



European Union Reference Laboratory for Fish and Crustacean Diseases

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

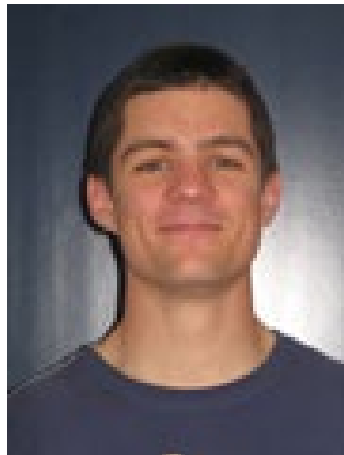
EURL-Fish and Crustacean work done in 2019/20

Niels Jørgen Olesen

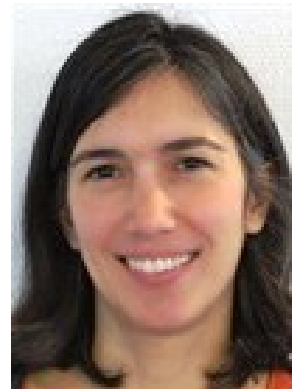




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EURL-Fish work program 2019-2020

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**

1-1 Organise and prepare for the 23rd Annual Workshop

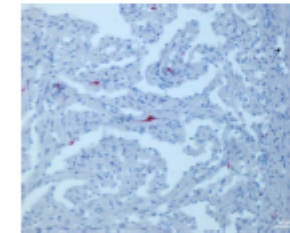


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Report of the 23rd Annual Workshop of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark

May 27th – 28th 2019



ISH staining of PRV-3 in Rainbow trout
heart tissue



European sea bass infected with VHS

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

1-2 Organise scientific working group meetings

A W.G. was organized on ISA with participation from UK, FO, NO and DK

Report from
 Meeting on
 Sampling and diagnostic procedures for the surveillance and confirmation of Infectious Salmon Anaemia ISA
 Lyngby December 5-6th 2019



DIAGNOSTIC METHODS FOR THE SURVEILLANCE AND CONFIRMATION OF INFECTION WITH HPR-DELETED INFECTIOUS SALMON ANEMIA VIRUS (ISAV)



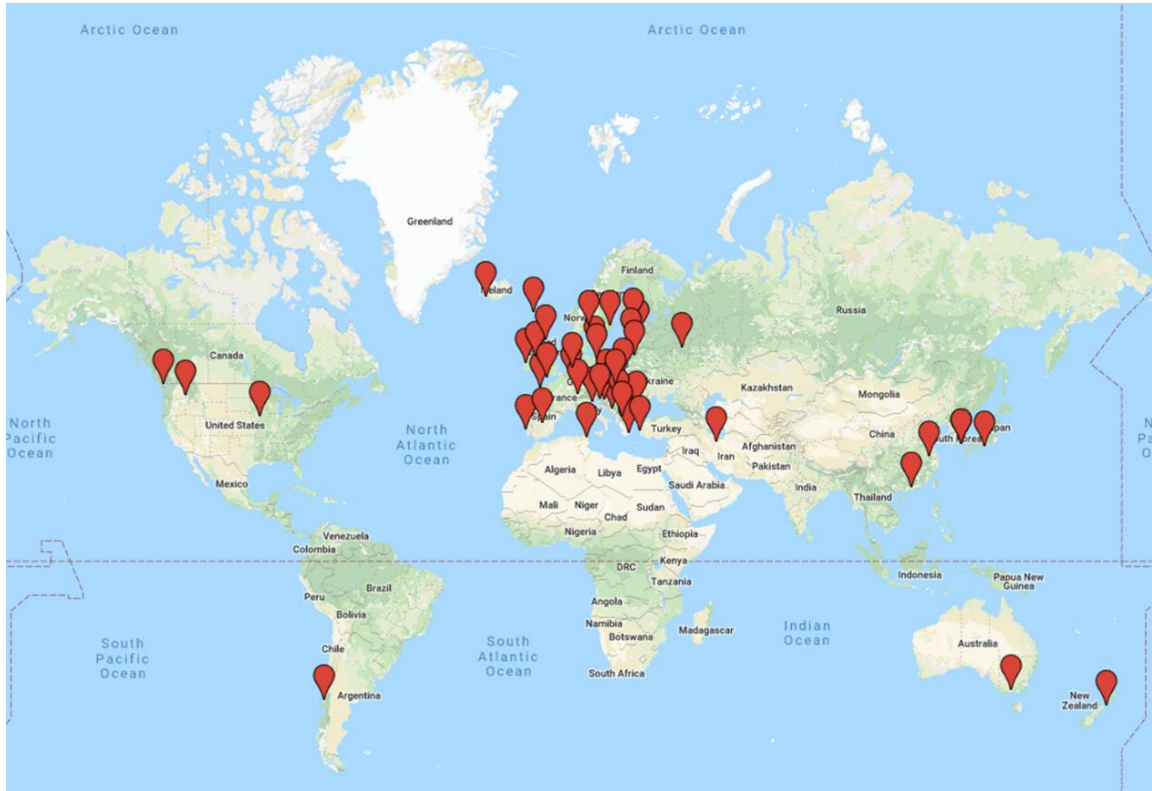
In the front page, on the top left, collection of clinically affected specimen in a net pen during fish farm visit.
 On the bottom right, necropsy of clinically affected Atlantic salmon during ISA outbreak, severe congestion of liver, enlargement of spleen, signs of anaemia of the heart.

1-3 Organise Proficiency tests

PT1 and PT2, 42 and 38 laboratories respectively participated



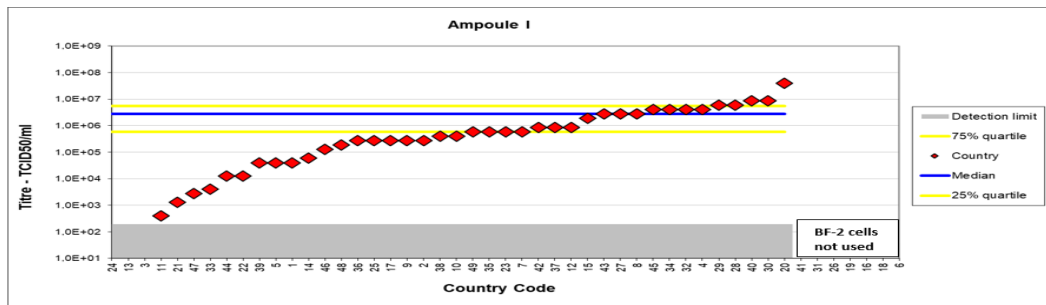
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EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2019
for identification and titration of
VHSV, IHNV, EHN, 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



1-4 Novel molecular methods

For the EURL to have molecular diagnostic methods of the highest scientific standards and to be able to provide these methods to all Member State NRLs.

1. qPCR for Candidatus *Midichloriaceae* – main aetiological agent of Red Mark Syndrome in Rainbow trout
2. RT-qPCR for Infectious Haematopoietic Necrosis Virus in one-step reaction has been implemented and validated including proficiency test with 8 participating NRLs.

Manuscript submitted:

“Analytical validation of one-step real-time RT-PCR for detection of infectious hematopoietic necrosis virus (IHNV)” Argelia Cuenca^{1*}, Niccolò Vendramin¹, Niels Jørgen Olesen¹

2-1 Training:

Facilitate and provide training in laboratory diagnosis:

EURL training courses Copenhagen, October 7th - 18th 2019

Course 1: Methods for implementation of surveillance procedures for listed fish diseases

Course 2: Introduction to histopathology in fish and crustacean diseases

The two courses are now accredited to grant ECTS at PhD level to the participants.

EURL Training Courses



Copenhagen, October 7th - 18th 2019

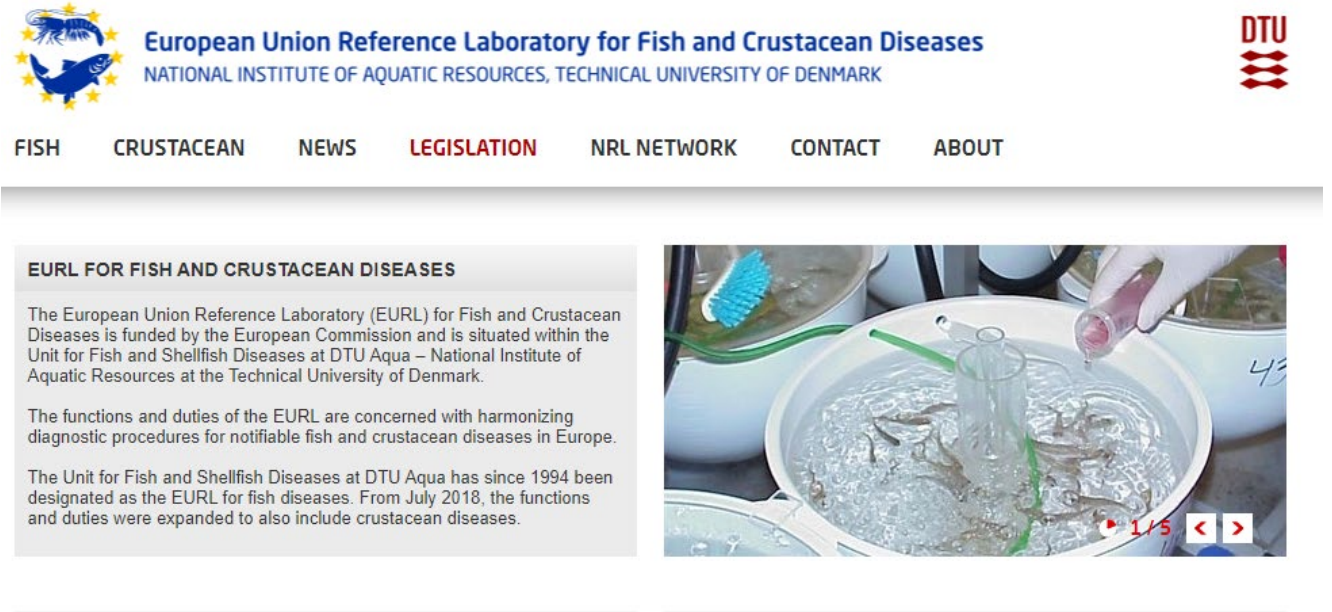
Hosted by the European Union Reference Laboratory for Fish and Crustacean Diseases

Sub-activity 2.2 Webpage

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

www.eurl-fish-crustacean.eu

The EURL website has gone through a substantial re-structuring and update. It now compiles the information on the activities by both the EURL for fish and crustacean diseases. The website has been accessed 4.448 times; 14.028 pages have been accessed from January to October 2020.



The screenshot shows the homepage of the European Union Reference Laboratory for Fish and Crustacean Diseases. The header features the DTU logo on the right and the EURL logo on the left, which includes a blue fish and a yellow star. The text reads: "European Union Reference Laboratory for Fish and Crustacean Diseases" and "NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK". Below the header is a navigation menu with the following items: FISH, CRUSTACEAN, NEWS, LEGISLATION, NRL NETWORK, CONTACT, and ABOUT. The main content area has a title "EURL FOR FISH AND CRUSTACEAN DISEASES" and three paragraphs of text. The first paragraph states: "The European Union Reference Laboratory (EURL) for Fish and Crustacean Diseases is funded by the European Commission and is situated within the Unit for Fish and Shellfish Diseases at DTU Aqua – National Institute of Aquatic Resources at the Technical University of Denmark." The second paragraph states: "The functions and duties of the EURL are concerned with harmonizing diagnostic procedures for notifiable fish and crustacean diseases in Europe." The third paragraph states: "The Unit for Fish and Shellfish Diseases at DTU Aqua has since 1994 been designated as the EURL for fish diseases. From July 2018, the functions and duties were expanded to also include crustacean diseases." To the right of the text is a video player showing a close-up of a laboratory setup with a petri dish containing water and small fish, a test tube, and a pipette.

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FISH CRUSTACEAN NEWS **LEGISLATION** NRL NETWORK CONTACT ABOUT

EURL FOR FISH AND CRUSTACEAN DISEASES

The European Union Reference Laboratory (EURL) for Fish and Crustacean Diseases is funded by the European Commission and is situated within the Unit for Fish and Shellfish Diseases at DTU Aqua – National Institute of Aquatic Resources at the Technical University of Denmark.

The functions and duties of the EURL are concerned with harmonizing diagnostic procedures for notifiable fish and crustacean diseases in Europe.

The Unit for Fish and Shellfish Diseases at DTU Aqua has since 1994 been designated as the EURL for fish diseases. From July 2018, the functions and duties were expanded to also include crustacean diseases.

2.3. FishRefLabNet.

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.

2.5. 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.

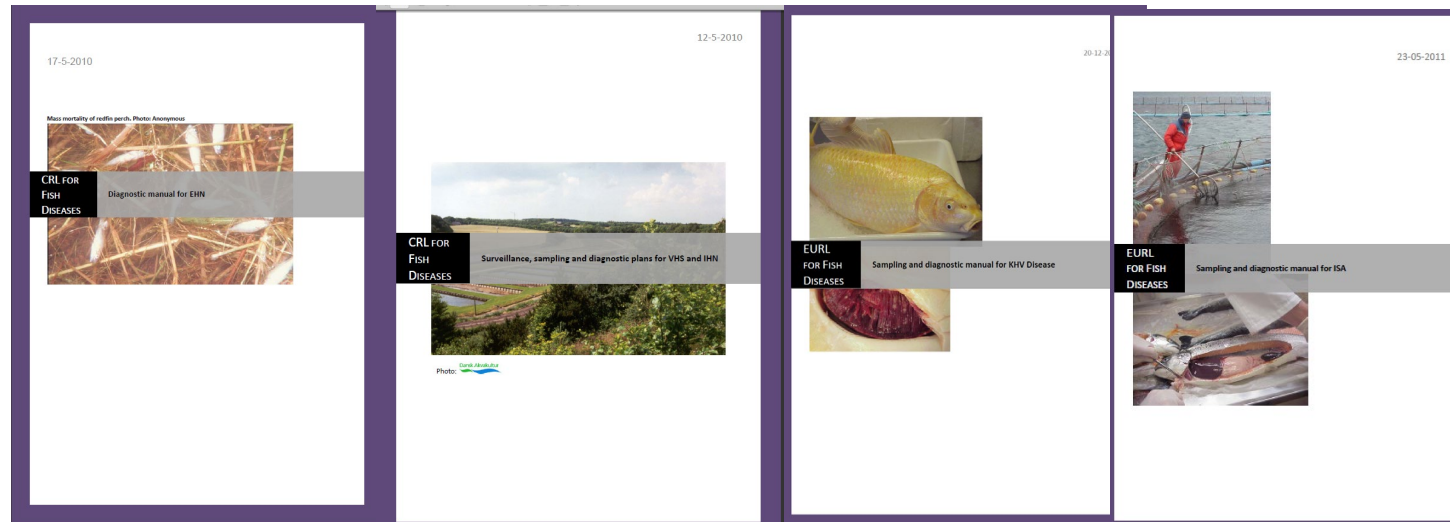
The EURL team has attended and contributed with scientific talks to a number of international conferences and meetings within the field.

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.

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

3.1. Diagnostic manuals.

To have updated diagnostic manuals for all listed fish diseases available for Member State NRLs on the EURL website www.eurl-fish.eu.



3.2. Survey and diagnosis. "collate and forward information on exotic and endemic diseases, that are potentially emerging in Community"

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Report on Survey and Diagnosis of Fish Diseases in Europe 2019



DISCLAIMER The EURL for Fish and Crustacean Diseases and the EU commission, have no liability for the accuracy of the information and cannot be held liable for any third-party claims or losses of any damages related to this report.



4. REAGENTS AND REFERENCE COLLECTIONS

4.1. Pathogen library. For the EURL for fish and crustacean diseases to have an updated library of crustacean pathogens relevant for the EURL and Member State NRLs.

Material received from 5 countries in 9 parcels and shipped to 15 in 20 parcels

Please help us updating the repositories, in order to maintain an EU wide repository of Fish pathogen isolates.

Annex 4.2 Reagents received at the EURL for fish and crustacean diseases in 2019

Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.	Purpose
Ireland	Felix Scholz	FishVet Group Ireland	16.01.2019	Fish tissue in RNA later	9 tubes	19-878	PMCV. Research. Screening of Salmon virus
Ireland	Felix Scholz	FishVet Group Ireland	07.03.2019	Hearts from Atlantic salmon	11 tubes	19-878	PMCV positive hearts for trial
Ireland	Felix Scholz	FishVet Group Ireland	07.03.2019	Gills from Atlantic salmon	6 tubes	19-5326	AGD - Ring Test Parafish Control
Sweden	Eva Blomkvist	National Veterinary Institute	13.03.2019	Cells supernatant infected with IPNV	2 tubes	19-3291	To have in stock
The Netherlands	Otga Haenen	Wageningen Bioveterinary Research of Wageningen UR	29.03.2019	Cells supernatant infected with IHNV	1 vial	19-5429	To have in stock (IHNV panel)
Serbia	Vladimir, Ivan Radosavljevic	Institute of Veterinary Medicine of Serbia Department of Fish Diseases	28.05.2019	Cells supernatant	1 tube	19-8000	Confirmation of IHNV
Norway	Torfinn Moldal	Norwegian Veterinary Institute	16.07.2019	Fish tissue in RNA later, Fish tissue homogenate	2 vials, 2 vials	19-9736	Verification of KHV
Norway	Britt Gjerset	Norwegian Veterinary Institute	24.09.2019	Fish tissue in RNA later	2 tubes	19-11951	Verification of CEV
Norway	Maren Gagnat	Cfeed AS	04.10.2019	Copepod in RNA later	2 tubes	19-12315	Diagnostic (PCR: TSV, WSSV and YHV)

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Annex 4.3 Reagents supplied by the EURL for Fish and Crustacean Diseases in 2019

Country	Name	Institute	Date of receipt	Material	Amount	Specifics
Sweden	Eva Hansen	National Veterinary Institute	04.02.2019	SNM-1 cells E-11 cells	2x 120 ml 2x 120 ml	2 small flasks (250ml) with SNM-1 cells 2 small flasks (250ml) with E-11 cells
Ireland	Felix Scholz	University of Limerick	26.02.2019	SNM-1 cells ASK cells	2x 120 ml 2x 120 ml	2 small flasks (250ml) with SNM-1 cells 2 small flasks (250ml) with ASK cells
France	François Jégu	INRA, Lappas Isest	12.04.2019	Spores	3 tubes	3 Eimeria/Isospora (VHS) isolates
Bosnia of Herzegovina	Adilbeković	University "Sa. Cyril and Methodius" Skopje	14.05.2019	FTA Cards	12 pk	• VHSV, • A. salmonicida, • Aeromonas (E, H, B, BL, D, G and GII) • IHNV, • Ictaluriosis (E and U) • SSV, 1. aeromonas (d), • IPNV, 1. aeromonas (V) • KHV, 6. Saprolegnia
South Korea	Hyunjin Kim	NFIS National Fishery Products Quality Management Service	12.06.2019	IPNV, IPNV, IPNV	3 tubes 2 ampoules	The control of new IPNV PCR, IPNV virus (B, C, D, H, and H2, (2b) of each) 2 ampoules with IPNV (Amp. II from PT3)
Spain	Onovillo Delgado	CSIC Instituto de Fomento de Tecnología	25.06.2019	Intestine from Gilthead sea bream	8 samples 1 box	Diseases control. Samples of homogenized intestine from Gilthead sea bream (Date no. 2018-3429). The proficiency test for Enteropneustosis analysis by real time PCR, (validation of diagnostic methods)
Italy	Maria Letizia Sorrentino	University of Bologna, Dept. Veterinary Medical Sciences	25.06.2019	Intestine from Gilthead sea bream	8 samples 1 box	Diseases control. Samples of homogenized intestine from Gilthead sea bream (Date no. 2018-3429). The proficiency test for Enteropneustosis analysis by real time PCR, (validation of diagnostic methods)
Croatia	Bozica Stokich	ZOOB Laboratory of Aquaculture, Institute of Oceanography and Fisheries	25.06.2019	Intestine from Gilthead sea bream	8 samples 1 box	Diseases control. Samples of homogenized intestine from Gilthead sea bream (Date no. 2018-3429). The proficiency test for Enteropneustosis analysis by real time PCR, (validation of diagnostic methods)

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Country	Name	Institute	Date of receipt	Material	Amount	Specifics
Sweden - UK	James Dowd	University of Stirling	25.06.2019	Intestine from Gilthead sea bream	8 samples 1 box	Diseases control. Samples of homogenized intestine from Gilthead sea bream (Date no. 2018-3429). The proficiency test for Enteropneustosis analysis by real time PCR, (validation of diagnostic methods)
Tunisia	Nadia Chérif	I.N.S.T.M Institut National des Sciences de l'Agriculture et de Médecine Vétérinaire	25.06.2019	BF-2 cells EPV cells FTA Cards - VHSV FTA Cards - IHNV	2x 120 ml 2x 120 ml 1 card 1 card	2 small flasks (250ml) with BF-2 cells 2 small flasks (250ml) with EPV cells FTA Cards - VHSV FTA Cards - IHNV
Northern Ireland	Paul Sweeney	Department of Aquaculture and Fisheries, Veterinary Services Division	25.06.2019	FTA Cards - PRV3	2 cards	PRV3 positive PCR samples on FTA cards * 2 used with four application on each. Each application contains approx. 30-40 mg of tissue infected with PRV3.
Bosnia	Mikaela Gocic	Institute for Diseases and Animal Health, Aquatic Animals and Aquaculture	02.07.2019	BF-2 cells EPV cells ASK cells	2x 120 ml 2x 120 ml 2x 120 ml	2 small flasks (250ml) with BF-2 cells 2 small flasks (250ml) with EPV cells 2 small flasks (250ml) with ASK cells
Bosnia	Mikaela Gocic	Institute for Diseases and Animal Health, Aquatic Animals and Aquaculture	02.07.2019	VHSV - Antibody IHNV - Antibody IPNV - Antibody	2x 1 ml 2x 1 ml	Physical antibody raised in Rabbit Dok Ant VHSV Dok Ant IHNV
Italy	Anna Taffari Francesca Sironi	Università del Piemonte Orientale	06.08.2019	Serum	144 tubes	Serum samples from Sea bass vaccinated with VLP - 91 tubes VLP and challenged with RCNSV - 53 tubes
Eswatini	Janet Dadi	Enose Fish and Veterinary Laboratory	03.09.2019	BF-2 cells EPV cells CHSE-24 cells IPNV cells SAV-2 PER	2x 120 ml 2x 120 ml 2x 120 ml 2x 120 ml 1x Amp. 1x Amp.	2 small flasks (250ml) with BF-2 cells 2 small flasks (250ml) with EPV cells 2 small flasks (250ml) with CHSE-24 cells 2 small flasks (250ml) with IPNV cells SAV-2 SAV-2, ampoules V1 from PT2016
Slovenia	Aljdanec Luka Fajfar	Institute of Microbiology and Parasitology	14.11.2019	CHSE-24 cells	2x 120 ml	2 small flasks (250ml) with CHSE-24 cells
Chile	Ricardo Estay Estay Soto	Universidad Austral de Chile	14.11.2019	BF-2 cells	2x 120 ml	2 small flasks (250ml) with BF-2 cells

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Country	Name	Institute	Date of receipt	Material	Amount	Specifics
Italy	Maria Caterina	Università del Lazio	25.11.2019	Ostrac in Formalin	-----	Ostracae fixed in Neutral Buffered Formalin 10% (Formaldehyde concentration 4%) from experimental trial against PRV3 (Paci Mark Sandrone) in rainbow trout.
Italy	Maria Caterina	Università del Lazio	16.12.2019	Ostracae in Formalin	-----	Ostracae fixed in Neutral Buffered Formalin 10% (Formaldehyde concentration 4%) from experimental trial against PRV3 (Paci Mark Sandrone) in rainbow trout.
Italy	Alessio Marini	Università Zootecnica e Veterinaria del Piemonte	15.01.2019	Shrimp, oysters	4 tubes	4 tubes with shrimp (glycerol in ethanol), some tubes contain material infected with WSSV and some vials contain material from sea specific pathogen free (SPF) shrimps. For isolations of internal SCP

4.2. Pathogen characterization.

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

Support to NRLs in molecular characterization of IHNV isolates occurring in their country.



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.

The databases are published but nobody beside the EURL upload isolates on it. And very few visit it.

Internal discussions and considerations whether the database should be closed or not.

If closed a full copy of the database will still be accessible on our Web but it will no longer be maintained and updated

FISH PATHOGENS DATABASE



F I S H P A T H O G E N S

The Fish Pathogens Database is a platform for sharing of information on isolates of fish pathogens.

[Go to the Fish Pathogens Database](#)



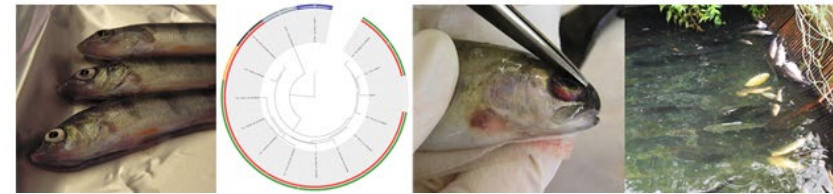
Fish Pathogens Database

Fishpathogens.eu offer a platform for sharing of available information on isolates of fish pathogens and their sequences to facilitate research on fish pathogens. The databases are free to use, but require subscription. One subscription covers all databases.

We encourage laboratories from all around the world to submit data of fish pathogens isolated in their laboratory, including as much isolate information as possible as well as genetic information if the isolate has been sequenced. It is not a requirement for upload to the database that the isolate has been sequenced.

For terms and conditions for using the database, please see [Terms and Conditions](#). For information on how to use Fishpathogens.eu, please see the [F.A.Q.](#)

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EURL Workplan 2021?

Most likely only a 1-year program

1. PT 1 and PT2: No major changes foreseen
2. Annual Workshop 2021: Hopefully face-to-face or combined with virtual access.
3. Training course(s) 2021 Hopefully face-to-face in wk 41 and/or 42
4. Emerging fish diseases (like POMV-infection, CMS in salmonids, SAV-2 in EU) will be studied
5. Scientific assessment of the effect of pooling samples for surveillance and diagnostics by PCR and provision of guidelines on how to pool.
6. All Diagnostic Manuals will be updated and ready on www.eurl-fish-crustacean.eu by 21st April 2021:
 - i. Infection with HPR-deleted ISAV is finalised,
 - ii. VHS and IHN will be separated into two and updated,
 - iii. Infection with KHV will be updated
 - iv. Infection with EHNV will be updated and aligned with the OIE Aquatic Manual

25th Annual workshop?

Hopefully physical meeting at DTU Campus in Kgs- Lyngby, Denmark **1st-3rd June 2021**

Two workshops back to back, on fish and crustacean diseases, respectively.

Large meetings may not 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).

Possibilities for organizing combinations of virtual and face to face meeting will be assessed



End of 24th Annual workshop of NRL's for Fish Diseases 2020

A Special joint session for NRL's Fish and Crustacean Diseases on the new EU Animal Health Law will follow at 11.40 to 13.00, Please access to this meeting through the Zoom invitation.

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
Hopefully face to face 😊 !

