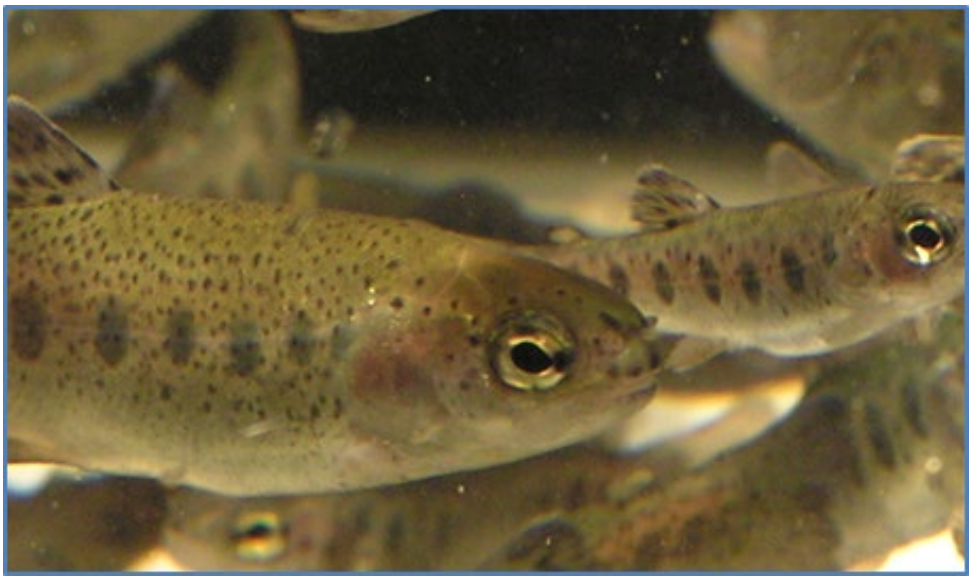
European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Report of the

28th Annual Workshop of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark

29th of May 2024



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 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.

The workshop was held back-to-back with the 15th 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 2023 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 2023.

The session continued with two presentations covering relevant topics in the field of fish diseases, covering the ISA outbreak in Iceland given by Árni Kristmundsson NRL of Iceland and next Britt Bang Jensen gave an presentation on the IHN surveillance and control in Denmark.

After a coffee break, we continued with session II Control and Surveillance of fish diseases in Europe. This session consisted of four talks. The first presentation were given by the representative of the Faroe Food and Veterinay Office, Maria Marjunardóttir Dahl, that presented the Surveillance and phylogenetic analysis of PMCV circulating in Atlantic salmon in Faroe Island.
Afterwards, the representative of Finnish Food Authority, Tuija Kantala, gave a presentation of the IHN outbreaks in Finland. The last presentation was on rapid differentiation of ISAV HPR0 from ISAV HPRΔ by multiplex RT-qPCR, given by Argelia Cuenca from the EURL.

After lunch break, we continued with session III results from ongoing research on listed and emerging fish diseases.
The first presentation was given by researcher Dagoberto Sepulveda describing susceptibility of Atlantic salmon to European IHNV isolate. Then Jacob Günther Schmidt, presented a summary of 8 years of experiments at DTU, with Red mark syndrome. Next presentation was on Detection of a betanodavirus in seahorses, given by representative of the Australian Centre for Diseases Preparedness, Nick Moody. Subsequently, Laurent Bigarré from ANSES, gave a presentation on reaching the Peak in the detection of fish pathogens.

The session was finished with a presentation from IZSVe given by Andrea Marsella describing how temperature influence on replication and virulence of European Infectious Hematopoietic Necrosis Viruses.

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 2024 were advertised. A resume of the Interlaboratory Proficiency test 2023 was presented by Teena Vendel Klinge, 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 2023 were presented and proposals for the EURL work plan for 2024 and 2025 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 2025. 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

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

9:30 – 9:45 Welcome and announcements
Britt Bang Jensen

SESSION I: Update on important fish diseases and their control

Chair: Britt Bang Jensen and minutes: Jacob Schmidt

9:45 – 10:15 Overview of the disease situation in Europe
Niccolò Vendramin

10:15 – 10:35 Overview of the disease situation in Norway
Torfinn Moldal

10:35 – 10:55 Update on ISA outbreak in Iceland and control
Árni Kristmundsson

10:55 – 11:15 Update on IHN surveillance and control in Denmark
Britt Bang Jensen

11:15 – 11:30 Coffee break

SESSION II: Control and Surveillance of fish diseases in Europe

Chair: Niccolò Vendramin and minutes: Argelia Cuenca

11:30 – 11:50 Surveillance and phylogenetic analysis of PMCV circulating in Atlantic salmon in Faroe Island
Maria Marjunardóttir Dahl

11:50 – 12:10 Development and application of a whole genome amplicon sequencing method of infectious salmon anemia virus (ISAV)
Bjørn Spilsberg

12:10 – 12:30 Update on control of the 2021 and 2022 IHN outbreaks in Finland
Tuija Kantala

12:30- 13.15 Rapid differentiation of ISAV HPR0 from ISAV HPRΔ by multiplex RT-qPCR
A.Cuenca

13:15 – 13:55 Lunch

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

Chair: Argelia Cuenca and minutes: Anna Luiza Farias Alencar

13:55 – 14:15 Susceptibility of Atlantic salmon to European IHNV isolate
Niels Lorenzen

14:15 – 14:35 Red mark syndrome: A summary of 8 years of experiments at DTU
Jacob Günther Schmidt

14:35 – 14:55 Detection of a betanodavirus in seahorses.
Nick Moody

14:55 – 15:15 No mix preparation, no freezer and a qPCR in 20 min: reaching the Peak in the detection of fish pathogens
Laurent Bigarré

15:15 – 15:35 Temperature influence on replication and virulence of European Infectious Hematopoietic Necrosis Viruses
Andrea Marsella

15:35-15:45 Coffee break

SESSION IV: Update from the EURL for fish diseases

Chair: Niccolò Vendramin minutes: Thomas Weise

15:45-15:55 EURL Training Courses. Topics and organization of courses 2024
Argelia Cuenca

15:55-16:15 Interlaboratory Proficiency test for fish diseases 2023
Teena Vendel Klinge and Niccolò Vendramin

16:15-16:35 EURL Work done in 2023, ongoing activities in 2024, plan for 2025
Niccolò Vendramin and Britt Bang Jensen

Next meeting and end of 28th Annual Workshop
Niccolò Vendramin and Britt Bang Jensen

End of fish 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 2023

Niccolò Vendramin

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Abstract
The questionnaire on Survey and Diagnosis of the listed fish diseases in Europe (S&D) for 2023 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 website (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 2022

The most update data on aquaculture production in Europe refer to 2022 on the website of Federation of European Aquaculture producers (draft produced by FEAP secretariat on December 2023). 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 2022 and is now at 2,865,072 t. The total production of EU countries had minimal increase from 2021 where production was 534,143 tons to 535,103 tons.

The 5 non-EU countries Iceland, Faroe Islands, Turkey, UK and Norway produced 2,329,969t and experienced a a slight reduction since 2021.

Number of fish farms in Europe

The total number of authorised/licensed fish farms in Europe is estimated in about **34676** 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

Germany provide the largest contribution when it comes to fish farm with 13453 farms, while the second contributor is Poland with 6020. In both cases, this high number of farms reflects a large number of small size farms.

When it comes to production, Norway has by far the largest production in Europe and has licensed 1179 farms/sites.

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 2023, a health status was assigned to 13924 farms with susceptible species to VHS, to 13761 farms with susceptible species to IHN, to 6556 farms with susceptible species to ISA.

Health status for VHS, 18% of fish farms are approved disease free; 1% is under eradication/control program; 11% under voluntary program; 70% is not approved disease free and not under eradication/control program

Health status for IHN, 17% of fish farms are approved disease free; 1% is under eradication/control program; 12% under voluntary program; 70% is not approved disease free and not under eradication/control program

Health status for ISA (Infection with HPRΔ ISAV), 68% of fish farms are approved disease free, 0% is under eradication/control program, 1% under voluntary program, 31% is not approved disease free and not under eradication/control program

Outbreaks of listed diseases in Europe
For **VHS**, 30 new outbreaks were reported in Europe in 2023, 10 in Germany, 6 of these were in Switzerland, the rest occurred in Romania (2), Poland (4), Austria (3), Latvia (1), Italy, (2), France (1), Czech republic (1).

For **IHN**, 48 new outbreaks were reported. The majority was in the Republic of North Macedonia (29), followed by Germany (21), Denmark (9), Belgium (6), Italy (2), Switzerland (1) and Austria (1).

For **ISA** (Infection HPRΔ ISAV) Norway reported 22 confirmed cases. A specific talk on the topic will be provided at the 26th Annual Workshop for National Reference Laboratories for Fish Diseases.

For **KHVD**, 47 outbreaks were reported in 2023, 29 in Germany, 6 in Hungary, 3 in Czech Republic, Denmark and Austria..

Other fish diseases problems in Europe
A whole range of other disease problems in 2021 were reported:

- In **rainbow trout** the major concerns remain flavobacteriosis (RTFS), Enteric Redmouth Disease (*Y. ruckeri*) with a concern for biotype 2, Bacterial kidney disease (BKD), Infectious Pancreatic Necrosis (IPN). Emerging concern has been attributed to Nodular gill disease and Lactococcosis. An unexplained syndrome in RAS farmed Rainbow trout was investigated in Denmark.
- In **salmon** farming the major concern is sea lice; after the ectoparasite, a number of disease problems cause concerns and include pancreas disease, heart and skeletal muscle inflammation, cardiomyopathy syndrome, infection with *Piscirickettsia salmonis*.
- In **Cyprinid,** it is primarily CEV.
- In **seabass** and **seabream,** the main challenges remain, *Photobacterium damselae* subsp. *Piscicida*, tenacibaculosis, *Vibrio harvey*, *Sparicotyle chrysophrii* infection, the first outbreak of Lactococcosis has also been reported.

Overview of the disease situation in Norway

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Abstract

The 'Norwegian Fish Health Report' has been published by the Norwegian Veterinary Institute annually since 2003. The report focuses on health and welfare of farmed fish, but wild fish are also included. 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 the majority of 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 2023 in Norwegian was published in March, and the English version is expected to be published in late May on https://www.vetinst.no/rapporter-og-publikasjoner/rapporter.

The main species in Norwegian aquaculture is Atlantic salmon. In 2023, approximately 307 million smolt were transferred to sea and more than 62 million farmed salmon died during the seawater phase of the production. The probability of a fish dying during the year was 16.7% at national level. However, there is great variation between the thirteen production areas with highest probability in the western part of the country. The main reasons for mortality are reported by fish health personnel to be mechanical injuries after delousing operations, infections caused by *Moritella viscosa* and *Tenacibaculum* spp., complex gill disease, CMS, HSMI, wounds, pasteurellosis and jelly fish attacks.

Among the notifiable diseases, infectious salmon anemia (ISA) was confirmed at 18 sites in 2023. Additionally, ISA was suspected at five sites, all of which were emptied of fish by the end of the year. A significant portion of the outbreaks and suspicions were on the west coast. ISA was confirmed at one land-based facility with brood-stock, while the remaining outbreaks were at sea sites. There are several cases of likely transmission from nearby sites, but there were no extensive epidemics or spread of infection caused by the same virus variant in 2023. In one instance, transmission may have occurred via well-boat, and several outbreaks can be linked to detections of ISAV HPR0 at the smolt facility or post-smolt facility that had supplied fish to the outbreak sites.

At national level, there has been a notable decline in cases of pancreas disease (PD) in recent years. In 2023, there were 58 cases compared to 98 cases in 2022. However, four PD outbreaks caused by salmonid alphavirus 2 (SAV2) outside the endemic zone was concerning. Rapid emptying of the sites was done with the aim of preventing further spread of the infection.

A risk-based surveillance programme for infectious hematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV) is in place, based on examination of samples submitted for routine diagnostic investigation. In 2023, brown trout at cultivation sites, rainbow trout at inland sites

as well as pink salmon captured in rivers in the very north of Norway were also included in the surveillance programme. Neither IHNV nor VHSV were detected in 2023.

Bacterial Kidney Disease (BKD) has been detected only sporadically the last 20 years. In 2023, BKD was detected at 12 sites, and suspicion was raised at additionally two sites based on PCR. Most of the sites are in Mid-Norway. The source has not been determined, but spread has likely occurred via well-boats as well as movement of fish.

Q&A

Q: Cat. G. What does that mean?
A: Based on legislation. National list. Reportable, but no measures taken.

Q: So equivalent to EU cat. E?
A: Well, it is not dependent on surveillance.

Q: Mycobacteria: Any connection between the cases. They are widespread.
A: No epidemiology studies. More cases on the west coast (area 3), but also area 10 up north.

Q: Did you check the mycobacterial species?
A: Not always typed to species.

Q: BKD: Appears a minimal problem in Norway compared to Iceland. I am puzzled about that. Do you have data on prevalence of BKD in wild populations? It is everywhere here in Iceland.
A: There have been few detections in wild fish. And mostly old detections. Generally limited knowledge of diseases in wild stocks. But could be overlooked in both farmed and wild fish.

Update on ISA outbreak in Iceland – reaction and preventive measures

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Abstract
In late November 2021, increased and unexplained mortality was observed in Atlantic salmon in one sea cage in Reyðarfjörður, in the Eastfjords of Iceland. Based on macroscopic clinical signs, suspicions arose of a notifiable disease. Samples were sent to the NRL in Iceland (Keldur) and ISAV-HPR-del was confirmed in these fish, which was the first time the pathogenic strain of the virus was observed in Iceland. Immediate reactions included a formal distribution ban, and a formation of an emergency slaughter plan as well as extensive ISAV-screening at other farming sites in Reyðarfjörður and nearby fjords. Following 4 months of extensive screening, ISAV-del was observed in April and May 2022, at two farming sites and Reyðarfjörður, and furthermore in Berufjörður, a nearby fjord. Phylogenetic analyses strongly suggested a primary outbreak in Reyðarfjörður, i.e. originating from a mutation/deletion in a wild-type/native strain of ISAV-HPR0. The later outbreaks in April and May proved to be secondary outbreaks, originating from the same original source.

Following this first ISAV outbreak in Iceland, the Minister of Food, Fisheries and Agriculture formed a working group of specialists which aims was to: (1) review the current legislation and regulatory framework in Iceland, in terms of reactions and prevention of serious fish diseases in aquaculture; (2) review legislations and regulatory frameworks in other countries, particularly in Norway and the Faroe Islands, and (3) weigh and assess whether there was a need for a changed approach, and if such changes were needed, come up with suggestions in that respect, i.e. how reactions and preventive measures related to serious diseases could be improved. The committee submitted the work to the Ministry in February 2023, which agreed with the committee´s suggestions. At present, a bill for a new wholistic law on aquaculture is under discussion in the Icelandic parliament - Alþingi. The chapters in these proposed laws concerning reactions and preventive measures of diseases, are based on the recommendations of the aforementioned working group. Although the laws have still not been accepted in the parliament, many farming companies have already started to employ some the suggested improvements.

In the presentation, the ISAV outbreak in Iceland will be reviewed in a broad sense, and present status and future plans for biosecurity in Icelandic aquaculture discussed.

Q&A

Q: Were the problematic algal blooms mainly diatoms?
A: (not answered)

Q: Did you look for ISAV in wildlife?
A: Have not looked at wildlife populations in the vicinity of the outbreak. Almost no wild salmon in the area – only sea trout and char.

Q: Are the farmers vaccinating for ISA?
A: Will probably mandatory. On the east coast they have started vaccinating all fingerlings.

Update on IHN surveillance and control in Denmark

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Abstract

Since the first outbreak of IHN in Denmark in May 2021, there has been a total of 39 cases of IHN. The majority of these (25) has been in freshwater farms, 10 have been in marine farms, and 4 in lakes and put&take lakes. The incidence in freshwater farms has been stable in the period 2021-2023, with approximately 4-5% per year. All outbreaks in marine farms were detected in 2022.

About half of the cases have been diagnosed based on initial clinical suspicion. 9 cases were diagnosed when investigating contact farms. The rest of the cases were found during surveillance and screening programs.

Since 2022, the Danish Aquaculture Producers Organization (DAPO) have coordinated a voluntary screening program, where all farmers have taken part, with only a few exceptions. Within this program, screenings for IHNV by PCR have been performed every spring and fall. This has lead to a good overview of the prevalence of IHNV in Denmark, and can perform a base for planning of a control and eradication program.

A small-scale study conducted in 2022 showed that the mortality was low in marine farms, and high in some freshwater farms, consistent with the disease normally affecting mostly young fish.

Q&A

Q: There has been an IHN outbreak in Germany after delivery of declared disease-free fish from Denmark. Enquiries to the relevant authorities remain unanswered. What can we do to prevent or clarify such incidents?
A: If the transports are not using TRACES, this is a case for the competent authorities. We can talk to our national CA, based on dialogues between the NRLs when there are ongoing outbreaks

SESSION II: Control and Surveillance of fish diseases in EU

Chair: Niccolò Vendramin

Surveillance and phylogenetic analysis of PMCV circulating in Atlantic salmon in the Faroe Island

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Abstract

Although increasing number of CMS outbreaks have been recorded in the last decade, there is only one publicly available whole genome and currently no way of cultivating the virus. Hence, understanding PMCV transmission mode and route is fundamental for proper management and mitigation strategies in salmon farming. Here we present a fast amplicon-based whole genome sequencing method of PMCV directly from field samples.

This study is based on a broad spatiotemporal representation of PMCV positive samples from Faroese salmon farming. Samples originating from 22 production sites in the Faroe Islands and from a returning wild salmon were collected for disease surveillance purposes by the authorities and the farming companies over a period of 12 years. Whole genome sequences were obtained, and phylogenetic analyses found PMCV to be highly homogeneous and revealed a monophyletic Faroese cluster comprising samples originating from farmed salmon. The genome obtained from the returning wild salmon differed significantly from samples from the Faroe Islands, Norway, and Ireland. To set the phylogenetic data in context, information on roe and smolt origin, sampling site and date, Ct values and CMS clinical signs was collected for all samples in this study. Combined, the phylogeny and metadata show no continuous reintroduction of PMCV to Faroese farmed salmon and no evidence of vertical transmission being the main transmission mode. Furthermore, the results show no apparent correlation between assigned CMS cases and potential virulence markers.

Q&A

Q: We need to be careful with the legislative aspects of detection of virus in water. For the EU it is mandatory to use fish tissue for pathogen surveillance. Have the veterinary authorities in the Faroe Islands agreed to use eDNA/eRNA for screening?
A: This is a voluntary screening that the farmers have adopted

Q: So, the pathogen presence in the eDNA(eRNA) will prompt tissue sampling on the farm?
A: Yes, we will take tissue samples before declaring the farm as infected

Discussion: There is some debate about the vertical transmission of PMCV. Although we cannot say that it is not happening, this does not seem to be what we see in the phylogenetic trees of PMCV in the Faroe Islands.

Development and application of a whole genome amplicon sequencing method of infectious salmon anemia virus (ISAV)

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Abstract
Infectious salmon anemia (ISA) is an infectious disease in farmed Atlantic salmon, *Salmo salar*, caused by the infectious salmon anemia virus (ISAV). ISAV belongs to the *Orthomyxoviridae* family. The disease is a serious condition that can lead to reduced fish welfare and high mortality. In this study we designed an amplicon based sequencing protocol for whole genome sequencing of ISAV. The method consists of eighty ISAV specific primers that cover 92% of the virus genome and was designed to be used on a Illumina MiSeq platform. The sequencing accuracy was investigated by comparing sequences with previously published Sanger sequences. The sequences obtained were nearly identical to those obtained by Sanger sequencing, thus demonstrating that sequences produced by this amplicon sequencing protocol had an acceptable accuracy. The amplicon based sequencing method was used to obtain whole genome sequence of 12 different ISAV isolates from a small local epidemic in the northern part of Norway. Analysis of the whole genome sequences revealed that segment reassortment had taken place between some of the isolates and could identify which segment that had been reassorted.

Q&A

Q: Two slightly different genomes have been found in the Galsvær isolate. Is this an artifact?
A: We sampled the same fish from the original Galsvær sequence, and we have 9 SNPs in the comparison, so we believe is an artifact.

Q: Are some of those SNPs in the primer binding sites? So, that would indicate that they are an artifact.

Update on control of the 2021 and 2022 IHN outbreaks in Finland

Tuija Kantala

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Abstract

In 2021, IHNV was detected in rainbow trouts in five fish farms, and in 2022, in rainbow trouts in one fish farm in Åland Islands. In 2021, the infection had spread from Denmark via fish movements to two sea-cage fish farms in Åland Islands during the spring. IHNV was detected in these two farms on May 26th and June 1st. Epidemiological investigation was carried out to find out where the infection had possibly been spread in Finland. A third rainbow trout sea-cage farm, located approximately 2 km from the first two infected farms, was found positive on June 4th. The infection had probably spread there via water from the first two farms.

IHNV was further detected in two more farms in 2021: in the fourth farm on October 5th, and in the fifth farm, on October 22nd. These two farms located further (approximately 6–20 km) from the first three positive farms. It is not clear how the virus spread to these farms, but transmission especially via boat traffic was suspected.

In 2022, IHNV was detected in a RAS farm in Åland Islands on June 21st. Distance from the RAS farm to the farms that were positive in 2021 was 50–60 km, and it is not clear how the virus spread to this farm. The fish in the farm originated from IHN free compartments in Denmark, Estonia, USA (roe) and South Africa (roe). Transmission from the farms that were positive in 2021 was considered unlikely because there was no contact between them and the RAS farm, and the RAS water intake is deep in the sea, over 50 km from the closest previously positive farm.

A restricted zone was immediately established around each of the infected farms after detection of IHNV. All IHN sensitive fish from the infected farms were euthanized or slaughtered. A total of approximately 750 000 kg of rainbow trout were euthanized, and 950 000 kg were slaughtered. The farms and equipment from them were washed, disinfected, and after disinfection, a fallowing period of at least six weeks was carried out.

The eradication in the farms where IHN was detected in 2021 was completed in March 2022. In the end of April 2022, surveillance program of two years started in the surveillance zone which corresponded to the original restricted zone. All surveillance samples tested negative for IHNV, and the zone was declared officially free of IHN on March 12th, 2024.

The eradication in the farm where IHN was detected in 2022 was completed in early January 2023. A surveillance program of two years started in the surveillance zone which corresponds to the original restricted zone in the beginning of April 2023. Currently, Finland is officially declared free of IHN, with exception of this one surveillance zone (a coastal compartment), where surveillance program is ongoing. So far, all the surveillance samples have tested negative for IHNV. The zone is expected to regain an official IHN-free status in summer 2025.

Q&A

Q: Where was the rainbow trout used for restocking farms in Finland from?
A: I don’t have that information, but you can get the email of the people who can answer that

Q: Who paid for the surveillance of IHNV in Finland?
A: The government. The producers don’t pay themselves.

Q: It seems that there are 2 different outbreaks. have the isolates been sequenced?
A: Yes, and we will be talking with the EURL soon about it.

Q: We know that it is very difficult to disinfect RAS farms. Is there any kind of discussion or working group on who to do so?
A: Yes, there are people working in our office who have contacted experts abroad to discuss how to do this in the most effective way

Rapid differentiation of ISAV HPR0 from ISAV HPRΔ by multiplex RT-qPCR

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Abstract
The work presented here has been published by Rounsville et al. 2024 and it constitutes a collaborative effort from the University of Maine, the U.S. Department of Agriculture–Agricultural Research Service, and the EURL for Fish and Crustacean Diseases. Most of the essay development, optimization, and testing have been done by our colleagues in the USA.

'Infectious salmon anemia virus (ISAV; *Isavirus salaris*) causes an economically important disease of Atlantic salmon (*Salmo salar* L.). ISA outbreaks have resulted in significant losses of farmed salmon globally, often with a sudden onset. However, 2 phenotypically distinct variants of ISAV exist, each with divergent disease outcomes, associated regulations, and control measures. ISAV-HPRΔ, also known as ISAV-HPR deleted, is responsible for ISA outbreaks; ISAV-HPR0, is avirulent and is not known to cause fish mortality. Current detection methodology requires genetic sequencing of ISAV-positive samples to differentiate phenotypes, which may slow responses to disease management. To increase the speed of phenotypic determinations of ISAV, we developed a new, rapid multiplex RT-qPCR method capable of 1) detecting if a sample contains any form of ISAV, 2) discriminating whether positive samples contain HPRΔ or HPR0, and 3) validating RNA extractions with an internal control, all in a single reaction. Following assay development and optimization, we validated this new multiplex on 31 ISAV strains collected from North America and Europe (28 ISAV-HPRΔ, 3 ISAV-HPR0). Finally, we completed an inter-laboratory comparison of this multiplex qPCR with commercial ISAV testing and found that both methods provided equivalent results for ISAV detection.'

Thomas F. Rounsville Jr, Mark P. Polinski, Alyssa G. Marini, Sarah M. Turner, Niccolò Vendramin, Argelia Cuenca, Michael R. Pietrak, Brian C. Peterson, Deborah A. Bouchard. 2024. Rapid differentiation of infectious salmon anemia virus avirulent (HPR0) from virulent (HPRΔ) variants using multiplex RT-qPCR. Journal of Veterinary Diagnostic Investigation, 36(3) 329–337. https://doi.org/10.1177/10406387231223290

There was an interesting discussion on this assay, which focused on the level of validation of the methods, its pros and cons. It was proposed that NRLs working routinely with surveillance for ISAV implement it to assess its performances. Further the EURL suggested that a specific proficiency test could be implemented to compare the two assays.

General discussion of the session

Question to the EURL: we would like to ask the EURL if there is some mechanism for you to help us get information from the Veterinary Authorities and work on how to prevent the spreading of IHNV in Europe.

For example, the Veterinary Authorities are asking what to do because they receive fish with no symptoms that later start dying of IHNV/VHSV.

Answer: it is the competent authorities who are responsible for seeing that, farms, producers, transport, etc. are living up to what is expected in the legislation, not us as NRLs (or EURL).

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

Chair: Argelia Cuenca

Susceptibility of Atlantic salmon to European IHNV isolate

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Abstract

In 2021, infectious haemorrhagic necrosis virus (IHNV) was detected in Denmark for the first time. Initially, the primary concern was its impact on Danish rainbow trout production, but there is also a potential risk of the virus being accidentally transmitted to Atlantic salmon populations in Denmark and/or in neighbouring countries. Based on the experience with devastating outbreaks of IHN among farmed Atlantic salmon in British Columbia, Canada, it would be important to determine whether the Danish IHNV variant is virulent to this species.

To address this question, a pathogenicity study was conducted using the Danish IHNV isolate (obtained from diseased rainbow trout) for the challenge of both rainbow trout and Atlantic salmon, employing different challenge models: immersion and injection. The initial results confirmed the expected high virulence to rainbow trout; the survival rate was 17.3% for the immersion challenge and 2.3% for the injection challenge. On the other hand, for Atlantic salmon, the immersion challenge did not cause disease, while the injection challenge resulted in approximately 75% survival.

Following this initial study, the present research aimed to further evaluate whether an increased virus challenge dose as well as whether challenge by co-habitation would result in increased frequency of clinical disease in Atlantic salmon. A clear dose-dependent effect was seen among fish challenged by injection, with the highest virus dose resulting in 40-70% mortality. Interestingly, approx. 20% of the corresponding cohabitant fish developed clinical disease, whereas this happened for only 5% of the fish exposed to a high virus dose by immersion.

In order to address whether the virus isolated from the naturally exposed diseased Atlantic salmon had gained virulence, a comparative co-habitation challenge trial in Atlantic salmon was performed with the virus passaged twice in Atlantic salmon as well as the parental virus isolated from rainbow trout. The passaged virus caused a faster onset of disease among injected and cohabitant naïve fish than the parental virus.

These findings stress that although IHNV derived from Danish rainbow trout has no/low immediate virulence to Atlantic salmon, it has the potential to quickly adapt and become more virulent to this fish species if given suitable conditions for such adaptation. This again underscores the need to keep a high-level control measures for IHNV in aquaculture, including exports/imports of live fish as keeping high hygiene standards for trucks and boats used for transporting fish.

Q&A

Q: Was the infectious dose the same for all experiments?
A: No, initially 105 and 104, then decided to increase dose and number of injected fish for the last experiment.

Q: What were the necropsy findings?
A: Did not see so much symptoms as the disease killed very fast. The survival rate was the main difference.

Red mark syndrome: A summary of 8 years of experiments at DTU

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Abstract

Red mark syndrome (RMS) is a skin disease only reported from farmed rainbow trout (*Oncorhynchus mykiss*). The disease is non-lethal but the conspicuous hall-mark pathology with large red skin lesions leads to down-grading in rainbow trout that are ready for the market (Schmidt et al., 2018).

In 2016 we established a cohabitation infection model of RMS, sourcing RMS-affected seeders from a commercial rainbow trout farm. At that time very little had been published regarding the disease, and what few studies existed used fish from natural outbreaks: Histological case definitions of RMS lesions had been defined (Oidtmann et al., 2013); few insights into RMS lesion immune responses had been gained (McCarthy et al., 2013); a likely causative agent (initially named *Rickettsia*-like organism (RLO), but later more often referred to as *Midichloria*-like organism (MLO)) had been discovered through 16S rDNA sequencing (Lloyd et al., 2008, 2011); and it was established that RMS was identical to strawberry disease described from the USA (Metselaar et al., 2010). There were also a few unpublished attempts at experimental infection by cohabitation as well as isolation and cultivation of the putative cognate pathogen (Matthijs Metselaar and Alexandra Adams pers. comm.).

The RMS cohabitation model of infection was maintained by regular addition of naïve rainbow trout, which subsequently became seeders for a new batch of naïve cohabitants. The infection model was discontinued in April 2024.

Through a number of experiments, we have used the infection model to learn more about the disease and the causative agent. We have published studies on the immune response (von Gersdorff Jørgensen et al., 2019), the effect of antibiotics (Schmidt et al., 2021), the association of MLO with the fish parasitic ciliate *I. multifiliis* (Pasqualetti et al., 2021) and the effect of temperature on RMS (Orioles et al., 2022). In addition, several studies are pending publication, but some of these have already been disseminated at conferences and workshops, including previous Annual Workshops of National Reference Laboratories for Fish Diseases. As yet unpublished studies include: effect of low temperature on RMS; firmly establishing the causative agent of RMS; susceptibility of brown trout to RMS; tissue tropism of MLO over the course of infection and development of pathology; shedding of MLO; development of protective immunity against RMS in fingerlings; effect of stress on RMS; effect of functional feeds on RMS; a more thorough dissection of the RMS immune response, and most recently ongoing attempts at whole genome sequencing MLO.

In the presentation I will try to gather the pieces to the puzzle that is RMS.

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Q&A

Q: Have you tried to use any antimicrobial compounds or phage treatment instead of antibiotic?
A: We don’t know much about the bacteria – only have 16s so we did not try it.

Q: Do you still use 16s PCR to verify?
A: Yes, a probe based PCR is being designed but it is not done yet.

Confirmation of infection of pot-bellied seahorses (*Hippocampus abdominals*) with betanodavirus

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Abstract

Over one month during 2022, twenty-nine 6–9-month-old pot-bellied seahorses (H. *abdominalis*), from a single tank of 2000 to 3000 animals in a breeding establishment exhibited clinical signs which included whirling, floating and inability to eat. Histopathological findings included prominent vacuolation in the grey matter of the brain and in the retina of the eye. Betanodavirus infection was detected by NNV RT-qPCR and confirmed by RT-PCR and amplicon sequencing. CPE typical of NNV was observed after 3 days in E-11 cell cultures after inoculaton with homogenates form brain and eye tissue. High throughput sequencing indetified both segments as the red spotted grouper nervous necrosis virus (RGNNV) genotype. RGNNV genotype of Betanodavirus has previously been detected in Australia in fish including Barramundi (*Lates calcarifer)* and Striped Trumpeter (*Latris lineata*). Immunohistochemistry using antibodies directed against the capsid protein of BNNV demonstrated intralesional virus antigen. This is the first case study describing the detection and confirmation of viral nervous necrosis in pot-bellied Seahorses (*Hippocampus abdominals*) in Australia.

Fish pathogens: reaching the PEAK of detection

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Abstract
Many fish infectious diseases are diagnosed using real-time PCR-based methods efficiently detecting an agent but implicating the preparation of mixes of reactants (often stored at -20°C) just before the reaction and a series of cycles during 1h-2h. We have tested a new technology based on disposable cartridges ('diaxxopods'), stored at room temperature, preloaded with all the amplification pathogen-specific reactants and adapted to a platform ('PEAK') that perform all the cycles in less than 45 minutes for RNA viruses and 20 minutes for DNA viruses. In preliminary assays with fish samples, the method has been successfully tested with three viruses of economic interest: sturgeon mimivirus-European, piscine reovirus (PRV3) and carp edema virus (CEV). Combined to a simplified nucleic acid extraction, this cheap and simple methodology has the potential to be used in the field (in farms) as a rapid on-site assay. It can speed up the detection of any pathogen, during a mortality event for instance, without the expedition to a specialized laboratory. The technology may also be used in laboratories, either in those with limited facilities or in those with very limited requests for testing specific pathogens. It can also speed up assays in routine researches: test the enrichment of virus during purification, confirm the positivity of a sample before genotyping, etc.

Q&A

Q: What is the oil used to cover the cartridge?
A: It is provided by the company.

Q: Have you tried to make dilutions of samples?
A: Not yet. We have tried different types of samples (tissues, swab etc).

Q: Have you tried to make serial dilutions to see the limit of detection?
A: There are only 5 wells in the cartridge, so it is difficult to test too many samples.

Temperature impact on replication and virulence of European Infectious Hematopoietic Necrosis Viruses

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Abstract

In recent years, Infectious Hematopoietic Necrosis is commonly recognized as one of the most relevant diseases threatening salmonid aquaculture in Europe. An *in vivo* assessment was performed by exposing rainbow trout juveniles to three different IHNV isolates originating from Italy and Denmark and showing differences in their *in vitro* temperature-related fitness. The trial provided evidences that IHNV can adapt to the environment by increasing its temperature-related fitness as well as being able to maintain its pathogenicity at warm water temperature. Interestingly, these features appears to be extremely variable and linked to the intrinsic characteristics of isolates. In the light of the climate change, these features increase the concern for the IHNV impact on salmonid aquaculture. Analysis at the peak of morbidity and of the survival curves showed that viral load in the coelomic organs reached a sort of upper limit in fish succumbing the infection regardless of the water temperature. Conversely, the temperature influenced the kinetics of the disease which proved to occur faster at warm water temperature. The mid-term evaluation of fish surviving the IHN outbreak following the *in vivo* trial suggested that the coelomic organs are more likely to test positive to RT-qPCR compared to the brain with low level of prevalence. In addition, the low level of detection observed generally correlated with negative viral isolation on cell culture meaning that IHNV RNA detected didn't correspond to viable particles.

Funded by the Italian Ministry of Health IZSVe RC 13/19 and by the Aquaexcel 3.0 TNA programme.

Q&A

Q: Does the high virulence isolate in high temperature correlate *in vivo* and *in vitro*?
A: Yes for one of the isolates but need to investigate more about it. Some of the isolates are very temperature sensitive. It is isolate dependent. It will be good to get the sequencing.

Q: When comparing the qPCR and peak of mortality there was more viral RNA than viral particles – is it possible that it is mRNA.
A: Yes, if you only do PCR you detect mRNA and also not viable viruses.

Q: Peak viral load is the same independent of the temperature or virus. Is the difference in the time to reach that point?
A: We have not done statistical analysis yet. Descriptively it seems similar, but we don't know yet.

SESSION IV: Update from the EURL

Chair: Niccolò Vendramin

EURL TRAINING COURSE FOR 2024

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Abstract
In 2024, two training courses 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 "Validation of diagnostic methods for disease diagnostic in aquatic animal health"

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, 2023

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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 2023.

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

42 laboratories participated in PT1 while 41 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
93% of the parcels were delivered by the shipping companies within two week and 100% was delivered within 22 days.

Overall, 39 out of 43 participants scored 100% success rate; out of the 4 laboratories which underperformed two participants scored <100% for the sole reason that they did not back up their concluding results of ampoule I (EHNV) with sequencing. 2 laboratories identified EHNV in another ampoule than the designated one, suggesting a contamination. Suggestions to improve on underperformance will be provided individually to each laboratory. One laboratory never handled in the result.

Résumé and concluding remarks PT2
43 laboratories participated in PT2, 39 obtained 100% success rate. Out of the 4 laboratories which underperformed, three obtained a lower score do to not providing sequencing-results for the ISAV isolate in ampoule IX. This point will be addressed directly with the participants that has underperformed. All 41 laboratories correctly identified the CyHV-3 (KHV) in ampoule VII.

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

42 laboratories correctly identified the ISA virus in ampoule IX, hereof three laboratories did not sequenced. One laboratory did not find the ISAV in this ampoule.

36 laboratories tested for SAV and 35 correctly identified the virus in Ampoule VIII, 7 laboratories did not test for SAV and one laboratory who tested for SAV did not find the SAV in this ampoule.

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.

EURL for Fish Diseases, work done in 2024

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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 27th Annual Workshop of the National Reference Laboratories for Fish Diseases was held in person, on 30th and 31st of May 2023.

The annual proficiency test for fish diseases (PT) was divided into PT1 and PT2 with 42 laboratories participating. The tests were sent from the EURL October 2023. 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 2023 and the EURL has focused on improving the diagnostic manual for EHN and KHV.

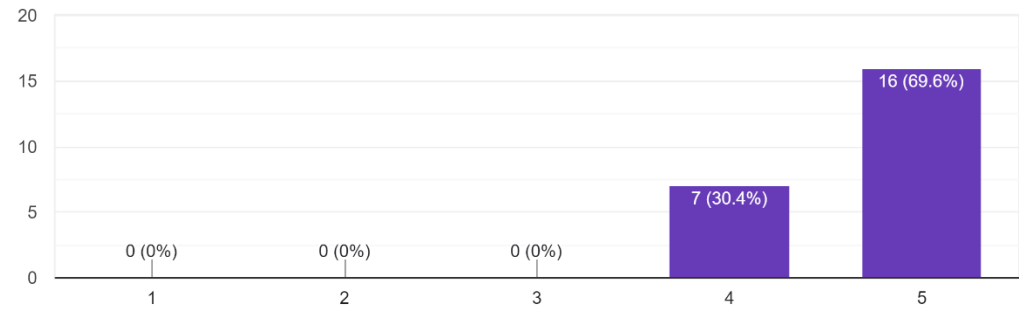
During 2023, resources were again used to collate data on surveillance, health categorisation and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to supply reference materials to NRLs; to provide training courses in 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 aspect of the workshop. An overview of the 23 questionnaires retrieved is shown below.

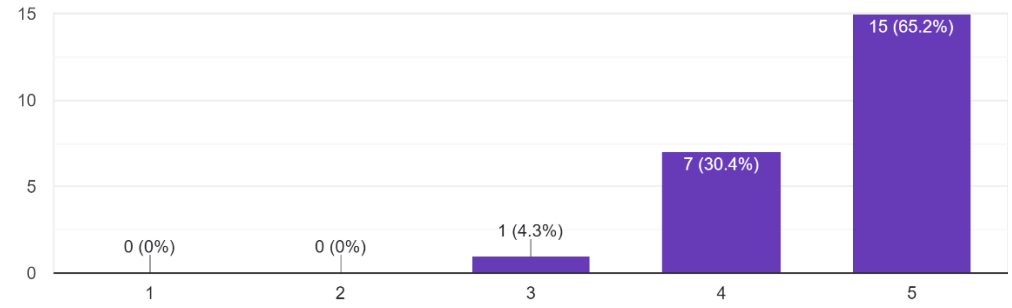
Session I: Update on important fish diseases and their control - Quality of presentations

23 responses



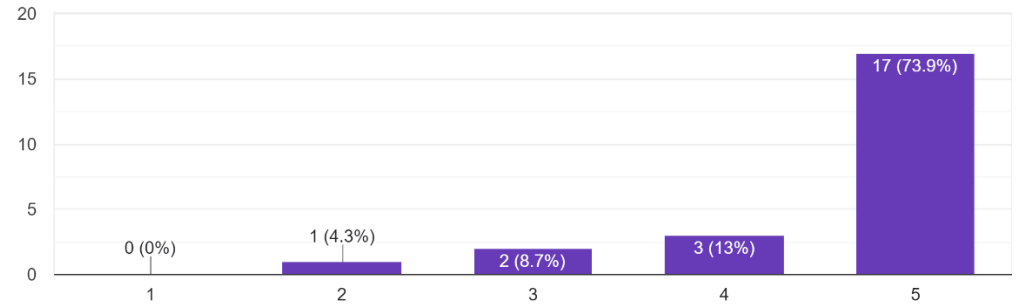
Session I: Update on important fish diseases and their control - relevance for you

23 responses



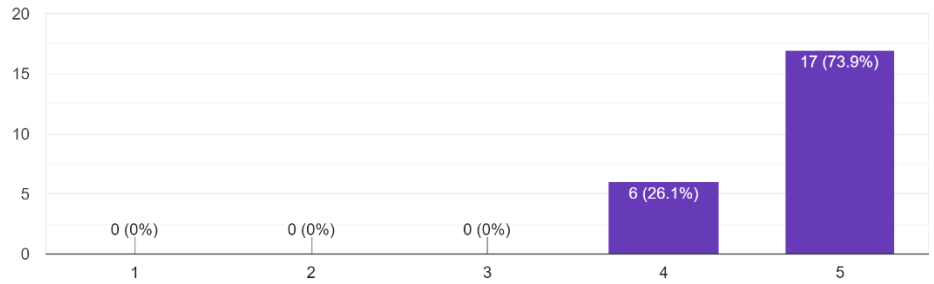
Session I: Update on important fish diseases and their control - increase of your knowledge

23 responses



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

23 responses

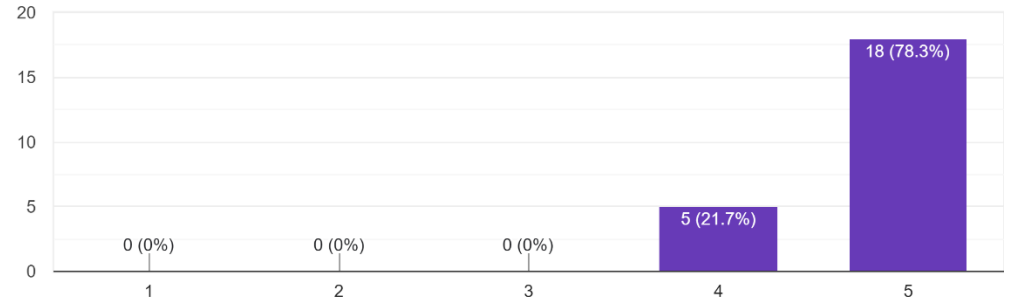


Session I: Update on important fish diseases and their control - comments, remarks, inputs

Informative to have this information summarised into one presentation each year.

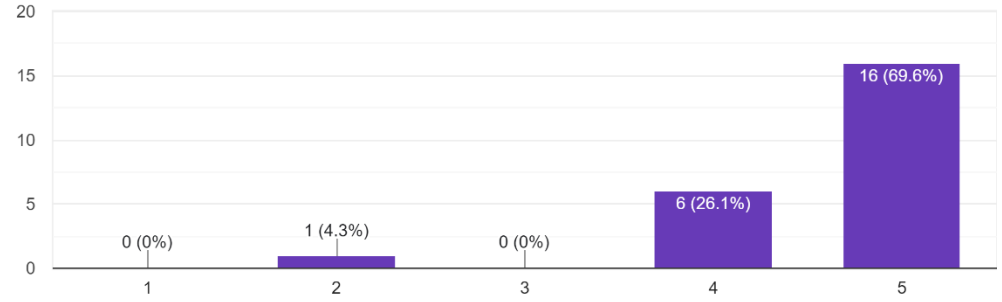
SESSION II: Control and Surveillance of fish diseases in Europe- Quality of the presentations

23 responses



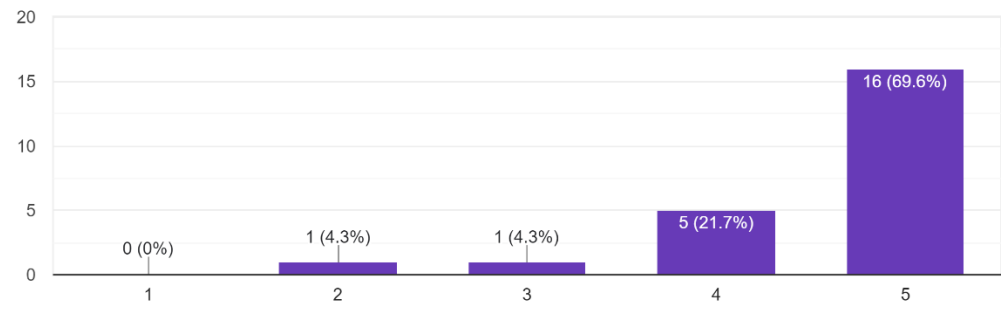
SESSION II: Control and Surveillance of fish diseases in Europe- relevance for you

23 responses



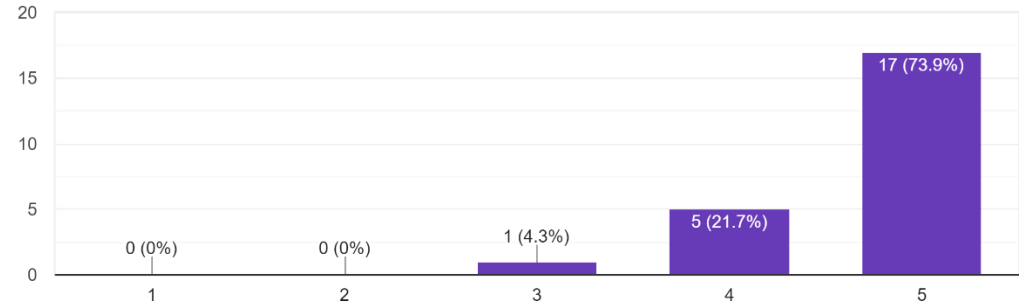
SESSION II: Control and Surveillance of fish diseases in Europe- increase of your knowledge

23 responses



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

23 responses

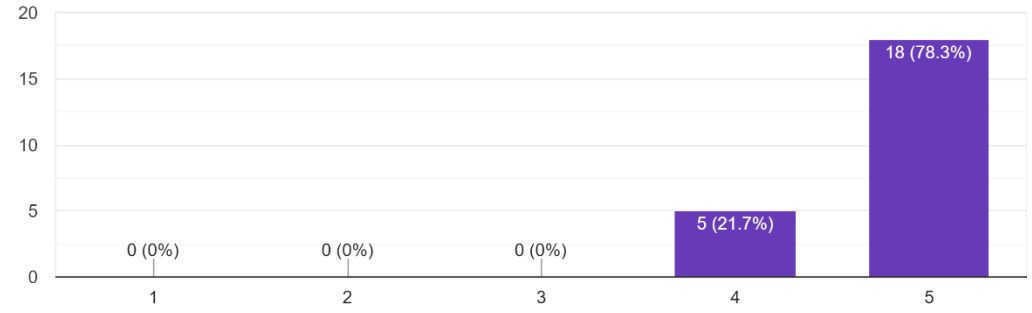


SESSION II: Control and Surveillance of fish diseases in Europe- comments, remarks inputs

Interesting presentations and good discussion of ISAV multiples RT-qPCR that has been developed. Loved the talks on PMCV and ISAV multiplex very applicable and interesting

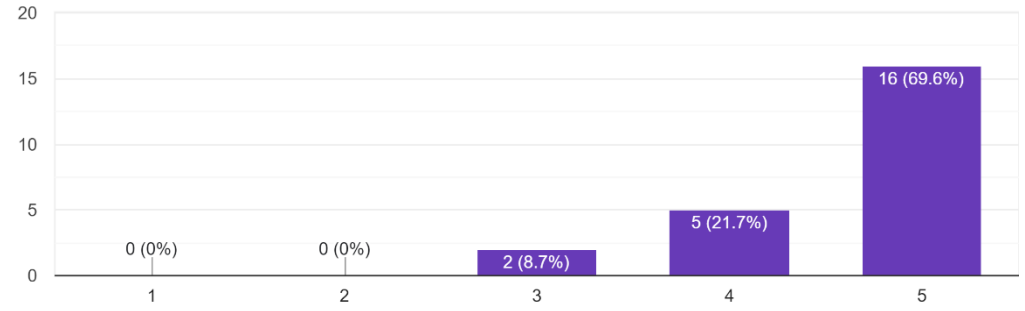
SESSION III: Results from ongoing research on listed and emerging fish diseases-quality of the presentations

23 responses



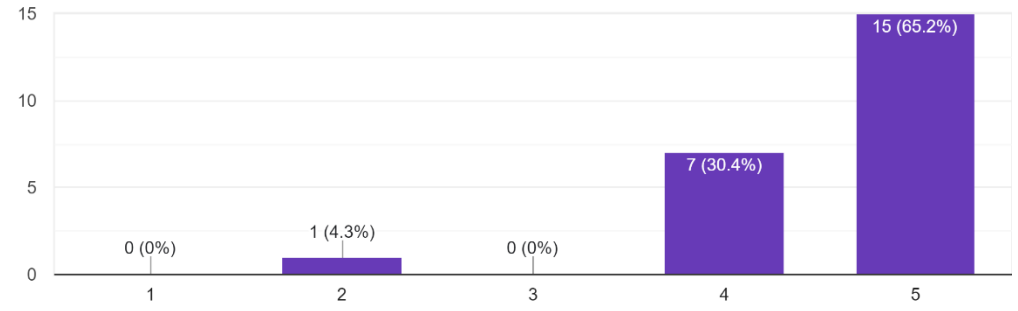
SESSION III: Results from ongoing research on listed and emerging fish diseases-increase of your knowledge

23 responses



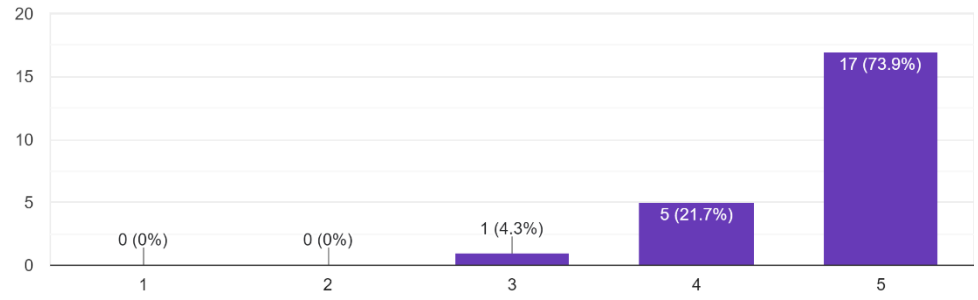
SESSION III: Results from ongoing research on listed and emerging fish diseases-relevance for you

23 responses



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

23 responses

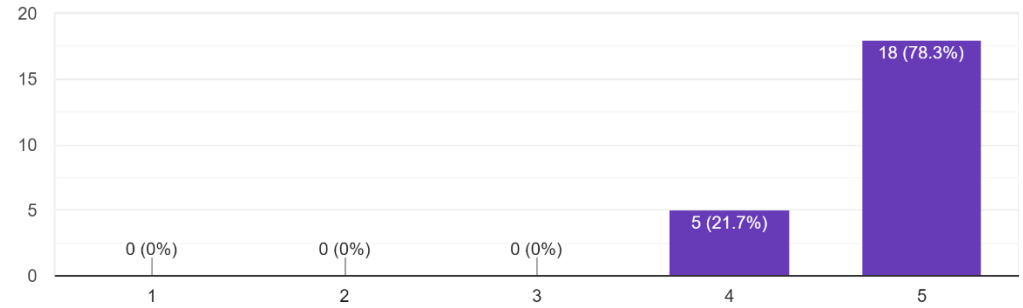


SESSION III: Results from ongoing research on listed and emerging fish diseases- comments, inputs, remarks

Great presentations on wide range of fish diseases
I didn't not find this sessions as useful or relevant as the others. The talk on seahorse was something new and added to the session

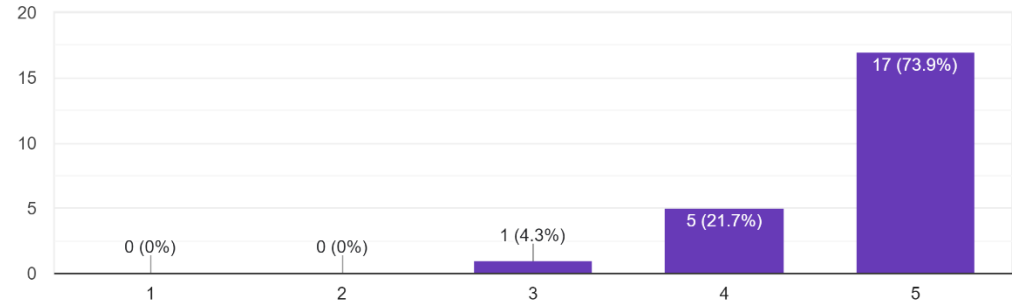
SESSION IV: Update from the EURL for fish diseases- quality of the presentations

23 responses



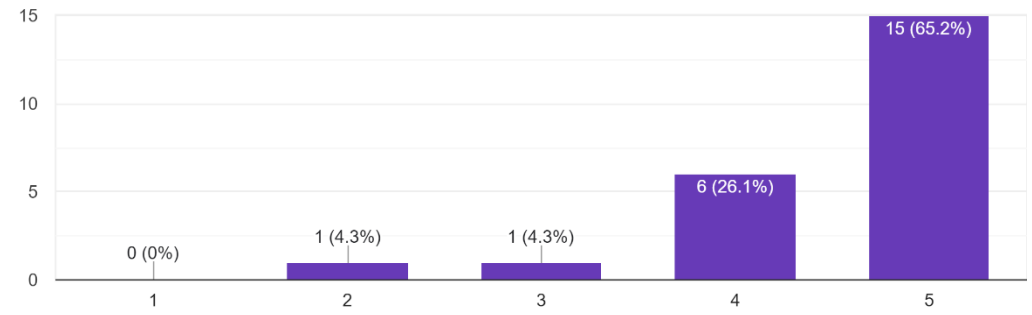
SESSION IV: Update from the EURL for fish diseases- relevance for you

23 responses



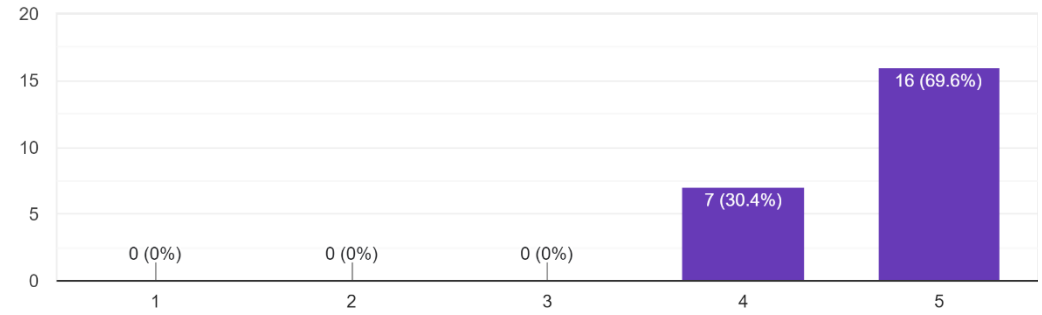
SESSION IV: Update from the EURL for fish diseases- increase of your knowledge

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

it is better to have the meeting in presence
Greatly appreciate the EURL providing the Ring test panels each year.
It hasn't been possible for me to attend this part of the meeting. But I have to give an answer to each question !
Useful information on upcoming courses.

Greetings and conclusions of the meeting

The tentative dates for the next meeting will be the in the end of May 2025. It will be organized as a on site physical meeting in Lyngby, Denmark. 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.