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

Report of the

11th Annual Workshop of the National Reference Laboratories for Crustacean Diseases

Kgs. Lyngby, Denmark

November 5th 2020

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

Contents

Introduction and short summary

The 11th Annual Workshop of the National Reference Laboratories for Crustacean Diseases was held virtually on 5th of November 2020. Because of the Covid-19 pandemic and the resulting limitations on travelling to and from Denmark, the workshop was held virtually using the Zoom platform.

The virtual organization of the meeting has allowed a significant expansion on the number of participants attending the workshop as well as for people in oversea countries to participate. In total, 66 participants from 39 countries attended the workshop.

The workshop was held back to back with the 24th Annual Workshop for National Reference Laboratories for Fish Diseases. There were two sessions with a total of 10 presentations.

On November 4th a special session dedicated only to the staff of NRLs in Europe was held to present the new Animal Health Law which is going to be implemented by April 21st, 2021, and includes implications for Aquatic animal health.

The workshop was opened with "Welcome and announcements" by Head of the EURL for Crustacean Diseases, Niels Jørgen Olesen. The first session had the title "Update on important crustacean diseases and their control", and the first speaker was Peter Mohr from Australian Centre for Disease Preparedness (ACDP) who talked about Yellow Head Disease diagnostics. This was followed by two talks by Kelly Bateman from Centre for Environment, Fisheries and Aquaculture Science (Cefas), the first one concerning paramoebiasis in edible crabs, and the next one presenting work done by the OIE Collaborating Centre for Emerging Aquatic Animal Diseases. Fiona Swords from The Marine Institute Ireland then presented a project on surveillance of crayfish plague and white-clawed crayfish in Ireland using environmental DNA, which was followed by a presentation of a project using ultrasonic treatment against crayfish plague by Trude Vrålstad from Norwegian Veterinary Institute. After a short break the session continued with a presentation of a project about conservation of *Austropotamobius pallipes* crayfish in Italy given by Andrea Basso from Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe). The session ended with a shared talk by Eann Munro from Marine Scotland Science and Christopher Evans from Centre for Environment, Fisheries and Aquaculture Science (Cefas) about an outbreak of IHHNV in a shrimp farm in Scotland and a shrimp farm in England in 2019.

Session II had the title "Update from the EURL for fish diseases" and started with EURL coordinator Morten Schiøtt giving two talks, the first on the disease and surveillance situation of crustacean diseases in EU countries, and the second on the interlaboratory proficiency tests for crustacean diseases in 2019 and 2020. The final talk was given by Niels Jørgen Olesen, presenting the EURL activities in year 2019 and 2020 and proposals for the EURL work plan for 2021.

Argelia Cuenca and Niccolò Vendramin from DTU Aqua took minutes from the meeting, and Morten Schiøtt assembled the report.

We would once again like to thank all the presenters for their great contribution, without them the meeting would not have been a success. The workshop and meeting was organized by a team consisting of Morten Schiøtt, Niccoló Vendramin and Niels Jørgen Olesen, with

the help from the rest of the fish disease section at the National Institute of Aquatic Resources, DTU AQUA. The meeting next year is tentatively planned to be held at beginning of June 2021, hopefully in a face to face meeting at DTU Aqua. More details will follow.

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

Niels Jørgen Olesen and Morten Schiøtt

Programme

Thursday November 5th
Annual Workshop of the National Reference Laboratories for Crustacean Diseases

9.30 – 9:40	Welcome and announcement *Morten Schiøtt and Niels Jørgen Olesen*
SESSION I:	**Update on important crustacean diseases and their control**
	Chair: Lone Madsen and minutes: Argelia Cuenca
09:40 – 10:00	Detection and pathogenicity of yellow head virus genotypes one and seven *Peter Mohr*
10:00 – 10:20	Emergence of paramoebiasis in edible crabs (*Cancer pagurus*) from UK waters *Kelly Bateman*
10:20 – 10:40	Recent discoveries of the OIE Collaborating Centre for Emerging Aquatic Animal Diseases *Kelly Bateman*
10:40 – 11:00	Environmental DNA (eDNA) Surveillance of Crayfish Plague and White-clawed crayfish, an Irish National Monitoring Program *Fiona Swords*
11:00 – 11:20	Testing ultrasonic treatment against crayfish plague *Trude Vrålstad*
11:20 – 11:30	***Coffee break***
	Chair: Morten Schiøtt and minutes: Niccoló Vendramin
11:30 – 12:50	LIFE18 NAT/IT/000806 – LIFE+ CLAW: Conservation of *Austropotamobius pallipes* in North-Western Apennine *Andrea Basso*
12:50 – 12:10	The first reported detection of infectious hypodermal haematopoietic necrosis virus (IHHNV) infection in the European Union *Eann Munro and Christopher Evans*
SESSION II:	**Update from the EURL for crustacean diseases**
12:10 – 12:30	EURL proficiency test for crustacean disease 2019 and 2020 *Morten Schiøtt*
12:30 – 12:45	Surveillance and diagnostics of crustacean diseases in Europe *Morten Schiøtt*
12:45 – 13:00	EURL for crustacean diseases workplan 2021 *Niels Jørgen Olesen*

SESSION I: Update on important crustacean diseases and their control

Chair: Lone Madsen

Detection and pathogenicity of yellow head virus genotypes one and seven

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Abstract

The yellow head virus complex consists of many genotypes demonstrating different host specificities and virulence to shrimp species. Yellow head virus genotype one (YHV1) causes the most severe, yellow head disease. Infection with YHV1 is listed by the OIE with an Aquatic Manual chapter dedicated to diagnostic tests. However, the PCR tests listed are conventional and specificity issues have been documented. In addition, the chapter lacks a real-time PCR assay to assist with more rapid screening of samples.

In recent years the number of published yellow head virus genotypes has expanded from six to eight with the discovery of YHV7 in Australia and YHV8 in China. Genotype YHV7 was initially an incidental detection from diseased *Penaeus monodon* broodstock. However, experimental YHV7 infection by injection, feeding or via infected water has demonstrated that disease can be induced in *P. monodon*. The onset of disease induced after YHV7 infection appears to be less rapid and more temperature dependent than yellow head disease caused by YHV1.

Questions and comments:

Q: When you look at the OIE manual it is only YHV genotype-1 that is reportable, whereas EU legislation includes the whole group of YHV. What is your perception about this? Should we talk about the whole group or only YHV-1? I am thinking that YHV-7 also seems to be quite pathogenic.

A: We know that YHV-1 is the most pathogenic and of major concern at this stage, whereas YHV-7 has only been found by association with other issues. Although GAV has been associated with disease, this is not usually an issue. Most of the genotypes of YHV have been found in healthy animals, and are not well defined at this stage. In my opinion, it is better to target YHV-1 instead of targeting a large group that is not well defined yet.

Comment: Yes, so we will need to look at the EU legislation since as it is right now the detection of YHV will cause immediate eradication regardless of which genotype of YHV is found.

Q: Do you think the differences in mortality between the two temperatures can tell us more about viral biology or the defenses of the host?

A: Yes, absolutely. At this point most of this is speculation and unknown, as only two groups within Australia are working with this issue. We think that this could be caused by a temperature dependent viral replication, but could as well be something related with the host instead.

Emergence of paramoebiasis in edible crabs (*Cancer pagurus*) from UK waters

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Abstract

The genus *Paramoeba* (including *Neoparamoeba*) (Amoebozoa, Dactylopodida) includes well known opportunistic pathogens associated with fish (*N. peruans*; amoebic gill disease), lobsters, molluscs, and sea urchins, but only rarely with crabs (grey crab disease of blue crabs). Following reports of elevated post-capture mortality in edible crabs (*Cancer pagurus*) captured from parts of the English Channel fishery in the UK, a novel disease (paramoebiasis) was detected in significant proportions of the catch. We present histopathological, transmission electron microscopy, and molecular phylogenetic data showing that this disease was associated with infection by multiple *Paramoeba* spp. The disease was defined by colonization of haemolymph, connective tissues and fixed phagocytes by amoeboid cells, leading to tissue destruction and presumably, death in severely diseased hosts. Four genetically distinct *Paramoeba* lineages were apparently associated with infection and diseases: two lineages had closest relation to *P. pemaquidensis*, one to *P. aestuarina*, and a fourth novel *Paramoeba* lineage, described herein for the first time. The novel parasite is a divergent and early branching lineage of the *Paramoeba* clade but is morphologically similar to other members of the genus. We name this novel parasite *Paramoeba canceri* n. sp. and provide evidence that it is associated with emerging paramoebiasis in *C. pagurus* from UK waters. The emergence of paramoebiasis in edible crabs from the UK is discussed relative to published historical health surveys for this species, and changes in the hydrological and environmental conditions of the English Channel in which the important UK fishery for *C. pagurus* is centred.

Questions and comments:

Q: How are you going to find out if there is only one of the amoeba causing disease, are you going to make infection trials?
A: We would like to do them, but it depends entirely on available funding.
Q: Can you cultivate the amoeba?
A: We did attempts early in the project, but we can't keep the cultures for very long, maybe just few days.

Recent discoveries of the OIE Collaborating Centre for Emerging Aquatic Animal Diseases

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Abstract

It is well known that global aquaculture production has increased and diversified rapidly in recent decades and has surpassed capture fisheries as a source of aquatic animal protein. This trend is set to continue, with the requirement estimated to be a doubling of production to meet global need by 2050 (FAO, 2020). A major constraint in achieving this goal are new and emerging aquatic animal diseases in aquaculture sectors globally. An **emerging disease** is defined as a new infection resulting from the evolution or change of an existing pathogen or parasite resulting in a change of host range, vector, pathogenicity or strain; or the occurrence of a previously unrecognized infection or disease. A re-emerging disease is considered an already known disease that either shifts its geographical setting or expands its host range, or significantly increases its prevalence. To mitigate the effects of these diseases it is critical to achieve rapid detection and characterisation of the causative agent(s), develop accurate diagnostic tests, understand their epidemiology, and to disseminate the information efficiently to raise awareness to facilitate control measures.

The OIE Collaborating Centre for Emerging Aquatic Animal Disease (CCEAAD) is based at the Cefas Weymouth Laboratory and heads a network of laboratories residing in major aquaculture producing regions globally. A key objective of this network is to harmonise and exchange information and expertise to improve emerging disease surveillance globally. Here we provide an overview of the CCEAAD and report on recent work in the field of crustacean diseases, a novel viral infection in giant freshwater prawn (*Macrobrachium rosenbergii*) from Bangladesh and a haplosporidian parasite in jelly shrimp (*Acetes sibogae australis*) from Australia.

Questions and comments:

Q: Where is the funding for all the work done by CEFAS with other countries coming from? Can you keep autonomous from the business?

A: Are you thinking in the logistics to work with people? We always need to inform and involve the competent authorities, in our case we need to involve DEFRAS, and we ask the different partners to inform their countries' competent authorities. And yes, we also involve the different associations of prawn producers. In some cases they can be commercial susceptibilities and we need to sign confidentially agreements with the different farms.

Q: You mention that this new virus is part of the nidovirales, but in your trees it is very close to yellow head. Could it be a yellow head genotype instead?

A: Yes, as I mentioned, this work has been done by my colleague Chantelle (Hooper), and she unfortunately is not here to answer this questions, but I guess that more work will need to be done to decide whether they are part of YHV or not.
Comment: It is my understanding that MrGV is very distantly related to the YHV complex of viruses. MrGV shares a similitude of 30-40% with YHV, so it must be within a different genus. In contrast, different genotypes of YHV share a similitude of around 80%, depending of which gene is used (for comparisons).
Q: What is next for this collaborative center?
A: If the conference for disease in aquaculture is open next year we would like to have a session where all participants can get together and meet each other. But we will continue publicizing the work, and strengthening our collaborations abroad.

Environmental DNA (eDNA) Surveillance of Crayfish Plague and White-clawed crayfish, an Irish National Monitoring Program

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Abstract

The National Crayfish Plague Surveillance Program (NCPSP) was established in 2018 in partnership with National Parks and Wildlife Services (NPWS) to monitor the occurrence of crayfish plague (CFP) outbreaks on a national level, following five confirmed outbreaks around Ireland (2015-2017). The main objectives of this program were to determine the prevalence of *Aphanomyces astaci* (*A. astaci*) nationwide, and also the distribution of white-clawed crayfish (WCC) in selected catchments using environmental DNA (eDNA) methodology as a surveillance tool. Catchment selection (28 total) for the 2018-2019 surveillance program was based on known WCC habitats as outlined by Biodiversity Ireland. The program also examined the possible introduction of *A. astaci* to Ireland through the intentional or accidental introduction of non-indigenous crayfish species (NICS).Filtered water samples were screened for the presence or absence of *A. astaci* from extracted eDNA, or DNA extracted from crayfish mortality specimen, by real-time qPCR (Vrålstad et al. 2009). eDNA screening identified eight additional catchments in which a confirmed outbreak of *A. astaci* had occurred. All catchments which tested positive for *A. astaci*, were also tested for the presence of non-indigenous crayfish species NICS listed under current legislation, SI 354/2018 (European Parliament 2014). Taken together, the results of the NCPSP 2018-2019 clearly demonstrate a rapid spread of *A. astaci* between catchments in Ireland, necessitating further investigation.

The NCPSP 2020-2022 is a continuation and refinement of the original program using eDNA methodology as a surveillance tool, focusing on prevalance and persistence of *A. astaci* in Ireland, and its potential vectors. Here we present our progress to date, future research, and discuss the advantages and drawbacks of eDNA surveillance techniques in the context of currently available *A. astaci* genotyping methods.

Questions and comments:

Q: Nice to see the program up and running, congratulations. Have you published reports about all this?

A: Yes, we have reports that we provide to our collaborators, and we are planning publication of results.

Comment: I thought to share experiences from a similar program in Norway, first take in consideration that it is very difficult to detect crayfish using eDNA, even in dense populations with high number of individuals. Negative results do not necessarily mean a missing population, so intensify the sampling, particularly in low density populations. The other thing is regarding the time of sampling, as summer is not a good time to detect eDNA. October, during the reproduction phase, is much better to detect crayfish eDNA. Regarding detection of *Aphanomyces astaci*, we found better results when the water is colder (to detect the spores in the water).

A: Just to specify that we needed to take this time frame for sampling in order to complete the study. The program is developed as it goes and we are hoping to include more testing and ecological variables.
Q: Thanks for the presentation. Maybe I missed it, but was there correlation between mortality outbreaks and *A. astaci* presence/isolation in the different basins?
A: *A. astaci* was detected in most of the mortality outbreaks, only in one case was *A. astaci* not detected, but that case was associated with weird environmental conditions in the lagoon.
Comment: Just to specify: a number of cases where mortality was negative were tested for *A. astaci*, but mortality has been attributed to environmental factors instead. Another negative case was found in the Grand Canal close to Dublin. So, not all events were caused by *A. astaci*, but in the cases where we found crayfish plague, we have also detected *A. astaci* in the water.
Q: My question is regarding sampling procedures. I can see that you need 3 x 5 liters per sampling point per site? Has that been optimized in any way?
A: We have followed the work done by Norway, the GMT and we did some optimization ourselves.
Comment: There is never enough water, we should always sample more. I know that this is a lot more than used, for example, to detect fish. We need lots of water to detect *Aphanomyces* and even more to detect crayfish.

Testing ultrasonic treatment against the crayfish plague pathogen *Aphanomyces astaci*

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Abstract

North American crayfish are healthy carriers of *Aphanomyces astaci*, and release infective zoospore to the ambient water. The *A. astaci* zoospores will re-infect adult crayfish (after molting) and infect the offspring. If carrier crayfish encounter a population of native and susceptible European crayfish, the *A. astaci* zoospores will infect and rapidly kill the naïve hosts, causing crayfish plague outbreaks. There is presently no feasible cure to mitigate or control *A. astaci* apart from waiting for all native crayfish to die, and eradicating the alien host using chemicals, with drastic impact on the habitat.

For mitigation and control of undesirable algae and cyanobacteria, a patented technology using ultrasonic sound waves can destroy algae, biofilm and even some parasites. Compared to chemicals, this is a more environmentally friendly approach. Although mainly used in industrial water systems, aquaculture basins, and cooling towers, some countries also use the applications in stagnant water bodies such as ponds and lakes. Oomycetes, comprising many serious pathogens including *A. astaci,* are phylogenetically related to algae and share many features on cell- and reproduction level. We therefore explored the potential of this technology for elimination of infective *A. astaci* zoospores.

Our overall objective was to explore in controlled experiments the potential of ultrasonic technology to eliminate *A. astaci* in the water and in carrier crayfish. We conducted three sets of experiments with multiple ultrasound treatments: 1) a co-habitation experiments with *A. astaci* positive signal crayfish and noble crayfish, 2) a moulting experiments with *A. astaci* positive signal crayfish, and 3) an *A. astaci* zoospore challenge experiment with noble crayfish. After several rounds of promising results but inconclusive results, we finally managed to document that we were not able to find any specific ultrasonic wavelength/program/treatment that was detrimental to viable *A. astaci* zoospores. We tend to forget and undercommunicate disappointing results. They are nevertheless important to make public so that others can learn from our resource-intensive experiences.

Questions and comments:

Q: What are the differences among the different methods?

A: I don't know. This is protected technology. The company produced a set of different testing, but we don't know the exact variables that they have used.

Q: If this approach had worked, how would you make it operational? I guess that if the crayfish are in a farm, the crayfish plague would be in the environment and will re-infect the farm after treatment?

A: In the picture shown, you can see a device placed in a lake. This is used to control algae, and it is sold by the company. It has a reach of 400 m and it is operated by solar energy. So, it would have been some kind of similar device that could be placed as a barrier to avoid spread – for example upstream- from a occurring outbreak. Of course it would have targeted not only the plague but also other microorganisms.

LIFE18 NAT/IT/000806 – LIFE+ CLAW: Conservation of *Austropotamobius pallipes* in North-Western Apennine

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Abstract

The last few decades marked a significant contraction and decline of the European Crayfish populations across Europe. Even though recent studies highlighted a hot spot of biodiversity in Northern Italy, the species complex *Austropotamobius pallipes* recorded one of the heaviest reduction in recent history, mainly due to mass mortality events often caused by the crayfish plague. The Life CLAW project aims to preserve the *A. pallipes* populations and their genetic variability among the Northwestern Apennine area monitoring the status of allochthonous and autochthonous specimens. The objectives of the project include i) a reduction of the allochthonous populations (i.e. *Procambarus clarkii* and *Pacifastacus leniusculus*) nearby to the endangered *A. pallipes* populations and ii) an improvement of the autochthonous populations located into the NATURA 2000 sites (SCI) with habitat restoration and restocking of new individuals obtained from ex-situ breeding. The prevalence of the causative agent of crayfish plague (*Aphanomyces astaci*) will be investigated with both non-invasive sampling (cuticular swabs) and environmental DNA (eDNA) approach. All the sampling methods (swabs and filter), storage methods (dry, ethanol and extraction buffer), and extraction protocols (commercial kit and CTAB) were standardized in the initial stages of the project. The efficacy of the eDNA assay was tested at first in experimental conditions considering both different filter porosity and filtered water volumes. Subsequently the eDNA method was applied in field sampling with good results on streams already investigated by cuticular swabs analysis. qPCR assay optimized by Strand (2013) and by Rush *et al*., (2020), was applied for its high sensitivity and specificity. Nevertheless, to avoid cross-amplification and misidentification with the closest related *Aphanomyces* species (i.e. *A. fennicus*), a nested PCR was set up in order to confirm the presence of *A. astaci* through sequencing.

Questions and comments:

Q: Did you find *A. fennicus*?
A: No, it was not detected.
Q: Did you have problems with clotting of the filters?
A: Filtration is done using a power drill with batteries. The lower the pressure the more we can filter the 20 liters.
Q: What about inhibition?
A: Inhibition is observed, but by taking four sample points this can be managed.

The first reported detection of infectious hypodermal haematopoietic necrosis virus (IHHNV) infection in the European Union

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Abstract

A population of 350,000 post-hatch larval whiteleg shrimps (*Litopenaeus vannamei*) were imported from a supplier in Texas, USA on 18 April 2019. A mortality of approximately 50% occurred during transport of the animals to Scotland. The animals were imported by a small, inland aquaculture site and placed in closed containment tanks, with artificial heated seawater and recirculation units. Site effluent as well as final discharge were collected and treated and no live animals were moved off the site. The aquaculture site was classified as an experimental pilot facility, distantly located from coastal areas and the waters surrounding the United Kingdom do not contain IHHNV susceptible species.

After arrival on site, the whiteleg shrimps demonstrated poor and extremely variable growth. Samples were sent for diagnostic investigation to a private laboratory who reported the detection of infectious hypodermal haematopoietic necrosis virus (IHHNV) by conventional PCR on 15 July 2019.

A Marine Scotland Science, Fish Health Inspector, attended the site on 16 July 2019 to initiate a disease investigation. The remaining stock of whiteleg shrimp were culled later that day. Samples were taken from 30 animals for histopathology examination and molecular screening. No clinical signs of disease or significant pathology were observed, however, 24/30 individuals tested IHHNV PCR positive (Tang et al., 2000; 389 F/R). DNA sequence analysis performed on two samples confirmed the detection of decapod Penstyldensovirus 1 (IHHNV).

A population of 300 animals for broodstock that were also imported from the same facility in Texas but at a different time point to the infected population were housed in a separate contained area of the facility and showed no clinical signs of disease or significant mortality. The broodstock population was also culled as a precautionary biosecurity measure by the company.

Immediately after culling, the site was fully disinfected by high test hypochlorite to achieve 500ppm free chlorine in the storage reservoir and pipework.

Infection of animals with IHHNV was subsequently confirmed at the export hatchery in Texas, USA after the transport of shrimp to Scotland.

References:

Tang K.F.J., Durand S.V., White B.L., Redman R.M., Pantoja C.R. & Lightner D.V. (2000). Postlarvae and juveniles of a selected line of Penaeus stylirostris are resistant to infectious hypodermal and hematopoietic necrosis virus infection. *Aquaculture*, 190, 203–210.

Questions and comments:

Q: Have you compared the IHHNV sequences to sequences from USA?
A: Yes they are almost 100% identical.
Q: Do you have plans for NGS?

A: Not really – it is a niche industry and disease investigation has finished.
Q: Has there been a follow up on the Scottish farm? Are they still producing shrimp?
A: It took time to re-start. We have not done disease testing ourselves.
Q: What is the diagnostic conclusion – was the slow growth caused by IHHNV?
A: Other things could also be relevant.
Q: There was a phased sanitation on 3 periods - how could you contain other sites?
A: The site is designed to do so. Let them grow stocks to send them to human consumption and then disinfect. And then continue with next batch. There was not really legal background to enforce culling.
Q: Do you believe you were successful in eradicating the virus?
A: It has not been found again. Samples were imports. Hard to detect disease, we follow up on annual basis on the site.

SESSION II: Update from the EURL for crustacean diseases

Surveillance and diagnostics of crustacean diseases in Europe

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Abstract

As part of being the EURL for crustacean diseases we see it as our obligation to collect and disseminate data on the disease situation for crustacean production in Europe. To that end we send out an inquiry to all European NRLs for crustacean diseases to answer the following questions:

1) What is the current status of crustacean production in your country (which species, how many farms and what is the amount of production)?

2) Are you performing regular disease surveillance on any of farms?

3) Has there in 2019 and till now been any incidents of disease in these farms (concerning both listed and non-listed diseases), and how were these incidents addressed?

Despite a short notice, 19 out of 26 NRLs have so far responded to our inquiry. 12 NRLs report to have crustacean farms in their country, with 8 countries having shrimp farms and the remaining mostly being small scale crayfish farms or lobster farms for restocking of natural populations. Only very few incidents of disease have been reported, and very few NRLs perform regular disease surveillance at the farms. All in all, crustacean farming is still in its infancy in Europe, but seems to be increasing.

2019 + 2020 Inter-laboratory proficiency test for crustacean diseases

Morten Schiøtt, Teena Vendel Klinge and Niels Jørgen Olesen

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Abstract

In November 2019 an inter-laboratory proficiency test for White Spot Syndrome Virus (WSSV) was organised by the EURL for Fish and Crustacean Diseases. The test material consisted of shrimp pleopods infected with WSSV or not. The participants were asked to identify the WSSV positive pleopods among six test samples. 22 laboratories in 20 EU member states received and answered the test.

In July/August 2020 an inter-laboratory proficiency test for White Spot Syndrome Virus (WSSV), and another inter-laboratory proficiency test for Taura Syndrome Virus (TSV) and Yellow Head Virus (YHV) was organised by the EURL for Fish and Crustacean Diseases. The test material for the WSSV test consisted of shrimp pleopods infected with WSSV or not. The participants were asked to identify the WSSV positive pleopods among five test samples. 25 laboratories in 18 EU member states received and answered the test. The test material for the TSV/YHV test consisted of shrimp pleopods infected with TSV, YHV or not. The participants were asked to identify the TSV and YHV positive pleopods among six test samples. 16 laboratories in 12 EU member states received and answered the test.

This presentation reports the results of all three tests and gives some recommendations for future inter-laboratory proficiency tests for crustacean diseases.

EURL for crustacean diseases workplan 2021

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Abstract

A status for the EURL for crustacean diseases workplan 2019-2020 and a tentative workplan for 2021 will be presented.

The COVID19 pandemic has caused some changes in our work plan, e.g. this Annual workshop was postponed to a physical meeting in November, and due to the second wave of COVID-19 is now rearranged to take place as a virtual meeting. On the same account the training course in histopathology in fish and crustacean diseases had to be cancelled. A scientific expert group meeting on susceptible and vector animal species to infection with the A and C listed crustacean diseases was done virtually. In contrast, the EURL has kept open and accessible to laboratory examinations and tank facilities during the whole pandemic, and the inter-laboratory proficiency tests for crustacean diseases have been completed as originally planned.

Ideas and plans for the work programme of 2021 are very welcome. From our side – beside all the work that will be done every year we would like to focus on:

- Scientific assessment of the effect of pooling samples for surveillance and diagnostics by PCR.
- Emerging crustacean diseases (like IHHNV infection)
- Establishment of SOP´s and Manuals for WSSV, YHD and TSV

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

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

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

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

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

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

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

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

Sub-activity 1.3 *(Scientific working groups)*

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

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

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

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

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

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

Sub-activity 1.6 (Diagnostic methods)

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

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Sub-activity 2.1 *(Training Courses)*

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

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

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

Sub-activity 2.3 *(EURL Contact Lists)*

Objectives: To ensure that relevant and important information rapidly can get from the EURL directly to the Member State NRLs. Description: We will aim to have three contact lists. 1) Member State NRLs for fish diseases, 2) Member State NRLs for Crustacean disease and 3) a general list which all interested in the work of the

EURL can subscribe to. The EURL use the mailing lists for important notifications i.e. meeting calls, training course calls and other relevant information such as information on upcoming conferences, new research findings and relevant reports and publications, emergency situations etc. Often the notifications will include links to the website or other sites for further and detailed information.

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

Duration: January – December 2019 and 2020.

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

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

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

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

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

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

Duration: January – December 2019 and 2020.

Sub-activity 2.6 *(Confirmatory diagnosis)*

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

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

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

Duration: January – December 2019 and 2020.

Sub-activity 2.7 *(Pathogen characterization)*

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

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

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

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

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

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

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

Duration: January – December 2019 and 2020.

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

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

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

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

Duration: January – December 2019 and 2020.

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

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

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

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

Duration: January – June 2019 and 2020.

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

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

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

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

Duration: January – December 2019 and 2020.

4 REAGENTS AND REFERENCE COLLECTIONS

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

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

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

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

Duration: January – December 2019 and 2020.

Sub-activity 4.2 *(Pathogen library)*

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

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

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

Duration: January – December 2019 and 2020.

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

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

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

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

Duration: January – December 2019 and 2020.

5 REQUIREMENTS RELATED TO OTHER LEGISLATION

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

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

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

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

Duration: January – December 2019 and 2020

Sub-activity 5.2 *(Listing susceptible species)*

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

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

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

Duration: January –March 2019.

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

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

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

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

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

Introduction to the Technical Report for the EURL in 2019

The Technical University of Denmark (DTU) was confirmed appointed as the EU Reference Laboratory (EURL) for Fish and Crustacean Diseases in November 2018 for the period 2019 and 2020, and granted the financing with the Commission Implementing Decision of 14.11.2018 C(2018) 7485 final.

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern the fish and crustacean diseases listed as exotic diseases: Epizootic haematopoietic necrosis (EHN), taura syndrome, and infection with yellow head virus genotype 1; and diseases listed as non-exotic diseases: Infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN), koi herpes virus disease (KHVD) and white spot disease (WSD).
The facilities supporting the activities of the EURL are placed in the new DTU Campus in Kgs. Lyngby, 15 km north of the capital. The EURL is now placed in DTU AQUA-National Institute of Aquatic Resources and it is in progress to further integrate the group with the ongoing activities in this institute for collaborating with research teams working in the field of aquaculture and fisheries.

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

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

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

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

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

During 2019, resources were again used to collate data on surveillance, health categorisation and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to develop, update and maintain the database containing information on fish pathogens (www.fishpathogens.eu); to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to update the EURL website (www.eurl-fish.eu) and finally to attend international meetings and conferences.

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

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

	Technical report for 2019
Work Programme for 2019-2020	
1.1. Annual workshop for fish diseases. *To ensure knowledge dissemination and sharing between the Member State NRLs on existing and emerging fish diseases and to agree on the future priorities of the EURL, by holding the 23rd and 24th annual workshop of the National Reference Laboratories (NRLs) for fish diseases in 2019 and 2020, respectively.*	The 23rd Annual Workshop of the National Reference Laboratories for Fish Diseases was held 27th – 28th of May 2019, at DTU Aqua, 2800 Kgs. Lyngby, Denmark. This annual workshop was the second to be held at our premises in Kgs. Lyngby. A total of 43 participants from 26 countries attended the two days' workshop. There were four sessions with in total 16 presentations, eight of which were given by invited speakers *Click here for the report of the 23rd Annual Workshop*

1.2 Annual workshop crustacean diseases. To ensure knowledge dissemination and sharing between the Member State NRLs on existing and emerging crustacean diseases and to agree on the future priorities of the EURL, by holding the 10th and 11th annual workshops of the National Reference Laboratories (NRLs) for crustacean diseases in 2019 and 2020, respectively.	The 10th annual workshop for crustacean diseases was held for the first time at the premises of DTU Aqua in Lyngby and organized back to back with the workshop for fish diseases on 29th of May. A total of 43 participants from 26 countries attended the one day workshop. There were four sessions with in total 16 presentations, eight of which were given by invited speakers *Click here for the report of the 10th Annual Workshop*
1.3 Scientific working groups. *To ensure that fast and reliable scientific advice on specific topics related to listed and emerging diseases and to legislative issues, is provided by organising expert meetings in order to solve arising challenges in EU.*	In 2019, a scientific working group meeting was organized to update the ISA diagnostic manual in compliance with new Animal Health Law. The diagnostic manual will be finalized in March 2020 *The report of the expert group meeting is located here*
1.4 Proficiency test fish diseases. *To assess the capabilities of all Member State NRLs to detect pathogens causing fish diseases and to harmonize the procedures used by an inter-laboratory proficiency test.*	An inter-laboratory proficiency test was provided by the EURL for Fish and Crustacean Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2). 49 laboratories participated in this activity. The interlaboratory proficiency tests for fish diseases were sent from the EURL 27th of September 2019 The ampoule content has been disclosed in December 2019. A full report will be provided to the participants as well as to the EU commission in march 2020. *The report of the Inter-Laboratory Proficiency Test 2019 for fish disease is located here*
1.5 Proficiency test crustacean diseases *To assess the capabilities of all Member State NRLs to detect pathogens causing diseases in crustacean and to harmonize the diagnostic procedures used by inter-laboratory proficiency tests*	The annual proficiency test for crustacean diseases was organized for the first time by our team in 2019. The test was delivered to 22 NRL's in Europe. The test was shipped 1st of November. The ampoule content has been disclosed in December 2019. A full report will be provided to the participants as well as to the EU commission in march 2020.

	The Ampoule content of the Inter-Laboratory Proficiency Test 2019 for crustacean disease is located here
1.6 Diagnostic methods *For the EURL to have diagnostic methods of the highest scientific standards and to be able to provide these methods to all Member State NRLs*	In 2019 the following 2 new diagnostic qPCR methods were introduced in the laboratory: qPCR for detection of Taura Syndrome Virus PCR for Yellowhead Disease Virus Genotype 1 qPCR for Candidatus Midichloriaceae – main aetiological agent of Red Mark Syndrome in Rainbow trout qPCR for White Spot Syndrome Virus has been implemented and validated qPCR for Infectious Haematopoietic Necrosis Virus in one-step reaction has been implemented and validated
1.7 Crustacean tank facilities For the EURL to be able to conduct infection trails with crustacean species.	In 2019 the team of the EURL for fish and crustacean disease has successfully planned and conducted an infectious trial in *P.vannamei* with White Spot Syndrome Virus to produce reference samples. Further optimization of the facilities will be conducted in 2020, in order to guarantee suitable source of SPF *P.vannamei* and increase the number of crustacean pathogens to be included in the experimental model.
2.1 Training Courses *To ensure that employees of the Member State NRLs have the highest scientific and excellent skills in diagnosis of fish and crustacean diseases*	Two training courses were successfully organized from October the 7th to 18th, 2019. The two courses prepared were: "Methods for implementation of surveillance procedures for listed fish diseases" and "Introduction to histopathology in fish and crustacean diseases" are now accredited to grant ECTS at PhD level to the participants. *The report of the 2019 training courses is located here*
2.2 Website www.eurl-fish-crustacean.eu *To provide the Member State NRLs with a fast entrance to information from the EURL.*	The EURL website has gone through a substantial re-structuring and constant update in spring 2019 and a new website launched at the Annual workshop. It now compiles the information on the activities by both the EURL for fish and crustacean diseases. It can be accessed through https://www.eurl-fish-crustacean.eu/ The new website has been accessed 4.465 times; 12.990 pages have been accessed since May 2019. *Link to the website:* https://www.eurl-fish-crustacean.eu/

2.3 EURL Contact Lists *To ensure that relevant and important information rapidly can get from the EURL directly to the Member State NRLs*.	The e-mail list FishRefLabNet have been continuously updated during 2019 and now contain 167 people with interest in our work. The list now includes all the NRL contacts for the Crustacean Diseases.
2.4 Missions to NRLs for fish diseases *To ensure a high standard of diagnostic capabilities of all Member State NRLs.*	Due to the high performances in 2018 no mission was scheduled in 2019.
2.5 International conferences and meetings *To keep the EURL updated on the newest scientific information on emerging and listed exotic and non-exotic fish and crustacean diseases, and to disseminate knowledge and scientific data provided by the EURL.*	The EURL team has attended and contributed with high profile scientific talks to a number of international conferences and meetings within the field. EURL employees and members of the fish and crustacean unit at DTU participated in 14 international meetings and conferences and gave 38 oral presentations. The Unit authored 20 publications in Peer-reviewed journals.
2.6 Confirmatory diagnosis *For the EURL to be able to identify and characterize isolates of listed viral fish and crustacean pathogens on request from the Member State NRLs*	The EURL has been involved in corroborating the diagnosis of a number of disease outbreak. In 2019 the EURL for fish disaeses has been involved in the confirmation of . KHVD outbreak in common carps in Norway. CEV outbreak in common carps in Norway. IHN outbreak in rainbow trout in Republic of north Macedonia.
2.7 Pathogen characterization *For the EURL to be able to characterize isolates of listed viral pathogens of aquatic animals as well as emerging pathogen and provide scientific based risk assessment to the scientific community and stakeholders*	In 2019 the EURL has been involved in characterizing the following pathogens: PMCV in Atlantic salmon from Ireland. Chimeric VHSV in Rainbow trout. Susceptibility of Sea bass to infection with VHSV isolates. IHNV isolates from rainbow trout in North Macedonia.
3.1 Diagnostic manuals fish diseases *To have updated* diagnostic manuals for all listed fish diseases available for Member State NRLs on the EURL website.	The sampling and diagnostic procedures for detection of VHS, IHN, ISA, KHV, EHN and EUS were kept and updated at our web site. In order to update the ISA diagnostic manual an expert meeting has been organized. It is expected that the work will be finalized by March 2020.

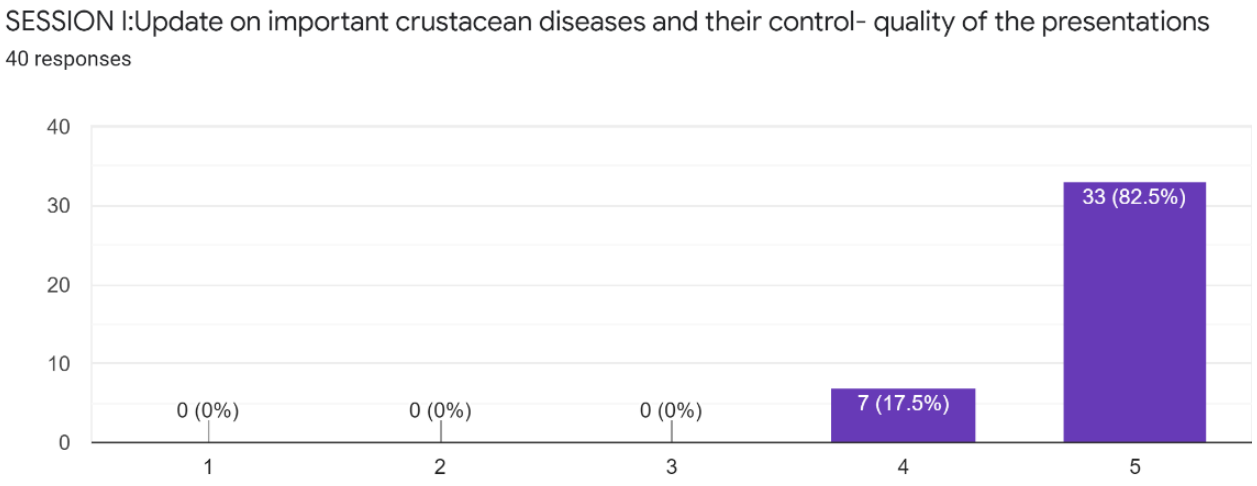
	Link to the manuals: *https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals*
3.2 Diagnostic manuals crustacean diseases To have updated diagnostic manuals for all listed crustacean diseases available for Member State NRLs on the EURL website.	The sampling and diagnostic procedures for detection of WSSV, TSV and YHDV are presented on the EURL website. *Link to the manuals:https://www.eurl-fish-crustacean.eu/crustacean/diagnostic-manuals*
3.3 Survey and diagnosis fish diseases *As part of our duties given in given in C.D. 2006/88/EC Annex VI, Part I.5 (f) to "collate and forward information on exotic and endemic diseases, that are potentially emerging in Community aquaculture" data on emerging and endemic fish diseases and fish health surveillance in Europe will be collated in order to ensure that the EU Commission, the Member State NRLs and the EU in general are updated on the fish diseases situation in aquaculture and natural fish populations in Europe.*	The report, based on data from the questionnaire on Survey and Diagnosis of the listed fish diseases in Europe (S&D) for 2018 send from the EURL to all NRLs was prepared and presented at the AW 2019 *The report for S&D 2018 can be downloaded at* *https://www.eurl-fish-crustacean.eu/fish/survey-and-diagnosis*

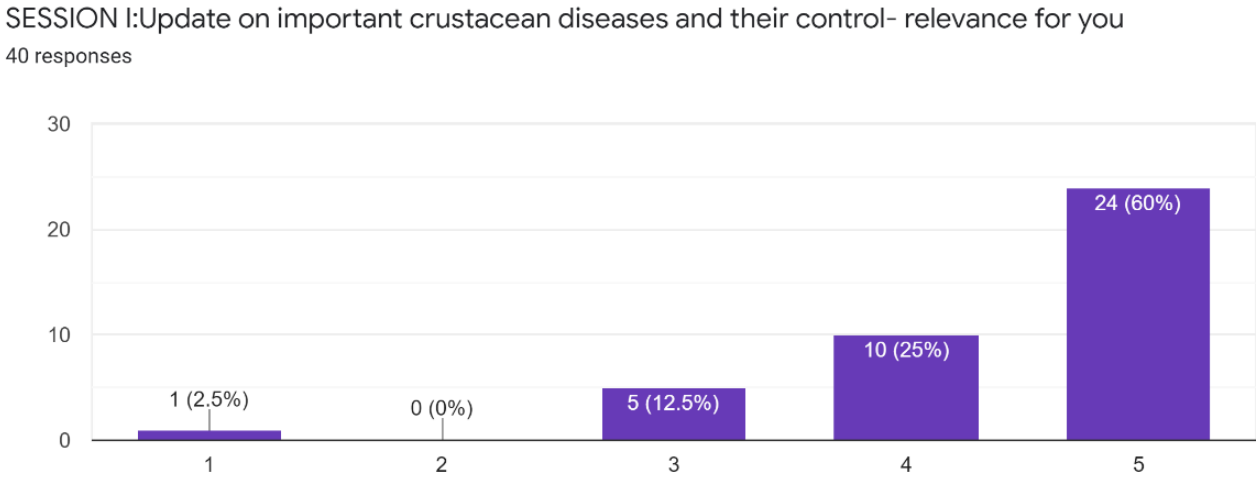
3.4 Risk assessment for emerging diseases For the EURL to have the most updated and highest scientific knowledge of emerging and re-emerging fish and crustacean diseases in Europe.	The EURL has initiated a study to assess infectivity and virulence of Piscine myocarditis virus (PMCV) in Atlantic salmon.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

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

Workshop evaluation

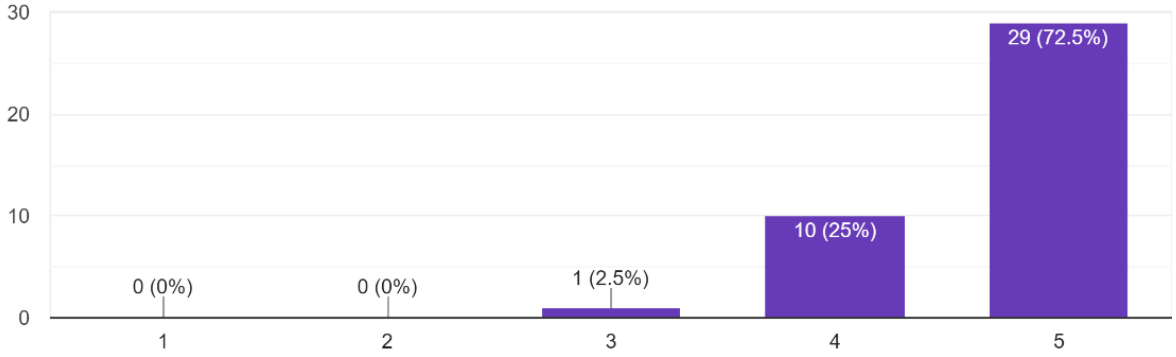
A questionnaire was delivered to the participants asking to evaluate various aspect of the workshop. An overview of the 40 questionnaires retrieved is shown below. Specific comments are going to be considered for the next annual workshop organization.





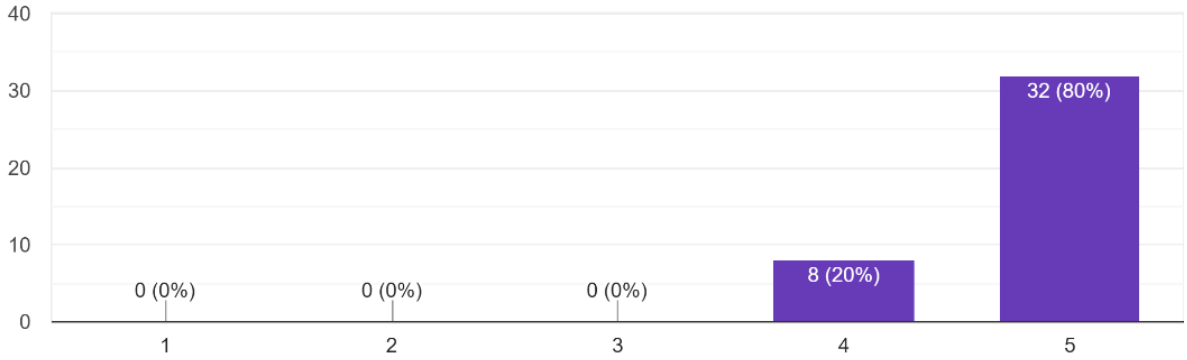
SESSION I:Update on important crustacean diseases and their control- increase of your knowledge

40 responses



SESSION I:Update on important crustacean diseases and their control- overall score

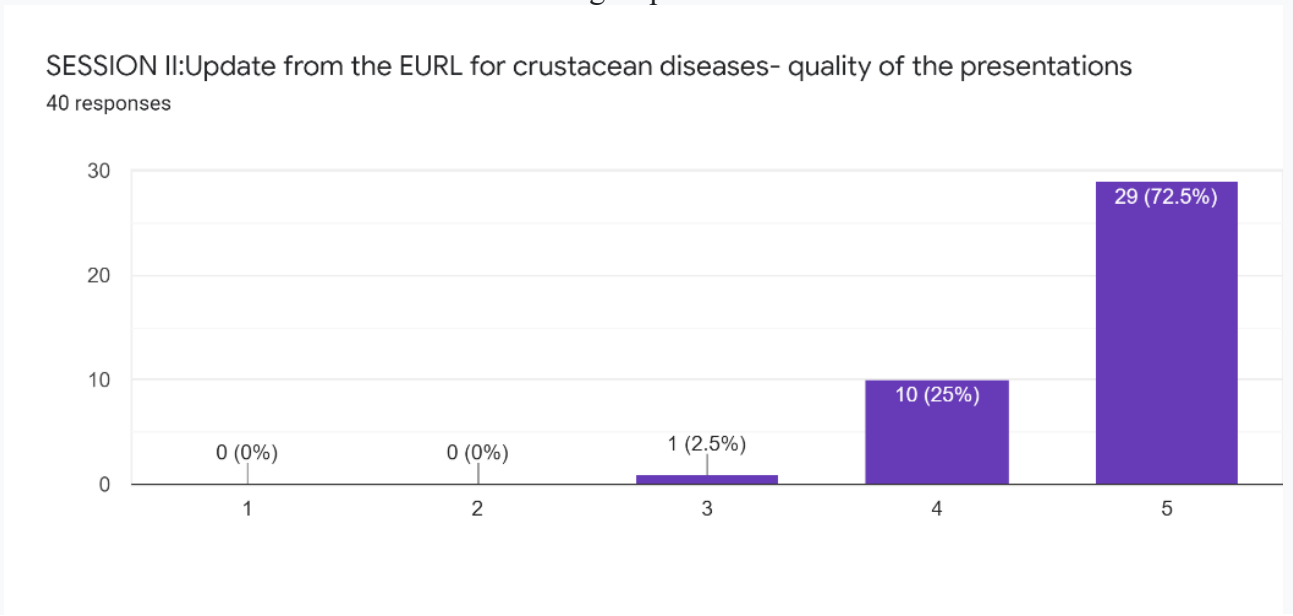
40 responses

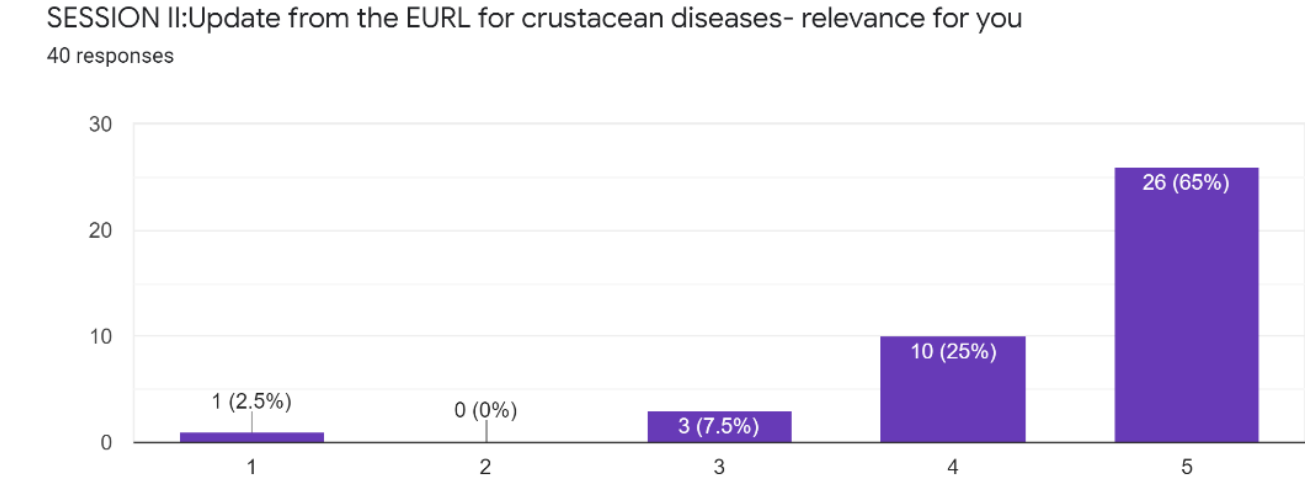


SESSION I:Update on important crustacean diseases and their control- comments, feedback, input

my knowledge on emerging crustacean diseases and especially on the criteria for screening when dealing with exotic species
I think a round table discussion on the use of eDNA as a monitoring tool would be useful
it was informative for me, Definitely, I increased my knowledge and I could share this information in my field.
Thank you for great meeting
no objections
nice scientific work
-
no comment

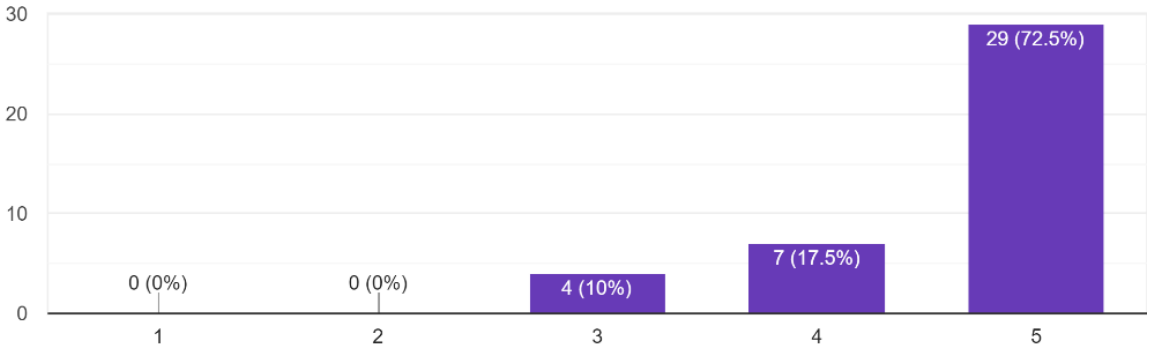
Excellent presentations and topics. Perhaps some break out groups (4-5 random people) for coffee between sessions? part of the amazingness of this network is the familiarity and friendly interaction and that can be fostered in small groups.





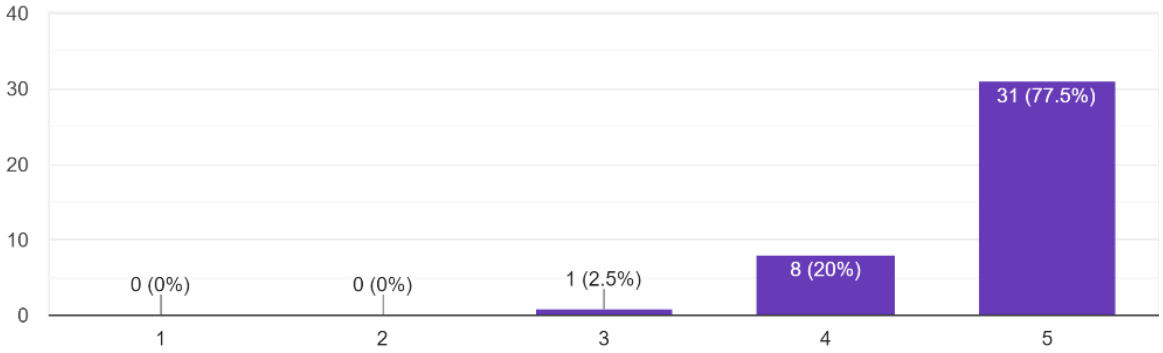
SESSION II:Update from the EURL for crustacean diseases- increase of your knowledge

40 responses



SESSION II:Update from the EURL for crustacean diseases- overall score

40 responses



SESSION II:Update from the EURL for crustacean diseases- comments, feedback, inputs

This increased my knowledge and network for crustacean diseases

Could you publish the most propriate protocol for YHV RT-qPCR much prior PT to have enough time to validate protocol, please?

maybe to increase collaboration between attendees, you may have a discussion group or round table. Everyone can share their experiences with this issue. Or you may add "ice breaker" time. İt seemed most of you know each other, for others who do not know you in person, it could be difficult to involve discussion or ask questions.

Thank you for great meeting

Thank you so much, altogether really well done with, although difficult online, still the idea of being part of the international expert group. More cameras of participants on in the breaks would be also nice, possibly. Cheers, Olga

no objections

thank you for the effort
-
no comment
I had retired before session II, so cannot actually say anything about it, but had to fill out the form in order to submit it.

Greetings and conclusions of the meeting

The next meeting will be held at the 1st – 3rd of June 2021. It will most likely be organized as a virtual meeting again. Thanks a lot to the people arranging the meeting as well as those of you who helped running the meeting by being chair, presenter and/or participant.

We are looking forward to seeing you all next year!

With kind regards,

The EURL fish and crustacean team