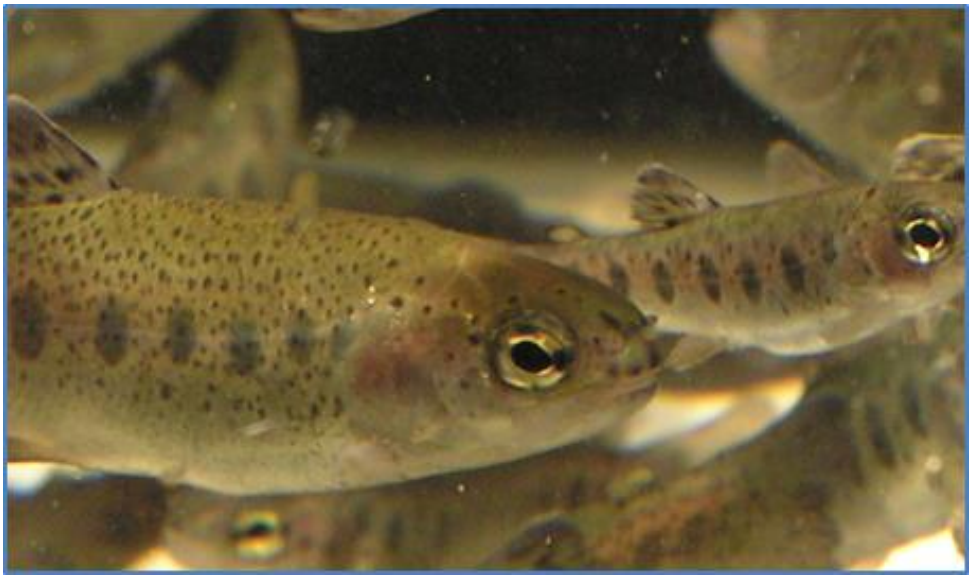
European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Report of the

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

Kgs. Lyngby, Denmark

26th 27th of May 2025



Organized by the European Union Reference Laboratory for Fish and Crustacean Diseases,

National Institute of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby

Contents

Introduction and short summary

The 29th Annual Workshop of the National Reference Laboratories for Fish Diseases was held on the 26th and 27th of May 2025. The meeting was held in person.

58 delegates from EU member states and EFTA countries attended the workshop. There were four sessions with a total of 18 presentations.

The workshop was held back-to-back with the 16th Annual Workshop for National Reference laboratories for crustacean diseases and a special session for NRL in EU and EEA on the implementation of the Animal Health Law.

The scientific programme of the Annual Workshop was again this year wide and covered many interesting topics.
The workshop was opened with "Welcome and announcements" by Head of the Section for fish and shellfish diseases, Britt Bang Jensen. The scientific part was opened with the traditional Session I "Update on important fish diseases and their control", in which participants had the opportunity to present new findings from their respective countries.

Initially, an overview of the disease situation and surveillance in Europe 2024 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire. A report compiling all information is made available for contributors on sharepoint.
Secondly, the fish disease situation in Norway was presented; a detailed report is available, see link Fiskehelserapporten 2024.

The session continued with two presentations covering relevant topics in the field of fish diseases, covering a systematic review of infectious disease reported to cause mortality in the mediterranean basin with focus on EU and WOAH listed diseases given by Andrea Marsella from the Italian NRL and next Britt Bang Jensen gave an presentation on a virtual tool based on R to gather and map disease history of fish farm in Denmark which experienced IHN outbreaks.

After a coffee break, we continued with session II Control and Surveillance of fish diseases in Europe. This session consisted of three talks. The first presentation was given by Argelia Cuenca who reported the experimental and field work conducted at DTU Aqua/EURL to develop validate and test methods to detect VHSV and IHNV in water samples to supplement surveillance for these diseases.
Afterwards, Annal Alencar, also from DTU Aqua/EURL, reported the results of a recent infectious trial conducted with Rainbow trout, European perch and EHNV. The last presentation was given from Alejandra Alonso also from DTU Aqua/EURL, on spread of *R. salmoninarum* based on phylogenetic analysis of a large repository of isolates.

After coffee break, we continued with session III results from ongoing research on listed and emerging fish diseases.
The first presentation was given by invited speaker Luana Cortinovis describing the recent outbreak and characterization of *Lactococcus garviae* in marine farmed species. Finally, Marine Baud from French NRL, provide an overview of recent VHS outbreak and initial characterization of isolates.

In the evening a social dinner at "Den Hvide Hest" was organized for all participants.
On Tuesday morning we resumed the workshop continuing session III Results from ongoing research on listed and emerging fish diseases.

First Debes Christiansen from the NRL in Faroe Island provided an update on disease situation in salmon farming in the country with focus on ISA and CMS.
Then Inga Piginka-Vjaceslavova described the recent outbreak of BKD occurred in re-stocking station in Latvia with focus on diagnostic challenges that this pathogen poses and collaboration with the EURL for corroborating the diagnostic.
Later on, Juliane Sørensen provided an overview of the validation of Ht-qPCR chip to detect all salmonids listed and non-listed viral and bacterial pathogens.
Hilde Sindre from Norwegian NRL provided an overview of the use of eDNA and eRNA to survey for ISAV HPR0 in inland facilities, and her colleague Åse Garseth presented the report on disease surveillance in wild fish in Norway. The last presentation of the session was given by Eva Lewisch from Austrian NRL on detection of AciHV-2 in Danube sturgeon in Austria.

After a coffee break it was time for the last session IV regarding updates from the EURL for fish diseases.
In this session the EURL the training course for 2025 were advertised. A resume of the Interlaboratory Proficiency test 2024 was presented by Niccoló Vendramin, summing up the results of the online workshop where results of the ILPT were presented and discussed by all participants, as well as the feedback on ILPT 2023 were presented. Furthermore, the EURL activities in year 2024/2025 were presented and proposals for the EURL work plan for 2025-2027 were discussed. It was informed that the work plan will include tasks for both fish and crustacean diseases.

Employees from DTU Aqua that took minutes from the meeting: Jacob Günther Schmidt, Argelia Cuenca, Anna Luiza Farias Alencar and Thomas Weise. Niccolò Vendramin assembled the report.
We regard this activity as a success and a great venue for knowledge sharing.
We would once again like to thank all the presenters for their great contribution; without them the meeting would not have been a success. The workshop was organized by Niccolò Vendramin, with the help from the rest of the fish disease section at the National Institute of Aquatic Resources, DTU AQUA. The Annual Workshop next year is planned to be held at end of May 2026. More details will follow.

We wish to thank all of you for participating and we are looking forward to seeing you next year.

Niccolò Vendramin

Programme

Monday May 26th

Annual Workshop of the National Reference Laboratories for Fish Diseases

13:00 – 13:20 Welcome and announcements

Britt Bang Jensen, DTU, Denmark

SESSION I: Update on important fish diseases and their control

Chair: *Britt Bang Jensen and* ***Minutes:*** *Jacob Schmidt*

13.20 – 13:50 Overview of the disease situation in Europe

Niccoló Vendramin, DTU, Denmark

13:50 – 14:10 Overview of the disease situation in Norway

Torfinn Moldal, NVI, Norway

14:10 – 14:30 Fish Diseases in the Mediterranean Sea: state of the art and future challenges

Marsella Andrea, IZSVE, Italy

14:30 – 14:50 The Map as a tool on outbreaks and epidemiology

Britt Bang Jensen, DTU, Denmark

SESSION II Control and Surveillance of fish diseases in Europe

14.50-15.10 Coffee Break

Chair: *Niccoló Vendramin and* ***Minutes:*** *Shyam K Uthaman*

15:10 – 15:30 Environmental RNA monitoring for early and non-invasive detection of salmonid Novirhabdoviruses VHSV and IHNV

Argelia Cuenca, DTU, Denmark

15:30 – 15:50 Experimental infection of rainbow trout (*Oncorhynchus mykiss*) and redfin perch (*Perca fluviatilis*) with Epizootic haematopoietic necrosis virus (EHNV).

Anna Luiza Farias Alencar, DTU, Denmark

15:50 – 16:10 Endemic reservoirs and aquaculture spread of *Renibacterium salmoninarum*: genomic insights from Norway and beyond

Alejandra Villamil Alonso, DTU, Denmark

16:10 – 16:30 **COFFEE BREAK**

SESSION III: **Results from ongoing research on listed and emerging fish diseases**

Chair: *Niccoló Vendramin and* ***Minutes:*** *Shyam K Uthaman*

16:30 – 17:00 Mediterranean Marine Lactococcosis

Luana Cortinovis, IZSVE, Italy

17:00 – 17:20 Sanitary situation in France: Update of the IHN and VHS eradication plan implement and challenging detection of VHSV strains involved in recent outbreaks

Marine Baud, ANSES, France

17:20 – 17:30 **Wrap up for the day**

From 18:45 **SOCIAL DINNER at Bakken**

Tuesday May 27th
Annual Workshop of the National Reference Laboratories for Fish Diseases

SESSION III: Results from ongoing research on listed and emerging fish diseases

Chair: *Argelia Cuenca and* ***Minutes:*** *Anna Luiza Farias Alencar*

09:00 – 09:10	***Welcome***
09:10 – 09:30	Update on the disease situation in the Faroes including the last ISA outbreak in 2024. *Debes Hammershaimb Christiansen, FFVA, Faroe Islands*
09:30 – 09:50	*Renibacterium* spp. outbreak in Baltic salmon (*Salmo salar*) in Latvia *Inga Piginka-Vjaceslavova, SIFS-BIOR, Latvia*
09:50 - 10:10	Validation of multi-target high-throughput qPCR for pathogen detection in salmonids *Juliane Sørensen, DTU, Denmark*
10:10 - 10:30	Using environmental DNA/RNA as a tool for monitoring and surveillance of pathogens in aquaculture *Hilde Sindre, NVI, Norway*
10:30 - 10:50	Norwegian Wild Fish health report 2024 *Åse Helen Garseth, NVI, Norway*
10:50 – 11:10	First report of ictavirus acipenseridallo2 (AciHV-2) in Danube sturgeons (*A. gueldenstaedtii*) in Austria *Eva Lewisch, VUW-Vienna, Austria*
11:10 – 11:30	**COFFEE BREAK**

SESSION IV: Update from the EURL for fish diseases

11:30 – 11:50	EURL Training Courses. Topics and organization of courses 2025 *Niccoló Vendramin, DTU, Denmark*
11:50 – 12:10	Interlaboratory Proficiency test for fish diseases 2024 *Niccoló Vendramin, DTU, Denmark*
12:10 – 12:30	EURL Work done in 2024, ongoing activities in 2025, plan for 2026 *Niccoló Vendramin, DTU, Denmark*
12:30 – 13:30	LUNCH BREAK

*****End of the fish disease Workshop*****

SESSION I: Update on important fish diseases and their control

Chair: Britt Bang Jensen

Overview of the Fish Diseases Situation and Surveillance in Europe in 2024

Niccolò Vendramin and Thomas Weise

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Abstract

The questionnaire on Survey and Diagnosis of the listed fish diseases in Europe (S&D) for 2024 is provided by the EU Reference Laboratory for Fish and Crustacean Diseases , it is collated annually and is the only comprehensive overview of the disease situation in fish farming in Europe. The information has been made available on the EURL web site (www.eurl-fish-crustacean.eu) , where all raw data can be obtained. The questionnaire comprises 4 parts:

1. General data on aquaculture fish production: Number of fish farms, and their health status according to AHL 2016/429, and information on national surveillance programmes.
2. Epidemiological data on the disease situation in each Member State with focus on the listed diseases (information on number of out breaks and increase or decrease in number of infected farms and severity of outbreaks) but also including other diseases of interest.
3. Laboratory data from the NRLs and other laboratories, including the numbers of samples examined, and diagnoses of fish diseases made.
4. A National report describing health and surveillance situation in general. These reports are compiled into one and can be found on https://www.eurl-fish-crustacean.eu/ .

Production data for 2023

The most update data on aquaculture production in Europe refer to 2023 on the website of Federation of European Aquaculture producers (draft produced by FEAP secretariat in 2024). We decided to refer to the dataset provided by FEAP .

The total fish production in aquaculture in Europe, including Turkey and Norway, remained somewhat stable from 2023 and is now at 2,789,559 t. The total production of EU countries has some fluctuations but is relatively stable with production of 532,599 tons.

The 5 non-EU countries Iceland, Faroe Islands, Turkey, UK and Norway produced 2,256,960t and experienced a slight reduction since 2022.

Number of fish farms in Europe

The total number of authorised/licensed fish farms in Europe is estimated in about **26760** farms. This estimates may suffer some bias:

1- In some instances put and take lakes are counted as farms
2- The figures received do not state whether a farm is active or not and whether the number account for registered or authorised farms
3- From some member states only partial response were received

Health status of fish farms

There are currently four possible health statuses:

1) Approved disease free
2) Under eradication/control program
3) In voluntary surveillance program
4) Non approved disease free and not under eradication/control program.

In 2024, a health status was assigned to 8454 farms with susceptible species to VHS, to 8186 farms with susceptible species to IHN, to 5074 farms with susceptible species to ISA.

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

Health status for IHN, 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

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

Outbreaks of listed diseases in Europe

For **VHS**, 17 new outbreaks were reported in Europe in 2024, 6 in Germany, 5 in Poland, 2 in Czech republic, 2 in Austria, 1 in France, 1 in Switzerland.

For **IHN**, 79 new outbreaks were reported. The majority was in the Republic of North Macedonia 36, followed by Germany 30, Belgium 11,Slovenia 1, Czech republic 1.

For **ISA** (Infection HPRΔ ISAV) Norway reported 20 confirmed cases and in 1 Faroe Island.

For **KHVD**, 23 outbreaks were reported in 2024, 4 in Croatia, 1 in Sweden, 1 in Slovenia, 1 in Slovakia, 3 in Poland, 8 in Hungary, 2 in France, 1 in Czech republic, 2 in Austria.

Q: Is it correct that you said you find BKD positively associated with RAS farms?

A: Yes, in Denmark, but not necessarily elsewhere

Overview of the disease situation in Norway

Torfinn Moldal, Jannicke Wiik-Nielsen, Victor Henrique Silva de Oliveira, Julie Christine Svendsen and Ingunn Sommerset

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Abstract

The Norwegian Fish Health Report has been published annually by the Norwegian Veterinary Institute since 2003. Since 2020, the Norwegian Veterinary Institute has gained access to data at site level for several non-notifiable diseases diagnosed at private laboratories. The agreements cover most active sites. With these data, the prevalence and geographical distribution of important diseases, such as winter ulcer, cardiomyopathy syndrome (CMS), and heart and skeletal muscle inflammation (HSMI), are reported in a representative way. The report for 2024 in Norwegian was published in March, while the English version will be published in May on https://www.vetinst.no/rapporter-og-publikasjoner/rapporter.

The main species in Norwegian aquaculture is Atlantic salmon. In 2024, approximately 387 million smolt were transferred to sea, and 57,8 million farmed salmon died during the seawater phase of the production. The probability of a fish dying during the year was 15.4% at national level. However, there was great variation between the thirteen production areas with highest probability in PA6 in Mid-Norway. The main reasons for mortality are reported by fish health personnel to be injuries after delousing operations, gill diseases, jellyfish attacks, infections caused by *Moritella viscosa*, unspecified wounds, HSMI and CMS. These results accomplish well with data from the industry initiative AquaCloud, where site personnel categorize causes of death daily.

Among the notifiable diseases, infectious salmon anaemia (ISA) was confirmed at 13 sites in 2024. Additionally, ISA was suspected at nine sites. A significant portion of the outbreaks and suspicions were in PA6. There are some cases of likely transmission from nearby sites, but there were no extensive epidemics caused by the same virus variant in 2024. There has been a notable decline in cases of pancreas disease (PD) in recent years. In 2024, there were 48 cases compared to 58 cases in 2023 and 98 cases in 2022. All cases were within the endemic area. Bacterial Kidney Disease (BKD) was detected at 12 sites in 2023 and eight sites last year. Whole genome sequencing has revealed different variants of *Renibacterium salmoniarum*. The bacteria have likely spread via well-boats as well as movement of fish.

An unusual warm summer with high water temperatures in Mid- and Northern Norway led to high levels of sea lice and more delousing treatments during the autumn. Amoebic gill disease is mainly a problem in Western and Mid-Norway, but the amoeba is also detected in the north. There were fewer detections of CMS and HSMI in 2024 compared to previous years. However, the viruses associated with the diseases were detected at a higher number of sites. The IPN situation remains stable, but the detection of IPN virus that appears to be adapted to fish resistant to IPN is concerning. Pasteurellosis has been a challenge in Norwegian aquaculture since 2018, mainly affecting Atlantic salmon in Western Norway. Outbreaks have been associated with mechanical and thermal delousing operations. Last year, pasteurellosis was detected further north than in previous years, and the spread may be linked to well-boats. Piscirickettsiosis has only been detected sporadically in Norway during the last years, but the disease was detected at several sites in Northern Norway last autumn. Whole

genome sequencing of *Piscirickettsia salmonis* from one of the sites that experienced outbreak last year and some older Norwegian isolates demonstrates high similarity.

Q: A third of the salmon in the south of Norway are reported to die of unknown causes. Why is that?

A: There could be several reasons. Not all personnel are equally trained. Some diseases are more easily recognized. But there is a general decline in reports of salmon mortality with unknown causes, so that is a good trend.

Q: In production area 2 and 3 mortality seems to have gone down. There is normally a lot of PD there. Has PD also gone down?

A: Some decline in PD, but it has stagnated in recent years. Perhaps related to production of larger post-smolts.

Q: Why does it appear that most infectious disease-related mortality is reported in the first third part of the year in all production areas (or at least in the three examples)?

A: I have no explanation for that.

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

Marsella A.[1], **Toffan A.**[1]

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Abstract

The Mediterranean Sea is a semi-closed basin, particularly vulnerable to the spread of diseases and to climate change effects. Warming water temperature prolongs the duration of environmental conditions favorable to most of the pathogens that affects aquatic animals in the area, potentially increasing their impact on marine aquaculture productions. Despite several endemic infectious diseases have a major impact on the Mediterranean marine finfish aquaculture, none is currently notifiable according to WOAH and EU legislation. No surveillance programme are in place and few data are available on the impact and distribution of the diseases among the countries. A systematic review has been performed in order to gather updated informations on pathogens causing diseases in the Mediterranean Sea from 2010 to 2024. Reports retrieved are scattered along the referred period and among countries. Informations and methods described were not consistent among the analyzed reports and some diagnostic issues have been elicited. A harmonized approach to the diagnostic workflow and to the surveillance in the area is paramount to strengthen the prevention and control of diseases in the Mediterranean Sea. This objective acquires further relevance in the light on the occurring changes driven by the global warming that are increasing the frequency of mortalities and disease outbreaks.

Q: Could you explain why some reports were excluded from the review?

A: Yes, in some cases the fish were not tested for other diseases than the reported disease. So difficult to say if that was the only cause.

Q: Do we have red seabream iridovirus in the Mediterranean?

A: I do not know. But I can tell you that Anna Toffan is starting to look into this and to make a challenge experiment in the near future, so we are ready if/when it comes.

Q: Did you see a relation between mortality and algal blooms?

A: No, because we were not looking for that. Not part of the scope of the review. But we know that in the northern part of the Mediterranean we see some problems with toxins from algae.

Comment: We should be prepared for the red seabream iridovirus. There are requests that tuna exported to China test negative for this virus.

The Map as a tool on outbreaks and epidemiology

Caitlin Yoo, Britt Bang Jensen

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As an NRLWe 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

The need: Know where the farm is located. Know if neighboring farms are infected with disease Know the history of the farm

The approach: Use data that is freely available. Use software that is freely available. Simple, but highly adaptive tool

Q: You have no historical data in your dataset. That is really important to have. What are you going to do about that?

A: Annoyingly, the FVST do not keep historical data of fish farms and fish diseases, but we have established a database that automatically downloads data from the CHR registry monthly. So in the future we will have historical data!

Q: Thank you very much. This was absolutely brilliant and should be done on a European level. Can you perhaps find traces of historical data on the internet?

A: Yes, perhaps with some detective work. About doing this on the European level, I agree that it would be very valuable.

SESSION II: Control and Surveillance of fish diseases in EU

Chair: Niccolò Vendramin

Environmental RNA monitoring for early and non-invasive detection of salmonid Novirhabdoviruses VHSV and IHNV

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Abstract

Infectious haematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV) are major pathogens in aquaculture, and they are typically detected through fish tissue analysis. However, this approach only identifies the pathogens once viral transmission has already occurred and infection is underway. As both viruses spread through water, an early and efficient environmental detection method could significantly improve disease management. In this study, we assessed the feasibility of detecting IHNV and VHSV environmental RNA (eRNA) in water during *in vivo* infection trials in *Oncorhynchus mykiss*, using RT-qPCR. By sampling at multiple time points post-infection, we could test the efficacy of detection at decreasing viral concentration in water over time. Viral eRNA was recovered using different methods: viral concentration with polyethylene glycol (PEG) precipitation, extraction from filter membranes, filtered water or unprocessed water samples. RT-qPCR values were compared to the quantification of infectious particles using viral titration, with RT-qPCR consistently detecting higher eRNA copies/mL than the cell-based assay to detect infectious particles ($TCID_{50}$/mL). eRNA detection from the filter membranes outperformed the PEG precipitation method, significantly enhancing eRNA recovery, particularly at lower viral concentrations. Notably, eRNA detection in water was still possible after the peak of mortality, for both viruses. Additionally, IHNV eRNA was successfully detected in farm water samples, even up to 50 days post initial fish tissue diagnosis, confirming the feasibility under real conditions. This study provides the first quantification of IHNV eRNA from aquaculture water and demonstrates the effectiveness of a filtration-based viral concentration method for environmental surveillance. These findings suggest that eRNA-based RT-qPCR detection of IHNV and VHSV from water could be a valuable addition to current diagnostic tools, potentially enabling earlier detection and prevention of transmission in aquaculture.

Q. Is the team planning to monitor samples from water exchange areas or water catchment areas for eRNA detection?

A: Currently, there are no IHNV-positive cases in Denmark, and we have not collected water samples from such areas. However, we are considering the possibility of testing samples from these locations in the future, if they become available.

Q. What volume of water from the farm was used in the study?

A: We used 250 mL of water collected from an infected farm for the study.

Q. If water samples are sent from distant farms, is the RNA stable by the time it reaches the lab? Is there any degradation due to RNases or related enzymes?

A: We stored the samples in the laboratory for three months and did not encounter any issues in detecting eRNA. This suggests that RNA remains sufficiently stable for detection under our storage and handling conditions.

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

Anna Luiza Farias Alencar[1*], Argelia Cuenca[1], David Cummins[2], Lachlan Coff[2], Nick Moody[2], Peter Mohr[2], Laura Hawley[3], Kyle Garver[3], Niccolò Vendramin[1]

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Abstract

Epizootic haematopoietic necrosis virus (EHNV) is a ranavirus responsible for high mortality events in redfin perch (*Perca fluviatilis*) and low severity disease in rainbow trout (*Oncorhynchus mykiss*). This disease, notifiable for the WOAH (World Health Organization for Animal Health), is endemic in wild redfin perch in southern Australia, and exotic to Europe. Currently there are no high-throughput diagnostic procedures for EHNV require sequencing of a fragment of the MCP gene to discriminate EHNV from other closely related ranaviruses. In collaboration with CSIRO (Commonwealth Scientific and Industrial Research Organisation, Australia) and DFO (Fisheries and Oceans Canada) an EHNV specific qPCR assay is being developed. To validate the method, a cohabitation trial was conducted with two EHNV isolates (EHNV 86/8774, isolated from rainbow trout and EHNV 24-00087, from redfin perch) in Specific Pathogen Free rainbow trout and redfin perch. The infection trial was carried out in 150L tanks, at 18°C and salinity of 20ppt where 50% of the redfin perch (30/60) and 37,5% (30/80) of the rainbow trout were injected with either MEM (Control) or EHNV 86/8774 or EHNV 24-00087. Sampling was conducted weekly (7, 14, 21 and 28DPI), and tissue homogenate samples were tested by virus isolation and qPCR. Survival and morbidity were recorded daily through the 28 days of experiment. While no significant difference in virulence was observed between the EHNV isolates, there were differences in host susceptibility. Only injected redfin perch developed clinical signs of disease resulting in significantly reduced survival compared to the cohabitants, and no mortality was observed in rainbow trout. However, it was possible to detect positive EHNV through qPCR in cohabitant redfin perch samples at 21DPI (EHNV 86/8774) and 28DPI (EHNV 24-00087). There was significantly reduced survival in the injected redfin perch compared to the cohabitants in both EHNV isolates. Rainbow trout never showed any clinical signs during the experiment. It was possible to detect EHNV through qPCR in 84% and 90% of injected and 15,4% and 20% of cohabitant redfin perch infected samples with EHNV 86/8774 and EHNV 24-00087, respectively, throughout the experiment. EHNV reisolation in BF-2 cells was successful in 43,5% (EHNV 86/8774) and 45,8% (EHNV 24-00087) of the samples from infected redfin perch but only in 1 sample (2,3%) from rainbow trout infected with EHNV 24-00087. These results are contributing to a better understanding of EHNV kinetics in both species and to the development of better diagnostic tools for this disease.

Q: Was randomization applied when selecting control samples for the infection trial? It was observed that the control group appeared weak during the trial.

A: Yes, the control fish were randomly selected from the quarantine stock. However, due to a limited number of available fish, only a small number could be included in the control group.

Endemic reservoirs and aquaculture spread of *Renibacterium salmoninarum*: genomic insights from Norway and beyond

Alejandra Villamil-Alonso[1], Torfinn Moldal[2], Cathrine Arnason Bøe[2], Birkir Þór Bragason[3], Lone Madsen[1], Argelia Cuenca[1], Karin Lagesen[2]

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Abstract

Renibacterium salmoninarum is the causative agent for bacterial kidney disease (BKD) and has been isolated from northern European salmonid farms since the 1980s. The bacterium has been only sporadically detected in Norway during the last decades, but different production areas within the country experienced a sudden increase of BKD incidence since December 2022. The ongoing BKD outbreaks on the west coast and in mid-Norway were explored using a whole genome sequencing approach to trace the patterns of transmission of *R. salmoninarum*. In the global context, gaining a broader overview of the phylogeography of the pathogen was allowed though sequencing and phylogenomic analysis of a collection of isolates from Norway, Iceland, Denmark, and the Faeroe Islands. The accuracy of our phylogeny-based genomic relatedness analyses allowed us to discriminate between two distinct active hotspots of BKD in Norway where the disease emerged and locally spread. Both clusters are part of the expanding, aquaculture-associated Lineage 1 of *R. salmoninarum*. The spread is consistent with contemporary aquaculture practices, raising critical questions on the effectiveness of current disinfection protocols for *R. salmoninarum*. Here, Lineage 3 is described for the first time consisting mainly of *R. salmoninarum* from Iceland, where BKD has been considered endemic since historical times. This study supports the hypothesis that different endemic reservoirs of *R. salmoninarum* exist in the European water systems, as suggested by Lineages 2 and 3, and underscores the importance of further investigating pathogen persistence in different environments, especially in those related to the aquaculture industry.

Q: Positive findings from wild fish can act as an environmental reservoir that may contribute to transmission into farms. Are there any specific cases?

A: The positive findings from wild fish date back to the 1990s. It is possible that the transmission occurred from farmed fish to wild populations.

Comment: In wild fish, we observed fewer positive cases, and they typically did not show clinical symptoms. This suggests that wild fish may act as a reservoir and could be more adapted to BKD. In contrast, farmed fish appear to be more susceptible to the infection. This pattern was particularly noted in Iceland.

Q: In Denmark, there has been a significant increase in BKD cases. Have you observed any link between these cases and those in Norway?

A: We need to investigate further to explore any potential links.

Comments: In Norway, the spread of BKD has primarily been associated with wellboats transporting fish from hatcheries, which may be a major contributing factor. As a preventive measure, we are planning to introduce disinfection protocols for wellboats before fish transfers.

In the Faroe Islands, there were major BKD outbreaks during the 1980s and 1990s. Since then, management practices have evolved, particularly in Atlantic salmon and related species, which may have helped mitigate the issue.

Q: How significant is the role of vertical transmission in the spread of BKD?

A: Vertical transmission plays a critical role and significantly complicates disease management. Once BKD is established in a farm, it became very difficult to control, and the possibility of vertical transmission increases the risk of persistent infections across generations.

SESSION III: Result from ongoing research on listed and emerging fish diseases

Chair: Argelia Cuenca

Mediterranean Marine Lactococcosis

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Abstract

Lactococcus garvieae is one of the most important infectious agents of temperate aquaculture, both of fresh and salt water species. Since its first description with the former name of *Enterococcus seriolicida*, in early 1970s, it has been reported causing disease in over 40 fish species. In southern European countries, the rainbow trout (*Oncorhynchus mykiss*) farming has been historically considered the sector most negatively affected by the spreading of this bacterium. However, since 2023, *L. garvieae* has shown its potential as an emerging pathogen of Mediterranean marine aquaculture, with multiple outbreaks registered in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) farms located along Italian Tyrrhenian coast, Balearic and Canary islands. In the same year, significant outbreaks have been recorded for the first time also in farmed Atlantic blue fin tuna (*Thunnus thynnus*) in farms located in the central eastern Mediterranean.

Diseased fish display erratic and unbalanced swimming, anorexia, hypermelanosis and severe ocular impairment, ranging from exophthalmia, to keratitis and eye ruptures. The anatomopathological findings are mainly referable to a massive haemorrhagic septicaemia, with enlarged spleen, multi-focal haemorrhages and epicarditis. Chronic forms may present with necrotic pockets in the skeletal muscle along the spine.

Lactococcosis is generally an acute or hyperacute infection, characterized by extremely high mortalities especially in sub-adults and adults specimens. The outbreaks generally occur when the water temperatures rise above 18 °C, as Lactococcosis can be considered a seasonal disease, with higher prevalences during late spring- early fall and sporadic winter cases mainly occurring in farms supplied by warm well water. Nevertheless, the rising temperatures of Mediterranean water may lead to a wider seasonality of the disease.

Diagnosis involves laboratory examination aimed at proper isolation and identification of the agent, mainly by molecular methods, to discriminate from *Lactococcus petauri*, a sibiling species hitherto related only to outbreaks in rainbow trout.

The management of the disease can be complicated by the high pathogenicity of *Lactococcus garvieae* associated with the heavy reliance of some farming systems (e.g. net pen farms) on

environmental conditions, which, if unfavourable, can lead to late and inefficient harvesting of dead animals, promoting the spread of the bacterium, and to discontinuous administration of medicated feed.

Pharmacological treatment with erythromycin and oxytetracycline have been applied with varying outcomes, but remain the main tool for dealing with the infection. Autologous vaccines have been produced in Italy, and their efficacy will be observed in the coming summer season.

To deepen the epidemiological knowledge on the distribution of Lactococcosis in Italian marine aquaculture, a monitoring plan will be conducted on Italian European sea bass and gilthead sea bream farms in June and July 2025. The results obtained will help assess the severity of the situation and predict future spatial developments of the disease.

Q: Have you observed any outbreaks of *Lactococcus garvieae* at temperatures below 18°C?

A: No, the observed cases were septicemic and involved co-infections with other pathogens. We monitored conditions during January and February but did not observe significant mortality, except for a few stress-related cases.

Q: What is the typical temperature for *L. garvieae* infections in rainbow trout, particularly in Italian freshwater and marine farms?

A: Infections typically occur around 18°C during the summer in the Mediterranean region. Different clusters of *L. garvieae* have been identified in marine rainbow trout farms. We are currently investigating the introduction of the pathogen into susceptible species. Isolating the bacterium has been challenging. There is also a possibility that waste from nearby bovine farms may contribute to contamination in trout farms.

Q: Is there any connection between infections in tuna farms and rainbow trout farms?

A: No, the infections were unrelated. The strain found in tuna was different and belonged to a distinct cluster. It was also found to be conservatively susceptible to tuna.

Q: Were any biosecurity measures implemented, and what was the follow-up period after the outbreak?

A: No formal biosecurity measures were in place. In tuna farms, following the outbreak (December to April–May), the stock was removed from the cages and the cages were cleaned. However, the underwater metal structures could not be cleaned. In Italian trout farms, no similar biosecurity cleaning procedures were carried out.

Q: Can L. garvieae be part of the normal gut microbiota in fish?

A: No, *L. garvieae* is not part of the normal gut microbiota. In outbreak-affected farms, the bacterium was isolated from the gills of infected fish.

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

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Abstract

Although our territory contains many zones and compartments free of IHNV and VHSV, these listed Rhabdoviruses have been occasionally detected in France since their first description in 1971 and 1989 respectively. In order to obtain a free status for those diseases, a National Eradication and Surveillance Plan, supported by European Union, was started in France in 2017. The amount of laboratory analysis has been consequently increased to reach around 2000 analysis per year, regardless of the context of analysis (eradication programmes, maintaining freedom status, or outbreak investigations).

Almost all the metropolitan territory is involved in this approach and more and more sites of production are committing to this program so that at the beginning of 2025, 73% of fish farms had acquired IHNV and VHSV free status, while 9% of the sites were involved in an eradication program. Nevertheless, despite a global healthy sanitary situation regarding listed viral diseases in France, a VHS outbreak was reported at the end of 2024 in the East of France, on rainbow trout. The virus was detected without any clinical sign, after sampling done for disease free status monitoring. In the laboratory, the virus grew slightly, only on RTG_2 cell line and its genome could finally be sequenced for further investigations regarding the outbreak origin. A few weeks later, VHSV was once again detected on rainbow trout in the Eastern part of France, after clinical signs such as petechia were observed. The latter strain was isolated on RTG_2 cell line, and displayed some difficulties to grow on BF-2 as well as on EPC cell lines. The late detection by RT-qPCR led the NRL to investigate and characterize the viral genome. Interestingly, the 2024 and 2025 isolates exhibited almost 100% nucleotide identity on G gene, and seemed to belong to a new cluster different from former strains isolated a few years before in the same area. However, regarding the N gene sequence, 2 and 1 Single Nucleotide Polymorphism(s) (SNPs) were observed at forward and reverse primers positions (Jonstrup's method), respectively. Further assays were carried out to assess the potential impact of those mutations on the RT-qPCR method analytical sensitivity.

Q: Considering the reintroduction of the disease, are there any new government plans or targeted strategies to reduce its spread?

A: At the moment, I cannot provide a definitive answer, as we are based away from the central government offices in Paris and have not received any official communication regarding new plans. However, during the disease-free status period, action plans similar to those in Norway were in place. If the government includes fish in the national monitoring program, we would be able to participate. Otherwise, we cannot proceed independently.

Comment: A similar system exists in Denmark, where fish are not imported or transferred from outside sources. It should be mandated that farms follow such protocols or that these measures be incorporated into national policy.

Q: When do you expect France to regain disease-free status for IHN/VHS?

A: I do not have a specific timeline. The declaration of disease-free status is solely under the authority of the government, which oversees the relevant testing and certification programs.

Q: Could the clinical signs observed in the second infection case be due to IPNV?

A: We are not certain. We did not examine the fish individually, and IPNV is not considered a significant issue in France, unlike in Denmark.

Comment: It would be valuable to test the isolate grown in RTG-2 or BF-2 cell lines and assess the virulence of the strain in an experimental setup. This could help to improve the RT-PCR results.

Q: In some cases, Ct values were high even when clinical signs were present. Could you explain the delayed Ct values observed?

A: This could be due to mismatches in the primers or probes used. Although the VHSV isolate showed mismatches, the PCR detection efficiency remained consistent. We observed similar mismatches in the isolates we tested.

Update on the fish health situation in the Faroe Islands with focus on ISA and CMS

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Abstract

The Faroese production of Atlantic salmon (*Salmo salar* L.) was in 2024 90.000 tons which is an increase of 10.000 tons from the 2023 but 5.000 tons lower than the record year in 2021. The mortality after sea transfer has for the last 10 year been on average 15% ranging from 10 to 20%. The most important causes of mortality reported by the farmers the last years has been 1. handling related to treatments for sea lice with heated water, 2. wounds/winter ulcers, 3. unknown, 4. smoltification 5. accidents, 6. Infectious diseases, 7. escapees and 8. seals. Within the last couple of years handling related mortality has decreased mainly due to production of large smolt which has resulted in fewer mechanical treatments and the shift from heated water to fresh water.

Because of the ISA epidemic in the Faroe Islands the industry started to vaccinate against ISAV in 2005 and have continued since. Although the production has increased significantly, we have only had three ISA outbreaks the last 2020 years, the first in 2014, second in 2016/2017 and the final one last year. The usage of non-lethal eDNA/eRNA as an alternative for lethal tissue sampling to monitor for infectious agents has been used in the Faroes for several years and the first detection of the ISAV-HPRΔ in the 2024 outbreak was in fact from an eRNA sample.

Since reintroduction of the piscine myocarditis virus (PMCV) in Faroese aquaculture in 2013 the cases of *Cardiomyopati syndrome* (CMS) have spread considerably and caused more than 60 minor and major outbreaks the last decade. Interestingly, we experienced a marked drop in CMS cases in 2024 concomitantly with a decrease in mechanical treatments.

Here I will give an overview of the most important fish health and welfare challenges in Faroese aquaculture with focus on the 2024 ISA outbreak and PMCV/CMS in the Faroe Islands.

Q: Is there a pattern for the disease spread?

A: We see a pattern that correlates with the risk of disease. The more HPR0, the more likely to see HPRΔ happen. HPR0 is not pathogenic so it is unsure if we can see a pattern.

Q: Was it discussed to take measures to get rid of the smolt farms?

A: In the smolt farms they have completely emptied RAS systems before and 1 month after the virus was found again. They clean and empty everything, but the same strain was detected. It is unsure if it stayed there or if it came after.

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

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In recent years, a significant decline in Baltic salmon population has been observed in Latvian rivers. In response, the Scientific Institute of Food Safety, Animal Health and Enviroment BIOR has continued its long-standing efforts to restore and restock wild salmon populations. As part of this restoration program, salmon spawners were collected from the River Venta, resulting in the collection of 250,000 eggs in 2022. These eggs were fertilized and incubated, and the hatched alevins were reared in 2023 in tanks with a water volume of 1.2 m^3, each containing approximately 3,000 larvae. In April 2024, one-year-old salmon smolts were released into the River Venta. Additionally, 25,000 juveniles were retained for further rearing and placed in 1.2 m^3 tanks at a stocking density of 1,500 fish per tank.

Increased mortality was observed starting in the second half of January 2025, initially at a rate of 1–3 fish per day. This rate rose to 2–22 fish per day in February and exceeded 200 fish per day in April. Clinical signs were nonspecific and appeared shortly before death. Affected fish exhibited apathy, occasional exophthalmia, ocular hemorrhages, distended abdomen, and skin discoloration; notably, appetite was largely preserved. Gross pathological examination of all fish (n=20) showing clinical signs revealed multifocal granulomas, with or without necrosis, primarily in the kidneys, and occasionally in the spleen and liver. The liver was usually pale, and effusion of abdominal cavity was present in some individuals. In March, necropsy of 35 clinically healthy fish revealed kidney granulomas in 17% of the specimens, with some also exhibiting granulomas in the spleen.

Histopathological analysis was conducted using hematoxylin and eosin staining, Gram-staining, PAS reaction, and Ziehl-Neelsen staining. Large granulomas with severe caseous necrosis and smaller granulomas without necrosis were observed by histological examination. Gram-positive rod-shaped bacteria were identified in the periphery of necrotic granulomas and diffusely within non-necrotic granulomas. Ziehl-Neelsen staining was negative, ruling out infections with *Mycobacterium* spp. and *Nocardia* spp.

The morphological findings were consistent with Bacterial Kidney Disease. This diagnosis was confirmed by the European Union Reference Laboratory for Fish and Crustacean Diseases through nested PCR, quantitative PCR (qPCR), and ELISA testing for *Renibacterium salmoninarum*, and *Piscirickettsia salmonis* was excluded as a differential diagnosis by real-time PCR.

This marks the first documented outbreak of *Renibacterium salmoninarum* in Baltic salmon population in both Latvia and the Baltic region.

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

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Abstract

Introduction: Since the 1990's, aquaculture production has increased steadily. Environmental regulations aimed at limiting pollution have led to increased implementation of recirculated aquaculture systems (RAS). In Denmark, this has led to a reduction in the total number of farms, but with more intensive production in the RAS farms. Consequently, new batches of eggs or juveniles are introduced at a higher rate than before, increasing the risk of potentially introducing pathogens. This may result in disease cases evolving from single-pathogen infections to more complex diseases involving multiple agents.

To ease the testing of multiple targets – both in time and reagent cost – we have developed and partially validated a multi-target high-throughput microfluidic qPCR (HT-qPCR) method. The method currently enables the detection of 20 different pathogens and putative pathogens. The method was used in a longitudinal field study in 2022, which coincidentally occurred during a disease outbreak with increased mortality.

Methodology: The validation of the HT-qPCR method was performed step-wise: 1) Testing specificity using known positive reference material, 2) determining limit of detection using artificial dsDNA and ssRNA, 3) testing with known positive tissue material, 4) assessing the impact of pre-amplification and exonuclease treatment, 5) comparing to current standard diagnostic methods in a field study, and 6) comparing of qPCR, dPCR and HT-qPCR (limit of detection).

In cases where it is relevant, the assays listed in the diagnostic manual (EURL for fish diseases) were used. Validation is ongoing.

Results: Overall, the method is robust, with results comparable to current diagnostic methods. The limit of detection is similar to that of qPCR, and pre-amplification of samples does not affect the detection of a target in low concentrations when a highly concentrated target is present in the same sample. During a longitudinal field investigation, a disease outbreak with increased mortality occurred (37% of the unit succumbed) in which *Piscine orthoreovirus* 3 and *Candidatus* Branchiomonas cysticola were detected in fish samples.

Conclusions: Multi-target HT-qPCR is a robust method which yields results that are comparable to current diagnostic methods. However, some assays targeting bacterial pathogens did not perform to standard and require revision. Furthermore, validation was performed using cell culture, bacterial cultures, and known positive tissue samples. Although the method was tested with water samples from the field, its application for environmental nucleic acids (eNA) needs to be validated. Interpretation of eNA data should be approached cautiously and while considering the production

system. Field investigation in 2022 revealed a constant low presence of several bacterial pathogens in the water which did not necessarily infect the fish.

Q: What are the thoughts to use this technology for the future? Would it be used in surveillance?

A: There is hope to implement it in diagnostic routine. It is the same assay that it is used in diagnostic and the validation is done in the same way.

Comment: It would be nice to receive samples from other laboratories to test/validate the assays.

Comment: the validation is done the same way as the diagnostic qPCRs, it is just a different platform.

Comment: Still skeptical about using this in routine diagnostics. Farmers usually request specific tests at a time, not a whole panel.

Comment: It is a useful tool for testing farm for export, for example. It is necessary to define the purpose of the test. It can also be automated to minimize errors.

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

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Abstract

Infectious diseases are a major challenge in aquaculture, causing mortality, impaired fish health and welfare, as well as economic losses. To prevent the spread of and reduce the negative consequences of disease, sufficient knowledge on the occurrence of pathogens during the production cycle is crucial. This monitoring has traditionally been based on lethal testing of fish. As fish farms grow larger, often housing millions of fish, detecting pathogens at low prevalence requires testing a large numbers of individuals — potentially resulting in the loss of many healthy fish.

In later years, the Norwegian Veterinary Institute has established and improved methods for sampling, concentration and detection of infectious agents in water, for parasites, bacteria, and viruses. Environmental samples have been successfully used for surveillance programs for both *Aphanomyces astaci* (crayfish plague pathogen) and *Gyrodactylus salaris* in Norwegian water ways, and through several research projects we have shown that these methods are also very useful in monitoring bacteria and virus in various aquaculture facilities in Norway. With this approach, healthy fish are not sampled, and an acceptable detection coverage can be achieved with a small amount of targeted environmental samples, reducing both sampling time and analysis cost. Although environmental samples cannot replace fish samples for diagnostic purposes, this provides a tool for the aquaculture industry to design targeted monitoring strategies without substantial costs.

Q: Would it be possible to start with the water sample and then look at the fish sample later?

A: We will do that in the future. Prevalence is low and therefore there is a lack of eDNA. Pathogens in mucous and skin are easier to detect than internal pathogens.

Q: Using eDNA to look for pathogens – one cannot know if they are pathogenic or not?

A: We are not looking for pathogens, we are looking for indicators.

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

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Abstract

In an Austrian fish farm, 100% mortality occurred in newly stocked Danube sturgeons (*Acipenser gueldenstaedtii*) in the course of two months after introduction. The most obvious lesions were skin hemorrhages and cutaneous hyperplasia. Histology of the gills and the skin showed epithelial hyperplasia and hypertrophy while in tissues of the spleen, the kidney, and the intestine, focal necrosis was observed. Moreover, cell-nucleus alterations indicating an infection by a herpesvirus were present. By electron microscopy, numerous typical herpes-viral particles at different developmental stages were demonstrated in different tissues. A newly developed conventional PCR protocol, targeting a fragment of the viral DNA polymerase gene, further confirmed the presence of a virus related to *Ictavirus acipenseridallo2* (formerly *Acipenserid herpesvirus-2,* AciHV-2) in the diseased fish. Sequencing of the amplification products showed 100% identity to a Siberian sturgeon herpesvirus (SbSHV) strain. We report for the first time the detection of herpesvirus in sturgeon in Austria and of SbSHV, a strain of *Ictavirus acipenseridallo2*, in Danube sturgeons.

C: It is surprising that the boat is going places.

A: The boat is a breeding station, it does not go around.

SESSION IV: Update from the EURL

Chair: Niccolò Vendramin

EURL Training Course for 2025

Argelia Cuenca, Britt Bang Jensen and Niccoló Vendramin

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Abstract

In 2025, two training activities will be hosted by the EURL

A short overview of the program and the activities will be presented.

An on-site course with physical participation is planned in week 41. The course is entitled "Implementation of surveillance procedures for listed fish diseases"

A webinar series will take place in Autumn focusing on "Epidemiology in aquatic animal health"

The content of the training courses and the procedure to register will be described.

More information is available on the EURL website

www.eurl-fish.eu

Results of the Proficiency Test, PT1 and PT2, 2024

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Abstract

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).

PT1 was designed to primarily assess the identification of the fish viruses causing the notifiable diseases: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), and epizootic haematopoietic necrosis virus (EHNV) or related rana-viruses and in addition the fish pathogenic viruses: other fish rhabdoviruses as pike fry rhabdovirus (PFR),spring viraemia of carp virus (SVCV) and infectious pancreatic necrosis virus (IPNV) by cell culture based methods. PT2 was designed for assessing the ability of participating laboratories to identify the fish pathogens: infectious salmon anaemia virus (ISAV), salmon alphavirus (SAV) and cyprinid herpesvirus 3 (CyHV-3) (otherwise known as *koi herpes virus* – KHV) by biomolecular methods (PCR based). As in previous years, Salmonid Alphavirus (SAV) was included in the panel of pathogens to be investigated should include. Since SAV is not a listed disease in the European legislation, testing for SAV was done on voluntarily base. The EURL would then take care of calculating the score accordingly.

Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043. This report covers both the results of PT1 and PT2. Participants were asked to identify the content of each ampoule by the methods used in their laboratory which should be according to the procedures described in EURL diagnostic manuals available on the website

Participants were asked to download an excel sheet from the EURL web site (http://www.eurl-fish.eu/) to be used for reporting results and to be submitted to the EURL electronically. Additionally, participants were requested to answer a questionnaire regarding the accreditation status of their laboratory.

The tests were sent from the EURL in September 2024.

The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).

44 laboratories participated in PT1 while 43 participated in PT2.

Each laboratory was given a code number to ensure discretion. The code number of each participant is supplied to the respective laboratories with this report. Furthermore, the providers of the

proficiency test have included comments to the participants if relevant. An uncoded version of the report is sent to the European Commission.

Résumé and concluding remarks PT1

95% of the parcels were delivered by the shipping companies within one week and 100% was delivered within 28 days.

Overall, 38 out of 44 participants scored 100% success rate. Out of the 6 laboratories which underperformed two participants scored <100% for the sole reason that they did not back up their concluding results of ampoule IV (ECV) with sequencing. Three laboratories incorrectly answered EHNV in 'Concluding Result' on ampoule IV of these, one correctly answered ECV in the sequencing sheet. Two laboratories answered Rana in 'Concluding Result' on ampoule IV but correctly answered ECV in the sequencing sheet. one laboratory correctly answered "Not EHNV" in 'Concluding Result' on ampoule IV but did not type any Genotype in the sequencing sheet. One laboratory identified IPNV in another ampoule than the designated one, suggesting a contamination. Two laboratories did not identify the present virus in all ampoules.

Suggestions to improve on underperformance will be provided individually to each laboratory.

Résumé and concluding remarks PT2

43 laboratories participated in PT2, 36 obtained 100% success rate.

37 laboratories tested for SAV and 36 correctly identified the virus in Ampoule VI, 6 laboratories did not test for SAV and one laboratory who tested for SAV did not find the SAV in this ampoule.

All 43 laboratories correctly identified the CyHV-3 (KHV) in ampoule VII.

42 laboratories correctly identified the ISA virus in ampoule VIII and all 43 laboratories correctly identified the ISA virus in ampoule IX. One laboratory did not sequence, one laboratory did not find the ISAV in ampoule VIII, five laboratories gave the wrong HPR-type for the ISAV isolate in ampoule VIII or IX and six laboratories answered ISAV in 'Concluding Result' on ampoule VIII and IX but correctly identified the isolates as HPR-deleted in the sequencing sheet.

One laboratory identified KHV in another ampoule than the designated one, suggesting a contamination.

One laboratory did not identify the present virus in all ampoules.

This point will be addressed directly with the participants that has underperformed

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they fare in relation to the other participants. It is up to the individual laboratory to assess if they perform according to their own expectations and standards. We take the opportunity to provide comments to participants regarding submitted results if relevant. Furthermore, we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

Comment: It would be nice to compare what methods we have available at the EURL and what is done in the NRLs – for example if the NRLs are doing the denaturalization step.

Comment: In real diagnostics/samples it is not necessary to do denaturalization step.

Q: The virus in PT1 was stable, but in PT2 the Ct values increased while the titers were stable. What happened?

A: We do not have an answer to that, the viruses used in the ringtest passed the criteria required to be in the proficiency test.

EURL for Fish Diseases, work done in 2025

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Abstract

The duties of the EURL are described in the REGULATION (EU) 2017/625 (OCR). The duties mainly concern the fish cat A, C and E diseases given in (EU) 2018/1882: Epizootic haematopoietic necrosis (EHN), Infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious hematopoietic necrosis (IHN), and koi herpes virus disease (KHVD).

The facilities supporting the activities of the EURL are placed in the DTU Campus in Kgs. Lyngby, and placed in the institute DTU AQUA, National Institute of Aquatic Resources.

The 28th Annual Workshop of the National Reference Laboratories for Fish Diseases was held in person, on 29th and 30th of May 2024.

The annual proficiency test for fish diseases (PT) was divided into PT1 and PT2 with 44 laboratories participating. The tests were sent from the EURL October 2024. The full report with the results and the identification of NRL has been submitted to the Commission, whereas each participant has received: 1- Coded version the report, 2- Certificate of performances indicating also the laboratory code, and if underperformances were observed, a comment explaining potential reasons for this and 3- An email with comments on sequencing and genotyping results.

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. In 2024 and the EURL has focused on reviewing of the diagnostic manual of fish diseases. Furthermore, the EURL did focus on reviewing list of susceptible and vector species for the listed fish diseases.

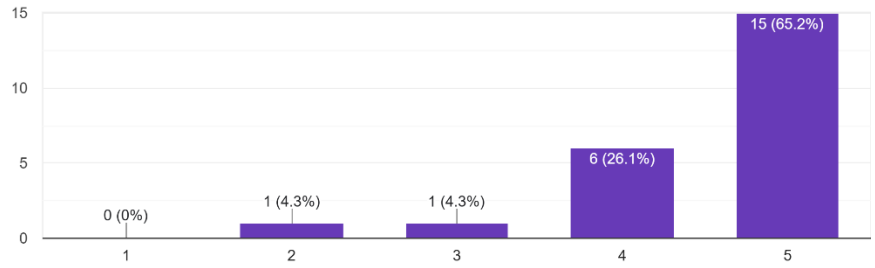
During 2024, resources were again used to collate data on surveillance, health categorisation and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to update the EURL website (www.eurl-fish.eu), to provide consultancy to NRL´s and finally to attend international meetings and conferences.

Workshop evaluation

A questionnaire was delivered to the participants asking to evaluate various aspects of the workshop. An overview of the 23 questionnaires retrieved is shown below.

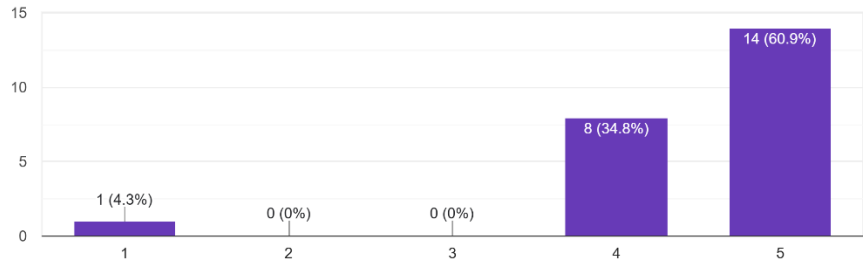
Session I: Update on important fish diseases and their control - Quality of presentations

23 responses



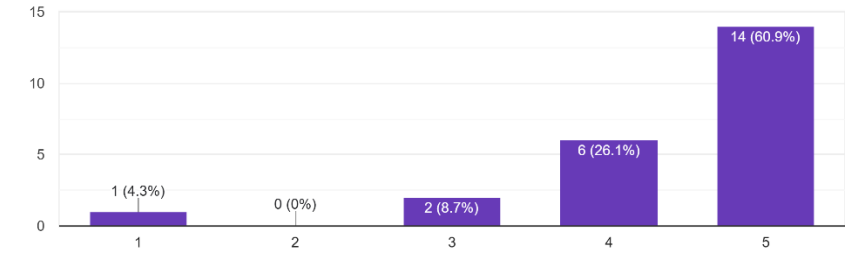
Session I: Update on important fish diseases and their control - relevance for you

23 responses



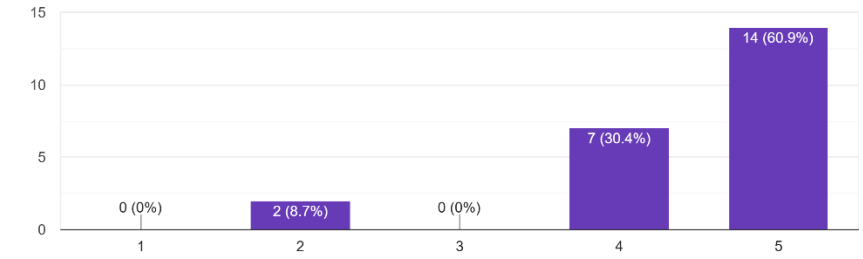
Session I: Update on important fish diseases and their control - increase of your knowledge

23 responses



Session I: Update on important fish diseases and their control - overall score

23 responses

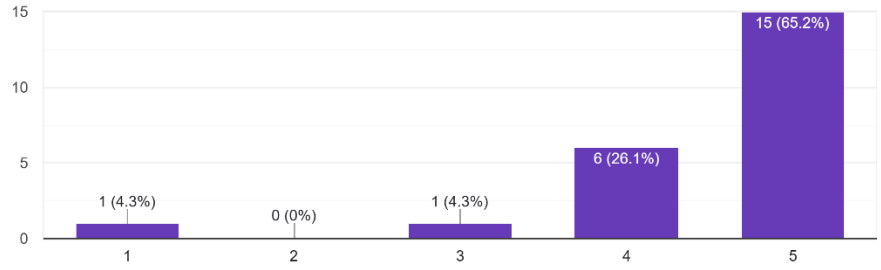


Session I: Update on important fish diseases and their control - comments, remarks

- Relatively low news value,
- I think it would be better to announce in advance the schedule of the sessions. This year it started at 13:00 but most of the participants thought it would start at 9:00. This would be important to organize the trip in advance
- Good session

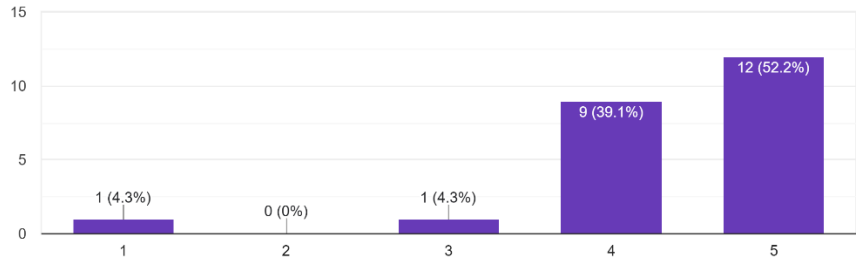
SESSION II: Control and Surveillance of fish diseases in Europe- Quality of the presentations

23 responses



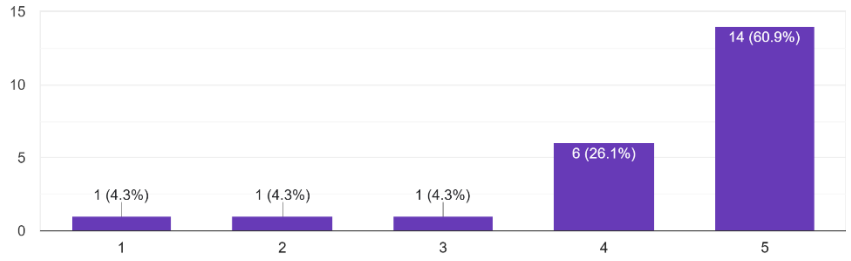
SESSION II: Control and Surveillance of fish diseases in Europe- relevance for you

23 responses



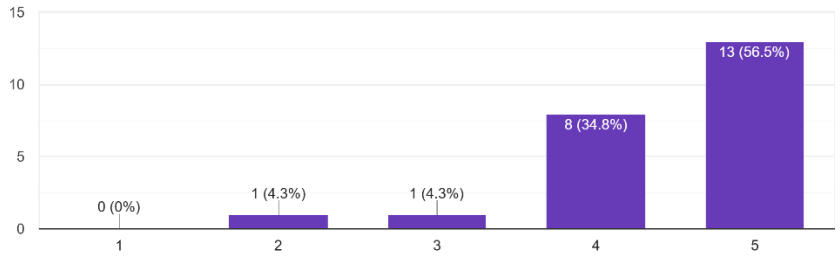
SESSION II: Control and Surveillance of fish diseases in Europe- increase of your knowledge

23 responses



SESSION II: Control and Surveillance of fish diseases in Europe- overall score

23 responses

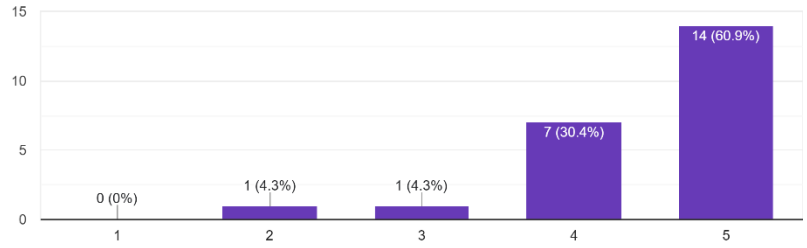


SESSION II: Control and Surveillance of fish diseases in Europe- comments

- Relatively low news value, Data regarding the EU audit in Germany and Denmark would be welcome. The illustrations and font were too small in most of the presentations.
- Diagnostic relevant

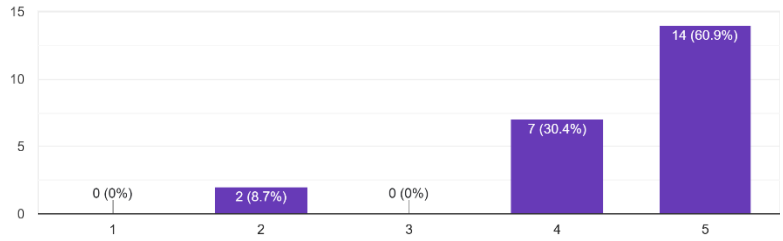
SESSION III: Results from ongoing research on listed and emerging fish diseases-quality of the presentations

23 responses



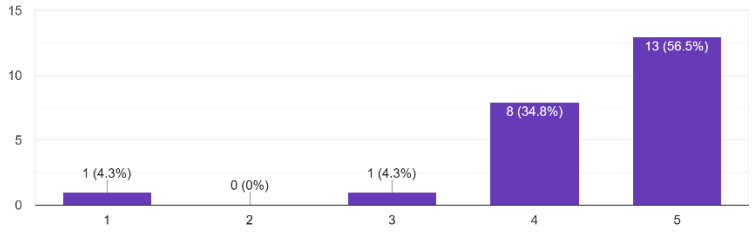
SESSION III: Results from ongoing research on listed and emerging fish diseases-increase of your knowledge

23 responses



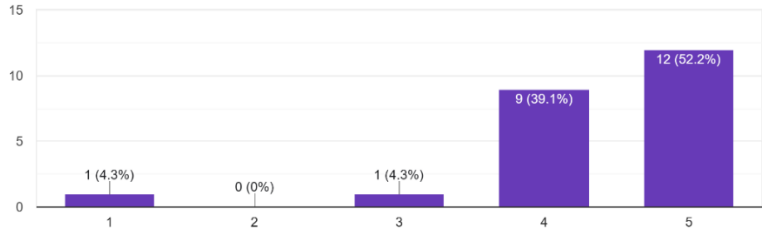
SESSION III: Results from ongoing research on listed and emerging fish diseases-relevance for you

23 responses



SESSION III: Results from ongoing research on listed and emerging fish diseases-overall score

23 responses

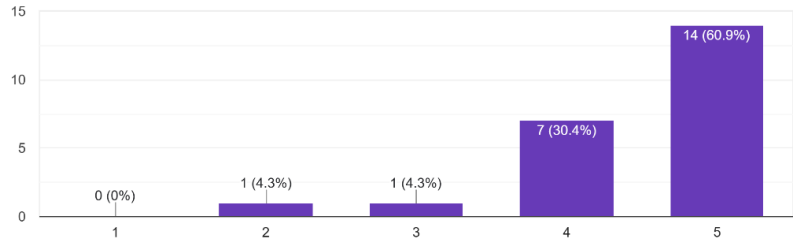


SESSION III: Results from ongoing research on listed and emerging fish diseases- comments, inputs

- Relatively low news value
- The information from Faroes was great

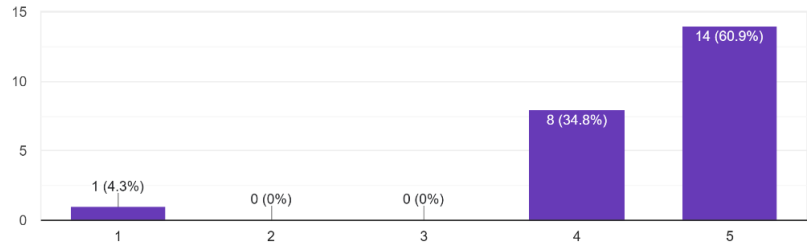
SESSION IV: Update from the EURL for fish diseases- quality of the presentations

23 responses



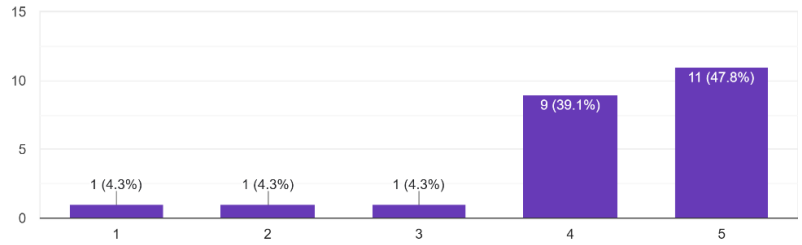
SESSION IV: Update from the EURL for fish diseases- relevance for you

23 responses



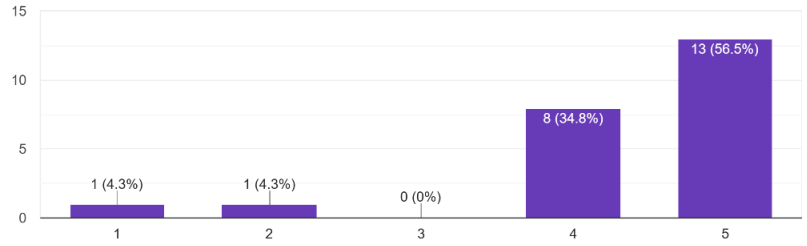
SESSION IV: Update from the EURL for fish diseases- increase of your knowledge

23 responses



SESSION IV: Update from the EURL for fish diseases- overall score

23 responses



SESSION IV: Update from the EURL for fish diseases- comments, inputs, remarks

- Very interesting meeting overall
- The illustrations and font were too small in most of the presentations.
- General comment: Please space the breaks out more evenly. Most of the breaks in the morning where just 1 hour before lunch.

Greetings and conclusions of the meeting

The tentative date for the next meeting will be the end of May 2026. It will be organized as a online meeting. Thanks a lot, to the people arranging the meeting as well as those of you who helped running the meeting by being chair, presenter and/or participant.

We are looking forward to seeing you all next year!

With kind regards,

The EURL fish and crustacean team.