



LIFE Project Number

LIFE20 NAT/IT/001122

Final Report

Covering the project activities from 01/10/2021 to 30/09/2025

Reporting Date¹

23/01/2026

LIFE PROJECT NAME or Acronym

LIFE PINNA

Data Project

Project location:	Italy, Slovenia
Project start date:	01/10/2021
Project end date:	30/09/2025 Extension date: N/A
Total budget:	€ 2,965,885
EU contribution:	€ 1,753,700
(%) of eligible costs:	59.13%
Data Beneficiary	
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¹ Include the reporting date as foreseen in part C2 of Annex II of the Grant Agreement

This table comprises an essential part of the report and should be filled in before submission

Please note that the evaluation of your report may only commence if the package complies with all the elements in this receivability check. The evaluation will be stopped if any obligatory elements are missing.

Package completeness and correctness check	
Obligatory elements	✓ or N/A
Technical report	
The correct latest template for the type of project (e.g. traditional) has been followed and all sections have been filled in, in English <i>In electronic version only</i>	✓
Index of deliverables with short description annexed, in English <i>In electronic version only</i>	
<u>Mid-term report</u> : Deliverables due in the reporting period (from project start) annexed <u>Final report</u> : Deliverables not already submitted with the MTR annexed including the Layman's report and after-LIFE plan Deliverables in language(s) other than English include a summary in English <i>In electronic version only</i>	✓
Financial report	
The reporting period in the financial report (consolidated financial statement and financial statement of each Individual Beneficiary) is the same as in the technical report with the exception of any terminated beneficiary for which the end period should be the date of the termination.	✓
Consolidated Financial Statement with all 5 forms duly filled in and signed and dated <i>Electronically Q-signed or if paper submission signed and dated originals* and in electronic version (pdfs of signed sheets + full Excel file)</i>	✓
Financial Statement(s) of the Coordinating Beneficiary, of each Associated Beneficiary and of each affiliate (if involved), with all forms duly filled in (signed and dated). The Financial Statement(s) of Beneficiaries with affiliate(s) include the total cost of each affiliate in 1 line per cost category. <i>In electronic version (pdfs of signed sheets + full Excel files) + in the case of the Final report the overall summary forms of each beneficiary electronically Q-signed or if paper submission, signed and dated originals*</i>	✓
Amounts, names and other data (e.g. bank account) are correct and consistent with the Grant Agreement / across the different forms (e.g. figures from the individual statements are the same as those reported in the consolidated statement)	✓
Mid-term report (for all projects except IPs): the threshold for the second pre-financing payment has been reached	
Beneficiary's certificate for Durable Goods included (if required, i.e. beneficiaries claiming 100% cost for durable goods) <i>Electronically Q-signed or if paper submission signed and dated originals* and in electronic version (pdfs of signed sheets)</i>	✓
Certificate on financial statements (if required, i.e. for beneficiaries with EU contribution ≥ 750,000 € in the budget) <i>Electronically Q-signed or if paper submission signed original and in electronic version (pdf)</i>	
Other checks	
Additional information / clarifications and supporting documents requested in previous letters from the Agency (unless already submitted or not yet due) <i>In electronic version only</i>	✓
This table, page 2 of the Mid-term / Final report, is completed - each tick box is filled in <i>In electronic version only</i>	✓

**signature by a legal or statutory representative of the beneficiary / affiliate concerned*

Instructions:

Please refer to the General Conditions annexed to your grant agreement for the contractual requirements concerning a Mid-term/Final Report.

Both Mid-term and Final Technical Reports shall report on progress from the project start-date. The Final Report must be submitted to the Agency no later than 3 months after the project end date.

Please follow the reporting instructions concerning your technical report, deliverables and financial report that are described in the document [Guidance on how to report on your LIFE 2014-2020 project](#), available on the LIFE website. Please check if you have the latest version of the guidance as it is regularly updated. Additional guidance concerning deliverables, including the layman's report and after-LIFE plan, are given at the end of this reporting template.

Regarding the length of your report, try to adhere to the suggested number of pages while providing all the required information as described in the guidance per section within this template.

1. Table of contents

1.	4
2.	5
3.	6
4.	8
5.	10
6.	12
7.	85
8.	87
8.1.	87
8.2.	114
8.3.	116
8.4.	116
8.5.	117

2. List of key-words and abbreviations

ARPAL - Agenzia Regionale per la Protezione dell'Ambiente Ligure
ASINARA - Parco Nazionale dell'Asinara
NIB - National Institute of Biology
Shoreline - Shoreline Società Cooperativa
Triton - Triton Research S.r.l.
UNIGE - Università degli Studi di Genova
UNISS - Università degli Studi di Sassari
IMC - International Marine Center
MATTM - Ministero dell'Ambiente e della Tutela del Territorio e del Mare
MASE - Ministero dell'Ambiente e della Sicurezza Energetica
ISPRA - Istituto superiore per la protezione e la ricerca ambientale
MEDPAN - Network of Marine Protected Areas managers in the Mediterranean
MPA - Marine Protected Area
GA - Grant Agreement
PA - Partnership Agreement
AB - Associated beneficiaries
CB - Coordinating Beneficiary
PR - Project Responsible
PM - Project Manager
FM - Financial Manager
SC - Steering Committee
HPF - hours post fertilisation
PCR - Polymerase Chain Reaction
qPCR - quantitative Polymerase Chain Reaction (operated by Real Time)
SSU - ribosomal Short SubUnit
LSU - ribosomal Long SubUnit
MME - Mass Mortality Event of *Pinna nobilis*
CT - Cycle threshold (at the Real Time during the qPCR)
COI - mitochondrial Cytochrome c Oxidase subunit I
STR - Short Tandem Repeat
ITS - ribosomal Internal Transcribed Spacer

3. Executive Summary

Project objectives

The Life PINNA project kicked off on 1/10/2021 with the aim of curbing the severe die-off of *Pinna nobilis*, which has spread throughout the Mediterranean since 2016, starting from southern Spain, leading to the near disappearance of the populations there. The main objectives of the project are extremely ambitious and involve, on the one hand, the reconstitution of part of the original populations of the species through the translocation of individuals from sites where there is still a good density of these animals to others where they have undergone a steep decline. On the other hand, the project aims to achieve successful captive breeding to create a substantial reservoir of individuals in a protected environment. Even from this brief description, it is evident that the project can be defined as high risk. However, it is equally evident how urgent it is to act promptly to arrive in time and prevent the extinction of the species

Achievements, deviations, problems and difficulties met.

At the project kick-off meeting (Dec 2021) the project coordination team was nominated:

- ARPAL: Daniela Caracciolo (project responsible) and Maria Grazia Pizzoni (project financial) supported by Istituto Delta (External assistance)
- TRITON: Valeria Pulieri and then Diego Mattioli as Project Manager

Furthermore, the Scientific Committee was composed by: Antonio Terlizzi, Alberto Castelli, and Jamila Ben Souissi.

The preparatory actions of the project, A1, A2, A3, were carried out smoothly.

Action A1 involved characterising the 2 donor sites envisaged by the project (Strunjan MPA, Miramare MPA) and assessing the suitability of the 4 receiving sites intended to host the new populations of *Pinna nobilis* (Capo Mortola MPA, Asinara MPA, Strunjan MPA, Miramare MPA). From this perspective, the molecular characterization of sentinel species carried out in action A2 was crucial. Sentinel species are filter-feeding bivalves species other than *Pinna nobilis* (e.g., mussels and oysters) that provide information on the presence or absence of pathogens in the potential intervention areas. The aim of these activities was to prevent both the transfer of *P. nobilis* individuals to areas where pathogens are (still) present and the transfer of *P. nobilis* individuals from areas still infected to other pathogen-free areas. The investigations focused on the search for *Haplosporidium pinnae*, a protozoan considered the main suspect in the decline of *P. nobilis*, as well as other microorganisms possibly involved as etiological agents in the disease, such as *Mycobacterium* spp., *Vibrio* spp., other protozoa belonging to the genus *Bonamia*, along with viruses including Picornavirus and Ostreid Herpesvirus Type 1.

Simultaneously, within action A3, a list of stakeholders was created, both through bibliographic research and by administering an online questionnaire to selected target subjects. This effort allowed for the compilation of a list of individuals, beneficial for networking actions aimed at collaborations and exchanges of experiences regarding the conservation of *Pinna nobilis*.

Finally, among the preparatory activities for the actions that represent the core of the project, the request for authorization under DPR 357/97 must be mentioned. In Italy, to manipulate Natura 2000 species such as *Pinna nobilis*, a special authorization must be obtained from the Ministry of the Environment and Energy Security (MASE), based on a highly detailed feasibility study. In particular, for the translocation of *Pinna nobilis*, a lot of information was requested to ensure the genetic compatibility of the populations present (currently or in the past) in the donor and recipient sites. Following numerous exchanges with MASE and ISPRA (the Higher Institute for Environmental Protection and Research), the scientific-technical body that MASE relies on, the authorization was issued on 24/5/2022 and subsequently, after the submission of some additions, on 30/3/2023.

The Conservation actions, C1-5, have been undertaken, leading to:

- Precisely identifying the implantation sites of *P. nobilis* within the receiving sites.
- Genetically characterising the living specimens of *P. nobilis*: all known individuals belonging to surviving populations have been genotyped, throughout the project duration by UNISS.

- Collection of a total of 34 specimens of *P. nobilis* in the Venice Lagoon and transferring them to the UNIGE laboratory (30 specimens) and Shoreline laboratory (4 specimens). During the years of the project, we carried out 20 spawning induction trials and of these we observed 16 spawning events: 7 caused by transport stress, 5 by thermal shock and 4 by a gradual temperature increase. Following these spawning events, we obtained eight fertilizations, 5 of which were self-fertilizations and 3 were controlled fertilizations.
- Some of these adult individuals have died, while for others, it has been possible to monitor their growth, collecting very interesting scientific data, and rear them for reintroduction purposes..On 14 December 2023 (4 specimens) and 31 July 2024 (6 specimens) some specimens of *P. nobilis* were transferred to the marine area of Capo Mortola. Furthermore, in July 2025 the surviving specimen in the Shoreline laboratory was transferred to Miramare MPA, and on September 20, 2025, five specimens were transferred from the Camogli laboratory to the Bergeggi MPA. This required the issuance of a special authorization by the Liguria Region, regarding the repopulation of species listed in Annex IV of the Habitat Directive (Dir. 92/43/EEC), such as *P. nobilis*, as provided by Italian legislation (Art. 12 of DPR 357/97), based on a feasibility study, drafted according to the indications provided in the April 2, 2020 Decree of MASE - Ministry of Environment and Energy Security. The first post-implantation monitoring, conducted on January 24, 2024, confirmed the good conditions of the animals.
- Coding every activity carried out to date, in order to implement action C5, which involves collecting protocols related to all phases of the project, to ensure their replicability and standardise activities and share them with as many stakeholders as possible.

The recruitment and translocation activities of individuals, foreseen respectively in actions C3 (the positioning and monitoring of larval collectors and the search for individuals of *P. nobilis* in dangerous conditions) and C6, were instead heavily compromised due to the drastic decline suffered by the populations of *P. nobilis* in the donor sites. This situation partially compromised action C6. This led to the preparation of the Contingency Plan, transmitted on January 31, 2024, within which the partnership has developed an alternative strategy to achieve the project's objectives. This strategy involves a series of activities including the identification of a greater number of donor sites and the opportunity to prioritise in situ movements of individuals in order to minimise stress, consequently limiting the mortality rate.

The monitoring activities, related to the assessment of water quality in reintroduction sites using sentinel organisms (D1) and the updating of information on the presence of *P. nobilis* in donor and recipient sites and surrounding areas (D3), were carried out according to the established program, running parallel to those described above. In this context, ARPAL has entered into an agreement with the IMC - International Marine Centre in Oristano to obtain further support for monitoring the species through Citizen Science activities, which are considered important for acquiring information on the presence of any living specimens of *P. nobilis*. In addition, it has formalised two agreements with the marine protected areas (E3) - Tuscan Archipelago National Park and Bergeggi Marine Protected Area - which have made it possible, in part, to transfer the results in terms of repopulation (Bergeggi) and, in part, to implement monitoring activities for the search for *P. nobilis* individuals in areas where the species was historically widespread (Tuscan Archipelago).

Action D2, concerning the monitoring of restocked individuals, experienced a delay due to the aforementioned difficulties, which prevented all planned repopulations from taking place before 2023 (February for Slovenia, March for Miramare, and December for Capo Mortola). Due to the delayed start of monitoring activities, only two out of the three monitoring reports originally foreseen could be completed within the project duration. The report for the first monitoring year was submitted twelve months after the translocation at the Capo Mortola site (i.e. in December 2024), while the second-year report covers the monitoring data collected from January 2025 until the end of the project.

4. Introduction

The project aims to conserve *Pinna nobilis* in the western Mediterranean and the Adriatic Sea, by acting specific conservation and repopulation actions in pilot areas, transferable to other regions.

The main objectives of the project are:

- Development and implementation of the most suitable techniques of repopulation through translocation of self-recruited juveniles and captive breeding of *P. nobilis* in order to release a large number of specimens in the wild in a few years;
- Selection and characterization (environmental and anthropogenic pressures, climate, and levels of pathogenic infection) of the marine or transitional areas appropriate for restocking actions on the basis of comparison with the area where the survivors are living, and molecular characterization of the best candidates to be reproduced (surviving specimens);
- Maintenance of a good level of genetic variation among the individuals used for restocking in order to obtain offspring that will be the founders of new future populations with a good fitness in the long term;
- Transfer and replicate of performed skills and methodologies (environmental characterization of best sites; molecular characterization of surviving specimens; juveniles capture; transport and logistical techniques of the juveniles to the growing plants; reproduction in captivity; techniques of regret in the natural environment) to the areas where fan mussel is decreasing

Involved sites

Receiving sites: MPA Capo Mortola, MPA Asinara, Park Strunjan, MPA Miramare

Donor sites: Park Strunjan, MPA Miramare, Venice Lagoon (included during project implementation)

Species and Habitat targeted

The project addresses specifically the fan mussel *P. nobilis**, which is a species characterising, in particular, the *Posidonia oceanica* meadows, which are acknowledged to represent one of the most important marine ecosystems in the Mediterranean coastal waters.

Main conservation issues

*P. nobilis** is nowadays listed as “Critically Endangered” by the IUCN Red List and it is a protected species under the Annex IV of EC Habitats Directive 92/43/EEC and the Annex II of the Barcelona Convention. In autumn 2016, a mass mortality event affected *P. nobilis** populations, starting from the south-western Mediterranean Sea (Vázquez-Luis et al., 2017). Since then, the situation has worsened, gradually affecting the coastal areas of Spain, France, Tunisia, Turkey, and Italy. In Italy fan mussels died from Sardinia to Sicily, from Puglia to Tuscany. The protozoan *Haplosporidium pinnae*, a pathogenic microorganism that affects the digestive system of the mollusc progressively reducing the feeding of the animal and causing its death, was initially imputed as the main cause of this mass mortality. However, several bacteria species have been also recently invoked as pathogens involved in the mass mortality of *P. nobilis** suggesting that the real causes of its mortality are not yet completely understood and that a multifactorial disease may be the most responsible factor.

When the LIFE PINNA project was conceived, the northern Adriatic still hosted *P. nobilis* populations in good condition. Specifically, along the Slovenian and Croatian coasts, there were adult individuals with high densities. In the Italian part, where the parasite *Haplosporidium pinnae* was first reported (Manfrin et al., 2023), young recruits were still abundant and easily found. Unfortunately, starting from the summer of 2020, the first signs of disease began to be observed in Slovenia and Croatia (Istria-Brioni), and within a year, *P. nobilis* populations in these areas were severely impacted (Mavrič, unpublished data; Bakran-Petricioli et al., 2023).

In the Slovenian part of the Gulf of Trieste (Northern Adriatic), based on data collected between 2007 and 2017, the average density of *P. nobilis* varied between 1 and 39 individuals per 100 m² (Hanžek-Turnšek H., 2018). The average density was higher in areas covered by seagrass meadows (22 individuals/100 m²) compared to areas without vegetation (4 individuals/100 m²). In these high-density areas of *P. nobilis*, the average mortality did not deviate significantly from 15%, with some areas showing mortality rates below 5% (Hanžek-Turnšek H., 2018). Based on a study conducted in 2012 (Lipej et al., 2012) in the pilot site of Strunjan (Strunjan Landscape Park), the average density

of live *P. nobilis* ranged from 14 to 33 individuals/100 m², depending on depth (maximum density: 38 live individuals/100 m²). The same study mentions an average density of 95 live specimens/100 m² in the area in front of the Marine Biology Station in Piran (MBP-NIB). In the monitoring carried out by NIB as part of the LIFE PINNA project in 2022, along the Slovenian coast, in a total area of 3300 m², 1506 empty shells were counted (average density 25 shells/100 m²), with only 2 live individuals.

The data indicate that, even in the Italian part of the Gulf of Trieste, *P. nobilis* populations followed a similar trend to those recorded in neighbouring areas along the Slovenian coast. During the 2018 monitoring activities, in the Miramare area, vital *P. nobilis* density values were, in one site (Sticco), exceeding 50 individuals/100 m², with an average value in the area of 25.9 ind/100 m². Additionally, the number of recorded dead organisms remained consistently below 1.5%.

The density values in the Miramare area are the highest among those recorded during the monitoring period of 2018-2020, which covered the coasts of 11 Italian regions, for a total investigated area of almost 50 hectares. Values lower by an order of magnitude but still exceeding 1 ind/100 m² were observed in Veneto and, on the Tyrrhenian side, in some sites in Tuscany, Sardinia, and Liguria, although these sites exhibited higher average percentages of dead organisms. These data confirm that the Miramare was the best donor site at the time when the LIFE PINNA project was taking shape.

In 2020 and subsequently in 2022 and 2023, the Miramare area underwent additional monitoring campaigns following the initial campaign in 2018. Results obtained through the collaboration of ARPA Friuli Venezia Giulia and ISPRA reveal a drastic reduction in pinna densities, decreasing from average values of 25.9 ind/100 m² to 0.44 (2020), 0.15 (2022), and 0.00 (2023).

On the Tyrrhenian side, monitoring conducted by ARPA Liguria in four areas along the Ligurian coast in 2022 and 2023 also confirmed the complete disappearance of *Pinna nobilis* populations that had been surveyed in 2018.

Expected longer term results

Here are some of the main expected results in terms of impact on the targeted species and replicability and transferability.

- 200 N. of individuals released through restocking actions
- Installation of at least 3- restocking plants
- 1 georeferenced map of the best pilot sites for the restocking actions
- Creation of 1 genetic database
- 5 Protocols for replicability validated by the National or Regional Authorities on:
 1. selection of restocking sites;
 2. best restocking techniques (methods of juveniles capture, controlled growth, transport, Installation of the specimens);
 3. best practices for controlled reproduction in captivity;
 4. agreement scheme for the juveniles capture;
 5. identification form for the transport specimens tracking (from protected areas with presence of survivors to areas with high *P. nobilis** mortality);
- 1 Map of the presence of specimens in all involved regions;
- 1 report of economic evaluation on the cost-efficient replicability and transferability of the project solutions on *P. nobilis** in 2 other sites: MPA Bergeggi and Tuscan archipelago
- 5 protocols on various aspects to protect *Pinna nobilis**, diffused among MPA authorities obtaining 50 expressions of interest to apply them that will be monitored

5. Administrative part

Project Management structure.

The CB ARPAL is the Project Responsible (PR) and ensures the coordination and performance of the project as a whole, maintaining contacts with the Agency/CINEA, with the Project Monitor and with the ABs. The PR coordinates the internal communication, the coherent management of the administrative aspects of the project, the drafting of the Partnership Agreements, the quality control on the delivered products. The CB ARPAL is also responsible for the monitoring and project-level coordination of the financial reporting (implemented through the external support of a sub-contractor – Istituto Delta Ecologia Applicata srl), for the monitoring of deviations/changes from the approved project and for the assessment of the achievement of the expected results with specific regard to the conservational aspects. The CB and PR constituted the Steering Committee (SC), which is the project decision-making body, led by the CB ARPAL and composed by a representative of each ABs. The Project Management is performed by the AB TRITON through the following figures: a Project Manager (PM) Diego Mattioli supported by a project assistant, retaining full control of the project under the supervision of the CB, that perform the project direction and overall coordination, reporting, communication supervision and coordination, actions and deliverables supervision.

The PM ensures the sound progress and quality of the project activities, coordinates the drafting of the technical reports and provides the quarterly update to the external monitoring team (until April 2023). The coordination and management of the project are organised and implemented taking into account the needs of a consortium of 7 Beneficiaries in two Member States. Regular quarterly meetings are held with the possibility of connecting via web conference, to ensure the participation of all the involved staff. Besides joint meetings, one-to-one meetings are organised for specific technical and financial issues raised by the ABs. Other than through meetings, the management is performed on a daily basis using the usual communication channels of emails and phone calls and specific in person meetings at the headquarters of the Beneficiaries depending on the agenda to be discussed.

Comment on the respect of the project workplan and time schedule

Deviations from the work plan and project schedule are shown in technical section 6.1 and in more detail in section 6.2. The present Report has been postponed from 19/12/2025 to 23/01/2026.

Added value of the partnership

The partnership has added value due to the presence of mixed and well-balanced types of organisations that are mutually complementary and synergistic: universities or research institutions that provide the necessary scientific component; Managers of MPAs that are directly involved in the activities; Private organisations with direct knowledge and experience of the area and communication/project management issues.

Communication with CINEA and the Monitoring Team

Communication with CINEA and the Monitoring Team is carried out according to official appointments and in relation to requests for clarification and/or communications by the PC, via email. Communication with the Project Monitor, Dr. Teresa Catelani, was performed via email and occasionally via direct phone. During 2023 the monitoring process changed, as detailed in communication Ares(2023)7727725 - 14/11/2023, with the setting up of the LIFE Monitoring Helpdesk. Five monitoring visits were conducted:

- 1st Monitoring Visit - 20/01/2022 by videoconference;
- 2nd Monitoring Visit - 29 - 31 March 2023 Sassari - University of Sassari - Porto Torres Asinara Parks- Asinara Island;
- 3rd Monitoring Visit - 6 October 2023 - Genoa - University of Genoa - Camogli Laboratories
- 4th monitoring visit – 24th January 2025 – Rome – Spazio Europa
- 5th monitoring visit – 24th/25th September 2025 – Genova – ARPAL headquarters. Savona – MPA Isola di Bergeggi

Amendment

During the project implementation period an amendment has been requested regarding two administrative modifications:

- change in the banking references of the Coordinating Beneficiary Regional Agency for the Protection of the Ligurian Environment – ARPAL starting November 28th 2022, due to the acquisition of the previous banking institute by BPER;
- addition of the co-financer Ministrstvo Za Naravne Vire in Prostor (Ministry of Natural Resources and Spatial Planning of Slovenia) for the Associated Beneficiary National Institute of Biology – NIB, through decision N. 5440-6/2022-2550-7 approved on 09/05/2023 following the opening of a national call for the co-financing of LIFE projects for 2020, 2021 and 2022 published on the Official Journal of the Republic of Slovenia on 25/11/2022. The Amendment request for the AB NIB therefore entailed the addition of Ministrstvo Za Naravne Vire in Prostor as co-financer of the action for a total amount of EUR 78,715, and the remodulation of the AB's own contribution amounting to EUR 90,095.

The Amendment Request package has been issued to CINEA on 06/10/2023 and the communication of approval of the proposed Amendment has been received via email on 14/11/2023 with Letter Amendment Nr. 1 to Grant Agreement LIFE20 NAT/IT/001122 - LIFE PINNA - Ares(2023)7727725.

6. Technical part

6.1. Technical progress, per Action

Action A1: Environmental status and health assessment of seagrass meadows and *Pinna nobilis* populations in donor and receiving areas

Foreseen start date: 10/2021

Actual start date: 10/2021

Foreseen end date: 05/2022

Actual end date: 03/2023

Action A1 Field surveys and collection of data from the literature were the two main activities carried out for the environmental characterisation of donor and receiving sites, and to obtain information regarding the health status of relict populations of *Pinna nobilis* in the donor sites of Miramare and Strunjan. In particular thanks to the **Sub action A1.1** the maps of the 2 areas and the location of the selected donor sites have been identified. At Miramare, the selected donor site was located 1 km far from the buffer zone of the MPA Zone B, at the area of the mussel farms where the collection of *P. nobilis* juveniles was planned (see fig.3 Deliverable A1.1). Although the donor site didn't fall within the boundaries of the Miramare MPA, the area was considered in a good ecological status according to the latest monitoring activities of ARPA. Similarly, the donor site identified in Strunjan was included in the Strunjan fishery reserve (see Fig. 6 Deliverable A1.1), at the area of the mussel farm. In order to identify pilot areas where specific sites for restocking have to be selected, an environmental and ecological assessment was carried out in the framework of the **Sub action A1.2**. All the 4 pilot sites, even the donor sites of Miramare and Strunjan, have been the subject of more detailed environmental investigations. In particular, in Capo Mortola, according to the ecological quality indices (PREI, AMBI, CARLIT) and the measure of water nutrients and contaminants all the area has resulted suitable for restocking of *P. nobilis* individuals (see fig. 3 Deliverable A1.2). Additionally in July 2022, 8 high-definition video surveys of a length of 500 m and 3 dives have been carried out to characterise the seabed and to verify the presence of surviving individuals: no live *P. nobilis* were found (see fig. 9 Deliverable A1.2). In Strunjan, a suitable receiving area has been identified in the Strunjan Landscape Park, within the Natura 2000 and fishing reserve area (45° 31 '56.22"N; 13° 35' 58.42"E) (see Fig. 19 deliverable A1.2). Indeed, the area was found to be the least affected by human activities such as fishing and anchoring that could negatively influence the restored *P. nobilis* population. Underwater monitoring conducted in May 2022 shows that only 2 living specimens were observed per 3300 m² of surveyed area. To assess the status of the *P. nobilis* population in the receiving area, 2 transects parallel to the coast were carried out at 3 m and 5 m depth and one transect perpendicular to the coast were conducted between 2 m and 5 m depth. Along parallel transects the density of dead specimens was 31 specimens/100 m² at 3 m depth, and 77 specimens/100 m² at 5 m depth. Along the depth transect the density of dead specimens was 42 specimens/100 m², with the density of dead specimens increasing with depth. Therefore the potential area of transplantation of new specimens has been set in deeper waters, between 5 and 8 m where the conditions are likely to be better due to reduced influence of waves, which can have a major impact on *P. nobilis* in shallow waters. The ecological status of the Miramare water body was characterised by values that always fluctuated between Good and High for both the Phytoplankton and Macroinvertebrate components (M-AMBI index) and physical-chemical and hydromorphological quality thus indicating all the area as suitable for the restocking of *P. nobilis*. The 2022 monitoring activities in the Gulf of Trieste led to the identification and mapping of numerous live individuals of *Pinna nobilis*. The distribution was diversified both in terms of space and size, generally showing a greater presence of live individuals in the northernmost areas. As far as juvenile individuals (max. 15 cm) are concerned, in summer 2022 more than 100 individuals were found in the stretch of sea between Barcola and Valle Cavanata, which were constantly monitored for viability. The number of live adult individuals identified, on the other hand, was about 30. In Miramare MPA monitoring activities were carried out on 3 survey sites (see Fig. 15-Miramare Deliverable A1.2) (square 1, 3 and 5: 100 m x 100 m cells). Within each cell chosen for monitoring, 3 transects (replications) were carried out, each 100 m long. On each of the two sides of the transect, a strip of 3 m was considered (100 x 6 altogether 600 m²). A total of 1578 specimens were counted and measured, with only 8 live specimens. In Asinara MPA the marine ecosystem was mainly characterised by a high diversity of flora and fauna communities, high biodiversity and water

quality. In particular the eastern sector that overlooks the Gulf of Asinara was characterised by a low-relief coast where sandy beaches alternate with promontories; the seabed was largely covered by *Posidonia oceanica* meadows resulting the most suitable area for *P. nobilis* restocking.

Deliverable name	Foreseen Deadline	Actual Deadline
D_A.1.1 Donor sites maps and environmental characterization	31/3/2022	31/3/2022 Delivered Open until the end of the project
D_A.1.2 Report on environmental suitability of receiving areas for <i>Pinna nobilis</i> restocking	31/5/2022	31/3/2023 Delivered Open until the end of the project

Milestone name	Foreseen Deadline	Actual Deadline
M_A.1 Identification of proper donor sites	31/12/2021	31/3/2022 Open until the end of the project

Comparison with planned output and time schedule: Delays in the subaction A1.2 caused a delay in delivering the final product until the following year. Nevertheless the planned outputs were all achieved.

Major problems: The delays in sub-action A1.2 were mainly related to action A2 and the need to define the four pilot sites suitable for restocking as pathogen-free. Difficulties in finding sentinels and the need to repeat molecular analyses led to delays in the action A2 and consequent delays in starting the activities on the field for the action A1.2. In the context of monitoring actions under SubAction A1.2, delays in concluding collaborations with external firms to support data collection in the field caused further delays.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action). As the initially identified donor sites (Miramare and Strunjan) were not sufficient to provide the required number of adult *P. nobilis* individuals for controlled reproduction trials, a new donor site has been identified in the Venice Lagoon (M_A.1), from which adult specimens can be sourced. Miramare and Strunjan have maintained their role as donor sites for naturally self-recruited juveniles collected from larval collectors. The action is formally concluded.

Outline the perspectives for continuing the action after the end of the project: This action does not need to be continued beyond the end of the project

Mention any complementary action outside LIFE. (ref to E1 Annex 8_ supporting documents.pdf)
None

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities. None

Attachments:

Deliverable A1.1 Donor sites Environmental characterization

Deliverable A1.2 Environmental suitability of receiving area for restocking

Action A2: Molecular characterization of sentinel species in the putative pilot sites of restocking

Foreseen start date: 10/2021

Actual start date: 10/2021

Foreseen end date: 09/2022

Actual end date: 07/2025

Activities undertaken and outputs achieved:

Action A.2. This preparatory action has been based on the fact that mass mortality of *Pinna nobilis* is associated with pathogen infection in combination with specific environmental conditions. In this context, researchers of the University of Sassari, in a study conducted shortly before the start of this project, demonstrated that the pathogens primarily associated with *Pinna nobilis* mortality, the protozoan *Haplosporidium pinnae* and various species of mycobacteria, are able to infect, albeit without signs of pathogenicity, molluscs, mainly bivalves, that share the habitat of *Pinna nobilis*.

For this reason, the presence of pathogens potentially lethal to *Pinna nobilis* was rapidly identified by analysing these sentinel organisms, both in the absence of *Pinna nobilis* individuals in the area, and in situations where it was impossible to analyse the internal tissues of *Pinna nobilis* individuals (without jeopardising their survival). This ensured an accurate assessment of the presence of pathogens in the water.

Sub action A2.2. The diagnostic analyses performed for a quick and reliable identification of pathogens in the sites involved in the project, allowed, not only to identify the presence of pathogens, but also to evaluate the genetic variability that these organisms had in the analysed areas. These analyses aimed to evaluate the progress of the waterborne infections and to provide predictions regarding the future expansion or contraction of these pathogens.

In this context, throughout the entire project duration, UNISS researchers selected several ribosomal molecular markers that could allow for rapid diagnostic analyses to detect pathogens in water through their isolation from sentinel organisms' tissues. These markers also enabled phylogeographic analyses of pathogens individuals/populations present at the intervention sites. To reach the aim, sentinel bivalves were analysed every six months from the intervention sites in Liguria, Sardinia, Friuli Venezia Giulia, Veneto and Slovenia. Recognizing the critical need for continuous pathogen monitoring across all project areas, a crucial decision was made in late 2023. Action A.2's water pathogen monitoring activity was extended to encompass all intervention sites for the project's entirety. This expansion served as a permanent health assessment for the entire western Mediterranean basin, ensuring rapid detection of any emerging pathogen expansions that could pose a significant threat to transplanted individuals.

For this reason, albeit on a smaller number of individuals, and not with the same frequency, all sites involved in the project were monitored for the occurrence of pathogens until July 2025.

Methods: Throughout the project duration, the Italian and Slovenian partners of the project provided UNISS with sentinel species collected at sites involved in the project. Sentinel sampling was carried out periodically, with up to 30 individuals per site and per sampling event. Biological samples were sent to the University of Sassari, from the sites of collection, by means of private couriers, transported in polystyrene boxes filled with dry ice to preserve tissue integrity. Overall, sentinel organisms were collected from intervention areas located in Sardinia, Liguria, Friuli Venezia Giulia, Veneto, and Slovenia. Sampling activities were conducted over five monitoring rounds between 2022 and 2025, covering a total of 13 sites by the end of the project. In addition to the core intervention sites, supplementary sampling was carried out in surrounding areas whenever feasible, in order to obtain a more comprehensive assessment of pathogen presence in waters adjacent to the intervention zones. Specifically, additional sites were investigated around Asinara Island MPA (Sardinia), Strunjan MPA (Slovenia), and Miramare MPA (Friuli Venezia Giulia). Furthermore, shortly before the third monitoring round, the Venetian Lagoon (Veneto, Italy) was identified as another promising candidate donor area for restocking activities. Consequently, sentinel species were also collected and analysed in this new area to assess the presence of pathogens.

In total, 1395 sentinel individuals belonging to several genera such as *Crassostrea*, *Mytilus* and *Ruditapes* were collected and analysed during the project, with sampling intensity varying among regions and monitoring rounds.

For each round of analyses, all biological samples were stored at the Conservation Genetics Laboratory of the Department of Veterinary Sciences of the University of Sassari, while the molecular diagnostic analyses were performed at the Population Genetics Laboratory of the Department of Biomedical

Sciences of the University of Sassari. For each sentinel organism, four different tissues were analysed: gills, adductor muscle, digestive gland, and mantle. Each tissue was analysed using standard PCR. The molecular markers employed targeted genes belonging to the ribosomal gene complex and included: 18S ribosomal DNA specific for *Haplosporidium pinnæ* and universal for other protozoa taxa; 18S and ITS ribosomal DNA markers for protozoa of the genus *Bonamia*; 16S ribosomal DNA markers universal for bacteria and specific for the genera *Mycobacterium* and *Vibrio*; specific primer sets for RNA and DNA viruses, including *Picornavirus* and *Ostreid herpesvirus* type 1. Positive PCR products were sequenced in both forward and reverse directions. To assess the diagnostic potential of standard PCR, a representative subset of samples from each monitoring campaign conducted on sentinel organisms was also analysed using quantitative real-time PCR (qPCR). Approximately 800 qPCR reactions were performed in total, and direct comparisons between the two methodologies were carried out using the same primer sets.

n° of reactions performed for 1,395 sentinel organisms	
PCR	~ 4,500
Sanger sequencing of PCR positive samples	600
qPCR	800
Total	5,900

Results: Comparison tests conducted by UNISS in 2023 demonstrated no significant discrepancies between PCR and qPCR results in terms of positive diagnoses. Consequently, during the 2024 and 2025 diagnostic campaigns, the choice between PCR and qPCR was based on the need for either rapid and cost-effective screening (PCR) or higher sensitivity in detecting low-level infections within sentinel tissues (qPCR).

Across all monitoring rounds, a generally low proportion of samples tested positive for protozoan, bacterial, and viral pathogens. This trend, in particular observed starting from 2023, was consistently confirmed during the 2024 and 2025 analyses. It is important to take into consideration that the low number of positive detections does not necessarily indicate the complete absence of environmental microorganisms in the sampled waters. Rather, it suggests that no significant infections were present within the tissues of the analysed sentinel organisms during the final years of the project. In particular, a decreasing trend in the frequency of *Haplosporidium pinnæ* detections was observed from 2022 onwards across the intervention areas. Similarly, detections of *Mycobacterium* spp. pathogens showed a marked reduction over time, with only sporadic positive cases recorded in later monitoring rounds.

Overall, the molecular screening of sentinel species throughout the project provided robust evidence of a low pathogen pressure in the investigated areas during the final phase of the project, supporting their suitability as potential donor or intervention sites for conservation and restocking actions.

The use of sentinel organisms combined with standardized molecular diagnostic markers allows for non-invasive, cost-effective, and scalable monitoring of pathogen presence in marine environments, particularly in situations where direct sampling of protected species is not feasible. This approach, which is based on the use of sentinel species for the first time in marine environments, can be adopted by other research groups and conservation practitioners to assess pathogen pressure, support risk evaluations prior to translocation or restocking actions, and inform adaptive management strategies. As such, this methodology represents a transferable tool that can contribute to broader conservation efforts, supporting evidence-based decision-making in the management of vulnerable marine populations.

Furthermore, in the following scientific papers (ref to E1 Annex 8_ supporting documents.pdf), the researchers of the University of Sassari are principal investigators in the first case and participated as co-authors in the second case. These studies are focused on topics that overall correspond to the main objectives of the action A.2. of the present project.

- F. Scarpa, D. Sanna, I. Azzena, P. Cossu, M. Casu. From dark to light and back again: is *Pinna nobilis*, the largest Mediterranean shellfish, on the brink of extinction? *Veterinaria*, 2021. doi.org/10.51607/22331360.2021.70.1.1
- Francesca Carella, Dušan Palić, Tomislav Šarić, Ivan Župan, Bartolomeo Gorgoglione, Patricia Prado, Karl B. Andree, Ioannis A. Giantsis, Basile Michaelidis, Athanasios Lattos, John A. Theodorou, Juan Luis Barja Perez, Sergio Rodriguez, Fabio Scarpa, Marco Casu, Elisabetta Antuofermo, Daria Sanna, Domenico Otranto, Rossella Panarese, Carmelo Iaria, Fabio Marino, Gionata De Vico. Multipathogen infections and multifactorial pathogenesis involved in noble pen shell (*Pinna nobilis*) mass mortality events: Background and current pathologic approaches. *Veterinary Pathology* 1–18, 2023. DOI: 10.1177/03009858231186737journals.sagepub.com/home/vet

Deliverable name	Foreseen Deadline	Actual Deadline
D_A.2: report on the molecular characterization of sentinel species and identification of free pathogens sites	05/2022	09/2025*

Milestone name	Foreseen Deadline	Actual Deadline
M_A.2.2:Identifications of the locations where pathogens are absent	05/2022	09/2025*
M_A.2.1:Definition of the complete suites of pathogens which cause the mass mortality of the fan mussel	05/2022	09/2025*

* *This action was extended to the whole project*

Comparison with planned output and time schedule: The planned outputs were all achieved. Furthermore, the sub action A.2.2 was extended to the end of the project to maintain a constant control of pathogen levels for all the sites involved in the project and further increase the genetic knowledge on the pathogens which have already been or will be associated with the mass mortality of *Pinna nobilis* such as new species of protozoans and bacteria or viruses.

Major problems: No significant issues have been experienced for this action.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action). NONE

Outline the perspectives for continuing the action after the end of the project:

After the end of the project, researchers from the University of Sassari continued to analyse sentinel species and to carry out the genetic characterisation of pathogens detected in these organisms, building on the methodologies and results developed within the LIFE PINNA project.

Mention any complementary action outside LIFE. NONE

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities. NONE

Attachments:

D_A.2: report on the molecular characterization of sentinel species and identification of free pathogens sites

Action A3: Survey on best practices for *P. nobilis* restocking concrete actions: SWOT analysis on restocking best techniques and available methodologies. Elaboration of a feasibility study and exit strategy

Foreseen start date: 01/10/2021
Foreseen end date: 30/06/2022

Actual start date: 01/12/2021
Actual end date: 31/08/2023

Activities undertaken and outputs achieved:

Arpal has engaged, by selection procedure, the Delta Institute of Applied Ecology srl. (assignment given by official act of Arpal N. 70 of 03/28/2022) to implement the activities foreseen by this action. The first activity involved the identification of the main stakeholders (D_A.3.1) represented by the organisations that have developed experiences, projects, aquarium cultivation or sea farming activities of *P. nobilis*. This research was conducted by collecting experiences outside the partnership as well, questioning a range of Key Stakeholders, including: Partners of other projects; MPA managers, with particular reference to the Medpan network of Mediterranean MPAs; Researchers, scholars from the world of research who have published articles on the subject in recognized scientific journals; and Institutes with expertise in the cultivation of large mussels at sea or in aquariums. Stakeholders were then divided into reproduction specialists and managers of sites with the presence of the species. Reproduction specialists clearly appear to be a limited number with equally limited experience, the most significant of which are in Turkey, Spain and Croatia. In contrast, marine managers are a significantly larger number, with the limitations set out in the introduction 396 Natura 2000 sites were surveyed. In the database, spreadsheet format, emails and reference websites.

A questionnaire, in the form of a google form (D_A.3.2), was then developed and, after sharing with project partners Shoreline, NIB, and UNIGE, disseminated to key stakeholders. The request for completion of the questionnaire was sent to 74 identified stakeholders through two mailings: one with the form in Italian language to the 32 stakeholders identified in Italy; and a second to the remaining 42 stakeholders from other origins in the Mediterranean. A total of 13 responses were obtained, reported in report D_A.3.3, which in quantitative terms may appear limited, they are in fact equal to 16% of the identified stakeholders, the quality of the responses, however, is very high, in terms of both possible experiences to be replicated and the availability of Organizations to be contacted to disseminate the results of the project. Considering the stakeholders identified, the results of the questionnaire, and the activities carried out, 12 key points were identified for SWOT analysis (D_A.3.4), including strengths and opportunities and weaknesses and threats. Specifically, the analysis conducted revealed 5 strengths and 2 opportunities versus 2 threats and 3 weaknesses. Therefore, based on this study, the elements that seemed to favour the achievement of the project's intended objectives, 7 in total, outweighed the elements that were dangerous to achieving the objectives.

Finally, a feasibility study (D_A.3.5) was carried out for the purpose of obtaining an exemption permit under Presidential Decree 357/97. This procedure was necessary because *Pinna nobilis* is included in Annex IV of the Habitats Directive, dir. 92/43/EEC, and Annex II of the SPA/BIO Protocol of the Barcelona Convention. Annex IV of the Habitats Directive lists the species for which strict protection measures must be taken and for which any form of collection, killing, possession or exchange for commercial purposes is prohibited. Italy transposed the above directive in 1997 through Regulation DPR No. 357 of 8 September 1997, as amended and supplemented by DPR 120 12/03/2023.

In accordance with the provisions of Article 16 of the Habitats Directive, Presidential Decree 357/97 established in Article 11 that the Ministry of the Environment (now the Ministry of Environment and Energy Security), having consulted the Ministry of Agriculture, Food Sovereignty and Forestry and ISPRA as far as it is concerned, may authorise derogations from the provisions of Articles 8, 9 and 10, paragraph 3 (a) and (b). The request for authorization allowed for a thorough feasibility analysis aimed at assessing the suitability of the populations chosen as donors, depending on the characteristics of the populations present at the recipient sites.

Arpal then initiated and concluded on 31/03/2023 the procedure that led to the granting of the authorization, provided, following the preliminary investigation carried out by ISPRA, for the handling of individuals of *P. nobilis*.

Deliverable name	Foreseen Deadline	Actual Deadline
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D_A.3.1:List of key stakeholders	12/2021	03/2023
D_A.3.2:Questionnaire produced	12/2021	03/2023
D_A.3.3:Report of interviews and Focus Group	04/2022	03/2023
D_A.3.4:Report with SWOT analysis	06/2022	03/2023
D_A.3.5: Feasibility study for the reintroduction with an exit strategy	09/2022	08/2025

Milestone name	Foreseen Deadline	Actual Deadline
M_A.3.1:Engagement of key stakeholders	12/2021	03/2023
M_A.3.2:Realisation of interviews/FG	03/2021	03/2023
M_A.3.3: Swot analysis	05/2021	03/2023
M_A.3.4: Feasibility study for the reintroduction with an exit strategy	07/2021	03/2023

Comparison with planned output and time schedule: None

Major problems: None

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action). None

Outline the perspectives for continuing the action after the end of the project: None

Mention any complementary action outside LIFE. None

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities. None

Attachments:

- Deliverable A.3.1 List of key stakeholders
- Deliverable A3.2 Questionnaire produced
- Deliverable A3.3 Report of interviews and Focus Group
- Deliverable A3.4 Report with SWOT analysis
- Deliverable A.3.5 Feasibility study for the reintroduction with an exit strategy

Action C1: Identification of the pilot sites for restocking

Foreseen start date: 1/04/2022

Actual start date: 1/04/2022

Foreseen end date: 30/6/2022

Actual end date: 31/03/2023

Activities undertaken and outputs achieved:

Based on the results of Actions A1 and A2, which aimed to identify locations free of pathogens and with ecological conditions suitable for *P. nobilis*, the best sites for restocking within each receiving pilot area were carefully identified and mapped.

In Liguria, Field activities to identify suitable sites for hosting *P. nobilis* individuals took place over three survey campaigns to investigate the seabed of the Capo Mortola Marine Conservation Area. All areas investigated were characterised by the presence of the seagrass *Posidonia oceanica*. As the seagrass was very dense, suitable sites were identified at sandy clearings and bare matte areas within the meadow to facilitate the identification of *P. nobilis* specimens once transplanted.

The 3 sites identified as suitable for *P. nobilis* restocking (see Table 1; Fig. 8 Deliverable C1) were located between 7 and 12 m depth. This choice has been made in order to: i) be aligned with the bathymetric range of the donor sites, where *P. nobilis* specimens survived ii) prevent that local

hydrodynamics affect the bottom mechanically, damaging the transplanted individuals and iii) facilitate logistics of transplanting and monitoring operations. In Strunjan, the sites identified as suitable for *P. nobilis* restocking were located between 5 and 8 m depth on a sediment bottom historically characterised by the presence of a *Cymodocea nodosa* bed (see Fig. 9 and Table 2 Deliverable C1). The choice has been made because: i) the sites were located in the Strunjan Landscape Park, so within an area where an active site management plan with protection criteria exists; ii) it hosted the higher density of *P. nobilis* before the mass mortality event; iii) the depth guarantee protection from waves and storm; iv) the impacts that caused a regression of *C. nodosa* bed have ceased. In Miramare, to determine the most suitable site for *P. nobilis* individuals, GIS analyses and 6 field activities were carried out. All the sites studied were characterised by the presence of coarse sands with fragments of sessile organisms and shells. The 3 sites identified as suitable for *P. nobilis* reintroduction were between 3 and 5 m depth (see Fig. 14 and table 2-Miramare Deliverable C1). This choice reflects the demonstrated presence of numerous *P. nobilis* individuals in the past and was made to prevent local hydrodynamics from mechanically affecting the soil and damaging transplanted individuals, and to facilitate the logistics of transplanting and monitoring activities. In Asinara MPA, two suitable sites were identified for *P. nobilis* transplanting, one within the Zone A of the MPA, where boating, mooring, and bathing are prohibited and one within the zone B, where navigation is allowed but restricted (see fig. 15 Deliverable C1). The two sites were also naturally protected from the maestrale wind and the bottom was characterised by sand and patches of *P. oceanica*.

Deliverable name	Foreseen Deadline	Actual Deadline
D_C1: Detailed georeferenced maps of the four specific pilot sites where restocking will be carried out	30/6/2022	31/03/2023 Delivered

Milestone name	Foreseen Deadline	Actual Deadline
M_C1: Identification of the four pilot sites for restocking	30/6/2022	31/03/2023

Comparison with planned output and time schedule:

Delays in the action C1 caused a delay in delivering the final product until the following year. Nevertheless the planned outputs were all achieved.

Major problems:

The delays in action C1 were mainly related to actions A1.2 and A2 and the need to define the four pilot sites suitable for restocking as pathogen-free and of ecological value. Difficulties in finding sentinels and the need to repeat molecular analyses led to delays in the action A2 with consequent delays in the actions A1.2 and C1.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action).

Due to the limited availability of both adult and juvenile *Pinna nobilis* individuals, and in order to avoid the stress associated with long-distance transport, it was decided not to use the Asinara pilot site for restocking activities. Consequently, restocking efforts have been focused on the remaining sites.

Outline the perspectives for continuing the action after the end of the project: This action does not need to be continued beyond the end of the project.

Mention any complementary action outside LIFE. None

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities. None

Attachments:

- D_C1: Detailed georeferenced maps of the four specific pilot sites where restocking will be carried out

Action C2: Molecular characterization of surviving individuals of *Pinna nobilis* UNISS

Foreseen start date: 10/2022
Foreseen end date: 06/2025

Actual start date: 10/2022
Actual end date: 06/2025

Activities undertaken and outputs achieved:

Sub action C.2.1. In 2022, the *Pinna nobilis* still surviving population from Friuli Venezia Giulia (Miramare, Gulf of Trieste), which was originally chosen as a donor area, was genotyped. The molecular characterization relied on a mitochondrial marker (Cytochrome c Oxidase subunit I gene, COI) which has been already used for all fan mussel populations so far investigated during the last 15 years, and for this reason, allows to make diachronic further comparison among Mediterranean populations. Additionally, individuals from a surviving population from Tunisian coastlines (Kerkennah) were also genotyped. Tunisian populations could potentially serve as a source of individuals for restocking activities if the western Mediterranean population face complete disappearance during the LIFE-PINNA project. Indeed, Tunisian individuals can provide a concrete operational "Plan B": the identification of this southern Mediterranean population as a compatible and viable backup source for restocking, ensuring project continuity even in the event of total loss of Western Mediterranean populations.

In parallel, a second and independent genetic study (partially funded by UNISS private resources) was developed to place the LIFE PINNA donor populations within a broader context to evaluate the genetic relationships between surviving donor populations and the past *Pinna nobilis* populations formerly inhabiting in Western Mediterranean. The main concrete output of this study was the scientific validation of the donor populations: by comparing modern DNA with samples dating back up to 300 years, we proved that surviving individuals still carry the ancient genetic lineages of the Western Mediterranean. This result is crucial for the project's application, as it guarantees that the founders selected for restocking are genetically capable of withstanding environmental stressors, preserving the species' long-term adaptive potential.

In particular, this study integrated mitochondrial COI sequences from ancient, modern and surviving *P. nobilis* populations across the Mediterranean basin, including material dating back up to three centuries. By combining newly generated data with published and unpublished reference datasets, this approach allowed the reconstruction of the evolutionary history and long-term mitochondrial genetic variability of the species. The results provided essential insights into the persistence of historical haplotypes in surviving populations and supported the genetic compatibility of donor populations selected within the LIFE PINNA project with the genetic makeup of Western Mediterranean populations that recently disappeared following the mass mortality event (MME). The objective was to select founders capable of giving rise to new populations, preserving the ancient mitochondrial lineages characterizing the Western Mediterranean. Over geological eras, these genetic lineages allowed the species to adapt to environmental conditions and withstand stressors, thanks to the high adaptive potential they appear to have provided over time.

Moreover, activities for an additional characterization of the Miramare population using a panel of nuclear markers (microsatellites) started in summer 2023. This additional analysis resulted in a high-resolution genetic map of the Miramare population and was requested by the Italian Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) to achieve a more detailed genetic assessment of this potential donor population. Microsatellites analysis would enlarge the knowledge on the genetic variability occurring within the Friuli Venezia Giulia population. Furthermore, the comparison with previous studies on the other two *Pinna nobilis* populations which have been genotyped by microsatellites in Spain and France (Balearic Islands, Spain by González-Wangüemert et al. 2015 <http://dx.doi.org/10.12681/mms.949> and Banyuls, Gulf of Lion, France by Peyran et al. 2020, 2022 <https://doi.org/10.1007/s11033-020-05338-1>, <https://doi.org/10.1002/ece3.8482>) would be possible. Furthermore, in August 2023, the unexpected death of *Pinna nobilis* individuals from the Venetian Lagoon (Veneto) population (just before their planned transplantation to the Ligurian restocking area of Capo Mortola) presented an opportunity for a fine genetic characterization of the surviving Venetian Lagoon population. Indeed, this unexpected mortality event in the Venetian Lagoon was transformed into a sampling opportunity, allowing the team to secure genetic data (collecting large amounts of biological samples for DNA extraction from the whole body of dead

individuals) from this specific population before its decline, thus preserving vital information for future restoration efforts.

Furthermore, in this context, a major technical achievement of this sub-action was the development and standardization of an innovative completely non-invasive sampling methodology based on cotton swabs. This protocol, developed by the University of Sassari, represents a concrete tool for future conservation: it allows for DNA collection without harming or stressing the few remaining critically endangered individuals. The successful validation of this method eliminates the need for invasive (even if no-lethal and minimally invasive) biopsies in sensitive monitoring programs.

In particular, with the genetic analyses described above, specific efforts were dedicated to optimising sampling strategies in order to minimise invasiveness while ensuring the collection of biological material suitable for genetic analyses of *Pinna nobilis*. Within the LIFE PINNA project, an innovative non-invasive sampling methodology based on the use of cotton swabs was therefore developed and standardised. Given the critically endangered status of the species and the reduced number of surviving individuals, conventional tissue biopsies, although acceptable and effective for specific analyses, were considered less suitable when a further reduction of handling stress and physical damage was required. The cotton swab approach was designed to provide DNA suitable for genetic characterisation of surviving individuals while limiting invasiveness, thereby supporting conservation-oriented genetic studies within the project. The entire methodological development, optimisation and validation process was carried out as a self-financed activity by the University of Sassari, outside the original LIFE budget, highlighting a strong institutional commitment to improving conservation-oriented genetic research tools for *Pinna nobilis*.

The following sections present the results of mitochondrial and nuclear genetic analyses performed on samples collected using both conventional tissue biopsies and, whenever possible, the standardised non-invasive cotton swab protocol described above.

Results of mitochondrial DNA

Mitochondrial sequences belonging to *Pinna nobilis* samples collected during this project from the surviving populations of Miramare (20), the Venetian Lagoon (13), and the Kerkennah Islands (Tunisia) (5) were combined with data from previously published studies on populations from Spain, Italy, France, Greece, and Tunisia (Katsares et al. 2008 <https://doi.org/10.2478/s11756-008-0061-8>, Rabaoui et al. 2011 <https://doi.org/10.1007/s10750-011-0827-9>, Sanna et al. 2013 <https://doi.org/10.1371/journal.pone.0067372>, Sanna et al. 2014 <https://doi.org/10.12681/mms.459>, Wesselmann et al. 2018 <https://doi.org/10.1038/s41598-018-23004-2>) that now went extinct. Researchers at the University of Sassari further enriched the dataset by incorporating molecular data from individuals sampled before the mass mortality began in Miramare (28) and Asinara Island (32). This comprehensive dataset was then analysed to improve the genotyping of modern donor populations from northern Adriatic (Miramare and Venetian Lagoon) and gain a deeper understanding of their genetic makeup in relation to fan mussels populations before the bottleneck caused by the mass mortality.

The analysis of the Adriatic population from Miramare and Venetian Lagoon revealed a significant finding: many of their current haplotypes were also present in the western Mediterranean before the mass mortality, particularly in the Sardinian site of Asinara Island which is included as intervention site in the project. Such a haplotype sharing between the Adriatic and western Mediterranean strengthens the expectation that the planned LIFE-PINNA restocking activities would originate new populations with minimal genetic drift and genetic variability similar to one which was present in the western Mediterranean restocking areas.

Furthermore, a comparison of genetic variation before and after the mass mortality event for Miramare and the Venetian Lagoon populations reveals notable diachronic differences for the Venetian Lagoon area. In particular, in the Gulf of Trieste (Miramare), there is substantial continuity, with the presence of the same haplotypes and comparable levels of genetic variation with data obtained for individuals sampled before mass mortality. In contrast, the modern Venetian Lagoon population shows a decrease in genetic variation in comparison with the pre-mortality period. Although the current Venetian population still shows the most common haplotypes for *Pinna nobilis* in the western Mediterranean before the mass mortality, it also exhibits the lowest level of diversity ever recorded for *Pinna nobilis*

populations. This phenomenon can be attributed to a selective sweep effect that may have acted on the Venetian Lagoon population during the mass mortality event, modifying its genetic makeup. Analysis of Tunisian individuals from the Kerkennah coast revealed a surprising finding: these individuals showed haplotypes that were commonly found in the western Mediterranean prior to the mass mortality event. This suggests the potential for using the Tunisian population as a donor for the LIFE-PINNA project, should the need arise. However, confirming this sharing of haplotypes requires further analyses with a larger sample size from Kerkennah.

A second genetic study (partially funded by UNISS private resources) was conducted to reconstruct the evolutionary history and mitochondrial genetic diversity of *Pinna nobilis* across long temporal scales, integrating ancient, modern and surviving populations from the Mediterranean Sea. The main objective was to assess whether populations that survived recent mass mortality events (MMEs) still retain the historical adaptive and protective mitochondrial lineages of the species and to evaluate their suitability as a genetic reservoir for conservation and restoration actions.

Mitochondrial COI sequences generated during the project from surviving populations in the northern Adriatic (Miramare and the Venetian Lagoon) and southern Mediterranean (Kerkennah Islands, Tunisia) were combined with an extensive dataset from previous studies, including samples collected before the MMEs and historical material dating back up to 300 years. A standardized protocol for DNA extraction from ancient byssus material was developed, allowing, for the first time, direct genetic comparisons across three centuries.

The analyses revealed that *Pinna nobilis* exhibits remarkable long-term mitochondrial stability. Two major evolutionary lineages were identified, originating during the early Pleistocene (approximately 2.5–1.5 million years ago). Importantly, several of the most common mitochondrial haplotypes detected in ancient and pre-mortality populations are still present in surviving individuals today, including in the northern Adriatic donor populations targeted by LIFE PINNA restoration actions.

Despite a reduction in population size caused by MMEs, surviving populations retain a substantial proportion of the historical mitochondrial diversity of the species. In particular, the Gulf of Trieste population shows strong genetic continuity with pre-mortality conditions, while the Venetian Lagoon population, although displaying reduced diversity, still harbours widespread and historically persistent haplotypes. These findings suggest that recent MMEs acted as a selective bottleneck rather than causing a complete loss of evolutionary lineages.

Phylogeographic analyses indicated high connectivity across the Mediterranean basin and only weak spatial genetic structuring, consistent with the species' larval dispersal capacity. No strong isolation-by-distance pattern was detected, supporting the genetic compatibility of donor and recipient populations involved in LIFE PINNA restocking actions.

Overall, this study demonstrates that surviving *Pinna nobilis* populations preserve key components of the species' historical genetic legacy. This genetic resilience provides a solid scientific basis for conservation and restocking strategies implemented within the LIFE PINNA project and highlights the importance of protecting even small remnant populations, as they may harbour genetic traits essential for the long-term recovery of this critically endangered Mediterranean species.

In general, the analysis on the mitochondrial DNA yielded a fundamental result for the project's restocking strategy: we confirmed a high degree of "haplotype sharing" between the Adriatic donor sites and the recipient sites in western Mediterranean sites. Concrete implications of these results include:

- **Suitability Confirmation:** The Adriatic populations are officially confirmed as genetically appropriate donors for the Western Mediterranean.
- **Restocking Safety:** Restocking activities will not introduce "alien" genetic makeup, maintaining the natural genetic integrity of the intervention areas.
- **Selective Bottleneck Identification:** We identified that while the Venetian Lagoon has suffered a reduction in diversity (selective sweep), it still hosts the most resilient and common haplotypes. This allows the project to prioritize individuals with the highest probability of survival for translocation.

In addition, the development of a standardized protocol for DNA extraction from gland tissue fragments of ancient byssus samples further allowed us to confirm that recent mass mortality events (MMEs) did not cause a total loss of evolutionary lineages, but rather a bottleneck that allowed to

maintain the most adaptive ancient lineages. This provides the scientific justification for protecting even small remnant groups, as they still harbor the historical genetic legacy of the species.

Results of microsatellites

The process of microsatellite characterization for the *Pinna nobilis* populations of Gulf of Trieste (Miramare) and Venetian Lagoon started in summer 2023 provided the first detailed nuclear genetic data for the species in the Northern Adriatic. In response to a specific request from ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale), microsatellite analyses were carried out to deepen the understanding of the nuclear genetic composition of surviving *P. nobilis* individuals from the Gulf of Trieste and the Venetian Lagoon, and to evaluate their similarity with western Mediterranean populations prior to the mass mortality events (MMEs).

An evaluation of previous studies was conducted to identify the most suitable microsatellite set for analysing Adriatic populations of *Pinna nobilis* during the present project. Six previous studies employed microsatellite markers to characterise two *Pinna nobilis* populations in the western Mediterranean coast of Spain and France (González-Wangüemert et al. (2015 <http://dx.doi.org/10.12681/mms.949>, 2019 <https://doi.org/10.1002/aqc.2976>), Wesselmann et al. (2018 <https://doi.org/10.1038/s41598-018-23004-2>), and Peyran et al. (2020 <https://doi.org/10.1007/s11033-020-05338-1>, 2021 <https://doi.org/10.1038/s41598-021-87493-4>, 2022 <https://doi.org/10.1002/ece3.8482>).

Based on these previous studies, a total of 30 specimens from the northern Adriatic were processed for microsatellite analyses. The analyses were based on a set of ten microsatellite loci organised into three multiplex panels. During this standardisation phase, the standard PCR with non-fluorescent primers was used to evaluate the amplification efficiency before proceeding with the more expensive fluorescent primers.

Due to variable DNA quality, reliable genotyping results were obtained for 15 individuals. Genetic data from Adriatic populations were compared with a reference dataset for western Mediterranean populations collected before the MMEs (Gulf of Lion, France; Peyran et al., 2021).

Results indicated a good overall level of genetic diversity in the analysed Adriatic populations, with mean observed heterozygosity values comparable between the Venetian Lagoon and the Gulf of Trieste. Genetic differentiation between Adriatic sites was low, indicating a high degree of genetic similarity within the northern Adriatic area and supporting their comparability as donor populations. Although a moderate genetic divergence was detected between Adriatic populations and the western Mediterranean reference population, this divergence remains within the range reported for the species at a broader geographic scale and does not limit the suitability of Adriatic populations for conservation and restoration purposes. Overall, this analysis provided the first nuclear genetic data for northern Adriatic *P. nobilis* populations within the LIFE PINNA project and confirmed that surviving individuals retain a measurable level of genetic diversity despite the severe demographic decline caused by MMEs. These findings support the suitability of these populations as donor sources for conservation and restoration actions, while also highlighting the importance of continued genetic monitoring.

The concrete output of this microsatellite analysis was the measurement of the "genetic health" of surviving populations.

Key practical findings include:

- Genetic Stability: Despite the demographic collapse, surviving individuals retain a good level of genetic diversity (heterozygosity), comparable to pre-MME populations.
- Population Connectivity: Low differentiation between Miramare and Venice confirms that these sites can be managed as a single, cohesive donor unit, simplifying the logistics of the restocking phase.
- Donor Validation: Although a moderate divergence from French populations was noted, it remains within the species' natural range. This provides the final "green light" for using Northern Adriatic individuals as founders for new colonies in the Western Mediterranean.

Furthermore, in the following scientific papers (ref to E1 Annex 8_ supporting documents.pdf), the researchers of the University of Sassari are principal investigators. These studies are focused on topics that overall correspond to the main objectives of the action C.2. of the present project.

- Daria Sanna, Ilenia Azzena, Chiara Locci, Pavel Ankon, Petar Kružić, Chiara Manfrin, Alberto Pallavicini, Saul Ciriaco, Marco Segarich, Edoardo Batistini, Fabio Scarpa, Marco Casu.

Reconstructing the Evolutionary History of *Pinna nobilis*: New Genetic Signal from the Past of a Species on the Brink of Extinction. Animals 2024, 14, 114. <https://doi.org/10.3390/ani14010114>

- Azzena, I., Locci, C., Pascale, N. et al. **Conservation hints for *Pinna nobilis* from a century-old genetic time capsule.** Sci Rep 15, 37660 (2025). <https://doi.org/10.1038/s41598-025-21574-6>

Deliverable name	Foreseen Deadline	Actual Deadline
D_C.2 Report on the genetic make-up of survival specimens	12/2022	12/2023

Milestone name	Foreseen Deadline	Actual Deadline
M_C.2.1 Identification of the genetic make-up of still alive specimens	12/2022	06/2025

Comparison with planned output and time schedule:

The outputs planned by the project were all achieved. Furthermore, the sub action C.2.1 proceeded until the end of the project to constantly improve the knowledge on the genetic make-up of surviving *Pinna nobilis* populations and understand how mass mortality acted on the genetic variation of the species.

Major problems:

No significant issues have been experienced for this action. However, the successful development of the sub action C.2.1 depends on the possibility to find and analyse surviving *Pinna nobilis* individuals. For this reason, a further sampling method without any impact on individuals has been tested as a supplement to the non-lethal sampling method originally planned for the project activities of sample collection.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action). NONE

Outline the perspectives for continuing the action after the end of the project:

At the end of the present project, researchers of the University of Sassari will continue to analyse the surviving *Pinna nobilis* individuals and perform genetic characterization of pathogens isolated from this species. In particular, the analysis of the genetic variation in Haplosporida species will be likely extended to other species of Pinnidae, both inside and outside the Mediterranean Sea.

Mention any complementary action outside LIFE. NONE

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities. NONE

Attachments:

- D_C.2.1:report on the genetic make-up of survival specimens

Action C3: Collection and growth of *Pinna nobilis* self-recruited individuals from Northern Adriatic Sea

Foreseen start date: 10/2021

Actual start date: 10/2021

Foreseen end date: 06/2025

Actual end date: 09/2025

Activities undertaken and outputs achieved:

During the project period, extensive monitoring activities were conducted across the Gulf of Trieste and the Venice Lagoon where, in September 2023, researchers reported a mass mortality event affecting the majority of the *Pinna nobilis* population, most likely attributable to a marine heatwave. Further surveys enabled the identification of a new site in the Grado and Marano Lagoon, where specimens of *P. nobilis* considered at risk of damage were retrieved. During the 2022–2023 and 2023–2024 periods, several targeted actions were carried out in collaboration with mussel farming operators.

Shoreline actions

The following individuals were recorded during monitoring activities (2022/2023) in the Gulf of Trieste and other areas, considering only “endangered” juveniles:

- Thirty-three live *P. nobilis* individuals were found in the Gulf of Trieste (30 adults and 3 juveniles);
- Twenty juvenile individuals were confirmed through sightings reported by the citizen science network;
- Fifteen juvenile *P. nobilis* individuals (3.5 - 5 cm in shell height) were detected inside larval collectors placed in mussel farms; these specimens were subsequently transferred to the aquarium facility;
- Twenty-one juvenile individuals were found in high-risk areas such as docks, mooring zones, and anchoring site;
- Seven juvenile individuals were initially detected and monitored in hazardous locations within the Grado Lagoon.

On 19 June 2023, based on information and support provided by CNR-Venice personnel and a private research company, eleven live *P. nobilis* individuals (28 - 43.5 cm in shell height) were collected from the Venice Lagoon and transported to the Camogli laboratory for controlled reproduction as part of the C4 - C5 activities. All individuals were collected following authorisation from ISPRA. Genetic analyses tested negative for the presence of *H. pinnae* and other pathogens. A total of 96 *P. nobilis* specimens were recorded during the first two years of the project, 57 of which were self-recruited juveniles. Following collection, each specimen underwent transferability testing, whether placed in aquaria (for small specimens measuring 3.5 - 5 cm in shell height), suspended in a lantern-net or inside a small protective sea cage. An acclimation phase was performed for every specimen prior to its placement in the aquarium. Lantern-nets, traditionally used in shellfish aquaculture, served as nursery systems in which *P. nobilis* individuals could grow; these structures were attached to mussel-farm longlines and remained suspended in the water column for the duration required for rearing. Specimens placed at sea were located within protected areas under Shoreline concessions. When mortality occurred and the cause was not immediately evident, such as in cases lacking mechanical damage already present at the time of collection, remains were examined over time to determine the cause of death. Seventy percent of the samples tested positive for *H. pinnae*.

During the 2024/2025 monitoring activities, a total of 25 *P. nobilis* individuals were found alive in the Grado Lagoon, 3 in the Bay of S. Bartolomeo (near Slovenia), and 2 within the Miramare MPA. Of the 25 individuals recorded in the lagoon, 8 were identified and confirmed thanks to the participation of divers involved in citizen science initiatives, who detected specimens in high-risk areas such as docks, mooring sites, and anchorage zones. In January 2024, 24 pinnids were found in 14 larval collectors placed at Shoreline facilities. Of the 25 specimens detected in 2024, only 16 were subsequently confirmed alive in the Grado Lagoon. In 2025 all the specimens found in Grado lagoon are all dead.

In 2025, an additional 12 *P. nobilis* specimens, measuring between 11 and 24 cm, were identified within the Grado and Marano Lagoon system. In 2025, 4 adult specimens of *P. nobilis* were collected from Venice lagoon and placed in 500-litre tanks in a vertical position for gonadal maturation testing in collaboration with University of Genova and Aquarium Pula (duration of the experiment 8 months). Genetic analyses tested negative for the presence of *H. pinnae* and other pathogens. The cleaning of the tanks took place 3 times a week, to remove pseudo-feces and any food residue from the bottom of the tanks, the volume of water removed is restored again. The water change was regularly performed partially (1/3 of the total volume) to avoid air exposure of the specimens. The water used to fill the

tanks was filtered (sand filter, biological filter, 2 cartridge filters of 10 and 1 micron) and sterilised with UV lamps. All the tanks were provided of air stones inside connected to a compressor for aerating the water. The specimens were fed 3 times a week with a mix of live microalgae (*Isochrysis galbana*, *Chaetoceros calcitrans* and *Tetraselmis suecica*) and an amount of Easy Reef artificial feed® (Najdek et al., 2013; Trigos et al., 2015; Prado et al., 2020; Hernandis et al., 2022). The different strains of microalgae were maintained and grown in the laboratory inside bioreactors of 40 litres each, to be able to produce microalgae in large quantities. The specimens that died during the period of maintenance in the tanks were sent to colleagues at the University of Sassari to test for the presence of the protozoan *H. pinnae*. At the end of the experiment, the two surviving specimens were returned to the sea, within the Miramare Marine Protected Area.

Another key point to collect and grow *P. nobilis* is the use of larval collectors to recruit new individuals. The larval collector device consists of a main rope deployed vertically in the water column onto which a certain number of “collector bags” are attached in succession. Each collector bag is made up of an outer net bag that encloses a filler material. The structure consists of a rope with a maximum length of 8 m, kept vertically with buoys and bags for collecting larvae positioned at 3 different depths: -2 m, -4 m, and -6 m. Outer bags for larval recruitment can be made from onion or potato bags. These bags have proven particularly effective for larval settlement. The filler inside the bags can be made of different materials (tangled nylon filaments, additional plastic bags, jute). This material provides a surface for the larvae to settle on and protect them from predators while allowing them access. As *Pinna nobilis* larvae are transported by currents, collectors should preferably be positioned in areas exposed to open water. However, they can also be installed in semi-enclosed sites, such as lagoons. Larval collectors can be anchored to a ballast fixed to the seabed and kept vertically with a floating buoy or attached to the long line of the mussel farm. The collector devices should be retrieved after a period of about 5 months, however autumn weather and sea conditions may cause delays. A protocol has been created for the construction, positioning and control of larval collectors (Attachments, C3 Annex 1 - “Larval collectors preparation, setup and positioning”).

Across the Gulf of Trieste, the number of larval collectors and collector bags increased from 2022 to 2025.

- In 2022, there were three lines of collectors at each of three sites (a total of 39 bags) along the Italian coast at depths of 1–8 m. No pinnids were found.
- In 2023, three lines of collectors were placed at each of three sites (a total of 39 bags) along the Italian coast (depth: 1–8 m). Three empty shells were found.
- In 2024, three lines of collectors were placed at each of three sites (a total of 81 bags) along the Italian coast (depth: 1–8 m). Twenty-four young *P. nobilis* were found.
- In 2025, two lines of collectors were placed at each of three sites inside the Miramare MPA (total of 63 bags, depth: 1–8 m), and six lines of collectors were placed on one site on the shoreline mussel farms (total of 63 bags, depth: 1–8 m). No pinnids were found.

Juvenile specimens of *P. nobilis* found in a risk situation, were maintained on lantern-net or in the aquarium at the Shoreline facilities, were fed with a mix of 6 live microalgae (*Dunaliella salina*, *Dunaliella tertiolecta*, *Diacronema lutheri*, *Chaetoceros calcitrans*, *Isochrysis galbana* and *Tetraselmis suecica*) and a quantity of Easy Reef® artificial feed (Najdek et al., 2013; Trigos et al., 2015; Prado et al., 2020; Hernandis et al., 2022).

The aquariums are equipped with a biological filter, a cooler and a bed of sand and live stones. The different strains of microalgae are maintained and grown in the laboratory.

In the laboratory, “stock cultures” of the 6 microalgae (100 ml or 250 ml per strain) are stored in the enclosure without aeration, with light 12h/12h. These stock cultures are used to start all cultures and are fed with F/2 culture medium (Guillard) about every 15 days.

NIB actions

Considering the Slovenian sea, in the first two years of monitoring, 82 pinnids were surveyed. Of these 82 individuals monitored, 39 are young self-recruited *P. nobilis* individuals. These are the data collected by National Institute of Biology (NIB) during the first two years of the project (2022/2023):

- A total of 13 alive pinnids and 16 empty shells found in collectors in 2022; 26 alive pinnids and 68 empty shells found in collectors in 2023.
- In 2022 no *P. nobilis* was found on musselfarm longlines. Fishermen reported the presence of juveniles of *Atrina fragilis* only, animals were checked and kept in the aquarium of Piran. In 2023 two specimens of *P. nobilis* were saved by fishermen from mussel farms and kept at MBP facilities.
- In 2022-2023, 11 alive juveniles were observed during dedicated and opportunistic monitoring by NIB staff. One individual of 3 cm was found in June 2022 in a location at risk (floating protections in the port of Koper) was collected and brought to NIB facilities but it died a few days after.
- In 2022-2023, 29 records of living specimens were collected through the Citizen science campaign; 8 of those have been confirmed, most of them died soon afterwards.
- : A total of 29 living specimens were found at 6 different locations for years 2021/23, thanks to the collaboration with the Institute Vodni Krog for *P. nobilis* monitoring. In September 2025 only one of them was still found alive.

In 2022 3 lines of larval collectors were placed at each of five sites along the Slovenian coast, for a total of 43 bags; in 2023, 15 collectors of 6 bags each were placed at the same sites, for a total of 90 bags. A total of 39 juveniles were found on these collectors. Additional 3 living pinnids were found on mussel longlines and moved to NIB facilities. The samples sent to UNISS confirmed they were *P. nobilis*.

Alive specimens ranged between 1.2 and 5.4 cm of length in 2022, and between 0.5 to 8 cm of length in 2023. Alive pinnids were kept in aquaria in open system, with UV sterilization, and fed with Easy Reef® artificial feed (Najdek et al., 2013; Trigos et al., 2015; Prado et al., 2020; Hernandis et al., 2022). In 2023, 4 juvenile specimens from collectors were put in lantern-nets, in 2024 15 juveniles from collectors were transferred to lantern-nets, unfortunately, none of these juveniles survived till the end of the project (see action D2). All individuals were analysed for cause of death when not evident due to signs of mechanical damage already present at the time of sampling. Tests confirmed positive for the pathogen *H. pinnae*.

In 2024/2025, 15 collectors of 6 bags each were placed at the same sites of the previous years, for a total of 90 bags. A total of 18 small pinnids were found in collectors and moved to NIB facilities. Six living individuals were identified and confirmed thanks to the citizen science activities in 2024 and 2025, for a total of 36 from the start of the project.

Total collection and growth of *P. nobilis* during the project for Shoreline = 72 individuals

- November 2022, 20 juveniles from larval collectors kept at the Shoreline mussel farms.
- July 2023, 8 individuals placed in lantern-net (found in dangerous areas in the Grado lagoon).
- October 2023, 8 juveniles found in areas considered dangerous along the Trieste coastline (placed in lantern-net).
- January 2024, 24 juveniles are found in larval collectors (kept in aquariums).
- June 2024, 4 individuals collected in the Venice Lagoon for the start of captive breeding.
- October 2024, 4 individuals found near the port of Grado and placed in lantern-net.
- January 2025, 4 individuals collected in the Venice Lagoon arrived at the Shoreline laboratories.

Total collection and growth of *P. nobilis* during the project for NIB = 60 individuals

- 13 juveniles found in larval collectors in 2022.
- 26 juveniles found in larval collectors in 2023.
- 18 juveniles found in larval collectors in 2024.
- 3 juveniles found in mussel farms and kept in an aquarium.

Of these specimens, 19 were kept in lantern-net.

Deliverable name	Foreseen deadline	Actual deadline
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D_C.3 Best practices protocol for manipulation, captation and growth of juveniles of <i>Pinna nobilis</i> .	31/08/2025	31/09/2025
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Milestone name	Foreseen deadline	Actual deadline
M_C.3.1 First complete setup of the nursery-harvesting process achieved.	31/07/2022	31/09/2022
M_C.3.2 Second complete setup of the nursery-harvesting process achieved.	31/07/2023	31/08/2023
M_C.3.3 Third complete setup of the nursery-harvesting process achieved.	31/07/2024	31/09/2025

Comparison with planned output and time schedule:

We expect to recruit a total of 70 ind./year of *P. nobilis* juveniles, shared between Shoreline (50 ind./year) and NIB (20 ind./year), for a total of 210 during the project. Between 2022/2023, 96 specimens of *P. nobilis* were found in two years. Of these 96 individuals, 57 are self-recruited juveniles and 39 are adults. In November 2024 we collected 24 juveniles from the larval collectors. As Shoreline and NIB, we collected a total of 132 *P. nobilis* individuals during the period of the project, instead of the planned 210. Analysis of the dead individuals revealed evidence of the presence of pathogens. Despite increased efforts to find new locations to increase the number of living (not infected) individuals, the mortality rate is still very high. The hope is to find new individuals in the new locations where there are no pathogens.

Major problems:

The situation has now changed due to the drastic reduction in *P. nobilis* populations, and recruitment activities present a twofold problem: first, the potential impact they could have on existing populations, which are becoming increasingly smaller, and second, the risk associated with transporting the specimens collected. In response to this problem, capture and monitoring activities have been intensified, with a considerable effort on the part of the partnership to identify as many specimens of *P. nobilis* as possible, paying particular attention to those at high risk of damage and therefore in need of protection, including through transfer.

In addition, most of the specimens already collected are infected and have died. The challenge is therefore to find new areas with a low incidence of pathogens that will allow us to bring suitable specimens to the destination areas for transplantation.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action).

No significant over-spending.

Outline the perspectives for continuing the action after the end of the project:

Activities after the project will be continued as the species will remain critically endangered and the scientific community has expended considerable efforts to study this species. Thanks to this LIFE project and other existing collaborations, it has been possible to establish a strong network with local, national and international scientific entities (SPA RAC collaboration). *Pinna nobilis* has become the subject of studies in several fields (ecological, genetic, physiological) that are increasingly intertwined at the level of methodologies in the field of breeding for food of other related species (mussels, oysters etc.). The positioning of the larval collectors in the Gulf of Trieste and along the Slovenian coast will continue, as will the monitoring of new *P. nobilis* individuals, including both juveniles and adults.

Mention any complementary action outside LIFE.

Complementary actions outside the Life Pinna project are carried out thanks to the TRECap project (Interreg Italy - Slovenia) and Marine Strategy Framework Directive – MSFD.

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities.

Attachments:

- D_C3: Best practices protocol for manipulation, captation and growth of juveniles of *Pinna nobilis*

- C3 Annex 1 - Larval collectors preparation, setup and positioning.

Action C4: Adaptation, breeding and reproduction for active restocking

Foreseen start date: 04/2022

Actual start date: 04/2022

Foreseen end date: 06/2025

Actual end date: 09/2025

Activities undertaken and outputs achieved:

Clearly describe all the activities undertaken in the relevant period taking into account what foreseen in the project application

The action started on time and developed through the setup of the rearing systems for adults and larvae, alongside the setup of microalgal cultures for feeding the *Atrina fragilis* and *P. nobilis* specimens.

As it was not possible to collect adult specimens of *P. nobilis* in Trieste in the summer of 2022, due to the severe depletion of adult stocks, in December 2022, rearing started using a model species, *Atrina fragilis*, with specimens from the Triest lagoon. These specimens were cultured from December 2022 to the arrival of *P. nobilis* specimens in June 2023. *A. fragilis*, also belonging to the family Pinnidae, is a large Pinnidae, which shares similar habits to *P. nobilis*, is widely distributed and is not a protected species.

The following year, these protocols were applied to *Pinna nobilis* specimens from the Venice lagoon. The Milestone_C.4.1 “First spawning induction achieved” (due on 09/2022) was achieved late due to the lack of breeding stock in summer 2023. This Milestone was achieved in June/July 2023, following the collection and transfer of adult specimens (Action C5). Consequently, the Milestone_C.4.2 “Second spawning induction achieved” (due on 09/2023) was achieved between May/July 2024. The Milestone_C.4.3 “Third spawning induction achieved” (due on 09/2024) was achieved between April and May 2024, following the gonadal maturation induction trials, in collaboration with Shoreline and Pula Aquarium. In addition, we carried out a fourth transport and spawning induction in July 2025. In each reproductive season, four consecutive phases are foreseen in the framework of the present action: 1) acclimatisation and sexual maturation; 2) spawning induction and eggs fertilisation; 3) larval rearing and metamorphosis; 4) juveniles rearing.

“Initial trials with model species, *Atrina fragilis*” (December 2022-June 2023)

1. Acclimatisation

As far as *Atrina fragilis*, on 1 December 2022, 9 specimens of *A. fragilis* (size range, 7 adults: 20-24 cm in height and 2 juveniles: 6.3 - 8.3 cm in height) were collected in the Gulf of Trieste and transported to the laboratory in Camogli. The 7 adult specimens were placed inside two 240 litre tanks, and the 2 juveniles in a 40 litre aquarium. In the laboratory tanks, all specimens (adults and juveniles) were placed vertically inside glass containers filled with sand (Annex C.4.1). The cleaning of the tanks took place 3 times a week, to remove pseudo-feces and any food residue from the bottom of the tanks, the volume of water removed is restored again. The water change was regularly performed partially (1/3 of the total volume) to avoid air exposure of the specimens. The water used to fill the tanks was filtered (sand filter, biological filter, 2 cartridge filters of 10 and 1 micron) and sterilised with UV lamps. All the tanks were provided of air stones inside connected to a compressor for aerating the water. The photoperiod was regulated using a LED lamp (12h light and 12h dark). The specimens were fed 3 times a week with a mix of live microalgae (*Isochrysis galbana*, *Chaetoceros calcitrans*, *Diacronema lutheri*) and an amount of Easy Reef artificial feed® (Table in annex C.4.2), (Najdek et al., 2013; Trigos et al., 2015; Prado et al., 2020; Hernandis et al., 2022).

After 9 months from the arrival in the laboratory, 8 on 9 *A. fragilis* specimens died, with a total survival rate of 11% (Annex C.4.3). The juveniles survived until September 2023, while the adults only survived until June 2023, when the last surviving adult specimen was returned to the sea in the Gulf of Trieste.

2. Spawning and fertilization

In May 2023, *Atrina fragilis* adult specimens were subjected to three spawning induction trials, by means of a thermal shock with a temperature change of approximately $\Delta=10^{\circ}\text{C}$ (Helm et al., 2006; Trigos et al., 2018), without obtaining any gamete release and consequently no fertilisation and larval development.

M_C.4.1: “First spawning induction” (June/July 2023)

1. Acclimatation

On 19 June 2023, 11 adult specimens of *P. nobilis* (between 28-43.5 cm height) were transported to Camogli laboratory from the Venice Lagoon, for controlled reproduction. To ensure the maintenance of these specimens in the laboratory, 2 tanks of 240 litres each and a 540 litre tank were set up, inside which the specimens were placed in a horizontal position resting on plastic boxes (Annex C.4.4). The tank cleaning activities were carried out as described above for *A. fragilis*. The photoperiod was regulated using a LED lamp (12h light and 12h dark). The specimens were fed 3 times a week with a mix of live microalgae (*Isochrysis galbana*, *Chaetoceros calcitrans*, *Diacronema lutheri*) and an amount of Easy Reef artificial feed® (Najdek et al., 2013; Trigos et al., 2015; Prado et al., 2020; Hernandis et al., 2022). The different strains of microalgae were maintained and grown in the laboratory inside bioreactors of 40 litres each, to be able to produce microalgae in large quantities. Table of annex C.4.5 shows the daily diet administered to adult individuals. After 6 months from the transport to the laboratory, 4 specimens survived and the survival rate is 36.4 % (Annex C.4.6). The specimens that died during the period of maintenance in the tanks were sent to colleagues at the University of Sassari to test for the presence of the protozoan *Haplosporidium pinnae*. The four survivors were transported on December 14th 2023 in Capo Mortola.

2. Spawning and fertilization

The first spawning of *P. nobilis*, induced by transport "stress" (Hernandis et al., 2023), occurred on June 20th, but between June and July 2023 we observed 5 spontaneous spawning events, 3 fertilizations, and two larval cultures were established. During the spontaneous spawning (Annex C.4.7), already fertilized eggs were released, which were collected, counted and the rapid development of the zygote until the formation of the larva was observed. Only the first two fertilizations were followed completely, the third unfortunately we were not able to count the number of eggs released, but these were inserted into the various tanks used for the other two fertilizations. A total of 14.623.800 eggs were released, with high fertilisation rates, between 95.31 and 99% (14.060.200 fertilised eggs). (Table in annex C.4.8).

3. Larval rearing and metamorphosis

The fertilized eggs showed rapid development, probably due to the high water temperature (25.5°C). The various stages of development of the zygote were observed up to the formation of the trochophore (first larval stage) and the early veliger (second larval stage). Daily, the larvae were fed a fixed dose of a mix of the three microalgae (*I. galbana*, *C. calcitrans*, and *D. lutheri*). Unfortunately larval development was blocked at the early veliger stage at 9 dpf (days post fertilization), consequently no metamorphosis and settlement of post larvae was observed (Annex C.4.9).

Data from 2022-2023 relating to transport, maintenance and reproduction protocol of *Atrina fragilis* and *Pinna nobilis* were published in Ecology and Evolution journal:

“Ferranti M.P., Azzena I., Batistini E., Caracciolo D., Casu M., Chiantore M., Ciriaco S., Firpo V., Intini L., Locci C., Montefalcone M., Oprandi A., Sanna D., Scarpa F., Segarich M., 2024. Handling of the bivalve *Pinna nobilis*, endangered and pathogen affected species, for controlled reproduction: precautions taken. *Ecology and Evolution*, 14: e70565. <https://doi.org/10.1002/ece3.70565>” (Annex C.4.10)

M_C.4.2: “Second spawning induction achieved” (May-July 2024)

1. Acclimatation

On 23 May 2024, 6 adult specimens of *Pinna nobilis* (between 31.2 - 60 cm height) were transported to Camogli laboratory from the Venice Lagoon, for controlled reproduction. The specimens were maintained inside 2 tanks of 540 litres each, in an oblique position on plastic boxes (Annex C.4.4). The specimens were fed 3 times a week with a mix of two live microalgae strains (*Isochrysis galbana*, *Chaetoceros calcitrans*), supplied several times a day using a peristaltic pump. In addition, we supplied them with a portion of Easy Reef artificial feed®, and decapsulated *Artemia salina* cysts. Table of annex C.4.11 shows the daily diet administered to adult individuals. After 2 months from the transport to the laboratory, all specimens survived (survival rate: 100 %). On 31 July 2024, the 6

surviving specimens, tested negative to *H. pinnae*, were reintroduced into the sea (Capo Mortola MPA).

2. Spawning and fertilization

Between May and July, 7 spawning induction trials occurred: 2 induced by transport stress and 5 by thermal shock. One of the two spawning events triggered by transport stress resulted in the release of already fertilized eggs (self-fertilization Pn_F1), while in the other we only observed the release of male gametes. As for the thermal shock trials, only 4 out of 5 induced a release of gametes: 3 times we obtained the release of only male gametes, while once we also observed the release of oocytes by 3 specimens (Annex C.4.12; Annex C.4.13). During this last spawning event, one of three specimens (specimen ID n° 6) also subsequently released male gametes, which were used for controlled fertilization (Pn_F2). The released eggs, fertilized or not, were filtered, collected in a known volume (11 L), counted and then (eventually) fertilized (in the case of Pn_F2) and the development from zygote to the larva was monitored and described (Annex C.4.14). The total number of embryos released in Pn_F1 was not quantified, but in the 11 L collected from the adult maintenance tank, 2.420.000 larvae were estimated. For controlled fertilization Pn_F2 a total of 44.280.000 eggs were counted, with a fertilization rate of 72.5 % and a trochophore rate of 37.5 % (ratio between trochophore and fertilised eggs, accounting for the rate of embryos which continue the development to the trochophore stage), resulting in 11.835.000 trochophores.

3. Larval rearing and metamorphosis

The larvae were reared at a water temperature of about 21°C (room temperature), according to Trigos et al. (2018), to avoid the rapid development due to the high temperature observed in Ferranti et al. (2024). The larvae were placed in tanks with different volumes, at the density of 2.5 and 5 larvae/mL. The larvae were fed daily with a mix of *I. galbana* and *C. calcitrans* and once they reached a size of 120 µm, *Tetraselmis suecica* was also added to the larvae's diet (Helm et al. 2006). For both fertilizations (Pn_F1 and Pn_F2), the larval development was monitored. In the self-fertilization Pn_F1 larval development stopped at 9 dpf at the D-larva stage, whereas the larvae obtained from Pn_F2 survived until 21 dpf, of these, 40% reached the umbonate larval stage at 16 dpf with a shell size of 150 µm, in accordance with Hoyos-Chairez and Chavez-Villalba (2023). This larval size was never achieved before for *P. nobilis* (Annex C.4.14; Annex C.4.15).

These data were submitted in Marine Biology journal, and now this paper is under review: “Ferranti M.P., Azzena I., Batistini E., Casu M., Ciriaco S., Di Napoli C., Farris S., Intini L., Locci C., Oprandi A., Prandoni L., Sanna D., Scarpa F., Segarich M., Chiantore M., under review. Controlled Reproduction of the Noble Pen Shell *Pinna nobilis*: Comparison of Larval Development from Self-Fertilization and Controlled Fertilization. Available at SSRN: <https://ssrn.com/abstract=5382258> or <http://dx.doi.org/10.2139/ssrn.5382258>” (Annex C.4.16)

M_C.4.3: “Third spawning induction achieved” (April-May 2025)

During this year, we conducted an induced gonadal maturation trial in collaboration with Shoreline and the Pula Aquarium. The experimental setup was designed based on the team's experience at the Pula Aquarium in 2023 and information found in the literature. The aim of this trial was to induce gonadal maturation in some specimens of *P. nobilis* in about 4/6 months (from January to May/July) to obtain early gamete release and increase the number of reproductive events. Four or eight specimens were kept in the three laboratories. The temperature increased from 11° to about 23 °C, and the photoperiod was increased from 10 to 18 hours of light. The specimens were fed a mix of three strains of algae (60% *Isochrysis galbana*; 30% *Chaetoceros calcitrans*; 10% *Tetraselmis suecica*). In addition, zooplankton was supplied in vivo (copepods, *Artemia salina* nauplii and rotifers) or as artificial feed (Bea zoo plus®; Gold pearl®).

1. Acclimatation

On 17 January 2025, 12 adult specimens of *Pinna nobilis* (8 UniGe and 4 Shoreline: 23.5-31 cm in height) were placed in a vertical position in tanks of about 540 litres (Annex C.4.4), and the sea water used was filtered and sterilized. The sea water temperature was maintained using chillers and heaters. Cleaning activities included removing faeces and food residues, with water changed three times a week.

After 6 months from the transport to the laboratories, the adult survival rate was low 8.3 % (UniGe: 0 specimens + Shoreline: 1 live specimens; Annex C.4.17). But all specimens (live and dead) were tested negative for *H. pinnae*. Histological analyses will be performed on the gonads of dead specimens to assess their level of maturation. In July, the only surviving specimen of *P. nobilis* (Shoreline laboratory) was transferred to the Miramare MPA.

2. Spawning and fertilization

In April, we observed 4 spontaneous spawning and in addition, in May, we carried out 4 thermal shocks, obtaining only a release of male gametes. From the spontaneous spawning, we carried out 3 fertilizations: 1 spontaneous fertilization and 2 controlled fertilizations and monitored 3 larval developments. A total of about 8.000.000 eggs were released in 3 spawning events. Unfortunately, fertilization rates were low (around 9 and 18%), (Table of annex C.4.18).

3. Larval rearing and metamorphosis

Larval development stopped at 8–10 dpf, reaching only the trochophore stage (Annex C.4.19).

Regarding this experience, the method still needs refining. In fact, few eggs were released, despite being the right size (50-55 µm), we obtained a low fertilization rate and larval development that stopped at the trochophore stage. Our results, along with those obtained by the Pula Aquarium, will be included in a scientific paper that we are currently writing.

In addition, “Fourth spawning induction” (July 2025)

Given the poor results obtained with gonadal maturation, we decided to carry out a fourth collection and transport (16 July 2025) of five specimens (26.5-42 cm in height) for reproduction in July.

1. Acclimatation

These specimens were maintained and fed in the same way as the other specimens. After a month in the laboratory, all specimens survived and tested negative for *H. pinnae*. As a result, they were transferred to the sea, in the MPA of the island of Bergeggi.

2. Spawning and fertilization

In July, we conducted two spawning induction trials using the thermal shock method. However, we did not observe any gamete release. These specimens were probably already in the sexual resting phase, also due to the high temperatures reached in July at sea in the donor site (Venice lagoon).

Deliverable name	Foreseen Deadline	Actual Deadline
D_C.4 Best practice protocol for <i>Pinna nobilis</i> reproduction in controlled conditions	30/6/2025	30/9/2025

Milestone name	Foreseen Deadline	Actual Deadline
M_C.4.1 First spawning induction achieved	30/9/2022	20/06/2023
M_C.4.2 Second spawning induction achieved	30/9/2023	30/09/2024
M_C.4.3 Third spawning induction achieved	30/9/2024	30/09/2025

Comparison with planned output and time schedule:

The action suffered of delays, caused by late due to the lack of breeders, was not possible in 2022 because of seriously depleted adult stock in Gulf of Trieste. However, the Milestone_C.4.1 (First spawning induction achieved, due on 09/2022) was achieved in June 2023, thanks to the availability of adult specimens from the Venice lagoon. This inevitably led to the postponement of the other two Milestones by one year respectively, as shown in the table above.

Major problems:

Management of adult specimens/larvae in the tank: initially not knowing whether the specimens were positive for the protozoan or not, to be on the safe side the wastewater was chemically sterilised in special storage tanks before being eliminated. This has made management of specimens (adults and larvae) in the laboratory more challenging. Furthermore, the limited information available in the literature on the reproduction and larval development of *P. nobilis* has made the management of this action challenging.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action).

Outline the perspectives for continuing the action after the end of the project:

Another reproduction induction trial will be carried out (summer 2026), depending on the availability of adults from healthy natural populations and facility availability (that is not assured after the end of the project for UNIGE). The resources (mostly human resources) will be made available jointly from UNIGE and SHORELINE.

Mention any complementary action outside LIFE.

Between November 2022 and June 2024, as part of the afterLife of the RELIFE project, we continued to maintain juvenile *Patella ferruginea* in the tank, obtained from controlled reproduction in November 2022. Every two months we monitor their survival rate, while monthly the growth rate is calculated.

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities.

Attachments:

- D_C.4: Best practice protocol for *Pinna nobilis* reproduction in controlled conditions
- C4 Annex C.4.1 Accommodation of *Atrina fragilis* specimens in laboratory tanks
- C4 Annex C.4.2 Daily diet of *Atrina fragilis* specimens
- C4 Annex C.4.3 Monthly survival rate of *Atrina fragilis* specimens
- C4 Annex C.4.4 Accommodation of *Pinna nobilis* specimens in the laboratory tank
- C4 Annex C.4.5 Daily diet of *Pinna nobilis* specimens maintained in the laboratory in 2023
- C4 Annex C.4.6 Monthly survival rate of *Pinna nobilis* specimens in the laboratory tanks in 2023
- C4 Annex C.4.7 Spontaneous spawning of *Pinna nobilis* specimens in 2023
- C4 Annex C.4.8 Table relating to the data of the spontaneous spawning events and fertilization in 2023
- C4 Annex C.4.9 Developmental stages of *Pinna nobilis* in 2023
- C4 Annex C.4.10 Ferranti et al., 2024
- C4 Annex C.4.11 Daily diet of *Pinna nobilis* specimens maintained in the laboratory in 2024
- C4 Annex C.4.12 Eggs release of *Pinna nobilis* specimen in 2024
- C4 Annex C.4.13 Data relating to spawning events of *Pinna nobilis* in 2024
- C4 Annex C.4.14 Larval developmental stages of *Pinna nobilis* in 2024
- C4 Annex C.4.15 Larval survival rate and average shell size of larvae of *Pinna nobilis* in 2024
- C4 Annex C.4.16 Ferranti et al., under review_ Marine Biology
- C4 Annex C.4.17 Monthly survival rate of *Pinna nobilis* specimens in the laboratory tanks in 2025
- C4 Annex C.4.18 Table relating to the data of the spontaneous spawning events and fertilization in 2025
- C4 Annex C.4.19 Larval developmental stages of *Pinna nobilis* in 2025

Action C5: Collection and transport of specimens from self-capture to receptor sites

Foreseen start date: 01/10/2021

Actual start date: 01/10/2021

Foreseen end date: 30/06/2025

Actual end date: 30/09/2025

Activities undertaken and outputs achieved:

This action, coordinated by Arpal, aims to collect all protocols used by beneficiaries for Collection and transport of specimens from self-capture to receptor sites. Action C5 is divided into 4 sub-actions, relating to the collection of samples (D_C5.1) and the transport of sentinels (D_C5.2), mantle fragments (D_C5.3) and *P. nobilis* individuals (D_C5.4). Protocols were initially acquired at the draft form and implemented as the actions progressed, leading to the completion of the final Deliverable D_C.5.2 *Final report on all transport forms*. The relevance of these Protocols is very high in that they are experimental procedures that have been refined and codified to enable their use with a view to replicability both within the LIFE PINNA project and in the future by other parties interested in undertaking similar activities.

C5.1: Collection of organisms (sentinel bio-indicators and resistant adults) and small fragments of the mantle tissue from adults of *Pinna nobilis*

As a first step, a reconnaissance activity was carried out to retrieve any existing protocols on the collection of organisms and mantle fragments of *Pinna nobilis*, also calling on the stakeholders identified in Action A3. This activity was unfortunately unsuccessful, as no response was obtained. It was therefore decided to rework all the protocols relating to the activities implemented in the Life PINNA project, as communicated during the second monitoring visit. This activity continued for the duration of the project, in order to systemise all the information acquired within the project.

In the end, the following were implemented:

- A protocol concerning the collection of sentinel individuals, which takes into account both wild and purchased individuals;
- A protocol for the collection of mantle fragments and other biological samples: following the non-lethal sampling authorisation received from the Ministry (MASE), alongside the transmission of a protocol based on a standard method for the removal of mantle fragments, UNISS prepared a less invasive protocol for genetic analysis and pathogen detection on tissue, based on the use of a cotton or a sampling brush swab

C5.2: Transport of sentinel organisms

This activity was carried out by ARPAL, using different species of bivalve molluscs. The first shipment of sentinel organisms from ARPAL to UNISS took place in April 2022, using the species *Mytilus galloprovincialis*. The same procedure was also used for sentinel organisms belonging to *Ostrea* sp. and for the species *Mytilus galloprovincialis*.

Following these experiences, which took place until May 2023, a first draft protocol for the shipment of sentinels was drawn up, which was subsequently refined following shipments made in subsequent periods.

C5.3: Transport of small fragments of the mantle tissue from adults of *Pinna nobilis*

For the transport of mantle tissue fragments to the University of Sassari for genetic analysis, the protocol developed for the transport of sentinel organisms was verified during the project and found to be fully suitable.

C5.4: Transport of specimens from donor to receiving sites and hatcheries

Since the translocation activities were considerably delayed due to the unavailability of the planned number of *P. nobilis* individuals, an initial experimentation was carried out with species *Atrina fragilis*, which allowed for preliminary transport and tank testing activities, as it is a related species to *P. nobilis*.

On the basis of this experience, a first draft of the Protocol was drawn up in March 2023, which was subsequently perfected following the transport and rearing in the UNIGE laboratory of some *P. nobilis* specimens collected in the Venice Lagoon.

The protocol has been implemented as the activities progressed in the second part of the project, until the final version of the document was prepared, which codifies each step related to the transport of individuals and their maintenance in captivity within the University of Genoa laboratory.

In addition, the University of Genoa has also prepared a protocol for the reproduction of *P. nobilis*, following the very first experience of reproduction of the species: starting with the first specimens transferred to the laboratory on 19/6/2023, which released gametes the day after their arrival and some of which resulted in fertilisation (the eggs were monitored until the formation of larvae, as described

in Action C4), up to the latest thermal shock tests applied to individuals arriving at the laboratory in July 2025, all activities were precisely codified (Annex 4.2).

Deliverable name	Foreseen Deadline	Actual Deadline
D_C.5.1 protocols for collection and transport of all the organism and small fragments tissue	12/2021	09/2025
D_C.5.2 final report on all the transport forms	05/2025	09/2025

Milestone name	Foreseen Deadline	Actual Deadline
M_C5.1: Collection & transports (9 times) of sentinel organisms for A2,D1;	04/2025	04/2025
M_C5.2: Collection & Transport of fragments of mantle tissue from adults (3 times) P. nobilis	11/2023	11/2023
M_C5.3: Transport of juveniles (3 times) from donor to receptor areas for C6	08/2024	08/2024
M_C5.4: Collection & Transport of adults (3 times, 10 units yearly) for C4	03/2024	03/2024
M_C5.5: Collection & Transport of larvae spawned in captivity (3 times) to the mussel farmers' nursery areas	12/2024	12/2024

Comparison with planned output and time schedule: None

Major problems: None

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action). None

Outline the perspectives for continuing the action after the end of the project: The protocols will be promoted and shared with all those involved in international research on Pinna nobilis in order to facilitate the activities codified therein, promote their application in new conservation projects and, where necessary, optimise them in line with the evolution of knowledge resulting from subsequent developments. The partners involved in preparing the various protocols will always be available for constructive discussion with other researchers interested in exploring the topics in greater depth.

Mention any complementary action outside LIFE. None

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities.

Attachments:

- D_C.5.1 protocols for collection and transport of all the organism and small fragments tissue
- D_C.5.2 final report on all the transport forms

Action C6: Installation of the specimens of *Pinna nobilis* at the pilot areas

Foreseen start date: 04/2022
Foreseen end date: 06/2025

Actual start date: 04/2022
Actual end date: 09/2025

Activities undertaken and outputs achieved:

During the first phase of the project, the transplantation activity developed with the preparation of useful materials for the journey. On several occasions we were able to experiment with the materials, first without live organisms, then with organisms other than *P. nobilis*, particularly *Atrina fragilis*. This allowed us to be able to test the entire transport phase without endangering live organisms of *P. nobilis*. Confirmed 100% efficiency of *Pinna nobilis* transport during all the transport activities C3/C4/C5/C6 actions. The first attempts of transport of *A. fragilis* to the Camogli laboratories took place in December 2022. Specifically, the materials used were a tank to transport without harming the individuals, bubbler to oxygenate the water, and filtered water (0.45µm) to change/fill the tank during the journey. Transfer was successful and confirmed the efficiency of the transport protocol.

Within the framework of the LIFE project, the translocation and transplantation of *Pinna nobilis* specimens in the designated pilot areas were carried out in accordance with the relevant conservation actions (C.3, C.4 and C.5). The activities are summarised below:

On 17 February 2023, four juvenile specimens obtained from larval collectors were transplanted in the Strunjan Marine Protected Area (MPA). This was followed, on 12 April 2023, by the installation of twelve juveniles from larval collectors in the Miramare MPA. Subsequently, on 4 July 2023, eight adult specimens originating from the Grado Lagoon were transplanted in the Miramare MPA. On 16 October 2023, a further eight juvenile specimens, recovered from high-risk areas along the Trieste coastline, were transplanted in the Miramare MPA. In July 2023, eleven *P. nobilis* individuals were translocated from the Venice Lagoon to Camogli, with the support of the University of Genoa, within the scope of Action C.4. In December 2023, four of these specimens were subsequently transplanted to Capo Mortola. These individuals represent the only self-recruited specimens that survived (see Action C.3) and were confirmed to be pathogen-free. The translocation and transplantation were successfully completed, and the specimens are currently alive and monitored at the Capo Mortola site. In order to increase the survival probability of these specimens, additional protective measures were implemented. Specifically, protective cages were transplanted, designed to ensure adequate water circulation while minimising the risk of mechanical damage under open-sea conditions. Further transplantation activities were carried out in 2024. On 6 March 2024, eighteen specimens obtained from larval collectors were transplanted in the Miramare MPA. On 2 April 2024, six juveniles from larval collectors were transplanted in the Strunjan MPA, followed by the transplantation of nine juveniles at the same site on 14 May 2024. On 23 May 2024, six specimens were transferred to the Camogli facilities, while four specimens were transferred to Trieste, in accordance with Action C.5. On 3 July 2024, three additional specimens were transplanted in the Miramare MPA. In July 2024, six specimens originating from the Venice Lagoon, following the completion of Action C.4, were transplanted at the Capo Mortola site. Subsequent installations included one specimen from larval collectors transplanted in the Strunjan MPA on 23 August 2024, and four specimens originating from the Grado Lagoon transplanted in the Miramare MPA on 15 October 2024. In 2025, two specimens from the Venice Lagoon were transplanted in the Miramare MPA on 11 August 2025. In September 2025, five adult specimens originating from the Venice Lagoon were transplanted in the Bergeggi MPA. Finally, in October 2025, four juvenile specimens obtained from larval collectors in Deboli rtič were transplanted in the Strunjan MPA.

A total of 14 transplant actions were carried out during the project: Seven in the Miramare MPA, four in the Strunjan MPA, two in Capo Mortola, and one in the Bergeggi MPA. A total of 94 individuals were successfully relocated to the receiving sites: 55 to the Miramare MPA, 24 to the Strunjan MPA, 10 to the Capo Mortola site and 5 to the Bergeggi MPA.

Thanks to this experimentation, it was possible to develop a transport protocol that allowed all phases to be studied, thus reducing the possibility of damage and stress to the organisms (*Attachments, C6 ANNEX 1 - "Capo Mortola Transplantation protection devices"*). During each action, video materials were collected for the completion of D_C6.2, making a DIY video "*Pinna nobilis: how to transplant*".

Deliverable name	Foreseen Deadline	Actual Deadline
D_C.6.1. Best practices protocol for transplanting <i>P. nobilis</i> juveniles.	31/8/2025	30/9/2025
D_C.6.2. Video “ <i>Pinna nobilis</i> : how to transplant” - A DIY video”.	31/8/2025	30/9/2025

Milestone name	Foreseen Deadline	Actual Deadline
M_C.6.1. First transplanting setup of <i>P. nobilis</i> in four Pilot areas.	31/07/2022	30/12/2023
M_C.6.2. Second transplanting setup of <i>P. nobilis</i> in four Pilot areas.	31/7/2023	30/9/2024
M_C.6.3. Third transplanting setup of <i>P. nobilis</i> in four Pilot areas.	31/7/2024	30/9/2025

Comparison with planned output and time schedule:

Expected results from the project: considering *P. nobilis* specimens of possible accidental deaths during previous actions (C.3, C.4, C.5) and transplantation operations (C.6), we expect to transplant 55 ind./year in four marine areas overall (Capo Mortola, Miramare MPA, Strunjan and Asinara Island), for a total of 165 transplanted individuals during a three year period. Instead of 165, we transplanted a total of 94 individuals during a three-year period. Genetic analysis to detect the presence of *H. pinnae* produced negative results both at the recipient sites and in the analysis carried out on the transplanted individuals.

Major problems:

During the first two-year of the project, Shoreline monitored and surveyed 96 individuals of *P. nobilis*, NIB monitored and surveyed 82 individuals. In total, 178 individuals of *P. nobilis* were surveyed. Of these 178 individuals, 135 were positive for *H. pinnae*. This would have made it impossible (according to the proposal) to transfer them to the receiving MPAs, even if they had survived. Even after the main problem was to find new individuals, adults and juveniles of *P. nobilis* to be transplanted. Even the young recruitment found in the larval collectors did not survive. In fact the individuals that were transferred to Capo Mortola and Bergeggi MPA were negative for *H. pinnae*.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action). None

Outline the perspectives for continuing the action after the end of the project:

The possibility of expanding the range of donor sites will allow us to find areas with more pathogens-free organisms.

Mention any complementary action outside LIFE.

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities.

Attachments:

- D_C6.1: Best practices protocol for transplanting *Pinna nobilis* juveniles
- D - C6.2 - Video *Pinna nobilis*: how to transplant” - A DIY video
- C6 ANNEX 1 - Capo Mortola transplantation protection devices.

Action D1: Monitoring of pathogens in restocking sites by using sentinel species UNISS

Foreseen start date: 07/2022

Actual start date: 08/2023

Foreseen end date: 12/2024

Actual end date: 09/2025

Activities undertaken and outputs achieved:

During the final year of the LIFE PINNA project, molecular diagnostic activities were completed to provide a consolidated and comprehensive assessment of pathogens associated with mass mortality events of *Pinna nobilis*. These analyses integrated data from sentinel mollusc species and from *P. nobilis* individuals that died during project activities, both in the wild and under controlled conditions, with the aim of drawing final conclusions on pathogen presence, diversity and epidemiological relevance.

Overall, the results confirmed a highly consistent pattern across all years of monitoring. Among the wide range of protozoan, bacterial and viral agents screened, *Haplosporidium pinnae* emerged as the only protozoan pathogen systematically associated with mortality in *P. nobilis*. Genetic analyses of *H. pinnae*, performed on both conserved (18S rRNA) and more variable (ITS) molecular nuclear regions, revealed a complete absence of detectable genetic variability. All sequences obtained during the project were identical to those previously reported from other Mediterranean regions. Screening for additional protozoan pathogens historically associated with bivalve diseases (*Bonamia* spp. and *Perkinsus* spp.) consistently yielded negative results. Bacterial pathogens belonging to the genera *Mycobacterium* and *Vibrio* were occasionally detected in deceased *P. nobilis* individuals, supporting their role as opportunistic or secondary infections rather than primary drivers of mass mortality. No evidence of viral pathogens was detected.

ADDITIONAL ANALYSES, FUNDED BY UNISS PRIVATE RESOURCES INDEPENDENTLY OF THE LIFE PINNA PROJECT, THAT HAVE CONTRIBUTED TO THE ACHIEVEMENT OF THE PROJECT'S OBJECTIVES

During the first 18 months of the LIFE PINNA project, it was not possible to fully implement Action D.1, as no dead or clinically infected individuals of *Pinna nobilis* were available for pathogen analyses. The action was therefore effectively developed starting from summer 2023, in parallel with the first restocking activities, and continued until the end of the project.

Between 2023 and 2025, a structured pathogen-monitoring programme was implemented to evaluate the role of *Haplosporidium pinnae* as a predictor of disease development and mortality, and to define operational infection thresholds applicable to conservation and restocking actions.

Overall, 30 individuals were monitored: 26 originating from the Venetian Lagoon and 4 from the Gulf of Trieste. Individuals were sampled repeatedly over time using non-invasive techniques when alive and comprehensive post-mortem sampling in the event of mortality. Infection presence and intensity were assessed using standard PCR and quantitative real-time PCR (qPCR), resulting in more than 780 molecular reactions performed during the project.

In order to support this monitoring effort while minimising stress and handling impacts on living individuals, a specific focus was placed on the optimisation of non-invasive sampling strategies.

To improve pathogen monitoring in *Pinna nobilis*, we developed and standardised a non-invasive cotton swab sampling protocol. This approach enabled repeated screening of living individuals for pathogen presence/absence while reducing cumulative stress associated with invasive sampling. DNA obtained from cotton swabs proved suitable for standard PCR and quantitative PCR analyses, supporting reliable detection of pathogens associated with mass mortality events. The entire development of this methodology was self-financed by the University of Sassari, highlighting a strong institutional commitment to advancing conservation-oriented diagnostic tools.

The adoption of the non-invasive sampling protocol significantly improved the feasibility of long-term pathogen monitoring and contributed to safer donor selection during restocking activities. When combined with qPCR analyses, the method supported early detection of infections and assessment of mortality risk, reducing the likelihood of translocating infected individuals. This methodological innovation directly strengthened the effectiveness of conservation and restoration strategies implemented within LIFE PINNA and provides a transferable framework for future monitoring and management of endangered marine bivalves.

On the basis of the non-invasive sampling strategy described above, the following section details the experimental conditions and molecular methods applied for pathogen detection and monitoring.

Methods: Health monitoring was conducted between 2023 and 2025 on *Pinna nobilis* individuals maintained under controlled conditions at the University of Genoa aquaria and, in 2025, also at SHORELINE facilities in Trieste. Monitoring consisted of repeated sampling events (three to five time points per individual, depending on the year), generally carried out on a monthly basis.

For living individuals, samples were collected using non-lethal and minimally invasive mantle biopsies and, from 2024 onwards, using completely non-invasive cotton swabs to collect biological traces from the valves of fan mussels (internal and, when required, external). These methods were selected to minimise stress and were approved by the competent Italian authorities. In the event of mortality, post-mortem examinations were carried out and tissues were collected from mantle, gills, adductor muscle and digestive gland.

All samples were first screened using standard PCR to detect the presence of *H. pinnae*

Quantitative real-time PCR was subsequently applied to estimate infection intensity through Cycle threshold (CT) values, used as a proxy for pathogen load. Based on the full dataset and survival outcomes, three operational infection classes were defined and applied throughout the project: CT ≤ 25 (high infection, high mortality risk), CT between 25 and 30 (intermediate infection), and CT > 30 (low infection, generally compatible with short-term survival). Positive and negative controls from reference collections were included in all analyses, and qPCR protocols were standardised and validated during the initial phase of the project to ensure reproducibility and comparability of results across years.

2023 – Pilot monitoring phase

In 2023, 11 individuals from the Venetian Lagoon were monitored between August and November. Three individuals died shortly after arrival, and three additional specimens died within the following month. Individuals that died early showed low CT values (approximately 19–25), often already detectable in mantle samples while alive, and post-mortem analyses revealed systemic infections affecting multiple tissues. In contrast, individuals that survived throughout the monitoring period consistently showed negative PCR results or high CT values (> 30), indicating low pathogen loads. Four of these individuals were subsequently transplanted as part of restocking actions.

2024 – Extended monitoring

In 2024, 7 individuals were monitored between May and July. Almost all specimens remained PCR negative and showed high CT values (> 30). One mortality event occurred, but post-mortem analyses did not reveal significant *H. pinnae* infection. This year was characterised by low prevalence and low infection intensity, highlighting that weak qPCR signals may occur in healthy individuals without leading to disease.

2025 – Venetian Lagoon and Gulf of Trieste

In 2025, 8 individuals from the Venetian Lagoon were monitored between January and May, with additional mortality events recorded later in summer, including transplanted individuals found dead in situ. While swab samples from live individuals generally showed low pathogen loads, post-mortem analyses in selected cases revealed marked increases in infection intensity, with CT values well below 25 in multiple tissues.

In parallel, 4 individuals from the Gulf of Trieste were monitored between February and July 2025. Two individuals remained alive until June and consistently showed low or absent infection. The other two died during the monitoring period and displayed a clear increase in *H. pinnae* infection over time,

confirmed by decreasing CT values in swabs and very low CT values in post-mortem tissues. By July 2025, only one individual remained alive.

The results obtained over the three-year monitoring period demonstrate that qPCR analysis of *Haplosporidium pinnae*, combined with repeated sampling over time, is an effective tool for assessing health status and mortality risk in *Pinna nobilis*. CT values ≤ 25 were consistently associated with high pathogen loads and mortality, whereas stable CT values >30 were generally observed in surviving individuals.

The definition of operational infection thresholds and the validation of non-invasive monitoring techniques represent a significant advancement for conservation management. Action D.1 contributed to improving donor population selection, reducing the risk of translocating infected individuals, and increasing the overall effectiveness of restocking actions implemented within the LIFE PINNA project. Furthermore, in the following scientific paper (ref to E1 Annex 8_ supporting documents.pdf), the researchers of the University of Sassari participated as co-authors. This study is focused on topics that overall correspond to the main objectives of the action D.1. of the present project.

- Stéphane Coupé, Ioannis A. Giantsis, Maite Vázquez Luis, Fabio Scarpa, Mathieu Foulquié, Jean-Marc Prévot, Marco Casu, Athanasios Lattos, Basile Michaelidis, Daria Sanna, José Rafa García-March, José Tena-Medialdea, Nardo Vicente, Robert Bunet. The characterization of toll-like receptor repertoire in *Pinna nobilis* after mass mortality events suggests. Ecology and Evolution, 2023. 13:e10383, DOI: [10.1002/ece3.10383](https://doi.org/10.1002/ece3.10383)

Deliverable name	Foreseen Deadline	Actual Deadline
D_D1.1 Report on the analysis of etiological agents at year one	06/2022	12/2023
D_D1.2 Report on the analysis of etiological agents at year two	06/2023	12/2024
D_D1.3 Report on the analysis of etiological agents at year three	06/2024	09/2025

Milestone name	Foreseen Deadline	Actual Deadline
M_D1.1 Data from first year monitoring collected	03/2022	10/2023
M_D1.2 Data from second year monitoring collected	03/2023	10/2024
M_D1.3 Data from third year monitoring collected	03/2024	09/2025

Comparison with planned output and time schedule: The outputs planned by the project were achieved. Major problems: No significant issues have been experienced for this action. However, the successful development of the sub action D.2 depends on the possibility to study in detail the tissues of *Pinna nobilis* individuals that have died from the disease.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action). NONE

Outline the perspectives for continuing the action after the end of the project:

At the end of the present project, researchers of the University of Sassari will continue to analyse the surviving *Pinna nobilis* individuals and to perform genetic characterization of pathogens isolated from this species. In particular, the analysis of the genetic variation in Haplosporidia species will be likely extended to other species of Pinnidae, both inside and outside the Mediterranean Sea.

Mention any complementary action outside LIFE. NONE

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities. NONE

Attachments:

- D_D1.1: Report on the analysis of etiological agents at year one
- D_D1.2: Report on the analysis of etiological agents at year two

- D_D1.3: Report on the analysis of etiological agents at year three

Action D2: Monitoring of implanted juveniles

Foreseen start date: 07/2022

Actual start date: 03/2023

Foreseen end date: 09/2025

Actual end date: 09/2025

Activities undertaken and outputs achieved:

Juvenile individuals of *P. nobilis* collected under Action C3 (i.e., *Collection and growth of Pinna nobilis self-recruited individuals from the Northern Adriatic Sea*), along with adult specimens used as breeders in reproduction trials, were placed in natural environments and regularly monitored to evaluate their survival, growth, and health status. Monitoring activities were conducted monthly—depending on sea and weather conditions—at three of the four pilot sites: Capo Mortola, Miramare, and Strunjan. This activity could not be performed at the Asinara MPA site due to the insufficient number of individuals in the nearest potential donor population (i.e. the Sant’Antioco lagoon) and the logistical constraints associated with the long transport time from the Adriatic Sea.

The monitoring activities envisaged in Action D2 were carried out thanks to the collaboration between the partners of the LIFE PINNA project. Data from Capo Mortola were collected by the University of Genoa, those from Miramare by Shoreline, and data from the Slovenian coast by NIB.

At the Capo Mortola site, two translocations were performed between December 2023 and July 2024. All translocated individuals (10 in total) were adult specimens originating from the Venice Lagoon and were used as breeders in reproduction trials conducted at the Camogli laboratory. Following the 2023 and 2024 breeding seasons, the specimens were reintroduced into the sea. Analysis of two years of monitoring data showed that the average survival time at sea was approximately eight months.

At the Miramare pilot site, seven translocation operations were carried out by Shoreline between April 2023 and August 2025. Both juvenile and adult individuals were restocked (17 adults and 38 juveniles). Juveniles mainly originated from larval collectors deployed in mussel farms, while adults were relocated from unsafe areas of the Grado Lagoon or, as in Capo Mortola, collected from the Venice Lagoon for breeding purposes. Analysis of two years of monitoring data showed that the average survival time at sea was approximately two months for the adults and four months for the juveniles.

Along the Slovenian coast, four translocations were conducted by the NIB team. Only juvenile *Pinna nobilis* individuals (20 in total) originating from larval collectors and mussel farms were transferred into lantern nets. On one occasion, a surviving juvenile was later relocated to the seabed, where it was monitored on a monthly basis. This specimen achieved a record survival duration of 15 months—three months in the lantern net and an additional twelve months on the seabed. On average, the survival time derived from monitoring data collected over two years was approximately seven months.

The number of translocations varied among the three pilot sites, depending on the availability of individuals. Miramare recorded the highest number of restocked specimens (55 in total), including both juveniles and adults. Only two adult *P. nobilis* remained alive at this site, having been reintroduced to the sea only in August 2025.

In the Adriatic Sea, juveniles demonstrated higher survival potential than adults, as evidenced by the exceptional survival time of the individual monitored along the Slovenian coast. Adult *P. nobilis* showed markedly different survival durations between basins: approximately eight months in the Ligurian Sea versus two months in the Adriatic. Predation can be ruled out as a major mortality factor in the Adriatic, since even individuals maintained in lantern nets survived only about two months. The discrepancy likely reflects local environmental conditions (i.e., depth, hydrodynamism and temperature), which require further investigation.

Deliverable name	Foreseen Deadline	Actual Deadline
D_D.2.1 Report on the status of the restocked individuals of <i>Pinna nobilis</i> at year one	30/9/2023	30/12/2024
D_D.2.2 Report on the status of the restocked individuals of <i>Pinna nobilis</i> at year two	30/9/2024	30/09/2025

D_D.2.3 Report on the status of the restocked individuals of <i>Pinna nobilis</i> at year three	30/9/2025	Not available*
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Milestone name	Foreseen Deadline	Actual Deadline
M_D.2.1 Data from first year monitoring collected	30/6/2023	30/12/2024
M_D.2.2 Data from second year monitoring collected	30/6/2024	30/9/2025
M_D.2.3 Data from third year monitoring collected	30/6/2025	Not available*

**the deliverable and milestone regarding the third year was not produced because the data from March 2023 and December 2024 were collected in the D.D.2.1 and the data from Jan to Sept 2025 in the D.D.2.2.*

Comparison with planned output and time schedule: Due to a one-year delay in the start of the action, it was produced just two “Report on the status of the restocked individuals of *Pinna nobilis*”, indeed data for the first year of monitoring, required for producing the first report, have been collected between March 2023 (following the first translocation along the Slovenian coast) and December 2024 (one year after the first translocation at Capo Mortola, which eventually took place in December 2023 following a delay caused by the absence of the authorizations from the competent authorities). Therefore, the data for the second year of monitoring, necessary for the second report, have been collected between January 2025 and September 2025 (end of the project). In addition, the last two adult individuals transferred to the sea at the Miramare Marine Protected Area in August 2025 died within two months. Consequently, neither time nor surviving specimens of *P. nobilis* were available for further monitoring.

Major problems: The main causes of delay in action D2 were the difficulty in finding adult individuals for reproduction, the failure to produce juveniles through controlled reproduction (action C4), and the delayed authorization from the competent authorities to release adult individuals into the sea at Capo Mortola, the last area where translocations took place, which in turn delayed the subsequent data collection for monitoring.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action). None

Outline the perspectives for continuing the action after the end of the project:

As the two adult individuals released into the sea in August 2025 died within two months of the translocation no further monitoring activities are foreseen.

Mention any complementary action outside LIFE.None

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities.None

Attachments:

- Deliverable D.2.1 Report on the status of the restocked individuals of *Pinna nobilis* at year one
- Deliverable D.2.2 Report on the status of the restocked individuals of *Pinna nobilis* at year two

Action D3: Monitoring of the project's impact on the *P. nobilis status**

Foreseen start date: 10/2021

Actual start date: 10/2021

Foreseen end date: 09/2025

Actual end date: 09/2025

Activities undertaken and outputs achieved:

Partners involved in action D.3 started gathering information about the presence of fan mussels in donor and receiving sites from the beginning of the project. Unfortunately, results depicted a situation dramatically different from previous data, in particular from donor sites where *P. nobilis* density collapsed to zero.

Additional efforts were made to gather information about *P. nobilis* in different areas: some comforting results were obtained by project partners and by citizen science activities.

In particular, within the scope of this action, it was planned to carry out a simplified monitoring conducted through Citizen Science (CS) campaigns aimed at implementing the analysis on the status of *P. nobilis* of restocking sites and to complete the knowledge framework in the absence of data that Arpal has entrusted to the IMC Foundation International Marine Center as a *third party*.

Main events for disseminating Life PINNA and, in particular, Citizen Science activities were:

- First public event of the LIFEPINNA project, December 2, 2022 (approximately 40 people online and 60 in person)
- Online event "Rimettiamoci le pinne!", May 10 (hosted by PADI): approximately 60 people [<https://www.lifepinna.eu/2023/04/20/rimettiamoci-le-pinne/>]
- S. Antioco "Immaginari possibili" event, Spiaggia di Maladroxia", August 26-27, 2023: approximately 50 people
- Fin search with group support from the Cagliari Carabinieri Underwater Unit and the Divers for Science group: approximately 15 people
- REGINA-MSP Conference on Regional Ocean Literacy Strategy for MSP, Cagliari, May 10, 2023 (Presentation with a generic title, which also discussed Life PINNA): approximately 50 people
- "Successione ecologica" congress 18-19 September (Presentation with a generic title where LIFEPINNA was also discussed): approximately 50 people
- Dozens of people reached on social media by tagging the Life PINNA page and "Ricerca la pinna" in the comments
- Posidonia Green Festival, July 11-13, 2025: approximately 200 people at the stand, approximately 20 people during the snorkeling trip.

See details of events in E1.

In addition, the project website was implemented with an *ad hoc* section on CS <https://www.lifepinna.eu/citizen-science/> where reports from citizens are collected.

Deliverable name	Foreseen Deadline	Actual Deadline
D_D3.1: Report on the <i>P. nobilis</i> status ex ante	31/08/2022	28/02/2023
D_D3.2: Report on the <i>P. nobilis</i> status ex post	30/09/2025	30/09/2025
D_D3.3: Report on citizen science campaigns	30/09/2025	30/09/2025

Milestone name	Foreseen Deadline	Actual Deadline
M_D3.1: Survey campaigns on the <i>P. nobilis</i> status ex ante in the donors sites and in the receptor sites	30/06/2022	31/12/2022
M_D3.2: Survey campaigns on the <i>P. nobilis</i> status ex post in the donors sites and in the receptor sites	31/08/2025	31/08/2025
M_D3.3: Citizen science campaigns	31/08/2025	31/08/2025

Comparison with planned output and time schedule:

The report concerning the ex ante campaign on the *P. nobilis* status in donor and receiving sites was delivered with about 6 months of delay basically due to the uncertainty on the eligibility of receiving sites after the analyses of sentinel organisms. The two deliverables relating to ex-post monitoring and citizen science activities are delivered together with the final report, thus respecting the project's timeline. However, since it was not possible to transplant *Pinna nobilis* specimens to the Asinara site, no ex-post monitoring was conducted there. The objective of the ex-post monitoring has also shifted from the original project (i.e. to verify positive impacts on the receiving sites), due to the few number of transplanted individuals. For this reason, the ex-post monitoring campaign has been focused also on finding surviving *Pinna nobilis* specimens, both through the use of scientific divers and remote-operated vehicles (ROVs).

Major problems:

The lack of a high-density, pathogen-free population of *P. nobilis* in donor sites shifted the scheduled first transplanting event and, consequently, ex-ante campaign. As stated above, the small number of

organisms transplanted in the receiving sites of Capo Mortola, Miramare, and Strunjan made the implementation of the ex-post campaign of little relevance, especially in the sites of Miramare and Strunjan, which in the project served as both donor and recipient sites: the *P. nobilis* populations in these two areas in fact recorded a dramatic decline during the project.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action). none

Outline the perspectives for continuing the action after the end of the project:

Institutional monitoring activities will eventually be carried out by project partners.

Mention any complementary action outside LIFE.

ARPAL carried out a monitoring campaign in 2022-2023 aimed to gain information about the evolution of previously observed *P. nobilis* population. Furthermore, during other underwater monitoring activities, particular attention was paid to the possible presence of specimens of *Pinna nobilis*.

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities. None

Attachments:

- D_D3.1: Report on the *P. nobilis* status ex ante
- D_D3.2: Report on the *P. nobilis* status ex post
- D_D3.3: Report on. citizen science campaigns

Action E1: Communication

Foreseen start date: 01/10/2021

Actual start date: 01/10/2021

Foreseen end date: 30/09/2025

Actual end date: 30/09/2025

Activities undertaken and outputs achieved:

Communication plan

The communication plan was completed and delivered in October 2022 by Triton Research. It was updated in March 2023.

The project logo: The logo, developed by AC Consulting with Triton Research indications, has been used in all the project documents, materials and tools according to the LIFE Communication requirements from April 2022.

Project website: The project website www.lifepinna.eu, developed by AC Consulting with Triton Research indications, is online from April 2022 and it will remain active for at least 5 years after the end of the project. The LIFE Pinna website was created for keeping track of the various project activities, objectives, results and products, enabling its wide-ranging awareness and dissemination. The website has been translated to English and constantly updated, **at least once a month**, with contributions from all the partners to increase the public understanding of the project and improve communication with scientists and the general public. It is divided into the following sections:

- **The project**, which gives an overview of the project with its main goals, providing detail with project actions too.
- **The species**, with a focus on *Pinna nobilis* biology and ecology.
- **Report Pinna**, devoted to citizen science activities, with a form to report *Pinna* species. This section has been developed with specific attention to be user friendly.
- **Photo contest**, with information to participate in the summer LIFE Pinna photo contest and winners of previous years.

- **Media** that shows Pinna photo stories, videoclips and final documentary (resident on youtube), material to download (Layman's Report, rollout and citizen science posters) and a selected press review.
 - **News**, with all the most important news related to the project.
 - **Partners**, with the list of beneficiaries and main tasks.
 - **Network**, which contains a list of projects with which LIFE Pinna has created synergies.
 - **Contact**, with a form to write a mail to Life Pinna (info@lifepinna.eu)
- At the end of the project 13,000 unique users had visited the website.

Social networks Facebook and Instagram are on-line since December 2021: they represent a very powerful tool to help the project to publish and communicate its status. Twitter/X was added later, after the website was developed, in September 2022. The contents are managed by Triton Research, with contribution from the project partners. They are updated at least once a week. All partners have been interviewed for the elaboration of social posts and provided photo/video materials. Followers number per social media platform are as follows (September 2025), with more than triple growth since the end of 2022:

- www.facebook.com/lifepinna - 2770 followers
- www.instagram.com/life.pinna/ - 2479 followers
- twitter.com/life_pinna - 44 followers

In August and September 2022 an advertisement campaign with the external assistance of AC Consulting was implemented to promote contents on the LIFE Pinna website and on social networks, with a strong focus on the promotion of the first edition of the LIFE Pinna photo contest. The adv campaign used banners on the web to promote interest in the project and to attract people to the website (Native Adv campaign) and promoted contents in Instagram and Facebook (sponsored contents). The same campaign was carried on in 2023 and 2024. Between June and September 2025 a new double advertisement campaign (Meta Ads and Google Ads) with the external assistance of GO UP Agency was implemented to promote to support the photo contest and increase social media coverage and website traffic on the one hand, and to increase views of the LIFE Pinna documentary (*Stop the extinction*) and related videoclips, on the other. With the new cookie policies (2025), Google Analytics only records users who accept tracking. This makes the numbers more reliable in terms of quality, but it also means that actual traffic may have been higher than reported. Nevertheless, considering the four-year period from 2022 to 2025, sponsorship campaigns resulted in total social network coverage exceeding 1,600,000.

(see "E1 Annex 1_supporting documents")

Promotional materials:

- **Roll up.** For the project's first public event in 2022, 9 project roll-ups were produced, each measuring 80x100 cm. These roll-ups were produced in three languages: Italian (6), English (2), and Slovenian (1) and distributed among partners.
- **Information panels.** In July 2024 7 indoor information panels (70 x 100 cm), Italian (6) and Slovenian (1), were produced to be placed in the beneficiaries' premises and in the visitor centers of the protected areas involved. (See "E1 Annex 3_Info panels.pdf")
- **Photo panels.** These panels were intended to exhibit photographs from the photo contest "Life among the reef". 6 pictures in 70x50 cm and an introductory panel have been used at different events in 2022 and 2023. (see "E1 Annex 1_supporting documents")
- **Citizen Science posters.** In July 2023 and then in July 2024, 100 Citizen Science posters in 70x50 cm were produced on durable, waterproof paper, to promote pinna reports form citizens. They were used at University of Genoa headquarters, ARPAL in Genoa, Shoreline Cooperative, AMP Miramare in Trieste, Festival della Scienza di Genova and other events for the general public. (See "E1 Annex 2_Citizen Science Poster")
- **Leaflets.** In June 2024 1,000 leaflets (Italian 800, English 200) and in August 2025 100 more (English) for the final event. Anyway, Citizen Science posters placed in locations of interest for

activities, such as diving centres, visitor centres, and universities, were considered more effective, less polluting, and cost-effective. (See “E1 Annex 4_Leaflet.pdf”)

- **Nature illustrations.** 2 Life Pinna tables were produced by illustrator Joanna Klepadło, for her PhD at Nicolaus Copernicus University in Toruń, Poland, with a focus on *Pinna nobilis* ecology and LIFE Pinna actions, which was exhibited at Festival della Scienza di Genova 2023 and other events with general public, as Posidonia Festival 2025. (See “E1 Annex 6_Nature illustrations.pdf”)

- **Layman’s Report.** A 16-page informative report, aimed at non-experts, was produced in two languages (English and Italian) in both paper and digital formats. 100 copies were printed and distributed mainly during the final event in Slovenia on September 17, 2025, at the NIB headquarters in Piran. The digital version is published on the project website: <https://www.lifepinna.eu/en/media-material-to-download/> (see “E1 Annex 5_Laymans Report.pdf”)

Gadget: t-shirts, rulers, eco-sustainable bags
Gadgets were produced as promotional tools in meetings and festivals and to award photo contest winners and most useful citizen science records. They were distributed to the partners during the first public meeting on 02/12/2022 and in 2023, 2024 and 2025 meetings, with each participant receiving many of each gadget produced.

- 400 LIFE Pinna pins
- 600 LIFE Pinna magnets
- 100 LIFE Pinna shopper bags
- 100 LIFE Pinna keychains
- 165 LIFE Pinna T-shirts, 3 sizes
- 100 USB pens

As a prize for the 2023 photo contest and for the most useful contributions received through the citizen science form, in July 2023, five copies of the book “Pinneggiando nei mari italiani” (Finning through the Italian seas) by Marco Bertolino and Maria Paola Ferranti, published by Hoepli Editore in 2018, were also purchased. Additional copies of marine atlases were also purchased and sent to the winners of the 2024 and 2025 photography competitions, respectively “Atlante di flora e fauna del Mediterraneo. Guida alla biodiversità degli ambienti marini” (Atlas of Mediterranean Flora and Fauna: Guide to the Biodiversity of Marine Environments), Ed. Il castello, by Egidio Trainito and Rossella Baldaconi, and “Guida all'identificazione dei pesci marini d'Europa e del Mediterraneo” (Guide to the Identification of Marine Fish of Europe and the Mediterranean), ed. Il Castello, author Louisy Patrick.

(see details and the material in “E1 Annex 1_supporting documents” and “E1 Annex 7_LIFE PINNA GADGET dissemination_2022-2025”)

Scientific papers

During 2022-2025 twelve scientific papers citing LIFE Pinna have been produced by partners. (see list in “E1 Annex 1_supporting documents”)

Articles and other media

LIFE Pinna had about 400 citations on different media so far (online, paper, radio, TV), including very popular TV shows like “Linea Blu” (Rai 1), “Geo” (Rai 3), “TG Leonardo” (Rai 3), TG5 (Canale 5), in addition to magazines and newspapers like Corriere, Repubblica, La Stampa, Focus, Vatican News, Il Secolo XIX, Osservatore Romano, Le Figaro, Focus, Rivista della Natura. Total combined number of readers and audience is superior to 10 million people (prudent estimate), not including LIFE Pinna social, website and videos. The press offices Boe Sas (2022-2023) and Green Factor srl (2024-2025) collaborating with Triton Research, provided help with general media, especially with newspapers. More details on Life Pinna selected press review: <https://www.lifepinna.eu/en/media-press-review> (see “E1 Annex 1_supporting documents” and “E1 annex 8_PRESS REVIEW”)

Videos and documentaries

A collection of 15 **video clips** in double language (15 in Italian, 15 in English) about Life Pinna activities were uploaded to the project web page in late 2023. All the video clips can be found in

<https://www.lifepinna.eu/en/media/>

In early 2024, the project documentary, developed by Marco Tessaro with Triton Research indications, was also produced in a first version (less than 10 minutes) in Italian and English, launching an advertising and media campaign to promote it.

Between May and July 2025, filming of the project's planned actions was completed, and in August 2025, the **final version of the project documentary** (23 minutes) was published, in Italian and with English subtitles. At the same time, a strong dual campaign (Meta Ads and Google Ads) was launched, which, in just a few weeks, allowed the documentary to garner over 41,000 views and to the 15 videoclips almost 6,000 views on the YouTube channel and lifepinna.eu website.

(see “E1 Annex 1_supporting documents”)

Conferences

Partners took part in some scientific conferences and congresses in 2022-2025, citing LIFE Pinna.

- **52nd Congress of the Italian Marine Biology Society, Messina, 12-15 June 2023 - [S.I.B.M. - Società Italiana Di Biologia Marina - Congressi \(sibm.it\)](#)**
 - Ferranti M.P., Ciriaco S., Meroni L., Cimini J., Firpo V., Intini L., Oprandi A., Segarich M., 2023. Preliminary protocol of transport and maintenance of Pinnidae (Bivalve Mollusc). 52° Congresso della Società Italiana di Biologia Marina (SIBM), Messina, 12-15 Giugno 2023. (Poster).
 - Bordoni R., Chiantore M., Ferranti M.P., Merotto L., Ruggeri F., Venturini S., Montefalcone M., Oprandi A., Paoli C., Rigo I., Vassallo P. 2023. Perdita di capitale naturale a seguito della moria di *Pinna nobilis* nel Mediterraneo. 52° Congresso della Società Italiana di Biologia Marina (SIBM), Messina, 12-15 Giugno 2023. (Poster).
- **81° Congresso Unione Zoologica Italiana, Trieste, September 2022 - [81 Congresso Unione Zoologica Italiana 2022 \(uzionlus.it\)](#)**
- **20° International Conference of disease of fish and shellfish, EAFF Virtual Congress, 20-23 September 2021 - [20 EAFF conference-first Announcement - EAFF](#)**
- **XXXII Congress of Italian Society of Ecology, Catania, 6-8 September 2023 - [XXXII Congresso della Società Italiana di Ecologia 6-8 Settembre 2023 \(cnr.it\)](#)**
 - Rigo I., Bordoni R., Chiantore M., Ferranti M. P., Merotto L., Mitreanu C., Ruggeri F., Venturini S., Vassallo P., Paoli, C., 2023. Natural capital lost as a result of *Pinna nobilis* die-off in the Mediterranean Sea. XXXII Congresso della Società Italiana di Ecologia (S.It.E), Catania, 6-8 Settembre 2023 (Talk).
- **Conservation of *Pinna nobilis* and strategies to avoid extinction, 29 November 2023 (promoted by LIFE Pinnarca as networking activity), attended by 20 people. [6e6b38_bec19bfd181a4bebb937ed0824eae27a.pdf \(lifepinnarca.com\)](#)**
- **9° International Symposium on Marine Sciences ISMS – Valencia, 10-12 July 2024**
 - M.P. Ferranti, Mariachiara Chiantore, Monica Montefalcone, Alice Oprandi, Daniela Caracciolo, S. Ciriaco, M. Segarich, E. Batistini, I. Azzena, C. Locci, N. Pascale, A. Ciccozzi, F. Scarpa, D. Sanna and M. Casu, 2024, Concrete actions for the restoration of *Pinna nobilis* within the european Life Pinna project: transport, captive breeding and transplantation - ISMS – Valencia, 10-12 July 2024 (talk)
 - S. Picchi, D. Caracciolo, M. Lazzari, C. Picetti, S. Ciriaco, M. Segarich, F. Tomasinelli and D. Grech, 2024, Engaging citizens in Life Pinna project: the strategic role of communication actions for the critically endangered species conservation - ISMS – Valencia, 10-12 July 2024 (talk)
 - V. Pitacco, D. Caracciolo, S. Ciriaco, A. Fortič, D. Trkov, L. Lipej, B. Mavrič, M. Orlando-Bonaca, M. Segarich, E. Batistini, T. de Lorenzi, C. Locci, I. Azzena, N. Pascale, A. Ciccozzi, F. Scarpa, D. Sanna, M. Casu, 2024, Larval collectors and ex-situ maintainance for juveniles of *Pinna nobilis* in the Gulf of Trieste (Northern Adriatic Sea) as part of european project Life Pinna - ISMS – Valencia, 10-12 July 2024 (talk)
 - C. Locci, I. Azzena, N. Pascale, A. Ciccozzi, P. Ankon, M.P. Ferranti, M. Chiantore, M. Montefalcone, A. Oprandi, D. Caracciolo, C. Manfrin, A. Pallavicini, M. Segarich, E. Batistini, S. Ciriaco, V. Pitacco, A. Fortič, D. Trkov, L. Lipej, B. Mavrič, M. Orlando-

Bonaca, 2024, Reconstruction of the evolutionary history of *Pinna nobilis*: implications for conservation and restocking activities - ISMS – Valencia, 10-12 July 2024 (talk)

- **54° Congresso della Società Italiana di Biologia Marina (SIBM), Napoli, 9-12 giugno 2025.**
 - M.P. Ferranti, L. Rodriguez, M. Mičić, E. Batistini, S. Ciriaco, M. Segarich, T. Coloretti, C. Di Napoli, A. Oprandi, L. Intini, M. Chiantore. Gonadal maturation protocol of the endangered species *Pinna nobilis* (Mollusca, Bivalvia), (Poster).
 - Oprandi, A., Ferranti, M.P., Segarich, M., Pitacco, V., Fortič, A., Trkov, D., Dagnino, A., Caracciolo, D., Montefalcone, M., 2025. First-year monitoring of *Pinna nobilis* survival in the wild: The Life Pinna Project. 54° Congresso Società Italiana di Biologia Marina (SIBM), Napoli, 9-12 giugno 2025. (Poster)
- **58th European Marine Biology Symposium (EMBS), Bodó, Norway, July 6-9, 2025**
Orlando-Bonaca, Martina, Lipej, Lovrenc, Trkov, Domen, Mavrič, Borut, Pitacco, Valentina, Fortič, Ana, Lokovšek, Ana, Donša, Daša, Ivajnsič, Danijel. Benthic vegetation distribution in the northern Adriatic: present and future. (Talk).
Fortič, Ana, Pitacco, Valentina, Trkov, Domen. Empty *Pinna nobilis* shells: refuge for mobile macroinvertebrates. (Talk)
Trkov, Domen, Pitacco, Valentina, Fortič, Ana. *Pinna nobilis* shells as key elements in the environment for coastal fish community. (Talk).

Events/exhibitions

The project has been presented at more than 10 national dissemination events, like public lectures, outdoor activities and science festivals to highlight its activities and results.

(see the material in “E1 Annex 1_supporting documents” and “E1 annex 9_Lists of dissemination and networking events”)

<i>Deliverable name</i>	<i>Foreseen Deadline</i>	<i>Actual Deadline</i>
D_E.1.1 Communication plan	10/2022	10/2022
D_E.1.2 Website and social network	05/2022	05/2022
D_E.1.3 First video-documentary	10/2023	02/2024

<i>Milestone name</i>	<i>Foreseen Deadline</i>	<i>Actual Deadline</i>
M_E.1 First public event	10/2022	12/2022

Comparison with planned output and time schedule: The documentary's original date (10/2023) has been postponed to 2/2024 to follow main action development. More in detail, the first translocation of captive *Pinna nobilis* in Capo Mortola, Liguria, in late December 2023 was a main topic to cover. Some interviews were recorded in January and February after this important activity, so the video was ready in late February 2024.

Major problems:

The communication strategy developed for Life PINNA involved presenting the project as a fight to save a unique and charismatic species from extinction in the Mediterranean Sea, thanks to the pioneering work of a group of talented scientists. This approach has had some success with the national press, which immediately showed interest in the topic. For this reason, while not neglecting communication activities on the website and social networks, given the generated interest, considerable efforts have been made to involve journalists both in Italy and in Slovenia and appear in a large number of media, including television (RAI1, RAI3, Rai News, Canale 5 in Italy, RTV SLO in Slovenia), and international press (Vatican News, Le Figaro). This approach was not highlighted in the initial project documents but is already included in the LIFE Pinna Communication Plan, under key messages.

Outline the perspectives for continuing the action after the end of the project:

Mention any complementary action outside LIFE.

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities.

For picture and tables see E1 Annex 8

Attachments:

- D_E.1.1:Communication plan
- D_E.1.2:Website and social network
- D_E.1.3:First video-documentary
- E1 annex 1_ supporting documents
- E1 Annex 2_Citizen Science Poster
- E1 Annex 3_Info panels
- E1 Annex 4_Leaflet
- E1 Annex 5_Laymans Report
- E1 Annex 6_Nature illustrations
- E1 Annex 7_LIFE PINNA GADGET dissemination_2022-2025
- E1 Annex 8_PRESS REVIEW
- E1 annex 9_Lists of dissemination and networking events

Action E2: Replication and transfer

Foreseen start date: 01/07/2023

Actual start date: 01/07/2024

Foreseen end date: 30/09/2025

Actual end date: 30/09/2025

Activities undertaken and outputs achieved:

Phase 1 – Development of the Transfer and Replicability Plan focused on defining a structured and realistic strategy to ensure that the tools, methodologies and results produced by LIFE PINNA could be effectively transferred and replicated beyond the project's pilot sites. The Plan, finalised on 29 December 2023, analysed the level of engagement and collaboration already established with national and international organisations and used this assessment to design a transfer strategy grounded in existing networks and institutional frameworks. Particular attention was given to identifying appropriate users, defining roles and responsibilities, and clarifying how the project outputs should be applied in different ecological and administrative contexts. The Plan was developed through a collegial, collaborative and interdisciplinary process involving all project partners, integrating scientific, technical and governance perspectives. To ensure long-term effectiveness and continuity, key elements of the Transfer and Replicability Plan were incorporated into the After LIFE Plan, thereby embedding the transfer of results within a sustained post-project strategy rather than treating it as a stand-alone dissemination activity.

Phase 2: Development of Protocols

Phase 2 of the Transfer and Replicability Plan focused on the elaboration and consolidation of methodological and operational protocols that could be effectively replicated in other Mediterranean contexts affected by the mass mortality of *Pinna nobilis*. This phase translated the concrete conservation actions implemented within LIFE PINNA into structured, transferable tools, building on the intense and continuous collaboration among project partners with complementary expertise in marine ecology, pathology, genetics, conservation management, and governance.

Through this collaborative process, five core protocols were defined and progressively refined, as documented in Deliverable D_E.2.1. These protocols address key aspects of *Pinna nobilis* conservation, including site selection, restocking and translocation procedures, transport and handling, captive reproduction, and inter-institutional coordination. Together, they represent an integrated framework for managing a critically endangered and strictly protected species under conditions of high ecological uncertainty and sanitary risk.

In December 2024, an article entitled “*Handling of the Bivalve Pinna nobilis, Endangered and Pathogen - Affected Species, for Controlled Reproduction Precautions Taken*”, dedicated to the protocols for transport, maintenance, and reproduction of two species of the Pinnidae family: *Atrina fragilis* and *Pinna nobilis*.

As originally foreseen in the project proposal, the dissemination of these protocols was expected to take place primarily through seminars and annual technical meetings, held both in person and remotely, in collaboration with national and international stakeholders such as UNEP SPA/RAC. This approach initially envisaged the collection of expressions of interest from at least 50 organisations at international level willing to apply the guidelines developed by the project.

However, the rapid and severe worsening of the mass mortality phenomenon, combined with the existence of well-established relationships with major international conservation organisations, made it evident at an early stage that postponing dissemination and uptake until the final phase of the project would significantly reduce the potential impact of the protocols. At the same time, scientific knowledge on *Pinna nobilis* pathology, reproductive biology and disease dynamics evolved rapidly, requiring continuous updates and refinements of the methodologies developed.

For these reasons, training, capacity-building and early application of the protocols were initiated from the outset of the project and progressively intensified over time, going well beyond the mere collection of individual expressions of interest. Protocols were not conceived as static outputs, but as living tools, continuously tested, adapted and improved in response to new scientific evidence and operational feedback emerging from the field.

Within this evolving context, the initially envisaged mechanism based on individual organisational commitments was effectively superseded by a more structured and impactful institutional process. In January 2022, SPA/RAC formally requested the LIFE PINNA project to elaborate, on its behalf, a Mediterranean Plan for the Restoration of *Pinna nobilis*. This request marked a decisive step in the transfer and replication strategy, shifting the focus from voluntary uptake by individual entities to coordinated, basin-wide institutional adoption.

The Mediterranean Restoration Plan prepared with the technical contribution of LIFE PINNA was subsequently discussed and refined through a series of scientific and technical exchanges involving researchers and authorities from across the Mediterranean region. This process culminated in its formal adoption by the Contracting Parties to the Barcelona Convention during the 23rd Ordinary Meeting (Decision IG.26/5, Portorož, Slovenia, 5–8 December 2023).

Please Ref to E2 Annex 2 Decision IG. 26/5 adopted by the Contracting Parties to the Barcelona Convention with regard to RAC/SPA field of intervention and the SPA/BD Protocol - Pag 82 specific reference to LIFE PINNA project.

This institutional endorsement, involving all Mediterranean Parties, represents a higher-level and more effective form of commitment than the originally planned expressions of interest, ensuring broad uptake, long-term implementation and strategic integration of LIFE PINNA methodologies at Mediterranean scale. It also provided a stable governance framework for the continued application, updating and harmonisation of the protocols beyond the project’s duration.

The SPA/RAC request and the subsequent institutional process also triggered strengthened collaboration with IUCN and other related initiatives, including LIFE PINNARCA, and led to the organisation of multiple technical and scientific events where LIFE PINNA protocols, methods and results were shared and critically discussed. It was within this framework that the idea of establishing a Pan-Mediterranean task force for *Pinna nobilis* conservation emerged.

This task force will be formalised in spring 2026 through the coordination of the IUCN offices.

Since then, the task force has operated at all institutional and technical levels, involving hundreds of participants with the shared aim of safeguarding *Pinna nobilis*. This commitment extends far beyond the signing of formal expressions of interest and has led to a Mediterranean-wide restoration plan

(https://rac-spa.org/sites/default/files/doc_pinna/pinna_nobilis_restoration_strategy.pdf *E2 Annex 3 Restoration Programme of Pinna nobilis*)

), approved by the 22 representatives of the parties to the Barcelona Convention, with all the resulting actions and future implications.

Phase 3 – Active Replication, Training and Capacity Building

Phase 3 of Action E2 focused on the active replication of LIFE PINNA methodologies and on strengthening technical and institutional capacity at Mediterranean level. Building directly on the protocols developed and consolidated during Phase 2, this phase aimed to ensure that the knowledge generated by the project was effectively transferred, operationally tested, and embedded within the practices of research institutions, Marine Protected Area managers and competent authorities across different countries.

A series of targeted training, dissemination and capacity-building activities were implemented throughout the project, progressively expanding in scope and geographical coverage. These activities were designed not only to present project results, but also to foster practical understanding of the protocols, stimulate peer-to-peer exchange, and support their application in diverse ecological and institutional contexts.

A first major milestone of Phase 3 took place in June 2022, when a regional training activity was organised in Tunisia, in the Kerkennah Archipelago, by SPA/RAC–UNEP/MAP in collaboration with the LIFE PINNA project. This event brought together 40 researchers and experts from Greece, Croatia, Lebanon, Egypt, Tunisia, Algeria and Italy. The training focused on the conservation challenges affecting *Pinna nobilis*, the emerging methodological approaches developed within LIFE PINNA, and the initial elements of protocol transfer, providing an early platform for Mediterranean-wide exchange at a time when mass mortality events were still rapidly evolving.

As the project progressed and the protocols became more structured and validated through field application, additional training and dissemination actions were organised to address specific operational themes. On 14 May 2025, an international webinar entitled “Restocking of *Pinna nobilis*” was held, with the participation of 63 attendees. This webinar specifically addressed restocking strategies, site selection criteria, sanitary precautions and post-restocking monitoring, offering participants a detailed overview of the protocol-based approach developed within LIFE PINNA.

This was followed, on 21 May 2025, by a dedicated training course entitled “Marine restoration: two case studies: LIFE PINNA and LIFE REEFforest”, which involved 37 participants. By comparing experiences from two LIFE projects, this course placed *Pinna nobilis* conservation within a broader marine restoration framework, highlighting common challenges, transferable methodologies and the importance of adaptive management and long-term monitoring in restoration initiatives.

The International Workshop held on 17 September 2025 in Piran represented a key stage of Phase 3 and of the overall Action E2. Organised as part of the final LIFE PINNA event, the workshop fulfilled Milestone M_E.2.1 (Workshop for the dissemination of good practices) and gathered 59 participants. This event provided a comprehensive forum for presenting consolidated protocols, discussing lessons learned from their application, and exploring future perspectives for replication and scaling up at Mediterranean level. The workshop also reinforced linkages between scientific, technical and institutional actors, supporting the long-term uptake of project results.

Collectively, these activities ensured that Phase 3 functioned as a concrete bridge between protocol development and real-world application. Through hands-on training, thematic webinars and high-level dissemination events, LIFE PINNA effectively translated its methodological outputs into shared operational capacity, contributing to a coordinated and informed Mediterranean response to the conservation crisis affecting *Pinna nobilis*. (*Details of these activities are provided in “E2 annex 4 – Supporting documents: Active replication and training”.*)

The sites selected to implement the *Pinna nobilis* project solutions were the Bergeggi Marine Protected Area (MPA) and the Tuscan Archipelago MPA, for which an economic assessment report was carried out on replicability and transferability in terms of cost efficiency. Arpal signed the agreement with the Bergeggi MPA in July 2024 and the second with the Tuscan Archipelago MPA in April 2025. Many other activities were carried out for the development of action E2:

- In October 2024, in Bergeggi MPA, UniGe researchers carried out the installation of the 3D models created by the Fablab, scientific lab of ICTP. This activity aimed at evaluating the hydrodynamics of the seabed and useful for defining the replication protocol of conservation and transfer actions.
- In December 2024 Arpal conducted an ex ante survey in three sites of the Bergeggi MPA, the same ones where, in 2018, a previous monitoring by UniGe had recorded 167 Fins (90% alive). In December 2024 Arpal divers found only 8 shells of dead animals.
- In April, May and August 2025, additional surveys in Bergeggi were carried out by ARPAL with a ROV (remote operated vehicle).
- In August 2025, a monitoring campaign was carried out in the Giannutri island utilizing both scientific divers and ROV.
- Meanwhile, AMP Archipelago Toscano organized a survey campaign with scientific divers in the other four islands of the archipelago, Montecristo, Pianosa, Capraia and Gorgona.
- In September 2025, a second monitoring campaign with scientific divers was carried out in the Bergeggi Posidonia meadow.
- In September 2025, 5 adults of *Pinna nobilis* were transplanted in the selected site of Bergeggi.

<i>Deliverable name</i>	<i>Foreseen Deadline</i>	<i>Actual Deadline</i>
D_E.2.1 Protocols for replication	12/2024	09/2025

<i>Milestone name</i>	<i>Foreseen Deadline</i>	<i>Actual Deadline</i>
M_E.2.1 Workshop for dissemination of good practises	12/2024	09/2025

Comparison with planned output and time schedule:

During the implementation of Action E2, the approach to transfer and replication evolved significantly compared to what was originally foreseen in the Application Form. Initially, the project envisaged the collection of at least 50 expressions of interest from individual organisations willing to adopt the protocols developed. However, the rapid and severe worsening of mass mortality events affecting *Pinna nobilis*, together with the urgent demand for coordinated guidance at Mediterranean scale, made it clear that a more immediate and structured mechanism was required.

As a result, the project shifted from a bilateral, organisation-based approach to a basin-wide institutional process. This change materialised through the formal request received in January 2022 from SPA/RAC to elaborate the Mediterranean Plan for the Restoration of *Pinna nobilis*, which was subsequently adopted in December 2023 by the Contracting Parties to the Barcelona Convention. This collective institutional endorsement effectively replaced the initially foreseen individual expressions of interest, ensuring a broader, more binding and longer-term commitment to the uptake and application of LIFE PINNA protocols across the Mediterranean.

Major problems:

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action).

Outline the perspectives for continuing the action after the end of the project:

Mention any complementary action outside LIFE.

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities.

Attachments:

- D_E.2.1: 5 Protocols for replication

- E2 Annex_1 Transfer and Replicability Plan
- E2 Annex_2 Decision IG 26_5
- E2 Annex_3 Restoration Programme of Pinna Nobilis
- E2 Annex_4 Supporting documents: Active replication and training

Action E3: Networking and synergies with other LIFE and/or non-LIFE projects

Foreseen start date: 01/10/2021

Actual start date: 01/10/2021

Foreseen end date: 30/09/2025

Actual end date: 30/09/2025

Activities undertaken and outputs achieved:

During years 2022-2025, Life Pinna developed several networking activities, also promoted on social network channel and website, in the pertinent section: <https://www.lifepinna.eu/network/>

Among the main activities, in 2022, LIFE PINNA researchers and SPA/RAC UN Agency defined the "Restoration Program of Pinna nobilis", the Mediterranean restoration plan of Pinna nobilis, later adopted by the Contracting Parties to the Barcelona Convention (5-8 December 2023 in Portorož, Slovenia)

In June 2024 in Miramare MPA (Trieste), LIFE PINNA organized its **first networking event** (Milestone) with researchers from Italy, Slovenia and Croatia working on the survival of Pinna nobilis in the Adriatic Sea. A visit to Pula Aquarium, Croatia, was also organised.

In July 2024 in Valencia, as part of the International Marine Sciences Symposium (ISMS24), LIFE PINNA organized a **second networking event**, joint with the LIFE PINNARCA project, involving international organizations such as SPA/RAC and IUCN.

Researchers joined in Valencia updated the priorities and they split into different research groups to act in following months and years with several meetings, in person and via videoconference. 1) to implement the study of pathogenic organisms and the genetic variability of surviving individuals. 2) to improve reproductive techniques adopting simultaneously LIFE Pinna protocol to drive the specimens to the reproductive stage. In January 2025 activities at University of Genoa, Shoreline Coop and Pula Aquarium started.

All researchers from other projects involved in the networking activities were invited to the final LIFE PINNA conference, which took place on September 17, 2025, in Piran, Slovenia.

The following conservation projects have been involved so far in networking activities. Main activities are also listed.

Project	Subject	Website	Activity
LIFE Pinnarca, 2021-2024	Protection and restoration of <i>Pinna nobilis</i> population as a response to catastrophic pandemic started in 2016, with a focus on Spanish waters	www.lifepinnarca.com	Involved in the first public meeting on 2 December 2022, first networking activity started in 2022. Social network posts by LIFE Pinna, section on LIFE Pinna website. LIFE Pinna organised a networking event on 29 November 2023. More than 60 people attended the meeting, including all LIFE Pinna partners. In July 2024 LIFE Pinna and LIFE Pinnarca organised a joint networking event in Valencia and Calpe, within the framework of the International Symposium on Marine Sciences

			(ISMS24). Furthermore, LIFE Pinnarca researchers participated in the international webinar organized by LIFE Pinna on May 14, 2025. Many activities of the research groups. Ref. Jose Rafael Garcia March, Francesca Carella
Pinna Hope, 2020-2025	Turkish conservation program on <i>P. nobilis</i> in the Sea of Marmara	https://www.umutpina.com.tr/en/	One meeting in March 2023. Social network posts by LIFE Pinna, section on LIFE Pinna website. In November 2025 Shoreline in Trieste and NIB in Piran hosted a delegation of Turkish researchers from Hope Pinna. Ref: Mustafa Sarı e Uğur Karadurmuş
LIFE REEF Forest, 2022-2026	Restoration of Cystoseira macroalgal FORESTs to enhance biodiversity along Mediterranean rocky REEFs	www.lifereeforest.eu	Involved in the first public meeting on 2 December 2022, networking in 2022. Social network posts by LIFE Pinna, section on Life Pinna website. Co-organization of the online training “Marine restoration: two case studies: LIFE Pinna and LIFE REEForest”, May 21, 2025. Ref. Valentina Asnaghi.
MPA Engage, 2019- 2022	(Interreg MED programme, 5216 5MED18_3.2_M23_007)Engaging Mediterranean key actors in Ecosystem Approach to manage Marine Protected Areas to face Climate Change	https://mpa-engage.interreg-med.eu/	Involved in the first public meeting on 2 December 2022, networking in 2022. Social network posts by LIFE Pinna, section on Life Pinna website. Ref. Daniele Grech
PINNA GARDEN project, 2024-2025	Local Pinna conservation project involving Salvatore Giacobbe (University of Messina) and Andrea Spinelli (Oceanogràfic Valencia)	-	First online meeting in March 2023. Ref. Salvatore Giacobbe, Andrea Spinelli.
Crisis management of Pinna nobilis in Croatia, 2022-2026	Local Pinna conservation project in Croatia	-	Annual meetings of partners in the project Conservation of the noble pen shell (<i>Pinna nobilis</i>) in the Adriatic Sea (Croatia). 1st meeting with Shoreline and Uniss on 16-17 March 2023; 2nd meeting with NIB and Shoreline on 11-12 April 2024; 3th meeting with NIB and Shoreline on 3-4 April 2025. Ref: Dora Klisovic and Alena Sprcic

SPAMI Twinning Programme "Ensure effective management of Specially Protected Areas of Mediterranean Importance (SPAMIs)" - A Sea of Synergy	Project launched by the UNEP/MAP - SPA/RAC and the Ministry of Environment and Energy Security of Italy (MASE)	https://spa-rac.org/en/article/214/the-spami-twinning-programme-fostering-peer-to-peer-collaboration-for-mediterranean-marine-conservation	On 22 November 2024 at the Miramare Marine Protected Area (Trieste) researchers presented best practices—effective management and restoration measures for species and habitats that can be replicated elsewhere. Specifically, in addition to LIFE Pinna ones, these included initiatives from projects such as TRECAP, REEFOREST, POSEIDONE.
ADRIATIC NETWORK Interreg TRECAP, Interreg Eco2Smart, Interreg POSEIDONE, CenSy4Sea	Northern Adriatic organizations and projects working for <i>Pinna nobilis</i> conservation.	https://www.ita-slo.eu/it/trecap https://www.ita-slo.eu/it/eco2smart https://www.ita-slo.eu/it/poseidone https://www.ampmiramare.it/progetto-censy4sea/	Many networking events and meetings among northern Adriatic organizations and projects working for <i>Pinna nobilis</i> conservation. For example, days dedicated to the preparation of "larval collectors" with experimentation of new materials. See "E1 annex 11_Lists of dissemination and networking events"
LIFE NATUREEF, 2023-2027	Project for restoration of native oyster and sabellariid reefs, seeding the native species in a rare non-urbanized coastal stretches of the northern Adriatic coasts	https://site.unibo.it/life-natureef/it	Between late 2024 and early 2025, social media shared posts have been planned to raise awareness of each project's readers.
Other projects involved: ReLife, Life Intermare, Lifeseposso, LIFE Adapts			News and updates on LIFE Pinna social networks.

Within the LIFE PINNA project, preparatory, operational and monitoring activities were implemented in the Bergeggi Marine Protected Area and the Tuscan Archipelago National Park to support the economic assessment of the replicability and cost-effective transferability of the project solutions for the conservation of *Pinna nobilis**.*

In accordance with the Grant Agreement, ex-ante seabed surveys were carried out in the selected pilot areas (Bergeggi Island and Giannutri Island) in collaboration with ARPAL and the University of Genoa, including the use of the ARPAL-owned Remotely Operated Vehicle (ROV). These surveys provided a baseline for habitat characterisation and for the comparison of different monitoring approaches.

Following the preparation of the initial report on the provision of financial support to third parties, coordination meetings were held with the involved MPAs, leading to the signing of dedicated Partnership Conventions under Action E3. The Convention with the Bergeggi MPA was signed on 5 July 2024, while the Convention with the Tuscan Archipelago National Park was finalised on 18 April 2025. Both agreements defined the planned activities, expected outputs, eligible costs in line with the LIFE Grant Agreement and compliance with national public procurement rules.

A pilot restocking attempt was planned in the two MPAs, coordinated by UNIGE with the support of ARPAL and the managing authorities, followed by ex-post monitoring activities carried out according

to the schedule defined under Action D2 and concluded with a final monitoring campaign, including ROV surveys.

While the widespread mass mortality events affecting *Pinna nobilis* significantly limited the availability of healthy specimens, resulting in a single, unsuccessful restocking attempt and delays in the original time schedule, Action E3 successfully achieved its main objectives. Ex-ante and ex-post monitoring activities were fully implemented, enabling the collection of robust technical and economic data and a comparison between traditional scientific diving and ROV-based surveys, including cost and amortisation aspects.

<i>Deliverable name</i>	<i>Foreseen Deadline</i>	<i>Actual Deadline</i>
D_E.3 Report describing purpose and means of providing financial support to third parties	06/2022	09/2025

<i>Milestone name</i>	<i>Foreseen Deadline</i>	<i>Actual Deadline</i>
M_E.3 First networking event	10/2023	06/2024

Comparison with planned output and time schedule:

The first LIFE Pinna networking event was moved to June 2024, instead of October 2023. This date has enabled us to share the most significant achievement in project actions, especially the result of the first Pinna introduction and monitoring in Capo Mortola and new larval collector in the Adriatic Sea. The difficulties encountered provided valuable insights for transferability, highlighting critical constraints, organisational and logistical challenges, and the need for flexible implementation strategies. Overall, Action E3 delivered a realistic, experience-based economic assessment of the transferability of LIFE PINNA solutions to other MPAs, particularly in complex and operationally demanding island contexts.

Major problems:

The implementation of the transferability activities was affected by external constraints primarily related to the conservation status of *Pinna nobilis**. The widespread mass mortality events recorded during the project period significantly reduced the availability of healthy specimens suitable for restocking activities. As a result, only a single pilot restocking attempt could be carried out, and this attempt did not lead to the establishment of a viable population in the selected pilot area.

These biological constraints required adjustments to the original implementation schedule, particularly with respect to the sequencing and timing of the restocking and subsequent monitoring phases. Despite the reduced biological output, the activities were not suspended and were refocused on maximising the collection of technical, operational and economic data through ex-ante and ex-post monitoring, thus ensuring the achievement of the core objectives related to economic assessment and transferability.

The results achieved under Action E3 provide a solid basis for the continuation and upscaling of similar initiatives beyond the end of the LIFE PINNA project. In particular, the economic assessment and the comparison between traditional scientific diving and ROV-based monitoring offer concrete guidance for future monitoring and conservation strategies targeting *Pinna nobilis* and other threatened benthic species.

The experience highlights the importance of flexible and adaptive management approaches, especially in contexts characterised by limited biological availability and high logistical complexity, such as small island MPAs. The methodologies tested within Action E3 can be readily applied in future restocking initiatives, once biological conditions allow, or integrated into long-term monitoring programmes implemented by MPAs.

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action).

Outline the perspectives for continuing the action after the end of the project:

Mention any complementary action outside LIFE.

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities.

Attachments:

- D_E.3: Report describing purpose and means of providing financial support to third parties
- E3 Annex_1 Preliminary and ex-ante Report_AMP
- E3 Annex_2 Supporting documents_networking events

Action F1: Project coordination and management, After LIFE plan and Green procurement

Foreseen start date: 01/10/2021

Actual start date: 01/10/2021

Foreseen end date: 30/09/2025

Actual end date: 30/09/2025

Activities undertaken and outputs achieved:

F.1.1 Project management

The project was launched on October 1 2021, and by official act on 3/11/2021 the lead beneficiary ARPAL approved the Grant Agreement and assigned Daniela Caracciolo as project responsible (RP). ARPAL chairs the Steering Committee, which is formed Project Manager Daniela Caracciolo (RP) by the Financial Manager Maria Grazia Pizzoni (FM), Project Manager Diego Mattioli, Communications Manager Alessandro Midlarz (CM), and a reference person for each beneficiary as indicated below (F.1.3 - F.1.8). The steering committee was formed at the kick off meeting on December 3 2021.

The project responsible in these months of work has:

- ensured constant coordination among beneficiaries to promote the smooth progress of the project
- ensured contacts with CINEA and Monitor
- developed the partnership agreements
- supervised and supported Project Manager, Financial Manager and Communicator all the activities
- continuously monitored the progress of project actions including scientific and conservation aspects.

The beneficiary TRITON RESEARCH s.r.l. has the role of project management, leading communication and replication and networking of the project. At the start of the project, the Project Manager (PM) was Ilaria Vielmini, who was succeeded by Valeria Pulieri from 14/01/2022, Diego Mattioli from 01/02/2023, Valeria Pulieri from 01/07/2024, and from 01/03/2025 the PM is Diego Mattioli, the Communication Manager (CM) of the project is Alessandro Midlarz who substituted Francesco Tomasinelli from 01/04/2024

The Project Manager has the role to:

- coordinate the whole project implementation;
- guarantee the correct implementation of the activities, the achievement of milestones and the production of deliverables;
- coordinate the preparation of quarterly technical reports; and
- organise and coordinate the monitoring visits.

The Financial Manager (FM) is Maria Grazia Pizzoni (see letter of assignment), an official of ARPAL's European Project Office, prepared and updated the Project's financial statements quarterly with external support from the external support of a sub-contractor – Istituto Delta Ecologia Applicata srl. The communications responsible created the project website and social networks, which are constantly updated, and has been in charge of all project communication activities.

The Scientific Committee chaired by Leonardo Tunesi and formed by Alberto Castelli, Antonio Terlizzi and Jamila Ben Souissi was formed and participated in the launch event on December 2, 2022.

F1.2 Green Public Procurement

The FM monitored the purchases made by the beneficiaries especially with regard to the application of GPP criteria. A LIFE PINNA_GPP_Application Procedure document has been prepared that collects through a register the procurement that followed the environmental criteria.

F.1.3 – F1.8

Associated beneficiaries project responsible who are member of the Steering Committee:

- UNIGE: Monica Monfalcone (Internal staff)
- UNISS: Marco Casu (Internal staff)
- Asinara MPA: Vittorio Gazale (Internal staff)
- NIB: Valentina Pitacco (Internal staff)
- Triton Research: Diego Mattioli
- SHORELINE Saul Ciriaco (internal staff)

The following meetings of the SC were organised in the period from 1/10/2021 to 31/12/2023:

- 1st Monitoring Visit 20 January 2022 on vdc

- 2nd Monitoring Visit 29 – 31 March 2023 – in Sassari – Porto Torres – Isola dell’Asinara
- 3rd Monitoring Visit 6 October 2023 in Genova – Camogli
- 4th Monitoring Visit 24 January 2025 in Roma - Spazio Europa
- 5th Monitoring Visit 24/25 September 2025 in Genova (Arpal) and MPA Isola di Bergeggi
- project meeting with all partners on vdc:
 - 06/09/2021 (preliminary meeting)
 - 19/01/2022
 - 13/05/2022
 - 23/06/2022
 - 14/02/2023
 - 02/05/2023
 - 17/07/2023
 - 04/08/2023
 - 25/09/2023
 - 17/11/2023
 - 22/01/2024
 - 22/03/2024
 - 22/04/2025
- project meeting with all partners in presence: 13/12/2023 in Genova, 23/02/2025 in Roma, 16/09/2025 in Pirano.

The After LIFE Plan has been developed by TRITON, discussed and approved by the SC, which plan the activities necessary to ensure the sustainability of the Project after its end, in order to increase its long-term effectiveness.

Deliverable name	Foreseen Deadline	Actual Deadline
D_F1:After Life Plan	09/2025	09/2025

Milestone name	Foreseen Deadline	Actual Deadline
M_F.1.1: Assignment of role to project manager PM, project responsible PR, technical administrative monitoring TAR and financial manager FM	12/2021	12/2021
M_F.1.2: Reports of Steering Committee meetings	09/2025	09/2025

Comparison with planned output and time schedule:

Following the third monitoring visit on October 6 in Genoa, the Technical Monitor Dr. Teresa Catelani, view the status of the progress of the LIFE PINNA project suggested to the partnership the drafting of a Contingency Plan (CP); this request was then acknowledged and confirmed by the project adviser Sarunas Zableckis and communicated to the lead beneficiary ARPAL on 26.10.2023 (by e-mail). Following this request the delivery of the Mid - Term Report was postponed from 01/12/2023 to 28/02/2024 (Ares(2023)7309971)

Major problems: None

If relevant, clearly indicate how actions were modified, and any correspondence with the EASME approving the changes (in particular this is required if there has been a significant over-spending of the foreseen budget for the action).None

Outline the perspectives for continuing the action after the end of the project:None

Mention any complementary action outside LIFE.None

Include tables, photographs etc. to illustrate the actions, such as (for LIFE Nature & Biodiversity, as well as LIFE Climate Action) land purchase and non-recurring management activities.None

Attachments:

D_F1_After Life Plan

F1 Annex 1_Index of deliverables with short description

6.2. Main deviations, problems and corrective actions implemented

The main difficulty the project has faced is due to the serious state of *Pinna nobilis* populations compared to what was declared at the application submission, where the decline of specimens was already taking place but not to the levels of severity reached in the years 2022 and 2023, which correspond to the first two years of LIFE PINNA's work. As a result of this situation, some project actions have suffered significant delays and even changes in the implementation of some activities.

For this reason, the partnership was invited to prepare a Contingency Plan (delivered to the Project Advisor on 31/01/2023) in order to "take a photograph" of the status of the project and propose solutions that would allow it to work with a deviation from the approved form but at the same time maintain project objectives. The following table details the actions with the changes implemented on the activities, and with an indication of the Contingency measures identified. As can be seen, the actions that have undergone the greatest changes are Actions C3 and C6 and consequently the actions related to them. These actions are in fact the ones that are directly affected by the reduced availability of *P. nobilis* individuals in the originally identified donor sites. The extensive monitoring carried out during the course of the project, together with the activities carried out in the context of citizen science, initially made it possible to identify additional populations of the species. Therefore, at that time, it was thought that by partially readjusting the planned activities, it would be possible to achieve the original objectives of the project. Unfortunately, the situation subsequently worsened, making it impossible to proceed with some of the planned activities. The decline of *Pinna nobilis* worsened, affecting all areas where large numbers of individuals were present.

The partnership therefore decided that it was not ethically appropriate to continue collecting individuals from the wild, especially adult breeders, as the species is in serious danger of extinction. Furthermore, it must be said that in order to ensure the success of the repopulation intervention, a substantial number of individuals need to be transplanted, while the translocation and implantation of one or two individuals makes no sense from a scientific point of view.

Similarly, there was a lack of individuals available for reproduction in captivity, which therefore could not continue with further trials to achieve complete larval development.

As proof of the validity of the choice made, it is necessary to refer to the mortality rate of individuals transplanted in Liguria (Capo Mortola and Bergeggi), which was 100%. All the relocated *Pinna nobilis* died, despite being apparently in good health and showing no evidence of infection by *Haplosporidium pinnae* or other pathogens sought during the analyses carried out prior to their release into the wild. Even the possibility of continuing the project by requesting an extension, which had been seriously considered by the partners at first, was, for obvious reasons, shelved in these dramatic circumstances. The project partners have put a great deal of effort into finding a solution that could give hope for the survival of the species. In this context, the networking activity that had been initiated with LIFE PINNARCA has developed considerably. In fact, after a face-to-face discussion following the workshop organised by PINNARCA on 9/29/2023, researchers from the University of Sassari and those from the University of Naples decided to work closely together to research pathogens and assess the genetic variability of Mediterranean populations. Additionally, researchers from the University of Genova, and Shoreline established a network with Spanish and Croatian researchers working on captive reproduction of the species. Finally, researchers from NIB and Shoreline together with other institutions established a Northern Adriatic network, with the aim of monitoring the recruitment potential and boosting the repopulation of the species, through the deployment of larval collectors in different areas. This network involves other institutions from Slovenia (Aquarium Piran, University of Primorska), Italy (CNR ISMAR Venice) and Croatia (PI Natura Histrica, Brijuni National Park). Some institutes from this network are also collaborating within the "Pan-Mediterranean Pinna Larval Collectors Network" established by Spanish researchers to standardise this approach to support large-scale larval monitoring efforts.

All those initiatives contribute to creating a broad multi-disciplinary collaborative network that is still active today, enabling a continuous exchange of information between scientists and representing the only hope of one day saving *Pinna nobilis* from extinction.

The partners obviously guarantee their utmost commitment in this regard, even during the After Life period and, in general, beyond the conclusion of the LIFE PINNA project.

(Projects funded under the Call 2014 onwards must use this format)

Action	Expected results from project	Obtained results	Problems	Contingency measures
A1	Donor sites maps and environmental characterisation	The two donor sites, Miramare (Gulf of Trieste) and Strunjan in Slovenia, have been characterised.	The identified sites are not sufficient to ensure the necessary number of <i>P. nobilis</i> individuals for reproduction and transplantation.	Reconfiguring source sites, in order not to modify the project objectives, as mentioned above. Potential new donor sites include the Grado Marano Lagoon, the Venice Lagoon, and Sant'Antioco (for which a genetic compatibility assessment has also been conducted), a potential donor site for the Asinara Park. The action is concluded, but with the described implementations, it will remain 'open' and updated at the end of the project.
C1	Identification of the pilot sites for restocking: on the basis of A1	The 4 pilot sites have been mapped based on Action A1: 1 in Liguria - Regional Protected Area Capo Mortola, 1 in Sardinia - Marine Protected Area Isola dell'Asinara, and 1 in Friuli Venezia Giulia - MPA Miramare, and 1 in Slovenia at Strunjan Park.	The individuals of <i>P. nobilis</i> may not be sufficient to ensure restocking in all 4 pilot sites.	The sites where restocking will take place could be fewer than those initially planned 4. To date, a first restocking attempt has occurred only in the receiving site of Capo Mortola in Liguria (conducted on December 14, 2023). Restocking actions will also be carried out in the Miramare site (MPA Miramare) by the summer 2024. Similarly, if sufficient <i>P. nobilis</i> individuals are found, restocking will take place at the Strunjan site in Slovenia in summer 2024.
C3	We expect to recruit a total of 70 ind./year of <i>P. nobilis</i> * juveniles, shared between Shoreline (50 ind./year) and NIB (20 ind./year), for a total of 210 individuals during the project.	Between 2022/2023, 96 specimens of <i>P. nobilis</i> were found by Shoreline in Italy. Of these 96 individuals, 57 are self-recruited juveniles and 39 are adults, 36 of them were collected. In the same period 82 specimens were found by NIB in Slovenia, 42 of them were collected. In 2024 Shoreline collected 36 individuals, 24 of them from the larval collectors and NIB collected 18 juveniles. In 2025 we didn't find pinnids inside the collector bags. As Shoreline and NIB, we collected a total of 132 <i>P. nobilis</i> individuals during the period of the project, instead of the planned 210. Analysis of the dead individuals revealed evidence of the presence of <i>H. pinnae</i> and other pathogens.	Limited or no availability of juvenile individuals in donor sites and in the larval collectors.	To maintain the original objectives of the project the following actions were implemented: ● Increase the number of larval collectors, doubling the number of collectors bags, along with a corresponding increase of the placement sites starting from the reproductive seasons of 2023/2024. ● Increase in the classification of risk situations. ● Intensification of sampling efforts to identify additional high-risk situations. Starting from February, two additional monthly samplings are foreseen with six operators in high-risk zones, such as ports, in the Friuli Venezia Giulia region. ● Increase sites following monitoring and Citizen Science activities. Bi-monthly meetings with local divers and sea operators are scheduled from February to support the identification of new potential sites. The partnership has covered the necessary expenses for sampling and analyses exceeding the initially proposed number of samplings and analyses in the proposal phase, using their own funds.

C4	The tests on controlled reproduction in captivity will be done in three different times, after having characterised the genetic characters of surviving adults and verified the absence of pathogens. These precautions will reduce the risk of failure, encouraging the calibration of the procedure and its continuous improvement in order to achieve an effective protocol. The 20 (max 30) adult specimens which will be collected for being maintained in captivity for controlled reproduction will be treated according to best established protocols for collection and transfer and will be transferred in three different occasions, in order to minimise stochastic mortality during collection and transfer.	Four controlled reproduction trials in a controlled environment were achieved during the project. 1. in the summer of 2023; however, we did not witness larval settlement and, consequently, the development of juveniles. 2. In the summer 2024; The larval cycle stopped at 21 days after fertilisation, reaching a size of 150 microns for the umbonate larva for the first time 16 days after fertilisation. However, we did not witness larval settlement 3. In spring 2025, after gonadal maturation induction trial. However, the larval cycle stopped at 8 or 10 days after fertilisation, reaching only the first larval stage (trochophore). 4. In summer 2025; however, we only obtained male gametes from one specimen, without being able to carry out fertilisation. During the years of the project, a total of 34 adult specimens were used for controlled reproduction trials	- Limited availability of adult specimens for reproduction. - Limited information in the literature on the larval cycle of this species - Greater efforts in maintaining specimens that tested positive for <i>H. pinnae</i> in the laboratory, to avoid contamination.	The breeders may come from different donor sites (Action A1) in order not to impact a single population. The partnership has covered the necessary expenses to ensure the implementation of activities related to controlled reproduction in the Camogli laboratory. During the years of the project, we have improved the protocol for maintaining adults in the laboratory. In addition, larval development management has been improved by attempting to supply a diet of microalgae suitable for larval growth.
C6	Considering <i>P. nobilis</i>* specimens possible accidental deaths during previous actions (C3, C4, C5) and installation operations (C6), we expect to transplant 55 ind./year in four marine areas overall (Capo Mortola, Miramare, Strunjan and Asinara Island), for a total of 165 transplanted individuals during a three year period.	A total of 14 transplant actions were carried out during the project: seven in the Miramare (MPA), four in the Strunjan MPA, two in Capo Mortola, and one in the Bergeggi MPA. A total of 94 individuals were successfully relocated to the receiving sites: 55 to the Miramare MPA, 24 to the Strunjan MPA, 10 to the Capo Mortola site and 5 to the Bergeggi MPA. Instead of 165, we transplanted a total of 94 individuals during a three-year period. Genetic analysis to detect the presence of <i>H. pinnae</i> produced	Absence of suitable conditions for the transplant of individuals due to the lack of individuals, their unavailability, and the high mortality rates of juveniles	the following activities were implemented: ● Prioritise movements <i>in situ</i> to minimise stress in the relocation of individuals. ● Identify a greater number of suitable sites from which to collect new individuals.

		negative results at the recipient sites and in the analysis carried out on the transplanted individuals.		
E3	• Evaluation of cost-efficient replicability and transferability of the project solutions on <i>P. nobilis</i> in MPA Bergeggi and in Tuscan archipelago • First event of networking	• completed task	Unavailable individuals for replication activities. Delays of project actions	Although the replicability activities did not yield positive results from a biological perspective, the activities carried out made it possible to perform a cost-benefit analysis that fully met the objectives of the transferability study. This has resulted in the creation of a useful tool for future activities that will hopefully be implemented by MPAs which, when conditions are favourable, wish to undertake <i>Pinna nobilis</i> repopulation measures. The first networking event took place on June 13-14, 2024 at Miramare Marine Protected Area (Trieste, Italy) and the Pula Aquarium (Croatia).

(Projects funded under the Call 2014 onwards must use this format)

6.3.Evaluation of Project Implementation

Methodology applied

Initially, the methodology applied seemed likely to yield good results. In fact, thanks to close interaction between the partners and constant collaboration with the project monitoring manager, any problems were promptly identified and discussed, and the necessary corrective actions were taken.

The problems listed and explained in the previous point were in fact identified promptly and discussed at specific technical meetings organised by the ARPAL and TRITON project management staff with the technical and scientific staff of the partners. A corrective plan was then drawn up and promptly implemented to ensure that concrete results were achieved, even under critical conditions.

Unfortunately, this was not enough, as the dramatic nature of the raging pandemic overwhelmed the partners, who were unable to stem the tide of death that affected our sea. However, most of the project's results were achieved, even if for some specific activities there was no way to reach the exact expected results, in terms of numbers. Below are the details of each action.

Comparison among results achieved against objectives expected results

Action	Foreseen in the revised proposal	Achieved	Evaluation
A1 Environmental status and health assessment of seagrass meadows and <i>Pinna nobilis</i> populations in donor and receiving areas	<u>Objectives:</u> Assessing the ecological status of seagrass meadows and the health status of <i>Pinna nobilis</i> populations in donor and receiving areas <u>Expected results:</u> A detailed description of the environmental characteristics and good conditions of each selected area. The selected donor sites have an adequate number (i.e., 30) of resistant individuals of <i>P. nobilis</i> still alive. Pilot receiving sites have characteristics that correspond to the requirements of <i>P. nobilis</i>.	All Except for the number of live individuals in the donor sites that were significantly less than those expected at the beginning of the project	Pilot receiving sites have a good ecological status and are suitable for transplantation.
A2 Molecular characterization of sentinel species in the putative pilot sites of restocking	<u>Objectives:</u> Development of molecular diagnostic protocols for the pathogens involved in the mass mortality of <i>Pinna nobilis</i> with the aim to evaluate the quality of the waters in the sites of the project. Identification of pathogens free site to the restocking activities foreseen by the project. <u>Expected results:</u> Molecular diagnostic protocols that are capable of rapidly detecting the presence of the pathogens associated with the mass mortality of <i>Pinna nobilis</i> in any biological tissue and identify the state of advancement of the ongoing infection in order to predict its evolution. Identification	All. The better pathogens free sites were identified to perform the first transplantation activities of the project. Furthermore, the activities of this action have been extended to the entire project to maintain a constant monitoring of the quality of the waters in all the sites involved in the project.	The molecular diagnostic analyses by means of sentinel species developed for this project showed to be effective in the quick identification of pathogens. The restocking sites were continuously evaluated in relation to the long-term persistence of the species. The use of sentinel species, for the first time in marine areas, helped to safeguard restoration efforts by promoting the establishment of populations in environments compatible with their

	of the better pathogens free sites for the restocking activities planned for the project.		survival, resilience and ecological function within Mediterranean ecosystems. Action A2 supported the conservation of <i>Pinna nobilis</i> by guiding the selection of suitable restocking sites and by providing continuous information on environmental conditions affecting population persistence. The integration of molecular monitoring into site assessment strengthened evidence-based restoration planning and contributed to improving the long-term viability of reintroduced populations within the LIFE PINNA project.
A3 Survey on best practices for <i>P. nobilis</i> restocking concrete actions: SWOT analysis on restocking best techniques and available methodologies. Elaboration of a feasibility study and exit strategy	<u>Objectives:</u> Identification of key stakeholders with whom a database of contacts and experiences was created and to whom the questionnaire was sent to engage networks interested in the project and elaboration of the feasibility study. <u>Expected results:</u> Sharing with beneficiaries of the results of the action products useful for networking actions and the development of the feasibility study.	In line the project provisions	The activities carried out were very positive, as they enabled good networking for networking activities and in-depth analysis of possible critical issues in the project through SWOT analysis. Also, they allowed preliminary verification of the sustainability of translocations in terms of genetic compatibility and health conditions and population densities at donor sites.
C1 Identification of the pilot sites for restocking	<u>Objectives:</u> Identify and map suitable sites for restocking within the receiving areas <u>Expected results:</u> Four maps of the pilot sites for restocking. Pilots sites are located within the putative receiving areas which have suitable environmental conditions for the survival of restocked individuals and a pressure regime (both natural and human induced) as low as possible.	All	The sites identified for repopulation in the designated areas have successfully accommodated <i>P. nobilis</i> individuals.

C2 Molecular characterization of surviving individuals of <i>Pinna nobilis</i>	<u>Objectives:</u> Molecular characterization of the surviving individuals of <i>Pinna nobilis</i> with particular reference to fan mussels belonging to the candidate donor populations. <u>Expected results:</u> A fine molecular characterization of surviving <i>Pinna nobilis</i> to evaluate the effect of mass mortality on the genetic make-up of still alive populations and assess the level of genetic homogeneity between past and present populations.	All.	A fine mitochondrial characterization of modern surviving <i>Pinna nobilis</i> populations was obtained. Results evidenced a genetic homogeneity between the Adriatic donor sites involved in the present project (Gulf of Trieste and Venetian Lagoon) and the western Mediterranean populations (that now went extinct), which inhabited the restocking areas of the project. In conclusion, Action C2 demonstrated that surviving <i>Pinna nobilis</i> populations retain genetic features representative of historical Mediterranean populations, confirming their suitability as donor sources for restoration actions. The observed genetic continuity between present-day Adriatic populations and extinct western Mediterranean populations strengthens the ecological coherence of restocking activities and supports the long-term conservation value of the populations involved in the LIFE PINNA project.
C3 Collection and growth of <i>Pinna nobilis</i> self-recruited individuals from Northern Adriatic Sea.	<u>Objectives:</u> Collect juvenile specimens of <i>P. nobilis</i> from healthy populations previously identified in A1 and to store them in locations where to control their growth. <u>Expected results:</u> We expect to recruit a total of 70 ind./year of <i>P. nobilis</i> juveniles, shared between Shoreline (50 ind./year) and NIB (20 ind./year), for a total of 210 individuals during the project.	Between 2022/2023, 96 specimens of <i>P. nobilis</i> were found by Shoreline in Italy. Of these 96 individuals, 57 were self-recruited juveniles and 39 were adults. 36 of them were collected. In the same period 82 specimens were found by NIB in Slovenia. 42 of them were collected. In 2024 Shoreline collected 36 individuals, 24 of them from the larval collectors and NIB 18 juveniles. As Shoreline and NIB, we collected a total of 132 <i>P. nobilis</i> individuals during the period of the project, instead of the planned 210.	Analysis of the dead individuals revealed evidence of the presence of pathogens. Despite increased efforts to find new locations to increase the number of living (not infected) individuals, the mortality rate is still very high. The next objective is to find new individuals in the new locations where there are less/no pathogens.

C4 Adaptation, breeding and reproduction for active restocking	Objectives: establish a protocol for the controlled reproduction of <i>Pinna nobilis</i>. After the collection and transfer of adult specimens (action C5), action C4 will be implemented in four consecutive steps: 1) acclimatisation and sexual maturation; 2) spawning induction and eggs fertilisation; 3) larval rearing and metamorphosis; 4) juveniles rearing Expected results: The tests on controlled reproduction in captivity will be done in three different times, after having characterised the genetic characters of surviving adults and verified the absence of pathogens. These precautions will reduce the risk of failure, encouraging the calibration of the procedure and its continuous improvement in order to achieve an effective protocol. The 20 (max 30) adult specimens which will be collected for being maintained in captivity for controlled reproduction will be treated according to best established protocols for collection and transfer and will be transferred in three different occasions, in order to minimise stochastic mortality during collection and transfer.	All 34 <i>P. nobilis</i> specimens were collected from the Venice lagoon. The first reproduction in a controlled environment was achieved between June/July 2023, using 11 adult specimens. A total of 14.623.800 eggs were released in 3 spawning events, with high fertilisation rates, between 95.31 and 99% (14.060.200 fertilised eggs). However, we reached only the second larval stage (D-larvae, at 9 dpf), but we did not witness larval settlement and, consequently, the development of juveniles. The second spawning induction was achieved between May/July 2024, using 6 specimens. A total of about 50.000.000 eggs were released in 2 spawning events and 2 fertilizations were performed: one self-fertilization and one controlled fertilization. For this last fertilization the fertilization rate was 72.5 %, reaching for the first time, the umbonate larval stage at 16 dpf with a shell size of 150 µm, but did not witness larval settlement. The third spawning induction was achieved between April/May 2025, using 12 specimens (8 UNIGE and 4 Shoreline), after a gonadal maturation induction trial. A total of about 8.000.000 eggs were released in 3 spawning events and 3 fertilizations were performed (1 spontaneous fertilization and 2 controlled fertilization). Fertilization rates were between 9-18%. Larval development stopped at 8–10 dpf at the trochophore stage.	The adult specimens, if mature, release gametes even after transport stress, so it is necessary to be prepared to handle the larvae even immediately after the arrival of the specimens in the laboratory. However, it is also possible to stimulate adult specimens using thermal shock, a method already used in aquaculture for other species of bivalves and also for <i>Pinna nobilis</i>. If possible, keep the larvae in an air-conditioned room (around 21°C) to prevent development from occurring too quickly due to the high temperatures. The reproduction of <i>Pinna nobilis</i> is extremely challenging and requires further studies and collaboration between experts to share knowledge to optimize the larval development.
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C5 Collection and transport of specimens from self-capture to receptor sites	<u>Objectives:</u> The action foresees collecting and transporting the sentinels, the tissue fragments, surviving adults from the donor to receiving sites with minimal stress to the specimens. <u>Expected results:</u> Constant codification of all activities aimed at translocation and reproduction of <i>Pinna nobilis</i> specimens.	In line the project provisions	The preparation of protocols containing timely coding of all the steps necessary to carry out certain activities is very useful for the replicability of those activities and for comparison with any other methods applied elsewhere. Therefore, it was decided to proceed with the coding of any activity carried out within the project, even if not originally planned in the submitted proposal. A total of seven protocols were produced as Annexes to Action C5. These, together with the cost-benefit study conducted under Action E3, represent a useful transferability tool for MPAs and other entities that decide to undertake <i>Pinna nobilis</i> repopulation initiatives.
C6 Installation of the specimens of <i>Pinna nobilis</i> at the pilot areas.	<u>Objectives:</u> The action aims to transplant the juveniles coming from C3 (in situ self-recruitment, implemented by Shoreline and NIB) and C4 (ex situ controlled reproduction, mainly implemented by UNIGE with the participation of Shoreline for back-up). Pula Aquarium will give its support in preparing and testing the protocol to transplant individuals, in light of its previous experience in <i>P. nobilis</i> restocking activities. <u>Expected results:</u> Considering <i>P. nobilis</i> specimens possible accidental deaths during previous actions (C3, C4, C5) and installation operations (C6), we expect to transplant 55 in./year in four marine areas overall (Capo Mortola, Miramare, Strunjan	Instead of 165, we installed a total of 94 individuals during a three-year period. Genetic analysis to detect the presence of <i>H. pinnae</i> gave negative results from receiving sites. During the project Shoreline monitored and surveyed 96 individuals of <i>P. nobilis</i> , NIB monitored and surveyed 82 individuals. In total, 178 individuals of <i>P. nobilis</i> were found alive. Of these 178 individuals, 135 were positive for <i>H. pinnae</i> . This would have made it impossible (according to the proposal)	The range of potential donor sites was expanded to find areas with more pathogens-free organisms. Most of the individuals collected from C3 activities died within a few months and were positive to <i>H. pinnae</i> . This would have made it impossible (according to the proposal) to transfer them to the receiving MPAs, even if they had survived.

	and Asinara Island), for a total of 165 transplanted individuals 'during a three year period (2022-2024).	to transfer them to the receiving MPAs, even if they had survived. In fact the individuals that were transferred to Capo Mortola were negative for <i>H. pinnae</i> .	
D1 Monitoring of pathogens in restocking sites by using sentinel species	<u>Objectives:</u> Constant monitoring of the presence of pathogens in restocking sites by using sentinel species <u>Expected results:</u> Development of molecular markers for conducting genetic variability analyses to depict the Mediterranean phylodynamic patterns of the etiological agents involved in the onset of the fan mussel's disease. Constant monitoring of the presence of pathogens in the restocking sites, further evaluating their evolutionary dynamics for a rapid identification of new outbreaks (if any).	All. The activities planned for this action proceeded for the entire duration of the project. These activities were characterised by a constant development of increasingly efficient and specific molecular markers for the etiological agents that would have been associated with the mass mortality of <i>Pinna nobilis</i>.	Action D1 directly supported the conservation of <i>Pinna nobilis</i> inferring on the microorganisms that could be involved as potential etiological agents in the development of the disease that provoked mass mortality. The molecular diagnostic analyses developed for this project showed to be effective in the quick identification of pathogens for every kind of biological sample and sampling collection methods. A major breakthrough of these activities was the development of the first predictive model for disease progression following ongoing infection by <i>Haplosporidium pinnae</i>. This tool allowed for the early detection of the pathology up to 30 days before the onset of symptoms or the death of the individual, providing a critical window for potential intervention. Furthermore, the project successfully established a positive association (not necessarily positive correlation) between the presence of <i>Haplosporidium pinnae</i> in the organs of <i>Pinna nobilis</i> and the development of the lethal disease, providing solid scientific evidence for future monitoring and mitigation strategies.
D2 Monitoring of implanted juveniles	<u>Objectives:</u> Regularly monitor <i>P. nobilis</i> juveniles transplanted at the pilot sites to assess their survival, growth and state of health.	In total, 85 <i>P. nobilis</i> individuals (27 adults and 58 juveniles) were returned to the sea and monitored for two years.	Overall, the project action produced relevant and informative outcomes, demonstrating the effectiveness of the monitoring activities. The results

	<u>Expected results</u> : three years data from monthly monitoring of <i>P. nobilis</i> individuals.		indicate a higher survival potential in juvenile individuals compared to adults, as highlighted by the exceptionally long survival time of the specimen monitored along the Slovenian coast. In addition, the action revealed marked differences between the investigated basins: adult <i>P. nobilis</i> showed substantially longer survival in the Ligurian Sea (approximately eight months) than in the Adriatic Sea (around two months)
D3 Monitoring of the project's impact on the <i>P. nobilis</i> * status	<u>Objectives</u>: Main objective of this action is to quantitatively describe the <i>P. nobilis</i> population in donor and receiving sites and its evolution after project activities. <u>Expected results</u>: Monitoring protocols have been shared among partners. The ex-ante monitoring campaign has been realised in donor and receiving sites and data have been reported in deliverable D.3.1. The status of pinna populations in the project sites has been summarized in deliverable D3.2, confirming pinna populations decline during the project period. Citizen science activities (deliverable D3.3) have contributed to spread the awareness of pinna decline among the population and to support the research of living organisms all along the project areas and also outside.	All	Activities of action D.3 are technically completed. Unfortunately, results from the ex-ante campaign unveiled a drastic reduction of <i>P. nobilis</i> densities in donor sites as compared to those expected from previous available data. Population data at the end of the project (ex-post campaign) confirmed the trend.
E1 Communication	<u>Objectives</u>: prepare a communication plan to raise awareness about <i>Pinna nobilis</i>, promote responsible behaviour among marine ecosystem users, engage environmentally conscious individuals in community outreach through the website and socials. <u>Expected results</u>: disseminate results of LIFE Pinna to a wide audience, reaching 850.000 people in total; reach more than two million people, considering TV, radio, online and traditional press.	The communications plan was developed, and the website and social media were constantly updated. Media and online advertising campaigns, videos, and project documentaries were sponsored with the help of specialized agencies. Life Pinna partners participated in more than 20 external dissemination events.	Project coverage was highly satisfactory, and the objectives were achieved. The dissemination results reached a total audience of over 1,600,000 people through social media and the project website, documentaries, and video clips. Including TV, radio, online, and traditional media, the audience exceeded 10 million.

E2 Replication and transfer	Objectives: multiply the impact of the project, through transfer and replication of good practices and effective methodologies used by the project to other areas Expected results: expression of interest from around 50 organisations at international level to use these guidelines and participation of 15 people at international level in the training	The replication plan has been produced by TRITON on 29 December 2023, and ARPAL has signed two agreements with the sites selected to implement the <i>Pinna nobilis</i> project solutions (with Bergeggi MPA in July 2024 and with the Tuscan Archipelago MPA in April 2025). Five protocols for the management and reproduction of <i>Pinna nobilis</i> have been defined.	Delays in conservation efforts caused by the species' deteriorating condition have postponed the actual start of the project's multiplication and transferability efforts until July 2024. During the implementation of Action E2, the approach to transfer and replication evolved significantly compared to what was originally foreseen in the Application Form. Initially, the project envisaged the collection of at least 50 expressions of interest from individual organisations willing to adopt the protocols developed. However, the rapid and severe worsening of mass mortality events affecting <i>Pinna nobilis</i>, together with the urgent demand for coordinated guidance at Mediterranean scale, made it clear that a more immediate and structured mechanism was required. As a result, the project shifted from a bilateral, organisation-based approach to a basin-wide institutional process. This change materialised through the formal request received in January 2022 from SPA/RAC to elaborate the Mediterranean Plan for the Restoration of <i>Pinna nobilis</i>, which was subsequently adopted in December 2023 by the Contracting Parties to the Barcelona Convention. This collective institutional endorsement effectively replaced the initially foreseen individual expressions of interest, ensuring a broader, more binding and longer-term commitment to the uptake and application of LIFE PINNA protocols across the Mediterranean
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E3 Networking and synergies with other LIFE and/or non-LIFE projects	<u>Objectives:</u> networking activities with other LIFE and other projects for the entire duration of the project, including the exchange of experiences and technical and scientific documents <u>Expected results:</u> involvement of at least 5 other projects operating in similar fields; networking events for the exchange of the methodologies <u>Transfer activities to AMP protected areas</u> <u>Objectives</u> To assess the economic replicability and transferability of LIFE PINNA solutions for the conservation and restocking of Pinna nobilis in other Marine Protected Areas; To test and compare operational and monitoring approaches in selected pilot MPAs under real management and logistical conditions. <u>Expected results</u> Implementation of ex-ante and ex-post monitoring activities in the selected MPAs; Execution of a pilot restocking attempt to support the economic assessment; Collection of technical, organisational and cost data, including a comparison between traditional scientific diving and ROV-based monitoring, to support a final cost–benefit analysis for transferability.	Several (14) projects were contacted, with 8 with a special focus on Pinna nobilis (LIFE Pinnarca, Pinna Hope, Pinna Garden, Crisis management of Pinna nobilis in Croatia, Interreg TRECAP, Interreg Eco2Smart, Interreg POSEIDONE, CenSy4Sea)	The success of networking activities is one of LIFE Pinna's main achievements. In recent years, collaboration with SPA/RAC, the United Nations Institute for Marine Biodiversity in the Mediterranean, has been consolidated, important training sessions have been organized, and the Mediterranean Action Plan for the Conservation of P. nobilis has been developed, subsequently adopted by the Barcelona Convention in 2023. The first networking event took place on June 13-14, 2024, at the Miramare Marine Protected Area (Trieste, Italy) and the Pula Aquarium (Croatia). In July 2024, a joint event with the LIFE Pinnarca project, which also involved the IUCN, was organized in Valencia and Calpe, Spain, as part of the International Marine Science Symposium. One of the LIFE Pinna's legacies is the creation of an international working group that today involves researchers from various disciplines, from ecology to reproduction, who study the Pinna nobilis throughout the Mediterranean basin, from Tunisia to the Balkans, and continue to transparently share current knowledge to develop new strategies. <u>Transfer activities to AMP protected areas:</u> Ex-ante and ex-post monitoring activities were carried out in the selected MPAs, providing a detailed characterisation of environmental conditions and operational contexts;
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			A pilot restocking attempt was implemented; however, due to the limited availability of healthy specimens, it did not result in the establishment of a viable population; Traditional scientific diving and ROV-based monitoring approaches were tested and compared, allowing the collection of robust technical and economic data; Dedicated Partnership Conventions with the involved MPAs were signed, enabling the structured implementation of transferability activities in compliance with LIFE and national administrative rules; A comprehensive set of cost, logistical and organisational data was collected, forming the basis for the final economic assessment of transferability.
F1 Project coordination and management, After LIFE plan and Green procurement	<u>Objectives</u>: the lead partner has the role of ensuring coordination among the beneficiaries from the technical, managerial and financial point of view in order to monitor the progress of the project and foster the progress of the actions also from the scientific point of view. <u>Expected results</u>: Coordination and constant monitoring of the progress of project activities, reducing possible critical issues encountered through a continuous exchange with all beneficiaries (Authorization in Derogation, population situation of <i>P. nobilis</i>)	In line with the project provisions.	To date, project monitoring and coordination have been problem-free, thanks to numerous meetings with the technical and administrative parts of each beneficiary. Delays in some deliverables are due to the status of <i>P. nobilis</i> populations.

Project results immediately visible

The main immediately visible results of the project included:

- **Validation of a Non-Lethal and Minimally Invasive Mantle Fragment Sampling Collection for DNA Isolation of *Pinna nobilis* and its Pathogens by means of biopsy:** The validation of mantle fragment collection via biopsy represents a crucial technical milestone for the conservation of *Pinna nobilis*. Unlike traditional sampling which often required the sacrifice of the animal, this protocol ensures the collection of high-quality genomic DNA and pathogen data while preserving the life of the individual.
- **Innovative Non-Invasive Sampling Protocol for DNA Isolation of *Pinna nobilis* and Pathogens by means of cotton swabs:** The development and standardization of the cotton swab methodology is an immediate success. It replaces invasive biopsies, allowing for DNA collection from fan mussels and their pathogens without harming the critically endangered individuals.
- **Scientific Validation of Donor Suitability:** The project has immediately confirmed that surviving populations in the Northern Adriatic (Gulf of Trieste and Venetian Lagoon) share the same ancient genetic lineages (mitochondrial haplotypes) as the extinct Western Mediterranean populations. This gives the "green light" for translocation activities
- **Predictive Diagnostic Tool for disease development:** The ability to predict mortality up to 30 days in advance through qPCR and non-invasive cotton swab screening is a functional result. This allows operators to constantly monitor individuals and detect dangerous *Haplosporidium pinnae* infection increasing before early symptoms appear.
- **Comprehensive Genetic Mapping:** A fine genetic map (mitochondrial and nuclear/microsatellites) for the Gulf of Trieste and Venetian Lagoon *Pinna nobilis* populations are now complete and available for ISPRA and the international scientific community.
- **Confirmation of Reduced Pathogen Pressure:** Molecular screening of 1,395 sentinel organisms has provided immediate proof of a present low pathogen pressure in specific intervention areas, justifying their use as restocking sites.
- **Effectiveness of "Sentinel Species" Monitoring as a Management Standard in Marine areas:** While this methodology was already established for the conservation of terrestrial fauna, this was the first time these methods were based on marine organisms (bivalves) and applied to Mediterranean-wide marine fauna conservation. The approach proved to be highly effective and powerful and successful thus being ready to be used for preventing future mass marine mortality outbreaks elsewhere.
- **Success of the transplanting of adult individuals** carried out at Capo Mortola in December 2023, **and of juveniles** in Miramare MPA and Slovenia; all transplanted individuals survived months after transplantation, confirming the suitability and efficacy of the applied protocols.
- **Acquisition of expertise** regarding captation of larvae through larval collectors, maintenance in ex-situ facilities, translocation, breeding and reproduction activities, by the partners, who are thus able to assess the best solutions to be undertaken for the continuation of the activities, to cope with the difficulties encountered (see proposals included in the CP);
- **Public awareness** of the ecological importance of the species and its role in the marine ecosystem, which serves as a stimulus for important knowledge input from citizen science;
- the success, albeit partial, of captive reproduction; Although the larval cycle has not been completed and juveniles have been obtained, several results have been achieved. During the years of the project, we have improved the protocol for maintaining adults in the laboratory. Additionally, we have improved larval development management by attempting to supply a microalgae-based diet suitable for larval growth. In fact, it was possible to keep the larvae in a controlled environment for 21 days after fertilisation, reaching a size of 150 microns at the umbonate larval stage (16 dpf). This size has never previously been reported for this larval stage of *Pinna nobilis*, though it has been for other species in the Pinnidae family (Hoyos-Chairez *et al.*, 2023). It should be noted that the reproduction of *P. nobilis* is extremely challenging and requires further studies and collaboration between experts to optimise larval development.

- **Creation of networks** with researchers working on different aspects of *Pinna nobilis* conservation, enabling to maintain a constant exchange of information and knowledge to improve conservation efforts.
- the development of **7 protocols** related to the following activities:
 - Non-lethal and minimally invasive mantle fragment sampling collection for DNA isolation of *Pinna nobilis* and its pathogens by means of biopsy;
 - Non-invasive sampling protocol for DNA isolation of *Pinna nobilis* and its pathogens by means of cotton swabs;
 - Transport of sentinel organisms;
 - Transport of small mantle tissue fragments;
 - Transport and maintenance of the pinna;
 - Breeding of the pinna;
- a report describing purpose and means of providing financial support to third parties

Results of the replication efforts.

Despite the lack of immediate positive biological outcomes, Action E3 has generated significant added value in terms of learning, capitalisation of experience and development of decision-support tools. The results provide a solid foundation for future conservation initiatives and for the design of new funding actions, contributing to the definition of more effective, selective and sustainable strategies for the conservation of *Pinna nobilis* and, more broadly, for the management of species and habitats under severe ecological stress.

Indicate the effectiveness of the dissemination activities and comment on any major drawbacks.

LIFE Pinna raised a surprising interest in the media, because the project storytelling has been appreciated: a group of scientists trying to save an important and endemic species from extinction in the Mediterranean Sea. The website and various social media platforms were developed quite early to inform the public and provide interesting news for the media. Besides this, in the first months of the project, a good image archive has been built, capable of capturing the public's interest and supporting journalists' activities, with quality photo and video material at their disposal. Across social platforms, the coverage grew quite fast, reaching 430,000 people in late 2023, and exceeded 1,600,000 in September 2025. The general public involved by different media could have been more than 10 millions, thanks to bigger newspapers and TV programs, both in Italy and in Slovenia.

Engaging people in the conservation efforts for a mollusk may pose challenges due to its perceived lack of charisma or immediate relevance to many individuals. However, during the project, conservation efforts were presented in a broader perspective, in terms of ecological health, biodiversity conservation, and ecosystem interconnection. This contributed to generating interest and support. Furthermore, to make the cause more compelling, the unique role that this mollusk plays in ecosystems was highlighted, such as its contribution to nutrient cycling or its role as indicator of environmental health. Part of the public engagement, aimed at a more motivated public of sea and nature lover in general, is due to the photography contests and the citizen science activity, which have enabled extensive dissemination within the scuba diving community. Citizen science projects promote public engagement with science and nature, empowering individuals to actively contribute to scientific research and conservation efforts. By involving volunteers in hands-on activities, citizen science fosters a sense of ownership and connection to the issues being studied. The project also benefited from dissemination through generalist paper, online media and television that have helped to introduce the general public to the issue. Those media serve as powerful agents for raising awareness about new issues owing to their multifaceted strengths. Their accessibility, credibility, and expansive reach make them effective conduits to disseminate information to diverse audiences. In some specific cases, like specialised magazines with a focus on nature and environment, their capacity for in-depth analysis allows for a comprehensive understanding of complex topics, fostering informed discussions and encouraging engagement of more passionate individuals.

The citizen science campaign "Report the Pinna" in search of survivors was carried out through all communication channels and achieved concrete results. On project channels (website, social networks

and general press) the campaign has been regularly highlighted, mostly during spring-summer, through press office work and ad hoc content creation (e.g. news and videoclips). Arpal's cartographers developed a database that collects reports of *Pinna nobilis* received from citizens, including those submitted via the LIFE Pinna website, and allows them to be viewed on a map. Thus preventing the harvesting of bivalves by malicious individuals, a margin of approximation has been maintained. Furthermore, a series of public live and online training initiatives, also with PADI divers collaboration, attracted over a thousand people engaging and educating with LIFE Pinna gadget awards assignment. This approach led to the collection of over 70 reports in 2023-2025, with over 60 specimens discovered. Among the most relevant, three surviving populations were discovered in the middle Adriatic and Sardinia Seas. As proof of the wide diffusion of the campaign, it is interesting the report received in summer 2025 from Egypt, in the Red Sea, of a Pinnidae probably attributable to the species *Pinna bicolor*. Citizens have so proven to be key players in *P. nobilis* conservation because these discoveries contributed to avoid a drastic revision of the project aims, which can thus count on new potential donor sites hopefully to be preserved as sanctuaries for the conservation of this species.

Besides this, LIFE Pinna partners were engaged in numerous conferences, scientific gatherings, and exhibitions, seizing the chance to illustrate the project. Scientific events serve as dissemination platforms for sharing issues and outcomes with a broader audience, including policymakers, industry professionals, educators, and the general public. Participating at important public conferences, like Festival della Scienza in Genoa, enhances the credibility of the activity within the scientific community. It provides a platform to showcase the work, build credibility, and attract attention from peers, funders, and the media. Networking activities were developed almost immediately with LIFE Pinnarca and with other projects operating in the upper Adriatic and then also with more distant projects such as Pinna Hope from Turkey.

The main drawback has been the decreasing of the species, which has made Citizen Science activities more challenging. Even organising live events with such a rare, delicate, and rapidly declining animal proved to be more complicated than initially anticipated. However, the participation of certain stakeholders, such as the diving community and fish farmers, has been still significant.

Policy impact

At the present the project has had no direct impact in terms of implementation of present legislation.

The main regulations and competencies, in Italy and Slovenia, are as follows:

Regional management of marine Natura 2000 sites;

National establishment of MPAs and entrusting their management to public bodies, scientific institutions, environmental associations; for Slovenia "Regulation for the protection of wild flora and fauna," in Slovenian is: Uredba o zavarovanih prosto živečih živalskih vrstah (Uradni list RS, št. 46/04, 109/04, 84/05, 115/07, 32/08 - odl. US, 96/08, 36/09, 102/11, 15/14, 64/16 in 62/19);

EU list of species of conservation concern and the levels of protection to be applied to them (Habitats Directive).

In any case, although it cannot be considered a 'law', the project experts were the authors of the 'Mediterranean Plan for the Restoration of *Pinna nobilis*' adopted by during the 23rd Ordinary Meeting of the Contracting Parties to the Barcelona Convention (Decision IG.26/5, Portorož, Slovenia, 5–8 December 2023). In January 2022, SPARAC requested LIFE PINNA to draw up, on their behalf, the Mediterranean Plan for the Restoration of Pinna, which was presented at a first technical meeting organised by SPARAC and LIFE PINNA in cooperation with IUCN in June 2022. This meeting brought together researchers from all Mediterranean countries to evaluate the plan prepared by the project's technicians.

Although the plan is not a law, it provides a framework for all conservation actions undertaken by SPARAC as part of their coordinated conservation activities in the Mediterranean within the Barcellona Convention umbrella.

The actions undertaken by the project and its results may positively influence sector legislation, particularly through the introduction of management measures to create the conditions for a repopulation of *P. nobilis*. This could likely take place as part of the updating of the Conservation Measures and Objectives for marine ZCS. In addition, since the pilot sites were mostly within or adjacent to protected areas (e.g. Miramare MPA, Strunjan NP), the results of this project will help to maintain and improve the conservation status of the species, even after the end of the project, through the regulations of marine parks and reserves. The Slovenian Ministry of Natural Resources and Spatial Planning was actively involved, co-financing the project from May 2023 (sklep št. 5440-/2022-2550-7). For this Ministry, NIB researchers produced once per year a report on the outcome of the project in Slovenian language.

Policy developments resulting from project activities

The creation of a permanent international networking group, with the involvement of the most competent scientists in the world and of entities such as SPA RAC and IUCN, represents an important result, which we hope will lay the foundations for the preparation of shared guidelines and directions at a global political level, for the protection of the species.

Impact of the project results foreseen in the Grant Agreement form B3 “EU ADDED VALUE OF THE PROJECT AND ITS ACTIONS”.

The LIFE PINNA, in line with the results foreseen in the Section B3 will impact on LIFE Sub-programme for Environment and in particular topic 3 for Nature, through the testing and implementation of conservation strategy aimed at stopping the decline of *Pinna nobilis*, priority species in the Annex IV of the Habitat Directive. Concrete conservation actions have consisted of all the activities undertaken to halt the decline of the *Pinna nobilis* and attempt to reproduce the species in the laboratory and transfer individuals from donor to recipient sites. All the information gathered represents an extremely important body of knowledge for science and future planning, regardless of the success of the interventions carried out during the project. Due to the species' tragically rapid and unexpected decline, these interventions did not achieve the desired results.

Thanks to the project approach and concrete actions the project is directly impacting on:

- the COM/2020/380 “EU Biodiversity Strategy for 2030 Bringing nature back into our lives” of the EU Commission with the overall objective to restore degraded terrestrial and marine ecosystems across Europe by 2030, including to protect the ecosystem services they generate, with their economic benefits;
- the Marine Strategy Framework Directive (MSDF), since it aims at setting a strategic approach to integrate monitoring of indicators for D1 (distribution and abundance of *Pinna nobilis* are maintained) and D4 (ensure the long-term abundance of *P. nobilis* and full reproductive capacity);
- the further development, implementation and management of the N2000 network, identifying important sites and risk areas and connectivity integrity.

The experience gained during the project will provide the tools to define priority conservation strategies within international planning. Furthermore, the scientific results achieved, in terms of specific innovative procedures developed for reproduction, genetic analysis, and transfers, will help identify guidelines for management policies at the level of individual member states and stakeholders actively involved in conservation policies.

A concrete example concerns the protocols developed under Action C5 and the transferability study developed under Action E3, which will provide valuable support in the preparation and updating of management plans for marine Special Areas of Conservation in the Mediterranean Sea.

6.4. Analysis of benefits

1. Environmental benefits

Conservation *P. nobilis* in the western Mediterranean and northern Adriatic Sea

Along the Italian coast a total of 96 specimens of *P. nobilis* were found in the first two years of the project. Of these 96 individuals, 57 are self-recruited juveniles. Each individual, after being secured in aquaria (small individuals, between 3.5 - 5 cm high), in suspended lantern-net or in the sea covered by small cages to provide additional protection, was tested for transferability. For each individual, an acclimation phase was performed before being placed in the aquarium. This phase takes approximately 30-40 minutes. Lantern-net are traditionally used in shellfish aquaculture and they play the role of nurseries where *P. nobilis* specimens grow. These baskets were attached to the mussel farm's longline and thus remained suspended in the aquatic medium for a period necessary for the growth and rear of fan mussel specimens. Each individual of *P. nobilis* placed in the sea, was inside Miramare MPA or inside protected areas under Shoreline concessions.

In the Italian sea the number of larval collectors and collector bags was increased from 2022 to 2025.

In 2022, 3 lines of collectors at each of 3 sites (total of 39 bags) along the Italian coast (depth: 1-8m).

In 2023, 3 lines of collectors at each of 3 sites (total of 39 bags) along the Italian coast (depth: 1-8m).

In 2024, 3 lines of collectors at each of 3 sites (total of 81 bags) along the Italian coast (depth: 1-8m).

In 2025, 2 lines of collectors at each of 3 sites inside Miramare MPA (total of 63 bags, depth: 1-8m) and 6 lines of collectors on 1 site on Shoreline mussel farms (total of 63 bags, depth: 1-8m).

Considering the Slovenian sea, from 2022 to 2025, 3 lines of collectors were installed at each of 5 sites.

The total number of bags was increased from 43 in 2022 to a total of 90 bags the following year. In the first two years of monitoring, 82 pinnids were surveyed, and 24 individuals were monitored in 2024/2025. Of these 106 individuals monitored, 60 are young self-recruited *P. nobilis* individuals (**13** juveniles found in larval collectors in 2022, **26** juveniles found in larval collectors in 2023, **18** juveniles found in larval collectors in 2024 and **3** juveniles found in mussel farms) and were first kept in aquarium. 19 of those juveniles were moved to lantern net at sea. Alive specimens ranged between 1.2 and 5.4 cm of length in 2022, between 0.5 to 8 cm of length in 2023, between 0.9 and 1.5 in 2024. Alive pinnids kept in aquaria (open system) and fed with commercial feed (Najdek et al., 2013; Trigos et al., 2015; Prado et al., 2020; Hernandis et al., 2022). Unfortunately, only 4 juveniles survived.

Considering the Italian sea, in 35 monitoring activities in 2024:

- a total of 25 individuals of *P. nobilis* were found alive in the Grado lagoon, 3 in the bay of S. Bartolomeo (near Slovenia sea) and 2 inside the Miramare MPA. Eight individuals of the 25 were identified and confirmed thanks to the participation of divers in citizen science activities (found in dangerous situations like docks, moorings and anchorages). Monitoring measures are currently underway, utilising the winter season with fewer leaves of phanerogams.
- In January 2024, 24 pinnids were found in 14 larval collectors (placed in the Shoreline facilities).
- Of the 25 specimens from 2024, only 16 were found alive in the Grado lagoon.
- In 2025, 12 new specimens of *P. nobilis*, between 11 and 24 cm, were identified in the Grado and Marano lagoon.

In 2025, 4 adult specimens were collected from Venice lagoon and placed in 500-litre tanks in a vertical position for gonadal maturation testing in collaboration with University of Genova and Aquarium Pula (duration of the experiment 8 months).

The cleaning of the tanks took place 3 times a week, to remove pseudo-feces and any food residue from the bottom of the tanks, the volume of water removed is restored again. The water change was regularly performed partially (1/3 of the total volume) to avoid air exposure of the specimens. The water used to fill the tanks was filtered (sand filter, biological filter, 2 cartridge filters of 10 and 1 micron) and sterilised with UV lamps. All the tanks were provided of air stones inside connected to a compressor for aerating the water. The specimens were fed 3 times a week with a mix of live microalgae (*Isochrysis galbana*, *Chaetoceros calcitrans*, *Diacronema lutheri*) and an amount of Easy Reef artificial feed® (Najdek et al., 2013; Trigos et al., 2015; Prado et al., 2020; Hernandis et al., 2022). The different strains of microalgae were maintained and grown in the laboratory inside bioreactors of 40 litres each, to be able to produce microalgae in large quantities. The specimens that died during the period of maintenance in the tanks were sent to colleagues at the University of Sassari to test for the presence of the protozoan *Haplosporidium pinnae*. At the end of the experiment, the two surviving specimens were returned to the sea, within the Miramare Marine Protected Area.

During the first phase of the project, the transplantation activity developed with the preparation of useful materials for the journey. On several occasions we were able to experiment with the materials, first without live organisms, then with organisms other than *P. nobilis*, particularly *Atrina fragilis*. This allowed us to be able to test the entire transport phase without endangering live organisms of *P. nobilis*. Confirmed 100% efficiency of *P. nobilis* transport during all the transport activities C3/C4/C5/C6 actions.

Number of translocation actions: 7 in Miramare MPA, 4 in Strunjan MPA, 2 in Capo Mortola, 1 in Bergeggi MPA.

- 55 individuals installed in the Miramare MPA.
- 24 individuals installed in Strunjan MPA.
- 10 individuals installed in Capo Mortola receiving site.
- 5 individuals installed in Bergeggi MPA.

Development and implementation of the most appropriate restocking techniques by creating 5 replicability protocols

The LIFE PINNA project has developed innovative scientific approaches to preserve residual genetic diversity, reduce health risks, and identify recovery strategies. The experience gained has produced operational knowledge transferable to other Mediterranean contexts.

Product D_E.2.1 "5 Protocols for Replication" contains five operational protocols designed to facilitate the replication of conservation and restocking methodologies for the species. They aim to translate research into practical tools, support management decisions, reduce translocation risks, and harmonize conservation practices. The document is intended for managers of Marine Protected Areas, environmental authorities, research institutions, and NGOs. The protocols, the result of interdisciplinary work, integrate ecological, health, genetic, and administrative aspects. The guiding principles include precaution, ethical responsibility, scientific transparency, and adaptability. The document's structure, divided into thematic chapters and cross-sections, offers a comprehensive toolkit to support conservation efforts and a coordinated response to the species' crisis.

Characterisation of appropriate marine areas for restocking:

As part of Action E3, a report was developed that provides an initial assessment for engaging third parties in conservation activities (E3_ annex 1_Preliminary and ex-ante Report AMP). This report analyzes the environmental, socioeconomic, and management characteristics of two Marine Protected Areas: Bergeggi Island and the Tuscan Archipelago National Park. Both are suitable for transferability of actions due to their ecological value and technical capacity. Bergeggi boasts valuable habitats and a long history of monitoring, while the Tuscan Archipelago, the largest in Europe, offers a diverse and representative context for Mediterranean systems. Ex-ante monitoring activities show a serious decline in the *Pinna nobilis* in both areas, with only one live individual detected in the archipelago. A methodology based on standardized tools, stakeholder engagement, and systematic data collection is proposed. This approach allows for the development of a transferability plan that can also be applied to other sites. The report confirms the strategic role of MPAs in translating scientific evidence into concrete management and conservation actions.

2. Economic benefits

The project has not a direct economic impact nevertheless there are several aspects that must be considered:

The project is supporting the MPA in the implementation of conservation activities of marine ecosystems and Natural Capital. In the replicability study for Action E3, a key component of the report is the economic analysis of transferability, based on actual costs incurred and systematized in the CostiFattibilità_LifePINNA_01 dataset. This analysis compared different types of activities, including underwater monitoring, ROV monitoring, captive breeding, health surveillance, transport, and transplantation. The results highlight significant differences in cost structure, technical complexity, and replication potential. For example, environmental and health monitoring emerges as the activity with the most favorable cost-benefit ratio and the highest transferability potential, while captive breeding and transplantation are characterized

by high fixed costs and a strong dependence on specific structural and health conditions. This information is valuable for MPAs that decide to undertake *Pinna nobilis* conservation activities.

The wide citizen science campaign carried out with the fundamental collaboration of the divers represent another impact in terms of economic benefit of the project. In fact *Pinna nobilis* is one of the best known species to divers and is one of the most interesting attractions of the seagrass beds. Conservation of this species is therefore also important to support this type of tourism linked to the natural heritage of the Mediterranean seabed.

In terms of FTE at the date of the Final Report the project has produced a total of 8,6 FTE, 7 in Italy and 1.6 in Slovenia.

3. Social benefits

The project's social benefits are mainly related to the effort in terms of information of the public on the biodiversity value and the risks related with biodiversity loss as well as on the stakeholders engagement through extensive citizen science campaigns.

In terms of Information to the public the project had a very wide impact on the different media channel:

- **Social Media:** Over the years of the project, to broaden the dissemination of LIFE Pinna content, digital marketing tools were used with the external assistance of specialized agencies, both using online banners to promote interest in the project and attract people to the website (Native Adv campaign) and through a dual sponsorship campaign (Meta Ads and Google Ads) on the content that allowed us to reach a specific audience with personalized ads. Total social network cover exceeded 1,600,000 in 2022-2025.
- **Media and broadcast:** LIFE Pinna had more than 400 citations on different media so far (online, paper, radio, TV), including very popular TV shows like “Linea Blu” (Rai 1), “Geo” (Rai 3), “TG Leonardo” (Rai 3), TG5 (Canale 5), and “Dobro jutro” (RTV SLO) in addition to magazines and newspapers like Corriere, Repubblica, La Stampa, Focus, Vatican News, Il Secolo XIX, Osservatore Romano, Le Figaro, Focus, Rivista della Natura, Delo (Slovenia) and Primorske novice (Slovenia).. Total combined number of readers and audience is superior to ten million people (prudent estimate), not including LIFE Pinna social, website and videos. The press offices Boe Sas (2022-2023) and Green Factor srl (2024-2025) collaborating with Triton Research, provided help with general media, especially with newspapers. More details on Life Pinna selected press review: <https://www.lifepinna.eu/en/media-press-review>

As for stakeholders engagement the basic strategy was **citizen science** aimed at a more motivated public of sea and nature lover in general, due to the photography contests and the citizen science activity, which have enabled extensive dissemination within the scuba diving community. Citizen science projects promote public engagement with science and nature, empowering individuals to actively contribute to scientific research and conservation efforts. By involving volunteers in hands-on activities, citizen science fosters a sense of ownership and connection to the issues being studied.

4. Replicability, transferability, cooperation:

The LIFE PINNA project provides a solid analytical basis for assessing the potential replication of its approaches beyond the project duration. While the severe sanitary crisis affecting *Pinna nobilis* continues to limit the immediate feasibility of large-scale biological restoration, the project delivers transferable technical knowledge and decision-support elements that can inform future conservation actions in comparable contexts.

From a technical application perspective, the highest transferability and cost-effectiveness are associated with environmental and health monitoring activities. These actions require limited infrastructural investments, rely on established methodologies and are fully consistent with the institutional mandates of Marine Protected Areas and environmental authorities. Their economic feasibility is therefore high in relative terms, as they can be implemented using existing resources and supported through ordinary public funding mechanisms. The likelihood of replication for this component is assessed as high, with potential for wide geographical dispersion at local, national and EU levels.

More complex interventions, such as experimental transplantation and specimen transport, present stronger limiting factors. Their feasibility is highly dependent on site-specific conditions, availability of healthy individuals, sanitary status and logistical capacity. These constraints reduce their immediate replicability and require targeted funding and careful planning. Replication of these actions is therefore assessed as selective, suitable only in well-evaluated contexts and under coordinated frameworks.

Captive breeding and advanced sanitary activities show limited transferability at local level, but retain strategic value when implemented by specialised research institutions. Their replication potential is considered low in terms of geographical dispersion, but relevant at regional or Mediterranean scale as support actions for multiple MPAs.

The suitability for additional funding from other streams is mainly linked to public instruments, such as EU structural funds, national biodiversity strategies and regional environmental programmes. The structured cost and feasibility data generated by LIFE PINNA increase the readiness of future initiatives to access such funding, even in the absence of direct economic returns.

The benefits for stakeholders are primarily institutional and societal. MPAs and managing authorities gain operational guidance, cost benchmarks and prioritisation tools; research institutions benefit from clearer identification of high-value specialised activities; policy-makers gain realistic evidence on drivers and obstacles for transfer. Public awareness and interest in the conservation of *Pinna nobilis*, an iconic and highly visible species, act as an important driver for continued support and replication.

Overall, the replication of LIFE PINNA results is primarily policy-dependent and not market-driven. The project's likelihood of replication is assessed as high for monitoring and management-related components, selective for experimental restoration actions, and low for highly specialised breeding activities. LIFE PINNA thus offers a realistic and adaptable framework for future applications in the same sector and, by extension, in other conservation contexts facing similar ecological constraints.

The results of LIFE PINNA clearly indicate that the long-term conservation of *Pinna nobilis* cannot rely on isolated or small-scale initiatives, but would significantly benefit from coordinated, large-scale European funding mechanisms. The ecological scale of the sanitary crisis affecting the species, combined with its wide geographical distribution across the Mediterranean, requires interventions that go beyond local capacities and short-term project cycles.

A European-level funding framework would enable the harmonisation of monitoring protocols, the consolidation of specialised infrastructures and the coordination of actions among Member States, ensuring continuity, comparability and efficiency. In particular, sustained funding could support the establishment of permanent monitoring networks, centralised sanitary surveillance and shared research facilities, reducing duplication of efforts and optimising resource allocation.

Such an approach would also enhance the effectiveness and transferability of conservation measures, allowing Marine Protected Areas to focus on core monitoring and management activities while relying on coordinated support for highly specialised actions. In this context, large-scale European funding represents a critical enabling condition for moving from experimental, project-based interventions to a structured and resilient conservation strategy for *Pinna nobilis* at Mediterranean scale.

5. Best Practice lessons:

In the project form, some best practices are indicated, aimed at meeting the recommendations of the IUCN (1. identification of high-density *Pinna nobilis* hotspots and targeted transfer from high-density areas to other high-density zones, paying attention to avoiding disease transmission; 2. Enhancement of larval recruitment from resistant individuals and unaffected areas for restocking actions).

The activities carried out up to now have fully adhered to the good practices indicated in the form, in particular:

- **Use of sentinel species as bioindicators for the first time in marine habitats (preparatory activities):** different species of filter-feeding mollusks (mainly bivalves) were used to monitor the water quality of donor and receiving sites. Within the framework of Action A2, UNISS has developed the first molecular protocol for the use of sentinel species as biomarkers for marine environments for the first time. Within action C5, the first protocols for obtaining the sentinels (both through collection in the natural environment and through purchase) and shipping them to the laboratory (UNISS) responsible for conducting the analysis of them have been collected
- **Two biological sample collection protocols for DNA isolation from *Pinna nobilis* and its pathogens** have been developed by UNISS within the framework of Actions C2 and D1. These

protocols — ranging from minimally invasive (by biopsies) to completely non-invasive (by cotton swabs) — allow for the genotyping of individuals without risking their survival, while enabling constant monitoring of the infection status in both juveniles and adults, whether they are healthy, severely infected and moribund, or deceased. In certain cases, forensic-grade DNA extraction and analysis protocols developed by UNISS have made it possible to genotype individuals and possibly assess the presence of severe pathogenic infections even when only empty shells or fragments, collected from former *Pinna nobilis* habitats, were available.

- **An analytical protocol that utilizes *Haplosporidium pinnae* infection in *Pinna nobilis* tissues as a molecular biomarker for the progression of the disease responsible for mass mortality** has been developed by UNISS within the framework of Action D1 . Using *H. pinnae* as a molecular marker allows for the identification of the early stages of the disease and the prediction of death approximately 30 days in advance. This methodology enables the selection of the best individuals for restocking activities, minimizing the risk of introducing pathogens into selected new areas and maximizing efforts by focusing restoring activities on individuals with the highest probability of long-term survival
- **Employment of the best restocking techniques:** all activities for the collection of juveniles, growth monitoring, transportation, and installation of *Pinna nobilis* specimens from donor sites to receiving sites have been carried out according to the best-known methods. Moreover, protocols of good practices have been constantly updated throughout the project to refine the available techniques to the maximum, ensuring the welfare of the animals and the successful completion of the translocation operations
- **Tested methodologies for sample manipulation and transportation:** In this case as well, the best available methodologies have been employed for conducting these activities. An existing protocol for harvesting mantle tissue fragments of *P. nobilis* was utilised. However, since the aim is always to minimise impact on the animals as much as possible, a completely non-invasive technique was developed during the project, involving the collection of mucus using cotton swabs. Again, activities were codified and documented in a specific protocol
- **Agreement framework for juvenile capture among the various management authorities of the MPAs:** this activity has not yet been implemented
- **Experimentation of a specific protocol for the captive breeding of *P. nobilis* (C4):** a protocol has been released following the several reproduction experiences in the laboratories of UNIGE. In this regard, the Deliverable_C.4: “Best practice protocol for *Pinna nobilis* reproduction in controlled conditions” was drawn up. This document describes the best practices for maintaining adult specimens, techniques for inducing spawning and fertilization, and finally, management of the larval cycle.
- **Use of larval collectors for larval recruitment:** three different types of collector bags were used, developed by the NIB (National Institute of Biology, Slovenia), CNR-ISMAR in Venice, and Natura Histrica, Croatia. The larval collector device consists of a main rope deployed vertically in the water column onto which a certain number of “collector bags” are attached in succession. Each collector bag is made up of an outer net bag that encloses a filler material. The structure consists of a rope with a maximum length of 8 m, kept vertically with buoys and bags for collecting larvae positioned at 3 different depths: -2 m, -4 m, and -6 m. Outer bags for larval recruitment can be made from onion or potato bags. These bags have proven particularly effective for larval settlement. The filler inside the bags can be made of different materials (tangled nylon filaments, additional plastic bags, jute). This material provides a surface for the larvae to settle on and protect them from predators while allowing them access. As *Pinna nobilis* larvae are transported by currents, collectors should preferably be positioned in areas exposed to open water. However, they can also be installed in semi-enclosed sites, such as lagoons. Larval collectors can be anchored to a ballast fixed to the seabed and kept vertically with a floating buoy or attached to the long line of the mussel farm. The collector devices should be retrieved after a period of about 5 months, however autumn weather and sea conditions may cause delays.

6. Innovation and demonstration value:

The innovative character of the project is linked to its two main groups of actions. These innovative elements are twofold, relating to molecular aspects of marine water and individual monitoring, as well

as to translocation and reproduction. The innovation introduced by this project in the molecular field lies in the first-ever successful use of sentinel species for pathogen monitoring in aquatic and marine environments. Furthermore, an additional innovative element is represented by the development of two sampling methods—minimally invasive and entirely non-invasive—which allow for the genotyping of *Pinna nobilis* individuals and the screening of microorganisms present in their tissues. In particular, in the case of the non-invasive sampling method based on the use of cotton swabs, it allows for sample collection even from moribund individuals without in any way compromising their chances of survival. For what concerns the innovation introduced by translocation and species reproduction, activities that have been completed, albeit with delays due to the difficulties previously mentioned, as highlighted in the preceding sections.

The demonstrative aspect lies in the fact that restocking activities consider diversified environments with different ecological conditions, as stated in the project form. For this reason, the project results offered a wide range of situations useful for replicating the actions. For example, from this perspective, it has been evaluated that enhancing in situ translocation could, on one hand, avoid complex authorization procedures aimed at obtaining permits, including international ones, for the collection and transportation of animals, and on the other hand, avoid long-distance transfers, which undoubtedly represent a significant stress factor for the individuals involved.

Furthermore, by adjusting the number of transfers, particularly favouring a greater number of translocations of a few individuals versus a few transfers of many individuals, it was possible to:

- Obtain useful information regarding translocation conditions, depending on seasonality, acquiring greater knowledge regarding optimal conditions for the success of such operations.
- Minimise negative effects related to stochastic events.

The information collected through these methods represented a useful source of knowledge for the transferability of the project.

In any case, each activity will be codified and included in specific protocols, which will be disseminated to the maximum extent possible during the After Life period.

7. Policy implications

The results achieved by the project will form the basis for implementing conservation measures for *P. nobilis* within the management tools of protected areas and Natura 2000 sites. The managing authorities of these areas should be able to guarantee continuous monitoring of the species populations.

Useful elements supporting species programming and management policies have emerged from the economic report, including cost-benefit evaluation, resulting from the activities planned in the Bergeggi MPA and the Tuscan Archipelago National Park.

Furthermore, based on the results achieved, during the After Life period, the project will promote better coordination and synergies between the EU environmental policy, fisheries, and regional policies, providing recommendations to be included in protocols and systematising projects dealing with similar issues (E3), demonstrating the value of linking biodiversity strategy with the blue growth pillars.

Among the obstacles to the project's implementation, Italian state authorisations are required for (i) the handling and translocation of *P. nobilis* and (ii) restocking actions. Within the project, the preparatory activities for obtaining these authorisations have been meticulously followed by the lead partner and have been obtained with considerable effort from the entire partnership. The fact that the outcome has been positive has laid the groundwork for the feasibility of these actions, not only during the project but also in the near future, providing a crucial element for post-project capitalisation and replicability.

7. Key Project-level Indicators

Indicator Values - 1.5. Project area/length

The calculation of the total general area at the end of the project is the sum of the all Natura 2000 sites (MPA Miramare - IT3340007 - 30 ha, MPA Asinara - ITB010082 - 17.192 ha, Park Strunjan - SI5000031 + SI3000249 - 242 ha, MPA Capo Mortola - IT1316175 - 339 ha) where project partners have realised concrete actions (C1, C2, C3, C4, C5, C6) and the replication site in Bergeggi AMP where concrete replication actions have been carried out (MPA Bergeggi - IT1323271 + IT1323202 - 390 ha).

In addition, CIS areas from Arcipelago Toscano (putative replication site of the project) where extensive monitoring activities have been completed during the project, have also been considered: SCIs of Pianosa (IT5160013 - 4500 ha), Gorgona (IT5160002 - 14608 ha), Montecristo (IT5160014 - 14441 ha), Capraia (IT5160006 - 16867 ha) and Giannutri (IT51A0024 - 10791 ha).

Indicator Values - 1.6. Humans (to be) influenced by the project

In the first version of the report, in the section “Indicator Values - 1.6. Humans (to be) influenced by the project” a single descriptor was envisaged to indicate both the number of people concretely involved in conservation activities by the project and the number of people more generally aware of the issue of the *Pinna nobilis* and LIFE Pinna.

So in that section we added the descriptor “Persons with improved capacity or knowledge due to project actions,” in which we indicated the number of people (researchers, ordinary citizens) who were involved in theoretical or practical activities for the conservation and restocking of *Pinna nobilis*.

In the second descriptor of the section, “Persons who may have been influenced via dissemination or awareness raising project actions (reaching),” we provided a conservative estimate of the people contacted by traditional media (TV, radio, online and paper newspapers, various websites) and by LIFE Pinna social networks – then a global estimate of users exposed to LIFE Pinna content. Traditional media estimates were calculated by applying coefficients between 0.1 and 1 to the audience data of individual TV programs/publications etc.

In particular:

1) Coefficient = 1

The total audience was considered just to those programs/publications that are highly targeted (Rai 3 GEO program, for example)

2) Coefficient = 0.1-0.5

For other more generalist media, such as general news services or newspapers (TG5 on Canale 5, for example), only a fraction (0.1 to 0.5) of the total users/public was considered, since in most cases the media dedicated limited space to the LIFE Pinna topic. This could be, for example, a 2-3 minute segment on a TV news program or an article in the environment section. See details in E1 annex 8_PRESS REVIEW.pdf

Indicator Values - 7.4. Wildlife species

- Species Area -

The project aims to conserve the very rare mollusc *Pinna nobilis* in the western Mediterranean and the Adriatic Sea. The area is calculated as indicated under 1.5 KPI: the total general area at the end of the project is the sum of the all Natura 2000 sites (MPA Miramare, MPA Asinara, Park Strunjan and Capo Mortola); the additional area is the replicated area of Bergeggi where transplantation activities have been carried out before the end of the project. In addition, CIS areas from Arcipelago Toscano (putative replication site of the project) where extensive monitoring activities have been completed during the project, have also been considered: SCIs of Pianosa (IT5160013 - 4500 ha), Gorgona (IT5160002 - 14608 ha), Montecristo (IT5160014 - 14441 ha), Capraia (IT5160006 - 16867 ha) and Giannutri (IT51A0024 - 10791 ha).

- Population count-

The populations of *Pinna nobilis* are near to extinction.

All the foreseen activities were highly challenging and with unpredictable results. All researchers' predictions regarding the spread of mortality and the possibility of maintaining hotspots, or ‘sanctuaries’

for survival, have been disproved in recent years. Due to the spread of the epidemic, the project researchers, in agreement with the scientific committee and after consulting many colleagues in the Mediterranean, decided to limit the areas in which to work, also restricting the removal of excessive numbers of individuals for reproduction purposes. This necessarily reduced both the areas and the numbers of individuals transplanted. Natural recruitment has also undergone a significant reduction in recent years, rendering the increased collection effort with larval collectors futile and further reducing the number of possible transplants. The strategy of searching for new potential donor areas has also failed to provide sufficient numbers to support transplantation actions. For example, the area of Sant'Antioco, which reported a large number of individuals at the start of the project over a two-year period, has seen its population more than decimated, forcing a reassessment of its possible use as a donor site. Despite this, the project's efforts have enabled the transplantation of 94 individuals in three different protected areas. All individuals survived for several months after transplantation but were subsequently found dead during monitoring in the following months. This highlights, if it were necessary, that mass mortality is still ongoing, albeit at a slower rate.

14.2.2. Operating expenses expected in case of continuation/replication/transfer after the project period – There had been an error in the previous method of compiling the indicator. The amounts considered are the costs foreseen in the different actions (C1, C3, C4, and D2) during the project implementation period. The values currently entered correspond to the after-life plan forecasts.

8. Comments on the financial report

The financial statements (available in the [LIFE reporting](#) section on the LIFE website) must be used and completed to prepare the financial report as explained in the [How to report on your LIFE project](#) guidance document. You may also consult the [Q&A on financial statements](#). This part of the technical report must include the following points:

- an overview of the costs incurred
- information about the accounting system and relevant issues from the partnership agreements
- allocation of costs per action

This information should include sufficient detail to establish a clear link between the technical activities on the one hand and costs declared in the financial forms on the other. Please note that – as set out in the General Conditions on the eligibility of costs – only costs that are necessary for and clearly linked to the activities carried out, are eligible. This section should justify and explain extraordinary cases, e.g. necessary costs not foreseen in the budget, persons changing status during the project from external consultants to employed staff (or vice versa), etc.

8.1. Summary of Costs Incurred

Complete the following table to show the project costs incurred compared to the approved budget and comment on each of the cost categories focussing particularly on discrepancies compared to the allowed flexibility of the 20% limit (cf. Article II.22 of the General Conditions).

PROJECT COSTS INCURRED			
Cost category	Budget according to the grant agreement in €*	Costs incurred within the reporting period in €	%**
1. Personnel	1.732.409	1.610.081,43	93%
2. Travel and subsistence	139.840	70.754,09	51%
3. External assistance	486.400	418.422,87	86%
4. Durables goods: total <u>non-depreciated</u> cost			
- <i>Infrastructure sub-tot.</i>	-	-	-
- <i>Equipment sub-tot.</i>	178.060	171.013,23	96%
- <i>Prototype sub-tot.</i>			
5. Consumables	50.750	50.592,41	100%
6. Other costs	184.400	161.348,62	87%
7. Overheads	194.026	173.753	90%
TOTAL	2.965.885	2.655.965,65	90%

*) If the Agency has officially approved a budget modification through an amendment, indicate the breakdown of the revised budget. Otherwise this should be the budget in the original grant agreement.
 **) Calculate the percentages by budget lines: e.g. the % of the budgeted personnel costs that were actually incurred

The expenditure level reached by 30/09/2025 for the whole project is EUR 2.655.965,65, 90% of the total foreseen budget per GA, with an expenditure of 189% of the first and second EU pre-financing instalments received, amounting to EUR 1.402.960. The reported expenditures include costs of:

- Personnel – the expenditure level reached by the consortium is 93% of the budgeted costs, amounting to EUR 1.610.081,43. Lower reported costs are mainly to be attributed to the reallocation of budget of the AB ASINARA from Personnel (Additional staff) to External assistance (temporary work agency for the contracting of external support) costs as detailed in the sub-section of the AB
- Travel costs were reduced due to a budget reallocation involving the AB NIB (reallocation of budget from ‘Travel and subsistence’ to ‘Other direct cost’, as detailed in the sub-section of the AB NIB) as well as due to an overall underspending of the partnership, leading to an expenditure level of 51% of the budgeted costs for the category by September 2025
- Equipment – a total of EUR 171.013,23 (96% of the budgeted costs) has been reported by the Consortium by 30/09/2025 in line with the foreseen costs for the category
- External assistance – the expenditure level reached for the cost category is 86% for a total amount of EUR 418.422,87. Lower costs were reported mainly due to procurement savings (entrustment of services to the best economic offer) and due to unassigned/partially assigned services (e.g., ASINARA – partially assigned service for the transport of juveniles to Porto Torres, support in the installation of *Pinna nobilis* specimens, sentinel monitoring campaigns; SHORELINE – consultancy for LIFE and After LIFE project management; UNIGE – reallocation to ‘Other direct costs’ of the contracts for the controlled reproduction facility and running)
- Consumables - the expenditure level reached for the cost category is 100% for a total amount of EUR 50.592,41
- Other direct costs – EUR 161.348,62 (87% of the budgeted costs) have been reported by the Consortium for the budget line. Savings in the cost category are related to both procurement procedures (entrustment to best economic offers) and unassigned services that proved not to be necessary or could not be carried out during the implementation of the project (e.g., collaboration of the AB NIB with Pula Aquarium, translation costs of the AB TRITON, etc.). Further details are provided in the individual sections of the Beneficiaries

The cost flexibility and compliance with art. II.22 of the General Conditions were constantly monitored at both partner and project level to ensure the respect of the 20% variance limit for budget deviations. Variance – as of 30/09/2025 – stands at 0%, well below the limit threshold identified by the programme.

Respect of the 2%-rule for public entities

Respect of the 2%-rule for public entities was monitored throughout the entirety of the project by the involved Beneficiaries – as requested in Issue 20, CINEA letter LIFE20 NAT IT 001122 MISMC4LET 250214 following the project visit held on 24th January 2025. By 30/09/2025 compliance with the 2%-rule is ensured as evidenced in the table below:

	A) reported by 30/09/2025	B) own co- financing	C) non- additional staff	D) 2%-rule compliance (B/C)
ARPAL	564.122,62	265.701,75	260.478,76	
ASINARA	122.944,52	49.177,81	17.407,48	
NIB	410.640,18	168.773,11	166.751,62	
UNIGE	491.131,32	192.409,59	197.283,80	
UNISS	450.678,66	184.012,10	183.231,69	
<i>total</i>		860.074,35	825.153,35	104%

8.1.1 Regional agency for the protection of the Ligurian environment

The CB ARPAL reported a total of EUR 564.122,62 on the project by September 2025, including costs for the budget categories Personnel, Travel, External Assistance, Equipment, Other Direct Costs and Overheads.

Personnel

The costs actually incurred by September 2025 amount to EUR 260.478,76 whereas the total personnel budget declared in the Grant Agreement amounts to EUR 257.594.

During the first part of the project period, 8 professional figures were accounted for on the project:

- A Professional Manager, involved in the administrative management of the project and in particular in the monitoring and implementation of actions A1, A3, C1, C2, C3, C4, C5, C6, D1, D3, E1, E2, E3, F1 – Daniela Caracciolo (from October 2021)
- Director for the sector Natural Risks, involved in the monitoring and implementation of actions A1, A3, C1, D3, E1, F1 – Fabrizia Colonna (from January 2022 to December 2022)
- A Professional Manager, technical and monitoring responsible for the project, involved in the monitoring and implementation of actions A1, A2, A3, C1, C2, C3, C4, C6, D1, D3, E1, F1 – Sonia Albanese (from January 2022)
- A financial responsible and communication officer involved in the implementation of actions A3, C1, C5, D3, E1, E2, E3, F1 – Maria Grazia Pizzoni (from October 2021)
- An administrative officer involved in the implementation of actions A3, C1, C5, E1, E3, F1 – Camilla Cambiaggio (from October 2021)
- A technical officer – marine strategy monitoring expert, involved in the monitoring and implementation of actions A1, A2, A3, C1, D3 – Alessandro Dagnino (from January 2022)
- A technical officer – data analyst, involved in the implementation of actions A1, A2, A3, C1 – Claudia Riccio (from January 2022)
- A technical officer – ROV monitoring expert, involved in the implementation of actions A1, A3, C1, D3, E3 – Federico Gaino from January 2022)

The total annual personnel costs of the employees are calculated by adding the following items:

- Annual gross salary, which includes the ordinary remuneration of the employee (with the thirteenth remuneration) and variable complements (bonuses paid in accordance with customary internal rules of the beneficiary)
- Annual obligatory social charges & other eligible statutory costs, which include the social security charges of the entities Inpdap ex Cpdel (23,80%), Inpdap ex INADEL (2,88%) and INAIL (0,50%).

Through Issue 21 (raised in CINEA letter LIFE20 NAT IT 001122 MISMC4LET 250214 following the project visit held on 24th January 2025), clarifications were asked on the eligibility of bonuses included in the calculation of the annual personnel costs of Albanese and Colonna. It is confirmed that the bonuses are part of the usual remuneration practices of the Beneficiary and are not triggered by the participation in the LIFE project, in line with the CB's internal regulation and National Collective Labor Agreement (CCNL). As for the productivity of the sector and management performance, Decree 159 of 10/09/2020 "Regolamento Sistema di misurazione e valutazione della performance di Arpal. Approvazione della revisione n.2" is provided together with the regulation of

performance and the contractual reference of the funds. On the National Collective Labor Agreement, the reference on bonus and performance funds are:

- CCNL 2019-2021 Sector, Section 103, Bonus Fund and Working Conditions
- CCNL 2019-2021 Local Functions, PTA Managers, Section 23, Differentiation and Variability of Performance-Related Pay
- CCNL 2019-2021 Healthcare Managers, Section 74, Performance-Related Pay Fund (*supporting docs\Issue 21\ARPAL_Personnel regulation*)

The annual costs inserted in the Financial Statement of the Individual Beneficiary are real and not estimated, even those referring to 2021, first incomplete year. The total annual costs for 2025 have been filled in using figures of the previous complete year (2024) proportional to the annual share included in the present report (annual costs 2025 = annual costs 2024/12*9). The annual cost of Cambiaggio in 2024 and 2025 has been included only in the share of five months/year to exclude the period spent on maternity leave during both project years.

The number of hours reported refers to the productive hours worked and can be deduced from the timesheets. The employee Colonna Fabrizia does not fill in the timesheets in accordance with the exception case per Annex X (Annex X to the Model LIFE Grant Agreement Financial and Administrative Guidelines – II.1.2 EXCEPTIONS): *b) Timesheets [...] are also not required for staff working on average less than 2 days per month in a given calendar year.* The yearly record of hours worked on the project is represented by the filling in and submission of the Financial Statement of the CB ARPAL. Given the exception to the maintenance of timesheets for Colonna, the standard figure- 1720 - is used to indicate the total productive hours worked in the year.

The daily rates reported in the Financial Statement for all the personnel of the CB exceed by more than 20% the daily rates foreseen in the application form. As the deviation is high and consistent for all the figures reported on the project, it is to be traced back to a clerical error in the proposal phase, when the gross annual salaries were considered instead of the total annual costs (annual obligatory social charges and other eligible statutory costs were not considered in the calculation).

Following the submission of the Mid-term Report, an additional issue has been raised (CINEA letter LIFE20 NAT IT 001122 MTR1LET 240315 KK SZ – Issue 10) on the excessively high daily rate and on the role of Fabrizia Colonna. As specified in reply to Issue 10, until the date of retirement, Colonna was the Director of the Saturn Environmental Status and Protection from Natural Risks Genoa Department and Head of the Water and Quality Status Operating Unit to which the ARPAL Sea Center belongs; Colonna was reported on the project only in 2022, to ensure the sound start of the project, and only for a total of 160 hours (20 working days).

Other deviations in the cost category stem from the breakdown of costs per action:

- Higher costs reported for project actions A1 (EUR 17.172,58 against a foreseen budget of EUR 13.310), A2 (EUR 4.284,43 against a foreseen budget of EUR 2.660), A3 (36.390,82 against a foreseen budget of EUR 33.418), D3 (37.498,47 against a foreseen budget of EUR 35.880), E2 (EUR 7.205,44 against a foreseen budget of EUR 4.740), F1 (EUR 88.189,58 against a foreseen budget of EUR 73.990), due to the higher daily rates of the personnel involved in their implementation, with a similar commitment in terms of person-days. The deviation is compensated through savings in the cost category ‘Travel and subsistence’.

Travel and subsistence

The CB reported EUR 6.981,22 for the budget line ‘Travel’ by September 2025, whereas the total budget foreseen amounts to EUR 11.600. The reported costs include:

- D3 – travels to Bergeggi and Giannutri for the monitoring activity of the MPAs - EUR 423
- E3 - travels to Miramare, Valencia and Bogliasco to participate in networking events and festivals – EUR 2.885,40. Higher costs are reported for action E3, due to the unforeseen participation in the LIFE Pinnarca event held in Valencia; though not envisaged in the proposal phase, the participation of the CB was paramount to exchange knowledge and lessons learned on the conservation of *Pinna nobilis*
- F1 - travels to Sassari, Porto Torres and Asinara, Rome, Pirano and Genoa to participate in the annual project coordination and monitoring meetings, including final project event (seq 1 – seq 3, seq 8 – seq 10, seq 15 – seq 20) – EUR 3.672,82

The CB shared its internal regulation for the reimbursement of travels and subsistence costs and reports its costs under the specific budget line in accordance with it.

External assistance

The reported costs by the CB for the category by September 2025 amount to EUR 100.881,36 and include the entrustment of the following services:

- Technical support for the best practices survey (drafting of the questionnaires and reporting) for the concrete actions aimed at the repopulation of *P. nobilis* (action A.3) by the supplier Istituto Delta Ecologia Applicata srl – EUR 16.050
- Technical support for the assessment of the replicability and transferability activity with the MPAs (action E.3) by the supplier Istituto Delta Ecologia Applicata srl – EUR 26.750
- Support in the coordination and project management for the monitoring of the project progress, the financial management and the consortium coordination (action F.1) – by the supplier Istituto Delta Ecologia Applicata srl – EUR 10.699. The supplier was selected through public tendering (open procedure), activated on 01/12/2021 (General Director Decree n.193), with the collection of eight offers by the established deadline, the approval of the report on the procedure on 01/03/2022 and the Entrustment Decision on 28/03/2022 (General Director Decree n.70)
- Technical support for the identification and mapping of the pilot sites (action C.1) by the supplier Ubica srl - EUR 9.394
- Technical support for the monitoring campaign and reporting activities (action D.3) by the supplier Ubica srl – EUR 30.988. The supplier was selected through public tendering (open procedure) activated on 18/03/2022 (Det. n.123) via PA electronic market, the collection of three offers, the drafting of the Commission Report on 09/05/2022 and the Entrustment Decision (Det. 221) on 24/05/2022
- ROV training course (action D.3) – EUR 5.730 by the supplier Eurosportos srl

The CB ARPAL abides by the rules of the Italian national legislation for the public procurement of external services and goods; in compliance with the programme provisions, the respect of the principles of best value for money and avoidance of conflict of interest is ensured by requesting a minimum of three economic offers also for purchases/contracts below the national threshold for direct awarding. Evidence of the request and of the collected offers is maintained and provided by the CB to ensure the transparency of the selection process.

Equipment

The CB ARPAL reported EUR 92.013,32 for the cost category whereas the total foreseen budget amounts to EUR 80.000. The reported costs include:

- Action D3 - purchase of the submarine remotely operated vehicle from the supplier Eurosportos srl, reported at 100% on the project. The unitary cost of the ROV at the time of the purchase was significantly higher than the foreseen cost at the time of the submission of the project proposal (estimated cost: EUR 59.000) due to a general increase of the costs of raw materials. As specified in reply to Financial Issue 16, CINEA Letter LIFE20 NAT IT 001122 MTR1LET 240315 KK SZ, following the submission of the Mid-term Report, during the purchase phase, a choice was made to opt for a professional-level ROV, characterized by low weight, high autonomy, battery power supply, high quality of videos and images and structural robustness – essential to ensure reliability in field activities. A crucial aspect of the project is the need to monitor specific areas of the seabed and populations of *Pinna nobilis*, requiring the return to predefined sites at regular intervals to evaluate the effectiveness of the actions undertaken. For this reason, a system that allows precise georeferencing of the images and videos acquired was essential. For this purpose, a USBL (Ultra-Short Baseline) acoustic positioning system was integrated, associated with a high-precision differential satellite receiver, already supplied by ARPAL. The USBL system allows the position of the ROV to be determined with centimetre precision, allowing georeferenced images to be obtained and the instrument's travel tracks to be recorded. This guarantees the possibility of finding specific points on the seabed with absolute certainty avoiding errors in the identification of organisms or habitats, especially in environments such as Posidonia meadows or sandy expanses, where visual references are scarce. The implementation of the USBL system entailed an additional cost but was considered a necessary expenditure to guarantee the success of long-term monitoring operations, as well as the repeatability and reliability of the results obtained. The combined use of the ROV and

the USBL system significantly reduces, if not eliminates, the need for verification operations by divers, this brings significant benefits in terms of operational safety, as well as simplifying the logistical organization. Furthermore, considering the costs associated with the rental and movement of vessels necessary for underwater operations with scientific personnel, the additional investment for the USBL system has proven to be cost-effective, allowing to amortize and potentially cover the entire extra cost over the duration of the project. The higher cost of the purchased ROV is also compensated through lower costs of the computer equipment purchased and through a reallocation of budget from the BL 'External assistance' (savings of the entrusted services to Istituto Delta Ecologia Applicata srl and Ubica srl). Financial Issue 24 – raised in CINEA letter LIFE20 NAT IT 001122 MISMC4LET 250214 following Project Visit of 24 January 2025, required clarification on the open selection procedure for the provision of the ROV, involving the participation of five different suppliers. It is confirmed that five suppliers were invited (EURSPORTOS SRL, IROV-UNDERWATER SERVICES DI STASI MAURO & C.S.-A.S., MACARTNEYITALY SRL, RATES SRL, THE SEA OPPORTUNITIES) and that only one supplier – EUROSPORTOS SRL – participated in the tender process by submitting its offer; the other four suppliers invited to participate in the procedure did not submit their offers within the established terms. The outcome of the selection process, including clarifications on the offer(s) received, is specified in Entrustment Determination n. 141 on 17/03/2023, provided with the previous procurement documentation and hereby attached. The conformity report of the RUP (Sole Responsible for the selection Procedure) attesting the reception of a single offer is also provided together with the signed contract (*Supporting documents:\supporting docs\Issue 24*)

- Action D3 - purchase of computer equipment – 5 laptops Lenovo ThinkBookL14 Gen6 from the supplier Converge srl, reported at 100% on the project.

The purchased durable goods are labelled (project and programme logos, Natura2000 logo and reference to the EU co-funded purchase) in compliance with the programme provisions and visibility rules. The certificate on the use of Equipment has been extracted from the Financial Statement of the CB and signed by the Legal Representative of ARPAL (General Director – Carlo Pepe) and is attached to the present Report (*Payment request > Certificates_NATURE_projects > 'Certificate_NATURE_projects_ARPAL'*).

Other direct costs

The CB ARPAL reported EUR 66.862,96 for the cost category whereas the total foreseen budget amounts to EUR 67.500. The reported costs include:

- Action D.3 – involvement of International Marine Center Foundation - Fondazione IMC (foreseen in the GA) in citizen science monitoring of *Pinna nobilis* – EUR 15.000
- Action E.1 – service for the organization of the opening conference (buffet, room rental, equipment) by GENOVA CULTURA SC (foreseen in the GA) – EUR 4.354,85
- Action E.1 – translation service for the opening conference by Maura Capelli (foreseen in the GA) – EUR 802
- Action E.1 – catering service organization for the final event (foreseen in the GA) by R&B SNC DI R. BARBIERI E B. BALDUCCI – EUR 285
- Action E.1 – expenditure reimbursement for the participation of the speaker Leonardo Tunesi in the final event – EUR 150,24
- Action E.1 – interpreting service for the final event by Multilingual pro (foreseen in the GA) – EUR 5.490
- Action E.1 Agreement on the replicability and transferability of results to other MPAs for the monitoring and repopulation of *Pinna nobilis* (foreseen in the GA) by Parco Nazionale dell'Arcipelago Toscano and Comune di Bergeggi – EUR 40.000
- Action E.3 – reimbursement for the participation in networking event in Valencia organized by LIFE Pinnarca, to Daniele Grech and reimbursement to Valentina Giussani for the participation in the Posidonia Green Festival (unforeseen but deemed necessary to exchange knowledge and lessons learned on the conservation of *Pinna nobilis*) – 780,87 EUR

The unforeseen costs for the participation in the networking events and conferences, and the higher costs sustained for the organization of the final event, are compensated through savings in the same

budget category from the unassigned services of sentinel shipping and Layman's report printing (carried out by the AB TRITON).

8.1.2 Parco Nazionale dell'Asinara

The AB ASINARA reported a total cost of EUR 122.944,52 on the project by September 2025, including costs for the budget categories Personnel, Travel, External Assistance, Other Direct Costs and Overheads.

Personnel

The costs actually incurred by September 2025 amount to EUR 17.407,48 whereas the total personnel budget declared in the Grant Agreement amounts to EUR 135.165.

During the project implementation, 4 professional figures were accounted for on the project:

- Project Manager and Coordinator, involved in the monitoring and implementation of actions C1, C6, D2, E1, F1 – Vittorio Gazale (from January 2022)
- a Financial and Administrative Coordinator, involved in the monitoring and implementation of action F1 – Gian Luca Idini (from January 2022)
- a Technical Coordinator, involved in the implementation of actions A1, C1, C6, D1, D3, E1 – Aldo Angelo Zanello (from January 2022)
- a Technical Assistant, involved in the implementation of actions A2, C1, C6, D1, D3 – Gavino Moro (January 2022)

The total annual personnel costs of the employees were calculated by adding the following items:

- Annual gross salary, which includes the ordinary remuneration of the employee (with the thirteenth remuneration)
- Annual obligatory social charges & other eligible statutory costs, which include the social security charges of the entities CPDEL, INAIL and severance indemnity

The annual costs inserted in the Financial Statement of the Individual Beneficiary are real and not estimated for all project years starting 2022, with the exception of the total annual costs for 2025 – partial year – that have been filled in using the figures of the previous complete year 2024 proportionally to the share of year reported on the project (annual costs 2025 = annual costs 2024/12*9). The annual costs of all the staff reported on the project did not vary between 2023 and 2024, therefore the same total annual costs (gross salary and obligatory social charges & other eligible statutory costs) are reported for the indicated project years.

The number of hours reported refers to the productive hours worked and can be deduced from the timesheets, filled in by all the reported personnel, as well as from the internal attendance detection system.

The daily rates reported in the Financial Statement are generally in line with the daily rates foreseen in the application form and don't exceed the 20% threshold of deviation with the sole exception of Zanello Aldo Angelo in 2022 (higher by 25%) due to a lower total number of productive hours worked on the year (mainly due to sick leave).

The AB ASINARA through a communication (ARPAL's protocol I.0004702.21-02-2022. h.09:46) to the CB, expressed the need for a budget reallocation (from Personnel costs to 'External assistance') entailing the transfer of part of the resources destined to cover for the foreseen Additional personnel (EUR 60.000) to the budget line 'External assistance' for the contracting of a temporary working agency, due to the legal restrictions to which the Park was subjected in hiring temporary personnel. The proposed adjustment, that ensured the continuity and actual implementation of the project activities as foreseen from a technical standpoint, has been assessed by the CB with respect to the financial/administrative compliance to the General Conditions (Art.II.22). The proposed reallocation complies with the 20% flexibility rule among budget categories assessed at project level, ensures the maintenance of the External assistance costs below the 30% threshold of the overall project action cost and does not affect the respect of the 2% rule. During the lifespan of the project the legal framework for the AB ASINARA did not change, and the AB – through communication (n. 5726 25.02.2025) to the CB ARPAL – anticipated the full reporting of the additional figure in external assistance through the contracting of a temporary working agency. The same checks (compliance with the General Conditions – Art.II.22, external assistance maximum share and 2% rule) have been repeated on the new foreseen budget adjustment to ensure its compliance.

Deviations in the breakdown of costs per action regard the higher personnel costs reported for action D2 (EUR 1.685,47 against a foreseen GA budget of EUR 455) due to the higher number of hours reported by the Project Manager and Coordinator (Gazale) following the increased monitoring effort of the AB ASINARA in 2024-2025.

Travel and subsistence

The AB reported EUR 2.822,90 for the budget line 'Travel' by September 2025, whereas the total budget foreseen amounts to EUR 9.500. The reported costs include:

- Action E1 - travel from Porto Torres to Genoa by Vittorio Gazale to participate in the first public project event – EUR 437,31
- Action F1 – travel from Porto Torres to Genoa and Piran by Vittorio Gazale, Gianluca Idini, Gavino Moro to participate in the annual monitoring visits and final project event – EUR 2.385,59

The AB shared its internal regulation for the reimbursement of travels and subsistence costs and reports its costs under the specific budget line in accordance with it.

External assistance

The AB reported a total of EUR 92.397,14 for the budget line 'External assistance' whereas the total foreseen budget for the cost category amounts to EUR 74.000. The reported costs include:

- contracting of two temporary work agencies (SYNERGIE ITALIA AGENZIA PER IL LAVORO SPA and OPENJOBMETIS S.p.A.) for the provision of a supporting figure to the AB's personnel in the implementation of the project actions. The AB kept track of the breakdown of costs per action based on the actual involvement of the supporting figure; the breakdown of costs per action is reported as follows: action C6 – EUR 40.352,80, action D1 – EUR 37.544,34, action D3 – EUR 4.500, action E1 – EUR 3.000, action E2 – EUR 1.500, action F1 – EUR 5.500. The sub-contractor SYNERGIE ITALIA AGENZIA PER IL LAVORO SPA was selected through direct award with Determination n. 277 on 28/06/2022. The procurement procedure was carried out via electronic market for PAs (SardegnaCAT) through the publication of an offer request on 08/06/2022 aimed at seven economic operators, the collection of five economic offers by the deadline on 17/06/2022 and the selection of the lowest economic offer under the same conditions and requisites met. The sub-contractor OPENJOBMETIS S.p.A. was selected through direct award with Determination n. 367 on 23/07/2024 following the publication on SardegnaCAT of the request for offer on 27/06/2024 aimed at three economic operators, the reception by the deadline on 12/07/2024 of two offers, and the selection of the lowest economic offer under the same conditions and requisites met
- Action D1 – sub-contracting for the service of technical and operational support to the sentinel monitoring campaigns (transportation, installation and monitoring of young specimens of *Pinna nobilis*) by MARC SUB SERVICES SRL – EUR 23.180. The contract with MARC SUB SERVICES SRL was terminated ahead of completion of the service due to the occurrence of technical constraints in proceeding with what was initially planned. Through official communication on 06/03/2025 the CB ARPAL attested the state of the art of the project, in response to ASINARA's request (prot. n.5726 25/02/2025): following the project meetings held on 17/10/2024, 08/01/2025 and 15/01/2025 the partnership unanimously decided not to proceed with additional translocations of *Pinna nobilis*, including the ones planned within the territory of the National Park, considering the severe state of sufferance of the residual populations of the species. Further collections of specimens from populations in critical conditions are deemed unsustainable, both for ethical reasons and for they would not ensure high percentages of success of the operation. In light of this decision, official communication has been given to the sub-contractor and the contract terminated on 03/04/2025 (prot.1517/2025 – via certified email)

Deviations for the cost category stem from the overall higher costs reported due to the reallocation of budget from 'Personnel' (ADDITIONAL) to 'External assistance' for the contracting of external staff via temporary work agencies. The deviation is more than compensated through savings in the cost category 'Personnel' and in the partially assigned services for the transport and installation of *Pinna nobilis* specimens.

The AB ASINARA abides by the rules of the Italian national legislation for the public procurement of external services and goods; in compliance with the programme provisions, the respect of the principles of best value for money and avoidance of conflict of interest is ensured by requesting a minimum of three economic offers, whenever possible, also for purchases/contracts below the national threshold for direct awarding. Evidence of the request and of the collected offers is maintained and provided by the AB to ensure the transparency of the selection process.

Other direct costs

A total of EUR 2.274 was reported by the AB for the budget line 'Other direct costs', whereas the total foreseen budget amounts to EUR 6.000. The reported costs, in line with the foreseen activities, include:

- Action E1 - catering service for the project meeting by the supplier Ditta Maria Piano – EUR 120
- Action E1 – organization (catering service) for the awareness raising meeting with decision-makers by the suppliers Ditta Emanuela Schiaffino and Ditta Massimo Dimeglio – EUR 2.154

8.1.3 National Institute of Biology

The AB NIB reported a total of EUR 410.640,18 on the project by September 2025, including costs for the budget categories Personnel, Travel and subsistence, Equipment, Consumables, Other Direct Costs and Overheads.

Personnel

The costs actually incurred by September 2025 amount to EUR 316.759,81 whereas the total personnel budget declared in the Grant Agreement amounts to EUR 299.751.

During the project, 11 professional figures were accounted for on the project:

- A researcher – marine strategy monitoring expert and data analyst, involved in the monitoring and implementation of actions C1, C3, D2, D3, E1, F1 – Marina Orlando Bonaca (from January 2022)
- A researcher – Project Responsible and coordinator of the communication activities, involved in the implementation of actions A3, C3, D2, D3, E1, E3, F1 - Valentina Pitacco (reported on the project from September 2022)
- A researcher – monitoring responsible, involved in the monitoring and implementation of actions C3, D2, D3, E1, F1 - Lovrenc Lipej (from January 2022)
- A researcher – involved in field and lab work and in the implementation of actions A1, C3, C6, D2, D3, F1 - Domen Trkov (additional staff, from January 2022)
- A researcher – involved in field and lab work and in the implementation of actions C3, D2, F1 – Simone Spinelli (additional staff, working on the project from May 2024)
- A technician involved in field work and lab, and in the implementation of actions A1, C3, D1, D2, F1 - Leon Lojze Zamuda (additional staff, from 16/03/2022). As specified in the reply to Issue 12 (CINEA letter LIFE20 NAT IT 001122 MTR1LET 240315 KK SZ following the submission of the Mid-term Report) the first contract of Zamuda, signed on 27/11/2020, started on 01/01/2021 as a fixed-term full-time contract until 15/03/2022. As per first temporary contract, until 15/03/2022 Leon Lojze Zamuda could not be reported on the project as 'Additional' personnel, considering the non-compliance with the first condition set out in Annex X (starting date of the contract before the project start date). Following the contract renewal, with the new contract starting on 16/03/2022, Zamuda falls under the indicated cases for employees to be reported as 'Additional' personnel. In the Financial Statement of the AB two separate lines are inserted for Zamuda in 2022 to signal the change of contract and type
- A technician involved in field work and lab, and in the implementation of actions A1, C3, D1, D2, F1 - Ana Fortič (additional staff, from January 2022 to December 2024)
- A technician – data analyst involved in the monitoring and implementation of actions C3, D3, F1 - Milijan Šiško (from January 2022)
- A researcher – involved in the planning of *P.nobilis* monitoring and in particular in the implementation of actions C1, C3, D3, E1, E3, F1 - Borut Mavrič (from January 2022)
- A field work technician and diver, involved in the monitoring and implementation of actions C3, C6, E1, F1 - Tihomir Makovec (from January 2022)

- An administrative officer, coordinating and monitoring the progress of the financial reporting, participating in action F1 - Anja Sergaš (additional staff, from January 2025)

The total annual personnel costs of the employees were calculated by adding the following items:

- Annual gross salary, which includes the ordinary remuneration of the employee
- Annual obligatory social charges & other eligible statutory costs, which include the severance indemnity and the pension fund

The annual costs inserted in the Financial Statement of the Individual Beneficiary are real and not estimated for all the reported project years, including partial year 2025.

The total number of hours reported refers to the productive hours worked and can be deduced from the timesheets, filled in by all the personnel of the AB.

The daily rates reported in the Financial Statement are generally in line with the daily rates foreseen in the Grant Agreement and do not exceed the 20% threshold of deviation, with the exception of:

- Martina Orlando Bonaca in 2023 (higher by 40%) to be traced back to a promotion starting 01/07/2023
- Ana Fortič in 2023 (higher by 40%) to be traced back to a promotion starting 01/07/2023
- Borut Mavrič in 2023 (higher by 27%) due to a salary increase for the new position held (Head of Department) starting 01/05/2023

Other deviations detected in the breakdown of costs per action are:

- Action A1 – significantly higher personnel costs reported (EUR 20.831,41 against a foreseen budget of EUR 7.976) stemming from the increased monitoring effort that emerged during the implementation of the action and could not have been foreseen in the proposal phase. The prolonged effort is reflected in the one-year delay of the action as detailed in the technical description of chapter 6;
- Action C3 – higher personnel costs reported (EUR 204.911,10 against a foreseen budget of EUR 168.910) due to the need for increase of the capture and monitoring effort aimed at identifying as many individuals as possible – placing special attention to those at high risk of being damaged therefore in need of protection through translocation - to respond to the reduction of *P.nobilis* populations
- Action D3 – higher personnel costs reported (EUR 16.374,61 against a foreseen budget of EUR 9.579) originated by an unforeseen higher need of person-days effort in the monitoring activity, due to the huge number of empty shells and very few living individuals found
- Action E1 – higher personnel costs reported (EUR 21.949,38 against a foreseen budget of EUR 5.511) due to a clerical error in the proposal phase when insufficient budget was envisaged for communication and networking activities considering the central technical role of the AB

Deviations in the breakdown of costs per action are compensated through savings in the same budget category (e.g., action A3, C1, C6, D1, D2, E3) as well as from lower costs reported for ‘Travel and subsistence’.

Travel and subsistence

The AB reported EUR 9.097,77 for the budget line ‘Travel and subsistence’ by September 2025, whereas the total budget foreseen amounts to EUR 29.580. The reported costs include:

- Action A1 – travels from Piran to Miramare and Strunjan for the visit to the breeding facilities of *P.nobilis* and for the participation in monitoring activities EUR 43,73
- Action C3 – travels from Piran/Ljubljana to Miramare, Izola and Ljubljana, to visit the breeding facilities and transfer samples of *P.nobilis* – EUR 50,29
- Action E1 – travels from Koper and Ljubljana to Bodo (Norway) to participate and lecture at the 58th European Marine Biology Symposium (EMBS) – EUR 3.946,42
- Action E3 – participation of the project staff in communication events and networking meetings in Genoa/Trieste/Pula/Valencia/Miramare – EUR 2.531,15
- Action F1 – participation in annual project coordination meetings and monitoring visits in Genoa and Rome - EUR 2.526,18

The reported costs are generally in line with the foreseen budget and activities; deviations in the cost category include:

- Higher travel costs reported for action E1 due to the unforeseen participation in the 58th European Marine Biology Symposium. Though not foreseen, the symposium represented an

opportunity to present the results and achievements of LIFE PINNA, as well as to network with other subjects involved in the conservation effort. The higher costs are compensated through savings in the same cost category for action F1 (EUR 2.526,18 reported against a foreseen budget of EUR 11.040)

- Transfer of a share of the foreseen travel costs, connected to the use of the AB's own boat, to the cost category 'Other direct costs' as per indications received via CINEA letter LIFE 20 NAT IT 001122 MISMC1LET 220210 SZ (Issue 6) following the first monitoring visit.

The AB shared its internal regulation for the reimbursement of travels and subsistence costs and reports its costs under the specific budget line in accordance with it.

Equipment

The AB NIB reported EUR 24.891,35 for the budget line 'Equipment' by September 2025, whereas the total budget foreseen amounts to EUR 25.200. The reported costs include:

- Action C3 – purchase of 3 aquarium facilities (reported at 100% on the project) and open system function (reported at 100% on the project) by the supplier Aquarium, d.o.o. Lesce – EUR 12.991,30
- Action C3 – purchase of a camera Nikon Z8 with objectives (reported at 100% on the project) by the supplier Fotospecialisti, spletna trgovina, d.o.o. – EUR 6.979
- Action D3 – purchase of three-channel multimeter (reported at 100% on the project) by the supplier Mikro+Polo d.o.o. – EUR 4.921,05. The purchase of the multimeter probe – though unplanned in the GA – was deemed necessary to obtain thorough data about sites where *Pinna nobilis* specimens were still found alive, in order to provide more detailed information on the parameters that were hypothesized as crucial for the survival of the species. The probe is equipped with sensors that allow it to measure additional parameters (e.g., turbidity). Measurements were performed during the period in which larval collectors were at sea, with results collected on temperature, salinity, oxygen (see chapter 6 for additional information).

Other deviations in the cost category are related to the higher total costs sustained under action C3 (EUR 19.970,30 against a foreseen budget of EUR 15.600). The higher costs stem from the increase in the unitary cost of the purchased durable goods compared to the unitary cost of the same items at the time of the project proposal - based on market research – due to the general price increase post pandemic emergency. The aquarium facilities – foreseen in the GA – were still considered fundamental for the implementation of action C3 notwithstanding the cost increase, as they allow for the setting of the collected juvenile individuals of *Pinna nobilis* to spend the initial growth period before being placed in lantern-nets in the identified locations. The exceeding amount and unforeseen cost sustained for the purchase of the multimeter probe are compensated through savings in Equipment – action F1, namely computer for data and image acquisition and processing, whose purchase was not necessary for the sound implementation of the project activities.

The purchased durable goods are labelled (project and programme logos, Natura2000 logo and reference to the EU co-funded purchase) in compliance with the programme provisions and visibility rules. The certificate on the use of Equipment has been extracted from the Financial Statement of the AB and signed by the Legal Representative of NIB (Director – Maja Ravnika) and is attached to the present Report (Payment request > Certificates_NATURE_projects > 'Certificate_NATURE_projects_NIB').

The AB NIB abides by the rules of the Slovenian national legislation for the public procurement of external services and goods; in compliance with the programme provisions, the respect of the principles of best value for money and avoidance of conflict of interest is ensured by requesting a minimum of three economic offers, whenever possible, also for purchases/contracts below the national threshold for direct awarding. Evidence of the request and of the collected offers is maintained and provided by the AB to ensure the transparency of the selection process.

Consumables

The AB NIB reported EUR 5.073,65 for the budget line 'Consumables' by September 2025, whereas the total budget foreseen amounts to EUR 4.800. The reported costs include:

- Action A1 - purchase of consumable materials for the collectors by the supplier OBI d.o.o., unforeseen – EUR 7,49
- Action C3 - purchase of various materials (feed, sediment, boxes, graduated cylinders, rope, wire ties, buoys, tubes, etc.) by the suppliers Merkur trgovina d.o.o., KZ Agraria Koper,

z.o.o., Planktovie SAS, Mikro+Polo d.o.o., Acqua&Co srl, KAMM d.o.o., Omega d.o.o., Aquariumline S.R.L., Norik-sub d.o.o., ECP d.o.o., OBI Italia Srl, Sanolabor d.d., foreseen for the implementation of the action in the GA – EUR 4.813,45

- Action D3 - purchase of various materials (disk for camera monitoring, batteries, callipers, transect tracks) by the suppliers Delta MM Izola d.o.o., Mimovrste d.o.o., OBI d.o.o., necessary for the monitoring activity, unforeseen in the GA – EUR 252,71

The unforeseen costs incurred for the purchase of materials for actions A1 and D3, were erroneously not foreseen in the proposal phase, but necessary for the on-field sound implementation of the project monitoring activities. The slightly higher costs reported under ‘Consumables’ due to unforeseen purchases are compensated through savings in the cost category ‘Equipment’.

Other direct costs

The AB NIB reported EUR 27.953,60 for the budget line ‘Other direct costs’ by September 2025, whereas the total budget foreseen amounts to EUR 24.500. The reported costs include:

- Action C3 – travel costs (21 travels with the boat of the institute) sustained through the use of the own boat for field work – EUR 8.016,01. As detailed in the reply to CINEA letter LIFE 20 NAT IT 001122 MISMC1LET 220210 SZ (Issue 6), the reported costs are calculated on an annual basis by adding up the following voices (net of VAT): fuel costs, insurance costs, annual service fee, other material and maintenance, annual costs of mooring, staff costs. The annual calculation is divided by the total number of days of use for the year to obtain the daily use cost of the boat of each project year. Track of the travels implemented for the LIFE PINNA project is maintained through navigation orders filled in by the personnel of the AB. A complete package with all the supporting documents produced and archived by the AB NIB for the use and payment of the own boat has been provided to the Project Monitor ahead of the third monitoring visit on 6th October 2023. Following the additional request for adjustments to the calculation expressed in CINEA letter LIFE20 NAT IT 001122 MISMC2LET 230418 SZ (Issue 6) and subsequent clarifications, VAT has been excluded not just from the internal invoicing of the AB, but also from the payments to external providers/subcontractors, even though it represents a cost for NIB (art. 63 of National Law on VAT-1). Invoices for 2022 were invalidated to exclude VAT and newly issued in 2023 for the sustained costs related to the use of the boat from 2022 onwards, for which the issuing date and date of payment are in 2023 /reply to Issue 6 re-raised in CINEA letter LIFE20 NAT IT 001122 MTR1LET 240315 KK SZ following the submission of the Mid-term Report)
- Action D1 – sentinel shipping costs by supplier DHL Ekspres d.o.o. – EUR 132,56
- Action D3 – travel costs (18 travels with the boat of the institute) and monitoring of the presence of living specimens/sample collection for genetic analyses and labelling of living specimens by the supplier Inštitut vodni krog. The genetic analyses and monitoring activity were not initially planned but were paramount for the additional information gathering effort that emerged given the drastic change in the presence of fan mussels in donor and receiving sites (as detailed in the technical description of chapter 6) – EUR 11.864,67
- Action E1 – translation costs for official documents (PA and co-financing decision) by suppliers TOLMAČ.SI d.o.o. and Mag. tadej Ian s.p. – EUR 944,62
- Action E1 – project meeting organization (coffee break) by supplier Gastur d.o.o. – EUR 35,80
- Action E1 – unforeseen costs for the participation in the 58th European Marine Biology Symposium (EMBS) for Ana Fortič in July 2025 (reported as project staff until 2023, therefore the participation is reported under ‘Other direct costs’ instead of ‘Travel and subsistence’) including insurance costs in case of cancellation for 3 flight tickets for the participation in the congress - EUR 2.061,62
- Action E1 – unforeseen costs for the publication of an article in open access – EUR 2.467,97. Ticket submitted on 10/09/2025 TCK008348 has been resolved entailing the opportunity to make use of savings in the cost category (budget voice ‘E1 – meeting organization’) for a publication on a scientific journal: [Diversity | An Open Access Journal from MDPI](#), published on September 23, 2025. The publication focused on “Recruitment and controlled growth of juveniles of the critically endangered *Pinna nobilis* in the Northern Adriatic”,

results of project action C3. The added value of the unforeseen publication is connected to the increased reach and dissemination within the scientific community, as well as to the transferability of the methodologies implemented within the project to potentially interested subjects

Deviations in the cost category other than the ones described above include:

- Reallocation of budget from the cost category 'Travel and subsistence' to 'Other direct costs' for the use of the own boat of the Institute, accounting for EUR 14.890,68
- Savings in the organization of meetings (action E1) and in the Agreement with a third party (action C3) Pula Aquarium, that was not subscribed due to the missing conditions for the implementation of the activity as initially planned in the GA.

8.1.4 Shoreline Società Cooperativa

The AB SHORELINE reported a total cost of EUR 247.797,86 on the project by September 2025, including costs for the budget categories Personnel, Travel and subsistence, Equipment, External assistance, Consumables, Other Direct Costs and Overheads.

Personnel

The costs actually incurred by September 2025 amount to EUR 166.440,69 whereas the total personnel budget declared in the Grant Agreement amounts to EUR 178.475.

During the project implementation, 5 professional figures were accounted for on the project:

- A senior researcher, involved in the management, coordination and implementation of actions A1, A2, A3, C3, C4, C5, C6, D3, E1, E2, F1 – Ciriaco Saul (starting from October 2021)
- A junior researcher, involved in the implementation of technical activities A1, A2, A3, C2, C3, C4, C5, C6, D3, F1 – Segarich Marco (starting from October 2021)
- A junior researcher, involved in the implementation of action C3 – Batistini Edoardo (starting from April 2023)
- A junior researcher, involved in the implementation of actions C3, C4 – Toti Elena (starting from January 2025)
- An administrative reference supporting the project management for the AB (action F1) – Stradi Cristina (starting from January 2022)

The total annual personnel costs of the employees were calculated by adding the following items:

- Annual gross salary, which includes the ordinary remuneration of the employee (with the thirteenth and fourteenth remunerations)

- Annual obligatory social charges & other eligible statutory costs, which include the social security charges of the entities INPS, INAIL, the severance indemnity and integrative pension fund.

Following CINEA letter LIFE20 NAT IT 001122 MISMC4LET 250214 –project visit of 24 January 2025 – Issue 21, it is confirmed that the included bonuses of Segarich and Ciriaco in 2021 ('premio di risultato') are usual remuneration practices of the AB, not triggered by the participation in the LIFE project. As a work production cooperative, based on the trend of the previous year, profits are redistributed among shareholders. The internal regulation on the personnel of the AB SHORELINE is provided as a further verification means. The salary slips, proof of payment and calculation table for Edoardo Batistini in 2024 are provided in the supporting documents folder. Note: the annual costs reported in the Financial Statement and based on the provided cost table are calculated as:

ANNUAL GROSS SALARY = Taxable income ('Imponibile sociale') + Indirect costs accrued ('Costi indiretti maturati') - Indirect costs paid ('Costi indiretti erogati')

ANNUAL OBLIGATORY SOCIAL CHARGES & OTHER ELIGIBLE STATUTORY COSTS = Company contributions ('Contributi c/ditta')

\supporting docs\Issue 21\SHORELINE_Personnel_seq 13_Batistini

\supporting docs\Issue 21\SHORELINE_Personnel regulation

The annual costs inserted in the Financial Statement of the Individual Beneficiary are real and not estimated, even those referring to the partial years 2021 and 2025.

The number of hours reported refers to the actual total productive hours worked, also for the incomplete year 2021, and can be deduced from the timesheets, filled in by all the personnel of the AB.

The daily rates reported in the Financial Statement are generally higher than the 20% threshold of deviation for all the reported staff (Ciriaco by 46% to 59% between 2022 and 2025, Segarich by 45% to 68% between 2022 and 2025, Stradi by 24% to 32% between 2022 and 2025; Batistini by 27% to 40% between 2023 and 2025; Toti by 33% in 2025). The consistency in the detected higher rates is to be traced back to the specific situation of the AB at the time of the project proposal: starting 2012 up until December 2021, the AB declared a state of ‘corporate crisis’ that was revoked following the Resolution of 15/11/2021 of the Board of Directors, after a careful evaluation of the company financial information. The daily rates defined at the time of the project proposal (February 2021) were calculated based on the costs of the latest available year (2020), falling under the ‘state of crisis’ period that provided for the non-payment of the 13th and 14th remunerations for all the employees and for a 15% pay cut for councillors and Director, resulting in much lower daily rates. Starting 2022, the abovementioned salary components have been regularly paid to all employees, leading to higher daily rates for the reported project years.

Other deviations for the budget category stem from the breakdown of costs per action:

- Lower costs reported for A-actions overall and actions C1, C6, D1, D2, D3
- Higher costs reported for actions C3 (EUR 119.006,32), C5 (EUR 5.270,98), E1 (EUR 4.846,49), E2 (EUR 4.604,64)

The higher number of hours reported for actions C3, C5, E1, E2 were erroneously not foreseen in the proposal phase, but were necessary for the on-field sound implementation of the project activities. No deviation is in any case detected in the overall budget of the cost category.

Travel and subsistence

The AB reported EUR 18.184,70 for the budget line ‘Travel’ by September 2025, whereas the total budget foreseen amounts to EUR 26.550. The reported costs include:

- Action C3 – reimbursement of travels for the transport of *Pinna nobilis* (from Trieste to Camogli, Venice, Fano, Genoa, Sant’Antioco) – EUR 6.717,27
- Action C4 – reimbursement of travels for the transplant of *Pinna nobilis* (from Trieste to Camogli) – EUR 635,74
- Action E2 – reimbursement of travels for the participation in communication and networking events (Genoa Science Festival, CREDIMA conference in Ancona, Brioni Conference in Croatia, networking with Pinnarca in Valencia and Pola, and final event in Pirano) – EUR 6.720,89. Action E2 erroneously did not foresee a ‘Travel and subsistence’ budget for the AB SHORELINE, whose participation in the networking and communication events was deemed necessary due to its technical contribution
- Action F1 – reimbursement of travels for the participation in the annual project coordination and monitoring meetings, including the kick-off meeting (from Trieste to Genoa, Porto Torres, Rome) – EUR 4.110,80

Deviations in the breakdown of costs per action are represented by higher costs reported for actions C3, E2, F1 due to the increased need of travels in the implementation phase for the transport of *Pinna nobilis*, participation in networking events and coordination/monitoring meetings. The higher costs reported are more than compensated through savings in ‘Travel and subsistence’ for actions C4, C5, E1, E3.

Travel and subsistence costs are reported in line with the Beneficiary’s own internal regulation, provided by the AB SHORELINE.

External assistance

A total of EUR 29.216,76 has been reported by the AB SHORELINE for the cost category ‘External assistance’ including services for:

- Action A1/E1 – support in the organization and hosting of the international training activities on behalf of the SPA and in the drafting of the report on the health status of the receiving site AMP Miramare, by the sub-contractor Fondazione WWF Italia – EUR 16.745,09
- Action C3 – support in the monitoring activity and research boat trips by the sub-contractors Caiffa Andrea and Caressa Stefano (unforeseen) – EUR 7.359,91. The unforeseen costs were necessary due to the need for increase of the capture and monitoring effort aimed at identifying as many individuals as possible – placing special attention to those at high risk of being damaged therefore in need of protection through translocation - to respond to the reduction of *P.nobilis* populations

- Action C5 – nautical support for the collection of reproductive specimens by the sub-contractor LAGUNA PROJECT SNC (unforeseen) - EUR 2.577. The unforeseen cost was necessary to sustain the increased collection effort of reproductive individuals by the AB as described in chapter 6
- Action C6 – DIY video production by the sub-contractor DIVULGANDO SRL -EUR 2.534,76. Though unforeseen in the budget of the AB, the cost was necessary for the achievement of the envisaged deliverable D.C.6.2: during the implementation of action C6 video materials were collected for the completion of the deliverable ‘Video “*Pinna nobilis*: how to transplant” – A DIY video’.

The higher/unforeseen costs sustained for actions C3, C5, C6 are compensated through savings in the budget category ‘External assistance’ for action E1 (lower costs necessary for the organization of project events in the MPA Visitor Center) and action F1 (unnecessary costs for consultancy for LIFE and After LIFE project management).

VAT for the AB SHORELINE is partially recoverable at 93%, therefore the percentage of non-recoverable VAT applied to the reported costs on the project is at 7%. As specified in the reply to Issue 9 of CINEA letter LIFE20 NAT IT 001122 MTR1LET 240315 KK SZ following the submission of the Mid-term Report, evidence was provided of the partial recovery of VAT by the AB in the form of: declaration on VAT scheme of the AB signed by the legal representative, VAT model of the national tax authority Agenzia delle Entrate for the years 2022-2023-2024 (referred respectively to project years 2021-2022-2023).

The AB SHORELINE, though not subjected – as a private entity – to the rules of the Italian national legislation for public procurement of external services and goods, ensures the respect of the principles of best value for money and avoidance of conflict of interest by requesting, whenever applicable, a minimum of three economic offers also for purchases/contracts below the national threshold for direct awarding. Evidence of the request and of the collected offers is maintained and provided by the AB to ensure the transparency of the selection process.

Equipment

The AB reported equipment costs for a total amount of EUR 10.571,96, against a foreseen budget of EUR 17.500. The reported costs for the budget category include the purchase of:

- Action A1 – PC Macbook PRO 13 M2 by the supplier UNIEURO SPA, underwater illuminator (scuba diving and underwater photographic equipment) by the supplier DIGITAL MEDIA SERVICE SRL, underwater regulator and dry suit (scuba diving and underwater photographic equipment) by the supplier BIGNAMI SUB di Martinelli F., dry suit by the supplier IDRA di Manlio Saporito – 4.620,73
- Action C3 – TK1000 wi-fi module, aerator, data logger HOBO USB, filter, eco cooler for aquarium, dosing pump, autoclave, scale, data logger HOBO USB, aquarium heater, icehouse Igloo Marine, air conditioner TECO, aquarium pump SICCE Syncra (aquarium facilities and laboratory equipment) by the suppliers TROPICAL ZOO, SELIN SRL, STARCAMP SRL, MARCHETTI MASSIMO, TopReef, FUNGUS PARADISE, NOVALBERGHIERA SRL, Shenzhen Aiku Pet Technology Co., BERNABÈ GROUP SRL, Mantovani Pet Diffusion Srl – EUR 5.633,23
- Action D2 – camera lenses (scuba diving equipment and underwater photographic equipment) by the supplier OPTIKA SRL – EUR 318

The purchased equipment is reported at 100% on the project, in compliance with the internal depreciation rules to which the AB is subjected.

A share of the foreseen ‘Equipment’ costs was not reported as such and has instead been included in the reported costs for the BL ‘Consumables’ as detailed in the dedicated paragraph below.

All the purchased goods are labelled (project and programme logos, Natura 2000 logo and reference to the EU co-funded purchase) in compliance with the programme provisions and visibility rules. The certificate on the use of Equipment has been extracted from the Financial Statement of the AB and signed by the statutory representative, and is attached to the present Report (Payment Request > Certificate_NATURE_projects > ‘Certificate_NATURE_projects_SHORELINE’).

Consumables

The AB SHORELINE reported EUR 5.517,57 for the budget line ‘Consumables’ by September 2025, whereas no budget was foreseen for the AB in the cost category. The unforeseen costs are

connected to the purchase of scuba diving equipment, aquarium items and laboratory equipment that had been erroneously included as 'Equipment' in the proposal stage. In consideration of the internal rules of the AB for inventory items, the above listed materials foreseen as 'Equipment' are reported as 'Consumables' due to their low unitary cost, and include:

- Action A1 – Reel Scubapro, diving materials, Dropbox subscription (software for mapping and graphic management of materials) by the suppliers BIGNAMISUB di Martinelli F., DROPBOX, OCEANIK d.o.o, MOTOMARINE B2C SRL – EUR 1.672
- Action C3 – electric materials, aquarium cartridge, biopsy forceps, ironmongery, fishing materials, panels and digital prints, polyester braid, various materials (aquarium facilities and laboratory equipment) by the suppliers OBI SRL, TROPICAL ZOO, DOCTOR SHOP SRL, FERRAMENTA TERCON SNC, SOLUZIONE MARE SRL, TACAMACO Officina grafica, Global Engineering Network srl, ELETTROVENETA SPA, SCUBLA SRL, INDOORLINE SRL, ZHONG SHAN SHI, Polsinelli enologia Srl, Merck Life Science Srl, TEPSA – EUR 3.285,89

The deviations listed above in the form of unforeseen 'Consumable' costs, are compensated through reallocations from the budget category 'Equipment' (where these costs were originally included) and the shift among budget categories is monitored and verified at project level in order to ensure its compliance with the 20% flexibility rule.

Other direct costs

The AB SHORELINE reported EUR 2.214,86 for the budget line 'Other direct costs', whereas the total budget foreseen amounts to EUR 4.500. The reported costs include:

- Action C3 – catering service and boat fuel by the suppliers FERDI SRL and PORTO SAN ROCCO SRL – EUR 267,26
- Action C4 – purchase of materials for transportation and transport costs by the suppliers AGRICOLA DI TRIESTE S.C., SOLUZIONE MARE SRL, THALASSIA SAS, SEMAR SRL, SME SPA, SIDERURGICA MORSELLI SAS, SUBSUPPLY – EUR 990,04
- Action C5 – boat rental by the supplier LAGUNA PROJECT SNC – EUR 559,68
- Action E3 – catering service by the supplier FOCUS SRL (unforeseen) – EUR 397,88. The unforeseen cost was sustained to support the network activities organized and held in Trieste on 13th and 14th of June: colleagues from LIFE PINNARCA and other EU projects involved in conservation of Pinna met firstly in Trieste to share knowledge and strategy to strengthen conservation of P. nobilis. The deviation is compensated through overall savings in the cost category.

8.1.5 Triton Research S.r.l.

The AB TRITON reported a total of EUR 368.650,50 on the project by September 2025, including costs for the budget categories Personnel, Travel and Subsistence, External assistance, Equipment, Other Direct Costs and Overheads.

Personnel

The costs actually incurred by September 2025 amount to EUR 202.375 whereas the total personnel budget declared in the Grant Agreement amounts to EUR 216.000.

During the project, 9 professional figures were accounted for on the project:

- A full time Project Manager/senior expert, involved in the monitoring and coordination of actions C3, C4, C6, D3, E1, E2, E3, F1 – Picchi Stefano (from October 2021 to December 2023), Vielmini Ilaria (from October to December 2021), Pulieri Valeria (from January 2022 to January 2023 – from September 2024 to February 2025), Mattioli Diego (from February 2023 to July 2024 – from March 2025 onwards)
- A Project Assistant/junior expert, involved in the monitoring and implementation of actions E1, E2, E3, F1 – Ledda Eleonora (from October 2021 to September 2023), Viglianisi Costanza (from October 2023 to February 2025), Mazzoli Alessandra (starting January 2025)
- A Communication Manager/technician, involved in the coordination and implementation of actions E1, E2, E3, F1 – Tomasinelli Francesco (from October 2021 to March 2024), Midlarz Alessandro (starting April 2024)

The annual costs inserted in the Financial Statement of the Individual Beneficiary are real and not estimated, based on the monthly invoices issued by the reported figures.

The number of days reported refers to the productive days worked and can be deduced from the timesheets. Following the reception of CINEA letter after the first monitoring visit LIFE20 NAT IT 001122 MISMC1LET 220210 SZ, Financial issue N. 5, regarding the use of scanned pictures of the signature for the validation of the timesheets, the AB, starting November 2021, modified its procedure for the validation consisting now of: print and handwritten signature of the timesheet by the staff member, forward of the scanned timesheet to the supervisor, print and handwritten signature of the supervisor, archival of the original document signed by both the staff member and the supervisor.

The daily rates reported in the Financial Statement are in line with the daily rates foreseen in the Grant Agreement for all the reported personnel and do not exceed the 20% threshold of deviation.

Deviations are detected in the subdivision of costs per action, in particular:

- Higher costs reported for action F1 (EUR 114.154,75 against a foreseen budget per action of EUR 77.050) due to an underestimation in the proposal phase of the person-days necessary for the role of project management of the AB TRITON. The reported higher costs for action F1 are in any case more than compensated through lower costs reported in the other foreseen project actions of the AB.

Travel and subsistence

The AB reported EUR 6.048,99 for the budget line 'Travel and subsistence', whereas the total budget foreseen amounts to EUR 33.560. The reported costs include:

- Action E1 – travel to Genoa to participate in Festival della Scienza and in the project launch event - EUR 856,04
- Action E2 – travel to Pirano to participate in the Final International Conference – EUR 666,15
- Action E3 – travels to Trieste and Valencia to participate in networking events - EUR 1.086,85
- Action F1 – travels to Genoa, Sassari and Rome to participate in coordination, steering and monitoring meetings - EUR 4.106,10

No deviations are detected in the breakdown of costs per action as significantly lower costs have been reported for all actions in the cost category 'Travel and subsistence'.

The AB shared its internal regulation for the reimbursement of travels and subsistence costs and reports its costs under the specific budget line in accordance with it.

External assistance

A total of EUR 128.980,97 has been reported by the AB TRITON for the costs category 'External assistance' in line with the foreseen services to be externalised, in particular:

- Action E1 – press agency service by the subcontractors Boe Sas di Santoro Elena & C. and Green Factor – EUR 21.000. Both sub-contractors were selected through a comparison of three offers, based on criteria of quality and lowest price to ensure the respect of the best value for money principle
- Action E1 – image collection for archive and documentary by the sub-contractor Marco Colombo – EUR 1.600
- Action E1 – subscription and renewal of Microsoft 364 by the sub-contractor Go Daddy – EUR 174,64
- Action E1 – video maker, shooting and editing of the project documentary by the sub-contractor Marco Tessaro – EUR 40.990. The sub-contractor was selected through a comparison of three offers, based on criteria of quality and lowest price to ensure the respect of the best value for money principle, under the same conditions and requisites met (e.g., including submission within the established deadline of an operative plan for the production of the requested outputs). As specified in the reply to Issue 8 (CINEA letter LIFE20 NAT IT 001122 MTR1LET 240315 KK SZ following the submission of the Mid-term Report) the video was edited to grant higher visibility to the EU funding and LIFE PINNA project
- Action E1 – web hosting (technical assistance, statistics, backups, updates), custom website and newsletter setting, and design of the project logo by the sub-contractor AC Consulting S.r.l. – EUR 35.583,33. The sub-contractor was selected through a comparison of three

offers, based on criteria of quality and lowest price to ensure the respect of the best value for money principle, under the same conditions and requisites met

- Action E1 – web marketing and social advertising by the sub-contractors The River S.R.L. and Go Up – EUR 20.228. Both sub-contractors were selected through offer request, with a selection based on quality of the service and lowest price offered, to ensure the respect of the best value for money principle
- Action F1 – consultancy for After LIFE Plan drafting and assistance for the elaboration of the mandatory project reports by the sub-contractor Stefano Picchi – EUR 9.400

Higher costs than foreseen were reported for the foreseen service under action F1 ‘Consultancy for After LIFE Plan elaboration’ due to the need of increased support that emerged in the implementation phase, specifically with regard to the drafting of the mid-term report and After LIFE Plan. The higher costs reported are compensated through savings from the entrustment of services in ‘External assistance’ stemming from the selection of the lowest economic offers.

The AB TRITON, though not subjected – as a private entity – to the rules of the Italian national legislation for public procurement of external services and goods, ensures the respect of the principles of best value for money and avoidance of conflict of interest by requesting, whenever applicable, a minimum of three economic offers also for purchases/contracts below the national threshold for direct awarding. Evidence of the request and of the collected offers is maintained and provided by the AB to ensure the transparency of the selection process.

Equipment

The AB reported EUR 450 against a foreseen budget of EUR 11.360. The reported costs for the budget category include the purchase of:

- Action E1 – Hp Elitebook 830 G5 Notebook from the sub-contractor Sokom s.r.l., as foreseen in the GA

Deviations in the cost category stem from the significant savings, as most of the foreseen equipment in the GA (e.g., platform app for video conferencing, cameras, tablets, etc.) was deemed not necessary for the sound implementation of the project during the implementation phase and was therefore not purchased.

The purchased good is labelled (project and programme logos, Natura 2000 logo and reference to the EU co-funded purchase) in compliance with the programme provisions and visibility rules. The certificate on the use of Equipment has been extracted from the Financial Statement of the AB and signed by the statutory representative, and is attached to the present Report (Payment Request > Certificate_NATURE_projects > ‘Certificate_NATURE_projects_TRITON’).

Other direct costs

The AB TRITON reported EUR 6.678,54 for the budget line ‘Other direct costs’ by September 2025, whereas the total budget foreseen amounts to EUR 77.900. The reported costs – foreseen in the GA - include:

- Action E1 – purchase of project gadgets (400 magnets, 300 pins, 150 t-shirts, 100 shoppers, 100 key rings, project labels, usb keys and stamps) by the suppliers Pins I Like, Pro&Fast srl, Stampa e Stampe, Customize srl, General Pen, Grafica Miloro, Viva R&D – EUR 2.601,32
- Action E1 – purchase of roll-ups, leaflets, panels by the supplier Utilgraph Trieste, Digital line web, Loreto Print, DigitalK – 1.805,78
- Action E1 – shipment costs for photo contest prizes, rental of diving materials for documentary field by the suppliers Alessandro Midlarz (refund) and Sirena Sardinia Diving School – EUR 231,65
- Action E1 - reimbursement of costs for the website images by the supplier Alamy Limited – EUR 126,99
- Action E1 - subscription to Canva PRO by the supplier Canva – EUR 90,16
- Action E1 – Layman’s report printing by the supplier Grimm – EUR 245
- Action E3 – transport/food/accommodation for the participation in networking events in Valencia by the suppliers AUTOBUSES VIALCO, S.L. and Stefano Picchi – EUR 1,185,64
- Action E3 – catering for the networking event held in Trieste by the supplier FOCUS – EUR 392

Significantly lower costs were reported for the cost category 'Other direct costs' due to the provision in the approved Grant Agreement budget of several services/supplies that proved not to be necessary, including: English translation of products, protocols and mandatory reports (EUR 24.000), dinghy and diving equipment rental for project documentation, participation fees for international events/conferences.

8.1.6 Università degli Studi di Genova

The AB UNIGE reported a total cost of EUR 491.131,32 on the project by September 2025, including costs for the budget categories Personnel, Travel and Subsistence, External assistance, Equipment, Consumables, Other Direct Costs and Overheads.

Personnel

The costs actually incurred by September 2025 amount to EUR 342.941,06 whereas the total personnel budget declared in the Grant Agreement amounts to EUR 339.974.

During the first period, 6 professional figures were accounted for on the project:

- A Professor/Project scientific coordinator involved in the coordination, monitoring and implementation of actions A1, A3, C1, C6, D1, D2, E1, E2, E3, F1 – Professor Montefalcone Monica (from October 2021)
- An Associate Professor, involved in the monitoring and implementation of actions A1, A3, C1, C4, C6, D1, D2, E1, E2, E3, F1 – Professor Chiantore Mariachiara (from October 2021)
- A Researcher RTD A, involved in the implementation of actions A1, A3, C1, C6, D1, D2, E1, E2, E3, F1 – Professor Asnaghi Valentina (from October 2021)
- A full professor, Director of the Department, involved in the coordination and monitoring of actions A1, A3, C1, C6, D1, D2, E1, E2, E3, F1 – Professor Mariotti Mauro (from October 2021)
- A post-doc researcher, involved in the implementation of actions C1, C4, C6, and D2 – Ferranti Maria Paola (additional personnel, from March 2022 to April 2023. Starting May 2023 Ferranti Maria Paola is contracted as self-employed worker/researcher and reported on the project as additional personnel, non-employee)
- A post-doc researcher, involved in the implementation of actions C1, C6 and D2 – Oprandi Alice (additional personnel, from May 2022)

The total annual personnel costs of the employees were calculated by adding the following items:

- Annual gross salary, which includes the ordinary remuneration of the employee (with the thirteenth remuneration)
- Annual obligatory social charges & other eligible statutory costs, which include the treasury withholding and the pension plan withholding, net of IRAP

The annual costs inserted in the Financial Statement of the Individual Beneficiary are real and not estimated, even those referring to the first project incomplete year (2021) for Montefalcone, Mariotti, Asnaghi and Chiantore. The total annual costs for 2025 have been filled in using figures of the previous complete year (2024) proportional to the annual share included in the present report (annual costs 2025 = annual costs 2024/12*9).

The number of hours reported refers to the productive hours worked and can be deduced from the timesheets. The actual annual productive hours for the first incomplete year could not be recovered for Prof. Mariotti and Prof. Montefalcone, due to the lack of an internal registration system of the University. The pro-rata increase of the actual productive time for the months of October, November and December (registered via timesheets) could not be applied as contracts for University Professors do not include an indication of the foreseen yearly productive time (financial issue 3 and 4 – letter LIFE20 NAT/IT/001122 – LIFE PINNA – First monitoring visit) therefore, only for the year 2021 and limited to the two abovementioned Professors, the standard figure of 1,720 has been used to indicate the total annual productive hours. Starting from 2022, the permanent personnel reported on the project for the University of Genoa make use of the timesheet registration system, and the total annual productive hours reported in the Individual Statement of the Beneficiary correspond to the actual productive hours reported in the timesheets.

Two people are not filling in the timesheets in accordance with the exception case per Annex X (Annex X to the Model LIFE Grant Agreement Financial and Administrative Guidelines – II.1.2 EXCEPTIONS):

- Post-doc researchers Ferranti and Oprandi, working on the project for a contractually fixed percentage of time (100%). In these cases, the total productive hours and the total hours worked on the project inserted in the Financial Statement (Annex VI) correspond and are filled in using the standard 1,720 figure

The daily rates reported in the Financial Statement are generally in line with the daily rates foreseen in the application form and do not exceed the 20% threshold of deviation, with the exceptions of:

- Prof. Mariotti Mauro (higher by 22% in 2021, by 21% in 2022, by 25% in 2023) due to an underestimation of the daily rate for the specific role at the time of the project proposal
- Prof. Chiantore Mariachiara (higher by 32%, 55% and 62% in 2023, 2024, 2025 respectively). The higher variation registered between 2023 and 2024 for Prof. Chiantore originates from the transition of Prof. Chiantore from Associate professor to Full professor. The decree certifying the transition (DR 4132) is provided within the supporting documents package (*supporting docs\Issue 23\DR 4132_20230912_Chiantore_art.18 c.1 BIO 07 DISTAV.pdf*). Financial-administrative issue 23, CINEA letter LIFE20 NAT IT 001122 MISMC4LET 250214 following project visit of 24 January 2025 was raised on the excessive daily rates of researchers Mariachiara Chiantore and Mauro Mariotti, against a foreseen daily rate for the role of either EUR 198 or EUR 238. The significant discrepancy between the foreseen daily rates and the reported ones for Mariachiara Chiantore and Mauro Mariotti stems from an incorrect attribution of roles in the project in the Financial Statement of the AB: Chiantore is an Associated Professor and Mariotti a Full Professor. The correct attribution of roles is reported as follows: Mariotti – full professor, management and historical data gathering from receiving sites, authorization management, SWOT analysis, management, coordination of staff recruitment, of collection of sentinel organisms, workshop organization, dissemination activities, overall management, financial and technical; Chiantore – associate professor, skilled in environmental status assessment of littoral benthic ecosystems, synthesis of pre-existing information about maintenance in captivity, controlled reproduction and larval capture, sampling design and monitoring plan, installation design, data analysis, implementation of training events for personnel engaged on restocking conservation action, networking events attendance, overall management, financial and technical. The correct roles of the two professors were corrected in the Financial Statement. The variance between the actual reported daily rates and the foreseen daily rates for both Professors is therefore significantly lower
- Prof. Monica Montefalcone (higher by 29% and 25% in 2024 and 2025 respectively) due to the appointment (DR 3276 on 12/07/2023) starting 01/01/2024 as Associate Professor at the Department of Earth, Environmental and Life Sciences – DISTAV for the scientific-disciplinary sector BIO/07 Ecology

Other deviations stemming from the breakdown of costs per action are detected in the overreporting of personnel costs for project actions:

- A1 (EUR 28.092,89 against a foreseen budget of EUR 25.206) due to the necessity of an increased monitoring effort that emerged during the implementation phase
- C4 (EUR 124.583,55 against a foreseen budget of EUR 110.512) stemming from the difficulties encountered in the challenging management of adult specimens/larvae in the tank, leading to the need for an increased number of working hours/days dedicated to the action
- E actions (EUR 39.731,46 against a cumulative foreseen budget of EUR 14.842 for actions E1, E2, E3) due to the poor initial planning of the necessary involvement of the AB in communication, dissemination and networking/knowledge transfer activities, in consideration of the role and technical contribution provided by the AB
- F1 (EUR 18.746,48 against a foreseen budget of EUR 15.116) due to the generally higher daily rates of the figures involved in the coordination and management of the project (Montefalcone, Chiantore, Mariotti, Asnaghi) on equal terms of hours worked on the action as per GA budget

The recorded increases in the personnel costs of actions A1, C1, E and F1 are compensated through savings in other actions of the same budget category as well as through savings in the cost category ‘Travel and subsistence’.

Travel and subsistence

The AB reported EUR 13.491,42 for the budget line 'Travel' by September 2025, whereas the total budget foreseen amounts to EUR 19.050. The reported costs, in line with the foreseen project activities, include travels of the AB's staff working on the project for:

- A1 – visits to the laboratories of other ABs (from Genoa to Trieste and Ventimiglia) - EUR 748,80
- A2 – collection of sentinel organisms from Genoa to Ventimiglia (unforeseen cost) - EUR 72,80
- C1 – implementation of action C1 through field visits in Capo Mortola - EUR 350,61
- C4 – setting up of the tanks and maintenance of the specimens (from Genoa to Camogli, Riomaggiore and Trieste) - EUR 1.713,95
- C6 – installation design and implementation, and data analysis (from Genoa to Ventimiglia) - EUR 82,67
- D1 – monitoring activity (from Genoa to Camogli and Mentone) - EUR 432,01
- D2 – monitoring, sampling design, and data analysis (from Genoa to Sanremo, Menton, Camogli, Ventimiglia, Napoli, Bergeggi, Pirano) – EUR 3.794,79
- E1 – tissue sampling for the shooting of the documentary and participation in dissemination workshop (from Genoa to Camogli and Naples) – EUR 2.375,61
- E2 – participation in networking event in Spotorno (unforeseen cost) – EUR 36,71
- E3 – participation in networking events in Trieste and Valencia – EUR 1.354,12
- F1 – participation in periodic coordination and monitoring meetings (from Genoa to Sassari, Oristano, Rome, Bergeggi) – EUR 2.529,35

No significant deviations are to be reported for the cost category 'Travel and subsistence' also from the breakdown of the costs by action, with the sole exception of the unforeseen costs for the collection of sentinel organisms (action A2) and for the participation in the networking event (action E2), compensated through overall savings in the cost category.

Travel and subsistence costs are reported on the project in line with the internal regulation of the University, shared at the start of the project.

External assistance

The AB UNIGE reported EUR 17.629 for the budget line 'External assistance' by September 2025, against a foreseen budget of EUR 54.000. The reported costs include services of:

- A1 - Citizen science activity for the mobilisation of local outdoor sport communities in the monitoring of *Pinna nobilis* in the areas of interest (unforeseen cost) by the sub-contractor OUTBE SRL soc. benefit (direct award) – EUR 6.100,00
- D2 - Support boat service for the sampling at sea by the sub-contractors Associazione sportiva dilettantistica pianeta Blu (direct award) and Centro Sub Sanremo Associazione sportiva dilettantistica (direct award) – EUR 11.529

Deviations from the foreseen budget in external assistance stem both from the unforeseen cost for the citizen science activity necessary to support the on-field data collection of action A1.2, and from the overspending of action D2 for the diving support (EUR 11.529 against a foreseen budget of EUR 5.000) due to the need for an increased monitoring effort.

Overall lower costs were reported in 'External assistance' due to the reallocation of the foreseen costs in action C4 (contract for 4 years for controlled reproduction facility and running) amounting to EUR 50.000 to the cost category 'Other direct costs'.

The AB UNIGE abides by the rules of the Italian national legislation for the public procurement of external services and goods; in compliance with the programme provisions, the respect of the principles of best value for money and avoidance of conflict of interest is ensured by requesting, whenever possible, a minimum of three economic offers also for purchases/contracts below the national threshold for direct awarding. Evidence of the request and of the collected offers is maintained and provided by the AB to ensure the transparency of the selection process.

Equipment

The AB UNIGE reported a total of EUR 25.160,94 for the budget line 'Equipment' against a total foreseen budget of EUR 26.000. The reported costs include:

- C1 – purchase of diving photo-video kit from the supplier Sub Evolution di Petricelli Fabio – EUR 7.747 reported at 100% on the project

- C1 – purchase of GPS Garmin from the supplier TOP SUB S.A.S. di Agostini Silvano – EUR 335,50 reported at 100% on the project
- C4 – purchase of a binocular microscope from the supplier Savatec Strumenti s.r.l. – EUR 9.760 reported at 100% on the project
- C4 – purchase of a bioreactors for algal crops from the supplier Scuba s.r.l. – EUR 7.318 reported at 100% on the project. The purchase of the bioreactor was not foreseen in the GA budget but deemed necessary for the maintenance and growth of the different strains of microalgae in the laboratories of the University, to be able to produce large quantities of them, as detailed in the technical description of action C4 (chapter 6). The unforeseen cost is compensated through lower costs reported in action C1, as a share of the diving equipment to be purchased, initially foreseen in the cost category ‘Equipment’ has been reported under the budget line ‘Consumables’, in line with the internal regulation of the AB UNIGE for inventory items (the unitary cost of part of the diving materials is lower than the identified threshold for inventory items).

All the purchased goods are labelled (project and programme logos, Natura2000 logo and reference to the EU co-funded purchase) in compliance with the programme provisions and visibility rules. The certificate on the use of Equipment has been extracted from the Financial Statement of the AB, signed by the statutory representative, and is attached to the present Report (Payment Request > Certificate_NATURE_projects > ‘Certificate_NATURE_projects_UNIGE’).

Consumables

UNIGE reported a total cost of EUR 8.468,24 for the cost category ‘Consumables’ whereas the total foreseen budget amounts to EUR 9.000. The reported costs include:

- Action A1 – purchase of diving equipment from the supplier TOP SUB sas, erroneously included in the original budget as ‘Equipment’ costs, as detailed in the paragraph above – EUR 3.156,52
- Action A2 - purchase of laboratory materials (slates/containers/rulers) from the supplier RAM Apparecchi Medicali (initially foreseen in the context of action C1) – EUR 29,90
- Action C4 – purchase of various consumables (spare parts for the aquarium tanks in the hatchery, containers/glassware/tubes, spatula, flask, pliers, laboratory growing tube, microscopy scissors, plankton mixture, canisters, insulating tape, Teflon, bioreagents, m.s feed and aeration taps, glass coverslips, laboratory consumables) from the suppliers Ecoplast 2000 sas Idrocentro spa-arboco sns, VWR International S.r.l., Scubla s.r.l., Fisher scientific s.a.s., SARSTEDT S.R.L., Biosigma S.p.a., SAVATEC STRUMENTI S.R.L., Aquariumline S.r.l., Ditta Zunino, Tecnomat, Parri Srl, Cabona, Merck Life Science S.r.l., Il triangolo srls, TOP SUB S.A.S. di Agostini Silvano – EUR 5.281,82

Other direct costs

The AB UNIGE reported a total cost of EUR 51.310,66 for the cost category ‘Other direct costs’ whereas the total foreseen budget amounts to EUR 2.000. The reported costs include:

- Action A1 – travel costs for staff not yet assigned to the project (Oprandi Alice) and unforeseen costs for the maintenance of diving equipment by the supplier PDE Attrezzature subacquee professionali di F Demoro & C. sas (unforeseen in the GA but necessary in the implementation phase) – EUR 893,99
- Action C1 – travel costs to Menton and Levanto for personnel of the University not specifically assigned to the project (Azzola Annalisa) – EUR 114,60
- Action C4 - unforeseen transport costs of specimens from the supplier SRC SERVIZIO RAPIDO CORRIERI SRL – EUR 151,77
- Action C4 – Agreement with Istituto di Biofisica del Consiglio Nazionale delle Ricerche CNR (Biophysics Institute of the National Council of Research) for the use of the laboratory of Camogli – EUR 50.000. The AB UNIGE proposed (ticket TCK005949 closed on 05/12/2024) the activation of a collaboration within the context of the foreseen activities for action C4 – adaptation, breeding and reproduction for active restocking. The Collaboration Agreement with CNR is based on the unicity of the facility for the purposes of the activity as well as on the scientific cooperation between the two entities for the realization of action C4. The laboratory in Camogli is the one facility in Liguria Region with access to sea water and a fully functional recirculation and sterilization system for sea water, and is equipped

with microalgal cultures for feeding larvae and adults of *Pinna nobilis*. CNR-IBF contributed to the scientific collaboration with analyses on seawater quality and specifically on dissolved and particulate organic matter, to characterize the quality of the feed provided to the cultured organisms. The proposed Agreement entailed the direct entrustment of the service to CNR, requiring the reallocation of the foreseen budget from the cost category 'External assistance' to 'Other direct costs'

- Action E1 – poster supply for congress/workshop organization from the supplier LAB PHOTO SERVICE SRL – EUR 150,30

Overall higher costs were reported by the AB UNIGE than foreseen in the GA for the Beneficiary, as anticipated via ticket TCK007300 to the Helpdesk submitted on 11/04/2025 and communication to the Project Advisor Dr. Aramburu on 14/04/2025. A request for transfer of budget between Beneficiaries was submitted, consisting in the use of part of the foreseen savings by the ABs ASINARA and TRITON. The proposed reallocation of savings, in line with Art.II.22 of the Financial Provisions (General Conditions, Grant Agreement), respects the 20% limit between budget categories at project level, and does not entail any changes in the foreseen % of EU co-financing (in any case within the limit of the maximum eligible amount). The reallocation proposal, formalized through the signature of an Addendum to the Partnership Agreement by all Beneficiaries, includes the use of savings by the ABs ASINARA and TRITON (detailed in the respective sub-chapters 8.1.2 and 8.1.5) to ensure the full coverage of the contracted service to CNR considering the AB UNIGE sustained higher/unforeseen costs regarding:

- Equipment - purchase of a bioreactor for algal crops (action C4) necessary for the maintenance and growth of the different strains of microalgae in the laboratories of the University, to be able to produce large quantities
- External assistance - unforeseen costs for the citizen science activity necessary to support the on-field data collection of action A1.2, and overspending of action D2 for the diving support due to the need for an increased monitoring effort

8.1.7 Università degli Studi di Sassari

The AB UNISS reported a total cost of EUR 450.678,65 on the project by September 2025, including costs for the budget categories Personnel, Travel, External assistance, Equipment, Consumables, Other Direct Costs and Overheads.

Personnel

The costs actually incurred by September 2025 amount to EUR 303.678,63 whereas the total personnel budget declared in the Grant Agreement amounts to EUR 305.450.

During the project implementation 8 professional figures were accounted for on the project:

- A Coordinator – Senior researcher, responsible for the management of the project, involved in the monitoring and implementation of actions A2, C2, C6, D1, E1, F1 – Associate Professor Casu Marco. As specified in the reply to CINEA letter LIFE20 NAT IT 001122 MTRLET 240315 KK SZ following the submission of the Mid-term Report, due to a clerical error in the filling-in of the Financial Statement of the AB, only the role of 'Coordinator – Senior researcher/management' was indicated for Prof. Casu. The actual involvement of Prof. Casu also includes the roles of: Associate Professor/supervising selection candidate sites based on sentinel species results, Associate Professor/supervising activities of molecular diagnostic analysis on sentinel species for monitoring the infection level of pathogens responsible for the mass mortality of *Pinna nobilis* – as evidenced in the provided Letter of Assignment
- A Researcher/Scientific coordinator involved in the monitoring and implementation of actions A2, C2, D1, E1, E3, F1 – Associate Professor Sanna Daria. Analogously to the situation of Prof. Casu described above, Prof. Sanna covers roles other than 'Researcher RTD B/monitoring diagnostic molecular surveys on sentinel species and molecular characterization of any surviving specimens of *Pinna nobilis*' including: Associate Professor/supervising molecular characterization of surviving specimens of *Pinna nobilis* to be used for the selection of the best individual to be reproduced, Associate Professor/supervising activities of molecular diagnosis analysis on sentinel species for monitoring the infection level of pathogens responsible for the mass mortality of *Pinna*

nobilis (foreseen person-days divided among Prof. Casu and Prof. Sanna), Associate Professor/Communication and disclosure of molecular results on sentinel species and *P. nobilis* aimed at increasing the awareness on the severity of *P. nobilis* mass mortality – as specified in the provided Letter of Assignment

- A Researcher, involved in the implementation of actions A2, C2, F1 – Scarpa Fabio (from January 2022 to April 2023)
- An Associate Professor, involved in the implementation of action A2 – Mereu Paolo (from April 2025)
- A Research fellow (additional figure, personnel non-employee, reported at 100% on the project), involved in the implementation of action A2 – Cossu Piero (starting May 2022 to May 2023)
- A Research fellow (additional figure, personnel non-employee, reported at 100% on the project), involved in the implementation of action D1 – Ciccozzi Alessandra (from 15/02/2024 to 30/09/2024)
- A Research fellow (additional figure, personnel non-employee, reported at 100% on the project), involved in the implementation of actions A2, D1 – Azzena Ilenia (from 01/03/2024 to 30/08/2025)
- A Research fellow (additional figure, personnel non-employee, reported at 100% on the project), involved in the implementation of action D1 – Locci Chiara (from 01/12/2024 to 30/09/2025)

The total annual personnel costs of the staff reported on the project were calculated by adding the following items:

- Annual gross salary, which includes the ordinary remuneration of the employee
- Annual obligatory social charges & other eligible statutory costs, net of IRAP, that include the entities Tesoro c.E. and Opera Previdenza c.E.

The annual costs inserted in the Financial Statement of the Individual Beneficiary correspond to the actual costs incurred by the Beneficiary for all the reported years, including partial year 2025. As evidenced in Issue 22 (raised in CINEA Letter LIFE 20 NAT IT 001122 MISMC4LET 250214), the annual costs of 2022 are maintained in 2023 for all the reported staff of the AB, therefore the same values are used both years to indicate the annual costs.

The number of hours reported refers to the productive hours worked and can be deduced from the timesheets. The four additional figures reported on the project are not filling in the timesheets in accordance with the exception case per Annex X (Annex X to the Model LIFE Grant Agreement Financial and Administrative Guidelines – II.1.2 EXCEPTIONS).

The actual hours worked on the project have been inserted to fill-in the Financial Statement of the AB for the 4 additional figures, in accordance with the internal report system of the AB UNISS (monthly reports) to monitor the actual person-days effort on the project actions.

The daily rates reported in the Financial Statement are generally in line with the daily rate foreseen in the Grant Agreement and do not exceed the 20% threshold of deviation, with the exception of the daily rate of Prof. Casu for 2022 and 2024 (higher respectively by 32% and 28%) due to a role change from "Associate Professor Law 240/10 - t. full - class 1" to "Associate Professor Law 240/10 - t. full - class 2" with a consequent increase of the gross annual cost, and all Research Fellows (varying between 31% and 97% due to an underestimation of the daily rates in the proposal phase as well as to the use, instead of the 1.720 standard figure, of the actual hours worked on the project that are generally lower.

Other deviations recorded in the breakdown of the personnel costs per action, regard:

- Higher costs reported for action A2 (EUR 89.159,43 against a foreseen GA budget of EUR 69.190) due to the need to extend sub-action A2.2 to the end of the project to maintain constant control of the pathogens levels for all the sites involved in the project and further increase the genetic knowledge on the pathogens associated with the mass mortality of *Pinna nobilis*, and to identify new possible pathogens associated with the mass mortality and study their infection and spread dynamics
- Unforeseen personnel costs reported for action E3 (EUR 5.483,66), connected to the participation of Prof. Sanna Daria in the networking activities. Personnel costs for action E3 were erroneously not included for UNISS in the proposal phase, as the participation of the

AB in the networking activities is core to its sound implementation and achievement of the expected results

The reported deviations are compensated through savings in other project activities of the same cost category, particularly for actions E1 and F1.

Travel and subsistence

The AB UNISS reported EUR 14.127,09 for the budget line ‘Travel’ by September 2025, whereas the total budget foreseen amounts to EUR 10.000. The reported costs include travels of the AB’s staff working on the project for:

- Action A2 – sentinel bivalve sampling (from Sassari to Porto Conte, Capo Caccia, Punta Giglio, Santa Maria Navarrese, Santa Gilla, Pula, Siniscola, Sant’Antioco) – EUR 1.251,66
- Action C2 – sentinel bivalve sampling and monitoring (from Sassari to Isola Rossa, Porto Conte, Costa Paradiso, Sant’Antioco, Santa Teresa di Gallura, Faro Messina) – EUR 1.075,25. A specific ‘Travel and subsistence’ budget was not included for action C2, due to a clerical error in the proposal phase, considering the actual involvement of the AB UNISS in the implementation of the project conservation action
- Action D1 – travels to Pula, Villasimius, Sant’Antioco to check reports on the presence of *Pinna nobilis*, non-lethal tissue sampling and research in other areas of the island - EUR 537,28
- Action E1 – participation in communication/dissemination events and conferences (in Genoa, 52nd Sibm conference in Messina, dissemination activity in Stintino, dissemination activity in Sant’Antioco, SIBM conference in Rome and Naples, 58th European Marine Biology Symposium in Bodo) to share knowledge and best practices on the conservation of *Pinna nobilis* - EUR 6.602,66
- Action E3 – participation in networking events in Valencia and Dorgali on the results of the project - EUR 750,92. The unforeseen cost is related to the involvement of Prof. Sanna and Azzena Ilenia in the activity, as detailed in the ‘Personnel’ section
- Action F1 – participation in the annual project coordination meetings and monitoring visits in Genoa, Trieste, Rome - EUR 3.909,32

Overall higher costs were reported for the cost category due to the increased need for the participation of the AB in project coordination meetings and annual monitoring visits as well as in networking and knowledge-sharing events (action E1) than expected at the time of submission of the proposal. Higher costs are compensated through savings in ‘Personnel’ costs and ‘Consumables’, thus the total costs reported by the AB do not exceed the foreseen GA budget.

Travel and subsistence costs are reported on the project in line with the internal regulation of the University, shared at the start of the project.

External assistance

The AB reported a total amount of EUR 49.317,64 for the budget line “External assistance”, against a total foreseen budget of EUR 50.000. The reported costs and external entrustments are in line with the foreseen budget and project actions as they include:

- Action A2 – molecular diagnostic analyses on sentinel species aimed at identifying pathogen-free sites, standardization and partial complete sequencing services of *Pinna nobilis* individuals and associated pathogens by the sub-contractor Macrogen Europe BV – EUR 15.000. The sub-contractor was directly awarded the foreseen service due to the specific technical requirements of the activity. The referent Prof. Sanna provided a declaration on the applied selection of Macrogen Europe B.V. (Meibergdreef 57, Amsterdam 1105 BA, Netherlands, Business Reg. No. 69716099, VAT ID NL857980944B01) for EZ-bag and EZ-seq sequencing services addressing the irreplaceability of the company and its role in ensuring the replicability of the tests and the high standards of the results
- Action C2 – standardization and partial and complete sequencing services of *Pinna nobilis* individuals and associated pathogens, and molecular diagnostic analysis services (rt-pcr and sequencing) by the sub-contractor Macrogen Europe BV – EUR 15.000
- Action D1 – standardization and partial and complete sequencing services of *Pinna nobilis* individuals and associated pathogens, and molecular diagnostic analysis services (rt-pcr and sequencing) by the sub-contractor Macrogen Europe BV – EUR 19.317,64. Via CINEA letter LIFE20 NAT IT 001122 MIS5LET 251013 following project visit on 24-25 September

2025 – Financial Issue 25, details were requested on the selection of the supplier Macrogen Europe BV for seq. 4-8 (contract amounting to EUR 45.555,16). The sub-contractor was selected via direct entrustment (Decision 137/2024 Prot.n.886 on 10/05/2024); the selection procedure was activated on 23/04/2024 via Rep. n. 123/2024 Prot. n. 764 in compliance with national legislation (D.lgs. 36/2023, art. 50, comma 1, lett. b) for the acquisition of the service of molecular diagnostic analysis (RT-PCR and sequencing). The procedure was carried out on the electronic market for public administrations – MEPA with the economic operator Macrogen Europe BV – RDO n. 4024365. The direct award procedure is compliant with the national legislation and internal regulation of the AB UNISS (art.55 comma 2 D.lgs. n.36/2023) and is based on the technical ability of the company to provide all types of services listed, ensuring an external sequencing service capable of achieving high-standard results while guaranteeing continuity in the acquisition of molecular data and the repeatability of experiments – requirements met and subject to the submission of an economic offer in line with the market price and GA foreseen budget. The economic operator presented the offer and supporting documentation within the deadline on 08/05/2024 for an overall amount of EUR 37.500 net-VAT. The offer was deemed adequate and enabling of an increased number of analyses compared to the initially foreseen amount due to the lower price offered. The stipulation between the AB UNISS and Macrogen Europe BV was carried out on the electronic market (n. 4024365 on 31/07/2024). Relevant supporting documentation (procedure, offer, entrustment decision, stipulation, checks, payments) is provided as per request, at ‘*supporting docs>Issue 25>UNISS_MACROGEN Europe BV_seq 4-8*’

The AB UNISS abides by the rules of the Italian national legislation for the procurement of external services and goods; in compliance with the programme provisions, the respect of the principles of best value for money and avoidance of conflict of interest is ensured by requesting a minimum of three economic offers also for purchases/contracts below the national threshold for direct awarding, whenever possible. Evidence of the request and of the collected offers is maintained and provided by the AB to ensure the transparency of the selection process.

Equipment

The AB UNISS reported a total amount of EUR 17.925,66 for the budget line “Equipment”, whereas the total foreseen budget amounts to EUR 18.000.

The reported costs and external entrustment are in line with the foreseen budget and project actions as they include:

- Actions A2/C2/D1 - purchase of Thermal Cycler, Centrifuge, Rotor, Pump Water bath with integrated internal recirculation, 2 rectangular floating tube racks 2 floating round tube racks, laboratory equipment (reported at 100% on the project) by the supplier MICROTEC SRL – EUR 17.925,66 (action A2 – EUR 7.800, action C2 – EUR 7.200, action D1 – EUR 2.925,66)

All the purchased goods are labelled (project and programme logos, Natura 2000 logo and reference to the EU co-funded purchase) in compliance with the programme provisions and visibility rules. The certificate on the use of Equipment has been extracted from the Financial Statement of the AB and signed by the statutory representative, and is attached to the present Report (Payment Request > Certificate_NATURE_projects > ‘Certificate_NATURE_projects_UNISS’).

Consumables

The AB reported a total amount of EUR 32.092,63 for the budget line “Consumables”, against a total budget of EUR 36.000. The reported costs, in line with the foreseen purchases, include:

- Action A2 – purchase of lab reagents, consumables for molecular analysis (DNA extraction kit) by the suppliers EUROCLONE SPA, BIOSIGMA S.P.A., BIOGENERICA S.R.L. S.I.A.L. SRL, LIFE TECHNOLOGIES ITALIA – EUR 14.603,93. Higher costs were reported for Action A2 (EUR 14.603,93 against a foreseen budget of EUR 12.000) due to the need to extend sub-action A2.2 to the end of the project to maintain constant control of the pathogens levels for all the sites involved in the project and further increase the genetic knowledge on the pathogens associated with the mass mortality of *Pinna nobilis*. The higher costs are compensated through savings in actions C2-D1, therefore the overall costs reported for the budget line ‘Consumables’ do not exceed the foreseen GA budget for the category

- Action C2 – purchase of consumables for molecular analysis (DNA extraction, PCR, qPCR, electrophoresis, DNA purification) by the supplier SIAL SRL – EUR 8.744,25
- Action D1 – purchase of consumables for molecular analysis (DNA extraction, PCR, qPCR, electrophoresis, DNA purification) by the supplier SIAL SRL – EUR 8.744,25

Other direct costs

UNISS reported a total amount of EUR 4.054 for the budget line “Other direct costs”, against a total budget of EUR 2.000. The reported costs include:

- D1 – reimbursement to Fabio Scarpa for the participation in the mission to Sant’Antioco for sampling and testing of *Pinna nobilis* specimens for analysis of genetic variability. The cost is reported under the budget line ‘Other direct costs’ as Dr. Scarpa though not reported as ‘Personnel’ on the project at the time of the travel, was still cooperating in the implementation of part of the project activities
- E1 – scientific publication “Reconstructing the Evolutionary History of *Pinna nobilis*: New Genetic Signal from the Past of a Species on the Brink of Extinction” on Animals MDPI. Erroneously a dedicated budget was not included in the proposal phase for the publication of scientific papers, that are paramount tools to reach and engage the scientific community on the conservation of the target species
- E1 – reimbursement to Dr. Ilenia Azzena for the participation in the UZI Congress in Cagliari (16th-19th September). The cost is reported under the budget line ‘Other direct costs’ as Dr. Azzena though not reported as ‘Personnel’ on the project at the time of the travel, was still cooperating in the implementation of part of the project activities
- F1 – reimbursement for the participation in project coordination meeting in Genoa (12th-15th/12/2023) to Dr. Fabio Scarpa and to Dr. Ilenia Azzena (monitoring visit in Genoa on 24th-25th September 2025). As Dr. Scarpa was reported on the project from 2022 to April 2023, and Dr. Azzena from 01/03/2024 to 30/08/2025 the cost for the participation in the meeting of respectively December 2023 and September 2025 could not be reported under the cost category ‘Travel and subsistence’

The unforeseen costs reported in the cost category are compensated through savings in the same cost category (unpurchased fuel for sampling in Asinara in the context of actions A2 and D1) as well as through savings in the cost category ‘Consumables’.

In the table below, a summary of the reported costs divided per action:

ALLOCATION OF COSTS PER ACTION									
Action N	1. Personnel	2. Travel	3. External assistance	4.b Equipment	6. Consumables	7. Other direct costs	GA budget	Incurred costs	%
A1	67.318,28	792,53	16.100	4.620,73	4.836,01	893,99	82.242	94.561,54	115%
A2	94.402,48	1.324,46	15.000	7.800	14.633,83		122.760	133.160,77	108%
A3	41.087,84		16.050				66.373	57.137,84	86%
C1	78.107,76	350,61	9.394	8.082,50		114,60	134.084	96.049,47	72%
C2	100.883,98	1.075,25	15.000	7.200	8.744,35		139.160	132.903,58	96%
C3	340.977,23	6.767,56	7.359,91	25.603,53	8.099,34	8.283,27	286.140	397.090,84	139%
C4	127.582,02	2.349,69		17.078,44	5.281,82	51.141,81	233.897	203.433,78	87%
C5	10.307,12	-	2.577			559,68	31.250	13.433,78	43%
C6	14.189,61	82,67	42.887,56				151.786	57.159,84	38%
D1	109.014,62	969,29	56.861,98	2.925,66	8.744,35	306,64	229.569	178.822,54	78%
D2	102.351,63	3.794,79	11.529	318			172.938	117.993,42	68%
D3	58.614,21	423	42.488,36	96.934,37	252,71	26.864,67	202.039	225.577,32	112%
E1	118.649,61	14.218,04	129.326,06	450		27.277,83	389.198	289.921,54	74%
E2	38.406,66	7.423,75	1.500				50.210	47.330,41	94%
E3	44.574,73	8.608,44	26.750			42.756,39	158.115	122.689,56	78%
F1	263.613,64	22.574,01	25.599			3.149,74	322.098	314.936,39	98%
Tot. per cost category	1.610.081,43	70.754,09	418.422,87	171.013,23	50.592,41	161.348,62	2.771.859	2.482.212,65	
Overheads							194.026	173.753	90%
Total incurred costs							2.965.885	2.655.965,65	90%

8.2. Accounting system

Include among other aspects:

- *Brief presentation of the accounting system(s) employed and the code(s) identifying the project costs in the analytical accounting system*
- *Brief presentation of the procedure of approving costs*
- *Type of time recording system used, i.e. electronic or manually completed timesheets*
- *Brief presentation of the registration, submission and approval procedure/routines of the time registration system*
- *Brief explanation on how it is ensured that invoices contain a clear reference to the LIFE project showing how invoices are marked in order to show the link to the LIFE project*

All money transfers from the European Commission towards the CB, from the CB to the ABs, as well as the recording of expenditure commitments and the payment of expenses, are recorded on specific budget headings of the involved entities, identified within the analytic accounting system with the code of the project. This system ensures the traceability of all the accounting movements linked to the project. The Project Identification Code (CUP) is B95F21001880002.

The accounting records of the CB and the ABs are filled in and updated according to the national accounting rules.

All beneficiaries have indicated the cost centres in which the financial flows of the project transit, as detailed below:

N°	Name of the beneficiaries	Cost account
1	ARPAL	PR0210202 – LIFE PINNA
2	ASINARA	LIFE PINNA-LIFE20 NAT/IT/001122 104210.0 102070.0 104200.0 105113.0
3	NIB	01PINNA
4	SHORELINE	Life Pinna 6900951
5	TRITON	LIFE PINNA Internal analytic accounting as a Private Entity
6	UNIGE	100022-2021-MM-LIFEPLUS_PINNA
7	UNISS	LIFEPINNA.LIFE20NAT/IT/001122.SANNAD LIFEPINNA.LIFE20NAT/IT/001122.CASUM

The individual cost statements of the beneficiaries are signed by Elisabetta Trovatore – General Director (ARPAL), Gianluca Mureddu - President (ASINARA), Maja Ravnikar – Director (NIB), Carlo Franzosini – President (SHORELINE), Alessandro Violi (TRITON), Federico Delfino – Rector (UNIGE), Gavino Mariotti - Rector (UNISS).

Timesheets are filled in electronic format by using the form requested by the Commission and downloadable from the LIFE website for all the Beneficiaries.

At the end of each month, the electronic sheet is printed and dated. When compiling the timesheet, the action - or actions - on which the worker has worked at the indicated hours are listed in order to be able to break down the personnel costs per action. At the beginning of each month the employee signs the timesheet reporting the hours worked the month before and sends it for the countersigning of the higher responsible.

As an internal time registration system, ARPAL and ASINARA are equipped with an automatic detection system of service attendance that does not include the detail of the time units worked on specific activities, therefore both Beneficiaries integrate the internal system with the filling in of the timesheet template provided by the programme. Monthly extracts of the service attendance are integrated to the supporting documents provided by the beneficiaries and coherence is ensured between the productive hours indicated in the internal registration system and in the timesheets filled in by the project staff of the two beneficiaries.

The supporting financial documentation of the expenditure is updated on a quarterly basis in accordance with the Partnership Agreements (art. 13.2 (v)) and uploaded in a cloud folders system, to which all the beneficiaries' representatives have differentiated access.

All beneficiaries equipped themselves with a project-related analytical accounting system and identified the responsible contact persons managing payments and spending commitments.

All beneficiaries are aware of the necessity to request for the specific reference to the project in the invoices of sub-contractors: all invoices must bear specific reference to the LIFE project (and action). If this does not happen, the connection to the project and to the specific action can be inferred from the determinations of payment of the expenditure, in which explicit reference is made to the details of the invoice. In some cases, the managing bodies communicate the code and acronym of the project in the invoice header. The procurement of external assistance for public bodies is assigned in accordance with the rules on credit lines provided for by the national legislation and by the internal regulations of the Bodies. Private entities, though not bound to the observation of national public procurement regulations, respect the programme principles of best value for money and no conflict of interest,

demonstrated through the collection and provision of supporting documents (market researches etc.) for the purchase of goods/services.

8.3. Partnership arrangements (if relevant)

Please briefly explain how financial transactions between the coordinating beneficiary and the associated beneficiaries have taken place. How is financial reporting implemented by each beneficiary and how is the consolidated cost statement prepared?

Transactions between the CB and the ABs are scheduled according to the timeline established in the Grant Agreement, with a first pre-financing of 40% granted when the individual beneficiary sends a specific request and demonstrates that it has started operations. The first pre-financing tranche was received by the CB on 11/11/2021 and then transferred on 24/01/2022 (TRITON), 26/01/2022 (Asinara and UNISS), 10/02/2022 (UNIGE), 18/02/2022 (SHORELINE) and on 21/03/2022 (NIB) to the Associated Beneficiaries. The second pre-financing tranche was received by the CB on 23/04/2024 and then transferred on 07/05/2024 to the Associated Beneficiaries.

The partnership agreement was signed between the CB and the six ABs; the seven legal representatives signed it: Carlo Emanuele Pepe – General Director (ARPAL), Gabriela Scanu – Special Commissioner (ASINARA), Maja Ravnkar – Director (NIB), Carlo Franzosini – President (SHORELINE), Alessandro Violi – Director (TRITON), Federico Delfino – Rector (UNIGE), Gavino Mariotti – Rector (UNISS).

An Addendum to the Partnership Agreement was signed by the CB and ABs (first signature on 12/08/2025) revision of Art. 8 on the financial contributions of each Beneficiary, allocation of the grant and co-financing, following the partners' decision to transfer budget among themselves. The reallocation proposal was submitted via ticket to the Helpdesk and via email to the Project Advisor Dr. Aramburu on 14/04/2025 to preliminarily assess its eligibility. The proposed reallocations of savings from one Beneficiary to another, in line with Art.II.22 of the Financial Provisions (General Conditions, Grant Agreement), respect the 20% limit between budget categories at project level, and do not entail any changes in the foreseen % of EU co-financing (in any case within the limit of the maximum eligible amount). As furtherly specified in the Addendum, the proposed reallocation of budget is provisional and the distribution of the final payment among the Beneficiaries is calculated on the basis of the actually reported expenses recognized as eligible and in any case within the limits of the maximum EU contribution recognized by the Agency for the whole project.

The financial report is filled in by each beneficiary and the Project Management structure (administrative reference of the Coordinating Beneficiary with the support of the external sub-contractors) verifies the compliance between the transmitted supporting documents, the Financial Statement of the Individual Beneficiary and the budget foreseen in the Grant Agreement. Only the incurred actual costs are recorded by each beneficiary during the financial reporting; the PM fills in the Consolidated Financial Statement based on the single financial reports submitted by the beneficiaries. The PM relies on the Work Breakdown Structure of the project to cross check the reported values as an additional means of verification; the WBS is an internal management tool outlining the financial progress of the project per partner, budget line and action.

8.4. Certificate on the financial statement

For the LIFE14-16 projects, in accordance with Art. II.24.2, the official registration number, organisation, full name and address of the approved auditor or competent and independent public officer who are to establish the certificate for the payment of the balance, shall be included in this section of the mid-term report. For the LIFE17 onwards projects, provision of the auditor's details is not required with the mid-term report. The auditor's report (to be included with the final financial report) must follow the format of the 'Terms of reference for the certificate on the financial statements' available on the LIFE website under the LIFE Reporting / Templates section.

As specified by the Programme Rules (Annex X – CERTIFICATE ON THE FINANCIAL STATEMENT AND ACCOUNTS) only for the beneficiaries for whom the total contribution in the form of reimbursement of the actual costs referred to in Annex III is at least EUR 750,000, a certificate concerning financial statements and the underlying accounts is necessary. In the specific case, no Beneficiary is bound to the provision of the Certificate on the Financial Statement and Accounts.

8.5. Estimation of person-days used per action

*In order to have an overview of the use of budgeted person-days by group of actions, it is **recommended to fill in the following additional table**. Please provide estimates of % of person-days spent compared to the budgeted numbers². This table will allow you and the Agency to monitor the actual absorption of budgeted time and will highlight any major deviations that should then be explained. When compiling the information you may refer to the number of days referred to in Form R2 of the proposal:*

Action type	Budgeted person-days	Reported person-days	% Of person-days spent
Action A: Preparatory actions	1.205	889	74%
Action B: Purchase/lease of land and/or compensation payment for payment rights	-	-	-
Action C – Concrete conservation actions	4.273	3.165	74%
Action D: Monitoring and impact assessment	2.505	1.446	58%
Action E: Communication and Dissemination of results	1.100	855	78%
Action F: Project management (and progress)	1.259	1.057	84%
TOTAL	10.342	7.413	72%

For A actions the percentage of person-days spent is 74%, in line with the overall effort of the staff reported on the project and with the incurred personnel costs up to 30/09/2025.

For C actions the achieved percentage of personnel-days reported against the budgeted ones is 74%, that adequately reflects the personnel effort on the conservation activities.

For D actions the achieved percentage of personnel involved is 58% of the budgeted person-days

For E and F actions the levels of reported persons-days, respectively at 78% and 84%, are in line with the actual progress of the activities implemented up to September 2025, considering both E and F actions began at the very start in December 2021 and continued until the end of the project.

² As we are only requesting estimations, those figures are not meant to be used for the financial reporting.

Instructions / guidelines for the submission of deliverables / annexes

- Please make a reference to the deliverables in the report text. In case the deliverables are presented in a national language other than English, please include a summary in English, in the deliverable, outlining the purpose, outcomes, results and conclusions.
- All the deliverables due in the reporting period shall be provided unless already submitted with previous report(s). Deliverables should only be resubmitted if a revised version has been requested by the Agency. For projects uploading their deliverables in BUTLER, please find the guidance on BUTLER under the LIFE Reporting webpage at https://cinea.ec.europa.eu/programmes/life/life-reporting_en.
- Please date deliverables with the actual date of completion (and the date of revision if applicable).
- You may annex any other document **only if** particularly useful to assess the success of the project but which is not part of the planned deliverables. For projects uploading their deliverables in BUTLER, if relevant, please contact your external monitor to request the addition of a specific deliverable in BUTLER to enable the submission of a ZIP file containing all those additional supporting documents that are necessary for a full reporting.
- Please note that For LIFE Nature & Biodiversity and LIFE Climate Action with land purchase you need to submit digital copies of the land register, including a "conservation clause" (in exceptional cases the purchase / lease acts can be accepted) as this is a prerequisite for the costs to be considered eligible. All land sections purchased or leased must be shown on a map, which also provides the boundaries of the project area and the Natura2000 site boundaries.
- Please be aware that the Layman's report and the After-LIFE Plan are **compulsory** deliverables and must be submitted with the Final report only:

Layman's report

Purpose: The layman's report is a document aimed at a broader target group and serves to inform decision-makers and non-technical parties on the objectives of the project and the results achieved. The layman's report will be distributed widely and will be available on the LIFE website via the project database. It is therefore **compulsory** for all projects.

Form: This document is an entirely self-standing document, often in the form of a leaflet or similar. It should be provided in English and in the language(s) of the beneficiaries.

Content: The length of the layman's report should normally be 5-10 pages, including supporting graphs, photographs etc. Since the target group is the general public, the technical details should not be excessive. However, it is normally advantageous to include some quantitative results to illustrate the impact of the techniques/methods demonstrated by the project. It should include the following points (adapted to the target group):

- Summary of project scope and objectives;
- Description of the techniques/methodology implemented and the results achieved;
- Assessment of the benefit and impact
LIFE Nature & Biodiversity: conservation benefits for the Natura 2000 (SCI/SPA) and species/habitat type targeted. Highlight briefly issues that may have important policy implications;
- Cost-benefit discussion on the results (economic and environmental benefits);
- Transferability of project results;
- Map indicating where the project takes place: NB please ensure that the project site is illustrated in a way that allows a broader public to know where in Europe and in the Member State the project is implemented.

After-LIFE plan – for LIFE Nature & Biodiversity, LIFE Environment & Resource Efficiency, LIFE Environmental Governance & Information and LIFE Climate Action projects

This compulsory plan (suggested length: 5 pages) shall set out how the beneficiary and partners plan to continue disseminating and communicating the results of the project after its end, and in particular how they plan both to continue applying the results themselves and to facilitate / encourage / ensure their

wider application by others; it shall be delivered in English and also in the language(s) of the beneficiaries.