EURL Training Courses

Copenhagen, October 7th - 18th 2019

Hosted by the European Union Reference Laboratory for Fish and Crustacean Diseases

Contents

General introduction

The training courses were organized by the EURL for Fish and Crustacean Diseases located in Kgs. Lyngby at the National Institute of Aquatic Resources, Technical University of Denmark, Kemitorvet, building 202, 2800 Kgs. Lyngby, Denmark, from October the 7th to the 18th, 2019. Two courses were prepared: the first one, with 12 trainees, was entitled "Methods for implementation of surveillance procedures for listed fish diseases" and took place from 7th to 11th October. The second course was entitled "Introduction to histopathology in fish and crustacean diseases" and took place from the 14th to 18th October 2018 with 18 participants. Five participants attended both training courses.

The overall purpose of the training courses was to provide an opportunity for the NRLs to send employees for training in techniques relevant when working with fish diseases. Concerning the course on surveillance of listed fish diseases, the staff of the EURL and DTU Aqua provided this training together with teachers from the Danish Veterinary and Food Administration. For the course, which focused on histopathology, staff from the EURL and DTU Aqua, in cooperation with NVI-Oslo, Cefas (UK) and Aquapri DK, constituted the tutor team. Knowledge-sharing and discussions between participants and teachers were important parts of both courses.

Course 1: Methods for implementation of surveillance procedures for listed fish diseases

The five-day course in "Methods for implementation of surveillance procedures for listed fish diseases" was based on practical work (hands on) in combination with theoretical presentations.

Day 1 was dedicated to a visit to the Danish Veterinary and Food Administration (DVFA) and to a fish farm. Professor Niels Jørgen Olesen and three laboratory technician from the EURL team participated from the EURL**.** In the morning, the participants were picked up at the hotel in a bus and driven first to the DVFA offices in Vejen – a three-hour drive. Here after a short introduction Morten Fruergaard-Andreasen from DVFA gave a talk about Danish aquaculture and disease surveillance after which Niels Jørgen Olesen gave a presentation on the control and eradication of VHS from Denmark. After lunch at the DVFA the participants were driven to Hesselho fish farm a 30 min drive from DVFA. The farm is a traditional earth pond farm fed by water from nearby Holme stream. The participants were shown around the farm by Morten Fruergaard-Andreasen and the owner Jens Jensen, who was kind to answer questions from the inquisitive participants as well as to provide coffee. During the walk, procedures for inspection and sample collection were demonstrated and diseased fish were caught and euthanized. Participants were taught fish necropsy techniques, and how these are performed in the field. All participants performed on site necropsy on diseased fish collected at the farm. They collected relevant samples, and the personally labelled samples were brought back to the laboratory in Lyngby for further examination the following days. After the return to the hotel, Kristi (one of our lab technicians) took some of the participants to the central station to sort out travel cards for the trips between the hotel and the EURL office at DTU in Lyngby. Some also continued with some light sightseeing and dinner.

On **day 2** an introduction and practicalities with a detailed description of the course programme was presented by Niels Jørgen Olesen and each participant presented their experience and expectations for the course. After the introduction, a lecture on "the legislative basis for aquaculture animal health and the sampling and diagnostic procedures to use" was given by Niels Jørgen Olesen. In addition, all

topics included in the compendium were presented as a preparation for the practical part of the course. In the afternoon, the participants were divided in to two groups where one after an initial demonstration prepared samples for cell cultivation, PCR and bacteriology on samples they collected Monday while the other followed practical cell culture passaging and production of 24-well plates. After the coffee break, the two groups shifted.

In the evening, there was a social dinner.

Day 3 started by theoretical lectures on PCR and real time PCR laboratory- lectures were hold by Molecular biologist of the EURL Dr. Argelia Cuenca. After coffee break all participants were involved in practical exercises and analysis of PCR results.
After lunch all participants were gathered in teaching laboratories and were involved in activities such as reading and inoculating the cells produced the day before with samples taken at the fish farm. The practical activities were followed by a presentation given by Niels Jørgen Olesen on "Use of cell culture in fish virology". After the coffee break practical demonstration of titration procedures, reading plates and calculating virus titres was conducted in the laboratory of DTU Aqua and supervised by Niccoló Vendramin. During this long session in the afternoon the participants were divided in two groups, while one group was proceeding with the activities in the laboratory the other group was taken for a tour of the institute showing the laboratory facilities and tank facilities of the EURL.

Day 4 was tutored by Argelia Cuenca and fully dedicated to PCR, sequencing, BLAST analysis and to phylogeny. The day started going over the flow in the diagnostic lab, and the requirements and routines that need to be ensured to avoid (cross-) contamination when performing PCR. After that, we had a small lecture about Sanger sequencing and how it works. For practical exercises, a couple of cases were done that needed to be followed during subsequent analyses. A session explaining how BLAST works and how to interpret BLAST results was conducted, followed by a series of practical exercises and discussion of the results obtained for each student. Finally, during the afternoon we focused in how to read, interpret and construct phylogenetic trees, with strong focus in the theory behind the phylogenetic analyses. This session included guided practical (computer) exercises.

At **day 5** in the morning, the participants were divided into two groups. One group were shown diagnostics concerning bacterial diseases in fish and some examples of bacterial pathogens from Lone Madsen, while the other group was reading their inoculated cell cultures and a number of cell lines inoculated with various fish viruses, including VHSV, IHNV, EHNV, IPNV with Niccoló Vendramin; afterwards the groups switched. After lunch all teams were given assignments on how to handle various cases. After one hour, each member should then present their results in new groups and finally all reports were presented and discussed in plenum, this session was supervised by Niels Jørgen Olesen. The course was closed up by discussing both results obtained by the participants and different methods for diagnosis and performing surveillance of listed fish diseases in their countries of origin. Finally, a questionnaire for the course evaluation was given and the participants were asked to fill it anonymously.

The methods taught were primarily focused on the protocols given in the EU legislation and on the OIE guidelines from the Manual of Aquatic Animal Diseases, and included how to select proper controls, the typical pitfalls, troubleshooting, etc. Every activity had a team of tutors in order to provide an

effective support to the trainees. For the practical activities Christina Flink Desler, Betina Lynnerup, Teena Vendel Klinge, Kristina Andkjær Andersen and Kári Karbech Mouritsen were assigned as tutors.

Course 2: Introduction to histopathology in fish and crustacean diseases

The five-day course in histopathology and immunochemical techniques was divided into two parts; a three and a half-day part on histopathology on fish and a one and a half day part on histopathology on crustacean. In both parts, theoretical lectures on relevant topics alternated with practical exercises both in necropsy room and microscopy room.

Day 1 started with an introduction to the course and practical information. Each participant had the opportunity to present themselves to the tutors and the other trainees. Practical demonstration on how to sample fish tissues and organs for proper histological examination followed, and each participant tried the technique on their own fish thereafter. Lectures given by Ole Bendik Dale from NVI-Oslo on the normal histology and artefacts followed after lunch break.

Day 2 was divided between practical observation of slides from confirmed cases at the microscope and theoretical lectures focusing on general and special pathology with focus on listed diseases. Ole Bendik Dale and Tine Moesgaard Iburg were in charge of the teaching.

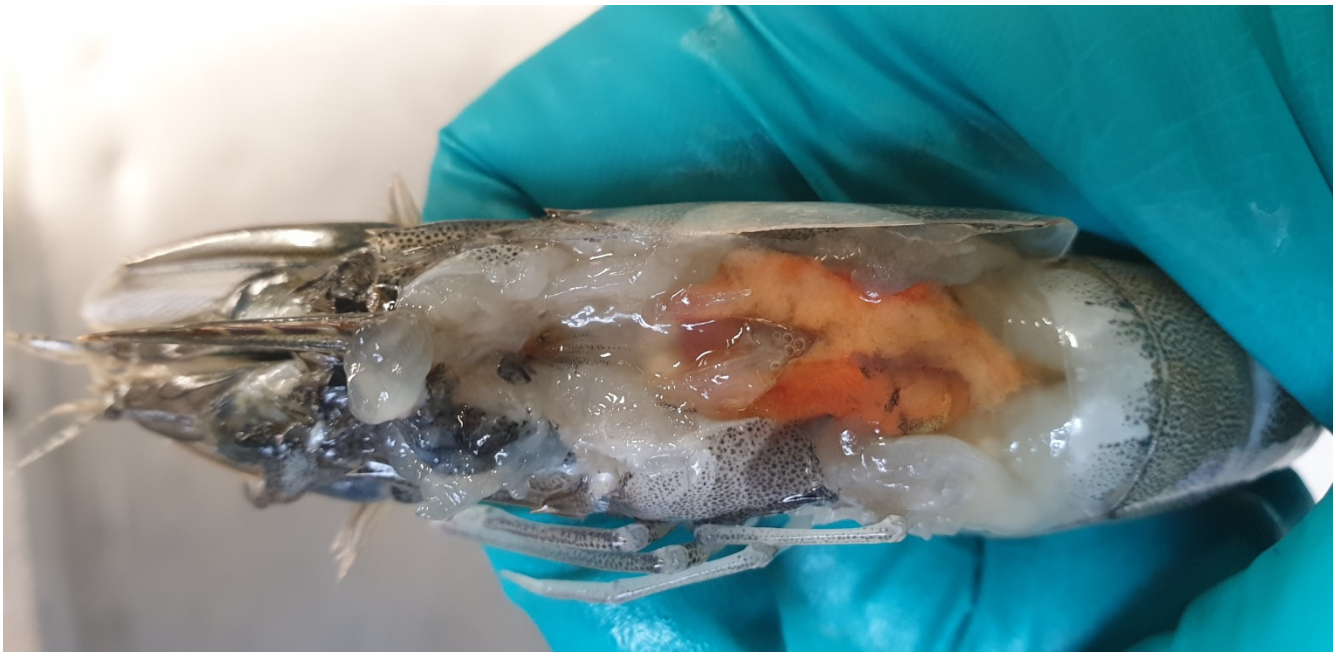
The first part of **day 3** was dedicated to lectures on Immunohistochemistry (IHC), the different phases of sample preparation for staining techniques and troubleshooting and pitfalls during the process were discussed. This part of the programme was conducted by Torsten Snogdal Boutrup. Theoretical exercises in IHC were used as a platform for discussions with tutors Tine Moesgaard Iburg, and Torsten Snogdal Boutrup. Also another session with cases in the microscopy room was done with Ole Bendik Dale and Tine Moesgaard Iburg.

Day 4 started directly in the lecture room with lectures and show and tell by Ole Bendik Dale of special pathology, mainly in listed diseases. Scanned slides were used on the big screen in the lecture room giving the same feeling as when case slides were shown on the teaching microscope in the microscopy room. After lunch, a session on crustacean anatomy started. Dr. Kelly Bateman (from the former EURL for crustacean diseases) provided an overview of the organs of relevance of disease diagnostics and surveillance in crustaceans and afterwards a practical exercise was conducted. Dr. Kelly Bateman demonstrated dissection procedures and organ localization; afterwards each participant could try by himself the dissection of the crustaceans.

Day 5 was dedicated to crustacean histopathology. The morning was filled with lectures on sampling issues and fixatives specific for crustaceans and special pathology for listed diseases. After lunch, introduction to scanned case slides was given and in the microscopy room, case slides from listed diseases could be examined in the microscope combined with working with scanned slides.
At the end of the day, the participants were provided with a questionnaire for the whole course to fill in as part of the evaluation. Furthermore, the participants were invited to give their opinions on the course. All in all, the course was very well-received, and the distribution of practical and theoretical lessons seemed well balanced.

Through lectures, exercises, discussions, and knowledge exchange between participants, the knowledge on histopathology, and troubleshooting related to this technique was increased. Discussion among participants and teachers during the course was fruitful, during boththe lectures, the breaks, the theoretical and practical exercises.

As get-together, a dinner event for all was held on day three.



Lymphoid organs of P. vannamei during the dissection exercise in crustacean anatomy. Photo by Mark John Fordyce.

Participant list

Name	Surname	Country	Affiliation	Course 1	Course 2
Aleksandra	Eriksen	Sweden	SVA/National Veterinary Institute, Section for Fish Diseases		x
Àrni	Kristmundsson	Iceland	Institute for Experimental Pathology at Keldur, University of Iceland		x
Àsthildur	Erlingsdóttir	Iceland	Institute for Experimental Pathology at Keldur, University of Iceland		x
Carina	Duarte	Scotland	Marine Scotland Science, Scottish Government	x	
Dimitar Petrov	Ivanov	Bulgaria	National Diagnostic Research Veterinary Medicine Institute		x
Ioana	Lupescu	Romania	Institute for Diagnosis and Animal Health	x	
Gerald N.	Misol Jr.	Greece	Department of Biology, University of Crete	x	x
Hampus	Hallbom	Sweden	National Veterinary Instititute / SVA	x	
Ivana Giovanna	Zupicic	Croatia	Croatian Veterinary Institute		x
Joanna	Pajdak-Czaus	Poland	Department of Epizootiology, Falculty of Veterinarian Medicine, University of Warmia and Mazury in Olsztyn	x	x
Joseph	Upstone	England	Fish Facility, Department of Biosciences, University College London		x
Karolina	Naumowicz	Poland	Department of Pathophysiology, Forensic Veterinary Medicine and adm. Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn	x	x
Lorena	Biasini	Italy	Istituto Zooprofilattico Sperimentale delle Venezie	x	
Maria Eugenia	Santos	Czech Republic	University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Behoemian Research Center of Aquaculture and Biodiversity of Hydrocenoses		x
Mark John	Fordyce	Scotland	Disease Diagnostics, Marine Scotland Science, Scottish Government		x
Massimo	Orioles	Italy	Udine University, Department of Veterinary Fish Pathology		x
Mavricija	Magister	Slovenia	Veterinary faculty of Ljubljana	x	
Melinda	Kocsis	Hungary	National Food Chain Safety Office (NEBIH), Veterinary Directorate, Laboratory of Virology	x	x
Mihaela	Costea	Romania	Institute for Diagnosis and Animal Health	x	
Pavlina	Ninikova	Slovakia	State Veterinary and Food Institute in Dolny Kubin, NRL for fish and crustacean diseases	x	
Samantha	White	Ireland	Marine Institute		x
Urvashi	Goswami	Hungary	Veterinary Medical Research Institute, Hungarian Academy of Sciences		x
Victória	Németh	Hungary	Laboratory of Parasitology, Fish and Bee Diseases of the Food Chain Safety Loboratory Directorate of the National Food Chain Safety Office in Hungary (NÉBIH)	x	x
Rezkar Jaafar	Mohammad	Denmark	KU		x
Asma	Mohammadkarami	Denmark	KU		x

Course description: Methods for implementation of surveillance procedures for listed fish diseases

Course content

This 5 day course is primarily based on practical work (hands on) in combination with theoretical presentations. This year the course will focus on the comparison between diagnostic techniques for listed fish diseases by evaluating pros and cons of cell culture and Real-Time PCR methods.

At the first day of the course all participants will take part in a fish farm visit. The trip will be supervised by the Danish Veterinary and Food Administration, Section for Aquaculture, that will provide guidelines and protocols for inspection, necropsy and sample collection. Organ material will be collected on site for processing in the lab the following days. During the farm visit sampling procedures will be demonstrated and afterwards conducted by each participant. Starting from day 2, theoretical class room teaching will be given on methodologies, pitfalls and troubleshooting. After the introduction, the participants will be divided into groups. Each group will start processing the samples collected at the farm. This process will include cell culture preparation, inoculation on monolayers, observation of cell culture and assessment of cytopathogenic effect. All cell cultures used for isolation of the listed viruses will be demonstrated and procedures will be conducted by the participants.

Participants will initially be introduced to basic cell culture work: 24-well plate preparation for diagnostic purpose as well as 96-well plate for titration and flask maintenance will be demonstrated and subsequently prepared by the participants. Inoculation of diagnostic samples on cell cultures will be practised. The CPE of different viruses will be shown and the participant will practise reading of diagnostic trays. Titration procedures will be demonstrated and afterwards participants will have the opportunity to practise by themselves. Finally, course participants will calculate virus titres.

The application of novel validated and accredited Real-Time PCR protocols suitable for surveillance will presented and discussed with the participants. This year there will be more focus on genetic characterization of listed fish viruses with theoretical lectures and practical exercises on sequencing, Blast tool and phylogenetic analysis. For this reason each participant is required to bring his/her own laptop.

The course is dialogue based and sufficient time will be given for discussions throughout the course and for evaluation of test results. Quality assurance, cleaning, disinfection, etc. will be an integral part of the practical demonstrations. The taught methods will primarily focus on the protocols given within the EU legislation and OIE guidelines from the Manual of Aquatic Animal Diseases, and include how to select proper controls, the typical pitfalls and troubleshooting, etc. A social dinner will be organized on the second evening. Further details are provided in the invitation letter.

General course objectives

The course aims to provide the participants with knowledge on the most used methods for diagnosis of important fish viruses. The course will focus on; 1) basic cell cultivation techniques, production of cells for different purposes (IFAT, diagnostic trays, titration, etc.), cell susceptibility testing, inoculation of samples and sub-cultivation procedures, reading of cell cultures (including CPE) and virus titration, 2)

providing the participants with knowledge on the most used methods for diagnosis of important fish pathogens, 3) Real-Time PCR protocols validated for surveillance of listed fish diseases, 4) genotyping the most important viral pathogens by sequencing and blasting and 5) information on the underlying principles of the tests and how to critically review them in order to assess pitfalls.

Learning objectives
The participants that have followed all the course objectives will be able to;

- Sample and process material for diagnostic purpose
- maintain, cultivate, inoculate and read most used cell lines (BF-2, EPC, CCB and ASK) for fish disease diagnostic purposes
- prepare cell cultures for different purposes, e.g. diagnosis, IFAT and virus titration
- inoculate and sub-cultivate diagnostic samples
- read diagnostic trays
- titrate virus
- apply Real-Time PCR for surveillance purposes
- genotype important viral isolates by sequencing and blasting
- assess pitfalls and errors in test performances and designs.

The major focus will be on the viral fish diseases using VHS as model. The course will provide a forum where pre-knowledge, experience and examples can be discussed between participants and teachers, and hereby raise the awareness of pitfalls when using the various techniques.

Intended learning outcomes
To increase the practical and theoretical knowledge of cell culture based and bio-molecular techniques used in fish virus diagnostics. The course also aims at providing a forum where (good and bad) experiences can be discussed among participants and teachers.

The core elements
Sampling and processing fish tissue for diagnostic purpose
Fish cell line cultivation
PCR / Real-Time PCR
Sequence analysis and use of BLAST tool
Identification and discussion of pitfalls and how to perform troubleshooting

Assessment
Each day will end with a short result and discussion follow-up in order to evaluate whether the participants picked up the treated core elements. Their results will be discussed. At the end of the course a questionnaire for course evaluation will be delivered to all participants.

The course material
Protocol for the hands-on exercises, PowerPoint presentations as well as the original scientific papers describing the applied assays/techniques is included in the course binder. The supervisors will collect the generated results and provide shared data overviews to be starting point of discussions.

The course participants

Since course attendants can come with very different backgrounds, during the general introduction (day 2), researchers and technicians will be asked to introduce themselves, their pre-experience in the laboratory and their expectations to the course in order to target the course content optimally, especially during the theoretical and discussion workshops. Their starting point will therefore be mixed as some may have limited theoretical or practical experience, while others may be highly experienced in some or all disciplines.

Course supervisors

Prof. Niels Jørgen Olesen (DVM, PhD): Diagnostic fish virology
Lone Madsen (DVM, PhD): Diagnostic fish bacteriology.
Argelia Cuenca Navarro (M.Sc., PhD): Molecular methods.
Jacob G. Schmidt (M.Sc., PhD): Course facilitator
Niccoló Vendramin (DVM, PhD): Diagnostics

Technical help and assistance for running the laboratory courses will be given by

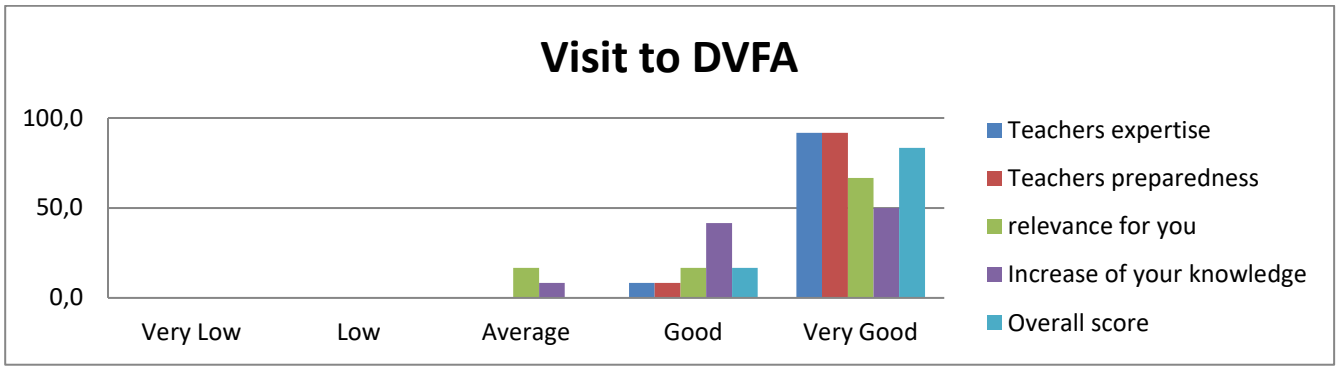
Betina Lynnerup Warming (cell culture)
Christina Flink Desler (sample preparation)
Anne Marie Nordvig Petersen (Real Time PCR)
Teena Vendel Klinge (Real Time PCR)
Kristina Andkjær Andersen
Kári Karbech Mouritsen (bacteriology)

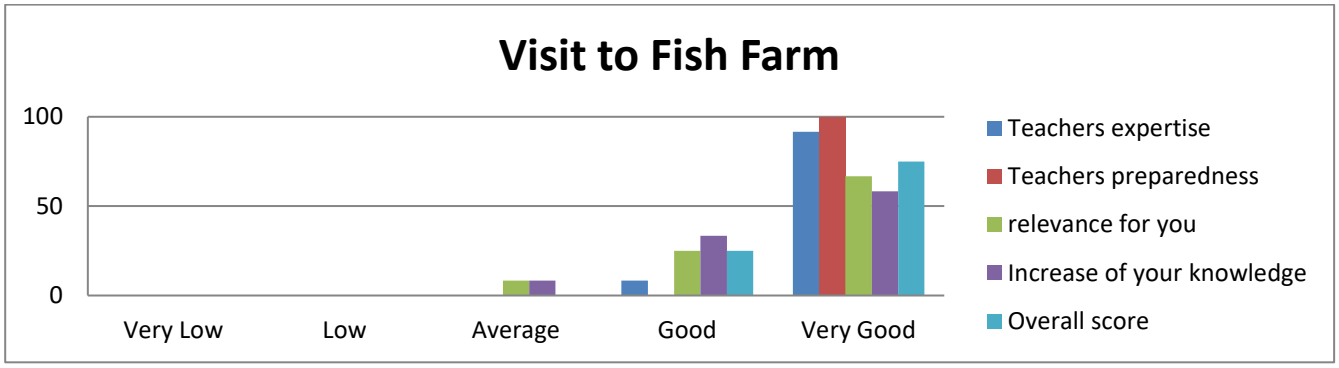
Programme: Methods for implementation of surveillance procedures for listed fish diseases

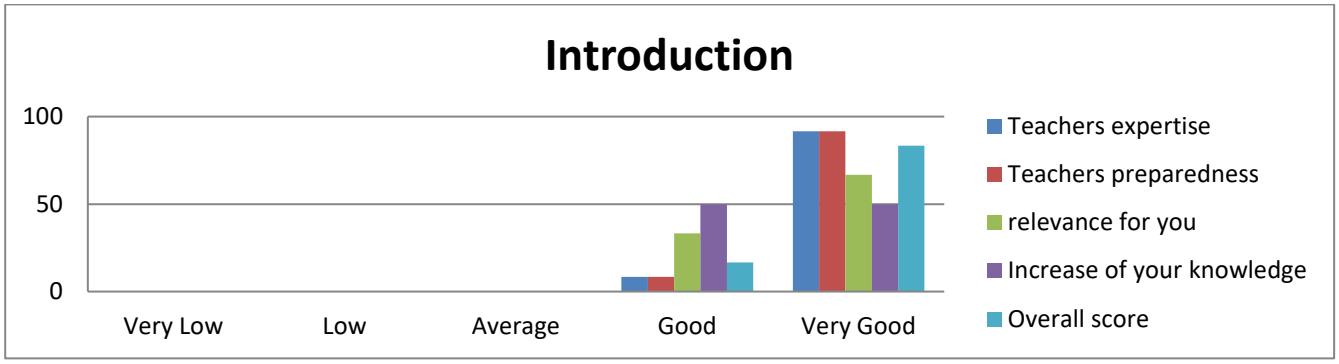
Day 1	Day 2	Day 3	Day 4	Day 5
Section 1 **Visit to fish farm and DVFA in Jutland**	**Section 2** **Laboratory introduction and sample preparation**	**Section 3** **qPCR analysis**	**Section 4** **Cell culture and phylogeny**	**Section 5** **Cell culture /bacteriology and evaluation**
8:00 – 11:00 **Transport by car to Danish Veterinary and Food Administration, DFVF Vejen in Jutland.** **Start in front of Cabinn Hotel** **11:00 – 12:15** **Aquaculture surveillance and sampling procedures in Denmark, By DVM Morten Fruergaard, DFVF. Control of VHS in DK by NJ Olesen**	**9:00 - 10:30** **Introduction.** **Participants experience and expectations** ***Coffee break 10:30 - 10:50*** **10:50 - 12:15** **Theoretical introduction to sample preparation, cell cultivation, virus ID and qPCR for surveillance programs for the non-exotic listed fish disease in Europe**	**9:00 – 10:30: PCR and real time PCR theory.** ***Coffee break 10:30 - 10:50*** **10:50 - 12:15 Result analysis Practical exercises**	**9:00 - 10:30** **PCR and Real Time PCR Troubleshooting.** **The diagnostic laboratory – PCR flow.** ***Coffee break 10:30 - 10:50*** **Sequencing theory and practical exercises**	**9:00 - 12:10** **Team 1,2 and 3 - Cell observation** **Team 4, 5 and 6 - Fish bacteriology demonstration** ***Coffee break 10:30 - 10:50*** **Team 4, 5 and 6 - Cell observation** **Team 1,2 and 3 - Fish bacteriology demonstration**
Lunch: 12:15 - 13:00	**Lunch: 12:15 - 13:00**	**Lunch: 12:15 - 13:00**	**Lunch: 12:15 - 13:00**	**Lunch: 11:45 - 12:30**
13:00 – 13.30 Transport to Hesselho Fish Farm **13:30 – 15:30** **Inspection and sampling** **15:30 – 19:00** **Transport by car to hotel Cabinn**	**13:00 - 14:30** **Team 1,2 and 3: Sample preparation for cell culture, PCR and bacteriology on samples collected Monday** **Team 4, 5 and 6: Practical cell culture passaging and production of 24 well plates** ***Coffee break 14:30 - 14:45*** 14:45- 16.45 Change **19:00 -Social dinner**	**13:00- 14:30 Reading cells and inoculation of samples** ***Coffee break 14:30 - 15:00*** **15.00—16.00 Titration procedure, viral titre calculation**	**13:00 – 14:30** **Blast analysis and practical exercise** ***Coffee break 14:30 - 15:00*** **15:00-17:00** **Introduction to phylogenetic analysis**	**12:30 – 14:45** **Assignment + presentation and assessment of data obtained by each group** **Discussion and recommendations** **Conclusion** **14:45-15:00** **Course evaluation, coffee and goodbyes**

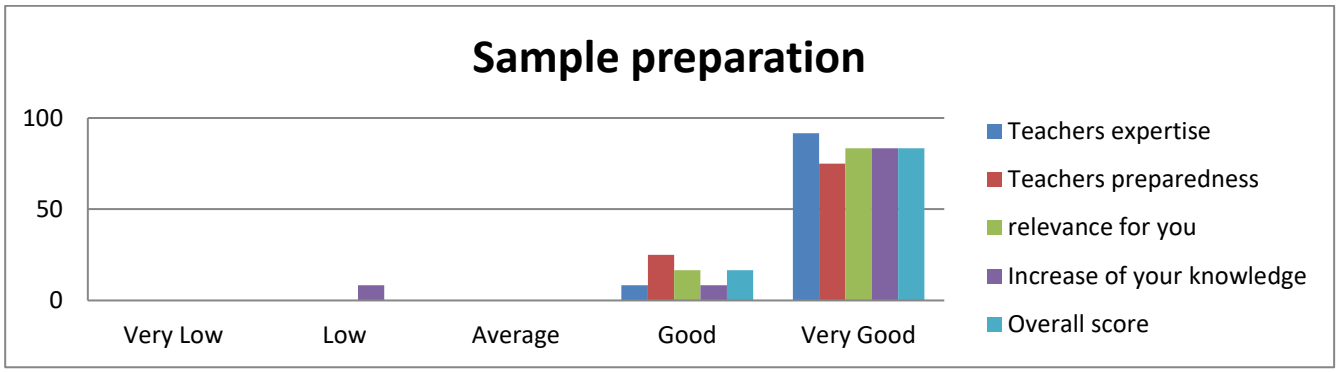
Evaluation: Methods for implementation of surveillance procedures for listed fish diseases

Participant satisfaction level for each respective section. The calculations are based on returned evaluation schemes from 12 participants.








Sample preparation
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Very Low
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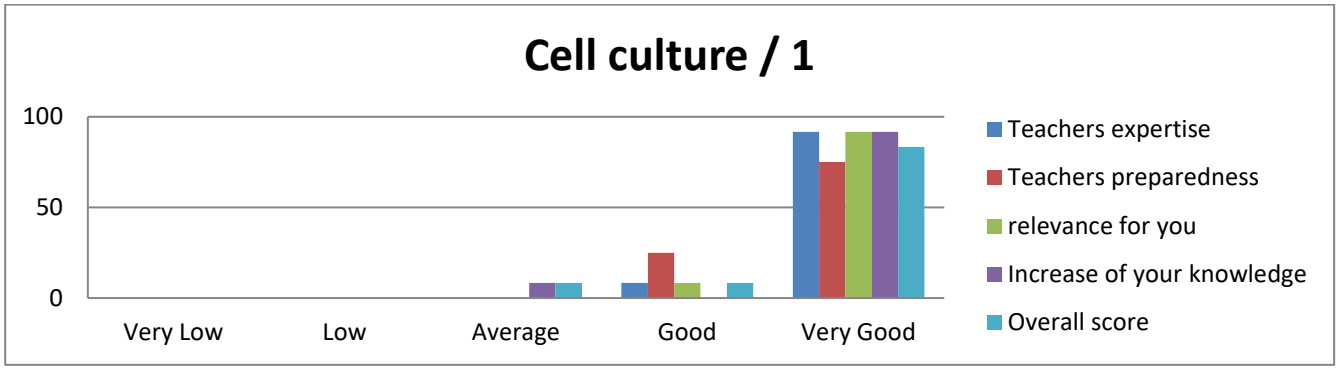
Average
Good
Very Good
Teachers expertise
Teachers preparedness
relevance for you
Increase of your knowledge
Overall score


Cell culture / 1
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Good
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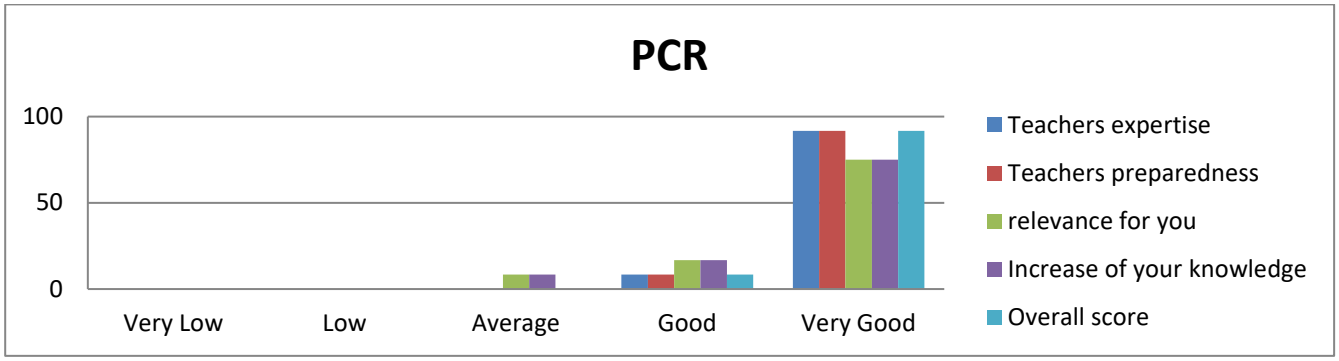
Teachers expertise
Teachers preparedness
relevance for you
Increase of your knowledge
Overall score


PCR
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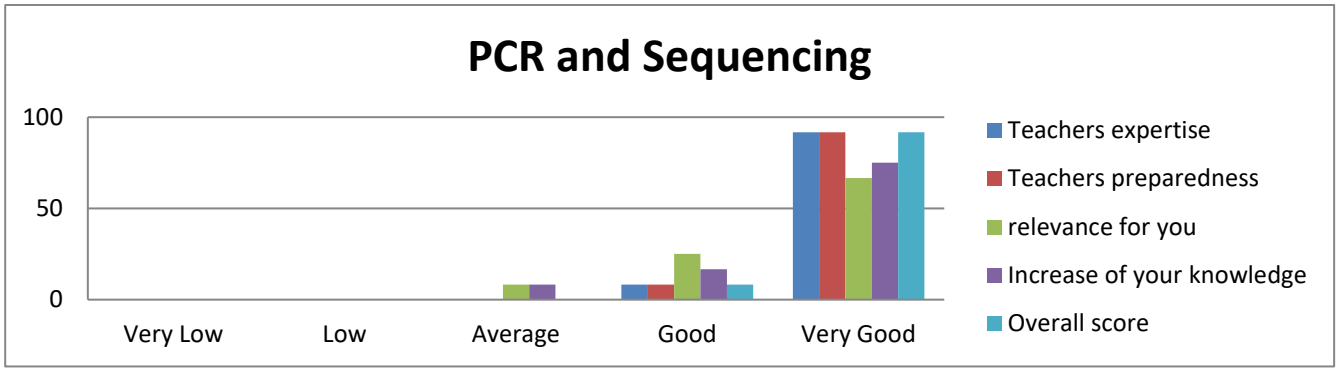
Teachers expertise
Teachers preparedness
relevance for you
Increase of your knowledge
Overall score


PCR and Sequencing
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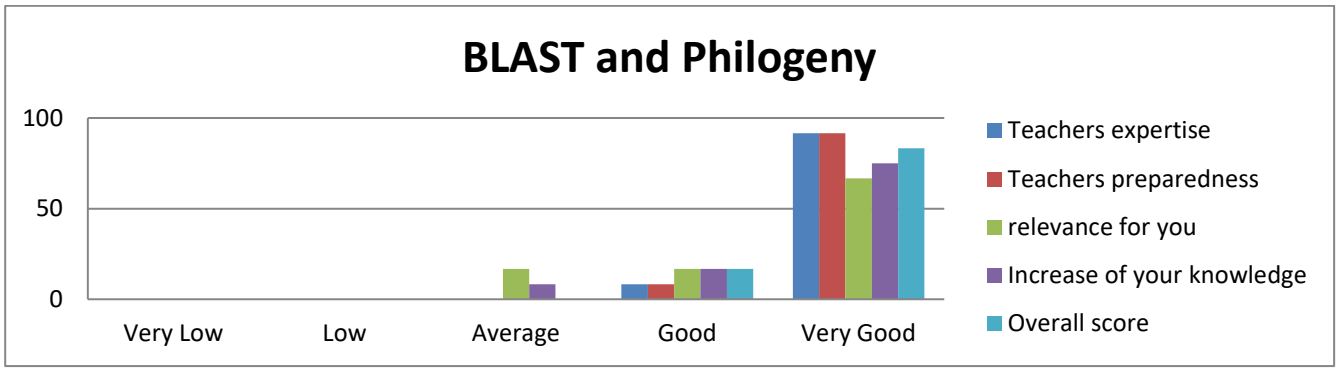
relevance for you
Increase of your knowledge
Overall score


BLAST and Philogeny
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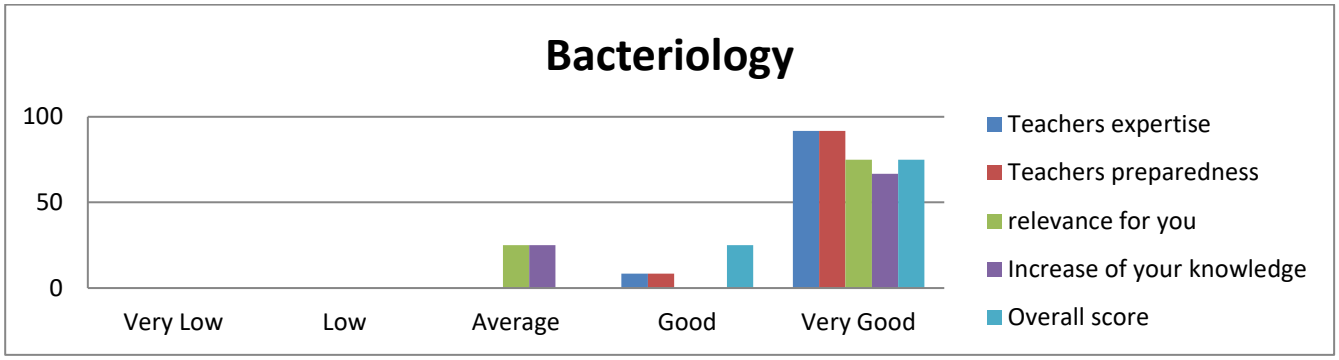
Bacteriology
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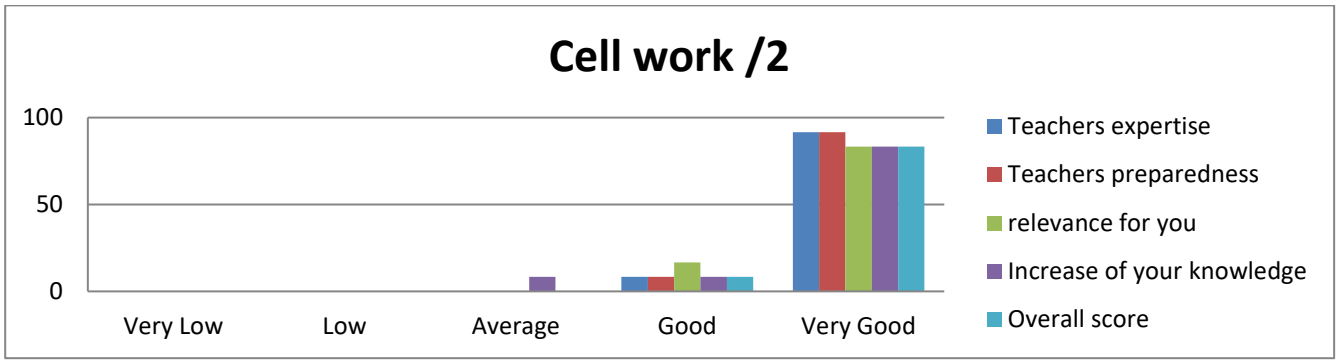
Teachers expertise
Teachers preparedness
relevance for you
Increase of your knowledge
Overall score


Cell work /2
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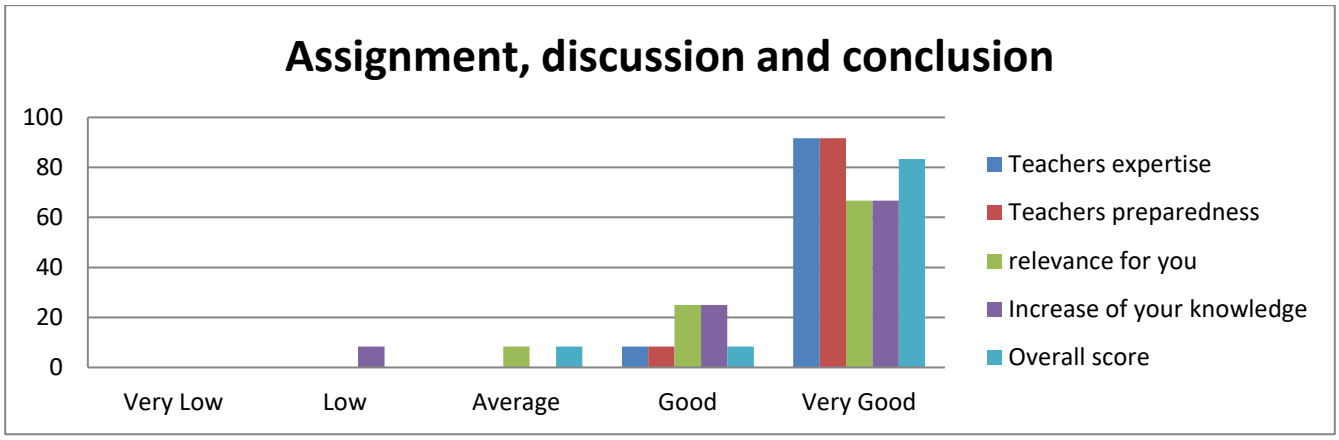
Teachers expertise
Teachers preparedness
relevance for you
Increase of your knowledge
Overall score


Assignment, discussion and conclusion
100
80
60
40
20
0
Very Low
Low
Average
Good
Very Good
Teachers expertise
Teachers preparedness
relevance for you
Increase of your knowledge
Overall score

Course description: Introduction to histopathology in fish and crustacean diseases

Course content

The 5-days course is primarily based on a combination of practical work (hands on) and theoretical presentations.

This course will focus on the use of histopathology in fish and crustacean diseases, combining a general histopathological approach with pathogen specific techniques such as Immunohistochemistry (IHC).

The first day participants will be shown how to take optimal samples for histopathological evaluation, considering different tissues and fish sizes. In the afternoon, lectures in pathology and histopathology will begin.

During the next days, the participants will continue the training track with a combination of lectures and practical work and will be introduced to special staining methods or pathogen detecting techniques like IHC. Part of the forth and the whole fifth day is dedicated to crustacean diseases using a mixture of lectures and practical work. The course gives an introduction to general pathology and the specific histopathological lesions and lesion pattern that occur as a consequence of disease. Focus is put on the understanding of general pathological processes and on training in histopathological diagnostic skills. The course is dialogue based and sufficient time will be given for discussion under way.

A social dinner will be organized the third evening. Further details are provided in the invitation letter.

General course objectives

The course aims to introduce participants to the use of histopathology in fish and crustacean diseases, combining technical knowledge on how to process samples including collection, fixation and the detection and description of lesions that can be observed during different disease stages of systemic infections.

The course will be structured on two main pillars: an overarching part on how to approach histopathology and combine theoretical knowledge on specific lesions to diseases patterns and a more specific part on Immunohistochemistry describing pitfalls and application of this technique to specific pathogens.

Lectures will include descriptions of the techniques with major focus on their application, pitfalls and trouble shooting. Practical sessions and show-and-tell sessions will allow participants to spend time on the microscope individually observing prepared slides, working with scanned slides, open discussion as well as one-to-one supervision with the tutors.

Participants are encouraged to bring their own slides to discuss the case with the other participants and tutors. If slides for the last day with open discussion have not been sent beforehand they should be handed in on the first day.

Learning objectives
This course aims to introduce the students to pathology and histopathology of fish and crustaceans with the main focus on the systemic infections in farmed fish and crustaceans.

The participants that have completed the entire course and fulfilled the course's objectives:

Will be able to:
- sample organs and tissue for histopathological examination and submit them in a correct way.

Will have gained knowledge:
- on how to discriminate between normal histology and artefacts that occurred during fixation and processing.
- on how to detect and describe pathological changes and patterns in a systematic and uniform way.
- on the technology for preparing IHC and how to assess pitfalls and errors in staining processes.

Overall the course will allow participants to understand the underlying principles of the histopathology and specific techniques such as IHC, thus increasing the ability to evaluate histological slides and critically review results based on histopathological examination. Furthermore the course will allow the participants to obtain a better understanding of specific staining methods thus increasing the ability to critically review these methods in order to assess pitfalls and to correctly interpret them

The major focus will be on systemic infections including listed fish and crustacean diseases.

The course will provide a forum where pre-knowledge, experience and examples can be discussed between participants and teachers, and hereby raise the awareness of pitfalls when using the various techniques.

Intended learning outcomes
To increase the practical and theoretical knowledge of histopathology of systemic fish and crustacean diseases including listed diseases. The course also aims at providing a forum where (good and bad) experiences can be discussed among participants and teachers.

The core elements
Histopathology of fish diseases
Histopathology of crustacean diseases
IHC applied to fish tissue

Assessment
During each day participants are encouraged to take part in the discussions on the subjects presented.
A specific session at the end of the course is allocated for discussion and evaluation of the course and at the end of the course a questionnaire for course evaluation will be delivered to all participants.

The course material
A course binder with practical information will be provided. The course binder will also be used for collection of hand-outs from the various lectures.

The course participants

Since course attendants can come from very different experiences, during the general introduction (day 1), researchers and technicians will be asked to introduce themselves, their pre-experience in the laboratory and their expectations to the course in order to target the course content optimally, especially during the theoretical- and discussion workshops. Their starting point will therefore be mixed as some may have limited theoretical or practical experience, while others may be highly experienced in some or all disciplines.

Course supervisors

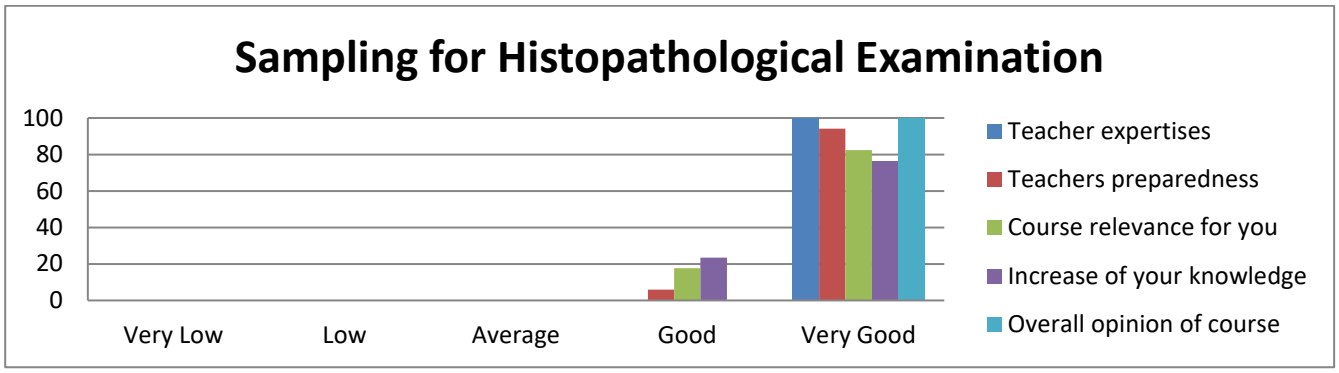
Ole Bendik Dale, tutor responsible for pathology and histopathology on fish
Kelly Bateman, tutor responsible for pathology and histopathology on crustacean
Torsten Snogdal Boutrup tutor responsible on IHC
Tine Moesgaard Iburg, tutor and course facilitator
Niccolò Vendramin, course facilitator
Linda Stuhr Christensen, secretary
Lis Vinther Elmsted, secretary

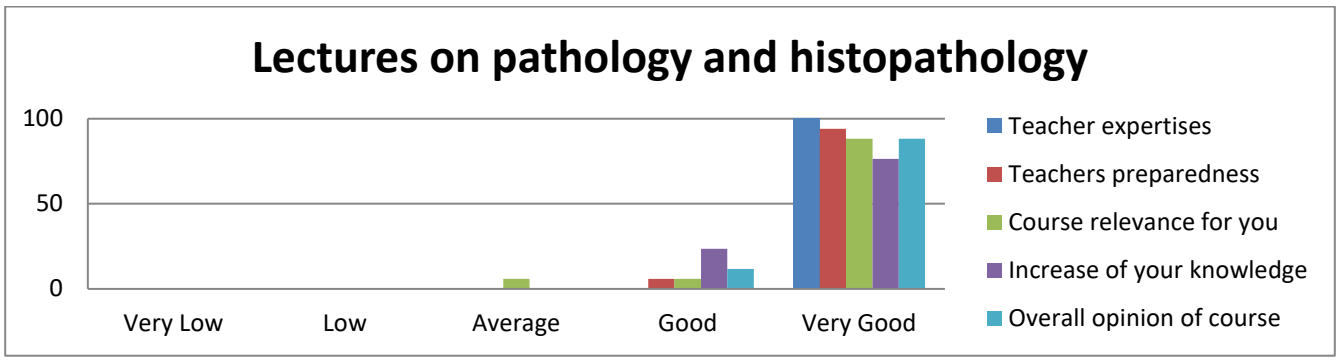
Programme: Introduction to histopathology in fish and crustacean diseases

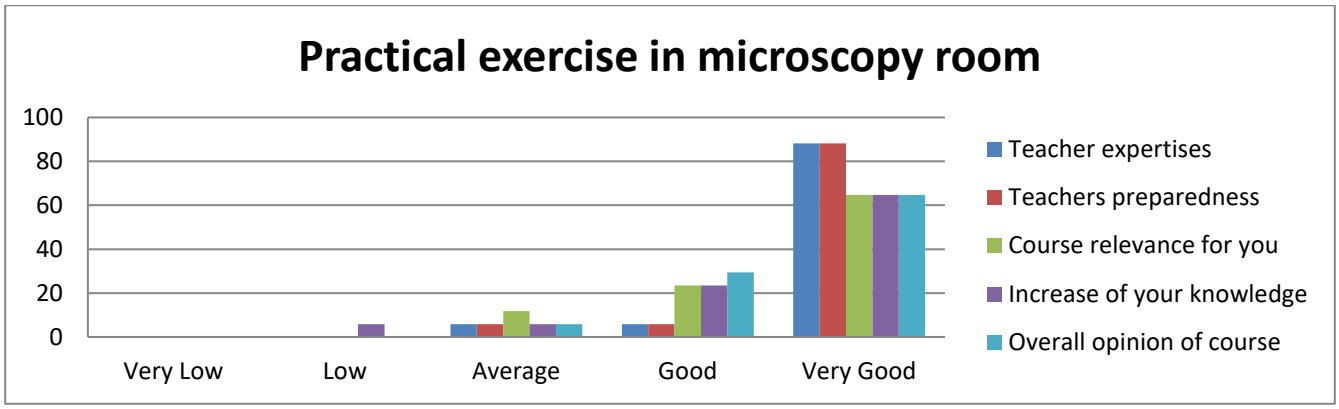
Day 1	Day 2	Day 3	Day 4	Day 5
Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**
9:00-10:30 **Course introduction** **Participants will present themselves** **Place: Auditorium** ***Coffee Break 10:30-11:00*** **11:00-12:30** **Sampling for histopathological examination. Theory and Practice** **Place: Necropsy room**	**9:00-10:30** **Lecture on pathology and histopathology** **Place: Auditorium** ***Coffee Break 10:30-11:00*** **11:00-12:30** **Microscopy room I** **Practical exercise**	**9:00-10:00** **Lecture on IHC I** **Place: Auditorium** ***Coffee Break 10:00-11:00*** **10:30-12:00** **Lecture on IHC II** **Place: Auditorium**	**9:00 - 10:00** **Microscopy Room/auditorium** **Show and tell of cases by Ole Bendik Dale with discussion and participation of course participants** ***Coffee Break 10:00-10:30*** **10:30- 12:00** **Microscopy room** **More show and tell**	**9:00 – 9:30** **Overview of WSSV** **9:30 – 10:30** **Microscopy practicals (scanned slides)** ***Coffee Break 10:30-11:00*** **11:00 – 12:30** **Overview of TSV and YHV** **Microscopy practicals (scanned slides)**
Lunch 12:30 -13:30	**Lunch 12.30-13.30**	**Lunch 12:00 -13:00**	**Lunch 12:00 -13:00**	**Lunch 12:30-13:30**
13:30 – 14:30 **Lecture on pathology and histopathology** **Place: Auditorium** ***Coffee Break 14:30-15:00*** **15:00 – 16:00** **Lecture on pathology and histopathology** **Place: Auditorium**	**13:15 – 13:30** **Lecture on pathology and histopathology** **Place: Auditorium** ***Coffee Break 13:30-14:00*** **14:00-16:00** **Microscopy room II** **Practical exercise**	**13:00-14:00** **Theoretical exercise IHC I** **Place: Auditorium** ***Coffee Break 14:00 – 14.30*** **14:30-16:30** **Microscopy room III** **Practical exercise** **16:30 – 17:15** **Theoretical exercise IHC II** **Place: Auditorium** **Social Dinner in the evening**	**13:00-14:30** **Crustacean Dissection and Sampling** **Place: Auditorium** ***Coffee Break 14:30 – 15:.00*** **15:00-16:00** **Shrimp Dissection Practical** **Place: Necropsy room** **16:00-17:00** **Overview of Crustacean Tissues - Structure and Function** **Place: Auditorium**	**13:30-14:00** **OIE listed diseases and Emerging Pathogens** **Microscopy Practicals (scanned slides)** **Place: Auditorium** **14.00 - Coffee, cakes and evaluation** **Place: auditorium**

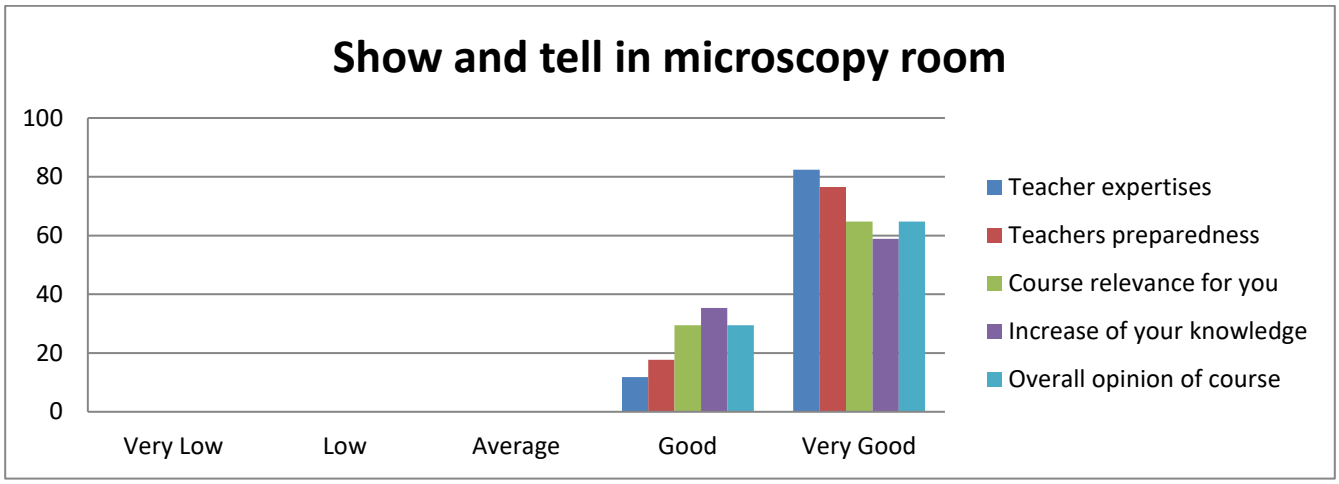
Evaluation: Introduction to histopathology in fish and crustacean diseases

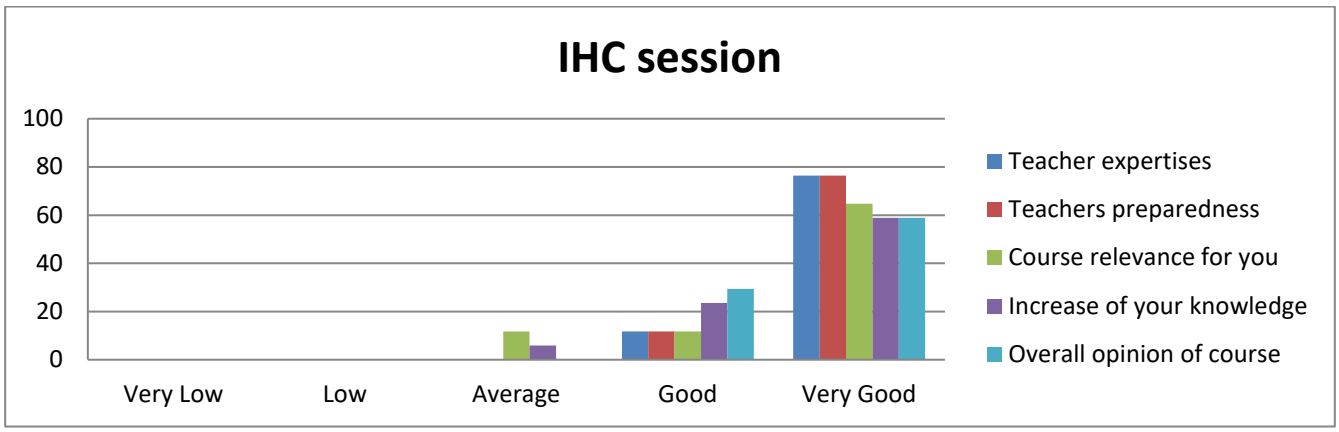
Participant satisfaction for each respective section. The calculations are based on returned evaluation schemes from 17 participants.

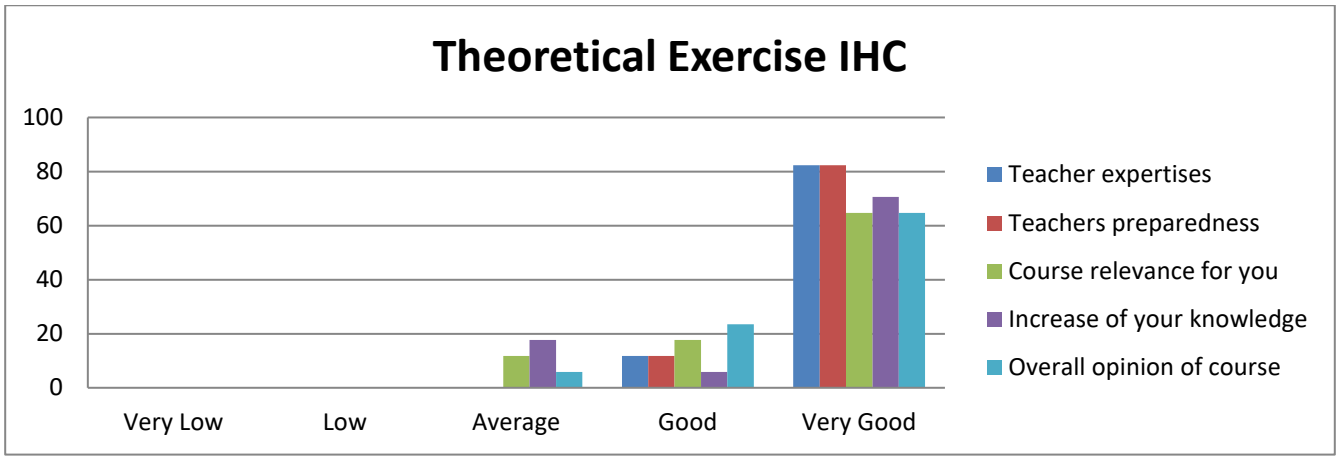


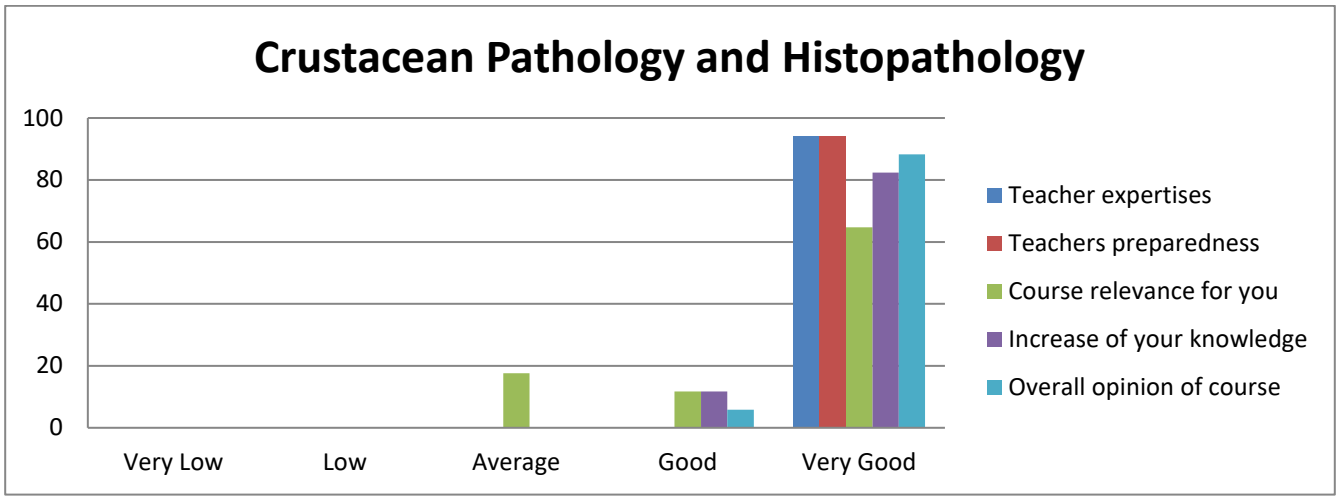


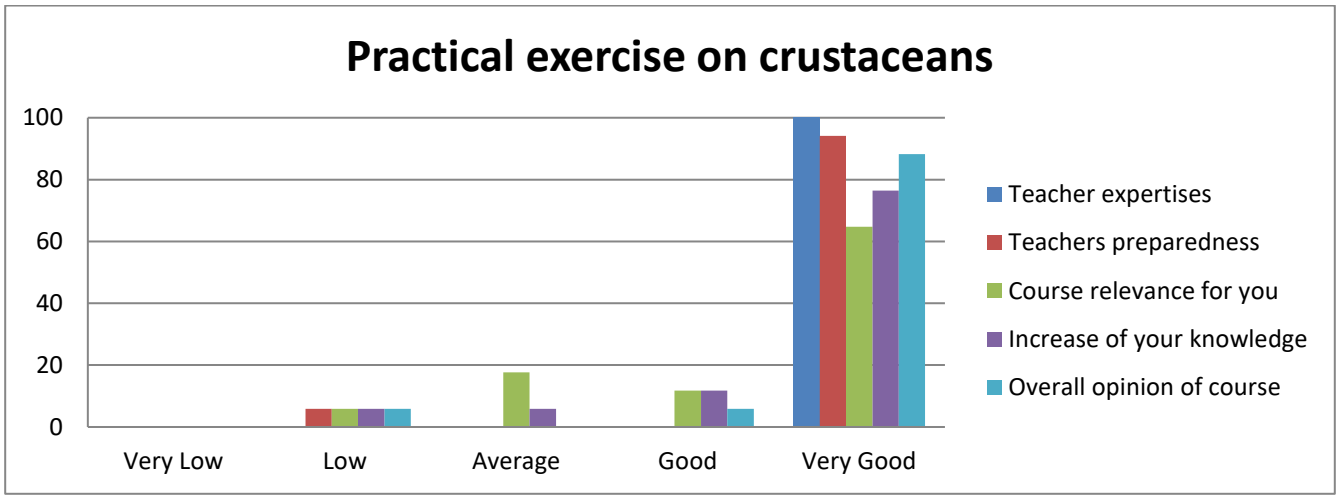



Show and tell in microscopy room
100
80
60
40
20
0
Very Low
Low
Average
Good
Very Good
Teacher expertises
Teachers preparedness
Course relevance for you
Increase of your knowledge
Overall opinion of course


IHC session
100
80
60
40
20
0
Very Low
Low
Average
Good
Very Good
Teacher expertises
Teachers preparedness
Course relevance for you
Increase of your knowledge
Overall opinion of course


Theoretical Exercise IHC
100
80
60
40
20
0
Very Low
Low
Average
Good
Very Good
Teacher expertises
Teachers preparedness
Course relevance for you
Increase of your knowledge
Overall opinion of course


Crustacean Pathology and Histopathology
100
80
60
40
20
0
Very Low
Low
Average
Good
Very Good
Teacher expertises
Teachers preparedness
Course relevance for you
Increase of your knowledge
Overall opinion of course


Practical exercise on crustaceans
100
80
60
40
20
0
Very Low
Low
Average
Good
Very Good
Teacher expertises
Teachers preparedness
Course relevance for you
Increase of your knowledge
Overall opinion of course

Comments from the participants (evaluation schemes from both courses)

"Awesome team: really helpful and eager to share knowledge"

"I learned a lot, what I need for my own work"

"Attitude of all tutors was amazing and I appreciate their engagement and preparation"

"Extremely valuable course, the lecture material was well complemented with practicals"

"the course had a nice balance of theory and practice. Very well organized. Teachers and participants are very friendly and approachable. Teacher very knowledgeable but relaxed"

"this course helped me to learn about immunohistopathology which was new to me. I got good suggestions for my histopathology method improvements"

Closing remarks

The EURL training course 2019 was - based on the feedback from the participants - considered a success. The evaluation schemes enabled the participants to evaluate each day and topic on the course. The majority of the participants still evaluate the courses "very good".

The possibility to provide financial support to participants made it possible to offer training to laboratories where the lack of funding usually makes it hard to find the resources to participate in such activities. This way of funding the training courses, therefore, holds the possibility to increase the expertise in all National Reference Laboratories within the EU.

Again, this year's course on "Methods for implementation of surveillance procedures for listed fish diseases", it was decided to include an inspection to a fish farm, to demonstrate the full process from sampling fish at a fish farm to final reading and interpretation of results, giving a holistic view and large span of topics that, according to the evaluation schemes, were well received.

DTU-Aqua is acknowledged for offering training course facilities for free. Morten Fruergaaard-Andreasen from the Danish Veterinary and Food Administration is deeply acknowledged for organizing the office and field visit and for teaching how to organize and implement surveillance and eradication programmes and how to inspect and sample on fish farms. Hesselho Fish Farm and Mr. Jens Jensen are deeply acknowledged for great hospitality and for providing all information and facilities needed during the farm visit.

External tutors, Dr. Ole Bendik Dale, Norwegian Veterinary Institute, Oslo, Norway, Dr. Torsten Snogdal Boutrup, Aquapri, Denmark and Dr. Kelly Bateman, Cefas, UK are deeply acknowledged for their very enthusiastic and excellent lectures.

Finally, all laboratory technicians and scientists in the unit for fish and shellfish diseases at DTU Aqua are deeply acknowledged for delivering excellent teaching and training and help with practical issues. Also technician Vibeke Bøgelund Hansen from Copenhagen University is thanked for all her practical help at Frederiksberg Campus on Copenhagen University in connection with course 2.

Copenhagen, Monday, 18 November 2019

Niels Jørgen Olesen, Niccoló Vendramin, Jacob Günther Schmidt, Tine Moesgaard Iburg, Argelia Cuenca and Lone Madsen

EURL for Fish and Crustacean Diseases