Deliverable D14.1
Title: Analysis of access provided by IFREMER infrastructure: types and users

Version 1

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Executive Summary

Objectives: IFREMER has offered access to three infrastructures, two rearing molluscs (PMMB and PMMLT) on the Atlantic coast of France and one rearing European seabass in the Mediterranean Sea (PEARS).

Main Results: In total, 9 Transnational Access (TNAs) were conducted and 19 persons were trained. Users were all able to conduct the experiment they had planned in coordination with the local teams and the TNA managers. For the installations, some users followed up their stay with other collaborations, and overall, the three infrastructures appreciated the visitors and also learned in return. Along with the users, we received three PhD students for a long stay of 3 months for molluscs experimental work, and several master students came for short stays at PEARS.

Authors/Teams involved: Marie-laure Bégout and Christophe Stavrakakis, Ifremer.

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1. Overview of TNA user's projects realized in TNA IFREMER

1.1.1. Installations (short description of each installation in the local INFRA)

IFREMER Aquaculture infrastructure is composed of 3 installations:
1. IFR-PEARS (Palavas Experimental Aquaculture Research Station in Palavas-les-Flots) offers one 4000m^2 infrastructure located on the Mediterranean seashore, which includes independent units for research on fish performance, all suited for experiments with new or established species in larval, juvenile, or grow-out phase. The infrastructure includes 3 sections: 1-MES: Marine Ecotolerance Section, 2-FLA: From Larvae to Adult Fish and 3-IMTA: Integrated Multi-Trophic Aquaculture platform will allow the evaluation of performances and bioremediation capacity of different species ("inorganic and organic extractors") associated in different compartments.

2. IFR-PMMLT (La Tremblade Experimental Aquaculture Research Station) offers 1200m^2 experimental facilities dedicated to aquaculture research, specifically to the determinisms of resistance of marine molluscs to pathogens (viruses, bacteria, and protozoa), the genomic plasticity of marine shellfish and the evolution and understanding of pathogen virulence. They are intended to produce experimental families of bivalve molluscs (cupped oysters, flat oysters, mussels) and to carry out experiments on animals in farming. A process has been undertaken to consolidate its experimental aquaculture research system in order to guarantee the safety of its research families, but also to protect the environment by managing and securing its discharges and confining animals according to the anticipated risk.

3. IFR-PMMB (Mollusc Experimental Platform of Bouin) offers a 2000 m^2 infrastructure located on the Bourgneuf bay, with independent units for research on shellfish, including experiments from the larval level to the adult size. PMMB is suitable for completing any kind of experiments in ecotoxicology as it is equipped with a specific retention device for effluent.

Website address: 1. IFR-PEARS:https://www.ifremer.fr/en/research-infrastructures/palavas-marine-experimental-platform; 2. IFR-PMMLT : https://www.ifremer.fr/en/research-infrastructures/marine-mollusc-platform-la-tremblade; 3. IFR-PMMB :https://www.ifremer.fr/en/research-infrastructures/marine-mollusc-platform-bouin.

1.1.2. User projects

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

	Unit of Access	N allowed	N realized
PEARS	m3.week	1872	703
PMMLT	tank.week	952	1656
PMMB	m2.week	3348	400

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Installation number	Installation code	Project title	Project acronym	Description about the experiment	Coordinator and users	Affiliation of the user	Country	Period	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
IFR-1.	PEARS	Cryoplankton as an alternative live feed for Seabass	CRYOBASS	Fish nutrition	Konstantinos TZAKRIS	SME Planktonic	Norway	Q4 2022 Q4 2023	yes	m^3.week	292	yes	2
IFR-2.	PMMLT	Optimization of the larval cryopreservation in the Pacific oyster *Crassostrea gigas*	LACRY	Mollusk reproduction	Antonina DE MARCO	University of Bologna	Italy	Q2 2023	yes	tank.week	220.5	no	2
IFR-1.	PEARS	Transgenerational Epigenetic Inheritance in the European sea bass	TEI-BASS	Fish genetic	Fransec PIFERRER	CSIC	Spain	Q1 2023	yes	m^3.week	201	yes	2
IFR-1.	PEARS	Establishment of seabass as recipient for European eel spermatogonia xenotransplantation	BASSEEL	Fish reproduction	Marta BLANES	UPV	Spain	Q2 2023 Q2 2024	yes	m^3.week	90	yes	2
IFR-1.	PEARS	Epigenetic regulation of the onset of sexual size dimorphism in the European sea bass.	SSD-BASS	Fish genetic	Fransec PIFERRER	CSIC	Spain	Q1 2024	yes	m^3.week	55	yes	2
IFR-3.	PMMB	Toxicity tests with seeds of European flat oyster and Pacific cupped oyster	TOXSeed	Mollusk ecotoxicology	Dominique NOETZEL	University of Rostock	Germany	Q2 2024	yes	m^2.week	400	yes	3
IFR-3.	PMMLT	Exploring the protective power of novel next-gen probiotics against bacterial diseases of Pacific oysters	NGP	Mollusk desease resistance	Valentina RAMBOLI	Wageningen University	The Netherlands	Q2 2024	yes	tank.week	1040	yes	1
IFR-2	PMMB	Optimizing Ultrafiltration Techniques for reducing *Bonamia ostreae* risk and enhancing aquaculture biosecurity in hatcheries environments	OUTBREAK	Mollusk rearing system	Thiviya NAIR	DTU Aqua	Denmark	Q2 2025	yes	m^2.week	395	yes	3
IFR-1	PEARS	Sea bass Outfits Detection by Artificial intelligence	SODA	Fish & IA	Petr CISAR	JCU	Czech Republic	Q2 2025	yes	m^3.week	65	yes	2

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2. TNA projects

2.1.1. TNA projects description

CRYOBASS:
Use of live feed is a key element of success in larval rearing of marine fish. The most commonly used are rotifers and artemia, which imply a significant workload and necessary infrastructure. Planktonic now produces an off-the shelf solution to this, with cryopreserved larvae of *Balanus crenatus* (Cryoplankton Small) and *Semibalanus balanoides* (Cryoplankton Large), which can be re-vitalized after thawing for a few minutes in seawater (see https://planktonic.no/). In this project, we compared a protocol combining Cryoplankton and dry feed to the classical "clear water" methodology with artemia and dry feed for larval rearing of sea bass, and with an intermediate protocol using Cryoplankton, enriched artemia and dry feed. Effects on larval survival, larval and juvenile growth, larval quality, sex-ratio and deformities were studied.
The main results were satisfactory with similar growth observed with the different regime and our facility has now modified protocol to use Cryoplankton for larval rearing of seabass.
Users: 2, a senior scientist and an engineer.

LACRY
Shellfish farming is negatively affected by climate change, anthropogenic pressure, and lethal diseases that cause mass mortalities, leading to seed unavailability. These factors also impact the research world involved in genetic breeding programs on shellfish, by threatening the selected lines, and worst, by the loss of the animals. One way to secure the selected lines is cryopreservation. While sperm cryopreservation is a useful method, it only provides 50% of the germplasm, and 5 generations of reproduction using daughters and cryopreserved sperm are needed to get more than 95% of the germplasm. So, it is necessary to use a faster method, such as cryopreserved embryos, that allows protecting 100% of the genetics. This method requires to be optimized for marine bivalve larvae, in order to make a useful tool for the stakeholders in their production cycle management, and also for the researchers to preserve safely the samples of genetic work. The purpose of this research was to improve the methodology for the Pacific oyster larvae cryopreservation by evaluating three larval stages and two cryoprotectant agents' solutions. The expected outcome was to upgrade the technique that could ensure at least 10% of survival beyond the larval stage of thawed oyster larvae. From the experiments and analyses that were conducted in this project, it will be possible to understand which oyster larval stage is best for cryopreservation, and which cryoprotective agents allow greater survival with survival and growth rates close to standard under control. Thanks to this knowledge, the experimental protocol could be improved, aiming to overcome the successes achieved so far by researchers and reach at least 10% of thawed larvae capable of developing in spat.
User: 1 PhD student trained over 3 months

TEI-BASS
The inheritance of environmentally-induced traits, epigenetic marks, holds great promise for animal production, particularly in fish production, since data available so far indicate that, in contrast to mammals, some fish species experience little epigenetic reprogramming during early development. However, testing this is time-consuming, as it involves creating and following three different generations; therefore, it has been demonstrated only in a couple of fish model species. This project builds upon the fact that in sea bass, we already have available and characterized the first two generations needed to address this important question. This proposal, therefore, represents testing epigenetic inheritance for the first time in a species of commercial relevance. This project builds upon and is the logical continuation of two previous

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and successful projects. The overall objective of this proposal was to determine the presence and actual extent of transgenerational epigenetic inheritance in the European sea bass in order to evaluate whether this could be applied in sea bass farming to pass environmentally-induced desirable traits into the offspring as a complement to traditional genetic selection. Sampling proceeded as planned and data are being analysed.
Users: 2, a senior scientist and a master student.

BASSEEL
The European eel (*Anguilla anguilla*) is a commercial species whose aquaculture cycle is not closed yet. Nowadays, it is possible to obtain eel gametes after long-term hormonal treatments, but there is a high variability in their quality and the eel's response to the process. Recently, the surrogate broodstock technology in fish has been developed as a tool to produce donor-derived gametes from a recipient species by the xenotransplantation of the donor germ cells. Besides, before transplanting the germ cells, it is necessary to consider that the recipient's germ cells should be suppressed to prevent the recipient's offspring. Using a recipient species with a well-known reproduction in captivity as the European sea bass would avoid the long-term hormonal treatment for the European eel. Therefore, the research included:
1. The xenotransplantation of European eel spermatogonia isolated from fresh testis into triploid European sea bass larvae (60 dpf).
2. The monitoring of the transplanted cells in the recipient species.
3. The evaluation of the success of xenotransplantation by histology, fluorescence, and molecular analysis.

The main results are published :
Blanes-García Marta, Marinović Zoran, Morini Marina, Vergnet Alain, Horváth Ákos, Asturiano Juan F. (2024). Xenotransplantation of European Eel (*Anguilla anguilla*) Spermatogonia in Zebrafish (*Danio rerio*) and European Sea Bass (*Dicentrarchus labrax*). *Fishes*, 9(7), 290 (21p.). https://doi.org/10.3390/fishes9070290.
Users: 3, a PhD and 2 master students.

SSD-BASS
Growth is one of the most or perhaps the most important trait to be selected for in farm animal production, including aquaculture. Many fish species have sexual size dimorphism (SSD), meaning that one sex, usually the females, grow more than the other sex. This is the case of the European sea bass, a major aquaculture species in Europe. In this species, which lacks a strong chromosomal sex determining mechanism and instead relies on a polygenic system, growth and sex seem to be the two sides of the same coin, since a relationship with higher growth and females is present since the very beginning of the process of gonadal sex differentiation. This suggests that sex-specific gene expression programs related to growth are established early. However, the underlying epigenetic regulation of growth is completely unknown. In this project, we sampled sea bass at different times and studied both DNA methylation, a major epigenetic modification, and gene expression in four tissues related to growth and reproduction: brain, liver, muscle and gonads. Furthermore, we also tested whether the blood epigenome may recapitulate the epigenome of other tissues. The potential applications of these findings to aquaculture are important. Thus, identifying the underlying epigenetic basis of SSD would open the door to affect this process through environmental manipulation, as can be done with temperature or by affecting major players of the epigenetic mechanisms, such as DNA methyltransferases, as already done, or by direct editing of specific loci in the future.
Users: 2, a senior scientist and a master student.

TOXSeed
In coastal areas, heavy metal pollution poses a significant issue, stemming from human activities like industrial effluents, mining, and atmospheric deposition. This raises a specific concern for marine ecosystems, particularly oyster reefs, given their pivotal role in biodiversity

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maintenance and the provision of fundamental ecosystem services. In Europe, there's a growing interest in conserving the endangered European flat oyster (*Ostrea edulis*), including in the German Bight. While the habitat suitability of the benthic life stage has been examined in the past, a deeper dive into restoration sites and potential dispersal corridors for the planktonic larval stages is crucial, considering the potential impact of heavy metal pollution. Even with lower concentrations today (BSH 2016), there's a potential threat to the larval stages, known for being more sensitive to pollutants compared to adults.
Information on metal toxicity specific to *O. edulis* larvae is limited, with only zinc, copper, and mercury being studied. The relevance of the zinc and copper studies is, however, compromised by the absence of standard toxicity test information (see Ecological Effects Test Guidelines OCSPP 850.1000 2016). Moreover, this study aimed to incorporate climate change into the toxicity tests. This addition is based on the understanding that an increase in temperature can potentially influence the impact of contaminants, as suggested by previous research. This approach acknowledges the interaction of temperature and pollutants to the responses of the oyster larvae, providing a more comprehensive understanding of potential ecological implications. Expanding the scope, a comparative analysis of how *O. edulis* and the non-native Pacific Oyster (*C. gigas*) larvae respond to copper and zinc exposure was included. This comparison aimed to explore whether the non-native species might possess advantages in dealing with contaminants over European flat oyster larvae. As the planktonic stages of molluscs play a crucial role in dispersal and population connectivity, understanding how heavy metal exposure affects larval viability is an essential step in selecting restoration sites for the successful reintroduction of *O. edulis* in the future. The expected knowledge outputs are Acute toxicity tests (48 h) and Chronic toxicity tests: Concentration-response curves for *O. edulis* and *C. gigas* three larval stages for heavy metals; Determination of point estimates for: Mortality and shell malformation using classical ecotoxicological endpoints (LC50/EC50, LOEC, NOEC).
User: 1, a PhD student trained over 3 months

NGP
Bacterial pathogens cause substantial losses in aquaculture, significantly impacting commercially important species. Recognized as a primary limiting factor for aquaculture growth and economic value, diseases have far-reaching consequences in the aquaculture industry. Among the vulnerable species, oysters frequently succumb to bacterial diseases, notably those attributed to Vibrio spp. In response to these challenges, probiotics have emerged as promising tools for antibiotic-free aquaculture. Defined as live beneficial bacteria administered to hosts, probiotics mitigate bacterial infections through different mechanisms, including direct pathogen inhibition, nutrient and adhesion site competition, immunomodulatory enhancement, and water quality improvement. While the use of probiotics is promising in fish and shellfish aquaculture, only one probiotic bacterium, *Pediococcus acidilactici* CNCM I-4622 – MA 18/5M (commercially available as Bactocell) is authorized in European fish and shellfish aquaculture. Two other bacterial strains, *Bacillus pumilus* RI06-9 and *Phaeobacter inhibens* S4, have shown significant promise in enhancing Eastern oyster (*Crassostrea virginica*) survival against *V. coralliilyticus* and *V. tubiashii*, relying on *B. pumilus* that also demonstrated a reduction in total Vibrio abundance in tank water and surfaces. However, the application of these probiotics remains largely unexplored, aside from not having been designed for Pacific oysters.
In prior *in vitro* experiments, we established a next-generation probiotic biobank specifically designed for Pacific oyster aquaculture throughout several life stages, protecting against bacterial pathogens that threaten the production. The term "next-generation" denotes a personalized probiotic development strategy crafted to address the distinct needs of Pacific oysters. The selection of probiotic strains was methodical, considering their potential beneficial attributes in mitigating bacterial pathogens associated with Pacific oyster diseases. Specifically, the study has evaluated the impact of probiotic administration on oyster survival at the larval and adult stages through experimental infection challenges. For larvae, probiotics were administered via seawater and challenged with their respective bacterial-threatening agents, which are *V. coralliilyticus*, *V. harveyi*, *V. tubiashii*, *V. neptunius*, *V. europaeus*, and *V.*

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crassostreae. For the oyster adult stage, a second experiment was conducted, with probiotics administered via seawater and then challenged with the bacterial agent *V. aestuarianus*. All experimental infections were performed in secured facilities of the Ifremer platform in La Tremblade. These experimental studies validated probiotic functionality in oyster aquaculture by assessing their efficacy in reducing mortality during challenging events. The research also provided insights into the optimal concentration and duration of protection conferred by probiotics against pathogenic challenges in oysters. Following *in vivo* experimentation, the study will be extended to microbiome profiling and meta-transcriptomics analysis to further validate health claims.
User: 1, a PhD student trained over 3 months

OUTBREAK
The European flat oyster (*Ostrea edulis*) is an OSPAR-protected species that historically formed extensive reef habitats across European coastal ecosystems. These reefs significantly contributed to biodiversity, water quality improvement, and ecosystem services such as nutrient cycling. However, the species has suffered a severe population decline due to multiple anthropogenic pressures, including the global spread of oyster pathogens. A primary biological threat to *O. edulis* populations is *Bonamia ostreae*, a highly pathogenic haplosporidian parasite measuring 2-3 µm in size. *B. ostreae* can infect flat oysters through both horizontal transmission in the water column and vertical transmission from parent oysters to larvae. The Native Oyster Restoration Alliance (NORA) has identified the urgent need for enhanced pathogen management strategies as a critical component of effective oyster reef restoration. To ensure a consistent supply of healthy, uninfected broodstock, hatcheries must implement rigorous biosecurity protocols, including seawater treatment, extended quarantine periods for incoming animals, repeated disease testing, and thorough disinfection of all effluents before environmental release. In this context, *B. ostreae* has been selected as the focal pathogen for this study due to its prevalence in both the applicant's and host's regions and its classification as the primary biosecurity threat to flat oysters in these areas. This project evaluated the efficacy of ultrafiltration (UF) membranes for decontaminating *B. ostreae*-contaminated effluent seawater, a significant challenge in hatchery operations. Since *B. ostreae* cannot be cultured *in vitro*, the study used naturally infected oysters as the pathogen source. An environmental DNA (eDNA) method developed by the applicant, incorporating propidium monoazide (PMA) treatment, was used to distinguish viable from non-viable parasite cells. Water samples were collected at multiple points within the filtration system, followed by DNA extraction and qPCR analysis to assess pathogen removal rates under varying operational conditions. The study aimed to validate UF (and potentially other additional treatments) as an effective method for removing *B. ostreae* from contaminated seawater, providing a scientifically robust solution to enhance biosecurity, fulfil the aforementioned criteria and support certification for *B. ostreae*-free operations in aquaculture.
User: 1, a PhD student trained over 3 months

SODA
This study is a continuation of a successful TNA project (AQUAEXCEL2020 - AE140007), which focused on exploring the feasibility of individual fish identification based on the unique biometric patterns of European sea bass (representative of the fish species without obvious skin patterns), such as the scale patterns determined by machine vision systems. The original project was performed in 2020. The project results demonstrated that the image-based individual identification of sea bass is possible. The results have been published as a research paper. The study was performed with the anaesthetized fish out of the water, which limited the usability of the method. The development of the hardware and software enables the application of the approach to the fish swimming freely in the tank. Therefore, we wanted to record data on free-swimming sea bass in the aquarium and develop a method for image-based identification under real conditions. The output of this study will enable individualized fish treatment, which will lead not only to the optimization of feeding, real-time disease detection

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and precise biomass estimation but also to a suitable substitute for invasive tagging methodology. Furthermore, it will enable fish farmers to implement eco-intensive farming to improve the health and welfare of the animals and ultimately reduce the putative adverse effects.
Users: 2 - a senior scientist and a postdoctoral fellow; on site an Indonesian PhD student was also involved in the experiment.

2.1.2. Selection of One exemplary project

BASSEEL Project – Publication Blanes-Garcia et al. https://doi.org/10.3390/fishes9070290
The European eel encounters challenges in achieving sexual maturation in captivity, which has been a concern for researchers. This study explores surrogate broodstock technology as an alternative approach for eel production. The present study aimed to evaluate zebrafish and European sea bass as potential recipients for European eel spermatogonia transplantation, given the abundance of eel type A spermatogonia (SPGA). Immature European eel testes were dissected and maintained at 4 °C or cryopreserved. SPGA were obtained by dissociation of fresh or post-thawed tissue, employing an enzymatic solution, and then labelled with fluorescent membrane marker PKH26. SPGA from fresh tissue were transplanted into wild-type zebrafish larvae and triploid European sea bass larvae, while SPGA from cryopreserved testis were transplanted into *vasa:egfp* transgenic zebrafish larvae. One-and-a-half months post-transplantation (mpt), fluorescent donor cells were not detected in the gonads of zebrafish or European sea bass. Molecular qPCR analyses at 1.5 or 6 mpt did not reveal European eel-specific gene expression in the gonads of any transplanted fish. The findings suggest that the gonadal microenvironments of zebrafish and European sea bass are unsuitable for the development of European eel spermatogonia, highlighting distinctive spermatogonial stem cell migration mechanisms within teleost species

3. Reflection on results of the TNA programme

PEARS facility was already in the previous AquaExcel projects and hence benefited from previous experiences, AE3.0 was nonetheless rich in new interesting projects.
For the mollusc's platforms, the TNA projects were very interesting and their topics widen the activities at PMMB and PMMLT. New collaborations are very likely to emerge after all these TNAs at all sites.

4. References

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