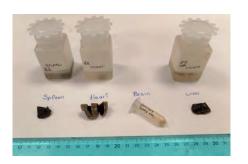


One week later









- Other tuna samplings were performed All tuna tested positive for *L. garvieae*
- Frozen baits and sea bream and sea bass examined tested negative for L. garvieae
- The pathogen was found also in a wild little tunny (Euthynnus alletteratus) captured inside the net-pens.

The episode ceased as temperatures dropped and the fish were harvested. New outbreaks appeared the following year



Piscine Lactococcosis

- Generally recorded as acute or hyper-acute haemorragic septicemia in fish
- Generally related to water temperatures > 18°C (but...)
- Mainly sub-adult and adult fish
- Clinical or sub-clinical infections have been reported in a wide range of hosts, including cultured and wild fish from fresh and marine environments, octopi, marine mammals, terrestrial ungulates, dogs, rabbits, reptiles, birds, and humans
- Zoonotic potential in immunocompromised patients (endocarditis)



Marine Lactococcosis: Clinical signs



Erratic swimming

Exophthalmia/
Ocular lesions

Hypermelanosis

Anorexia

The **LD50** in Mediterranean saltwater species is still **unknown**

onset of the disesase among cages/tanks and even closest farms





Marine Lactococcosis: Lesions









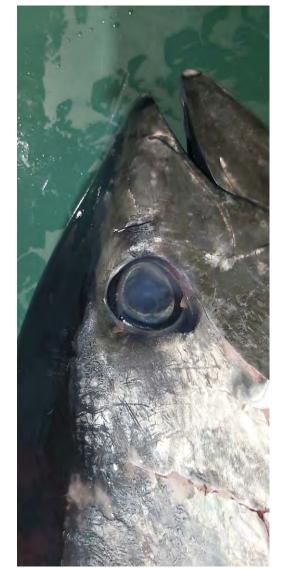




Marine Lactococcosis: Lesions





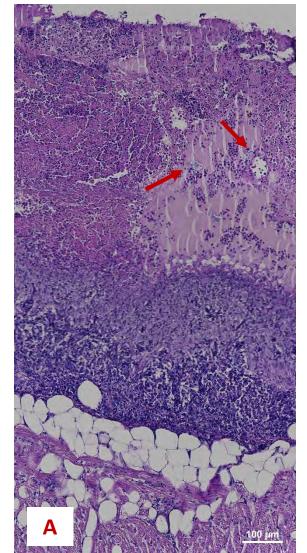


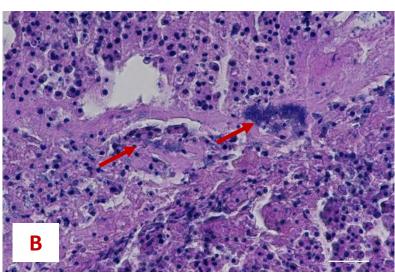


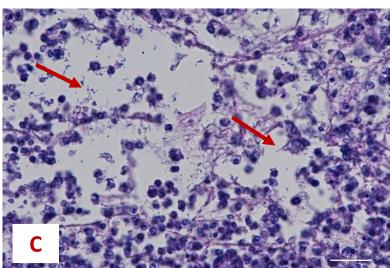


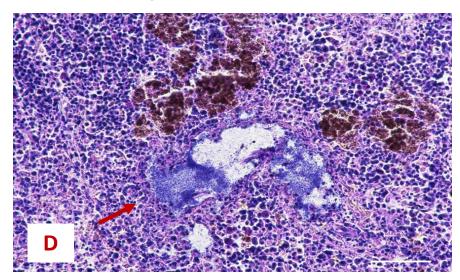


Histological examination (Atlantic bluefin tuna samples)









A: HEART: **Severe epicarditis** with mononucleate cell inflammation and multifocal foci of bacterial aggregates (Gram + cocci) (arrow). Underlying **compact myocardium appears unaffected**. H&E, 10X

B: HEART: Detail of the epicardium with clusters of Gram + bacterial aggregates (arrow). H&E, 60X

C: ENCEPHALON: Detail of Gram + cocci spread in the grey matter (arrow). H&E, 60X

D: SPLEEN: Clusters of Gram + bacterial aggregates in the splenic parenchyma (arrow). H&E, 40X

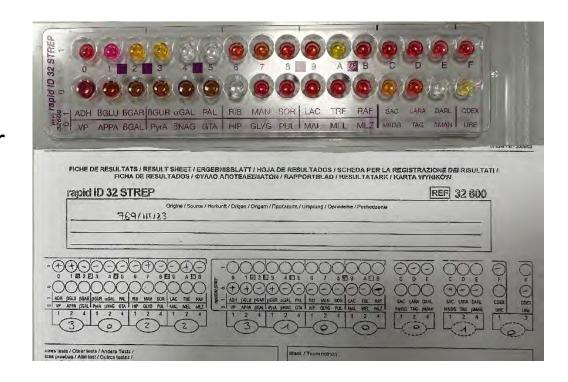


Marine Lactococcosis: Diagnosis



Classical bacteriology: white, circular, α - or non-hemolytic colonies on 5% sheep blood agar. Short gram-positive chains or pairs, facultatively anaerobic, non-motile, catalase and oxidase negative. Clindamycin test (?)

API systems (Biomerieux): several profiles for the API rapid ID 32strep (two miniaturized tests – namely **acidification of sucrose and hydrolysis of hippurate - may differentiate** among *L. garvieae* and *L. petauri*).





Marine Lactococcosis: Diagnosis

MALDI-tof: available libraries cannot distinguish among L. garvieae and L. petauri

Molecular methods:

several available methods (and many newly published) to correctly identify Lactococcus species such as:

- Amplification and Sanger sequencing of portion of the gyrB gene (Barbanti et al., 2024)
- Discriminant SNPs detection in the ITS1 region (Stoppani et al., 2023)
- Whole genome sequencing



	16S-23S Region position									
	219	358	440	442	443	469	478			
	63	200	282	284	285	311	320			
L. garvieae ATCC49156	-	G	Т	Т	Т	С	G			
L. petauri 159469	А	Т	G	А	ı	А	Т			
L. petauri spain rbw trout	А	Т	G	А	ı	А	Т			
L. garvieae sea bass	=	G	Т	Т	Т	С	G			
L. garvieae sea bream	-	G	Т	Т	Т	С	G			
L. garvieae Atl bluefin tuna	-	G	Т	Т	Т	С	G			
L. petauri water Eastern Med	Α	Т	G	Α	-	Α	Т			
L. garvieae Little tunny	-	G	Т	Т	Т	С	G			



Available literature





Case Report

The Characterisation of Lactococcus garvieae Isolated in an Outbreak of Septicaemic Disease in Farmed Sea Bass (Dicentrarchus labrax, Linnaues 1758) in Italy

Cristian Salogni ¹¹⁰, Cristina Bertasio ¹¹⁰, Adelchi Accini ², Lucia Rita Gibelli ¹, Claudio Pigoli ¹, Francesca Susini ³, Eleonora Podavini ¹, Federico Scali ^{1,*}¹⁰, Giorgio Varisco ¹ and Giovanni Loris Alborali ¹

January 2024

- Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", 25124 Brescia, Italy; cristian.salogni@izsler.it (C.S.); cristina.bertasio@izsler.it (C.B.); luciarita.gibelli@izsler.it (L.R.G.); claudio.pigoli@izsler.it (C.P.); eleonora.podavini@gmail.com (E.P.); giorgio.varisco@izsler.it (G.V.); giovanni.alborali@izsler.it (G.L.A.)
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- ³ Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", 00178 Roma, Italy; francesca.susini@izslt.it
- Correspondence: federico.scali@izsler.it



Journal of Fish Diseases





RESEARCH ARTICLE (CHEN ACCESS

Expanding Horizons: The First Reported Outbreak of Piscine Lactococcosis in Farmed Gilthead Seabream Sparus aurata in the Northern Tyrrhenian Sea

Giuseppe Esposito^I | Giorgia Bignami² | Silvia Colussi^I | Paolo Pastorino^I | Fabio Bondavalli^I | Marialetizia Fioravanti² | Elena Bozzetta^I | Pierfulgi Acutis^I | Andrea Valentino³ | Renzo Rizzi^I | Andrea Gustinelli² | Marino Prearo^I

³Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy | ²Department of Veterinary Medical Sciences, Ozzano dell'Emilia, Italy | ³Independent Veterinarian, Gaeta, Italy | ⁴Independent Biologist, Orbetello, Italy

March 2025

March 2025



Italian monitoring plan

Objective: To assess the epidemiological situation at the national level in farms of target species (Gilthead sea bream and European sea bass) and in wildlife (marine species) in order to identify appropriate prevention and control measures.

Sampling period: 1st – 30rd June 2025

End of analysis: 30rd July 2025

Involved authorities: Italian Ministry of Health; Regional veterinary services; ASL; IIZZSS

Methods: sampling of 30 animals from each farm (representative of the species held, ongoing or past mortalities) and evaluation by culture techniques of heart, brain and eye.

Wildlife will be sampled in case of mass mortality events (MME).

Allegato 1 Pag. 1

Piano di monitoraggio nazionale lattococcosi - scheda accompagnamento campioni

DATI DEL VERBALIZZANTE									
Veterinario Ufficiale Azienda Sanitaria									
DATA PRELIEVO									
NOME E COGNOME									
INDIRIZZO									
COMUNE			CAP		PROV.				
TELEFONO (CELLULARE):		E-MAIL							
LUOGO DEL PRELIEVO									
DENOMINAZIONE STABILIMENTO									
CODICE STABILIMENTO:									
Campo obbligatorio, solo nel caso di allevamento ITC									
INDIRIZZO	COMUN	NE		CAP	PROV.				
TEL.	FAX			E-MAIL					
ORIENTAMENTO PROD	OVITTUO			SPECIE ALLEVATE	PRESENTI				
☐ AVANNOTTERIA		□ ORATA							
☐ ALLEVAMENTO CON RIPRODUTTORI		□ SPIGOLA							
□ ALLEVAMENTO SENZA RIPRODUTTORI		□ ALTRO:							
☐ INGRASSO PER CONSUMO UMANO									
□ VALLE DA PESCA CON ATTIVITA' SEMINTEN									
D ALTRO:									
RILIEVI ISPETTIVI		CASIDI	MALATTIA	A/MORTALITÁ PREG	RESSI				
Sintomatologia in atto:** presente i	assente	Indicare se riscontrati casi nell'estate-autunno del 2024							
Descrizione sintomi:	□ NO □ 5i Indicare il mese								
T0 C 200000	Diagnosi effettuata:								
T° C acqua:	□ NO □ Si: specificare								
Ultima Introduzione nuovi animali:		Indicare se riscontrati casi nei 6 mesi precedenti la data odierna:							
□ NO	□ NO								
□ Si: Deta,	□ Si: Indicare: il mese:								
Specie	L'identificativo (ID) unità produttiva/e***								
Codice stabilimento di provenienza:	Diagnosi effettuata:								
ID Unità produttive dove sono stati introdotti	□ NO □ Si: Specificare								
* vall' che svolgono un'affixità semindensiva (pesci introdotti de albri stabilimenti, comfinati in vasche in cui i pesci sono alimentati). ** Sintomi su cui concentave le indagini: melamosi, esofatimo, nuoto erratico superficiale, isolati dal resto del gruppo e individui monbondi. ***Per un'dia produite e intesce a larginale usecalgabilo.									

Utilizzare la pagina successiva per identificazione e anamnesi del/del campioni prelevati



Luana Cortinovis - Lyngby - May 27th 2025

To conclude

• Lactococcosis is a major threat to Mediterranean aquaculture (both freshwater and saltwater species).

• To date, Italian and Atlantic bluefin tuna are affected by Lactococcus garvieae, but there is recent evidence of the circulation of L. petauri in saltwater fish species reared in the Mediterranean basin and in seawater.

 Isolates from European sea bass and sea bream differ from those of Atlantic bluefin tuna in some phenotypic and genetic characteristics. New species?



Forecast for next years

- The spread of Lactococcosis to new farming sites may be facilitated by the rising temperature of Mediterranean waters.
- Infect wild specimens may act as vectors.
- Inadequate biosecurity measures and disinfection protocols may maintain the pathogen at the farming site.
- High waters temperatures compromise the homeostasis and immune system of fish, increasing susceptibility to pathogens (high pathogenicity of *L. garvieae*, low capability to counter coinfections...).
- Increasing water temperature promotes unfavourable environmental conditions (algae blooms, low oxygen levels...) that act as stress factors.

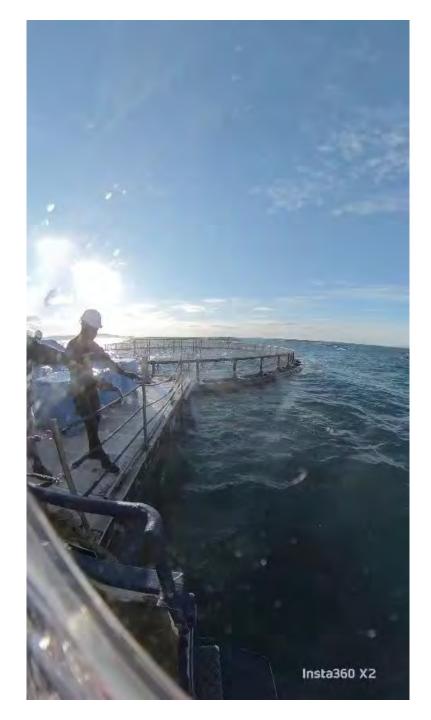


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- Heckman, T. I., Yazdi, Z., Older, C. E., Griffin, M. J., Waldbieser, G. C., Chow, A. M., ... & Soto, E. (2024). Redefining piscine lactococcosis. Applied and Environmental Microbiology, 90(5), e02349-23.
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- Stoppani, N., Colussi, S., Pastorino, P., Prearo, M., Sciuto, S., Altinok, I., ... & Fernández-Garayzábal, J. F. (2023). 16S-23S rRNA Internal Transcribed Spacer Region (ITS) sequencing: A potential molecular diagnostic tool for differentiating Lactococcus garvieae and Lactococcus petauri. Microorganisms, 11(5), 1320.



Thank you for your attention



Questions?









Sanitary situation in France: Update of the IHN and VHS eradication plan implement and challenging detection of VHSV strains involved in recent outbreaks

M. Baud, L. Pallandre, P. Grippon, E. Rolland, L. Bigarre, G. Lefebvre, I. Guerry, T. Morin, L. Louboutin

French Agency for Food, Environmental and Occupational Health & Safety

Ploufragan-Plouzané-Niort Laboratory, Unit Virology, immunology and ecotoxicology of fish

NRL for listed fish diseases, Plouzané, France

In close relation with:

Direction Générale de l'Alimentation,

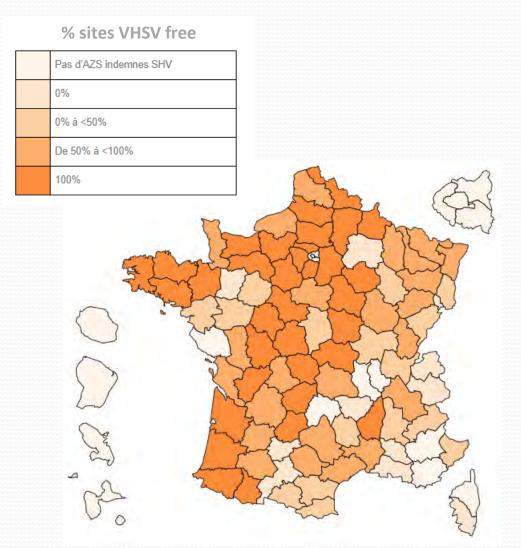
Service des actions sanitaires,

Sous direction de la santé et du bien-être animal, Bureau de la santé animale, Paris, France

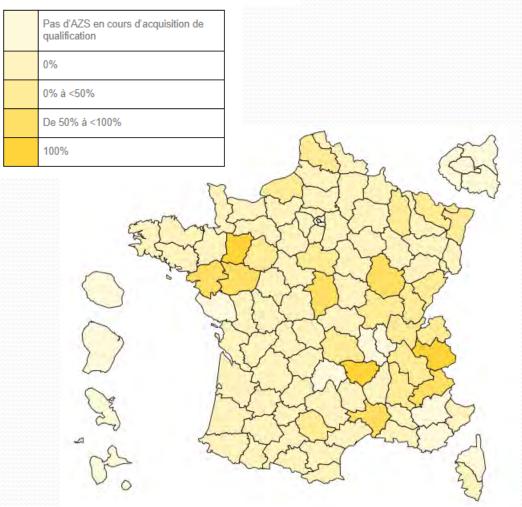
Situation in France in 2025:

anses

IHNV and VHSV plan for eradication since 2017

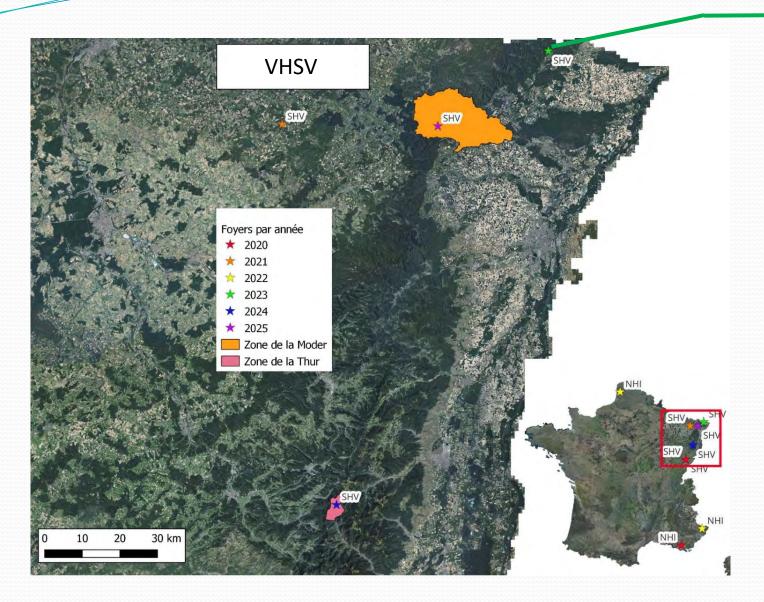


% sites with eradication plan in progress





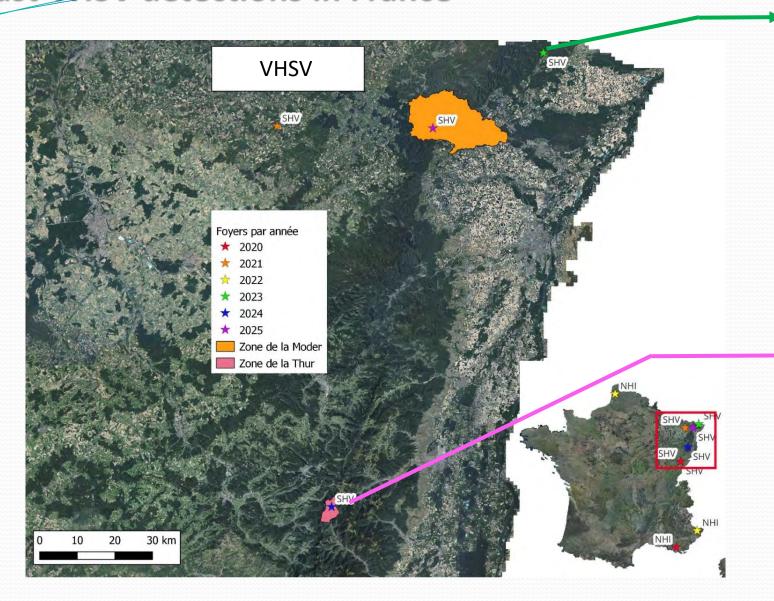




2023_23-087_FR67

Supplier with undetermined status?



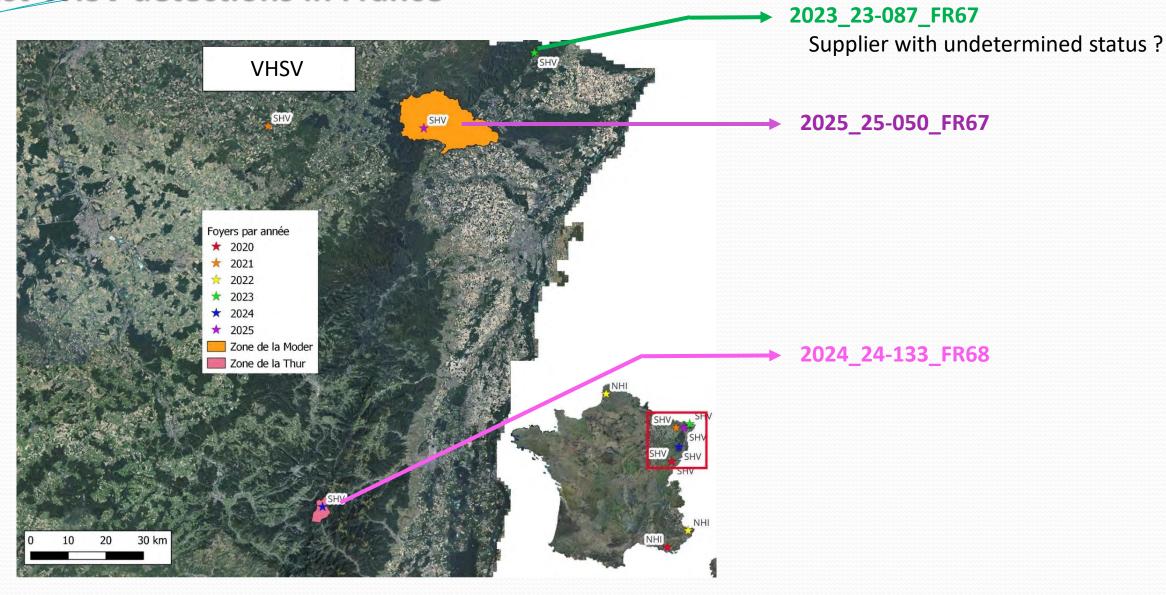


2023_23-087_FR67

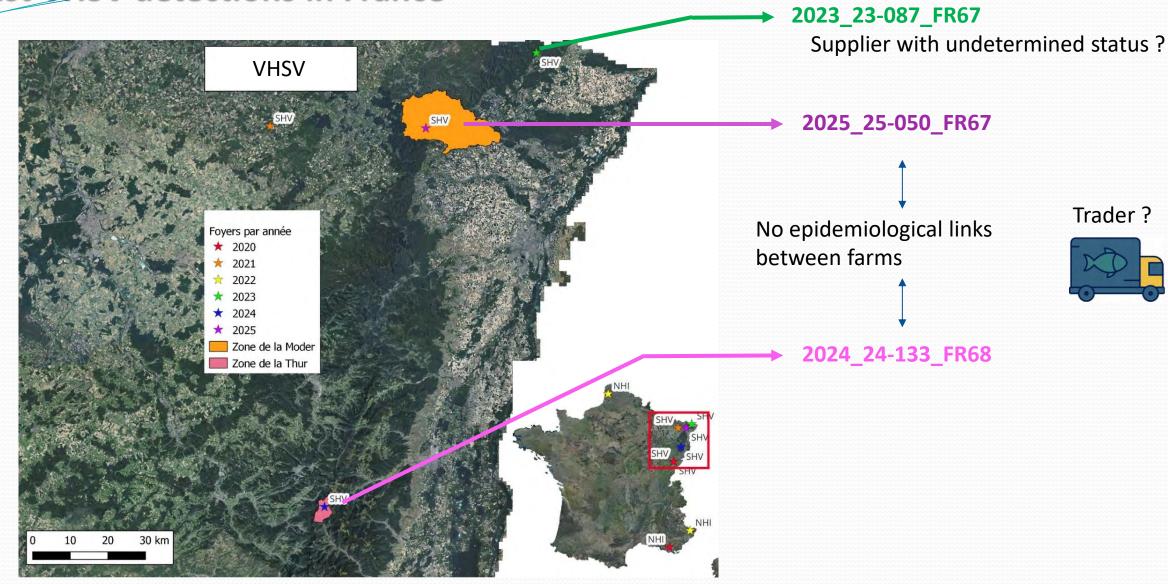
Supplier with undetermined status?

2024_24-133_FR68











Three outbreaks in the North Eastern France detected by a routine laboratory and sent to NRL:

- ✓ Middle of 2023 in department 67
 - Rainbow trout 200 to 600g, water temperature 13°C
 - No clinical signs
 - Analysis performed during the eradication plan
 - Detection by RT-qPCR with late Ct ~ 35
 - Cell culture -
 - Sequencing (partial G gene)

→ 2023_23-087_FR67



Three outbreaks in the North Eastern France detected by a routine laboratory and sent to NRL:

✓ Middle of 2023 in department 67

→ 2023_23-087_FR67

- ✓ End of 2024 in department 68
- → 2024 24-133 FR68 Rainbow trout 50g, water temperature 9°C
 - No clinical signs
 - Detection by RT-qPCR with late Ct ~ 35
 - Cell culture + only on RTG₂ cells
 - Sequencing (complete G gene) on the cell culture production (Sanger and NGS)



Three outbreaks in the North Eastern France detected by a routine laboratory and sent to NRL:

- ✓ Middle of 2023 in department 67
- ✓ End of 2024 in department 68
- ✓ Beginning 2025 in department 67
 - Rainbow trout adults, water temperature 5°C
 - High mortality, petechiae, suspected Yersiniose or VHSV
 - Detection by RT-qPCR with late Ct ~ 35
 - Cell culture in the first assay (presence of IPNV)
 - Partial G gene sequencing (286bp)
 - Cell culture + in the second assay
 - Sanger sequencing (complete G gene) on the cell culture production



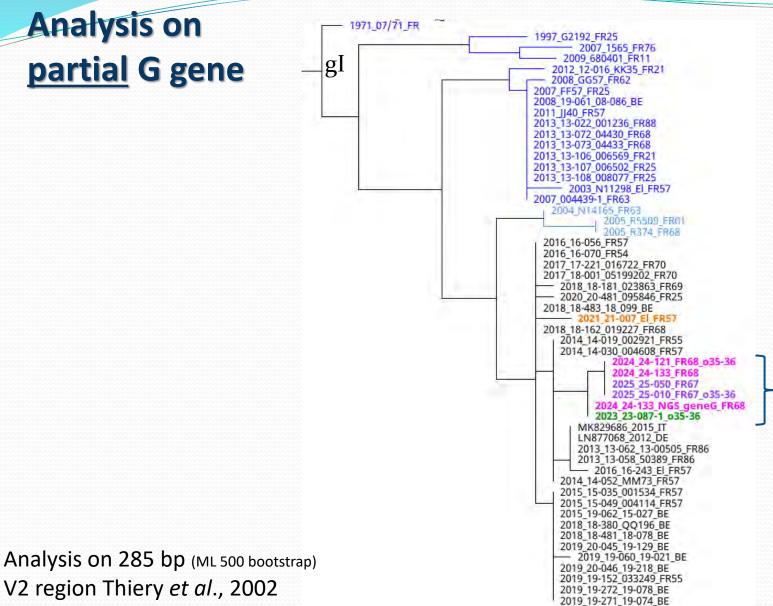
→ 2024_24-133_FR68

→ 2025_25-050_FR67



Sequencing: **Analysis** on partial G gene



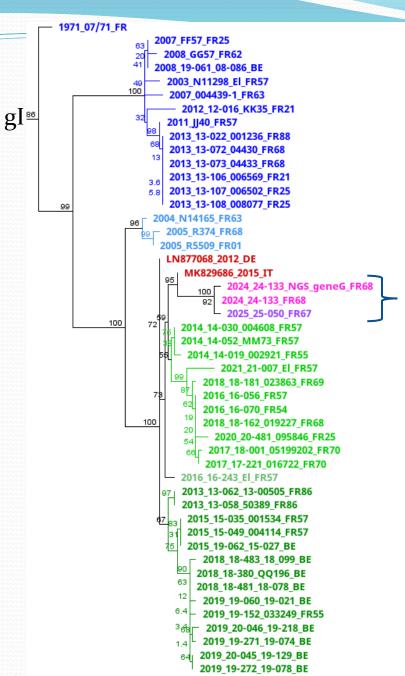


New branch with the 3 last sequences from 2023 to 2025 on the partial G gene (sequencing from cell culture supernatant or organs):

99.7 to 100% identity between them (≠ 1 nucleotide)

V2 region Thiery et al., 2002

Sequencing: Analysis on complete G gene





New branch with the 2 last sequences from 2024 and 2025:

- 97.7 to 99.9% identity between them
 (≠ 2 to 4 nucleotides)
- 99.1% identity (≠14 nucleotides)
 with a 2015 Italian sequence (nearest in
 Genebank)

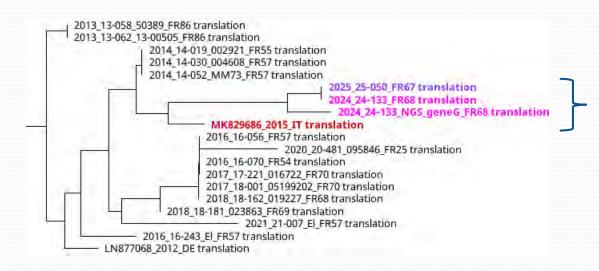
Analysis on 1524 bp (NJ 1000 bootstrap)

Sequencing:

anses

Analysis on complete G gene

PROTEINS



Confirmation of a new strain circulating in the East part of France

- Clinicals signs not always present
- Late detection with molecular biology tools
- Behaviour in cell culture different from previous isolates (no or slight growth on EPC and BF2 cells respectively)



Cell line RTG₂ is the most recommended



Would be interesting to compare with recent European sequences

Analysis on 1524 bp



Analysis of the sequence produced by NGS

Tools for G gene sequencing

(Einer-Jensen et al., 2004)



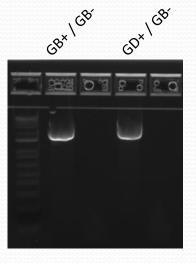




Many mismatches between primers and the NGS sequence

However, PCR products could be amplified on the cell culture supernatant with primers:

- GB+/GB-
- GD+ / GB-

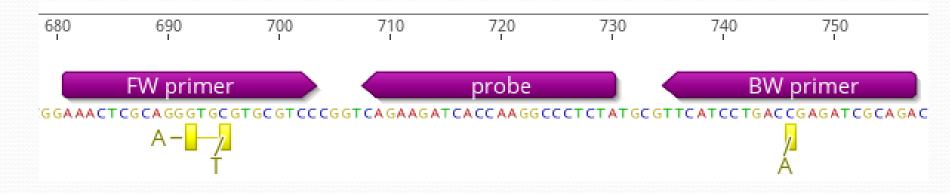




Analysis of the sequence produced by NGS

Tools for N gene RT-qPCR

(Jonstrup *et al.,* 2013)





3 mismatches between the primers and the NGS sequence

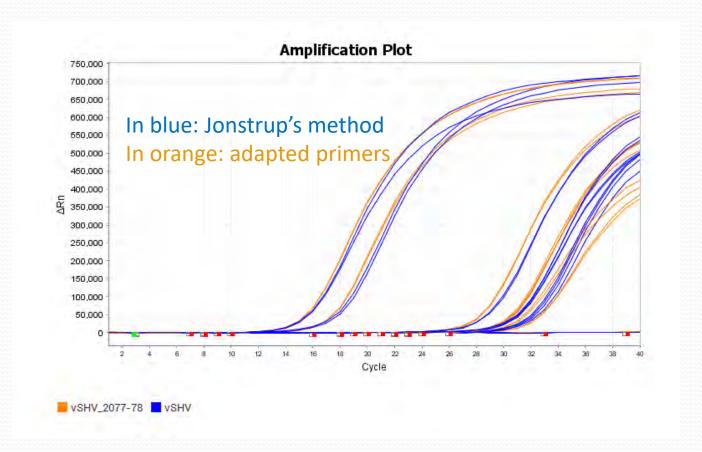
- Design of new primers adapted to the sequence
- Test by RT-qPCR with 2 methods: Jonstrup compared to adapted primers



Analysis of the sequence produced by NGS

Tools for N gene by RT-qPCR

(Jonstrup *et al.,* 2013)



6 samples from organs or cell supernatants tested:

All were detected with both methods with an average difference of 0.25 Ct



Conclusion for detection by RT-qPCR: **NO PROBLEM**!



Thanks for your attention!



If you wish to share more information or sequences, please contact lnr.poissons@anses.fr



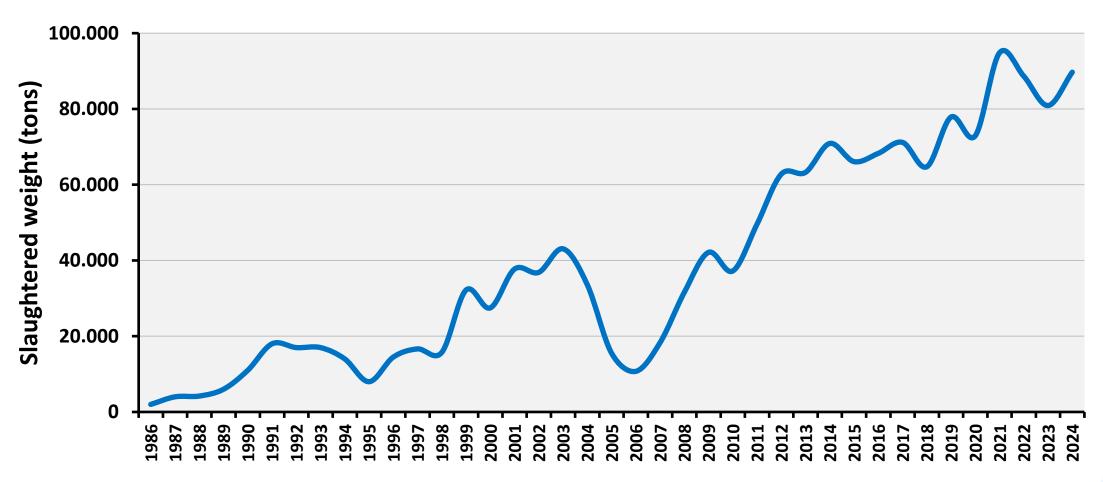
Agenda

Production Data

Infectious diseases in the Marine environment

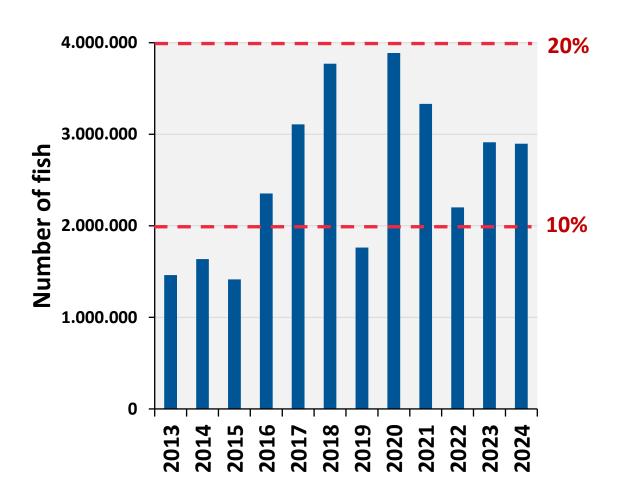
- Winter Ulcers
- Infectious salmon anaemia (ISA)
- Cardiomyopati syndrome (CMS)

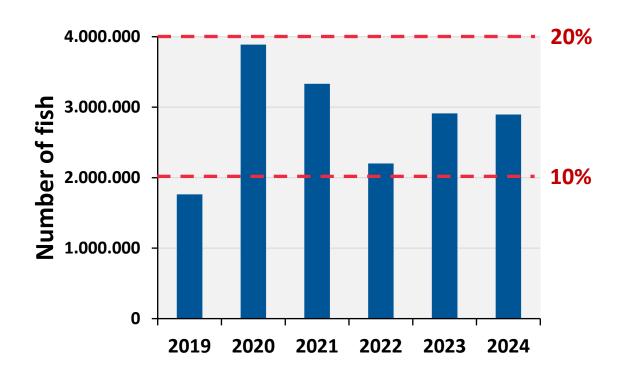
Production of Atlantic salmon

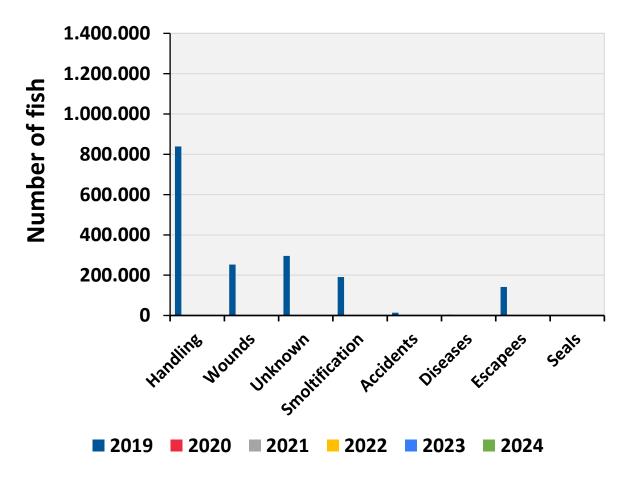


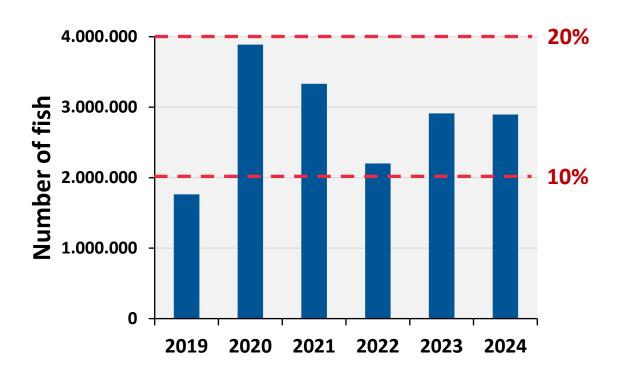


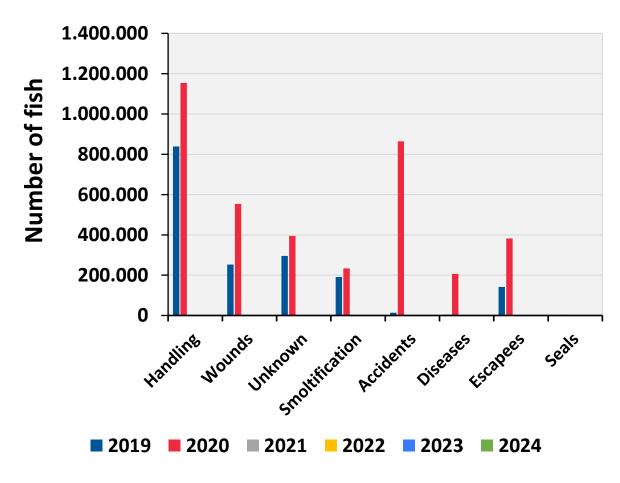
Yearly Mortality

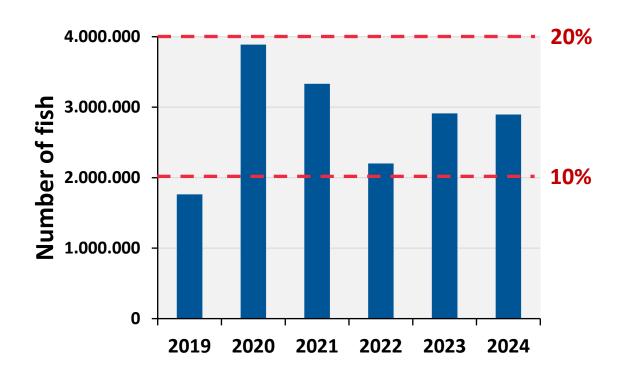


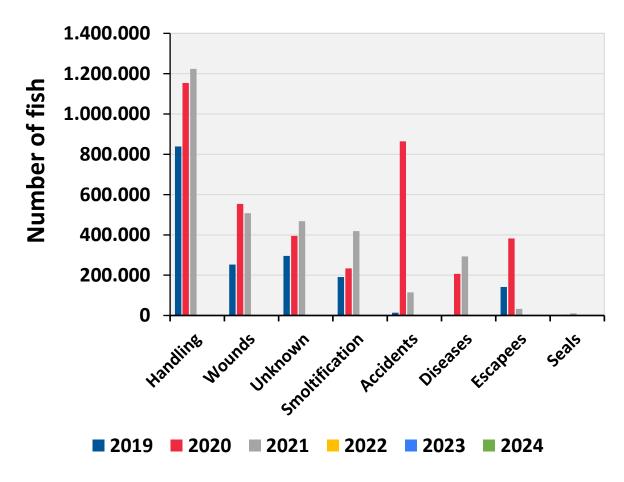


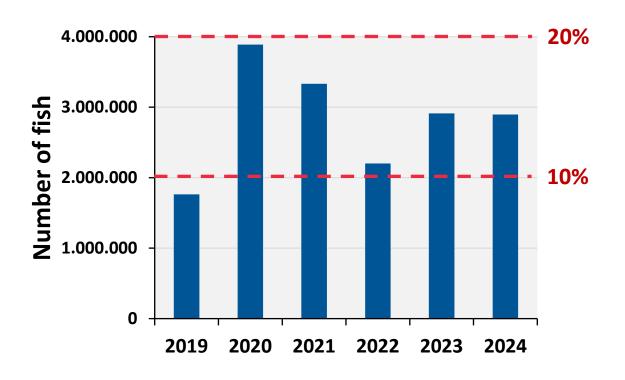


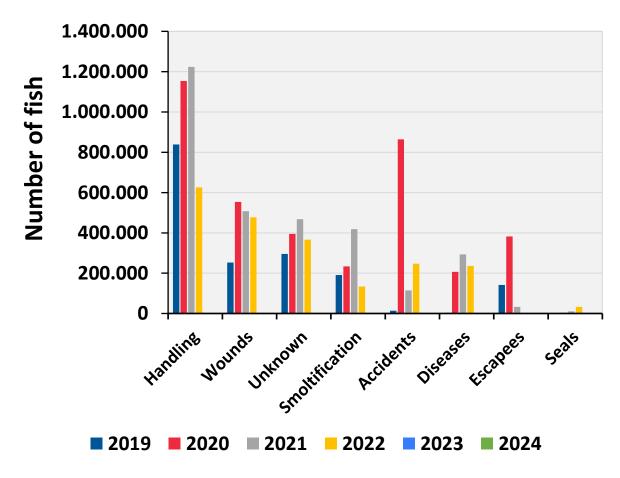


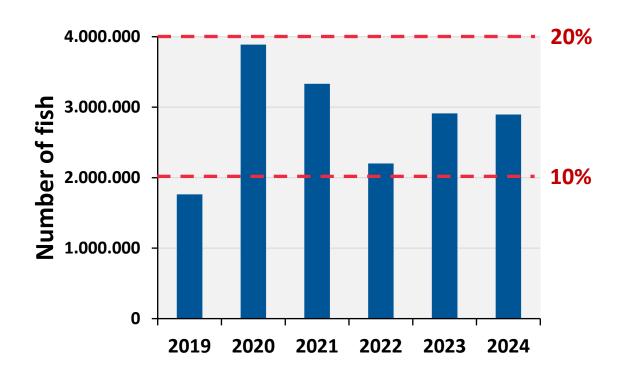


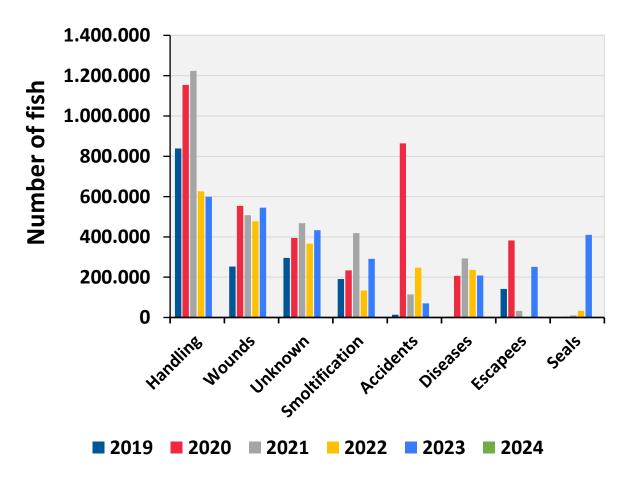












Kópur

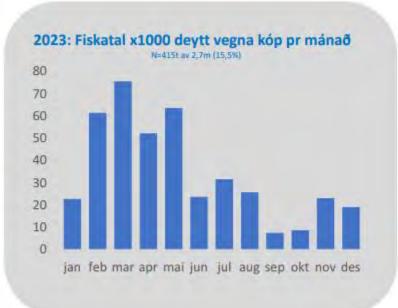
Nú verður tað ólógligt at veiða kóp í Føroyum Ein eranie virtual en fyre ar tigergar prifestering menn en bake til erak, og tall skull komber tæng til ar liger i greger eran t

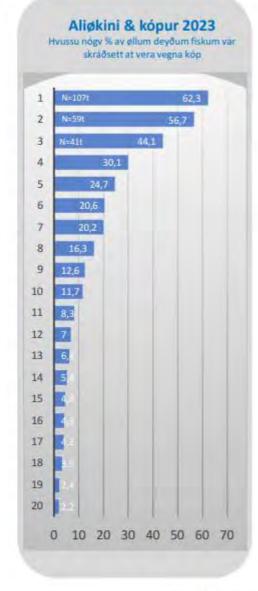
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mai yeur tud werden obligdigt at vende old slag av koopt i Ferrogram, og tverse tutern ner year two werees reagant as the enter upprobably images Heydral, land ortains businessessiblem, be at leggin byt Lagungs's investion.

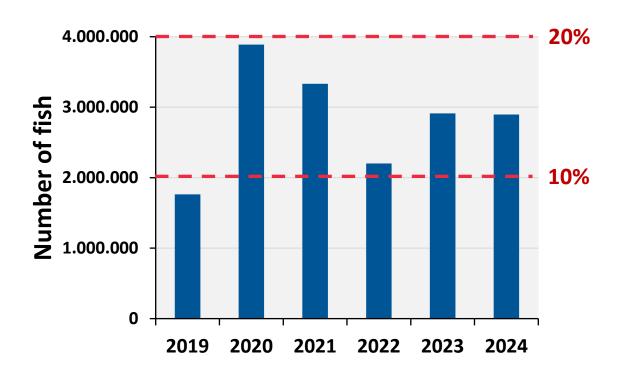


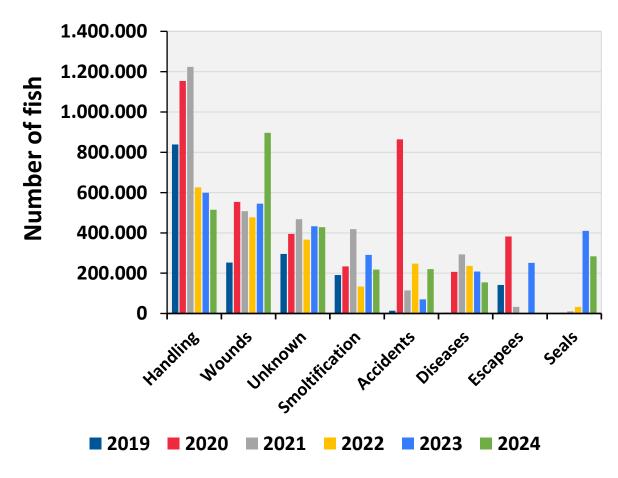




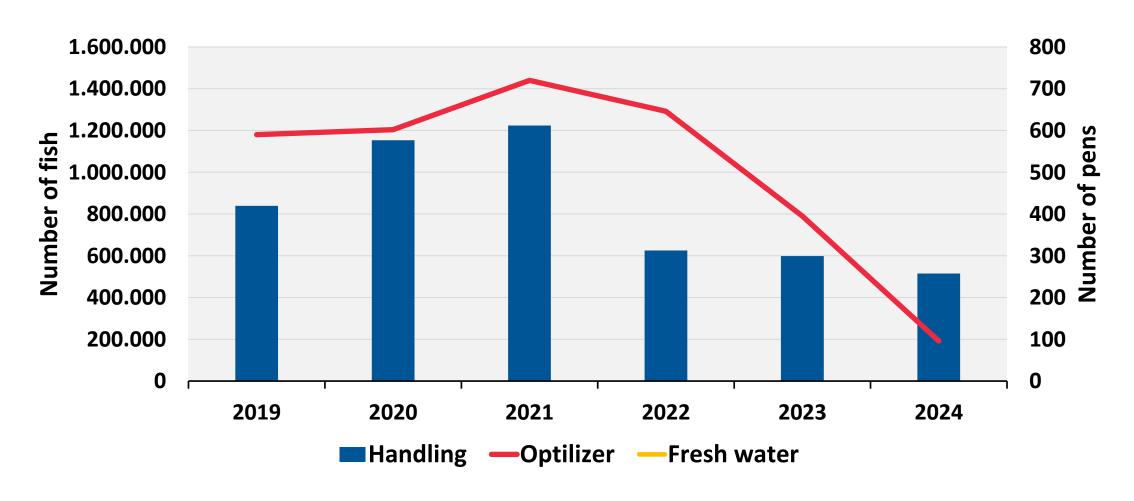


JAVRIK

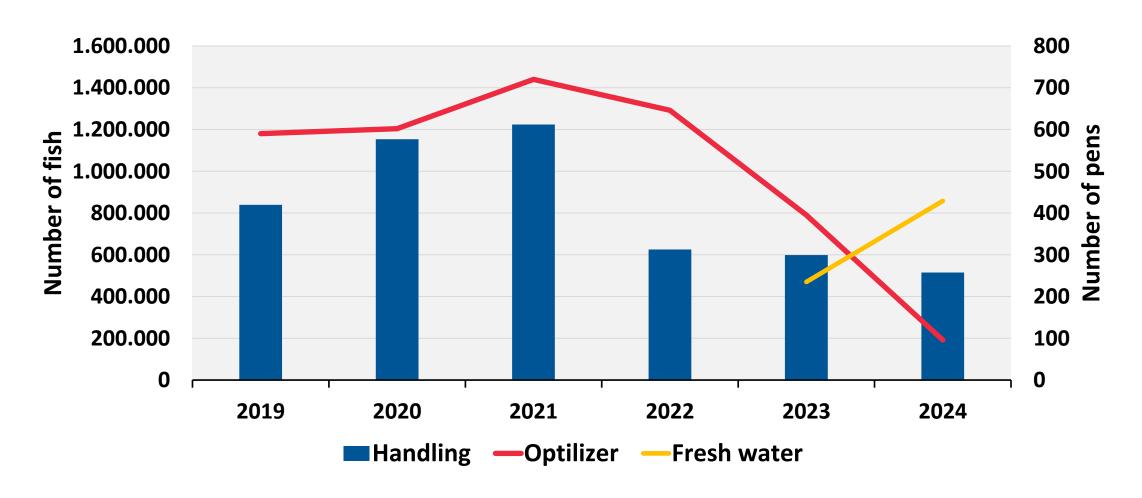




Handling related morts and mechanical treatments

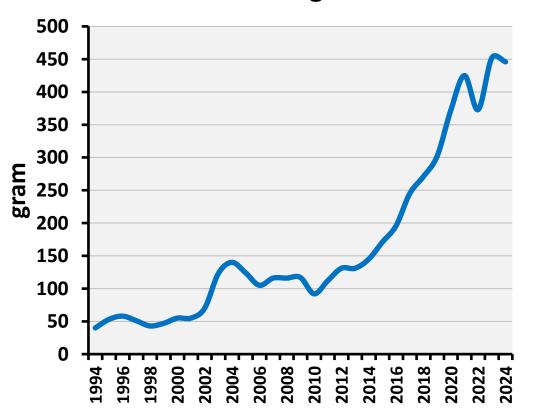


Handling related morts and mechanical treatments

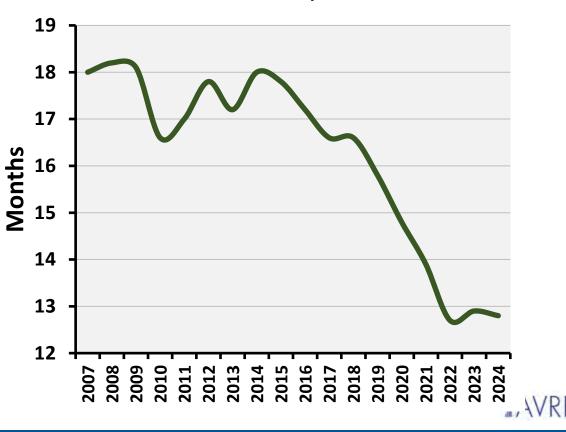


Production of large-smolt is one of the main strategies to reduce handling and disease related mortalities at marine sites

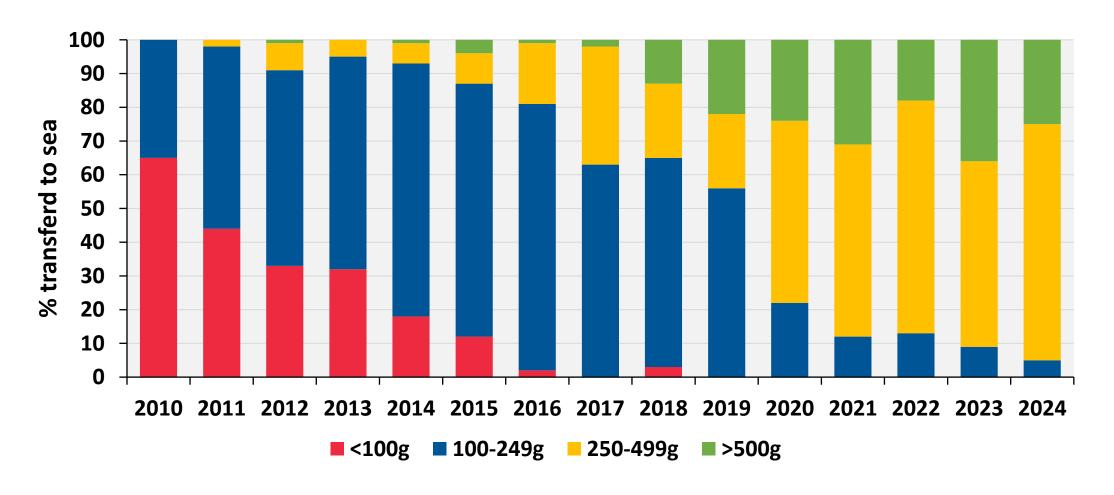
Produktion of large smolt



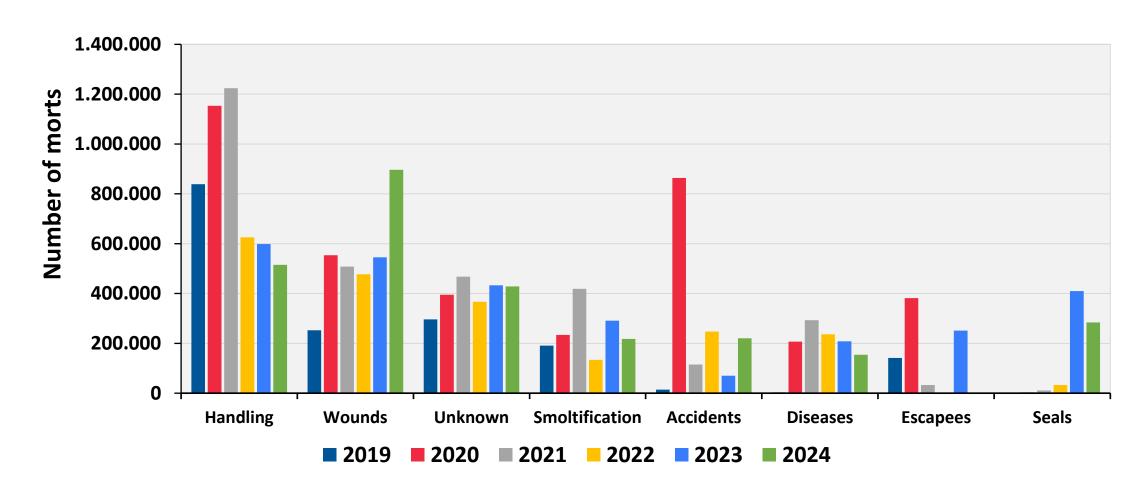
Duration of marine production



Production of large-smolt is one of the main strategies to reduce handling and disease related mortalities at marine sites



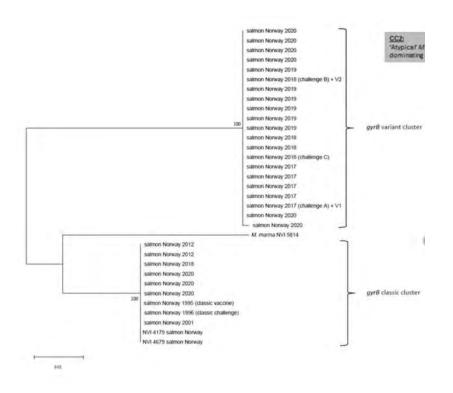
Almost 1/3 of the morts in 2024 were related to wounds or winter ulcers



Prevalence of Moritella viscosa

	Samples tested	Moritella viscosa	Cq-values
2019	24	9	31 – 38
2020	21	15	24 – 36
2021	42	8	30 – 35
2022	101	40	21 – 34
2023	70	23	26 – 36
2024	177	88	21 – 35
2025	248	108	18 – 35

New variant Moritella viscosa

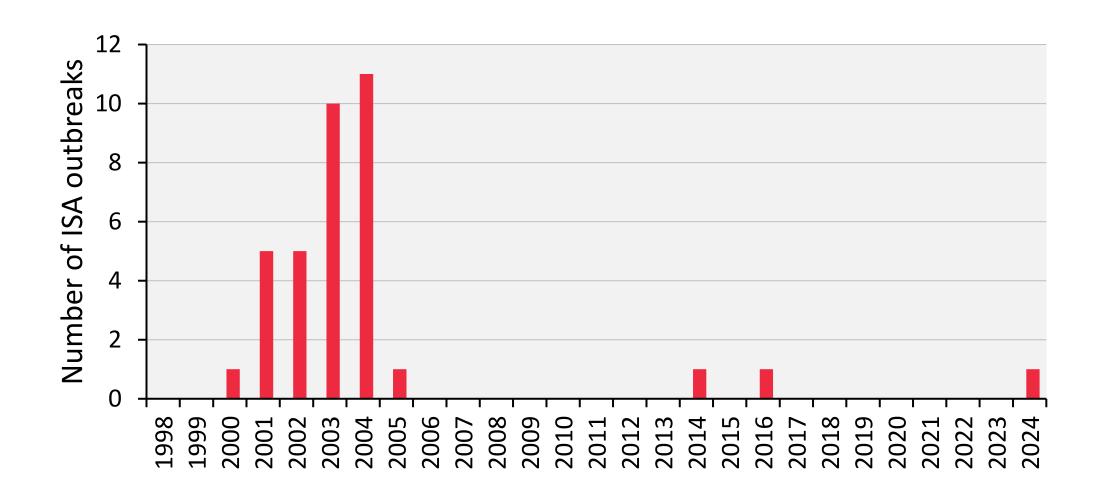


 We see a switch from classical moritella to variant mortella in the Faroe Islands

 classical moritella vaccine does not cross-protect against variant moritella

Infectious salmon anaemia

ISA outbreaks in the Faroe Islands

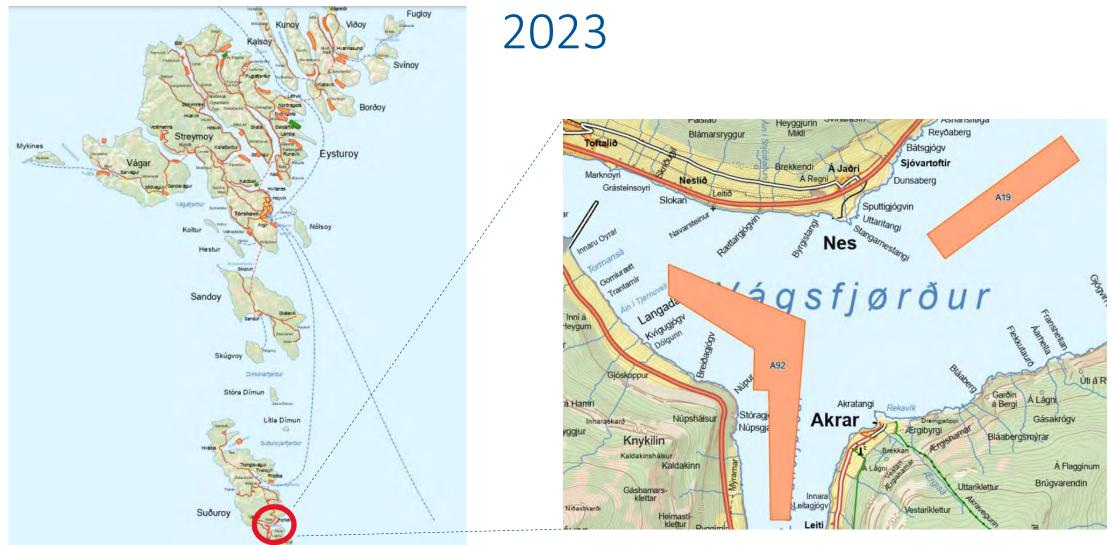


Epidemiological and genetic characterization of the

outbreak Sandoy

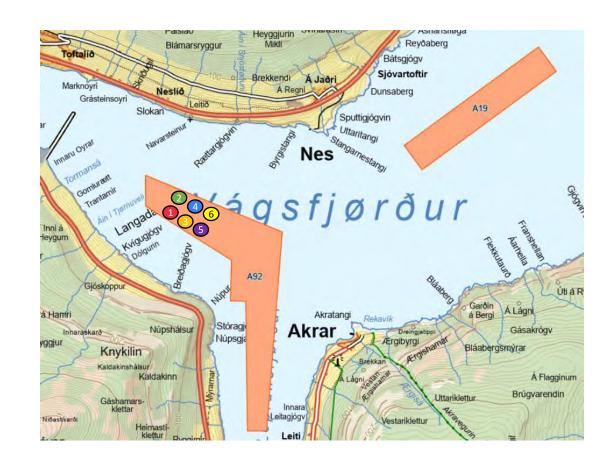


Fish were transferred to the sea site in October



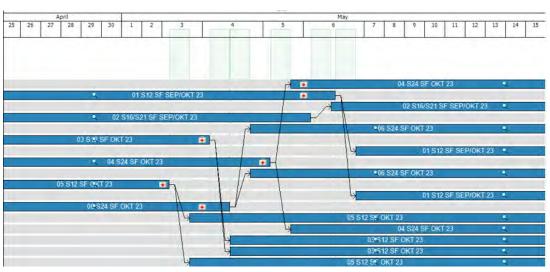
Fish were transferred to the sea site in October 2023

Cage	Smolt supplier	Number of fish
1	Α	194.971
2	B + C	116.015
3	А	204.633
4	D	173.716
5	А	218.664
6	D	188.135
Total		1.096.134



Fish were treated in FW and moved from A-92 to A-19





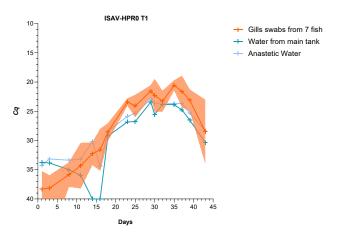


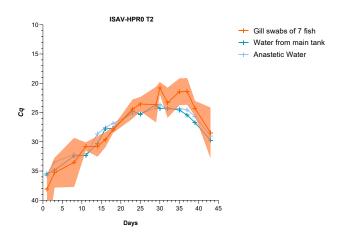
Monitoring for ISAV in fish tissue

Date	Sample type	Samples	ISAV positive	HPRΔ	HPR0	Comments
29-09-2023	Tissue	8	0			
15-11-2023	Tissue	25	0			
17-01-2024	Tissue	14	1		1	
22-02-2024	Tissue	34	0			
05-04-2024	Tissue	9	2		2	

Monitoring for ISAV in environmental RNA

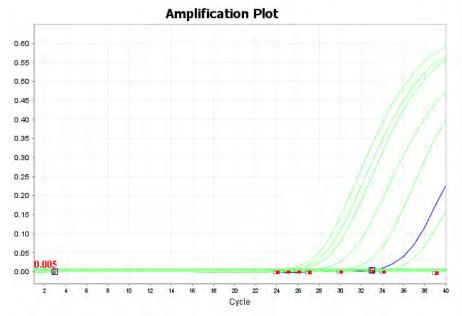
Date	Sample type	Samples	ISAV positive	HPRΔ	HPR0	Comments
29-09-2023	Tissue	8	0			
15-11-2023	Tissue	25	0			
17-01-2024	Tissue	14	1		1	
22-02-2024	Tissue	34	0			
05-04-2024	Tissue	9	2		2	
13-05-2024	eRNA	4	1			

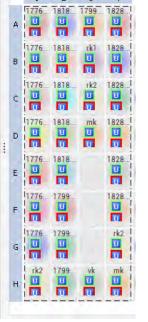


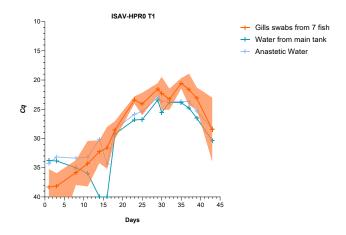


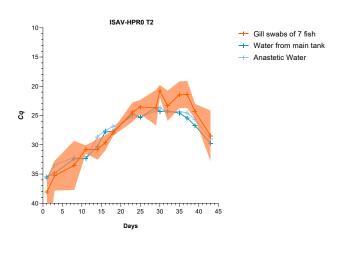
Detection of ISAV in eRNA

Date	Sample type	Samples	ISAV positive	HPRΔ	HPR0	Comments
29-09-2023	Tissue	8	0			
15-11-2023	Tissue	25	0			
17-01-2024	Tissue	14	1		1	
22-02-2024	Tissue	34	0			
05-04-2024	Tissue	9	2		2	
13-05-2024	eRNA	4	1			



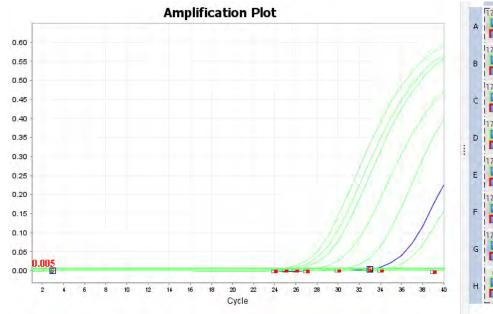




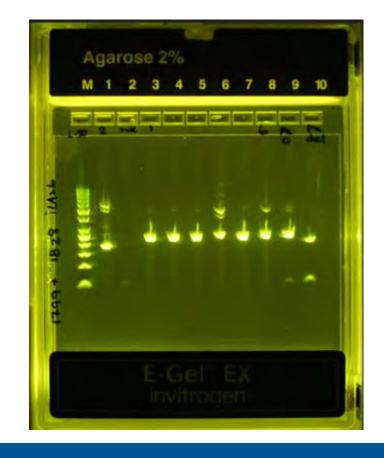


Detection of ISAV-HPRΔ

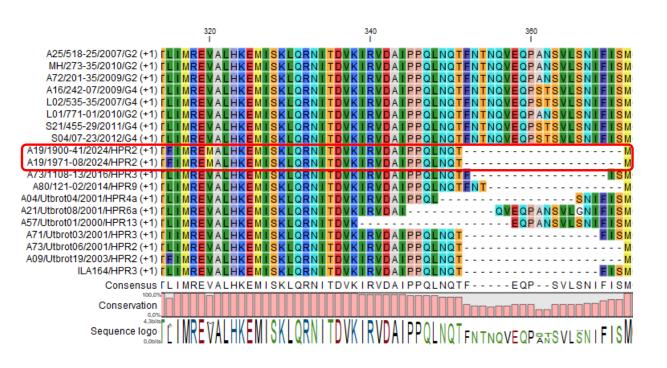
Date	Sample type	Samples	ISAV positive	HPRΔ	HPR0	Comments
29-09-2023	Tissue	8	0			
15-11-2023	Tissue	25	0			
17-01-2024	Tissue	14	1		1	
22-02-2024	Tissue	34	0			
05-04-2024	Tissue	9	2		2	
13-05-2024	eRNA	4	1	1		First detection in C04



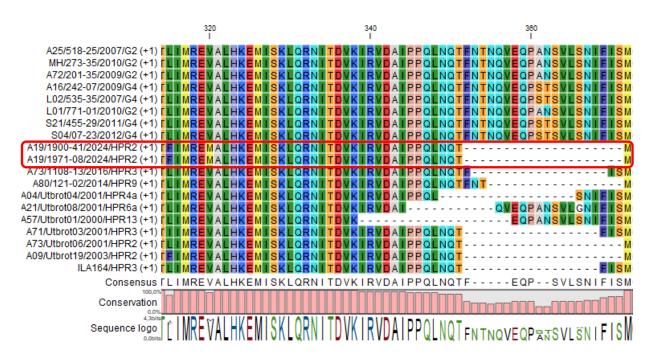


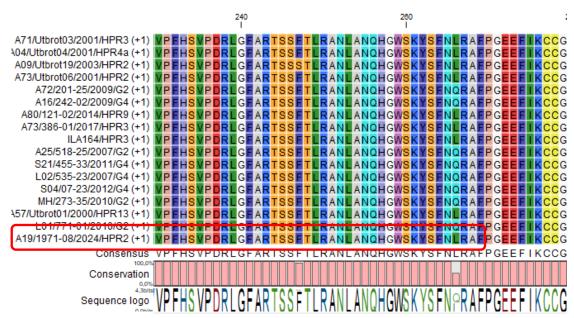


Confirmation of HPR∆ in the *HE*-gene by NGS



Confirmation of Q266L in the F-gene by NGS



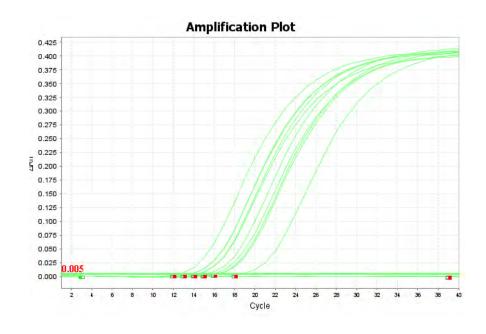


Confirmation of ISA

Date	Sample type	Samples	ISAV positive	HPRΔ	HPR0	Comments
29-09-2023	Tissue	8	0			
15-11-2023	Tissue	25	0			
17-01-2024	Tissue	14	1		1	
22-02-2024	Tissue	34	0			
05-04-2024	Tissue	9	2		2	
13-05-2024	eRNA	4	1	1		First detection in C04
26-05-2024	Tissue	13	10	10		ISA clinics in C04 og C06





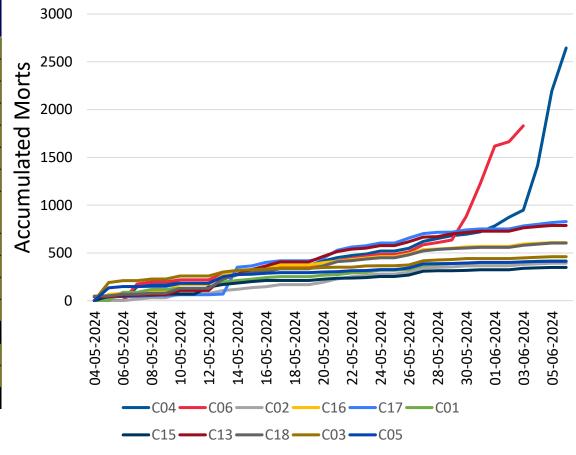


Screening for ISAV in all 11 cages

Date	Sample type	Samples	ISAV positive	HPRΔ	HPR0	Comments
29-09-2023	Tissue	8	0			
15-11-2023	Tissue	25	0			
17-01-2024	Tissue	14	1		1	
22-02-2024	Tissue	34	0			
05-04-2024	Tissue	9	2		2	
13-05-2024	eRNA	4	1	1		First detection in C04
26-05-2024	Tissue	13	10	10		ISA clinics in C04 og C06
27-05-2024	Tissue	408	45	39	6	All cages. HPRΔ in C04 & C06 only
30-05-2024	Tissue	13	13	13		C06
31-05-2024	Tissue	12	12	12		C06
04-06-2024	Tissue swab	47	3		3	All but C04 & C06
04-06-2024	eRNA	9	0			All but C04 & C06
04-06-2024						C06 Empty
04-06-2024	Tissue	6	6	6		C04
05-06-2024	Tissue	12	12	12		C04
06-06-2024						C04 Empty

Significant increase in ISA related mortality in C04 and C06 only

Date	Sample type	Samples	ISAV positive	HPRΔ	HPR0	Comments
29-09-2023	Tissue	8	0			
15-11-2023	Tissue	25	0			
17-01-2024	Tissue	14	1		1	
22-02-2024	Tissue	34	0			
05-04-2024	Tissue	9	2		2	
13-05-2024	eRNA	4	1	1		First detection in C04
26-05-2024	Tissue	13	10	10		ISA clinics in C04 og C06
27-05-2024	Tissue	408	45	39	6	All cages. HPR∆ in C04 & C06 only
30-05-2024	Tissue	13	13	13		C06
31-05-2024	Tissue	12	12	12		C06
04-06-2024	Tissue swab	47	3		3	All but C04 & C06
04-06-2024	eRNA	9	0			All but C04 & C06
04-06-2024						C06 Empty
04-06-2024	Tissue	6	6	6		C04
05-06-2024	Tissue	12	12	12		C04
06-06-2024						C04 Empty



Surveillance of the 9 remaining cages

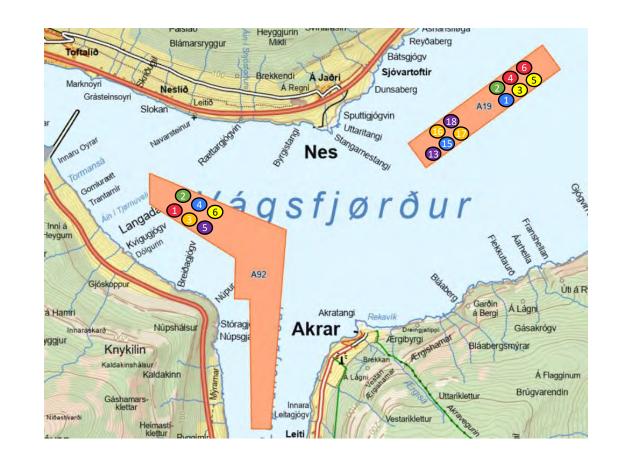
Date	Sample type	Samples	ISAV	HPRΔ	HPR0	Comments
06-06-2024			positive			C04 Empty
10-06-2024	Tissue swab	79	1		1	All cages
12-06-2024	eRNA	9	0			All cages
18-06-2024	Tissue	124	0			All cages
19-06-2024	eRNA	9	0			All cages
26-06-2024	eRNA	18	0			All cages
26-06-2024	Tissue	30	0			4 of 9
28-06-2024	Tissue	224	0			All cages
04-07-2024	Tissue swab	56	0			All cages
10-07-2024	eRNA	9	0			All cages
10-07-2024	Tissue swab	60	0			All cages
11-07-2024	eRNA	4	0			All cages
11-07-2024	Tissue swab	30	0			All cages
12-07-2024	eRNA	8	0			All cages
16-07-2024	Tissue swab	75	0			All cages
24-07-2024	Tissue swab	61	0			All cages
26-07-2024	eRNA	8	0			All cages
02-08-2024	eRNA	8	0			All cages
05-08-2024	eRNA	8	0			All cages
09-08-2024	Tissue	40	0			All cages
13-08-2024	eRNA	7	0			All cages
15-08-2024	Tissue swab	35	0			All cages
46		1516	106	93	10	

When did HPR0 mutate to HPR Δ ?

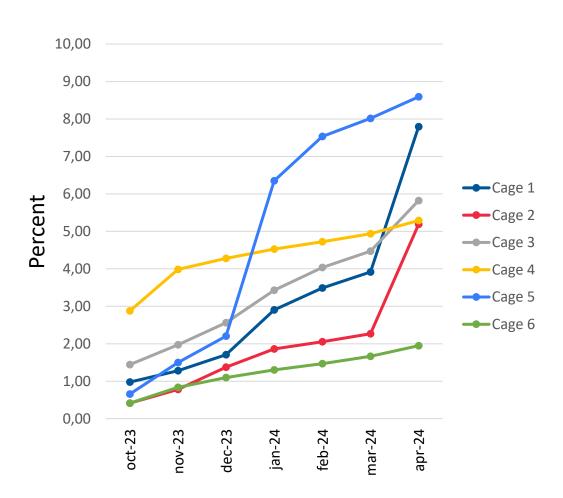


When did HPR0 mutate to HPR Δ ?

Cage	Smolt supplier	Number of fish
1	Α	194.971
2	B + C	116.015
3	Α	204.633
4	D	173.716
5	Α	218.664
6	D	188.135
Total		1.096.134



When did HPR0 mutate to HPR Δ ?

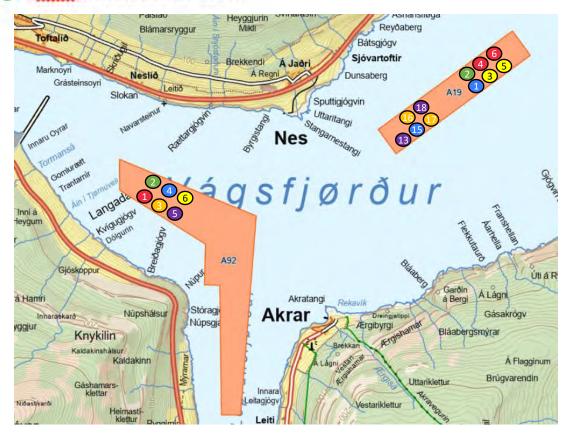




When did HPR0 mutate to HPR Δ ?

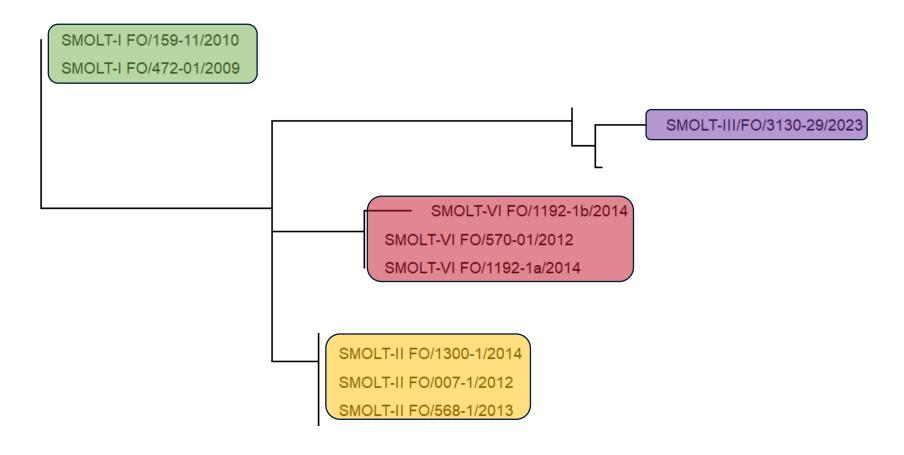
Table 4 Vindmátingar frá LV av Porkerishálsi - dagarnir undan fráboðan um økt felli (08-04-2024) er nógvur vindur á økinum av landnyrinngin snarandi niður í landsynning og sunnan (ikki góðar ættir á A-92). Serliga innastu ringar á økinum verða ávirkaðir R01 og R02, við sárfiskum og øktum felli eftirfylgjandi. Allarhelst er hetta dag0 í mun til mutation av ILAV.



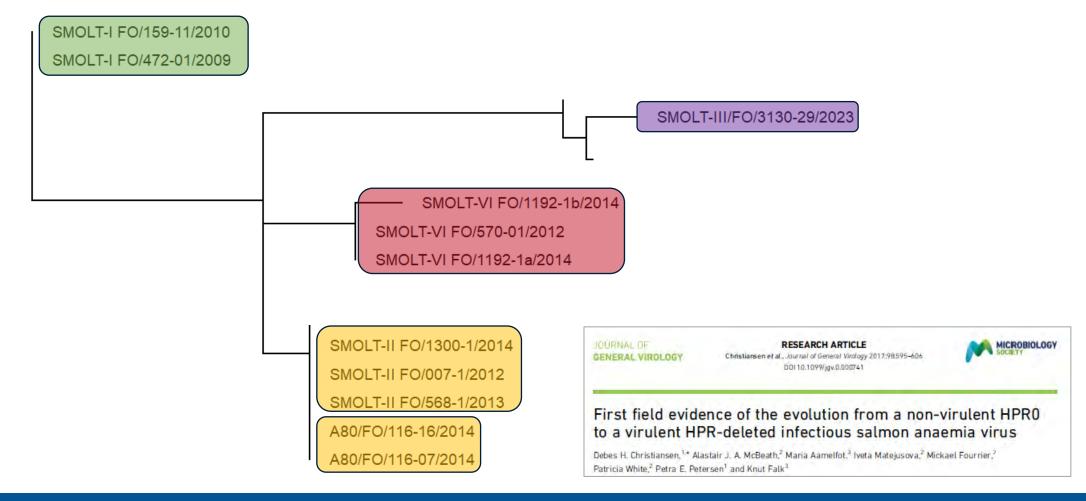


What is the origin of the three last ISA outbreaks?

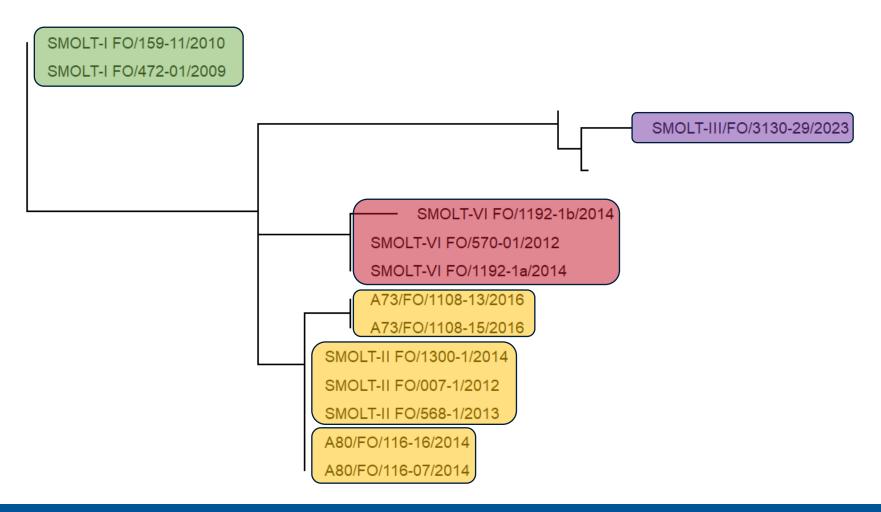
NGS shows that different HPRO are circulating in each smolt farm



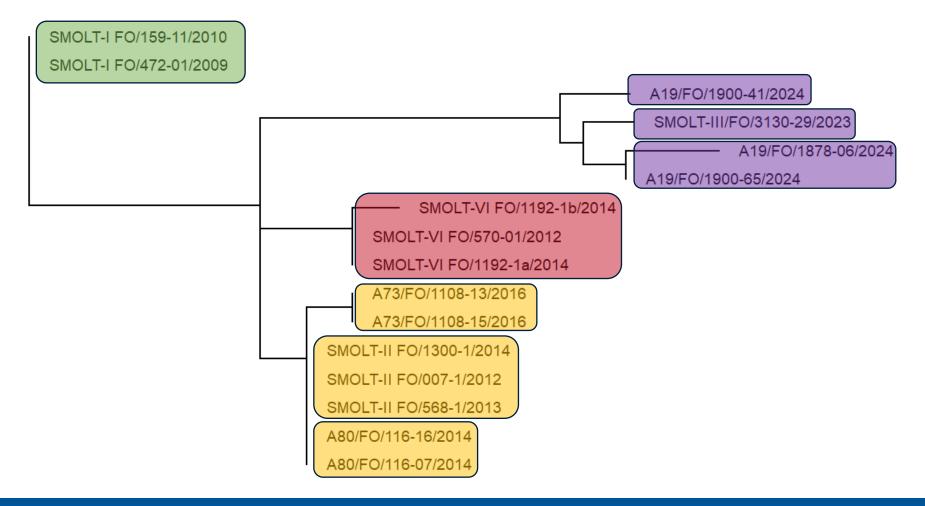
HPRΔ in 2014 originated from HPR0 in smolt farm II



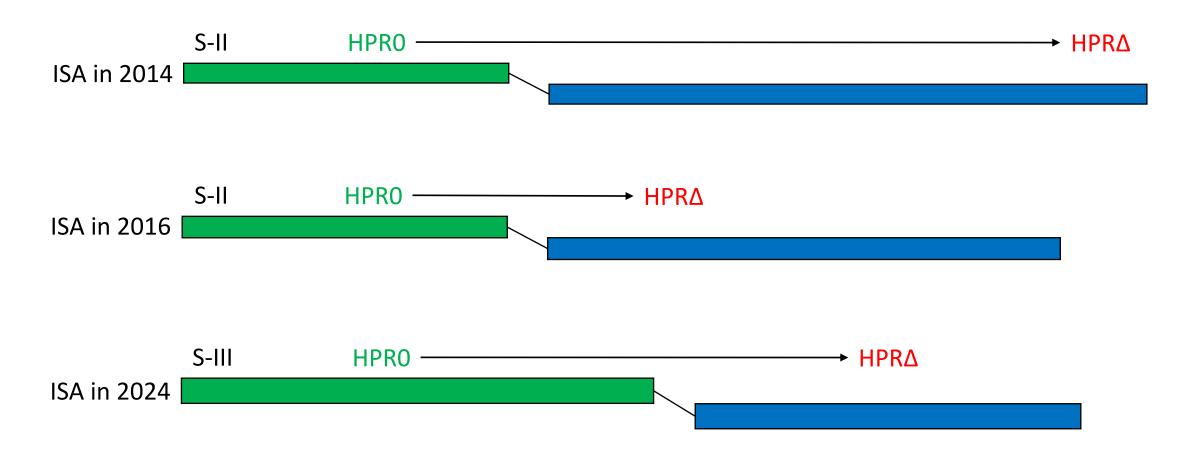
HPRΔ in 2016 originated from HPR0 in smolt farm II



HPRΔ in 2024 originated from HPR0 in smolt farm III



HPRΔ at the marine sites originated from HPR0 in the smolt farms



Was the first HPRO > HPR∆ conversion in an unvaccinated fish?

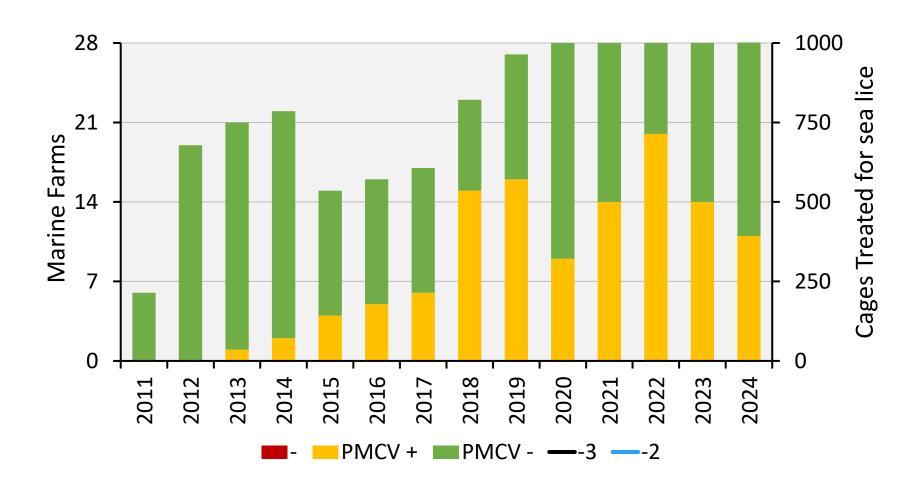
	No gross pathological signs of ISA		Gross pathological signs of ISA	
	N	%	N	%
Vaccinated	846	93,6	89	83,2
Not vaccinated	58	6,4	18	16,8
Total	904	100	107	100

Cardiomyopathy syndrome (CMS)

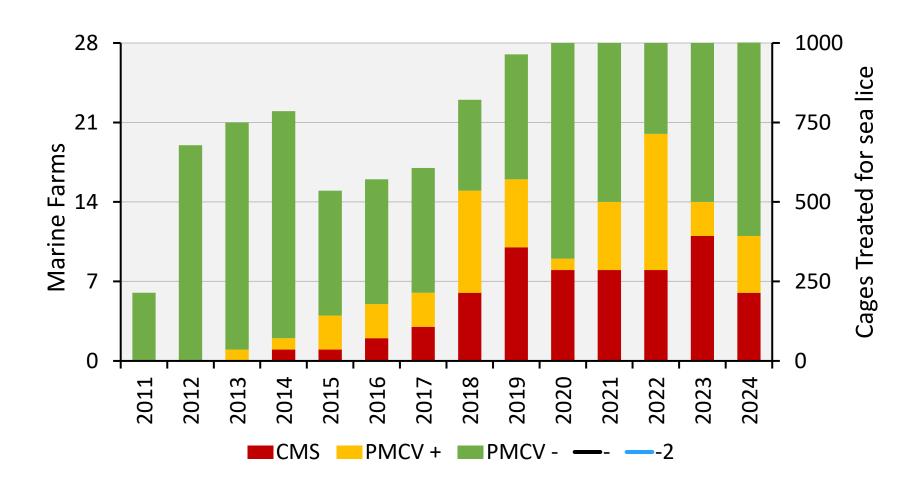
Prevalence of PMCV and CMS since 2013

	Total N
Farming sites	28
Production cycles	198
PMCV positive	118
CMS	64

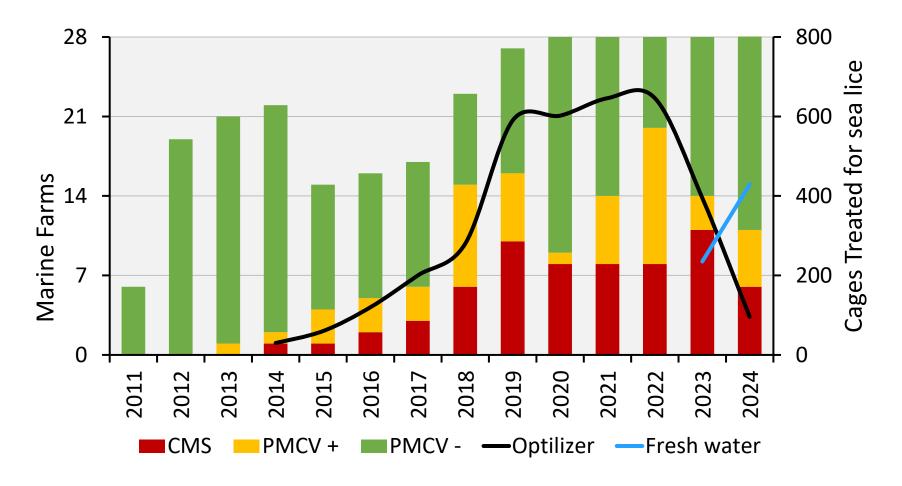
PMCV annual prevalence



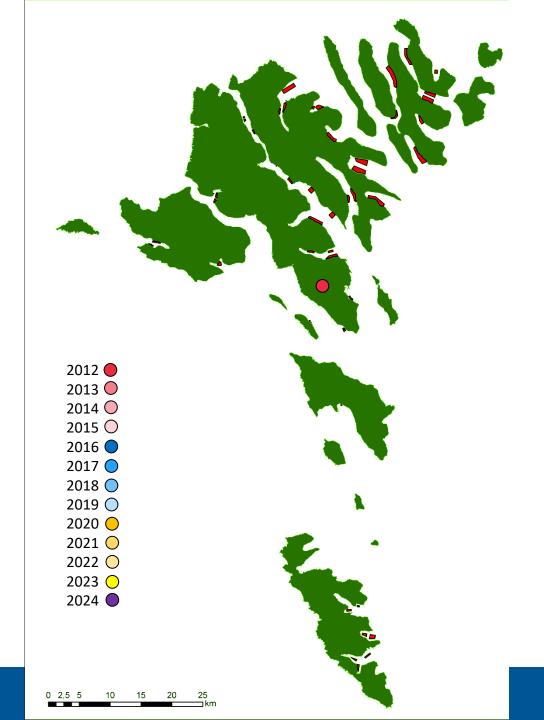
CMS annual prevalence



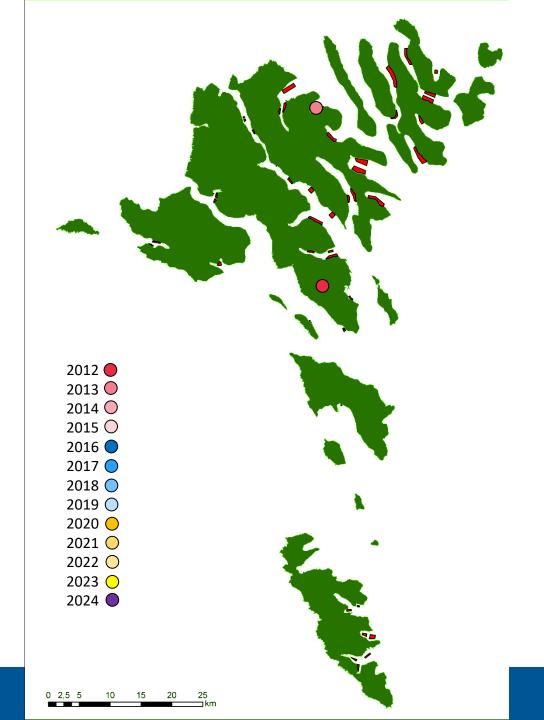
Shift in treatment from heated water to freshwater and/or shorter marine production time



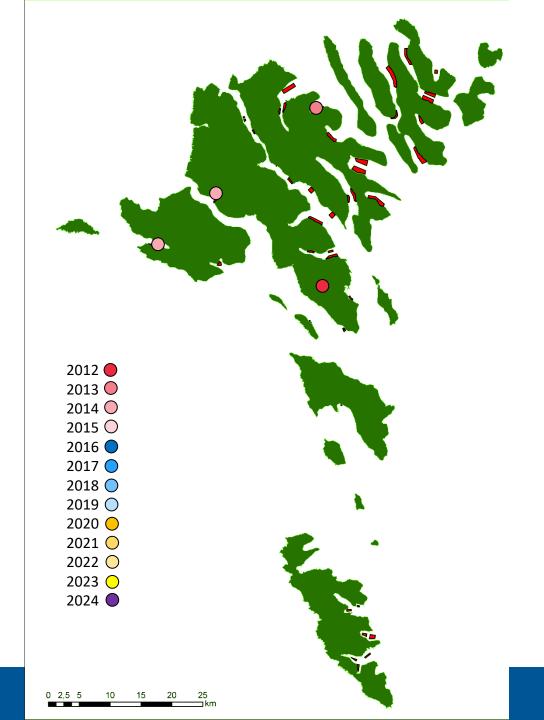
	PMCV positive
2012	1



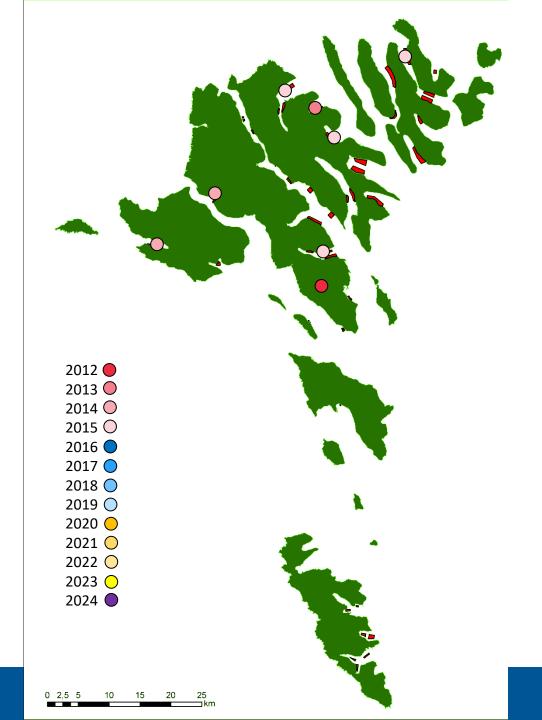
	PMCV positive
2012	1
2013	1



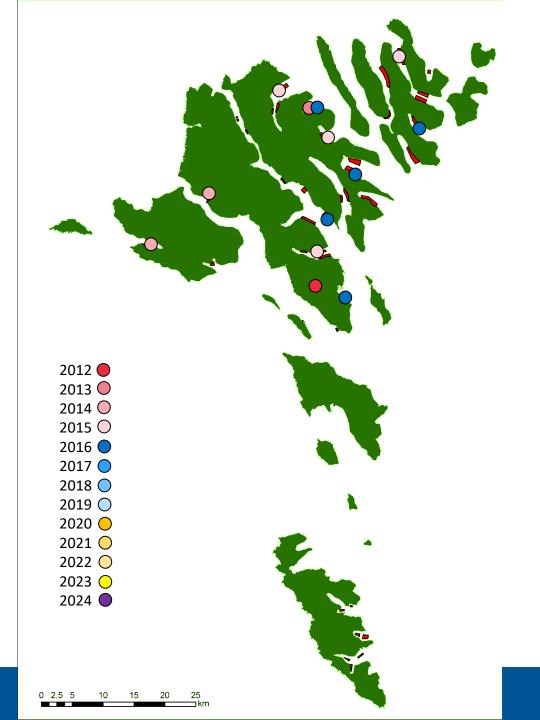
	PMCV positive
2012	1
2013	1
2014	2



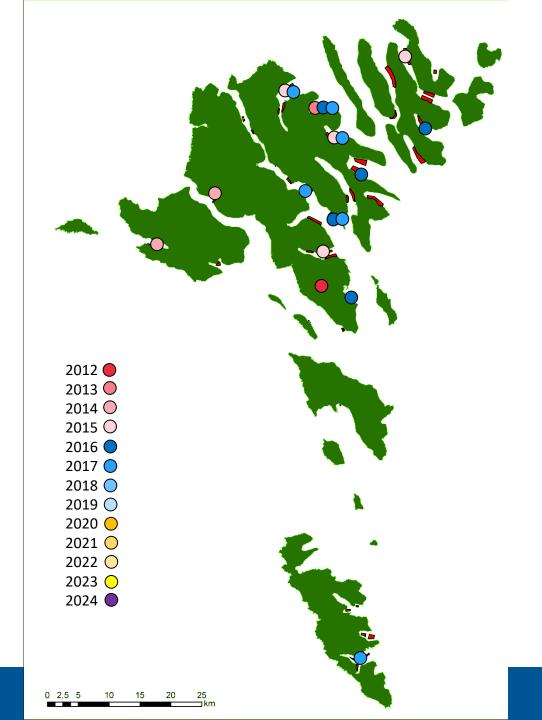
	PMCV positive
2012	1
2013	1
2014	2
2015	4



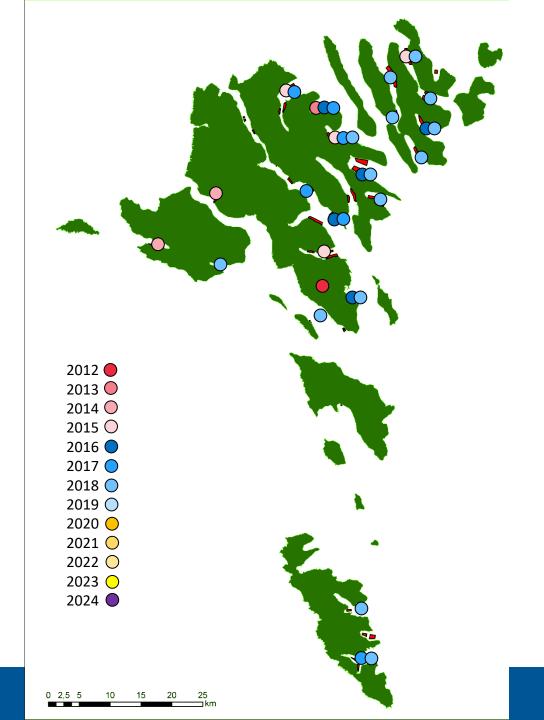
	PMCV positive
2012	1
2013	1
2014	2
2015	4
2016	5



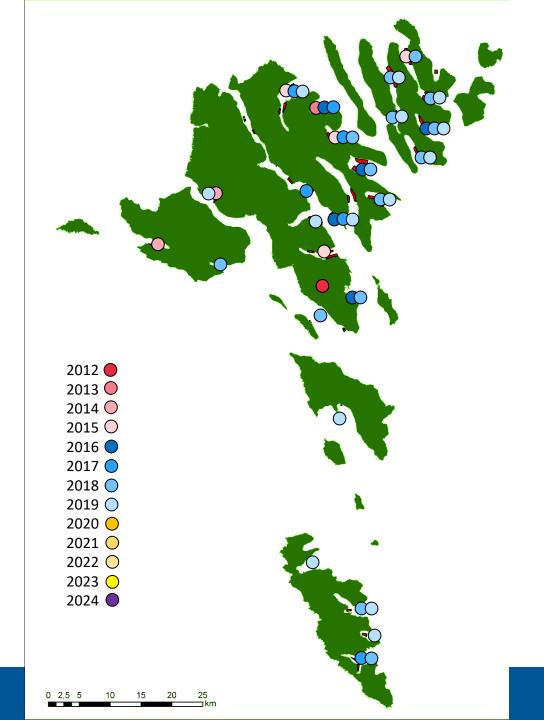
	PMCV positive
2012	1
2013	1
2014	2
2015	4
2016	5
2017	6



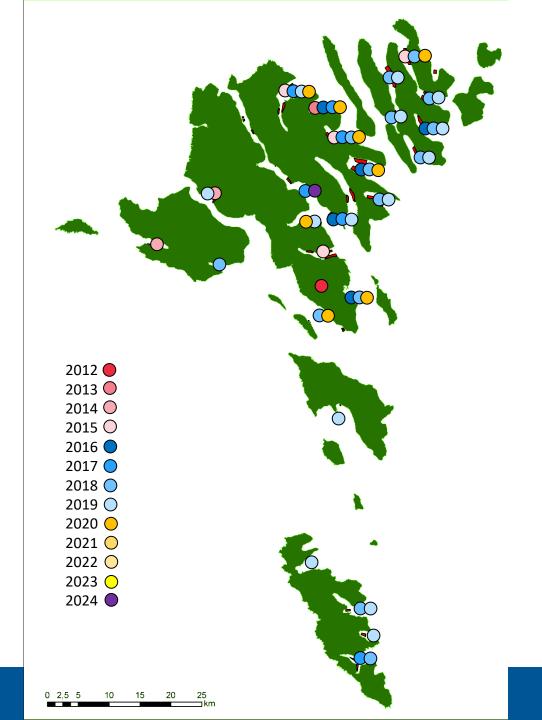
	PMCV positive
2012	1
2013	1
2014	2
2015	4
2016	5
2017	6
2018	15



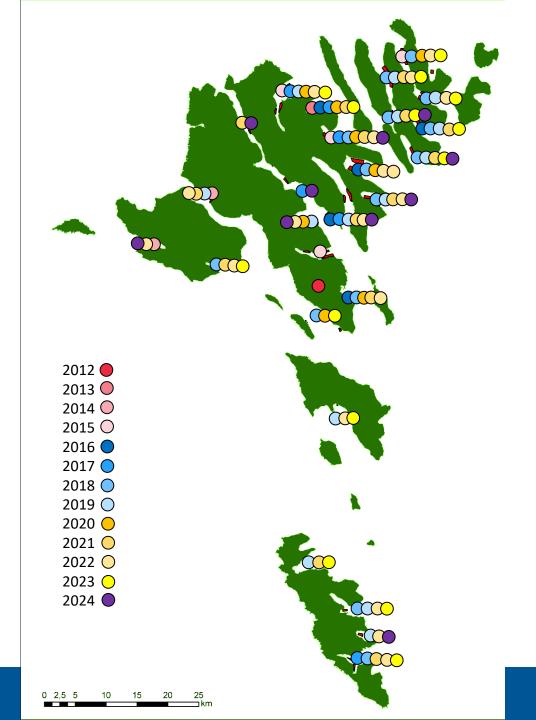
	PMCV positive
2012	1
2013	1
2014	2
2015	4
2016	5
2017	6
2018	15
2019	15



	PMCV positive
2012	1
2013	1
2014	2
2015	4
2016	5
2017	6
2018	15
2019	15
2020	9



	PMCV positive
2012	1
2013	1
2014	2
2015	4
2016	5
2017	6
2018	15
2019	15
2020	9
2021	14
2022	22
2023	15
2024	11



JOURNAL OF GENERAL VIROLOGY

RESEARCH ARTICLE

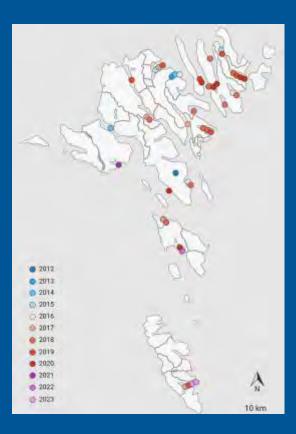
Dahl et al., Journal of General Virology 2025;106:002068 DOI 10.1099/jgv.0.002068

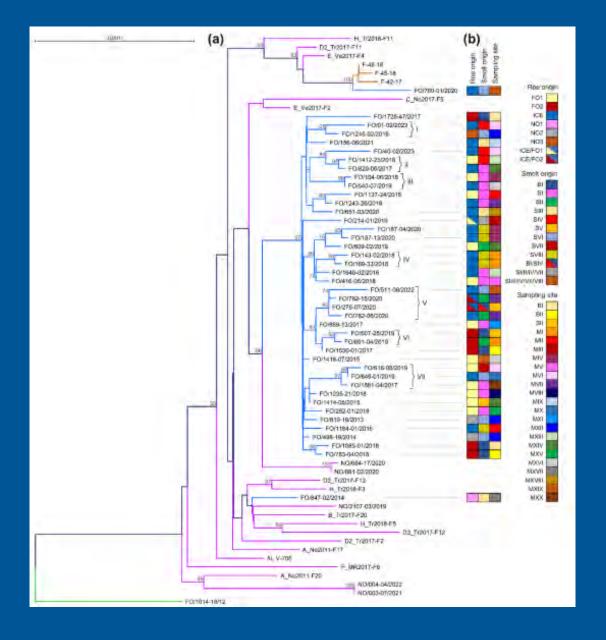


8 ACCESS

Genomic analysis reveals low genetic diversity and no continuous reintroduction of piscine myocarditis virus in farmed Atlantic salmon in the Faroe Islands

Maria Marjunardóttir Dahl, Petra Elisabeth Petersen and Debes Hammershaimb Christiansen*









Renibacterium spp. outbreak in Baltic salmon (Salmo salar) in Latvia

Inga Piginka-Vjaceslavova

Olga Revina

Anete Niemi

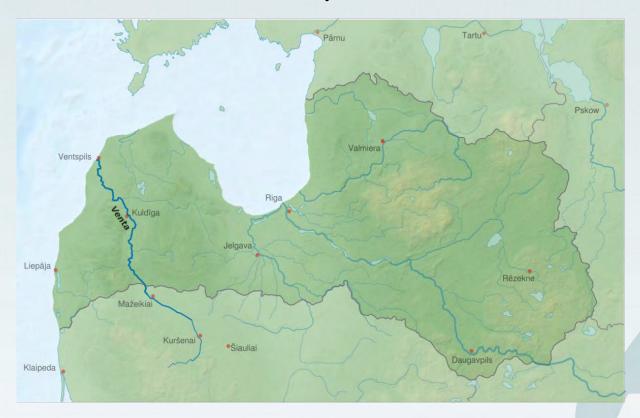
Liga Ansonska

Zanete Steingolde

26.05.25.-27.05.25.

Overview

- Institute BIOR provides restore and restock wild salmon population
- Baltic salmon spawners were collected from the River Venta





Eggs collection

- In 2022
 - 250,000 eggs, fertilized and incubated
 - fisher receive bacteriologically tested fish fillets
- In 2023 hatched alevins were reared in tanks with a water volume of 1.2 m³, each containing approximately 3,000 larvae
- In 2024, April
 - One part of one-year-old salmon smolts were released into the River Venta
 - Second part of one-year-old salmon smolts (n=25,000) were retained for further rearing and placed in 1.2 m³ tanks at a stocking density of 1,500 fish per tank

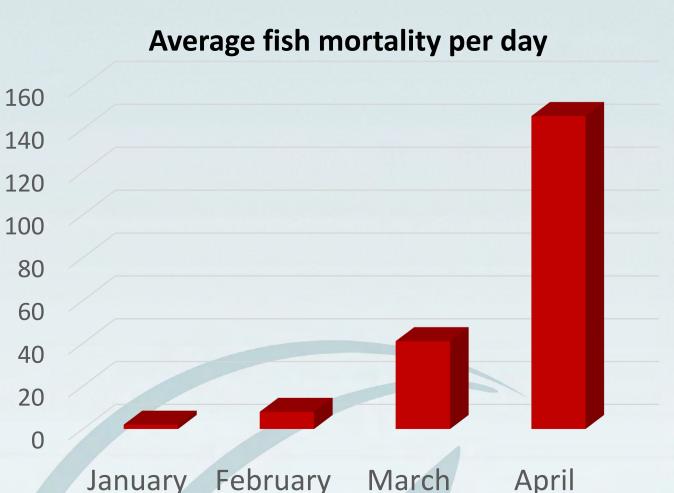




Outbreak in 2025

Mortality:

- in January (second half) –
 1 to 3 fish per day
- in February 2 to 22 fish per day
- In March 3 to 107 fish per day
- in April 101 to 214 fish per day
- Mortality increases when water temperatures reach 15 °C



Clinical signs

- Appeared shortly before death
- Non-specific exhibited apathy, appetite was preserved
- External examination:
 - Exophthalmia -> ocular hemorrhages -> eyeball rupture
 - Distended abdomen
 - Skin discoloration and hemorrhages









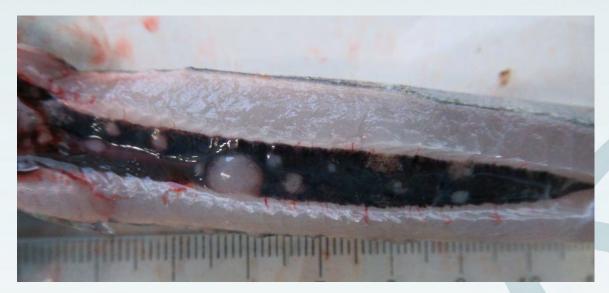


Gross changes

Kidney









Gross changes



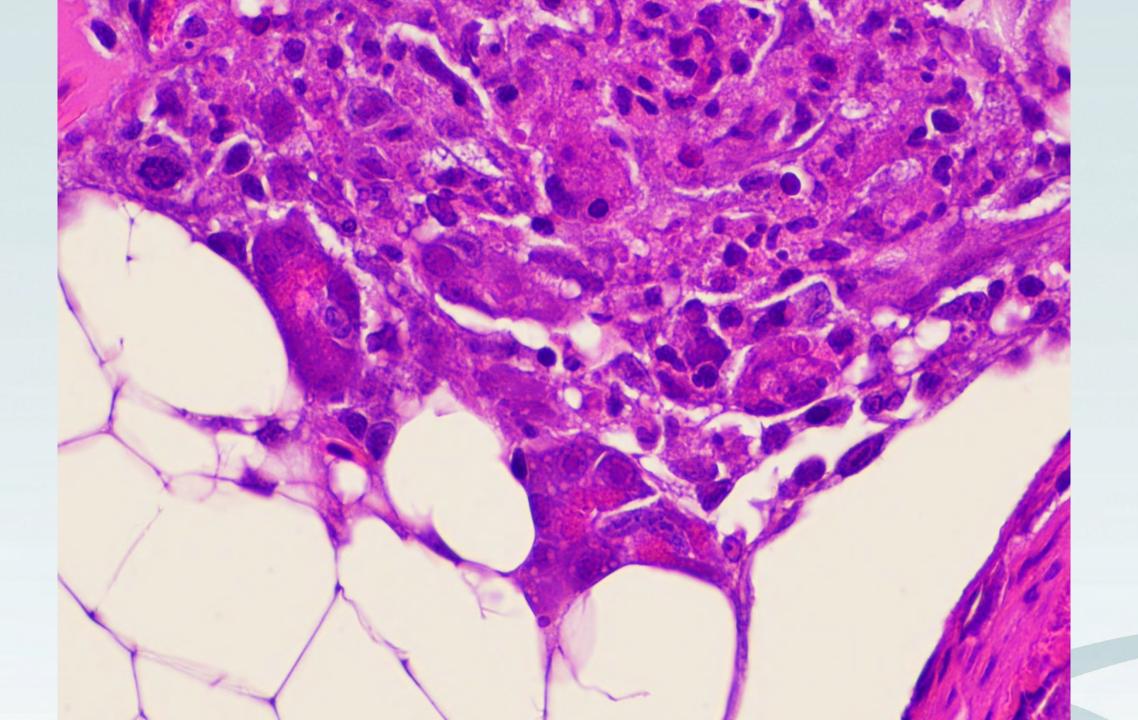


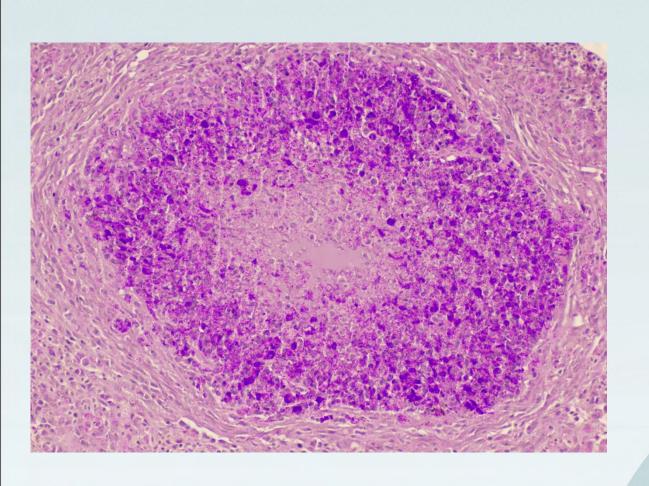
Spleen

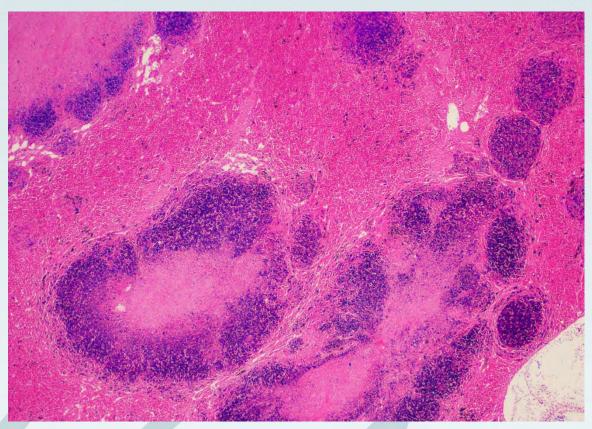
• Liver



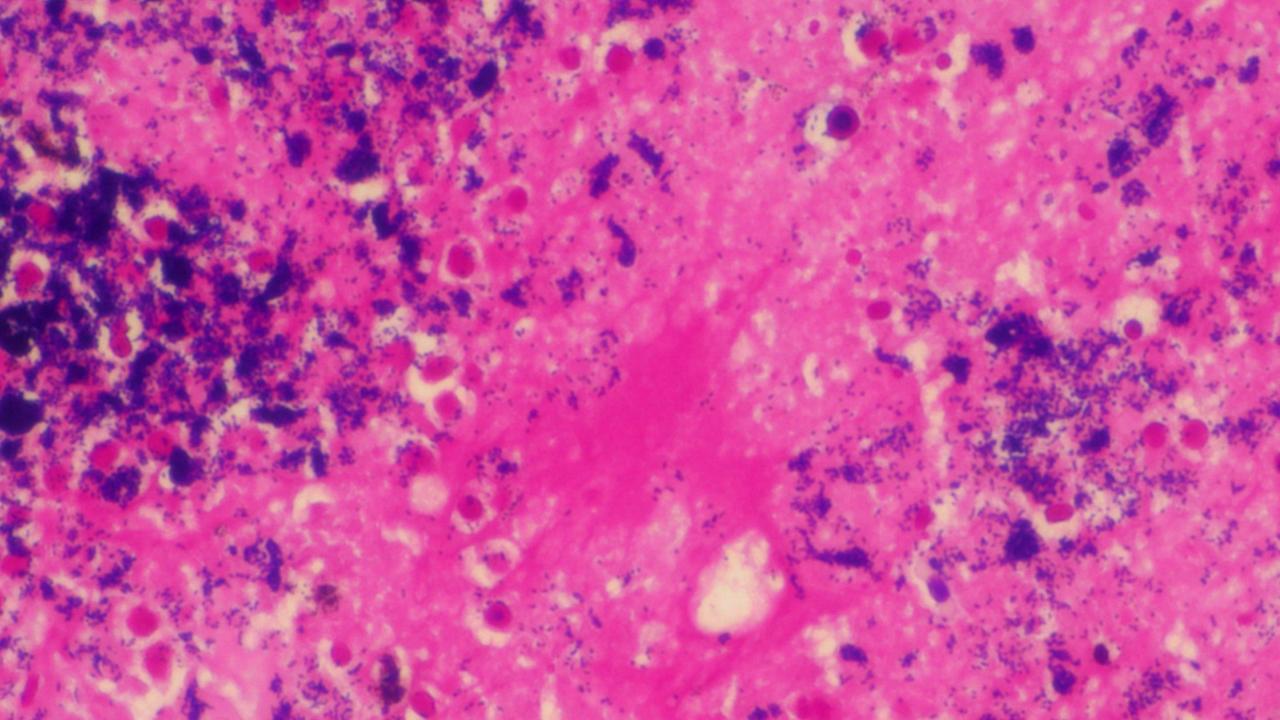








PAS, x200 Gram, x40



Gross changes – internal examination

- 20 fish **showing clinical signs** revealed:
 - multifocal granulomas, with or without necrosis, in the kidneys
 - occasionally in the spleen and liver
 - rare peritonitis
 - liver was usually pale or jellow discoloration
 - rare effusion in abdominal cavity
- 35 **clinically healthy** fish revealed:
 - kidney granulomas in 17% of cases
 - few also exhibiting spleen granulomas

Laboratory tests

Institute BIOR:

- Bacterilogical investigation (exclude *Renibacterium* and *Piscirickettsia*)
- Histopathological analysis
 - HE, Gram-staining, PAS reaction, and Ziehl-Neelsen staining
- PCR -
 - Viral haemorrhagic septicaemia virus (VHSV), Infectious haematopoietic necrosis virus (IHNV), epizootic haematopoietic necrosis and infectious salmon anaemia virus

European Union Reference Laboratory for Fish and Crustacean Diseases:

- nested PCR, quantitative PCR (qPCR), and ELISA testing for Renibacterium salmoninarum
- real-time PCR for *Piscirickettsia salmonis*

EURL results

Sampling:

- bacterial swabs from kidney, with transport media, and sterile plastic tubes with kidney samples from 10 salmon
- fish with no clinical signs, but fish number 1,3,6,9 and 10 had granulomas in the kidney

Sample	ELISA for BKD	PCR for BKD	RT PCR for BKD	RT PCR for Piscirickettsia
1	Positive	Positive	Positive	Negative
2	Positive	Positive	Positive	Negative
3	Positive	Positive	Positive	Negative
4	Negative	Negative	Positive	Negative
5	Negative	Positive	Suspect	Negative
6	Suspect	Positive	Positive	Negative
7	Negative	Positive	Positive	Negative
8	Negative	Positive	Positive	Negative
9	Positive	Positive	Positive	Negative
10	Suspect	Positive	Positive	Negative

Activities

- Institute BIOR adapted real time PCR testing for Renibacterium salmoninarum
- Institution BIOR all 6 farms were tested for *Renibacterium*, only in one farm 2+ Baltic salmon tested positive for *Renibacterium*
- All 2+ Baltic salmon were culled at the farm
- Farm contain other fish:
 - Sea trout smolts, Baltic salmon smolts, Baltic salmon fry, and sea trout fry all tested negative for *Renibacterium*
 - Sea trout and Baltic salmon smolts were released in May 2025
- New spawners and their eggs will be tested for Renibacterium
- Eggs will be incubated separately from each spawners
- During salmonids rearing, regular monitoring for Renibacterium will be conducted

Thank you for your attention!









Juliane Sørensen, Niccolò Vendramin, Lone Madsen, Argelia Cuenca Annual workshop 2025

Validation of multi-target high-throughput qPCR for pathogen detection in salmonids



Background

Why HT-qPCR?

Field cases with more than one pathogen present

Complex disease

Export cases (testing of multiple targets)

Development of a high-throughput real-time PCR system for detection of enzootic pathogens in pigs

Nicole B. Goecke, ¹ Charlotte K. Hjulsager, Jesper S. Krog, Kerstin Skovgaard, Lars E. Larsen Journal of Veterinary Diagnostic Investigation 2020, Vol. 32(1) 51-64 © 2019 The Author(s) Article reuse guidelines: sagepub.com/journals-permissions DOI: 10.1177/1040638719890863 jvdi.sagepub.com

Report on the Performance Evaluation of the Fluidigm BioMark Platform for High-Throughput Microbe Monitoring in Salmon

Kristi M. Miller¹, Ian A. Gardner², Raphaël Vanderstichel², Timothy Burnley², Angela D. Schulze¹, Shaorong Li¹, Amy Tabata¹, Karia H. Kaukinen¹, Tobi J. Ming¹, Norma G. Ginther¹

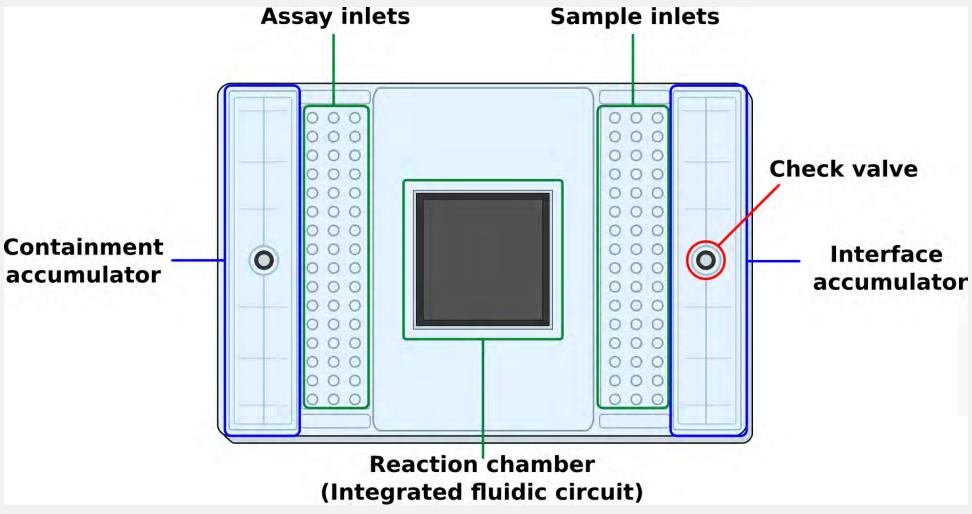
Effectiveness of Adding a Mask Recommendation to Other Public Health Measures to Prevent SARS-CoV-2 Infection in Danish Mask Wearers: A Randomized Controlled Trial PREE

Authors: Henning Bundgaard, DMSc , Johan Skov Bundgaard, BSc , Daniel Emil Tadeusz Raaschou-Pedersen, BSc , Christian von Buchwald, DMSc , Tobias Todsen, MD , Jakob Boesgaard Norsk, MD , Mia M. Pries-Heje, MD, Christoffer Rasmus Vissing, MD , Pernille B. Nielsen, MD, Ulrik C. Winslow, MD, Kamille Fogh, MD, Rasmus Hasselbalch, MD , Johas H. Kristensen, MD , Anna Ringgaard, PhD, Mikkel Porsborg Andersen, PhD , Nicole Bakkegård Goecke, PhD , Ramona Trebbien, PhD , Kerstin Skovgaard, PhD , Thomas Benfield, DMSc , Henrik Ullum, PhD , Christian Torp-Pedersen, DMSc , and Kasper Iversen, DMSc , and Kasper Iversen, DMSc , and William, Abticle, & Disclosure Information

Publication: Annals of Internal Medicine • Volume 174, Number 3 • https://doi.org/10.7326/M20-6817



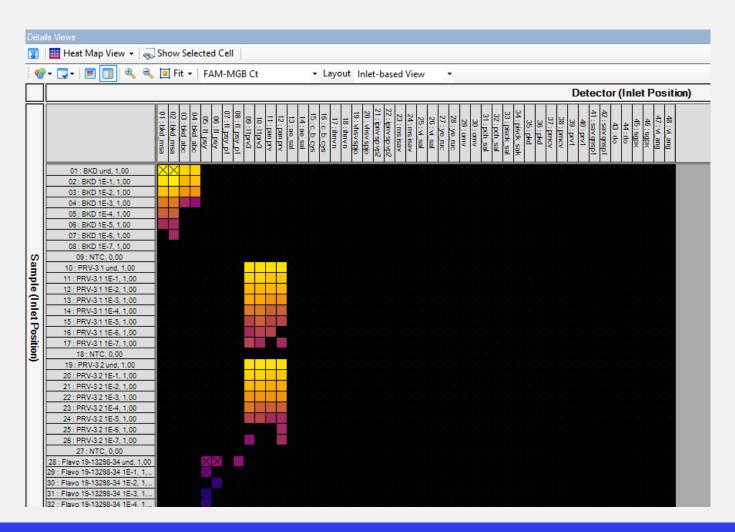
What is high-throughput qPCR (HT-qPCR)?

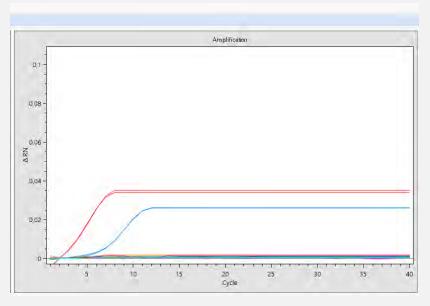


System: BioMark (Fluidigm/Standard BioTools)



Example of data







HT-qPCR

• Benefits of HT-qPCR:

Screening of a large panel of samples against large panel of targets

Saves time and reagents (192.24 chip corresponds to 48x 96-PCR plates)

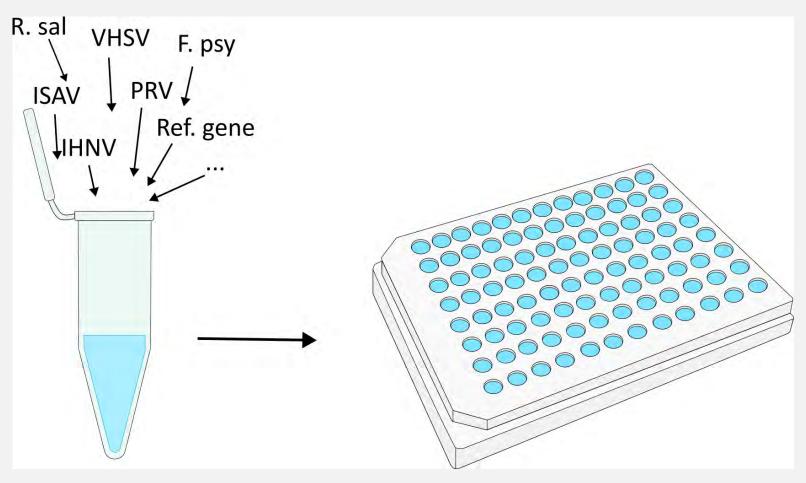
Challenges:

Small reaction volume – pre-amplification required

Same conditions for all assays



Pre-amplification



10 nM final concentration of each primer pair



Validation

- Assay criteria
 - Amplicon size 70-150 bp
 - Universal cycling conditions
 - All known sequence variants
 - No amplification of related species
 - Assays from diagnostic manual where relevant
- Samples
 - Synthetic dsDNA (Gblocks, IDT)
 - Synthethic ssRNA (synthetized in house)
 - Isolates
 - Known positive tissue
 - Known negative tissue



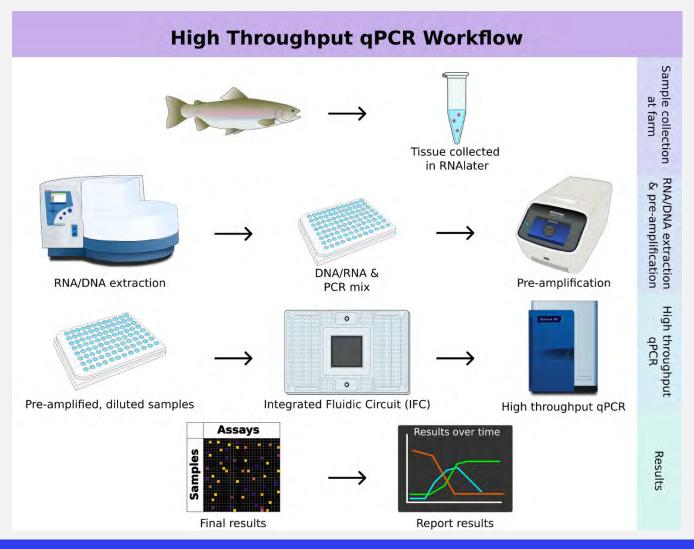
Assays

- Viral and bacterial agents
- Focus on rainbow trout, with inclusion of salmon specific pathogens

IHNV (2)	PRV-3 (1)	Candidatus Branchiomonas cisticola (1)	V. anguillarum (1)
IPNV (3)	Salmon gill pox virus (1)	Candidatus Piscichlamydia salmonis (1)	V. salmonicida (1)
ISAV (2)	SAV (1)	F. psychrophilum (2)	Y. ruckeri (1)
OMV (1)	VHSV (1)	MLO (1)	
Pan-PRV (1)	A. salmonicida (1)	Piscirickettsia salmonis (1)	Internal reference (elongation factor)
PRV-1 (1)	R. salmoninarum (1)	PKD (1)	



Work flow





Chip setup

- Duplicate reactions (assay or sample)
- Controls:

Negative purification control

Positive purification control

Non-template control, pre-amplification

Positive controls, pre-amplification (synthetic)

Non-template control, HT-qPCR

27-05-2025 Technical University of Denmark Validation of HT-qPCR

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Limit of detection

Limit of detection, synthetic DNA

14, 16 and 18 cycles of pre-amplification

Samples tested in triplicates

Best sensitivity with 18 cycles of pre-amplification

Non-pre-amplified samples were only amplified if highly concentrated (>2000 copies)



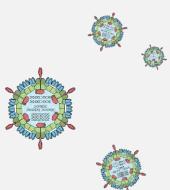
Sensitivity

Virus assays	14 cycles	16 cycles	18 cycles	
IPNV SP VP2	1.5	1.5	1.5	
IPNV Tapia	150	15	1.5	
L1PRV3	15	15	1.5	
Pan PRV	215	21	21	
PMCV	150	15	1.5	
MS ISAV	15	15	1.5	
PRV-1	15	15	15	
SAV Qnsp1	15	15	1.5	
IHNV N	40	40	4	
VHSV SPJO	40	40	4	

Bacterial assays	14 cycles	16 cycles	18 cycles
Ye_ruc	275	27	27
Vi_sal	275	27	2.7
RS msa	27	27	2.7
Pisck_sal	24	2.4	2.4
Fl_psy	24	2.4	2.4
C_b_cys	2.4	24	24
Vi_ang	242	24	2.4
MLO	24	24	24
RS abc	242	242	24
Pch_sal	25	25	NA
Fl_psy_p1	25	25	NA
Ae_sal	256	256	NA







Test with known positive material (isolates)

Noted cross-reactivity was attributed to highly positive samples → Cross-contamination.

No amplifications in non-template controls (pre-amp and qPCR)

Test with known positive tissue

No cross-reactivity was noted

No amplifications in non-template controls

Test with negative tissue samples

No amplifications in any assays



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Validation

- Exonuclease
 No detection of samples with or without exonuclease in mastermix with only probe (no primers)
- Impact of PA (low abundant target in presence of high abundant target)

Samples/assay	ISAV	PRV3
ISAV 1E+6 & PRV-3 1E+2	11.9	21.3
L1PRV3 1E+2	No Ct	19.6

Samples/assay	BKD	ISAV
BKD 1E+8 & ISAV 1E+2	3.6	23.4
ISAV 1E+2	No Ct	25.0

Samples/assay	F. psy	BKD	
F. psy 1E+6 & BKD 1E+3	13.2	19.4	
BKD 1E+3	No Ct	18.9	



Validation – comparison to diagnostic methods

Farm samples

Tested RAS farm once per month for 7 months total

The method showed consistent and robust results compared with diagnostic methods

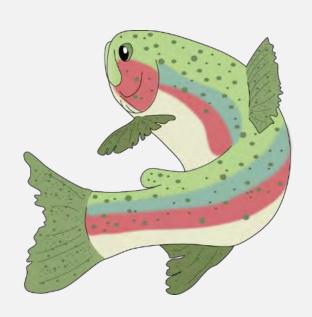
Revealed points for improvement

	Y. ruckeri	F. psychrophilum	PRV-3	Branchiomonas cysticola	Piscichlamydia salmonis
Diagnostic methods	+	+	+	N/A	N/A
HT-qPCR (internal organs)	+	- (Extraction issue)	+	+	+
HT-qPCR gills	+	- (Extraction issue)	+	+	+



Validation - ongoing

- Inclusion of new assays & optimization of current
- Limit of detection using tissue samples
- Test more assays with tissue samples from experimental challenges
- Comparison to qPCR (BioRad) and digital PCR
- Inter-laboratory comparison?



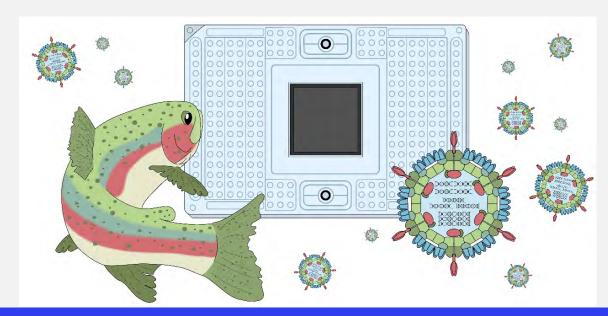


Thank you

Contact: jusor@aqua.dtu.dk

Testing and usage of HT-qPCR chip on farm samples:

A novel high-throughput qPCR chip for solving co-infections in RAS farmed rainbow trout (Scientific Reports)

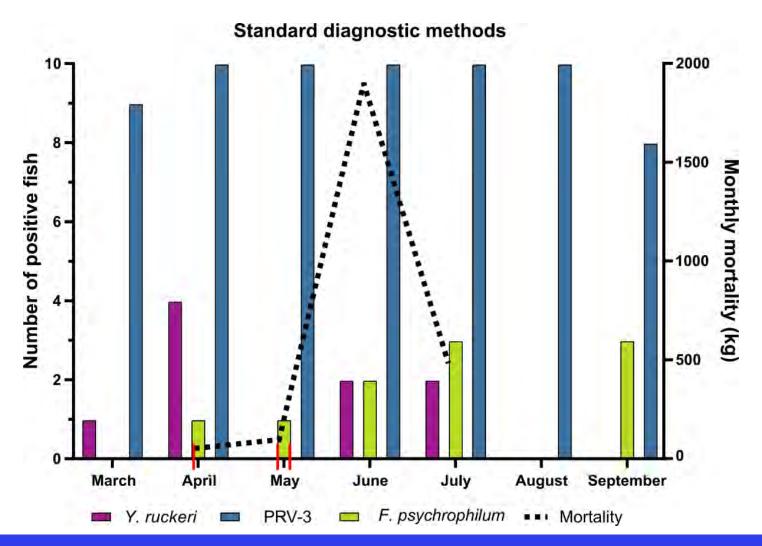


27-05-2025 Technical University of Denmark Validation of HT-qPCR

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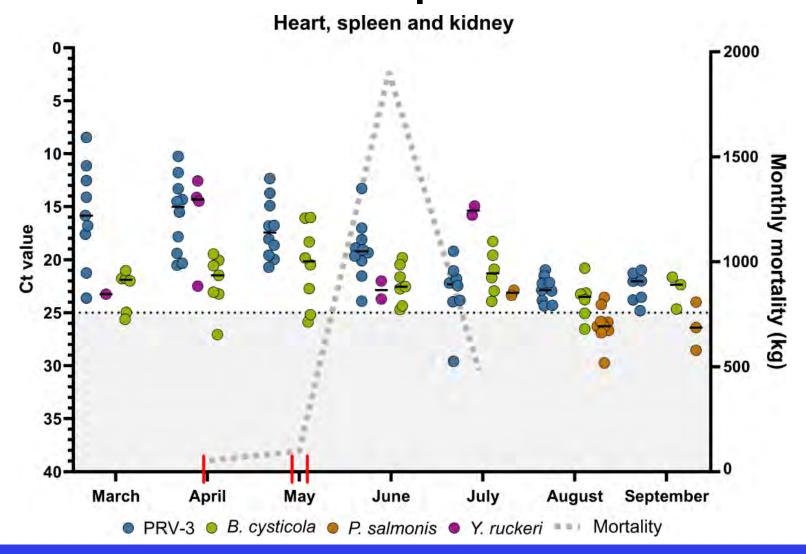


Farm surveillance



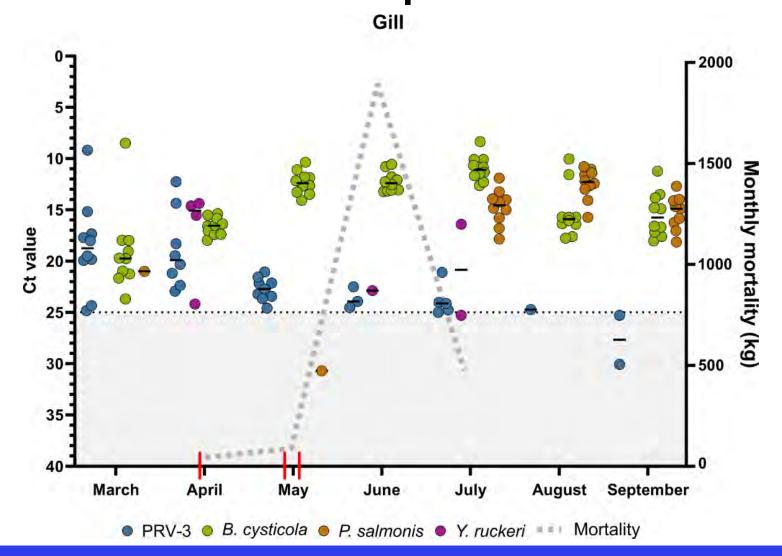


Farm surveillance – HT-qPCR





Farm surveillance – HT-qPCR



Using environmental DNA/RNA for monitoring and surveillance of pathogens in aquaculture





What is e-DNA/RNA (eNA)?

WOAH's definiton*:

 «Environmental-DNA/RNA: Nukleic acids from pathogen agents extracted from 'true'environmental samples (like water, soil, sediment, biofilm)»

Material like faeces and mucus is often included in less strict definitions

The use of environmental DNA methods for detection of WOAH listed aquatic animal diseases

Original: English (EN) February 2022

A discussion paper developed by the WOAH Aquatic Animal Health Standards Commission (Aquatic Animals Commission).

1. Summary

The monitoring of aquatic systems using environmental DNA (eDNA) is a rapidly advancing research field that will provide opportunities for cost-effective, non-destructive methods to screen for pathogenic agents, including those of wild aquatic populations where samples may be difficult or undesirable to obtain.

The Aquatic Animals Commission is aware that eDNA methods are being applied for detecting the causative agents of several WOAH listed diseases. As these methods are available and currently in use, the Commission has agreed that it would be advisable for guidance to be provided on appropriate application of eDNA methods and potential limitations.

The Commission notes that, as accurate estimates of diagnostic performance are not available for designing surveillance programmes using eDNA assays, data obtained from eDNA methods are not suitable to support declarations of freedom from listed diseases. Confirmation of infection with listed diseases could also not be made using eDNA methods because a positive result does not demonstrate that a susceptible host animal(s) is infected.

Positive eDNA results could, however, provide evidence amounting to suspicion of infection such as the presence of the pathogen DNA/RNA in the sample. This application of eDNA methods may be particularly useful for the monitoring of high-value or rare animals as an alternative to collection of tissue samples. It has a potential role in early detection of disease incursion in wild populations or under circumstances when infection is not likely to result in observable clinical signs. However, following suspicion, based on positive eDNA, samples obtained directly from aquatic animals need to be tested as described in the relevant disease-specific chapters of the *Manual of Diagnostic Tests for Aquatic*

*The use of environmental DNA methods for detection of WOAH listed aquatic animal diseases



e-NA methods eDNA/eRNA monitoring of pathogens and hosts Validated nucleic acid extraction Swabs of biofilm Adjusted Concentration by filtering MinIon 3rd generation seq Metagenomikk Work in progress Threshold Validated qPCR/ddPCR (specificity& Veterinærinstituttet sensitivity)

The mortality in Norwegian aquaculture is too high:

Table 2.4.2 The table provides an overview of the proportion of registered dead fish for salmon within the six main categories (level 1) per year, based on data from the "Fish Health Dalabase." Note: The figures do not represent the actual prevalence of the various categories. The data basis will change if the number of participating companies changes, and historical data may also be adjusted as a result.

Level 1	2021*	2022*	2023*	2024
Infectious diseases (A)	21,9 %	28,1 %	38,0 %	32,9
Environmental impacts (B)	0,2 %	0,4%	2,7 %	8,81
Injuries (trauma) (C)	24,7 %	32,2 %	33,1 %	26,6
Physiological causes (D)	5,9 %	4,5 %	4,1 %	70-61-7 8 6
Other causes (E)	2,8 %	1,6 %	2,1 %	Table 6.1 h
Unknown cause (F)	44,4 %	33,2 %	19,9	made avai
5.00	2000	222.0	122.	(Chapter 1

Infectious diseases are a major challenge

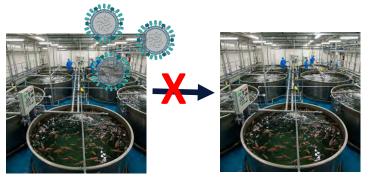
Table 6.1 Number of sites with salmonids with detected viral diseases in the period 2015-2024. For the period 2015-2019, he number of sites with CMS, HSMI, and IPN is based on samples sent to the Norwegian Veterinary Institute, while data nade available from aquaculture companies through private laboratories have been included in the count since 2020 Chapter 1 Stastistical basis for the report!)

	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
ISA	15	12	14	13	10	23	25	15	18	13
PD	137	138	176	163	152.	158	100	98	58	48
CMS	105	90	100	101	82	154	155	131	129	78
HSMI	135	101	93	104	79	161	188	147	184	115
IPN	30	27	23	19	23	22	20	12	12.	13

Source: Fish health report 2024

Why is it important to detect pathogens early?

Prevent spread between parts of same site



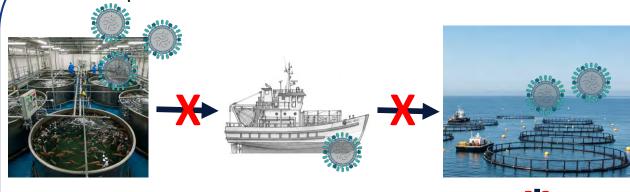
Reduce impact of infection/disease





Limit handling

Prevent spread from one site to other sites



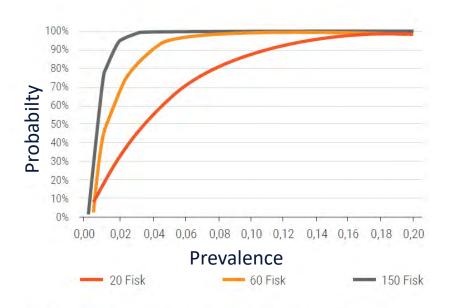
Measures:

Increase biosecurity/early slaughtering

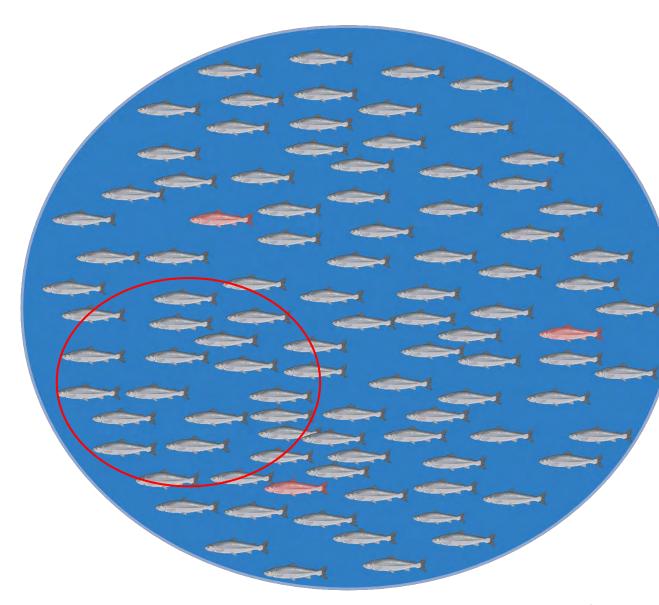




Challenges in surveillance of pathogens at low prevalence

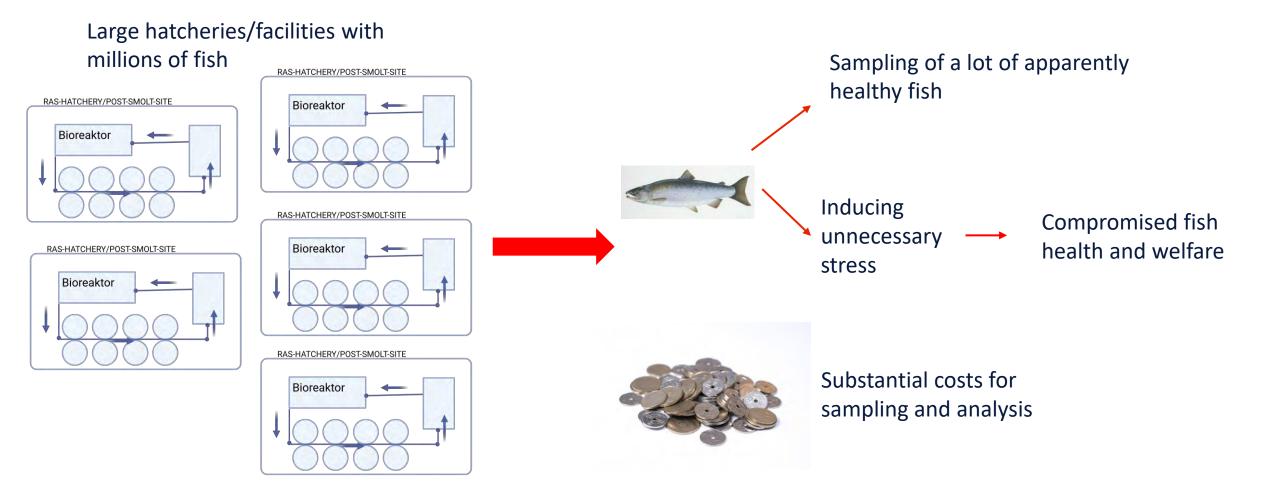


Figur 2. Sammenheng mellom antall prøver til laboratorieanalyse og sannsynlighet for å påvise minst en smittet fisk ved ulike prevalens når testen som benyttes er perfekt (Se=Sp = 1) . Anne Gerd Gjevre, Veterinærinstituttet, Norsk Fiskeoppdrett 4, 2018



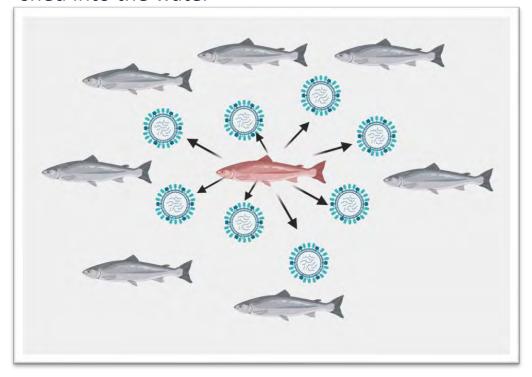


Challenges in surveillance for pathogens:

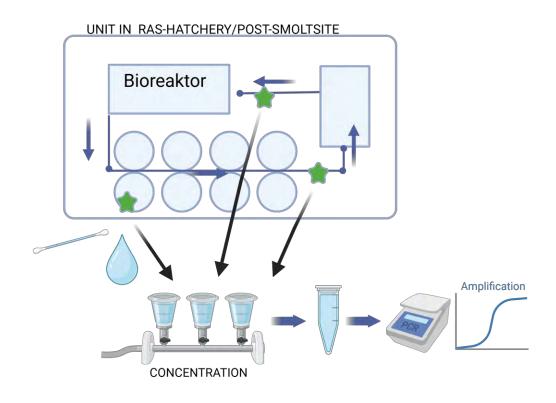




During the viremic phase large amounts of virus shed into the water







- + LIMITED AMOUNT OF SAMPLES
- + DECREASED STRESS DURING SAMPLING
- + NO HEALTHY FISH SACRIFISED
- + REDUCED COSTS



Background for the ILA-SAFE project:



Torfinn Moldal





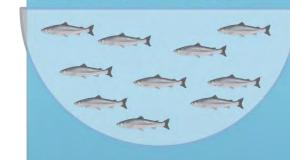
Well boats

Sea lice treatment in well boats

ISA-free production site

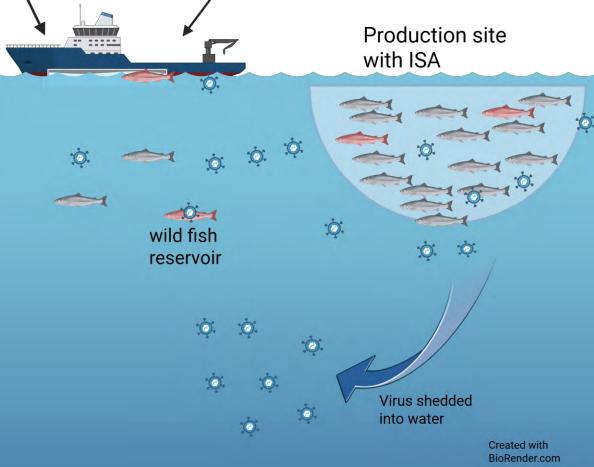


Hilde Sindre

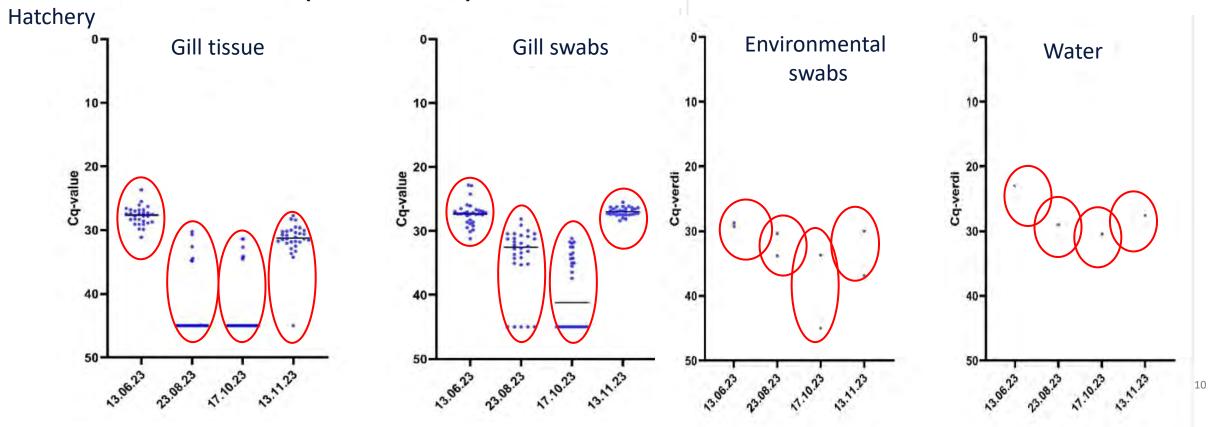


Ottavia Benedicenti





Experiences with environmental sampling for ISAV from hatcheries (ILA-SAFE):



Detection of ISAV HPRO on site level - independent of sample type



Graphs made by Torfinn Moldal

Example eDNA use for detection of bacteria:



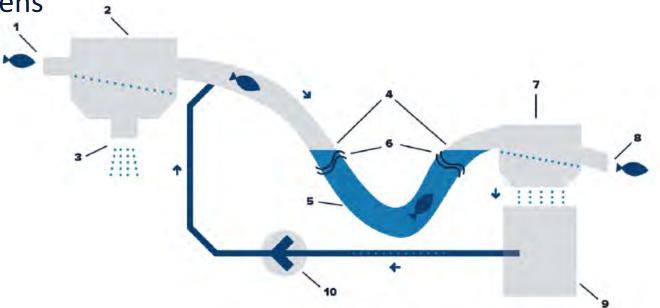
Duncan Colquhoun

Thermal delousers are ideal targets for eDNA surveillance:

Many thousands of fish treated in small volum of water

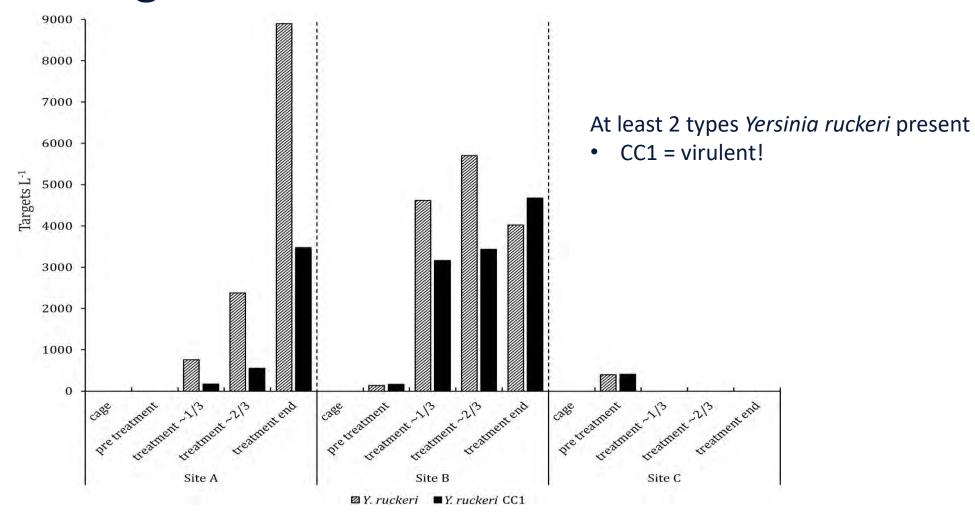
• The fish is stressed and releases pathogens

• Possible pathogen concentration??





Thermal delousing - Yersinia ruckeri

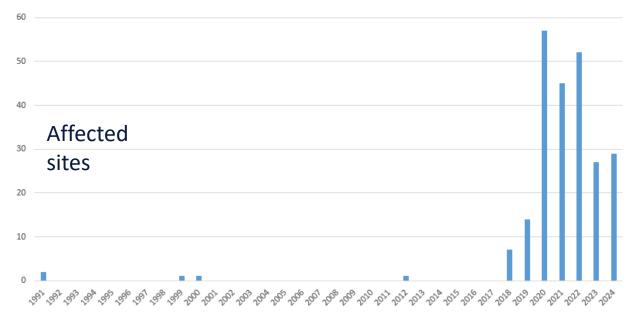


Journal of Fish Diseases, Volume: 45, Issue: 8, Pages: 1211-1224, First published: 01 June 2022, DOI: (10.1111/jfd.13656)



Pasteurella (Phocoenobacter) status 2024







JOURNAL OF **FISH DISEASES**

Associations Between Delousing Practices and Pasteurellosis in Farmed Atlantic Salmon

Leif Christian Stige ⋈, Duncan J. Colquhoun, Victor H. S. Oliveira

First published: 27 January 2025 | https://doi.org/10.1111/jfd.14085

Funding: This study was funded by the Norwegian Seafood Research Fund through the project 'Pasteurellosis in Norwegian salmon' (project no. 901680).





Some de-licing methods can increase disease risk, study finds

By Vince McDonagh - 31st October 2023



















Some sea lice treatments can increase the risk of bacterial infection for salmon. according to research by the Norwegian Veterinary Institute.

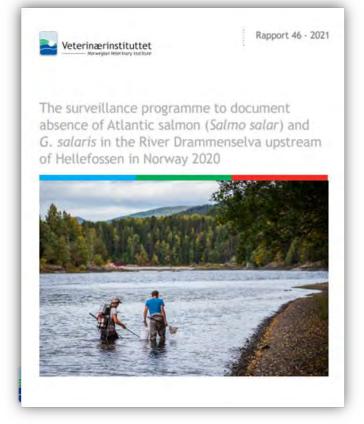


The Drammen River system –combat of G. salaris (Haakon Hansen et al)



Haakon Hansen

- Using Chlorid-based method to kill *G. salaris*
- Monitoring has been carried out on behalf of the Norwegian Environment Agency since 2021.
- Uses classical methods and environmental DNA (eDNA).











Marit Måsøy Amundsen



David A. Strand



David A. Strand



Trude Vrålstad



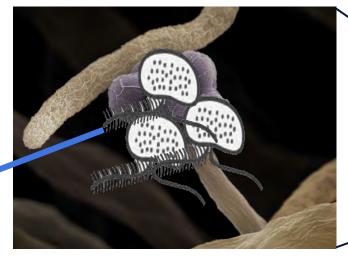
Marit Måsøy Amundsen

Noble crayfish, crayfish plague & signal crayfish

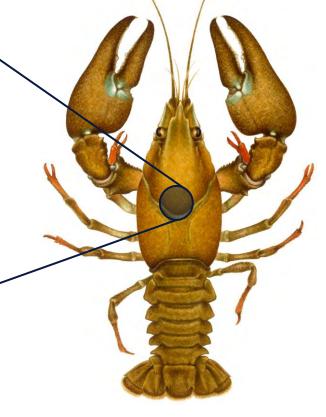


Noble crayfish

SEM Microscopy



Water mold Aphanomyces astaci

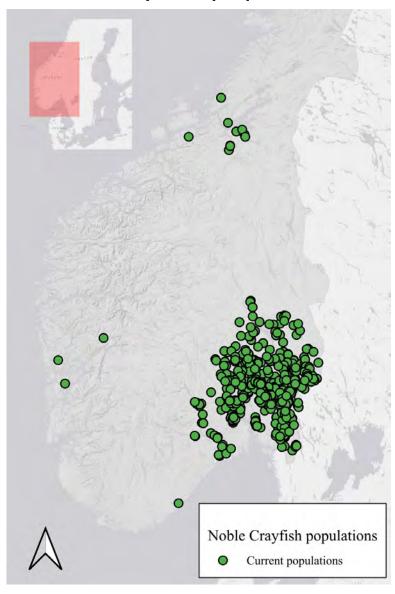


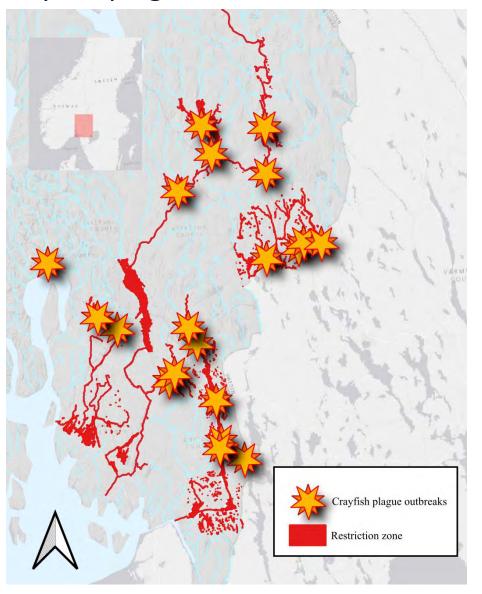
Signal crayfish

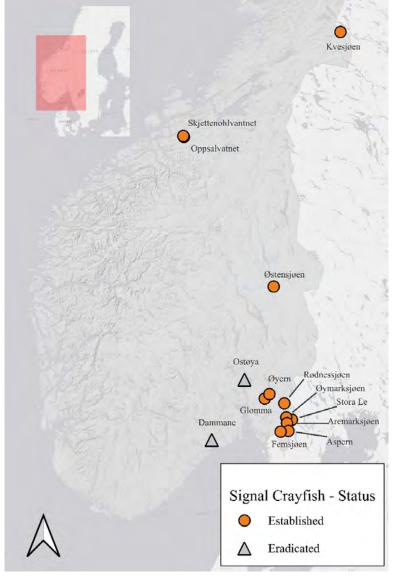


Noble crayfish populations

Crayfish plague outbreaks 1971-2024 Signal crayfish populations



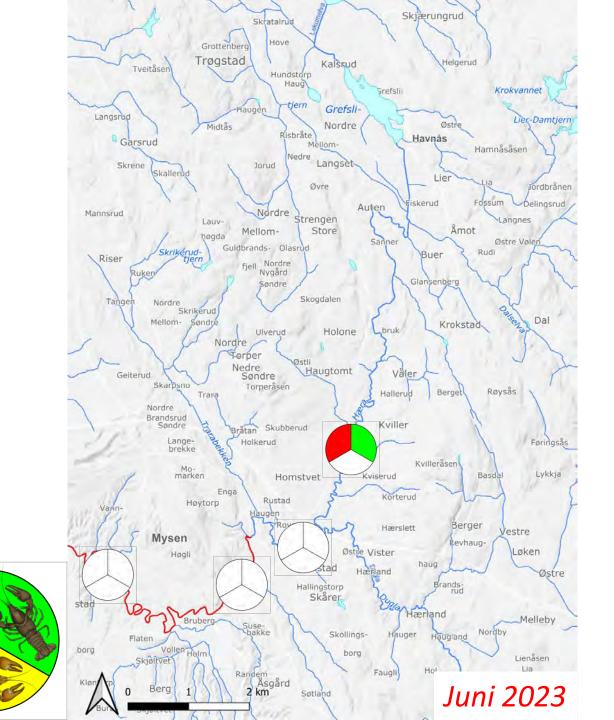






Early warning of crayfish plague outbreak with eDNA surveillance

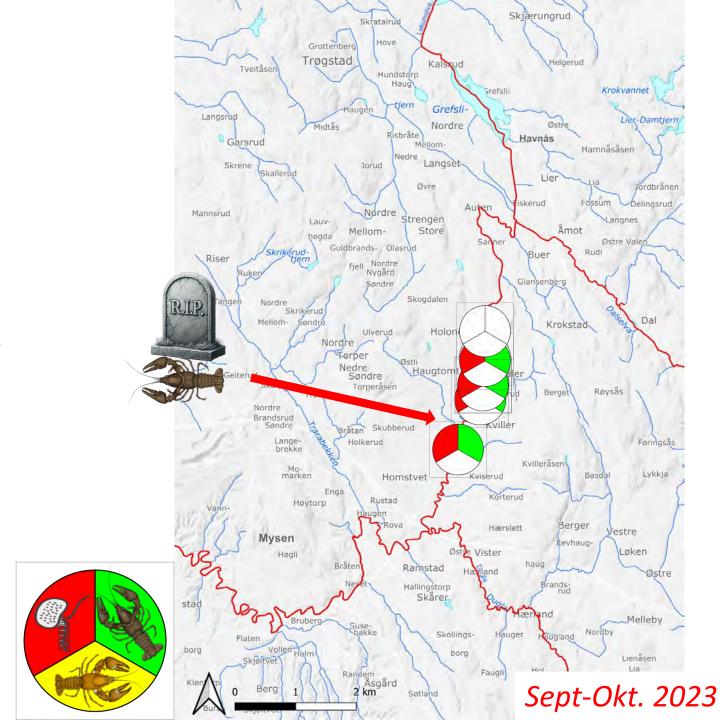
• e-DNA sample positive for *A. astaci* in an upstream restriction zone.





Early warning of crayfish plague outbreak with eDNA surveillance

- e-DNA sample positive for *A. astaci* in an upstream restriction zone.
- Surveillance for dead crayfish and additional e-DNA sampling
- Dead crayfish detected, sampling confirmed crayfish plague
- e-DNA shows slow up-stream spread of the pathogen





BIOSECURITY-PROJECT: «WATERSAFE» Sea water Sufficent water treatment important to prevent introduction and spread of pathogens Water treatment/ disinfection Lack of good protocols for field testing of Irene Roalkvam Land-based disinfection-systems Use methods developed for e-NA to **POST-SMOLT SITE** Fertilized eggs Hatchery investigate water samples for suitable (from broodfish site) indicator agents to measure disinfection effect Transport of fish Lice-treatment Well boat Water reservoir Water treatment/ disinfection Outlet to sea Veterinærinstituttet Hilde Sindre

Summary:

 Environmental samples promising for surveillance of virus and bacteria, especially in closed facilties



- Positive from a 3R perspective and in reducing costs
- Early detection increases the possibility for implementing biosecurity measures

Important:

- •The optimal e-NA sampling points will vary between sites.
- •Further optimization might be required for certain pathogens.
- •The significance of a detection must be interpreted carefully:
 - Is it due to contamination, vaccination, or an active infection?
- •Environmental samples are suitable for surveillance purposes NOT for diagnostics!









Åse Helen Garseth, Editor/Scientific coordinator of wild fish health, Norwegian Veterinary Institute 29 th Annual Workshop of the National Reference Laboratories for Fish Diseases, 26 th to 27th of May 2025

Wild fish health report 2024

- Health in wild fish and noble cray fish



Foto: Audun Richardsen

Monitoring Wild Fish Health is a Mandated Role of the Norwegian Veterinary Institute

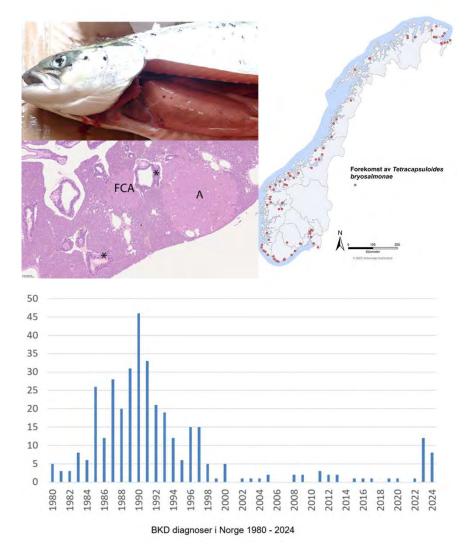
<u>Letter of Allocation from Ministry of Trade and Fisheries</u>:

«Effective preparedness depends on having a **good overview of the national health status** of both farmed and **wild fish**, and is built on surveillance, necessary access to health data, high-quality diagnostics of risk-based material, and research».



Objective

- More attention to wild fish health
- Provide Quality-assured Information on Major Diseases
- Address
 - Risks and Threats
 - Knowledge Gaps
 - Need for Measures
- Raise Public Awareness = preparedness





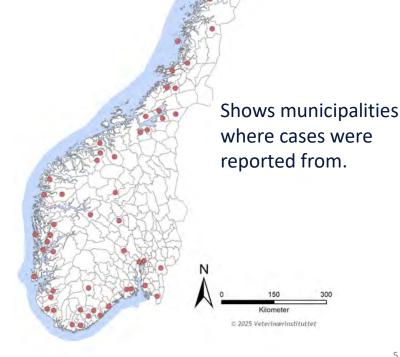


Notification system

- for mortality and disease in wild fish

«Notify the Norwegian Veterinary Institute immediately....»*

- Listed diseases
- «New» diseases
- Reduced welfare
- www.vetinst.no/syk-villfisk





Map: Attila Tarpai, NVI

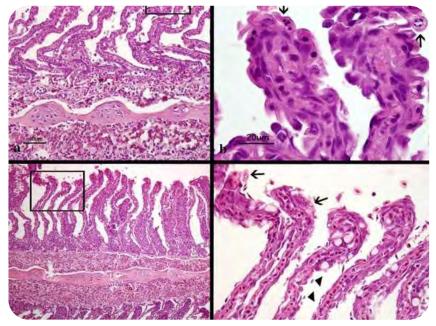
^{*} Norwegian Food Safety Authority web-page

Diagnostics at Norwegian Veterinary Institute (NVI)

«.. high-quality diagnostics of risk-based material.."



Saprolegnia parasitica from wild salmon Photo: Ellen Christensen, NVI



Salmon gillpox in wild salmon from River Nidelva, Trondheim Photo: Mona C. Gjessing, NVI



Active surveillance programs – organized health monitoring

Program	Executor
Gyrodactylus salaris	Norwegian Veterinary Institute
Health monitoring in wild fish (impact from aquaculture)	Norwegian Veterinary Institute Institute marine research
Salmon lice (NALO)	Institute marine research
Crayfish plague	Norwegian Veterinary Institute

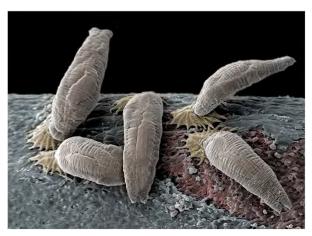


Photo: Jannicke W Nielsen, NVI



Health control of wild anadromous broodfish in stock enhancement and genebank programs



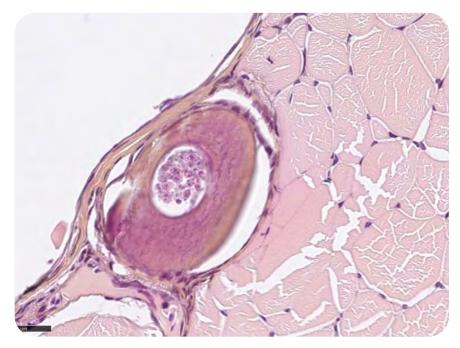




Research, Investigations and Risk assessments



Papillomatosis in salmon parr Photo: Jonas Havn, NVI



Myxobolus cerebralis i ribs od parr from River Mandalselva. Photo: Åse Helen Garseth, NVI



Published studies

Received: 15 October 2018 | Revised: 20 November 2015 | Accessed: 20 November 2018 | OCt 50.1111/(6.12942 ORIGINAL ARTICLE Piscine orthoreovirus-3 is prevalent in wild seatrout (Salmo WILEY Fish Diseases en Garseth¹ | Torfinn Moldal¹ | Siri Kristine Gasnes¹ | A survey of the distribution of the PKD-parasite Jankowska Hjortaas¹ | Vegard Pedersen Sollien¹ | Anne-Gerd Gjevre^{1,2} Tetracapsuloides bryosalmonae (Cnidaria: Myxozoa: Malacosporea) in salmonids in Norwegian rivers additional information gleaned from formerly collected fish Abstract in 2017, a PCR-based survey for Piscine orthoreovirus-3 (PRV-3) was conducted in T A Mo and A Jorgensen wild anadromous and non-anadromous salmonids in Norway. In seatrout (anadro-Norwegian Veterinary Institute, Orlo, Norway mous Salmo Brutto L.L. the virus was present in 16.6% of the fish and in 15 of 21 invesmust some outside LLs the virtual was present an according to the tent and at tigated rivers. Four of 221 (1.8%) Atlantic salmon (Salmo salm L) from three of 15 en garzech@vesinst.no beginner merca. Pour of 444 14-born makenia, patenter souther and 44 in from some or 44 inverse were also PCR positive, with Covalues indicating low amounts of viral RNA. All anadromous Arctic char (Solvelinus alpinus L.) were PCR-negative. Neither non-anadromous trout (brown trout) nor landlocked salmon were PRV-3 positive. Atogether. these findings aggest that in Norway PRV-3 is more prevalent in the marine environ-Abstract
The milescoperan Trenscopolide type-dimension was discreted in lidency from Atlantic coltions part to 40 ft sampled Norwegian tives. Using real-time PC, then parasit was found to be present as the part of the storage of the process of the parasit was found in Malman and mountain administration of the control of the European antifact In conclusion. The protections are suited as In the control of the European antifact In conclusion of the European antifact In control of the European and the control of the European and the control of ment. In contrast, PRV-3 is present in areas with intensive inland farming in continen-Okamura, 1999) (Cnidaria: Myxozoa) is the case tal Europe, PRV-3 genome sequences from Norwegian seatout grouped together Okamura, 1999) (Cnidaria: Myxonos) is the cau-sative agent of proliferative kidney disease (PKD), a widespread and severe condition in salmonids in the Northern Hemisphere (Okamura et al. 2011). In Farrage, IPCN has media with sequences from rainbow trout (Oncorhynchus mykas Walloaun) in Norway and a wakespread and severe conditions in admonth's in the Nontriberral Hemisphere (see a 2011). See a 2011). See a 2011). See a 2011 of the superior of the Nontriberral Period (see a 2011). See a 2011 of the See a manus experience from removing to their concentration in prices transcent on two may muc. Cohe salmon (Oncorhynchus kitutch Walbaum) in Chile. At present, the origin of the virus remains unknown. Nevertheless, the study highlights the value of talleguarding native figh by upholding natural and artificial barriers that hinder introduction and spread, on a local or national scale, of alien fish species and their pathogens. aprecially, further investigations of freshwater reservoirs and interactions with farmed salmonids are warranted. KEYWORDS olpinus L.I. Atlantic salmon (Solmo salar L.I. landlocked, PRV-3, trout. (Salmo trutta L.), wild RODUCTION stress and various forms of handling (Hjebner, Bangulansen, Bornel, Haukuas, & Wallie, 2018). Over the past few years, research efforts and new bioinformatics stools have increased our incrudedge about kaletal Muscle Inflammation (HSMI) in Atlantic salmon Raseza inuscie incammation intomi in poanue, samuoli Jivas first recorded in 1999 (Kongtorp, Kjerstad, Kjerstad, Jok, & Falk, 2004; Kongtorp, Taksdal, Taksdal, & Lyngov, Keynwede andtropogenic translocation, climate change, fish disease, geographic distribution. in, & Falk, 2004. Kongton, Taksail, Taksail, & Lyngov, sociated with a picene orthogonist winas (PAV) in 2020. A 2020, Taksy, EMBI is one of common viral immed Assartic salmon in Norway, causing major economic viral immed Assartic salmon in Norway, causing major economic viral immed Assartic salmon in Norway, causing major economic viral immed Assartic salmon in Norway, causing major economic viral immed Assartic salmon in Norway, causing major economic viral immediate vira and new promises and the disease. Accordingly, the causal relationship be oon of the write and one or create. Accordingly, who create i resolutionly do trues DRV and HSM was firmly established in 2017 (Westell et al., 2017), and new subtypes of DRV have been described and linked to ADL/1 and nevy sucrypes or HYV have been described and sinked to several disease conditions. The association between Enythrocytic in-2014; Jonië et al. 2014).

In Nerways, the presence of PKD and the associated, previously named PKX organism. Ixa been known is allowed harderies since the 196th (T. Hastein, pers. comm. Laboratory Information Managament System at the Norwegian Veterinary Institute). However, the number of northesis is servina ossape coronocis. I ne association desveen cryptrocybo in-clusion body syncirone (EISS) in Coho salmon (Oncontrinctus Essats) Walbaum) and PRV2 was described in 2016 (Takano et al., 2016), and (Canning, Curry, Feist, Longshaw & Subin License, which permits use, distribution and reproduction in any medium, Management System at the Norwegian Veterinary Institute. However, the number of outbreak in these hurcherine has declined rover the past 20 years due to closure of operational changes. In 1989 and 1991, were outbreaks of PKD were observed among wild Atlantic salmon part in the River

Pukkellaks (Oncorhynchus gorbuscha) tatt på Vestlandet. - Hvilke parasitter og infeksjoner bærer de på?



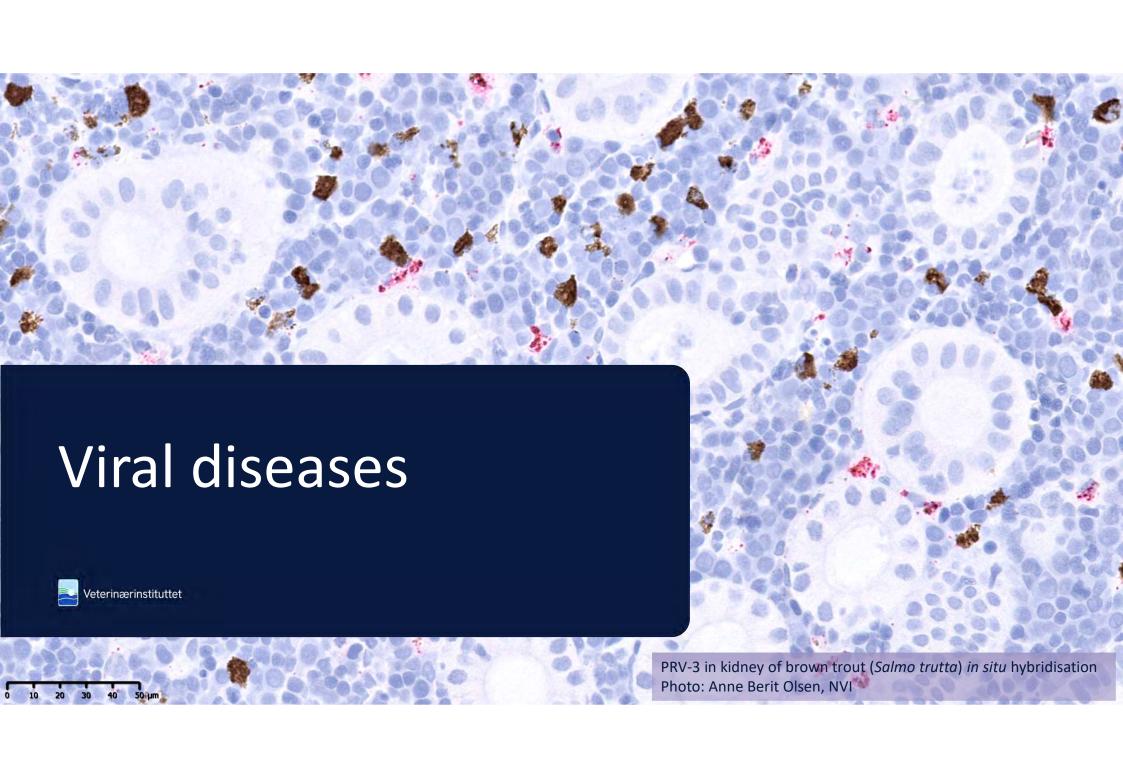
Marte Andrea Fjær

Masteroppgave i fiskehelse



Universitetet i Bergen Det matematisk-naturvitenskapelige fakultet Institutt for biologi Juni 2019

Hout incompany
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Nations Link
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Results from testing of wild anadromous broodfish 2024

The table shows the number of PCR-positive fish and the number of tested broodfish

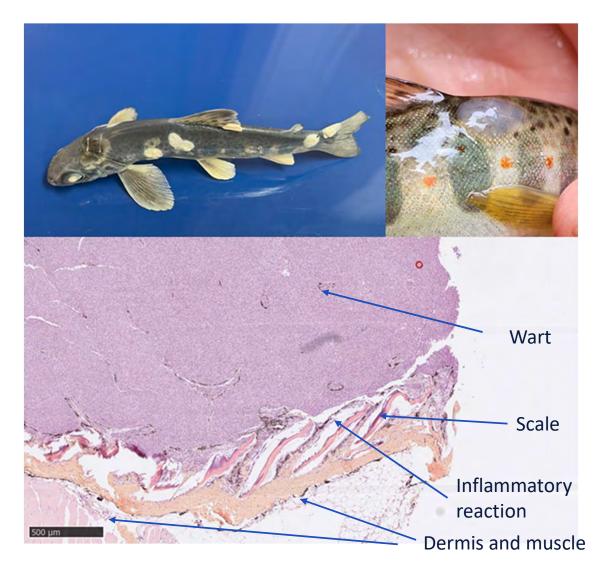
Region/County	Species	IPNV	ISAV	PRV1 (salmon)	PRV-3 (seatrout)
Nordland	Laks	0/23	0/	1/23	-
Møre og Romsdal	Sjøørret	0/8	0/8	-	0/1
	Laks	0/56	0/0	0/56	-
Vestland	Laks	0/39	0/34	1/34	-
Hardanger	Sjøørret	0/56	0/56	-	12/56
	Laks	0/37	0/37	4/37	-
Drammen	Sjøørret	0/113	0/113	-	9/113
	Laks	0/29	0/29	0/29	-
Number of test positives/		0/361	0/300	6/179	21/170



Papillomatosis

- •Common in fingerlings/parr, but not found in all counties
- Large variation in
 - Prevalence (# of infected individuals per river)
 - Extent at the individual level
- •Are warts a health problem in themselves?
- Or a sign of compromised health?





Occurence of papillomatosis in parr/fingerlings

Counties	Vassdrag
Finnmark	Alta, Vestre Jakobselv, Stabburselva, Karpelv, Munkelva, Neiden, Komagelva, Storelva Kunes, Børselva, Lakselva, Repparfjordelva
Troms	Reiselva, Skibotn, Signaldalselva, Salangen
Nordland	Leirelva, Drevja, Fusta, Bjerka
Trøndelag	Stordalselva, Figga, Homla
Møre og Romsdal	Surna, Isa (Henselva), Innfjordelva, Rauma, Måna
Vestland	Gaula i Sunnfjord, Nausta
Rogaland, Agder,	Papillomatosis not detected
Vestfold, Buskerud	
Akershus	Gjersjøelva, Hølenelva, Lysaker



Typical furunculosis – no detections in 2024

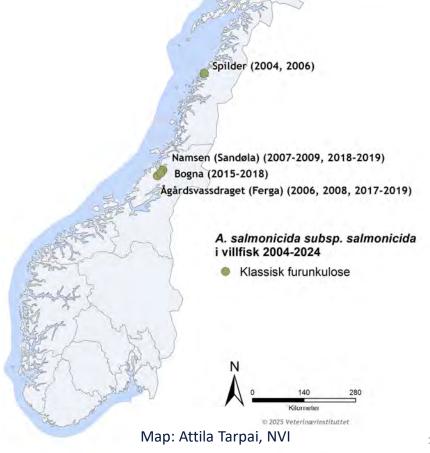
Aeromonas salmonicida subsp. salmonicida

Categori F – notifiable

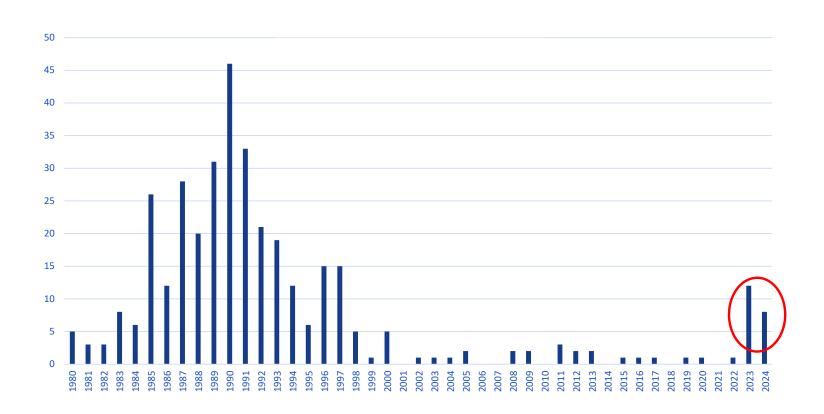


Photo: Anton Rikstad (County Governor) and Geir Bornø, NVI



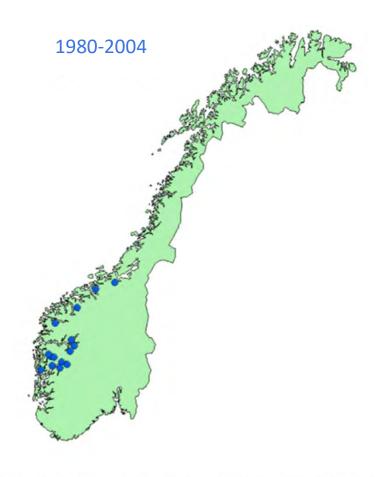


BKD cases in farmed fish 1980-2024

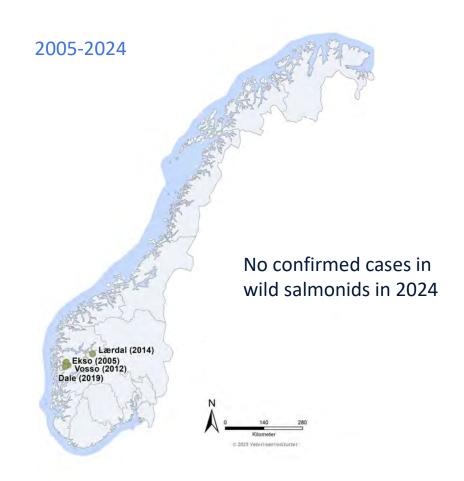




Bacterial kidney disease in wild fish in Norway



BKD påvisninger i norske elver. Kilde: Tore Håstein, Veterinærinstituttet



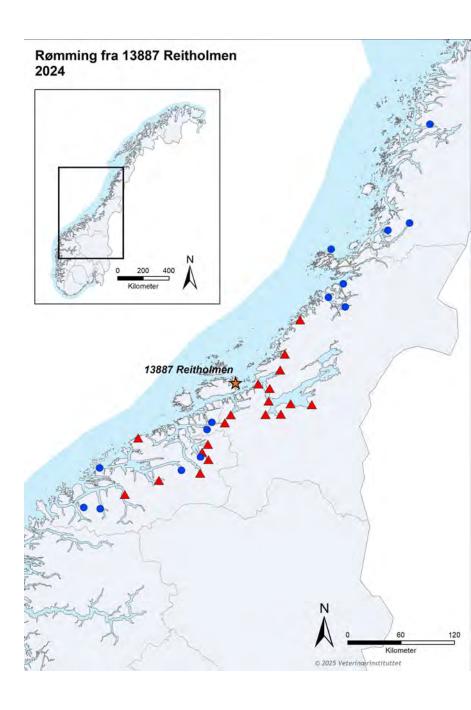
Map: Attila Tarpai, Veterinærinstituttet

May 5th 2024:

_

Escape of 8400 salmon from BKD-infected seasite (Reitholmen).





Fish farmer required to test recaptured salmon

Collaboration between several organisations: Skandinavisk naturovervåking, NINA, Lerøy, Sporbarhet AS, River owner associations

Monitoring and capture



Sampling and analyses



Tracing by genetic analyses



Foto: Mari Skjøstad Berger, Åse Helen Garseth

Development of gonads and reduced condition





Time





Foto: Åse Helen Garseth and Tine S.Tønder, NVI



Results – PCR and cultivation

294 salmon traced* to Reitholmen were tested

	PCR-pos.	Male	female
Sea	3	0	3
River Nordelva	2	1	1
Rivr Gaula	1	1	0
Total	6		

- PCR positive: 2,04% (95%CI: 0,75-4,39)
- *R. salmoninarum* was cultivated from two escaped salmon









Francisellosis in cod

Francisella noatunensis subsp. noatunensis

Categori F – notifiable

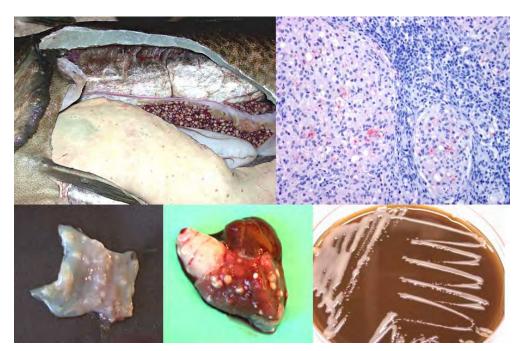
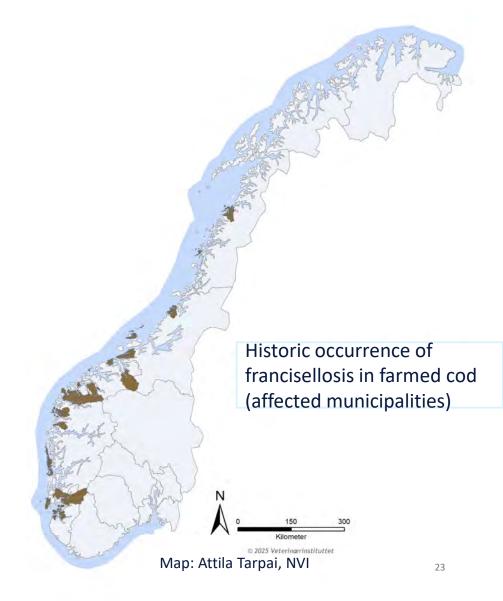


Photo: Anne Berit Olsen, Duncan Colquhoun, Mona C. Gjessing, NVI





Mycobacteriosis

Not notifiable

- Difficult to distinguish from francisellosis
 - Three spleens with francisellosis
 - Spleen with mycobacteriosis

Report findings consistent with francisellosis and mycobacteriosis in wild fish:

www.vetinst.no/syk-villfisk







Saprolegniosis

- a significant problem in several rivers

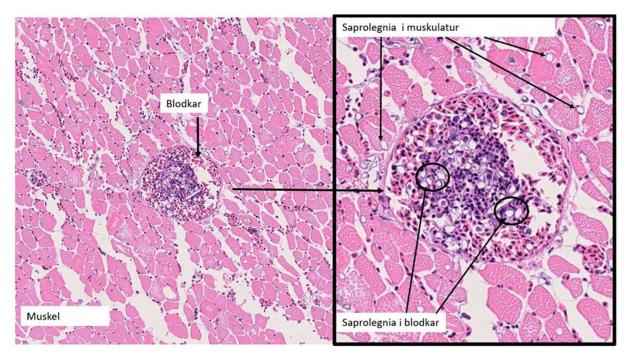


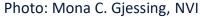


Saprolegniosis - caused by Saprolegnia parasitica

Often complex cases comprising environmental challenges and host vulnerability

- Need for:
 - Surveillance
 - More and better data
 - Research
- •Knowledge regarding risk factors and possible measures





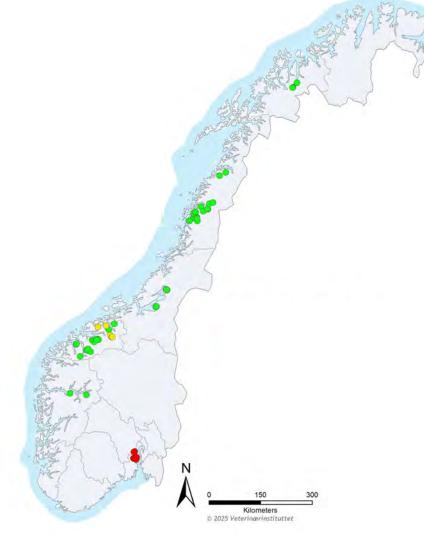




Gyrodactylus salaris

Category F - notifiable

- One new infected river in 2024:
 - Bergerelva (Drammenregion)
- In total, 54 Norwegian rivers have been infected:
 - 43 declared free
 - 5 under surveillance after treatment O
 - 6 not treated

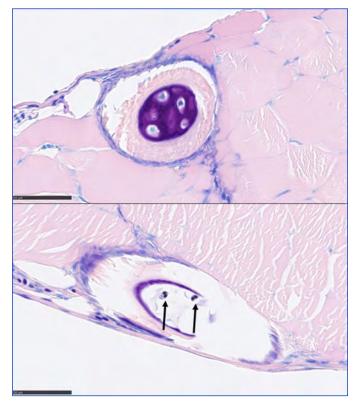




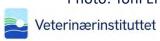


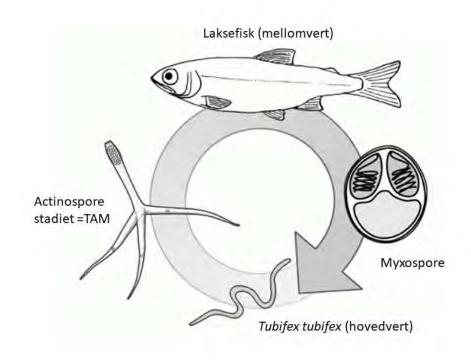
Whirling disease – *Myxobolus cerebralis*

Not notifiable and not detected in Norway for several years, until 2024....

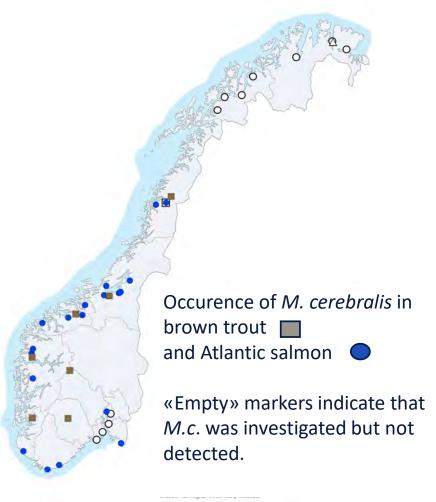






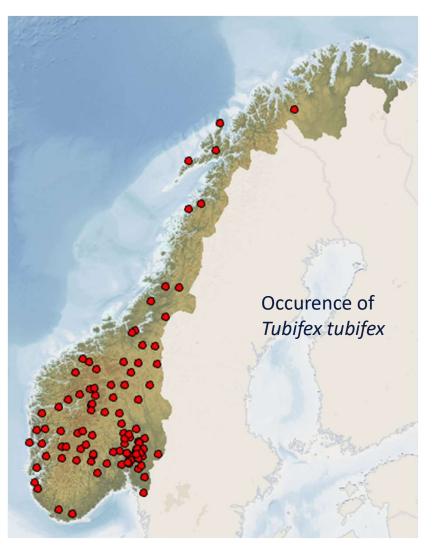


Illustr.. Stephen Atkinson, Fishpatogens.net



Map: Attila Tarpai, Veterinærinstituttet





Illustr.: Artsdatabanken

Proliferative kidney disease

- Tetracapsuloides bryosalmonae





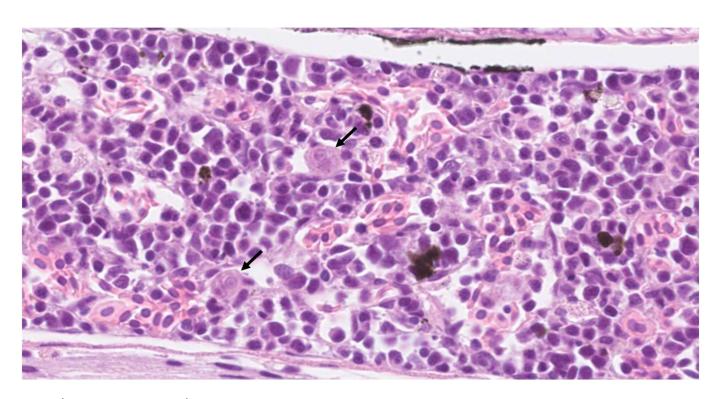


Photo: Lisa Furnesvik, NVI

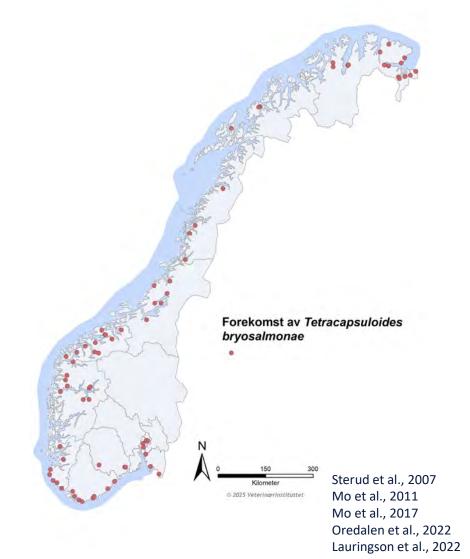


Proliferative kidney disease

- Tetracapsuloides bryosalmonae

Not notifiable in Norway

- Severe consequences in other countries
- Parasite is widespread in Norway
- Development of disease is temperature dependent (>15°C for 14 days)
- Prognosis: several populations will be impacted in the years to come





Kart: Attila Tarpai, Veterinærinstituttet



Other examples

Eustrongylides i brown trout and Arctic char

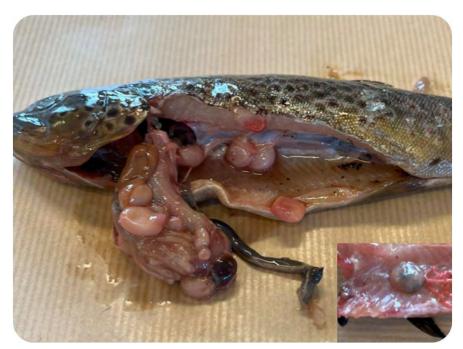


Photo: Åse Helen Garseth, NVI

Plaice with Stephanostomum baccatum



Photo: Pål Eilertsen





Red skin disease in Atlantic salmon





Photo: Geir Jensen, AJFF

Mass mortality of krill (Meganyctiphanes norvegica) and velvet belly lanternshark (Etmopterus spinax)



Photo: Brynjar Berg



Photo: Ingunn Sommerset, NVI



Mass mortality of whitefish Coregonus lavaretus



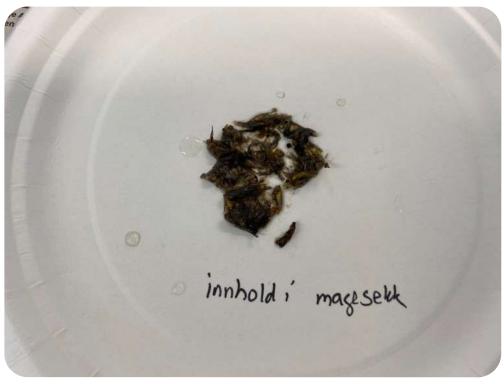
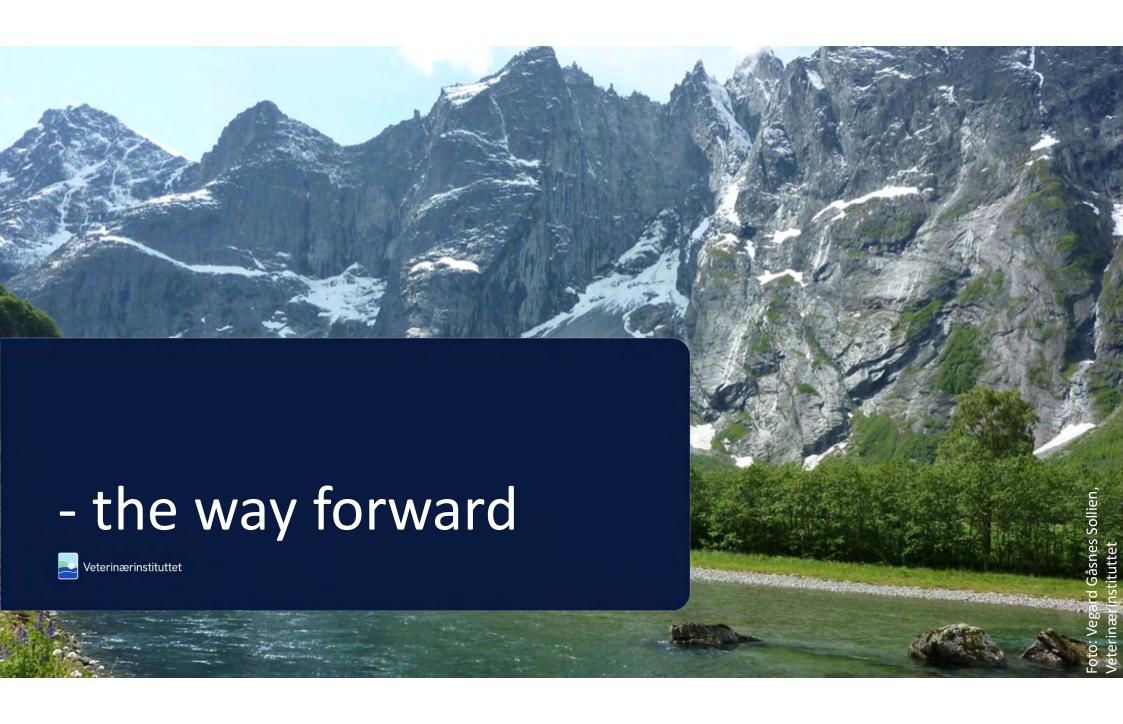




Photo: Hanne Nilsen, NVI



Thank you for your attention!

And thanks to the editorial team and co-authors





Scientifically ambitious, forward-looking and collaborative — for One Health





First report of ictavirus acipenseridallo2 (AciHV-2) in Danube sturgeons *Acipenser gueldenstaedtii* in Austria

Hella Schwegler¹, Laurent Bigarré², Karoline Lipnik¹, Nora Dinhopl³, Astrid Holzer¹,

Eva Lewisch^{1*}

¹Clinical Division of Fish Health, University of Veterinary Medicine, 1210 Vienna, Austria

²Laboratoire de Ploufragan-Plouzané-Niort, ANSES, 29280 Plouzané, France

³Institute of Pathology, University of Veterinary Medicine, 1210 Vienna, Austria



Why sturgeon?

- A few caviar and sturgeon meat producers in Austria
- Some small producers with yard sales
- * Hobbyists and pet fish in garden ponds
- EU stocking program for river Danube: LIFE- Boat for Sturgeon Breeding of 4 species:
- > Sterlet (A. ruthenus)
- Danube/Russian sturgeon (A. gueldenstaedtii)
- > Starry sturgeon (A. stellatus)
- > Beluga sturgeon (Huso huso)



lb4storgeon.eu

Introduction



Danube sturgeon (Acipenser guldenstaedtii)

native to the Black Sea, Azov Sea, Caspian Sea, and associated waters

Up to 2.35 m long, >100 kg body weight Seawater, brackish water, freshwater IUCN Red List Status (2024): Critically endangered

Conservation and restocking projects worldwide Stocked mostly with unknown health status



Danube sturgeon production: 213 t globally

(global aquaculture fish production 2022: 61.6 million t)

Largest sturgeon producers: China, Russia, Armenia; Italy, Poland, Bulgaria

Danube sturgeon: 1/5 of global caviar production

Introduction



Sturgeon herpesviruses (HV):

Large, double standed DNA virus; family *Alloherpesviridae* 3 genotypes:

- *Acipenserid herpesvirus-1 (AciHV-1): white sturgeon (A. tranmontanus)
- ❖ Ictavirus acipenseridallo2 (formely AciHV-2): white sturgeon, shortnose sturgeon (A. brevirostrum)

Siberian sturgeon herpesvirus (SbSHV): Siberian sturgeon (*A. baerii*), bester (beluga *Huso huso* x sterlet *A.ruthenus* hybrid): 2 lineages, distinct genotypes of AciHV-2

*Acipenserid herpesvirus-3 (AciHV-3): lake sturgeon (A. fluvescens); integration into genom of many sturgeon species

AciHV-1 and AciHV-3: genatically close to each other ACiHV-2 more close to ictalurid herpesviruses

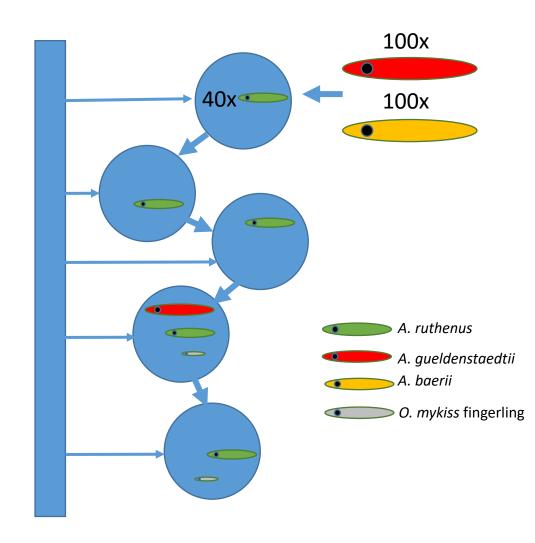
The case – background



Hobby and meat production fish farm 5 adjacent ponds

Two months before presentation: purchase of 100 Siberian sturgeons (*Acipenser baerii*) and 100 Danube sturgeons (*Acipenser gueldenstaedtii*) All TL 30-40 cm

Stocking of the upper pond, already stocked with 40 Sterlets (*Acipenser ruthenus*) Water drainage partly via lower ponds Lower ponds: Sterlets (TL 50-60 cm), one old Danube sturgeon (TL 140 cm), rainbow trout fingerlings



Water temperature: 13°C

The case – background



5 weeks after stocking: some Danube sturgeon listless, stopped feeding

Increasing mortality
No problems at the source farm

At date of submission:

13 Danube sturgeon dead Approximately 50 showing symptoms of illness All others OK

Submission of 4 live Danube sturgeon



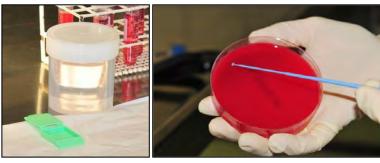
Materials and Methods



Submission of 4 lethargig fish

- Euthanasia: buffered MS222
- Skin and gill smears for parasitology
- Gross exteral examination
- Necropsy
- Bacteriological swabs from kidney and spleen cultivation on COS incubation at 22°C for 48h
- Bacteria identification with MALDI-TOF
- Samples for histology, H&E stain
- Samples for TEM (tissues in glutaraldehyde)



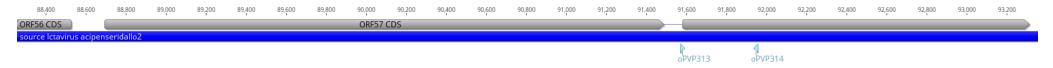


Materials and Methods



Molecular detection of viruses:

- ❖ Mimivirus: gills, skin, skin lesions, kidney, intestine → conventional PCR protocol, generic primers oPVP339 and oPVP344 (Bigarré et al. 2017)
- ❖ Nodavirus: eye, CNS in RNAlater →1-step real-time qPCR (Baud et al. 2015)
- ❖ Herpesviruses: gills, skin, skin lesions → new conventional PCR protocol targeting DNA polymerase gene of white sturgeon herpesvirus 2 Sanger sequencing of amplification products



Position of the PCR primers on the DNA polymerase gene of the WSHV2 genome. The genome is the one of the UC Davis strain of Ictavirus acipenseridallo2 (GenBank PP622675) (Doszpoly et al. 2024). Primers are inside the catalytic unit of the DNA polymerase encoded by ORF57 and an ORF downstream separated by an intron. One primer (oPVP313) is at the border of the 3' end of the intron.

Results



- **❖ Total length:** 31-35 cm
- **Parasitological examination**: no disease-relevant parasites
- Gross examination: Skin and fins: disseminated multiple skin hemorrhages ventral of the body, epidermal hyperplasia
- Necropsy: no food in the slightly distended digestive tract;
 2x oligofocal petechial hemorrhages in the serosa, gills and viscera pale, spleen enlarged







Results



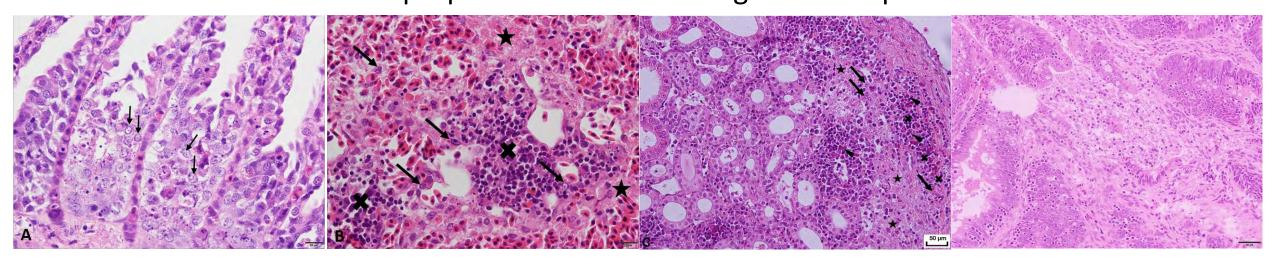
- **Bacteriology**: identification of *Aeromonas sobria* in kidney and spleen of one fish
- Histology:

Gills: diffuse generalized moderate hyperplasia, focally moderate hypertrophy of the epithelium

Spleen: severe disseminated necrosis in the white pulp

<u>Kidney</u>:oligofocal necrotic spots in the hematopoietic tissue

All three organs: karyopycnosis and karyorrhexis or chromatin margination Intestine: generalized diffuse mild infiltration of the villi with inflammatory cells, oligofocal necrotic areas in the lamina propria. No relevant changes in the epithelial cells.



Results



Molecular examiation

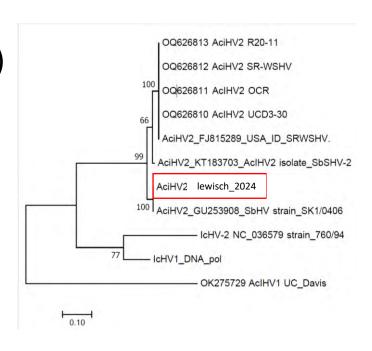
Mimivirus: negative

Nodavirus: negative

* Herpesviruses: 349 bp amplification product in each of the 4 individual samples

Alignment: 100% identity to SbSHV strain SK1/0406 (GU253908) and 94.84–98.28% identity to HVs from lake sturgeon and white sturgeon

GenBank accession number PQ619818





Electron microscopy

Herpes nucleocapsids at different stages of development

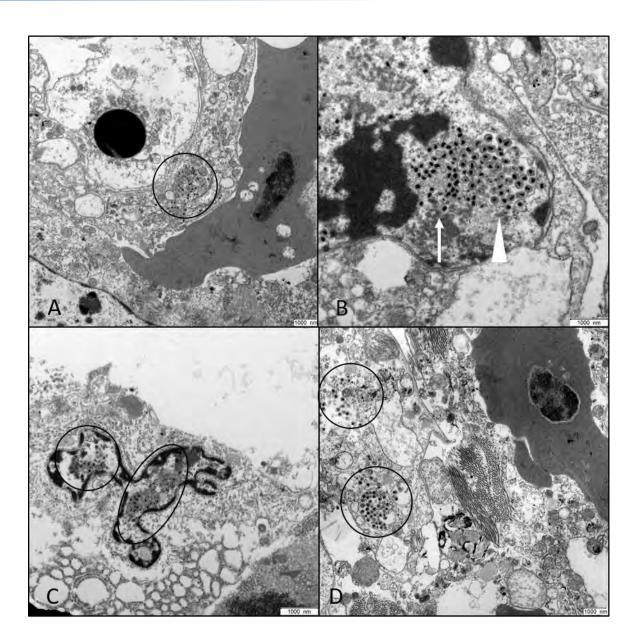
Enveloped herpesviruses in endothelial cells and other cells in the gills, spleen, and digestive tract

(A) Intestinal tissue: HV nucleocapsids (circled) in the cytoplasm of an endothelial cell. (B) Intestinal tissue: nucleocapsids

in different developmental stages (arrow) and enveloped HV (arrowhead) in a cell nucleus. (C) Spleen: HV nucleocapsids

in different developmental stages and enveloped HV (circled) in a nucleus; note the electron-dense, marginal chromatin.

(D) Gills: different developmental stages of HV (circles) in the cytoplasm



Conclusions



- First report of AcHV-2 homologue in Austria and in Danube sturgeon
- > Herpesvirus disease with high mortality in non-fingerling specimens
- > Systemic infection with virus replication also in other than epithelial cells
- > Outbreak after stressful transportation
- > Siberian sturgeons of same shipment succumbed 2 wks later to the disease
- Cohabitated sterlets and old Danube sturgeon were not affected
- ➤ Intense global trade for aquaculture and wild waters
- > Routine testing for selected viruses seems appropriate



First report of ictavirus acipenseridallo2 (AciHV-2) in Danube sturgeons Acipenser gueldenstaedtii in Austria

Hella Schwegler¹, Laurent Bigarré², Karoline Lipnik¹, Nora Dinhopl³, Astrid Holzer¹, Eva Lewisch1,*

> Clinical Division of Fish Health, University of Veterinary Medicine, 1210 Vienna, Austria ²Laboratoire de Ploufragan-Plouzané-Niort, ANSES, 29280 Plouzané, France ³Institute of Pathology, University of Veterinary Medicine, 1210 Vienna, Austria

the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 871029.

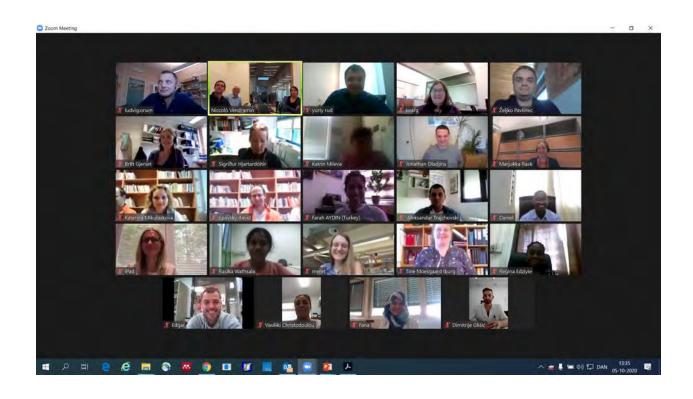
Special thanks to Anna Toffan!



EURL-Fish training course







In 2022/2023 we get back to in person participation





In 2024 we tried something new



European Union Reference Laboratory for Fish and Crustacean Diseases



NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Introduction to validation of Diagnostic methods for Aquatic animal diseases

7th - 9th of October 2024

Day 1	Day 2	Day		
Monday 7 th October	Tuesday 8 th October	Wednesday 9th October		
9:00-9.30	9:00-9:30	9:00-9:45		
Introduction and presentations of participants Argelia Cuenca Niccoló Vendramin	Pooling of samples & Case studies crustacean Peter Mohr	Diagnostics of crayfish plague David Strand		
Martin Statement	9:30-10:20	9:45-10:30		



Training course in 2025



- 3 years workprogram (2025,2026,2027) with opportunity for better planning
- The EURL plans the following events
 - "Methods for implementation of surveillance procedures for listed fish diseases" in week 41 of 2025 – October 6th to 10th
 - Webinar on Aquatic animal health epidemiology in 2025 (DATES TO BE DEFINED)
 - Training course on validation of diagnostic methods in 2026 (DATES TO BE DEFINED)
 - Training course on sampling and diagnostic procedures for crustacean diseases in 2027 (DATES TO BE DEFINED)

Both for the course in 2026, and 2027 we anticipate to hold them "as usual" in week 41

- Within june announcement and invitation out
- By August collection of applications
- In early September communication to the participants and programs
- STAY TUNED ☺

Day 1	Day 2	Day 3	Day 4	Day 5
Section 1	Section 2	Section 3	Section 4	Section 5
Visit to fish farm and Veterinary sevices in Jutland	Laboratory introduction and sample preparation	PCR analysis and Cell culture methods	PCR, blast and phylogeny	Cell culture /bacteriology and evaluation
8:00 – 11:00	9:00 - 10:30	9:00 – 10:30 : PCR and real time PCR theory.	9:00 - 10:30	9:00 - 10:30
Transport by bus to Danish Veterinary and Food Administration, in Jutland. 11:00 – 12:15 Introduction to surveillance programs Aquaculture surveillance and sampling procedures in Denmark	Introduction and practicalities. Participants experience and expectations Coffee break 10:30 - 10:50 10:50 - 12:15 Theoretical introduction to sample preparation, cell cultivation, virus ID and qPCR for surveillance programs for the non-exotic listed fish disease in Europe	Coffee break 10:30 - 10:50 10:50 - 12:15 Result analysis Practical exercises.	PCR and Real Time PCR Troubleshooting. The diagnostic laboratory – PCR flow. Coffee break 10:30 - 10:50 Sequencing theory and practical exercises	Cell culture observation with different reference viral isolates Coffee break 10:30 - 10:50 10:50-12:15 Fish bacteriology demonstration
Lunch: 12:15 - 13:00	Lunch: 12:15 - 13:00	Lunch: 12:15 - 13:00	Lunch: 12:15 - 13:00	Lunch: 12:15 - 13:00
13:00 – 13.30 Transport to Fish Farm	13:00 - 14:30 Sample preparation for cell culture, PCR and	13:00- 16:30 13:00-13:45 Reading cells and inoculation of samples 13:45-14:30 Use of cell culture in fish virology	13:00 – 17:00 Blast analysis and practical exercise	13:00 – 14:45 Assignment + presentation and assessment of data obtained by each group
13:30 – 15:30	bacteriology on samples collected Monday	<u>Coffee break 14:30 - 15:00</u> <u>15:00-16:00</u>	<u>Coffee break 14:30 - 15:00</u>	Discussion and recommendations
Inspection and sampling $15:30-19:00$	Coffee break 14:30 - 14:45 14:45-16.45 Practical cell culture passaging and production of 24 well plates	Titration procedure, viral titre calculation. Barcoding cell lines	Introduction to phylogenetic analysis	Conclusion 14:45-15:00 Course evaluation, coffee and goodbyes
Return to Hotel	19:00 -Social dinner			





Webinar on epidemiology

Modules:

Module I	Wednesday, September 9th					
Introduction	10.00	Introduction.				
		Introduction to course				
Epidemiological		material.				
principles	10.30	Basic epidemiological				
Outbreak		concepts				
investigation	11.00	Outbreak investigation				

Module II	Wednesday, September 17th						
Surveillance in	10.00	Surveillance fit for purpose					
Aquaculture	10.30	Roles in surveillance					
	11.00	Designing a program					

Module III	Wednesday, September 24th						
Sampling and	10.00	Sampling fit for purpose					
testing for	10.30	Sample sizes					
aquatic	11.00	Interpretation					
diseases							

Module IV	Wednesday, October 1st						
Diagnostic test	10.00						
evaluation	10.45	Validation of tests					

Module V	Wednesday	Wednesday, October 8th						
Risk factor	10.00	Formulating hypothesis						
analyses	10.20	Data for purpose						
	10.40	Analyses and interpretation						

Module IV	Wednesday, October 22nd		
Evaluation of	10.00	Surveillance sensitivity	
surveillance	10.20	Compliance with EU	
programmes		regulations	
	11.00	Q&A	
Follow-up		Further resources	



Résumé of the Inter-Laboratory Proficiency Test 2024

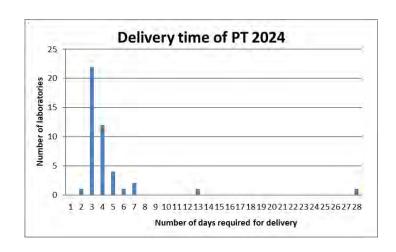
Identification of VHSV, IHNV, EHNV, SVCV and IPNV (PT1) and CyHV-3 (KHV), ISAV and SAV (PT2)

Teena Vendel Klinge, Argelia Cuenca and Niccolò Vendramin



IL-PT 2024

- The IL-PT2024 was shipped to 44 laboratories
- 24 EU Member States
- 11 other European countries
- 9 outside Europa
- 44 participated in PT1
- 43 participated in PT2
- 98% of the packages were delivered within the first two weeks

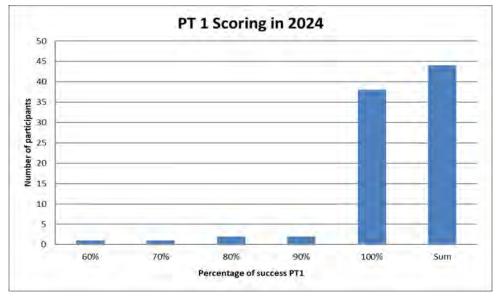


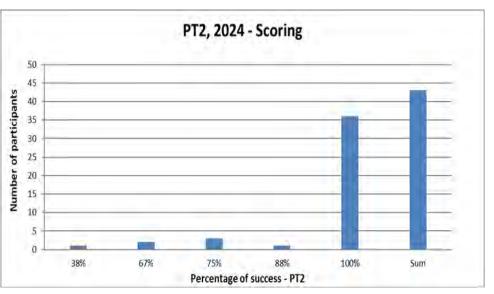


Laboratory scoring

"Underperfomance"

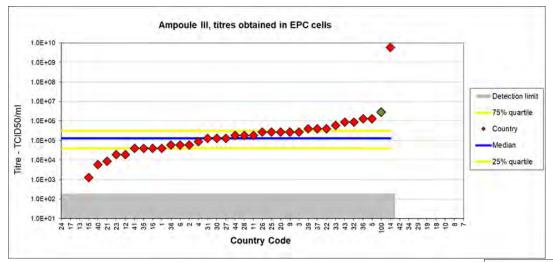
- Answering Ranavirus without corroborating the finding with sequence analysis.
- Answering EHNV (even though sequencing shows ECV)
- Answering ISAV without discriminate between listed HPRΔ ISAV and nonlisted HPR0 ISAV.
- Wrong identification of HPR-type
- Not identify the viral content of the ampoule.
- Contamination with another pathogen.



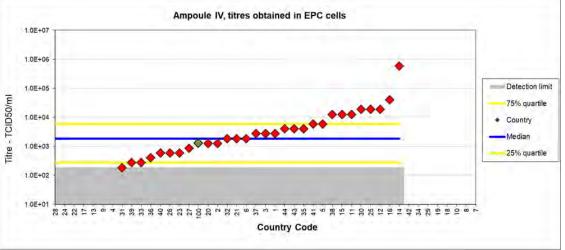




IL-PT 2024 - PT1, viral titration



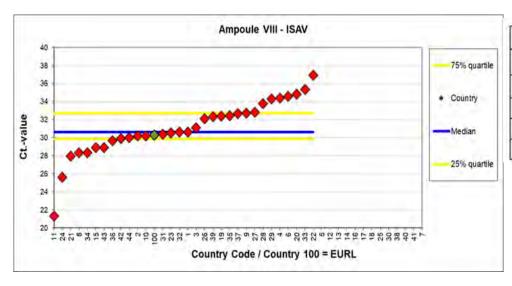
← Viral titration – Amp. III (SVCV) on EPC cells



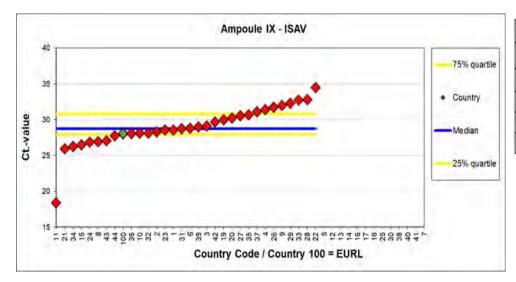
Viral titration – Amp. IV (ECV) on EPC cells \rightarrow



IL-PT 2024 – PT2, Ct values



Number of laboratories	32
Median Ctvalue	30.6
Maximum Ctvalue	36.9
Minimum Ctvalue	21.3
25% quartile Ctvalue	29.8
75% quartile Ctvalue	32.7



Number of laboratories	32
Median Ctvalue	28.8
Maximum Ctvalue	34.5
Minimum Ctvalue	18.4
25% quartile Ctvalue	27.9
75% quartile Ctvalue	30.8



On-line meeting on 28th of March 2025

- 52 participants Attended the meeting, with participants from Europe and oversea.
- We appreciate that so many participants took the time to participate in the meeting
- It seems like most participants find the online meeting useful and it allows more people from the same laboratory to participate.
- I will take the opportunity to encourage every participants to ask questions both theoretically and practically during as well as after the meeting.



Participant-feedback 2024

41% of the participating laboratories completed the questionnaire with feedback.

The questionnaire will be included in the documentation for our quality assurance which is a demand from DANAK, so a great thanks to the 18 participants for support and contribution.

You are still welcome to send the questionnaire with your feedback, they will still be included in the documentation as long as they are received before July.



Feedback from participants 1/2

- 1. A check box "denaturation step" in the result spreadsheet for PCR methods.
- 2. It could be useful to add some information regarding PCR methods used by EURL for PT1 in the report
- 3. It could be interesting/challenging to include other viruses than SVC or IPN.
- 4. When genotyping ISAV, is it sufficient to distinguish between ISAV HPR0 and HPRdel, or is the specific HPR genotype also required in the EURL-PT?
- 5. Since cq values and titers should correlate, it would also make sense to determine the cq values accordingly
- 6. The PT2 report states that cq values are not directly comparable due to the use of different methods, reagents and equipment. This statement may lead to misunderstandings. The EURL has published the diagnostic manuals on its website. The aim should be standardized diagnostics in the NRL. If other methods are used, they should guarantee the same sensitivity and specificity.
- 7. While the titers of the PT1 pathogens were relatively stable or only slightly reduced after lyophilization/storage, the cq values of the PT2 pathogens are significantly increased (= the titers are significantly reduced.) Is there an explanation for why the PT2 pathogens do not survive the treatment so well?



Feedback from participants 2/2

- 8. Is it possible to optimise the lyophilisation process of the PT2 pathogens?
- 9. Can only the amino acid sequence be used to determine the HPRdel genotype? For colleagues with limited experience in sequence analysis, a separate training course on sequencing/analysis/genotyping would be useful. An alternative would be for the EURL to provide both the PCR sequences of ISAV HPR0 and various ISAV HPRdel types, together with instructions on how to analyze them. I am aware that this is not easily possible as not all NRLs have the same software for evaluating and analyzing sequences.
- 10. It was not possible to isolate the pathogens (ISA) in cell culture in our laboratory. Could the EURL please make these pathogens available to the NRL on request on the basis of an MTA?
- 11.I would like to suggest that the EURL add further details on assigning ISAV HPR-deleted/HPR0 to the diagnostic manual with respect to alignment and paper reference for ISAV deletions (highlighted at EURL PT meetingplanning and coordination of dates for interlaboratory test evaluation meetings).
- 12. What is the temperature required for transportation by FedEx? I remember that the package didn't contain any ice packs



Feedback - EURL

- Rana isolate included shall be sequenced, distinguishing EHNV from the non-listed Ranavirus.
- Correct answer in 'Concluding results' for Ranavirus is 'EHNV' or 'Ranavirus Not EHNV'.
- ISA isolate included shall be sequenced, distinguishing HPRΔ and HPR0. Interpret sequencing data and alignments.
- Correct answer in 'Concluding results' for ISA is 'HPR-deleted ISA' or 'HPR0 ISA'.
- Correct answer in 'Concluding results' for a blank is 'Not ...and all the pathogens tested for'.
- Fill in the N/A prefilled cells with "+" (tested **positive**); "-"(tested **negative**) and only leave the N/A if you did not perform the test.
- Testing of the ampoules by molecular methods should be directly from the re-suspended material and not from the viral isolates.
- Filling the spreadsheet in standardized way please use the instruction how to fill in the spreadsheet.

May 2025 Technical University of Denmark

10



Proficiency Test 2025

- The test is scheduled to be shipped in September 2025
- PT1: For identification of VHSV, IHNV and EHNV and in addition SVCV, differentiating from other viruses as IPNV, Rana-viruses etc.
- PT2: Identification of ISAV, KHV and SAV
- We will have an online meeting in March/April 2026 for discussing the results. Please fill
 free to handle in any suggestion for topics to discuss at the meeting.

May 2025 Technical University of Denmark

11



Acknowledgements

The EURL team:

- Argelia Cuenca
- Niccolò Vendramin
- Teena Vendel Klinge



- KHV- Received from: Dr. Sven M. Bergmann, FLI, Germany
- SAV- Received from: Dr. Hilde Sindre, Veterinary Institute, Norway
- ISAV Received from: Norwegian Veterinary Institute, Norway
- **ECV-Received from:** Dr. G. Bovo, ISZ-Ve, Padova, Italy

12



European Union Reference Laboratory for Fish and Crustacean Diseases

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

EURL-Fish work done in 2024 and 2025 – ideas

for 2026

Niccoló Vendramin, Shyam Shyam Kokkattunivarthil Uthaman Argelia Cuenca, Teena V. Klinge, Britt Bang Jensen



The present Fish and Shellfish Diseases team

• 28 employees

7 PHD Students!



Britt Bang Jensen



Niccolò Vendramin



Argelia Cuenca



Niels Jørgen Olesen



Lone Madsen



Niels Lorenzen



Tine Solvoll



Christina Behrens Ladime



Anna Alencar



Shyam





Dagoberto Sepulveda Jacob G. Schmidt.



Jeong In Yang





Lise

Christensen

Juliane Sørensen Valentina Donati



Lakshan Warnakula



Sophia Carolir Windeballe Dahl



Charlotte Larsen



Irina **Vardia**



Teena Vendel Klinge



Lone Jessen



Kristina Andkjær Andersen



Tune Øst-Jacobsen



Thomas Weise



Kári **Karbech** Mouritsen





EURL-Fish work program 2023-2024 and now **2025-2027**

5 main objectives:

- 1. TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs.
- 2. TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs
- 3. TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS
- 4. REAGENTS AND REFERENCE COLLECTIONS
- 5. REQUIREMENTS RELATED TO OTHER LEGISLATION



Sub-activity 1.1 Proficiency test fish diseases. To assess the capabilities of all Member State NRLs to detect pathogens causing fish diseases and to harmonize the procedures used by an inter-laboratory proficiency test

An inter-laboratory proficiency test on fish diseases was provided in 2024 by the EURL. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).

43 laboratories participated in this activity in 2023. The inter-laboratory proficiency tests for fish diseases were sent from the EURL in October 2023.

The content in the ampoules was disclosed in January 2025. A full report was provided to the Commission and the participants March 2025. In April 2025 a specific online meeting was held with all participants to discuss the findings, the report and underperformances.



EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2024
for identification and titration of
VHSV, IHNV, EHNV (fish ranaviruses), SVCV and IPNV (PT1)
and identification of
CyHV-3 (KHV), SAV and ISAV (PT2)

Organised by the European Union Reference Laboratory for Fish and Crustacean Diseases, National Institute of Aquatic Resources, Technical University of Denmark Kgs. Lyngby, Denmark





Sub-activity 1.3 Diagnostic methods. For the EURL to have diagnostic methods of the highest scientific standards and to be able to provide these methods to all Members state NRLs

In 2024, the qPCR for detection of Nidovirus was introduced in the laboratory and tested with positive control provided.

IHNV was revalidated to include new probe

The analytical characteristics (specificity and sensitivity) of the following methods were further assessed by participating in interlaboratory proficiency test provided by CSIRO

One RT-PCR for detection of SVCV

One qPCR for Nodavirus

One qPCR for KHV

A qPCR for detection of red sea bream iridovirus



Sub-activity 1.5 Support NRLs in obtaining accreditation. To support NRLs in obtaining accreditation for diagnosing listed fish and crustacean diseases in Europe

A number of "ad Hoc" online meetings with NRLs to provide assistance on diagnostics of IHN, ISA, etc.

Sub-activity 2.1 Annual workshop for fish diseases.

To ensure knowledge dissemination and sharing between the Member State NRLs on existing and emerging fish diseases and to agree on the future priorities of the EURL by holding the 28th annual workshop of the National Reference Laboratories (NRLs) for fish diseases in 2024.

The 28th Annual Workshop of the National Reference Laboratories for Fish Diseases was held on the 29th of May 2024. The meeting was held online.

The number of participants reached 83 participants from all EU member states, associated countries and Ukraine, Australia and USA. There were four sessions with a total of 16 presentations





Sub-activity 2.3 Scientific working groups. To ensure that fast and reliable scientific advice on specific topics related to listed and emerging diseases and to legislative issues, is provided by organising expert meetings in order to solve arising challenges in EU

In 2024 and 2025 one working group to address sampling and diagnostic procedures for IHN was organized and finalized a report

The established working groups on diagnostic manuals were addressed for manual revisions

European Union Reference Laboratory for Fish and Crustacean Diseases



NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Introduction and background

Infection with Infectious Haematopoietic Necrosis virus (IHNV) occurred in Denmark for the first time in May 2021. Previously free of IHNV, the disease and control of this novel IHNV outbreak in Denmark triggered a series of questions on the diagnostic procedures for the detection of IHNV by the European Union Reference Laboratory for Fish and Crustacean Diseases.

To address these questions, a scientific working group was established between the EURL for Fish and Crustacean Diseases and the WOAH Reference Center for IHN.

The working group started with an online meeting on December 2023, followed by a series of preparatory meetings leading up to the culmination of a hybrid workshop in October 2024 that was hosted by the EURL for Fish and Crustacean Diseases and held at the Danish Technical University.Dr. Kyle Garver (WOAH Ref. Expert for IHNV and VHSV) attended the meeting inperson. This report contains the outcome of the meeting and a list of actions to be implemented in the respective manuals.

Aim: compare and contrast the current chapter 2.3.5 of WOAH Diagnostic Manual with the Eu diagnostic methods and procedures for the surveillance and confirmation of infection with vhsv and ihnv.

Information gaps and/or inconsistencies across the two documents, were identified, discussed and resolved, when possible, based on peer review published articles, grey literature, experience and knowledge from the two laboratories.

Working group participants:

Members of the EURL for fish and crustacean diseases at the section for fish and shellfish diseases at DTU AQUA, and the WOAH reference laboratory for infection with infectious haematopoietic necrosis virus and infection with viral haemorrhagic septicaemia virus at DFO.

Note: If in the process of the assessment of the EU manual, significant revisions are identified, the NRL experts involved in drafting the original version will be consulted for approval of the revised version.



Sub-activity 2.4 Training Courses

To ensure that employees of the Member State NRLs have the highest scientific and excellent skills in diagnosis of fish and crustacean diseases

A 3 days training course on **Introduction to validation of Diagnostic methods for Aquatic animal diseases**

With 16 trainees was held in week 41





Sub-activity 2.5 Website www.eurl-fish-crustacean.eu

To provide the Member State NRLs with a fast entrance to information from the EURL

The EURL website https://www.eurl-fish-crustacean.eu/has been periodically updated with relevant information regarding Annual workshop, training courses, diagnostic manuals and so forth.

In 2023-2024 the website was visited 5,179 times and a total of 21,145 pages were opened.

Please note that the actual traffic on the site is somewhat higher, as presumably about 40 % of users do not accept cookies and therefore are not registered as visitors.

Link to the website: https://www.eurl-fish-crustacean.eu/

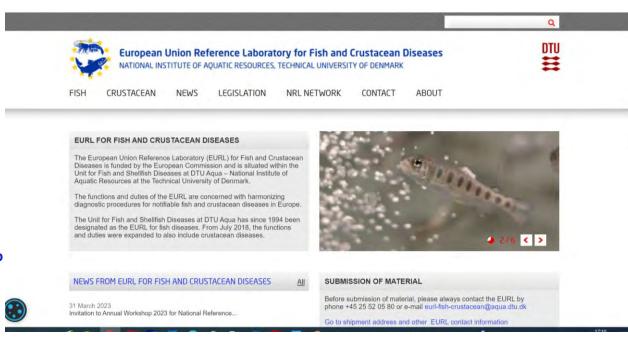
The website is now supplemented with a sharepoint with restricted access to NRL of EU and EEA for ad hoc updates.

Sub-activity 2.6 EURL Contact Lists

To ensure that relevant and important information rapidly can get from the EURL directly to the Member State NRLs

Sub-activity 2. 7 Missions to NRLs for fish diseases

To ensure a high standard of diagnostic capabilities of all Member State NRLs.





Sub-activity 2.8 International conferences and meetings

To keep the EURL updated on the newest scientific information on emerging and listed exotic and non-exotic fish and crustacean diseases, and to disseminate knowledge and scientific data provided by the EURL.

The EURL team has attended and contributed with high profile scientific talks to a number of international conferences and meetings within the field.

The Section authored 19 publications in Peer-reviewed journals.

Numerous participation at international conferences and workshops

- EFSA FEEDAP Panel (EFSA Panel on Animal Health and Welfare), Nielsen, S. S., Alvarez, J., Calistri, P., Canali, E., Drewe, J. A., Garin-Bastuji, B., Gonzales Rojas, J. L., Gortazar, C., Herskin, M. S., Michel, V., Miranda Chueca, M. A., Padalino, B., Roberts, H. C., Spoolder, H., Stahl, K., Velarde, A., Viltrop, A., Winckler, C., . . . Bicout, D. J. (2023). Assessment of listing and categorisation ofanimal diseases within the framework of the Animal Health Law (Regulation (EU)2016/429): Infectionwith salmonid alphavirus (SAV). EFSA Journal, 21(10), 1–80. https://doi.org/10.2903/j.efsa.2023.8327
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 Calistri, P., Canail, E., Drewe, J. A., Garin-Bastuij, B., Gonzales Rojas, J. L., Gortázar, C.,
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 Ståhl, K., Velarde, A., Viltrop, A., Winckler, C., ... Bicout, D. J. (2023). Assessment of listing
 and categorisation of animal diseases within the framework of the Animal Health Law
 (Regulation (EU) 2016/429): Bacterial kidney disease (BKD). EFSA Journal, 21(10), 1–76.
 https://doi.org/10.2903/jefsa.2023.8326
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen, S. S., Alvarez, J.,
 Calistri, P., Canali, E., Drewe, J. A., Garin-Bastuji, B., Gonzales Rojas, J. L., Gort_azar, C.,
 Herskin, M. S., Michel, V., Miranda Chueca, M. A., Padalino, B., Roberts, H. C., Spoolder, H.,
 St_ahl, K., Velarde, A., Viltrop, A., Winckler, C., . . . Bicout, D. J. (2023). Assessment of listing
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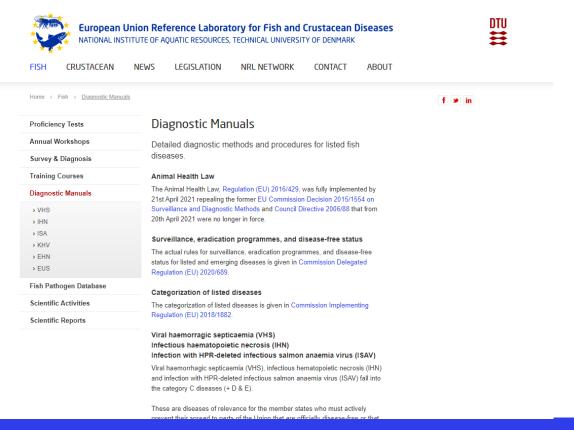


Sub-activity 2. 9 Diagnostic manuals fish diseases To have updated diagnostic manuals for all listed fish diseases available for Member State NRLs on the EURL website.

Diagnostic manuals for all fish diseases listed in EU legislation are now uploaded on our website

https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals

Work in progress to periodically update them





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Sub-activity 3.1 Survey and diagnosis fish diseases

To collate and forward information on exotic and endemic diseases, that are potentially emerging in Community aquaculture" data on emerging and endemic fish diseases and fish health surveillance in Europe will be collated in order to ensure that the EU Commission, the Member State NRLs and the EU in general are updated on the fish diseases situation in aquaculture and natural fish populations in Europe



Niccolò Vendramin and Thomas Weise 29th AW of the National Reference Laboratories for Fish Diseases, May 26th 2025

Survey & Diagnosis of fish diseases in 2024



European Union Reference Laboratory for Fish and Crustacean Diseases

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK



Sub-activity 3.2 *Risk assessment for emerging diseases*

For the EURL to have the most updated and highest scientific knowledge of emerging and re-emerging fish and crustacean diseases in Europe

The EURL has conducted a study on EHNV





EHNV pilot infection trial - Results

- · European perch
- Darkening of skin and lethargy
- Tail erosion (fin rot)
- Exophtalmia
- Enlarged spleen
- Liver lesions/degeneration





EHNV pilot infection trial - Results



- · Loss of control group in EP
- Indetermined cause no virus growth in samples, bacterial growth in BA
- · No development of disease in RT
- No clinical signs
- Development of disease in EP shedders (injected)

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Sub-activity 3.3 *Confirmatory diagnosis*

For the EURL to be able to identify and characterize isolates of listed viral fish and crustacean pathogens on request from the Member State NRLs

Sub-activity 3.4 Pathogen characterization

For the EURL to be able to characterize isolates of listed viral pathogens of aquatic animals as well as emerging pathogen and provide scientific based risk assessment to the scientific community and stakeholder

IHNV in Atlantic salmon and Rainbow trout (in vivo and sequencing and phylogeny)

IHNV in Rainbow trout (temperature adaptation and virulence resistance)

Systemic Granulomatosis in Rainbow trout

A panel of IHNV isolates have been full genome sequenced:

IHNV isolates from outbreak in Belgium (2023) NGS

IHNV isolates from North Macedonia

Exotic pathogens:

In Vivo characterization of EHNV from Australia in RT and EP

VHS/IHN confirmation from the Republic of North Macedonia

P. Salmonis from Poland

VHS confirmation from Armenia

Viral diagnostic (PRV-3, IPNV) Lithuania

RSIV in TUNA Malta







Sub-activity 4.1 *The database* www.fishpathogens.eu

To have an updated database of all serious viral fish pathogens in the EU

DISCONTINUED

Sub-activity 4.2 Pathogen library For the EURL to have an updated library of fish and crustacean pathogens relevant for the EURL and Member State NRLs

Sub-activity 4.3 Production and supply of reagents

For the EURL to be able to provide Member State NRLs with diagnostic reagents

Dato for modtagels e	EVT. UN- numm er	Materiale	Modtaget af (initialer)	Antal/str.	Transpor t firma		Afsender			ESTER Nr	Formål	Bemærkninger	Email and Phone
						Navn	Institut	Adresse					
15.03.2024		Kidney in RNA Later	Jytra	10				ul. Spacerowa 12 77-100 Dabie	Poland			The package has been standing for 2 days at room temperature. It was not written on the package that it should be kept refrigerated	E-mail: kgr@lkra-dabie.pl Phone: + 48 795 655 480
25.04.24		Rainbow Trout, organ pool from 5 fish	Jytra	5	DHL	Hovhanne s Zurabyan	RasAqua	Masisi Hayway 26 Masis, 0099 Ararat	Armenia	135	RT-PCR,	No request form was send with the samples, so saple information was obtained from the consulting veterinarian, Thomas Clausen	E-mail: - Phone: -

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06.02.202 4			2 bottles/25c m2	NIVEN	Thomw ei		Betty van	Wageningen Bioveterinary Research - WBVR, Fish Diseases Laboratory	Houtribweg 39 8221 RA Lelystad The Netherlands	Netherlands		2 small bottles (25cm ²) with FHM cells	Phone: +31 320 238373 E-mail: visdiagnostiek.bvr@wur.nl
21.02.202 4	1845	Extracted DNA	80 tubes/1,5 ml	ARCUN	Thomw ei	000	Novogene Sample Collection Team	Novogene GmbH	Am Klopferspitz 19a, 82152 Planegg-Martinsried, Munich, Germany	Germany	106702, E-1	80 x 1,5 ml tubes with extracted DNA	Phone: +49 89 3704 0454 E- mail: saly.khaled@novogene- europe.com or thais.fernandagodoy@novog ene-europe.com
09.04.202 4			2 bottles/25c m2	NIVEN	Thomw ei		Betty van Gelderen	Wageningen Bioveterinary Research - WBVR, Fish Diseases Laboratory	Houtribweg 39 8221 RA Lelystad The Netherlands	Netherlands	111608, X-4	2 cells	Phone: +31 320 238373 E-mail: visdiagnostiek.bvr@wur.nl
09.04.202 4			4 bottles/25c m2	NIVEN	Thomw ei		Erlingsdótt	The Institute for Experimental Pathology at Keldur, University of Iceland	Keldnavegur 3 112 Reykjavík Iceland	Iceland	111608, X-4		Phone: +354 585 5100 E-mail: asthildure@hi.is
30.04.202 4		Cell culture	4 bottles/25c m2	NIVEN	Thomw ei	QCS		Marine Institute Fish Health Unit	Rinville, Oranmore Co. Galway H91 R673	Ireland	111608, X-4		Phone: +353 91 387248 samantha.white@marine.ie
30.04.202 4			54 x 1,5 ml tubes	ARCUN	Thomw ei	220	Collection Team	Novogene GmbH	Am Klopferspitz 19a, 82152 Planegg-Martinsried, Munich	Germany		54 x 1,5 ml tubes with extracted DNA	Phone: +49 8937040403 E-mail: shipping@novogene.com
21.05.202 4		Monoclonal antibody IHNV	1 tube/3 ml 1 tube/1 ml	СВЕМА	Thomw ei	QCS	Silvia	Marine Scotland - Science Scottish Government Marine Laboratory Diagnostic Group	375 Victoria Road Aberdeen AB11 9DB	Scotland	E-1		Phone: +44 (0) 131 2444304 Silvia.Soares@gov.scot
28.05.202 4			2 bottles/25c m2	NIVEN	Thomw ei		Thorbjorg Einarsdotti r	SVA DOA Fisksektionen	Travvägen 20 756 51 Uppsala	Sweden	111608, X-4	2 small bottles (25cm²) with	Phone: +46 70-105-4895 E-mail: thorbjorg.einarsdottir@sva.s e

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Sub-activity 5.1 Scientific advice in relation to aquatic animal health legislation

For the EU commission and Member States to access scientific based advice on interpretation and implementation of aquatic animal health law

The experts of the EURL have been part of a working group established by the European Food Safety Authority (EFSA) to produce guidelines for defining vector species of listed aquatic animal diseases. These guidelines will be used in commissioned work to search the scientific literature for evidence of aquatic animal species working as vector species for the relevant diseases. The work was finalized in 2024.

The experts of the EURL were involved in giving advice to the content of delegated act, lists of susceptible species to the listed diseases and consultancy concerning specific questions raised by the Member states to the Commission. 18 inquires were journalised in DTU AQUA system in 2024 and 42 in 2023.

Sub-activity 5.2 *Listing susceptible species*

For the EU Member States to have an updated list of susceptible species for the listed fish and crustacean diseases

The EURL is producing documentation of susceptibility of fish species, and asked to revised periodically the list of susceptible species.

Furthermore the EURL finalized a wg on vector species chaired by EFSA

26 May 2022 DTU Aqua EURL work done and planned

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Sub-activity 5.3 *Listing diseases for notification*

For the EU commission and Member states to access scientific based advice on criteria for including or excluding infectious diseases in new Aquatic animal health law

The EURL have attended in the Working group organized and chaired by EFSA on assessment of infectious diseases Infectious Pancreatic Necrosis (IPN), Bacterial Kidney Disease (BKD), infection with Salmonid Alphavirus (SAV), infestation with Gyrodactilus salaris, Spring Viraemia of Carp (SVC). To conduct risk assessment to support decision on their categorization

The work is now finalised. And published.

The EURL is now participating in a working group looking at Vertical transmission of Cat C diseases and mitigating measures.



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SCIENTIFIC OPINION

ADOPTED: 27 September 2023 doi: 10.2903/j.efsa.2023.8326

Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) 2016/429): Bacterial kidney disease (BKD)

EFSA Panel on Animal Health and Welfare (AHAW),
Søren Saxmose Nielsen, Julio Alvarez, Paolo Calistri, Elisabetta Canali, Julian Ashley Drewe,
Bruno Garin-Bastuji, José Luis Gonzales Rojas, Christian Gortázar, Mette S Herskin,
Virginie Michel, Miguel Ángel Miranda Chueca, Barbara Padalino, Helen Clare Roberts,
Hans Spoolder, Karl Ståhl, Antonio Velarde, Arvo Viltrop, Christoph Winckler, James Bron,
Niels Jorgen Olesen, Hilde Sindre, David Stone, Niccolò Vendramin, Sotiria Eleni Antoniou,
Inma Aznar, Alexandra Papanikolaou, Anna Eleonora Karagianni and Dominique Joseph Bicout



EURL Workplan 2025-2027

3-years program

- **1. PT 1 and PT2**: No major changes maybe change of material from cell culture to tissue from infected fish.
- 2. Annual Workshop 2026: online
- **3. Training course(s) 2025-2026-2027**: Alternating traditional training courses on site, with online series of webinars for ah hoc topic (e.g. accreditation, validation, update on diagnostic manuals)
- 4. Quality assurance: provide help for implementing in NRL's (SOPs, visits etc.)
- **5. Emerging fish diseases** (What to come?) The dramatic development of IHN in EU shall be addressed carefully and will be studied- hereunder update of the diagnostic procedures (RT-qPCR)
- 6. Further update of Diagnostic manual
- 7. Proposals for topics are VERY welcome!



30th Annual workshop 2026 (week 22)

26th and 28th May 2025 Remote

Three workshops back to back, on fish and crustacean diseases, respectively and a closed session for the NRLs in EU and EEA.







End of 29th Annual workshop of NRL's for Fish Diseases 2025

Important! Please give us feed back as soon as possible by filling the evaluation scheme send to all of you.

Upon request we will send you a signed certificate of participation.

Thank you for all the excellent presentations, valuable questions and contributions and for participating in this workshop

We are looking forward seeing you soon again