European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark, Copenhagen

Technical Report 2013

from the European Union Reference Laboratory for Fish Diseases

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National Veterinary Institute
Technical University of Denmark
Section for Virology
Copenhagen, Denmark

Content

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Introduction

The National Veterinary Institute, Technical University of Denmark (DTU-VET) is appointed as the European Union Reference Laboratory for Fish Diseases (EURL), according to Commission Implementing Regulation (EU) No 135/2013 of 18 February 2013 and to Commission Implementing Decision of 22 March 2013 as regards a Union financial aid for the year 2013 to European Union reference laboratories (2013/155/EU)

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern fish diseases listed as exotic diseases: epizootic haematopoietic necrosis (EHN); and fish diseases listed as non-exotic diseases: infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and koi herpes virus (KHV) disease. This report follows the format of the work programme adopted for the EURL for 2013, describing activities and sub-activities and the status of on-going projects. The list of functions and duties of the EURL follows this introduction.

The 17th Annual Meeting of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 29-30 May 2013 at the premises of the Section for Fish Diseases at DTU Veterinary. A total of 52 participants from 28 countries attended over the two days period. There were five sessions with a total of 32 presentations, 7 of which were given by invited speakers, and two round table discussions. A report was submitted in August 2013.

Again this year an inter-laboratory proficiency test was distributed to the NRLs mainly within the EU but there were also participants from countries outside EU. For the fourth year, the proficiency test consisted of two tests, PT1 and PT2. PT1 was designed to primarily assess the ability of participating laboratories to identify VHSV, IHNV and EHNV + SVCV and IPNV (upon request from laboratories being accredited for these pathogens). PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV and in addition also spores of the oomycete *Aphanomyces invadans* causing EUS. The proficiency test is covering all 5 listed exotic and non-exotic diseases. 43 National Reference Laboratories (NRLs) participated in the proficiency test. A report was submitted primo March 2014. Most laboratories performed well.

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. Diagnostic manuals for VHS, IHN, ISA, KHV and EHN are available at the EURL web page. Diagnostic manual for EUS was uploaded in 2012. But as the disease was delisted again in autumn 2012 only the most recent update of the OIE chapter on EUS is linked to the description of EUS on the webpage. Especially on KHV and ISA significant changes have been made. The manual on sampling and diagnostic procedures for the listed diseases are expected to be adapted by the Commission primo 2014 and will be in force as soon as accepted.

Another important focus area was the development, implementation and validation of diagnostic tools for identification of the listed and emerging diseases and their accreditation. One outcome of these efforts was the implementation of real time RT-PCR´s for detection of PMCV and PRV the causative agents og CMS and HSMI, respectively.

During 2013, resources were also 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 produce antisera; to update the EURL webpage (www.eurl-fish.eu); and finally to attend international meetings and conferences.

In 2013 the fish diseases activities of DTU Veterinary were moved from Arhus to Copenhagen, since DTU decided to close the department in Aarhus and move some of the activities to the headquarter in Copenhagen. Among the consequences were that almost all from the old permanent staff were substituted by new colleagues that had to be trained. New facilities were built for us (laboratories and aquaria) and had to be

equipped and organised, unfortunately our research group conducted by professor Niels Lorenzen and his 6 co-workers was transferred from DTU to Aarhus University in order for them to be able to stay in town. The fish diseases group therefore now only consist of 5 academics and 4 technicians in permanent positions. The transfer, however, also resulted in a close localization together with scientists conducting the function as NRL for mollusc diseases and who are internationally recognised researchers in fish bacteriology, as well as close distance to other research facilities in the Copenhagen area.

This report was prepared and collated in a close collaboration between Anemone Ojala, Niccoló Vendramin, Susie Sommer Mikkelsen, Torsten Snogdal Boutrup and undersigned with contributions from all the former academic staff in the Section for Fish Diseases.

Frederiksberg, 27 March 2014

Niels Jørgen Olesen
Professor, DVM
Head of EURL for Fish Diseases

The functions and duties for the European Union Reference Laboratory for Fish Diseases

According to Council Directive 2006/88/EC of 24 October 2006 - Annex VI.
Period: 1 January 2013 – 31 December 2013

The functions and duties for the European Union Reference Laboratory for Fish Diseases (EURL)

The European Union reference laboratories shall:

(a) coordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing the disease concerned, specifically by:

(i) typing, storing and, where appropriate, supplying strains of the pathogen of the relevant disease to facilitate the diagnostic service in the Community,

(ii) supplying standard sera and other reference reagents to the national reference laboratories in order to standardize the tests and reagents used in each Member State, where serological tests are required, L 328/48 EN Official Journal of the European Union 24.11.2006

(iii) organising periodic comparative tests (ring tests) of diagnostic procedures at Community level with the national reference laboratories designated by the Member States, in order to provide information on the methods of diagnosis used and the results of tests carried out in the Community;

(iv) retaining expertise on the relevant disease pathogen and other pertinent pathogens to enable rapid differential diagnosis;

(b) assist actively in the diagnosis of outbreaks of the relevant disease in Member States by receiving pathogen isolates for confirmatory diagnosis, characterisation and epizootic studies;

(c) facilitate the training or retraining of experts in laboratory diagnosis with a view to harmonising diagnostic techniques throughout the Community;

(d) collaborate, as regards methods of diagnosing animal diseases falling within their areas of competence, with the competent laboratories in third countries where those diseases are prevalent;

(e) collaborate with the relevant OIE reference laboratories with regard to exotic diseases listed in Part II of Annex IV under their responsibility;

(f) collate and forward information on exotic and endemic diseases, that are potentially emerging in Community aquaculture.

Work programme for 2013

Technical report

1. Coordination and training

1-1,1-2 Organise and prepare for the 17th Annual Meeting for the National Reference Laboratories for Fish Diseases (NRLs) and produce a report from the Annual Meeting

Organization of the 17th Annual Meeting

The 17th Annual Meeting of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 29-30 May 2013 at the premises of the Section for Fish Diseases at DTU Veterinary.

A total of 52 participants from 28 countries attended over the two day period. There were five sessions with a total of 32 presentations, 7 of which were given by invited speakers. The scientific programme of the Annual Meeting was diverse and covered many topics of current interest. The meeting was opened with the traditional session on update of fish diseases in Europe, where once again participants from the member states had the opportunity to present new findings from their home countries. Initially an overview of the disease situation and surveillance in Europe 2012 were provided on the basis of the results from the Survey & Diagnosis questionnaire.

Then the fish disease situation in Norway was presented; a detailed report is available at: http://www.vetinst.no/eng/Publications/Fish-Health-Report.

The results of a survey on the impact of fish disease in the Mediterranean basin, achieved thanks to the collaboration with a panel of acknowledged fish pathologist experts, were presented.

This was followed by an update on KHV, discussing diagnostic methods for surveillance and issues related to atypical strains; after the presentation an open discussion on this disease took place with very active participation of many experts.

Then the appearance of a VHS outbreak in Scotland in a new susceptible species, the wrasse (cleaner fish) was described.

The importance of preparation and harmonisation of legislation for fish disease surveillance and control within the EU territory was addressed with the following talk, where the Croatian Member state representative described the work in progress for the entry of Croatia in the EU.

Then the relevance of zoonoses in aquaculture was underlined by the next presentation describing *Vibrio vulnificus* outbreaks in aquaculture and their implication on human health.

This was followed by an update on non/low pathogenic HPR0 ISAV, a current issue for the salmon farming industry; the output of an EFSA specific working group on the topic was described.

Two more talks complete the first session.

First an overview on the diagnostic manuals: the tool to develop surveillance and diagnostic method for the listed fish diseases in EU was delivered.

To conclude the session the findings of a new potential emerging disease that occurred in Sweden were described.

This year the second session was dedicated to Emerging diseases. Three topics and related talks were presented.

First the Salmonid Alpha Viruses and their geographic distribution were

described. These pathogens represent one of the major constraints for salmon farming in Norway.
Secondly Viral Encephalopathy and Retinopathy the main viral problem for the Mediterranean Mariculture was described.
Thirdly the outbreak of Hirame Rhabdovirus, a pathogen detected for the first time in Europe. This virus was found in connection with increased mortality in farmed grayling (*Thymallus thymallus*). The virus was identified in cooperation between laboratories as Hirame Rhabdovirus by use of molecular and immunochemical techniques.

The third session, on control and surveillance of relevant pathogens in the EU, started by a presentation on health categorization of fish farms in Europe in 2012 based on answers from the questionnaire "survey and diagnosis" that is delivered every year to all national reference laboratories.

A funded EFSA project focusing on risk ranking in aquaculture was presented and the results obtained from the questionnaire were given.
A presentation on Aquatic Animal Health Law, a piece of framework legislation, was delivered emphasizing on the issues of interest and importance for aquatic animal health.
Finally a presentation concerning EUS was given addressing how to deal with this pathogen after its delisting from the group of notifiable diseases in the EU.

The second day was opened by the session on scientific research update; this fourth session faced several different topics covering molecular characterization of pathogen, a present and future core topic for all the laboratories involved in fish disease diagnosis, vaccination as a strategy to prevent disease and breeding program to select resistant fish to pathogen

The annual meeting ended with the traditional update from the EURL. The results of the two proficiency tests sent out in 2012, PT1 and PT2, were presented. A report from the annual training course provided by the EURL in January/February 2013 was given and topics for next year's training course were discussed. The planned EURL activities in year 2013 were presented and proposals for the EURL work plan for 2014 were discussed.

The final report, including programme and minutes of the meeting is enclosed as Annex 1

1-3 Collect and report data on the fish diseases situation in EU, including all the listed non-exotic fish diseases given in Council Directive 2006/88/EC Annex IV Part 2

Survey and diagnosis of fish diseases in Europe in 2012
The Questionnaire on Surveillance and Diagnosis (S&D) which is collated annually is the only comprehensive overview of the disease situation in aquaculture in Europe. The information has been made available on the EURL web site (www.eurl-fish.eu), where all raw data can be obtained. The S&D have evolved over the years, for 2012 it comprise 3 parts (not 4 parts as for 2011):

1. General data on production type and size, health categorization of fish farms 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 but also including other diseases of interest.
3. Laboratory data from the NRLs and other laboratories, including number of samples examined diagnoses of fish diseases made.

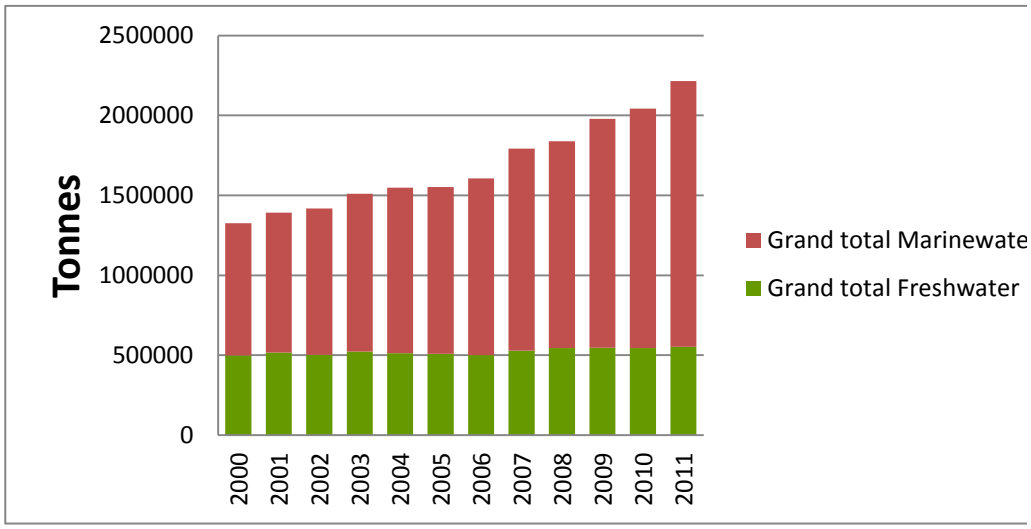
The data on the European aquaculture production were obtained from the FIGIS database. This database does not include information on the number and size of fish farms, and therefore these data had to be obtained directly in the questionnaire. The production has increased quite significantly from 2010 to 2011. The increase primarily account for the Atlantic salmon production, especially in Norway. With a raise from 1.6 mill in 2001 to > 2.2 mill ton in 2011, Europe is following the global development towards increased aquaculture production (Figur 1). Data from 2012 will only be available from May 2014. The farm sizes vary a lot between countries, e.g. the majority of farms in Germany produced < 5 tonnes, and for Spain the number of farms producing < 5 tonnes, 5-100 tonnes and > 100 tonnes is nearly equal.

The Atlantic salmon production has increased significantly while the rainbow trout production has stabilised in Europe in 2011. The carp production is still mainly in the Eastern part of Continental Europe and had a slight decrease compared to the years before. Both the production of sea bream and sea bass increased in the Mediterranean countries but not at the same space as in 2002-2007. Among other fish species of interest are pike-perch (499t), eel (6.720t), sturgeon (5.281t), cod (16.126 t), turbot (11.161t), and halibut (2.883t). Pike-perch have not yet obtained the expected increase, while the sturgeon production seems to grow significantly. The cod production decreased dramatically.

Concerning the epidemiological data, obviously, there is still a severe underreporting of VHS, IHN and KHV in many countries. Only 56 of 12.741 farms are considered VHS infected and 61 of 8.363 are considered IHN infected, while 53 of 10.403 farms are considered KHV infected in the reporting countries. There were no ISA infected farms in Europe 31.12.2012! VHS the infection status in only known for 33% of the farms, for IHN the situation is known in 37% of the farms. While for KHV the disease situation is unknown on 95% of the farms! For farms producing Atlantic salmon and categorised for ISA, the infection status for ISA is known for 66% of the farms. The findings of Isavirus HPR0 pose some problems regarding how to health categorise salmon farms.

Many countries have surveillance programmes for SVC (19 of 35 countries), BKD (14 of 35 countries), IPN (18 of 35 countries) and Gyrodactylus salaries (7 of 35 countries), for which they are seeking “additional guaranties” according to §42 in CD 2006/88/EC. The number of farms in the programmes varies from very few farms to many farms. In northern European countries the most common problems are sea lice, pancreas disease, Amoebic gill disease in the salmon production, in continental Europe it is primarily bacterial diseases like ERM and Aeromonas infections, AGD and RTFS, while problems in the Mediterranean countries are the same as in continental except that Nodavirus infection in mariculture seem to play an increasing role.

There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. The total number of samples increased since last year and PCR is coming up in most countries. The large number of PCR-tests conducted in some countries mostly reflects the KHV and ISA testing.



Total production of fish in aquaculture in Europe 2000 to 2011 (www.fao.org/figis)

A summary of the results for 2012 is presented on our website:

www.eurl-fish.eu/Activities/survey_and_diagnosis.aspx

1-4 Facilitate and provide training in laboratory diagnosis. The yearly training courses in methods used for diagnosis of fish diseases will be offered at the EURL laboratory facilities. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants.

Training, missions and scientific collaboration

The EURL Fish is offering a yearly 2 weeks training course in diagnostic techniques for identification of listed fish diseases.

The training course took place at DTU National Veterinary Institute, Hangøvej 2, DK-8200 Aarhus N, 21/1-31/1 2013. The course was divided in two parts where one or both parts could be followed. Part 1 “Diagnostic procedures” took place

21/1-25/1 and 5 persons participated. Part two "Advanced bio-molecular techniques and bio-informatics" took place 28/1-31/1 and 13 persons participated. 1 person participated in both parts of the training course.
The overall purpose of the training course was to provide an opportunity for the NRLs to send employees for training in techniques relevant when working with fish diseases. Staff of the EURL provided this training, together with teachers from the Danish Veterinary and Food Administration; CLC-bio, Denmark; FLI, Germany; and CVI, The Netherlands. Also, knowledge-sharing and discussions between participants and teachers were important parts of the courses.

Course I: Sampling and diagnostic procedures for surveillance of listed fish diseases
The 5-days course in "Sampling and diagnostic procedures for surveillance of listed fish diseases" was primarily based on practical work (hands on) in combination with theoretical presentations.

Day 1 was dedicated to a fish farm inspection.
As it is not possible to visit a fish farm after working in our laboratory it was decided to meet with the participant's downtown in Aarhus and to drive to the FVO offices in Vejle, where we were received by Dr Korsholm. After the training course introduction by NJ Olesen and presentations on Danish surveillance plan for fish diseases held by Dr Korsholm, the participants visited a rainbow trout farm, Vingsted Dambrug, approx. 25 km from Vejle. Here it was demonstrated how to inspect a farm and to collect relevant samples and taught how fish necropsy techniques are done in the field. All participants collected their own samples, which were brought back to the laboratory in Aarhus for further examination.

On day 2 the participants were divided into two small groups. As an assignment each group received 4 blinded ampoules containing lyophilized putative fish pathogenic viruses to be identified during the course. The processing of fish samples collected the day before as well as opening and preparing the proficiency test ampoules was demonstrated before the participants were asked to do it themselves. Later, each group were introduced to basic cell culture work, and then produced their own flasks, 24-well trays, and 96-well plates for titration and immunofluorescence. The participants were then introduced to cell freezing- and thawing procedures followed by mycoplasma testing. Inoculation of diagnostic samples on cell cultures was also practised. The CPE of different viruses was shown and the participants practised reading of diagnostic trays. Quality assurance, contamination, cleaning and disinfection etc. was an integral part of the practical demonstrations.

On Day 3 ELISA techniques were addressed; each group designed and performed the practical testing in order to be able to identify the distributed virus isolates, following theoretical classroom teaching on methodologies, pitfalls and error findings.
The course was dialogue-based and sufficient time was given for discussion under way and for evaluation of test results.

On Day 4 real-time PCR and bio molecular techniques were targeted. Participants had the possibility to test two protocols for disease surveillance, testing the ampoules for VHSV and IPNV.
While the amplification process was running, each group received a collection of slides for studying characteristic IFAT results for listed disease pathogens and other relevant viruses.

On day 5 titration procedures were demonstrated and the participants were asked to read viral load on titration plates prepared in advance. Finally results sheet were filled in.
Production of medium, cell sensitivity tests and test of calf serum batch before general use in cell medium was discussed. In the end time was allocated for course evaluation.

The methods taught were primarily focused on the protocols given in EU legislation and on the OIE guidelines from the Manual of Aquatic Animal Diseases, and included how to select proper controls, the typical pitfalls, trouble shooting, etc.

Course II: Advanced bio molecular techniques and bioinformatics.

The 4-day course in molecular techniques and bio-informatics was devoted to lectures on relevant topics, as well as theoretical exercises.
The course was built up so that it followed a logical progression from the basic techniques of PCR and real-time PCR, to sequencing of the PCR products, both by Sanger sequencing and next-generation sequencing and finally using the sequences for phylogeny and seeing presentations of real case-stories.
The participants started by learning about PCR and real-time PCR on the first day, followed by theoretical exercises. The lectures included information on the general theory behind the techniques, the use of controls, prevention of contamination of samples as well as suggestions for troubleshooting

The following day, the theory behind Sanger sequencing and next-generation was explained, as well as lectures on trouble-shooting and pitfalls concerning these techniques. It also included a lecture on the sequence analysis software Main bench from the CLC-bio company.

On the third day, participants were introduced to other software used for sequence analysis and phylogenetic studies and lectures were given on phylogenetic theory. Later in the day the participants were introduced to some theoretical exercises where they got hands-on experience with the before-mentioned software for phylogenetic studies.

On the last day (day four) they were introduced to the www.fishpathogens.eu database, which facilitated a discussion on guidelines for use of the database. Furthermore, the participants were introduced to phylogenetic case stories on rhabdoviruses, herpesviruses and nodaviruses, respectively, as well as a lecture on molecular epidemiology.
Through lectures, exercises, discussions, and knowledge exchange between participants, the knowledge on molecular techniques, bio-informatics and troubleshooting related to these was increased.

For the full training course report is included as Annex 2

Many requests from colleagues for training were postponed to take place at this yearly course. The following colleagues visited the institute during 2013 for scientific meetings, project collaboration or training:

EURL training course in fish diagnostics, part 1: Laura Valls – Spain; Perla Tedesco – Italy; Renate Johansen – Norway; Susan Schares – Germany; Tobia Pretto - Italy	21/01 to 25/01 2013

EURL training course in fish diagnostics, part 2: Mona Saleh - Austria; Ekaterina Mileva - Bulgaria; Tomáš Veselý - Czech Republic; Mihkel Mäesaar - Estonia; Tobia Pretto – Italy; Torfinn Moldal, Trude Marie Lyngstad, Britt Bang Jensen, Alf Dalum Frøyse, Anne Berit Olsen – Norway; Hongan Duan , Zhou Yi - P.R.China; Vladimir Ivan Radosavljevic - Serbia	28/01 to 31/01 2013
Takafumi Ito, Ph.D. Tamaki Station, Aquatic Animal Health Division, National Research Institute of Aquaculture, Fisheries Research Agency Hiruta 224-1, Tamaki, Watarai, Mie 519-0423, Japan	10/03-14/03 2013
UNIVERSITY of Prince Edward ISLAND **David B. Groman**, BA, MSc, PhD Section Head **Aquatic Diagnostic Services** **Atlantic Veterinary College** University of Prince Edward Island 550 University Avenue, Charlottetown Prince Edward Island, Canada C1A 4P3 T (902) 566-0830 LAB (902) 566-0864 F (902) 566-0723 E aquaticdx@upei.ca **upei.ca/aquatic**	18/03-20/03 2013

Master and PhD students:

M.Sc. Sekar Larashati, Bandung Institute of Technology, Indonesia. Ph.D. study at DTU-VET in the field of "delivering small RNAs to fish" from 1 November 2009 to February 2014. Supervisor: Niels Lorenzen; Co-supervisor: Brian Dall Schyth

M.Sc. Dennis Bela-Ong, Manilla University, The Philippines. Ph.D. study at DTU-VET on "The role of RNA interference in host-virus interactions in a fish model" from 1 February 2011 to 28 February 2014. . Supervisor: Niels Lorenzen; Co-supervisor: Brian Dall Schyth

Dagoberto Sepúlveda, Biochemist, Pontifical Catholic University of Valparaíso, Chile. Ph.D. study at DTU-VET Århus on “Functional characterization of protective immunity following DNA vaccination against a lethal viral disease in fish” from June 2012 to May 2015. Supervisor: Niels Lorenzen, PhD; Co-supervisor: Niels Jørgen Olesen, PhD.

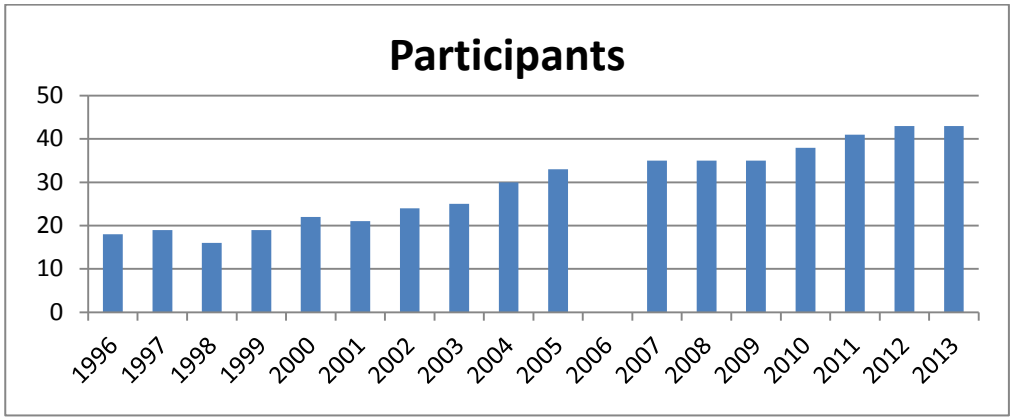
OIE collaboration: N.J. Olesen **is** designated expert of the OIE reference laboratory for VHS, and in this function had consultancies and requests worldwide concerning VHS. The OIE chapter on VHS and related diseases in the diagnostic manual for aquatic animal diseases is revised and updated by the OIE reference laboratory for VHS.

2. Proficiency test

2-1 Prepare the Annual Inter-laboratory Proficiency Test year 2013 for the NRLs. The test will include IPNV, VHSV, IHNV, EHNV, ISAV, KHV and Aphanomyces Invadans.

The inter-laboratory Proficiency Test 2013

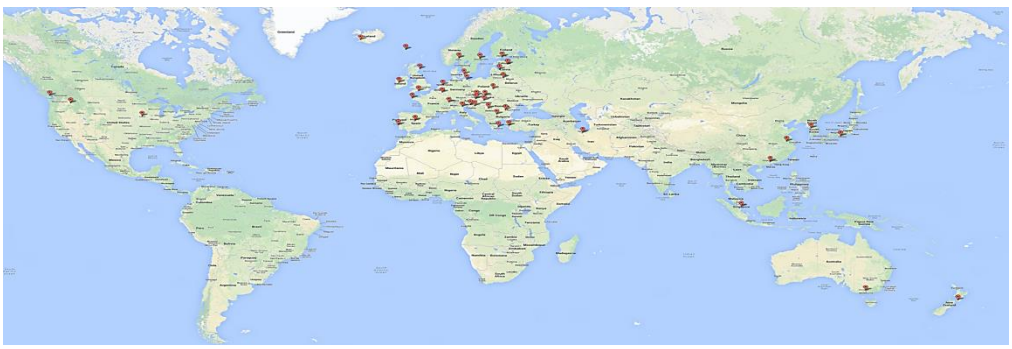
Since 1996, seventeen inter-laboratory proficiency tests have been organised by the EURL. The number of participants has increased from 18 to 43. The goal of these tests is to harmonise diagnostic methods between national reference laboratories and to ensure that the examination of a given sample leads to the same conclusions in any laboratory.



PT1 was designed as the proficiency tests provided by the EURL in previous years to primarily assess the identification of viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), epizootic haematopoietic necrosis virus (EHNV), spring viraemia of carp virus (SVCV), and infectious pancreatic necrosis virus (IPNV) by cell culture based methods. PT2 was structured with the aim of assessing the ability of participating laboratories to identify the fish pathogens: infectious salmon anaemia virus (ISAV), Cyprinid herpesvirus 3 (CyHV-3) (otherwise known as *koi herpes virus* - KHV) and *Aphanomyces invadans* the causative agent of epizootic ulcerative syndrome (EUS) by biomolecular methods (PCR based). The number of National Reference Laboratories (NRLs) participating in PT1 and PT2 was 43.
The tests were sent from the EURL in the beginning of October 2013.

Proficiency test 1, PT1

Five ampoules with lyophilised cell culture supernatant were delivered to all NRLs in the EU Member States, including Denmark, and likewise to the NRLs in Australia, Canada, Croatia, Faroe Islands, Iceland, Japan, New Zealand, Norway, 2 in P.R. China, Serbia, Switzerland, Turkey, Republic of Korea, Singapore, and 2 in USA. The Belgian NRL covers both Belgium and Luxembourg and the Italian NRL covers Italy, Cyprus and Malta for identification of all listed diseases. The figure on next page shows the worldwide distribution of the participating NRLs.



PT1 consisted of five coded ampoules (I-V). These ampoules contained IPNV, EHNV, SVCV, IHNV and VHSV, respectively, see table 1. The proficiency test

was designed to primarily assess the ability of participating laboratories to identify the listed fish viruses VHSV, IHNV and ENHV (Council Directive 2006/88/EC) and the non-listed viruses SVCV and IPNV if present in the ampoules, bearing in mind that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses). In addition the participants were asked to quantify the viruses in PT1 by titration on cell cultures in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Decision 2001/183/EC using fish cell cultures followed by e.g. ELISA, PCR, immunofluorescence (IFAT) or neutralisation test.
If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals 2009. Laboratories were encouraged to identify VHSV and IHNV isolates as far as possible by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et *al*. (2004) for VHSV and in Kurath et *al*. (2003) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT2 consisted of five coded ampoules (VI-X). The ampoules contained ISAV and KHV. Furthermore, one ampoule contained *Aphanomyces invadans* spores and one sterile pyrogen-free water, see table 9. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV and KHV (listed in Council Directive 2006/88/EC). *A. invadans* was in 2012 delisted from the Council Directive but was nevertheless included also this year in PT2 following open discussions and agreement at the Annual Workshop held the 29th and 30th of May 2013. Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus, it might have been possible to replicate them in cell cultures. Only inactivated *A. invadans* was included in the ampoules.

2-2 Collate and analyse information gained from the Inter-laboratory Proficiency Test

Outcome of Inter-laboratory Proficiency Test 2013

Within two days, the tests were delivered to 26 participants, 18 participants in EU and 8 participants outside EU; due to a mistake in the delivery process by the shipping company 6 laboratories received their own parcels after 7 – 9 days. 2 laboratories (outside EU) received the test after 33 days; 1 participant collected the test directly from Denmark, finally one participant received an extra test because the first package was damaged during transport. 43 laboratories received the proficiency tests, and all participants submitted results within the deadline.

Concluding remarks PT1
The inter-laboratory proficiency test 2013 was conducted without major constraints. Despite the fact that the shipping company caused a delay in the delivery of the parcels 93% of parcels reached the respective laboratories of destination within 9

days after submission. Once again shipment to China demonstrated to be difficult and laborious taking about a month to reach the laboratories primarily due to border controls.
The overall performance of the participating laboratories was very high, and the fact that we this year included an ampoule with both IPNV and VHSV did not trouble most of the laboratories. It was, however, quite worrying that 6 of 43 laboratories detected virus in Ampoule V that only contain MEM without virus, and these laboratories should consider revising their procedures in order not to cross-contaminate their samples.

This year 40 participants were able to identify the EHNV isolate correctly using either sequencing or REA; however 2 laboratories sequenced the isolate but retrieved a sequence that was not correct. One laboratory did correctly isolate the virus but did not characterize it as they have indicated in their contingency plan that in case of EHNV suspicion they will forward the isolate to the EURL for Fish diseases.

All the viral titres submitted were compared according to cell line and ampoule, respectively. The titres obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory can thereby compare the sensitivity of its cell lines to the sensitivity of those used by the other participants. We recommend all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they perform 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 also took the opportunity to provide a comment 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.

Concluding remarks PT2

Considering that this was the fourth time that the EURL provided a proficiency test on ISAV and KHV identification, and the third time that the EURL provided a proficiency test on *A. invadans*, we consider that most participants obtained satisfying results.
Out of 36 laboratories testing for *A. invadans* 32 identified the pathogen in ampoule VI.
Out of 42 laboratories performing KHV identification, 41 laboratories identified KHV in ampoule VII and 42 correctly identified KHV in ampoule VIII.
Out of 43 laboratories 40 laboratories identified Not *A.invadans*, KHV or ISAV in ampoule IX.
Out of 42 laboratories performing ISAV identification 40 correctly identified ISAV in ampoule VI while 1 laboratory described co infection ISAV and KHV scoring 1 point for this ampoule. Very significant improvement in the proficiency of identifying and typing these pathogens has been observed during these 4 years. In autumn 2012 the European Commission decided to de-list EUS and it is officially no more considered as an exotic disease in the Union. However we find that a certain level of preparedness for the introduction of this disease in European aquaculture should be maintained. As agreed at the Annual Workshop held in May 2013 this pathogen was included in 2013 but will not be included in 2014.

It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterizing the isolates through sequence analysis as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains. It was not described according to what notification the genotype of viruses should be performed reflecting the various way of reporting isolate genotypes. In future tests we will clarify which notification the genotyping should follow.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they perform 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.

The results presented in this report will be further presented and discussed at the 18th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 3rd and 4th of June 2014 in Copenhagen, Denmark.

The full report is included as Annex 3

3. Reagents and products

3-1 Supply reference reagents to the NRLs in Member States

Materials supplied by the EURL

On request, the EURL supplies material to other laboratories in Member States and Third Countries to aid in the diagnosis and characterisation of fish diseases. The number of laboratories receiving material and the specific material and number of units supplied by the EURL in 2013 are listed in Annex 3.

Further details of the supplied materials are listed in Annex 4

3-2. Production of antisera against selected isolates when necessary

Production of antisera

In 2013 antisera against Nodavirus the causative agent of Viral Nervous Necrosis was produced in rabbits. In addition new stocks of supernatants from Hybridoma cells producing monoclonal antibodies against VHSV (IP5B11) were produced.

3-3 Update and maintain a library of isolates of ISAV, VHSV and IHNV, KHV, EHNV and A. Invadans.

Virus library

Isolates of the listed viruses VHSV, IHNV and KHV were received and stored in our library during 2013.
The EURL received 6 samples for PCR testing for KHV and 1 sample for IPNV and 2 samples were tested for Sarcocystidae infection. PRV, positive control material for PCR was received and our library have continuously been updated and maintained.

Further details of the received materials are listed in Annex 5

3-4 Maintain a library of tissue material from fish infected with listed pathogens

Library of tissue material from fish infected with listed pathogens

For use as tissue library of positive naturally infected tissue from VHSV, IHNV and IPNV infected fish, organ pieces has been collected, stored and maintained, as well as organ material from negative controls.
Tissue material from rainbow trout infected with IPNV, salmonid alphavirus (SAV), *Oncorrhynchus masou* virus (OMV) and *Aphanomyces invadans* (EUS) have been stored as well.

4. Scientific advice and activities

4-1 Update the webpage for the EURL, www.eurl-fish.eu

Update the webpage of the EURL

The EURL website (www.eurl-fish.eu) is a notice board, where NRLs and other interested parties can access relevant information and previous reports concerning the activities coordinated by the EURL and relevant upcoming events in the Community.

The diagnostic manuals for VHS, IHN, ISA, KHV, EHN disease and EUS have been updated and uploaded on the web page.
Furthermore, reports of the EURL, e.g. of the results of the proficiency test, the Annual Meeting of the NRLs, result of questionnaire on "Survey & Diagnosis", Training courses etc. are launched at the web page immediately after release.

4-2 Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, EHN and EUS on the EURL web page

Diagnostic manuals

The diagnostic manuals for VHS, IHN, ISA, KHV, EHN disease and EUS have all been prepared and are available from the EURL web page. The diagnostic manuals for VHS and IHN are updated and modifications of Commission Decision 2001/183/EC made. The diagnostic manual for ISA was prepared based on

Commission Decision 2003/446/EC. In all five manuals with the latest information on test developments as analytical sensitivity and specificity are included.
The diagnostic manual for EUS has been uploaded.

4-3 Fishreflabnet: Establish an interactive network with the NRLs, Fishreflabnet , in order to promote a more proactive data sharing and communication with and between reference laboratories in member states.

Fishreflabnet:

A new e-mail group was created in 2012: VET-EURL with approximately 100 colleagues subscribing. Official communication and updates of interest to the scientific community are delivered periodically.
Furthermore this tool is used for newsletters, scientific updates and announcements from the EURL Fish like announcements and invitations for the Annual Workshop or publication of content in the ampoules from the proficiency test or on the final Inter-laboratory Proficiency test report.

4-4 Pathogen characterization: Identify and characterise selected isolates of listed viruses (pathogenicity testing in vivo and in-vitro, serological and genetic characterisation).

Studies conducted on pathogen characterization:

VHSV: The full length G-genes of a large number of Danish VHSV isolates (>170) were sequenced by FLI, Germany (Dr Heike Schuetze) and aligned for molecular tracing of passed VHS outbreaks in Denmark.

KHV: A study primarily conducted by the NRLs in NL and UK, respectively, revealed that CyHV-3 might be more widely prevalent than previously anticipated, as the most used specific PCRs do not identify several subtypes regularly present in healthy carp. Only by using generic CyHV primers the viruses could be identified. A manuscript on the topic was published in Diseases of Aquatic Organisms: *Marc Y. Engelsma, Keith Way, Melanie J. Dodge, Michal Voorbergen-Laarman, Valentina Panzarin, Miriam Abbadi, Mansour El-Matbouli, Helle Frank Skall, Søren Kahns, David M. Stone* (**2013**) Detection of novel strains of cyprinid herpesvirus closely related to koi herpesvirus.

Identification and characterisation of selected virus isolates:
In 2013 a number of virus isolates were received for further characterisation at the EURL and for storing in our virus library as shown in the table beneath.

Member States and countries outside EU		
Material received	**Laboratories**	**Units**
Diagnostic material for virology	3	9 samples
Diagnostic material for PCR	1	6 samples
Diagnostic material for bacteriology	1	6 samples
PCR control material	1	1 sample
Serum	1	119 samples
Other material	1	2 samples

Further details are listed in Annex 5

Below is listed samples, isolates and reagents received for identification, characterization and update of the virus library and diagnostic procedures applied for the relevant cases:

Veterinary Institute, Croatia (Snjezana Zrncic): Rainbow trout, Supernatant from EPC and BF-2 cells for Virus examination. The isolate was confirmed to be Viral hemorrhagic septicaemia virus (VHSV) by ELISA and real-time PCR, and was subsequently sequenced. Sequencing placed the isolate in genotype Ia, subtype 2, and it grouped into subclade Pol II. This is the first case of VHS in Croatia. (DTU-VET 2013-50-182).

Saare District Pihtzla Parish, Estonia (Timo Kolk): Whitefish swab for laboratory examination. Non-specific mixed flora was isolated. (DTU-VET 2013-50-175).

Saare District Pihtzla Parish, Estonia (Timo Kolk): Whitefish swab for laboratory examination and resistance. *Aeromonas salmonicida sp. salmonicida* (Furunculosis) was isolated and susceptibility testing was performed. Isolates were susceptible to all antibiotics tested. (DTU-VET 2013-50-199).

Friedrich-Loeffler-Institut, Germany (Sven Bergman): Sera from rainbow trout, received for testing for anti VHSV and anti IHNV by plaque neutralization tests (50% PNT) and ELISA. (DTU-VET 2013-50-144).

National Food Chain Safety Office, Hungary (Adam Dan): PCR testing for KHV. KHV examination by PCR and real time PCR, KHV was detected. (DTU-VET 2013-50-165).

Veterinary Institute, Norway (Ingeborg Modahl): Salmon, Piscine Reovirus (PRV), positive control material for RT-PCR. Used for implementation of a real-time PRV RT-PCR in the laboratory. (DTU-VET 2013-50-285).

National Veterinary Institute, Sweden (Eva Blomkvist): Rainbow trout, Supernatant from BF-2 cells, testing for IPNV. Virological examination and neutralization test were performed. The IPNV was serotyped as an Ab virus, and sequencing placed the isolate as a "wild-type"-Ab-like IPNV with closest relation to EEV an IPNV from eel. (DTU-VET 2013-50-142).

National Veterinary Institute, Sweden (Eva Blomkvist): Rainbow trout, Supernatant from BF-2 cells, testing for IPNV. Virological examination and neutralization test were performed. The IPNVs were serotyped as Ab viruses, and sequencing showed that the four strains have 94% similarity with the reference strain IPNV Ab and 89% similarity with the reference strain IPNV Sp. (DTU-VET 2013-50-195).

National Veterinary Institute, Sweden (Eva Blomkvist): Rainbow trout, Supernatant from BF-2 cells, testing for Sarcocystidae infection. (DTU-VET 2013-50-239).

Central veterinary Institute Wageningen, The Netherlands (Olga Haenen): Rainbow trout, Supernatant from BF-2 cells for virus examination and real-time SAV-PCR. No specific causal organisms were found. (DTU-VET 2013-50-229).

4-5 Update and expand www.fishpathogens.eu with more pathogens.

The pathogen database www.fishpathogens.eu

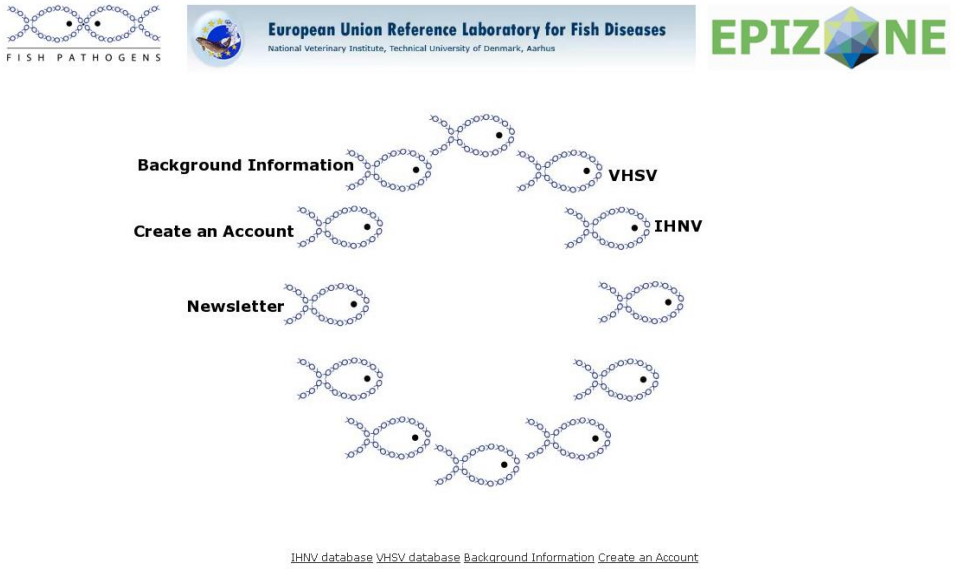
The whole interface for Fishpathogens.eu has been re-designed into a more user-friendly version with a new visual design, a F.A.Q. page, and a combined log-in system that removes the need to log-in for each database. Several bugs have been fixed in the coding.

Furthermore, multiple pages have been added, including a new end-user license agreement and a cookie politic according to EU law.

The new design will go live primo April, at which point the new betanoda database and the new sequence matcher will also be available.

The VHSV database has been expanded and now contains information on 755 isolates, up from 607 last year, and 414 sequences, up from 408.

A meeting was held in June 2013 in Norway with the Norwegian Veterinary Institute regarding the development of two new databases, one for SAV and one for ISA. This work has been commenced.



4-6 Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens.

Molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens

Two papers from the EURL on molecular epidemiology were published in 2013: *S. Kahns, H.F. Skall, R.S. Kaas, H. Korsholm, B. Bang Jensen, S.P. Jonstrup, M. J. Dodge, K. Einer-Jensen, D. Stone, and N.J. Olesen* (**2012**) European freshwater VHSV genotype Ia isolates divide into two distinct subpopulations. Diseases of Aquatic Organisms 99(1):23-35 and Reichert *M., Matras M., Jonstrup SP, Olesen NJ, Kahns S* (**2013**) Trade practices are main factors involved in the transmission of VHS in Polish aquaculture. Journal of Fish Diseases, volume 36, issue 2, pp. 103-114.

A fundament for studying molecular epidemiology is the access to significant amounts of reliable data on virus isolates and corresponding sequences data, and to connect these with classical epidemiological data such as prevalence, GIS, sampling strategies and number of positive samples in relation to negative samples. Part of this information is most accessible in our database www.fishpathogens.eu and efforts have primarily been devoted to expand and refine this database in order to use the most updated tools for conducting the studies. A close collaboration between partners in Europe on the topic has been established through the "MOLTRAQ" FP7 project.

4-7 Real-time PCR:

Assessment and standardisation of real-time PCR tests for diagnosis and

Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of the listed non-exotic fish diseases.

identification

Two new real-time PCR's has been has been assessed for the detection of the emerging diseases PMCV (Piscine myocarditis virus) and PRV (Piscine Reovirus).

PRV has recently been associated with heart and skeletal muscle inflammation (HSMI), a disease that has led to large mortalities in the Atlantic Salmon farming industry. The affected fish show lesions in the heart and the mortality rate may reach 20%. Therefore it was decided to implement a real-time RT-PCR in the lab to be able to detect PRV as well as conduct a study on the prevalence of PRV in the wild salmon population. The assay was developed using an adapted protocol by Palacios et al. (2010). Positive control material was obtained from Norway, but due to the small amount of materiel received a full validation was not possible. During the testing of wild salmon, several positive fish were found, providing material for further validation.

Cardiomyopathy syndrome (CMS) is another serious cardiac disease that affects wild and farmed salmon. Recently PMCV has been linked to CMS as the causative agent. Therefore, a real-time RT-PCR was implemented and assessed in the lab. Positive control material was obtained from Norway, but again, due to the small amount received, a full validation of the assay was not possible. Further collection of positive material will have to be conducted in the future to allow for full validation and accreditation.
The assay was conducted according to an adapted protocol from Løvoll et al. (2010).

4-8 Emerging diseases: In collaboration with specialised experts WW to review selected emerging fish diseases in Europe and assess their potential listing as exotic or non-exotic diseases (e.g. using discontools and similar tools)

New putative emerging disease in Sweden:

By letter of 15.04.2013 the National Reference Laboratory for Fish Diseases (NRL) in Sweden informed the European Union Commission the detection of an unknown parasite in rainbow trout in a farm in Sweden. Therefore the case was considered as a putative emerging disease case (Council directive 2006/88/EC article 41, Annex 3) and a working group on increased mortality in rainbow trout at farms in continental Sweden was established having a meeting in Brussels the 05/07/2013.
In the conclusion paper from this meeting the EURL Fish was asked to collaborate closely with the NRL in Sweden and with other interested Member States in order to produce additional information on the suspected pathogen and the epidemiology of the disease and for the development of readily available, practical and robust diagnostic tools to detect the pathogen or to measure the in-vivo response of the host to it.

A scientific working group consisting of Anders Alfjorden (AA), Charlotte Axén, and Anders Hellström from SVA, Tor Atle Moe, from VI Norway, Stephen Feist, CEFAS, UK, and Torsten Snogdal Boutrup (TSB) and Niels Jørgen Olesen (NJO), EURL Fish, DTU Vet was established, and a workshop was organized at SVA, Uppsala 10-11 October 2013.

The main goal of the workshop was

- To establish an overview of the disease case or cases.
- To assess if the disease is caused by infection with a specific parasite and if all cases can be attributed to the same parasite.
- To describe morphology of the parasite
- To propose how to conduct experimental trials for pathogen production and pathogenesis studies
- To propose how to develop specific diagnostic tests that could be universally used

- To recommend the disease to be listed and eradicated/controlled or to leave it as an interesting finding, with no direct legislative implication in EU.

No final conclusions were made as the studies performed till now had a number of gabs that should be filled before final recommendations can be given, cause-effect studies were not performed and a causative pathogen was not finally determined. But the situation did not prove to be alarming and no restrictions in trade are foreseen.

The full workshop report is included as Annex 6

It has been questioned whether or not *Aphanomyces invadans* is present in EU and during autumn the EU Commission decided based on the EFSA Report of the technical hearing meeting on Epizootic Ulcerative Syndrome (EUS) to delist EUS from the list of exotic diseases. As the EURL has put considerable efforts into establishment and harmonization of diagnostic procedures for this disease and as the question still is a matter of discussions (OIE have taken the position based on the same report to keep EUS as a listed disease), the EURL have decided to keep upgraded on diagnostic preparedness for this disease if it should enter and give rise to severe problems in EU. *Aphanomyces invadans* was thus included in 2013 PT2, and the oomycetes that cannot be frozen have been passaged and sporulated every 3 months and kept for long term storage in order to keep them alive.

Other diseases of interest is pancreas disease and sleeping disease caused by the salmonid alphaviruses, SAV-1/ SAV-3 and SAV-2, respectively. SAV has recently been decided to be included in the list of notifiable disease in the OIE Code and the Diagnostic Manual for Aquatic Animal Diseases but the implementation have not been conducted yet. Discussions are undergoing in order to assess if these diseases should be listed or not in EU (In collaboration with colleagues in Norway and Member States)

VHSV strain IVb is a major problem to wild fresh water fish species in Canada and United States and could be a serious threat for European wild fish stocks, therefore measures to reduce the risk of introducing this virus should be taken. The EURL have developed fast methods for discriminating between European and American genotypes.

5. Missions

5-1 Missions: Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial.

Missions to relevant laboratories

In 2013 no missions were conducted to NRL´s in EU. The reason for this was primarily that we moved our laboratories from Aarhus to Copenhagen in July 2013 and laboratory technologist Nicole Nicolajsen was replaced with Anemone Ojala in April with 2-3 months overlap. Before missions can be conducted personnel must be fully trained in the wide range of methodologies included in our functions as EURL for Fish Diseases.
Therefore the Commission was asked and accepted that the budget for missions instead were used for organising a workshop in Sweden in October 2013 on a new putative emerging diseases (see 4-8: Emerging diseases)

5-2 International meetings. Attending missions, international meetings and conferences in order to be updated on emerging and listed exotic and non-exotic fish diseases.

International meetings and conferences attended.

Contact with colleagues from other laboratories is a channel for exchange of information in the field of fish diseases, and an opportunity to keep abreast with new developments in the field. Of special interest are of course the activities relating to VHS, IHN, KHV, ISA, EHN and EUS. Scientists at the EURL participated in the following international meetings and conferences in 2013:

Participation and presentations at international conferences and meetings

17th Annual Meeting of the National Reference Laboratories for Fish Disease. Copenhagen, 29-30 May, 2013.
PARTICIPANTS: Niels Jørgen Olesen, Torsten Snogdal Boutrup, Morten Sichlau Bruun, Susie Sommer Mikkelsen, Niccoló Vendramin

DAFINET Workshop, Copenhagen 2013: Diagnosis and Control of Fish Diseases April 9th to 11th, 2013
PARTICIPANTS: Niels Jørgen Olesen, Torsten Snogdal Boutrup, Morten Sichlau Bruun, Susie Sommer Mikkelsen, Niccoló Vendramin

Fish Immunology: From Egg To Adult Fish. November 12-14, 2013
PARTICIPANTS: Niels Jørgen Olesen, Niccolò Vendramin

EAFP 16th International Conference on Diseases of Fish and Shellfish Tampere, september 2-6 2013
PARTICIPANTS: Niels Jørgen Olesen, Torsten Snogdal Boutrup, Morten Sichlau Bruun, Susie Sommer Mikkelsen, Niccoló Vendramin

Aquaexcel courses:
Course 1: Recirculating Aquaculture Systems (RAS) Technology Location: Wageningen University, the Netherland Date: 22 – 25 April 2013
PARTICIPANT: Niccolò Vendramin
Course 2: Contribution of Genomic Approaches to the Development of Sustainable Aquaculture for Temperate and Mediterranean Fish. Location:Rennes,France Date: 16 - 18 October 2013
PARTICIPANT: Susie Sommer Mikkelsen

5th Meeting on Global Microbial Identifier: Copenhagen 27 - 28 Feb 2013
PARTICIPANT: Susie Sommer Mikkelsen, Niels Jørgen Olesen

Workshop on “Certifying Disease Status for Safe Trade in Aquaculture” 29 – 31 October 2013, Fort Collins, Colorado USA. PARTICIPANT: Niels Jørgen Olesen

Conference of the International Society of Fish and Shellfish Immunology 25-28 June 2013, Vigo, Spain PARTICIPANT: Niels Jørgen Olesen

7th EPIZONE Annual Meeting "Nothing permanent, except change" Brussels, Belgium 1-4 October PARTICIPANT: Niels Jørgen Olesen

NADIR FP7 final workshop, Tour, France, 4-6 November. PARTICIPANT: Niels Jørgen Olesen, Torsten Snogdal Boutrup

TargetFish FP7 1st progess meeting, 22-25 June 2013,Vigo Spain

Presentations and posters

Presentations and posters

1. Key note: N. J. Olesen Fish Virology: Mechanisms And Evolution Of Virulence 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

2. T. Ito and N. J. Olesen: Viral hemorrhagic septicemia virus (VHSV) genotype IVb is a potential threat to wild fresh water fish in Japan 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

3. L. Bellec, J. Cabon, M. Engelsma,T. Morin, N.J. Olesen, H. Schutze, K. Way and L. Bigarré: Genetic diversity of *Anguillid rhabdovirus* using N, P and G genes. 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

4. B. Bang Jensen, A.K. Ersbøll, H. Korsholm, H.F. Skall and N.J. Olesen: spatio-temporal risk factors for viral haemorrhagic septicaemia in Danish aquaculture. 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

5. N. Sandlund, R. Johansen, B. Gjerset, I. Modahl, M. Hjortaas, H. Sindre, N. Jørgen Olesen, T. Taksdal, M. McLoughlin and Ø. Bergh: Viral screening of wild fish along the Norwegian coast. 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

6. N.J. Olesen, H.F. Skall, B. Bang Jensen, N.H. Henriksen, S. Mellergård and H. Korsholm: Eradication of viral haemorrhagic septicaemia in Danish aquaculture. 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

7. D.Vázquez, C. López-Vázquez, H. Skall, S.S. Mikkelsen, N.J. Olesen and C.P. Dopazo: a multiplex real time RT-PCR for direct genotyping of VHSV. 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

8. F. Borghesan, N. Vendramin, G. Bovo, R. Quartesan, E. Cappellozza, N. Lorenzen, C. Terregino and A. Toffan: whole inactivated virus vaccine prototype protects against viral encephalopathy and retinopathy in european sea bass (*d. Labrax*). 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

9. Castro, R.; Martínez-Alonso, S.; Fischer, U.; Álvarez de Haro, N.; Soto-Lampe, V.; Lorenzen, Niels; Lorenzen, Ellen; Wang, T.; Secombes, C. J.; Tafalla, C DNA vaccination in fish promotes an early chemokine-related recruitment of B cells to the muscle. /. In: Fish and Shellfish Immunology, Vol. 34, No. 6, O-124, 2013, p. 164

10. K. Einer-Jensen, A. Harmache, S. Biacchesi, M. Bremont, A. Stegmann and N. Lorenzen* High virulence differences between phylogenetically distinct isolates of the fish rhabdovirus vhsv is not associated with variability of the surface glycoprotein g nor the nonvirion protein nv. 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European

Association of Fish Pathologists, 2013. P.14 O-3

11. K. Einer-Jensen, S. Biacchesi, M. Bremont, and N. Lorenzen* Changes in N-glycosylation affects pathogenicity of viral haemorrhagic septicaemia virus (VHSV). 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013. p. 16 O-5

12. B. Bang Jensen, P.A. Jansen, N.J. Olesen, S.S. Mikkelsen L. Bigarré, H. Schuetze, S.M. Bergmann, T. Renault, J.-C. Avarre, M. Aldrin and E. Brun: molecular tracing of viral pathogens in aquaculture -a multidisciplinary trans-European research project. 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

13. T. Johansson, H.F. Skall and N.J. Olesen phylogenetic studies of the glycoprotein and polymerase gene of perch rhabdoviruses. 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

14. V. Panzarin, S.S. Mikkelsen, S.P. Jonstrup, L. Bigarré, M. Baud, T. Gray, P.M. Agapow and N.J. Olesen: Fishpathogens .eu/noda : a free and handy online platform for Betanodavirus targeted research and data sharing. 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

15. R. Johansen, T. Snogdal Boutrup, H.F. Skall, N. Sandlund, B. Gjerset, I. Modahl, Ø. Bergh and N.J. Olesen: European herring shows high mortalit y rate in bath challenge with viral haemorrhagic septicaemia virus (VHSV). 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013

16. Bela-Ong, Dennis; Schyth, Brian Dall; Lorenzen, Niels. 2013 Do microRNAs induced by Viral *Hemorrhagic Septicemia* virus in rainbow trout (*Oncorhynchus mykiss*) possess anti-viral activity?. / DAFINET Workshop and Ph.D. Course. Conference abstract in Proceedings. DAFINET, 2013

17. Bela-Ong, Dennis; Schyth, Brian Dall; Lorenzen, Niels 2013 Evaluation of the potential anti-viral activity of microRNAs in rainbow trout (*Oncorhynchus mykiss*). /.In: Fish and Shellfish Immunology, Vol. 34, No. 6, 2013, p. 1639-1639.

18. Bela-Ong, Dennis; Schyth, Brian Dall; Lorenzen, Niels. 2013.Evaluation of the potential roles of microribonucleic acids in the interaction of rainbow trout (*Oncorhynchus mykiss*) with *Viral hemorrhagic septicemia virus*. / Abstract from 16th International Conference on Diseases of Fish and Shellfish, Tampere, Finland.

19. Bela-Ong, Dennis; Schyth, Brian Dall; Lorenzen, Niels 2013MicroRNA expression in rainbow trout (Oncorhynchus mykiss) vaccinated with a DNA vaccine encoding the glycoprotein gene of Viral hemorrhagic septicemia virus. /.. Abstract from 7th International Conference and Exhibition on Gene Regulation by Small RNAs (RNAi 2012), Oxford, United Kingdom.

20. Sepulveda, Dagoberto; Lorenzen, NielsTesting the ability of viral haemorrhagic septicaemia virus to evade the protective immune response induced in rainbow trout by DNA vaccination. /. In: Fish and Shellfish Immunology, Vol. 34, No. 6, 2013, p. 1737-1737.

21. Rasmussen, Jesper Skou; Lorenzen, Ellen; Kjær, Torben Egil; Einer-Jensen, Katja; Lorenzen, Niels Use of DNA vaccination for determination of onset of adaptive immunity in rainbow trout fry. In: Fish and Shellfish Immunology, Vol. 34, No. 6, 2013, p. 1674-1674.Publication: Research - pe Conference abstract in journal – 2013

22. Borghesan, F.; Vendramin, Niccolò; Bovo, G.; Quartesan, R.; Cappellozza, E.; Lorenzen, Niels; Terregino, C.; Toffan, A. Whole inactivated virus vaccine prototype protects against viral encephalopathy and retinopathy in european sea bass (*D.labrax*). / 16th International Conference on Diseases of Fish and Shellfish: Abstract Book. European Association of Fish Pathologists, 2013. p. 124 O-115.

23. Olesen, Niels Jørgen; Skall, Helle Frank; Jensen, Britt Bang; Henriksen, Niels Henrik; Mellergaard, Stig; Korsholm, Henrik. Eradication of viral haemorrhagic septicaemia in Danish aquaculture. / 2013. Abstract from DAFINET workshop, København, Danmark.

Peer reviewed publications

Peer reviewed publications

1. *Søren Peter Jonstrup, Søren Kahns, Helle Frank Skall, Torsten Snogdal Boutrup, Niels Jørgen Olesen* (**2013**) Development and validation of a novel Taqman based real time RT-PCR assay suitable for demonstrating freedom from Viral Haemorrhagic Septicaemia Virus. Journal of Fish Diseases volume 36, issue 1, pp. 9-23

2. *Reichert M., Matras M., Jonstrup SP, Olesen NJ, Kahns S* (**2013**) Trade practices are main factors involved in the transmission of VHS in Polish aquaculture. Journal of Fish Diseases volume 36, issue 2 , pp. 103-114

3. *Olesen, N.J. and Skall Helle Frank* (**2013**) Viral Haemorrhagic Septicaemia Virus. Chapter 20 Mononegaviruses of Veterinary Importance Vol. I: Pathobiology and Molecular Diagnosis (ed. M. Munir)

4. *Marc Y. Engelsma, Keith Way, Melanie J. Dodge, Michal Voorbergen-Laarman, Valentina Panzarin, Miriam Abbadi, Mansour El-Matbouli, Helle Frank Skall, Søren Kahns, David M. Stone* (**2013**) Detection of novel strains of cyprinid herpesvirus closely related to koi herpesvirus. Diseases of Aquatic Organisms, Vol. 107: 113–120, 2013

5. *Britt Bang Jensen, Annette Kjær Ersbøll, Henrik Korsholm, Helle Frank Skall and Niels Jørgen Olesen* () Spatio-temporal risk factors for Viral Haemorrhagic Septicaemia (VHS) in Danish aquaculture. Accepted for publication in Diseases of Aquatic Organisms 2013 http://www.int-res.com/prepress/d02706.html

6. *Borzym E., Matras M., Maj J., Baud M., Olesen NJ, Talbi C., Bigarré L.* (**2013**) First isolation of hirame rhabdovirus (HIRRV) in Europe. Journal of Fish diseases, doi:10.1111/jfd.12119

7. *Ito, Takafumi and N.J. Olesen* (**2013**) Susceptibility of various fresh water fish species in Japan to an isolate of viral haemorrhagic septicaemia virus (VHSV) genotype IVb, Diseases of Aquatic Organisms, 107: 1–8.

8. *Castro R, Martínez-Alonso S, Fischer U, Haro NA, Soto-Lampe V, Wang T, Secombes CJ, Lorenzen N, Lorenzen E, Tafalla C.* (**2013**) DNA vaccination against a fish rhabdovirus promotes an early chemokine-related recruitment of B cells to the muscle. Vaccine. 2013 Nov 27. pii: S0264-410X(13)01596-X. doi: 10.1016/j.vaccine.2013.11.062. [Epub ahead of print]

9. *Schönherz AA, Lorenzen N, Einer-Jensen K.* (**2013**) Inter-species transmission of viral hemorrhagic septicemia virus (VHSV) from turbot (Scophthalmus maximus) to rainbow trout (*Onchorhynchus mykiss*). J Gen Virol.;94(Pt 4):869-75. doi: 10.1099/vir.0.048223-0.

10. ***Vendramin N**, Toffan A, Mancin M, Cappellozza E, Panzarin V, Bovo G, Cattoli G, Capua I, Terregino C* (**2013**) Comparative pathogenicity study of ten different betanodavirus strains in experimentally infected European sea bass, Dicentrarchus labrax (L.). J Fish Dis. doi: 10.1111/jfd.12117.

11. *Fichi G, Cardeti G, Cocumelli C, **Vendramin N**, Toffan A, Eleni C, Siemoni N, Fischetti R, Susini F.* (**2013**) Detection of Cyprinid herpesvirus 2 in association with an Aeromonas sobria infection of Carassius carassius (L.), in Italy. J Fish Dis.;36(10):823-30. doi: 10.1111/jfd.12048. Epub 2013 Mar 11.

12. ***Vendramin N**, Patarnello P, Toffan A, Panzarin V, Cappellozza E, Tedesco P, Terlizzi A, Terregino C, Cattoli G.* (**2013**) Viral Encephalopathy and Retinopathy in groupers (Epinephelus spp.) in southern Italy: a threat for wild endangered species? BMC Vet Res.;9:20. doi: 10.1186/1746-6148-9-20. Epub 2012 Dec 12.

13. *N T K Vo, A W Bender, L E J Lee, J S Lumsden, N Lorenzen, B Dixon and N C Bols* (**2013**) Development of a walleye cell line and use to study the effects of temperature on infection by viral haemorrhagic septicaemia virus group IVb. Journal of Fish Diseases. Article first published online : 21 NOV 2013, DOI: 10.1111/jfd.12208

14.

Participation in international scientific collaborative studies

Participation in international scientific collaborative studies

NADIR FP7 The Network of Animal Disease Infectiology and Research Facilities. Project number: 228394 -Integrating activity.

NADIR aimed to facilitate the development of Europe's high level bio-containment facilities for which there is a strong demand from both the public and private sectors in the field of medical and veterinarian research, which have to respond to upgraded ethical and safety regulations whilst providing reliable answers in term of physiopathology for emerging infectious diseases (diagnosis, transmission conditions, risk analysis, therapeutic targets) or for vaccines and therapeutic trials.

Three transnational trials conducted on VHS: Evaluating pathogenicity and pathogenesis of Turkish VHSV isolates in turbot and rainbow trout. Assessment of oral vaccination against VHS in rainbow trout and Assessment of susceptibility of

herring to VHS infection. **INRA**- (France), **AU**- (Denmark), **ANSES**– (France), **CReSA**- (Spain), **FLI**- (Germany), **IAH**- (United Kingdom), **INIA**- (Spain), **KVI**- (Israel), **MRI**- (United Kingdom), **DTU**–Vet (Denmark), **VESO**- (Norway), **VLA**- (United Kingdom), **CVI**- (Netherlands), **PTP**- (Italia), **UNOTT**- (United Kingdom), **IT**– INRA Transfert (France), **UEDIN**– (United , Kingdom).

Danish Fish Immunology Research Network DAFINET

5 years. Production of immunological tools for studying the immunity of rainbow trout. A total of 15 participants affiliated with public and private institutions and companies University of Copenhagen, Faculty of Life Sciences; Danish Technical University, National Veterinary Laboratory and Institute of Aquatic Resources; University of Southern Denmark; University of Aarhus; Friedrich Loeffler Institute, Insel-Riems, Germany; Marine Laboratory, University of Aberdeen, Aberdeen, Scotland; Norwegian School of Veterinary Science, Oslo, Norway.

Co-evolutionary genomics of fish resistance and virulence in an epidemic virus.

4 years. Identify the process of adaptation to the fish host that makes the VHSV capable of causing epidemic outbreaks in rainbow trout and use the trout's own genetic variants in combination with targeted vaccine development to cope with this adjustment. National Veterinary Institute department in Aarhus, Aarhus University, University of Victoria in Canada and the University of Washington, USA.

MOLTRAQ: Molecular tracing of aquatic animal diseases

The group is partner in the FP7 EMIDA-ERA Net project MOLTRAQ and work package leader of WP6: Dissemination and exploitation.
The purpose of the project is to increase knowledge on transmission, prevention and control of viral diseases in aquaculture and to develop a generic approach to viral disease control by using information on epidemiological and phylogenetic attributes from several important aquatic animal viruses.
We will provide isolates from our large collection for sequencing at other institutes and we will also sequence a large number of isolates ourselves. Data will be used to generate phylogenetic trees and to identify important factors in the evolution and spreading of viruses.
Furthermore, all isolate and genetic information will be uploaded to the EURL database for fish pathogens: www.fishpathogens.eu.
Further information is available at the public project website www.moltraq.wordpress.com.

Identification of virulence markers in marine VHS virus and use in diagnostics for aquaculture.

Identification is conducted by in vivo imaging of VHSV propagation in fish and identification of virulence marker(s) in VHSV by generation and virulence testing of recombinant viruses. DTU Vet, Denmark

TargetFish FP7.

Targeted disease prophylaxis in European fish farming Targeted disease prophylaxis in European fish farming. Wageningen Universiteit, DTU, The University of Aberdeen, Marine Scotland, FLI, INIA, Universitat Autonoma De Barcelona, Universita Degli Studi Della Tuscia, UTUS, INRA, Norges Veterinaerhogskole, NSVC, The University of Stirling , IZSVe , UCPH , VRI , The Hebrew University of Jerusalem. HUJI , Universidad De Murcia Um , Tethys Aquaculture Limited Tethy, Patogen Analyse As, Fishlab, Osauhing Naxo, Naxo, Ridgeway Biologicals Limited Rbl.