



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

**Report of the  
15<sup>th</sup> Annual Workshop of the National Reference  
Laboratories for Crustacean Diseases**

Kgs. Lyngby, Denmark

May 30<sup>th</sup> 2024



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

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## Introduction and short summary

The 15<sup>th</sup> Annual Workshop of the National Reference Laboratories for Crustacean Diseases was held on 30<sup>th</sup> of May 2024. There were 68 participants attending the workshop in person, representing 34 countries.

The workshop was held back-to-back with the 28<sup>th</sup> 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. The first session had the title “Update on EU listed crustacean diseases and their control” and started with Head of the EURL for Fish and Crustacean Diseases Niccoló Vendramin 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 from NRL of the Netherlands Marc Engelsma on investigations of recurring mass mortalities in crabs in the Netherlands.

The second session had the title: “Results from ongoing research on crustacean diseases”. David Strand from Norwegian Veterinary Institute gave an update on molecular methods for diagnosing Crayfish plague and eDNA detection. Later on, invited speaker Peter Mohr from CSIRO in Australia provided an overview for emerging crustacean viruses in imported prawns. Finally, Professor Hans Nauwynck from Gent University gave two talks, one on behavioural fever as defence mechanism for shrimp infected with WSSV, and another on Hanamora an optimised cell culture medium for crustacean cells cultivation in vitro.

Session III had the title “Update from the EURL for Crustacean Diseases” and started with Senior scientist Argelia Cuenca, who gave a talk on the participation and expected results of the interlaboratory proficiency tests for crustacean diseases in 2024. Finally, Head of EURL for Fish and Crustacean Diseases Niccoló Vendramin presented the EURL activities in 2023-2024 and plans for 2025.

Lone Madsen and Anna Luisa Farias Alencar from DTU Aqua took minutes from the meeting, and Niccoló Vendramin 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 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 planned to be held in person at the end of May 2025.

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

Niccoló Vendramin

## Programme

*Thursday May 30<sup>th</sup>*

*Annual Workshop of the National Reference Laboratories for Crustacean Diseases*

- 9.30 – 9:40      Welcome and announcement  
*Britt Bang Jensen*
- SESSION I:      Update on EU listed crustacean diseases and their control**  
*Chair: Britt Bang Jensen and minutes: Lone Madsen*
- 09:40 – 10:00      Survey and diagnosis of shrimp production and diseases in Europe 2023  
*Niccoló Vendramin*
- 10:00 – 10:20      Investigations of recurring Mass mortalities in crabs in the Netherlands  
*Marc Engelsma*
- SESSION II:      Results from ongoing research on crustacean diseases**  
*Chair: Lone Madsen and minutes: Anna Luiza Farias Alencar*
- 10:20 – 10:40      Update on Crayfish plague diagnostics and eDNA detection  
*David Strand*
- 10:40 – 11:00      Surveillance of imported prawns for DIV1, CMNV and EHP  
*Peter Mohr*
- 11:00 – 11:20      Coffee Break**
- 11:20 – 11:40      Behavioral fever protects shrimp against white spot syndrome'  
*Hans Nauwynck*
- 11:40 – 12:00      Development of a new cell culture medium (HaNaMoRa medium) for the growth of organ explants and cell cultures to study viral infections'  
*Hans Nauwynck*
- SESSION III:      Update from the EURL for crustacean diseases**  
*Chair: Niccoló Vendramin and minutes Teena Klinge*
- 12:00 – 12:25      EURL proficiency test for crustacean diseases 2024

12:25 – 12:45

EURL Work done in 2024 and ideas and plans for 2025

Next meeting and end of 15<sup>th</sup> Annual Workshop

**End of meeting**

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

Chair: Britt Bang Jensen

## Surveillance and diagnostics of crustacean diseases in Europe 2023

Niccoló Vendramin

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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:

- 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 19 countries have so far been obtained and will be compared to the data received for since 2019.

An overview of health situation of crustacean farmed in Europe and trends will be discussed.

Tobia Pretto from NRL in Italy was asked to comment on the situation of *Callinectes sapidus* in Italy. It was reminded from DG Sante that WOAHP has revised standards for some diseases of Aquatic animals including WSS.

**Investigations of mass mortalities in crabs and lobsters in the Netherlands**  
**Marc Engelsma<sup>1</sup>, Betty van Gelderen<sup>1</sup>, Michal Voorbergen-Laarman<sup>1</sup>, Kelly Bateman<sup>2</sup>**

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*2 World Organisation for Animal Health (WOAH) Collaborating Centre for Emerging Aquatic Animal Diseases, CEFAS, Weymouth, UK*

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**Abstract**

In September 2023 divers reported notable numbers of dead crabs and lobsters in the Oosterschelde area in the Netherlands. Subsequently, shellfish farmers noted a considerable number of dead crabs on their mussel beds and lobster fisherman reported observing mortalities in caught lobsters. In early October crabs and lobsters were collected for investigation of the mortalities at WBVR using histology, bacteriology and molecular tools. The samples tested negative for the listed pathogen White Spot Syndrome Virus (WSSV). Further investigation, with assistance from CEFAS, did not reveal a consistent veterinary cause of the mortalities. In the crabs a number of pathogens were observed: *Sacculina carcini*, *Hematodinium* sp. and the recently described Amoebic Crab Disease, *Janickina feisti*. The latter was observed in 14 out of 20 crabs but it is not clear to what extent the amoeba infection contributed to the mortalities. In the collected lobsters there were no indications for the presence of pathogens. In conclusion, no known infectious disease agents were identified in the lobster samples.

In the spring of 2024, after the opening of the lobster fisheries season the population of commercial size lobsters seemed diminished. As weakened lobsters were caught, they were collected for disease and toxicological investigations. Results of these investigations are pending.



## **SESSION II: Results from ongoing research on crustacean diseases**

Chair: Lone Madsen

## Update on crayfish plague diagnostics and eDNA detection

David A. Strand

Norwegian Veterinary Institute

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### Abstract

Crayfish plague is a lethal disease for European freshwater crayfish, caused by the parasitic oomycete *Aphanomyces astaci*. The parasite is listed among the 100 of the world's worst invasive species and is largely spread through human mediated translocation of North American crayfish that acts a vector for the parasite. Crayfish plague diagnostic is commonly based on *A. astaci* specific qPCR, followed by sequencing for confirmation. However, a newly discovered and describes oomycete, *Aphanomyces fennicus*, is amplified with the WOH recommended qPCR, causing false positive detection. We have developed and published an improved *A. astaci* assay that does not amplify *A. fennicus*. The improved *A. astaci* qPCR assay has been verified across three different national reference laboratories for fish and crustacean diseases, using the respective laboratories workflow. The improved assay shows similar sensitivity with the WOH recommended qPCR and was highly specific for *A. astaci*, not amplifying *A. fennicus* or other closely related *Aphanomyces* spp. Additionally, sequences from environmental DNA samples collected in Norway, suggest the presence of *A. fennicus* in Norwegian waters.

Environmental DNA (eDNA) monitoring is rapidly becoming an important and non-invasive approach to monitor aquatic micro and macroorganisms. From a single watersample one can detect species of interest or even whole communities. Environmental DNA can also be used to monitor the presence of pathogens and its hosts in aquatic environments. We have developed and used eDNA monitoring tools for *A. astaci* and freshwater crayfish. We have implemented eDNA monitoring in lakes or rivers hit by crayfish plague. In addition to screen the collected water samples for *A. astaci*, the samples are analysed for the native noble crayfish (*Astacus astacus*) and the invasive signal crayfish (*Pacifastacus leniusculus*), giving compliment information on the habitat status of freshwater crayfish. Using eDNA monitoring we have revealed ongoing crayfish plague outbreaks and are able to follow the spread of the pathogen through the water course.

I will present results from our work with the improved *A. astaci* assay and from our eDNA monitoring of *A. astaci* in Norway.

### Q&A

Q. You have been using the improved assay since 2023?

A. We have been using both assays in parallel to see how it works. There could be inhibition in the qPCR in difficult samples.

## Surveillance of imported prawns for DIV1, CMNV and EHP

Peter Mohr

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Geelong, Victoria, Australia*

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### Abstract

To inform the “Review of the biosecurity risks of prawns imported from all countries for human consumption (the Prawn Review)”, the Department of Agriculture, Fisheries and Forestry (DAFF) contracted the ACDP Fish Diseases Laboratory (AFDL) to test 90 batches of imported commodity prawns for decapod iridescent virus 1 (DIV1), covert mortality nodavirus (CMNV) and *Enterocytozoon hepatopenaei* (EHP). Each batch consisted of 65 prawns, which were tested as 13 pools of 5 prawns (n=1170 samples). All 90 batches were tested negative for DIV1 and CMNV by real-time PCR. More than three quarters of the batches had at least one sample test positive for EHP by real-time PCR. A total of 66 batches generated conventional PCR amplicon nucleotide sequences that shared 100% identity with EHP reference sequences. The PCR and sequencing results confirmed that EHP nucleic acid was present in the samples, but not whether the EHP detected was viable or infectious.

### Q&A

Q. Can you elaborate about the pooling approach?

A. We follow approach guided by competent authorities, effect of pooling for surveillance of crustacean diseases is assessed in an internal project.

Q. How did you select the specific diseases to be tested?

A. There was a review that covered some diseases and the information for those are already available. For these 3 the information was lacking.

# **Behavioral fever protects shrimp against white spot syndrome**

## **Mostafa Rakhshaninejad, Liping Zheng and Hans Nauwynck**

*Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*  
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### **Abstract**

Both endotherms and ectotherms may raise their body temperature to limit pathogen infection. Endotherms do this by increasing their basal metabolism; this is called ‘fever’. Ectotherms do this by migrating to warmer places; this is called ‘behavioral fever’. White spot syndrome virus (WSSV) is the most lethal pathogen of cultured shrimp. This study examined the existence of behavioral fever in WSSV-infected *Penaeus vannamei* shrimp. Shrimp weighing  $15 \pm 0.5$  g were inoculated intramuscularly with WSSV and kept in a four-compartment system (4-CS) with all the chambers at 27 °C or with a thermal gradient (27–29–31–33 °C). During the first 4 days post-inoculation, 94% of the WSSV-inoculated shrimp died in the 4-CS with a fixed temperature (27 °C), while only 28% died in the 4-CS with a temperature gradient. The inoculated animals clearly demonstrated a movement towards the warmer compartments, whereas this was not the case with the mock- and non-inoculated animals. With primary lymphoid organ cell cultures, it was demonstrated that the increase of temperature from 27–29 °C to 31–33 °C inhibits virus replication. It is concluded that behavioral fever is used by shrimp to elevate their temperature when infected with WSSV. Behavioral fever prevents WSSV infection and mortality.

### **Q&A**

Q. Have you tested the animals to see viral load?

A. No, tested by immunofluorescence to see if positive or negative.

Q. Have you tried to expose the animals to WSSV to see if they are protected against infection

A. This has been done in a previous study.

Q. Wouldn't this design of pond put the animals at risk for constant infection unless they are cleared out occasionally?

A. If animals are exposed, they have to be checked if they develop some immunity. It has to be investigated in the field.

# Development of a new cell culture medium (HaNaMoRa medium) for the growth of organ explants and cell cultures to study viral infections

Mostafa Rakhshaninejad, Liping Zheng and Hans Nauwynck

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## Abstract

Based on the composition of hemolymph, a new cell culture medium was composed for shrimp. It got the name HaNaMoRa medium. This medium is patented and will be commercialized. It will be distributed worldwide by a private partner under a license. In addition, other solutions were produced for shrimp research: HaNaMoRa anticoagulant, HaNaMoRa PBS and HaNaMoRa transfection medium. With these products, several studies were already performed, demonstrating their value for research. (i) White spot syndrome virus (WSSV) replication was examined in organ explants from the cuticular epithelium of shrimp and cell cultures of ovaries and lymphoid organs. (ii) DNA and RNA transfections were successful in ovary and lymphoid organ cell cultures. (iii) Cytotoxicity was demonstrated with non-adherent lymphocyte-like haemocytes against xenogenous cells and WSSV-infected homogenous ovary and lymphoid organ cells. Videos of the technologies of how to make the different organ explants and cell cultures will be launched on the internet in the future, helping scientists to work *in vitro* with shrimp cells. In a long term, these products will boost shrimp research.

## Q&A

Q. Do you have antibodies for WSSV?

A. Yes, it was brought by a Korean student we have collaboration with.

Q. How can one get the media?

A. It is currently being produced. The company is being made as well. Contact if you need some media and it hasn't been made available yet.

Q. How many days are the cells kept from the explant?

A. Weeks. They need to proliferate. There is a problem using fetal calf serum and it has to be replaced.

Q. What are the species from explants you have tried so far?

A. Vannamei, monodon and some lobsters.

Q. Is the Osmolarity related to the marine environment? Would it be the same for freshwater species?

A. We tried to adapt the salinity in L15 media. This was not tested in this media.

Q. Which kind of support did you use for cell adhesion? What temperature do the cells grow at?

A. The cells grow in the temperature of 27C. We use adherent plastics and sometimes gelatin coated plastic. All the information will be available in the company website.

## **SESSION III: Update from the EURL for crustacean diseases**

Chair: Niccoló Vendramin

**2024 Inter-laboratory proficiency test for crustacean diseases**  
**Argelia Cuenca, Jytte Rasmussen, Teena Vendel Klinge, and Niccoló Vendramin**

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**Abstract**

In April 2024 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 nine test samples. 24 laboratories in 18 EU member states signed up for the test. The expected pathogen in each FTA card will be presented.



## **EURL for Crustacean Diseases, work done in 2023**

**Niccoló Vendramin and the EURL team**

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### **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 14<sup>th</sup> Annual Workshop of the National Reference Laboratories for Crustacean Diseases was held on site at DTU premises, physically on 1st of June 2023. There were 38 participants attending the workshop in person, representing 22 countries. There were three sessions, with 12 presentations.

The annual proficiency test for crustacean diseases (PT) was divided into a WSSV test panel and a TSV/YHV test panel with 25 laboratories including 18 NRLs of EU Member States accepted the invitation to participate and send in their test results for diagnostic assays not derogated to other laboratories. 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.

During 2023, 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](http://www.eurl-fish.eu)), to provide consultancy to NRL's and finally to attend international meetings and conferences.

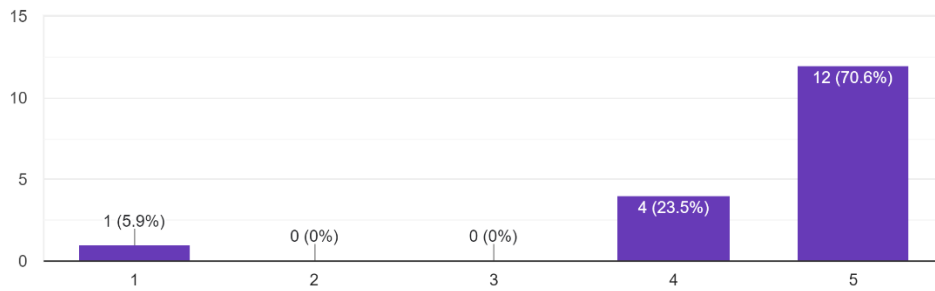
Significant efforts were put into the technical advisory to EFSA in working groups for vector species for listed crustacean diseases. From the laboratory side, the EURL further assessed the analytical characteristics (specificity and sensitivity) of the following methods by participating in Inter-Laboratory proficiency test provided by CSIRO in IMNV, AHPND, IHNNV.

## 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.

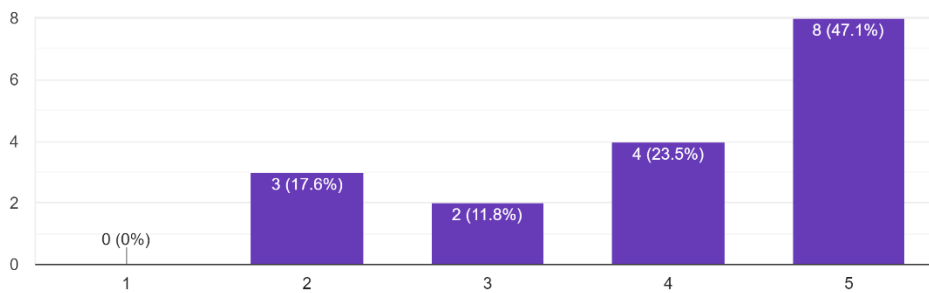
### SESSION I:Update on EU listed crustacean diseases and their control- quality of the presentations

17 responses



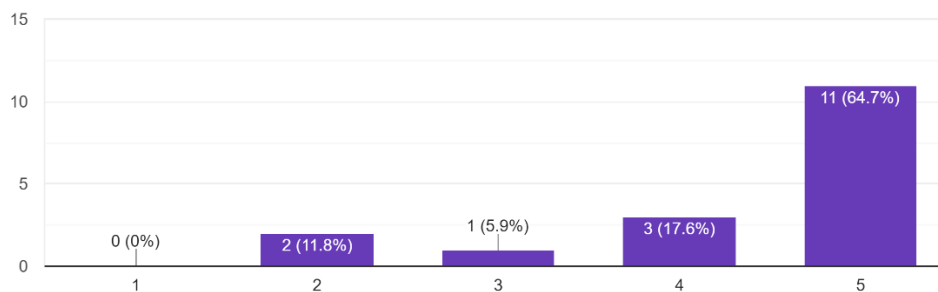
### SESSION I:Update on EU listed crustacean diseases and their control- relevance for you

17 responses



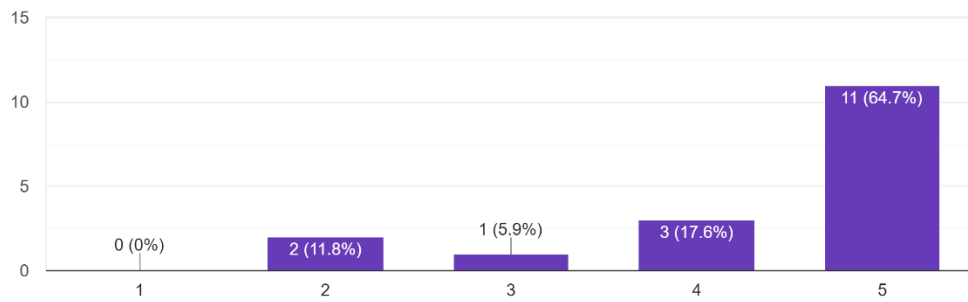
### SESSION I:Update on EU listed crustacean diseases and their control- increase of your knowledge

17 responses



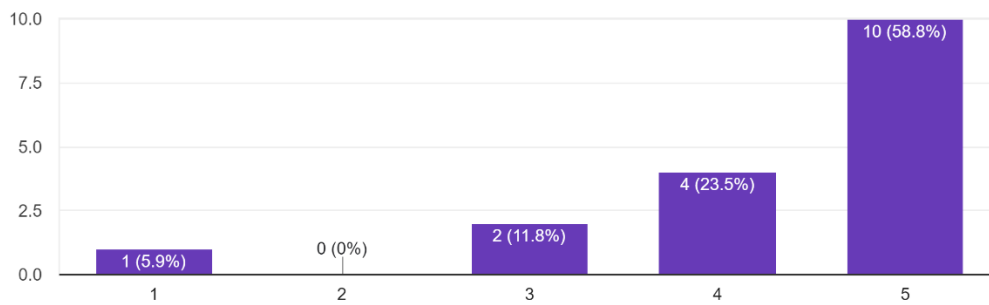
SESSION I:Update on EU listed crustacean diseases and their control- increase of your knowledge

17 responses



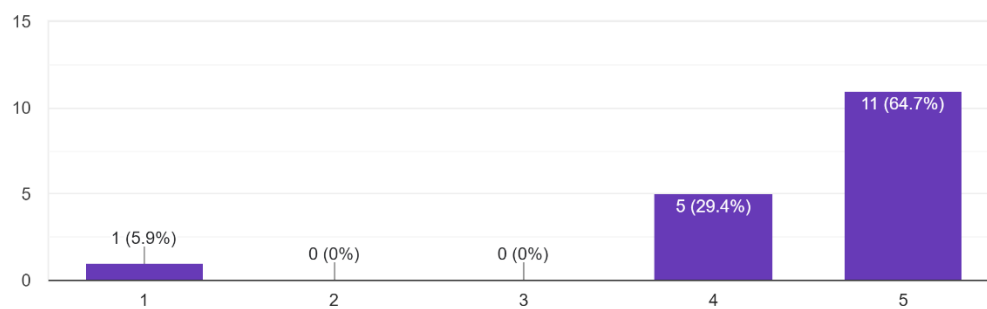
SESSION I:Update on EU listed crustacean diseases and their control- overall score

17 responses



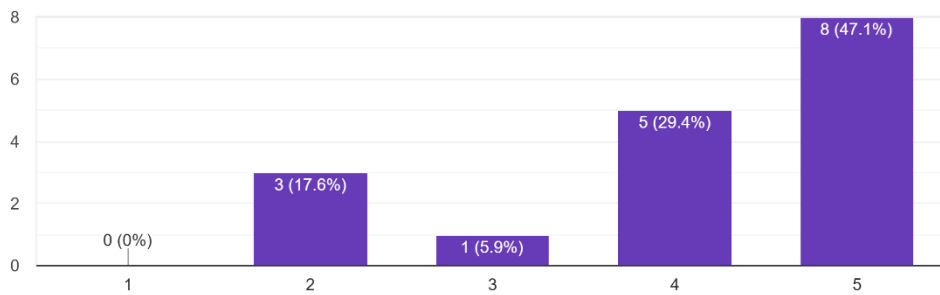
SESSION II:Results from ongoing research on crustacean diseases- quality of the presentations

17 responses



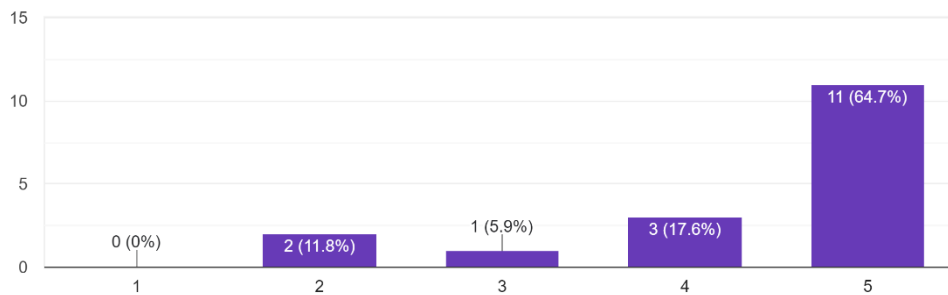
SESSION II:Results from ongoing research on crustacean diseases- relevance for you

17 responses



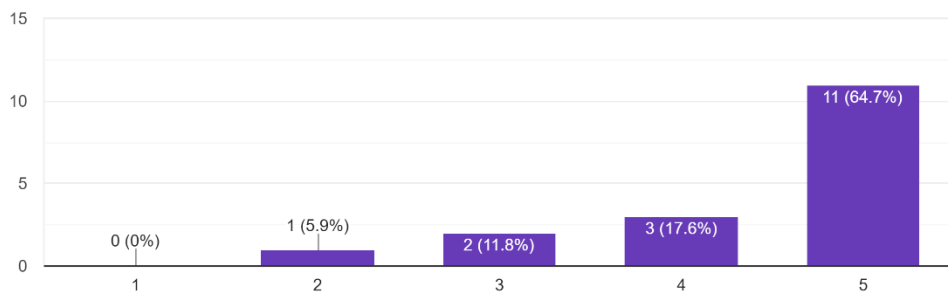
SESSION II:Results from ongoing research on crustacean diseases- increase of your knowledge

17 responses



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

17 responses

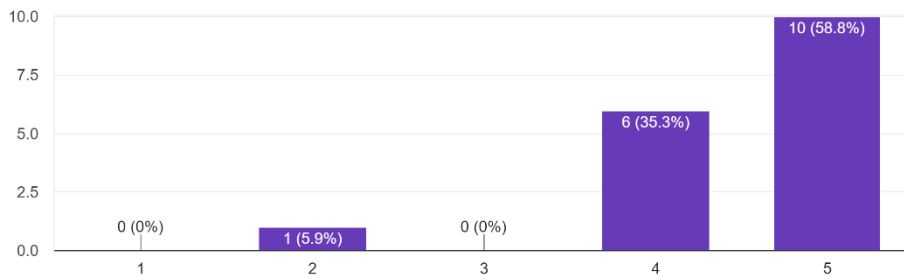


COMMENTS:

All topics covered were well presented and of interest.  
We don't have any crustacean breeding in Slovakia.  
it would be very much appreciated if the sessions would not be scheduled on a catholic holiday, which is also an official holiday in several member states

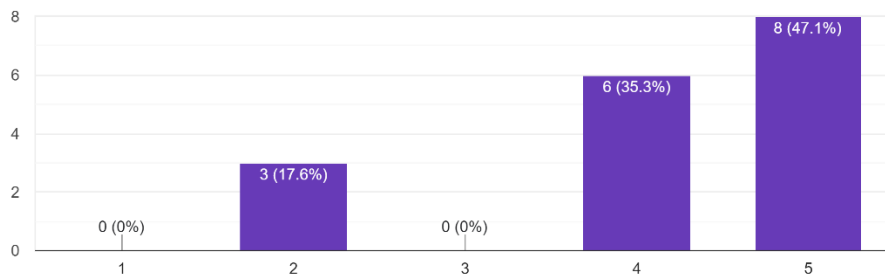
SESSION III: Update on EU listed crustacean diseases and their control- quality of the presentations

17 responses



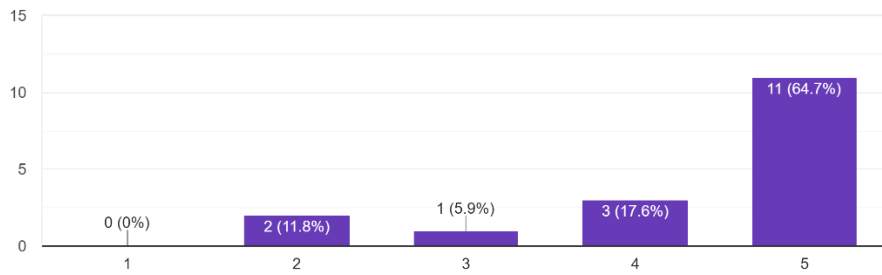
SESSION III: Update from the EURL for crustacean diseases- relevance for you

17 responses



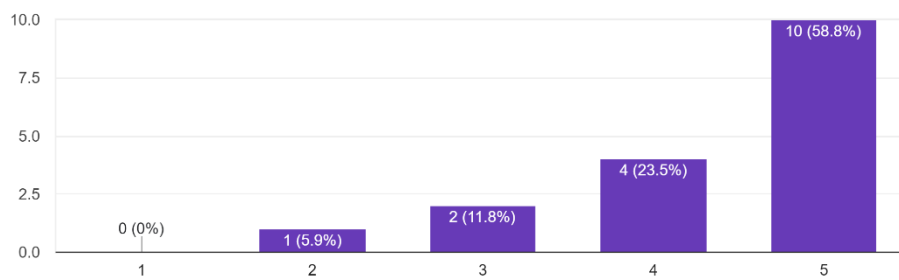
SESSION III: Update from the EURL for crustacean diseases- increase of your knowledge

17 responses



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

17 responses



### COMMENTS

Interesting discussion about PT program experiences

We don't have any crustacean breeding in Slovakia.

it would be very much appreciated if the sessions would not be scheduled on a catholic holiday, which is also an official holiday in several member states

## Greetings and conclusions of the meeting

The tentative dates for the next meeting will be the in the end of May 2025. It will be organized as a on site physical 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.