

# Deliverable 12.1

## Analysis of access provided by HCMR Mediterranean aquaculture experimental facilities: types and users

---

Version 2

WP 12
Deliverable 12.1
Lead Beneficiary: HCMR
Call identifier:
Biological and Medical Sciences - Advanced Communities: Research infrastructures in aquaculture
Topic: INFRAIA-01-2018-2019
Grant Agreement No: 871108
Dissemination level: PU
Date: 30.04.2025



## Executive Summary

The HCMR installations within the AQUAEXCEL3.0 project provided advanced research infrastructure supporting a wide range of transnational access (TNA) activities in Mediterranean aquaculture. The Aqualabs and Omics-Bioinformatics platforms enabled research on nutrition, welfare, genetics, and sustainability in aquaculture, focusing on key species such as *Dicentrarchus labrax* and *Sparus aurata*. A total of 15 user projects were supported, spanning nutritional innovations, alternative protein sources, stress mitigation strategies, and genomic studies. These activities not only resulted in scientific advancements and publications but also fostered training for students and technicians. The infrastructure facilitated hands-on experience in experimental procedures, histological analysis, and bioinformatics, thereby strengthening the research capacity of user institutions. The TNA programme contributed to knowledge generation, promoted sustainable aquaculture practices, and reinforced the role of HCMR installations as hubs for international collaboration and innovation.

### Objectives of the Local Infrastructure and Each Installation

The objectives of the local infrastructure were:

- To support high-quality research in aquaculture through access to state-of-the-art experimental and analytical platforms.
- To enable studies on fish growth, nutrition, welfare, and genetics across multiple life stages and environments.
- To foster training and collaboration between European researchers and SMEs in sustainable aquaculture technologies.

**Aqualabs** was designed to perform nutrition, behavior, and early life-stage experiments under both flow-through and recirculation systems. It includes specialized tanks for digestibility, weaning, and behavioral studies, as well as mesocosm units and sea cages for realistic pilot-scale trials.

**Genomics/Bioinformatics (OMICS-BIOINFO)** offers tools for population genetics, genomics, and transcriptomics. Equipped with advanced sequencing and analytical platforms, it supports molecular studies that inform breeding, stress responses, and functional genomics.

Both installations aimed to offer access to cutting-edge infrastructure for external users, facilitating high-level experimental and analytical work under the TNA framework.

### Main Results: Accesses, Training, Outcomes

- **Total accesses provided:**
  - **Aqualabs:** 203 tank-weeks (vs. 167 planned)
  - **OMICS-BIOINFO:** 64 weeks (vs. 48 planned)
- **Training activities:**



- At least **26 individuals** (mainly students and technicians) were trained across projects.
- Training involved experimental setup, data collection, molecular and histological analyses, and bioinformatics.
- **Scientific outcomes:**
  - All projects plan to publish results.
  - Topics included:
    - Alternative feed ingredients (e.g., wheat protein, *Nereis* sp.)
    - Innovative additives and feed technologies (e.g., +POP, GHRP-6)
    - Stress mitigation strategies (e.g., botanical extracts, post-transport stress)
    - Behavioral studies under sound exposure
    - gene expression and gut microbiome variations in response to alternative feed compositions in gilthead sea bream
    - Transcriptomics and genome assembly for under-studied species.
    - antimicrobial activities of freshwater fish-pond microbes against strains of marine pathogens

**Impact:** Users improved research capacity, developed feed formulations, validated biotech solutions, and expanded knowledge on fish behavior and physiology. The infrastructure also benefited through expanded scientific networks and enhanced visibility.

---

## Authors/Teams Involved

### Authors from AE3.0:

- **Dr. Nikos Papandroulakis** – Hellenic Centre for Marine Research (HCMR)
- **Dr. Stavros Chatzifotis** – Hellenic Centre for Marine Research (HCMR)
- **Dr. Costas Tsigenopoulos** – Hellenic Centre for Marine Research (HCMR)
- **HCMR Aqualabs and Genomics-Bioinformatics Team** – experimental and genomic support

### Other contributors (User PIs from projects):

- Rebeca Martinez Rodriguez
- Hans Boon
- Linn Danielsen Evjemo
- Konstantinos Tzakris
- Chiara Cardillo
- Hijran Yavuzcan
- Silvia Gomez Suarez
- Mahougnon Simeon Fagnon
- Ramon Fontanillas
- Daniel Garcia de la Serrana
- Giulia Riccioni
- Mbarsid Racaku
- Felix Kuebutornye

(Each affiliated with their respective research institutions or companies as listed in the TNA table.)



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 871108 (AQUAEXCEL3.0). This output reflects only the author's view and the European Commission cannot be held responsible for any use that may be made of the information contained therein.



## Contents

Executive Summary (half a page max) .....	3
1.    Overview of TNA users projects realized in TNA HCMR.....	7
1.1.1.    InstallationS (short description of each installation in the local INFRA) .....	7
1.1.2.    User projects .....	9
2.    TNA projects .....	15
2.1.1.    TNA projects description .....	15
2.1.2.    Selection of One exemplary project.....	19
3.    Reflection on results of the TNA programme .....	23
4.    References.....	23



## 1. Overview of TNA users projects realized in HCMR

### 1.1.1. Installations

The HCMR infrastructure covers up-to-date aquaculture needs, enabling multidisciplinary research on all stages of aquaculture production (broodstock, eggs, larvae, juveniles, grown-out fish and genetic breeding) of the most commonly cultured Mediterranean species like *D. labrax*, *S. aurata*, *A. regius* and *S. dumerili*. It is composed of: 1. Aqualabs consists of intensive (automated feeding system based on PC and monitoring systems with video, DO and T sensors) and mesocosm tanks, feed chain zone for artemia and rotifer culture as well as photobioreactors for phytoplankton production. It has also a behavioural and nutrition unit equipped with closed recirculation tanks with thermoregulation, monitoring systems (video, DO and T sensors) and demand feeders. A biochemical laboratory provides analytical support. The installation has also a net-pen cage unit (Souda), which provides access to experimentation under pilot production in the sea. The Aqualabs installation offers the following facilities: a) A behavior-nutrition facility comprising of 50-L tanks (weaning experimentation), 250-L tanks (digestibility experimentation) and 500-L tanks (nutrition-behavior experimentation) in a flow-through system with an option for recirculation with controlled temperature, video camera monitoring and temperature-oxygen sensors. b) An intensive hatchery facility, where there are 500-L tanks connected to biofilters. Temperature and photoperiod are controlled and feeding with microalgae, rotifers, and artemia can be adjusted through a computerized system. The facility employs video camera monitoring systems and there is additional continues monitoring of DO and temperature with specific sensors. The facility has also six mesocosm tanks of 40m<sup>3</sup> capacity used for rearing larvae with high survival rates, even larvae of most "difficult" species like amberjack are produced. In the live feed zone of the facility, microalgae (photobioreactors), rotifers and artemia are produced in industrial scale under automated feeding and controlled temperature. c) A sea farm with net-pen cages facility provides access to pilot scale cages of 100m<sup>2</sup>, as well as experimental 3x3x3m or 1x1x1.5m cages. The unit has also a controlled submersible cage down to 40 m.

2. Genomics/Bioinformatics facility has modern equipment, essential for the design and elaboration of research projects in the fields of populations genetics, molecular ecology, phylogeography, genetic improvement and genomics of Mediterranean aquaculture fish, and it is able to perform any necessary genetic analysis under the context of the proposed activity. The Genomics-Bioinformatics installation include next generation high-throughput genetic analysers [454 FLX & Junior, (Roche) and MiSeq (Illumina)], a capillary sequence analyzer ABI 3730xl (for DNA sequencing and genotyping), an arsenal of gradient and real-time PCR machines, a microarray scanner, a TaqMan OpenArray Genotyping System, a QIAcube (Qiagen) and in general equipment which facilitates and ensures the appropriate conditions for the isolation and the qualitative and quantitative study of the genetic profile of an organism at the level of DNA, RNA and proteins. Last, IMBBC operates a bioinformatics platform (BioCluster) with 108 Xeon 3.3GHz cores (9 Nodes), 784GB RAM, NAS 30 TB storage with quota (18TB /home and 12TB for data resources) and Dual 10Gbps ethernet optical interconnection.





**(Aqualabs-Souda Cages: Photo Dr. N. Papandroulakis)**



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 871108 (AQUAEXCEL3.0). This output reflects only the author's view and the European Commission cannot be held responsible for any use that may be made of the information contained therein.

### 1.1.2. User projects

Min. quantity of access units to be provided according ther DoA: HCMR-Aqualabs: 201 HCMR-OMIC-BIOINFO: 77  
 Total number of access units (sum of access units in the table): HCMR-Aqualabs: 203 HCMR-OMIC-BIOINFO: 64

Installation number	Installation code	Project acronym	Description about the experiment	Coordinator	Country	Institution	Period of access	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	HCMR-AQUA LABS	PID_34962 GHRP-6Feed	Evaluation of GHRP-6 secretagogue peptide included in the feed, impact on the growth of sea bass ( <i>Dicentrarchus labrax</i> ) fry	Rebeca Martinez Rodriguez	Cuba	Center for Genetic Engineering and Biotechnology	July 2025- October 2025	NO	tank.week	36	YES	2
2	HCMR-AQUA LABS	PID_27078 MULSAQ UA3	Improving feed efficiency by using a hydrophilic emulsifier in seabass diets reducing the dependence of fish oil and environmental impact	Hans Boon	Netherlands	Nucamel-Aquaculture Experience,	April 2024 - July 2024	YES	tank.week	12	YES	2



3	HCMR-AQUA LABS	PID 29151 FACTS : Fish reACTION To Stimuli	Marine Models at Aqualabs' with the proposal 'Measuring the change in behaviour of farmed sea bass when exposed to sounds within the fish cage: A comparison with farmed Atlantic salmon in Norway	Lin	Norway	SINTEF,	June 2025-July 2025	YES	tank.week	5	YES	2
4	HCMR-AQUA LABS	PID_3259 9 Wheat Protein Concentrate in Seabream Diets	Development of Wheat Protein Concentrate as a new sustainable protein source in diets for Gilthead Seabream (Sparus aurata)	Hans Boon	Netherlands	Aquaculture Experience,	March 2025-June 2025	YES	tank.week	15	YES	2
5	HCMR-AQUA LABS	PID_2986 6	Cryoplankton as an alternative live feed for seabream	Konstantinos Tzakris	Norway	Plantonic,	May 2024-July 2024	YES	tank.week	15	YES	2
6	HCMR-AQUA LABS	PID_2628 7 +POP	Studying +POP, a new technologies that allows to functionalise aquafeed, by evaluating both its delivery/protection ability with different molecules as well as	Chiara Cardillo	Italy	Aquatrade	Jun 2023-September 2023	YES	tank.week	25	YES	2



			the involvement of the coating itself in improving aquafeed palatability on <i>Sparus aurata</i>									
7	HCMR-AQUA LABS	PID_25677 IF WeLFaRe	Improving Gilthead Sea Bream ( <i>Sparus aurata</i> ) Welfare through Innovative Feed Additive	Hijran Yavuzcan	Turkey	Ankara University	January 2025-March 2025	YES	tank.week	20	YES	2
8	HCMR-AQUA LABS	PID_22768 IF Meagrefeed	Evaluation of <i>Nereis</i> sp as an alternative ingredient in meagre aquaculture feed	Silvia Gomez Suarez	Chile	Catholic University of Valparaiso	February 2023-May 2023	YES	tank.week	35	YES	2
9	HCMR-AQUA LABS	PID_20305 Phymucold	Natural botanical compounds and cold-water stress in European Gilthead Sea Bream <i>Sparus aurata</i> : effect on growth performances, immunological and physiological responses	Mahougnon Simeon Fagnon	France	Phytosynthese	January 2023-June 2023	YES	tank.week	20	YES	2



10	HCMR-AQUA LABS	PI_20680 Experimental assessment of the fish meal content requirements for Meagre (Argyrosomus regius) feeds.	Ramon Fonatanillas	Norway	Skretting	November 2022 - February 2023	YES	tank.week	20	YES	2	
11	HCMR-OMICS - BIOINFO	PID 20338 - MuSkeDev	Identifying the molecular networks involved in the development of the components of the muscle skeletal system and the molecules involved in their coordination	Daniel Garcia de la Serrana	Spain	University of Barcelona	February - September 2025	YES	week	14	YES	2



12	HCMR-OMICS - BIOINFO	PID 21646 - PHYGENC OLD	Natural botanical compounds and cold-water stress in European Gilthead Sea Bream <i>Sparus aurata</i> : effect on microbial community and immune-related gene expression	Mahougnon Simeon Fagnon	France	PHYTOSYNTHÈSE	February 2023 – April 2025	YES	week	13	YES	2
13	HCMR-OMICS - BIOINFO	PID 24291 - TransClam	Transcriptome analysis of <i>Chamelea gallina</i> reared at different conditions of temperature and pH to detect genes involved in environmental change responses for conservation and farming purposes	Giulia Riccioni	Italy	Catholic University of Sacred Heart	March - October 2024	YES	week	7	NO	



14	HCMR-OMICS - BIOINFO	PID 25255 - Genome assembly of mullets for sex-associated SNPs detection	Liza aurata (Chelon auratus) and Liza ramada (Chelon ramada) genome assembly for the identification of sex-determining loci.	Mbarsid Racaku	Italy	University of Padova	March 2024 – July 2025	YES	week	18	YES	1
15	HCMR-OMICS - BIOINFO	PID 35187 - MicroPhyte-AVAX	Antibacterial properties of pond microbiota against fish pathogens: genomic insights and in vivo anti-virulence evaluation	Felix Kuebutornye	Czechia	Jihočeská univerzita v Českých Budějovicích	February – September 2025	YES	week	12	YES	1

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



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 871108 (AQUAEXCEL3.0). This output reflects only the author's view and the European Commission cannot be held responsible for any use that may be made of the information contained therein.

## 2. TNA projects

### 2.1.1. TNA projects description

#### **PID\_34962 – Evaluation of GHRP-6 secretagogue peptide included in the feed, impact on the growth of sea bass (*Dicentrarchus labrax*) fry**

The study objective is to evaluate the most effective dose of GHRP-6 (Growth Hormone Releasing Peptide-6) in seabass feed to improve growth and increase feed efficiency and reduce feed cost. Sea bream/bass juveniles of 20 g initial weight will be fed for 3 months 1 (one) of 4 (four) experimental diets with grated levels of GHRP-6 (0,100, 250 and 500 ug/kg of feed) to establish which dose gives the best response. At the end of the feeding trial, biometric parameters (survival, SGR, FCR) will be determined and tissue samples (liver, intestine) will be collected for histopathological analysis.

The results will be published in a scientific journal. Two students will be trained.

#### **PID\_27078 – Improving feed efficiency by using a hydrophilic emulsifier in seabass diets reducing the dependence of fish oil and environmental impact**

The study objective is to evaluate the most effective dose of a hydrophilic emulsifier "Volamel Aqua" in fish feed to improve fat and total feed digestibility and, thus, efficiency in sea bream diets and reduce feed cost. Sea bream/bass juveniles of 10 - 15 g initial weight were fed for 3 months 1 (one) of 4 (four) experimental diets with a low inclusion of fish oil and fish meal and thus a suboptimal level of essential fatty acids and 4 inclusion rates of Volamel (500, 750, 1000 and 1250 ppm) to establish which dose gives the best response. At the end of the feeding trial, biometric parameters (survival, SGR, FCR, etc.) were determined and tissue samples (liver, intestine) were collected for histopathological analysis. Additional tissue samples were collected for future transcriptomic analysis of promising dietary groups, as well as skin and gut mucus for microbiome analysis.

The results will be published in a scientific journal. Two students were trained.

#### **PID\_29151 – Marine Models at Aqualabs' with the proposal 'Measuring the change in behaviour of farmed sea bass when exposed to sounds within the fish cage: A comparison with farmed Atlantic salmon in Norway**

This project aims to study the behavior of Mediterranean farmed fish within a fish cage when exposed to external impact factors, such as unfamiliar objects and sounds. The motivation for this study is to use sonars and cameras to map out the distribution of farmed fish within a fish cage, and to study how this distribution and the global and local behavior of the fish changes when an unfamiliar structure emitting sounds of different frequencies is introduced in the fish cage. The aim of this project is to perform these tests on European sea bass (*Dicentrarchus labrax*) and compare them with ones used for Atlantic salmon (*Salmo salar*).

The results will be published in a scientific journal. Two technicians will be trained.

#### **PID\_32599 – Development of Wheat Protein Concentrate as a new sustainable protein source in diets for Gilthead Seabream (*Sparus aurata*)**

This study aimed to evaluate the potential use of IDS' Wheat Protein Concentrate (IWPC) as protein sources in diets for gilthead seabream. IWPC is a unique by-product generated during wheat-based alcohol production process. For this research, seabream with an average weight of approximately 30 grams individual bodyweight will be fed for a period of 3 months a control



diet or one of 3 experimental diets, which will be formulated to contain graded levels (0 – 5 – 10 and 15%) of IWPC. All diets were be iso-nitrogenous and will be formulated according to industry standards with a fixed content of fish meal and fish oil with the alternative protein source (IWPC) at the different inclusion rates. At the end of the feeding trial survival rate, specific growth rate, feed conversion ratio and immunological status were determined. Additionally, the economic potential of IWPC as a feed ingredient was evaluated. The results will be published in a scientific journal. Two students were trained.

#### **PID\_29866 – Cryoplankton as an alternative live feed for seabream**

The successful rearing of marine fish larvae hinges on the provision of live feed. The most common options, rotifers and brine shrimp (*Artemia*), demand substantial labor and infrastructure. Planktonic has addressed this challenge by introducing ready-to-use cryopreserved larvae of *Balanus crenatus* (Cryoplankton Small) and *Semibalanus balanoides* (Cryoplankton Large), which can regain their vitality within minutes after thawing in seawater (see <https://planktonic.no/>). This project aimed to compare various protocols that combine Cryoplankton and dry feed with the traditional "pseudogreen water" methodology, involving rotifers, *Artemia*, and dry feed for sea bream larval rearing. Fish performance of the three Cryoplankton protocols were evaluated against the control. The Cryoplankton protocols include co-feeding with rotifers with different replacement ratios (low – high), while in one high replacement ratio protocol, dry feed was introduced earlier. The impact on larval survival, growth, and quality was meticulously examined.

The results will be published in a scientific journal. One technician and one student were trained.

#### **PID\_26287 – Studying +POP, a new technologies that allows to functionalise aquafeed, by evaluating both its delivery/protection ability with different**

A test on an innovative micro and nanotechnology method to introduce strategic supplements and additives for dry feed functionalisation in aquaculture was performed. A new coating method was created that reduces all the adverse side effects of traditional coating (oil leaching, waste of product and organic matter, long and complex procedures), keeping intact the benefits and freedom of the method. Through the present project, this new technology was tested by evaluating both its delivery/protection ability of different molecules as well as the involvement of the coating itself in improving aquafeed palatability. Specifically using Aquatrade's microencapsulation technology (+POP), standard diets were functionalized in situ and tested on a significant number of sea bream (*Sparus aurata*) juveniles at Aqualabs facilities for testing both the effects of +POP as a vehicle of its content and +POP as feed attractant coating. The data from the trial provided new information about functionalized aquafeed related to fish performance and helped to formulate a commercial product ready to be used by fish farmers and research centres.

The results will be published in a scientific journal. One technician and one student were trained.

#### **PID\_25677 – Improving Gilthead Sea Bream (*Sparus aurata*) Welfare through Innovative Feed Additive**

In order to promote fish welfare and improve the long-term sustainability of aquaculture production, novel and environmentally friendly feed additives can be utilized. The proposed project aimed to mitigate the adverse effects of post-transport stress in fish by introducing an innovative additive to their diet. The project was divided into two main phases. First, a transport simulation experiment was conducted at HCMR, followed by feeding the fish with the stress-reducing additives (components: glutamine, Vit C, fennel extract, and MOS). Second, stress indicators (plasma cortisol, glucose, lactate electrolytes, hematocrit, blood cell counts) in the fish exposed to transport stress will be analyzed at the University of Crete. The results were evaluated for the stress-reducing effect of the novel feed additive in the case of post-transport stress in gilthead sea bream. Through this project, the potential for the stress-reducing effect



of the novel mixture of additives (components: glutamine, Vit C, fennel extract, and MOS) was revealed. This may lead to its use for welfare improvement and the elimination of post-transport stress in sea bream aquaculture.

The results will be published in a scientific journal. One technician and one student were trained.

#### **PID\_22768 – Evaluation of *Nereis* sp as an alternative ingredient in meagre aquaculture feed**

The increasing scarcity of ingredients commonly used in aquaculture feeds and the efforts to achieve sustainable growth have made the search for new alternative nutrients a priority for European aquaculture. The culture of marine polychaetes, with high protein and fatty acid content, may provide a partial solution to this challenge. This proposal aimed to evaluate the potential of a formulated aquaculture feed for juvenile meagre (*Argyrosomus regius*), one of the most important emerging species, by introducing as an alternative ingredient the biomass of *Nereis* sp in different inclusion percentages with respect to a control feed. The nutritional content of the formulated feeds, consumption indices and growth rates, digestibility and fish performance will be analysed. The results obtained are expected to contribute knowledge to the EATIP sustainable food production area, analysing the performance of a new promising material from sustainable sources.

The results will be published in a scientific journal. Two students were trained.

#### **PID\_20305 – Natural botanical compounds and cold-water stress in European Gilthead Sea Bream *Sparus aurata*: effect on growth performances, immunological and physiological responses**

*Sparus aurata* is an important and sensitive farmed species in Mediterranean regions to low temperatures. Natural compounds are identified to mitigate stressful conditions. This project aims to assess the effect of Green propolis (GP) and turmeric and their combination on winter thermal stress. Thus, HCMR is identified to host this trial. 4 dietary groups were tested (triplicate). Diets were isonitrogenous and isocaloric for all groups. Trials were performed over 75 days (60d: optimal temperature at 20°C and 30d: low temperature at 13°C). Growth performances were assessed. Blood and liver were sampled for biochemical and histological analysis. At the end of this trial, natural compounds are expected to alleviate stress induced by low temperature. This study was a starting point for highlighting the potential synergistic effect of GP and turmeric in aquaculture. Results shall be published in a scientific journal and will be shared with EFSA for registration in the welfare category. Two students were trained during the execution of the project.

#### **PID\_20680 – Experimental assessment of the fish meal content requirements for Meagre (*Argyrosomus regius*) feeds.**

A growth performance trial was performed with juvenile meagre with novel feed formulations to evaluate the potential fishmeal replacement with plant proteins. Fish meal was replaced by a combination of plant protein raw materials in a dose response manner, with balanced amino acid, minerals and vitamins. The measured responses were fish performance, feed utilization, intestinal and liver structure and several haematological stress indicators, in order to identify what is the possible limiting factor for fish meal substitution.

Two students were training during the execution of the project

#### **PID\_20338 – Molecular regulation and coordination of the muscle skeletal system development (MuSkeDev)**

The present TNA application endeavours to respond to several inquiries previously raised, encompassing the identification of potential cytokines emitted by diverse cellular models of the muscle-skeletal system during two distinct phases of development. Additionally, it seeks to explore the role of lincRNAs and miRNAs in the differentiation of each cell type. The proposed methodology to achieve this objective entails the total RNA sequencing from proliferative and



fully differentiated myoblasts, osteoblasts, and adipocytes. These cells were obtained from primary cell cultures derived from skeletal muscle, vertebrae, bone, and visceral adipose tissue. The cells will be obtained from the gilthead sea bream (*Sparus aurata*), a species of considerable relevance to the Mediterranean aquaculture industry. This species is susceptible to skeletal anomalies and issues related to quality. Consequently, enhancing our comprehension of the molecular networks that govern cell growth and differentiation, as well as the cross-talk between tissues, could prove to be a pivotal strategy for augmenting this species' production.

#### **PID\_21646 – PHYTOBIOTIC & GENE EXPRESSION (PHYGENCOLD)**

In this current project, we have laid the groundwork for exploring variations in gene expression and the gut microbiome in response to alternative feed compositions in gilthead sea bream. We carried out 3'UTR RNAseq of 48 distal intestine samples in total comprising two different experiments and four different feeds. The average read count of each sample was about 11 million reads. The overall outcome of the RNAseq study revealed that the transcriptomic profile of the feed containing turmeric additives changed the most in comparison with the TL and Prolin additives in the first experiment. This research is particularly significant as it follows previous studies involving diverse feed formulations, allowing for a comparative analysis to understand the effects of varying feeds. Our investigation into the gut microbiome represents a novel aspect of our lab's research on feed experiments, adding extra value to our work. Furthermore, having standardized and evaluated immune-related qPCR genes regarding different feeds has enriched our molecular diagnosis toolbox in the gilthead sea bream.

#### **PID\_24291 – Transcriptome analysis of *Chamelea gallina* reared at different conditions of temperature and pH to detect genes involved in environmental change responses for conservation and farming purposes (TransClam)**

Tissue samples from 48 individuals of the Venus clam species *Chamelea gallina* (family Veneridae) were sent to HCMR for total RNA extraction, transcriptome library preparation, sequencing and bioinformatic analysis. The main objective was to study the transcriptomic changes induced due to the differential response of striped Venus clam to varying levels of temperature and salinity. Eight batches of Venus clam individuals, each batch containing six samples, were subjected to various pH and temperature parameters, and had already been acquired in a previous TNA project in ASSEMBLE-PLUS (EMBRC). At HCMR, we utilized custom and new tissue homogenization and RNA extraction methods but unfortunately, each sample exhibited either completely degraded RNA, appearing as a low molecular weight smear, or partially degraded RNA, characterized by a smear without clear rRNA bands. Therefore, none of the clam samples used for RNA extraction have produced the high-quality RNA necessary for subsequent reactions, such as constructing and sequencing an RNA library, and the project was prematurely ended. No scientists were trained during the execution of the project.

#### **PID\_25255 - *Liza aurata* (*Chelon auratus*) and *Liza ramada* (*Chelon ramada*) genome assembly for the identification of sex-determining loci (on-going).**

Samples of the two species collected close to the Venice lagoon were shipped to HCMR in March 2024; four for *Liza aurata* (one male and three females) and six for *Liza ramada* (three males and three females). Flash-frozen muscle tissues were used for high-quality DNA extractions and library preparations for PacBio (HiFi) and Hi-C sequencing. Sequencing results became available in early 2025, and preliminary remote bioinformatic analyses show that the HiFi was of excellent quality. The use of Hi-C data has only slightly improved genome assembly. For *Liza ramada*, 24 Scaffolds were identified at the chromosome level (presence of 94 unplaced contigs), with a mean HiFi reads coverage 38X, and a mean Hi-C reads coverage 47X; BUSCO genome score was 99.1%, and the Genome length 670,488,702 bp, L50=11, and N50=29,495,128 bp. Similarly for *Liza aurata*, 24 Scaffolds were identified at the



chromosome level (presence of 70 unplaced contigs), with a mean HiFi reads coverage 40X and a mean Hi-C reads coverage 42X; BUSCO genome was C:99.3% and the Genome length 685,127,071 bp, L50=11, and N50=30,197,303 bp. A scientist will be trained at the end of June for Genome Annotation, SNP calling analyses, and the search for sex determination gene(s).

**PID\_35187 - Antibacterial properties of pond microbiota against fish pathogens: genomic insights and *in vivo* anti-virulence evaluation (MicroPhyte-AVAX) (on-going).** The scientific objectives of the proposed research are i) to investigate the antimicrobial effects of the isolates against broad-spectrum fish pathogens to assess their potential for disease control, ii) sequence the whole genome of the isolated pondwater microbiota, enabling comprehensive genetic analysis, iii) identify and characterize gene clusters responsible for producing the enzymes and antimicrobial compounds within the isolates, contributing to their functional understanding, and iv) assess the safety and anti-virulence potential of the isolates through *in vivo* co-culture with larvae and genomic screening for virulence factors, ensuring their suitability for use in aquaculture. Genomic DNA has already been extracted from the isolates and is currently processed for whole-genome sequencing. The scientist will be trained in early June for sequence assembly using PATRIC platform, then the annotation using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) to identify gene clusters responsible for producing antimicrobial compounds and valuable enzymes using AntiSMASH and other bioinformatics tools.

### 2.1.2. Selection of One exemplary project

#### **PID\_20680 – Experimental assessment of the fish meal content requirements for Meagre (*Argyrosomus regius*) feeds.**

**Presented at:** Innovation Forum “Innovations in Nutrition and Feeding” European Aquaculture Society-Aquaexcel3

**Presenters:** a) Ramon Fontanillas, Skretting Aquaculture Innovation (Norway) b) Chatzifotis Stavros, Hellenic Centre for Marine Research (Greece)

**Objective:** To reduce the inclusion of fish meal in diets for grower meagre (*Argyrosomus regius*), while maintaining optimal growth performance and health through nutritional balance.

**Context:** Meagre aquaculture production has seen rapid growth, more than doubling since 2014. Fish meal, though nutritionally valuable (rich in phosphorus, lysine, EPA, and DHA), poses sustainability and cost concerns. The aim is to minimize fish meal use without compromising fish performance.

**Approach:** Development and testing of alternative diets with reduced fish meal content. Emphasis on maintaining balance in essential amino acids, vitamins, and



minerals. Focused on nutritional adequacy and practical application in commercial farming.

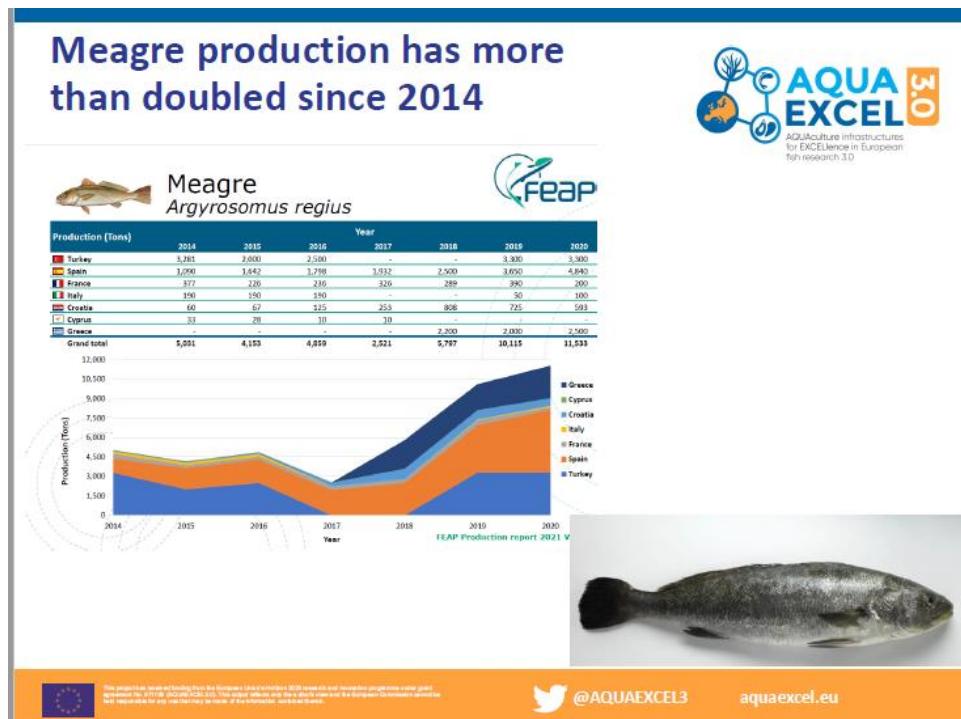
**Results:** Meagre diet has been produced with a 62% less use of fish meal

**Impact:** Supports the EU's sustainability goals in aquaculture. Potential to guide feed formulation strategies across the Mediterranean aquaculture sector.

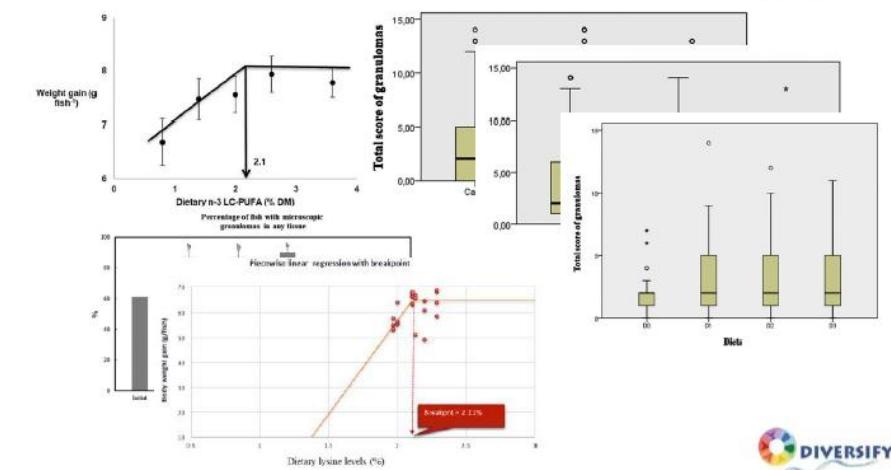


The slide features the AQUA EXCEL 3.0 logo and tagline: "AQUAculture infrastructures for EXCELlence in European fish research 3.0". Below the logo, the title "Fish meal reduction for grower Meagre (*Argyrosomus regius*)" is displayed. Underneath, the text "Innovation Forum ‘INNOVATIONS IN NUTRITION AND FEEDING’" is shown, followed by the names of the speakers: "Ramon Fontanillas, Skretting Aquaculture Innovation (Norway)" and "Chatzifotis Stavros, Hellenic Center for Marine Research (Greece)". At the bottom, there are logos for "hcmr EAKE GE" and "SKRETTING a Nutreco company". The slide also includes the European Union flag and the text "This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 871108 (AQUAEXCEL3.0). This output reflects only the author's view and the European Commission cannot be held responsible for any use that may be made of the information contained therein." Social media icons for Twitter and the URL "aquaexcel.eu" are also present.

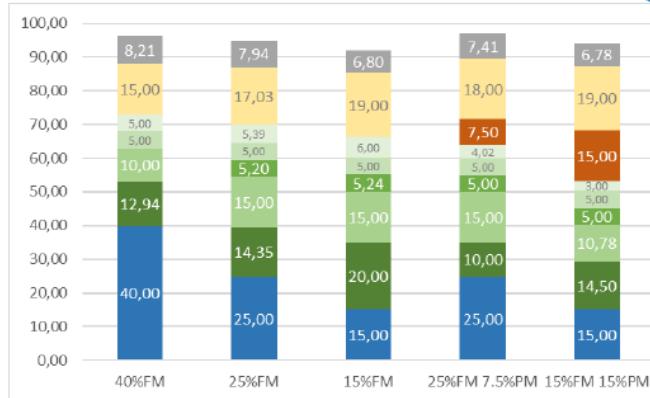




## Fish meal is a source of essential nutrients like P, Lys or EPA & DHA



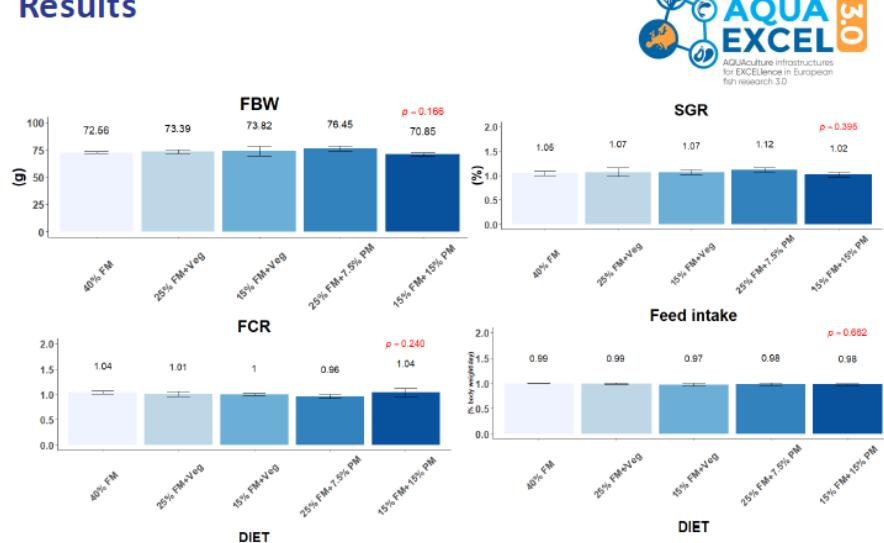
## Diet composition



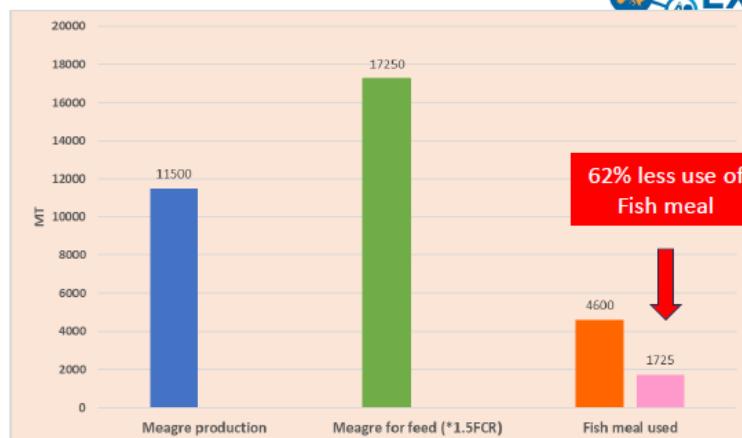
Balanced diets in terms of amino acids, minerals and vitamins



## Results



## IMPACT



@AQUAEXCEL3

aquaexcel.eu

Typically, 20-30 lines per project, potentially with a figure

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

Transnational Access (TNA) programs play an important role in fostering innovation, and collaboration in scientific research. By enabling researchers and small enterprises to access high-quality research infrastructures across borders, TNA promotes the exchange of ideas and expertise that might otherwise remain siloed within national or institutional boundaries. One of the key benefits of TNA is its ability to open new avenues for research as scientists from different backgrounds and institutions bring fresh perspectives and methodologies. Moreover, TNA significantly enhances the research capacity of smaller companies and early-career researchers. Many of these stakeholders lack their own sophisticated experimental facilities or testing environments. By gaining access to world-class installations through TNA initiatives, such as those supported by the AQUAEXCEL3.0 project, they can validate innovations, conduct advanced trials, and participate in high-level scientific work that would otherwise be out of reach.

### 4. References



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 871108 (AQUAEXCEL3.0). This output reflects only the author's view and the European Commission cannot be held responsible for any use that may be made of the information contained therein.

## 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°	D12.1	Title	Analysis of access provided by HCMR Mediterranean aquaculture experimental facilities: types and users
Work Package	N°	12	Title	TA5: Transnational Access to HCMR-Mediterranean aquaculture experimental facilities
Work Package Leader	HCMR			
Work Participants	HCMR			

Lead Beneficiary	Stavros Chatzifotis
Authors	Stavros Chatzifotis, HCMR, stavros@hcmr.gr
Reviewers	Jaime Perez Sanchez, CSIC, jaime.perez.sanchez@csic.es

Due date of deliverable	30.04.2025
Submission date	13.06.2025
Dissemination level	PU
Type of deliverable	R

Version log			
Issue Date	Revision N°	Author	Change
06.05.2025	1	Stavros Chatzifotis	First version
26.05.2025	2	Marc Vandeputte	Revision/validation

