

# Deliverable 8.1

## Analysis of access provided by INRA Aquaculture infrastructure: types and users

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## Executive Summary

### Objectives of the local infrastructure, of each installation of it, and (generally) for offering accesses

The main objective of the four INRAE installations offered is to support cutting-edge research in aquaculture by providing high-quality experimental platforms to external users through Transnational Access (TNA). Each installation targets specific scientific themes:

- **INRAE-PEIMA** focuses on trout life cycle studies, genetics, and aquaponics;
- **INRAE-STPEE** specializes in fish nutrition and feed technology;
- **INRAE-IERP** enables disease and immunology studies in fish with high biosecurity;
- **INRAE-LPGP** supports genomics and physiology, including GMO experiments and genome editing.

These facilities collectively promote collaborative research, innovation, and training in aquaculture science.

### Main Results: number of accesses, persons trained, what did this bring for users and for installations

During the reporting period, 11 TNA projects were hosted, using a total of **1,111 access units**: PEIMA (188), STPEE (521), IERP (6), and LPGP (156). At least **12 users** were trained in fish nutrition, feed formulation, immunology, metabolism, or genome editing, both on-site and virtually. Scientific outcomes include **3 peer-reviewed publications** and more papers in progress. The TNAs led to new models (e.g., non-lethal VHSV infection), improved knowledge (e.g., on plant-based diets, feed additives like rutin), and established long-term collaborations. Installations enhanced their protocols, training capacity, and visibility in the research community.

### Authors/Teams involved: Specify the list of AE3.0 people and other contributors that have worked on this Deliverable (name of authors and organisation)

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## Contents

Executive Summary .....	2
1. Overview of TNA users projects realized in TNA at INRAE .....	4
1.1.1. Installations .....	4
1.1.2. User projects .....	6
2. TNA projects .....	9
2.1.1. TNA projects description .....	9
2.1.2. Selection of One exemplary project .....	12
3. Reflection on results of the TNA programme .....	13
4. References .....	13



## 1. Overview of TNA users projects realized in TNA at INRAE

### 1.1.1. Installations

1. INRA-PEIMA is the reference experimental unit for research on all stages of the life cycle of trout performed within several INRA research departments, mostly in physiology (reproduction, growth, behaviour, adaptation, etc.), genetics and nutrition. It offers a hatchery, 156 fry rearing tanks of 200-400L, 156 tanks of 2 m<sup>3</sup> and 26 tanks of 28 m<sup>3</sup>, a behavioural study room with 20 tanks of 500 litres and 16 video cameras, a semi-industrial recirculating aquaculture systems (RAS) of 10 tanks of 6 m<sup>3</sup> linked with an aquaponic system, and a RAS unit of 3 independent thermoregulated rooms (6°C to 18 °C). This RAS is linked with an aquaponics green house.

INRA-PEIMA also has a world unique collection of trout lines with original characteristics like growth, sex-ratio, fat content, spawning date, adaptation to plant-based diets, disease resistance, including 20 isogenic lines of rainbow trout. They are available for TNA, under a collaboration agreement in the case of isogenic lines.

2. INRA-STPEE consists of 3 complementary platforms dedicated to nutrition of rainbow trout throughout the whole life cycle:

- Platform 1: Donzacq fish farm (17°C constant) has a feed manufacturing plant with a twin-screw extruder and wet labs, large (160 m<sup>3</sup>) and small scale (5000 and 200L) flow-through raceways, 36 50L tanks, 48 1m<sup>2</sup> tanks, 18 2m<sup>2</sup> tanks, the latter with computer controlled feeders.
- Platform 2: Lees Athas fish farm (7°C constant) comprises a hatchery for up to 400 groups of eggs; a UV-treated thermoregulated system for the production of eggs and fry; 84 self-cleaning tanks for juveniles, of special interest for studies on nutrient-genotype interactions, 16 tanks of 200L, 32 tanks of 500L; 6 concrete tanks and 8 raceways of 12 to 20m<sup>3</sup> for studies with broodstock nutrition.
- Platform 3: Specialised facilities, located at St Péé-sur-Nivelle, including:
  - 3.1 Feed intake and feeding rhythms: 2 independent RAS systems each with 12 tanks, equipped with self-feeders to (i) monitor feeding rhythms, (ii) control feed distribution and (iii) evaluate the amount distributed. With fecal collectors, accurate knowledge of feed intake is gathered over long periods. The setup also enables feed choice experiments
  - 3.2 Digestibility: 3 series of 6 cylindro-conical tanks in RAS with automatic faeces collector (Choubert et al. 1982), recognised by EIFAC as the most valid method for in vivo evaluation of apparent digestibility coefficients (ADC) of diets and ingredients, and the estimation of suspended matter loss of dietary origin.

INRA-STPEE can undertake all types of nutrition research experiments, using rainbow trout (freshwater) as the main study-model. It has been actively used in experiments from EU projects from the 5thFP to H2020 (PEPPA, RAFOA, GUTINTEGRITY, FINEFISH, AQUAMAX, PROMICROBE, LIFECYCLE, AQUAEXCEL, ARRAINA, AQUAEXCEL2020). An added strength to the experimental infrastructure is the proximity of nutrition researchers which provides a healthy environment for scientific interaction and exchange



3. INRA-IERP is the INRAE experimental facility dedicated to fish infectiology. Located at Jouy en Josas, close to Paris, it supports a number of long-term research programs on fish pathology, immunology, vaccinology and genetic of the resistance/susceptibility to diseases. EOPS fish (rainbow trout and carp) is produced in the 'clean' area, and are available for infectious challenges. Trout are from controlled genetic origin, either from a standard population (INRAE reference strain) or from isogenic lines with contrasted resistance to a range of pathogens produced at INRA-PEIMA (collaboration agreement). IERP also has access to stable inbred carp families from WU. The fish installation (1000m<sup>2</sup>) consists in rooms for breeding pathogen free fishes with 4 RAS, 344 incubators or 86 aquaria, 18 tanks of 200L. The infectiology part has 104 aquariums in recycled or flow-through water, 14 tanks of 300L in recycling or in lost water. A number of animal genetic origin, age, route of inoculation, water quality, etc... are available.

INRA-IERP produces and supplies specific animals (population, families, and isogenic lines) of rainbow trout and carp with specific sanitary status, and realizes experimental infections using injection or immersion. Pathogenic agents studied include most classical and emerging viruses and bacteria (trout: VHSV, IHNV, ISAV, Alpha virus SDV and SPDV, Flavobacterium psychrophilum; carp: SVCV). Experimental set-up benefits from the expertise of INRA labs Molecular Virology and Immunology (VIM) and Animal Genetics and Integrative Biology (GABI). INRA-IERP also comprises an imaging platform (clearing; biphotonic microscopy ...) in which new protocols are developed, including the usage of fluorescent pathogens to better monitor infections. The facility is part of the EMERG'IN research infrastructure and is also a partner of the VetBioNet infrastructure project.

4. INRA-LPGP indoor experimental facility of Fish Physiology and Genomics is unique in France as it accredited for the rearing of GMO fish for experiments on species of agronomic interest such as trout. It offers unique research capacities including (i) genome editing (ii) germ cell grafting into surrogate larvae (iii) the ability to experiment over the entire life cycle by varying environmental parameters. Our 1,000 m<sup>2</sup> facility has 10 rearing rooms with 20 recirculating water systems and contains more than 100 large tanks (trout, pike, goldfish...). It is adapted to the specific breeding conditions of each fish species (photoperiod and temperature) whatever their stage of development. Our facility also contains a room dedicated to eggs microinjection for production of transgenic or genome edited fish. INRA-LPGP proposes the production of transgenic or genome edited trout (GET service) that includes egg injection, genotyping and fish rearing up to reproductive stage. It also proposes a service for germ stem cell grafting (GCGraft service) into recipient embryos and the rearing of the surrogate fish up to one year.



### 1.1.2. User projects

**Min. quantity of access units to be provided according the DoA: PEIMA: 522 STPEE 647 IERP 32 LPGP 156**

**Total number of access units (sum of access units in the table): PEIMA: 188 STPEE 521 IERP 6 LPGP 156**

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 were trained by this procedure ?
1	INRAE-PEIMA	Preventative care: Co-application of fungal and herbal stimulants in rainbow trout ( <i>Oncorhynchus mykiss</i> ) to promote beneficial immune effects	ImmunoFeed	Incorporating feed additives such as generally recognised as safe (GRAS) fungal and herbal compounds is a promising strategy to improve fish immune health such as <i>T. versicolor</i> and <i>G. lucidum</i> fungi. Besides, capsaicin, the active pungent compound in chili peppers, promotes appetite and nutrient uptake. Both are believed to act on epithelial transcription, subsequently regulating the innate immune system. We propose using the diverse infrastructure access at INRAE-PEIMA to investigate the mechanism of action by which these novel feed additives regulate digestion and the immune response in trout.	Alyssa JOYCE, Univ. Gothenburg, Sweden	yes	tank.day	128	Julia Mougin, Victor Lobanov, Morgane Danion, Roxane Roquigny, Lionel Goardon, Thierry Grard, Thierry Morin, Laurent Labb��, and Alyssa Joyce. Effects of dietary co-exposure to fungal and herbal functional feed additives on immune parameters and microbial intestinal diversity in rainbow trout ( <i>Oncorhynchus mykiss</i> ). Fish and Shellfish Immunology, 137:108773, April 2023. doi: 10.1016/j.fsi.2023.108773.	1
1	INRAE-PEIMA	Innovative environmental enrichment for the health and welfare of farmed trout	EnriFish	The search for preventive and sustainable methods that reconcile pathogen control and fish welfare is becoming a priority for the fish farming industry. Environmental enrichment aims to improve the psychological and physiological needs of a captive animal by making its environment more complex. Although this practice already exists in terrestrial animals, the fish farming industry has not yet adopted it, probably due to a lack of scientific knowledge and specific regulations on fish welfare. In addition, enrichment by adding complex structures to the tank requires maintenance and can create a bacterial reservoir that affects fish health,	Uros LJUBOBRATOVIC, UNI-MATE, Hungary	yes	tank.day	60	O��c��ane Amichaud, Thomas Lafond, Georgina Lea Fazekas, Aude Kleiber, Thierry Kerneis, Axel Batard, Lionel Goardon, Laurent Labb��, Sophie Lambert, Sylvain Milla, and Violaine Colson. Air bubble curtain improves the welfare of captive rainbow trout fry and fingerlings. Aquaculture, 586:740828, March 2024. doi: 0.1016/j.aquaculture.2024.740828.	1



				which would also explain the industry's reluctance to implement these practices. In this project, we will study the effects of an innovative enrichment consisting of introducing a pipe generating a curtain of bubbles into the tank from the first stages of life, since preliminary observations have shown the attraction of fish in areas where bubbles are diffused. We will evaluate the short impact of these bubble curtains on growth, parasitism, fish behaviour and stress response. The focus will be on the observation of aggressive behaviours which we believe can be defined as they can be a source of injury and stress on the immune system. This type of enrichment would make it possible to integrate the notion of "positive welfare" in breeding by encouraging the expression of playful behaviour, while guaranteeing easy maintenance and a good state of health for the fish.					
2	INRA-STPEE	Role of dietary antioxidants selenium and pyridoxine in stress resistance of rainbow trout	SEPYS	Experiment about nutrition – robustness interaction using functional micronutrients known for their antioxidant properties	P Wischhusen, UoS, UK	Yes	tank.week	156 U	1 paper in Aquaculture : Vitamin B6 and selenium supplementation induce contrasting effects in the transsulfuration pathway of juvenile rainbow trout ( <i>Oncorhynchus mykiss</i> ) with interactive effects in stressed fish <a href="https://doi.org/10.1016/j.aquaculture.2024.741354">https://doi.org/10.1016/j.aquaculture.2024.741354</a>
2	INRA-STPEE	Decipher the regulation of nutrient sensing and metabolism of rainbow trout fed from first feeding with total plant based diet.	Ontosensing trout	Experiment to better understand the impact of new aquafeed in nutrient sensing and use at first feeding	A Blanco, Univ Vigo, Spain	Yes	tank.week	140 U	1 paper in Aquaculture: Effects of a plant-based diet from first feeding on the intestinal expression of nutrient sensors in rainbow trout ( <i>Oncorhynchus mykiss</i> ) <a href="https://doi.org/10.1016/j.aquaculture.2024.742093">https://doi.org/10.1016/j.aquaculture.2024.742093</a>
2	INRA-STPEE	Role of CMA in the control of food intake in rainbow trout.	CMAppetit	Experiment about mechanisms of regulation of feed intake linked to chaperone-mediated autophagy (CMA)	A Blanco, Univ Vigo, Spain	Yes	tank.week	90 U	1 paper in progress
2	INRA-STPEE	Rutin application in trout feed	Rutin Trout	Experiment about impact of a flavonoid – rutin – (an anti-inflammatory product) on dietary carbohydrates use	S Rahimnejad, Univ Murcia, Spain	Yes	tank.week	81 U	1 paper in progress



2	INRA-STPEE	To determine the effect of krill meal on rainbow trout under chronic hypoxia	KM against hypoxia	Experiment about nutrition – robustness (resistance to chronic hypoxia) interaction using dietary krill meal rich in antioxidants	K Kaur, Aker Biomarine Antarctic AS, Norway	Yes	tank.week	180 U	Analysis in progress	1
3	INRA-IERP	Deciphering EPIgenetic sexual dimorphisms after INFECTions	EPINFECT	Establish a non-lethal viral infection model in juveniles during the sex differentiation period (from 12 to 18 days post-fertilization). Three experiments have been conducted in Phase 1 only.	RIBAS Laia, Institute of Marine Sciences, Spain	YES	circuit.week	6 AU		1
3	INRA-LPGP	Foxl2 paralogs and their role in sex determination in rainbow trout	FUNCFOX	Generation (F0, mosaic) of CrispR/Cas9 knockout rainbow trout for foxl2b2 alone and foxl2b1 and foxl2b2 together to determine whether both paralogs are needed to fulfil the function of the gene on ovarian development in rainbow trout	Anna Troedsson-Wargelius, IMR, Norway	no	tank.week	156	Analysis in progress	2

\* 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

**ImmunoFeed:** The project aimed at testing the inclusion of immunostimulants from fungal and herbal compounds as an alternative to antibiotics in aquaculture. Humoral, cellular and molecular immunity was analyzed in a study on rainbow trout, along with intestinal diversity after treatments. Uptake of herbal and fungal compounds influenced the expression of immune related genes, without generating an inflammatory response. Significant differences were detected in the spleen-tlr2 gene expression. Supplementation with herbal additives correlated with structural changes in the fish intestinal microbiota and enhanced overall intestinal microbial diversity. Results demonstrated that the different treatments had no adverse effect on growth performance and survival, suggesting the safety of the different feed additives at the tested concentrations. Results from this experiment were published in 2023 in Fish and Shellfish Immunology (<https://doi.org/10.1016/j.fsi.2023.108773>). The applicant participated in all aspects of the study in conjunction with the staff from INRAE PEIMA, including experimental design, fish rearing and monitoring, as well as data analysis.

**EnriFish:** In this project, the participant studied the effects of an innovative and easy-to-maintain environmental enrichment consisting of introducing a pierced pipe connected to an air pump into the tank at the early stages, thus generating a bubble curtain. Two experiments were conducted in parallel, one short-term (7 weeks), and one long-term (21 weeks). It was found that bubble diffusion decreased aggressive and abnormal behaviors during diffusions in both the short-term and the long-term experiments. Bubbles were found to be attractive for young fish subjected to a motivation test. When subjected to the emotional reactivity test, Bubble fish seemed less fearful, exhibiting a lower maximum velocity than Control fish in the long-term experiment only. Growth parameters and fin erosion index did not differ between treatments. Altogether these results suggest that repeated bubble diffusion can act as environmental enrichment for fish, combining physical, occupational and sensory enrichment. Results from this experiment were published in Aquaculture (<https://doi.org/10.1016/j.aquaculture.2024.740828>). The applicant participated in all aspects of the study in conjunction with the staff from INRAE PEIMA, including experimental design, preparation of the equipment, fish rearing and monitoring, as well as video and data analysis.

#### **SEPYs: Role of dietary antioxidants selenium and pyridoxine in stress resistance of rainbow trout**

The data collected in this project will generate new knowledge on the role of dietary antioxidants selenium and pyridoxine in the stress metabolism of fish. This can support the ongoing transition from traditional to more sustainable feed ingredients for aquafeed formulations. The health and welfare of fish can be improved, especially in response to environmental/anthropogenic stress. The results should help the assess the effect of a co-supplementation of different feed supplements and identify possible interactive effects between selenium and pyridoxine in the metabolism of fish. Dr Pauline Wischhusen, a post-doc at the university of Stirling, UK, was trained on site about fish nutrition and feed formulation. Publication: Vitamin B6 and selenium supplementation induce contrasting effects in the transsulfuration pathway of juvenile rainbow trout (*Oncorhynchus mykiss*) with interactive effects in stressed fish

<https://doi.org/10.1016/j.aquaculture.2024.741354>

#### **Ontosensingtrout: Decipher the regulation of nutrient sensing and metabolism of rainbow trout fed from first feeding with total plant based diet.**



The data obtained during this study will generate new knowledge in the regulation of nutrient sensing in fish farmed from first feeding, an essential step in establishing feeding behavior that can influence the entire life cycle of fish. Besides improving the knowledge on the mechanisms regulating feeding efficiency in fish at early stage, knowing how different nutrients impact on gut nutrient sensing mechanisms is of great interest for aquaculture sector as it could help identify specific nutrient that could be added to fish feeds in order to improve feed efficiency. Also, the data and knowledge obtained will enable studies in other fish species to have improved designs to better characterize their dietary needs, at molecular levels, related to new feed formulations, to ensure a normal and healthy growth of these species from the very first meal. The results will be of interest for feed formulations related considerations in research and industry. The joint expertise of the two partners involved on the investigated topic also opens possibilities for further research collaborations. Pr Soengas (professor at the university of Vigo, Spain) and Dr Ayalen Blanco (a post-doc at the university of Vigo, Spain) were trained on site and virtually (Pr Soengas) about fish nutrition and feed formulation.

Publication: Effects of a plant-based diet from first feeding on the intestinal expression of nutrient sensors in rainbow trout (*Oncorhynchus mykiss*)

<https://doi.org/10.1016/j.aquaculture.2024.742093>

### **CMAppetit : Role of CMA in the control of food intake in rainbow trout.**

This study aimed to characterize the putative effects of rapeseed and palm oil on the mRNA abundance of several lipid sensors and appetite-regulatory hormones in the GIT of rainbow trout (*Oncorhynchus mykiss*). For this, fish were fed ad libitum with a diet containing high levels of either fish oil (control), rapeseed oil or palm oil, and samples of proximal and distal intestine, as well as plasma, were collected at 5 days, 3 weeks and 12 weeks for analysis (Nov 23). Main Results : • Ad libitum feeding with rapeseed oil- and palm oil-enriched diets required a significantly higher amount of feed to reach fish satiety, which indirectly suggests increased feed intake levels compared to fish fed a high fish oil diet, especially during the last weeks of the trial. • Fish fed with both diets rich in a vegetable lipid source showed significantly higher plasma triglyceride and fatty acid levels compared to control (fish oil) fish. • Changes in the mRNA abundance of gut lipid sensors in response to rapeseed and palm oil were more notorious in the distal intestine, where increased levels of mRNAs encoding the fatty acid transporter Cd36, free fatty acid receptor 1, members of the Ffar2 family, and the G-protein coupled receptor 119 were observed compared to fish fed a high fish oil diet. • Interestingly, rapeseed oil- or palm oil-evoked changes in the mRNA abundance of gastrointestinal appetite-regulatory hormones (colecistokinin, peptide tyrosine tyrosine, and glucagon- like peptide 1) showed an increase during the first weeks of trial (suggesting anorexigenic potential) but a decrease during the last weeks (suggesting orexigenic potential). In conclusion, results from this research offer a view of physiological appetite-regulatory mechanisms activated upon feeding vegetable diets, which can serve for better and more efficient aquafeed formulations. Dr Ayalen Blanco, a post-doc at the university of Vigo, Spain, was trained on site about fish metabolism.

Analysis is in progress.

### **Rutin Trout : Rutin application in trout feed**

The objective was to improve the utilization of dietary carbohydrates in trout by adding rutin as a feed additive (at 2 concentrations). The 12-week feeding trial was successfully completed, followed by the final sampling (June 24). Liver and intestines are currently being analyzed. Promisingly, initial data showed a hypoglycemic effect of rutin in carbohydrate-fed fish. Dr Samad Rahimnejad, a post-doc at the university of Murcia, Spain, was trained on site about fish nutrition and feed formulation.

Analysis is in progress..



### KM against hypoxia : Krill meal against chronic hypoxia

The feeding trial was successfully conducted, testing three krill meal levels (5, 7.5, and 10%) in rainbow trout juveniles (37g, 60 fish per tank) for 12 weeks, followed by a 3-week hypoxia challenge ( $O_2$ : 9.2 to 5.9 mg/L). Growth progressed as expected, and all planned samples were collected (March 25). Upcoming analyses will assess the potential of krill meal to mitigate hypoxia-induced effects on fish health. Dr Aker Kaur, from the company Biomarine Antarctic AS, Norway, was trained virtually about fish metabolism.

Analysis is in progress.

**EPINFECT** aimed to test the hypothesis that, in zebrafish, the epigenetic memory underlying disease susceptibility is sex-dependent. The overarching objective of this project was to shed light on the DNA methylation mechanisms found in the gonads in response to repetitive activation of the immune system. This project has to be carried out in different phases with go/no-go decisions at each stage:

Phase 1: Establish a non-lethal viral infection model in juveniles during the sex differentiation period (from 12 to 18 days post-fertilization).

Phase 2: Use the established model to investigate the impact of viral infection on gonadal differentiation.

In Phase 1, we compared Spring viremia of carp virus (SVCV) and Viral hemorrhagic septicemia virus (VHSV) models of infection, injected at different doses and incubated at different temperatures based on IERP expertise (Souto et al. 2024). To establish non-lethal viral infection model, IERP proposed to shift the infected fish at the beginning of the infectious process to non-permissive temperature for viral propagation as previously reported by Ludwig et al (PMID: 21304884). Whatever the dose of SVCV inoculum used in the assay, fish died of viral infection even after a shift to suboptimal temperature of viral replication. Because of the too high virulence of SVCV, we changed the model to VHSV virus. In this case, we similarly apply temperature shift few hours post infection and succeeded to establish the non-lethal viral infection model needed to continue the project. Due to a technical failure of the temperature regulation system, the next phases were not done at the time of writing of this deliverable, but will be implemented before the end of AQUAEXCEL 3.0. None of the TNA users has been trained hands on during this 1st phase of the project, however they were involved in experimental design, interpretation of the results taking into account the constraints of the model ( $T^\circ$ ) and discussion on the planning and design of the next experiments. This was done online with S. Biacchesi, virologist at VIM Unit, collaborator with IERP and provider of the viral strains. At this step, the results are preliminary and could not be published.

### FuncFox: Foxl2 paralogs and their role in sex determination in rainbow trout

This project explored to what extend salmonid-specific whole genome duplication (Ss4R) paralogs can be redundant, and if they can eventually backup themselves. This has been carried out within this project using a CrispR/Cas9 targeted gene inactivation approach in rainbow trout using the foxl2 gene as a case-study. This foxl2 gene is a well-known conserved gene involved in ovarian development in vertebrates and this gene is present in rainbow trout as two foxl2 Ss4R paralogs stemming from the whole genome duplication of salmonids (Ss4R). More specifically this project worked toward the generation of single (one foxl2 paralog) and double (the two foxl2 paralogs) knockout rainbow trout lines that will be explored to understand what are the respective roles of each paralogous copy and if their double dosage is still required to fulfill their function.

First generation (F0, mosaic) CrispR/Cas9 knockout rainbow trout individuals have been generated for foxl2b2 alone and foxl2b1 and foxl2b2 together, but not for foxl2b1 alone. This result will be now followed by a collaboration with the Norwegian collaborators in order to explore whether these foxl2 paralogs are still both physiologically important. This will be a very important knowledge that goes far beyond the simple analysis of these rainbow foxl2 paralogs



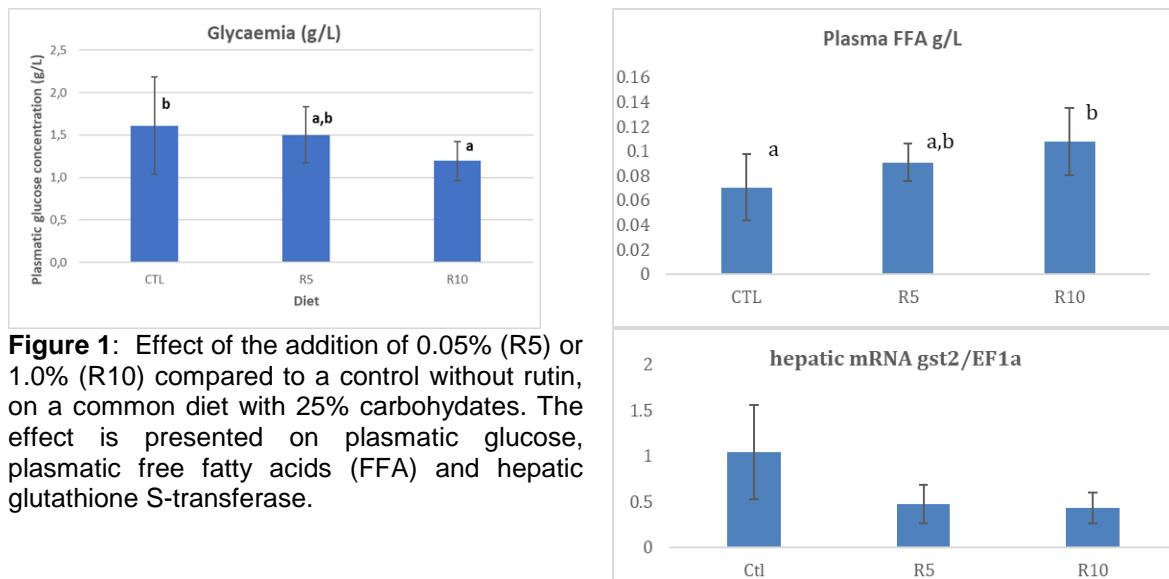
as many salmonid paralogs have similar Ss4R duplicate retention and contrasted expression profiles. We then expect a high value and significance of the results of this research that has never been attempted yet in a salmonid species and even in a fish species with a recent WGD (the analysis of double KO paralogous copies stemming for the teleost specific duplication have been obtained but the context here is completely different as these salmonid duplicated paralogs are recent paralogs not yet completely rediploidized and with similar expression pattern).

Dr Anna Troedsson-Wargelius and Rolf Brudvik Edvardsen from the Institute of Marine Research (Norway) visited to the LPGP fish facility and collected samples from KO trout. They were also trained to the microinjection and genotyping.

### 2.1.2. Selection of One exemplary project

#### Rutin Trout: Rutin application in trout feed

A specialist in feed additives contacted us about an experiment with trout fed on carbohydrates. Rainbow trout fed with high levels of carbohydrates have some difficulties in utilising them due to metabolic disorders. Dr Samad Rahimnejad (University of Murcia, Spain) suggested that we test a feed additive - a flavonoid - rutin, known for its antioxidant and anti-inflammatory properties. 3 diets with 25% carbohydrates were prepared in our feed production system to incorporate 3 doses of rutin: 0% (control) (R0), 0.05% (R5) and 0.10% (R10). The fish were fed for 9 weeks and then sampled with Samad. The first results showed a clear effect of dietary rutin on post-feeding glycaemia: the lower glycaemia (as expected in our hypothesis) (see figure below) was also associated with an increase in plasma free fatty acids (see figure below). In the liver, there was no change in gene expression for intermediary metabolism, but a significant decrease in gene expression for enzymes involved in antioxidant metabolism (such as glutathione S-transferase) (see figure below), suggesting an antioxidant effect of dietary rutin. These preliminary data are very promising for validating the flavonoid as a feed additive to increase the utilisation of dietary carbohydrates in carnivorous fish, which remains a limiting point to formulate more sustainable diets.



**Figure 1:** Effect of the addition of 0.05% (R5) or 1.0% (R10) compared to a control without rutin, on a common diet with 25% carbohydrates. The effect is presented on plasmatic glucose, plasmatic free fatty acids (FFA) and hepatic glutathione S-transferase.



### 3. Reflection on results of the TNA programme

TNA visits were successful and yielded positive outcomes for both the PEIMA installation and the visitors. We regret that not all accesses were used for the structure (few in fact), which may be a sign that visibility and communication need to be improved for future access programs. Still, many TNA options were discussed with potential partners but did not bear fruition. An additional difficulty was that in October 2023, all fish in PEIMA had to be euthanized as a sanitary measure following infection by lactococciosis, and it took some time to resume the experimental capacities to a suitable level:

to reduce the experimental capacities to a suitable level. For INRA STPEE, the 5 TNAs were successful. There were no problems with any applicants. All the topics were really related to the capacities of the installations offered. All the steps (application, experimentation, user feedback, etc...) were done correctly. We already have 2 scientific publications (in the journal Aquaculture) from the first 2 TNAs. There is no doubt that at least 3 more papers will be produced with the last 3 TNAs. So the implementation was fully positive.

At INRA-IERP, the EPINFECT project led to the establishment of non-lethal infection model of VHSV in juvenile zebrafish, which was not available at IERP at the beginning of the project. This provides an opportunity to continue the project with Dr Laia Ribas' team and to strengthen collaboration with Dr Stéphane Biacchesi, provider of the viruses. Another TNA project was planned but could not be implemented due to an unexpected closure (technical failure of water temperature control system) of the installation in summer 2024. We did not manage to resume its implementation in 2025 as this was a long project (24 units of access) and the system only went back to normal operational capacity in May 2025

## 4. References

Julia Mougin, Victor Lobanov, Morgane Danion, Roxane Roquigny, Lionel Goardon, Thierry Grard, Thierry Morin, Laurent Labb  , and Alyssa Joyce. Effects of dietary co-exposure to fungal and herbal functional feed additives on immune parameters and microbial intestinal diversity in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology*, 137:108773, April 2023. doi: 10.1016/j.fsi.2023.108773.

Océane Amichaud, Thomas Lafond, Georgina Lea Fazekas, Aude Kleiber, Thierry Kerneis, Axel Batard, Lionel Goardon, Laurent Labb  , Sophie Lambert, Sylvain Milla, and Violaine Colson. Air bubble curtain improves the welfare of captive rainbow trout fry and fingerlings. *Aquaculture*, 586:740828, March 2024. doi: 0.1016/j.aquaculture.2024.740828.



## Document Information

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Deliverable	N°	D8.1	Title	Analysis of access provided by INRA Aquaculture infrastructure: types and users
Work Package	N°	8	Title	X
Work Package Leader				
Work Participants				

Lead Beneficiary	INRAE (Partner 1)
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