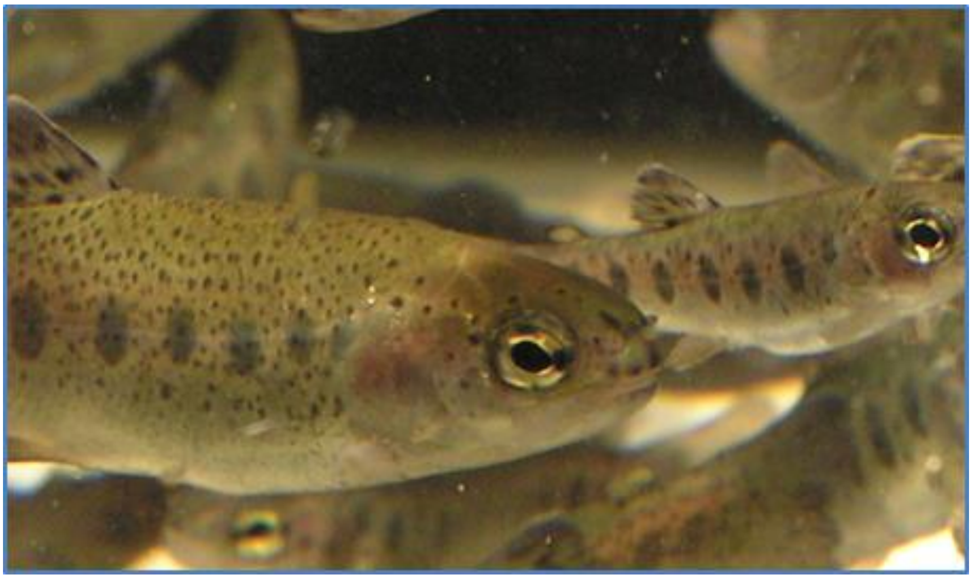
European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Report of the

25th Annual Workshop of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark

June 1st 2021



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 25th Annual Workshop of the National Reference Laboratories for Fish Diseases was held virtually on June 1st 2021.
Because of the Covid-19 pandemics and the limitation to travel to and from Denmark the workshop was held virtually using zoom platform.

The virtual organization of the meeting has allowed a significant expansion of the number of participants attending the workshop as well as the number of oversea countries participating.

The number of participants has reached 103 participants from 45 countries attending over the two days period. There were four sessions with a total of 14 presentations.

The workshop was held back to back with the 12th Annual Workshop for National Reference laboratories for crustacean diseases.

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 EURL for fish diseases, Niels Jørgen Olesen. The scientific part was opened with the traditional Session 1 "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 2020 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire. A report compiling all information is available at the EURL website https://www.eurl-fish-crustacean.eu/ .
Secondly, the fish disease situation in Norway was presented; a detailed report (currently in Norwegian, soon in English) is available at https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2021/fiskehelserapporten-2020
Due to the increased number of cases of ISA in 2020 in Norway, two presentations followed the overview of the fish health report 2020 in Norway. The first presentation held by Torfinn Moldal described the sequencing of ISA isolates in the attempt to trace source of infection. Afterwards Dr. Ole Bendik Dale provided insights in the pathogenesis of ISA and in the pathological picture that is observed in the outbreaks of 2020.
The session finished with a presentation on the screening program of CEV in Czech republic by Dr. Lubomir Pojezdal.
After a short break, session II Control and Surveillance of fish diseases in EU started.
This session consisted of four talks. The first presentation given by Dr. Marsella from IZSVe, and followed up on the presentation given at the AW 2019, the results of the field trial for testing DNA vaccination for preventing outbreaks of VHS and IHN in farmed rainbow trout in Italy were presented.
Afterwards, Dr. Shutze from FLI presented novel molecular RT-qPCR assays for surveillance of salmonid viruses VHSV, IHNV and IPNV.
Then, Dr. Toffan from IZSVe from Italy, presented the finding of a recent outbreak of VHS in Marble trout (*Salmo marmoratus*) in Italy.
Finally, prof. Niels Jørgen Olesen presented an update on the recent outbreak of IHN in Denmark.

After lunch break session III took place with presenting results from ongoing research on listed and emerging fish diseases.
The session started with an exhaustive presentation from Dr. Bergmann on pathogenesis of KHV infection in Common carp.
Then Dr. Laura Donati who recently finalized her PhD project at DTU Aqua, gave a talk on "prevention and control of infection with *F.psychrophilum* with bacteriophages". Finally Sofie Barsøe provided some further insights in the use of VLP based vaccine to prevent VNN outbreaks in European sea bass.

Afterwards, Session IV Update from the EURL for fish diseases took place.

In this session the EURL training courses scheduled for October 2021 were advertised. Furthermore the EURL activities in year 2020 were presented and proposals for the EURL work plan for 2021and 2022 were discussed. It was informed that the work plan will include tasks for both fish and crustacean diseases.

Employees from DTU Aqua took minutes from the meeting: Argelia Cuenca, Jacob Günther Schmidt, Niccolò Vendramin and Morten Schiøtt. Niccolò Vendramin assembled the report.

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 and meeting was organized by a team consisting of Morten Schiøtt, Niccoló Vendramin and Niels Jørgen Olesen, with the help from the rest of the fish disease section at the National Institute of Aquatic Resources, DTU AQUA. The meeting next year is tentatively planned to be held at end of May 2022, hopefully in a face to face meeting at DTU Aqua. More details will follow.

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

Niels Jørgen Olesen and Niccolò Vendramin

Programme

Tuesday June 1st

Annual Workshop of the National Reference Laboratories for Fish Diseases

9:30 – 9:40 Welcome and announcements

Niccoló Vendramin and Niels Jørgen Olesen

SESSION I: Update on important fish diseases and their control

Chair: Niels Jørgen Olesen and minutes: Morten Schiøtt

9:40 – 10:10 Overview of the disease situation in Europe

Niccoló Vendramin

10:10 – 10:30 Overview of the disease situation in Norway

Ingunn Sommerset

10:30 – 10:50 Molecular tracing of ISAV in Norway in 2020

Torfinn Moldal

10:50 – 11:10 Clinical and histopathological characterization of ISA outbreaks in Norway in 2020

Ole Bendik Dahle - Geir Bornø

11:10 – 11:30 Surveillance of CEV in Czech republic

Ľubomír Pojezdal

11:30 – 11:40 ***Coffee break***

SESSION II: Control and Surveillance of fish diseases in EU

Chair: Niccoló Vendramin and minutes: Jacob Schmidt

11:40 – 12:00 DNA vaccination field trial to contain VHS and IHN in rainbow trout farmed in freshwater in Italy

Andrea Marsella

12:00 – 12:20 Surveillance methods for infection with VHSV, IHNV and IPNV by qPCR in Germany

Heike Schütze

12:20 – 12:30	Viral Hemorrhagic Septicemia (VHS) outbreak in marble trout (*Salmo marmoratus*) *Anna Toffan*
12:30-12:40	First outbreak of Infectious Haematopoietic Necrosis (IHN) in Denmark *Niels Jørgen Olesen*
12:40- 13:10	*LUNCH BREAK*

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

Chair: Morten Schiøtt and minutes: Niccoló Vendramin

13:10 - 13:30	Update on KHV research *Sven Bergmann*
13:30 – 13:50	Prevention and control of infection with *F. psychrophilum* with bacteriophages *Valentina Laura Donati*
13:50 – 14:10	A virus like particle inducing protection in European sea bass against viral nervous necrosis *Sofie Barsøe*

SESSION IV: Update from the EURL for fish diseases

14:10 – 14:30	EURL Training Courses. Topics and organization of courses 2021 *Niccoló Vendramin and Tine Moesgaard Iburg*
14:30 – 14:50	EURL Work done in 2020, plan for 2021 and ideas and plans for 2022 *Niels Jørgen Olesen*
	Next meeting and end of 25th Annual Workshop *Niels Jørgen Olesen*

END OF FISH WORKSHOP

SESSION I: Update on important fish diseases and their control

Chair: Niels Jørgen Olesen

Overview of the fish diseases situation and surveillance in Europe in 2020

Niccolò Vendramin, Teena Vendel Klinge and Niels Jørgen Olesen

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Abstract

This report is based on the data from the questionnaire on Survey and Diagnosis of the listed fish diseases in Europe (S&D) for 2020. The Questionnaire 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 the health categorization according to Council Directive 2006/88/EC, 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 2020

The most update data on aquaculture production in Europe refer to 2019 both on the website of Federation of European Aquaculture producers and FAO Figis. We decided to refer to the same dataset of last year, provided by FEAP , it is expected that some changes in the production have occurred due to COVID-19 pandemics.
At global level, the pandemics is considered to affect significantly aquaculture production. According to FAO data, the Covid-19 pandemics have caused an estimated drop in overall output of 1.3 percent in 2020. Although this figure is yet to be confirmed, it would be the first annual decline in global aquaculture production in almost 60 years. It is expected that large variations occur across the production of different species.
The data available from FEAP report still refer to 2019, so this changes cannot be appreciated in the current report.

Number of fish farms in Europe

The total number of authorised/licensed fish farms in Europe was reported to be around **33260** farms, with the largest contingency in Germany with 10.813 farms having a high number of small producers. Compare to 2019 data there is an important decrease in number of fish farms in the coutnry, (13911 were reported in 2019). Possibly another effect of the Covid-19 pandemics on small producers. From Austria, the numbers of registered farms were included in the report for the first time, thus cumulating to 4862. The estimate number of fish farm is considered to be conservative. When it comes to production, Norway has by far the largest production in Europe and has licensed 1249 farms/sites. An overview of the number in each country can be found in Annex 1. It has to be ackowledged that it was not possible to retrieve the total number of active farms for all participants in the survey (2 missing).

Health Categorization of fish farms

Almost all Member States did reply to the questionnaire and provided very clear and correct answers.
In 2020, 11700 farms with species susceptible to VHS were reported in categorized zones, 11502 to IHN, 6.601 to ISA and 12578 farms with cyprinids susceptible to KHV.
69% of the authorised trout farms in Europe are situated in category III zones for VHS and 69% for IHN, with 27% respectively in Category 1 for both diseases. For both diseases the remaining of the farms are situated in category II, IV or V.

73% of the authorised farms in Europe with susceptible species for ISA are in category I, whereas 23% are in Cat. III (including 1042 farms in Norway and 34 farms in Faroe Island).
Only very few carp farms are approved KHV free in Category I (40 farms reported in Europe) and almost all are placed in Category III (94%) or in Category II 6%.
With the implementation of the new AHL law, a new categorisation system is to be set up, this will be reflected in the S&D report of next year.
For the time frame which this report refer to, Commission Decision 2015-1554 provide the guidelines for obtaining disease-free health statuses with regard to ISA and to contain infection with HPR deleted ISAV, saying that detection of isavirus HPR0 will not compromise the health status of a fish farm and is not notifiable to the EU (in contrast to OIE where detection of ISAV HPR0 is still notifiable). Some Member states do not include small registered APBs in the categorisation (e.g. hobby farms) but according to 2006/88/EC Annex III health categorisation comprise all APBs in the Member states, zones and compartments for each category. Only fish species listed as susceptible for the given listed disease shall be included in the categorization. Therefore important aquaculture species as sea bass, sea bream, meagre, eel and pike-perch are not included in the European health surveillance for specific diseases.
The new Animal Health Law is now adopted and includes all aquatic animals; in this connection the categorisation system will be simplified and be made more transparent on the other hand more lists will be adopted compared to the present lists of exotic and non-exotic diseases (from present 2 to 5 lists). Annex 2 provides the full list of farms in categorized zones.

Outbreaks and severity of listed diseases in Europe

Only few participants reported that they observed major changes in the epidemiological situations in their respective countries. For **VHS**, 11 new outbreaks were reported in Europe in 2020, 5 of these were in Germany. The remaining outbreaks were observed in Belgium (5), Czech Republic (1), France (2), Italy (1).
For **IHN**, 24 new outbreaks were reported. The majority was in Germany (21). The remaining outbreaks were in Croatia (1), Slovenia (1) and France (1)
For **ISA** Norway was the only country reporting outbreaks, and reported 23 new infection with ISAV HPRΔ in 2020. Due to the steep increase in number of outbreaks 2 specific talks on the topic will be provided at the 25th Annual Workshop for National Reference Laboratories for Fish Diseases.
For **KHVD**, 71 outbreaks were reported in 2020. The vast majority (42) in Germany, and England (11) in the latter case specifically in recreational fisheries and ornamental imports. The virus was reported also in Czech Republic (4), Hungary (4), Romania (2), Scotland (1), Croatia (5), Switzerland (1) and Denmark (1). Outbreaks of KHVD were reported from 9 countries in all. Annex 3 provides the full list of reports.

Other fish diseases problems in Europe

A whole range of other disease problems in 2020 were reported:

- In **rainbow trout** the major concerns remains flavobacteriosis (RTFS), Bacterial Gill disease (BGD), red mark syndrome, enteric redmouth (*Y.ruckerii*), Foruncolosis (*A.salmonicida*) and IPN, with an increase of clinical outbreaks.
- In **salmon** farming the major concern is sea lice; after the ectoparasite a number of disease problems cause concerns and includes pancreas disease, heart and skeletal muscle inflammation, cardiomyopathy syndrome, amoebic gill disease and complex gill disease CGD (amoebic gill disease, salmon gill poxvirus, *Paranucleospora theridion* etc.) and winter ulcers.
- In **Cyprinid** it is primarily CEV, SVCV, *Aeromonas hydrophila* and CyHV-2 in *carassius* species
- In **seabass** and **seabream** it is primarily VNN/VER, tenacibaculosis, *Vibrio harvey*, *Sparicotyle chrysophrii, Aeromonas veronii* and *Lernathropus kroyeri* infection.

The Health Situation in Norwegian Aquaculture 2020

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Abstract

The Norwegian Veterinary Institute publishes the annual 'The Health Situation in Norwegian Aquaculture" report. The report focuses on health and welfare of farmed fish, but wild fish are also included. The report covering 2020 was published 9th March 2021, and a short summary will be presented.

Mortality is a crude, but measurable, indicator of fish health and fish welfare. 52.1 million Norwegian farmed salmon were lost from production between sea-transfer and harvest during 2020, which is close to the "record" of 53.2 million in 2019. The calculated percentage mortality, based on monthly mortality rates, was 14.8% for farmed in 2020. However, regional differences were large ranging from 27.2% in production area 4 (west) to 6.7% in production area 13 (north). In contrast to the 2019 toxic algal bloom, we are not able to identify any particular event linked to the high mortality of farmed salmon in 2020. The losses of rainbow trout were similar to previous years.

Of the notifiable diseases, infectious salmon anaemia (ISA) was diagnosed in 23 new salmon farms in 2020. This is a clear increase in number compared to previous years (10-15 annual outbreaks). The number of farms affected by pancreas disease (PD) remains at a high level. With 158 new PD/SAV-positive locations in 2020, this represents a slight increase from 2019 (152).

For the first time, the Norwegian Veterinary Institute has gained access to data at the farm level for several non-notifiable diseases from private diagnostic laboratories. The agreements covered approximately 74% of active farming localities in 2020. With these data, the prevalence and geographical distribution of important diseases, such as heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS), are reported in a more representative way.

With a total of 2983 non-medicinal delousing treatments registered in 2020, this represents an increase of 21% compared to 2019. Thermal delousing (warm water) remains the most common treatment. Mortality following non-medical delousing treatments probably represents a considerable contribution to the overall mortality experienced during the sea phase of culture of both salmon and rainbow trout. There was a reduction in the number of cleaner fish, particularly lumpfish, transferred to sea in 2020. Emaciation, skin wounds and injuries following delousing events continues to be important welfare problems for cleaner fish kept in salmon sea cages. Reliable numbers of losses/mortalities for cleaner fish are still not available.

Q: does region 4 have a particularly high biomass of farmed A. salmon compared to other areas?

A: region 3 has highest biomass. Don't remember the biomass of region 4

Q Do you have a good handle on cleanerfish mortalities once they are deployed on salmon farms?

A: mechanical delousing gives highest mortality. Some die because they do not eat or are scared

Q: Pasteurellosis, origin?

A: two outbreaks genome sequencing showed similar to old isolate in Scotland not the new isolates.

Molecular tracing of ISAV in Norway in 2020

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Abstract

Infectious salmon anemia (ISA) has been detected in the Norwegian aquaculture industry since the mid-1980s. The virus that causes ISA is an orthomyxovirus that exists in two main forms – an avirulent form called ISAV HPR0 and a virulent form that is characterized by the loss of one or more amino acids in the hyperpolymorphic region (HPR) on segment 6 called ISAV HPRΔ. It is widely accepted that ISAV HPR0 can develop into ISAV HPRΔ, but it is not known how often this change occurs.

In 2020, ISA was detected at 23 marine sites with Atlantic salmon (*Salmo salar*) in Norway. In addition, ISA was suspected at five sites based on the detection of ISAV HPRΔ. Phylogenetic studies based on sequences for segment 5 and segment 6 kept together with information about smolt suppliers and movement of fish have proven to be useful for tracing infection and investigation of connections between outbreaks.

Historically, about half of all ISA outbreaks have been linked to cases at neighboring sites (horizontal infection), while an outbreak without any known source of infection often are referred to as a primary or isolated outbreak. In recent years, several ISA outbreaks at marine sites have been linked to hatcheries that have supplied the marine sites with smolt. In several cases, the genomic sequence of ISAV HPR0 detected at a given hatchery was closely related to ISAV HPRΔ detected at the respective sea sites.

Suggested reading:

1. Aldrin M. *et al*. Modelling the spread of infectious salmon anaemia among salmon farms based on seaway distances between farms and genetic relationships between infectious salmon anaemia virus isolates. J. R. Soc. Interface 2011;8:1346-1356.
2. Christiansen D.H. *et al*. First field evidence of the evolution from a non-virulent HPR0 to a virulent HPR-deleted infectious salmon anaemia virus. J. Gen. Virol. 2017;98:595-606.
3. Lyngstad T.M. *et al*. Use of Molecular Epidemiology to Trace Transmission Pathways for Infectious Salmon Anaemia Virus (ISAV) in Norwegian Salmon Farming. Epidemics 2011;3:1-11.
4. Lyngstad T.M. *et al*. Low virulent infectious salmon anaemia virus (ISAV-HPR0) is prevalent and geographically structured in Norwegian salmon farming. Dis. Aquat. Org. 2012;101:197-206.

Q: Is it realistic to have HPR0 free hatcheries?

A: Some hatcheries try to have HPR0 free fish.

Q: Is there any chance that HPR deleted in hatchery below detection limit?

A: One case of that, but investigated later

Comment: Tried to find deleted variant in hatchery but did not detect any

Q: What is your criteria for defining detections as closely related; is this based on WGS or segments?

A: Only segment 5 and 6, 1 – 2 nucleotides per segment = closely related,

Clinical and histopathological characterization of ISA outbreaks in Norway in 2020

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Abstract

Infectious salmon anemia (ISA) is an orthomyxovirus infection of salmonid fish that in farmed Atlantic salmon has caused devastating disease outbreaks. Thus, ISA is a listed disease and surveillance and control measures are important to successful Atlantic salmon farming. Norway managed to obtain control over ISA in early 1990ies, but a low number of cases are detected yearly, and clusters of outbreaks occurs – as now. A challenge for diagnostics can be the insidious start of the disease as the underlying anemia and severe pathology develop gradually. In this presentation, we review the typical signs of ISA and possible early signs of ISA, and discuss the implications of these observations.

Q: Have you ever observed HPR0 and HPRd in the same fish?

A: No, not in same fish, but using sanger sequencing may bias towards the most prevalent

Q: thoughts on where the HPRO in hatcheries is originating from? Directly from ova as not all hatcheries were associated with sea water?

A: Brood fish may be the source, HPR0 sheds an enourmous amount of virus, so maybe they circulate for a long time in hatcheries

Surveillance of CEV in the Czech Republic

Pojezdal L[1], **Reschova S**[1], **Motlova J**[1], **Matejickova K**[1], **Minarova H**[1,2], **Palikova M**[2], **Piackova V**[3].

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Abstract

Common carp (*Cyprinus carpio*) is the most important farmed fish in the Czech Republic, with a stable yearly production of around 20 000 tones, representing around 85 % of Czech aquaculture. Thousands of interconnected shallow ponds of varying sizes supply both local and international markets, especially in the Christmas season. Additionally, an increasing number of both professional and hobby breeders work with the ornamental koi carp reared in various conditions, from indoor tanks to garden ponds.

While the presence of koi herpesvirus (Cyprinid herpesvirus-3, KHV) in the country is established since at least 2009 in the form of several outbreak sites per year, in the last few years, attention is also given to carp edema virus (CEV). Although this poxvirus and its ability to infect both koi and common carp were already described in the 1970's in Japan, its presence in European aquaculture has been of concern only since about the year 2014. Our laboratory obtained the primers for CEV detection in the year 2015 thanks to our colleagues in CEFAS and the surveillance of the virus started in the same year. Initially, fresh and archived samples from KHV-negative mortality events with typical necrotic gill lesions in carp were examined, with three, one and two sites showing the presence of CEV in the samples from the years 2013, 2014 and 2015, respectively. After research funding for this topic was secured in 2017, larger scale surveillance followed, with the help of the Czech State Veterinary Administration. From 2017 to 2020, nearly 350 locations were examined along with the mandatory KHV surveillance, with 39 ponds tested positive for the presence of CEV. All of the positive samples were either from common carp farms or from koi breeders. Although samples from several different species including sander, sturgeon, burbot, chub, grass carp, crucian carp and crucian x common carp hybrids were also examined, none of the animals tested positive for the presence of CEV.

Phylogenetic analysis of the partial P4a protein gene of the obtained virus isolates confirmed the presence of all three proposed CEV genogroups in the Czech Republic: I and IIa in both koi and common carp and IIb only in koi, mostly recently imported from Asia.

This study was supported by the Ministry of Agriculture of the Czech Republic MZE-RO0518 and NAZV QK1710114; and the project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869 financed by ERDF in the operational program VVV MŠMT.

SESSION II: Control and Surveillance of fish diseases in EU

Chair: Niccoló Vendramin

Field testing of DNA vaccines against Viral Hemorrhagic Septicemia (VHS) and Infectious Hematopoietic Necrosis (IHN) in rainbow trout

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Abstract

The efficacy of DNA vaccines in protecting rainbow trout from VHS and IHN has been widely demonstrated in the past, at least under laboratory conditions. The first field tests of a VHS DNA vaccine in Denmark suggested that the vaccine was able to provide protection under traditional earth pond farming conditions. While a DNA vaccine against IHN licensed for use in Atlantic salmon has been commercially used for more than a decade in Canada, protection under intensive rainbow trout freshwater farming conditions still remains a feature that needs to be further investigated.

Recent Italian VHSV and IHNV glycoprotein gene sequences have been used to design two DNA vaccines tailored towards current virus variants that have been causing disease in rainbow trout farms in the Trento region (northeastern Italy). Following batch production in a GMP-certified facility, an experimental trial was first conducted at the Experimental Aquarium located at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) aimed at obtaining efficacy baseline data. VHS and IHN vaccines at the dose of 1 □g/fish resulted to be effective in protecting 10 grams rainbow trout juveniles challenged 60 days post vaccination (dpv).

Based on the results obtained under controlled condition, the Italian Ministry of Health granted the authorization to perform a field trial in a commercial farm classified as category V for both VHS and IHN. At the beginning of October 2020, a VHS and IHN free hatchery supplied 15,000 rainbow trout juveniles weighing approximately 8 grams. They were divided into three experimental groups: one injected with PBS as negative control (Group 1), one injected with 1 □g/fish of VHS vaccine (Group 2) and the remaining group given 1 □g/fish of both VHS and IHN plasmids (Group 3). Following anesthesia, the fish were injected in the epaxial muscles with 50 □l of vaccine solution. Fish were then housed in the hatchery for 60 days and only 1-2% of mortality occurred during this period. At 60 dpv, the fish were moved to a raceway in the infected facility and positioned in the following order: first the negative control, followed downstream by the VHS vaccinated group and finally by the VHS/IHN vaccinated group. Water temperature ranged from 9 to 11°C for the whole duration of the trial. Mortality began 7 days post transfer, with different trends according to the group they belonged to. Ten moribund fish of Group 1 were collected on a weekly basis for complete anatomopathological examinations, while fish from Group 2 and 3 were individually subjected to real time RT-PCR for VHSV and IHNV identification in order to determine the viral agent causing mortality. Both VHSV and IHNV were isolated during the trial and resulted to be the only pathogenic agents involved in the mortality. At 30 days post transfer, the cumulated mortality was 10.6 % in Group 1, 3.3% in Group 2 and 1.0% in Group 3 resulting in a Relative Percentage of Survival (RPS) of 69.3% and 90.5% for the VHS and VHS/IHN vaccines, respectively. After 60 days from the transfer, cumulative mortality resulted to be 18.4%, 7.6% and 2.5% respectively in Group 1, Group 2 and Group3. The calculated RPS for Group 2 was 59.6% and for Group 3 87.1%.

These results provide evidence that 1□g/fish DNA vaccine is safe and effective in reducing the impact of VHS and IHN also under intensive rainbow trout farming conditions.

Research project IZSVe RC 09/18 was funded by the Italian Ministry of Health and also supported by a grant from the Independent Danish Research Fund (9041-00227B)**.**

Q: Did you investigate the antibody response?

A: Yes, in the lab, but not in the field.

Q: Was the mortality from other causes similar across treatment groups?

A: Yes.

Q: Was there and effect on other parameters – e.g. growth?

A: We weighed the fish, but found no statistical difference between groups. We did not register any difference between groups except for mortality.

RT-qPCR for genome detection of the listed fish diseases, IHN and VHS

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Abstract

In salmonid aquaculture, IHN is one of the most dangerous viral diseases along with VHS. The causative agents of IHN (IHNV) and VHS (VHSV) belong to the family *Rhabdoviridae* within the order Mononegavirales. Based on genome organization and NV protein formation, IHNV and VHSV are taxonomically assigned to the genus Novirhabdovirus. Because the host range of IHNV is predominantly restricted to salmonids, this pathogen is assigned to the species Salmonid Novirhabdovirus. In contrast, VHSV has been isolated from numerous fish species of different taxonomic families and thus assigned to the species Piscines Novirhabdovirus.

Pathogens of IHN and VHS have been detected several times in Asia, the Americas, and Europe. In 2016, IHN outbreaks were reported for the first time in Kenya. Both diseases cause enormous losses in aquaculture farms in Germany every year.

Early detection and identification of viral pathogens is the main prerequisite to prevent the diseases.

The gold standard in diagnostics of IHN and VHS pathogens is virus isolation in cell culture with subsequent confirmatory tests. Meanwhile, genome detection methods are now an integral and indispensable part of the diagnosis of viral diseases in animals. Since April 2016, IHNV and VHSV genome detection assays have been officially approved in the European Union (EU 2015/1554).

Several RT-(q)PCR assays have been developed to amplify the genome of IHNV or VHSV (IHNV: Overturf et al. 2001, Dhar et al. 2008, Liu et al. 2008, Purcell et al. 2006, 2013, Hoferer et al. 2019, Cuenca et al. 2020; VHSV: Chico et al. 2006, Cutrin et al. 2009, Liu et al. 2008; Garver et al. 2011; Jonstrup et al. 2013).

The OIE currently recommended only the IHNV RT-PCR published by Emmenegger et al. (2000) and for VHSV genome detection the RT-PCR published by Snow et al. 2004 as well as the RT-qPCR by Jonstrup et al. 2013. Other assays have not been adequately tested and validated for isolates of different genotypes, according to the OIE (2020).

This presentation will provide a brief overview of the development, validation, and application of the RT-qPCR method of Hoferer et al. 2019 for the detection of IHNV and VHSV genomes.

Q: Are sequences available from the isolates?
A: No, but we plan it.
Comment from Niels Jørgen Olesen: We have tried your probe and it works well. Your changes are small, but effects significant, so we will try to incorporate these changes.

Viral Hemorrhagic Septicemia outbreak in marble trout (*Salmo marmoratus*)

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Abstract

An outbreak of VHS involving a trout farm inside a VHS-IHN free compartment located in Veneto region is described. The farm is the unique establishment inside the compartment and it breeds rainbow trout (*Oncorhyncus mykiss*) and marble trout (*Salmo marmoratus*) for restocking purposes. In February 2021 an unusually high mortality was reported in both species and therefore an official investigation started. The Compartment is separated from the surrounding hydrological system by 3 dams (2 upstream and 1 downstream). The farm's water supply derives from a small river and at the time of mortality the water temperature was 6-8 °C.

At clinical and pathological examinations, marble trout showed less pathological signs and less mortality, compared to rainbow trout. On the other hand, rainbow trout showed all the VHS disease typical signs such as melanosis, anemia and external as well internal hemorrhages. The VHS virus was isolated by cell culture and genetically characterized. The isolate belonged to genotype Ia2 (cluster D) according to classification proposed by Abbadi and colleagues (2016). The viral cluster D is the most commonly detected in Italy.

Epidemiological enquires identified a generally low level of implementation of biosecurity measures (due to economic problems of the farm) and the incorrect use of the truck, which has been used also outside the compartment itself.

In accordance to what reported in a previous work (Pascoli et al., 2015) it was observed that marble trout appeared more resistant to VHS disease compared to rainbow trout, and the latter probably has acted as an amplifier host of the viral infection.

Q: You mention zones of 5 and 10 km, but what do you do about down-stream farms farther away? Do you test?

A: No we do not. We rely on an EFSA report, which recommends that 5 km is enough for sufficient dilution of virus.

Comment from Niels Jørgen Olesen: We have experienced secondary outbreaks much more than 5 km downstream in the past.

First outbreak of IHN in Denmark

Niels Jørgen Olesen, Argelia Cuenca, Niccoló Vendramin, Tine Iburg

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Infectious hematopoietic necrosis virus was detected for the first time in Denmark, 17th of May 2021. A Danish fish farm, in Stouby municipality, was put under suspicion of IHNV infection following a notification from a German fish farmer claiming that a recent IHN outbreak originated from fish imported from Denmark. Based on this the Veterinary authorities inspected the farm and sampled organ and brain material from 30 fish and shipped them to the Danish NRL. IHNV was identified by cell culture and RT-PCR in organ material (spleen, kidney, heart) and to a lesser degree in brain. An epidemiological investigation was instigated and 6 putative contact farms was put under public supervision and inspected with targeted sampling for laboratory analysis. From that investigation, another farm, located in the southern part of Denmark, came out as IHNV positive. At the current moment further contact farms and fisheries are investigated and until the situation is clarified these farms will remain as suspected and will not be allowed to trade fish. Preliminary genetic analysis of the IHNV isolates have been conducted and will be presented.

Q: What raised their suspicion in Germany if no mortalities? Did they screen as a standard practice after receiving live fish on site?

A: Not quite sure. They were IHN negative at the time. Argelia Cuenca: It was a routine screening.

Q: Do you know when the fish batches (IHNV) was imported.

A: No there was no import

Q: Was case 3 also one of the 6 contact farms of the first farm ?

A: Niels Jørgen Olesen: No it was not. And we have not sequenced the virus isolate yet.

Comment from Heike Schütze: In Germany we also have cases without clinical disease signs. Previously when we have had IHNV cases the owners have said that they imported Danish fish, but I have excluded this possibility since Denmark was IHNV negative. Perhaps IHNV has been in Denmark for longer? Perhaps we can cooperate. Sometimes we find that viral load is very low. Isolation on cell culture has sometimes failed, while PCR has been positive.

Comment: Niccolò Vendramin: Background mortality probably masks the IHN clinics if these are not very prominent.

Q, Olga Haenen: I saw Dutch isolates in the clade (from 2008) you showed in the slide. The Netherlands do not do surveillance for IHNV, and a few years ago, like 2011, we had the last outbreak. This was in put&take fish ponds. What are the implications of this? Could the IHNV have come from The Netherlands? Was there contact with NL?

A, Argelia Cuenca: Most recent isolate sequences available are from 2015. A lot of things can have happened since then, so it is very difficult to say much about the epidemiology in relation to our present findings.

Comment from Heike Schütze: Other member states should be included.

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

Chair : Morten Schiøtt

Koi herpesvirus disease – soon forgotten?

Sven M. Bergmann and German as well as international co-workers

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Abstract

Koi herpesvirus disease (KHVD) presents still a threat for the carp and koi aquaculture worldwide. It was replaced from the list of notifiable diseases of EU at April 24 2021 and grouped into the category E (only outbreaks registered) but not from the list of the OIE. While over the last century, knowledge on the disease, its pathogenesis and immunogenesis in fish accumulated, many details are still unknown today. Different working groups, worldwide, discuss controversy, the entrance into the fish, the virus replication in different organs at different temperatures and hosts, the way of latency or persistence of KHV in different cells or tissues as well as the immune response from the fish. Prophylaxis mainly by vaccination and metaphylaxis as well as to cure the disease was one of the bullet points over the last years.

To close the knowledge gaps we carried out investigations on virus entry, virus distribution during the early and late phase of infection, virus replication in different organs of different fish at different temperatures as well as the humeral immune response in combination with the detection of molecular markers for the cellular immune regulations.

Contrary to published investigations, we found that KHV is mainly entering the fish via gill, mouth and gut tissues if the skin is not damaged and the mucosal barrier is working well. Obviously, the virus is infecting mononuclear cells and is than implicated into the second barrier, the leukocytes which can be infected and is afterward found in the blood stream. From here, KHV is transferred to the spleen, to the kidney and to other organs, always via infected leucocytes in the blood stream. After three to five days post infection (dpi), KHV will appear in the skin part above the basal membrane of the epidermis or wrapped into C cells in debris of the upper gill tissues. In both cases, it is releases into the water and can now infect other fish. It can be retained to the skin and gill mucus that can simulate a replication. During the next 14 days, the virus can dispatch the fish or the fish is able to combat successfully against the disease but always without virus eradication. From the approximately 14 to 16 dpi, fish starts to develop antibodies that will become strong a neutralizing ability after 30 to 35 dpi. While the antibodies develop well over the next 100 days, the virus is still present in the fish. The latent or better persistent phase of the infection will take place in polymorphic granulocytes in the blood but also in granulocytes in gill tissues and in epithelial cells of the skin, short above the basal membrane. If the virus re-appears in the leucocytes, it is re-activated. While neutralizing antibodies against KHV are detectable up to eight weeks post infection, other varieties of antibodies can be measured over 12 month post infection. Due to the persistence of the agent, infected fish will be boostered all its lifetime if it is not over replicating the virus that can have a fatal end. Over the last years, several vaccines are produced. In Germany two way were taken for development, once genetically and twice naturally attenuated. In both cases, no mortality occurred after vaccination by immersion with a 100% survival rate after challenge. Additionally, the naturally attenuated KHV-T was delivered orally with a booster by immersion or visa versa. Successfully was carried out the oral vaccination and the immersion twice, respectively.

Q. it was mentioned Giant Gourami and Stingray were detected positive with KHV.

A: yes we confirmed that with sequencing

Q: the detection of viral RNA with ISH , just a few hours after exposure, could it be phagocytosis rather than replication?

A: we have done EM and can show the capsid, we believe is replication

Q: in relation to vaccination, do you think that the vaccinated fish can carry the virulent virus after challenge?

A: it is possible.

Prevention and control of *F. psychrophilum* infections with bacteriophages

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Abstract

Flavobacterium psychrophilum is responsible for economic losses in salmonid aquaculture [Rainbow Trout Fry Syndrome (RTFS) and Bacterial Coldwater Disease (BCWD)]. Phage therapy, a novel environment-friendly potential alternative to antibiotics for prevention and control of infections, relies on the bactericidal activity of bacteriophages (phages), natural entities that infect and kill their specific host-bacteria. Here, we present the recent advances on the use of phages to control and/or prevent *F. psychrophilum* in rainbow trout [Donati et al. (2021, a and b); Donati V.L. (2021)].

In the first part of the work [published in Donati et al. 2021(a)], we focused on *F. psychrophilum* infections in rainbow trout fry. Fish experimental trials were set up with phages delivered by three methods: oral (prophylactically and continuous delivery; phages applied on feed pellets), bath (repeated procedures) and intraperitoneal injection (IP; 3 days after bacterial challenge). For the oral delivery trials, the effects on fish health of phages on feed pellets were evaluated and, bacteria and phages re-isolated from fish organs. A survival analysis was conducted for the three trials. No adverse effect on fish health was observed during oral delivery and phages were constantly detected in the intestine. For the oral and bath delivery experiments, no significant increase was observed on fish survival during *F. psychrophilum* infection (IP bacterial challenge). When phages were delivered by IP, a significant increase was observed in the infected group (80.0 %; control group = 56.7 %) (IP bacterial challenge).

In the second part of the work [published in Donati et al. 2021(b)], we focused on *F. psychrophilum* infections in eyed eggs. A bacterial bath challenge method was at first set up and the effects of phages on eggs assessed (i.e.: eggs' survival and interactions phages/eggs). Then, bacterial-challenged eggs were exposed to phages. Phages did not appear to affect the survival of the eyed eggs negatively. Further, phages were able to control *F. psychrophilium* in the short term (24h).

To conclude, the results obtained support the potential of using phages for *F. psychrophilum* prevention and control but also reveal challenges that should be evaluated in the future studies.

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A virus-like particle (VLP) vaccine inducing protection in European sea bass against viral nervous necrosis

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Abstract

Viral nervous necrosis (VNN) is a devastating disease of European sea bass, compromising the aquaculture production in the Mediterranean. In European sea bass, the disease is caused by red spotted-grouper nervous necrosis virus (RGNNV), which belongs to the genus *betanodavirus* (also called Nervous Necrosis Virus (NNV)). NNV is a group of small ichosahedral RNA(+) viruses with a bi-segmented genome of RNA1 and RNA2. NNV can be found globally and a reservoir exist in the wild fish stocks, with virus detected in up to 120 different marine and freshwater species. The rising water temperatures of the oceans result in migrations and movement of fish to new habitats, which will most likely further increase the prevalence, distribution and disease of NNV.

Vaccination of farmed fish is a potential way of reducing the VNN outbreaks. For this, safe and efficacious vaccines are needed. During the EU project Targetfish (GA 311993) an innovative virus-like particle (VLP) vaccine against viral nervous necrosis in European sea bass was developed. The coat protein (CP) encoded by the RNA2 of RGNNV was expressed in a eukaryotic expression system (*Pichia pastoris*). After disruption of cells, CP auto-assembled into VLPs which could be purified by selective precipitation. Similar vaccines have previously been shown to induce protection against challenge with NNV a few months post vaccination. However, in relation to use under farming conditions, long-term protection is required and we here tested whether immunity to VNN could still be demonstrated 3 and 7.5 months post vaccination.

European sea bass (average size: 5g) from a commercial breeder with NNV free status were vaccinated by intraperitoneal injection with 50ul of either VLP (40µg/fish), a commercial vaccine or phosphate buffered saline (PBS) (n ~ 350 fish/group). The fish were kept in aerated artificial seawater (10‰ salinity) and at 19ºC (+/- 1ºC) during immunization period. Monitoring of immunity included examination of the antibody response in ELISA and serum neutralization, as well as survival in experimental NNV challenge with RGNNV.

RGNNV specific and neutralizing antibodies were detectable in serum from VLP vaccinated fish until at least 8.5 months post vaccination, which was the latest sampling time point, with neutralizing titers from 160 – ≥640, the majority being above 640. In serum from the fish vaccinated with the commercial vaccine, the titers declined with time, until there was almost no detectable antibodies in ELISA at 8.5 months post vaccination, and neutralizing titer at this point was below 80.

Survival at 3 and 7.5 months post vaccination, showed superior survival of the VLP vaccinated fish compared to the non-vaccinated controls at three months post vaccination in both bath (RPS = 69) and injection challenge (RPS = 84). In addition, the fish was still protected 7.5 months post vaccination (RPS = 87), which corroborates with the high level of neutralizing antibodies at this time. RPS values for the commercial vaccine after injection challenge 3 and 7.5 months post vaccination was 29 and 3, respectively, also corroborating with the reduced antibody titers.

These results underline the applied potential of the VLP as an efficacious vaccine against VNN disease, since it after only one dose induces a long lasting protective immune response. Under these experimental conditions, the VLP vaccine even performed superior to the experimental vaccine. However, comparison should be made with caution as several factors, such as virus strain used for vaccine production and antigen content will most likely influence the results, and these are not known for the commercial vaccine.

This research has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 727315.

Q where did you source the Anti seabass IGM?

A : from IZSVE

Q: did you detect live virus in internal organs?

A: yes it is in the fish surviving the challenge, so fish exposed to virulent virus.

SESSION IV:Update from the EURL

EURL for Fish Diseases, work done in 2020

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Abstract

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern the fish diseases listed as exotic diseases: Epizootic haematopoietic necrosis (EHN), and diseases listed as non-exotic diseases: Infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious hematopoietic necrosis (IHN), koi herpes virus disease (KHVD).

The facilities supporting the activities of the EURL are placed in the DTU Campus in Kgs. Lyngby, 15 km north of the capital. The EURL is now placed in the institute DTU AQUA, National Institute of Aquatic Resources, and is in the progress of further integration with other ongoing activities in this institute in the field of aquaculture and fisheries.

Due to Covid-19 pandemic and related travelling restrictions, meeting activities had to be converted to on-line events, whereas the activities that required travelling were cancelled.

The 24th Annual Workshop of the National Reference Laboratories for Fish Diseases was held virtually, using the zoom platform, on 3rd and 4th of November 2020.The virtual organization of the meeting has allowed a significant expansion of the number of participants attending the workshop as well as the number of oversea countries participating.The number of participants has reached 107 participants from 39 countries attending over the two days period. There were four sessions with a total of 15 presentations. On November 4th a special session dedicated only to the staff of NRLs in Europe was held to present the new Animal Health Law which will be fully implemented in April 2021, and its implications for Aquatic animal health.

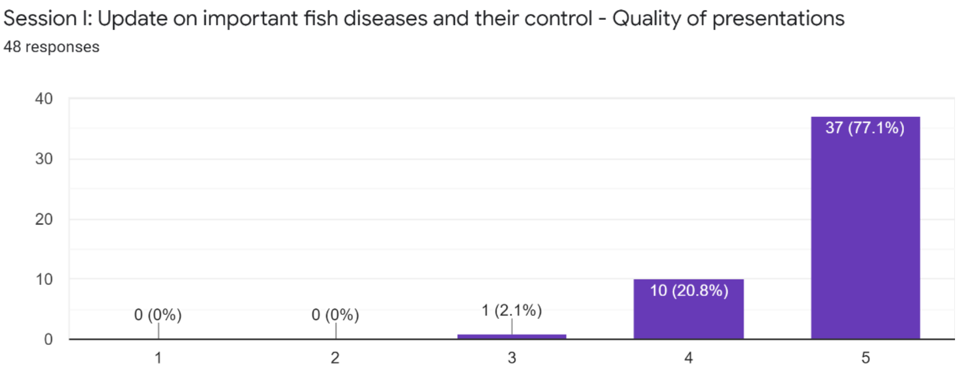
The annual proficiency test for fish diseases (PT) was divided into PT1 and PT2 with 45 laboratories participating. The tests were sent from the EURL on 25th of September 2020. The full report with the results and the identification of NRL has been submitted to the Commission, whereas each participant 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 .

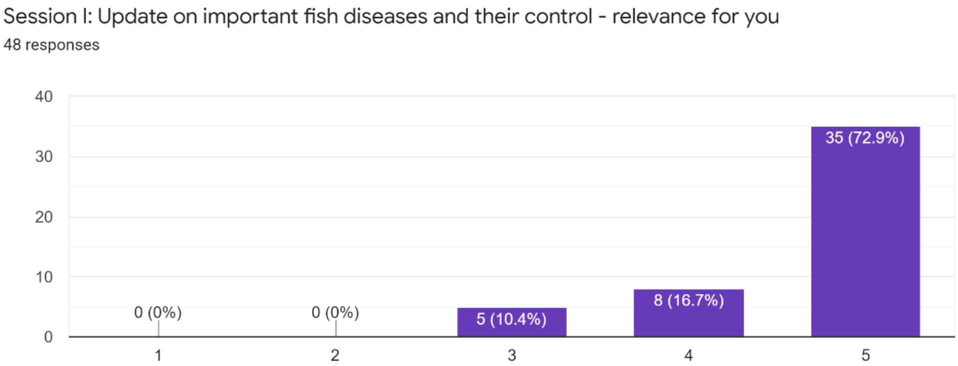
An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. In 2020 the EURL has focused on improving the diagnostic manual for ISA finalizing the work initiated in 2019.

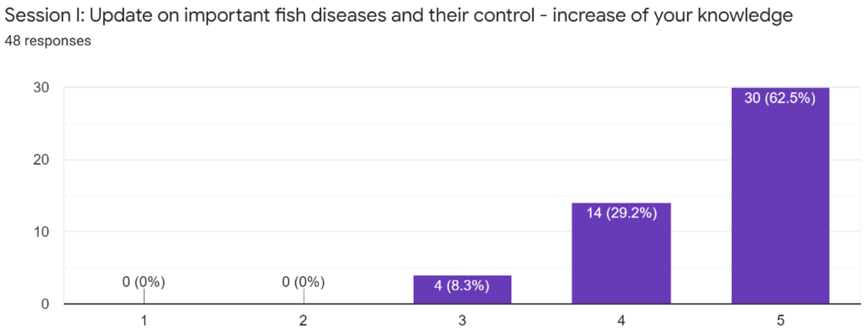
During 2020, resources were again used to collate data on surveillance, health categorisation and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to develop, update and maintain the database containing information on fish pathogens (www.fishpathogens.eu); 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.

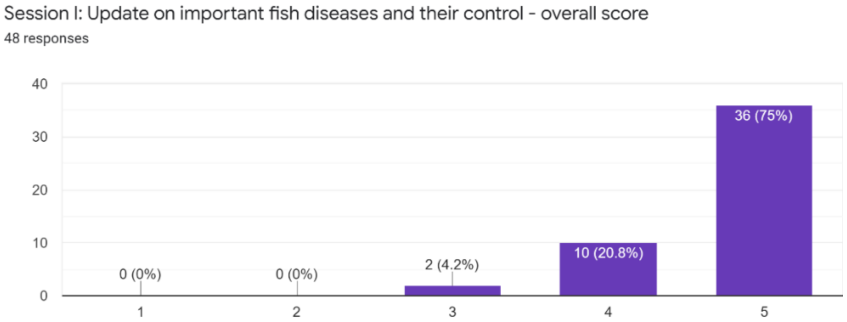
Workshop evaluation

A questionnaire was delivered to the participants asking to evaluate various aspect of the workshop. An overview of the 48 questionnaires retrieved is shown below. Specific comments are going to be considered for the next annual workshop organization.







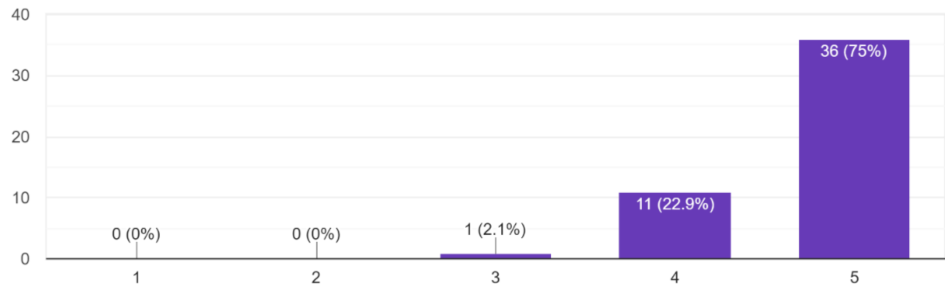


Session I: Update on important fish diseases and their control - comments, remarks, inputs10 responses

- Very informative presentation and easy to follow. The areas covered were relevant and informative.
- very interesting to understand other diseases
- Very important knowledge in order to be up to date.
- Very informative session regarding sanitary situation in Europe
- Relatively too much on the Norwegian fish diseases, too detailed to my idea. But I can imagine it depends on who likes to present, voluntary. Lectures on overview disease in Europe and CEV lecture were excellent. I was too late to get my questions in time in view, just to realize, just wait a bit longer when providing the floor, but there was not time for discussion left. I just put them in the chat and did get some reaction though. Thanks.
- We don´t have any salmon here, so informations about ISA were of little relevance. But I understand the importance of the disease and the presentations were well done and interesting nonetheless.
- mark 5
- Excellent organization
- For the next meeting a discussion on the role of cell culture is suggested
- very usefull

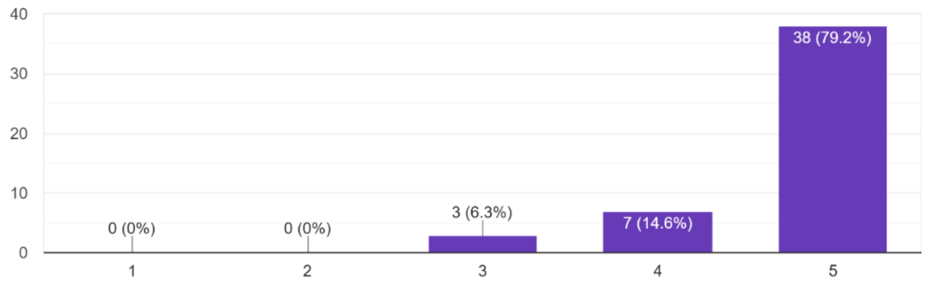
SESSION II: Control and Surveillance of fish diseases in EU- Quality of the presentations

48 responses



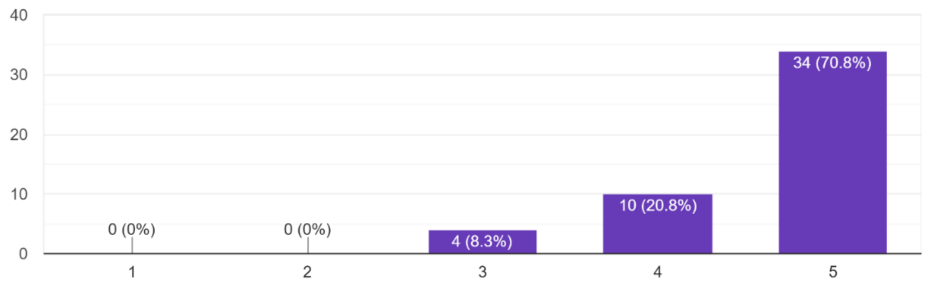
SESSION II: Control and Surveillance of fish diseases in EU- relevance for you

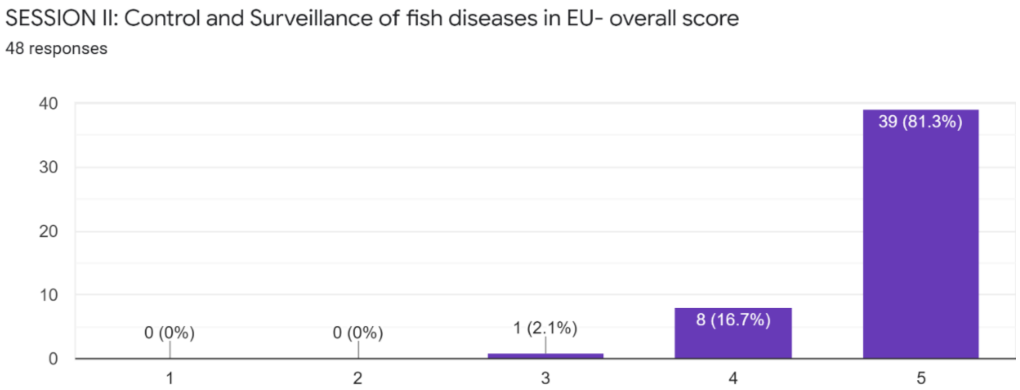
48 responses

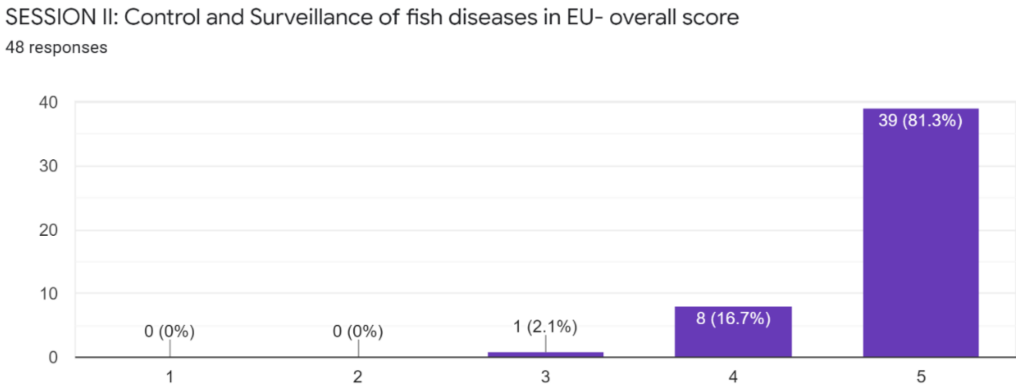


SESSION II: Control and Surveillance of fish diseases in EU- increase of your knowledge

48 responses






SESSION II: Control and Surveillance of fish diseases in EU- overall score
48 responses
40
30
20
10
0
0 (0%)
0 (0%)
1 (2.1%)
8 (16.7%)
39 (81.3%)
1
2
3
4
5

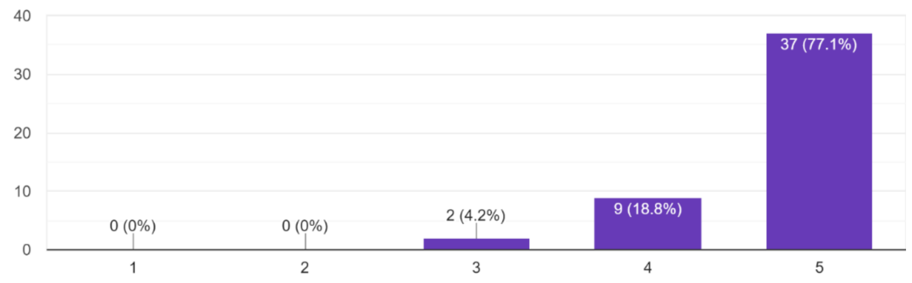
SESSION II:

Control and Surveillance of fish diseases in EU- comments, remarks inputs8 responses

- Flow of topics was good. I found the talk on development of qPCR for IHNV/VHS/IHNV very useful and interesting. I would like some more ppts based on method development as I find this very relevant and applicable to our roles as NRLs. It is good to see what others are trying. The talk on IHNV outbreak was very interesting and extremely relevant to see the actions put in place. Really enjoyed this session
- Excellent presentations.
- interesting to have an update on DNA vaccination work done in Italy. And very intesresting presentation regarding the first outbreak of IHN in Denmark... looking forward to have news on investigations focused on the potential origin of this outbreak...
- Very important issues, the lectures of Heike and of Niels as being very important for the Dutch situation. A pity, we could not discuss around on the IHN outbreak more, it was too short for discussion.
- Interesting talks, wery well put together
- mark 5
- Great workshop
- Very useful information/update about newIHN detection in Denmark. Thanks!

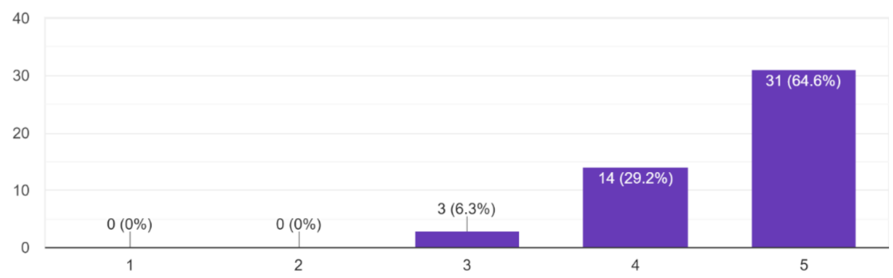
SESSION III: Results from ongoing research on listed and emerging fish diseases-quality of the presentations

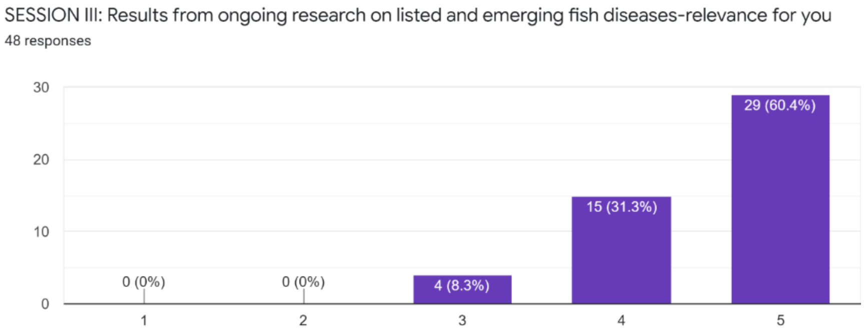
48 responses

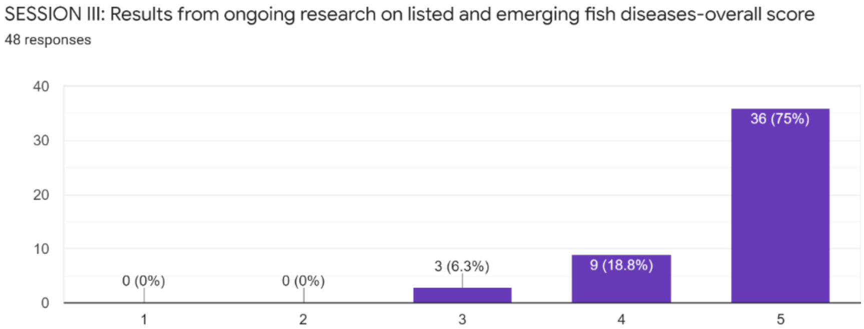


SESSION III: Results from ongoing research on listed and emerging fish diseases-increase of your knowledge

48 responses



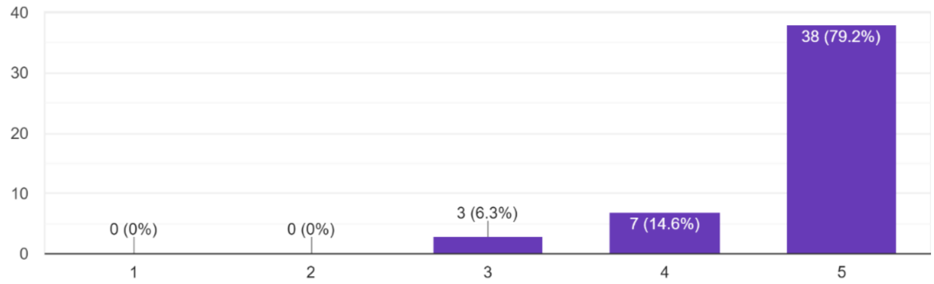




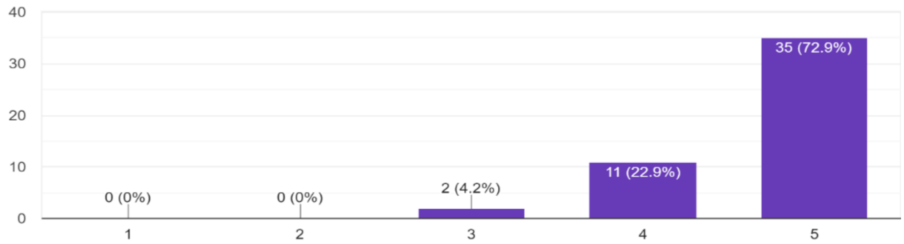
SESSION III: Results from ongoing research on listed and emerging fish diseases- comments, inputs, remarks7 responses

- Good presentations. Informative in terms of work being done at the NRL.
- really interesting
- The new research results are going to help us in our daily routine.
- very interesting overview of KHV research from Sven!
- Although the lecture of Sven was too long and detailed (we know him...) it was impressive in content. Other lectures were innovative, I learnt a lot!
- mark 5
- Excellent organization

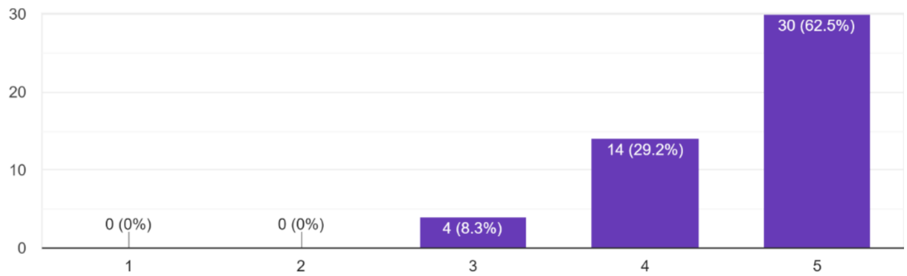
SESSION IV: Update from the EURL for fish diseases- quality of the presentations
48 responses



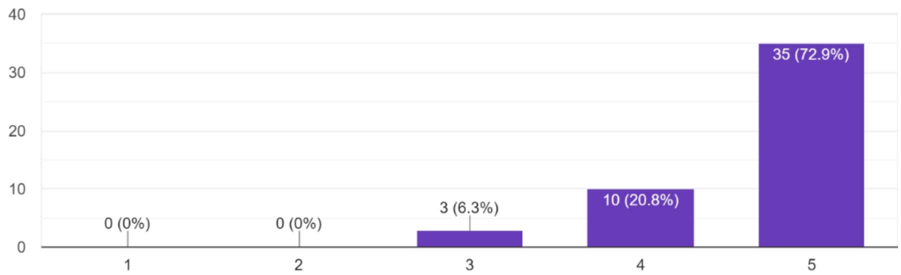
SESSION IV: Update from the EURL for fish diseases- relevance for you
48 responses



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



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



SESSION IV: Update from the EURL for fish diseases- comments, inputs, remarks10 responses

- I think a proper one hour lunch break would be better
- This part was a little rushed due to time constraints but all topics were adequately covered. Overall, would have been nice to have had a round up of what was talked on throughout the day at the end of the meeting. Take home messages if you like from all participants? Provision of powerpoints will be useful to reflect on all the information provided as this was a lot in one day.
- I hope to meet all of you in person next year
- It was a very educational workshop and very well organised. I had not attended before and it was a great opportunity for me. Thank you.
- Extremely helpful information.
- Thanks a lot for scheduled dates announced for next training courses. And of course for your Scientific support, your availability, kindness, ... to help us when we are faced with technical issues or questions about legislation!
- As ever, this session underlines the work you all do, great and impressive! Thanks so much!
- again mark 5
- Excellent presentation
- Only partly participation in session IV but not able to skip the rating and submit

Greetings and conclusions of the meeting

The next meeting will be held at 30th and 31st May 2022 likely a combination of virtual and face to face. 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