



EURL Training Courses



Copenhagen, October 10th – 21st 2022

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

Contents

General introduction.....	3
Course 1: Methods for implementation of surveillance procedures for listed fish diseases	3
Course 2: Introduction to histopathology in fish and crustacean diseases.....	5
Participant list	7
Course description: Methods for implementation of surveillance procedures for listed fish diseases	8
Programme: Methods for implementation of surveillance procedures for listed fish diseases	11
Evaluation: Methods for implementation of surveillance procedures for listed fish diseases	12
Course description: Introduction to histopathology in fish and crustacean diseases.....	12
Programme: Introduction to histopathology in fish and crustacean diseases	36
Evaluation: Introduction to histopathology in fish and crustacean diseases.....	37
Comments from the participants (evaluation schemes from both courses)	Error! Bookmark not defined.
Closing remarks	64

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 10th to the 21st, 2022. Two courses were prepared: the first one, with 13 trainees, was entitled “Methods for implementation of surveillance procedures for listed fish diseases” and took place from 10th to 14th October. The second course was entitled “Introduction to histopathology in fish and crustacean diseases” and took place from the 17th to 21st October with 11 participants. Two participants attended both training courses.

The overall purpose of the training courses was to provide an opportunity for the employees of NRLs to obtain training in techniques relevant when working with listed fish and crustacean 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 (NO) and IZSVE (IT), constituted the tutor team. For the course in histopathology, NVI and CEFAS kindly allowed access to their respective databases with scanned slides. 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. The head of the EURL for fish and crustacean Niccoló Vendramin, DVM Therese Birgitte Christoffersen and four 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, Jesper Valbak from DVFA gave a talk about Danish aquaculture and disease surveillance after which Niccoló Vendramin gave two presentations, one on the control and eradication of VHS from Denmark and another one on the laboratory findings of IHNV detected in Denmark in 2021. 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 on the farm by Jesper Valbak and Morten Lund from DVFA together with the owner Jens Jensen, who was kindly answering questions from the participants. During the visit, 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 Lyngby, participants were welcomed at the institute, where dinner was served.

On **day 2** an introduction and practicalities with a detailed description of the course programme was presented by Niccoló Vendramin and each participant presented their experience and expectations for the course. After the introduction, the section leader Britt Bang Jensen gave a lecture on “Epidemiological concepts of sampling and testing”. Afterwards Niccoló Vendramin gave a presentation on “the legislative basis for aquaculture animal health and the sampling and diagnostic procedures to use” as well as an overview of the methods for performing diagnostic and surveillance of listed fish diseases. 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 brought to the teaching laboratory and after an initial demonstration they prepared and processed the samples they collected on Monday. These were samples for cell cultivation, PCR and bacteriology on samples they collected Monday. After a coffee break all participants were introduced to cell culture techniques, and had the opportunity to prepare their own 24-well plates. In the evening, there was a social dinner in Lyngby.

Day 3 started by theoretical lectures on PCR and real time PCR laboratory- presented 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 again in the teaching laboratory 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 Niccoló Vendramin 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.

Day 4 was tutored by Argelia Cuenca and Morten Schiøtt the EURL coordinator for crustacean diseases. The day was fully dedicated to PCR, sequencing and BLAST analysis. 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. A session explaining how BLAST works and how to interpret BLAST results was conducted, followed by a practical exercise about using sequencing and BLAST searching for strain identification.

At **day 5** in the morning, the participants were divided into two groups. One group was shown diagnostics concerning bacterial diseases in fish and some examples of bacterial pathogens by senior researcher 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, nodavirus 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 Niccoló Vendramin. The course was closed 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. Later on an online questionnaire was distributed.

The methods taught were primarily focused on the protocols given in the EU legislation and on the WOAH (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, Lise Christensen, 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 crustaceans. In both parts, theoretical lectures on relevant topics alternated with practical exercises both with necropsy sessions and digital slide microscopy sessions.

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 necropsy and 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 and Raoul Kuiper from the Norwegian Veterinary Institute on the normal histology and artefacts followed after lunch break.

Day 2 was divided between practical work with digital slides from confirmed cases and theoretical lectures focusing on general and special pathology with focus on listed diseases. The histology exercises with digital slides were done in small groups using a large screen for discussion of possible lesions, furthermore the participants could work with the slides individually and also a few microscopes and physical slides were accessible in the group rooms. Ole Bendik Dale, Raoul Kuiper and Tine 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. After lunch there were a lecture on *in situ* hybridisation and a lecture on the use of digital pathology both now and in the future. This part of the programme was conducted by Raoul Kuiper and Tine Iburg. A theoretical exercise in IHC was used as a platform for discussions with tutors Tine Iburg, and Raoul Kuiper. Another histology exercise with the last fish cases in a similar way as day 2 Ole Bendik Dale, Raoul Kuiper and Tine Iburg.

Day 4 started directly in the lecture room with lectures and show and tell by Ole Bendik Dale and Raoul Kuiper of special pathology, mainly in listed diseases and some Norwegian experiences. Scanned slides from cases were used on the big screen in the lecture room to illustrate the cases. After lunch, a session on crustacean histopathology started. Tobia Pretto (from the National Reference Laboratory for Italy for fish and crustacean diseases) started with an overview of the organs of relevance of disease diagnostics and surveillance in crustaceans and afterwards a practical exercise in necropsy was done. Tobia Pretto demonstrated necropsy procedures and organ localization using noble crayfish (*Astacus astacus*) as model; how to sample a shrimp was also demonstrated. Afterwards the participants tried themselves to perform a necropsy of crayfish and shrimp.

Day 5 was dedicated to histopathology of crustacean diseases. In the morning, Tobia Pretto was giving lectures on special pathology for listed crustacean diseases in EU, followed by group work on scanned

case slides for each of the relevant diseases. After lunch, Tobia Pretto gave a lecture on non-listed crustacean diseases, again followed by group work on scanned case slides for some of the diseases.

At the end of the day, the participants were asked to provide suggestions and comments for the course, while an online questionnaire was provided later on. 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 teachers and participants was held on day three.

Participant list

Name	Surname	Country	Affiliation	Course 1	Course 2
Dimitrije	Glišić	Serbia	Serbian Institute of Veterinary Science (SIVS)	x	
Doriane	Flores	France	ANSES, Ploufragan-Plouzané-Niort Laboratory, Unit Virology, immunology and ecotoxicology of fish, French reference laboratory for regulated fish diseases	x	
Hana	Minarova	Czech Republic	Veterinary Research Institute, Brno, Czech Republic	x	
Stephanie	Linehan	Ireland	National Reference Laboratory, Fish Health Unit (FHU), Marine Institute, Oranmore	x	
Thomas	Lips	Switzerland	National Fish Disease Laboratory, Centre for Fish and Wildlife Health (FIWI), University of Bern	x	
Alessia	Vetri	Italy	Istituto Zooprofilattico Sperimentale delle Venezie (Italy) NRL for fish, mollusc and crustacean diseases		x
Deborah	Cheslett	Ireland	Marine Institute		x
Killian	Coakley	Ireland	Fish Health Unit (FHU), Marine Institute, Oranmore		x
Luana	Cortinovis	Italy	Istituto Zooprofilattico Sperimentale delle Venezie (Italy) NRL for fish, mollusc and crustacean diseases		x
Toni	Eterovic	Bosnia Herzegovina	National reference laboratory for fish diseases, Veterinary faculty in Sarajevo, Bosnia and Herzegovina		x
Katerína	Mikulášková	Czech Republic	Department of Virology, State Veterinary Institute Jihlava	x	
Sultan Nurhan	Ayikol	Turkey	Ankara University Faculty of Health Science, Department of Veterinary Pharmacology and Toxicology	x	
Olga	Bellot Zaragoza	Spain	Laboratory Assistant		x
Begul	Karakoc	Turkey	Phd student		x
Evgenia	Gourzioti	Greece	official veterinarian	x	x
Cathrine	whitby	Scotland	Project manager	x	
Baris	CISDIK	Czech Republic	phd		x
Nana	Karaoulani	Greece	Greek NRL	x	
Jonathan	Oladjins	Scotland	Scottish NRL		x
Furkan	KUTLU	Turkey	Ankara University Faculty of Health Science, Department of Veterinary Pharmacology and Toxicology	x	
Hanxi	Li	Denmark	DTU Sund	x	x
Therese	Christoffersen	Denmark	DTU AQUA	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. 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 organized in collaboration with veterinary services and aquaculture association. Protocols for inspection, necropsy and sample collection will be demonstrated. Sample collection will involve mandatory sampling for testing listed disease (virology and molecular assay) as well as testing for differential diagnosis (bacteriology).

Starting from day 2, theoretical 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 accredited Real-Time PCR protocols for surveillance will be presented and discussed with the participants. Furthermore, focus will be put 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 concept of test specificity and sensitivity will be explained, and the students will learn how this affects validity of surveillance systems.

The course is dialogue-based and sufficient time will be given for discussions throughout the course and for the 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 WOAH 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 commonly used methods for diagnosis of important viral fish diseases. 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 commonly 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 and 6) epidemiological aspects to consider when designing surveillance for viral diseases in fish aquaculture.

Learning objectives

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

- Explain the basic principles of the legislative framework for surveillance and control of listed fish disease in EU
- Describe basic principle of surveillance schemes or programs? for listed aquatic fish disease
- 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
- understand basic principles of PCR and real time PCR techniques and their use in diagnostics and surveillance
- Genotype viral isolates by sequencing and blasting
- Troubleshoot test performances and designs.
- Demonstrate the implications of test sensitivity and specificity for surveillance programs

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

Niccoló Vendramin (DVM, PhD), Fish virology and diagnostics

Argelia Cuenca Navarro (M.Sc., PhD): Molecular methods

Britt Bang Jensen (DVM, PhD) Epidemiology

Lone Madsen (DVM, PhD): Fish bacteriology and diagnostic

Jacob G. Schmidt (M.Sc., PhD) Course facilitator

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

Christina Flink Desler (sample preparation and cell culture)

List Christensen (cell culture)

Kári Karbech Mouritsen (bacteriology)



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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 Veterinary services in Jutland	Section 2 Laboratory introduction and sample preparation	Section 3 PCR analysis and Cell culture methods	Section 4 PCR, blast and phylogeny	Section 5 Cell culture /bacteriology and evaluation
<p>8:00 – 11:00 Transport by car to Danish Veterinary and Food Administration, in Jutland.</p> <p>11:00 – 12:15 Introduction to surveillance programs</p> <p>Aquaculture surveillance and sampling procedures in Denmark</p>	<p>9:00 - 10:30 Introduction and practicalities. Participants experience and expectations</p> <p><u>Coffee break 10:30 - 10:50</u></p> <p>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</p>	<p>9:00 – 10:30 : PCR and real time PCR theory.</p> <p><u>Coffee break 10:30 - 10:50</u></p> <p>10:50 - 12:15 Result analysis Practical exercises.</p>	<p>9:00 - 10:30 PCR and Real Time PCR Troubleshooting. The diagnostic laboratory – PCR flow.</p> <p><u>Coffee break 10:30 - 10:50</u></p> <p>Sequencing theory and practical exercises</p>	<p>9:00 - 10:30 Cell culture observation with different reference viral isolates</p> <p><u>Coffee break 10:30 - 10:50</u></p> <p>10:50-12:15 Fish bacteriology demonstration</p>
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
<p>13:00 – 13.30 Transport to Fish Farm</p> <p>13:30 – 15:30 Inspection and sampling</p> <p>15:30 – 19:00 Return to Hotel</p>	<p>13:00 - 14:30 Sample preparation for cell culture, PCR and bacteriology on samples collected Monday</p> <p><u>Coffee break 14:30 - 14:45</u></p> <p>14:45- 16.45 Practical cell culture passaging and production of 24 well plates</p> <p>19:00 -Social dinner</p>	<p>13:00- 16:30 13:00-13:45 Reading cells and inoculation of samples</p> <p>13:45-14:30 Use of cell culture in fish virology</p> <p><u>Coffee break 14:30 - 15:00</u></p> <p><u>15:00-16:00</u></p> <p>Titration procedure, viral titre calculation. Barcoding cell lines</p>	<p>13:00 – 17:00 Blast analysis and practical exercise</p> <p><u>Coffee break 14:30 - 15:00</u></p> <p>Introduction to phylogenetic analysis</p>	<p>13:00 – 14:45 Assignment + presentation and assessment of data obtained by each group</p> <p>Discussion and recommendations</p> <p>Conclusion</p> <p>14:45-15:00 Course evaluation, coffee and goodbyes</p>

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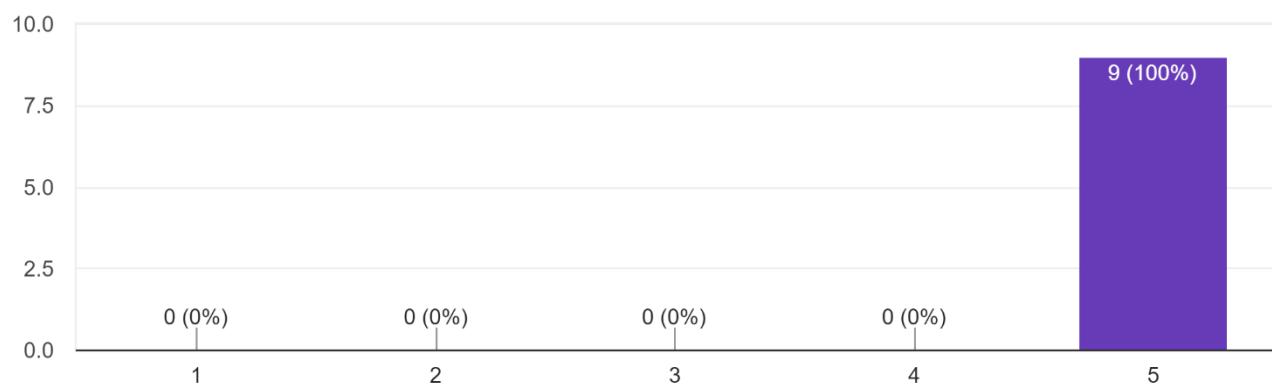


Evaluation: Methods for implementation of surveillance procedures for listed fish diseases

Participant satisfaction level for each respective section on a scale from 1 (very low) to 5 (very good). The calculations are based on returned evaluation schemes from 9 participants.

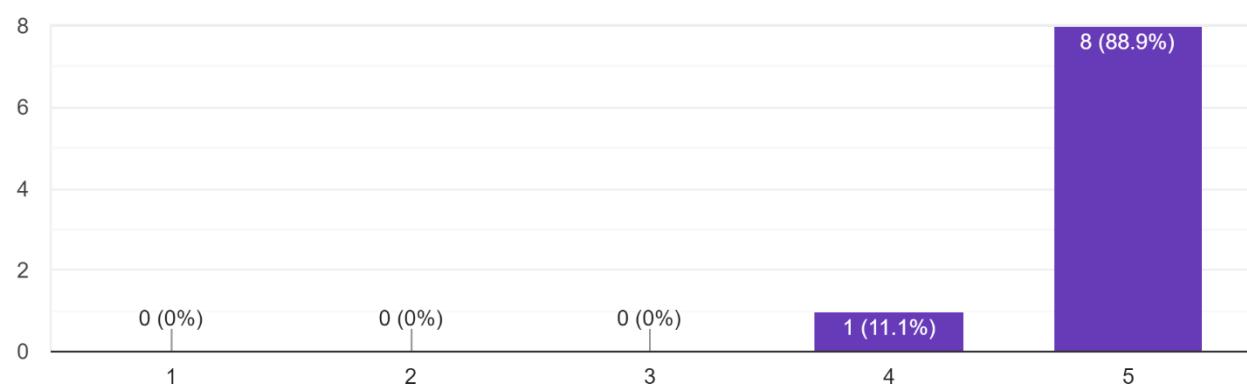
Day 1 Fish farm inspection and sampling - teacher's expertise

9 responses



Day 1 Fish farm inspection and sampling - teacher's preparedness

9 responses



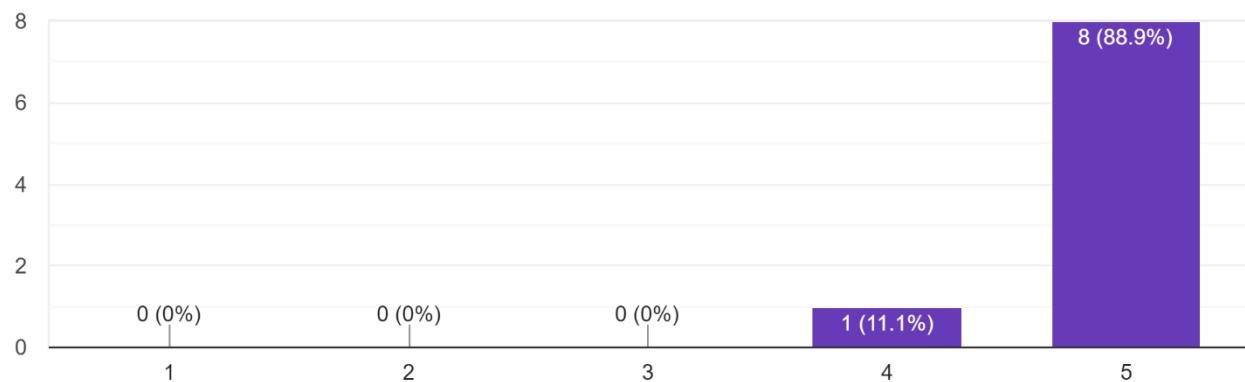
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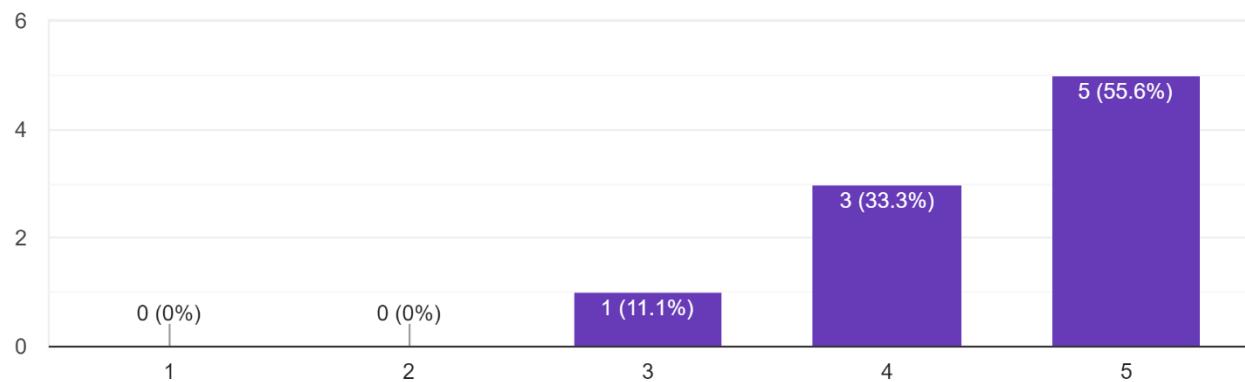
Day 1 Fish farm inspection and sampling - relevance for you

9 responses



Day 1 Fish farm inspection and sampling - increase of your knowledge

9 responses



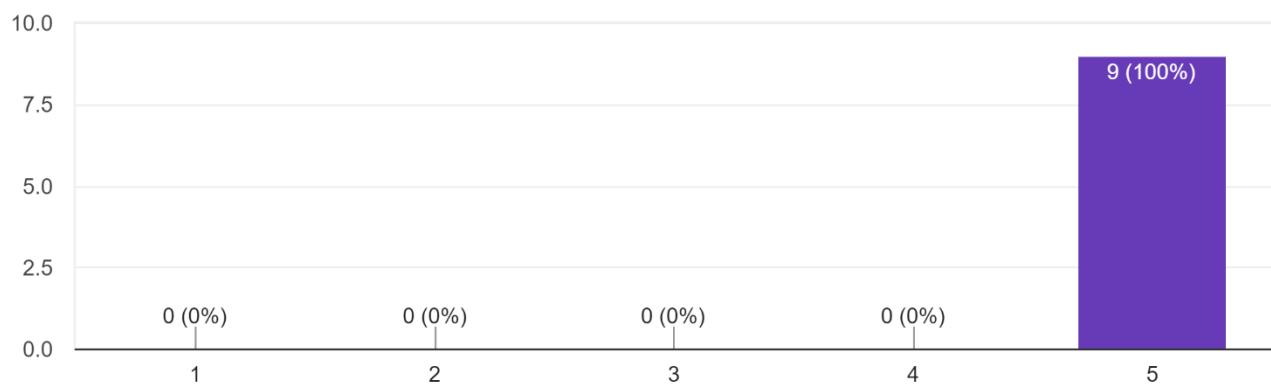
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Day 1 Fish farm inspection and sampling - overall score

9 responses



Day 1 Fish farm inspection and sampling - general comments

All my expectations were fulfilled, the visit to the farm was very interesting.

The fish farm inspection and sampling was an interesting experience and a worthwhile effort since we got to experience some field work outside of the laboratory.

Good insight into field sampling. I think it would have been good practice to also collect molecular samples.

Staff on fish farm very open to answering questions. DTU staff very organized and helpful during the sampling.

Very well

It was a satisfying and enjoyable experience.

It was a very good experience for me, seeing the farm is very beneficial for me, combining the field with the laboratory is a very good idea

A long but interesting day

I appreciate the opportunity to see a local fish farm and sampling in the field

very interesting to see a working fish farm and helped put the rest of the course into context

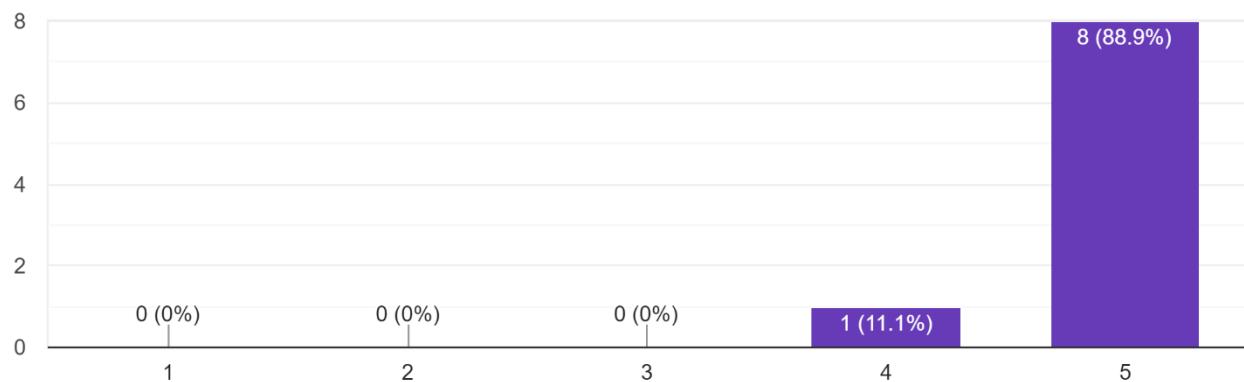
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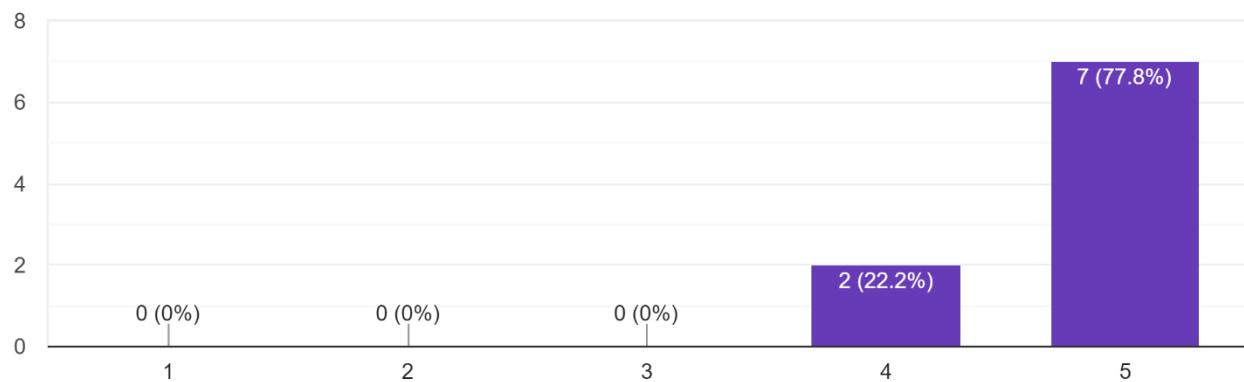
Day 2 concepts of epidemiology - teacher's expertise

9 responses



Day 2 concepts of epidemiology - teacher's preparedness

9 responses



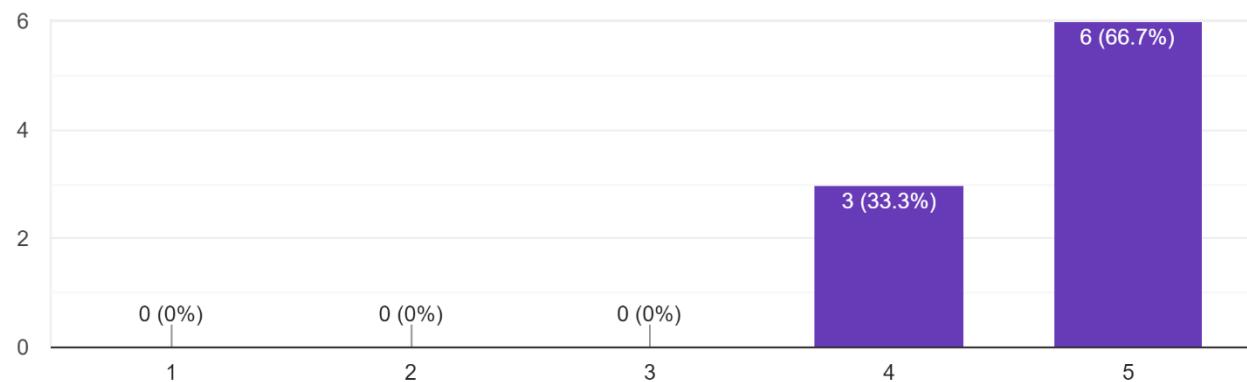
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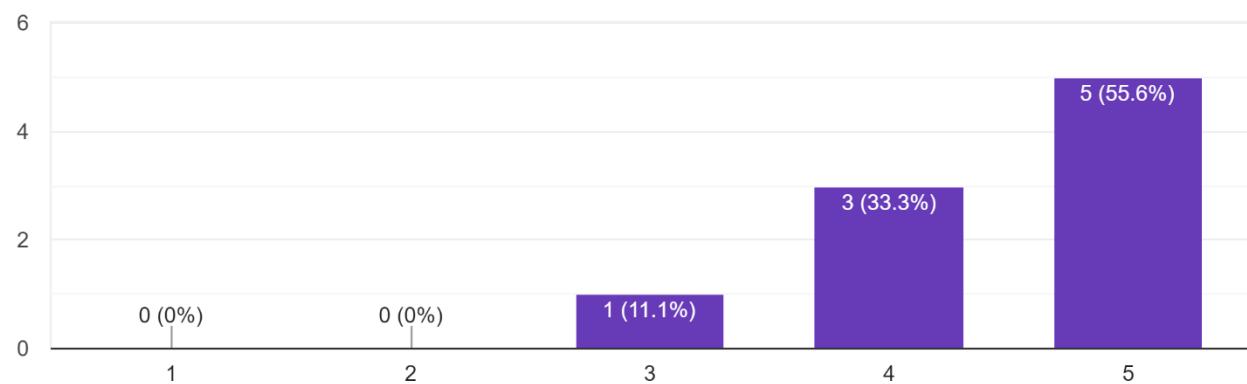
Day 2 concepts of epidemiology- relevance for you

9 responses



Day 2 concepts of epidemiology - increase of your knowledge

9 responses



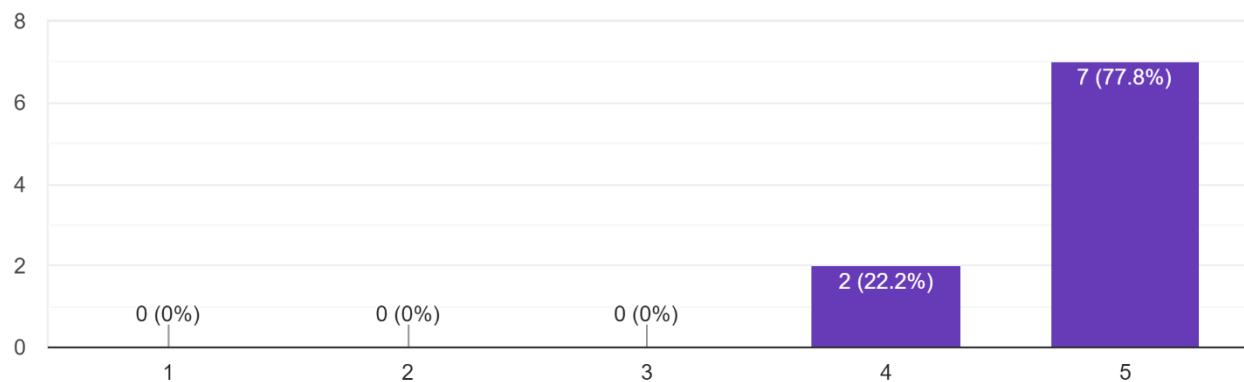
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Day 2 concepts of epidemiology - overall score

9 responses



Day 2 concepts of epidemiology - general comments

Very interesting and useful lecture.

We understood the general concepts regarding epidemiology in fish aquaculture and the teacher explained the significance well enough so that we could grasp it.

Lots of interesting information particularly about the importance of sample size and test sensitivity & specify.

Very well

Nice.

It was a really interesting part for me because I had no notion of the epidemiological concept

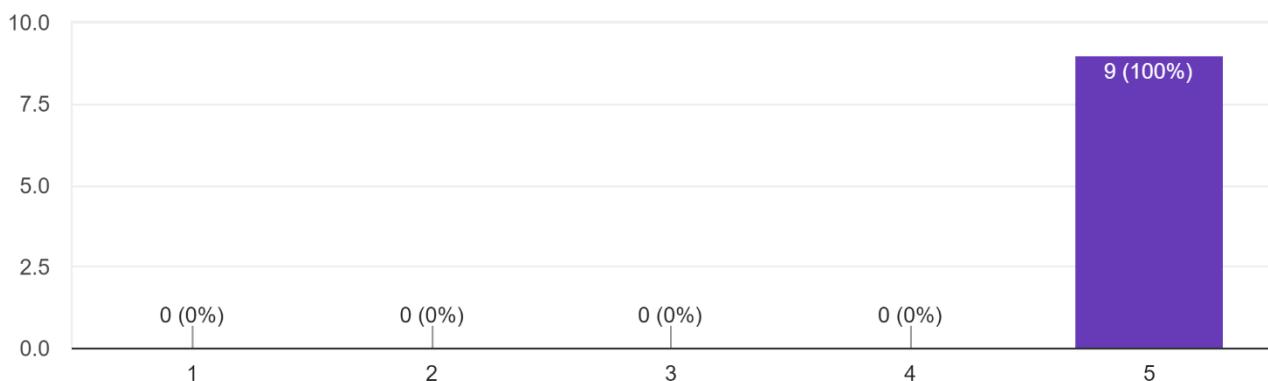
Interesting topic and very good to have exercises regarding sensitivity and specificity.

good overview and repetition

Good

Day 2 and 3- legislative background for surveillance and control of listed fish diseases, use of cell culture for surveillance of listed fish diseases- teacher's expertise

9 responses



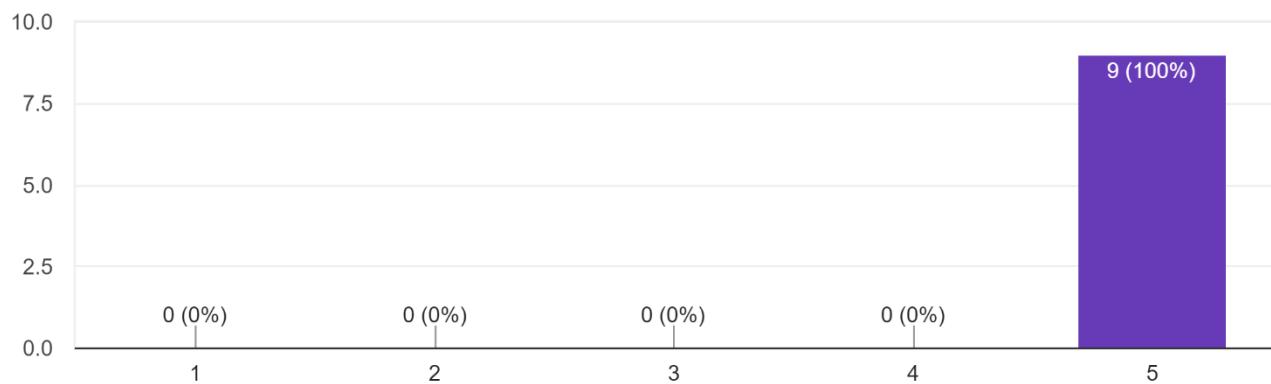
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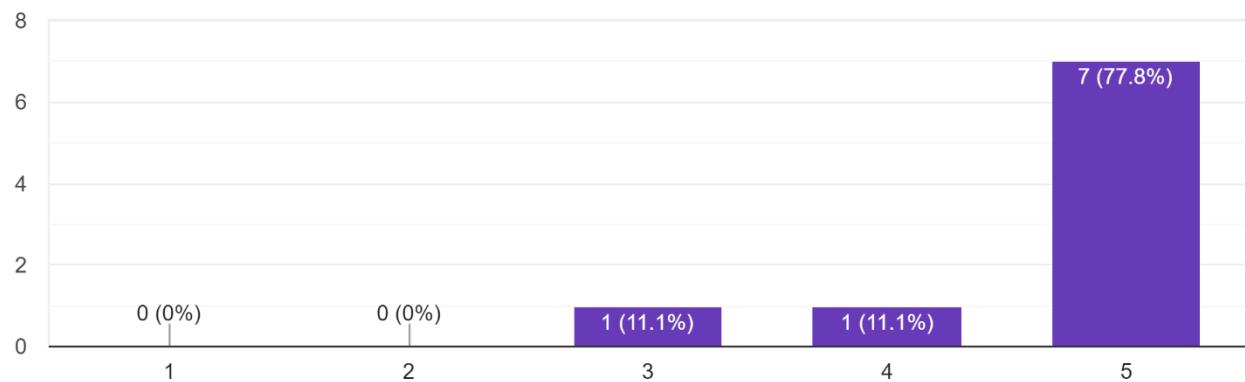
Day 2 and 3 - legislative background for surveillance and control of listed fish diseases, use of cell culture for surveillance of listed fish diseases - teacher's preparedness

9 responses



Day 2 and 3 - legislative background for surveillance and control of listed fish diseases, use of cell culture for surveillance of listed fish diseases - relevance for you

9 responses



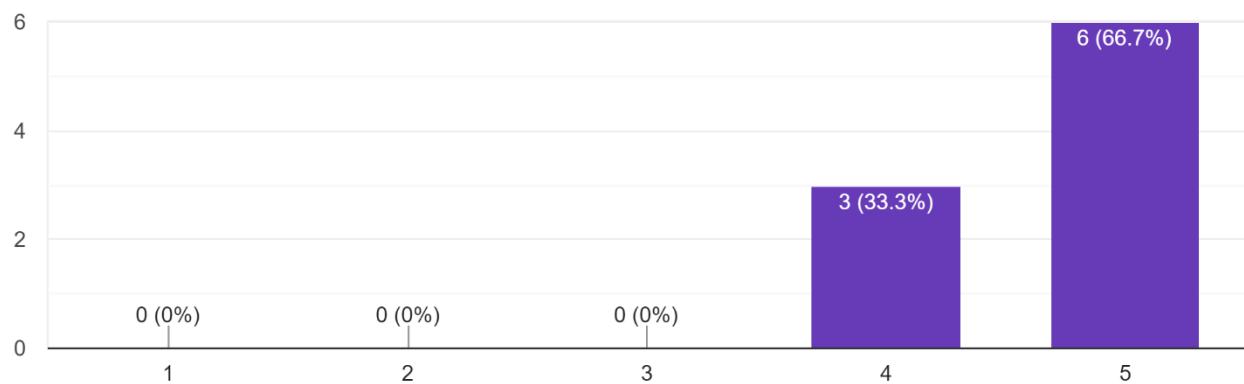
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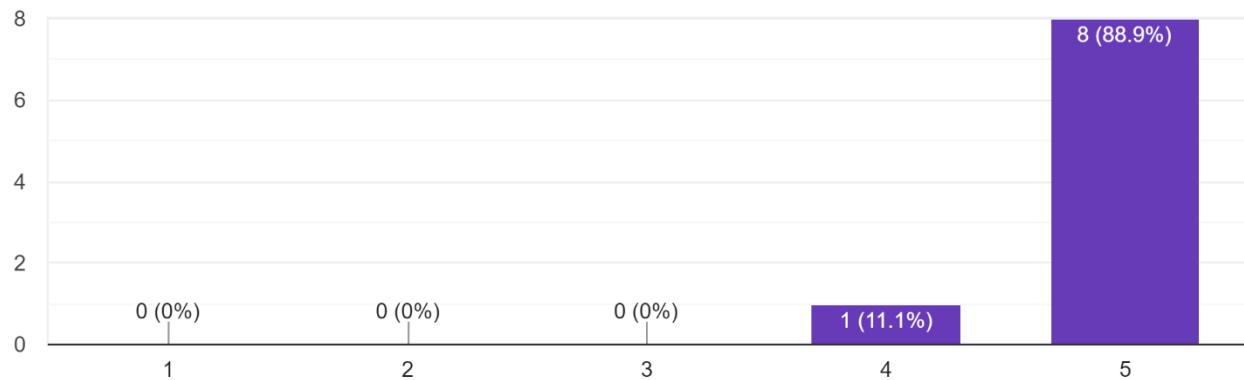
Day 2 and 3 - legislative background for surveillance and control of listed fish diseases, use of cell culture for surveillance of listed fish diseases - increase of your knowledge

9 responses



Day 2 and 3 - legislative background for surveillance and control of listed fish diseases, use of cell culture for surveillance of listed fish diseases overall score

9 responses



Day 2 and 3 - legislative background for surveillance and control of listed fish diseases, use of cell culture for surveillance of listed fish diseases - general comment

Difficult topic, but important and was very well put.

Legislature is always a difficult topic but a necessary one. The teacher was prepared for this part of the course and made it easier to digest and understand, with frequent breaks it was easier to remain concentrated on the topic.

Gained a very good understanding of the animal health law and legislation. Niccolo made the legislation very easy to understand and it explained why we collect and test samples a certain way.

Very informative

Every instructor was very experienced and knowledgeable about their branch of expertise. They all were very welcoming and open to our questions and discussions as well. Overall, awesome experience.

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The regulations are very complex but the teachers teach them very well, a good understanding of the regulations for me

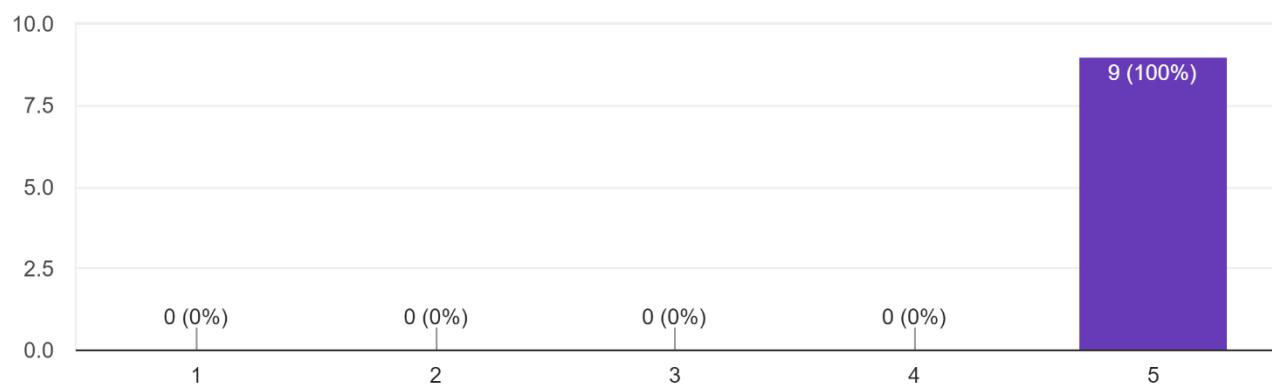
Very informative - perhaps an introduction to EUR-lex

good overview of complicated legislation; I appreciate the possibility to see a daily work with cell culture lines and your lab experiences

Did a good job of making quite a heavy subject of legislation interesting and concise

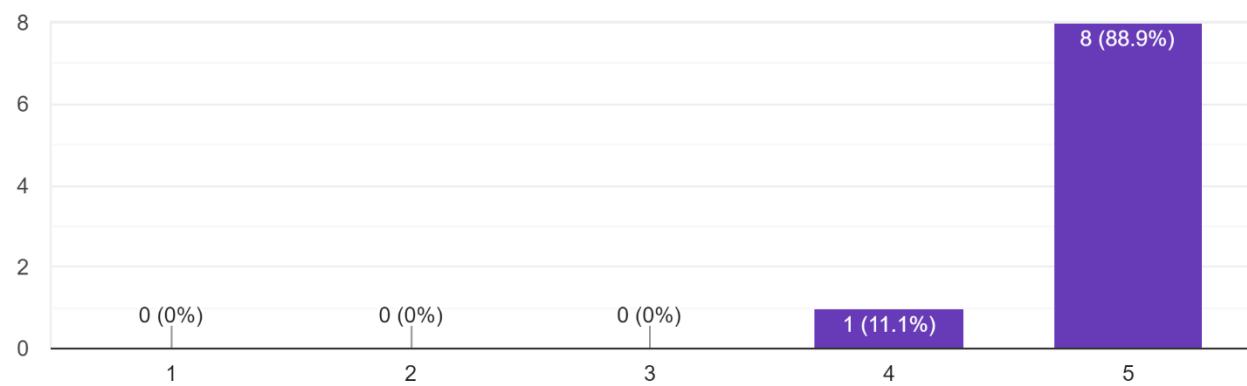
Day 3 and 4 - molecular methods for implementation of surveillance of listed fish diseases-
teacher's expertise

9 responses



Day 3 and 4 - molecular methods for implementation of surveillance of listed fish diseases-
teacher's preparedness

9 responses



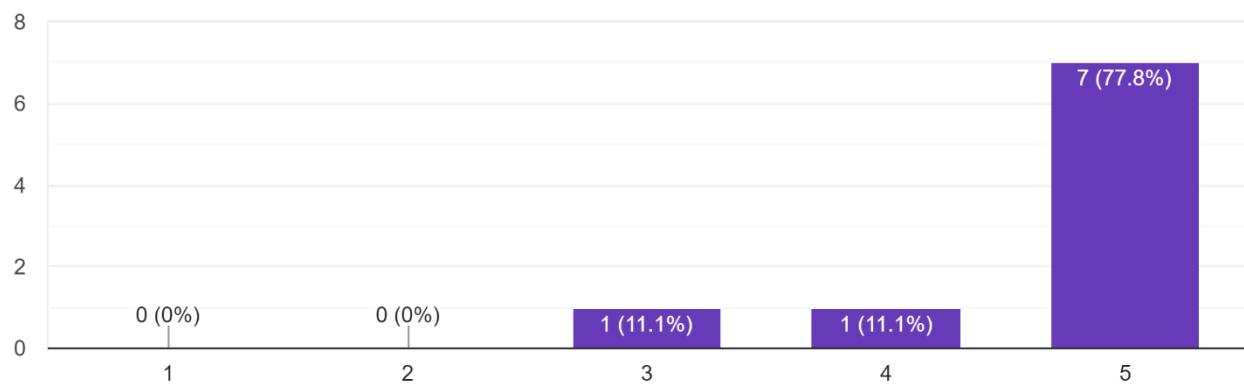
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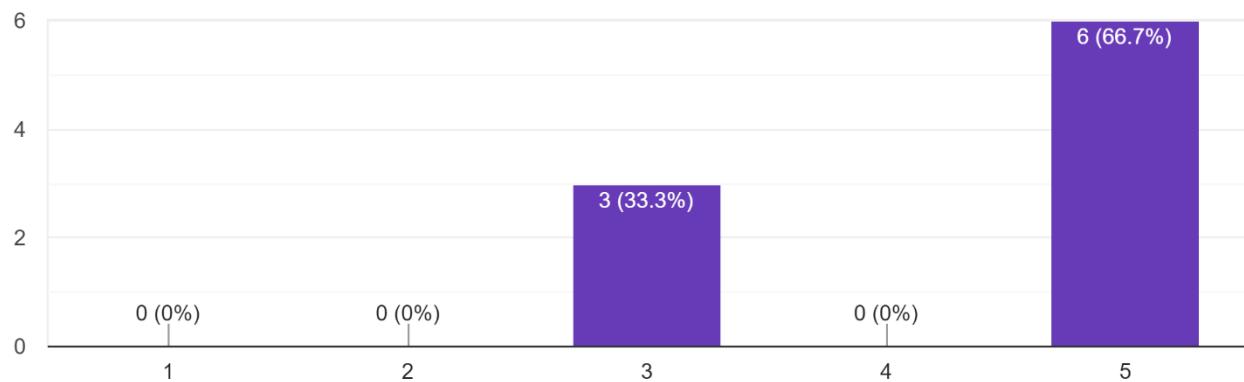
Day 3 and 4 - molecular methods for implementation of surveillance of listed fish diseases-
relevance for you

9 responses



Day 3 and 4 - molecular methods for implementation of surveillance of listed fish diseases-
increase of your knowledge

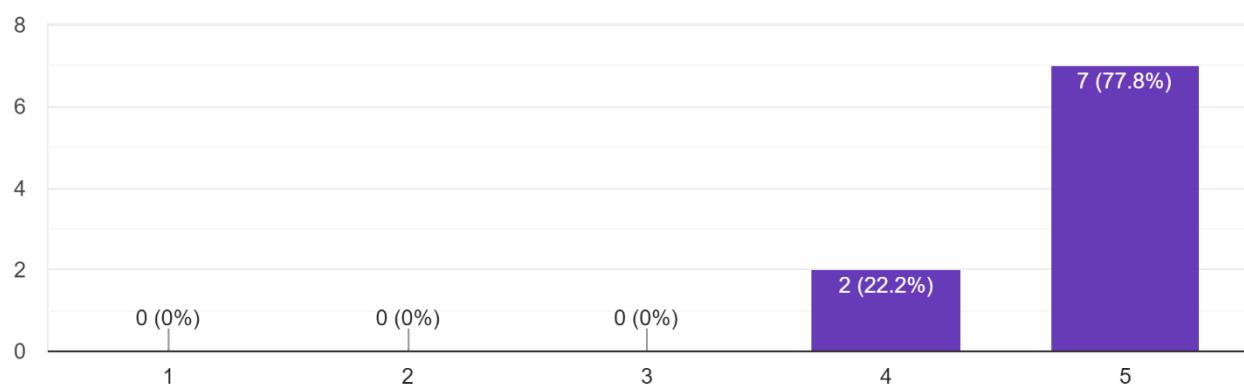
9 responses





Day 3 and 4 - molecular methods for implementation of surveillance of listed fish diseases- overall score

9 responses



Day 3 and 4 - molecular methods for implementation of surveillance of listed fish diseases- general comment

I learned a lot, all my expectations were met.

The first day for molecular methods was quite dry and difficult for me, but all in all a lot of information was given to us regarding the use of molecular methods. There was maybe too much biochem for my tastes, but I understand that the course is made for people with different backgrounds.

It was very useful going through the basics of molecular biology as it gives you a better understanding on the work you are carrying out.

Very well. I would like more practical exercise. We have different knowledge background with the participants, so I found it difficult few times to absorb all the theoretical information

I am familiar to molecular methods but still I was able to learn nice tips and tricks.

I already knew the molecular biology part, it is good to review the basics, an experimental part could be added to the training, if possible

With limited experience in molecular methods, it would have been nice to have the power point presentations beforehand for self-study.

very nice presentations for beginner in a molecular biology field

Good refresher of molecular background information - still made it interesting even though for me it wasn't new information

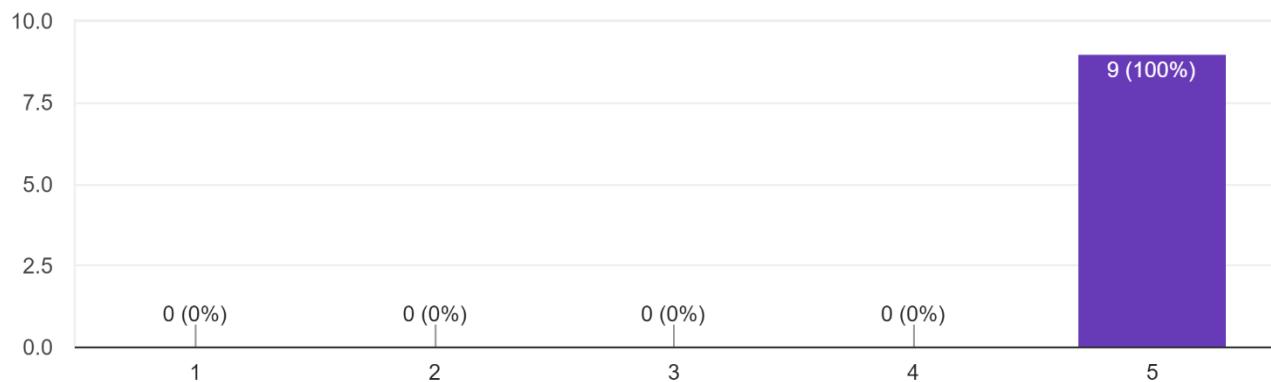
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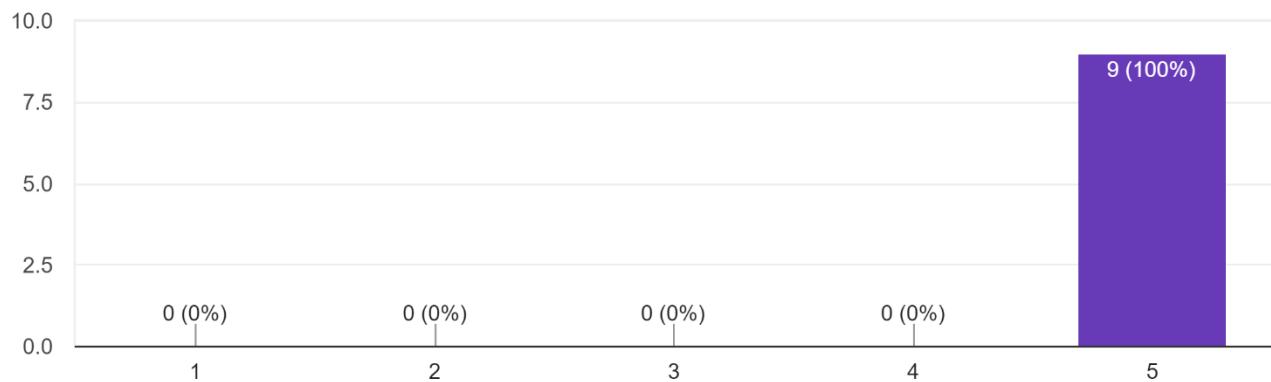
Day 4 (afternoon): sequencing, blast and phylogenetic analysis - teachers' expertise

9 responses



Day 4 (afternoon): sequencing, blast and phylogenetic analysis - teachers' preparedness

9 responses



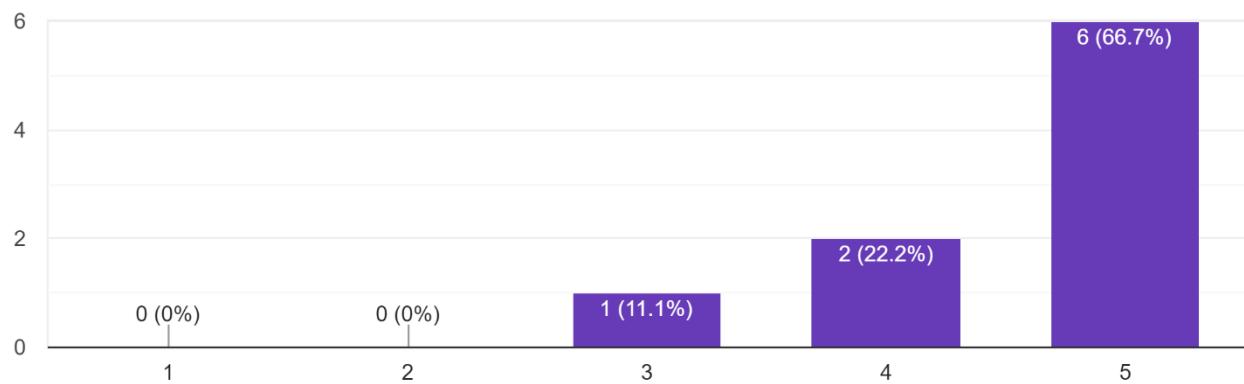
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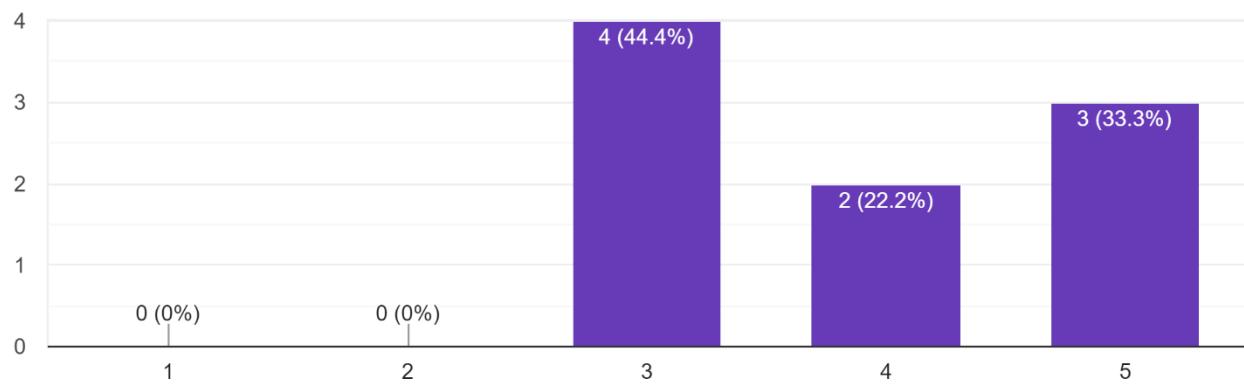
Day 4 (afternoon): sequencing, blast and phylogenetic analysis - relevance for you

9 responses



Day 4 (afternoon): sequencing, blast and phylogenetic analysis - increase of your knowledge

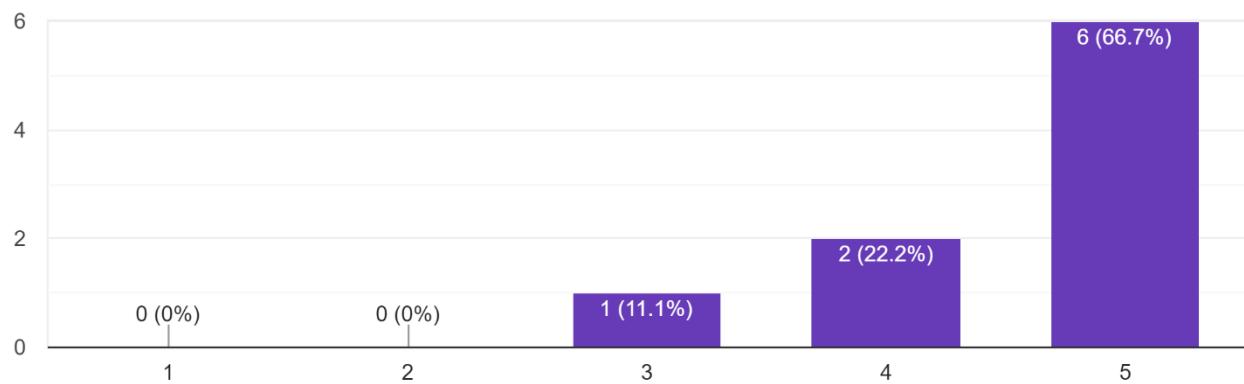
9 responses





Day 4 (afternoon): sequencing, blast and phylogenetic analysis - overall score

9 responses



Day 4 (afternoon): sequencing, blast and phylogenetic analysis- general comment

Very interesting and well put, we did not get to the phylogenetic analysis but it was not that relevant to me, so I am glad we covered the rest more thoroughly.

The teacher was very understanding of our lack of knowledge and helped us through the exercise.
It was a good exercise however the options of using different alignment softwares were a little confusing as everyone was doing it a slightly different way. I think it would be good to go into detail into a particular alignment software also.

Very well. It would be great to have time for a small presentation on phylogenetic analysis
Again, I am familiar to those topics as well. But adapting them to surveillance was a good experience for me.
I already knew the sequencing and phylogeny part, interesting exercise
With limited experience in molecular methods, it would have been nice to have the power point presentations beforehand for self-study.
the exercise was small challenge for me as a beginner, I was little bit lost at the beginning
Good to have the practical exercise. Would be good to know before the course that laptops wold be needed as i didn't bring mine as i didn't know

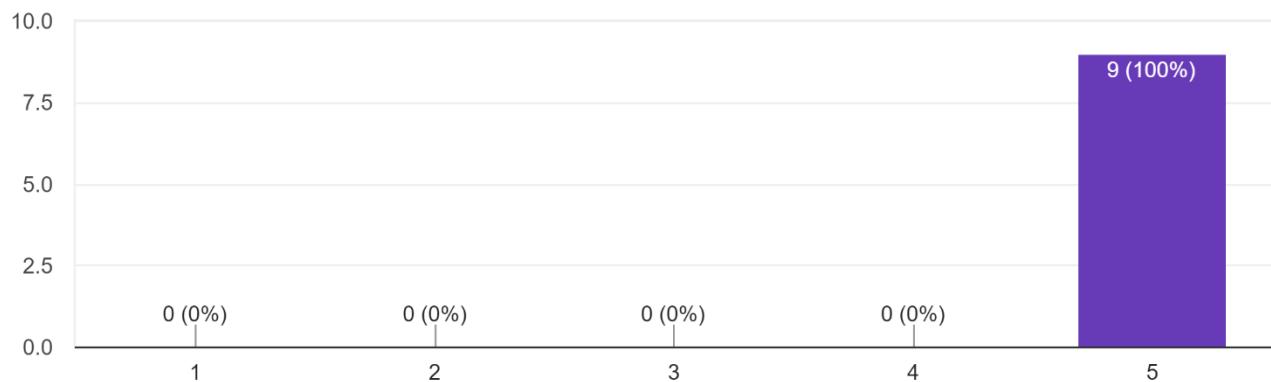
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Day 5 - fish bacteriology lab - teachers' expertise

9 responses



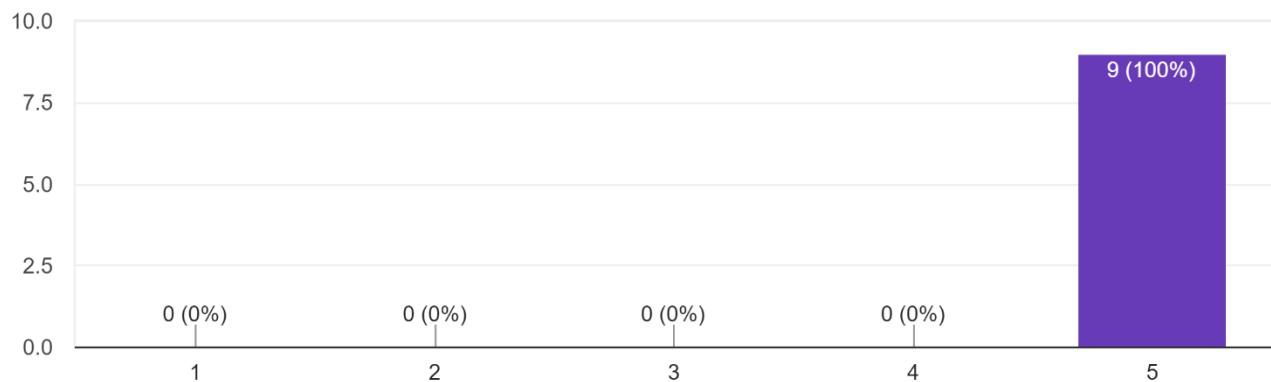
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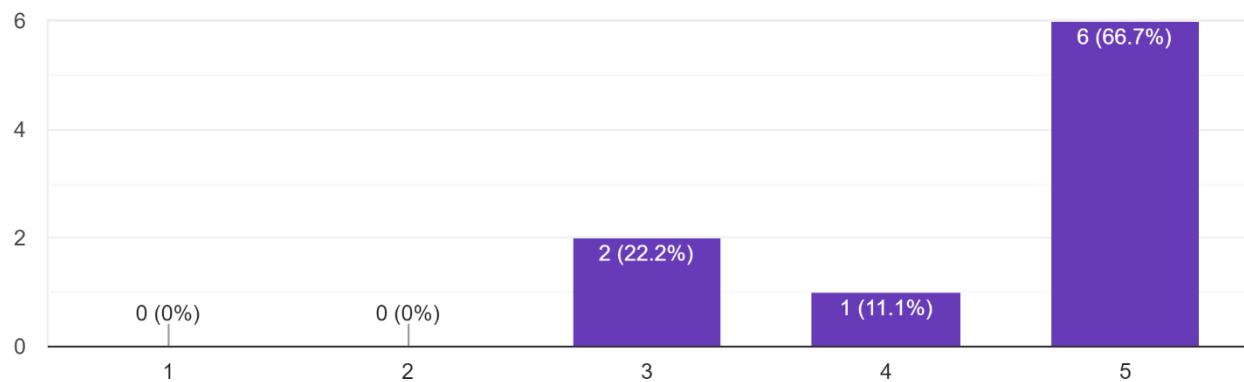
Day 5 - fish bacteriology lab - teachers' preparedness

9 responses



Day 5 - fish bacteriology lab - relevance for you

9 responses



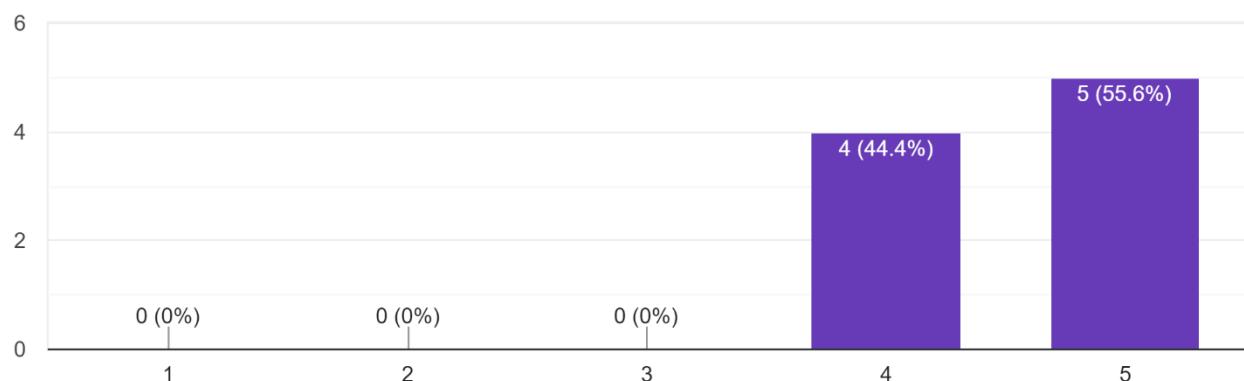
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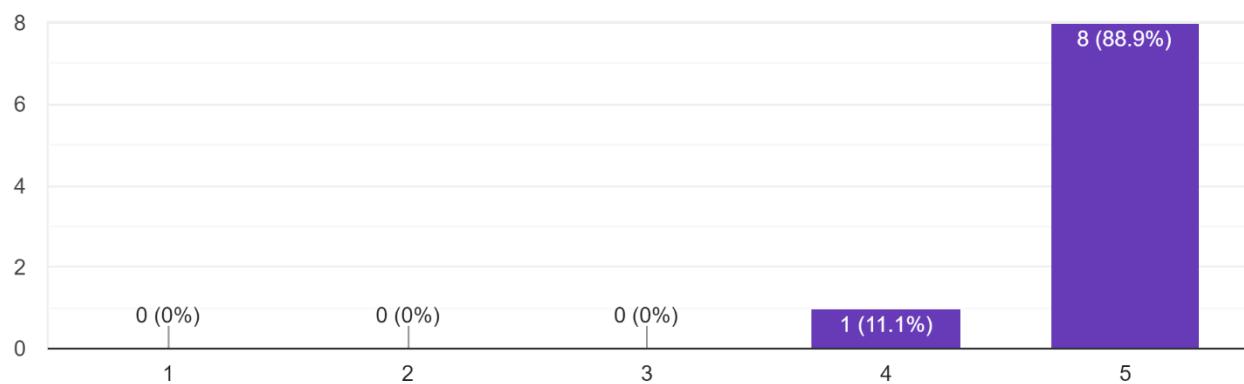
Day 5 - Fish bacteriology lab - increase of your knowledge

9 responses



Day 5 - Fish bacteriology lab - overall score

9 responses



Day 5 - Fish bacteriology lab - general comments

Very interesting.

Even though bacterial pathogens that cause fish diseases aren't included in listed fish diseases I am glad that at least a small part of bacteriology was included so that we can see the full picture more clearly.

Tutors very helpful and knowledgeable and showed us many different bacteria isolates.

Very well

I did discover devices that I wasn't aware of.

Very good teachers, very good explanations, very nice laboratory

Nice presentation of the lab.

nice opportunity to see results also from another field (bacteriology)

Was good to see the bacteriology to complement the other techniques

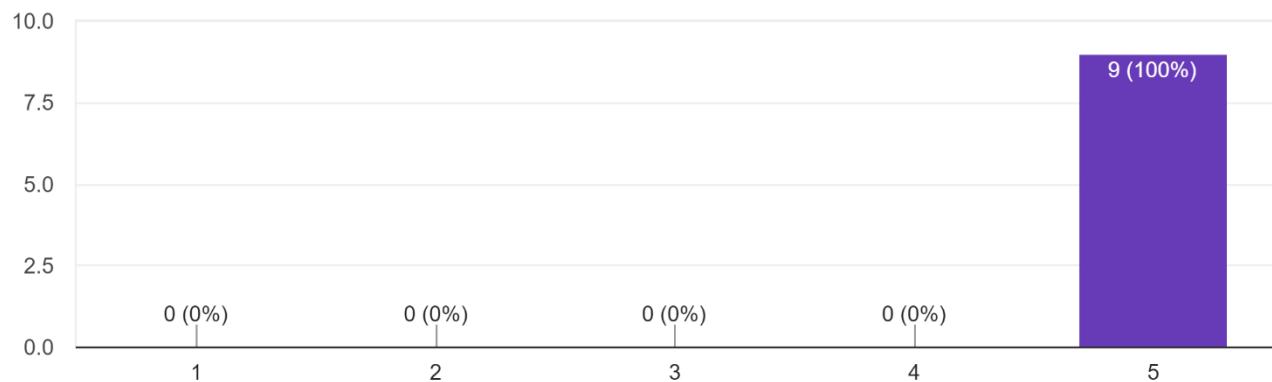
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Day 5 - Assignment, discussion and conclusions - teachers' expertise

9 responses



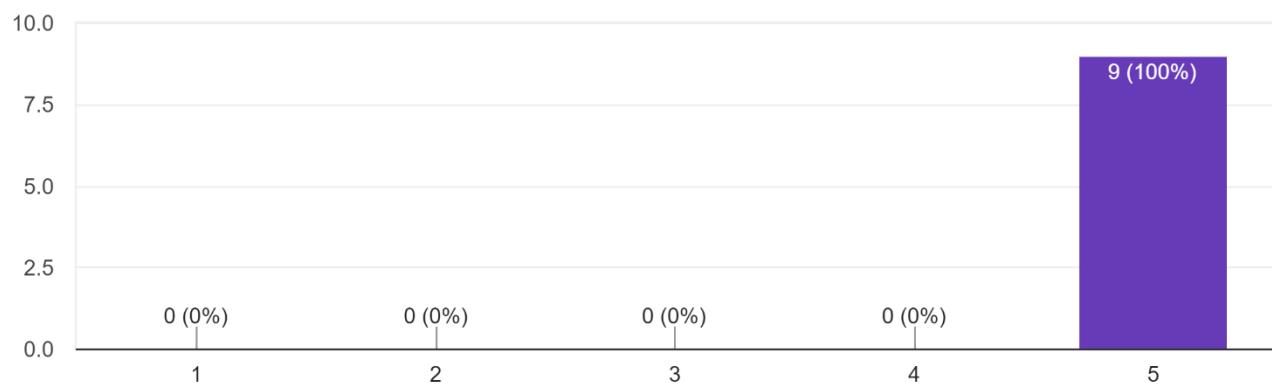
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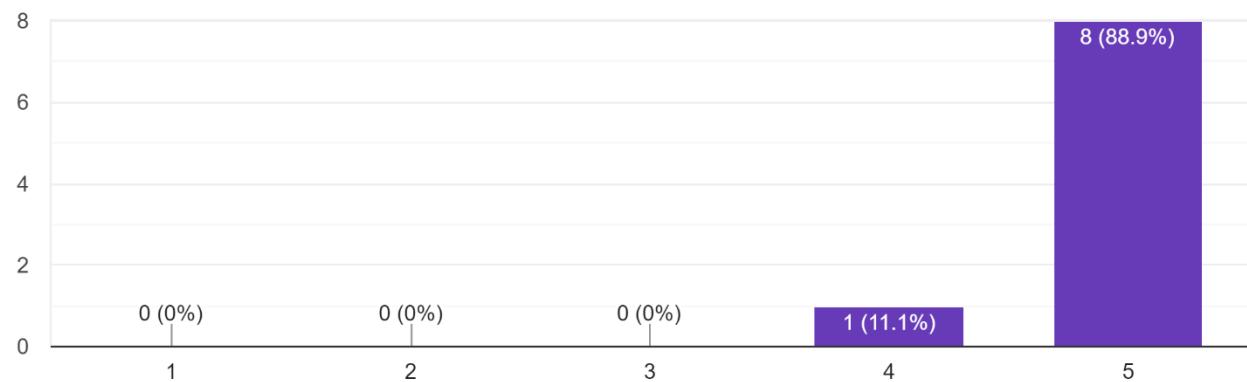
Day 5 - Assignment, discussion and conclusions - teachers' preparedness

9 responses



Day 5 - Assignment, discussion and conclusions - relevance for you

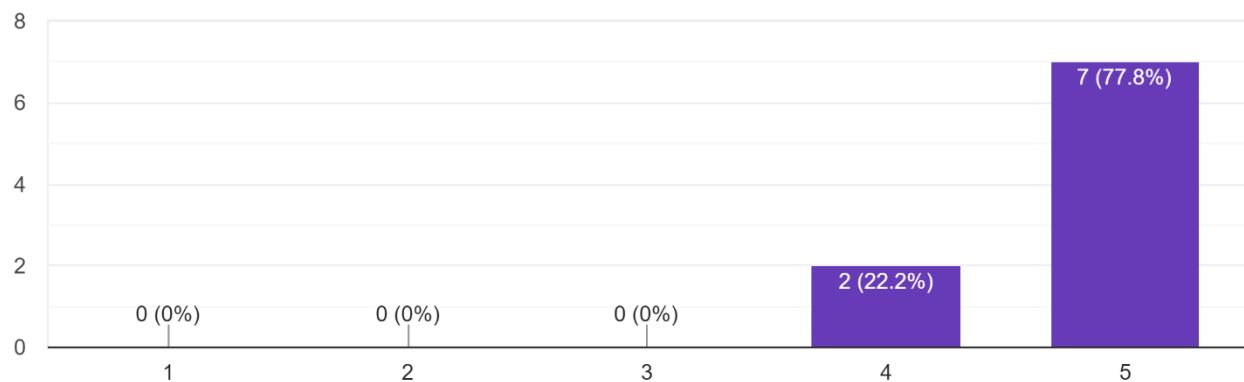
9 responses





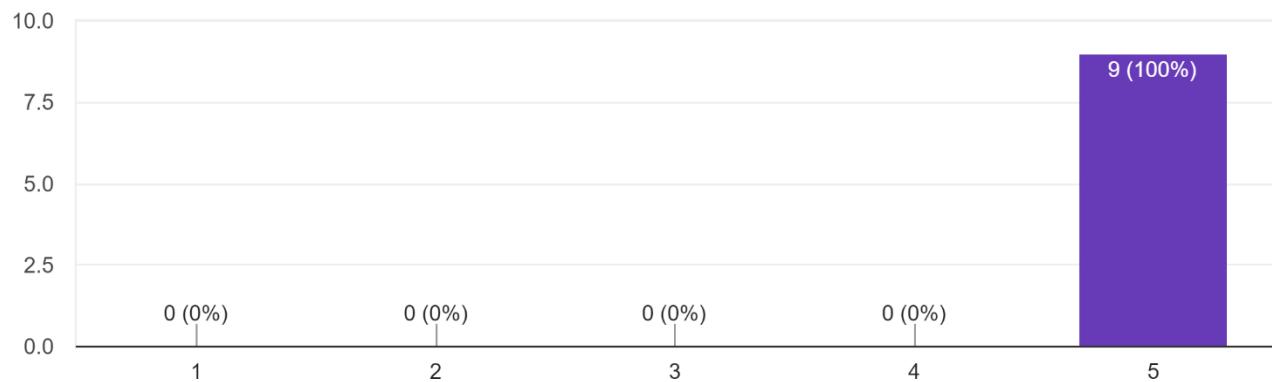
Day 5 - Assignment, discussion and conclusions - increase of your knowledge

9 responses



Day 5 - Assignment, discussion and conclusions - overall score

9 responses



Day 5 - Assignment, discussion and conclusions - general comments

I think this part was great and it might be a good idea to do something similar at the beginning of the course (something simple because we would not have the knowledge yet) because it is also a great way to get to know the other participants. Overall, the course was amazing.

The final discussion was very informative and it helped us clear away any misconceptions we might have had. Regarding the assignment, it was quite interesting and it helped us think back through the course and connect the dots, so to speak.

The assignment was a great way to bring all of our knowledge together in a practical way.

Very well.I would like the most important marine fish diseases to be included, if time can make it possible. It was all training course very well organized and all the teachers gave their best self for the courses

It was a very nice course overall, I am very satisfied with the course. I want to present my thanks to everyone involved.

It was a very good course overall

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Too little time for the assignment



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) and perhaps in situ hybridization (ISH). The first day, participants are 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. In the second half of the course specific topics regarding crustaceans will be addressed. The course gives an introduction to sampling, 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 is based 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 and perhaps in situ hybridization describing pitfalls and application of these techniques to specific pathogens.

Lectures will include descriptions of the techniques with major focus on their application, pitfalls and trouble shooting. Practical 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.

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. The participants that will 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 perhaps ISH 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 and ISH, 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 and crustacean diseases
IHC applied to fish tissue
(ISH applied to fish tissue)

Assessment

During each day participants are encouraged to take part in the discussions on the subjects presented. 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 in fish
Raoul Kuiper, tutor responsible for pathology and histopathology and IHC in fish
Tobia Pretto, tutor responsible for pathology and histopathology in crustaceans
Tine Moesgaard Iburg, tutor and course facilitator

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Niccoló Vendramin, course facilitator
Morten Schiøtt, course facilitator



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Programme: Introduction to histopathology in fish and crustacean diseases

Day 1	Day 2	Day 3	Day 4	Day 5
Monday	Tuesday	Wednesday	Thursday	Friday
9:00-10:00 Course introduction Participants will present themselves <u>Coffee Break 10:00-10:30</u> 10:30-12:00 Sampling for histopathological examination. Theory and Practice	8:30-9:30 Lecture on pathology and histopathology <u>Coffee Break 9:30-10:00</u> 10:00-11:30 Practical exercise (slides)	9:00-10:00 Lecture on IHC I <u>Coffee Break 10:00-10:30</u> 10:30-11:30 Lecture on IHC II	9:00 - 10:00 Show and tell of cases by Ole Bendik Dale/Raul Kuiper <u>Coffee Break 10:00-10:30</u> 10:30- 11:30 More show and tell fish diseases	9:00-10:15 Overview of WSSV Microscopy practicals <u>Coffee Break 10:15-10:45</u> 10:45-12:00 Overview of TSV and YHV Microscopy Practical
Lunch 12.00 -13:00	Lunch 11.30-12:15	Lunch 11.30 -12:15	Lunch 11:30 -12:30	Lunch 12:00-13.00
13:00 – 14:30 Lecture on pathology and histopathology <u>Coffee Break 14:30 – 15:00</u> 15:00-16:00 Lecture on pathology and histopathology	12:15 – 13:30 Lecture on pathology and histopathology Place: Auditorium <u>Coffee Break 13:30-14:00</u> 14:00-16:00 Practical exercise (slides)	12:15-13:15 Theoretical exercise on IHC 1 <u>Coffee Break 13:15 – 13.45</u> 13:45-15:45 Practical exercise (slides) 15:45 – 16:30 Theoretical exercise on IHC 2	12:30-14:30 Crustacean Dissection and Sampling. Theory and practice <u>Coffee Break 14:30 – 15:00</u> 15:00-16:00 Overview of Crustacean Tissues - Structure and Function Part I 15:00-16:00 Overview of Crustacean Tissues - Structure and Function Part II Slide session	13:00-15:00 OIE listed diseases and Emerging Pathogens 15.15 - Coffee, cakes and evaluation of the crustacean day Place: auditorium

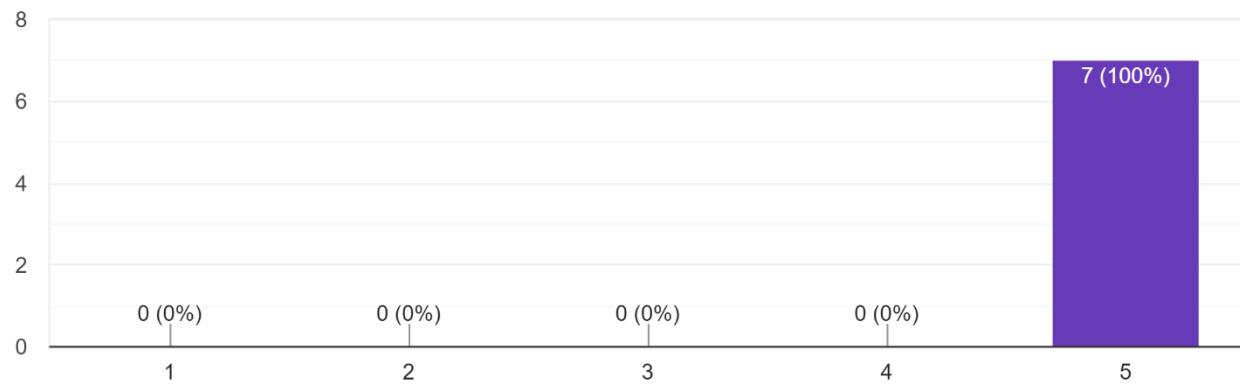


Evaluation: Introduction to histopathology in fish and crustacean diseases

Participant satisfaction for each respective section. The calculations are based on returned evaluation schemes from 7 participants.

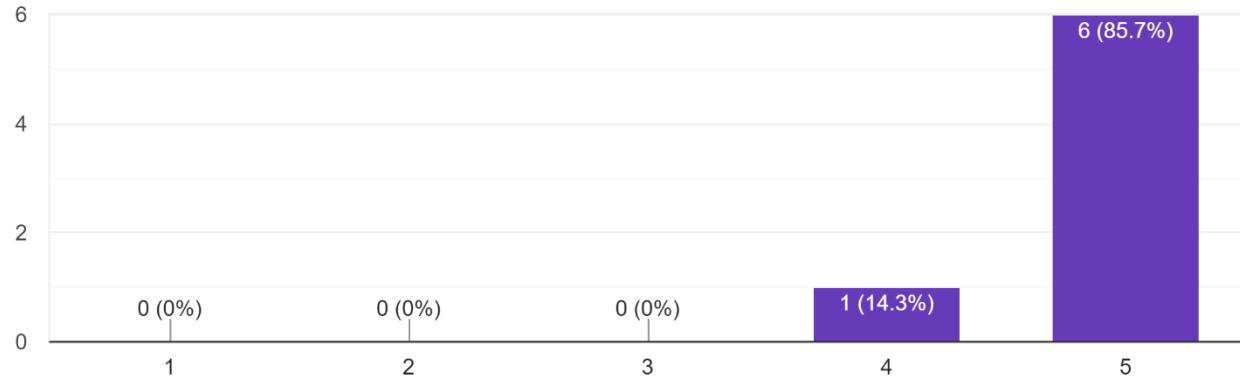
Day 1 Lecture and practical exercise in sampling for histopathological examination - teacher's expertise

7 responses



Day 1 Lecture and practical exercise in sampling for histopathological examination - teacher's expertise

7 responses



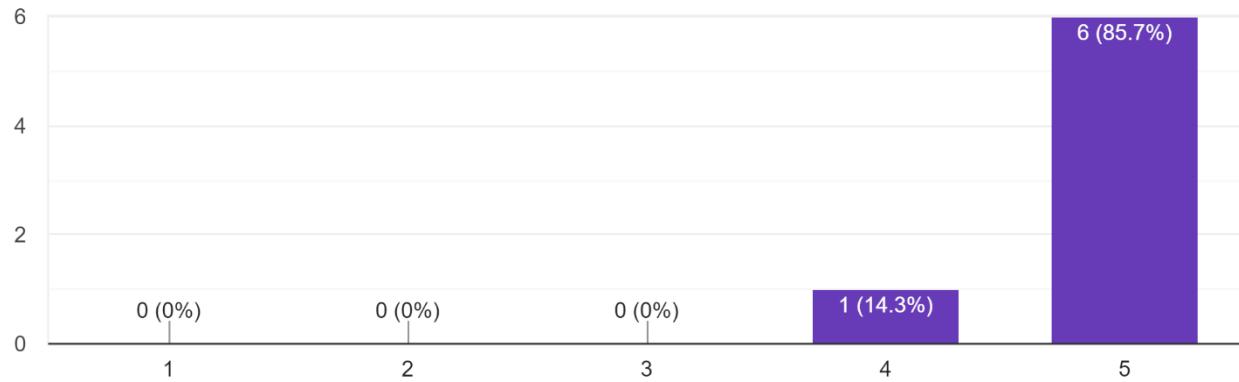
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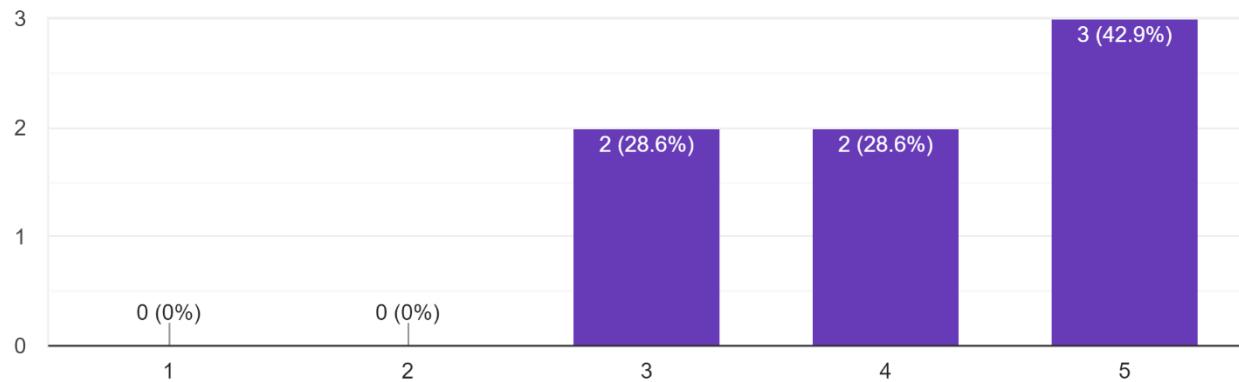
Day 1 Lecture and practical exercise in sampling for histopathological examination - teacher's preparedness

7 responses



Day 1 Lecture and practical exercise in sampling for histopathological examination - relevance for you

7 responses



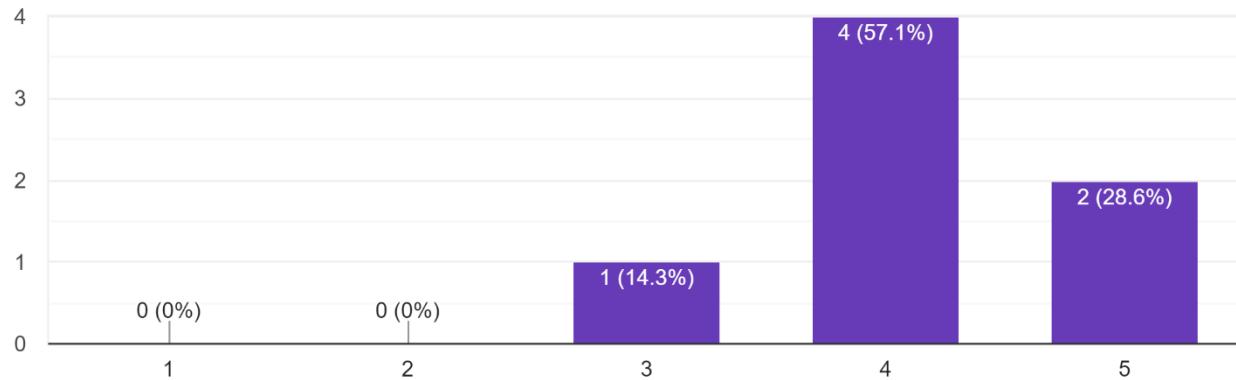
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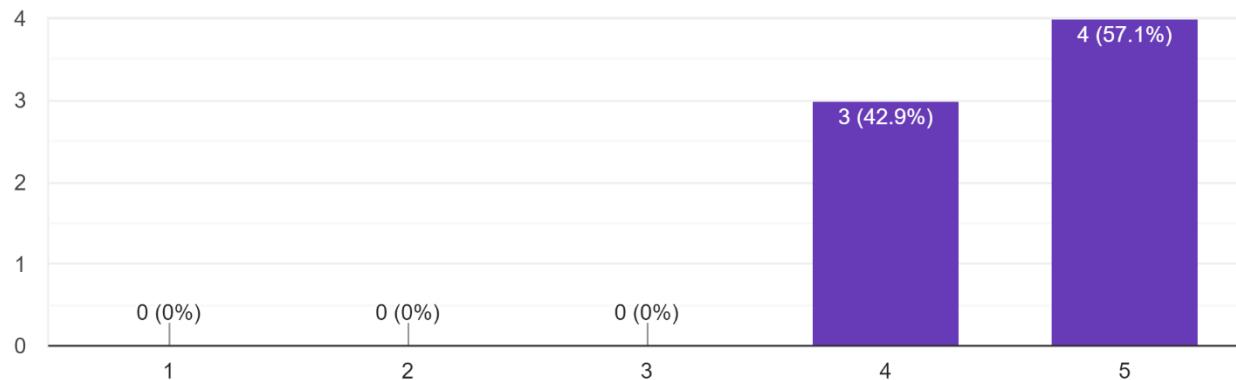
Day 1 Lecture and practical exercise in sampling for histopathological examination - increase of your knowledge

7 responses



Day 1 Lecture and practical exercise in sampling for histopathological examination - overall score

7 responses



Day 1 Lecture and practical exercise in sampling for histopathological examination - general comment

7 responses

The fish dissection exercise in the lab was a little too short. When questions and answers are factored in, it felt quite rushed. However, I understand that this is difficult to plan around. I think that the lecturer should advise the class that questions can wait until after so that more benefit can be gained from the valuable practical lab.

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Only, I wish the practical exercise in sampling for histopathological section was longer because I like to study samples in detail.

A very interesting practical exercise of fish dissection

Good

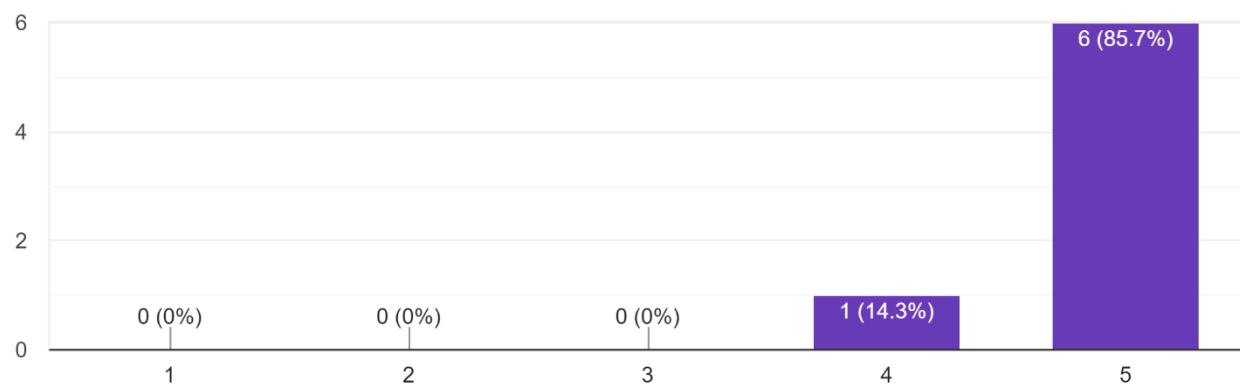
The histopathology lecture should be improved

Very well presented and organised - However, practical was a little bit rushed. A lot of content covered in limited time.

Very well

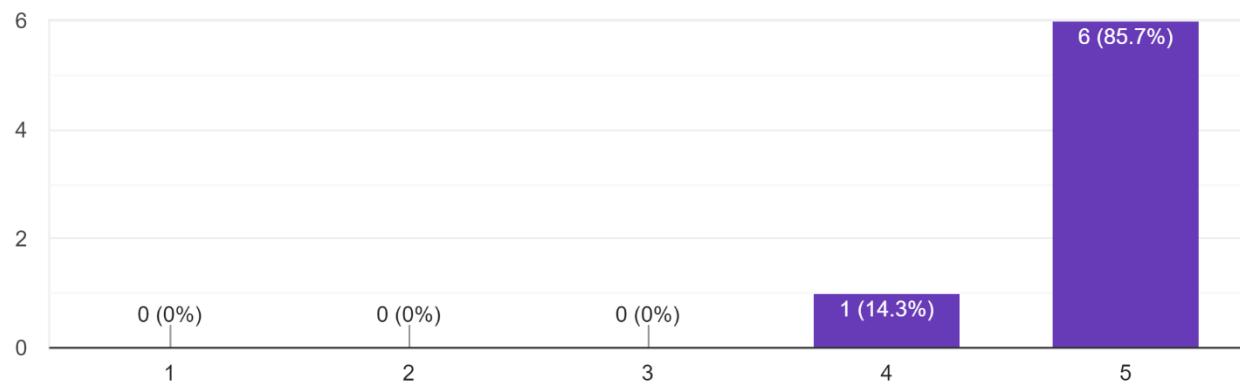
Day 1,2 and 4 Lectures in Fish Pathology and Histopathology - teacher's expertise

7 responses



Day 1,2 and 4 Lectures in Fish Pathology and Histopathology - teacher's preparedness

7 responses



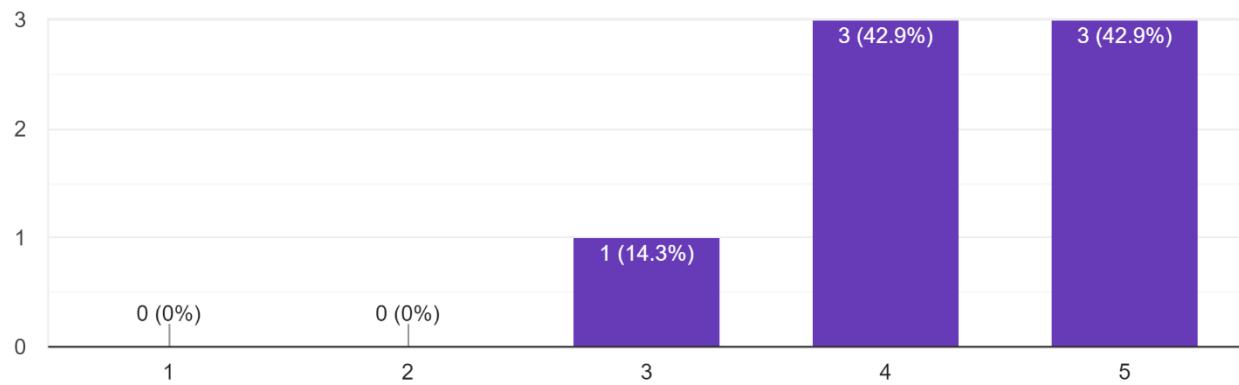
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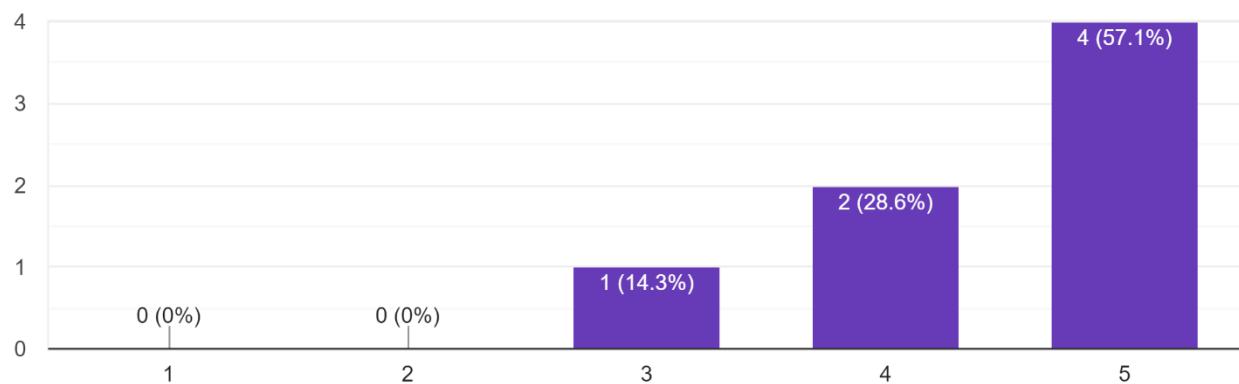
Day 1,2 and 4 Lectures in Fish Pathology and Histopathology - relevance for you

7 responses



Day 1,2 and 4 Lectures in Fish Pathology and Histopathology - increase of your knowledge

7 responses



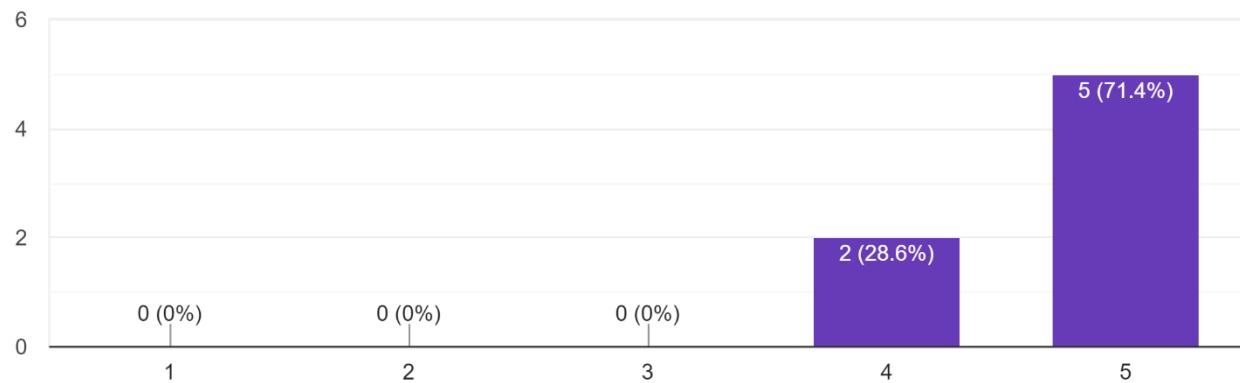
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Day 1,2 and 4 Lectures in Fish Pathology and Histopathology - overall score

7 responses



Day 1,2 and 4 Lectures in Fish Pathology and Histopathology - general comment 7 responses

I was mesmerized by the vast wealth of knowledge demonstrated by Tina, Ole-Bendik and Raoul. They were extremely thorough and thoughtful in the topics they chose and delivered the information in a consumable manner. Could not be more impressed.

It was quite fine, thanks.

Lectures very well described

good really well presented

lectures should be implemented with other fish species and pathologies

Very well presented and organised - However, a little bit rushed. A lot of content covered in limited time.

Very well

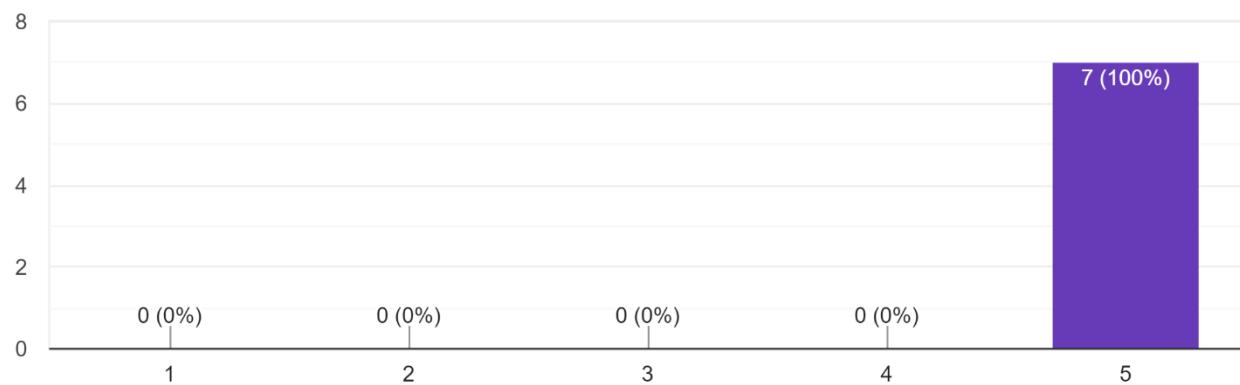
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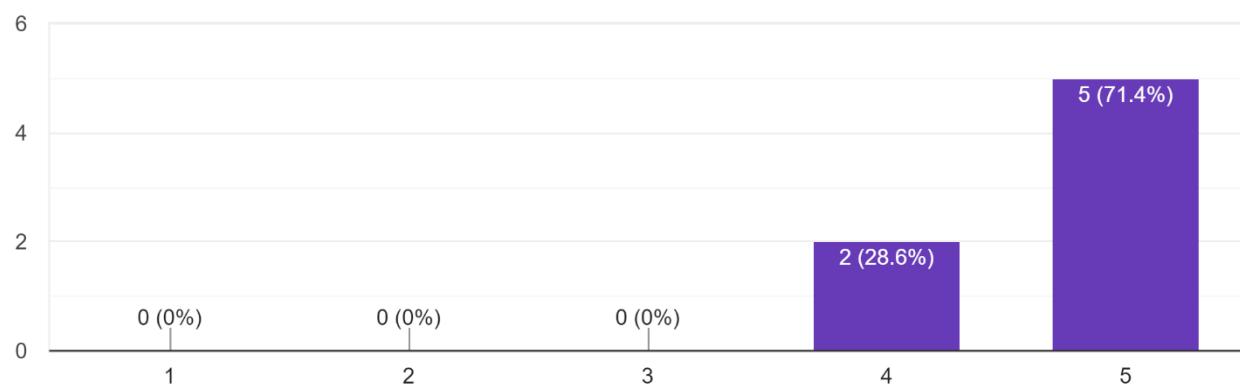
Day 2 and 3- Practical exercises in histopathology of fish - teacher's expertise

7 responses



Day 2 and 3- Practical exercises in histopathology of fish - teacher's preparedness

7 responses



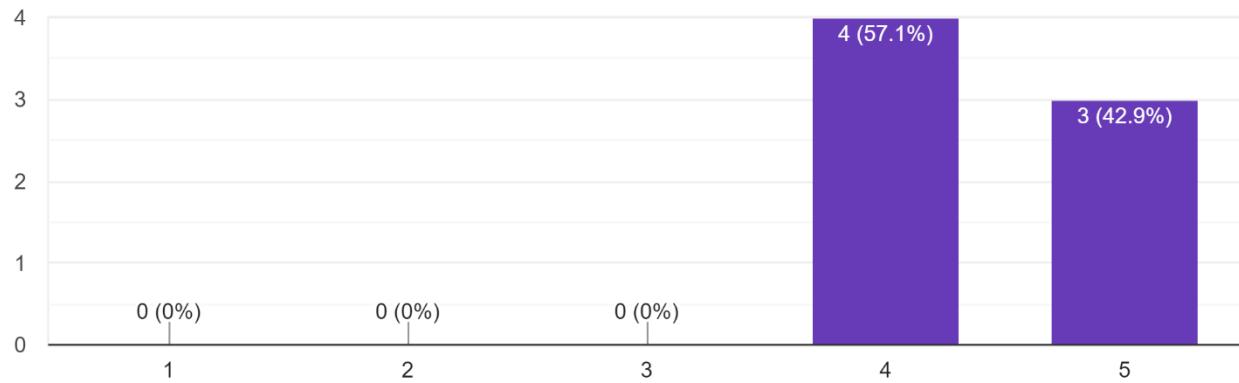
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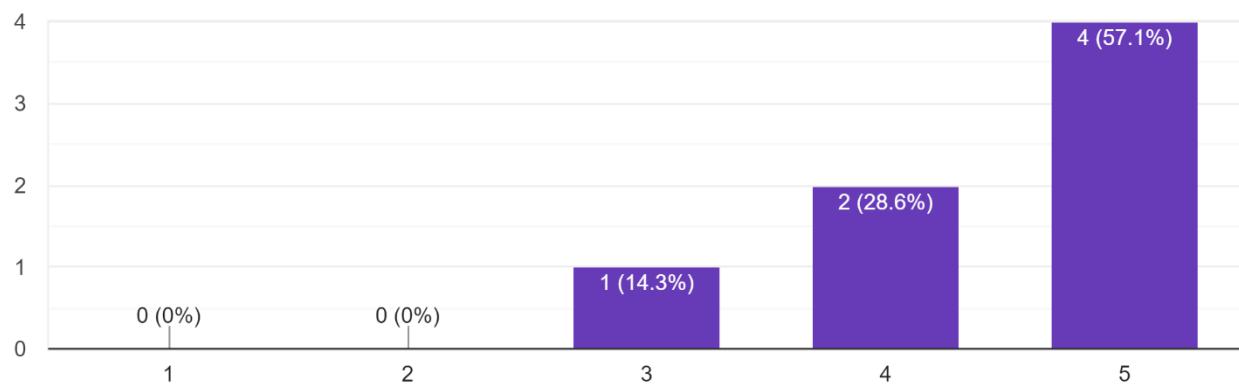
Day 2 and 3- Practical exercises in histopathology of fish - relevance for you

7 responses



Day 2 and 3- Practical exercises in histopathology of fish - increase of your knowledge

7 responses



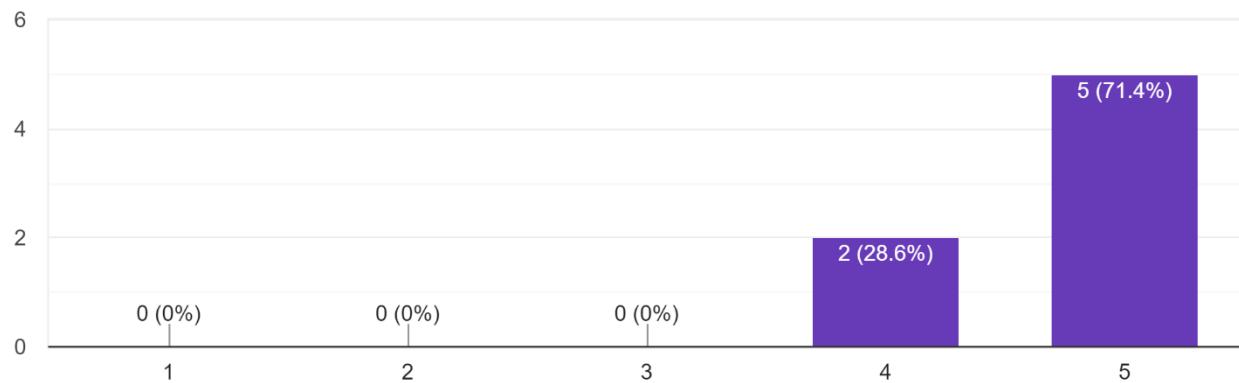
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Day 2 and 3- Practical exercises in histopathology of fish - overall score

7 responses



Day 2 and 3- Practical exercises in histopathology of fish - general comment

7 responses

It was my first time experiencing scanned slides. I found them to be immensely helpful due to the ease at which you can zoom in and out and move around the tissue, all while there is group input, rather than using only light microscopy and taking turns viewing the tissue. I felt overall learning experience from these sessions was very helpful.

Practical exercise time should have been longer

Interpretation of slides has been a bit difficult for me. I think I need more training. I will try to go deepness in this area

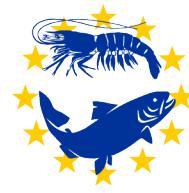
Really enjoyed this and learnt a lot

Good for those who have experience in fish pathology, lacking for beginners

Exercise was well organised but tight for time. Some minor issues with laptops connecting to network (maybe an idea to have a DTU provided laptop/PC already connected to the big screens in each room and logged into the slide image site ready to go).

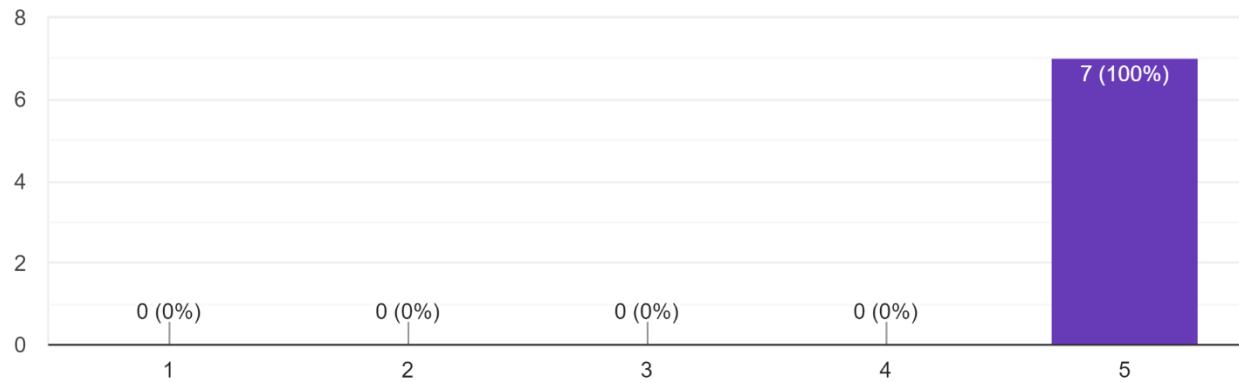
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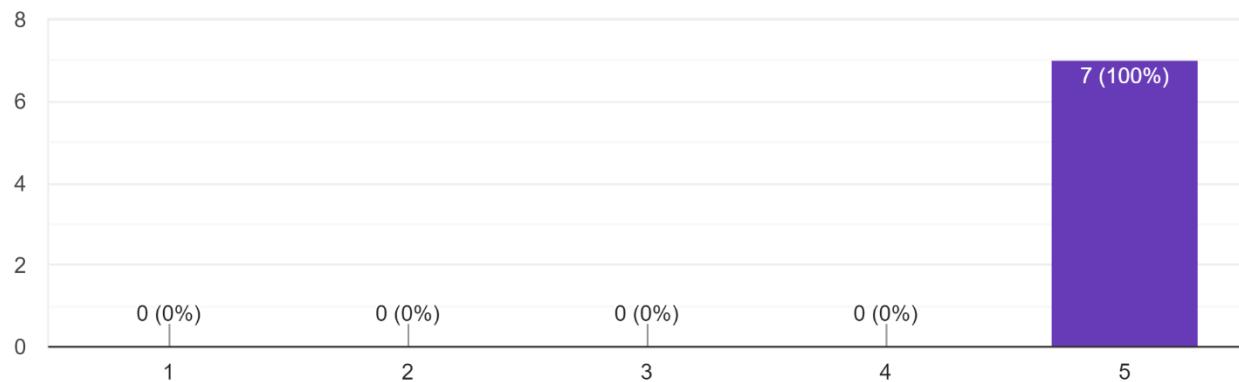
Day 3- Immunohistochemistry IHC - teacher's expertise

7 responses



Day 3- Immunohistochemistry IHC- teacher's preparedness

7 responses



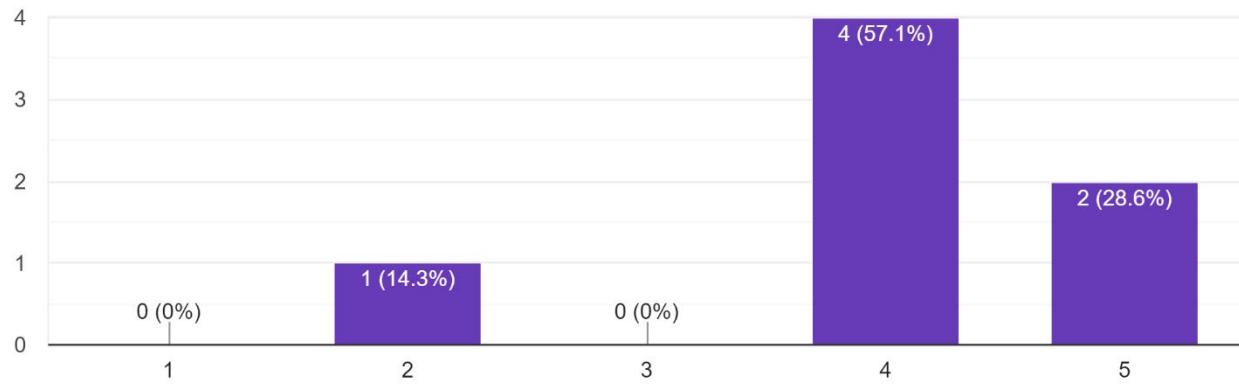
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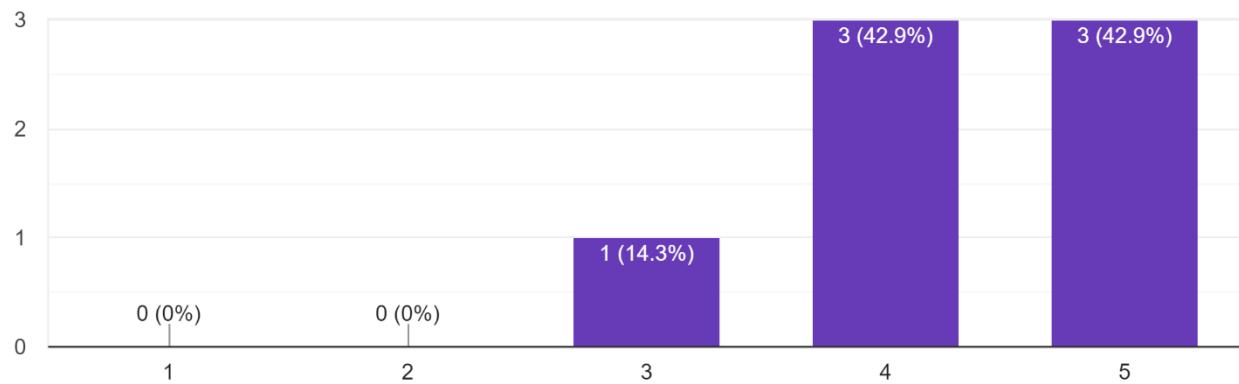
Day 3- Immunohistochemistry IHC- relevance for you

7 responses



Day 3- Immunohistochemistry IHC- increase of your knowledge

7 responses



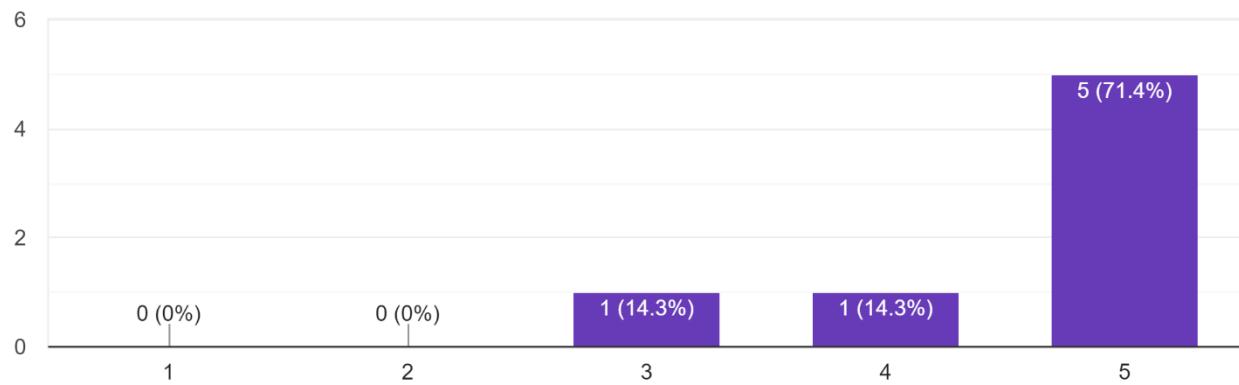
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Day 3- Immunohistochemistry IHC- overall score

7 responses



Day 3- Immunohistochemistry IHC- general comment

7 responses
Very interesting and informative. My knowledge of IHC was very limited before the course. Concepts were explained well and the benefits of IHC were made very apparent.

It was good, but it would be better if the lecture time was longer.

We don't expect to use this technique but good to know about it.

Already a good knowledge of IHC

Excellent immunoistochemistry overview

Well presented and informative. Might have been nice to have some practical element.

Very well

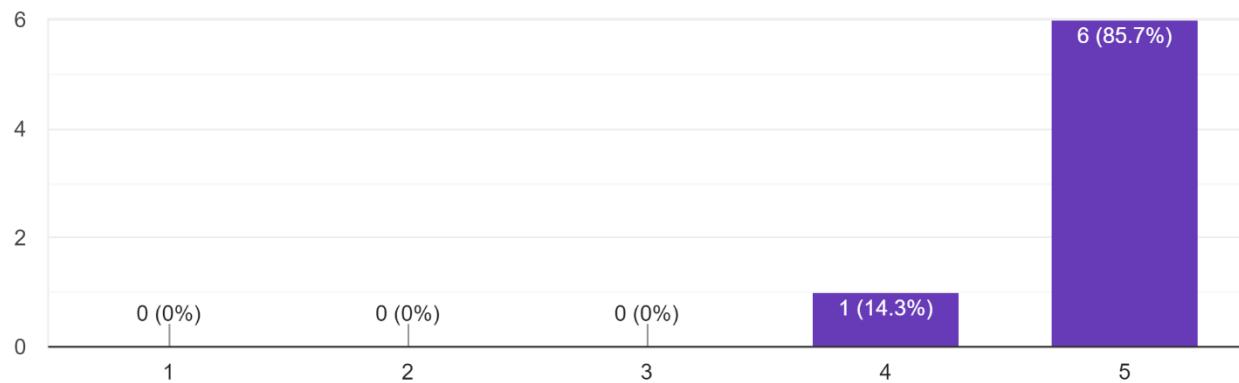
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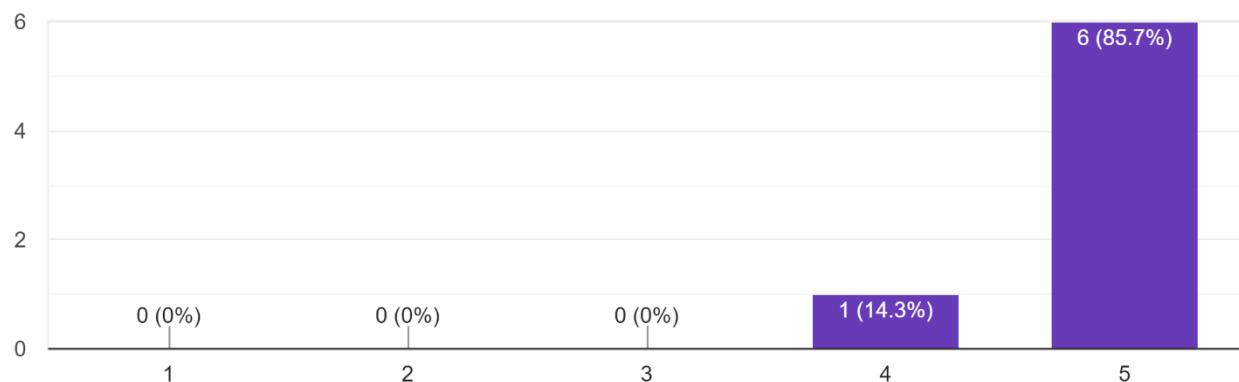
Day 3- In Situ hybridization and digital pathology - teacher's expertise

7 responses



Day 3- In Situ hybridization and digital pathology - teacher's preparedness

7 responses



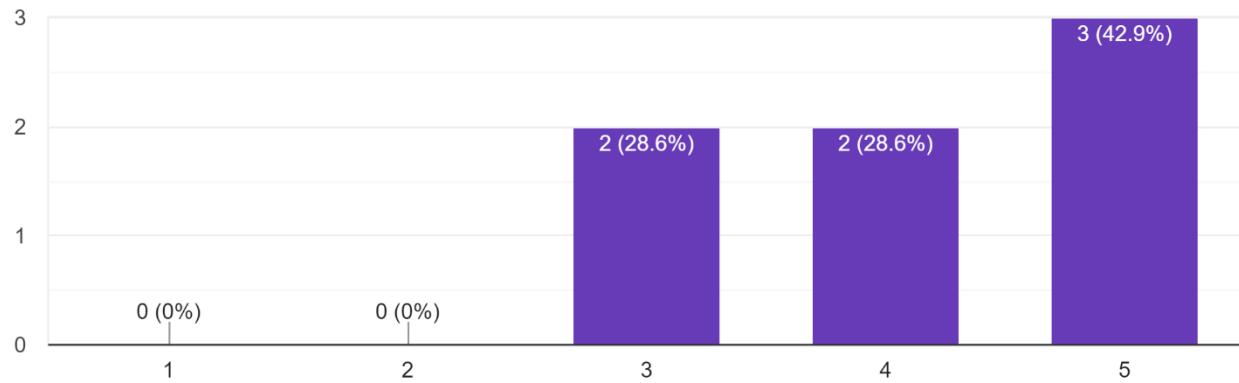
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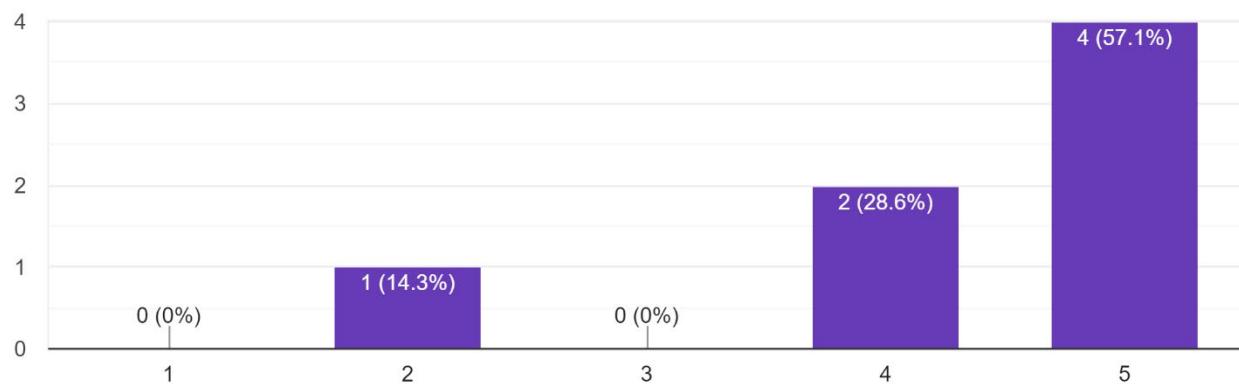
Day 3- In Situ hybridization and digital pathology - increase of your knowledge

7 responses



Day 3- In Situ hybridization and digital pathology - relevance for you

7 responses



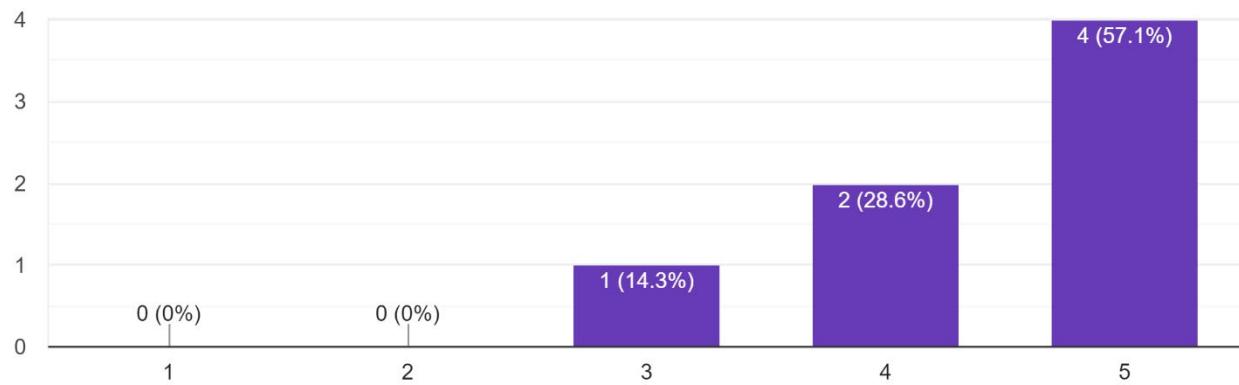
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Day 3- In Situ hybridization and digital pathology - overall score

7 responses



Day 3- In Situ hybridization and digital pathology - general comment

7 responses
Very interesting and informative. My knowledge of ISH and digital pathology was very limited before the course. Concepts were explained well and the benefits of ISH were made very apparent.

it was a different topic for me so it was quite interesting

We don't expect to use this technique but good to know about it.

very familiar with ISH already

I would have spent more time on ISH

Well presented and informative. Might have been nice to have some practical element.

Very well

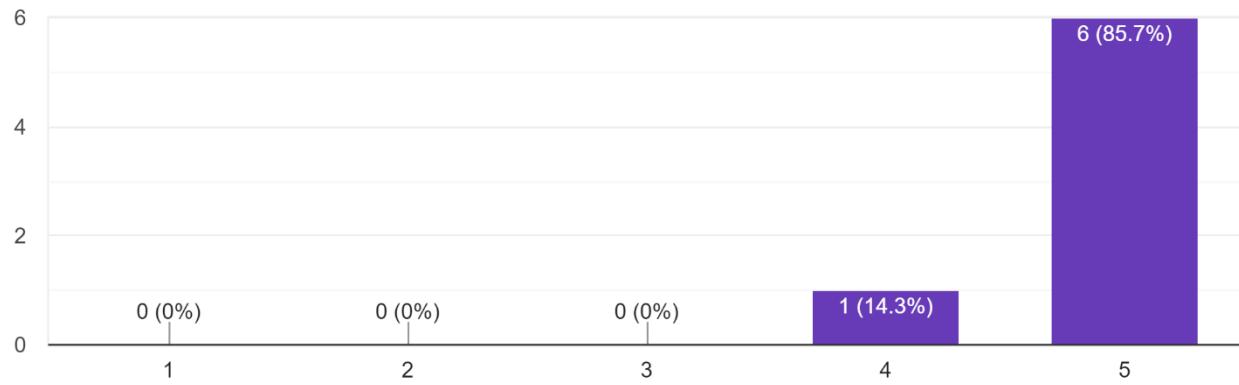
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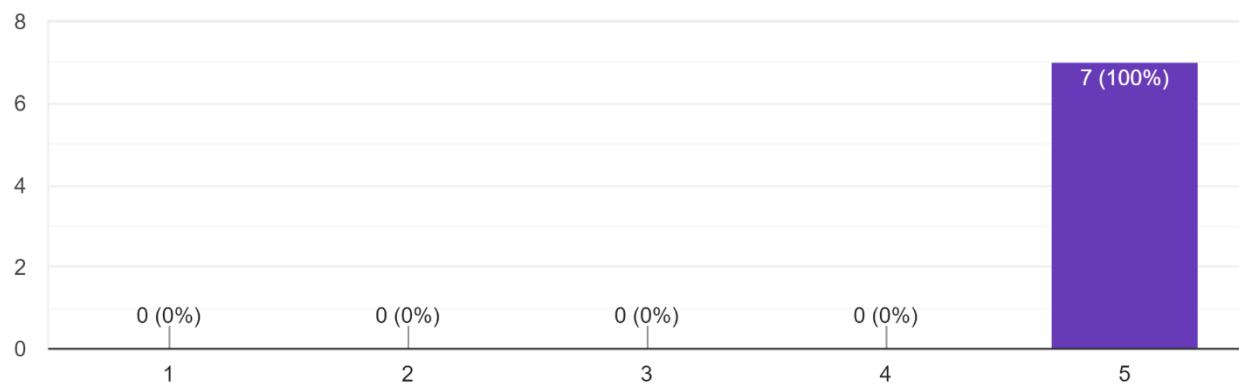
Day 3 Theoretical exercise in IHC - teachers' expertise

7 responses



Day 3 Theoretical exercise in IHC . teachers' preparedness

7 responses



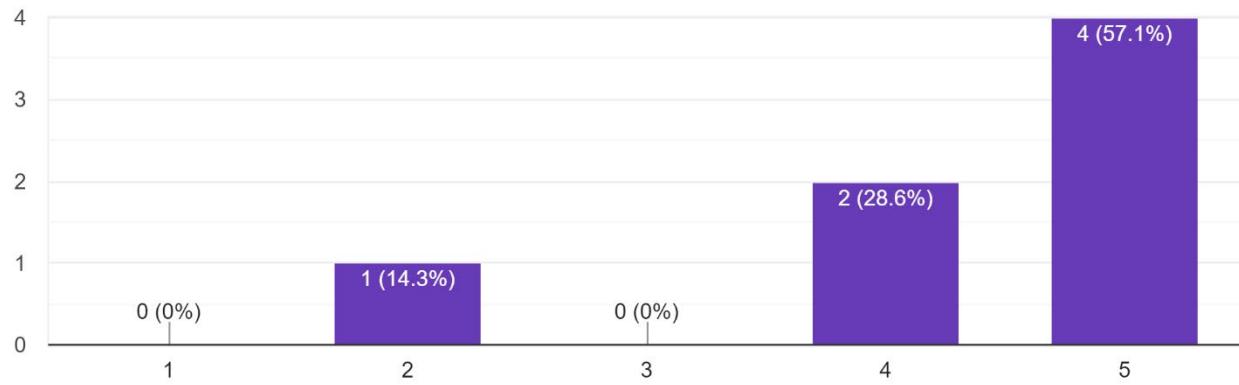
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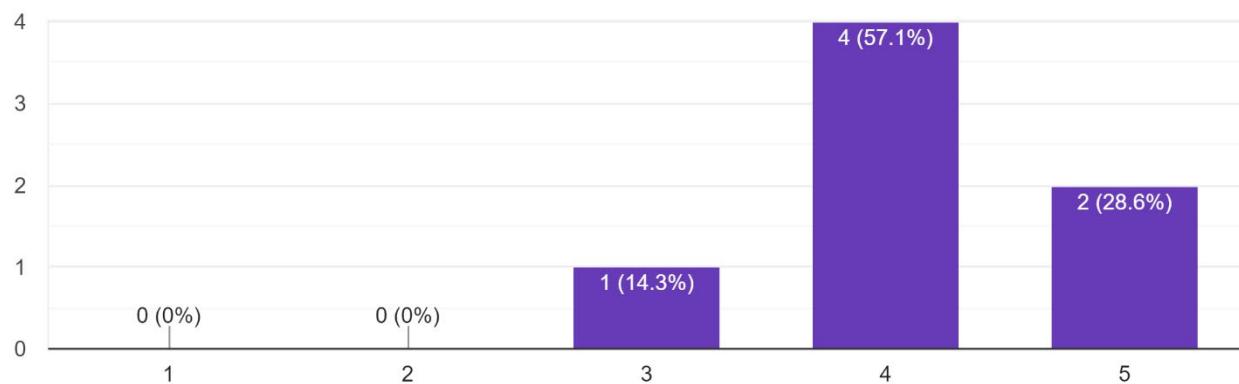
Day 3 Theoretical exercise in IHC - relevance for you

7 responses



Day 3 Theoretical exercise in IHC - increase of your knowledge

7 responses



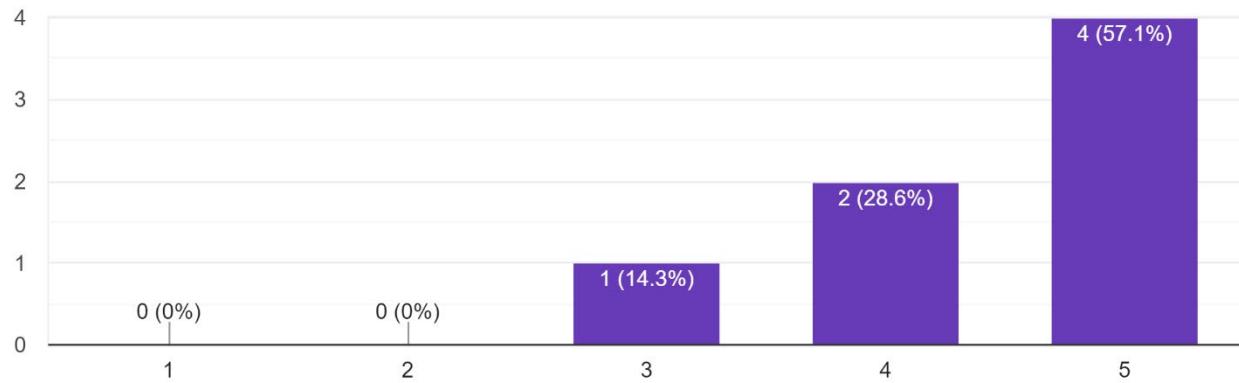
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Day 3 Theoretical exercise in IHC - overall score

7 responses



Day 3 Theoretical exercise in IHC - general comment

7 responses

Very good.

It was fine.

We don't expect to use this technique but good to know about it.

Good

I would have spent more time on ISH

Useful exercise but a little rushed through.

Very well

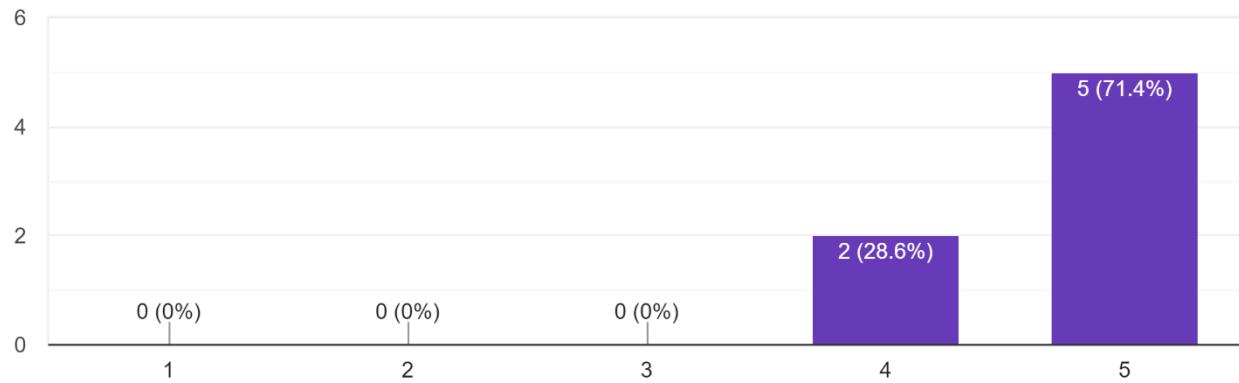
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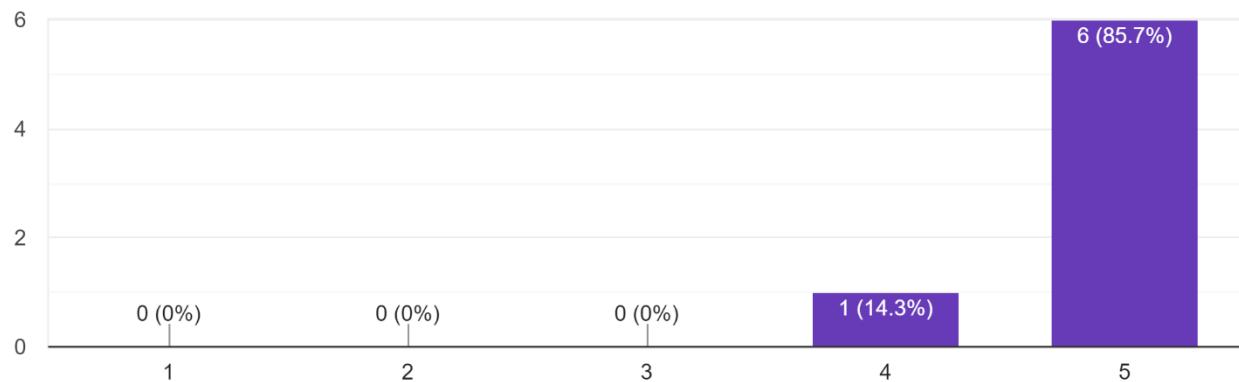
Day 4 - Show and tell of cases teacher's expertise

7 responses



Day 4 - Show and tell of cases- teacher's preparedness

7 responses



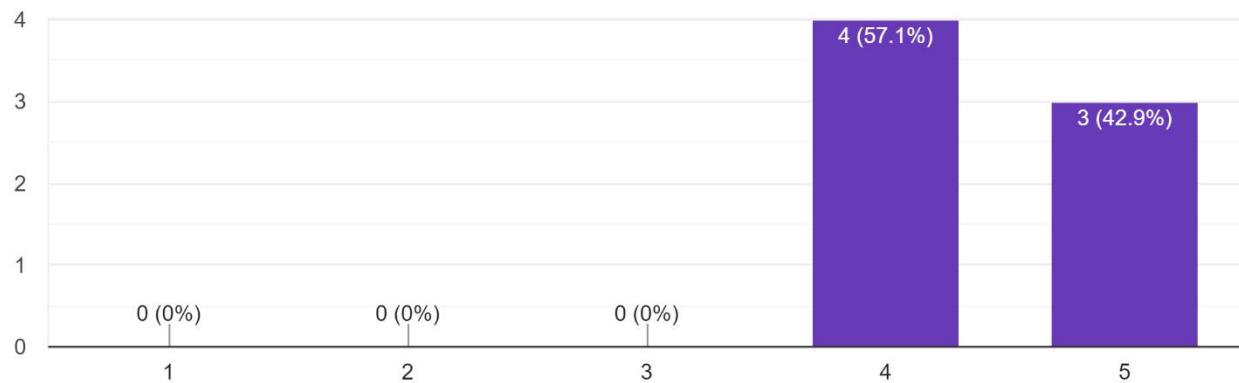
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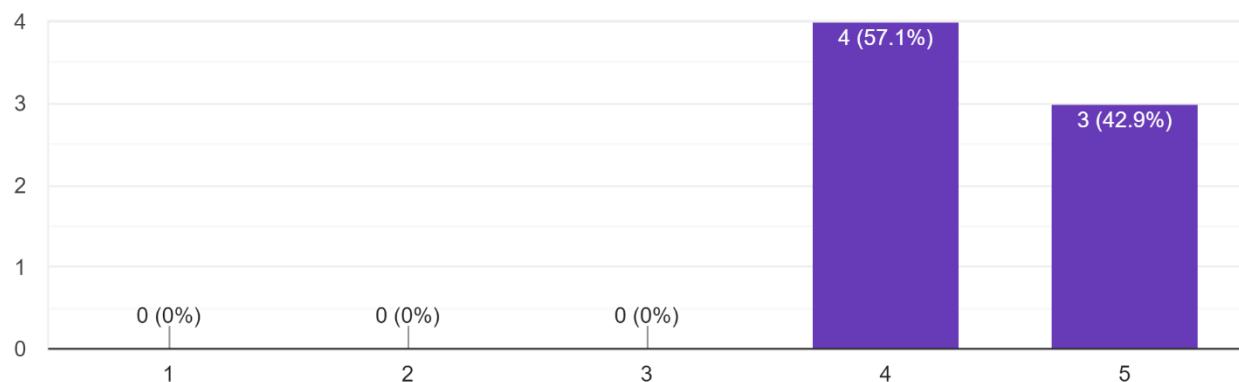
Day 4 - Show and tell of cases- relevance for you

7 responses



Day 4 - Show and tell of cases- increase of your knowledge

7 responses



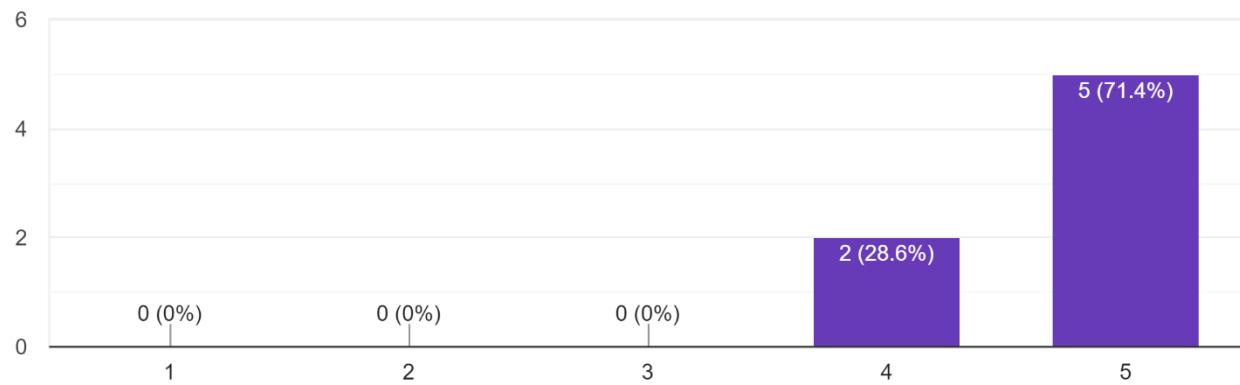
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Day 4 - Show and tell of cases- overall score

7 responses



Day 4 - Show and tell of cases- general comment

7 responses

Very good.

I have always loved to talk about the case and find solutions. It was quite enjoyable for me.

More knowledge about fish diseases, very well explained/described

Good

I would have used a wider case study

Very interesting and informative. Limited time.

Very well. I would suggest to include the Show and tell of cases in Day 3.

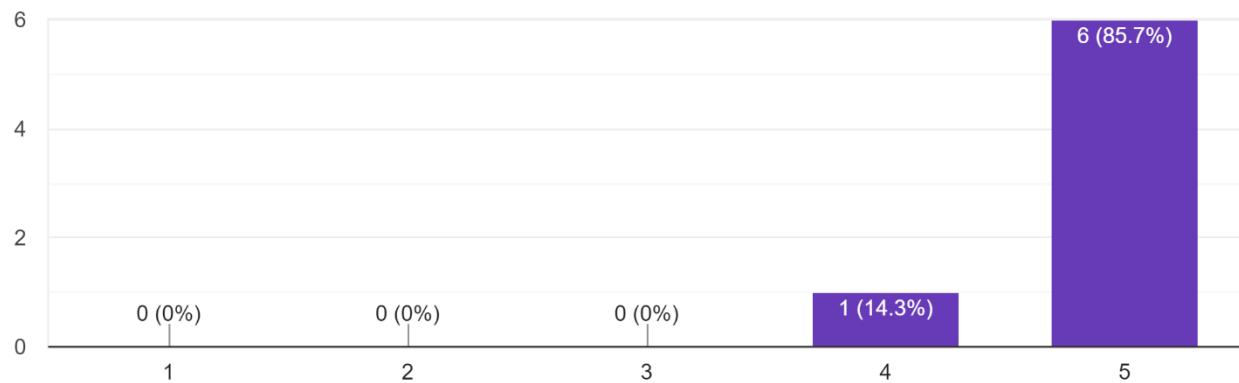
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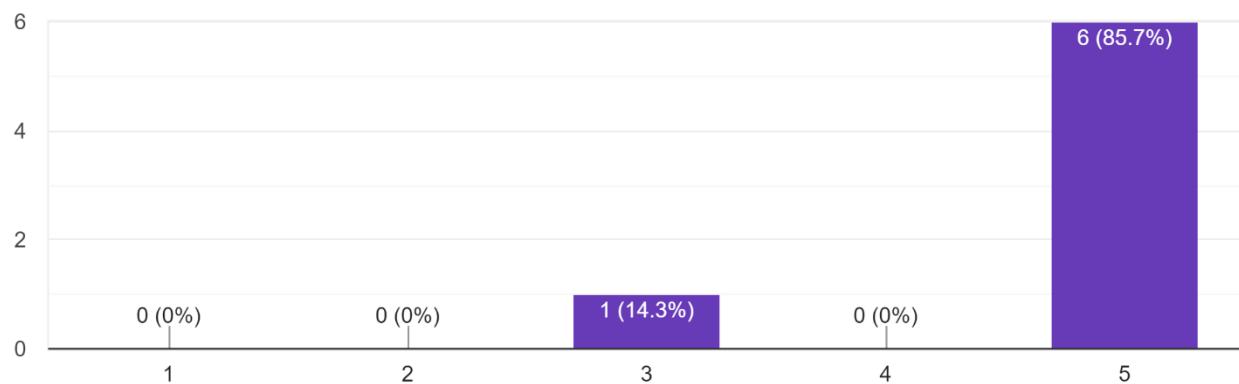
Day 4 and 5 - Crustacean diseases - teachers' expertise

7 responses



Day 4 and 5 - Crustacean diseases - teachers' preparedness

7 responses



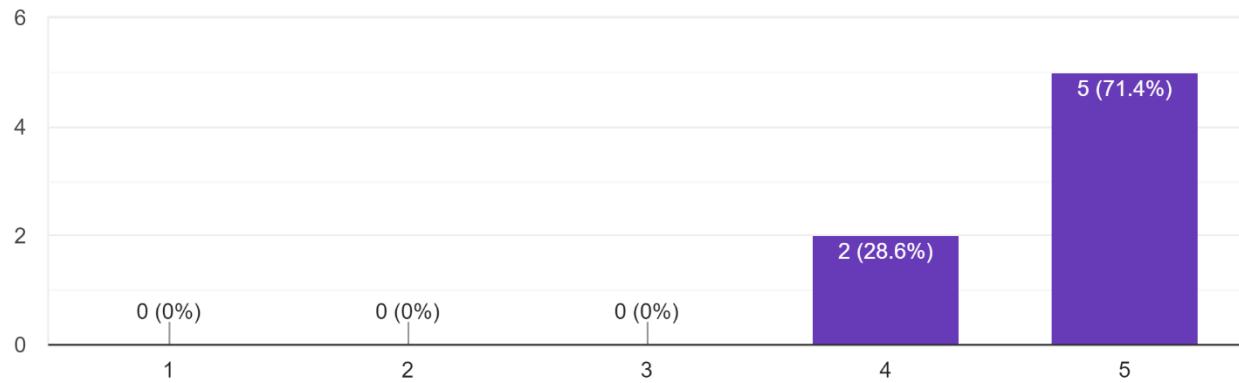
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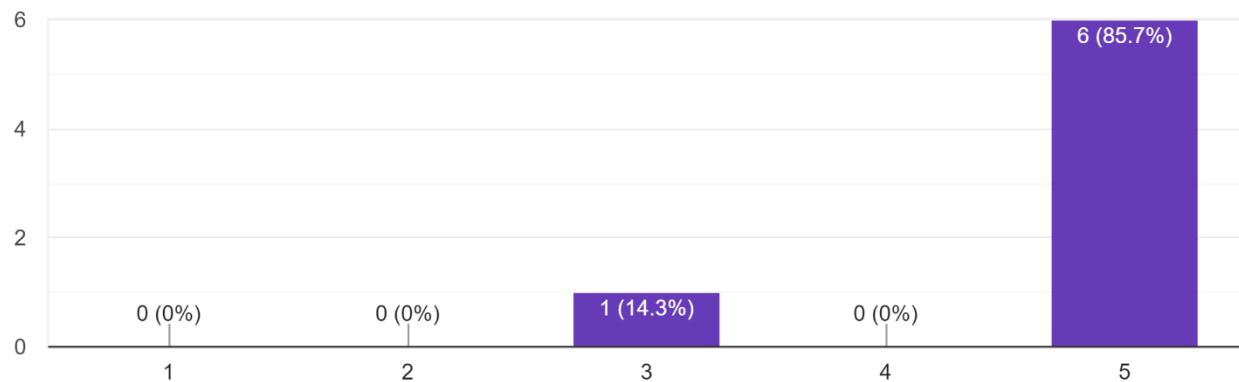
Day 4 and 5 - Crustacean diseases - relevance for you

7 responses



Day 4 and 5 - Crustacean diseases - increase of your knowledge

7 responses



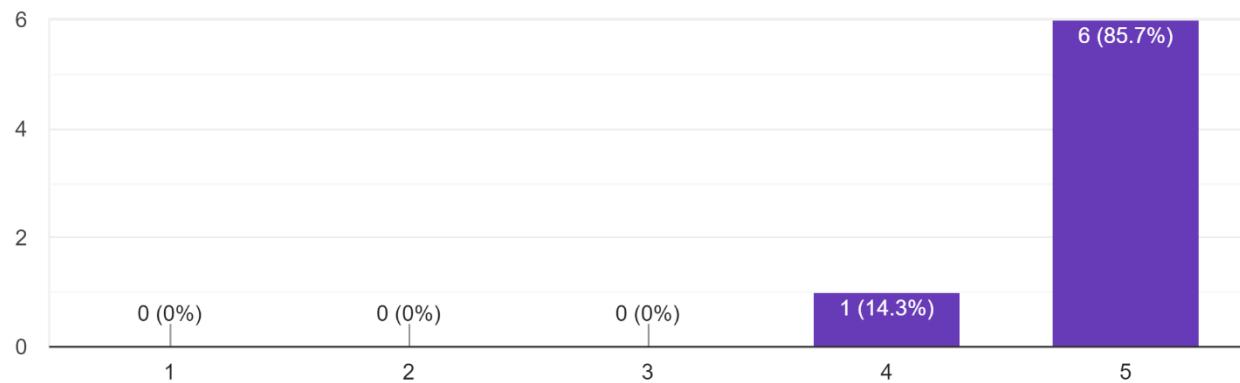
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Day 4 and 5 - Crustacean diseases - teachers' expertise - overall score

7 responses



Day 4 and 5 - Crustacean diseases - teachers' expertise- general comment

7 responses
Unfortunately, I found the material for crustaceans quite rushed and I also found Tobia somewhat difficult to understand. However, he was still very knowledgeable.

It was a very different and interesting.

Very professional teacher

Tobia has good knowledge and understanding of the subject matter and communicated this very well.

Very knowledgeable teacher

Very informative and lots of information. But a bit rushed through.

Very interesting and new knowledge. More time for practise if it is possible. All Day 4 and 5 to Crustacean diseases

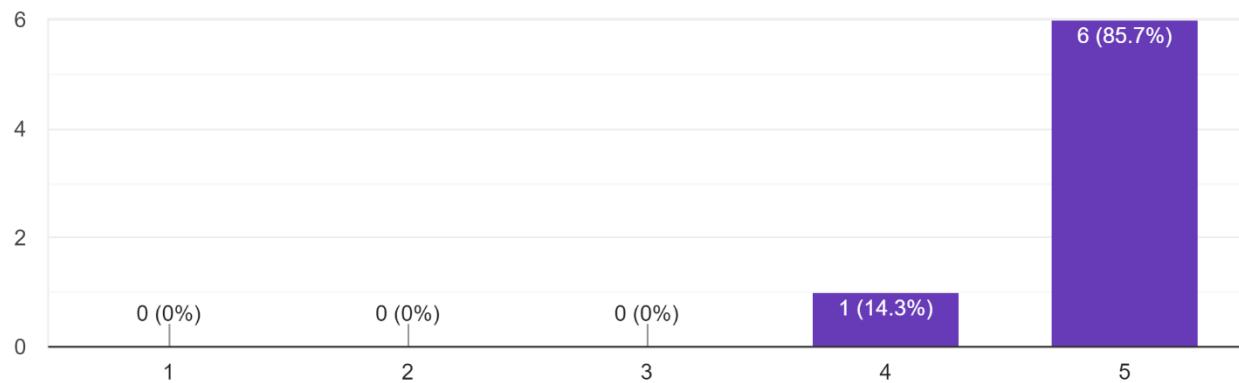
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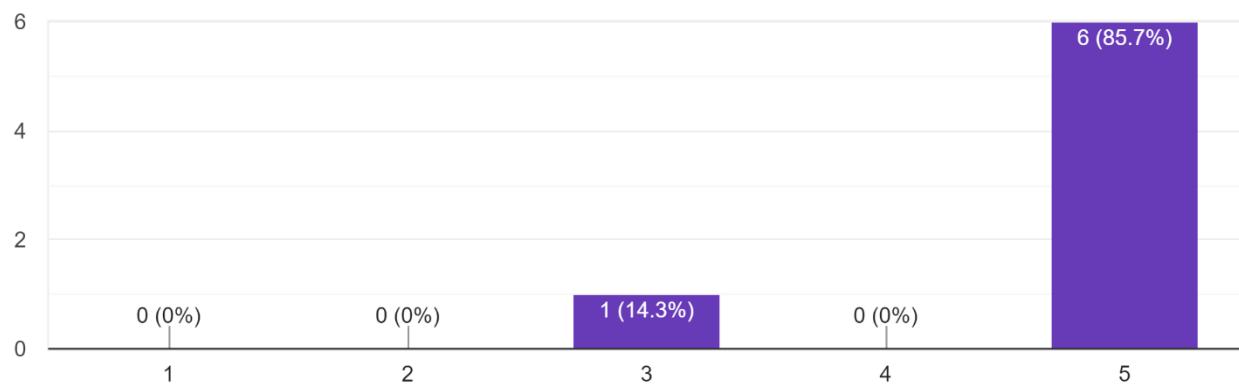
Day 4 and 5 Practical exercises in histopathology of crustaceans - teachers' expertise

7 responses



Day 4 and 5 Practical exercises in histopathology of crustaceans - teachers' preparedness

7 responses



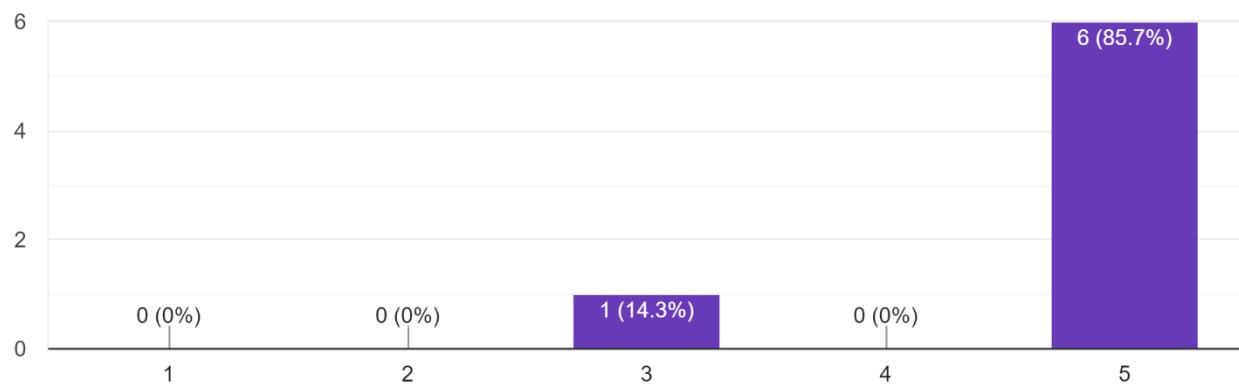
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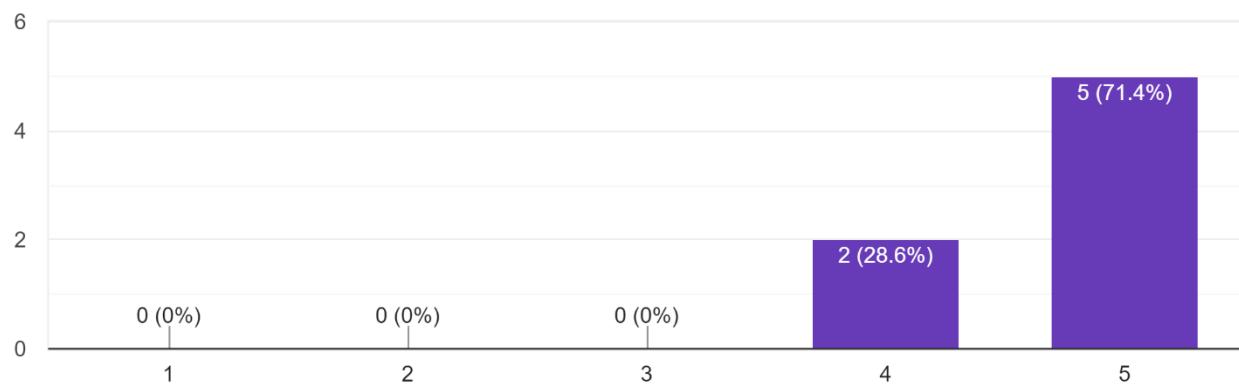
Day 4 and 5 Practical exercises in histopathology of crustaceans - increase of your knowledge

7 responses



Day 4 and 5 Practical exercises in histopathology of crustaceans - relevance for you

7 responses



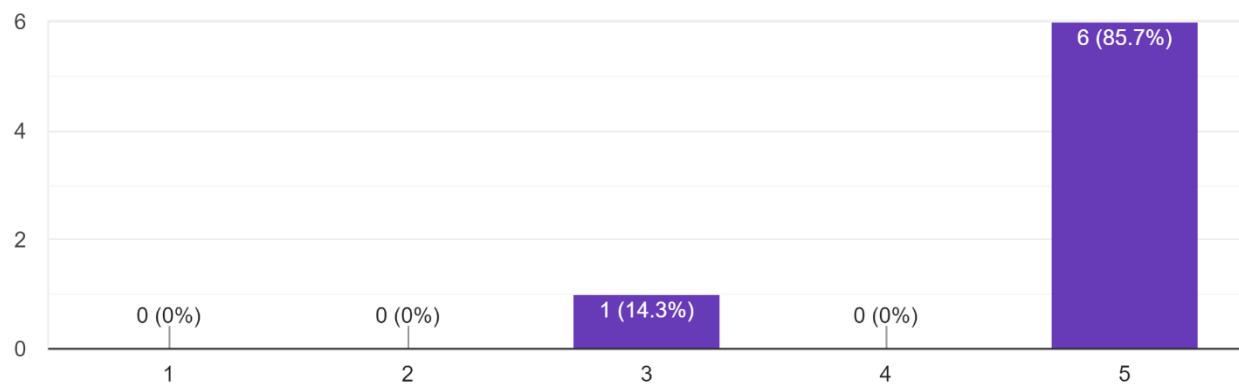
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Day 4 and 5 Practical exercises in histopathology of crustaceans - overall score

7 responses



Day 4 and 5 Practical exercises in histopathology of crustaceans - general comment

7 responses

The dissection was very difficult to observe because Tobia was working on the opposite side to which most people was observing. However, he did make an effort to bring the specimen to everyone and point out the organ/tissue he was speaking about. The session felt quite rushed, especially due to many people not being too familiar with crustaceans in comparison to fish.

I learned about crustaceans that I will never forget. Thanks a lot.

I understood the way of dissecting crustaceans, and it was very necessary for me because of my job. We will apply this knowledge right now because we have a contract with whiteleg shrimp and we also expect to get more crustacean projects so all about this area is very useful for our department. I also want to thank you everyone in the course, teachers and organisation.

Missed some of this therefore scoring based on material provided

Clear and useful practical exercises. Well organized lectures before practical part.

Very well presented and organised - However, practical was a little bit rushed. A lot of content covered in limited time.

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Closing remarks

The EURL training course 2022 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 evaluate the courses with the highest mark.

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. Jesper Valbak 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 and Dr. Raoul Kuiper Norwegian Veterinary Institute, Oslo, Norway, Dr. Tobia Pretto IZSVE IT are deeply acknowledged for their very enthusiastic and excellent lectures. Furthermore, NVI and CEFAS are acknowledged for allowing access to their respective databases of scanned histology slides of cases from fish and crustaceans.

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, Tuesday, 06 December 2022

Niccoló Vendramin, Morten Schiøtt, Tine Moesgaard Iburg, Argelia Cuenca, Lone Madsen and Britt Bang Jensen.

EURL for Fish and Crustacean Diseases