Deliverable 22.1
Analysis of access provided by UL Experimental Platform in Aquaculture: types and users

Version 2

WP 22
Deliverable 22.1
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Executive Summary (half a page max)

Objectives

The EPA of UL located in Nancy is a recent and modern indoor infrastructure dedicated to research on freshwater fish culture with facilities adapted to the different fish developmental stages (eggs, larvae, juveniles and breeders). These facilities are located in isotherm boxes to allow a very precise regulation and management of environmental factors (water temperature, photoperiod, light intensity, dawn and dusk simulation …). In relation to water management, the EPA UL-Nancy is able to rear all freshwater species, from cold water species like salmonids to warm water species like tropical species. The water temperature can vary between 4 and 30°C whatever the season is. This allow to conduct in vivo experiments in a wide range of freshwater fish species with interest for aquaculture.
This infrastructure is mainly used for experiments related to the domestication of new freshwater fish species requiring optimization of the external factors for improving the rearing performances (e.g. larval rearing and growth, fish welfare, reproduction). The sophisticated automatized control of these factors allows the conduction of rigorous experiments with high level of traceability of the experimental features. Once accepted, each visiting user is linked to a local group of researchers depending on the technical and scientific skills (e.g. fish production, larval development, reproductive physiology, molecular biology).

Main Results:
PID: 22222, VID: 38128. Effect of domestication on fish reproduction and welfare.
Number of units: **65**
Persons trained: Pr. Bahram Falahatkar
This brought knowledge on fish stress for the installations. Conversely this brought knowledge on fish transcriptomics for the user.

PID: 30329, VID: 52394. The influence of wintering temperatures on the epigenome & transcriptome of the Eurasian perch oocyte
Number of units: **90**
Persons to be trained: Dr. Mark Wossidlo
This will bring knowledge about studies in oocyte epigenetics for the installations. This will bring knowledge on fish reproduction for the user.

PID: 30424, VID: 52558. Impact of the fish life history (antibiotic therapy) and polyculture on the spread of antibiotic resistant microbial communities in RAS
Number of units: **168**
Persons to be trained: Dr. Carole Rougeot
This will bring knowledge on aquaponics systems for the installations.

Authors/Teams involved:)

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Different researchers and engineers from UL (team DAC, Domestication in Inland Aquaculture, AE.3.0) have been involved: Pr. Pascal Fontaine, Pr. Sylvain Milla, Dr. Bérénice Schaerlinger, M. Yannick Ledoré.

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1. Overview of TNA users projects realized in UL

1.1.1. Installations

This installation is composed of two RAS (Recirculating Aquaculture System) for eggs incubation (each containing 60 racks), two RAS for larval rearing (5 tanks of 700 L each) and 16 individual, autonomous and identical RAS (tanks of 2m3) for juveniles and breeders called ecotrons. A LED lighting allows to simulate dawn and dusk to cope with current research work. This is completed by a specific area (4 RAS of 1700 L each) for fish acclimatization step. These facilities are located in isotherm boxes to allow a very precise regulation and management of environmental factors (water temperature, photoperiod, light intensity, dawn and dusk simulation …). In addition to these facilities dedicated to aquaculture, the EPA contains 3 zebrafish racks containing three autonomous farming batteries with a capacity of 5 000 adults, each rack enclosing 50 aquarium. These aquaculture facilities are also linked to:

- Technical installations (200m2) with climatic engineering equipment (temperature regulating unit, air handling unit, blowers and boilers)
- Live prey rearing room.
- Laboratory dedicated to water physical and chemicals analyses and to experimental sampling.
- Laboratory dedicated to biochemical, histological and molecular biology techniques.
- Computer station for Centralized Technical Management allowing the control of environmental parameters and remote monitoring of functioning to achieve optimal security of animals and proper management of experimental protocols. The equipment controlled by CTM allows a highly accurate regulation of key factors for fish reproduction such as temperature (±0,1°C regulation) and light (photoperiod and light intensity ±1lux). These parameters can be individually controlled all over the 20 rooms of the platform.
- In an adjacent building, an annex contains two rooms, one, thermoregulated, is occupied by five mini hatcheries individually controlled, the other is dedicated to the study of behavior (larvae and juveniles).

Ecotron

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1.1.2. User projects

Min. quantity of access units to be provided according the DoA:216
Total number of access units (sum of access units in the table): 323

Installation number	Installation code	Project title	Project acronym	Description about the experiment	Coordinator	User/date of coming	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 ?
1	UL-EPA	Domestication and fish phenotype	SYNDOM	The aim is to examine the effect of domestication on zebrafish welfare and reproduction	Pr. Bahram Falahatkar	B. Falahatkar (University of Guilan, Iran) 2 visits: June/July 2023 and June/July 2024	No	rack.week	65	yes	1
1	UL-EPA	Wintering influence on perch eggs	EPIWINTER-PERCHEGGS	The aim is to examine the effect of different wintering temperatures on the chromatin accessibility, the DNA methylome, and transcriptome of perch oocytes.	Dr. Mark Wossidlo	M. Wossidlo (University of Vienna, Austria) 1 visit: September 2025	No	tank.week	90	yes	2
1	UL-EPA	Fish polyculture, antibiotic therapy and microbial communities	MICROBIOFISH	The aim is to investigate the effects of polyculture of pike-perch and Siberian sturgeon and of antibiotic treatment on the zootechnical performance and physiological state of the pike-perch and on the microbial communities structure of the RAS	Dr. Carole Rougeot	C. Rougeot (University of Liège, Belgium) 1 visit: September 2025	No	tank.week	168	yes	1

** 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

SYNDOM (completed). This study investigated, for the first time in teleost fish, a multigenerational and multi-trait approach to monitor phenotypic changes during the first generations of domestication, starting with the wild population. The hypothesis was that rapid changes should appear, reflecting the adaptation of populations to the new environment. The multi-generational and multi-trait study of this experiment enabled to highlight the adaptation of zebrafish populations during the domestication process. In response to the new environment imposed by captivity (but compatible with zebrafish requirements), traits linked to growth, reproduction and welfare were modified, more or less rapidly, over the generations. These results give new insights in the domestication effects observed and measured along the domestication process and inform about potential physiological modifications that could interfere with the product quality (higher growth, less stressed fish with potential impact on the flesh quality, modifications of the fish shape with potential effect on the consumer appeal due to a modified body shape) in an aquaculture context. The results highlight the importance of effectively managing the initial stages of the domestication process to optimize aquaculture success and enhance fish diversification.

One or two publications will stem from that project.
One Full professor came twice in the EPA facility during 6 weeks to perform the *in vivo* experiment.

EPIWINTER-PERCHEGGS (running). Temperate fish species well respond to environmental variations (e.g. water temperature or photoperiod), controlling reproduction. Unfortunately, even if RAS technology allows obtaining a high quantity of offspring for many species, the reproduction of several of them remains unpredictable and highly variable. One key question that remains is to understand more accurately the relationships between environmental variations and the control of fish reproduction. In that context, the dynamic and programmed changes to the epigenome, among which modifications of DNA methylation are a key driver of development, may act as an intermediate player to foster successful reproduction. In this TNA, we proposed to examine the effect of different wintering temperatures on the chromatin accessibility, the DNA methylome, and transcriptome of perch oocytes at the single oocyte level.
To this end, the Eurasian perch is a perfect model system because its oogenesis is controlled by environmental variations, especially temperature. This freshwater species can easily be adapted to RAS conditions but with still unstable reproduction success. Finally, perch oocytes spawn with a protective gelatinous ribbon, allowing localizing single oocytes along the antero-posterior axis of the ovary. Such information may give important information to understand potential gametes quality fluctuation within a spawn in relation to their direct microenvironment. In this study, breeders were submitted to three different wintering temperatures. Regular fish samplings are performed during the progression of oogenesis and spawn to compare individual oocytes within the same spawn or between spawns of females submitted to the same wintering temperature or a different temperature. Isolated and contamination-free single perch oocytes subjected to scNMT-seq, a multiomic next-generation sequencing method, which enables the simultaneous analysis of chromatin accessibility, the methylome, and the transcriptome of the same single oocyte. Obtained data will provide new insights to better understand the molecular mechanisms controlling proper oocyte development in response to environmental fluctuation, potentially allowing a prediction of fish reproduction success.

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One publication may stem from that project.
One senior researcher and one young researcher will come 2 times (around one week) in the EPA facility to perform the oocyte sampling.

MICROBIOFISH (running). In recent decades, RAS pikeperch aquaculture with RAS systems has developed in Europe and Central Asia as part of a classic monoculture approach. However, the future of this young industry remains fragile for a number of reasons, including the pikeperch's sensitivity to stress and its sub-optimal use of available resources. The sensitivity of pikeperch often leads farmers to appeal to antibiotic treatments, inducing the development of diseases and Antibiotic Resistant Bacteria (ARB) carrying Antibiotic Resistant Genes (ARGs). One solution could lie in the development of polyculture in RAS based on the complementarity of species; this approach would constitute a biological enrichment of the environment that could improve the pikeperch's welfare and make better use of the volume of water and trophic resources (biofilms). However, the rearing of several species of fish should affect the microbial communities structure and dynamic that play a major role in the functioning of the RAS. It should also be noted that the effects of antibiotic treatment on microbial communities in RAS are not well documented. In this context, this study investigates the effects of polyculture of pikeperch and Siberian sturgeon and of antibiotic treatment on the zootechnical performance and physiological state of the pikeperch and on the microbial communities structure of the RAS. On the latter point, particular attention has been paid to the risks of spreading ARB and ARB in the systems.

One publication may stem from that project.
One senior researcher came once and will return at least once in the EPA facility to perform the oocyte sampling.

2.1.2. Selection of One exemplary project

SYNDOM To ensure the sustainability of aquaculture, it is of major importance to understand and control the routes of fish domestication. Domestication is a continuous process that spans multiple generations, resulting in a population of animals adapting and evolving to live and reproduce under human-controlled conditions, namely “captive conditions”. The process of domestication is associated with a change in the evolutionary trajectory of the target species, which has to acclimatise to a new environment, more or less different from its natural habitat. Numerous changes have been characterised in mammals and birds, and also in teleost fish. Interestingly, some of these phenotypical and physiological changes occur very quickly (one generation is sufficient) while others can be visible only after several generations. However, biological mechanisms underlying this evolution process are still unknown. Studies of teleost fish have been limited to a small number of species and have often focused on a single biological function. In addition, comparisons are regularly made between wild individuals and so-called ‘domesticated’ individuals, without any real knowledge of the number of generations or the domestication pathway. As a result, we currently have no information on the adaptation of the first generations or on the kinetics of the changes that have taken place.

This study investigated, for the first time in teleost fish, a multigenerational and multi-trait approach to monitoring phenotypic changes during the first generations of domestication, starting with the wild population.

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The wild population (F0) was sampled using nets in June 2022 in a radius of 10 km around Bangladesh Agricultural University, the fish were then transported to the EPA. After the acclimatisation phase, ten randomly chosen pairs of fish were mated between January and March 2023 to produce the first generation born in captivity (F1). The offspring of each F0 pair remained unseparated, creating ten replicates (composed of siblings) of F1. For each replicate of F1, one male and one female were randomly mated with one individual of the opposite sex from another replicate in order to create the F2 generation, and then the F3 and F4 generation. For each generation, and at the same age, sampling was performed to make the morpho-anatomical, physiological, behavioural and transcriptomic analysis. Additionally to the first generations of domestication, a domesticated population was used as a more advanced generation in the domestication process, this was called Lab population.

While the process effectively improved fish growth and lessened stress, the study also revealed unexpected results: improved reproductive performances were observed in the populations more advanced in the domestication process (with higher levels of sex hormones, better sperm quality, higher fertilisation rate). A more precocious puberty, decrease of glycemia, change of the fish shape and depigmentation were also observed. Also more than 1000 genes were showed to be significantly differently expressed in the brain between the wild population and the captive generations (F1-F2-F3 and lab) which might explain why the domesticated populations (especially “lab”) was also more aggressive, active and bolder the F0 generation. When comparing with domesticated populations obtained from many decades (notably the “lab” population), the third generation does not display major differences with them.
All together, that TNA revealed major phenotypic and molecular changes along the domestication process, changes that might explain the zootechnical performances observed in the first generations of domestication.

3. Reflection on results of the TNA programme

TNA users provide technical and scientific skills complimentary to those of the host team. This is a major point for the success of TNA programmes. For future access programmes, such complementarity should be analysed before the onset of the TNA programmes.

4. References

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Lead Beneficiary	UL
Authors	Sylvain MILLA, UL, Sylvain.Milla@univ-lorraine.fr
Reviewers	Marc Vandeputte, INRAE, marc.vandeputte@inrae.fr

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