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

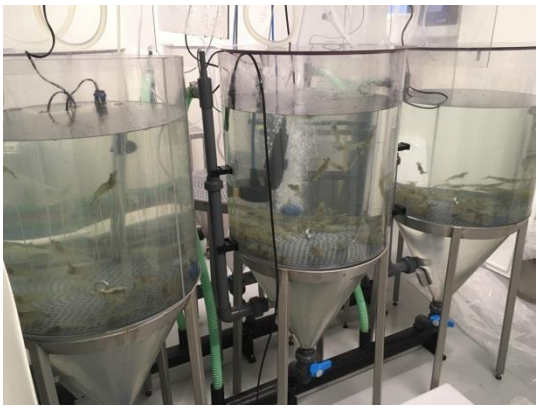
Report of the

10th Annual Workshop of the National Reference Laboratories for Crustacean Diseases

Kgs. Lyngby, Denmark

May 29th 2019

Sampling pleopods of *P.vannamei*



P.Vannamei in experimental tank facilty

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 10th Annual Workshop of the National Reference Laboratories for Crustacean Diseases was held 29th of May, at DTU Aqua, 2800 Kgs. Lyngby, Denmark. This was the first Annual workshop for crustacean diseases held at our premises in Kgs. Lyngby.

A total of 43 participants from 26 countries attended the one day workshop. All presenters arrived to the workshop, thus, no last minute changes were made in the programme. Unfortunately, the expert from Sweden, dr. Anna Aspan was not able to attend the workshop and chairman for the 3rd session was therefore changed. There were four sessions with in total 16 presentations, eight of which were given by invited speakers;

1) Tobia Pretto, from Italy, (Istituto Zooprofilattico Sperimentale delle Venezie), gave two talks, one on co-infection by *Mycobacterium gordonae* and CqBV in red-claw crayfish *Cherax quadricarinatus* and one on Crayfish plague outbreak in *Austropotamobius pallipes*: application of genotype-specific PCR for strain identification in clinical samples.

2) Trude Vrålstad from Norway, (Norvegian Veterinary Institute) provided two talks, one on update on e-DNA for detection of presence of *A. Astaci* and one on optimized qPCR that discriminate the new *Aphanomyces fennicus.*

3) Kelly Bateman from UK (CEFAS - former EURL for Crustacean Diseases) provided four talks: Emerging diseases in crustacean; NGS technologies and survey of a shrimp pond before and during a disease outbreak; The inter-laboratory proficiency test for crustacean diseases in 2018 organized by CEFAS; Update on the OIE Collaborating Centre for Emerging Aquatic Animal Diseases and future collaboration with the EURL and NRLs.

The scientific programme of the Annual Workshop covered many topics. The workshop was opened with "Welcome and announcements" by Head of the EURL for crustacean diseases, Niels Jørgen Olesen. The scientific part was opened with Session I Update on crustacean disease issues in Europe. After coffee break, Session II on Emerging diseases hosted two presentations and a round table discussion. In this last part, a PowerPoint presentation with all crustacean diseases listed in the OIE diagnostic manual had been prepared. All three invited speakers and dr. Satu Viljamaa-Dirks were asked to comment on different aspects of diseases, including their impact in Europe and the recommended diagnostic methods.
After lunch Session III on research and network activities took place. This included a broad spectrum of issues, including relevant legislative framework for listed crustacean diseases in Europe, the use of NGS to monitor microbial community disease outbreaks in shrimp farming, the establishment of a new OIE collaborating center for Emerging Aquatic Animal diseases at CEFAS; and finally the use of advanced challenge methods to study WSSV in penaeids. The workshop was concluded with Session IV Update from the EURL. This session included two presentations on Inter-laboratory proficiency tests for crustacean diseases (2018 and 2019), one presentation on Validation of qPCR for WSSV, as well as a presentation on the EURL crustacean diseases workplan for 2020.
In in the evening, participants were taken to Tivoli in Copenhagen, and a social dinner at restaurant "Færgekroen" was organized.

Employees from DTU Aqua (Juliane Sørensen, Argelia Cuenca, Camilla Priess, Niccolò Vendramin and Lone Madsen) took minutes from the meeting. Niccoló Vendramin and Lone Madsen have

assembled a draft of the report, which was sent to all the presenters and participants, who asked and answered questions during the presentations, for correction in order to avoid misunderstandings.

We would once again like to thank all the presenters for their valuable contributions, without them the meeting would not have been a success. The workshop and meeting was organized by a team consisting of Teena Vendel Klinge, Lone Madsen, Argelia Cuenca, Niccoló Vendramin and Niels Jørgen Olesen, with the help from the rest of the fish and crustacean diseases unit at the National Institute of Aquatic Resources, DTU AQUA. The meeting next year is tentatively planned to take place back to back with the Fish Diseases AW the 3rd and 4th of June 2020, also 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.

Lone Madsen, Niccolò Vendramin and Niels Jørgen Olesen

Programme

Wednesday May 29th
Annual Workshop of the National Reference Laboratories for Crustacean Diseases

08:50 – 9:20	**Registration**
09:20 – 09:30	Welcome address and local announcements *Lone Madsen, Niccoló Vendramin and Niels Jørgen Olesen*
09:30 – 09:40	Introduction to the new EU reference laboratory for fish and crustacean diseases *Niels Jørgen Olesen*
SESSION I:	**Update on Crustacean disease Issues in Europe**
	Chair: Lone Madsen *and minutes: Niccoló Vendramin*
09:40 – 10:00	Co-infection by *Mycobacterium gordonae* and CqBV in red-claw crayfish *Cherax quadricarinatus*. *Tobia Pretto*
10:00 – 10:20	Update on e-DNA for detection of presence of *A. Astaci* *Trude Vrålstad*
10:20 – 10:35	Crayfish plague outbreak in *Austropotamobius pallipes*: application of genotype-specific PCR for strain identification in clinical samples *Tobia Pretto*
10:35 – 10:50	Optimized qPCR that discriminate the new *Aphanomyces fennicus* *Trude Vrålstad*
10:50 – 11:10	***Coffee break***
SESSION II	**Emerging diseases** *Chair: Amedeo Manfrin and minutes: Jacob Schimdt*
11:10 – 11:30	Overview of emerging disease issues in crustacean including Hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei* (EHP) and shrimp hemocyte iridescent virus (SHIV). *Kelly Bateman*
11:30 – 11:50	Bacterial health in Dutch insect culture*Olga Haenen*
11:50 – 12:30	Listing infectious diseases in crustaceans and selection of diagnostic method (Plenum discussion). Chaired by *Niels Jørgen Olesen*

12:30 – 13:30 ***Lunch***

SESSION III Research and network activities

Chair: Anna Aspan and minutes: Lone Madsen

13:30 – 13:50 NGS technologies and survey of a shrimp pond before and during a disease outbreak. *Diana Minardi*, presented by *Kelly Bateman*

13:50 – 14:20 State of play of recent EU aquatic animal health legislative developments and their implementation
Laszlo Kuster

14:20 – 14:40 Update on the OIE Collaborating Centre for Emerging Aquatic Animal Diseases and future collaboration with the EURL and NRLs. *Kelly Bateman*

14:40 – 15:00 Challenge models for experimental infections in crustaceans
João Lima- To be confirmed

15:00 – 15:30 ***Coffee break***

SESSION IV: Update from the EURL-1

Chair Niccoló Vendramin and minutes Juliane Sørensen

15:30 – 15:50 The inter-laboratory proficiency test for crustacean diseases in 2018 organized by CEFAS – *Kelly Bateman*

15:50 – 16:10 EURL activities 2018 -Accreditation of WSSV diagnosis
Camilla Priess

16:10 – 16:30 The proficiency test 2019
Argelia Cuenca

16:30 – 16:50 EURL Work Plan 2019-20
Niels Jørgen Olesen

16:50 – 17:10 Next meeting and end of 10th Annual Workshop

17:30 – ***Bus transport to Hotel Cabinn City***

19:15 - ***TIVOLI***

19:30 – ***BANQUET dinner at Tivoli***

SESSION I: Update on Crustacean disease Issues in Europe

Chair: Lone Madsen

Co-infection by *Mycobacterium gordonae* and CqBV in red-claw crayfish *Cherax quadricarinatus*

Nadav Davidovich[1], Tobia Pretto[2] & Rona Grossman[3]

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Abstract

Chronic mortalities in red-claw crayfish raised in an Israelian RAS hatchery were investigated. Broodstock and juveniles showed lethargy, tendency to lie on the side and melanized spots on the cuticle. Cytological examination of the hepatopancreas (Hp) revealed melanized foci with acid fast bacilli (Z-N stain), while bacteriological analysis succeeded in isolating Mycobacteria colonies that were identified as *Mycobacterium gordonae* by amplification of 23S rRNA gene, followed by reverse hybridization (Richter et al., 2006). Histological examination confirmed melanised haemocytic aggregations in haemal spaces of the Hp and gills, seldom revealing numerous acid-fast bacilli. Moreover, intranuclear inclusion bodies were recorded in cells of the tubular epithelium of the Hp and were referable to *C. quadricarinatus* bacilliform virus (CqBV) (Anderson & Prior, 1992). Cuticles from moribund specimens tested negative for *Aphanomyces astaci* by real-time PCR. It could be speculated that the sub-optimal thermal condition of the facility (21°C) and the viral infection may promote the proliferation of *Mycobacterium gordonae*, which is mostly saprophytic but occasionally described in immunocompromised poikilothermic animals and humans.

Questions and comments

Questions about survival and transmission were asked, and Tobia Pretto answered that there is a low pathogenic infection present in natural populations in Australia, and he forwarded the question regarding transmission to Kelly Bateman, who answered that the infections happens when there are external damages of the animals. It was commented that the virus can also be found in cyprinids, wherefore the question was if the virus had also been found in connection with open water. Tobia Pretto answered that the virus only had been found in connection with cherax. A question regarding the zoonotic potential of this mycobacterium was raised, and Tobia Pretto answered that Mycobacterium gordonae is low virulent. The final question concerned the relationship between the two pathogens. Tobia Pretto answered that the virus was found in every specimen, all age classes, but with different intensities. Spot-like lesions in the hepatopancreas caused by the mycobacterium were found in 7-9 cherax with granuloma.

Update on environmental DNA (eDNA) monitoring of *Aphanomyces astaci* and freshwater crayfish

Trude Vrålstad[1], David A. Strand[1], Stein Ivar Johnsen[2], Johannes C. Rusch[1,3], Sune Agersnap[4], William Brenner Larsen[4], Steen Wilhelm Knudsen[4], Peter Rask Møller[4]

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[2] *Norwegian Institute for Nature Research*

[3] *University of Oslo*

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Abstract

Environmental DNA (eDNA) methodology has become an important non-invasive tool to monitor freshwater micro- and macro-organisms. From a single water sample, it is possible to detect several species of interest or even whole communities. eDNA can be used to reveal elusive targets, such as alien invasive species at an early stage or rare and endangered species. eDNA can also be used to investigate presence/absence and relative quantities of pathogens in water. Over years, we have developed and used eDNA monitoring tools for *Aphanomyces astaci*, the causative agent of crayfish plague, in combination with eDNA monitoring of susceptible- and tolerant freshwater crayfish.

In a recently published study, we used eDNA monitoring to follow a crayfish plague outbreak in a large water course, and compared it to traditional crayfish plague monitoring that uses cages with live crayfish as "canaries in a coalmine". We show that eDNA- monitoring corresponds well with the biological status measured in terms of crayfish mortality and trapping results. It reveals the presence of *A. astaci* in the water up to 2.5 weeks in advance of the cage- method. eDNA estimates of *A. astaci* concentration and noble crayfish numbers increased markedly during mortality, and vanished quickly thereafter, demonstrating the dynamics during an outbreak of crayfish plague. The eDNA monitoring also provided a snapshot of the presence, absence or disappearance of crayfish regardless of season, and constitutes a valuable supplement to the trapping- method that relies on season and legislation.

One of the main benefits of eDNA monitoring is the possibility for temporal and spatial monitoring of several organisms from the same water samples. The simultaneous eDNA monitoring of *A. astaci* and relevant native and invasive freshwater crayfish species is well-suited for early-warning of invasion or infection, risk assessments, habitat evaluation and surveillance regarding pathogen and invasive/native crayfish status. In Norway, eDNA monitoring have replaced the traditional cage dependent crayfish plague monitoring in the national surveillance program of crayfish plague, and was recently also implanted as a supplement method in the national surveillance program for noble crayfish and spread of signal crayfish.

Questions and comments

Questions focused on the strategies to eradicate the disease. Trude Vrålstad answered that Norway have eradicated crayfish in two areas with BETAMAX and heavy environmental impact. It can be difficult to do a stamping out and repopulation, as crayfish stay below the mud, wherefore it is needed to dry the area. Another question was on if there existed breeding lines free from crayfish plaque in Norway, where to Trude Vrålstad answered no.

Crayfish plague outbreak in *Austropotamobius pallipes*: application of genotype-specific PCR for strain identification in clinical samples.

Tobia Pretto

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Abstract

A crayfish plague episode in white-clawed crayfish, characterized by rapid and high mortality, was recorded in a restocking facility in Tuscany (Foreste Casentinesi National Park) in October 2018. Broodstocks collected from nearby Apennine creeks and maintained in concrete tanks, provided with river water (10-12°C), showed increased mortality after one month from the introduction, reaching 100% mortality after 20 days. Clinical symptoms referable to *Aphanomyces astaci* infection were recorded: ataxia, paralysis and dead specimens lying supine. Molecular analyses for *A. astaci* were performed on specimens dead during the outbreak based on real-time PCR (Vrålstad et al., 2009) with positive results, confirmed by end-point PCR (Oidtmann et al., 2006) and sequence analysis. End-point PCR with genotype-specific primers (Minardi et al., 2018) and sequence analysis revealed the presence of *A. astaci* genotype D carried by the allochthonous species *Procambarus clarkii*. The most probable route of introduction and case-history of previous outbreaks in restocking facilities will be discussed.

Questions and comments

There were no questions.

Update on the on-going development and validation of a new *Aphanomyces astaci* specific qPCR method that excludes the recently described species *Aphanomyces fennicus* sp.nov.

Trude Vrålstad[1], David A. Strand[1], Elin Rolén[1], Sirpa Heinikainen[2], Satu Viljamaa-Dirks[2]

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Abstract

The first quantitative qPCR method for specific detection of *Aphanomyces astaci* (the crayfish plague pathogen) targeting the ITS (internal transcribed spacer) region was published ten years ago (Vrålstad et al. 2009). The method was included in the OIE Manual of Diagnostic Tests for Aquatic Animals in 2012 due to its demonstrated specificity and sensitivity. It has since then been widely used for crayfish diagnostics, carrier status analyses of introduced American crayfish, and in environmental DNA (eDNA) monitoring of crayfish plague. Recently, a new species - *Aphanomyces fennicus* - was isolated and described from noble crayfish (*Astacus astacus*) in Finland. The natural distribution of this species is unknown, and there are no reports of it outside Finland. However, this new species is not discriminated by the current OIE recommended *A. astaci*- specific qPCR method, nor by any of the other published *A. astaci* specific PCR methods. Thus, it is a great need for alternative methods that rapidly discriminate *A. astaci* from *A. fennicus* in tissue samples and environmental samples. Here, we present an adjusted qPCR assay designed for species-specific detection of *A. astaci* that excludes *A. fennicus*, and at the same time maintains the discrimination against other closely related species. The adjusted assay also target the ITS-region and a 136 bp motif unique to *A. astaci*. The validation is not yet completed, so we present and discuss only preliminary results.

Questions and comments

There was agreement on that an international project on e-DNA would be a good initiative.

SESSION II: Emerging diseases

Chair: Anna Toffan

Overview of Emerging Disease Issues and New Discoveries

Dr Kelly Bateman

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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, 2016). A major constraint in achieving this goal are new and emerging aquatic animal diseases in aquaculture sectors globally. Pathogen characterisation in aquatic animals (wild and farmed) remains a core function at Cefas, here we present an overview of new and emerging diseases within shrimp aquaculture, highlighting the pathology and diagnostic testing currently available and an overview of novel pathogens from wild animals.

Questions and comments

SHIV (shrimp iridescent virus) is a problem in China and seems to be emerging. It is now renamed decapod iridovirus.

Bacterial health in Dutch insect culture

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Abstract

Insect culture develops fast, for protein for feed and food. Insects can be cultured on various left over streams. The Dutch Council on Animal Affairs (2018) warned for health risks for insects and man, and urged to support veterinary healthy, contact- and food-safe insect culture. The lectureship INVIS (Healthy, sustainable and safe insect culture for fish feed and food) of HAS cooperates with fish disease experts and epidemiologists of WBVR.

So far few bacteria are identified as pathogens for cultured insects. The aims of this pilot were bacteriology of crickets (*Gryllodes sigillatus* and *Acheta domesticus*) and morio worms (*Zophobas morio*) and their substrates and housing, during the production cycle, in the insect farm Kreca BV in the Netherlands. Isolated bacteria were identified by biochemical methods, and by MALDI-TOF (matrix assisted laser desorption/ionisation time-of-flight).

Various species of bacteria were detected, mostly commensals to humans and animals. Regarding insect pathogenic bacteria, in the morio worms, *Bacillus pumilus*, *Enterobacter cloacae*, *E. kobei*, and *Klebsiella pneumoniae* were detected. In the house cricket, *A. domesticus*, and *Lysinibacillus sphaericus* were detected in its drinking water. Some of the commensal bacteria may turn zoonotic in rare cases, only when humans are strongly immunocompromised. It was concluded, that no alarming results were found, given the fact, that standard hygiene measures are practiced to prevent for infections.

This pilot is a basis for further (inter)national research of the bacterioflora at insect farms, to support a healthy insect production chain, for healthy aquaculture and chicken feed, food, for education, and for science.

Data from literature will be presented as well.

Reference:

Dutch Council on Animal Affairs (2018). The emerging Insect industry: Invertebrates as production animals. https://english.rda.nl/publications/publications/2018/09/03/the-emerging-insect-industry

The pilot study was kindly sponsored by national research organization NWO.

Questions and comments

Comments on links between insect pathogens and crustacean diseases. According to Olga Haenen, so far, no fish and crustacean pathogens have been recorded during surveillance of insect culture in the Netherlands, but further studies are needed. A question regarding how likely it would be that pathogens found in insects would still be pathogenic after cooking was put forward. Olga Haenen answered that bacteria forming spores might be interesting, as spores are resistant to cooking treatment.

Listing infectious diseases in crustaceans and selection of reference diagnostic methods (Plenum discussion).

Niels Jørgen Olesen[1], Lone Madsen[1], Tobia Pretto[2], Kelly Batemann3

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Abstract

The OIE Aquatic Code and Aquatic Manual list 11 reportable diseases in crustaceans, while the EU list 2 diseases as Category A (Exotic) and 1 disease as Category 3 (non-exotic) but is this the most correct decision? During the session, we will present the diseases in question and discuss their listing.

	DISEASES OF CRUSTACEANS
Chapter 2.2.0.	General information
Chapter 2.2.1.	Acute hepatopancreatic necrosis disease
Chapter 2.2.2.	Infection with *Aphanomyces astaci* (Crayfish plague)
Chapter 2.2.3.	Infection with *Hepatobacter penaei* (Necrotising hepatopancreatitis)
Chapter 2.2.4.	Infection with infectious hypodermal and haematopoietic necrosis virus
Chapter 2.2.5.	Infection with infectious myonecrosis virus
Chapter 2.2.6.	Infection with *Macrobrachium rosenbergii* nodavirus (White tail disease)
Chapter 2.2.7.	Infection with Taura syndrome virus (EU List A)
Chapter 2.2.8.	Infection with white spot syndrome virus (EU List C)
Chapter 2.2.9.	Infection with yellow head virus genotype 1(EU List A)
Chapter 2.2.10.	Spherical baculovirosis (*Penaeus monodon*-type baculovirus)
Chapter 2.2.11.	Tetrahedral baculovirosis (*Baculovirus penaei*)

1. **Acute hepatopancreatic necrosis disease (AHPND)** means infection with strains of *Vibrio parahaemolyticus* (VpAHPND) that contain a ~70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB. Although there are reports of the isolation of other Vibrio species from clinical cases of AHPND, only VpAHPND has been demonstrated to cause AHPND.
2. **Infection with Aphanomyces astaci** means infection with the pathogenic agent *A. astaci* of the Family *Leptolegniaceae*, Phylum *Oomycota (*water moulds). The disease is commonly known as **crayfish plague**.
3. **Infection with Hepatobacter penaei** means infection with the pathogenic agent *Candidatus Hepatobacter penaei*, an obligate intracellular bacterium of the Order α-Proteobacteria. The disease is commonly known as **necrotising hepatopancreatitis** (NHP).

4. **Infection with infectious hypodermal and haematopoietic necrosis virus** means infection with the pathogenic agent infectious hypodermal and haematopoietic necrosis virus **(IHHNV)** Family *Parvoviridae*, Genus *Penstyldensovirus*.. The International Committee on the Taxonomy of Viruses has assigned IHHNV with the species name of Decapod penstyldensovirus 1 (King et al., 2012).
5. **Infection with infectious myonecrosis virus** means infection with the pathogenic agent infectious myonecrosis virus **(IMNV)** that is similar to members of the Family Totiviridae.
6. **Infection with Macrobrachium rosenbergii nodavirus** means infection with the pathogenic agent *Macrobrachium rosenbergiinodavirus* (MrNV), (Family Nodaviridae). The disease is commonly known **as white tail disease (WTD)**.
7. **Infection with Taura syndrome virus** means infection with the pathogenic agent Taura syndrome virus **(TSV)**, Genus *Aparavirus*, Family *Dicistroviridae*, Order *Picornavirales*.
8. **Infection with white spot syndrome virus** means infection with the pathogenic agent white spot syndrome virus **(WSSV)**, Genus *Whispovirus*, Family *Nimaviridae*.
9. **Infection with yellow head virus genotype 1** means infection with yellow head virus genotype 1 **(YHV1)** of the genus *Okavirus*, Family *Roniviridae* and *Order Nidovirales*.
10. **Spherical baculovirosis** is considered to be infection with Penaeus monodon-type baculovirus. Synonyms: MBV from P. monodon was designated PmSNPV. Although PemoNPV may be the most correct name for the virus, the term **P. monodon baculovirus (MBV)** will be used in most instances to designate this virus
11. **Tetrahedral baculovirosis** is considered to be infection with Baculovirus penaei. Synonyms: **PvSNPV** (singly enveloped nucleopolyhedrovirus from *Penaeus vannamei*).

Questions and comments

WSSV

According to Satu Viljamaa-Dirks it is difficult to list WSSV, as most countries do not have farming of the susceptible species but instead have susceptible species in the wild. Targeted surveillance of wild species is problematic. Trude Vrålstad raised the potential problem with frozen scampi and bait as well as conducting screening in wild populations. According to Kelly Bateman, CEFAS have found a number of viable pathogens when screening crustaceans from supermarkets and fish markets, and although WSSV is classified as a tropical disease, it can be viable at water temperatures of 18 degrees. David Stone said that CEFAS had done a survey in the wild without finding WSSV, wherefore he thought that the risk for the disease is low. Kelly Bateman said that when using frozen shrimp as bait, crayfish will be the first target of the disease. Laszlo Kuster asked how the import would be affected in e.g. volume, if frozen products were included in the EU legislation. Kelly Bateman answered that products should be cooked before import. The latest outbreak in Australia happened due to illegal trade.

TSV

When it comes to TSV, there are lots of species with incomplete evidence of being susceptible species though with positive PCR reaction but with no active infection. Kelly Bateman said that TSV is no longer a big issue in Asia, as they are using TSV resistant shrimps in aquaculture. Tobia Pretto commented that the only susceptible species in Europe is the blue crab, which is also an

invasive species for the area, and he argued that the list of susceptible hosts is limited. Niels Jørgen Olesen said that according to the new animal health law TSV is now a category A disease in Europe, and all European countries need to have emergency plans for exotic diseases, which has a cost. David Stone argued that one has to be consistent with the trade commodity. Trude Vrålstad argued that there seems to be a limited risk due to shrimp limitations, but Satu Viljamaa-Dirks said that there were some farms with susceptible hosts.

YELLOW HEAD DISEASE

For Yellow Head Disease, there are multiple genotypes where only one of them is associated with mortality. Niels Jørgen Olesen argued that laboratories shall be able to discriminate between genotypes. Trude Vrålstad asked if the diagnostic tests were able to make this discrimination. Argelia Cuenca and others answered that currently methods exist that either detect all the genotypes without discriminating between them and then the product can be sequenced or there are methods that specifically and only detect genotype 1. Kelly Bateman told about multiple publications regarding the matter.

AHNPD (Acute hepatopancreatic necrosis disease)

Kelly Bateman told about sampling the stomach, as the hepatopancreas is affected by toxins. AHNPD is causing issues in Thailand, but not as much as earlier due to improved management strategies. Niels Jørgen Olesen talked about the specific plasmid in the pathogen, and Kelly Bateman said that there is a PCR method specific for the plasmid. This is one of the pathogens causing EMS (Early Mortality Syndrome), which has caused massive problems in Asia. Laszlo Kutser said that discussion of EU listing or delisting should be avoided, as it is a huge task. Priorities had been given to diseases in 2006-88 and no changes had been done for crustacean in the new legislation.

CRAYFISH PLAQUE

Crayfish plaque has been an EU listed disease but is now delisted. Satu Viljamaa-Dirks said that there are no areas, where it is possible to declare freedom. Australia and New Zealand are free for the disease, so they have to be protected against invasion of the pathogen. Infection with Aphanomyces astaci shall be reported to the OIE, but American crayfish are always carriers, therefore it is a question what should be reported. Reports of the disease can be found in the scientific literature but not in the veterinary systems. Many countries do not report the finding of the pathogen to the OIE due to trading issues, e.g. Ireland reported crayfish plaque to the OIE, where after Saudi Arabia stopped import of fish from Ireland. Trude Vrålstad commented that the map is not completely covered with red as there are compartments that are free of the disease. Therefore, there should be a possibility of controlling the disease. Apparently, it is only Norway that is trying to control the disease. Thomas Wahli said that there are no measures implemented in Switzerland regarding the disease but that there is an intention to prevent American crayfish.

NHP (necrotising hepatopancreatitis)

Shrimp that are going to be farmed in Europe are tested specifically for NHP according to Kelly Bateman.

IHNNV

Kelly Bateman had the same comment for IHNNV as for NHP. The disease affects the growth but does not cause high mortalities. If the imported crustaceans are SPF certified it is fine. Kelly Bateman questioned where the pathogens originates from – maybe a wild reservoir.

IMNV

Tobia Pretto raised concern of a possible risk of introduction in the Mediterranean basin from Northern Africa, as those areas can import juveniles and broodstock from Asia and Africa without necessarily requiring a cerficate of freedom. There exists an anecdotal report of WSSV in Egypt, and host and temperatures can maintain vira in the natural populations. The direct import from high biosecurity facilities is not an issue but import from illegal or legal import from Southeast Asia is. Ornamental shrimp trade should also be considered as a risk. The latter imply all diseases. Niels Jørgen Olesen said that if regulations against importation are wanted then the justification for claiming disease freedom shall be in place for the different countries.

White tail disease

No comments due to running out of time.

General comments

It was commented that the presented list was a good overview. The question was raised: what should be prioritized in the future? Comments on that was that at least one laboratory in EU shall have in its repository all the positive materials as well as diagnostic methods. The necessity of acquiring positive controls and sharing material was stressed.

SESSION III Research and network activities

Chair Niccolò Vendramin

From seeding to emergency harvest: temporal microbiome analysis of Whiteleg shrimps *Penaeus vannamei* in a Thailand shrimp farm

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Abstract

In shrimp aquaculture, infectious disease outbreaks are considered one of the principal limiting factors to high production (Stentiford *et al*., 2017). Studies in aquatic and terrestrial animals show a strong relationship between the microbiome composition of an organ (e.g. gut, hepatopancreas) and health status of the animal (Hooper *et al*., 2002; Li *et al*., 2018). In previous studies, it has been observed that the gut microbiome of *Penaeus vannamei* (whiteleg shrimp) is mostly formed by the Proteobacteria, which does not change with diet and/or the shrimp growing environment, and by Actinobacteria, Bacteroidetes, and Firmicutes, whose proportions can instead change based on development stage, diet, environmental factors, and shrimp health status. Opportunistic and pathogenic bacteria and eukaryotes colonising the shrimp tissues can thus change depending on the composition of the microbiome, which may in turn directly influence the health status of the shrimp and the appearance and prevalence of diseases and therefore mortalities. However, temporal analysis of animals and environmental microbiome in shrimp aquaculture is still lacking. To better understand the association of microbiome changes and disease outbreaks in shrimp aquaculture, weekly whiteleg shrimp tissues samples and filtered water were collected from a pond in a Thailand aquaculture system from day zero (seeding the post larvae in the pond) to end of production cycle (day 56, emergency harvest day due to high mortalities likely caused by a yellow head virus outbreak). The weekly microbiome changes from individual organs and from filtered water were analysed by high throughput sequencing. We present the temporal microbiome changes of the shrimps organs leading up to an emergency harvest.

Questions and comments

It was asked if the microbiome changes were higher in water than in the animals, whereto the presenter Kelly Bateman answered that this was the case and it could be seen in one of the figures of the presentation.

State of play of recent EU aquatic animal health legislative developments and their implementation

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Abstract

In recent years two significant, overarching EU Regulations were adopted, which are relevant, *inter alia*, for aquaculture, aquatic animal health, for the competent authorities in the EU member states who are managing those and associated offical laboratories, as well as for economic operators running aquaculture production businesses. These Regulations will become applicable in the near future, will replace current key EU rules and will affect one way or another, the aquaculture sector and the relevant players in it.

One of these is Regulation (EU) 2016/429, the EU Animal Health Law[1]. This will be applicable from 21 April 2021. Currently several delegated and implementing Commission acts are being prepared with further details necessary for its future implementation. The discussions on these are getting close to their end, while the Commission has not yet officially adopted those acts, with a couple of exceptions. The details in those acts will cover key concepts such as zones, compartmens, criteria for freedom, risk-based surveillance, elements for control and eradication of diseases, conditions for movements of live aquaculture animals and products and so on. A selected few of those elements, which are more relevant for diagnosis of diseases and for NRLs and official laboratories, will be shared with the participants.

Details and summaries of Animal Health Law related expert group discussions are publicly available at the following page: https://ec.europa.eu/food/animals/health/expert_group_en .

The other new legislation is Regulation (EU) 2017/625, on official controls and other official activities peformed to ensure the application of food and feed law, rules on animal health and welfare etc.[2]. Some of its provisions are directly related to EURLs, NRLs, official laboratories, methods used for sampling, analysis, tests and diagnosis and so on. A selected few of these, which are more relevant for diagnosis of aquatic diseases and for NRLs and official laboratories, will be shared with the participants.

[1] https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1557495911818&uri=CELEX:02016R0429-20160331
[2] https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1557496471588&uri=CELEX:02017R0625-20170407

Questions and comments

Currently the legislation only allow import of shrimp larvae from USA, therefore it was questioned if there are any other countries where it is allowed to import from. Laszlo Kuster answered that one country (Thailand) has put in a formal application (not official yet) – if CVO's do not agree, it will not be granted. A quarantine facility in Europe will only be a possibility, if it is under strict regulations. It was commented that this was the same for fish for 25 years ago, so what kind of solutions does the EU suggest? Laszlo Kuster commented that EU has always raised its level under crisis. Lots of shrimp are being imported and are not cultured in the EU. If the crustacean sector starts to grow, the problem will be more visible, and then the EU will raise its level of efforts within this area. It was also commented that the EU cannot restrict trade. In Norway, it is mostly conservation of crustaceans but not trade, which means that Norway would not be able to have an NRL within the field if they were supposed to set up systems for controlling disease. It was questioned if there were designated laboratories in all EU countries, and the answer was that this is not the case. It is up to the CVO in each country to make sure that there is a designated laboratory in the country.

Proposed OIE Collaborating Centre for Emerging Aquatic Animal Disease

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Abstract

The World Organisation for Animal Health (OIE) defines an emerging disease as 'a new infection resulting from the evolution or change of an existing pathogen resulting in a change of host range, vector, pathogenicity or strain; or the occurrence of a previously unrecognized infection or disease'. 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, 2016). A major constraint in achieving this goal are new and emerging aquatic animal diseases in aquaculture sectors globally. To mitigate the effects of these diseases it is critical to achieve rapid detection and characterization 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 Cefas Weymouth Laboratory has proposed a new OIE Collaborating Centre for Emerging Aquatic Animal Disease, building on the historic expertise of Cefas Weymouth Laboratory in pathogen systematics, disease diagnosis and surveillance in aquatic animals and, its strong international relations with other expert centres. A network of laboratories residing in major aquaculture producing regions globally will be developed, seven laboratories have been identified, (China, Thailand, India, South Africa, Canada and North and South America) in addition to the two European Union Reference Laboratories (EURL) for Fish and Crustacean Diseases (DTU, Denmark) and for Mollusc Diseases (Ifremer, France). A key objective of the network will be to harmonise and exchange information and expertise to improve emerging disease surveillance globally.

Questions and comments

The first comment was that it is great that CEFAS will take on this task. Kelly Bateman answered the question regarding the definition on an emerging disease that it will be up to the experts at the laboratory when a case comes into the laboratory, if this is emerging or not.

A general question on how many crustacean farms there are in Europe was put forward. Comments from several lead to the following; Germany (2?), Switzerland (2), Belgium, Latvia, Bulgaria, Spain (at least 1), Netherlands (1 or 2). This lead to the question if all farms were importing post larvae. It was commented that there might be one farm in Spain producing post larvae. A further comment was that such farms should be authorized/registered in the countries in the EU.

Standard models for challenging shrimp *Penaeus vannamei* with white spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND) under laboratory conditions

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Abstract

IMAQUA is a contract research organization and a spin-off company of Ghent University (Belgium), which provides professional research services mainly on the development of shrimp health and nutrition products and improvement of shrimp breeding lines.

We have developed standard models for challenging juveniles of shrimp *Penaeus vannamei* with white spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND), which create a controlled and reproducible laboratory testing environment. This serves as a platform for screening, testing and precisely validating the impact of products, concepts and genetic selection on the resistance of shrimp to disease, with the possibility of high throughput operation mode. These are valuable tools for developing shrimp health products and disease resistant shrimp breeding lines.

Important elements of these challenge models are a unique experimental setup using standard conditions and a custom-made disease inoculation procedure. WSSV is inoculated by oral route while AHPND by immersion route. Shrimp are housed and inoculated individually in tanks equipped with individual biological/mechanical filters. All animals are treated, monitored and scored individually throughout the entire duration of the challenges. By completely isolating each animal, we eliminate variables such as heterogenicity of disease inoculation dosages, horizontal transmission of disease, competition for food/treatments, cross-contamination between experimental products and others. This also allows the collection of a considerable amount of reliable individual data for each challenged shrimp, which opens possibilities on data analysis.

These challenge models can be adapted to other shrimp viral and bacterial diseases as well as other shrimp species. As an example, we have recently adapted the WSSV oral infection model to juveniles of *Penaeus monodon*. We are starting a collaborative multidisciplinary research project where we will try to adapt this infection model to *Palaemon serratus*, a marine cold-water shrimp species autochthonous to several European coastal areas. We have also done preliminary work on development of similar infection models for other shrimp viruses.

Questions and comments

The presentation gave rise to questions, which was answered by Joau Lima, who started out with telling that the work is done by permanent staff and not by students due to the fact that it has to be done under standard conditions. The disinfection of water is done with per-acetic acid and H2O2. The disease challenges include 30 replicates, one shrimp in each replicate. If community tanks were used, there would be fight for food etc. The initiative started, when Joae Lima was a post doc at the university, where he had contact with companies.

SESSION IV:Update from the EURL

Chair: Niccoló Vendramin

2018 Inter-Laboratory Proficiency Test for Crustacean Diseases

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Abstract

An inter-laboratory proficiency test for crustacean diseases was organized by Cefas in May 2018. Testing was organised to ensure the National Reference Laboratories (NRL's) were fully supported in maintenance of their accredited systems for diagnosis (presence/absence) of EC-listed pathogens, White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV) and Yellowhead Virus (YHV). The invitation to participate in the proficiency testing was sent to 26 NRL's in 24 Member States. Samples for WSSV testing were sent to 24 NRL's in 22 Member States, samples for TSV/YHV testing were sent to 14 NRL's in 13 Member States. Results were received from all participating laboratories, here we present the expected results and compare with the actual results received in the 2018 proficiency test.

Questions and comments

It was commented that the cross contamination seen in some results of the crustacean proficiency test is also something that can be seen in the fish proficiency test. Kelly Bateman said that the viral load in the positive material is very high, making contamination easy, but if no contamination is seen then it also shows that the laboratory has good procedures.

EURL activities 2018 -Accreditation of WSSV diagnosis

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Abstract

White spot disease (WSD) has emerged globally as one of the most prevalent, widespread and lethal diseases for shrimp population. The causative agent of WSD is white spot syndrome virus (WSSV), a double strand DNA virus with a circular genome of approx. 293 Kb, belonging to the genus Whispovirus, Family Nimaviridae, and infecting all species of decapods.

WSD has been listed as a notifiable disease in Office of International Epizootic (OIE) Aquatic Animal Health Code since 1997. For all situations where infection with WSSV diagnosis is required, they recommend the use of a nested PCR (Lo et al. 1996. Dis. Aquat.Org. 27, 215–225) or a Taqman based real-time PCR (Durand, SV and DV Lightner. 2002. J. Fish Dis. 25, 381–389) for target surveillance, presumptive diagnosis and confirmatory diagnosis of WSD.

In the current European Legislation (EU Commission Decision 2015/1554 on Surveillance and Diagnostic Methods), diagnostic procedures to obtain or maintain disease-free status for white spot syndrome, rely on the use of a modified nested PCR in order to detect DNA from WSSV, and positive results have to be verified by sequencing. Although the real-time PCR method recommended by OIE has the advantage of being faster and less prone to cross contamination than the nested PCR, it is not mentioned in the diagnostic manuals of the European Union.

One of the points included in the work plan for the EURL for fish and crustacean diseases, refers to the validation and accreditation of diagnostic methods for detecting WSSV. To fulfil this, we carried out the first part of in the analytical validation of both the nested PCR (modified version) and the Taqman based real-time PCR in parallel. As the sensitivity for detection of WSSV seems to be slightly better in the Taqman based real-time PCR, we continue assessing the robustness, repeatability and reproducibility only for this essay. We aim to have at least one of these methods validated and accredited during 2019.

Questions and comments

Real-time PCR is considered more as a surveillance assay wherefore the question was if other tests (used as a confirmatory test) are being accredited. Argelia Cuenca answered that it was decided to start out with the real-time PCR assay, also to have something to test against, but other assays will also be accredited.

The proficiency test 2019

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Abstract

This is the first time than the newly appointed EURL for Fish and Crustacean Diseases will organize the proficiency test (PT) for the notifiable crustacean diseases included in the European legislation. To do so, we are following procedures and recommendations kindly provided from our colleagues at CEFAS, UK, where the EURL for Crustacean Diseases was previously placed.

This year we decided to focus in the detection White Spot Syndrome Virus (WSSV). In order to get enough material for the PT, 77 *Penaeus vannamei* of approximately 9 grams in average, were inoculated using WSSV positive material transferred from CEFAS. Two experimental groups were injected with supernatant from WSSV positive tissue homogenates and the negative control group was injected with sterile saline. Within 48 hours, all virus challenged shrimp succumbed whereas no mortality was observed in negative controls. Currently, we are finishing the last testing of material that will be included in the proficiency test.

Additionally, we are planning to test the stability of WSSV samples in Whatman FTA cards, to evaluate whether FTA cards are appropriate to be included in future PTs of viral diseases.

It is expected that samples for the crustacean PT will be shipped in autumn 2019.

Questions and comments

The timing if the PT is the same as for the fish PT, but no one in the audience objected to that. The possibility of a histology test was put forward, and the EURL answered that this has not been discussed, and the question is if a diagnosis can be made only by histology. It will be looked into if a histology test can be an option in the future. In October, there will be an EURL training course in histopathology, where one day will be dedicated to crustacean histology. Kelly Bateman said that CEFAS had slides for a PT test, when they were the designated EURL in crustacean diseases, but none of the member countries had ever requested it.

Technical report of EURL for Crustacean Diseases

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The Technical University of Denmark (DTU) was confirmed appointed as the EU Reference Laboratory (EURL) for Fish Diseases in February 2018, and was in June 2018 granted an increase of its scope to Fish and Crustacean Diseases in accordance with the Amendment n° 1 to the grant decision for an action regarding the EU Reference Laboratory for Fish and Crustacean Diseases– SI2.777824 (Ref. Ares (2018)3294875 - 21/06/2018).

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern the fish and crustacean diseases listed as exotic diseases: Epizootic haematopoietic necrosis (EHN), taura syndrome, and infection with yellow head virus genotype 1; and diseases listed as non-exotic diseases: Infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN), koi herpes virus disease (KHVD) and white spot disease (WSD). The technical report follows the new format of the work programme adopted for the EURL for 2018, describing activities and sub-activities and the status of on-going projects. In contrast to previous years almost all technical descriptions are given in Annexes making the report shorter and more easily accessible.

The entire unit for fish and shellfish diseases and all its functions and duties including the EURL functions were moved from DTU Veterinary to DTU National Institute of Aquatic Resources (DTU Aqua) in 2018 when the closing of the DTU Veterinary institute became a reality in January 2018. The transfer to the new institute has given us a number of new opportunities for collaborating with research teams working in the field of aquaculture and fisheries.

The annual workshop for crustacean diseases was organised by the former EURL at CEFAS in UK and held in Italy in 2017 before the transfer of the function to DTU.

In 2018, Dr. Nikolaj Reducha Andersen had until December 1st the responsibility as the Coordinator of the EURL for Fish Disease. Upon an international call for the position as EURL coordinator for crustacean diseases he successfully obtained this position – taking the tasks of organising workshop and training courses, updating our website, conducting in-vivo viral characterisations and strengthen our statistical capabilities. Unfortunately for personal reasons Nikolaj decided to leave the position again pr April 1st 2019 for a job closer to his home. The position as senior scientist/ scientist and coordinator of the Crustacean Diseases part of our EURL function was launched again internationally and the candidates for the position are currently under evaluation, it is our hope that the vacancy will be filled early autumn, in the mean while m.sc. Camilla Priess is filling the gap on validation and accreditation of the diagnostic method for the listed crustacean diseases.

Work Programme for 2018 including the Crustacean diseases part from 1. July

1.1. Obtain accreditation. *To obtain accreditation for the listed crustacean diseases.*	The work to obtain accreditation for the three listed crustacean diseases was launched in 2018. DTU Aqua did not manage to obtain the accreditation for all diseases in 2018. We currently expect to be fully accredited in May 2019.
1.2. Visit to Cefas. *To acquire relevant information from the former EURL for crustacean diseases (Cefas).*	During the planning of the 2018 half year crustacean diseases Work Plan, a working visit to the former location of the EURL for Crustacean Diseases Cefas, was planned. Before the function as EURL was transferred 1. July to DTU Aqua, Kelly Bateman visited DTU Aqua for a small seminar on the transfer. Presentations and discussions on this seminar clarified many of the concerns that a trip to Cefas should have contributed with. The trip to Cefas was therefore cancelled.
1.3. Literature review. *To acquire the most recent scientific information on crustacean diseases and crustacean aquaculture production.*	The latest relevant literature on crustacean diseases has been review. This work will of course continue, however, it was important to allocate hours for this task during the first months of the transfer of the function and duties of the Crustacean Diseases EURL.
1.4. Laboratory facilities. *To organize the EURL for fish and crustacean diseases laboratories for diagnosis of crustacean diseases.*	The laboratory facilities have been updated and an area has been allocated to the work with crustaceans and crustacean diseases. Currently 200 live shrimps of the species Pacific White Shrimp (*Litopenaeus vannamei*) are kept in the bio secured facilities at DTU Aqua to support the work of the EURL.
2.1. Training course. *To ensure that employees of the Member State NRLs have the highest scientific and excellent skills in diagnosis of crustacean diseases.*	Two training courses were successfully organized from October the 8th to 19th, 2018. The two courses prepared were: "Methods for implementation of surveillance procedures for listed fish diseases" with 11 participants and "Introduction to histopathology in fish and crustacean diseases" with 15 participants. The majority of the participants evaluated the courses "very good". *The report of the 2018 training courses is located in **Annex 4***
2.2. Website. *To provide the Member State NRLs with a fast entrance to*	The EURL website was constantly updated during 2018 with reports and news from the EURL. The website has been accessed 6098 times; in total 18882 pages of the website has been accessed during 2018. A new website

information from the EURL.	including both fish and crustacean diseases is under construction 
2.3. Email list. *To ensure that relevant and important information rapidly can get from the EURL for fish and crustacean diseases directly to the Member State NRLs.*	The e-mail list FishRefLabNet have been continuously updated during 2018 and now contain 145 people with interest in our work. The list now includes all the NRL contacts for the Crustacean Diseases.
3.1. Diagnostic manuals. *To have updated diagnostic manuals for all listed crustacean diseases available for Member State NRLs*	Diagnostic manuals for the three listed crustacean diseases where transferred from Cefas to DTU Aqua at 1. July 2018. These manuals where reviewed and changed to our accredited diagnostic methods (this work is still ongoing). The manuals will be uploaded to our webpage during 2019.
3.2. Advise and support to the Commission. *To support the Commission with state of the art scientific advice on crustacean diseases.*	Annex to the Delegated Act on sampling and diagnostic procedures for the two List A crustacean diseases Taura Syndrome and Yellowhead disease have been conducted.
3.3. Emerging diseases. *For the EURL to have the most updated and highest scientific knowledge of emerging and crustacean diseases in Europa.*	The EURL for fish and Crustacean Diseases did not have any requests concerning emerging crustacean diseases in 2018 (1 July-31 December)
4.1. Pathogen library. *For the EURL for fish and crustacean diseases to have an updated library of crustacean pathogens relevant for the EURL and Member*	During the transfer of the EURL for Crustacean Diseases from Cefas to DTU Aqua, reference material where also handed over. This material has been organized in -80 degrees and a database for Crustacean Diseases Reference Material has been constructed. The database continues to expand in the future.

State NRLs.	

Questions and comments

Niels Jørgen Olesen asked if there will be a need for a specific training course in crustacean diseases in the future and more than half of the participants at the meeting were interested in such a course. Topics suggested were sampling of target organs for diagnostics, necropsy. It was stressed that crayfish plaque should not be neglected, but it might be a bit out of scope, as it is not a listed disease. Another question from the EURL was if there is a need for specific crustacean network (email group), but as the majority are involved in both fish and crustaceans, one email group might be enough. One of the first important steps in survey and diagnosis of crustaceans in the EU will be to map if and where there is a production. It was considered to be a good idea to include crayfish plaque in the PT, and Norway was willing to supply with reference material of this disease. Niels Jørgen Olesen asked if it might be an idea to expand the PT to overseas countries. Kelly Bateman commented that Thailand were planning to make one themselves, so it might be an idea to contact them, as well as that she did not know if the laboratory in Arizona was still providing a PT.

Work programme of EURL for Fish Diseases

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

Sub-activity 1.2 *(Annual workshop crustacean diseases)*

Objectives: To ensure knowledge dissemination and sharing between the Member State NRLs on existing and emerging crustacean diseases and to agree on the future priorities of the EURL, by holding the 10th and 11th annual workshops of the National Reference Laboratories (NRLs) for crustacean diseases in 2019 and 2020, respectively.

Description: These workshops will be held back-to-back with the Annual Workshops on Fish Diseases. It is the first time DTU Aqua will host these workshops, since we were designated as the EURL for crustacean diseases in July 2018. We will continue the numbering of the workshop from the earlier EURL for Crustacean Diseases at Cefas, and these workshops will therefore be the 10th and 11th Annual Workshop of the NRLs for Crustacean Diseases. All Member State NRLs are strongly recommended to participate, as it is an important opportunity to be updated on the newest scientific knowledge of crustacean pathogens, diagnostics, legislation, etc. Several talks of high scientific standard will be given and discussions at group and plenum level will be facilitated during the one day workshop.

Expected Output: Successful preparation and completion of the annual workshops comprising one full day in May 2019 and 2020 back-to-back with the Annual Workshops of the NRLs for Fish Diseases. Based on previous experience it is expected that 20 participants will attend the workshop including EU member states, associated countries and invited speakers. From the EURL team 4 members will attend the workshop full time A technical and financial report of the workshops will be produced. The technical reports will contain abstracts and minutes from all presentations and discussions and will after acceptance be made publicly available through the EURL website.

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

Sub-activity 1.3 *(Scientific working groups)*

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

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

Expected Output: We expect to organise four scientific working groups in 2019 and 2020 with the duration of one to two days each. A working group on 1) susceptible fish species to listed diseases in EU, 2) assessing fish and crustacean diseases for possible listing in EU legislation, 3) crustacean diseases and 4) emerging diseases. The topic of the emerging disease working group will be defined in relation to ad hoc request. From each meeting, a scientific report including recommendations will be delivered to the relevant Member State NRLs and the European Commission and will be available on our website www.eurl-fish.eu.

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

Sub-activity 1.5 *(Proficiency test crustacean diseases)*

Objectives: To assess the capabilities of all Member State NRLs to detect pathogens causing diseases in crustacean and to harmonize the diagnostic procedures used by inter-laboratory proficiency tests.
Description: The EURL is going to prepare Annual Inter-laboratory Proficiency Tests to all Member State NRLs. The tests will include the viral crustacean pathogens; White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV) and Yellowhead Virus (YHW). The participation is mandatory for all NRLs in EU. After submission of test results from the NRLs to the EURL, we will collate and analyse information gained from the proficiency test and publish the anonymous data to all participants as a report on a restricted site of our website www.eurl-fish.eu. A non-coded version will be provided to the EU Commission with information on performances and under performances. The results will be presented and discussed at the Annual Workshops in 2019 and 2020.
Expected Output: Preparation and shipping the test and subsequently provide a report on the proficiency test 2019 and 2020. It is expected that 24 laboratories are participating with a success rate of > 90 percentage for both tests. Underperformances will be addressed by direct communication with the participant. Underperforming laboratories will be considered for mission from the EURL.
Duration: January – December 2019 and 2020. The samples included in the test will be shipped from the EURL in the fall and the final report will be submitted February the following year.

Sub-activity 1.6 (Diagnostic methods)

Objectives: For the EURL to have diagnostic methods of the highest scientific standards and to be able to provide these methods to all Member State NRLs.
Description: Novel molecular methods are highly sensitive and specific tools for diagnosis and surveillance of a number of listed pathogens. In 2019 and 2020, the EURL will focus on four techniques; 1) PCR for detection of genomic RNA/DNA from pathogens, 2) In-situ Hybridization (ISH) for pathogen localization in paraffin embedded tissue, 3) Next Generation Sequencing for full genome sequencing and 4) Improved cell culture techniques. Concerning the crustacean diseases White Spot Disease, Taura Syndrome and Yellowhead Disease accreditation according to ISO 17025 will be obtained during 2019. Furthermore, the

area of crustacean emerging diseases will be given priority, e.g. the disease Acute Hepatopancreatic Necrosis Disease (AHPND).
Expected Output: Four new diagnostic methods implemented in the two year period. Four diagnostic molecular methods validated according to the recommendations given by the OIE.
Duration: January – December 2019 and 2020.

Sub-activity 1.7 *(Crustacean tank facilities)*

Objectives: For the EURL to be able to conduct infection trails with crustacean species.
Description: Bio-secured laboratory facilities to rear and infect crustacean with listed and non-listed diseases are a prerequisite for providing Member State NRLs with proficiency tests, confirmatory diagnosis, pathogen characterization etc. The facilities will consist of a clean facility for SPF crustaceans and a bio-secured establishment to conduct infection trials.
Expected Output: A high quality crustacean rearing and infection facility at DTU Aqua.
Duration: January – December 2019.

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Sub-activity 2.1 *(Training Courses)*

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

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

Objectives: To provide the Member State NRLs with a fast entrance to information from the EURL.
Description: The EURL are administrating the webpage, www.eurl-fish.eu, by uploading relevant material such as updated lists of NRLs, annual workshop presentations, training course reports, sampling and diagnostic procedures, newest update on legislation, general news from the community, etc. The website has daily visitors from a great number of countries from around the world and are, therefore, a substantial part of disseminating the work of the EURL for fish and crustacean diseases. Due to the inclusion of crustacean diseases in the EURL we will 2019 launch a new and updated website. The new website will in the future be located at www.eurl-fish-crustacean.eu and the old one www.eurl-fish.eu will close. The

website will be further developed including a "restricted access area" where reports and information which are specific for targeted stakeholders will be uploaded.
Expected Output: A constantly updated webpage for the Member State NRLs. Establishment of a restricted area and provision of guidelines to all Member States NRLs for access to the restricted area.
Duration: The new website will be up running primo 2019 and maintenance will be from January – December 2019 and 2020.

Sub-activity 2.3 *(EURL Contact Lists)*

Objectives: To ensure that relevant and important information rapidly can get from the EURL directly to the Member State NRLs.

Description: We will aim to have three contact lists. 1) Member State NRLs for fish diseases, 2) Member State NRLs for Crustacean disease and 3) a general list which all interested in the work of the EURL can subscribe to. The EURL use the mailing lists for important notifications i.e. meeting calls, training course calls and other relevant information such as information on upcoming conferences, new research findings and relevant reports and publications, emergency situations etc. Often the notifications will include links to the website or other sites for further and detailed information.

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

Duration: January – December 2019 and 2020.

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

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

Description: Missions are only planned to Member State NRLs for fish diseases, however, we will be able to conduct missions to NRLs for crustacean diseases if it is found necessary. NRLs chosen for a mission are primarily based on performance in the yearly proficiency test. However, if missions to other countries, both EU Member States but also 3rd countries, will be able to provide important scientific knowledge for the EURL to pass on to Member State NRLs, missions to such countries will be conducted. This will ensure EU Member States to be updated with excellent scientific skills and knowledge.

Expected Output: As the decision for appointing target laboratories for missions is based on performances of the proficiency test- no final decision can be taken at this stage. Two missions per year conducted from the EURL, first draft of the report of each mission provided to the host institution within 1 month from the mission

Duration: April and/or November 2019 and 2020.

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

Objectives: To keep the EURL updated on the newest scientific information on emerging and listed exotic and non-exotic fish and crustacean diseases, and to disseminate knowledge and scientific data provided by the EURL.

Description: The EURL staff is able to provide consultancy to Member State NRLs on emerging and listed fish and crustacean diseases, and attending conferences are an important way of the EURL to keep the

excellence of this function. Conference participation therefore ensures up-to-date knowledge within the EURL.

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

Duration: January – December 2019 and 2020.

Sub-activity 2.6 *(Confirmatory diagnosis)*

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

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

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

Duration: January – December 2019 and 2020.

Sub-activity 2.7 *(Pathogen characterization)*

Objectives: For the EURL to be able to characterize isolates of listed viral pathogens of aquatic animals as well as emerging pathogen and provide scientific based risk assessment to the scientific community and stakeholders.

Description: The EURL every year contributes to characterize relevant pathogens for aquaculture in Europe as an emergency response to avoid unwanted spreading of new pathogens in EU. The EURL describe these strains by pathogenicity testing in-vivo. The experimental trial contribute to establish reference material to be used as positive controls and standards enabling diagnostic validation of new diagnostic methods.

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

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

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

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

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

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

Duration: January – December 2019 and 2020.

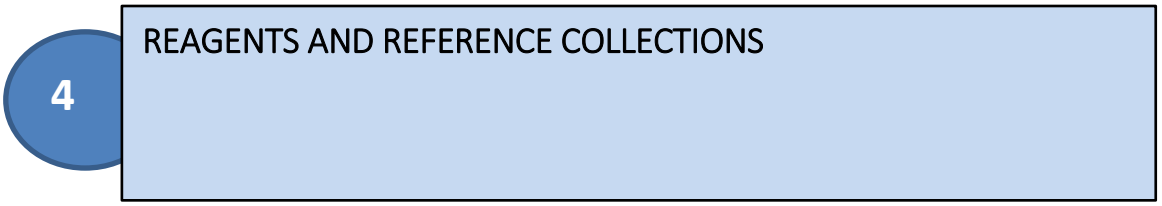
Sub-activity 3.4 *(Risk assessment for emerging diseases)*

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

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

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

Duration: January – December 2019 and 2020.



Sub-activity 4.2 *(Pathogen library)*

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

Description: The EURL are going to update and maintain a library of isolates of the viral fish pathogens infectious salmon anaemia virus (ISAV), viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), koi herpes virus (KHV), enzootic hematopoietic necrosis virus (EHNV) and other relevant putative emerging fish pathogens. Furthermore, the crustacean pathogens White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV) and Yellowhead Virus (YHW) will be included in the library.

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

Duration: January – December 2019 and 2020.

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

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

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

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

Duration: January – December 2019 and 2020.

5 REQUIREMENTS RELATED TO OTHER LEGISLATION

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

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

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

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

Duration: January – December 2019 and 2020.

Sub-activity 5.2 *(Listing susceptible species)*

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

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

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

Duration: January –March 2019.

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

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

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

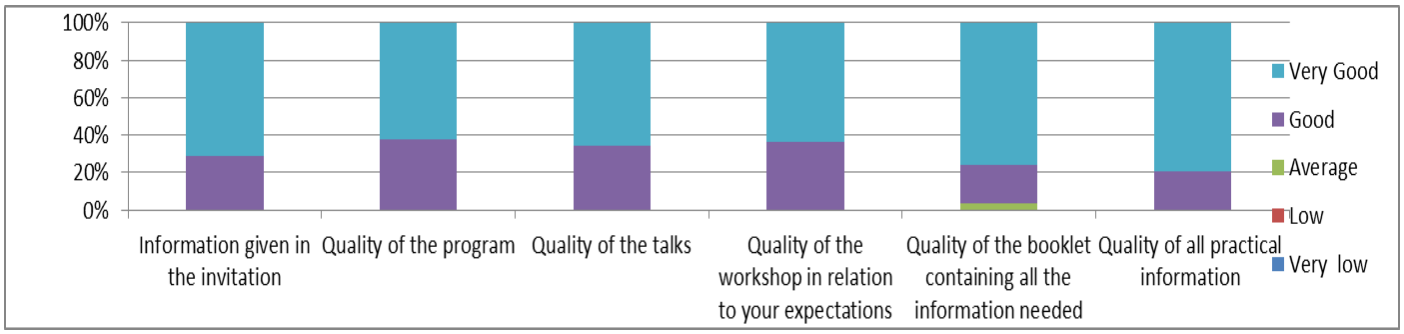
Expected Output: The EURL expect to assess two diseases per year, and provide scientific recommendation for including or exclusion them from the legislation.

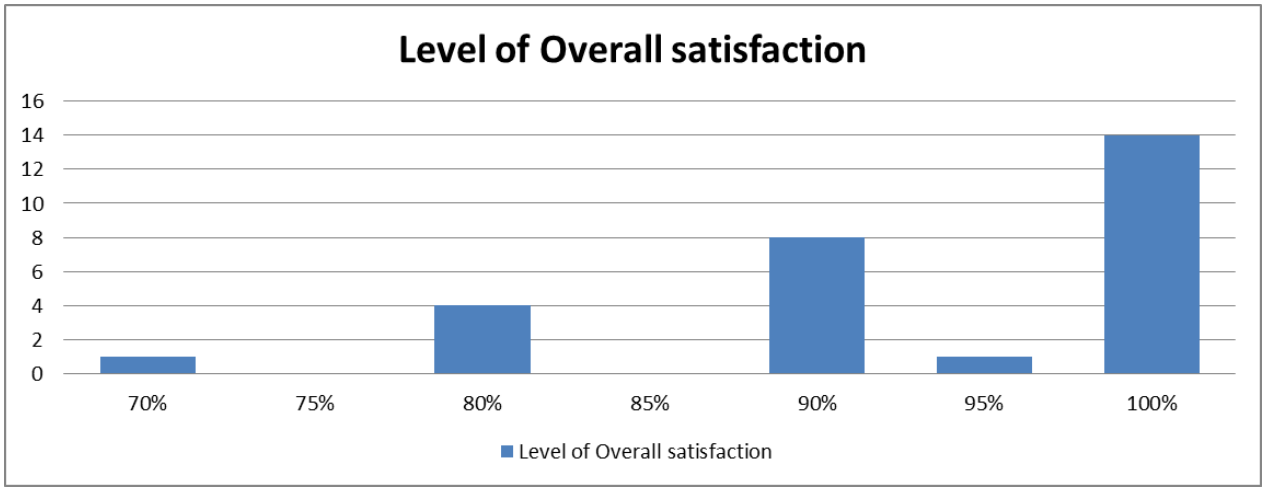
Duration: Upon request from the Commission in 2019 and 2020.

Questions and comments: None

Workshop evaluation

A questionnaire was delivered to the participants asking to evaluate various aspects of the workshop. An overview of the 38 questionnaires retrieved is shown below. Specific comments are going to be considered for the next annual workshop organization.





Greetings and conclusions of the meeting

The next meeting will be held 4th and 5th June, 2020 back to back with the AW for fish diseases. It will be organized at our facilities in Kgs. Lyngby. Thanks a lot to the people arranging and reporting 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