Deliverable 11.1
Analysis of access provided by CSIC Research facilities: types and users

Version 1

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Deliverable 11.1
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Executive Summary

Objectives

For the research facilities involved (i.e., CSIC-IATS-EXP, CSIC-IATS-ANA, CSIC-IEO-ICAR-MAP and CSIC-IEO-AquaCOV), to provide access for aquaculture research on topics like fish nutrition, pathology, immunology, reproduction, and microbiome studies.

Main Results:

Access units have been fully used. Twenty-four TNA projects have been approved and to the date of presentation of this deliverable 18 of them have been carried out. Each project has typically trained 1-2 visiting researchers, with hands-on access to experimental setups, analytical techniques, and data analysis. Some TNA projects have already produced Open Access publications and for most of them manuscript and other presentations are being prepared. Request of applicants have strengthened the technical capabilities and methodologies at the hosting institutions. New protocols (such as clam reproduction techniques or FMT in gilthead sea bream) have been developed. Skills have been transferred to visitors in experimental design, data analysis, molecular biology, microbiome analysis, and fish physiology.

Authors/Teams involved:

By CSIC-IATS-EXP and CSIC-IATS-ANA infrastructures, personnel coordinating the activities involved in TNAs have been Jaume Pérez-Sánchez, Óscar Monroig, Carlos Saavedra, Carla Piazzon, Ana Gómez, Oswaldo Palenzuela and Federico Moroni.

By CSIC-IEO-ICAR-MAP, the persons involved in the TNAs have been Aurelio Ortega, Fernando de la Gándara, Amanda Miras, Maria José Arenas, Pedro Mendoza, Pilar Baixauli and Pedro José Mayol

By CSIC-IEO-AquaCOV, the persons involved in the TNA have been Montserrat Pérez, Fiz da Costa, María José Justo, Xoana Blanco and Aitor Sotelo.

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1. Overview of TNA users projects realized in CSIC

1.1.1. Installations

CSIC-IATS-EXP (Instituto de Acuicultura Torre de la Sal / experimental tanks): The IATS-EXP infrastructure provides access to experimental tanks designed for a variety of aquaculture research projects that cover disciplines such as marine fish parasites, fish pathology, fish immunology, nutrigenomics, genomics, biochemistry, and cellular and molecular biology, to study and control fish reproduction, food intake and growth, and Artemia and fish larviculture. This installation includes research holding tanks located in different units at IATS, with a total surface of 2,100 m^2. Approximately 250 tanks, with different shapes and capacities (from 3,000 L to 30 L), along with associated wet labs and sampling rooms, are available. These installations are suitable for conducting experiments in most of the disciplines involved in aquaculture research: health and welfare (parasite and bacterial challenges), physiology and energy metabolism (hypoxia priming and pre-conditioning, swimming exercise tests), reproduction, nutrition and growth, live prey, and larval rearing. Water quality (salinity, temperature, filtration, etc.) and light conditions (photoperiod, intensity, etc.) vary depending on the type of project and specific tanks in use. The open sea flow provides 90,000 m^3/h, and water temperature ranges naturally from 11 to 28 ºC. Tanks with recirculation and heating/cooling systems are available in some units. Biosensor technology, based on the use of the AEFishBIT datalogger developed in AQUAEXCEL^{2020}, is also available for individual and minimally invasive monitoring of respiratory frequency and jerk acceleration in juvenile and adult fish. Experimental studies can be conducted with a great variety of species: gilthead sea bream, European sea bass, sole, turbot, mussel, clam, and Artemia, with access to one of the largest Artemia cysts collections available in Europe. Visiting scientists are integrated into a research group at IATS, promoting collaboration throughout the research process, including report and article writing and publishing. Users receive training and support from experienced technical and scientific personnel in methodologies, experimental design, and data analysis. Support is tailored to the project and the user's autonomy. The unit of access is defined as person-weeks, representing the number of weeks a person uses experimental tanks and associated lab units. A

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typical access period is 12 units, including preparatory work, fish acclimatization, and sampling support.

CSIC-IATS-ANA (Instituto de Acuicultura Torre de la Sal / Analytical Labs): IATS-ANA comprises analytical laboratories located within IATS, with scientific equipment for a wide range of techniques and analyses relevant to aquaculture research. They are able to conduct most of the techniques and analyses involved in research in aquaculture: microscopy, histology, histochemistry, ISH, immunoassays, gas and liquid chromatography, PCR and RT-PCR and other molecular techniques for gene expression analysis, epigenetic mapping, microbiome sequencing and analysis, in vitro cell and eukaryotic culture, isotopic assays, micromanipulation, etc. These methodologies are applied in the fields of: genetic marker analysis of molluscs and fish, fish health and welfare, fish nutrition and growth, marine larviculture, ecotoxicology, fish reproduction, fish neuroendocrinology and energetic metabolism, biotechnology, and environmental and organism monitoring. Users of the IATS-ANA facilities receive training and support from experienced technical and scientific personnel. This support covers methodologies and data analysis, ensuring that researchers can effectively utilize the available equipment and interpret their results. The level of support provided is tailored to the specific requirements of each project and the user's level of autonomy. Visiting scientists are incorporated into IATS research groups. This promotes a collaborative approach throughout the research process, encompassing not only laboratory work but also the stages of data analysis, reports and manuscripts redaction. The aim is to facilitate the transfer of knowledge and expertise, enabling visitors to effectively apply the insights gained during their TNA to enhance their own research infrastructures and practices. Users also benefit from access to a comprehensive collection of full-text journals and databases, streamlining literature reviews and information acquisition. The unit of access for IATS-ANA is defined in person-weeks. This refers to the number of weeks that a researcher utilizes the analytical labs and associated equipment. A typical access period consists of 4 units, which includes time for preparatory work and technical support for the analyses conducted.

CSIC-IEO-ICAR-MAP (Instituto Español de Oceanografía / Marine Aquaculture Facilities of Murcia): IEO-ICAR-MAP provides access to marine aquaculture facilities aimed at developing techniques for bluefin tuna juvenile production, as well as breeding and juvenile production of other Mediterranean fish species, including several areas, such as breeding, hatchery, nursery, phyto and zooplankton cultures and pre- and on growing, and several available on-site biological laboratories: wet labs, genetic, histology, nutrition, chemistry, and biochemistry as well as rooms for feeding preparation. The infrastructure includes a broodstock area with various square and rectangular-based tanks,

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an incubation room with cylinder-conical tanks, water recycled units for larval rearing with cylindrical tanks, a larval rearing area with cylindrical tanks, two experimental isotherm rooms with cylindrical tanks, a weaning and pre-ongrowing area with cylindrical and square-based tanks, and a room for pathology challenges, fully isolated to carry out challenges with pathogens. IEO-ICAR-MAP is used as a research infrastructure by IEO scientific staff and other Spanish institutes and universities, and has also hosted many experiments from private companies. Each visiting scientist will be linked to a local research group with expertise in the same or a closely related research field, and visitors planning experiments at IEO-ICAR-MAP will provide an experimental plan in collaboration with IEO researchers, with standard procedures and general maintenance performed by trained staff. Access will include the use of tanks (including maintenance, water supply, feeding, and husbandry), manipulation and sampling of fish, and access to all dry laboratory facilities, with other infrastructural, logistical, technical and scientific support offered, as well as access to internet, desk, fax and printing service, and copy machine. Users will receive technical assistance, training and advice on methodologies, experimental design and data analysis, and IEO-ICAR-MAP provides standardized experimental protocols, documentation of results, and support for sampling and sample conservation. The unit of access is one week (7 days) per person, and a typical access period consists of 10 units, including preparatory work and technical support during the project.

CSIC-IEO-AquaCOV has an area of 1,950 m^2 comprising offices, labs and hatcheries (tanks between 100-10,000 L) and on-growing facilities (tanks between 1,000-90,000 L) with a total volume of 550 m^3. The Marine Aquaculture Group from IEO-AquaCOV is a multidisciplinary team that currently consists of 4 Scientists, 2 Technicians and 3 Assistants.

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1.1.2. User projects

Min. quantity of access units to be provided according ther DoA: IATS-CSIC-EXP: 96; IATS-CSIC-ANA: 32; CSIC-ICAR-MAP: 32; CSIC-IEO-AQUACOV: 20; CSIC-IEO-PAU: 20
Total number of access units (sum of access units in the table): IATS-CSIC-EXP: 84; IATS-CSIC-ANA: 37.35; CSIC-ICAR-MAP: 52; CSIC-IEO-AQUACOV: 10; CSIC-IEO-PAU: 0

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 ?
1	IATS-EXP	Emulsifier in sea bream feed	EMULSAQUA2	Evaluation of a hydrophilic emulsifier's effect on digestibility and efficiency in sea bream diets with reduced fish oil	Evi Croes (Nukamel)	No	Tank.week	12	Yes	1
1	IATS-EXP	Venus clam rearing	VENUSREAR	Development of a rearing protocol for the striped venus clam to produce juveniles for stock enhancement, evaluating optimal conditions for growth and survival	Chiara Papetti (Padova University)	No	Tank.week	12	Yes	2
1	IATS-EXP	Diet and rearing conditions effects on seabream microbiota	CarboMicroSea	Examination of the effect of dietary carbohydrates on gilthead sea bream growth and mucosal microbiota under different water refreshment rates in aquaculture systems	Fotini Kokou (Wageningen University)	No	Tank.week	12	Yes	1
1	IATS-EXP	Delivery of immunostimulants directly to fish eggs	Direct2Egg	Development of a method for efficiently delivering immunostimulants directly to European sea bass eggs via bath immersion to increase larval disease resistance	Ana Maria dos Santos Rocha (CIIMAR)	No	Tank.week	12	No	1

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1	IATS-EXP	Microfibre exposure in seabass	RAYON2BASS	Assessment of the effects of dietary rayon microfibers on European sea bass health and nutritional value by analyzing fish growth, nutrient utilization, and tissue composition	Ricardo Silva Matias (CIIMAR)	No	Tank.week	12	Yes	1
1	IATS-EXP	Antiparasitic Activity of Essential Oils against *Sparicotyle chrysophrii*	COTYLEO	Development of functional feeds with essential oils to mitigate *Sparicotyle chrysophrii* infections in gilthead sea bream through *in vivo* challenge trials	Teresa Pirollo (Bologna University)	No	Tank.week	12	Yes	1
1	IATS-EXP	Faecal Microbiota Transplantation in Sea Bream	FMTBREAM	Determination of the effects of faecal microbiota transplantation on growth performance and stress response in gilthead sea bream under different conditions	Silvia Natale (Bologna University)	No	week	12	Yes	1
2	IATS-ANA	Insect oil in seabream diets	INSECTOIL	Evaluation of insect oil as a dietary component for gilthead seabream juveniles, analyzing its potential to replace fish oil in aquaculture feeds	Sara Patrícia Pinto Moutinho (CIIMAR)	No	week	4	Yes	1
2	IATS-ANA	Diet and rearing conditions effects on seabream microbiota	Breamucosa	Analysis of TNA CarboMicroSea samples to determine how diet and water refreshment rates affect gilthead seabream's gut, skin, and gill microbiota	Fotini Kokou (Wageningen University)	Yes	week	4	Yes	1
2	IATS-ANA	Boosting fish robustness	FISH+	Analysis of fish gastrointestinal and immune systems to determine how functional additives in fish diets enhance gilthead seabream robustness	Cláudia Aragão (CCMAR)	No	week	4	Yes	1
2	IATS-ANA	Fish microbiota bioinformatic analysis	COLOSSEUM	Analysis of fish intestinal microbiomes using the SAMBA tool to understand host-microbe interactions and improve feed utilization in aquaculture	Genciana Terova (Insubria University)	Yes (AQUAEXCEL^{2020})	week	4	Yes	1
2	IATS-ANA	Rayon effects on seabass gene expression	RAYON2BASS-GENEXP	Tissue gene expression analysis of samples generated in TNA RAYON2BASS	Ricardo Silva Matias (CIIMAR)	Yes	week	4	Yes	1

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2	IATS-ANA	Tilapia Microbial dynamics in RAS systems	TILAPIAMICRORAS	Exploration of microbial communities in tilapia aquaponics and recirculation systems by using Oxford nanopore sequencing to improve sustainable aquaculture management practices	Luisa Villamil (Universidad de la Sabana)	No	week	4	Yes	2
2	IATS-ANA	Fecal Microbiota Transplantation on Sea Bream Gut Microbiota	FMTMICROBREAM	Analysis of TNA FMTBREAM samples to determine how faecal microbiota transplantation affects gut microbiota functionality and structure in gilthead sea bream	Silvia Natale (Bologna University)	Yes	week	4	Yes	1
2	IATS-ANA	Investigation of salmon lice microbiome by Illumina and Nanopore sequencing	SLM	Analysis of the microbiome of salmon lice using Illumina and Nanopore sequencing to better understand host-pathogen interactions in aquaculture	Bela Klimesova (Atlantic Technological University)	No	week	4	Yes	1
2	IATS-ANA	*D. labrax* fed with *Hermetia illucens*	Hermetia BASS	Evaluation of black soldier fly larvae meal as a sustainable substitute for fishmeal in European seabass diets, analyzing effects on metabolism and gut health	Efstratios Roussos (HCMR)	No	week	5.35	Yes	1
3	CSIC-ICAR-MAP	Acoustic particle motion measurements on bluefin tuna in aquaculture tanks	TUNAMOTION	Monitoring of acoustic recordings in bluefin tuna tanks to understand their behavior in confinement by analyzing sound and particle motion	Sérgio Manuel Machado Jesús (Algarve University)	No	week	1	No	1
3	CSIC-ICAR-MAP	Closing the feeding gap in the Atlantic Bluefin Tuna feeding program	ABT Feeding	Establish the benchmark for artificial weaning and feeding of Atlantic bluefish tuna with artificial diets.	Paul Sindilariu (Next Tuna)	No	week	10	Yes	2
3	CSIC-ICAR-MAP	Thermal adaptation of Atlantic bluefin tuna	AB Endo	Characterization of the development of regional endothermy in Atlantic bluefin tuna reared at two temperatures, using histological, physiological, and metabolomic analyses	Alejandro Buentello (Ichthus Unlimited)	No	week	15	Yes	2

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3	CSIC-ICAR-MAP	Physiological energetics of Atlantic Bluefin tuna	TUNAPHYS	Development of protocols for swimming tunnel respirometry with cardiac biologging of live ABFT juveniles using a swim tunnel and heart rate logger before release	David McKenzie (UMR MARBEC)	No	week	5	Yes	2
3	CSIC-ICAR-MAP	Automatic sorting of tuna eggs	ASTUNE	Exploration of an automated AI approach to classify tuna eggs based on visual characteristics, potentially improving hatchery quality assurance	Ana Hernando (Bionomous)	No	week	4	Yes	2
3	CSIC-ICAR-MAP	Pattern of behaviour of juveniles Atlantic Bluefin under routine rearing conditions and estimation of routine energetics costs	ABBE	Analysis of the behaviour of juvenile tuna in tanks, by mean of stereo cameras, and in a swim tunnel to study swimming costs and kinematics	Daphne Cortese (IFREMER)	YES	week	5	Yes	2
3	CSIC-ICAR-MAP	Closing the feeding gap in the Atlantic Bluefin Tuna feeding program, replacing yolk-sack larvae as live feed and applying artificial feed	ABT FEEDING GAP	The TNA consist of two experiments: a first experiment on the replacement of bream yolk-sack larvae (YSL) through other means of commercially available feed, reduce the use of YSL to a minimum in Tuna feeding;and a second experiment to feed ABT from 200 g to 1 kg	Paul Sindilariu (Next Tuna)	Yes	week	12	Yes	1
4	CSIC-IEO-AquaCOV	Effect of a *Chroomonas* strain on broodstock conditioning of the clam *Ruditapes decussatus*	CHROMOCLAM	*Chroomonas* spp.'s effect on *Ruditapes decussatus* broodstock conditioning was be investigated through the study of gametogenic scale, gonad fatty acid composition, and gamete quantity	Rania Azirar (Université Abdelmalek Essaâdi)	Yes	week	10	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

IATS infrastructure (IATS-EXP and IATS-ANA installations):

EMULSAQUA2 (VID32806): This project aimed to improve feed efficiency in gilthead sea bream aquaculture by using a hydrophilic emulsifier. The experimental design involved feeding trials with four different diets: a control diet with fish oil, a diet with reduced fish oil, and two diets with reduced fish oil supplemented with the emulsifier at different concentrations. The dietary trial was conducted in the exceptionally hot summer of 2022, and growth performance, nutrient digestibility, and tissue composition of the fish was considered. After the final sampling, user Steven Cools from Nukamel visited our infrastructures and discussed the results. Additionally, the impact of the diets on the fish microbiome was determined. Microbiome analysis of gut samples generated in the dietary trial, combining different fat levels with/without emulsifier supplementation revealed the presence of genus *Brevinema* as a marker of heat stress. Addition of emulsifier turned down the microbiota dysbiosis and mimicked the microbiota changes associated to low energy diets. Results were presented at Vienna EAS2023 Conference, they merited the Student Spotlight Award to CSIC PhD student Ricardo Domingo-Bretón, and have resulted in an Open Access publication: Domingo-Bretón et al. Intestinal microbiota shifts by dietary intervention during extreme heat summer episodes in farmed gilthead sea bream (*Sparus aurata*). Aquaculture Reports 40:102566 (2025).

INSECTOIL (VID35044): This project was conducted along October-November 2022, with the visit of CIIMAR PhD student Sara Moutinho. This project investigated the potential of insect oil as a dietary component for gilthead seabream juveniles by RT-PCR analysis in liver and intestine tissue samples. Replacement of vegetable oil for insect oil in gilthead sea bream diets did not result in any adverse effects on the expression profile of genes related to lipid metabolism. This work has been published as Moutinho et al. Effects of black soldier fly larvae oil on lipid metabolism, liver fatty acid composition, and plasma metabolite profiles in gilthead seabream juveniles. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 277, 111069 (2025).

VENUSREAR (VID39439): This project was conducted from March to June 2023, and Massimiliano Babucci was trained in a 4 weeks stay. Group leader Chiara Papetti also visited the infrastructures for discussion of results. The project was focused on developing effective rearing protocols for the striped venus clam (*Chamelea gallina*). Different factors such as temperature, salinity, and diet were tested to determine their effects on growth, survival, and overall well-being of the clams. The physiological responses of the clams to different rearing conditions were also observed. A procedure for reproduction in captivity of striped venus clam was accomplished and temperature was revealed as one of the key variables for success. To date results have not been disseminated, although they are intended to be part of communications in upcoming meetings and a publication in an Open Access journal.

RAYON2BASS (VID38824) and **RAYON2BASS-GENEXP (VID42627)**: The RAYON2BASS project investigated the effects of dietary rayon microfibers on the health and nutritional value of European sea bass. Fish were fed diets containing different concentrations of rayon microfibers, and their effects were determined on growth, nutrient utilization, and tissue composition. The feeding trial was conducted in April-July 2023, PhD student Ricardo Matias was trained at our infrastructures and participated in the final sampling. Some samples were sent for further analysis to applicant institution, and additional tissue samples (liver, skeletal muscle, anterior intestine, head kidney) kept in IATS infrastructure were analysed for gene

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expression profiling of genes related to oxidative stress, immune response, energy and lipid metabolism and muscle growth were analyzed in the framework of RAYON2BASS-GENEXP. This TNA project ended in November 2023, and Ricardo Matias was trained in the RT-PCR array technique and analysis. Significant hepatic transcriptional changes were detected, such as the activation of lipogenesis and mitochondrial respiration uncoupling in combination with the overexpression of markers of cholesterol metabolism, and triacylglycerol- and fatty acid catabolism. RF exposure also affected the transcriptomic profile of white skeletal muscle, revealing enhanced regulation of both positive and negative modulators of muscle growth. Likewise, a number of pro-inflammatory markers was altered by RF exposure, denoting an opposite regulation of the immune response at the systemic and local level. The multi-tissue target approach identified three distinct transcriptional signatures corresponding to different dietary levels of RF, driven by 43 discriminant genes. The intestine and muscle had a major contribution on this group separation. These findings indicated that dietary exposure to semi-synthetic microfibres induces physiological changes in seabass, potentially impacting fish health. Preliminary results of RAYON2BASS and RAYON2BASS-GENEXP were presented in as an oral communication in the AQUA 2024 EAS meeting. A manuscript of these works has been submitted to Journal of Hazardous Materials and it is currently under evaluation. Further analysis have been conducted addressing the impact of RF on gut, skin and environmental microbiota, and they have been submitted as on oral communication for the upcoming Aquaculture Europe 2025 EAS meeting to be held in Valencia (Health & welfare management – microbiome section).

CarboMicroSea (VID42610) and **Breamucosa (VID45042)**: Both projects were complementary, comprising the trial in tanks (IATS-EXP) and microbiome sequencing analysis (IATS-ANA), and were conducted in July-October 2023. Visitor PhD student Elyza Syropoulou was trained to assess the impact of dietary carbohydrates on the growth and mucosal microbiota of gilthead sea bream in aquaculture systems. The trial examined the effects of varying levels and types of carbohydrates in the diet on the growth performance, nutrient utilization, and mucosal microbiota composition of fish. The experiment also considered the influence of different water refreshment rates in the aquaculture systems, including RAS, to assess how environmental conditions interact with dietary factors. Breamucosa aimed to explore the interaction between diet and water refreshment rates on the mucosal microbiota of gilthead sea bream. Initial growth performance results were promising, with the achievement of low feed conversion rates regardless of the culture system. Further dissemination of results in conferences and Open Access publications are in progress.

COLOSSEUM (VID42117): The COLOSSEUM project focused on the functional analysis of fish intestinal microbiomes using the bioinformatic tool SAMBA (Soriano et al., 2023. Genes 14, 1650), developed by the Nutrigenomics group of IATS-CSIC. User Genciana Terova was remotely assisted in the use of SAMBA along December 2023 to analyze and compare different datasets of fish intestinal microbiome data. The purpose of these analysis were to elucidate the complex interactions between host and microbial populations, providing insights into how the microbiome influences feed utilization, nutrient absorption, and overall fish health. Main conclusions of this study are intended to be disseminated in upcoming aquaculture conferences and the writing of an Open Access publication.

DIRECT2EGG (VID49713/VID49716): This project aimed to develop a novel bath immersion method for delivering immunostimulants directly to European sea bass. The effectiveness of this method in enhancing larval disease resistance was expected to be evaluated by challenging the larvae with pathogens, and also assess the impact of the treatment on larval survival and growth performance. The proposed work was dependent on a reliable and sufficient supply of good quality sea bass eggs by the established in-house sea bass broodstock of IATS-CSIC, with breeders maintained in dedicated facilities in a semi-open

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aquaculture system with natural photoperiod and temperature. They are reproductively mature hatchery-produced sea bass, with optimal ages for breeding (females between 5–8 years, and males around 2–4 years). Photoperiod and temperature are the main environmental factors controlling the process of sea bass reproduction and even though the photoperiod acts as a prime cue for synchronization of an endogenous rhythm of reproduction, water temperature above 17°C inhibits ovulation in sea bass. During the visit of Ana Rocha to the research infrastructure in February 2024, the sea water temperature experienced a continuous increase that led to a gradual decrease in the quality of the unfertilized eggs during the second week of the visit, and the incubation protocols optimized during the project, before the visit, were not successful as the obtained eggs could not be fertilized anymore. Thus, a new trial was envisaged for the spawning season in 2025, but the same temperature increase and bad spawn quality happened. As a result, the planned work could not be completed.

FISH+ (VID45366): Visitor PhD student Ibon García-Gallego was trained at IATS-ANA infrastructure in February-March 2024. The FISH+ project investigated the potential of dietary functional additives to enhance fish robustness in gilthead sea bream. This analysis aimed to evaluate the potential benefits of butyrate and taurine dietary supplementation on fish performance and gut microbiota composition of gilthead seabream juveniles after 10 weeks of feeding and after crowding stressor exposure. DNA from mucosal adherent bacteria from the anterior intestine was collected for the amplification and sequencing of 16 rRNA V1-V3 regions, using the Oxford Nanopore MinION. Altogether, the results reflect the important driving role of the environment upon gut microbiota, reinforcing the dynamic nutrition, environment, and microbiota interactions in farmed fish. Results were presented in as an oral communication in the AQUA 2024 EAS meeting, and a manuscript is being prepared for submission to an Open Access journal.

COTYLEO (VID31246): This TNA proposal aimed to evaluate the antiparasitic activity of vegetal essential oils against *Sparicotyle chrysophrii.* An *in vivo* trial testing the effect of 3 phytogenetic essential oils as alternative therapeutic treatments for infections by *Sparicotyle chrysophrii* in gilthead sea bream was conducted in August-November 2024. Visitor PhD student Teresa Pirollo was trained for this trial. Hosting researcher Oswaldo Palenzuela has been invited to co-direct the Ph.D. thesis of the visiting scholar. This thesis will be anchored on the scientific exchanges facilitated in part by this TNA. At least one Open Access publication is envisaged.

FMTBREAM (VID63552) and **FMTMICROBREAM (VID63547)**: Both projects are complementary, comprising the trial in tanks (IATS-EXP) and microbiome sequencing analysis (IATS-ANA), and they are currently ongoing and expected to be finished in July 2025. PhD student Silvia Natale is conducting a visit for training at IATS-CSIC infrastructure. The FMTBREAM project explores the use of faecal microbiota transplantation (FMT) to improve the growth performance and stress response of gilthead sea bream. The gut microbiota plays a crucial role in fish health and physiology, and manipulating it through FMT can be a promising strategy to enhance aquaculture production. The project will optimize the FMT technique for gilthead sea bream and investigate its effects on the fish growth performance and stress response under different conditions. Fish will receive FMT from donor fish, and their growth, feed efficiency, and physiological responses to stress will be compared to control groups. The study will also analyze the changes in the gut microbiota composition and function following FMT. FMTMICROBREAM will analyze how FMT affects the gut microbiota of gilthead sea bream, focusing on both the composition of the microbial community and its functional activity. The study will provide insights into how FMT can be used to modulate the gut microbiome to enhance fish health, improve feed utilization, and reduce the need for antibiotics in aquaculture.

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HermetiaBASS (VID67611): This proposal has been approved and will be conducted in June-July 2025. A training visit of PhD student Stratos Roussos is programmed. The HermetiaBASS project evaluates the use of black soldier fly (*Hermetia illucens*) larvae meal as a sustainable substitute for fishmeal in European seabass diets. The study will analyze the impact of the experimental diets on fish metabolism, growth performance, and gut health by means of PCR arrays of liver and anterior intestine samples of fish be fed diets with varying levels of black soldier fly larvae meal. The results are expected to provide valuable information on the potential of black soldier fly larvae meal as a sustainable and nutritious feed ingredient for European seabass aquaculture. To date, applicant have sent the frozen tissue samples to IATS-ANA infrastructure, and high-quality RNA has been extracted for further analysis.

SLM (VID53811): This proposal has been approved and will be conducted in June-July 2025. A training visit of PhD student Bela Klimesova is programmed. The SLM project investigates the microbiome of salmon lice (*Lepeophtheirus salmonis*) using Illumina and Nanopore sequencing. This will provide a comprehensive view of the microbial community associated with the lice. The study will investigate how the microbiome influences lice biology, virulence, and interactions with the host fish. The results are expected to contribute to the development of novel and targeted control strategies for salmon lice in aquaculture.

TILAPIAMICRORAS (VID52272): This proposal has been approved and will be conducted in July-August 2025. It is programmed the visit of PhD student Diego Cárdenas and group leader Luisa Villamil for a training stay. The TILAPIAMICRORAS project explores the microbial dynamics in tilapia aquaponics and recirculation systems. Understanding the microbial communities in these systems is crucial for optimizing their efficiency and sustainability.The project will characterize the microbial communities in tilapia aquaponics and RAS using advanced sequencing technologies. The study will investigate the composition, diversity, and function of the microbial communities and how they influence water quality, nutrient cycling, and fish health. The results are expected to provide valuable information for improving the management and design of aquaponics and RAS, promoting more sustainable aquaculture practices.

CSIC-IEO-ICAR-MAP infraestructure:

Acoustic particle motion measurements on bluefin tuna in aquaculture tanks (TUNAMOTION): Date: 01/05/2022 to 7/05/2022
This research aimed at planing, executing and monitoring an acoustic recording experiment in a bluefin tuna aquaculture tank. The analysis of the acoustic recordings, together with other biotic and abiotic data, gathered during the experiment should allow to provide or complement the understanding of the behavior of bluefin tuna in a confined environment. In particular it will provide recordings of the particle motion field at close range and low frequency where it significantly differs from the acoustic field.

Closing the feeding gap in the Atlantic Bluefin Tuna (*Thunnus thynnus*) feeding program, establishing a benchmark for future feeding trials (ABT Feeding):
Date: 20/07/2023 to 11/10/2023
The target of the project was to establish the benchmark for artificial weaning and feeding of ABT. The aim is to establish the baseline, describing the state of the art in ABT weaning to artificial diets and the on-growing of ABT with the available artificial diets under the existing protocols. Having a benchmark for future comparison is essential in proving the benefit of any development in Tuna rearing based on artificial diets.

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Thermal adaptation of Atlantic bluefin tuna: Implications for hatchery and restocking (AB Endo):
Date: 16/08/2023 to 15/12/2023
The objective was to characterize for the first time the ontogeny of the regional endothermy of ABFT reared at two different temperatures. Three different sizes were sampled for histological, physiological and metabolomic analyses and some tunas were logged with temperature data loggers. Two persons were trained on the handling and surgery of tuna juveniles to implant the loggers. A related publications is being produced.

Physiological energetics of Atlantic Bluefin tuna (TUNAPHYS):
Date: 16/10/2023 to 17/11/2023
The project aimed to use the Steffensen-type swim tunnel and hearth rate logger to develop protocols for swimming tunnel respirometry with cardiac biologging of live ABFT juveniles, which were measured when releasing back into the rearing tanks. Two persons were trained on the handling and surgery of tuna juveniles to implant the loggers. A short communication and one publication are being produced.

Automatic sorting of tuna eggs (ASTUNE):
Date: 12/09/2024 to 10/10/2024
This study explored a novel approach to classify tuna eggs based on visual characteristics using automation and AI, which has the potential to improve hatchery quality assurance. The impact on egg viability, hatching rates, and operational processing speed were all assessed. Results demonstrated no significant adverse effects on hatching rates or larval normality when comparing sorted and control groups, but further optimization in processing speed is necessary.

Pattern of behaviour of juveniles Atlantic Bluefin under routine rearing conditions and estimation of routine energetics costs (ABBE):
Date: 04/11/2024 to 05/12/2024

The project aimed to investigate behaviour of individual juveniles in their rearing tank (spontaneous swimming speed, activity patterns, response to external stimuli such as feeding and schooling behaviour) using stereo cameras. A second objective was to pursue studies using the Steffensen-type swim tunnel transported to IEO-ICAR in the previous TNAs. Building on the previous experience, smaller juveniles were tested to gain more information about swimming costs and kinematics in the tunnel. And finally to investigate about heart rate by biologging.

Closing the feeding gap in the Atlantic Bluefin Tuna (Thunnus thynnus) feeding program, replacing yolk-sack larvae as live feed and applying artificial feed until the fish reach 1kg (ABT FEEDING GAP): This TNA is currently being conducted. It was started last year but some problems prevented it from being completed, which will take place this June-July. The TNA consist of two experiments: a first experiment on the replacement of bream yolk-sack larvae (YSL) through other means of commercially available feed, reduce the use of YSL to a minimum in Tuna feeding to be completed this summer and a second experiment to feed ABT from 200g to 1 kg was carried out between October and November 2024.

<u>CSIC-IEO-AquaCOV infraestructure:</u>

Effect of a Chroomonas strain on broodstock conditioning of the clam Ruditapes decussatus (CHROOMOCLAM):
The project aimed to investigate the effect of *Chroomonas* spp. during broodstock conditioning of the clam *Ruditapes decussatus* by means of the study of the gametogenic scale and the fatty acid composition of gonads, and gamete quantity produced. The experiment was carried

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out with a strain of *Rhodomonas lens* that has a similar biochemical profile to a *Chroomonas* strain because the *Chroomonas* spp. did not perform well in terms of cell concentration to be produced at enough quantity to feed clam broodstock. The effect of two microalgal diets were test on *R. decussatus* broostock conditioning: the experimental diet *Rhodomonas lens* (R) + *C. neogracile* (C) and the control diet *Tisochrysis lutea* (T) + *C. neogracile* (C) during 2 months. Slightly higher gonad condition index (GCI) was observed in the clams fed the R+C diet compared to the clams fed the control diet after 60 days of conditioning. Also, clams fed R+C released more oocytes in the spawning induction carried out. Histological and fatty acid analysis are in progress. A publication will be finished once all the data is available. A PhD student was the coordinator of the project and was trained in person in the clam conditioning protocols, we interacted in the experimental set up, and in clam gametogenic scale determination.

TNA Acronym	Visitor name	Visitor Affiliation	Affiliation country	Accession dates	Number of units used
EMULSAQUA2 (VID32806)	Steven Cools	Nukamel	Belgium	11/6/22 - 2/9/22	12
INSECTOIL (VID35044)	Sara Moutinho	CIIMAR	Portugal	6/10/2022 - 4/11/2022	4
VENUSREAR (VID39439)	Massimiliano Babucci	Padova University	Italy	7/4/23 - 30/6/23	12
CarboMicroSea (VID42610)	Elyza Syropoulou	Wageningen University	Netherlands	8/7/23 - 30/9/23	12
RAYON2BASS (VID38824)	Ricardo Matias	CIIMAR	Portugal	8/5/23 - 31/7/23	12
Breamucosa (VID45042)	Elyza Syropoulou	Wageningen University	Netherlands	2/10/23 - 29/10/23	4
FISH+ (VID45366)	Ibon García	CCMAR	Portugal	10/2/24 - 16/3/24	4
COLOSSEUM (VID42117)	Genciana Terova	Insubria University	Italy	22/11/23 - 20/12/23	4
RAYON2BASS-GENEXP (VID42627)	Ricardo Matias	CIIMAR	Portugal	23/10/23 - 17/11/23	4
Direct2Egg (VID49713/VID49716)	Ana Rocha	CIIMAR	Portugal	8/1/24 - 25/3/24	12
TILAPIAMICRORAS (VID52272)	Luisa Villamil	U. Sabana	Colombia	to be conducted in August 2025	4
COTYLEO (VID31246)	Teresa Pirollo	University Bologna	Italy	27/9/24 - 20/12/24	12
FMTBREAM (VID63552)	Silvia Natale	University Bologna	Italy	8/4/25 - 1/7/25	12
FMTMICROBREAM (VID63547)	Silvia Natale	University Bologna	Italy	2/7/25 - 28/7/25	4
SLM (VID53811)	Anita Talbot	Atlantic Technological University	Ireland	2/6/25 - 30/6-25	4
HermetiaBASS (VID67611)	Stratos Roussos	HCMR	Greece	3/5/25-30/5/25	5.35
TUNAMOTION (PID19099)	Sergio Jesus	Universidade do Algarve	Portugal	20/4/22 - 7/5/2022	2.43
ABT Feeding (PID22213)	Paul Daniel Sindilariu	Nextuna GmbH	Germany	20/7/23 - 11/10/23	10
AB Endo (PID20269)	Alejandro Buentello	Icthius unlimited	U.S.A	16/8/23 - 19/12/23	15
TUNAPHYS (PID24609)	David Mackenzie	CNRS	France	16/10/23 - 17/11/23	5
ASTUNE (PID30724)	Ana Hernando	Bionomous SA	Switzerland	12/9/24 - 8/10/24	4
ABBE (PID32382)	Daphne Cortese	IFREMER	France	2/11/24 - 30/11/24	5
ABT FEEDING GAP (PID30748)	Paul Daniel Sindilariu	Nextuna GmbH	Germany	13/10/24 - 8/12/24; 16/6/25 - 14/7/25	12
CHROMOCLAM (PID30423)	Rania Azirar	University of Abdelmalek Essaadi	Morocco	5/9/24 - 14/12/24	10

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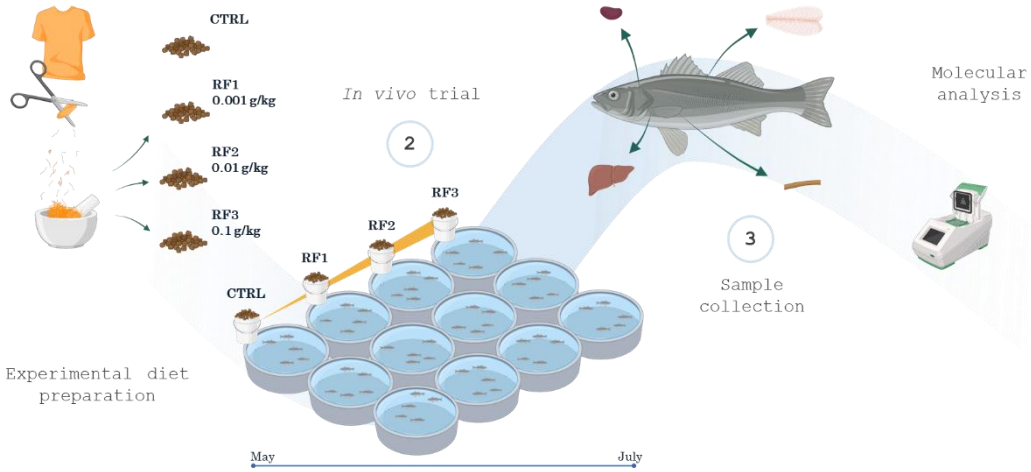
2.1.2. Selection of One exemplary project

RAYON2BASS (VID38824) and **RAYON2BASS-GENEXP (VID42627)**:



The effects of cellulosic microfibres on fish health remain understudied. The impacts of dietary viscose-rayon microfibres (RFs) on European seabass were evaluated on the growth performance and feed efficiency, as well as on gene expression patterns using tissue-specific customised PCR-arrays. Triplicate groups of juveniles (5.8 g) were fed with a diet without RFs (CTRL) or with increasing RFs levels: RF1 (0.001 g/kg), RF2 (0.01 g/kg), RF3 (0.1 g/kg). The exposure trial lasted 68 days with fish fed to visual satiety. While growth performance remained unaffected by RF exposure, the hepatosomatic index increased progressively from 1.49 in CTRL_RF1 fish to 1.98 in RF3 fish ($p = 0.037$). The rise of hepatosomatic index was related to significant hepatic transcriptional changes, such as the activation of lipogenesis and mitochondrial respiration uncoupling in combination with the overexpression of markers of cholesterol metabolism, and triacylglycerol- and fatty acid catabolism. RF exposure also affected the transcriptomic profile of white skeletal muscle, revealing enhanced regulation of both positive and negative modulators of muscle growth. Likewise, a number of pro-inflammatory markers was altered by RF exposure, denoting an opposite regulation of the immune response at the systemic and local level that contributed to discriminate between CTRL_RF1 and RF2_RF3. The multi-tissue target approach identified three distinct transcriptional signatures (CTRL_RF1, RF2, and RF3), driven by 43 discriminant genes. The intestine and muscle had a major contribution on this group separation. These findings indicate that dietary exposure to semi-synthetic microfibres induces physiological changes in seabass, potentially impacting fish health.

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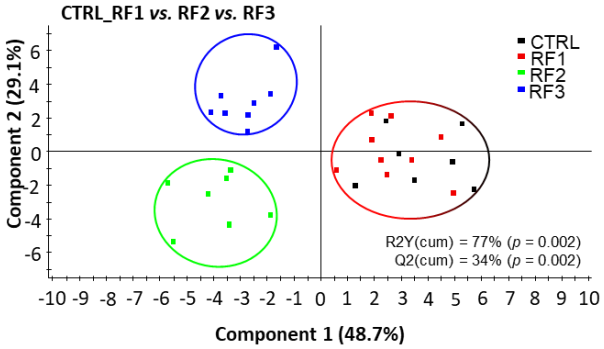

Two-dimensional PLS-DA score plot of multi-target (anterior intestine, head kidney, liver and muscle) gene expression, representing the distribution of the samples between the first two components in the model.

3. Reflection on results of the TNA programme

The TNA programme at CSIC research infrastructures yielded highly positive outcomes, strengthening technical expertise, enhancing infrastructure visibility, and fostering scientific collaborations that led to increased publication output and researcher training. The integration of visiting scientists into host research teams promoted knowledge transfer and skill development in experimental methodologies and data analysis, with several projects already resulting in open-access publications and conference presentations. For future access programmes, it is recommended to ensure a clear timing for the reviewing process of the proposal, as timing for approved proposal is critical, especially for the experiments with fish.

4. References

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Document Information

EU Project	No 871108	Acronym	AQUAEXCEL3.0
Full Title	AQUAculture infrastructures for EXCELlence in European fish research 3.0		
Project website	www.aquaexcel.eu		

Deliverable	N°	D11.1	Title	Analysis of access provided by CSIC Research facilities: types and users
Work Package	N°	11	Title	Transnational Access to CSIC Research facilities
Work Package Leader	Jaume Pérez-Sánchez			
Work Participants	Jaume Pérez-Sánchez, Carlos Saavedra, Carla Piazzon, Ana Gómez, Oswaldo Palenzuela, Federico Moroni, Óscar Monroig			

Lead Beneficiary	4-CSIC
Authors	Jaume Pérez-Sánchez, IATS-CSIC, jaime.perez.sanchez@csic.es Josep Calduch-Giner, IATS-CSIC, j.calduch@csic.es Aurelio Ortega, CSIC-IEO-ICAR-MAP, aurelio.ortega@ieo.csic.es Montserrat Pérez, CSIC-IEO-AquaCOV, montse.perez@ieo.csic.es Fiz Da Costa, CSIC-IEO-AquaCOV, fiz.dacosta@ieo.csic.es
Reviewers	Iris de Cesare, INRAE transfert, iris.decesare@inrae.fr Marc Vandeputte, INRAE, marc.vandeputte@inrae.fr

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