Deliverable 23.1
Analysis of access provided by DTU-AQUA Infrastructure: types and users

Version 2

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Deliverable 23.1
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Executive Summary

Objectives

DTU-AAH main objective was to provide access to the high containment research infrastructures to study infectious diseases in fish, including listed diseases. This includes not only infection dynamic studies but also preventive measures and other studies with a focus on host-pathogen interaction and implementation of biosecurity measures.

DTU-DSC objectives were to provide both infrastructure with a modulable, newly built state-of-the-art hatchery, as well as expertise from academics and technicians on low trophic species aquaculture in various phases of production: from hatchery to grow out.

Main Results:

DTU-AAH had 7 TNAs during the project period. Training has been provided to 5 PhDs (internal and external), 3 researchers and 2 lab technicians (internal). For users, we have provided the opportunity to work with fish pathogens that are not possible to be studied in other fish facilities or with well-standardized disease models. For the installation, we use the TNAs to strengthen collaboration and training of internal PhD /lab techs associated with helping the TNA user.

DTU-DSC managed only one access out of the three proposed. One was rejected due to the training aspect and the other was too slow in the process, thus upon acceptance, the trainee could not attend it anymore. Access provided a stronger collaboration network. New experiments were conducted that would not have been possible without AE3.0 support.

Authors/Teams involved:

Argelia Cuenca, Jacob Schmidt, Niccolò Vendramin and Camille Saurel, all from DTU aqua.

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1. Overview of TNA users projects realized in DTU

1.1.1. Installations

DTU-AAH is located in Lyngby, Copenhagen, Denmark. Besides fully equipped laboratory facilities, it includes:
1. A contained experimental unit capable of conducting infection and challenge trials with all known fish pathogens and bioengineered organisms under both fresh- and saltwater conditions
2. A quarantine unit for purchased fish, vaccination trials and trials with non-infectious reagents
3. A specialized facility for housing crustaceans and
4. A closed facility for supply of experimental specific pathogen free (SPF) rainbow trout.

The facilities can operate at various temperatures and salinities. This guarantees that well-controlled laboratory and tank trials can be conducted with a wide range of fish pathogens on most freshwater fish species and some saltwater fish species. The highly contained facilities enable us to conduct experiments with exotic and highly infectious pathogens, as well as GMO. The Unit for Fish and Shellfish Diseases is accredited according to ISO 17025. The service team has in-depth experience in the handling, management and care of fish at all stages. The laboratory is the European Union Reference Laboratory for Fish and Crustacean Diseases and the OIE Reference Laboratory for VHS and is leading within research and diagnostics on listed viral fish diseases.
Learn more about the facilities in this video: https://www.youtube.com/watch?v=9f4r8G_Cwhg

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DTU-DSC offers 3800 m^2 of building and lands, and off coast long-line culture units dedicated to low trophic aquaculture (LTA) including shellfish, crustacean, microalgae and macroalgae production.

The inland infrastructure consists of standard equipped laboratories and stand-alone hatchery units for research on shellfish and macroalgae, from larvae/spore to adult stage, as well as producing microalgae for shellfish production.

By mid-2021 our infrastructure was upgraded including new 750m^2 hatchery and 400m^2 nursery buildings. Current laboratories (275 m^2) include microscopes, autoclave, water quality instruments. The new hatchery laboratory (50 m^2) includes autoclave, laminar flow workstation, climate chambers, flow cytometer.

There are 4 units:
1) Bivalve hatchery + micronursery
Comprise a quarantine area. The unit has both recirculation and flow through systems. All rooms are supplied with min 1 µm filtered UV treated seawater, controlled water and room temperature.

- Quarantine (50 m^2) with wastewater treatment UV + 0.2µm filtration
- Flat oyster hatchery room (187 m^2) will include 12 x 100L and 12 x 30L broodstock tanks, 10 x 30L + 6 x 60L + 6 x 120L larval tanks, 20 x 50L settlement and 28 x 50L micronursery tanks as well as modular setups.
- Experimental room: X-ROOM (86 m^2) with modular tanks adapted to all kinds of bivalve experimentation
- Microalgae production room (82 m^2) constant temperature 20ºC

2) Macroalgae hatchery - 53 m^2 climate room at 10ºC and 30 m^2 non climate room + access to experimental X-ROOM

- Both batch, recirculation, and flow-through systems are available at various experimental and production scales.
- The hatchery is supplied with 0.2 - 1 µm filtered UV treated seawater with the option of different water temperatures.
- The infrastructure consists of a range of fixed and flexible setups at various scales to cover activities of both research and production

3) Nursery units: 60 µm or 1µm filtered seawater in 10 x 50L tanks x 6 raceways + new 400m^2 building with filtered seawater at 60 µm or 1 µm. Tank configuration as required for experiments.

4) grow-out unit
Two longline farms of 5 and 15ha. The large unit can hold 16 x 3 x 100m longlines. Spat collectors or socked mussels can be hung at different density, as well as grow-out system for flat oysters (e.g. lanterns, trays, cages) and seaweed.

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A small platform in the DSC harbor is also available for easy access grow out experiments.

1.1.2. User projects

Min. quantity of access units to be provided according the DoA: AAH: 180
Total number of access units (sum of access units in the table): AAH: 130

Installation number	Installation code	Project title	Project acronym	Description about the experiment	Coordinator	Already used installation (Yes/No)	Nature of the access unit*	Number of used access units during the project	(Potential) paper	How many people was trained by this procedure ?	User name, affiliation and allocated units
31	DTU-AQUA AAH	Characterization and transfer of RSD-MLO in Atlantic salmon	RSDslm	The experiment aimed at elucidating the causative agent of «red skin disease» (RSD) affecting wild Atlantic salmon. This entailed injecting homogenate of RSD-affected skin into naive atlantic salmon and monitoring for pathology.	David Persson/Jacob Schmidt	Yes	Tank.week	25	No	0	Hampus Hällbom. Swedish University of Agricultural Sciences, SLU. Sweden. 25 units
31	DTU-Aqua AHH	Inhibiting BAFF and APRIL signaling to treat red mark syndrome (RMS) in rainbow trout	RMScontrol	This TNA attempted to use injection of plasmids coding for decoy receptors to disrupt B cell signalling in red mark syndrome (RMS)-affected rainbow trout and thus hopefully reduce RMS pathology.	Carolina Tafalla/Samuel Vicente-Gil/Jacob Schmidt	Yes	Tank.week	35	yes	1	Samuel Vicente Gill Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria: Madrid, Spain 35 units
31	DTU-Aqua AAH	Combined used of camera technology	CeDNA	This work aimed at using video monitoring and	Massimo Orioles/Petr	Yes	Tank.week	30	Yes, potential	1	Petr Cisar

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		and eDNA for early stage detection and prediction of RMS in rainbow trout		eDNA for early detection of RMS in rainbow trout.	Cisar/Jacob Schmidt						University of South Bohemia. Czechia 30 units
31	DTU Aqua AAH	Temperature influence on IHN pathogenesis in rainbow trout	IHNTempo	IHNV virulence experiments were conducted with three different isolates (cold, intermedia and warm water) in rainbow trout at 2 different temperatures	Nicclo Vendramin	yes	Tank week	40	Yes, in review	1	Andrea Marsella Instituto Zooprofilattico Sperimentale delle Venezie. Italy 40 units
32	DTU-DSC	Flat and Pacific Oysters in a Warmer Sea	PID26356 - FLOWS	Compare the heat tolerance of flat oysters from different European populations and Pacific oysters (Magallana gigas) to determine which species can better withstand predicted marine heatwaves in Scandinavian waters.	C. Saurel	yes	70	70	Yes, but still working on results.	1	Chloé Robert University of Gothenburg Sweden 70 units

** Access units describe how accesses are calculated, typically 1 day x 1 pot, 1 season x 1 microplot, etc …*

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2. TNA projects

2.1.1. TNA projects description

2.1.1.1. CeDNA

With increasing computer power, camera technology coupled to image processing techniques are becoming an important tool in many aspects of society, not least in aquaculture. In this project we coupled imaging with eDNA monitoring for early warning of the skin disease red mark syndrome. The TNA provided a very fruitful collaboration between three institutions each with expertise in eDNA, imaging and the disease in question. The results of the TNA was that eDNA was not suitable for early detection of this disease, while video monitoring was able to detect very early symptoms of the disease. This automated detection was validated by blind annotation of video snippets and still images by an expert on red mark syndrome. The results were so promising that we are now working on getting the video surveillance of this disease implemented on one or two test farms with a known history of red mark syndrome. This early warning could be a beneficial tool for rainbow trout farmers. In addition, this mode of action can potentially also be applied to other fish diseases with any external signs of a specific disease - pathological or behavioural. Finally, the TNA has led to other cross-disciplinary collaboration between the partners. In conclusion, a very successful collaboration. We expect the work to result in a publication. One person trained in the user group.

2.1.1.2. RMScontrol

The TNA tested the effect of manipulation of B cell-specific cytokines during red mark syndrome (RMS) development in rainbow trout. RMS is characterized by severe histiolymphocytic infiltration into the dermis with B lymphocytes dominating. We attempted to manipulate the development of pathology through injection of plasmids coding for important B cells cytokine receptors. The hypothesis was that when incorporated into cells and transcribed, the cytokine receptors would act as decoy receptors for the cytokines. Unfortunately, we did not observe an effect of the manipulation. However, the collaboration with the TNA user resulted in more detailed knowledge about the B cell response during red mark syndrome development in general. The TNA will not result in a publication directly, but collaboration deriving from the user visit has resulted in the recent submission of a manuscript for publication. One PhD trained in the user group.

2.1.1.3. RSDslm

The TNA boldly aimed at identifying the causative agent of a disease that in recent years have affecting wild Atlantic salmon. The disease is simply called “red skin disease” or RSD for short. Since all previous attempts to detect an obvious cognate pathogen from salmon with RSD had failed, we instead decided to attempt to infect naïve salmon with material from wild-caught salmon with RSD. We knew from the onset that it would be a difficult task and all stars would have to align. Unfortunately, we already suffered difficulties in the initial steps, as we were struggling to obtain salmon with pathology, as there was very low levels of returning spawners in general across Northern Europe in 2024, and there only few salmon reported with RSD pathology. We were only able to obtain samples from salmon with doubtful pathology. We injected this into naïve salmon and waited for any disease signs, but no pathology was detected. No publication will come from this TNA, but we will continue to pursue the cause of RSD together with the TNA user and other collaborators.

2.1.1.4 IHNTempo

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The aim of the proposal is to evaluate the development and progression of the Infectious Hematopoietic Necrosis (IHN) in rainbow trout at two different water temperature. IHN is a viral disease affecting mainly salmonids and is widely considered a coldwater disease. However, field evidences and few studies suggest a wider temperature range. Rainbow trout (Oncorhynchus mykiss) fingerlings reared at two different water temperatures were bath challenged with selected IHNV strains and monitored for 60 days post challenge for clinical signs or reduced survival related to IHN. Virus re-isolation on cell culture and molecular methods have allowed to describe with high resolution viral load associated with reduced survival in experimentally infected fish. As well it has been possible to document that some isolates are limited by water temperature in causing diseases whereas other are adapted to a broader temperature range. The study is submitted to Aquaculture for publication. One person trained in the user group.

2.1.1.5 FLOWS: Flat and Pacific Oysters in a Warmer Sea.

Flat oysters (Ostrea edulis) are bivalves of considerable economic and cultural importance in Europe. Unfortunately, populations have been severely depleted over the past century. Furthermore, projected global changes pose a threat to the well-being of the remaining individuals. For restoration projects, many countries have translocated oysters, mainly from Northern Europe to new locations, while recent publications have highlighted the genetic difference between the European population and biosecurity issues. It has been hypothesised that oysters could be translocated from the Mediterranean Sea to higher latitudes, such as the North Sea, in Skagerrak and Kattegat to replenish the stocks with heat-tolerant bivalves. However, it is currently unknown whether Mediterranean flat oysters can survive the heatwave levels experienced in these water bodies. This project aimed to address this question by comparing the performance of flat oysters originating from different European populations when exposed to predicted marine heatwaves due to global warming in Scandinavian environment. Our objective was to determine whether genetically distinct populations have an increased tolerance to extreme heat events. Additionally, we aimed to compare the performance of flat oysters with that of Pacific oysters (*Magallana gigas*), to determine which species is more likely to withstand extreme heat events. Anticipating the behaviour of invasive species like Pacific oysters is crucial as the seas change. M. gigas originate from the Pacific Ocean and may be better suited to warmer environmental conditions than flat oysters, potentially leading to range expansions and increased reproductive success. One PhD Student trained with access to DTU-DSC facility. Student organised experimental design and setup the experiment with DSC team. Student conducted part of the experiment and DSC team did the maintenance, building of the setup and conducted part of the experiment when student was not present

2.1.2. Selection of One exemplary project

Climate change appears to impact aquatic viruses abundance and distribution. In particular, elevated water temperature appears to favour some viral species by increasing viral production rate and infectivity, as well as contact rate between the pathogen and hosts. Under this perspective, Infectious Haematopoietic Necrosis (IHN) is an interesting example. The disease is listed both as category C disease in the EU by Regulation (EU) 2016/429, for which control measures are needed to prevent its spreading to parts of the Union which are officially disease free, and as a WOAH notifiable disease. National Reference Laboratories in Europe have reported an increase of IHN incidence in recent years, as well as the spread of the infection to previously disease-free northern European countries (https://www.eurl-fish-crustacean.eu/fish/survey-and-diagnosis).

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Our study has provided documentation for a series of important aspects related to IHNV infection in Rainbow trout.
Within Genogroup-E which includes strains of IHNV circulating in Europe; some isolates have restricted temperature range both in vitro when it comes to replication capacity and in vivo with regards to pathogenicity, others have a broader temperature range.
Interestingly, in vitro replication capacity at higher temperatures correlates with in vivo pathogenicity.
In vivo, there is a “lethal” associated dose of virus, which is independent from strain or temperature.
Our study suggests that putative molecular markers for temperature adaptation may also reside in the L gene (which encodes for the polymerase).
Finally we provide practical and relevant documentation for tissue tropism in terms of sampling for surveillance of apparently healthy population, addressing whether brain shall be sampled or not in this circumstance.

3. Reflection on results of the TNA programme

We have very positive experiences with the TNA programme. It gives very nice opportunities for collaboration across borders and subject areas.
We have experienced that one the most important things to be aware of for a successful TNA is very clear communication about the responsibility for the different tasks already in the planning phase of the TNA.
A related take-home message is that we as partners always use a lot more hours on the TNAs than initially anticipated, this accounts for the persons involved in the training and direct collaboration in the TNAs, but also installation managers.

Due to the nature of the TNA installation on low trophic aquaculture (LTA), carried on in the DTU AQUA_DSC installation, it unfortunately did not attract many applicants, although we did advertise it. The nature of training was rejected during one application where applicant wanted to learn about the facility and protocols used rather than doing complicated publishable experiments. This element could be added in the future as an important training tool, such as the TTM was. It was difficult to get LTA reviewers for the application and the process was too long for PhD students who are limited in time and might not be able to include experiments in their thesis.

4. References

Marsella A, A. Buratin, F. Pascoli, M. Abbadi, M. Toson, A. Cuenca, A. Toffan, N. Vendramin. 2025. Temperature impact on replication and virulence of European infectious hematopoietic necrosis viruses. Aquaculture 609 (2025) 742786

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