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

Report of the

24th Annual Workshop of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark

November 4th – 5th 2020

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 24th Annual Workshop of the National Reference Laboratories for Fish Diseases was held virtually on 3rd and 4th of November 2020.
This year 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 107 participants from 39 countries attending over the two days period. There were four sessions with a total of 15 presentations.

The workshop was held back to back with the 11th Annual Wokrshop for National Reference laboratories for crustacean diseases.

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 is going to be implemented in April 2021, and implications there will be for Aquatic animal health.

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 2019 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 in english is available at https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2020/fish-health-report-2019 .
Afterwards a presentation on the first outbreak of IHN in saltwater in marine farmed rainbow trout in Croatia was provided by Snjezana Zrncic from the Croatian NRL. The monitoring and surveillance of sea louse in Danish salmonids was presented by prof. Kurt Buchman. Finally, Dr. Peter Mohr from Australian Centre for Disease Preparedness (ACDP) presented an overview of a newly described Orthomyxovirus, named Pilchard Orthomyxovirus (POMV) which has caused disease in farmed Atlantic salmon in Australia.

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, presented the field trial for testing DNA vaccination for preventing outbreaks of VHS and IHN in farmed rainbow trout in Italy. Afterwards, Dr. Axen from SVA gave an overview of a new syndrome – Red Skin Disease- observed in wild Atlantic salmon returning to the river to spawn in spring. Then, Dr. Kantala from Finland, presented an update on eradication measures implemented to re-gain health status category 1 for IHN in Finland.
Finally, phd student Sofie Barsøe presented the results on experimental vaccination with an innovative nodavirus vaccine prototype based on virus like particle in Europea sea bass (*D. labrax*).

The second and last day was opened with the research update on ongoing projects on listed and emerging fish diseases in Europe.
The two first talks were related to infection with ISA-virus. The first talk given by Dr. Fosse from Norwegian Veterinary Institute dealt with the interaction with ISAV and erythrocyte in fish. The second talk given by Dr. Dverdal-Jansen presented a model the spread of the infection depending on control measures applied. Finally phd student Juliane Sørensen from DTU Aqua presented her findings on surveying and characterize PRV-3 in Denmark.
Afterwards, Session IV Update from the EURL for fish diseases took place.

In this session presentations of the last training course, held online due to Covid-19 pandemics, the results of the inter-laboratory proficiency test for listed fish diseases; the EURL activities in year 2019 were presented and proposals for the EURL work plan for 2021 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. Niccoló 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 beginning of June 2021, 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 Niccoló Vendramin

Programme

Tuesday November 3rd
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 disease situation in Norway *Ingunn Sommerset*
10:30 – 10:50	IHN outbreak in rainbow trout farmed in Saltwater in Croatia *Snjezana Zrncic*
10:50 – 11:10	Salmon lice in Danish salmonid populations *Kurt Buchman*
11:10 – 11:30	POMV a novel orthomyxovirus infection in Atlantic salmon farmed in Australia *Peter Mohr*
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	Red skin disease – a novel syndrome affecting migrating wild Atlantic salmon in northern Europe *Charlotte Axen*
12:20 – 12:40	Update on control and management of IHN outbreak in Finland *Tuija Kantala*
12:40- 13:00	A virus like particle inducing protection in European sea bass against viral nervous necrosis *Sofie Barsøe*

Wednesday November 4rd
Annual Workshop of the National Reference Laboratories for Fish Diseases

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

Chair: Morten Schiøtt and minutes: Niccoló Vendramin

09:30 – 09:50	Salmon erythrocytes sequester infective ISAV particles during infection *Joanna Fosse*
09:50 – 10:10	Modelling scenarios for control and mitigation of Infectious Salmon Anemia (ISA) *Mona Dverdal Jansen*
10:10 – 10:30	Epidemiology and Pathogenesis of Piscine orthoreovirus Genotype 3 *Juliane Sørensen*

SESSION IV: Update from the EURL for fish diseases

10:30 – 10:50	EURL Training Courses. Topics and organization of courses 2019 *Niccoló Vendramin and Tine Moesgaard Iburg*
10:50 – 11:10	Results of the Proficiency Test, PT1 and PT2, 2019 *Niccolò Vendramin and Teena Vendel Klinge*
11:10 – 11:30	EURL Work Plan for 2020 and ideas and plans for 2021 *Niels Jørgen Olesen* Next meeting and end of 24th Annual Workshop *Niels Jørgen Olesen*
11:30 – 11:40	***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 2019

Niccolò Vendramin, Teena Vendel Klinge and Niels Jørgen Olesen

[1]*DTU Aqua National Institute of Aquatic Resources, Kemitorvet 202, Kgs. Lyngby,*

niven@aqua.dtu.dk

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 2019. 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 the website and in the present booklet.

Production data from FEAP

The data on the European aquaculture production was this year obtained from data provided by FEAP. It was observed during the preparation of the report, that some discrepancies between the two sources were present. This will be discussed at the Annual Workshop. The report does not include information on the number of fish farms, and therefore these data were obtained directly in the questionnaire.

The total fish production in aquaculture in Europe, including Turkey and Norway, increased slightly from 2018 and is now at 2.574.333 t. Among the EU Member states the production has been almost horizontal in the past 10 years whereas has observed an increase of about 50.000 t in 2019 with a total production of 712.648 t.

The 4 non-EU countries Iceland, Faroe Islands, Turkey and Norway produce 1.861.685 t and also experienced a significant increase since 2018.

The Atlantic salmon production, accounts for 1.664.541 t in 2019, and is by far the largest contingency in Europe. The production of large rainbow trout in sea water accounts now for 160.165 t while the production of portion rainbow trout is of about 225200 t in 2019 .Turkey is still the largest contributor of rainbow trout production with 103.000 t. The carp production is mainly in the Eastern part of Continental Europe, the data from FEAP reports approximately 62.000 t. Both the production of sea bream and especially sea bass also increased in the Mediterranean countries with a production of 208.197 t and 199476 t, respectively. Among other fish species of interest are eel (with 4.478 t in 2019 in decline from 2016); also sturgeon

represents a promising species (2724 t in 2019) especially in view of its caviar production (166,5 t in 2018, no data updated in 2019).

Turbot production appear in slight increase (11.423 t in 2019 and 10.229 in 2018), the production of other "so called" minor species includes halibut (1.594 t), Arctic charr (6.915 t), sole (1.211 t) and meagre (8.115 t).

The production of cleaner fish as lumpfish and wrasse for lice control is increasing significantly. In 2019, 49,1 million of cleanerfish were deployed at sea. This figure consist of booth wild caught Cleaner fish and farmed ones. Cleanerfish currently used are lumpsuckers (*C.lumpus*) and various species of Wrasse. According to the Directorate of Fisheries 22.6 million lumpsucker wereproduced in 2019. In Scotland production of lumpfish was assessed as equivalent to 660.000 fish and 4 sites producing 4 tonnes of wrasse with 59,000 fish.
Number of fish farms in Europe

The total number of authorised/licensed fish farms in Europe was reported to be around 30.704 farms, with the largest contingency in Germany with 13.911 farms having a high number of small producers. This estimate is considered to be very conservative. Norway having by far the largest production in Europe license almost 1.400 farms/sites. An overview of the number in each country can be found in Annex 1. It has to be ackowledged taht it was not possible to retrieve the total number of active farms for all participants in the survey (4 missing) and for 3 member states, number of farm has been inferred by the categorisation.

Health categorization of fish farms

Almost all Member States did reply to the questionnaire and provided very clear and correct answers.

In 2019, 13.000 farms with species susceptible to VHS were reported in categorized zones, 12.601 to IHN, 6.666 to ISA and 10.871 farms with cyprinids susceptible to KHV.

71% of the authorised trout farms in Europe are situated in category III zones for VHS and 69% for IHN, with 26% and 28% respectively in Category 1. For both diseases the remaining of the farms are situated in category II, IV or V.

71% 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%.

In Europe there are still several different views on how categorisation shall be performed, e.g. should VHS free marine rainbow trout farms be placed in Category III or I considering the risk of infection with VHSV from the marine environment?

It is envisaged that some of these issues will be solved with the implementation of the new Animal Health Law starting on 21st April 2021.

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**, 32 new outbreaks were reported in Europe in

2019, 13 of these were in Germany, importantly a number of confirmed VHSV infection in Austria (5) Belgium (5), Czech Republic (3), France (2), Poland (2), Switzerland (1), Italy (1).

For **IHN**, 30 new outbreaks were reported. The majority was in Germany (20), two countries with 2 outbreaks each (Austria, Estonia) and six countries with one outbreak each (Czech republic, Poland, Slovakia, Slovenia, Switzerland, Italy).

For **ISA** Norway was the only country reporting outbreaks, and reported 10 new sites with ISAV HPRΔ in 2019.

For **KHVD**, 96 outbreaks were reported in 2019. The vast majority (49) in Germany, 17 in UK. The virus was reported for the first time in Norway, in total KHVD was reported from 14 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 2019 were reported:

- In **rainbow trout** the major concerns are flavobacteriosis (RTFS), red mark syndrome, enteric redmouth, and infectious pancreatic necrosis but also, lactococcosis, ichthyophtariasis, saprolegniosis. More and more report BKD (bacterial kidney disease) are reported.
- 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.). Ulcers from *Moritella viscosa* and *Aliivibrio*.
- In **Cyprinid** it is primarily CEV, *Aeromonas hydrophila*,
- In **seabass** and **seabream** it is primarily VNN/VER, tenacibaculosis, *Vibrio harvey*, *Sparicotyle chrysophrii, Aeromonas veronii* and *Lernathropus kroyeri* infection. Of a certain significance Red Rash syndrome in gilthead sea bream.

Laboratory examinations

There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. Annex 5 provide the total number of laboratory examinations conducted in Europe in 2017 on VHSV, IHNV, ISAV, KHV, SVCV, CEV, IPNV, SAV, and Nodavirus, respectively.

ISAV, KHV, SVCV, CEV, IPNV, SAV, and Nodavirus, respectively.

Questions and comments:

Q: It was asked how the situation was for losses due to sea lice – if it was completely managed.
A: It appears that treatment against sea lice is very severe to the fish as it involves hot water and mechanical cleaning, but also cleaner fish.
Comment from NVI: there was conducted a study to measure the impact on mortality of treatment for sea lice and publication is about to be submitted. It was found that mortality was

up to 5 times higher in treated fish than in non-treated fish, and up to 10 times higher for hot water treatment. Up to 2 weeks after treatment mortality was still affected.
Q: It was asked if there were cases of Red Rash in the Mediterranean sea,
A: Yes these are confirmed
Q: It was asked what had caused the increase in disease outbreaks in Germany last year
A: There are increased issues with IHN and VHS
Q: It was asked if the impact on production due to disease is known
A: This is a very relevant topic but it is widely underinvestigated.

The Health Situation in Norwegian Aquaculture

Sommerset I, Walde C S, Bang Jensen B, Bornø B, Haukaas A and Brun E

Norwegian Veterinary Institute

ingunn.sommerset@vetinst.no

Abstract

The Norwegian Veterinary Institute (NVI) publishes annually the 'Health Situation in Norwegian Aquaculture report. The report describes the health and welfare situation of farmed fish in Norway, as well as the state of health of wild Norwegian salmonids. The statistics presented are mainly obtained from four different sources; official data, data from the NVI, data submitted by private laboratories and data based on responses to a survey sent out to Fish Health Services and the Norwegian Food Safety Authority. A summary of the 2019-report will be presented as well as a short update on the health situation so far in 2020.

The total losses within the industry for 2019 were higher than normal, as approximately 8 million salmon died during an algal bloom (*Chrysochromulina leadbeaterii)* in Northern Norway. In total 52.8 million Norwegian farmed salmon died between sea transfer and harvest and the overall percentage of mortality, based on monthly rates, was 16.2%. There were however large differences between different counties, ranging from 8.7% to 20.7%.

For the notifiable viral diseases infectious salmon anaemia (ISA) and pancreas disease (PD), 152 farms were registered as PD positive and 10 farms were diagnosed with ISA in 2019, which is similar to the numbers for 2018. Besides PD, two other viral diseases dominated: cardiomyopathy syndrome (CMS) was diagnosed in 82 farms and heart and skeletal muscle inflammation (HSMI) was diagnosed in 79 farms at NVI. When diagnoses made by private laboratories were included, the total number reached 237 for CMS and 197 for HSMI. However, as private laboratories reported their numbers anonymously to the NVI, the same farm can be counted more than once.

The bacterial disease situation for farmed salmonids remained favorable in 2019, with low antibiotic use and an extremely low frequency of antibiotic resistance among tested bacterial isolates. One notable change in the situation regards pasteurellosis in salmon, where a significant increase in the number of outbreaks related to infection with an as yet un-named *Pasteurella* spp is seen, with zero cases 2013-2017, seven in 2018 and 14 in 2019.

The most noticeable change so far in 2020, is an increase in ISA cases with 19 confirmed and 5 suspected ISA positive farms in Norway per 1st October. The majority of affected farms are located in Northern Norway. The number of salmon farms affected by *Pasteurella* spp. infection continues in 2020 and per 26th October almost 30 sites are registered at NVI. *Pasteurella skyensis*, which has caused major problems in salmon farms in Scotland, was for the first time confirmed in diseased salmon in Norway in October 2020.

Questions and comments:

Q: It was asked if ISAV could be interacting with other viruses,
A: We have applied for a research project looking at the different factors that interact during ISA outbreak and if some risk factors trigger higher mortality.
Q: It was asked why the fish were not resistant to IPN,
A: Fish are less susceptible but not completely resistant
Q. It was asked how big a loss was caused by Pasteurella
A. That outbreaks were mainly affecting big fish of 2 – 3 kg, and that mortality was not high but that due to wounds, the fish were downgraded at slaughtering.

IHN outbreak in rainbow trout farmed in Saltwater in Croatia

S. Zrnčić[1], D.Oraić[1], Ž. Pavlinec[1], I.G. Zupičić[1], A. Cuenca[2], N. Vendramin[2]

[1]Croatian Veterinary Institute, Zagreb, Croatia

[2]DTU Aqua National Institute of Aquatic Resources, Kgs. Lyngby, Denmark

zrncic@veinst.hr

Abstract

In the past fifteen years, there were several attempts to establish farming of rainbow trout in the Adriatic Sea. So far as during the summer temperatures are reaching 25ºC and more with low oxygen saturation, a particular area, Velebitski Kanal was evaluated as being acceptable for this project. This is the area with an abundant inflow of submerged rivers supplying marine environment with cold freshwater making this site suitable for rainbow trout farming.

A rainbow trout fry intended for on growing in the marine cages was imported from two different hatcheries in EU and situated in the rainbow trout farm in the hinterland for several months. The water temperature on the farm was 10 ºC. In the early spring of 2020, both batches were transferred into sea cages. At that time the sea temperature was almost the same, about 10.5 ºC. Soon after putting fish in marine cages, mortalities have started. Swabs of the kidney were taken from the kidneys of moribund fish, placed into transport media and delivered to the laboratory for analysis.

Upon receipt in the laboratory, swabs were streaked onto TSA and BA bacteriological media and viral RNA was extracted on KingFisher Duo Prime Purification System (Thermo Scientific) using MagMAX CORE Nucleic Acid Purification Kit following the manufacturers' simple workflow instructions for processing animal swab samples with no changes. RNA was tested for VHSV and IHNV using RT qPCR standard protocols.

All samples tested negative for VHSV and positive for the presence of IHNV. At the same time, bacterial colonies appeared on the solid media and were identified as *Vibrio* spp. using API20E. The affiliation to *Vibrio anguillarum* was confirmed by PCR using amiB gene amplification and 16S rRNA protocols and sequencing.

Received samples were not appropriate for confirmation of listed diseases and therefore competent authorities performed a sampling of fish from the cages. Rainbow trout weighing 50-300 grams were received and submitted specimen showed loss of scales, dark gills, haemorrhages in the mouth, on the fin basis and skin of opercula and vent. Necropsy revealed haemorrhages in the fat tissue, pyloric caeca, peritoneum and intestines, swollen and dark spleen and liver. Examination of organ pools on the cell culture resulted by virus isolation and identification was performed by ELISA followed by RT qPCR and amplification and sequencing of „mid-G" region of the G gene of the IHN virus. The obtained sequence belongs to the IHNV genotype E. Phylogenetic analysis of sequences available at the NCBI database as well as sequences from previous IHNV outbreaks in Croatia showed the presence of "Croatian clade". It means that sequence from this outbreak clustered with sequences from other IHNV outbreaks in Croatia from 2005, 2014 and 2015. It may be concluded that the virus has been circulated in the country rather than being imported from other countries.

Questions and comments:

Q: It was asked if the pathogen survived in reservoir species or at the farm.

A: The outbreaks did not take place in the same farms but in the same water systems. Maybe the farms worked as reservoirs.

Salmon lice in Danish salmonid populations

Kurt Buchmann

University of Copenhagen, Faculty of Health and Medical Sciences,

Department of Veterinary and Animal Sciences, Frederiksberg C, Denmark

kub@sund.ku.dk

Abstract

The salmon louse *Lepeophtheirus salmonis* is a crustacean ectoparasite parasitizing the external surfaces of salmonid fishes. It was originally described as *Caligus salmonis* by the Danish zoologist Henrik Nicolai Krøyer in 1838 in his treatise "About the parasitic crustaceans – especially with regard to the Danish fauna" in Naturhistorisk Tidsskrift (Journal of Natural Science). He mentioned that this crustacean ectoparasite occurred rather commonly on wild salmon during summer time but the author did not specify the investigated localities. It is well described that *L. salmonis* is a marine organism with predilection for high salinities and has a reproductive optimum between 28 and 35 ppt although it can survive at lower salinities and even in freshwater for up to 14 days. The Danish marine areas display a huge salinity variation from more than 33 ppt in the North Sea to less than 7 ppt in the Baltic east of the island Bornholm. This determines the distribution of *L. salmonis* on Danish salmonids. Wild Atlantic salmon returning from their high sea-migrations in the North Atlantic are heavily parasitized when reaching the Danish coastline (western, northern and north eastern part of Jutland). Danish seatrout display limited migrations but obtain infections during their stay at higher salinities such as the North Sea, Skagerrak and the Northern part of Kattegat. They may carry the parasites into areas with lower salinities when they reach the East coast of Jutland, the North coast of Funen, the North coast of Zealand, the Great Belt and Øresund. Baltic salmon (*Salmo salar*) and sea-trout (*Salmo trutta*) in the Baltic along the coast of Bornholm are not infected at all - emphasizing the low tolerance *L. salmonis* to low salinity. Investigations up untill now on the occurrence of this parasite species on maricultured rainbow trout in Danish marine waters have suggested that salmon louse reproduction is absent from mariculture systems in Denmark. The parasite is absent from most farms and only the northernmost located facilities in Kattegat may be exposed to infection as surveys show a few juvenile and preadult stages in these farms. High salinity currents from northern areas may carry infective copepodid stages into the area and thereby infect rainbow trout in net-pens although the infection success is limited. Restocking measures may influence fish densities and thereby transmission success. In addition, due to the changing environmental conditions, including climate changes affecting salinity and temperature, it is recommended, also in the future, to perform regular monitoring of *L. salmonis* infections of salmonids in Danish waters.

Questions and comments:

No questions

Pilchard orthomyxovirus (POMV) - An emerging pathogen in farmed Atlantic salmon in Australia

Peter Mohr

CSIRO, Australian Centre for Disease Preparedness, Geelong, Victoria, Australia.

Peter.Mohr@CSIRO.au

Abstract

The isolation of pilchard orthomyxovirus (POMV) from healthy pilchards and farmed Atlantic salmon in Australian waters was considered incidental and of minimal significance until 2012. Since that time, the virus has been associated with multiple outbreaks of disease in Atlantic salmon farms in Tasmania. Collaborative research funded by the Fisheries Research and Development Corporation at CSIRO's Australian Centre for Disease Preparedness and the Tasmanian Centre for Aquatic Animal Health and Vaccines has been critical to characterizations of the virus and the disease it causes in Atlantic salmon.

The assembly of POMV genomes from pilchards and farmed Atlantic salmon revealed minimal sequence diversity since its first isolation in 1998. The genome consists of eight negative-sense RNA segments that encode 10 putative proteins of which four are unique to POMV. Phylogenetic analysis of the *PB1* gene demonstrates that POMV isolates are most closely related to, but distinct from, *Isavirus* and *Mykiss* (proposed) genera within the *Orthomyxoviridae*. A POMV-specific real-time PCR has been developed to screen samples, as well as a nested PCR for confirmatory testing by amplicon sequencing. Preliminary validation of analytical and diagnostic characteristics of both PCR assays demonstrated that they are highly sensitive and specific for POMV detection.

Salmon orthomyxoviral necrosis (SON) disease in farmed Atlantic salmon is caused by infection with POMV and has resulted in significant production losses. In the early stages of disease, fish appear lethargic and exhibit a darker than normal colouration and reduced appetite. Following the appearance of these initial signs, the disease progresses rapidly to a loss of swimming ability and eventually death. In severe outbreaks, cumulative stock losses of up to 50% of fish in individual pens have occurred. Externally, the affected fish typically present with petechial haemorrhages on the ventral skin, and upon abdominal palpation, expel large quantities of clear or opaque mucus from the vent. Internally, SON is characterised by splenomegaly, mucus in the stomach and distal intestine and widespread vascular disturbances, including congestion and haemorrhage in the peritoneum, visceral fat and pyloric caeca.

Questions and comments:

Q: It was asked what control measures were taken in relation to the POMV outbreak.
A: Fish movements were limited in the affected areas.
Q: It was asked if the vaccine used was made using the whole virus or only part of it.
A: These data are not available
Q: It was asked if the stability of amino acids across all segments over time was surprising.
A: The similarity between isolates is surprising.
Q: The use of NGS for diagnosing an unculturable virus.
A: Without susceptible cell it would take a really long time to assemble the genome as there were no information in Genbank that could be used.

SESSION II: Control and Surveillance of fish diseases in EU

Chair: Niccoló Vendramin

Efficacy and Safety of DNA vaccine against Viral Hemorrhagic Septicemia and Infectious Hematopoietic Necrosis in rainbow trout: preliminary results from the field trial

Marsella[1] A., Toffan[1] A., Pascoli[1] F., Pretto[1] T., Manfrin[1] A., Rasmussen[2] J. S., Sepulveda[2] D., Vendramin[2] N., Lorenzen[2] N.

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*

[2]*Technical University of Denmark, Lyngby, Denmark*

Corresponding author: amarsella@izsvenezie.it

Abstract

Italian rainbow trout farming industry is strongly affected by Viral Hemorrhagic Septicemia (VHS) and Infectious Hematopoietic Necrosis (IHN). Both are regulated according to EU Directive 2006/88 and the notification of outbreaks is mandatory. Despite eradication efforts, both VHS and IHN are endemic in Italy except for a few compartments declared free of disease. Vaccination is allowed in the infected territories and as part of an eradication program. However, commercially licensed products are currently missing. The availability of vaccines is a key point in controlling and reducing the impact of VHS and IHN on rainbow trout production. Since the late '90s, DNA vaccines against both diseases have been designed and tested through *in vivo* challenge procedures under controlled conditions. Results obtained both in terms of efficacy and safety seemed to be extremely promising. Moreover, earlier field testing of a VHS targeted DNA vaccine in Denmark suggested that the vaccine provided protection under traditional earth-pond farming conditions, but few data are available regarding efficacy and applicability of DNA vaccines in commercial trout farming. Notably, the use of DNA vaccination has been successfully implemented in the control of IHN in Atlantic salmon production in Canada, and recently a DNA vaccine for prevention of Pancreas Disease in Atlantic salmon in Norway has been authorized.

In order to investigate this aspect, recent Italian VHSV and IHNV glycoprotein gene sequences were used to design two DNA vaccines tailored towards current virus variants causing disease in rainbow trout farms in the Trento Province. Following batch production in GMP-certified facilities, an experimental trial was first conducted at the IZSVe Experimental Aquarium, in order to obtain efficacy baseline data. A Potency and a Safety Tests were set up by intramuscularly injecting rainbow trout juveniles with 0.1 µg/fish and 1 µg/fish of VHS and IHN DNA vaccines, both singularly and in combination. Sixty days after the vaccination, Potency tanks were bath-challenged with selected strains of VHS and IHN and monitored for 30 days. Vaccine at the dose of 1 µg/fish induced the highest protection, reaching a RPS of 78.93% against VHS and 73.14% against IHN. Combination of the two plasmids resulted in a RPS of 48.76% in co-infected groups.

Based on these results, the Italian Ministry of Health granted the authorization to perform a field trial in a commercial category V farm. At the beginning of October 2020, 15,000 rainbow trout juveniles, provided by a VHS and IHN category I hatchery and weighing approximately 8 grams, were divided into three experimental groups: one injected with PBS as negative control, one injected with 1 µg/fish of VHS vaccine and the remaining group treated with 1 µg/fish each of each plasmid. Only 0.5% of mortality occurred following vaccination procedures. Fish are currently healthy and will be housed in the hatchery for approximately 60

days. At the beginning of December, they will be moved to the infected site and monitored for the onset of mortality. Due to the complexity of the authorization procedures, no active sampling will be performed. Depending on the results obtained, all fish will be monitored until they reach a marketable size, and the survivors will be used for human consumption as authorized by the Italian Ministry of Health (Authorization already obtained).

The research project IZSVe RC 09/18 was funded by the Italian Ministry of Health

Questions and comments:

Q: Would it be possible to bring a subsample of the field experimental fish to the lab and challenge under controlled conditions?
A: We will try if we can get authorization.
Q: Are patents for IHN DNA vaccines a problem?
A: Present vaccine is intellectual property of DTU.
Q: What is the outlook for public perception of DNA vaccination?
A: DNA vaccination approved in EU 2016. Is not GMO. Will make easier to approve. Stakeholders (farmers) do not have issues with vaccinating with DNA. Campaign necessary to explain to consumer that it is not GMO.
Comment: Niels Jørgen Olesen: DNA vaccination is not possible as part of control program in disease free areas, but will also become possible in relation to eradication programs.

Red skin disease – a novel syndrome affecting migrating wild Atlantic salmon in northern Europe

Charlotte Axén[1], Perttu Koski[2], Joachim Sturve[2]

[1]Swedish National Veterinary Institute (SVA), Uppsala, Sweden; [2]Finnish Food Authority, Oulu, Finland; [3]Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

charlotte.axen@sva.se

Abstract

In the summer of 2014, reports about diseased and dead fresh run, multiple sea winters (MSW) salmon started coming from Swedish rivers emptying into the heavily polluted Baltic Sea. Disease signs mainly included erythema or hemorrhage in the abdominal area, ulceration of the fin bases and secondary fungal infections. If fish with disease signs were caught, they were usually experienced as weak, even if fungus was not present. Sometimes even fish without any obvious disease were experienced as weak. Problems ceased as water temperatures reached about 20°C. Sea trout seemed to be unaffected. However, in the period around spawning, a new wave of fungal infections and a lot of dead spawners – both salmon and sea trout - were reported. This disease pattern has been occurring every year since.

In 2016, SVA conducted the first larger investigation in salmon from four rivers (Mörrumsån, Torneälven, Umeälven, Ljungan) and the inlet to lake Mälaren, Stockholm. Necropsy and sampling for histopathology, bacteriology and virology was performed during June, July and October. Torne river was sampled in association with the Finnish Food Authority, who also collected samples from other Finnish rivers (Kemijoki, Iijoki, Oulojoki). Torne river salmon mainly had mechanical injuries, whereas salmon from the other rivers mainly had skin hemorrhage and UDN-like injuries. A few fish were infected with bacteria and one fish was infected with PRV-1, but no common bacterial or viral cause could be identified. Whole genome sequencing of eight fish with skin hemorrhage indicated presence of herpes and/or iridovirus, but this has not yet been confirmed. Moderate to severe dermal and hypodermal inflammation was common in hemorrhaging skin. UDN was histologically confirmed in about 50 % of the fish with clinical signs of the disease.

In 2018 SVA conducted a second investigation together with Gothenburg University to look at biomarkers. Biomarkers are bodily factors (e.g. detoxification enzymes) commonly used in environmental surveillance. In addition, cytology, histopathology, thyreoid hormones (T3, T4), some vitamins and antioxidants were included and a case control trial to investigate the effect of thiamine injections was performed. Mörrumsån, Torneälven and Umeälven (June-July), Indalsälven (Sept) and Lagan (west coast, October) were sampled. Fish from Umeälven were severely ill at sampling whereas the Torneälven salmon appeared to be at good health. Mörrumsån salmon had mild disease signs. Hyperglycemia, hemodilution, elevated EROD, T3 and T4 levels, a high percentage of neutrophils and/or monocytes/macrophages and increased erythropoiesis were identified. Vitamin levels were significantly lower in spawning females (Lagan). Although total thiamine was not lower in Umeälven, free thiamine was low, indicating a high level of turnover to the active metabolite TDP. Multivariate analysis of the data associated hyperglycemia, hemodilution and a high % of monocytes/macrophages or neutrophils with disease[1]. The case control trial did show a positive effect of thiamine injection in MSW fish.

In 2019 the disease seemed to spread, as countries outside the Baltic Sea started to experience the same disease patterns. Reports came from Norway, Scotland, England, Ireland and Denmark. Reports have also come from Russia, but there UDN was reported as the main problem. In November 2019, a gathering was held in Oslo and the disease was named Red Skin Disease. The group has been updating each other about the development in 2020, and hopefully this group can collaborate to find the underlying factors of this disease manifestation, whether there is a common cause for the Baltic and Atlantic salmon or not

[1]Weichert, F. et al (2020). A multi-biomarker study on Atlantic salmon (*Salmo salar* L.) affected by the emerging Red Skin Disease in the Baltic Sea. J fish Diseases. Accepted 7 Oct 2020. DOI: 10.1111/jfd.13288

Questions and comments:

Q: Why the biphasic profile of reported cases? Only few cases in August.
A: Stress at summer temperatures approaching 20 degrees. They are then susceptible to disease. Then when spawning approaches they are immunosuppressed and have not been eating for a long time. They succumb to fungal infection.
Q: What about seals? The population has increased. Is this related to the symptoms you see?
A: In Torne river a lot of seal bites are reported, but not otherwise. But these are not related to RSD.
Comment: The same symptoms we observed among brown trout spawners in Poland. Examination is in progress; however, the first publication already appeared this year in Mycotoxin Research (https://doi.org/10.1007/s12550-020-00395-8). *Fusarium* detected on skin in the beginning of this year. We should cooperate and share information.
Q: Have you been trying to transmit the disease experimentally?
A: No. We do not have the facilities to keep adult salmon, and have no money to do it.
Q: Will you also try virus isolation on homologous cells, like from salmon? Might make a difference.
A: Already used a lot of different cell cultures, but would like to try different homologous cells of different kinds. We have also considered making a primary culture of skin from affected salmon. Maybe epidermal cells are better than fibroblasts.
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Update on control and management of IHN outbreak in Finland

Tuija Kantala and Tuija Gadd

Finnish Food Authority

tuija.kantala@foodauthority.fi

Abstract

In November 2017, infectious hematopoietic necrosis virus (IHNV) was detected for the first time in Finland. Until that, Finland had maintained an official IHNV-free status. A containment area was established around the positive winter storage net cage farm producing rainbow trout for food, located in Northern Ostrobothnia in the Gulf of Bothnia. Sampling and testing of contact farms and other production units of the positive farm started immediately. The second IHNV case was detected in the neighbouring winter storage net cage farm, located in the same containment area, in the beginning of December 2017. Later in December 2017, third case was detected in a government-owned broodstock and nursery facility in continental Finland in Northern Savonia (municipality of Tervo) in the River Kymi basin. When testing the contact farms of this facility, the fourth case was detected in a catch and release fishing pond close to the positive facility in the end of December 2017. In January 2018, the fifth and sixth cases were detected in samples from two different fishing ponds in River Vuoksi basin.

In total, four containment zones were established: in the municipality of Ii in Northern Ostrobothnia, in the municipality of Tervo in the River Kymi basin, and in the municipality of Kaavi and the city of Nurmes in the River Vuoksi basin. All six affected farms and ponds were emptied and about 230 t fish were slaughtered by 14.2.2018. All farms were disinfected by the end of October 2018, following a fallowing period of at least six weeks before taking new fish.

Increased number of official control visits and sampling was continued in the contact farms and other production units of the six positive farms, nearby units, units within the same water area, and units located near the units with planted fish that originated from the positive facility in Tervo. In addition, wild fish from areas with high risk of infection were sampled and tested.

As of July 2018, Finland, except the four zones where IHNV was detected, has had an official IHNV-free status. The aim was to eradicate IHN as quickly as possible, and by surveillance program of two years, to regain an official IHNV-free status for the whole country. By far, three of the containment zones have been be converted into surveillance zones, the one in Nurmes in September 2019, the one in Ii in July 2019, and the one in Tervo in February 2010. The containment zone in the municipality of Kaavi still remains, for there are no fish farms in the area. Surveillance program of two years is ongoing in the four zones, including inspection and testing of production units of farmed fish twice a year, and sampling and testing of wild fish.

The surveillance zone in Ii is expected to regain an official IHNV-free status by the end of 2020, and the zones in Tervo, Nurmes and Kaavi in summer 2021.

Questions and comments:

Q: Can you speculate on the source of the virus?
A: We do not know the original source, but we have an idea of some of the spreading internally in Finland.
Q: Could you elaborate a little bit on the efforts to eradicate the virus?
A: It took a long time and was very time consuming. For example in one case the sea cages were under a cover of ice when the virus was detected and they had to build an ice road before even being able to access the cages.

Experimental testing of a virus like particle (VLP-) vaccine against Viral Nervous Necrosis in European sea bass

Sofie Barsøe[1], Ansgar Stratmann[2], Niccolò Vendramin[1], Anna Toffan[3], Francesco Pascoli[3] & Niels Lorenzen[1]

[1]*DTU Aqua National Institute of Aquatic Resources, Kemitorvet 202, Kgs. Lyngby, Denmark*

[2]*W42 Industrial Biotechnology GmbH, Dortmund, Germany*

[3]*Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Padua, Italy*

sofhan@aqua.dtu.dk

Abstract

Viral Nervous necrosis remain to be one of the largest health treats to Mediterranean aquaculture, affecting European sea bass (*Dichentrarchus labrax*), amongst many other marine species (Munday et al., 2002; Muniesa et al., 2020). The etiologic agent is a small icosahedral RNA virus called Nervous Necrosis Virus (NNV) or betanodavirus, with a simple genome of two segments: RNA1 coding for the viral RNA-polymerase and RNA2 encoding the capsid protein (Mori et al., 1992). The capsid protein can be expressed in various expression vectors, such as a bacteria, virus, yeast or plants (Lin et al., 2001; Zeltins, 2013). After expression and release, the capsid protein auto-assembles into virus like particles (VLPs) with same size and morphological structure as the virions. The VLPs are promising vaccine candidates, as they have the same immunogenicity and antigenicity as virus particles but are unable to replicate and cause disease (Crisci et al., 2012; Jeong and Seong, 2017). VLPs of the NNV have shown promising protection and seroconversion in many fish species affected by NNV, but only few studies has been conducted in European sea bass (Gonzalez-Silvera et al., 2019; Marsian et al., 2019; Thiéry et al., 2006).

This presentation will include the preliminary results of vaccination and challenge experiments of sea bass with a new promising RGNNV VLP vaccine prototype. The vaccine was developed during the EU project Target Fish and is produced by W42 Biotech GmbH, in a *Pichia pastoris* expression system. Antibody response profiles as well as protection against disease following challenge at different time points and by different routes were examined.

This research has received funding from the European Commission Horizon 2020 (H2020) Framework Programme through grant agreement no 727315 MedAID project (Mediterranean Aquaculture Integrated Development).

Questions and comments:

Q: What was the antigen dose/fish in the commercial vaccine?
A: This is not know, but the commercial vaccine is based on a different strain than in the VLP. We used the VLP strain for challenge. We used the recommended dose for the vaccine.
Q: How relevant do you think i.m. challenge is to a field situation?
A: That is a general debate. Of course it is not a natural route of infection. However, it is a good model. Reproducible. If vaccine works with i.m. challenge, it is likely to work also in the field. In an experimental setting it can be hard to get disease from noda virus with immersion challenge. But I have included it in upcoming experiments.
Q: Were the challenge groups mixed or in separate tanks?
A: The groups were in separate tanks.

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

Chair : Morten Schiøtt

Salmon erythrocytes sequester infective ISAV particles during infection

Johanna Hol Fosse[1], Tonje Sønstervold[1], Anita Solhaug[1], Simon Weli[1], Argelia Cuenca[2], Petra E. Petersen[3], Maria Marjunadóttir Dahl[3], Niccolò Vendramin[2], Debes Christiansen[3], Maria Aamelfot[1], Knut Falk[1]

[1]Norwegian Veterinary Institute, Oslo, Norway; [2]National Institute of Aquatic Resources, Technical University of Denmark, Kongens Lyngby, Denmark; [3]Faroese Food and Veterinary Authority, National Reference Laboratory for Fish Diseases, Tórshavn, Faroe Islands

johanna.hol.fosse@vetinst.no

Abstract

Infectious salmon anaemia virus (ISAV) infects endothelial cells and binds red blood cells. However, it is not known how this affects the course of infection or development of disease. One hypothesis is that the irreversible binding of ISAV to red blood cells makes the cells fragile and contributes to anaemia. We have mapped the occurrence of ISAV-coated red blood cells in material from experimentally and naturally infected fish. We present preliminary findings that show that ISAV-coating is a common occurrence that preceeds the development of anaemia and other signs of disease. On rare occasions, we also observed ISAV protein production by a minor fraction of red blood cells. However, this was not required for development of disease and mortality. The majority of infectious particles in the blood appears to be associated with the cellular fraction. In conclusion, salmon red blood cells are targeted by ISAV and sequester infective virus particles during infection. While red blood cells do not appear to contribute significantly to viral replication, we discuss how their interactions with ISAV may influence other aspects of infectious salmon anaemia.

Questions and comments:

Q: Have you noticed the formation of haemagglutinins in the blood of the ill fish?
A: Not a major feature. Not tried in the trial challenge. We have difficulty to access samples form field trial
Q: Are the cells fixed? Have you tried to stain cells that are not fixed?
A: Will be interesting to do flowcytometry, a quantitative approach to be investigated. Likely the virus sits on the outside of the rbc. ISAV might be internalized to some extend. Further work need to be done to prove this.
Q: Hemoagglutinin does not happen in vivo, are you sure there is no clotting?
A: Not sure that doesn't happen, could be, we don't know. Not a prominent feature in experimental trial; need to do the Coomb's test

Modelling scenarios for control and mitigation of Infectious Salmon Anemia (ISA)

Britt Bang Jensen[1], Magne Aldrin[2], Ragnar Bang Huseby[2], Mona Dverdal Jansen[1]

1: Norwegian Veterinary Institute, 2: Norwegian Computing Central

Britt-Bang.jensen@vetinst.no

Abstract

With the planning of implementation of the new animal health directive, different ways to mitigate and control listed diseases are being explored. Control of Infectious Salmon Anemia (ISA) in Norway is currently by use of a stamping-out strategy: if a farm experiences a clinical ISA outbreak today, the farmer is usually required to slaughter all fish at the farm within a few weeks.

We have developed a spatio-temporal stochastic model for the spread of infectious diseases between and within marine aquaculture farms in Norway. The estimated model is used for scenario simulation, or what-if analysis, to investigate the effects of potential strategies to combat ISA, including screening, vaccination and culling.

From the model, we found the estimated relative importance for each of the various sources of infection. For spread of ISA, infection from infected neighbouring farms accounts for around 50 % of the infections directly. Infection from ”non-specified sources” accounts for around 40 % of the infections, and for ISA we believe that the most important of these are viruses mutating from HPR0 to the virulent HPRdel. Infected cohorts moved from another farm accounts for 8 %, and most of these are presumably originally either infected by other farms or by ”non-specified sources” before they were relocated. Infection as a result of stocking smolts infected by the virulent HPRdel accounts for around 1% of all infections in the model.

From the scenario simulations, we found that omitting the mandatory culling without any other preventive measure will increase the number of outbreaks dramatically, and is probably a non-viable option. Introducing mandatory vaccination may be equally effective as today's culling strategy alone depending on the obtained field-efficacy of a vaccine. Introducing mandatory screening combined with culling will bring the number of outbreaks to a low number, because infected cohorts usually are slaughtered before an outbreak occurs.

However, the selection of a new strategy also involves both economic and political considerations. Thus, using scenario simulations of different strategies provides one valuable input to support decision making.

Questions and comments:

Q: From previous talk, do you think HPR0 transmitted from FW or infection occurs once they are at sea?
A: Highly debated, we have a number of cases where there is high homology between HPR del and HPR0 of origin. But we don’t have overview. We miss the large screening of FW sites. There is also HPR0 in SW we don’t know.
Q: How about the vaccine, can you model 100% protection in the model?
A: We have also model different level of protection at different stages of the production.
Q: What is the vaccine?
A: Only Pharmaq whole inactivated virus.
Q: How easy will it be to screen for isa in sites with no increased mortaily?

A: We could use same samples for SAV screening.
Q: Did the model consider cost of intervention?
A: Yes we have done that, it is available to competent authorities.
Q: Regular screening in FW, what strategies when HPR0 is detected?
A: Farmers screen but we don't have data, a lot of farmers don't know how. Some plans to depopulate and disinfect, there is a lot of uncertainty. Hard to assess how the HPR0 will affect the outbreak of ISA.

Epidemiology and Pathogenesis of *Piscine orthoreovirus* Genotype 3

Juliane Sørensen, Niccolò Vendramin, Niels Jørgen Olesen and Argelia Cuenca

Unit for Fish and Shellfish Diseases, National Institute of Aquatic Resources, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

jusor@aqua.dtu.dk

Abstract

A new genotype of *Piscine orthoreovirus* (PRV) – genotype 3 (PRV-3) – was discovered for the first time in 2015 in Norway, and in 2017 PRV-3 was detected in Denmark in association with complex disease cases in rainbow trout in recirculating aquaculture systems (RAS). To explore the epidemiology of PRV-3 in Denmark, a surveillance study was conducted from late 2017 to early 2019. Fifty-three farms, including flow-through and RAS, were screened for PRV-3. Of the examined farms, PRV-3 was detected in thirty-eight (71.7%), with the highest prevalence in RAS. Notably, in Denmark disease outbreaks associated with PRV-3 were only observed in RAS. Additionally, wild Atlantic salmon and brown trout populations were included in the screening, and PRV-3 was not detected in the three years in which samples were obtained (2016, 2018, and 2019).

Historical samples in the form of archived material at the Danish National Reference Laboratory for Fish Diseases were also tested for the presence of PRV-3, allowing us to establish that the virus has been present in Denmark at least since 1995.

Sequence analyses of segment S1 and M2, as well as full genome analyses of selected isolates, did not reveal any clear association between genetic makeup in these two segments and virulence in the form of disease outbreaks in the field. Sequence isolates associated with disease outbreaks were identical or very similar to those not associated with disease. Importantly, sequence isolates from 1995 were identical or very similar to contemporary sequence isolates, indicating that the virus has not changed in the S1 and M2 segment the past 23 years.

Put together, these data and observations point to a need for certain external factors in order for PRV-3 to be associated with disease outbreaks. As farmers generally report more issues related to PRV-3 during the colder months, an infection trial with temperature at its core was therefore conducted. Preliminary results show a difference in haematocrit during the time course of an infection depending on the water temperature.

Questions and comments:

Q: Only outbreaks in RAS farms, can you speculate on additional factors?
A: Could be dose effect with the virus, level of stress that fish are exposed to in this farming system. Further work is needed.
Q: HCT correlation with virus?
A: Work on the PCR data.
Q: Could RAS have a water quality challenge that pre-dispose fish to PRV-3?
A: Work ongoing.

SESSION IV:Update from the EURL

RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2019

Teena Vendel Klinge, Niccoló Vendramin, Argelia Cuenca and Niels Jørgen Olesen

EU Reference Laboratory for Fish and Crustacean Diseases,

DTU AQUA National Institute of Aquatic Resources, Kemitorvet, Bygning 202, 2800 Kgs. Lyngby, Copenhagen,

niven@dtu.vet.dk

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 Commission Decision 2015-1554.

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 2019.

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

49 laboratories participated in PT1 while 47 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

The parcels were submitted on a Friday and 51% of parcels were delivered by the shipping companies the following Monday, 86% was delivered within 1 week and 98% was delivered within 23 days. One parcels took 38 days due to internal clearance problems.
Overall 39 out of 49 participants scored 100% success rate; 4 participant scored 90% due to sequencing of the content in ampoule II (ECV) or contamination in ampoule III (VHSV) and 2 participants scored 80% due to not finding one of the virus. 4 participants scored below 80% due to not finding one of the virus or/and contamination of the ampoule with another virus.
These points have addressed directly with the single participants that has underperformed.
For the first time this year we have scored the sequencing results of all participating laboratories.
For each ampoules 2 points were given. Ampoule II, which contained the ranavirus, was not included in this exercise being the sequence of virus already assessed in the main scoring.
Overall it is acknowledged that the majority of participants are putting more and more effort in this exercise, however it appears that sequencing efforts across the laboratories varies significantly spanning from laboratories which sequenced and genotyped all isolates in the panel of PT1 (15 out of 49) to laboratories which do not sequence any of the isolates included in the ampoule (13 out of 49). Within the results collected there is also somewhat a variation in the nomenclature, highlighting the need for further harmonization on nomenclature used in genotyping and this is reflected by examples of providing serotype identification instead of genotype in the case of IPN virus or using genotype M instead of E in case of IHNV.

Résumé and concluding remarks PT2

Starting from 2020, participants will have to report in the "concluding result" if the ampoule contain ISAV HPR0 or ISAV HPRΔ, as per methods provided in the diagnostic manual for ISA. Concluding ISAV HPR0 instead of ISAV HPRΔ will reduce the score to 0, considering that ISAV HPR0 is not listed according to EU legislation. The EURL will update the example in the spreadsheet accordingly.
It is acknowledged that more and more laboratories have increased efforts in genotyping the viral isolates included in the ampoules, and therefore for the first time this year, we have decided to score the genotyping results. Out of 47 participating laboratories, 33 perform sequencing of ISAV to determine HPR type, 23 for KHV and 26 for SAV. 32 laboratories out of 33 correctly sequenced the ISAV in ampoule VI, 28 correctly typed the isolate as HPRΔ and only 1 as HPR0. It has to be observed that the sequencing of the ISAV isolate in ampoule IX has created some challenges for the participants, in this case 4 laboratories typed it as HPR0.
It is generally highly appreciated the development observed in the quality of the analysis provided by the NRL and with small improvements in the nomenclature an harmonized system will be implemented within the NRL network.

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 TRAINING COURSES FOR 2020, Ideas and plans for 2021

Niccoló Vendramin and Tine Moesgaard Iburg

EURL Fish Diseases, National Institute of Aquatic Resources, Technical University of Denmark, Denmark

niven@aqua.dtu.dk and timi@aqua.dtu.dk

Abstract

In 2020, due to Covid-19 pandemics the EURL for fish diseases has organized one training course, which has been held virtually.

The course **Methods for implementation of surveillance procedures for listed fish diseases** was held in week 41 from Monday the 5th to Friday the 9th of October, a series of lectures, practical demonstrations, assignments and group work was organized.

Being the course organized remotely it was possible to raise the limit for participation, and the course was made accessible to participants oversea.

Plans and ideas for 2021 will be discussed with the floors.

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

More information are available on the EURL website

www.eurl-fish.eu

EURL Work Plan for 2020

Niels Jørgen Olesen, Niccoló Vendramin

EURL Fish and Crustacean Diseases, DTU

njol@aqua.dtu.dk

Compared to previous years the work programmes of the EU reference laboratories have become much more detailed specifying objectives, resources, outputs and durations. The work programme for the EURL for Fish and Crustacean Diseases for 2020 is given in the following:

1 TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs.

Sub-activity 1.1 *(Annual workshop fish diseases)*

Objectives: To ensure knowledge dissemination and sharing between the Member State NRLs on existing and emerging fish diseases and to agree on the future priorities of the EURL, by holding the 23rd and 24th annual workshop of the National Reference Laboratories (NRLs) for fish diseases in 2019 and 2020, respectively.

Description: These workshops are organised as annual event and all Member State NRLs are strongly recommended to participate in them, as it is an important opportunity to be updated on the newest scientific knowledge of fish pathogens, diagnostics, legislation, epidemiology etc. Several talks of high scientific standard will be given and discussions at group and plenum level will be facilitated during the two days of the workshop.

Expected Output: Successful preparation and completion of the 23rd and 24th annual workshop comprising two full days in May 2019 and 2020. Based on previous experience it is expected that 50 participants will attend the workshop including EU Member States, associated countries and invited speakers. From the EURL team six members will attend the workshop full time. A technical and financial report of the workshops will be produced. The technical reports will contain abstracts and minutes from all presentations and discussions and will after acceptance be made publicly available through the EURL website.

Duration: The workshop is to be held ultimo May 2019 and 2020. Preparation in February – April and finalizing of the reports in May – August.

Sub-activity 1.3 *(Scientific working groups)*

Objectives: To ensure that fast and reliable scientific advice on specific topics related to listed and emerging diseases and to legislative issues, is provided by organising expert meetings in order to solve arising challenges in EU.

Description: In case of critical fish or crustacean disease related problems within EU Member States, we will organize specific scientific meetings by collating international experts.

Expected Output: We expect to organise four scientific working groups in 2019 and 2020 with the duration of one to two days each. A working group on 1) susceptible fish species to listed diseases in EU, 2) assessing fish and crustacean diseases for possible listing in EU legislation, 3) emerging diseases. The topic of the emerging disease working group will be defined in relation to ad hoc request. From each meeting, a scientific report including recommendations will be delivered to the relevant Member State NRLs and the European Commission and will be available on our website www.eurl-fish.eu.

Duration: Working group 1 and 2 in 2019 and working group 3 in 2020; the timing of working group 4 held will be decided depending on specific need. The meetings will comprise one to two days in Copenhagen and time for organising and reporting.

Sub-activity 1.4 *(Proficiency test fish diseases)*

Objectives: To assess the capabilities of all Member State NRLs to detect pathogens causing fish diseases and to harmonize the procedures used by an inter-laboratory proficiency test. Description: The EURL is going to prepare Annual Inter-laboratory Proficiency Tests for all Member State NRLs. The tests will include the viral fish pathogens; Viral haemorrhagic septicaemia virus (VHSV), Infectious haematopoietic necrosis virus (IHNV), Epizootic haematopoietic necrosis virus (EHNV), Infectious salmon anemia virus (ISAV) and Koi herpes virus (KHV), and will also address other common viral pathogens in fish farming Infectious pancreatic necrosis virus (IPNV), Spring viraemia of carp virus (SVCV), Salmonid alphavirus (SAV), Ranaviruses, etc. The participation is mandatory for all NRLs in EU. After submission of test results from the NRLs to the EURL, we will collate and analyse information gained from the proficiency test and publish the anonymous data to all participants as a report on a restricted site of our website www.eurl-fish.eu. A non-coded version will be provided to the EU Commission with information on performances and under performances. The results will be presented and discussed at the Annual Workshops in 2019 and 2020. The tests are accredited according to ISO 17043 and are indispensable for maintaining accreditations at the NRLs. Expected Output: Preparation and shipping the test and subsequently provide a report on the proficiency tests 2019 and 2020. Based on previous experience it is expected that 45 laboratories are participating with a success rate of > 90 percentage for both tests. Underperformances will be addressed by direct communication with the participant. Underperforming laboratories will be considered for mission from the EURL. Duration: January – December 2019 and 2020. The samples included in the test will be shipped from the EURL in the fall and the final report will be submitted February the following year.

Sub-activity 1.6 (Diagnostic methods)

Objectives: For the EURL to have diagnostic methods of the highest scientific standards and to be able to provide these methods to all Member State NRLs. Description: Novel molecular methods are highly sensitive and specific tools for diagnosis and surveillance of a number of listed pathogens. In 2019 and 2020, the EURL will focus on four techniques; 1) PCR for detection of genomic RNA/DNA from pathogens, 2) In-situ Hybridization (ISH) for pathogen localization in paraffin embedded tissue, 3) Next Generation Sequencing for full genome sequencing and 4) Improved cell culture techniques. In 2020 the EURL will establish a repository of reference viral strains for Infectious salmon anemia virus (ISAV) and implement diagnostic qPCR able to discriminate virulent ISA strains HPRΔ and non-pathogenic ISA strains HPR0. With the ISH technology established in 2019, the main pathogens targeted in 2020 will be VHSV and the emerging pathogen PRV-3. Expected Output: Four new diagnostic methods implemented in the two year period. Four diagnostic molecular methods validated according to the recommendations given by the OIE. Duration: January – December 2019 and 2020.

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Sub-activity 2.1 *(Training Courses)*

Objectives: To ensure that employees of the Member State NRLs have the highest scientific and excellent skills in diagnosis of fish and crustacean diseases. Description: The EURL yearly provides two training courses in methods used for diagnosis of fish and crustacean diseases. These courses are primarily offered to participants of the Member State NRLs. The content is mainly based on the opinion of the EURL on what is required in the Member State NRLs. The course contents are also discussed during the annual workshops, where the Member State NRLs are able to provide specific input. Expected Output: Two training courses of 5 days in 2019 and 2020, with 10-15 participants in each course; more than 90 % of the participants were satisfied with the course based on the 2018 evaluation. Duration: September – October, 2019 and 2020.

Sub-activity 2.2 *(Website www.eurl-fish-crustacean.eu)*

Objectives: To provide the Member State NRLs with a fast entrance to information from the EURL. Description: The EURL are administrating the webpage, www.eurl-fish.eu, by uploading relevant material such as updated lists of NRLs, annual workshop presentations, training course reports, sampling and diagnostic procedures, newest update on legislation, general news from the community, etc. The website has daily visitors from a great number of countries from around the world and are, therefore, a substantial part of disseminating the work of the EURL for fish and crustacean diseases. Due to the inclusion of crustacean diseases in the EURL we will 2019 launch a new and updated website. The new website will in the future be located at www.eurl-fish-crustacean.eu and the old one www.eurl-fish.eu will close. The website will be further developed including a "restricted access area" where reports and information which are specific for targeted stakeholders will be uploaded. Expected Output: A constantly updated webpage for the Member State NRLs. Establishment of a restricted area and provision of guidelines to all Member States NRLs for access to the restricted area. Duration: The new website will be up running primo 2019 and maintenance will be from January – December 2019 and 2020.

Sub-activity 2.3 *(EURL Contact Lists)*

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

Description: We will aim to have three contact lists. 1) Member State NRLs for fish diseases, 2) Member State NRLs for Crustacean disease and 3) a general list which all interested in the work of the EURL can subscribe to. The EURL use the mailing lists for important notifications i.e. meeting calls, training course calls and other relevant information such as information on upcoming conferences, new research findings and relevant reports and publications, emergency situations etc. Often the notifications will include links to the website or other sites for further and detailed information.

Expected Output: The EURL usually prepare and submit around 10-15 notifications per year via the contact lists to ca. 130 subscribers.

Duration: January – December 2019 and 2020.

Sub-activity 2.4 *(Missions to NRLs for fish diseases)*

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

Description: Missions are only planned to Member State NRLs for fish diseases, however, we will be able to conduct missions to NRLs for crustacean diseases if it is found necessary. NRLs chosen for a mission are primarily based on performance in the yearly proficiency test. However, if missions to other countries, both EU Member States but also 3rd countries, will be able to provide important scientific knowledge for the EURL to pass on to Member State NRLs, missions to such countries will be conducted. This will ensure EU Member States to be updated with excellent scientific skills and knowledge. Expected Output: As the decision for appointing target laboratories for missions is based on performances of the proficiency test- no final decision can be taken at this stage. Two missions per year conducted from the EURL, first draft of the report of each mission provided to the host institution within 1 month from the mission Duration: April and/or November 2019 and 2020.

Sub-activity 2.5 *(International conferences and meetings)*

Objectives: To keep the EURL updated on the newest scientific information on emerging and listed exotic and non-exotic fish and crustacean diseases, and to disseminate knowledge and scientific data provided by the EURL.Description: The EURL staff is able to provide consultancy to Member State NRLs on emerging and listed fish and crustacean diseases, and attending conferences are an important way of the EURL to keep the excellence of this function. Conference participation therefore ensures up-to-date knowledge within the EURL.

Expected Output: The EURL expect to participate in 4 to 6 international conferences e.g. the 19th International Conference on Diseases of Fish and Shellfish, Porto, Portugal 9th-12th September 2019, OIE international conference on aquatic animal health, Santiago, Chile 3-4, April, 2019, The 11th International symposium of virus of lower vertebrates and the 5th Nordic RAS Workshop 7-8 October 2019, Berlin.

Duration: January – December 2019 and 2020.

Sub-activity 2.6 *(Confirmatory diagnosis)*

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

Description: Every year the EURL receives strains of pathogens for corroboration of diagnostic results in the EU Member States. Regularly these strains must be characterized properly as an emergency response to avoid unwanted spreading of new pathogens in EU. The EURL describe theses strains by serological and genetic characterization, including bioinformatics.

Expected Output: Based on experience from the previous year, the EURL expects to corroborate the diagnosis for five new outbreaks and sequence the isolates yearly

Duration: January – December 2019 and 2020.

Sub-activity 2.7 *(Pathogen characterization)*

Objectives: For the EURL to be able to characterize isolates of listed viral pathogens of aquatic animals as well as emerging pathogen and provide scientific based risk assessment to the scientific community and stakeholders. Description: The EURL every year contributes to characterize relevant pathogens for aquaculture in Europe as an emergency response to avoid unwanted spreading of new pathogens in EU. The EURL describe these strains by pathogenicity testing in-vivo. The experimental trial contribute to establish reference material to be used as positive controls and standards enabling diagnostic validation of new diagnostic methods.

Expected Output: The EURL expect to characterize two pathogens per year. A report of each single infectious trial included in a risk assessment report and/or published in peer review journals. Duration: January – December 2019 – 2020.

3 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

Sub-activity 3.1 *(Diagnostic manuals fish diseases)*

Objectives: To have updated diagnostic manuals for all listed fish diseases available for Member State NRLs on the EURL website.

Description: The diagnostic manual for sampling and detection of listed non-exotic diseases was finally adopted in 2015. However, as the diagnostic procedures for identification and surveillance of the listed diseases is rapidly evolving new procedures will be assessed and validated for inclusion in the first revision of the diagnostic manuals.

Expected Output: Updated sampling and diagnostic manuals for the viral fish diseases viral haemorrhagic septicaemia (VHS), infectious hematopoietic necrosis (IHN), infectious salmon anaemia (ISA), koi herpes virus (KHV) and epizootic haematopoietic necrosis (EHN) on the EURL website.

Duration: January – December 2019 and 2020.

Sub-activity 3.2 *(Diagnostic manuals crustacean diseases)*

Objectives: To have updated diagnostic manuals for all listed crustacean diseases available for Member State NRLs on the EURL website.

Description: The diagnostic manual for sampling and detection of listed non-exotic diseases was finally adopted in 2015. However, as the diagnostic procedures for identification and surveillance of the listed diseases is rapidly evolving new procedures will be assessed and validated for inclusion in the first revision of the diagnostic manuals.

Expected Output: Updated sampling and diagnostic manuals for the viral crustacean diseases White Spot Disease, Taura Syndrome and Yellowhead Disease on the EURL website.

Duration: January – December 2019 and 2020.

Sub-activity 3.3 *(Survey and diagnosis fish diseases)*

Objectives: As part of our duties given in given in C.D. 2006/88/EC Annex VI, Part I.5 (f) to "collate and forward information on exotic and endemic diseases, that are potentially emerging in Community aquaculture" data on emerging and endemic fish diseases and fish health surveillance in Europe will be collated in order to ensure that the EU Commission, the Member State NRLs and the EU in general are updated on the fish diseases situation in aquaculture and natural fish populations in Europe.

Description: The EURL collect data on emerging and endemic fish disease outbreaks from NRLs in all European countries by submitting a questionnaire and disseminating the information gathered in a report and at the Annual Workshop. The data are collated in a "Survey and diagnosis" report, which is made available for the Commission, Member State NRLs and for approved users on our website. This report includes information on the presence of all the listed non-exotic fish diseases given in Council Directive 2006/88/EC Annex IV Part 2, on emerging diseases, and on all surveillance programmes on fish diseases conducted in EU.

Expected Output: A report on "Surveillance and diagnosis of fish diseases in Europe". The report will be presented at the annual workshops and uploaded in the restricted area of the website. The report will be accessible for relevant stakeholders including NRLs and EU commission

Duration: January – June 2019 and 2020.

Sub-activity 3.4 *(Risk assessment for emerging diseases)*

Objectives: For the EURL to have the most updated and highest scientific knowledge of emerging and re-emerging fish and crustacean diseases in Europa.

Description: Due to increased international trade of fish and crustaceans, focus will be given to emerging diseases and rapid response to Member State NRLs and EU in case of outbreaks. An assessment of risk for contracting and spreading specific emerging and re-emerging diseases in EU will be conducted. In collaboration with specialised experts the EURL foresee to work e.g. with the emerging fish pathogens Infectious Salmon Anemia virus (ISAV), Tilapia Lake Virus (TiLV), Salmonid Alphavirus (SAV) and Piscine Myocarditis Virus (PMCV) in Europe to be able to assess their potential listing as exotic or non-exotic diseases in the future.

Expected Output: The EURL will have relevant and updated scientific knowledge on emerging fish diseases in EU and be able to provide immediately consultancy to all Member State NRLs, the European Commission and stakeholders. Scientific knowledge on specific emerging diseases will be disseminated through oral and written presentations in scientific journals (1 publication per year), at annual workshops, conferences (1 oral presentation per conference) etc. The EURL aims to assess diagnostic methods and establish reference material for validating diagnostic methods. Two diseases will be addressed yearly.

Duration: January – December 2019 and 2020.

4 REAGENTS AND REFERENCE COLLECTIONS

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

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

Description: The database www.fishpathogens.eu is a valuable tool for all Member State NRLs for virus characterisation and molecular epidemiology of listed and non-listed fish pathogens. The more isolates included the stronger the tool for the EURL and Member State NRLs. The database code is, however, more than 10 years old, and an urgent update is needed. This update, together with the addition of new tools to handle full genomes, is already in process and will continue during 2019.

Expected Output: During 2019, around 110 full genome sequences of VHSV will be included in the database, as well as around 30 full genomes of IHNV. Both SAV and Betanodavirus databases will be modified to include full genome data, as well with tools to detect/identify reasserting strains in betanodavirus (2020). In addition, collaboration with groups in Norway will be initiated in order to stablish a new database of infectious salmon anaemia virus (ISAV) isolates (2019-2020).

Duration: January – December 2019 and 2020.

Sub-activity 4.2 *(Pathogen library)*

Objectives: For the EURL to have an updated library of fish and crustacean pathogens relevant for the EURL and Member State NRLs.

Description: The EURL are going to update and maintain a library of isolates of the viral fish pathogens infectious salmon anaemia virus (ISAV), viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), koi herpes virus (KHV), enzootic hematopoietic necrosis virus (EHNV) and other relevant putative emerging fish pathogens.

Expected Output: The library will be updated yearly, furthermore, infected tissue material originated from the infectious trial conducted within the “Pathogen characterization” sub activity (two tissue libraries per year) will be made available upon request to Member State NRLs as positive control material (expected to ship five panel per year).

Duration: January – December 2019 and 2020.

Sub-activity 4.3 *(Production and supply of reagents)*

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

Description: Diagnostic reagents (i.e. polyclonal antibodies raised in rabbit, monoclonal antibodies from stored hybridoma cells or in situ hybridization (ISH probes) will be produced according to demand form the Member State NRLs.

Expected Output: The EURL expect request of diagnostic reagents from around 15 Member State NRLs yearly. However, we are able to provide more reagents if there is a need from more Member State NRLs.

Duration: January – December 2019 and 2020.

5 REQUIREMENTS RELATED TO OTHER LEGISLATION

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

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

Description: To harmonize implementation and interpretation of aquatic animal health law across the different Member States.

Expected Output: The EURL expect to receive 10 specific request per year from EU or Member States. First reply within five working days. Final deliver of official reply may change according to the entity of the request.

Duration: January – December 2019 and 2020

Sub-activity 5.2 *(Listing susceptible species)*

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

Description: With implementation of the new Animal Health Law, there is an acute demand for scientifically assessing the fish and crustacean species susceptible to the listed diseases. Therefore, an increased workload for the EURL will be to assess the listing of susceptible fish and crustacean species, e.g. assess susceptibility of cleaner fish (wrasse and lumpfish), sea bass and sea bream to VHS and IHN, etc.

Expected Output: Provide a report with a list of which fish and crustacean species are susceptible to the listed diseases, to be recommended for adaptation in the new legislation.

Duration: January –March 2019.

Sub-activity 5.3 *(Listing diseases for notification)*

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

Description: The EURL provides scientific based advice assessing new putative listed diseases for inclusion or exclusion from the EU legislation. Criteria for including a disease are clear knowledge of aetiological agent, possibility to controlling and limiting the spread of the disease, diseases with severe impact on animal welfare and economy on aquaculture production in EU.

Expected Output: The EURL expect to assess two diseases per year, and provide scientific recommendation for including or exclusion them from the legislation.

Duration: Upon request from the Commission in 2019 and 2020.

EURL Work Plan for 2020 and ideas and plans for 2021

Niels Jørgen Olesen, Niccolò Vendramin

EURL Fish and Crustacean Diseases, DTU

njol@aqua.dtu.dk

The workplan 2019-2020 and the work done for the fish diseases part in 2019 is given in the report presented below.

In general the pandemic have resulted in some significant alterations in our work plan, e.g. this Annual workshop was postponed to a physical meeting in November, and due to the second wave of COVID-19 is now rearranged to a virtual meeting. The same account for our training courses, were one was cancelled and the other changed into a virtual meeting. Two scientific expert groups were established who only met virtually, one on susceptible and vector animal species to infection with the list A, C and E fish diseases and one on the A and C listed crustacean diseases. On the other hand the EURL have kept open and accessible to laboratory examinations and tank facilities during the whole pandemic, and kept a vigilant contingency plans in case of severe disease outbreaks in EU and the proficiency tests have been produced and shipped as originally planned.

Ideas and plans for the work programme 2021 are very welcome.

From our side – beside all the work that shall be done every year we would like to focus on:

- Scientific assessment of the effect of pooling samples for surveillance and diagnostics by PCR.
- Emerging fish and crustacean diseases (like POV infection, IHHNV infection in crustaceans, CMS in salmonids, SAV-2 in EU).
- Refinement and update of the Diagnostic Manual for EHN, VHS, IHN, ISA and KHV.
- Establishment of SOP´s and Manuals for WSS, YHD and TVD.

Next Annual Workshop: Hopefully physical meeting at DTU Campus in Kgs- Lyngby, Denmark 1st - 3rd June 2021 – with two workshops back to back, on fish and crustacean diseases, respectively. With the latest update from the Commission it is likely that AW´s cannot be held face to face in the first half of 2021, therefore alternative dates could be September 21-23, 2021 (EAFP Conference 30th August-2nd September).

Work done in 2019 of the EURL for Fish and Crustacean Diseases

Introduction to the Technical Report for the EURL in 2019

The Technical University of Denmark (DTU) was confirmed appointed as the EU Reference Laboratory (EURL) for Fish and Crustacean Diseases in November 2018 for the period 2019

and 2020, and granted the financing with the Commission Implementing Decision of 14.11.2018 C(2018) 7485 final.

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 and crustacean diseases listed as exotic diseases: Epizootic haematopoietic necrosis (EHN), taura syndrome, and infection with yellow head virus genotype 1; and diseases listed as non-exotic diseases: Infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN), koi herpes virus disease (KHVD) and white spot disease (WSD).
The facilities supporting the activities of the EURL are placed in the new DTU Campus in Kgs. Lyngby, 15 km north of the capital. The EURL is now placed in DTU AQUA-National Institute of Aquatic Resources and it is in progress the further integration of the group with the ongoing activities in this institute in for collaborating with research teams working in the field of aquaculture and fisheries.

The 23rd nd Annual Workshop of the National Reference Laboratories for Fish Diseases was held 27th–28st of May, at DTU Aqua, 2800 Kgs. Lyngby, Denmark. A total of 58 participants from 32 countries attended over the two days period. There were six sessions with a total of 30 presentations three of which were given by invited speakers.

The 10th annual workshop for crustacean diseases was held for the first time at the premises of DTU Aqua in Lyngby and organized back to back with the workshop for fish diseases on 29th of May. A total of 43 participants from 26 countries attended the one day workshop. There were four sessions with in total 16 presentations, eight of which were given by invited speakers

The annual proficiency test for fish diseases (PT) was divided into PT1 and PT2 with 49 laboratories participating. The tests were sent from the EURL 27th of September 2019. The preliminary observation of the results confirmed that the vast majority of the laboratories had high scores. However due to the anticipation of the delivery date of this technical report, the full report of the proficiency test results is not finalized, as planned in the work program a full report will be shipped to all participants and the EU commission in March 2020.

The annual proficiency test for crustacean diseases was organized for the first time by our team in 2019. The test was delivered to 22 NRL's in Europe. The test was shipped 1st of November.

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. In 2019 the EURL has focused on improving the diagnostic manual for ISA organizing an expert group meeting. Further work will follow for other fish and crustacean listed diseases.

During 2019, 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) and finally to attend international meetings and conferences.

On November 1st 2019, Senior Scientist Morten Schiøtt has been employed as Coordinator of the EURL for Crustacean diseases, replacing Dr. Nikolaj Reducha Andersen.

After finalizing his PhD defending his thesis in March 2019, DVM Niccolò Vendramin, is now back full time in the position as Coordinator of the EURL for fish diseases.

	Technical report for 2019
Work Programme for 2019-2020	
1.1. Annual workshop for fish diseases. *To ensure knowledge dissemination and sharing between the Member State NRLs on existing and emerging fish diseases and to agree on the future priorities of the EURL, by holding the 23rd and 24th annual workshop of the National Reference Laboratories (NRLs) for fish diseases in 2019 and 2020, respectively.*	The 23rd Annual Workshop of the National Reference Laboratories for Fish Diseases was held 27th – 28th of May 2019, at DTU Aqua, 2800 Kgs. Lyngby, Denmark. This annual workshop was the second to be held at our premises in Kgs. Lyngby. A total of 43 participants from 26 countries attended the two days' workshop. There were four sessions with in total 16 presentations, eight of which were given by invited speakers *Click here for the report of the 23rd Annual Workshop*
1.2 Annual workshop crustacean diseases. To ensure knowledge dissemination and sharing between the Member State NRLs on existing and emerging crustacean diseases and to agree on the future priorities of the EURL, by holding the 10th and 11th annual workshops of the National Reference Laboratories (NRLs) for crustacean diseases in 2019 and 2020, respectively.	The 10th annual workshop for crustacean diseases was held for the first time at the premises of DTU Aqua in Lyngby and organized back to back with the workshop for fish diseases on 29th of May. A total of 43 participants from 26 countries attended the one day workshop. There were four sessions with in total 16 presentations, eight of which were given by invited speakers. *Click here for the report of the 10th Annual Workshop*
1.3 Scientific working groups. *To ensure that fast and reliable scientific advice on specific topics related to listed and emerging diseases and to legislative issues, is provided by organising expert meetings in order to solve arising challenges in EU.*	In 2019, a scientific working group meeting was organized to update the ISA diagnostic manual in compliance with new Animal Health Law. The diagnostic manual will be finalized in March 2020. *The report of the expert group meeting is located here*

1.4 Proficiency test fish diseases. *To assess the capabilities of all Member State NRLs to detect pathogens causing fish diseases and to harmonize the procedures used by an inter-laboratory proficiency test.*	An inter-laboratory proficiency test was provided by the EURL for Fish and Crustacean Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2). 49 laboratories participated in this activity. The interlaboratory proficiency tests for fish diseases were sent from the EURL 27th of September 2019. The ampoule content has been disclosed in December 2019. A full report will be provided to the participanst as well as to the EU commission in march 2020. *The report of the Inter-Laboratory Proficiency Test 2019 for fish disease is located here*
1.5 Proficiency test crustacean diseases *To assess the capabilities of all Member State NRLs to detect pathogens causing diseases in crustacean and to harmonize the diagnostic procedures used by inter-laboratory proficiency tests*	The annual proficiency test for crustacean diseases was organized for the first time by our team in 2019. The test was delivered to 22 NRL's in Europe. The test was shipped 1st of November. The ampoule content has been disclosed in December 2019. A full report will be provided to the participants as well as to the EU commission in march 2020. *The Ampoule content of the Inter-Laboratory Proficiency Test 2019 for crustacean disease is located here*
1.6 Diagnostic methods *For the EURL to have diagnostic methods of the highest scientific standards and to be able to provide these methods to all Member State NRLs*	In 2019 the following 2 new diagnostic qPCR methods were introduced in the laboratory: qPCR for detection of Taura Syndrome Virus PCR for Yellohead Disease Virus Genotype 1 qPCR for Candidatus Midichloriaceae – main aetiological agent of Red Mark Syndrome in Rainbow trout qPCR for White Spot Syndrome Virus has been implemented and validated qPCR for Infectious Haematopoietic Necrosis Virus in one-step reaction has been implemented and validated
1.7 Crustacean tank facilities For the EURL to be able to conduct infection trails with crustacean species.	In 2019 the team of the EURL for fish and crustacean disease has successfully planned and conducted an infectious trial in *P. vannamei* with White Spot Syndrome Virus to produce reference samples. Further optimization of the facilities will be conducted in 2020, in order to guarantee suitable source of SPF *P. vannamei*

	and increase the number of crustacean pathogens to be included in the experimental model.
2.1 Training Courses *To ensure that employees of the Member State NRLs have the highest scientific and excellent skills in diagnosis of fish and crustacean diseases*	Two training courses were successfully organized from October the 7th to 18th, 2019. The two courses prepared were: "Methods for implementation of surveillance procedures for listed fish diseases" and "Introduction to histopathology in fish and crustacean diseases" are now accredited to grant ECTS at PhD level to the participants. *The report of the 2019 training courses is located here*
2.2 Website www.eurl-fish-crustacean.eu *To provide the Member State NRLs with a fast entrance to information from the EURL.*	The EURL website has gone through a substantial re-structuring and constant update in spring 2019 and a new website launched at the Annual workshop. It now compiles the information on the activities by both the EURL for fish and crustacean diseases. It can be accessed through https://www.eurl-fish-crustacean.eu/ The new website has been accessed 4.465 times; 12.990 pages have been accessed since May 2019. *Link to the website:* https://www.eurl-fish-crustacean.eu/
2.3 EURL Contact Lists *To ensure that relevant and important information rapidly can get from the EURL directly to the Member State NRLs.*	The e-mail list FishRefLabNet have been continuously updated during 2019 and now contain 167 people with interest in our work. The list now includes all the NRL contacts for the Crustacean Diseases.
2.4 Missions to NRLs for fish diseases *To ensure a high standard of diagnostic capabilities of all Member State NRLs.*	Due to the high performances in 2018 no mission was scheduled in 2019.
2.5 International conferences and meetings *To keep the EURL updated on the newest scientific information on emerging and listed exotic and non-exotic fish and crustacean diseases, and to disseminate knowledge and scientific data provided by the EURL.*	The EURL team has attended and contributed with high profile scientific talks to a number of international conferences and meetings within the field. EURL employees and members of the fish and crustacean unit at DTU participated in 14 international meetings and conferences and gave 38 oral presentations. The Unit authored 20 publications in Peer-reviewed journals.

2.6 Confirmatory diagnosis *For the EURL to be able to identify and characterize isolates of listed viral fish and crustacean pathogens on request from the Member State NRLs*	The EURL has been involved in corroborating the diagnosis of a number of disease outbreak. In 2019 the EURL for fish disaeses has been involved in the confirmation of . KHVD outbreak in common carps in Norway. CEV outbreak in common carps in Norway IHN outbreak in rainbow trout in Republic of north Macedonia
2.7 Pathogen characterization *For the EURL to be able to characterize isolates of listed viral pathogens of aquatic animals as well as emerging pathogen and provide scientific based risk assessment to the scientific community and stakeholders*	In 2019 the EURL has been involved in characterizing the following pathogens: PMCV in Atlantic salmon from Ireland Chimeric VHSV in Rainbow trout Susceptibility of Sea bass to infection with VHSV isolates IHNV isolates from rainbow trout in North Macedonia
3.1 Diagnostic manuals fish diseases *To have updated* diagnostic manuals for all listed fish diseases available for Member State NRLs on the EURL website.	The sampling and diagnostic procedures for detection of VHS, IHN, ISA, KHV, EHN and EUS were kept and updated at our web site. In order to update the ISA diagnostic manual an expert meeting has been organized. It is expected that the work will be finalized by March 2020. *Link to the manuals:* *https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals*
3.2 Diagnostic manuals crustacean diseases To have updated diagnostic manuals for all listed crustacean diseases available for Member State NRLs on the EURL website.	The sampling and diagnostic procedures for detection of WSSV, TSV and YHDV are presented on the EURL website. *Link to the manuals:https://www.eurl-fish-crustacean.eu/crustacean/diagnostic-manuals*
3.3 Survey and diagnosis fish diseases *As part of our duties given in given in C.D. 2006/88/EC Annex VI, Part I.5 (f) to "collate and forward information on exotic and endemic diseases, that are potentially emerging in Community aquaculture" data on emerging and endemic fish*	The report, based on data from the questionnaire on Survey and Diagnosis of the listed fish diseases in Europe (S&D) for 2018 send from the EURL to all NRLs was prepared and presented at the AW 2019.

diseases and fish health surveillance in Europe will be collated in order to ensure that the EU Commission, the Member State NRLs and the EU in general are updated on the fish diseases situation in aquaculture and natural fish populations in Europe.	*The report for S&D 2018 can be downloaded at* *https://www.eurl-fish-crustacean.eu/fish/survey-and-diagnosis*

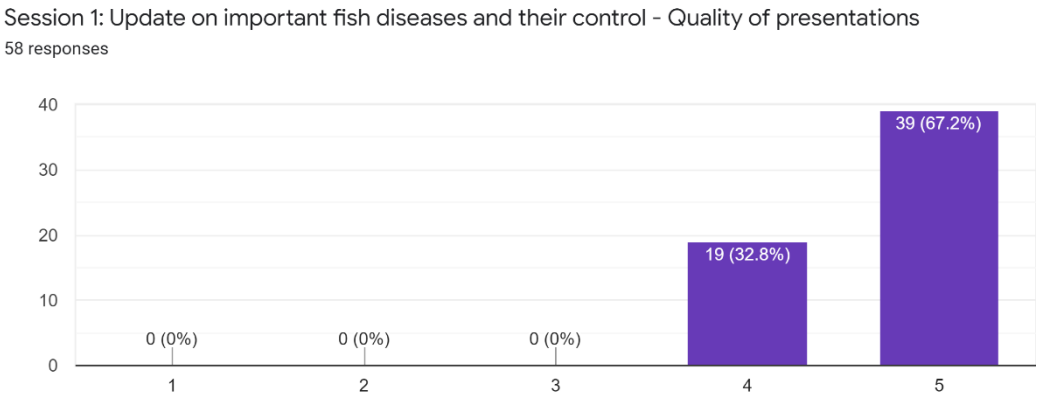
3.4 Risk assessment for emerging diseases For the EURL to have the most updated and highest scientific knowledge of emerging and re-emerging fish and crustacean diseases in Europe.	The EURL has initiated a study to assess infectivity and virulence of Piscine myocarditis virus (PMCV) in Atlantic salmon. This virus is suspected to cause CardioMyopathySyndrome (CMS). The analysis are expected to be finalized in 2020. The EURL has continued to investigate the disease linked to the PRV-3 infection in salmonids and has enrolled a new PhD student Juliane Sørensen in a project on this disease and the development of innovative diagnostic methods based on Fluidigm technology.
4.1 The database www.fishpathogens.eu To have an updated database of all serious viral fish pathogens in the EU.	A number of full genome sequences for VHSV (around 50) were added to the database, along with continuing in the process of curating the existing records in the VHSV database. Discussions about the SAV database have been carried on with colleagues from the Norwegian veterinary institute, considering whether the database should be closed or not.
4.2 Pathogen library For the EURL to have an updated library of fish and crustacean pathogens relevant for the EURL and Member State NRLs.	All Reagents and samples received by the EURL in 2019 were included in **Annex 4.2**
4.3 Production and supply of reagents For the EURL to be able to provide Member State NRLs with diagnostic reagents.	All Reagents and samples supplied by the EURL in 2019 are included in **Annex 4.3**
5.1 Scientific advice in relation to aquatic animal health legislation For the EU commission and Member States to access scientific based advice on interpretation and implementation of aquatic animal health law.	The experts of the EURL were involved in giving advice to the content of delegated act, lists of susceptible species to the listed diseases and consultancy concerning specific questions raised by the Member states to the Commission.
5.2 Listing susceptible species For the EU Member States to have an updated list of	N. J. Olesen is participating in an ad hoc working group of the OIE assessing the susceptible fish species to the OIE listed fish diseases. The list and the outcome of this work will be inserted in the list of susceptible species given the Animal Health Law and its delegated acts of EU.

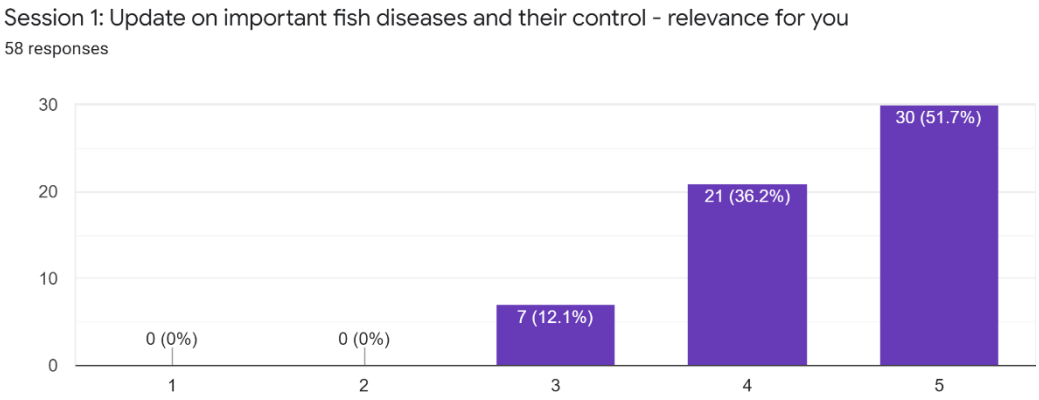
susceptible species for the listed fish and crustacean diseases	A study for assessing the susceptibility of European sea bass to VHS/IHN circulating in Europe has been finalized and will be published in 2020.
5.3 Listing diseases for notification For the EU commission and Member states to access scientific based advice on criteria for including or excluding infectious diseases in new Aquatic animal health law.	The EURL has been involved in the following topics in relation to listing diseases for notification: -assessment of CardioMyoPathy Syndrome (CMS) in Atlantic salmon , caused by Piscine Myocarditis virus and measures for control - Listing KHVD in category E - Including SAV in the annual proficiency test, based on the listing of this disease at OIE level, to ensure preparedness of the NRL network for diagnosis of this pathogen.

Workshop evaluation

A questionnaire was delivered to the participants asking to evaluate various aspect of the workshop. An overview of the 58 questionnaires retrieved is shown below. Specific comments are going to be considered for the next annual workshop organization.

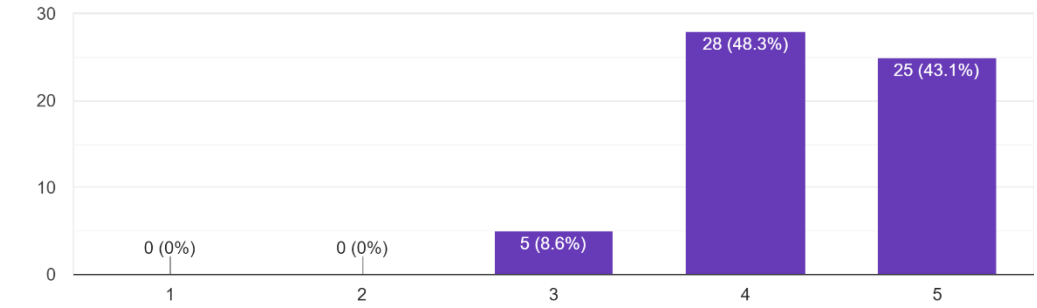
Evaluation graph 24th AW for National Reference Laboratories for Fish Diseases





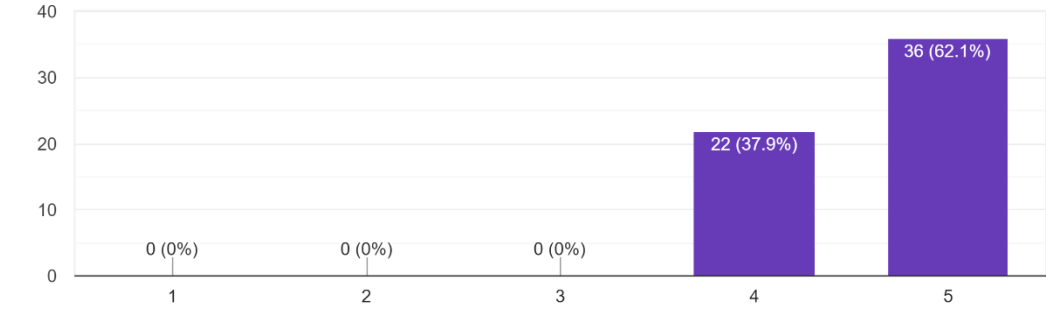
Session 1: Update on important fish diseases and their control - increase of your knowledge

58 responses



Session 1: Update on important fish diseases and their control - overall score

58 responses



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

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update on more emerging/important diseases of non-salmonid fish could be presented.

no remarks.

I would have preferred that the Annual Workshop would have been reseved to personnel of NRL only. There were too many participants, some of them not directly involved involved in fish disease diagnosis.

excellent.

it was nice to follow up on the new techniques for disease control or identification. yet maybe you may have another session for the bacterial and parasitical diseases.

no comment.

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Great meeting. Lots of interesting information.

Lecture of K. Buchmann was interesting, but too much detailed info with too many slides. Other lectures were very good.

Useful overviews.

Very nice meeting. It went very well. The zoom platform is OK even if it of course is better to be there in person and interact.

Very interesting material presented. For a new comer it provide me with a lot of reference report material that I previously unaware of.

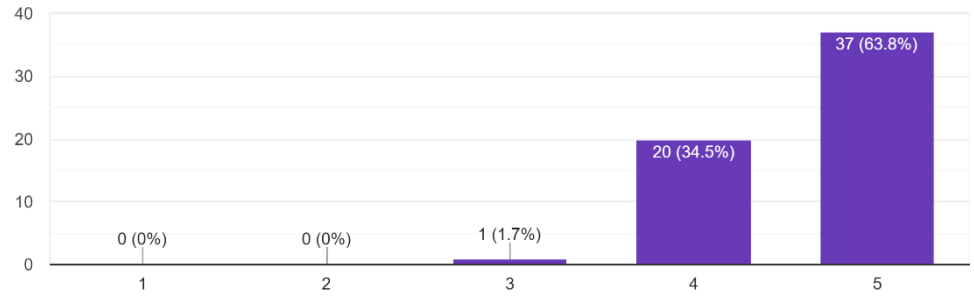
many new information about fish diseses, secialy outbreaks of IHNV in Finland.

The session was organised well with appropriate presentations and timely correct.

I think it should be considered to allocate different time to the presentations. In my opinion the reoccurence of IHN in Croatia and salmon lice in Danish salmonid populations do not need as much time as the overview of the disease situation in Europe and Norway.

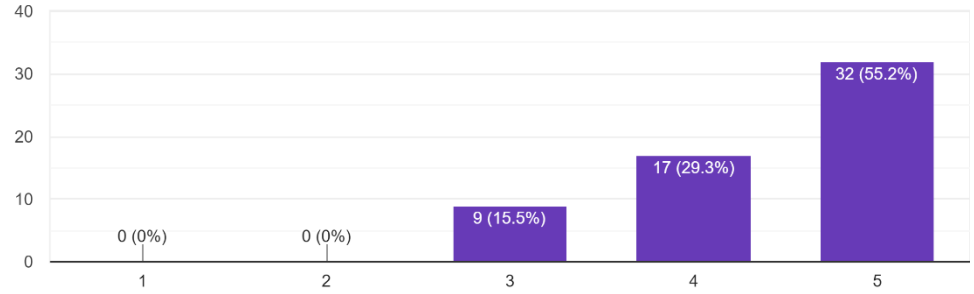
SESSION II: Control and Surveillance of fish diseases in EU- Quality of the presentations

58 responses



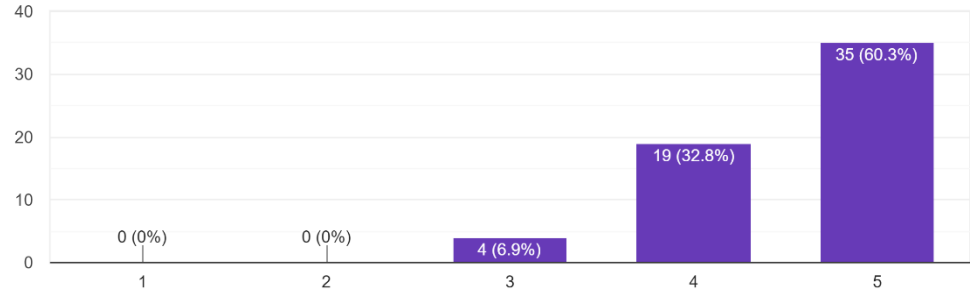
SESSION II: Control and Surveillance of fish diseases in EU- relevance for you

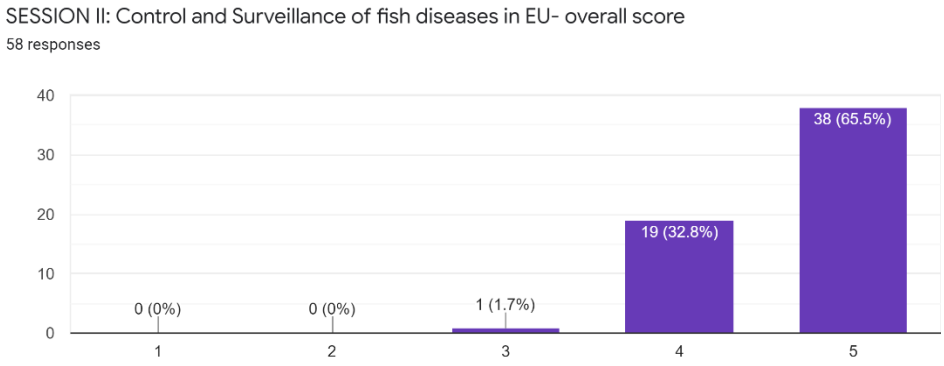
58 responses



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

58 responses





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

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no remark.

I would have preferred that the Annual Workshop would have been reseved to personnel of NRL only. There were too many participants, some of them not directly involved involved in fish disease diagnosis.

keep going.

The workshop mainly focused on viral diseases and their control or surveillance, but for the Mediterranean area, it's also important to control bacterial diseases or parasitical diseases. It has a huge impact on the economy.

no comment.

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Great meeting. Lots of interesting information.

Also nice, we could have a small discussion.

interesting and useful.

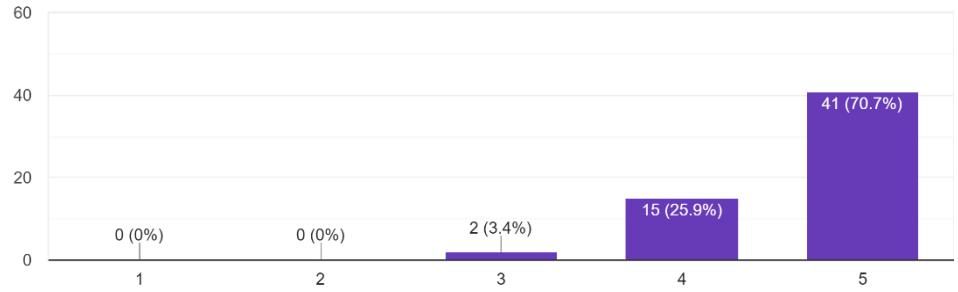
Fine meeting.

Particular enjoyed the presentation on RSD.

Obviously all presenters were good skilled professionals with very interesting and informative talks.

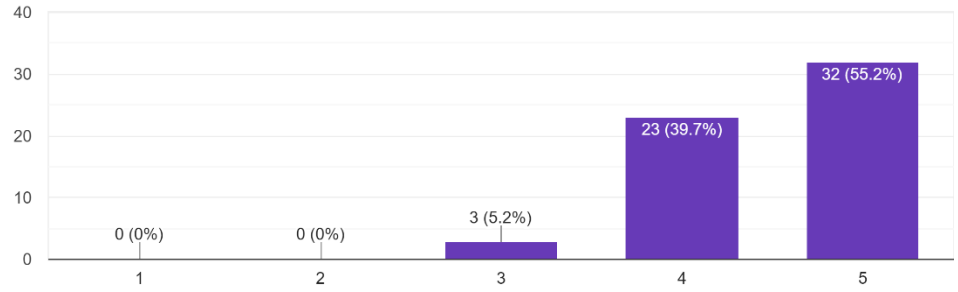
SESSION III: Results from ongoing research on listed and emerging fish diseases-quality of the presentations

58 responses



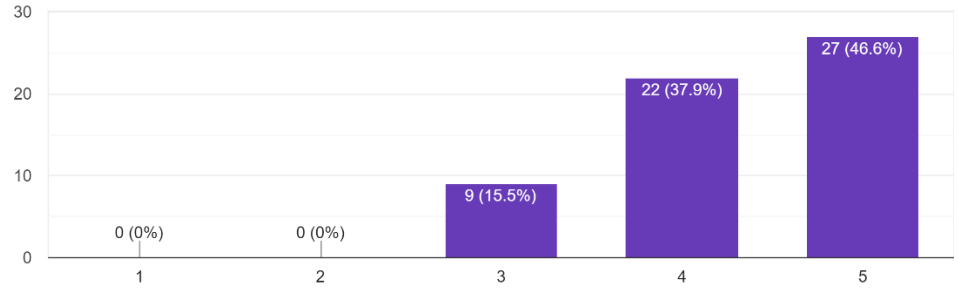
SESSION III: Results from ongoing research on listed and emerging fish diseases-increase of your knowledge

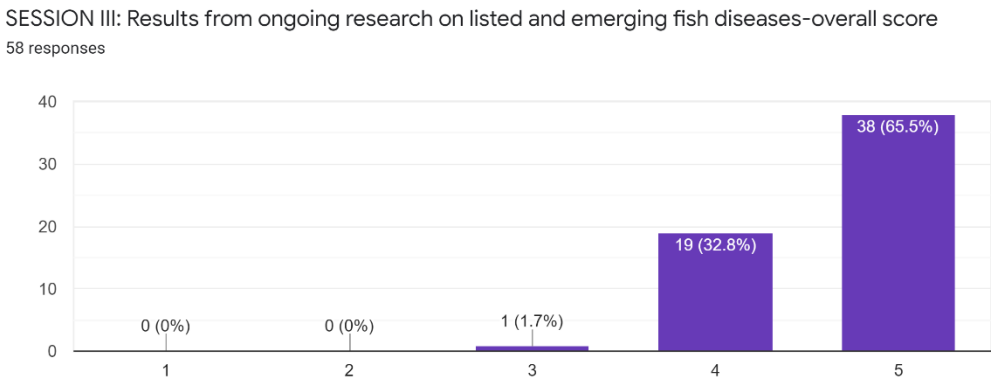
58 responses



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

58 responses





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

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I would have preferred that the Annual Workshop would have been reseved to personnel of NRL only. There were too many participants, some of them not directly involved involved in fish disease diagnosis.

We are note impacted with ISAV.

Particularly enjoyed presentation on modelling of ISAV.

Congratulations to the (young) scientists!

As always, it was good to know how it's done or should be done and how to set about to implement it at the national level.

no comment.

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Unfortunately, I could not attend section III.

It has been too focused on ISA. It could be extended to other diseases.

Very interesting talks on new emerging viruses and various methods used to characterize them...

Good to see more data on PRV-3, which has been detected in various European countries, but not all European countries have a test for this virus yet. Would it be an idea to make a kind of info flyer on this virus and disease for at the EURL Fish website, with set up like in the booklets "What should I do" so including diagnostic methods with reference, pictures etc.? Thank you.

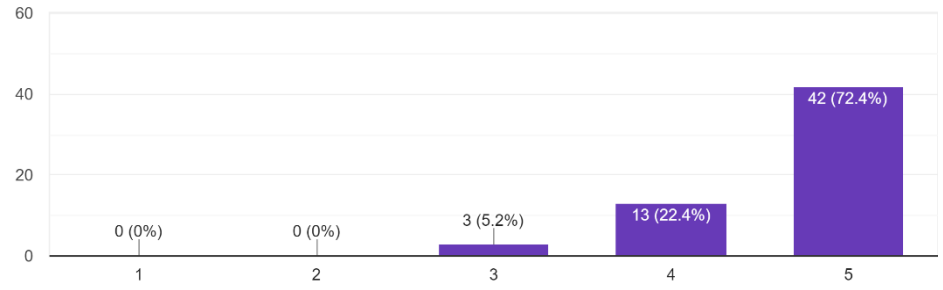
Fine meeting.

Presentation were informative and easy to follow.

Well organized.

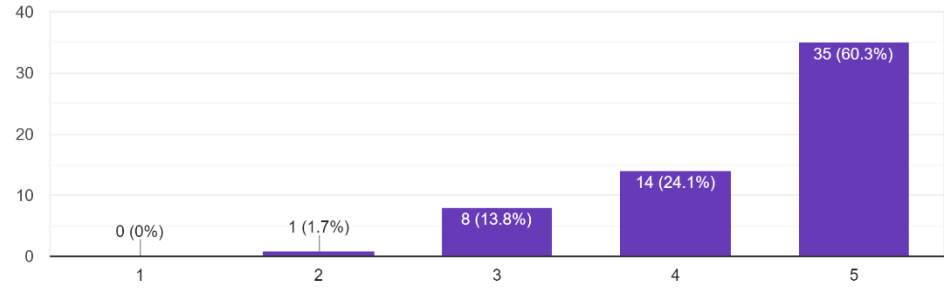
SESSION IV: Update from the EURL for fish diseases- quality of the presentations

58 responses



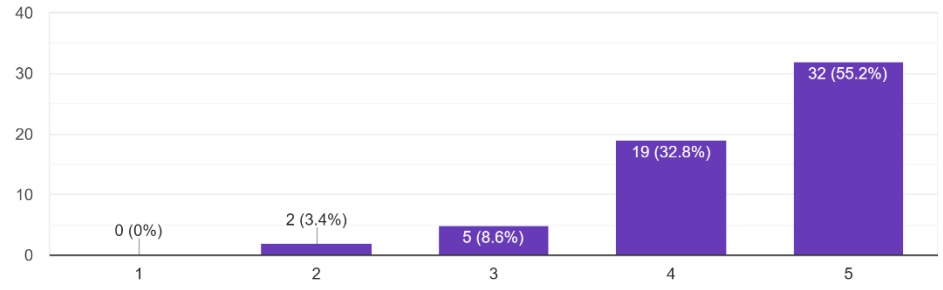
SESSION IV: Update from the EURL for fish diseases- relevance for you

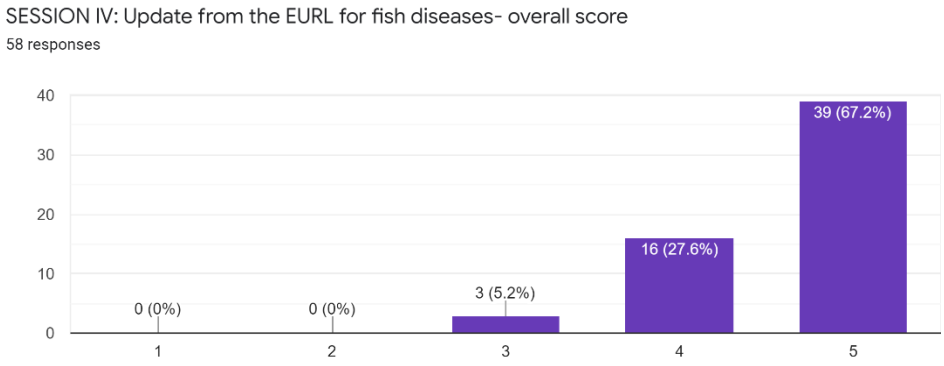
58 responses



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

58 responses





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

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no remark.

I would have preferred that the Annual Workshop would have been reseved to personnel of NRL only. There were too many participants, some of them not directly involved involved in fish disease diagnosis.

keep going.

Overall, I was hoping to follow up a little bit more about bacterial diseases and their treatment strategies or parasitical diseases or their economic impact on the aquaculture sector. For me, it was a big opportunity to attend online. Due to the time of the year or economical reason, it was difficult but this time I was able to part of this organization. Thanks for the informative workshop.

no comment.

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Unfortunately, I could not attend section IV.

Great meeting but unfortunately zoom restricts the opportunity for interactions between participants and ability to ask and discuss questions.

Thank you very much for your scientific support and all the work done in fish disease field to improve our knowledge and skills!! The virtual way of organization enabled technical staff to attend the presentations and this was really appreciated!!

Overall well done in this online setting, even with some discussion via the chat and live. Thank you!

Thanks for a well run meeting under difficult circumstances. Not quite as good as face to face meeting (less interaction and discussion) but a fair substitute uncder the circumstances.

Sorry I lost some of the lectures in the end but all I heard were just fine.

Due to time restrictions not all topics were covered but organizers did as much as possible for excellent on line workshop.

Greetings and conclusions of the meeting

The next meeting will be held at the 1st – 3rd of June 2021. It will most likely be organized as a virtual meeting again. 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