LIFE20 NAT/IT/001122

LIFE PINNA

Conservation and re-stocking of the Pinna nobilis in the western Mediterranean and Adriatic sea

Best practices protocol for transplanting P. nobilis juveniles

Deliverable C6.1

LIFE20 NAT/IT/001122 is a project co-funded by the European Union under the Life programme.

Co-funded by
the European Union

Best practices protocol for transplanting P. nobilis juveniles

LIFE20 NAT/IT/001122

LIFE PINNA

Conservation and re-stocking of the *Pinna nobilis* in the western Mediterranean and Adriatic sea

Contractual date of Delivery	August 2025
Actual date of delivery	September 2025
Deliverable number	C6.1
Deliverable name	Best practices protocol for transplanting P. nobilis juveniles
Status and Version	Delivered
Action contributing to the deliverable	C6
Responsible	SHORELINE
Other contributors	All partners

This comprehensive scientific protocol outlines rigorous procedures for transplanting juvenile Pinna nobilis, the critically endangered fan mussel. Drawing on international conservation frameworks such as the LIFE PINNA, MERCES, RESTORFAN and other projects, this document provides detailed operational steps to ensure maximum survival rates during mass mortality events caused by Haplosporidium pinnae.

These are the steps required to achieve a successful transplantation.

1. Site Selection and Ecological Assessment

The identification of a receiving site is a multi-layered process that must prioritize long-term habitat stability and sanitary safety.

- Sanitary Verification: The site must be confirmed pathogen-free (specifically regarding H. pinnae and Vibrio spp.). This is achieved by deploying sentinel organisms (healthy bivalves) or conducting preliminary analyses on existing bioindicators to ensure no pathogenic agents are active in the area.
- Geomorphological and Habitat Compatibility: Priority is given to sites with historical presence of the species, as this indicates naturally favorable conditions.
    - Substrate Types: Optimal habitats include Posidonia oceanica meadows (particularly those with a healthy "matte" of intertwined rhizomes), Cymodocea nodosa or Zostera spp. beds, and coarse sandy or detrital substrates.
    - Bathymetry and Hydrodynamics: The target depth should be between 10 and 18 meters. This range provides a vital compromise: sufficient light intensity for the ecosystem and low hydrodynamics to prevent unpredictable sea storms from undermining or uprooting newly planted individuals.
- Protection Status: To minimize anthropogenic pressures (anchoring, illegal fishing), sites within Marine Protected Areas (MPAs) are preferred.

2. Specimen Selection and Genetic Screening

- Optimal Size Class: Specimens measuring between 5.5 and 10 cm (approx. 6–18 months old) are the preferred candidates for transplantation. At this "escape size," they are

significantly less vulnerable to stress and predation compared to younger juveniles or aged adults.

- Health and Genetic Characterization: Before transfer, individuals must undergo non-invasive genetic analysis.
    - Sampling Techniques: Divers should aspirate pseudofeces (mucus secreted by the gills) or fecal pellets using 60 ml syringes. Alternatively, cotton swabs can be inserted gently between the valves to collect biological material.
    - Laboratory Testing: Samples are preserved in 80° alcohol and tested to confirm the absence of H. pinnae and other pathogens before the move.

3. Logistics, Handling, and Acclimatization

1. The transition from the donor site (or laboratory) to the sea is the most critical phase for physiological stability.
2. Handling Precautions: Extreme care must be taken to avoid any damage to the fragile shell valves or the byssus gland. An intact shell is vital because it allows the mussel to close hermetically, preserving its internal water and protecting its tissues during the installation.
3. Transport Conditions: Specimens should be kept in containers with seawater, air circulation, and stabilized with jute blankets.
4. The Acclimatization Window: Upon arrival at the destination, a 30-40 minute acclimatization period is mandatory. This involves gradually mixing water from the receiving site into the transport containers to equalize temperature and salinity, thereby preventing lethal osmotic or thermal shock. For large batches, individuals should be split into sub-groups to facilitate managed acclimatization sessions.

4. Technical Execution of Implantation

Scientific divers must execute the implantation with precision to mimic the natural anchoring of the species.

1. Excavation: A hole must be dug in the sediment (manually or using a sorbonne) to a depth of at least 1/3 to 1/2 of the total shell length.

2. Vertical Positioning: The individual is inserted vertically into the substrate. For adults, a density of one individual per square meter is recommended to balance resource competition with reproductive proximity.

3. Byssus Stimulation and Anchoring: It is a "best practice" to place small stones or shell fragments inside the hole next to the mussel. This provides a hard substrate for the animal to anchor its byssus threads, creating a stable "transplant unit" without damaging the gland itself.

4. Anti-Predator Protection: Newly implanted individuals—especially juveniles—must be covered with appropriately sized cages or netting. This protects them from predators like Octopus vulgaris and provides additional stability against currents until the byssus is fully attached.

5. Monitoring and Survival Metrics

To ensure the success of the restoration, a rigorous monitoring schedule must be established.

- Georeferencing: Each specimen must be tagged and its exact location recorded via GPS.
- Underwater Visual Census: Monthly dives should be conducted to record:
    - Survival vs. Mortality rates (distinguishing between predation, infection, or mechanical failure).
    - Growth rates (measuring the height of the unburied shell with 0.1 cm precision).
    - Environmental Status: Assessing the health of the surrounding seagrass and identifying any new anthropogenic pressures.
- Documentation: Continuous video and photographic records of each stage are essential for evaluating the replicability and cost-efficiency of the protocol.

A video for supporting transplantation have been recorded and is available at

https://www.youtube.com/playlist?list=PLbjN2qbQYFpVpSeTkfT3kk2RDkJJ-rJp5