European Union Reference Laboratory for Fish and Crustacean Diseases
National Institute of Aquatic Resources, Technical University of Denmark

EURL Training Courses

Copenhagen, October 8th - 19th 2018

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 8th to the 19th, 2018. Two courses were prepared: the first one, with 11 trainees, was entitled "Methods for implementation of surveillance procedures for listed fish diseases" and took place from 8th to 12th October. The second course was entitled "Introduction to histopathology in fish and crustacean diseases" and took place from the 15th to 19th October 2018 with 15 participants. A single person participated in 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 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 EURL coordinator stand-in Jacob G. Schmidt participated from the EURL**.** In the morning, the participants were picked up at the hotel in mini buses 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. A pond of fish was being size sorted during the visit, so the participants were able to see this procedure performed. 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 very "hyggelig" social dinner in the burrow of Vesterbro (close to the hotel) in which all participated.

Day 3 started by reading and inoculating the cells produced the day before with samples taken at the fish farm. Followed by a presentation 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 wet laboratory of DTU Aqua.

The afternoon was dedicated to PCR and real-time PCR theory followed by practical exercises and result analysis. The participants attended presentations explaining the theoretical principles behind the different PCR techniques, and the pros and cons of the use of the different techniques in the diagnostic laboratory. There was a session addressing the troubleshooting of PCR methods, sampling treatment and documentation. The next session consisted in methods of data analysis in real time PCR. This was split in a theoretical part and in and practical part where exercises for the analysis and interpretation of qPCR results were carried on.

Day 4 was 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 bacteriology techniques and visited the EURL laboratory and tank facilities and the other group were reading their inoculated cell cultures and a number of cell lines inoculated with various fish viruses, including VHS, IHN, EHN, IPN and KHV - the groups thereafter switched. After lunch all teams we 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. 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, and Kristina Andkjær Andersen 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 four-day part on histopathology on fish and a one-day part on histopathology on crustacean. In both parts, theoretical lectures on relevant topics alternated with practical exercises both in necropsy room and microscopy laboratory.

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 thereafter tried the technique on their own fish. Lectures given by Ole Bendik Dahle 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 Dahle 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 Tine Moesgaard Iburg, and Torsten Snogdal Boutrup. Theoretical exercises in IHC were used as a platform for discussions.

Day 4 started directly in the lecture room with show and tell by Ole Bendik Dahle 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, diagnostic cases brought by participants were discussed and presented in an open forum in the microscopy room, with supervision of tutors Ole Bendik Dahle and Tine Moesgaard Iburg. This was followed by a small round of show and tell of different, both emerging and more common, diseases to give a broader perspective on histopathology of fish diseases by Ole Bendik Dahle. At the end of the day the fish part was closed and participants were given the opportunity to raise points that could be improved.

Day 5 was dedicated to crustacean histopathology. The morning was filled with lectures on sampling issues and fixation 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 both the lectures, the breaks, the theoretical and practical exercises.

As get-together, a dinner event for all was held on day two. Furthermore, the participants having major focus on crustacean diseases were invited for a dinner meeting on day 4 in order to increase the networking.

Participant list

Name	Surname	Country	Affiliation	Course 1	Course 2
Alexandra	Hughes	England	Centre for Environment, Fisheries and Aquaculture Science (Cefas)	x	
Isabel	Aguirre Gil	Chile	Laboratory of Aquatic Pathology and Biotechnology, Universidad Austral de Chile	x	
Ivana	Giovanna Zupičić	Croatia	Croatian Veterinary Institute, Zagreb	x	x
Johan	Lundgren	Sweden	National Veterinary Institute (SVA)	x	
Laleh	Moazzami Goudarzi	Iran	NRL of Iran Veterinary Organization	x	
Laura	Ruicēna	Latvia	Institute of Food Safety, Animal Health and Environment "BIOR"	x	
Laurane	Pallandre	France	French Agency for Food, Environmental and Occupational Health & Safety	x	
Patricia	White	Scotland	Marine Scotland Science	x	
Per	Walter Kania	Denmark	Department of Veterinary and Animal sciences	x	
Rita	Vorobjoviene	Lithuania	National Food and Veterinary Risk Assessment Institute	x	
Triin	Tedersoo	Estonia	Estonian Veterinary and Food Laboratory	x	
Charlotte	Axén	Sweden	National Veterinary Institute (SVA)		x
Farah	Gonul Aydin	Turkey	Ankara University Faculty of Veterinary Medicine		x
Daniela	Stamate	Romania	Institute for diagnostic and Animal Health		x
Handan	Cetinkaya	Turkey	Department of Parasitology, University of Istanbul		x
Isabel	Gonzalo Pascual	Spain	Laboratorio Central de Veterinaria		x
Lisa	Furnesvik	Norway	Norwegian Veterinary Institute		x
Louise	von Gersdorff Jørgensen	Denmark	University of Copenhagen		x
Magdalena	Stachnik	Poland	National Veterinary Research Institute		x
Michaela	Brincko Cervenska	Slovakia	State Veterinary and Food Institute		x
Nichola	Still	Scotland	Marine Scotland Science		x
Riikka	Holopainen	Finland	Finnish Food Safety Authority, Evira		x
Tobia	Pretto	Italy	Istituto Zooprofilattico Sperimentale delle Venezie		x
Toni	Erkinharju	Norway	Norwegian Veterinary Institute		x
Tomas	Krivosudsky	Slovakia	State Veterinary and Food Institute		x

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

Course content

This five-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

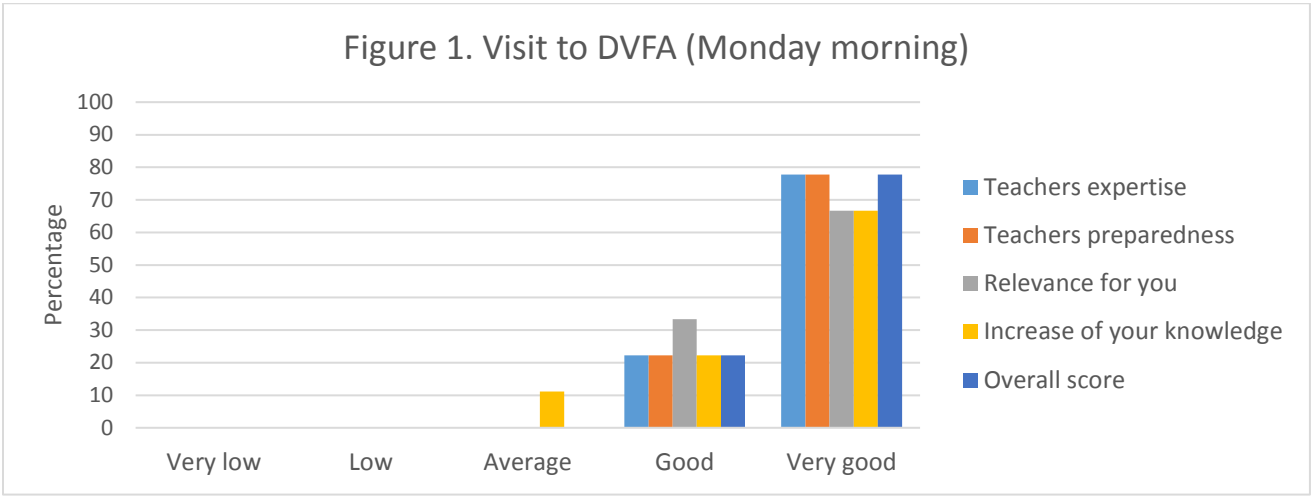
Technical help and assistance for running the laboratory courses will be given by
Betina Lynnerup (cell culture)
Christina Flink Desler (sample preparation)
Troels Secher Rundqvist (Real Time PCR)
Teena Vendel Klinge (Real Time PCR)

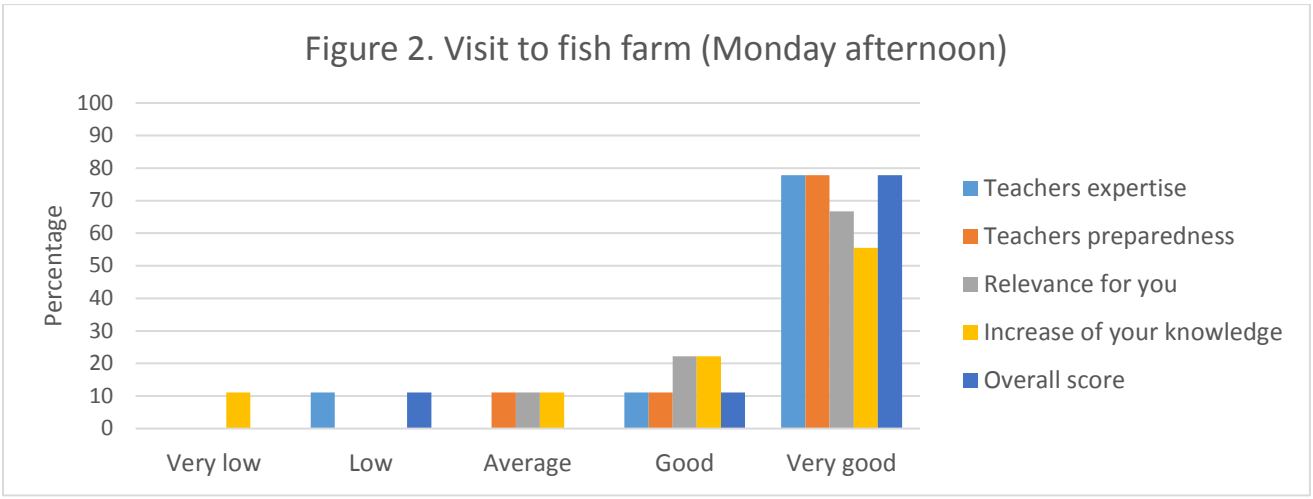
Programme: Methods for implementation of surveillance procedures for listed fish diseases

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** **Cell culture methods and PCR analysis**	**Section 4** **PCR, blast 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 and practicalities. 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 – 9:45: Reading cells and inoculation of samples** **9:45 – 10:30 Use of cell culture in fish virology** ***Coffee break 10:30 - 10:50*** **10:50 - 12:15 Titration procedure, viral titre calculation. Barcoding cell lines**	**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: 12:15 - 13:00**
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, Frederiksberg**	**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- 16:30** **PCR and real time PCR theory.** ***Coffee break 14:30 - 15:00*** **Result analysis** **Practical exercises.**	**13:00 – 17:00** **Blast analysis and practical exercise** ***Coffee break 14:30 - 15:00*** **Introduction to phylogenetic analysis**	**13:00 – 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

Figure 1-11. Participant satisfaction level for each respective section. The calculations are based on returned evaluation schemes from nine participants.





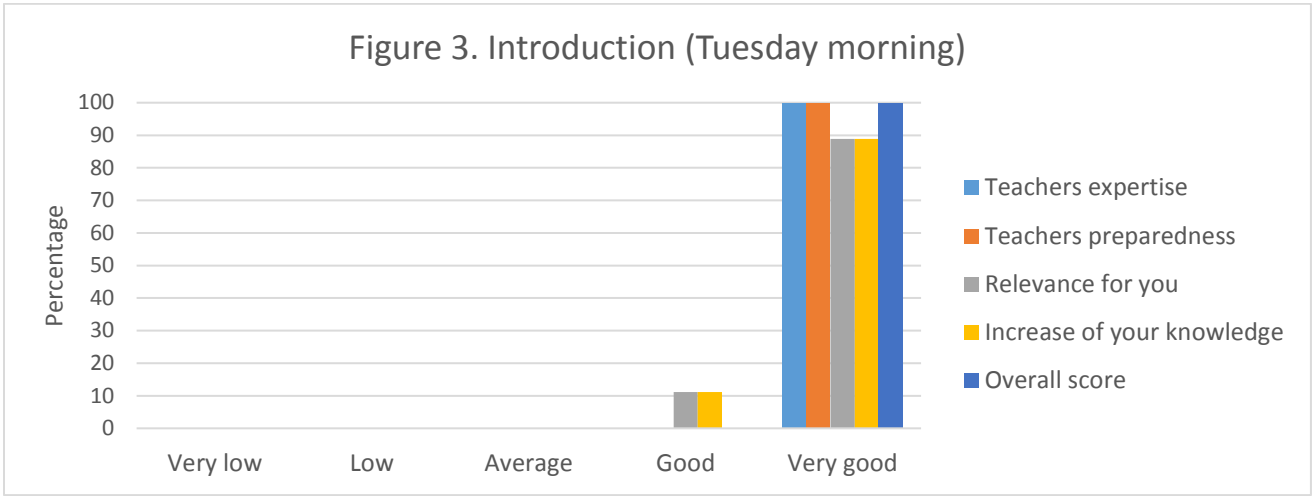


Figure 3. Introduction (Tuesday morning)
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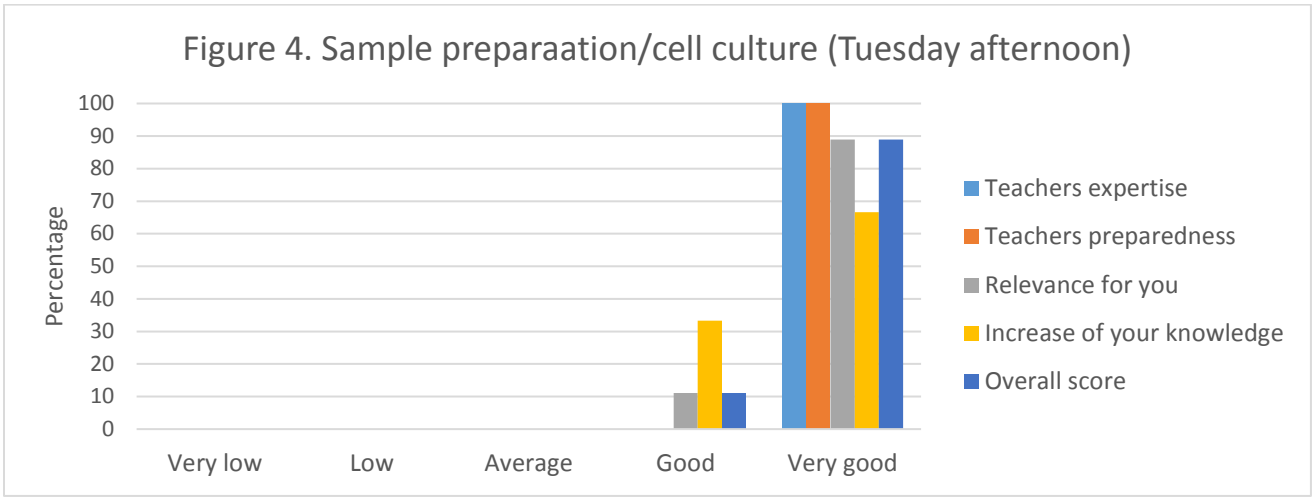
Figure 4. Sample preparaation/cell culture (Tuesday afternoon)
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Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

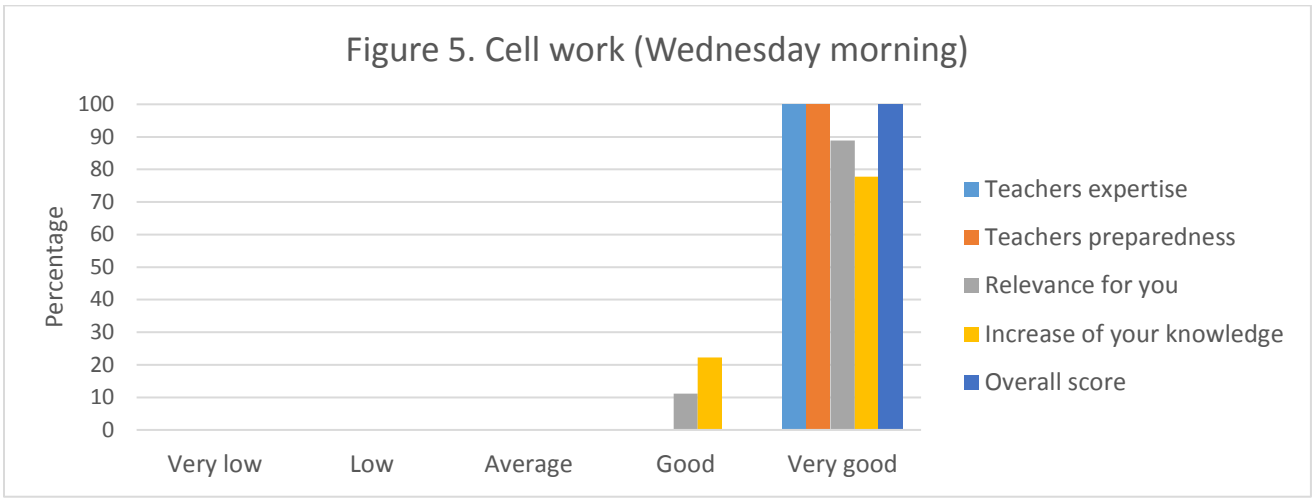
Figure 5. Cell work (Wednesday morning)
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Teachers expertise
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Increase of your knowledge
Overall score

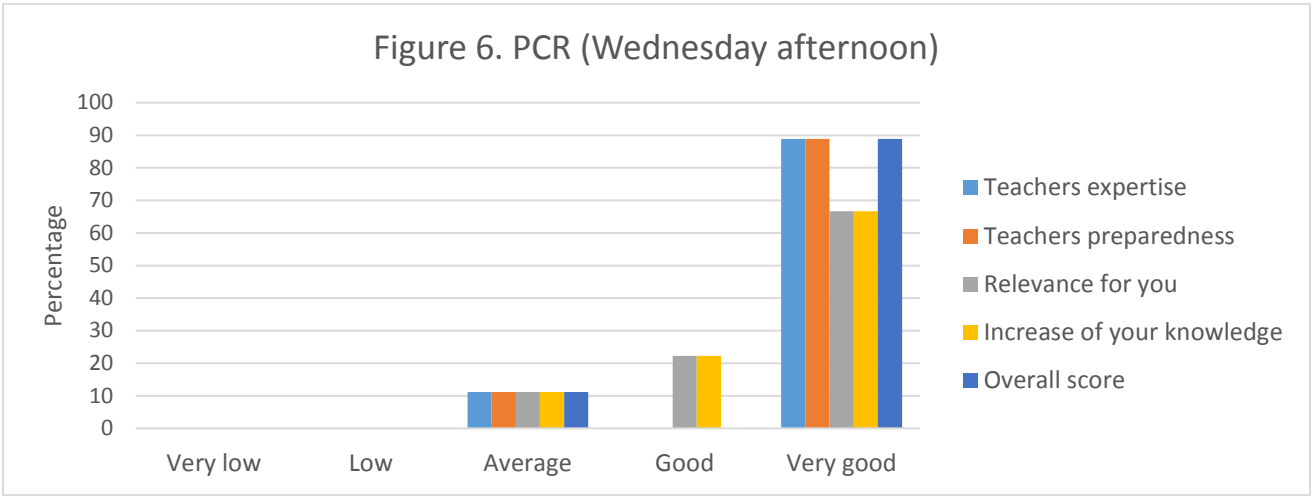
Figure 6. PCR (Wednesday afternoon)
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Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

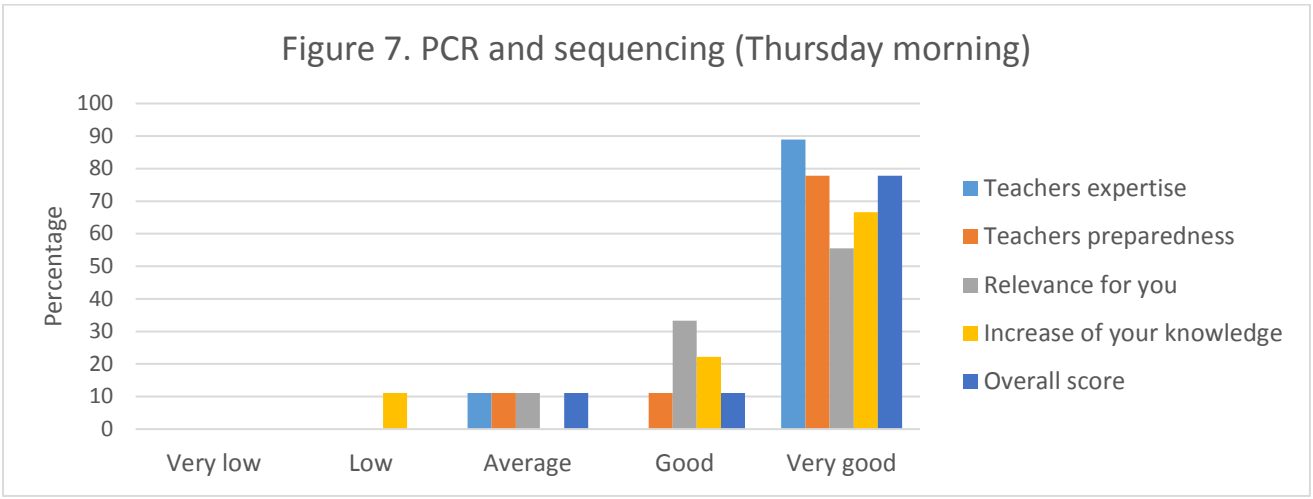
Figure 7. PCR and sequencing (Thursday morning)
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Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

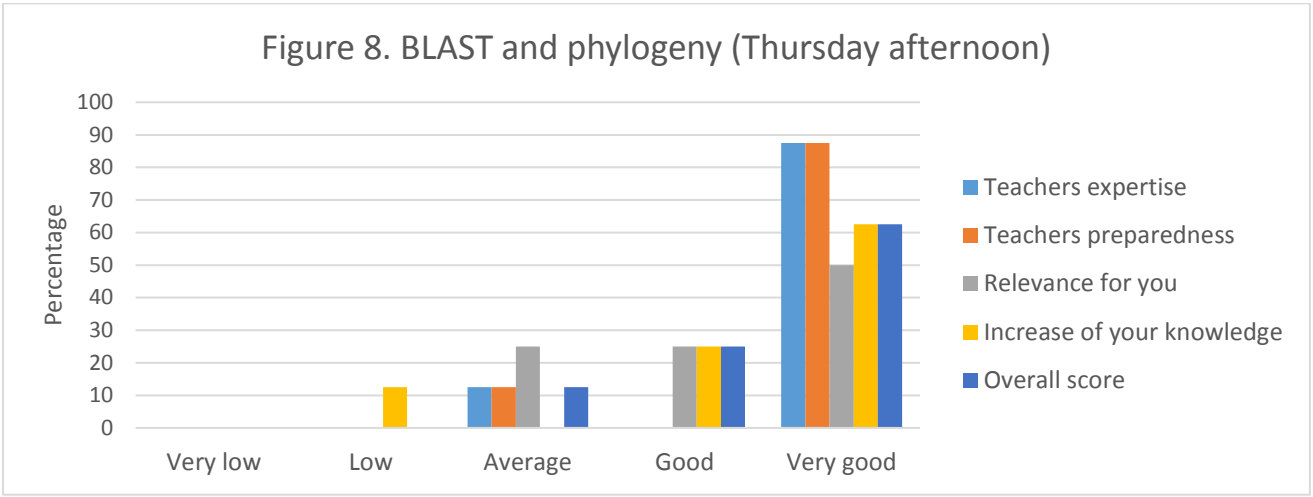
Figure 8. BLAST and phylogeny (Thursday afternoon)
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Teachers preparedness
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Increase of your knowledge
Overall score

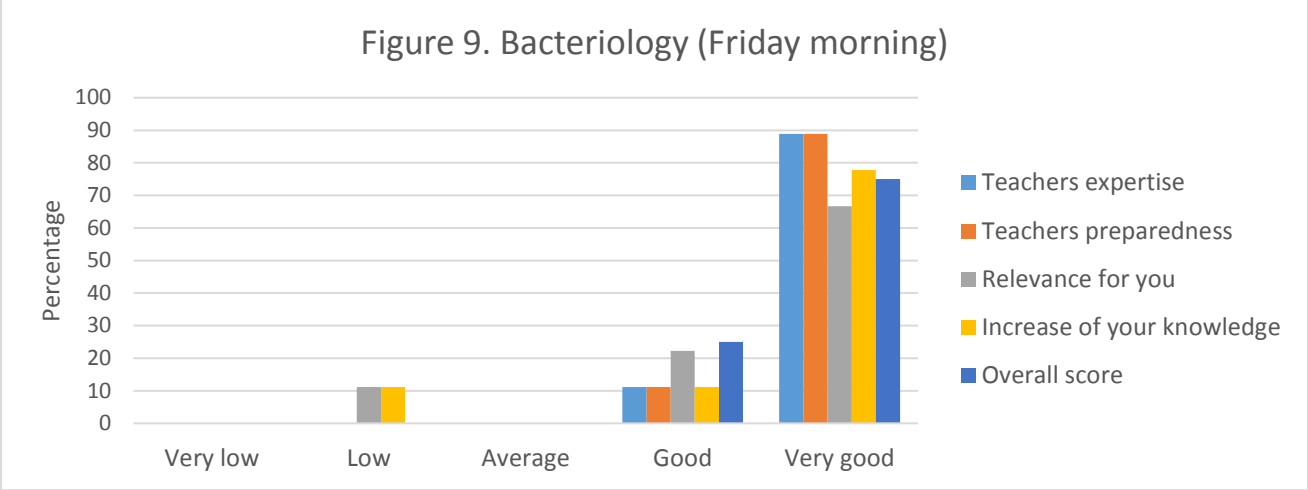
Figure 9. Bacteriology (Friday morning)
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Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

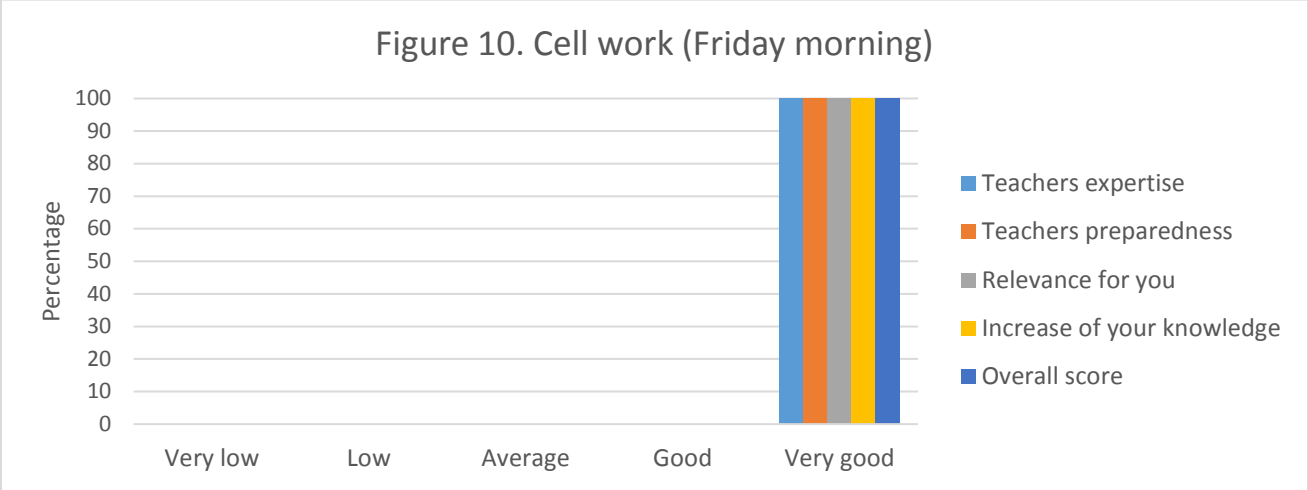
Figure 10. Cell work (Friday morning)
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Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

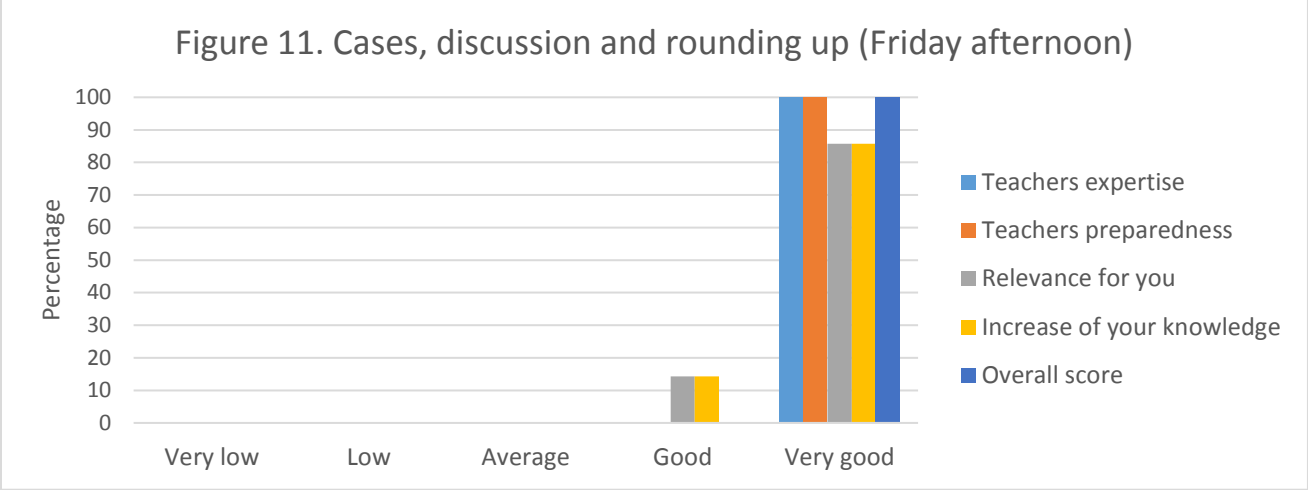
Figure 11. Cases, discussion and rounding up (Friday afternoon)
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Average
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Very good
Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

Course description: Introduction to histopathology in fish and crustacean diseases

Course content

The five-day 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. The 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 second 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, 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.

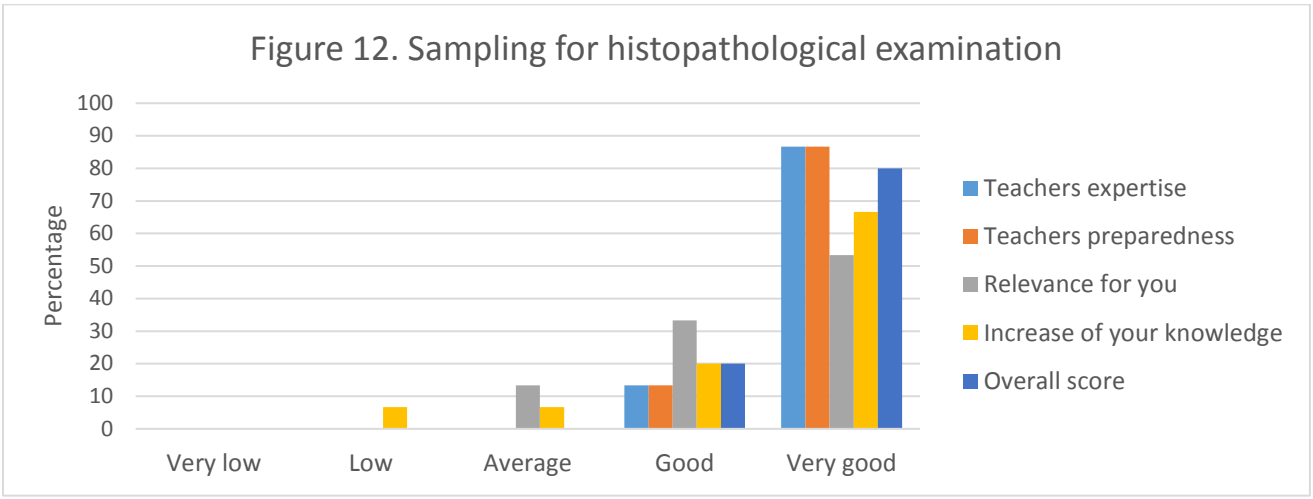
Course supervisors
Ole Bendik Dale, tutor responsible for pathology and histopathology on fish
Kelly Bateman, tutor responsible for pathology and histopathology on crustaceans
Torsten Snogdal Boutrup tutor responsible on IHC
Tine Moesgaard Iburg, tutor and course facilitator
Jacob G. Schmidt, course facilitator
Linda Stuhr Christensen, secretary
Lis Vinther Elmsted, secretary

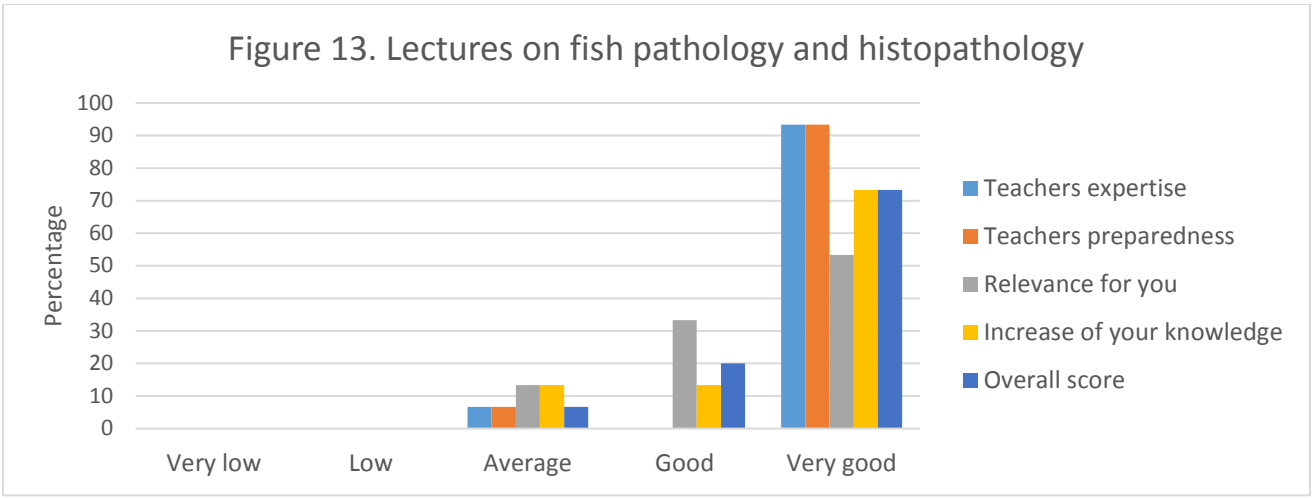
Programme: Introduction to histopathology in fish and crustacean diseases

Monday	Tuesday	Wednesday	Thursday	Friday
8:00-9:45 **Course introduction** **Participants will present themselves** **Place: Auditorium** **<u>Coffee Break 9:45-10:15</u>** **10:30-12:00** **Sampling for histopathological examination. Theory and Practice** **Place: Anatomy room same building**	**8:00-9:30** **Lecture on pathology and histopathology** **Place: Auditorium** **<u>Coffee Break 9:30-10:00</u>** **10:00-11:30** **Microscopy room I** **Practical exercise**	**8:00-8:30** **Introduction to DTU e-forms reimbursement** **8:30-9:30** **Lecture on IHC I** **Place: Auditorium** **<u>Coffee Break 9:30-10:00</u>** **10:00-11:30** **Lecture on IHC II** **Place: Auditorium**	**8:00 - 9:30** **Microscopy Room** **Show and tell of cases by Ole Bendik Dale with discussion and participation of course participants** **<u>Coffee Break 9:30-10:00</u>** **10:00- 11:30** **Microscopy room** **More show and tell**	**8:00 – 15:30** **Overview of Crustacean Tissues - Structure and Function** **Overview of WSSV & Overview of TSV and YHV** **<u>Coffee Break 9:30-10:00</u>** **OIE listed diseases and Emerging Pathogens** **Microscopy Practical**
Lunch 12.15 -13:00	**Lunch 11.30-12:15**	**Lunch 11.30 -12:15**	**Lunch 11:30 -12:15**	**Lunch 11:30-12:15**
13:00 – 15:30 **Lecture on pathology and histopathology** **Place:** **Auditorium**	**12:15 – 13:30** **Lecture on pathology and histopathology** **Place: Auditorium** **<u>Coffee Break 13:30-14:00</u>** **14:00-16:00** **Microscopy room II** **Practical exercise**	**12:15-13:15** **Theoretical exercise on IHC 1** **Place: Auditorium** **<u>Coffee Break 13:15 – 13.45</u>** **13:45-15:45** **Microscopy room III** **Practical exercise** **15:45 – 16:30** **Theoretical exercise on IHC 2** **Place: Auditorium**	**12:15-15:15** **General discussion on selected cases brought by participants** **Place: Microscopy room** **15:15-15:30** **Coffee, cakes and evaluation of the fish days** **Place: Auditorium**	**12:15-15:15** **Crustacean Dissection and Sampling** **Microscopy Practical and Demonstration of Slide Scanner** **Approx: 15.15 - Coffee, cakes and evaluation of the crustacean day** **Place: auditorium**

Evaluation: Introduction to histopathology in fish and crustacean diseases

Figure 12 – 20. Participant satisfaction for each respective section. The calculations are based on returned evaluation schemes from 13 participants.





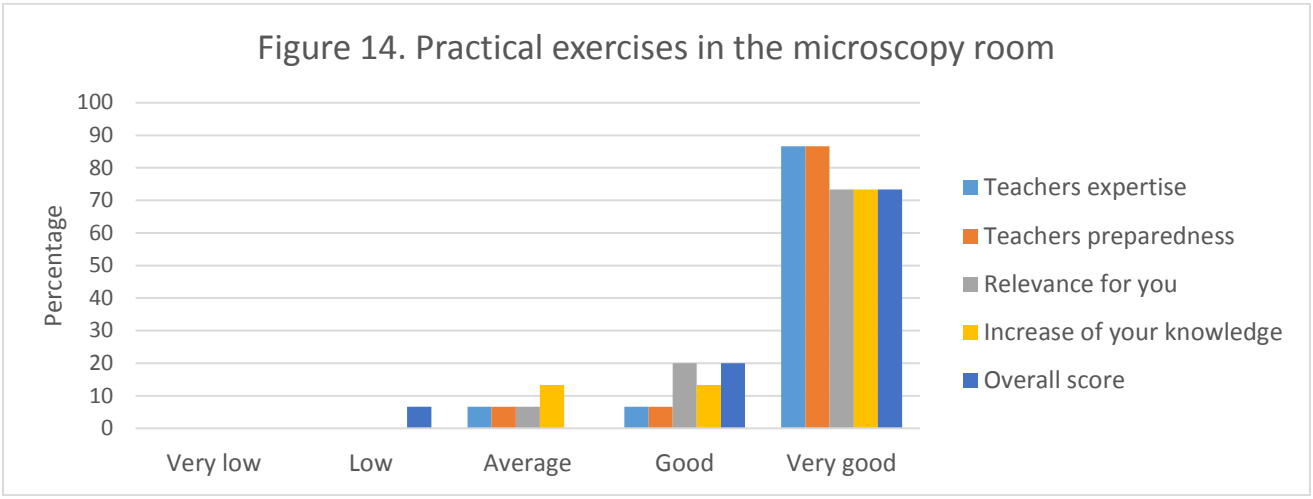


Figure 14. Practical exercises in the microscopy room
Percentage
100
90
80
70
60
50
40
30
20
10
0
Very low
Low
Average
Good
Very good
Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

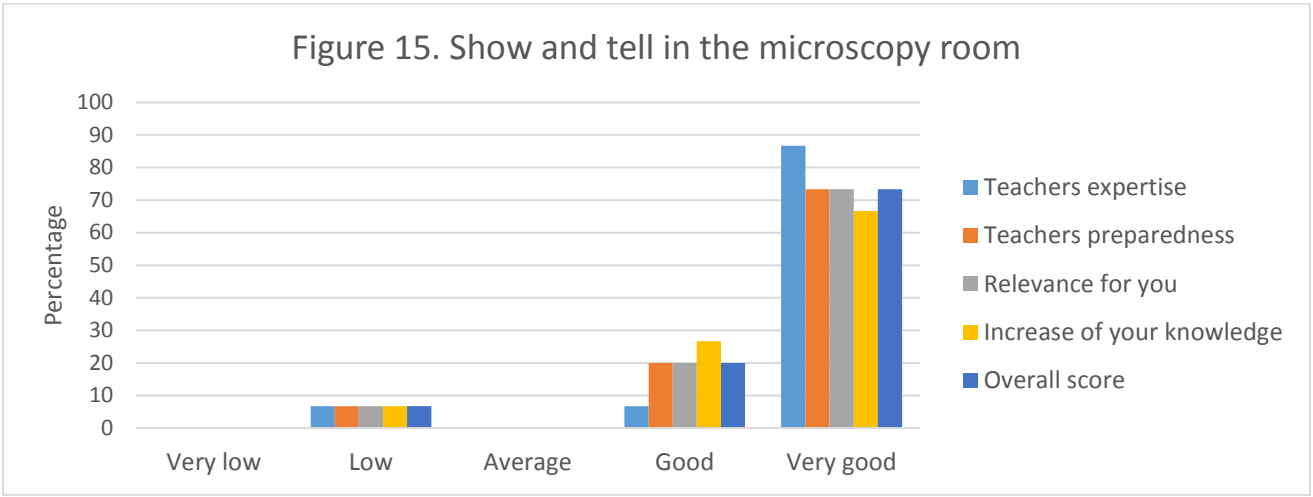


Figure 15. Show and tell in the microscopy room
Percentage
100
90
80
70
60
50
40
30
20
10
0
Very low
Low
Average
Good
Very good
Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

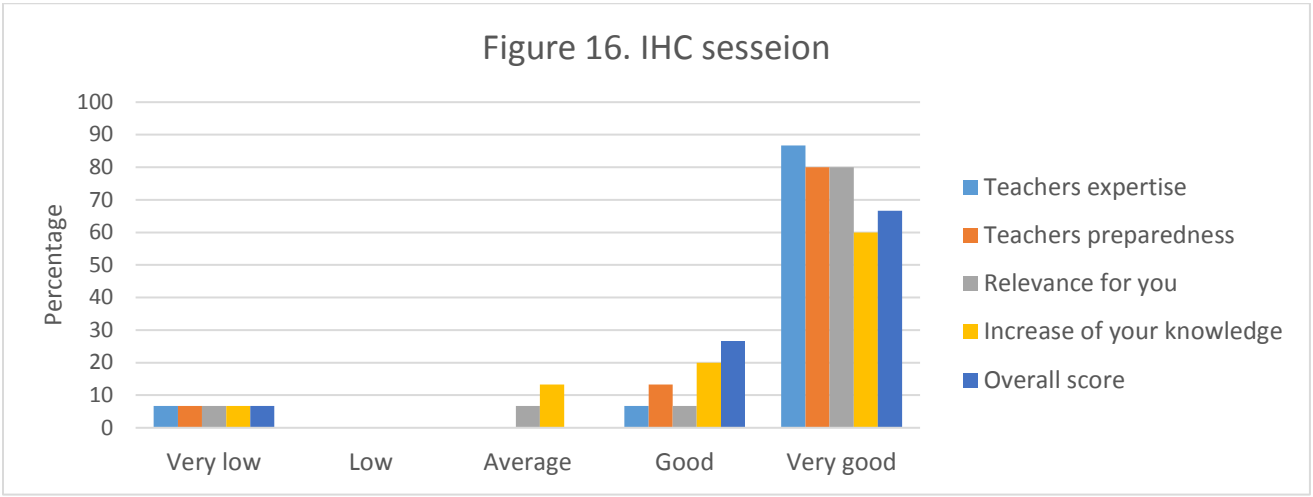


Figure 16. IHC sesseion
Percentage
100
90
80
70
60
50
40
30
20
10
0
Very low
Low
Average
Good
Very good
Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

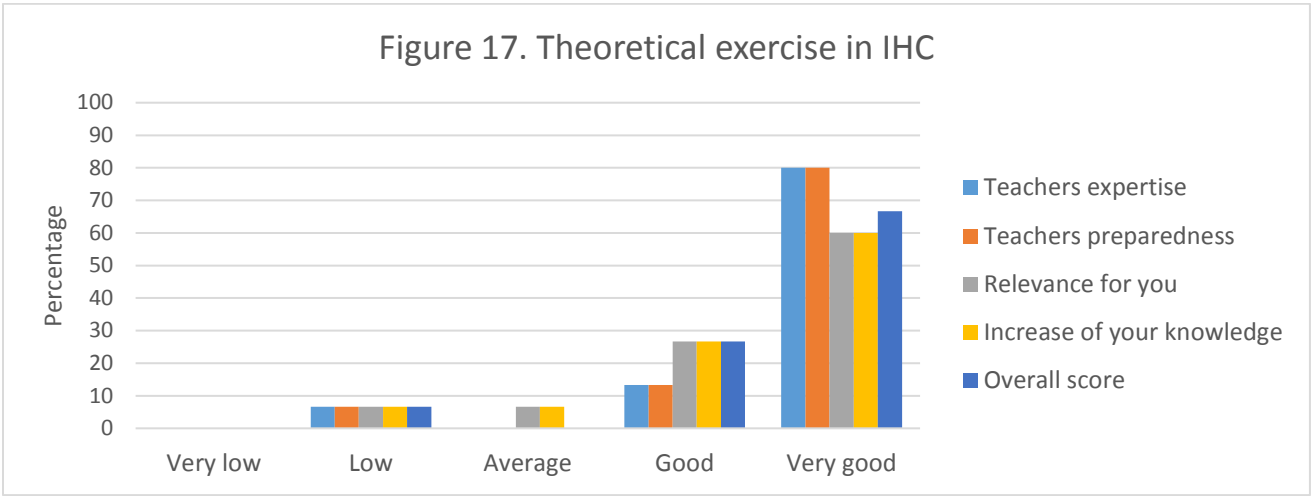


Figure 17. Theoretical exercise in IHC
Percentage
100
90
80
70
60
50
40
30
20
10
0
Very low
Low
Average
Good
Very good
Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score

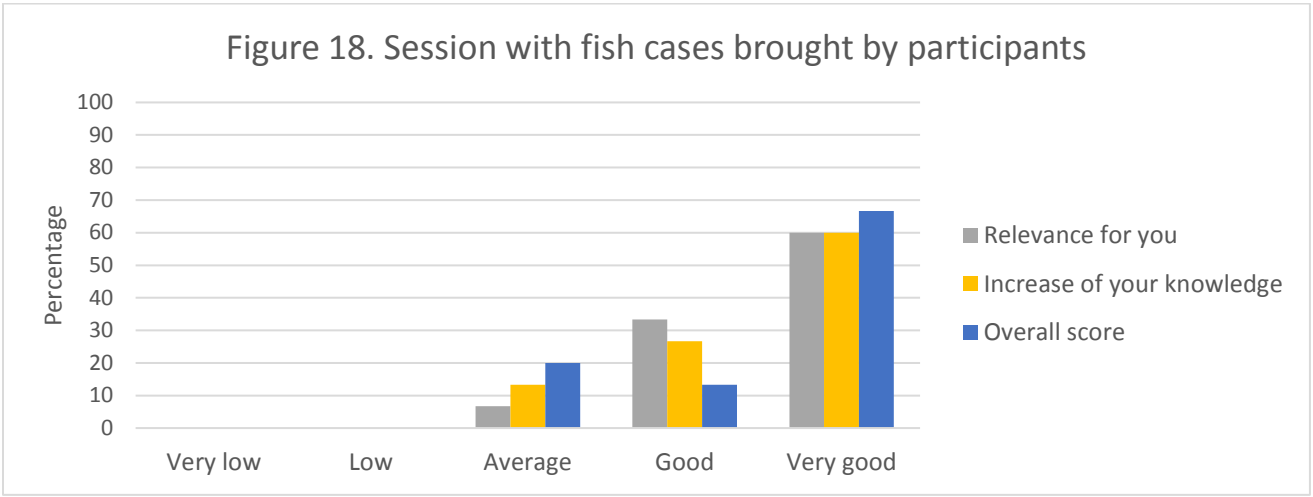


Figure 18. Session with fish cases brought by participants
Percentage
100
90
80
70
60
50
40
30
20
10
0
Very low
Low
Average
Good
Very good
Relevance for you
Increase of your knowledge
Overall score

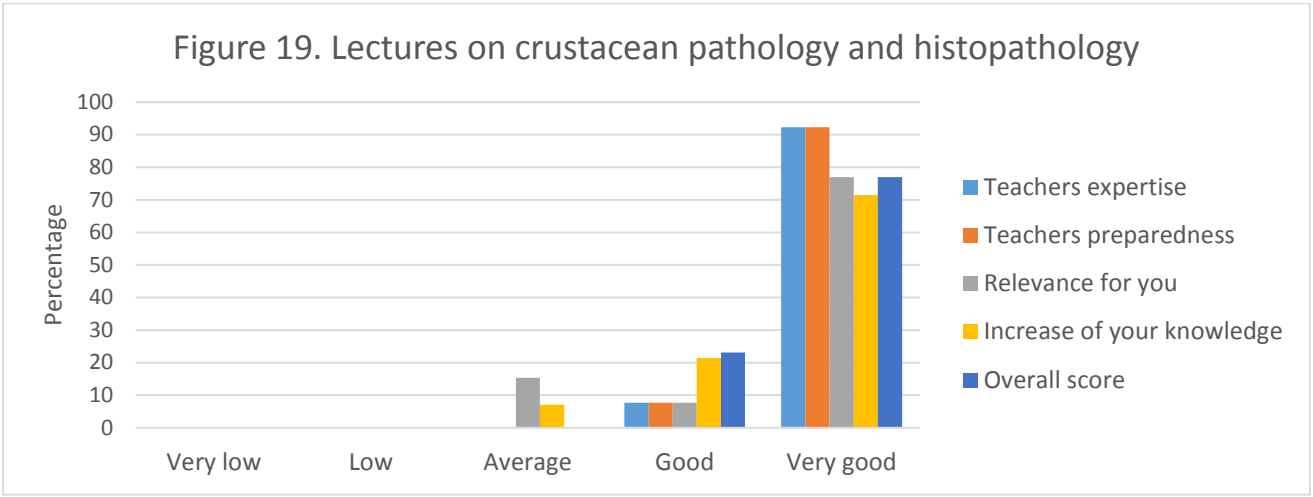
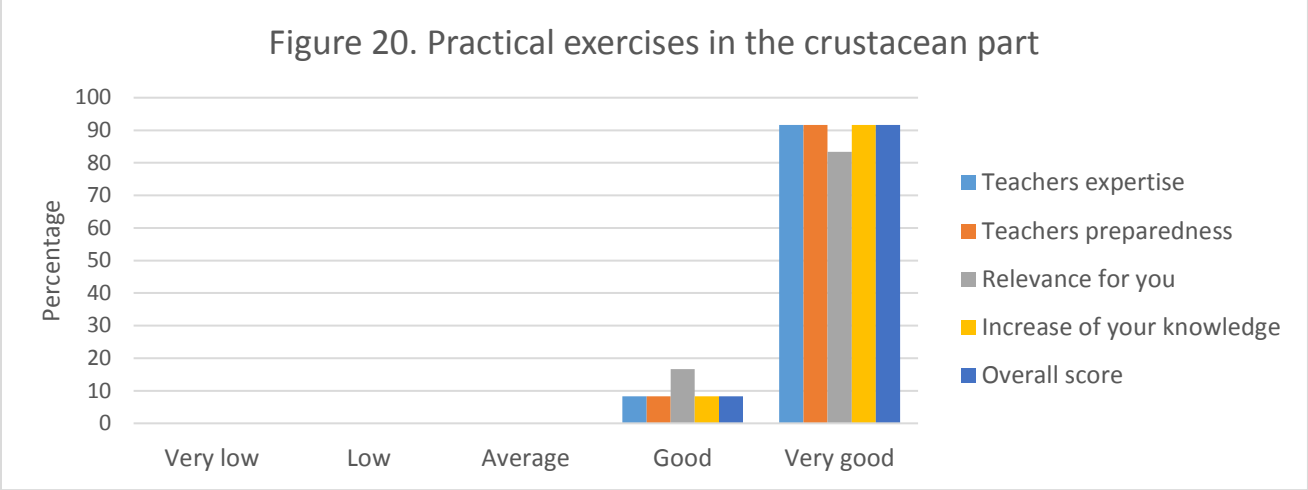


Figure 19. Lectures on crustacean pathology and histopathology
Percentage
100
90
80
70
60
50
40
30
20
10
0
Very low
Low
Average
Good
Very good
Teachers expertise
Teachers preparedness
Relevance for you
Increase of your knowledge
Overall score



Comments from the participants (evaluation schemes from both courses)

"I learned very useful information about sample preparation that I definitely am going to incorporate in my practice"

"I have learned handy tricks to improve my daily work"

"The course was really interesting and taught me a lot, I thoroughly enjoyed it! I think the content was varied and very interesting. There was a good balance of theory and practical"

"Loved finding out how same things are done in different labs. Highly skilled staff. Everyone very welcoming"

"Increasing my overall knowledge"

Closing remarks

The EURL training course 2018 was - based on the feedback from the participants - considered a success. The evaluation schemes for the first course was this year increased in details, enabling 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 is deeply acknowledged for great hospitality and for providing all information and facilities needed during the farm visit.

External tutors, Dr. Ole Bendik Dahle, 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.

Copenhagen, Monday, 07 January 2019

Niels Jørgen Olesen, Jacob Günther Schmidt, Tine Moesgaard Iburg, Argelia Cuenca and Nikolaj Reducha Andersen

EURL for Fish and Crustacean Diseases