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

Report of the

13th Annual Workshop of the National Reference Laboratories for Crustacean Diseases

Kgs. Lyngby, Denmark

June 1st 2022

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 13th Annual Workshop of the National Reference Laboratories for Crustacean Diseases was held virtually on 1st of June 2022. Because of the Covid-19 pandemic and the resulting limitations on travelling to and from Denmark, the workshop was held in hybrid form: 30 participants attended the workshop in person, and 45 were registered to attend 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, 73 participants from 33 countries attended the workshop. There were three sessions with a total of 10 presentations.

The workshop was held back to back with the 26th Annual Workshop for National Reference Laboratories for Fish Diseases and a a special session for NRL in EU and EEA on the implementation of the Animal Health Law. The workshop was opened with "Welcome and announcements" by 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 Morten Schiøtt giving an update 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 Natasja Cox from IMAQUA in Belgium giving a presentation on WSSV experimental infection models.

The second session had the title: "Results from ongoing research on crustacean diseases". Kelly Bateman from CEFAS in UK gave an online presentation on the OIE collaborating centre for emerging aquatic animal diseases hosted by CEFAS. Next, Anna Aspán from the National Veterinary Institute of Sweden and Valentina Paolini from IZSVe gave a shared presentation on a newly developed qPCR assay for the detection of *Thelohania contejeani* in crayfish. This was followed by a presentation by Fiona Swords from The Marine Institute Ireland giving an update on the Irish National Crayfish Plague Surveillance Programme. After a break, Peter Bossier from Ghent University talked about the use of essential oils to combat vibriosis in crustacean larvicultures. Next, Yussian Rovi Alfiansah from Alfred Wegener Institute gave an overview of shrimp production in EU, and presented a study on the gut microbiome of shrimp. The last speaker of the session was Evelien Swaef from IMAQUA in Belgium who gave a presentation about the optimization of a qPCR procedure for the detection of IHHNV in shrimp.

Session III had the title "Update from the EURL for fish diseases" and started with EURL coordinator Morten Schiøtt giving a talk on the results of the interlaboratory proficiency tests for crustacean diseases in 2022. Finally, the workshop ended with Morten Schiøtt presenting the EURL activities in year 2021 and 2022, and proposals for the EURL work plan for 2023.

Lone Madsen and Morten Schiøtt 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 and crustacean 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 2023, 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

Wednesday June 1st
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 EU listed crustacean diseases and their control**
	Chair: Lone Madsen and minutes: Morten Schiøtt
09:40 – 10:00	Surveillance and diagnostics of crustacean diseases in Europe *Morten Schiøtt*
10:00 – 10:20	Recent advances on WSSV experimental infection models *Natasja Cox*
SESSION II:	**Results from ongoing research on crustacean diseases**
	Chair: Morten Schiøtt and minutes Lone Madsen
10:20 – 10:40	OIE collaborating centre for emerging aquatic animal disease *Kelly Bateman*
10:40 – 11:00	Telohania diagnostics *Tobia Pretto/Anna Aspan*
11:00 – 11:20	Update on the Irish National Crayfish Plague Surveillance Programme *Fiona Swords*
11:20 – 11:40	***Coffee break***
11:40 – 12:00	The use of essential oils to combat vibriosis in crustacean larviculture *Peter Bossier*
12:00 – 12:20	Shrimp production in Europe & diversity of potential pathogenic bacteria in digestive tracts of shrimp *Yustian Rovi Alfiansah*
12:20 – 12:40	Optimization of a qPCR procedure for detection of IHHNV in shrimp *Evelien Swaef*
SESSION III:	**Update from the EURL for crustacean diseases**
	Chair: Niccoló Vendramin and minutes Argelia Cuenca
12:40 – 13:00	EURL proficiency test for crustacean diseases 2022 *Morten Schiøtt*

13:00 – 13:20 EURL Work done in 2021, plan for 2022 and ideas and plans for 2023
Niels Jørgen Olesen
Next meeting and end of 13th Annual Workshop
Niels Jørgen Olesen

End of meeting

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

Chair: Lone Madsen

Surveillance and diagnostics of crustacean diseases in Europe

Morten Schiøtt and Niels Jørgen Olesen

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

Questions and comments:

No questions or comments

Recent advances in WSSV experimental infection models

Natasja Cox[1*], Evelien De Swaef[1], Mathias Corteel[1], Wim Van Den Broeck[4], Peter Bossier[3], João J. Dantas-Lima[1], Hans J. Nauwynck[2]

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Abstract

White spot syndrome virus (WSSV) can cause a cumulative mortality up to 100% within 3-10 days on shrimp farms (Dey et al., 2020). Although progress has been made, a better understanding of the pathogenesis and transmission route(s) of WSSV is still necessary. This work relies heavily on the establishment of standardized *in vivo* experimental infection models that can be used as a tool for studying WSSV pathogenicity and for evaluating the efficacy of WSSV mitigation strategies. The objective of this study was to develop standardized and reproducible individual and group challenge models for experimental WSSV infection studies in *Litopenaeus vannamei* whereby 100% of clinical WSSV infection can be induced. By testing different experimental conditions to obtain this clinical outcome, we aim to learn more about WSSV transmission.

When individually housed shrimp received either 4, 5, 6 or 7 doses of WSSV positive solid inoculum within 24h, this did not result in a significantly different mortality (A1 = 16.7±4.7%; B1 = 20.0±9.4%; C1 = 20.0±9.4%, D1 = 20.0±18.9%). However, in case of daily re-inoculation (up till 4 days), a significantly lower mortality was observed in group A2 (28.9±16.8%, one day) compared to group D2 (57.8±3.8%, 4 consecutive days). When shrimp were housed in groups, the accumulated mortality reached 100%. In none of the trials, mortality was observed in the Mock treatment. For each experiment, WSSV infection was confirmed by qPCR in a sample of the dead shrimp. WSSV was absent in sampled survivors and negative controls.

The probability or risk of infection in the individually housed population increased when the inoculation procedure was repeated on subsequent days. Increasing the number of doses that are given within 24h did not significantly raise the chances of a clinical infection. When shrimp were individually housed, a clinical infection of 100% was not observed. This level could only be reached during the group challenge. This was a striking result, because the individually housed shrimp and the group-housed shrimp consumed the same amount of inoculum. In addition, during the group challenge cannibalization of sick shrimp was virtually absent. How WSSV transmission exactly occurs remains unknown. Proposed routes include consumption of infected tissue, water-borne transmission, and entry via the antennal gland during urination (de Gryse et al., 2020). After analysis of the results, it was hypothesized that shrimp behaviours associated with cohabitation and high stocking densities, such as increased aggression and urination, play a major role in a WSSV outbreak.

De Gryse et al., (2020) *Proc. Natl. Acad. Sci. U.S.A., 117*(45), 28374–28383.
Dey, B. K. et al., (2020). *Rev. Aquac.*, *12*(2), 822–865.

Questions and comments:

Q: Was WSSV found in the water?
A: No quatitative measuring of WSSV in water was performed, but they are looking into to optimizing the current method.

Q: Are there any difference between genetic lines when it comes to the effect of disease?
A: Seems like there are some differences, but has not been checked in details.

SESSION II: Results from ongoing research on crustacean diseases

Chair: Morten Schiøtt

Recent activities 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. 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 disease surveillance globally. Here we provide an overview of the CCEAAD and report on recent work in the field of crustacean diseases, a novel haplosporidian parasite in jelly shrimp (*Acetes sibogae australis*) from Australia and Amoebic Crab Disease in edible crabs (*Cancer pagurus*) from the English Channel.

Questions and comments:

Q: Is there a PCR method for identification of *Janickina feisti*?
A: Yes, there is – it will be available in the DAO publication Bateman et al. (soon to be released).

Q: Could shore crabs in Holland be infected too, and have populations in other countries been screened?
A: Kelly has checked crabs from other countries (among them France) by PCR but not found any positive. Until now the finding of *Janickina feisti* has only been seen in this specific batch of crabs in the UK.

qPCR detection of *Thelohania contejeani* and its application on clinical samples of *Austropotamobius pallipes* complex and other NICS

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Abstract

In European crayfish species, "cotton-tail" disease is caused by a microsporidium, *Thelohania contejeani*, which infects and multiplies mainly in crayfish muscle tissues (skeletal, cardiac, intestinal musculature) and in the nerve cord. Heavily infected tissues appear porcelain white, which has given the disease its common name. *Thelohania contejeani* can be a growth-limiting factor in crayfish populations, so the development of a sensitive and specific detection method is desirable.

Nowadays, the detection of this pathogen relies often on histologic examination. This approach is less sensitive than molecular biology techniques. Furthermore, similar macroscopic clinical signs in the *Austropotamobius pallipes* complex can be produced by other microsporidian species such as *Nosema* (*Vairimorpha) austropotamobii*, and new *Astathelohania* species have been recently described in North American crayfish (Stratton et al., 2022).

Here we present our evaluation of a qPCR method developed in 2020 by Anna Aspán and Tomas Jinnerot (National Veterinary Institute, Sweden). We tested 44 DNA samples extracted from abdominal muscle tissue of macroscopically infected and unaffected specimens from different crayfish species: *Austropotamobius pallipes* complex, *Pacifastacus leniusculus, Procambarus clarkii*, *Orconectes limosus* and *Cambaroides japonicus.* Samples were previously characterized using two different end-point PCR methods specific for *T. contejeani*, both targeting the small subunit ribosomal RNA gene: a PCR with primers from El-Matbouli and Soliman, 2006 and a nested PCR according to Imhoff et al., 2010. For most of the samples, also histological examination was performed.

This qPCR proved to be an effective method to detect *T. contejeani,* allowing to identify the presence of the pathogen also in co-infection with another microsporidian parasite *Nosema (Vairimorpha) austropotamobii* in 9 samples. The specificity for *T. contejeani* was confirmed obtaining at least one negative result analysing an *A. pallipes* highly infected with *Nosema (Vairimorpha) austropotamobii.* Further analyses are needed to verify the sensitivity and specificity of this method on DNA extracted from environmental samples and complex matrices.

Questions and comments:

No questions or comments

Results from the second Irish National Crayfish Plague Surveillance Programme

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Abstract

Austropotamobius pallipes (White-clawed crayfish; WCC) is the only crayfish species indigenous to Ireland. While the Irish population of WCC is still considered the healthiest in Europe, the latest species assessment under Article 17 of the EU Directive on the Conservation of Habitats, Flora and Fauna (92/43/EEC), reports their future prospects and overall status as bad. The main threats to this endangered and protected species are habitat destruction, the introduction of non-native species and the risk posed by *Aphanomyces astaci* (*A.astaci*), the causative agent of crayfish plague.

Since 2015, several outbreaks of crayfish plague have decimated crayfish populations in some Irish river systems. In response to the spreading plague, an environmental DNA (eDNA) based National Crayfish Plague Surveillance Programme was established in 2018. Following the successful first 2-year cycle in 2018-2019, a new collaboration between the National Parks and Wildlife Service and the Marine Institute was established with a second 2-year surveillance programme beginning in July 2020. The results from the 2020-2021 survey will be presented here with details of the temporal spread of crayfish plague in Ireland and estimated WCC population densities from DNA detection in the environment.

One point of note, the pathogen was not detected in previously confirmed crayfish plague-positive catchments for the first time in 2021, while detections of the WCC eDNA signatures remained steady. The experimental outcomes of additional sampling time points and concurrent eDNA and field ecology surveillance will also be discussed, along with future research plans to further understand and monitor the outbreaks of crayfish plague in Ireland, its vectors, and its impact on the native WCC. We hope to aid the relevant national bodies in developing disease control measures to protect the endangered WCC in Ireland.

Questions and comments:

Q: Do some of the white-clawed crayfish survive an outbreak of CFP?
A: Yes, they do.

Q: Are any mitigation measures considered in Ireland?
A: Right now – no bans regarding transfers between water catchments exists, but instead an awareness campaign of how to avoid transfer of CFD.

Q: If there are no invasive crayfish species in Ireland, then how did the agent come to Ireland?
A: Probably by human transferring/recreational things. This might also be the reason for the transfer of the agent between water catchments.

C: It has not been possible to genotype *A. astaci* based on eDNA samples. This is on the list of priorities.

Essential oil against vibriosis in crustacean cultures

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Abstract

Vibriosis is still an important problem in crustacean cultures, as also aggravated by the AHPND disease.

There are many attempts to work out alternative strategies for treating or preventing vibriosis. One of them is the use of essential oils.

Essential oils are complex mixtures of compounds produced by living organisms and isolated by physical means only (pressing and distillation) from a whole or plant part of known taxonomic origin The main compounds are mainly derived from three biosynthetic pathways only, (i) the mevalonate pathway leading to sesquiterpenes, (ii) the methylerythritol pathway leading to mono- and diterpenes, and (iii) the shikimic acid pathway leading to phenylpropenes. Overall, the main chemical classes of EOs are classified as aliphatic (e.g. neral, citronellal), aromatic (e.g. cinnamaldehyde), carboxylic acids (e.g. isovaleric acid), coumarins (e.g. coumarin), diterpenes (e.g. phytol, taxadiene), diterpenoles (e.g. sclareol), ester (e.g. linalyl acetate), ketones (e.g. pule.g.one), lactones (e.g. alantolactone), monoterpenes (e.g. limonene ocimene), monoterpenoles (e.g. linalool, thujanol), oxides (e.g. 1,8-cineol), phenols (e.g. carvacrol, thymol), phenol methyl ethers (e.g. methyl chavicol), phthalides (e.g. sedanolide), sesquiterpenes (e.g. chamazulene), sesquiterpenoles (e.g. viridiflorol, carotol), others (e.g. allicin).

In order the assess the effectivity of essential oils and the mode of action in vitro and in vivo a series of tests were developed to verify essential oils, essential oils compounds and mixtures of essential oil compounds.

We evaluated the antibacterial activity of essential oils (n = 22) or essential oil components (EOCs, n = 12) against Vibrio strains belonging to the harveyi clade. It was verified by three different approaches, e.g., (i) a bacterial growth assay, comparing Vibrio growth with or without EO(C)s at various concentrations; (ii) a vapor-phase-mediated susceptibility assay, comparing the e_ect of EO(C)s on bacterial growth through the vapor phase; and (iii) a quorum sensing-inhibitory assay, based on specific inhibition of quorum sensing-regulated bioluminescence. The results showed that, in the bacterial growth assay, EOs of Melaleuca alternifolia and Litsea citrata at 0.0001%, Eucalyptus citriodora at 0.01% can inhibit the growth of Vibrio campbellii BB120.

To determine in vivo EOs' potential protective effect towards gnotobiotic brine shrimp Artemia franciscana, challenged with V. campbellii. The study showed that brine shrimp larvae supplemented with EOs of M. alternifolia (0.0008%) and L. citrata (0.002%) displayed significantly increased survival against V. campbellii. The results indicated that supplementation of these EOs increased the expression of immune-related genes (either in the presence or absence of the pathogen), probably contributing to enhanced protection. Furthermore, in vitro studies indicated that some EOs modulated the expression of virulence factors including swimming motility, biofilm formation, and gelatinase and lipase activity, while flow cytometry data and regrowth assay indicated that these EOs do not exhibit antimicrobial activity as V. campbellii grew at the tested concentrations [M. alternifolia (0.0008%) and L. citrata (0.002%)]. Our findings suggest that EOs extracted from M. alternifolia and L. citrata, can modulate virulence factor production and immunological responses.

At full scale cinnamaldehyde was tested in larvae and postlarval stages of *L. vannamei*. The LD50 of cinnamaldehyde at the Protozoea I stage (7.70 μM) and Mysis I stage (6.78 μM) were lower ($P<0.05$) than the LD50 at postlarval stages (28.97 -30.94 μM). In a luminous vibriosis outbreak at shrimp hatchery (Yaguacam, Cienfuegos, Cuba), the *L. vannamei* postlarvae that received cinnamaldehyde treatment had a higher survival rate (35.8±15.4%) than the postlarvae without cinnamaldehyde treatment (20.6±6.4%).

Questions and comments:

Q: Have you also done the experiment using multiresistent Vibrios?
A: There exists one paper with *Vibrio parahaemolyticus* where that strain is found to be less sensitive than *V. campbellii*. Many laboratory strains of *V. campbellii* are resistant to many antibiotics.

Q: How is the gut microbiome affected by the essential oils?
A: This has not been investigated, but Peter Bossier thinks that because of the very low concentration, it might have very low effect on the gut microbiome.

Q: Are the essential oils costly?
A: No, so it might be an idea to use more than one at a time.

Shrimp production in Europe and diversity of potential pathogenic bacteria in digestive tracts of shrimp

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Abstract

Pacific white-leg shrimp (*Penaues vannamei*), blue shrimp (*Litopenaeus stylirostris*) and kuruma shrimp (*Marsupenaeus japonicus*) are the most cultured shrimp species in Europe. In 2020, European shrimp production reached up to 450 tonnes, produced by 25 aquaculture companies using bio-flocs and clear water systems. In addition, 9 companies in Europe serve as hatcheries and produce post-larvae for European shrimp farms. Productions of market-size shrimps as well as post-larvae in Europe are predicted to be increased due to consumer's preference. No mass mortality of cultured shrimp due to bacterial diseases has been reported from the European shrimp farms. However, effort to minimize bacterial disease outbreaks which may devastate the European shrimp farming is still challenging. Therefore, it is important to understand bacterial community composition in the digestive tract of shrimps including potential pathogens, and compare them to the disease status. Moreover, a robust detection method is needed to predict the pathogenic bacteria rapidly and precisely. We examined bacterial community composition in healthy and diseased shrimp samples covering fresh-water and saline shrimps such as *Macrobrachium nipponense, P. vannamei*, *L. stylirostris* and *M. japonicus*. We found that pathogenic bacteria of the genus Aeromonas, Alteromonas, Flavobacterium, Photobacterium, Pseudoalteromas, and Vibrio are the most common in the intestines of shrimps. We developed specific primer pairs to detect the thermolabile hemolysin (*tlh*) gene, a toxin inherent but not exclusively to the Vibrio genera to predict the risk of *Vibrio*-related disease outbreaks. Here, we developed a SYBR®Green qPCR assay to simultaneously target the *tlh* gene. Primers were experimentally validated against *V. alginolyticus*, *V. campbellii*, *V. harveyi*, *V. parahaemolyticus* and *V. vulnificus*, as well as *V. anguillarum* and *Bacillus subtilis* to constrain their taxonomic coverage and determine their specificity for *Vibrio*, thereby enabling the quantification of pathogenic *Vibrio* without the need of multiple species-specific markers. We obtained a couple of primers (tlh-G-vibrio-0515-a-S-22: GCTGGTTCTTRGGDCAYTTCTC, tlh-G-vibrio-0771-a-A-22: TGGAACGCYACGGTTRTAGT TC) as the best primer pair candidate that is able to amplify *tlh* with a melting temperature range of 83.5 - 85°C, a limit of quantification of log 3 gene copies/ng genomic DNA, and a limit of detection at qPCR cycle 34-36. Then, we tested the system over 113 shrimp samples and obtained 55 positive samples with a range of 3.5 ± 1.5 to 4.3 ± 1.6 log copies tlh gene/ng genomic DNA, which equal to 3,200-20,000 Vibrio cells. Our approach offers versatile applications for monitoring and disease prevention management in commercial aquaculture. In addition, this primer pair is also suitable for detecting *tlh* via conventional PCR.

Questions and comments:

Q: Is the tlh gene supposed to be a marker gene or is it a virulence gene?
A: It is not known to be a virulence gene.

Q: Are the reference strains proven to be pathogenic to shrimp?
A: Yes.

Optimization of a qPCR procedure for detection of IHHNV in shrimp

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Abstract

Decapod penstyldensovirus-1 (PstDV-1), formerly infectious hypodermal and hematopoietic necrosis virus (IHHNV), is causing frequent infections in shrimp hatcheries and grow out ponds. PstDV-1 can be detected throughout all life stages of *Litopenaeus vannamei* and transovarian transmission has also been reported. PstDV-1 is replicated in ectodermal and mesodermal tissue. The target organs are therefore gills, epithelium, connective tissues, haematopoietic or lymphoid organs, the antennal gland and nervous system (Lightner, 2001). Infections can be acute or more chronic of nature. In an acute fase, mortality can reach quickly to 100%. In more selected families, the infection can be very subtle, resulting in chronic disease and runt-deformity syndrome, which causes growth suppression and deformities rather than mortality. When breeding is performed with infected broodstock, a reduced hatching of the eggs is observed, as well as a lower survival and performance of the resulting (post) larvae (Motte et al., 2003). Detecting PstDV-1 infection is therefore of crucial importance for the quality of animals and progeny. But in the chronic phase, PstDV-1 infections are not always easy to observe. Determination of (the number of) viral genomes in infected animals by PCR has become one of the most important means of monitoring shrimp diseases, and it is especially useful for detecting viral infections in asymptomatic individuals.

At IMAQUA, diagnostic PCRs are performed on a regular basis. In addition, qPCRs are available for both pathogens and a set of immune related genes. Mainly from an economical point of view, all PCR procedures are SYBR green based and therefore needed to be optimized starting from the often TaqMan based protocols described in literature. Recently, for PstDV-1, the IMAQUA (q)PCR protocol was further improved to ensure results with a higher degree of certainty and to allow detection of the pathogen in situations with low pathogen pressure (chronic infection). First, looking at the melting curve, it was observed that primers were amplifying material with a melting temperature very close to the melting temperature of the target amplicon. It was expected that this was linked to excessive primer concentrations or the presence of inhibitors in the tissue of the *L. vannamei* shrimp. Reducing the primer concentrations did not show any difference in the melting curve. Diluting tissue samples 1:10 however, did remove the undesired peak in the melting curve as well as low amplification observed in negative samples across the qPCR run. Diluting 1:100, resulted in insufficient amplification in positive tissue samples. Secondly, in some cases, a melting curve with a clear peak at the correct melting temperature was observed, but Ct-values were undetermined. It was suspected that the PstDV-1 virus was present, but at levels that could not be detected by the qPCR assay. This was solved by subjecting the animals to non-lethal temperature stress prior to sampling. Animals were kept at a lower temperature for 24 h followed by normal husbandry conditions for 48 h. This induced viral replication and consequently higher viral loads were detected in target tissues when using our optimized qPCR procedure.

This case study shows how the continuous optimization of our qPCR procedures could improve sensitivity and certainty in routine PstDV-1 detection.

References:

Motte et al. (2003). Aquaculture, 219, 57–70. , Lightner & Redman (1998). Aquaculture, 164, 201– 220.

Questions and comments:

Q: Is the (out of line) PCR fragment sequenced?
A: Yes, and it was negative.

Q: Why develop a new PCR, when there is a fully validated qPCR already?
A: That is done because the laboratory use SYBR green PCR methods.

Q: How often do you get requests from other laboratories on using the method?
A: At least once a month.

Q: Which tissues are collected and analyzed from stressed shrimp?
A: Gills and muscle tissues are used.

SESSION III: Update from the EURL for crustacean diseases

Chair: Niccoló Vendramin

2022 Inter-laboratory proficiency test for crustacean diseases

Morten Schiøtt, Teena Vendel Klinge and Niels Jørgen Olesen

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Abstract

In April 2022 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 are asked to identify the WSSV positive pleopods among five test samples. 25 laboratories in 18 EU and 2 EFTA member states signed up for the test. The test material for the TSV/YHV test consisted of FTA cards incubated with tissue extracts of TSV infected shrimp, YHV-1 infected shrimp or non-infected shrimp. The participants are asked to identify the TSV and YHV positive samples among six test samples. 17 laboratories in 12 EU member states signed up for the test.

The results from the tests will be presented in the talk.

Questions and comments:

No questions or comments

EURL for Crustacean Diseases, work done in 2021

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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.

Due to Covid-19 pandemic and related travelling restrictions, most meeting activities had to be converted to on-line events, whereas the activities that required travelling were cancelled.

The 12th Annual Workshop of the National Reference Laboratories for Crustacean Diseases was held virtually, using the zoom platform, on 2nd of June 2021. The virtual organization of the meeting allowed for a significant expansion of the number of participants attending the workshop as well as the number of oversea countries participating. The number of participants thus reached 77 participants from 40 countries. There were two sessions with ten presentations in total. On May 31st, a workshop on the new Animal Health Law (AHL) was organised. 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 introduce the new legislative framework, 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 26 laboratories participating in the former and 17 in the latter. The tests were sent from the EURL 17th of June 2021. 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 2021 the EURL uploaded a diagnostic manual for WSSV on the EURL website and initiated the drafting of diagnostic manuals for TSV and YHV.

During 2021, 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.

Questions and comments:

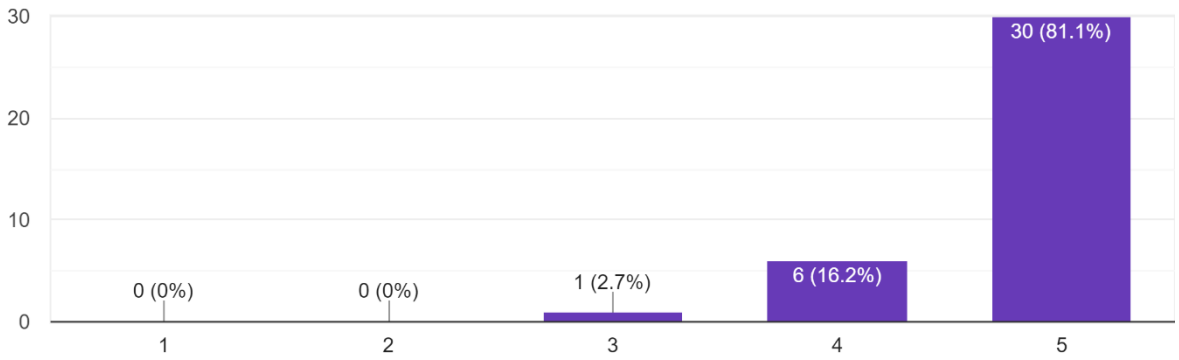
No questions or comments

Workshop evaluation

A questionnaire was delivered to the participants asking to evaluate various aspect of the workshop. An overview of the 37 questionnaires retrieved is shown below. Specific comments are going to be considered for the next annual workshop organization.

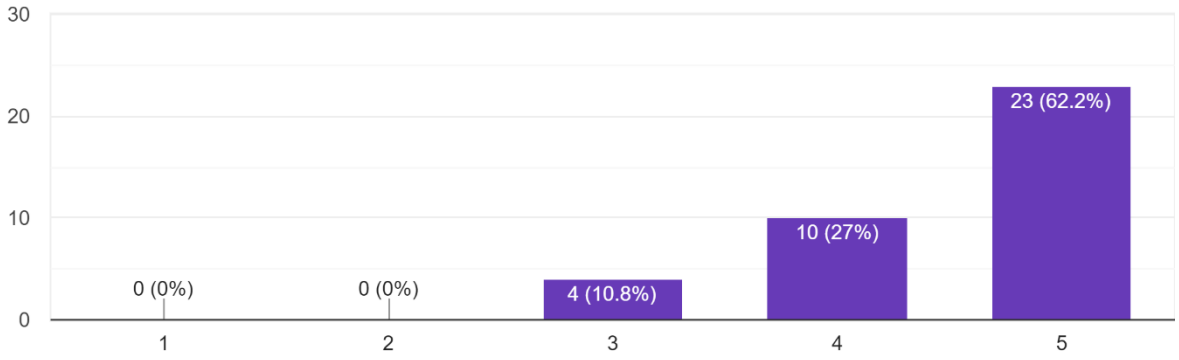
SESSION I:Update on important crustacean diseases and their control- quality of the presentations

37 responses



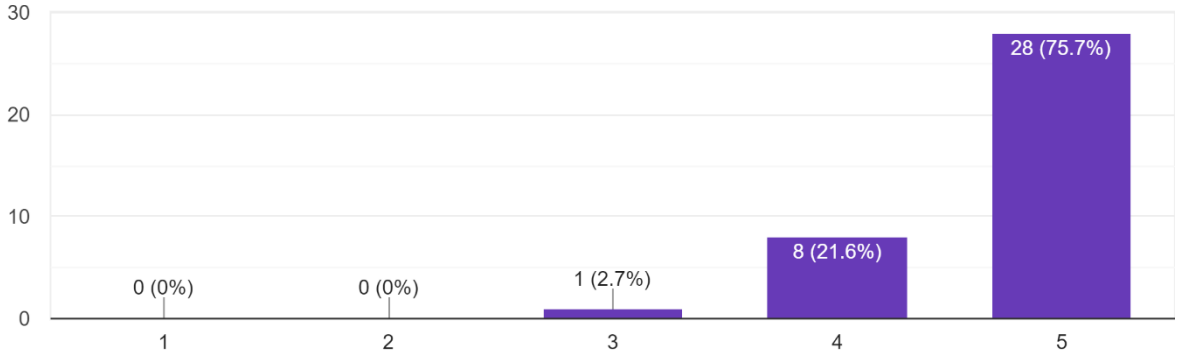
SESSION I:Update on important crustacean diseases and their control- relevance for you

37 responses



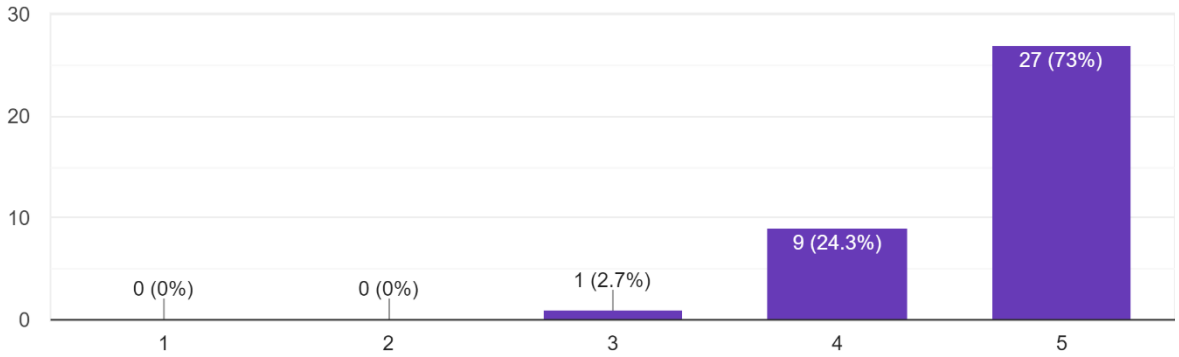
SESSION I:Update on important crustacean diseases and their control- overall score

37 responses



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

37 responses



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

7 responses

Quality of the images in TEM and histology was excellent

Very useful. There were both general and very specific topics included

Thanks againa

nice and interesting presentation

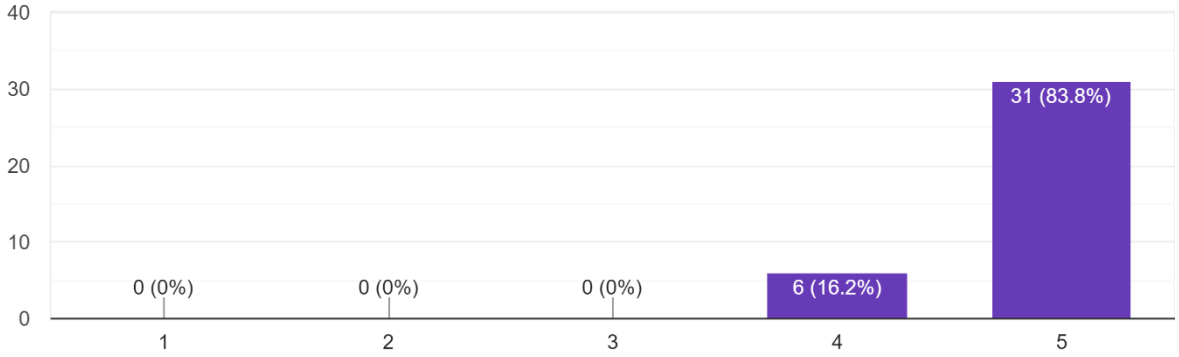
excellent workshop

Excellent organization

Very interesting lectures, although presentation of Fiona too detailed regarding locations in Ireland, but in general of interest (eDNA)!

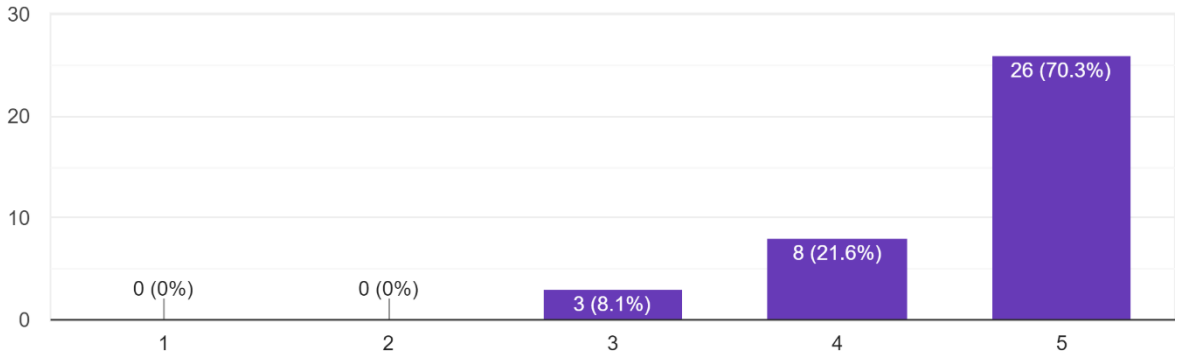
SESSION II:Update from the EURL for crustacean diseases- quality of the presentations

37 responses



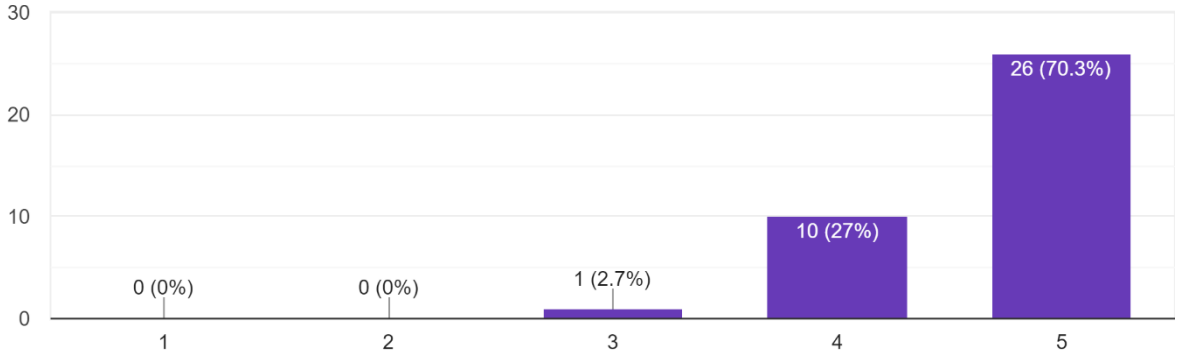
SESSION II:Update from the EURL for crustacean diseases- relevance for you

37 responses



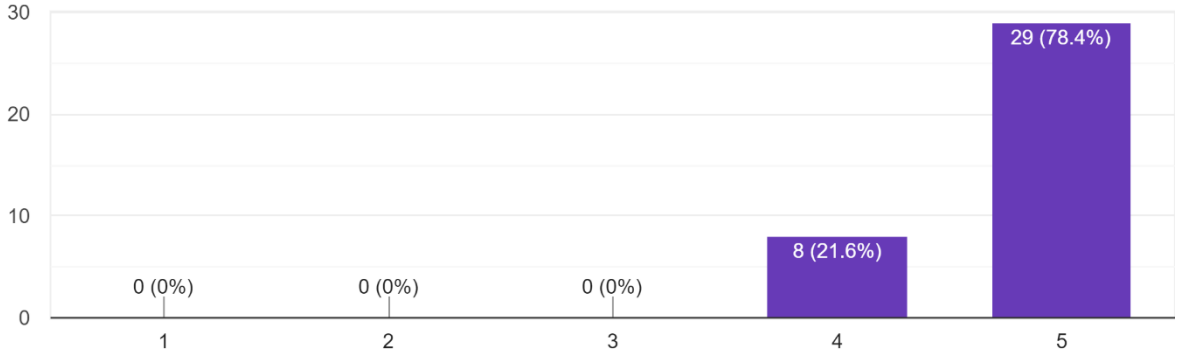
SESSION II:Update from the EURL for crustacean diseases- increase of your knowledge

37 responses



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

37 responses



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

8 responses

To hear about other diseases explained so clearly was very engaging. The speakers employed really good diagrams and the topics were interesting even if not immediately relevant to my job - it is always stimulating to hear others talk enthusiastically about their work. Wonderful workshop.
Lovely, informative presentation. Definitely increased my knowledge in crustacean research and advancements.
nice and interesting presentation
excellent workshop
Excellent presentations

The Surveillance data are for the first time collected. Next year we can see the dynamics. The ILPT announcement was very clear, important.
keep going!

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 most likely be organized as a 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