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

Report of the

14th Annual Workshop of the National Reference Laboratories for Crustacean Diseases

Kgs. Lyngby, Denmark

June 1st 2023

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 14th Annual Workshop of the National Reference Laboratories for Crustacean Diseases was held physically on 1st of June 2023. There were 38 participants attending the workshop in person, representing 22 countries.

The workshop was held back-to-back with the 27th Annual Workshop for National Reference Laboratories for Fish Diseases and a special session for NRLs in EU and EEA on the implementation of the Animal Health Law.
The workshop was opened with "Welcome and announcements" by section leader for fish and shellfish diseases at DTU Aqua, Britt Bang Jensen and Coordinator of the EURL for Crustacean Diseases, Morten Schiøtt. The first session had the title "Update on EU listed crustacean diseases and their control" and started with Coordinator of the EURL for Crustacean Diseases Morten Schiøtt giving an update on the disease and surveillance situation of crustacean diseases in EU countries based on the results obtained from the Survey and Diagnosis questionnaire. This was followed by a talk by WOAH expert Hyoung Jun Kim giving a presentation on Development of standard positive plasmid DNA for diagnosis of crustacean diseases based on real-time PCR to prevent false-negative and false-positive reactions.

The second session had the title: "Results from ongoing research on crustacean diseases". Magdalena Stachnik from the Polish NRL gave an online presentation on Microsporidium *Hepatospora eriocheir* - an emerging pathogen of aquatic invader Chinese mitten crab from Vistula Lagoon, bay of the Baltic Sea.
Next, Shana Genavia, a newly employed PhD student at the Section for Fish and Shellfish Diseases at DTU Aqua, gave the presentation "RNA Interference of Toll3 in *Litopenaeus vannamei* and Expression Analysis of MyD88, TRAF6, and Dorsal gene in response to WSSV infection".
After a break, Tiina Korkea-Aho from the Finnish NRL talked about "Identification of crayfish plague agent Aphanomyces astaci with MALDI-TOF MS".
Next, Simone Pisano from Swiss NRL gave an overview of Epidemiology and ecology of crayfish plague and genomics of *Aphanomyces astaci*.
Afterwards David Strand from the Norwegian NRL gave a presentation on the "Development of an improved species specific qPCR assay for *Aphanomyces astaci"*.
The last speaker of the session was senior scientist at the Section for Freshwater Ecology DTU Aqua Magnus W. Jacobsen who gave a presentation on "Mapping the distribution of crayfish and crayfish plague in Denmark using eDNA".

Session III had the title "Update from the EURL for Crustacean Diseases" and started with EURL coordinator Morten Schiøtt giving a talk on the results of the interlaboratory proficiency tests for crustacean diseases in 2023. Finally, the workshop ended with Morten Schiøtt presenting the EURL activities in year 2022 and 2023, and proposals for the EURL work plan for 2024.

Lone Madsen and Niccoló Vendramin from DTU Aqua took minutes from the meeting, and Niccoló Vendramin and Morten Schiøtt assembled the report.

We would once again like to thank all the presenters for their great contributions, without them the meeting would not have been a success. The workshop and meeting was organized by a team consisting of Morten Schiøtt and Niccoló Vendramin, with the help from the rest of the fish and shellfish diseases section at the National Institute of Aquatic Resources, DTU Aqua. The meeting next year is tentatively planned to be held at beginning of June 2024 and will be held virtually.

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

Niccoló Vendramin and Morten Schiøtt

Programme

Thursday June 1st
Annual Workshop of the National Reference Laboratories for Crustacean Diseases

9.30 – 9:40	Welcome and announcements *Morten Schiøtt*
SESSION I:	**Update on EU listed crustacean diseases and their control**
	Chair: Morten Schiøtt and minutes: Niccoló Vendramin
09:40 – 10:00	Surveillance and diagnostics of crustacean diseases in Europe *Morten Schiøtt*
10:00 – 10:20	Development of standard positive plasmid DNA for diagnosis of crustacean diseases based on real-time PCR to prevent false-negative and false-positive reaction *Hyoung Jun Kim*
SESSION II:	**Results from ongoing research on crustacean diseases**
	Chair: Morten Schiøtt and minutes Lone Madsen
10:20 – 10:40	Microsporidium Hepatospora eriocheir - an emerging pathogen of aquatic invader Chinese mitten crab from Vistula Lagoon, bay of the Baltic Sea. *Magdalena Stachnik*
10:40 – 11:00	RNA Interference of Toll3 in *Litopenaeus vannamei* and Expression Analysis of MyD88, TRAF6, and Dorsal gene in response to WSSV infection *Shana Genavia*
11:00 – 11:30	***Coffee break***
11:30 – 11:50	Identification of crayfish plague agent Aphanomyces astaci with MALDI-TOF MS *Tiina Korkea-Aho*
11:50 – 12:10	Epidemiology and ecology of crayfish plague and genomics of *Aphanomyces astaci* *Simone Pisano*
12:10 – 12:30	Development of an improved species specific qPCR assay for *Aphanomyces astaci* *David Strand*

12:30 – 12:50 Mapping the distribution of crayfish and crayfish plague in Denmark using eDNA
Magnus W. Jacobsen

SESSION III: Update from the EURL for crustacean diseases

Chair: Morten Schiøtt and minutes Niccoló Vendramin

12:50 – 13:05 EURL proficiency test for crustacean diseases 2023
Morten Schiøtt

13:05 – 13:20 EURL Work done in 2022, plan for 2023 and ideas and plans for 2024
Morten Schiøtt

Next meeting and end of 14th Annual Workshop
Morten Schiøtt

End of meeting

SESSION I: Update on EU listed crustacean diseases and their control

Chair: Morten Schiøtt

Surveillance and diagnostics of crustacean diseases in Europe

Morten Schiøtt

EURL for Fish and Crustacean Diseases,

National Institute of Aquatic Resources, Kemitorvet, Bygning 202, 2800 Kgs. Lyngby, Denmark

mosch@aqua.dtu.dk

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:

1) Report the number of farms belonging to each health status according to COMMISSION DELEGATED REGULATION (EU) 2020/689.

2) Report any outbreaks in the country of EU listed crustacean diseases, as well as health problems related to other crustacean diseases.

3) Report the number of samples tested for OIE listed crustacean diseases and how many of these gave a positive result.

4) Describe the current status of crustacean aquaculture in the country, as well as the strategy used for surveillance of crustacean diseases.

Data from 22 countries have so far been obtained and will be compared to the data received for 2019, 2020 and 2021.

Questions and comments:

Q: (NRL Hungary) we have no farming of crustacean at all. Only wild crayfish.

A: (EURL): Ok answers will be updated.

Development of standard positive plasmid DNA for diagnosis of crustacean diseases based on real-time PCR to prevent false-negative and false-positive reaction

Kim HJ[1*], Schiøtt M[2], Olesen NJ[2], Ku BK[3], Lee KK[3], Jeong HY[3], Hwang MH[3], Choi HS[1], Cho MY[1], Kim YC[4]

[1] WOAH reference laboratory for VHS in NIFS, [2] EU reference laboratory for crustacean disease in DTU Aqua, [3] Animal disease diagnostic division in APQA, [4] Department of Aquatic life medicine in GWNU

The development of recombinant plasmid-based standard materials is a significant advancement in the field of infectious disease diagnosis, as it can prevent false negatives and false positives in molecular diagnostic technologies. To ensure the accuracy and reliability of molecular diagnostic methods for infectious disease diagnosis, it is essential to establish a system to prevent false negatives and false positives due to gene contamination. This study introduces a novel approach to enhance the reliability and accuracy of molecular diagnostic technologies in the field of infectious disease diagnosis. By developing recombinant plasmid-based standards and utilizing the J assay as a benchmark, we provide a valuable tool for verifying the sensitivity of various diagnostic methods for crustacean diseases. This tool can apply to diagnosis all infectious disease including aquatic and terrestrial animals. This innovative approach ensures accurate diagnosis and prevention of false-negative and false-positive reactions, crucial for effective disease management and control. Using these tools, we can build up the WOAH collaboration centre for provision of all diagnostic positive control DNA of WOAH listed diseases in aquatic and terrestrial animals between Korea and Denmark.

Questions and comments:

Q: How does the contamination probe work?

A: Checks for contamination by positive control.

Q: When do you expect to have controls ready for all WOAH listed disease?

A: The plan is to add all listed diseases.

Q: Do you have made a patent for this? What are the financial plan?

A: No financial plans – the idea is the provide the plasmids to everybody for free.

SESSION II: Results from ongoing research on crustacean diseases

Chair: Morten Schiøtt

Microsporidium *Hepatospora eriocheir* - an emerging pathogen of aquatic invader Chinese mitten crab from Vistula Lagoon, bay of the Baltic Sea.

Magdalena Stachnik[1] & Monika Normant-Saremba[2]

1. National Veterinary Research Institute, Department of Fish Diseases, Al. Partyzantów 57, 24-100 Puławy, Poland

e-mail address: magdalena.stachnik@piwet.pulawy.pl

2. University of Gdańsk, Faculty of Oceanography and Geography, Department of Experimental Ecology of Marine Organisms, Al. M. Piłsudskiego 46, 81-378 Gdynia, Poland

Emerging diseases can be defined as infectious diseases that recently expanded their geographic or host range, or prevalence. The role of biological invasions in the emergence of diseases is still under debate. Many invasive species lose their pathogens during the invasive process, other taxa introduced novel parasites into colonised areas. Here, we describe first record of a microsporidian parasite *Hepatospora eriocheir* infection in invasive Chinese mitten crabs (*Eriochier sinensis*) from Vistula lagoon, a brackish waterbody on the Baltic Sea. The microsporidia are a diverse parasite phylum infecting hosts from all major taxa in all environments, ranging from the beneficial insects and aquatic animals, to important parasites of humans. *Hepatospora eriocheir* was first identified in 2007 in the cultured *E. sinensis* from China, as a pathogen suspected of causing hepatopancreatic necrosis disease with a high mortality rate. In Europe this parasite was described in 2011 in Chinese mitten crabs from the River Thames, as morphologically similar to the microsporidium in native Asian populations. Our present study describes a microsporidium infecting non-native Chinese mitten crabs from the Baltic Sea. Phylogenetic analysis based on DNA coding conserved regions of microsporidian small subunit ribosomal revealed that the parasites had almost 100% sequence identity to that of *H. eriocheir* from UK and indirectly from China. This supports the theory that they were introduced with the invader crab during its first invasions to Europe and Baltic Sea in the early 1900s.

Questions and comments:

Q: Which crabs in UK were infected?

A: Also Chinese mitten crab.

Q: Would you expect transfer to other crab species?

A:Probably not as the parasite is very specialized to this species of crab.

RNA Interference of Toll3 in *Litopenaeus vannamei* and Expression Analysis of MyD88, TRAF6, and Dorsal gene in response to WSSV infection

Genavia, S.F.[1,2] & Maningas, M.B.B.[1,2,3]

[1] The Graduate School, [2] Aquatic Molecular Biology & Biotechnology Laboratory, Research Center for the Natural & Applied Sciences, and [3]Department of Biological Sciences, College of Science, University of Santo Tomas, Espana 1015, Manila, the Philippines

sgen@dtu.dk

Abstract

Shrimp aquaculture is a thriving global industry, yet it faces significant challenges posed by various diseases, primarily caused by bacteria and viruses. Understanding the immune response of shrimp species is crucial for the development of effective treatment strategies and optimal farming practices. In this study, we focus on a newly identified shrimp Toll-like receptor 3 (TLR3) gene, LvToll3 (*Litopenaeus vannamei* Toll3), which plays a critical role in regulating immune molecules involved in the IRF-Vago-JAK/STAT pathway. Our aim is to investigate the function of LvToll3 in the signaling of Toll pathway molecules in *L. vannamei* in response to viral infection. We employ RNA interference (RNAi) to silence LvToll3 transcripts, followed by experimental infection of shrimp with white spot syndrome virus (WSSV) in vivo. Using semi-quantitative gene expression analysis, we examine the levels of LvMyD88, LvTRAF6, and LvDorsal at seven time points post-infection (3hr, 9hr, 12hr, 24hr, 48hr, 72hr, and 120hr) in the gills. Our results demonstrate successful knockdown of LvToll3 expression throughout all time points, as compared to the upregulation observed in the GFP-dsRNA treatment, which was significantly higher than that of the PBS treatment. Importantly, LvToll3 silencing led to increased expression of WSSV infection. Furthermore, the suppression of LvToll3 significantly impacted the overall expression of LvMyD88, LvTRAF6, and LvDorsal compared to the control groups. These findings highlight the vital role of LvToll3 in the recognition of WSSV and its subsequent stimulation of immune-related molecules.

Questions and comments:

Q: Did you run Toll4 in your assay?

A: No, this has not been done but would like to.

Q: Why focus on this toll receptor?

A: Because of previous evidence of Toll3 being involved in immunity – they also silenced Toll3.

Q: Are toll receptors in shrimp also intracellular like in mammalian?

A: Yes, they are.

Q: Why do you not call them toll receptors and not just toll?

A: Because that is what it has been called earlier

Q: Can your research be used in terms of vaccines?

A: Maybe, but the problem is the delivery method.

Q: Any speculations on the in vivo model to be used in further research?

A: Introduction of in vivo challenges and double stranded DNA – it is a matter of finding a delivery model – you cannot just inject lots of shrimp.

Identification of crayfish plague agent *Aphanomyces astaci* with MALDI-TOF MS

Tiina Korkea-aho1, Jussa-Pekka Virtanen1, Sirpa Heinikainen1, Satu Viljamaa-Dirks1

[1]Istituto 1Finnish Food Authority, Finland

tiina.korkea-aho@ruokavirasto.fi

Abstract

Crayfish plague agent Aphanomyces astaci causes a lethal disease to most freshwater crayfish species, except for North American crayfish species, which function as natural carriers of the crayfish plague agent. Detection of the oomycete A. astaci used to be dependent on the isolation of the agent, followed by pathogenicity test, until molecular diagnostic methods became available. Today, quantitative real-time PCR (qPCR) methods are most used for the diagnosis of crayfish plague directly from tissue samples. However, the new discoveries of Aphanomyces species, such as A. fennicus, have demonstrated that false positive results are possible with the established internal transcribed spacer (ITS) based qPCR. WOAH recommends sequencing the qPCR product to reliably confirm the species.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is widely used to identify bacterial pathogens and fungi in both clinical settings and research. MALDI-TOF MS identifies species from pure culture by its unique protein mass spectrum, which is compared to reference spectra, usually from commercial databases. MALDI-TOF MS is considered a cost- and time-efficient diagnostic method in microbiology. However, no reference spectra have been established for oomycetes, including A. astaci, nor oomycetes are present in any commercial databases supplied by device manufacturers.

In this study, we created a preliminary main spectral profile (MSP) for A. astaci and other oomycetes with MALDI-TOF MS. For the analysis, A. astaci strains of genotype Ps1 (B) were used to create the MSP against which A. astaci strains of several other genotypes, and other oomycete species in Finnish Food Authority culture collection were compared to assess the specificity of the method. Using the MALDI-TOF MS pure cultures of different A. astaci genotypes, they were reliably identified as A. astaci and differentiated from other Aphanomyces species as well as several other oomycete species tested. Furthermore, MALDI-TOF MS successfully differentiated between A. astaci and A. fennicus species, which have been difficult to separate with most of the qPCR methods and could prove an alternative to sequencing at species confirmation. Although molecular methods are widely used and recommended for quick diagnosis, isolation of A. astaci by cultivation is recommended for further research. MALDI-TOF MS appeared suitable as an additional diagnostic tool for A. astaci identification of isolated pure cultures and recognizing A. astaci from other oomycetes present in crayfish..

Questions and comments:

Q: How long does the test take?

A: Takes time to culture *Aphanomyces* but the actual MALDI-TOF time is 1 h.

C: Great with the start of using MALDI-TOF as a method for *A. astaci* – a nice tool – very good with the potential of a database of *A. astaci* for identification.

Q: Have you considered testing from tissue without purification of oomycete?

A: It requires a pure culture to get a reliable MALDI-TOF result.

Epidemiology and ecology of crayfish plague in Switzerland

Fiona Swords; Bogna Griffin; Simone Roberto Rolando Pisano1, Tatiana Zingre1, Jonas Steiner1, Manon Zürcher1, Elodie Cristina1, Zoe Delafortrie1, Simone Oberhäsli1,2, Pamela Nicholson2, Heike Schmidt-Posthaus1

1Institute of Fish and Wildlife Health, Vetsuisse Faculty, University of Bern

2Next Generation Sequencing Platform, Vetsuisse Faculty, University of Bern

simone.pisano@unibe.ch

Abstract

Aphanomyces astaci is the causative agent of crayfish plague in native European crayfish, leading to high mortality. Invasive North American crayfish serve as carriers of the agent. Although disease severity depends on genotypes (A-E) involved, detection and genotyping methods are complex and so far unsatisfying and disease dynamic in rivers is largely unknown.

Our aims were to (1) optimise detection and genotyping methods, (2) monitor A. astaci genotypes in Swiss crayfish populations, native and invasive, and (3) characterise disease dynamics in natural waters following crayfish plague outbreaks.

DNA was extracted from the exoskeleton of 30 crayfish plague affected native crayfish, using various commercial kits specific for animals (Ak), plants (Pk) and insects (Ik). DNA concentration, fragment length and quality, as well as A. astaci DNA detection probability by qPCR and PCR were compared. DNA originating from pure cultures of A. astaci genotypes A-E were sequenced using Pac-Bio HiFi-Long-Read-Sequencing. Whole genome comparative analysis was performed to identify sequences suitable for A. astaci detection and/or genotyping.

A retrospective study using archive material from 1960 till 2020 aimed to investigate A. astaci occurrence and genotypes by qPCR, PCR and Sanger sequencing on formalin-fixed paraffin-embedded (FFPE) material.

A Prospective study was performed between 2020 and 2023. 15-20 individuals per population were sampled, A. astaci occurrence and genotypes were investigated as described above.

After a crayfish plague outbreak, eDNA was investigated over a period of 12 months by filtering water (1800-2400ml) followed by qPCR.

Total DNA concentration was higher in samples extracted with Ak or Pk. Fragment length was highest using Ik. While qPCR detected A. astaci in all samples, PCR showed higher detection probability in samples extracted by Ik or Pk.

In the retrospective study, crayfish plague was diagnosed in 40/212 populations, with a first case from 1980. Three different genotypes were identified (B, D, E), as early as 1994.

In the prospective study, 1020 crayfish were sampled. So far, crayfish plague was confirmed in 4 native crayfish populations.Preliminary eDNA results indicate strong influence of season and river characteristics on A. astaci eDNA.

Questions and comments:

Q: Possible with a specific amplification with Aeromonas sp – which annealing temperature was used? The one given in Oidtmann 2006?

A: Used the annealing temperature given in the Oidtmann paper.

C: Annealing temperature has recently been changed.

Q: Have you considered a cut-off value when it comes to eDNA data (from the pond)?

A: Yes, but age of carcasses will be influencing the results.

Development of an improved species specific qPCR assay for *Aphanomyces astaci*

David A. Strand1*, Tomas Jinnerot2, Anna Aspan2, Satu Viljamaa-Dirks3, Sirpa Heinikainen3, Elin Rolen1, Trude Vrålstad1

1 Norwegian Veterinary Institute, Oslo, Norway . 2 National Veterinary Institute, Uppsala, Sweden ,3 Finnish Food Authority, Kuopio, Finland

*david.strand@vetinst.no

Abstract

The parasitic oomycete Aphanomyces astaci is the causative agent of crayfish plague, a devastating disease for European freshwater crayfish. Species-specific quantitative real-time PCR (qPCR) is commonly used for rapid detection and in molecular diagnostics. The recent discovered and described tentative novel species Aphanomyces fennicus is amplified by the recommended and well established A. astaci qPCR, potentially resulting in false-positive results if present.

We have developed an improved A. astaci specific qPCR assay that does not amplify A. fennicus. The assay has been tested and validated at three different national reference laboratories for fish and crustacean diseases, using the respective labs commonly used master mixes and workflow. Genomic DNA from A. astaci, A. fennicus and closely related Aphanomyces spp. was analysed and compared with both the improved and established assay. We also analysed DNA from crayfish tissue and environmental samples with both assays. The improved assay showed similar sensitivity with the established assay for all sample types, while proving highly specific for A. astaci avoiding amplification of A. fennicus and the other tested Aphanomyces spp.. However, for one of the tested master mixes we observed delayed amplification (13-18 cycles) of A. fennicus for very high DNA concentrations.

Environmental DNA (eDNA) samples from one river in Norway, amplified with the established assay but not with the improved assay. We were able to sequence a 530 bp fragment of the ITS region from these eDNA samples. The consensus sequence from the eDNA samples showed 99.9-100 % pairwise identity with A. fennicus and 97.2-98 % pairwise identity with A. astaci. This indicates that the occurrence of A. fennicus is not limited to Finland, where it was first discovered.

Questions and comments:

Q: How did you manage to amplify from eDNA samples?

A: I was only able to do it once on a sample with CT 33.

Q: What threshold levels did you use for eDNA?

A: Threshold C_T was 41, at least two out of three should be amplified.

Mapping the distribution of crayfish and crayfish plague in Denmark using eDNA

Magnus W. Jacobsen, Brian K. Hansen, Stig Pedersen & Søren Berg

DTU Aqua - Technical University of Denmark

lmvj@aqua.dtu.dk

Abstract

In this presentation we describe the results of a nationwide mapping of the distribution of freshwater crayfish and crayfish plague in the fresh waters of Denmark. The project was carried out from May 1st 2020 to December 1st 2021. The general monitoring method used was collecting and filtering water samples at site. The filtrate was subsequently used for analysis of presence of DNA – so-called eDNA – from crayfish and crayfish plague. Water samples were collected in streams and interconnected lakes throughout Denmark including the following larger islands that contains significant streams: Mors, Als, Ærø, Langeland, Lolland, Falster, Møn og Bornholm.
Analysis of the samples resulted in positive detection of noble crayfish in 57 sites, situated in 35 catchments, of these two were lakes, both located on Møn. Signal crayfish was detected in 106 sites situated in 49 catchments, of these three were found in lakes on Als. Narrow-clawed crayfish was only detected in six sites in five catchments, four on Zeeland and one on Lolland. Crayfish plague was detected in 54 sites in 21 catchments. Marbled crayfish and red swamp crayfish were not detected in the 192 samples analysed for these species.
In general, it was possible to detect crayfish by eDNA analysis in localities where presence of one of the three species of crayfish was known prior to this monitoring. Thus, eDNA monitoring is a suitable method for monitoring crayfish and crayfish plague distribution in Danish freshwater. The monitoring method reviled that the invasive signal crayfish is present in all parts of Denmark and must today be considered the most common crayfish in the country. Noble crayfish is also found in most parts of Denmark. However, several populations are threatened by either signal crayfish or crayfish plague. The presence of narrow-clawed crayfish is generally limited to Zeeland. Crayfish plague is also found throughout the country, most often associated with the presence of signal crayfish.

Questions and comments:

Q: What about sequencing the positive findings, due to what has just been said in talks on different *Aphanomyces* species?

A: Project has ended, so no money for doing it for – although some samples have been checked for *A. fennicus* with negative results.

Q: When was the sampling done – which temperatures?

A: Temperature ranges depend if it is ground water, lake water etc., so roughly in the temperature range 10-20 degrees.

Q: Shall a finding of *Aphanomyces astaci* be notified, when it cannot be confirmed clinically (the eDNA question)?

A: Not sure, but if signal crayfish are found in an area, then the finding of *Aphanomyces astaci* is also expected.

Q: For notification, it has to be done with an accredited method, as a positive sample (by another PCR method) was negative with Oidtmann et al. 2006, it cannot be dealt with as being positive and thereby reported.

C: A positive finding is reported, if it is found in a new area, where it has not been reported from before.

SESSION III: Update from the EURL for crustacean diseases

Chair: Niccoló Vendramin

2023 Inter-laboratory proficiency test for crustacean diseases

Morten Schiøtt, Teena Vendel Klinge, Argelia Cuenca and Niccoló Vendramin

EURL for Fish and Crustacean Diseases,

National Institute of Aquatic Resources, Kemitorvet, Bygning 202, 2800 Kgs. Lyngby, Denmark

mosch@aqua.dtu.dk

Abstract

In April 2023 an inter-laboratory proficiency test for White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV) and Yellow Head Virus (YHV) was organized by the EURL for Fish and Crustacean Diseases. The test material consisted of FTA cards incubated with tissue extracts of WSSV infected shrimp, TSV infected shrimp, YHV-1 infected shrimp or non-infected shrimp. The participants are asked to identify the WSSV, TSV and YHV positive samples among eight test samples. 25 laboratories in 18 EU member states signed up for the test. The results from the test will be presented in the talk.

EURL for Crustacean Diseases, work done in 2022

Morten Schiøtt

EURL for Fish and Crustacean Diseases,

National Institute of Aquatic Resources, Kemitorvet, Bygning 202, 2800 Kgs. Lyngby, Denmark

njol@aqua.dtu.dk

Abstract

The duties of the EURL are described in the REGULATION (EU) 2017/625 (OCR). The duties mainly concern the crustacean cat A and C diseases given in (EU) 2018/1882: White Spot Disease (WSD), Taura Syndrome (TS) and Yellow Head Disease (YHD).

The facilities supporting the activities of the EURL are placed in the DTU Campus in Kgs. Lyngby, and placed in the institute DTU AQUA, National Institute of Aquatic Resources.

The 13th Annual Workshop of the National Reference Laboratories for Crustacean Diseases was held as a hybrid of virtual and physical attendance on 1st of June 2022. The number of participants thus reached 73 participants from 33 countries. There were three sessions with ten presentations in total. On May 31st, a workshop on legislative issues for NRLs. This session was attended only by the staff of the National Reference Laboratories in EU and EFTA countries. The aim of this session was to clarify issues related to the new animal health law, which was adopted on 21st of April 2021, and to provide an overview of the methods used for diagnostics of the diseases listed in the new AHL.

The annual proficiency test for crustacean diseases (PT) was divided into a WSSV test panel and a TSV/YHV test panel with 25 laboratories participating in the former and 17 in the latter. The tests were sent from the EURL 4th of April 2022. The full reports with the results and the identification of NRLs have been submitted to the Commission, whereas each participant has received a coded version of the report and a certificate of participation with an indication of performance.

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. In 2022 the EURL uploaded diagnostic manuals for Taura syndrome (TS) and Yellow Head Disease (YHD) on the EURL website.

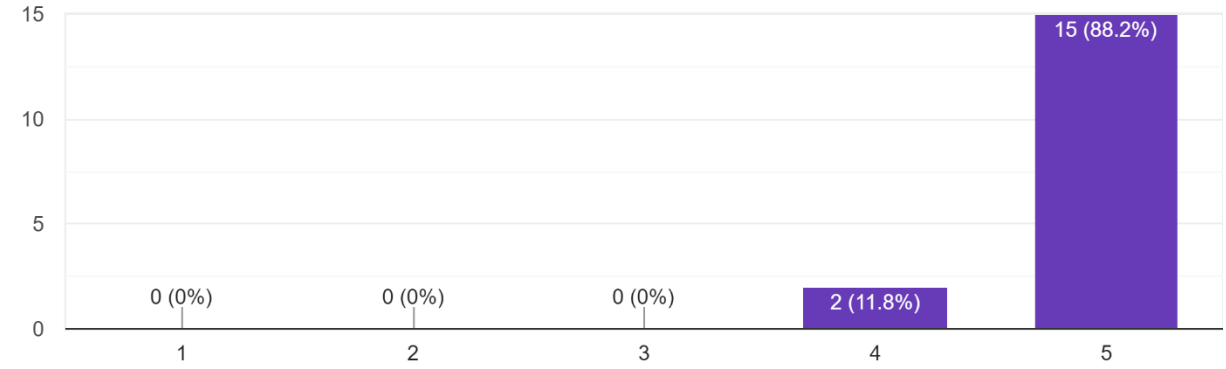
During 2022, resources were again used to collate data on surveillance, health categorisation and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to update the EURL website (www.eurl-fish.eu), to provide consultancy to NRL´s and finally to attend international meetings and conferences.

Workshop evaluation

A questionnaire was delivered to the participants asking to evaluate various aspect of the workshop. An overview of the 17 questionnaires retrieved is shown below. Specific comments are going to be considered for the next annual workshop organization.

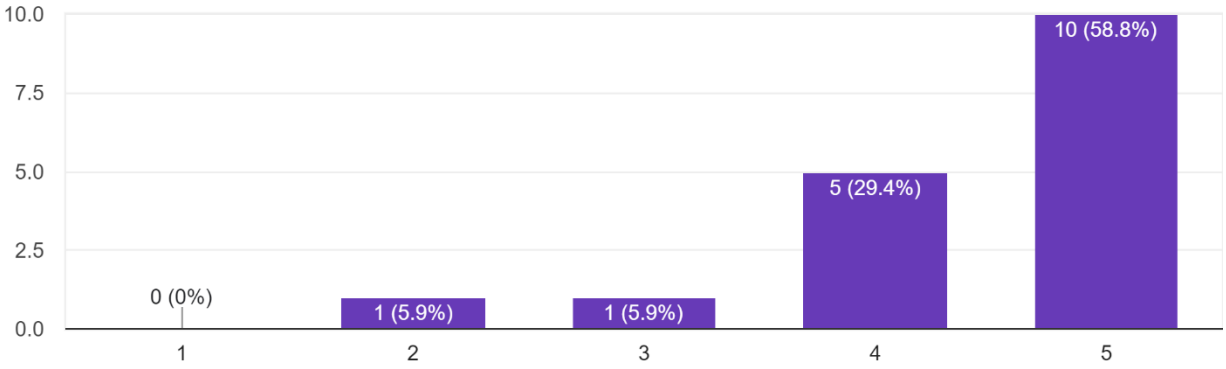
SESSION I:Update on EU listed crustacean diseases and their control- quality of the presentations

17 responses



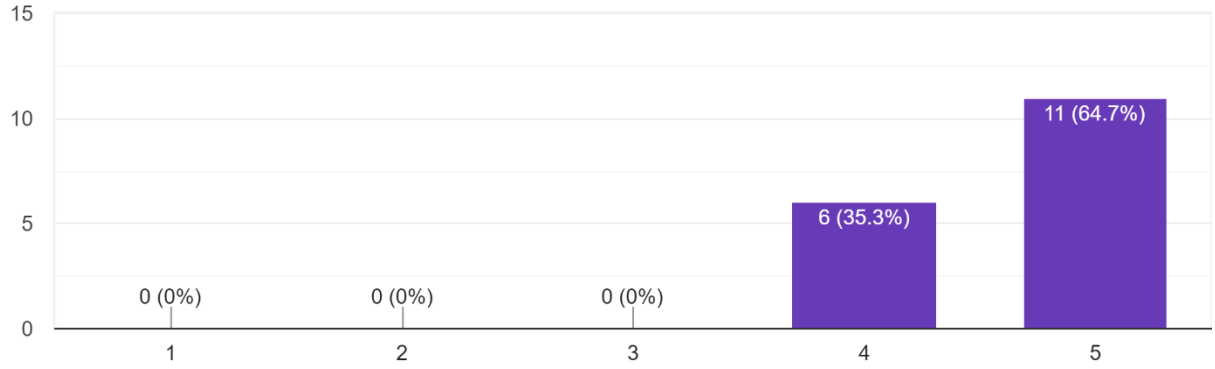
SESSION I:Update on EU listed crustacean diseases and their control- relevance for you

17 responses



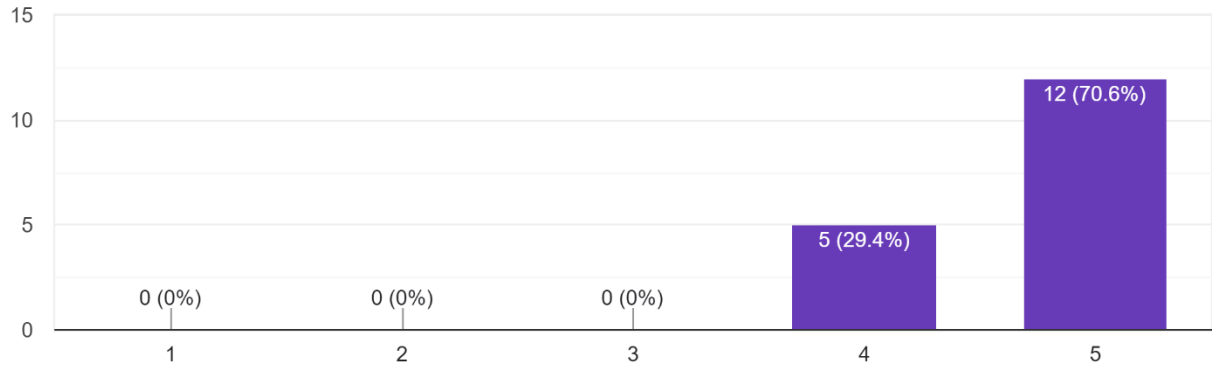
SESSION I:Update on EU listed crustacean diseases and their control- increase of your knowledge

17 responses



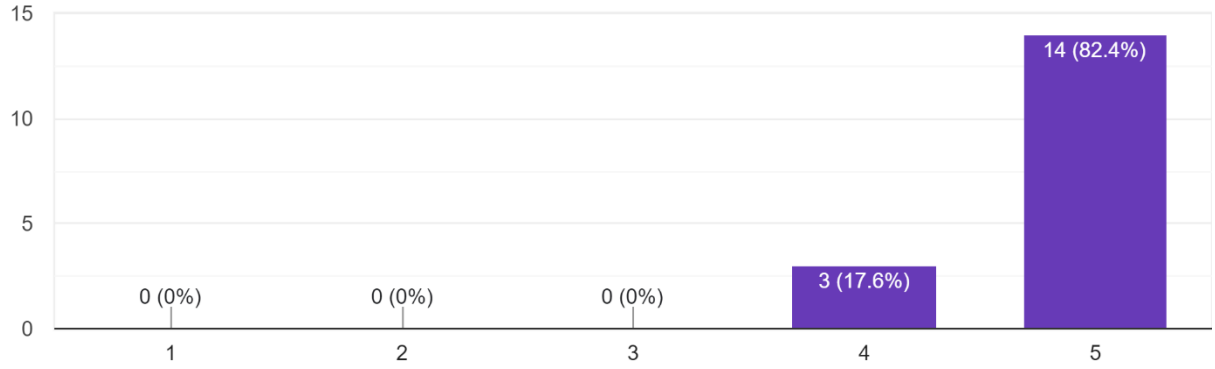
SESSION I:Update on EU listed crustacean diseases and their control- overall score

17 responses



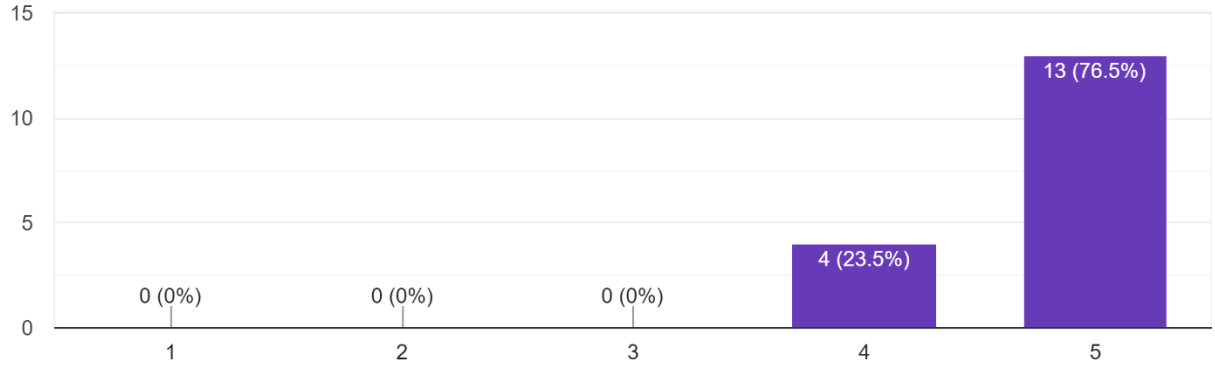
SESSION II:Results from ongoing research on crustacean diseases- quality of the presentations

17 responses



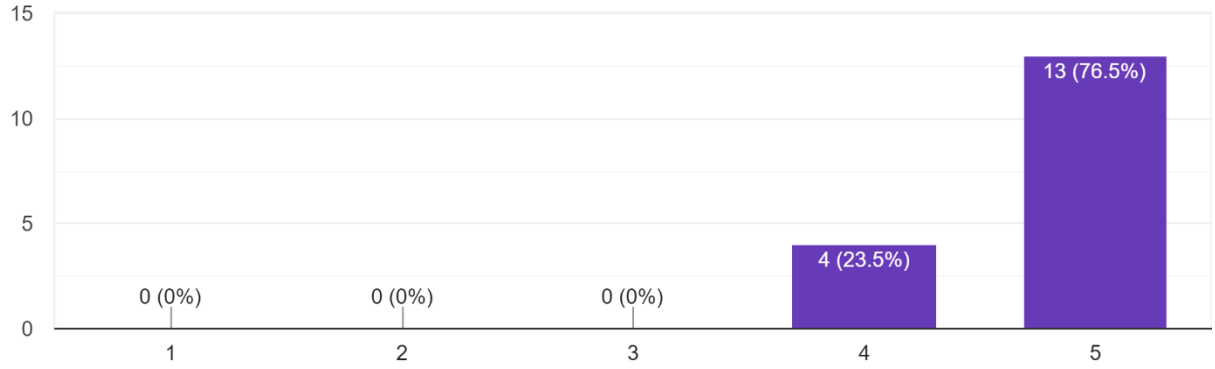
SESSION II:Results from ongoing research on crustacean diseases- increase of your knowledge

17 responses



SESSION II:Results from ongoing research on crustacean diseases- overall score

17 responses



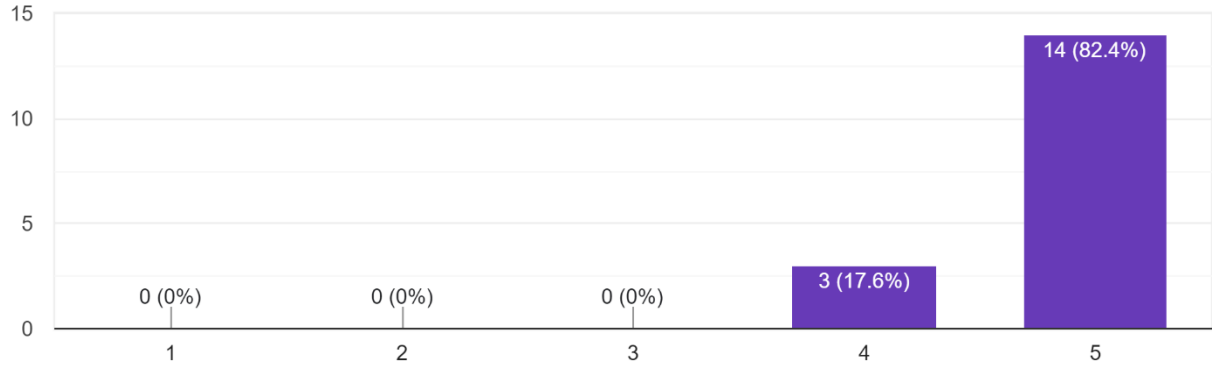
SESSION II:Results from ongoing research on crustacean diseases- comments, feedback, inputs2 responses

Very well organized

No

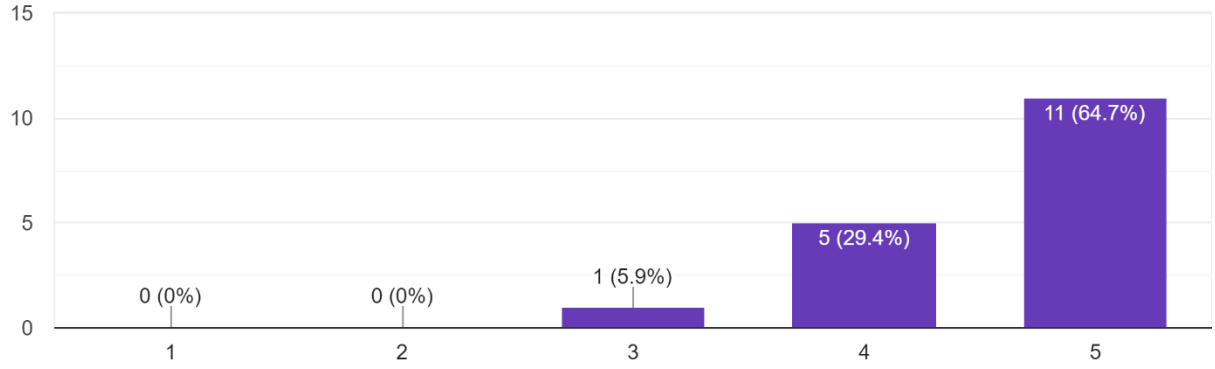
SESSION III:Update on EU listed crustacean diseases and their control- quality of the presentations

17 responses



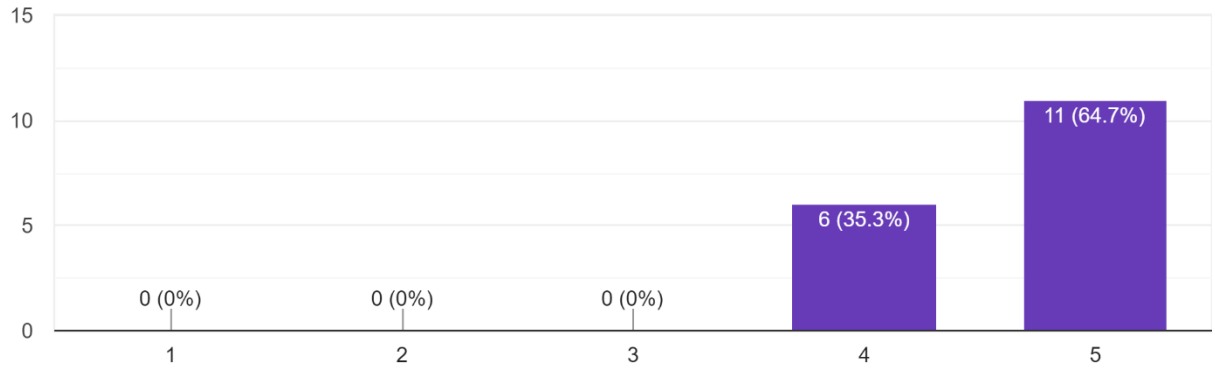
SESSION III:Update from the EURL for crustacean diseases- relevance for you

17 responses



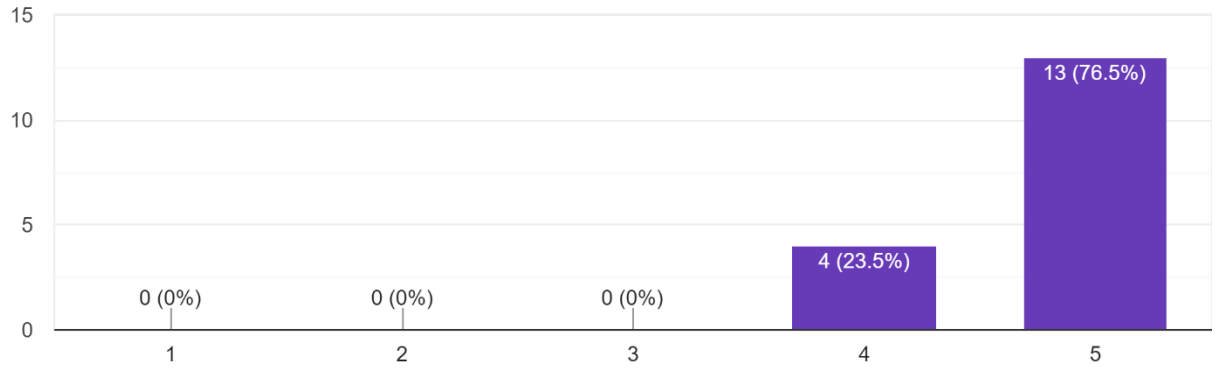
SESSION III:Update from the EURL for crustacean diseases- increase of your knowledge

17 responses



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

17 responses



SESSION III:Update from the EURL for crustacean diseases- comments, feedback, input3

responses

Very informative

No

We had no internet, and I could not donwnload the abstracts pdf during the day. It would be nice to have them printed again next year.

Greetings and conclusions of the meeting

The tentative dates for the next meeting will be the 30th of May – 1st of June 2023. It will be organized as a virtual meeting in Lyngby, Denmark. 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