

# Deliverable D24.1

## Analysis of access provided by CCMAR Infrastructure: types and users

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## Executive Summary

### Objectives

Located in Faro and Olhão, Portugal, the CCMAR and linked third-party IPMA infrastructure integrates three complementary installations: CCMAR Ramalhete Experimental Station, CCMAR Labs and Platforms, and the IPMA Aquaculture Research Station (EPPO). These installations provide advanced experimental setups, state-of-the-art laboratory platforms, and aquaculture facilities. The objective is to offer transnational access to high-quality research facilities that support marine biotechnology and aquaculture sciences.

### Main Results:

CCMAR and IPMA delivered 566 access units to support seven transnational research projects under the Aquaexcel3.0 program. This total included 383 access units at Ramalhete Station, 169 at CCMAR Labs & Platforms, and 14 at IPMA's Aquaculture Research Facilities. Access units were defined as tank.weeks for marine stations and person.days for labs.

The supported projects covered a wide range of topics, including fish development under light regimes, hormonal applications, invasive species, bivalve cancers, reproductive biology, thermal stress, and acoustic telemetry. Nine users received assistance from local scientists and technicians. There was significant participation from early-career scientists and a gender-balanced representation among the principal investigators (four female-led and three male-led projects). Participants hailed from Spain, Argentina, South Africa, Canada, Mexico, and Chile, highlighting the international scope of the program. Three projects integrated multiple CCMAR facilities, reflecting collaborative, cross-platform research approaches.

Two projects from China and Belgium were approved but had not yet started when this deliverable was written.

The visits contemplated the development of innovative research methodologies, data collection, and the advancement of experiments in marine biology, aquaculture, and biotechnology. For the infrastructure, the program strengthened international collaborations, enabled testing and refinement of new technologies—enhanced operational flexibility, improved visibility, and contributed significantly to scientific outputs, consolidating CCMAR and IPMA's roles as leading research infrastructures within the European Marine Research Area.

### Authors/Teams involved:

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

### 1.1.1. Installations

#### CCMAR Ramalhete Experimental Station (Faro, Portugal)

The Ramalhete Experimental Station is a specialized marine facility located by the Ria Formosa coastal lagoon. It offers indoor and outdoor tank systems with flexible experimental setups, tailored for research on marine species such as fish (seabass, seabream, sole), cephalopods (cuttlefish), and macroalgae. The station includes 500 m<sup>2</sup> of indoor facilities (wet and dry labs, environmentally controlled rooms) and a 900 m<sup>2</sup> outdoor area for larger-scale experiments. It supports studies on fish physiology, nutrition, behavior, aquaculture innovation, and the effects of environmental changes like acidification. Researchers can conduct experiments on early life stages of marine organisms, monitor long-term aquatic studies, and use a range of laboratory support services. The infrastructure allows customized experimental design and environmental control, crucial for precision marine biology research.

#### CCMAR Labs and Platforms (Gambelas Campus, Faro, Portugal)

The CCMAR Labs and Platforms facility provides access to advanced analytical platforms and cutting-edge technologies supporting marine and molecular research. It includes facilities for molecular biology (DNA/RNA sequencing, proteomics), analytical chemistry (HPLC, GC-MS, NMR, etc), imaging (fluorescence, light-sheet microscopy, etc), electrophysiology, and bioinformatics. The Labs and Platforms are essential for projects requiring high-resolution biological analysis, from cellular studies to ecosystem-level observations. Users benefit from well-equipped labs, dedicated technical support, and access to a vibrant research environment. This installation enables multidisciplinary approaches, combining experimental biology with analytical science to address key questions in marine biotechnology, physiology, and ecology.

#### IPMA Aquaculture Research Station (EPPO, Olhão, Portugal)

The IPMA Aquaculture Research Station (Estação Piloto de Piscicultura de Olhão, EPPO) is dedicated to research and technological development in marine aquaculture at a pre-industrial scale. Covering about 7 hectares, EPPO includes hatchery facilities (1,500 m<sup>2</sup>), support buildings (600 m<sup>2</sup>), experimental ponds, and facilities for pre-fattening and multitrophic aquaculture systems. The station maintains breeders of marine fish species, molluscs, and microalgae, providing opportunities for

experimental production cycles and technological testing. Research topics at EPPO include species diversification, integrated aquaculture systems, nutrition, and health of aquatic organisms. Access to EPPO enables users to perform large-scale experiments, develop new aquaculture techniques, and work within facilities that closely simulate commercial production environments.

### 1.1.2. User projects

As of April 2025, CCMAR has provided 383 access units at the Ramalhete Experimental Station and 169 access units at the CCMAR Labs & Platforms, while IPMA has provided 14 access units at the Aquaculture Research Facilities. Altogether, the infrastructures delivered a total of 566 access units across their installations, supporting seven transnational projects. Access units were defined as tank-weeks for experimental facilities (Ramalhete Station and IPMA-EPPO) and person-days for laboratories (CCMAR Labs & Platforms).

Six TNA projects received support at CCMAR, with one at IPMA, covering diverse areas such as fish development under different light regimes (Devsole), kisspeptin hormone application (KisspeptSole), invasive species impacts (BLUEIMPACT), contagious bivalve cancers (AQUA-CANCER), mussel reproductive hormones (Mussel GnRH), thermal stress biomarkers (AQUAMIR), and acoustic telemetry for welfare monitoring (SmartFishPonds).

Nine users, most of whom were early-career researchers, received local support from scientific and technical staff for experimental setup, fieldwork, laboratory techniques, telemetry, and welfare monitoring, which contributed to capacity building. Participation was gender-balanced, with four projects led by female researchers and three led by male researchers.

Users came from institutions across Spain, Argentina, South Africa, Canada, Mexico, and Chile, highlighting the strong international dimension of the TNA program.

Two additional projects involving two users have recently been approved: “Deorphanization of zebrafish olfactory receptors responding to alarm pheromone (DZORRAP)” and “Heat Shock Effects on Fatty Acid Metabolism in Artemia: A Degradation Analysis (T-FAMA).” However, the corresponding visits have yet to begin. When executed, the list of participating countries will

expand to include P.R. China and Belgium, contributing to an increase in the total number of access units delivered, particularly CCMAR Labs & Platforms.

Three of the projects involved the combined use of different CCMAR installations (e.g., Ramalhete Station and Labs & Platforms).

### 1.1.2.1 Summary of Transnational Access (TNA) at CCMAR and IPMA access units (2023 – April 2025):

	1- CCMAR-IPMA (Aquaria Facilities)	1- CCMAR (Ramalhete Exp Station)	2-CCMAR (Labs & Platforms)
Min. quantity of access units to be provided according to the DoA:	<b>288</b>	<b>672</b>	<b>112</b>
Total number of access units (sum of access units in the table)*:	<b>14</b>	<b>383</b>	<b>169</b>

\*Excluding the two approved projects whose visits have not yet started  
(PID:33508; PID: 36417)

### 1.1.2.2 Overview of Transnational Access (TNA) at CCMAR and IPMA: Research Installation Usage and Project Outcomes Under TNA(2023 – April 2025)

Installation number	Installation code	Project title	Project acronym	Description about the experiment	Coordinator /users	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-CCMAR	CCMAR (Ramalhete Exp Station)	Development and growth of Senegalese sole exposed to different light regimes or endocrine transcriptional regulators	PID: 18805 Devsole	Studied how light spectra and endocrine regulators affect early development and growth of Senegalese sole	Inigo Novales Flamarique/ Kennedy Bolstad	yes	tank.week	132		1

1-CCMAR	CCMAR (Ramalhete Exp Station)	The use of slow-release kisspeptin implants for hormonal induction in Senegalese Sole	PID: 28948 KisspeptSole	Tested slow-release kisspeptin implants to stimulate reproduction and improve gamete quality in Senegalese sole	Alejandro Sebastián Mechaly	yes yes	tank.week	44	yes (a) b)	1
2-CCMAR	CCMAR (Labs & &Platforms)						person.day	46		
1-CCMAR	CCMAR (Ramalhete Exp Station)	Potential impacts of the Invasive Blue Crab on the bivalve aquaculture of Ria Formosa lagoon: feeding preferences and environmental constraints	PID: 28840 BLUEIMPACT	Analyzed feeding behavior and environmental limits of invasive blue crabs to assess impacts on bivalve aquaculture	Kurt Manna/ Carlos Rosas Vázquez	yes	tank.week	180	Yes (in preparation)	1
2-CCMAR	CCMAR (Labs & &Platforms)					yes	person.day	19		
1-CCMAR	CCMAR (Ramalhete Exp Station)	Managing Contagious Cancers in Marine Bivalves: Aquaculture tool	PID: 28698 AQUA-CANCER	Screened bivalve species for contagious cancers (BTN) to develop disease monitoring tools for aquaculture	Alicia L. Bruzos	yes	tank.week	27	yes c)	1
2-CCMAR	CCMAR (Labs & &Platforms)					yes	person.day	20		
2-CCMAR	CCMAR (Labs & &Platforms)	Gonadotropin-releasing hormone-like signalling systems in the edible mussel: requisite information for aquaculture	PID: 30839 Mussel GnRH	Investigated reproductive hormone systems in mussels to support growth and breeding strategies in aquaculture	Heather Marco/ Gerd Gäde	yes	person.day	44	Yes (in preparation)	2
2-CCMAR	CCMAR (Labs & &Platforms)	In vitro functional validation of mRNA-miR interactions with relevance in aquaculture	PID: 34208 AQUAMIR	Validated miR-mRNA interactions linked to climate stress to identify molecular	Laura Calvo Rodriguez	yes	person.day	40	Yes (in preparation)	1

				biomarkers for aquaculture resilience						
1 -IPMA	CCMAR-IPMA (Aquaria Facilities)	Applied technology for studying behaviour and welfare of fish cultivated in ponds	PID:29873 SmartFishPonds	Improve welfare and behaviour monitoring of fish reared in extensive earthen-ponds by acoustic telemetry	Esther Hoyo Alvarez/ Andrea Martínez Villalba	yes	tank.week	14	Yes (in preparation)	2

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

**a)**Peer-reviewed article Mechaly, A.S., Awruch, C., Cabrita, E., Costas, B., Fernandes, J.M.O., Gallego, V., Hirt-Chabbert, J., Konstantinidis, I., Oliveira, C., Ramos-Júdez, S., Ramos-Pinto, L., Fatsini, E. (2024). *Cutting-edge Methods in Teleost and Chondrichthyan Reproductive Biology. Reviews in Fisheries Science and Aquaculture*, 33(1), 77-112. <https://doi.org/10.1080/23308249.2024.2377999>

**b)**Conference presentation: Mechaly, A.S., Awruch, C., Cabrita, E., Costas, B., Fernandes, J.M.O., Gallego, V., Hirt-Chabbert, J., Konstantinidis, I., Oliveira, C., Ramos-Júdez, S., Ramos-Pinto, L., Fatsini, E. (2024). *Métodos Innovadores en la Biología Reproductiva de Teleósteos y Condrictios. I Jornadas Latinoamericanas. Una Salud / Una Acuicultura. Buenos Aires, Argentina. 13–15 August 2024.*

**c)**Peer-reviewed article (in submission phase). Title: *Prevalence of hemic neoplasia in cockles and co-habiting bivalve species in the western coast of France and the southern coast of Portugal*; Authors: Alicia L. Bruzos, Angèle Moulin, Géo Bujard, Anthony Sturbois, Alejandro Viña-Feás, Ana Amaral, Camille Détrée

### 1.1.2.3 Overview of Transnational Access (TNA) Users at CCMAR and IPMA (2023 – April 2025):

TNA user	TNA user affiliation		User-project acronym	Installations used by the User		
	Name	Country		Infrastructure Short Name	Number of access units provided	User Access Period
Kennedy Bolstad	Simon Fraser University	Canada	PID: 18805 Devsole	CCMAR (Ramalhete Exp Station)	132 tank.week	05/05/2022 to 29/07/2022
Alejandro Sebastián Mechaly	Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina)	Argentina	PID: 28948 KisspeptSole	CCMAR (Ramalhete Exp Station)	44 tank.week	02/04/2024 to 11/06/2024
				CCMAR (Labs & &Platforms)	46 person.day	
Carlos Rosas Vázquez	Universidad Nacional Autónoma de México	México	PID: 28840 BLUEIMPACT	CCMAR (Ramalhete Exp Station)	180 tank.week	06/10/2024 to 31/10/2024
				CCMAR (Labs & &Platforms)	19 person.day	
Alicia L. Bruzos	Université de Caen Normandie	France	PID: 28698 AQUA-CANCER	CCMAR (Ramalhete Exp Station)	27 tank.week	08/07/2024 to 02/08/2024
				CCMAR (Labs & &Platforms)	20 person.day	
Heather Marco	University of Cape Town	South Africa	PID: 30839 Mussel GnRH	CCMAR (Labs & &Platforms)	22 person.day	01/10/2024 to 30/10/2024
Gerd Gäde				CCMAR (Labs & &Platforms)	22 person.day	
Laura Calvo Rodriguez	Universidad de León	Spain	PID: 34208 AQUAMIR	CCMAR (Labs & &Platforms)	40 person.day	17/02/2025 to 11/04/2025
Esther Hoyo Alvarez	Mediterranean Institute of Advanced Studies (IMEDEA-UIB/CSIC)	Spain	PID: 29873 SmartFishPonds	CCMAR-IPMA (Aquaria Facilities)	14 tank.week	01/05/2024 to 31/07/2024
Andrea Martínez Villalba	University Complutense of Madrid					30/04/2024 to 01/08/2024

## 2. TNA projects

### 2.1.1. TNA projects description

#### **PID 18805 – Development and growth of Senegalese sole exposed to different light regimes or endocrine transcriptional regulators (Devssole)**

This project studied the effects of different light spectra (blue, green, red, white, and darkness) and endocrine regulators (thyroid hormone and retinoic acid) on early development, retinal formation, and metamorphosis in Senegalese sole (*Solea senegalensis*). The larvae were exposed from fertilized eggs under controlled light conditions, and hormonal treatments were applied separately to assess their impacts on visual system development and growth performance.

The experiment aimed to “1) identify an optimal light spectrum for rearing *Senegalese sole* larvae, with focus on growth and 2) describe the effects of thyroid hormone and retinoic acid on development and growth of *Senegalese sole* larvae, with focus on eye development”

“From the results obtained we were able to conclude that the most suitable light to rear Senegalese sole larvae were blue and green wavelengths, since in these groups larvae showed the best growth and survival rates. In what concerns the chemicals tested on the larvae, we observed that the effects on total length, were inconclusive. However, in metamorphosis, L-thyroxine seemed to have a more evident effect. Due to larval mortality in retinoic acid prior to metamorphosis it was not possible to evaluate its effect. More studies on the effect of these chemicals are needed, especially on their effect on all larval development.”

The possibility of publication is high, given the relevance of the results to developmental biology, fish aquaculture, and hatchery management optimization. However, due to the PhD student's departure from the home institution, the analysis is currently on hold until a new student is assigned to continue the work.

#### **PID 28948 – The use of slow-release kisspeptin implants for hormonal induction in Senegalese Sole (KisspeptSole)**

This project explored the use of slow-release kisspeptin-12 implants to hormonally induce reproduction in *Senegalese sole*. Kisspeptin is known to regulate reproductive functions, but its delivery via implants in fish was previously untested. Researchers evaluated the effects on sex steroid levels and gamete (sperm) quality and obtain good results.

Possibility of new publication is high and findings already contribute to Innovative methods in the reproductive biology of Teleosts and Chondrichthyans. One Peer-reviewed article was already published Mechaly, A. S., Awruch, C., Cabrita, E., Costas, B., Fernandes, J. M. O., Gallego, V., Hirt-Chabbert, J., Konstantinidis, I., Oliveira, C., Ramos-Júdez, S., Ramos-Pinto, L., & Fatsini, E. (2024). *Cutting-edge methods in teleost and chondrichthyan reproductive biology. Reviews in Fisheries Science & Aquaculture*.

<https://doi.org/10.1080/23308249.2024.2377999> and one Conference oral presentation in Argentina was performed (Mechaly, A.S., Awruch, C., Cabrita, E., Costas, B., Fernandes, J.M.O., Gallego, V., Hirt-Chabbert, J., Konstantinidis, I., Oliveira, C., Ramos-Júdez, S., Ramos-Pinto, L., Fatsini, E. (2024). *Métodos Innovadores en la Biología Reproductiva de Teleósteos y Condrictios. I Jornadas Latinoamericanas. Una Salud / Una Acuicultura*. Buenos Aires, Argentina. 13–15 August 2024).

**PID 28840 – Potential impacts of the Invasive Blue Crab on the bivalve aquaculture of Ria Formosa lagoon: feeding preferences and environmental constraints (BLUEIMPACT)**

The study assessed the feeding preferences and environmental tolerances of the invasive blue crab (*Callinectes sapidus*) in the Ria Formosa lagoon. Experiments tested prey selection of the invasive blue crab in relation to a group of bivalves with economical value in aquaculture and harvest in Ria Formosa lagoon and evaluate the impacts of environmental fluctuation (temperature, salinity and oxygen dissolved) on the blue crab metabolic physiology.

“We used two types of set-ups for the prey selection experiments in which a single crab was tested per trial – a) open arenas where a mix of different species of bivalves were available to each crab, and b) Y-mazes, with running flumes, where different individual bivalves were presented in each arm. First selection, number of bivalves handled and/or eaten per species, handling time, etc were our main parameters in the arena, while arm selection, and consumption were used in Y-mazes.”

Results showed clear prey selectivity. Handling and feeding times varied significantly among species. The findings indicate the species’ strong adaptability and potential impact on local bivalve aquaculture, supporting the development of targeted management and mitigation strategies. Data analysis is ongoing.

This study has a high probability of being published. It addresses a relevant and timely topic, the impact of an invasive species on aquaculture ecosystems and provides both behavioral (prey selection) and physiological (metabolic and oxygen tolerance) data. These results are valuable for fields such as invasion biology, aquaculture management, and conservation ecology.

**PID 28698 – Managing Contagious Cancers in Marine Bivalves: Aquaculture tool  
Name/Acronym: AQUA-CANCER**

The project aimed to detect and characterize contagious cancers—specifically Bivalve Transmissible Neoplasia (BTN)—in three bivalve species collected from the Ria Formosa and Alvor estuaries. Sampling was conducted across multiple sites along the southern coast of Portugal, focusing on common cockles (*Cerastoderma edule*) and cohabiting species (*Ruditapes* spp. and *Scrobicularia plana*). Diagnosis to date has been based on hemolymph cytology. In addition, morphometric analyses were carried out to compare the size and weight of infected and uninfected cockles.

“The primary objective of this project was to identify and characterize the presence of hemic neoplasia in three bivalve species from the Alvor estuary and Ria Formosa. A secondary objective was to evaluate the prevalence of hemic neoplasia in common cockles over the past decade, comparing our findings to previous studies.”

“Sampling was carried out in summer 2024 taking into account weather and tidal conditions. Bivalves of different sizes were collected from natural sandbeds in intertidal zones using manual sampling methods to ensure representative sample sizes for prevalence assessment. The number of samples varied slightly due to natural differences in bivalve populations, but a minimum of 100 individuals per site was targeted for each region (Alvor vs. Fuzeta – Ria Formosa – Olhão).”

“Hemic neoplasia in bivalves is a contagious cancer characterized by the transmission of cancerous cells from one individual to another within a population; in certain cases, it can even spread to other species, highlighting the complex dynamics of disease spread in marine

ecosystems. The AQUA-CANCER project aimed to identify and characterize hemic neoplasia in three bivalve species from the Alvor estuary and Ria Formosa while evaluating historical prevalence in cockles. A total of 685 bivalves were collected, and the methodology included morphometric analyses and cytological diagnoses. Findings indicate a decrease in hemic neoplasia prevalence in cockles from Ria Formosa, with rates of 23.1% in 2017, around 14% in 2020, and 9.3% in 2024. No cases were found in cohabiting clam species, suggesting potential resistance. Morphometric data is being digitized for future analysis and will be made available to enhance our understanding of contagious cancers in bivalves and improve aquaculture management. Access to CCMAR was essential for the success of this project."

The possibility of publication is very high and one Peer-reviewed article is in phase of submission. Title: Prevalence of hemic neoplasia in cockles and co-habiting bivalve species in the western coast of France and the southern coast of Portugal; Authors: Alicia L. Bruzos, Angèle Moulin, Géo Bujard, Anthony Sturbois, Alejandro Viña-Feás, Ana Amaral, Camille Détrière.

**PID 30839 – Gonadotropin-releasing hormone-like signalling systems in the edible mussel: requisite information for aquaculture (Mussel GnRH).**

This project aimed to characterize the gonadotropin-releasing hormone (GnRH) system in the Mediterranean mussel (*Mytilus galloprovincialis*), focusing on gene identification and functional studies of reproduction-related pathways.

"We wanted to establish basic information about the control of reproduction and growth in the Mediterranean mussel. Given the short period for research, we focused our research stay on investigating possible role players in mussel reproduction.

The first scientific objective was to elucidate the gonadotropin-releasing hormone (GnRH)-like structure from *Mytilus galloprovincialis*. The second objective was to amplify the GnRH-like receptor of the mussel. Finally, because it is very likely that other peptide hormones may be modulators of GnRH activity, a third objective was to identify other role players and their cognate receptors (where possible) to elucidate the regulatory pathways involved in molluscan reproduction.

"We are very pleased that we achieved our major objectives and have paved the way for more applied experiments using our basic data sets. The knowledge is new for *M. galloprovincialis* and for other mussel species. It expands on the little information that is known from other molluscs. Our work is not publishable in its current form, we need time for certain test components to arrive, e.g. peptides in synthetic form".

**PID 34208 – *In vitro* functional validation of mRNA-miR interactions with relevance in aquaculture (AQUAMIR)**

This project validated predicted interactions between microRNAs (miRs) and mRNAs in rainbow trout (*Oncorhynchus mykiss*) under thermal stress, using *in vitro* co-transfection assays with reporter constructs. The goal was to establish molecular biomarkers for climate resilience in aquaculture.

Data analysis is ongoing. The study has a high publication potential.

**PID 29873 – Applied technology for studying behaviour and welfare of fish cultivated in ponds (SmartFishPonds)**

This project aims to enhance the monitoring of fish welfare and behaviour monitoring of fish welfare and behaviour in extensive earthen ponds through the application of acoustic telemetry. The use of this technology allows for non-invasive, real-time observation of fish movement patterns, contributing to a better understanding of welfare indicators under semi-natural conditions.

The results of this work are currently under analysis and have not yet been published. The project involved two Transnational Access (TNA) users from the Mediterranean Institute of Advanced Studies (IMEDEA-UIB/CSIC) and from the Complutense University of Madrid. The users carried out their research at the Aquaculture Research Station (EPPO) of the Portuguese Institute of the Sea and Atmosphere (IPMA) over a period of approximately three months.

The study has strong publication potential, particularly in the field of aquaculture technology.

### 2.1.2. Selection of One exemplary project

#### **PID 28948 – The use of slow-release kisspeptin implants for hormonal induction in Senegalese Sole (KisspeptSole)**

Findings from this TNA project have already contributed to the advancement of innovative methods in the reproductive biology of teleosts and chondrichthyans. One peer-reviewed article has been published:

Mechaly, A. S., Awruch, C., Cabrita, E., Costas, B., Fernandes, J. M. O., Gallego, V., Hirt-Chabbert, J., Konstantinidis, I., Oliveira, C., Ramos-Júdez, S., Ramos-Pinto, L., & Fatsini, E. (2024). *Cutting-edge methods in teleost and chondrichthyan reproductive biology. Reviews in Fisheries Science & Aquaculture*. <https://doi.org/10.1080/23308249.2024.2377999>

Additionally, the results were presented in an oral presentation at a regional conference in Argentina:

Mechaly, A. S., Awruch, C., Cabrita, E., Costas, B., Fernandes, J. M. O., Gallego, V., Hirt-Chabbert, J., Konstantinidis, I., Oliveira, C., Ramos-Júdez, S., Ramos-Pinto, L., & Fatsini, E. (2024). *Métodos Innovadores en la Biología Reproductiva de Teleósteos y Condrictios. I Jornadas Latinoamericanas: Una Salud / Una Acuicultura*, Buenos Aires, Argentina, 13–15 August 2024.

These results were generated through experimental work conducted at CCMAR's research facilities, within the framework of the AQUAEXCEL3.0 transnational access program, and contribute to the broader methodological context outlined by Mechaly et al. (2024).

“Reproduction is a fundamental biological process for producing offspring and ensuring the continuation of the species. With nearly 35,000 fish species worldwide exhibiting a very diverse range of reproductive systems and strategies, the study of fish reproduction is a significant challenge. Research into various fish reproductive models is driven by their use in human biomedical applications, improving the diversification and sustainability of aquaculture species, and developing strategies for biodiversity conservation. These models must be approached

from an animal ethics perspective, considering the welfare of the species under study and applying the 3Rs principle (Replacement, Reduction and Refinement). This principle focusses on replacing animals with alternative methods wherever possible, reducing the number of animals used and refining procedures to minimize suffering. While destructive sampling of wild aquatic species should be minimized, the need remains to provide information on reproduction as an important parameter for delineating management and conservation programs. One of the practices that helps reduce and replace the use of animal testing is the scientific study of individuals obtained through bycatch. This is the case for some chondrichthyans, and endangered fish species included in conservation programs. Regarding fish species that are kept under human care (commonly called captivity), studies on reproduction are essential to improve well-being and increase production of farmed species. Research on fish reproduction requires expanding basic knowledge of broodstock management tools, such as gamete quality assessment, reproductive behavior studies, the use of hormone therapies to stimulate reproduction, and the development of non-lethal or less invasive methods using body fluids (e.g., mucus, seminal plasma, blood plasma, or urine) to study reproductive status, as well as novel markers from state-of-the-art omics research. This review describes a multidisciplinary approach that includes the aforementioned reproductive management tools, indicators of welfare, and next-generation sequencing techniques using samples collected by minimally invasive methods. (Mechaly *et al.* (2024).)

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

The TNA programme at CCMAR and IPMA effectively supported seven projects, advancing research in fish biology, aquaculture technology, and environmental resilience. These projects expanded expertise in acoustic telemetry, validation of molecular biomarkers, and welfare monitoring. Collaborations with international partners reinforced scientific networks and raised the profile of the facilities.

The projects showcased the effectiveness of cutting-edge techniques, such as real-time monitoring in pond systems. Users expressed strong appreciation for the high quality of the facilities, technical assistance, and collaborative atmosphere. The programme improved operational flexibility and diversified the services provided.

Future initiatives should continue to encourage early user engagement and emphasise technological capabilities. Overall, the TNA programme made significant contributions to scientific excellence and capacity building.

### 4. References

Mechaly, A. S., Awruch, C., Cabrita, E., Costas, B., Fernandes, J. M. O., Gallego, V., Hirt-Chabbert, J., Konstantinidis, I., Olivera, C., Ramos-Júdez, S., Ramos-Pinto, L., & Fatsini, E. (2024). Cutting-edge methods in teleost and chondrichthyan reproductive biology. *Reviews in Fisheries Science & Aquaculture*.

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Work Package Leader				
Work Participants				

Lead Beneficiary	CENTRO DE CIENCIAS DO MAR DO ALGARVE (CCMAR), Partner 19
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