



European Union Reference Laboratory for Fish and Crustacean Diseases

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

EURL-Fish work done in 2022 and 2023 – ideas for 2024

*Niccoló Vendramin,
Argelia Cuenca, Teena V. Klinge, Morten Schiøtt
Britt Bang Jensen*





The present Fish and Shellfish Diseases team

- 25 employees
- 6 Veterinarians
- 4 Biologist
- 11 Master of Science
- 7 Technical engineers
- 7 Nationalities

- 2 new employees

From June 1st!



Britt Bang Jensen



Niels Jørgen Olsen



Niels Lorenzen



Niccolò Vendramin



Argelia Cuenca



Tine Iburg



Lone Madsen



Søren Dalsgaard



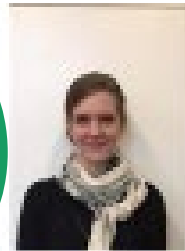
Dagoberto Sepulveda



Jacob G. Schmidt



Morten Schiøtt



Juliane Sørensen



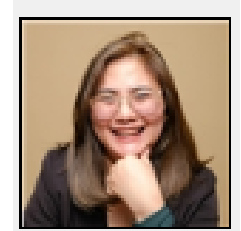
Valentina Donati



Alejandra V. Alonso



Giulia Zarantonello



Shana Genavia



Jeong In Yang



Maria Jacobsen



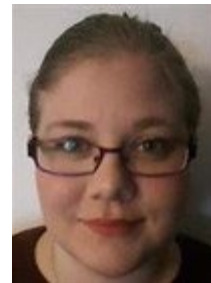
Irina Vardia



Teena Vendel Klinge



Jeanne Vinther



Kristina Andkjær Andersen



Lise Christensen



Tune Øst-Jacobsen



Mikkel Hviid Larsen



Kári Karbech Mouritsen

EURL-Fish work program 2021-2022

5 main objectives:

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

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

An inter-laboratory proficiency test on fish diseases was provided in 2022 by the EURL. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).

42 laboratories participated in this activity in 2022. The inter-laboratory proficiency tests for fish diseases were sent from the EURL in October 2022 respectively.

The content in the ampoules was disclosed in December 2022. A full report was provided to the Commission and the participants March 2023. In April 2023 a specific online meeting was held with all participants to discuss the findings, the report and underperformances.



EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2022
for identification and titration of
VHSV, IHNV, EHNV, SVCV and IPNV (PT1)
and identification of
CyHV-3 (KHV), SAV and ISAV (PT2)

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



In 2021-2022 the following new diagnostic PCR methods were introduced in the laboratory:

Pan PRV RT-qPCR for detection of all genotypes of Piscine orthoreovirus

One RT-PCR for detection of SVCV

A modified RT-qPCR for detection of a new variant of IHNV circulating in Europe

two different RT-qPCR for detection of Tilapia Like Virus

One confirmatory RT-qPCR for Tilapia Like Virus

A conventional PCR for detection of TiLV

A qPCR for detection of red sea bream iridovirus

Two qPCR for detection and quantification of Renibacterium salmoninarum

A RT-qPCR with improved sensitivity in detecting some isolates of IPNV

Article

Pan-Piscine Orthoreovirus (PRV) Detection Using Reverse Transcription Quantitative PCR

Julie Zhao ¹, Niccolò Vendramin ², Argelia Cuenca ², Mark Polinski ^{1,†}, Laura M. Hawley ¹ and Kyle A. Garver ^{1,*}



**European Union Reference Laboratory
for Fish and Crustacean Diseases**

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

NRLs for fish diseases in Europe
CC FVST Fødevarestyrelsen, DG Sante

UPDATED LABORATORY DIAGNOSTIC PROCEDURES FOR DETECTING IHNV RNA BY RT-qPCR

Dear colleagues,

In relation to the recent outbreak of IHN in Denmark occurred on May 18th 2021 <https://www.foedevarestyrelsen.dk/english/Animal/AnimalHealth/Animal%20diseases/IHN/Pages/default.aspx>, it is necessary to inform that we have observed unsatisfactory performances of the RT-qPCR protocols currently described in the diagnostic manual on the EURL website <https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals/ihn>. Both recommended methods, the validated RT-qPCR described in the publication by Purcell et al., 2013 (two-steps method) and Cuenca et al., 2020 (one-step method), have shown low sensitivity in detecting the IHNV variant present in Denmark in May 2021

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

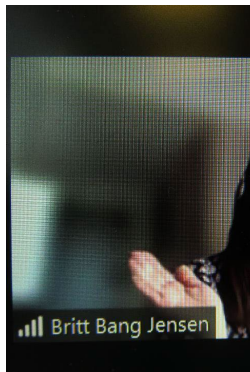
A number of “ad Hoc” online meetings with NRLs to provide assistance on diagnostics of IHN, ISA, etc.

Sub-activity 2.1 Annual workshop for fish diseases.

To ensure knowledge dissemination and sharing between the Member State NRLs on existing and emerging fish diseases and to agree on the future priorities of the EURL by holding the 26th annual workshop of the National Reference Laboratories (NRLs) for fish diseases in 2022.

The 26th Annual Workshop of the National Reference Laboratories for Fish Diseases was held in hybrid form on 30th and 31st of May 2022. There were 35 participants who attended the meeting in person and more than 60 registered to attend online. 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 95 participants from 38 countries attending over the two days period. There were four sessions with a total of 18 presentations.



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

In 2021 and 2022 two working groups to draft diagnostic manuals for VHS, IHN and infection with EHNV were held. Invited experts were NRL from Italy, France and Germany. Meeting were held online with no need for physical or online meetings.

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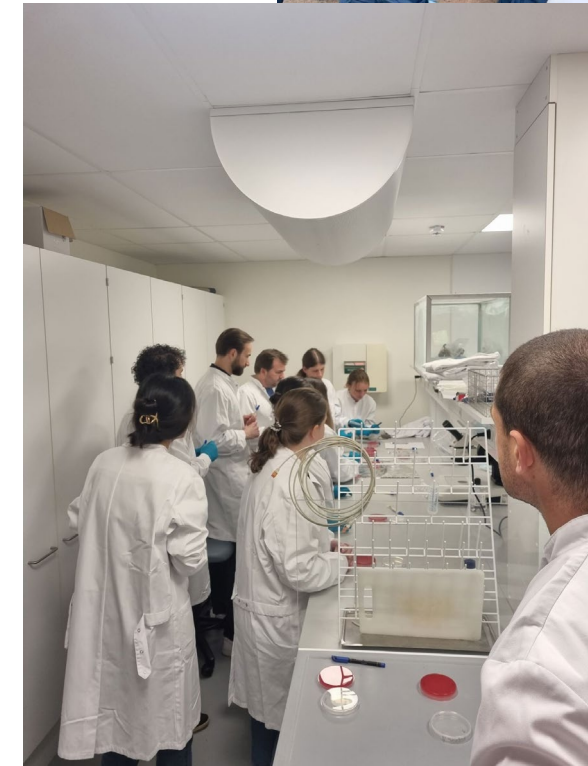
DIAGNOSTIC METHODS FOR THE
CONFIRMATION AND SURVEILLANCE OF
Category A disease
Epizootic haematopoietic necrosis (EHN)

V2022.1

Sub-activity 2.4 Training Courses

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

The courses given were: “Methods for implementation of surveillance procedures for listed fish diseases”, and “Introduction to histopathology in fish and crustacean diseases”. In 2022 the two courses were successfully organized as on-site courses at DTU Lyngby



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



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

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

In 2022 the website was assessed 2,732 times and 11,062 pages were opened. As regards 2021, we only have access to data from November and December. Based on these two months we assume that the level of traffic on the website was the same in 2021 as in 2022”.

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

Sub-activity 2.6 EURL Contact Lists

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

Sub-activity 2.7 Missions to NRLs for fish diseases

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

The screenshot shows the homepage of the European Union Reference Laboratory for Fish and Crustacean Diseases (EURL). The header features the EURL logo, the text "European Union Reference Laboratory for Fish and Crustacean Diseases NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK", and the DTU logo. A navigation menu includes links for FISH, CRUSTACEAN, NEWS, LEGISLATION, NRL NETWORK, CONTACT, and ABOUT. The main content area has a section titled "EURL FOR FISH AND CRUSTACEAN DISEASES" with text describing the EURL's role and a video player showing a fish. Below this is a "NEWS FROM EURL FOR FISH AND CRUSTACEAN DISEASES" section with a date of 31 March 2023 and a link to an invitation to an annual workshop. To the right is a "SUBMISSION OF MATERIAL" section with contact information for the EURL.

Sub-activity 2.8 International conferences and meetings

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

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

EURL employees and members of the fish and crustacean unit at DTU participated in 11 international meetings and conferences and gave 37 oral presentations. The Unit authored 26 publications in Peer-reviewed journals.

2022

1. Donati, V.L., Madsen, L., Middelboe, M., Strube, M.L. & Dalsgaard, I. (2022). The gut microbiota of healthy and *Flavobacterium psychrophilum*-infected rainbow trout fry is shaped by antibiotics and phage therapies. *Frontiers in Microbiology*, 13:771296. doi:10.3389/fmicb.2022.771296
2. Fosse, J.H., Aamelfot, M., Sønstevoid, T., Weli, S.C., Vendramin, N., Petersen, P.E., Solhaug, A., Amundsen, M.M., Heffeman, I.A., Cuenca, A., Christiansen, D.H. & Falk, K. (2022). Salmon Erythrocytes Sequester Active Virus Particles in Infectious Salmon Anaemia. *Viruses* 14 (2), 310.
3. Jørgensen, J., Sundell, K., Castillo, D., Dramshøj, L. S., Jørgensen, N.B., Madsen, S.B., Landor, L., Wiklund, T., Donati, V.L., Madsen, L., Dalsgaard, I. & Middelboe M. (2022). Reversible mutations in gliding motility and virulence genes: a flexible and efficient phage defence mechanism in *Flavobacterium psychrophilum*. *Environ. Microbiol.* Accepted Author Manuscript. doi:10.1111/1462-2920.16126
4. Marsella, A., Pascoli, F., Pretto, T., Buratin, A., Biasini, L., Abbadi, M., Cortinovis, L., Berto, P., Manfrin, A., Vanelli, M., Perulli, S., Rasmussen, J.S., Sepúlveda, D., Vendramin, N., Lorenzen, N., Toffan, A. Efficacy of DNA Vaccines in Protecting Rainbow Trout against VHS and IHN under Intensive Farming Conditions. *Vaccines* 2022, 10, 2062. https://doi.org/10.3390/vaccines10122062
5. Radosavljevic, V., Jazic, A., Milicevic, V., Bozidar, S., Zuko, A., Glisic, D., Maksimovic-Zoric, J. & Olesen, N. J. (2022) First confirmation of salmonid alphavirus infection in rainbow trout in Bosnia and Herzegovina and Serbia. *Diseases of Aquatic Organisms*, 150, 153-159.
6. Scholz, F., Vendramin, N., Olesen, N. J., Cuenca, A., Moesgaard Iburg, T., Mirimin, L., O'Connor, I., Ruane, N.M., Rodger, H.D. & MacCarthy, E. (2022). Experimental infection trials with European North Atlantic ranavirus (Iridoviridae) isolated from lumpfish (*Cyclopterus lumpus*, L.). *Journal of Fish Diseases*, 45 (11), 1745-1756.
7. Sepúlveda, D., Hansen, M. J., Dalsgaard, I., Skov, J. & Lorenzen, N. (2022) Antigenic variability of *Vibrio anguillarum* serotype O2a: A hurdle for vaccine efficacy against vibriosis in *Oncorhynchus mykiss*. *Fish and Shellfish Immunology*, 131, 300-311

In yellow: Members of the EURL team and the DTU Aqua Unit for Fish and Crustacean Diseases

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

The sampling and diagnostic procedures for detection of VHS, IHN, ISA, EHN were kept updated at our web site.

The ISA diagnostic manual was updated twice.

Two working groups were established:

The first to finalize the VHS/IHN diagnostic manual, which was published and updated twice.

The second to finalize the EHN diagnostic manual which was published.

Link to the manuals:

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

Sub-activity 3.1 *Survey and diagnosis fish diseases*

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

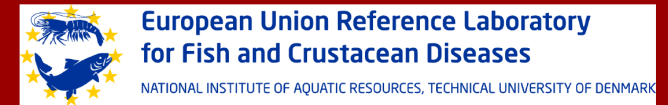
Report

- The report is collated, will be submitted to all of you for validation.
- Please check if the information given is correct!



Niccolò Vendramin, Niels Jørgen Olesen
26th AW of the National Reference Laboratories for Fish Diseases, May 31st 2021

Survey & Diagnosis of fish diseases in 2021



Sub-activity 3.2 Risk assessment for emerging diseases

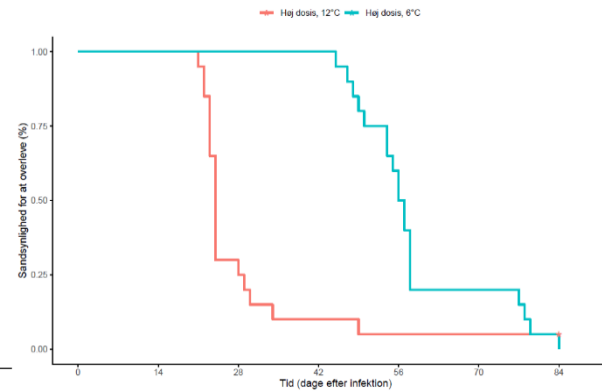
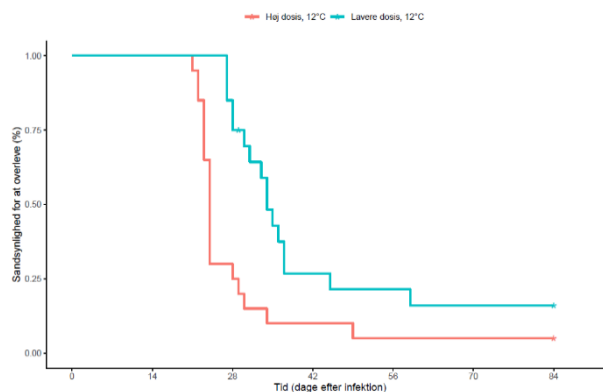
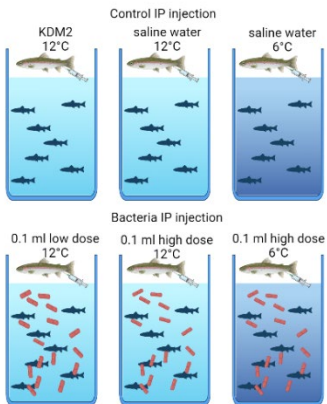
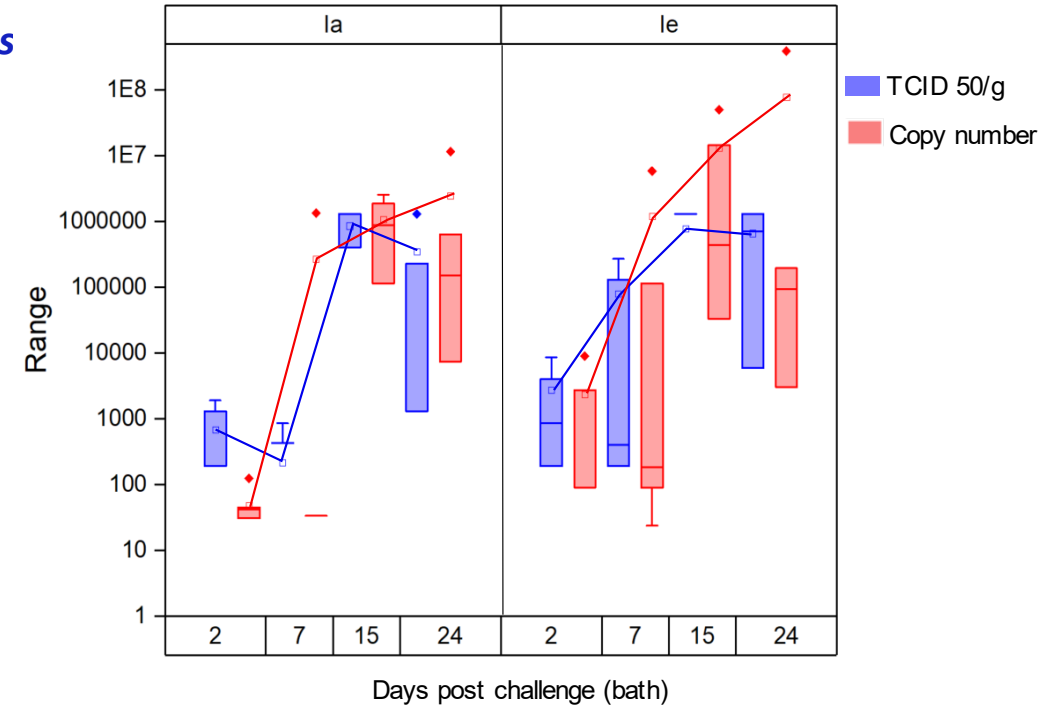
For the EURL to have the most updated and highest scientific knowledge of emerging and re-emerging fish and crustacean diseases in Europe

The EURL has finalized the study to susceptibility of European sea bass to VHSV, a 3 week study with repeated sampling to investigate viral replication has been conducted.

The EURL has also run a large infection trial in rainbow trout and Atlantic salmon to investigate virulence of newly discovered IHNV variant in Denmark.

Finally the EURL has conducted infectious study in rainbow trout to investigate BKD pathogenesis:

Viral load in sea bass after bath challenge with VHSV



Sub-activity 3.3 Confirmatory diagnosis

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

RSD wild salmon Finland Sweden

KHV Sweden

Unexplained increased mortality in Rainbow trout The Netherlands (PRV-3)

Sub-activity 3.4 Pathogen characterization

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

Unexplained increased mortality in eels

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

VHSV in European sea bass (in vivo)

IHNV isolates from rainbow trout in North Macedonia (sequencing and phylogeny)

A panel of IHNV isolates have been full genome sequenced:

IHNV isolates from Finland outbreak (2017-2018)

IHNV isolates from Croatia (2013,2015)

IHNV isolates from Republic of North Macedonia (2020)

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

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

DISCONTINUED

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

Sub-activity 4.3 *Production and supply of reagents*

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



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Annex 4.3 Reagents supplied by the EURL for Fish and Crustacean Diseases in 2021

Country	Name	Institute	Date of receipt	Material	Amount	Specifics
Belarus	Natalia Sai	The Belarusian State Veterinary Center - ISTC Department of Virology and Cell Culture	14.01.2021	FTA Cards	1 card	1 FTA-card: • EHNV
Iceland	Þorbjörg Einarsdóttir	University of Iceland Institute for Experimental Pathology	02.02.2021	SAV inf. Cell sup.	1 tube	1 ml of cell supernatant infected with MR-N1-2011, SAV2. Ct. value on 20. Journal No.: 2017-10023
Serbia	Vladimir, Ivan Radosavljevic	Institute of Veterinary Medicine of Serbia Department of Fish Diseases	19.02.2021	Non infected fish cells	6x 2 flasks	2 bottles (2x 25 cm ³) of each with non-infected cells of: EPC, BF-2, FHM, RTG-2, CCB and CHSE-214
Norway	Anne Berit Olsen	Norwegian Veterinary Institute	10.05.2021	Organs in Formalin	268 beakers	Ester no. 2020-2762 PRV-3 trial in rainbow trout at different temperatures: organ material fixed in formalin, collected from an infectious experiment conducted in rainbow trout challenged with PRV-3b (from Denmark) for histopathological examination.
Bulgaria	Nelly Milenova Vilhelmova-Ilieva	Bulgarian Academy of Sciences The Stephan Angeloff Institute of Microbiology Department of Virology	01.06.2021	Non infected fish cells	3 flasks	3 bottles (2x 25 cm ³) of non-infected cells of CCB
Norway	Torfinn Moldal / Lone Egerdahl	Norwegian Veterinary Institute Seksjon for immunologi og virologi	21.06.2021	Cell supernatant infected with IHNV	1 tube	1 ml of cell supernatant infected with IHNV, from the IHNV outbreak Case No.: 2021-4120
The Netherlands	Olga L.M. Haenen	Wageningen Bioveterinary Research - WBVR, Fish Diseases Laboratory	16.07.2021	Non infected fish cells	2 flasks	2 bottles (2x 25 cm ³) of non-infected cells of BF-2

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

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

The experts of the EURL have since September 2022 been part of a working group established by the European Food Safety Authority (EFSA) to produce guidelines for defining vector species of listed aquatic animal diseases. These guidelines will be used in commissioned work to search the scientific literature for evidence of aquatic animal species working as vector species for the relevant diseases. The work will continue in 2023.

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. 44 inquires were journalised in DTU AQUA system.

Sub-activity 5.2 Listing susceptible species

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

Two working groups were established in 2020 in order to assess the fish and crustacean species susceptible to infection with the listed pathogens and provide recommendations for revision of regulation EU/2018/1882. In 2022 the EURL was asked to follow up on this work to deliver lists of common names for the aquatic animals listed as susceptible species.

The EURL is involved in a working group organized and chaired by EFSA on assessment of vector and reservoir species for listed fish, crustacean and mollusc diseases.

Sub-activity 5.3 Listing diseases for notification

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

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

EURL Workplan 2023-2024

2-year program

- 1. PT 1 and PT2:** No major changes maybe change of material from cell culture to tissue from infected fish.
- 2. Annual Workshop 2023:** face to face
- 3. Training course(s) 2023/2024:** In 2023 one training course on diagnostic/surveillance, one online webinar in 2024 on epidemiology in aquatic animal health and one course on validation of diagnostic methods
- 4. Quality assurance:** provide help for implementing in NRL's (SOPs, visits etc.)
- 5. Emerging fish diseases** (What to come?) The dramatic development of IHN in EU shall be addressed carefully and will be studied- hereunder update of the diagnostic procedures (RT-qPCR)
- 6. Further update of Diagnostic manual**
- 7. Proposals for topics are VERY welcome!**

28th Annual workshop 2024 (week 22)

29th and 30st May 2024 ONLY Virtual

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



End of 27th Annual workshop of NRL's for Fish Diseases 2023

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

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

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

We are looking forward seeing you soon again