



EUROPEAN UNION OF AQUARIUM CURATORS

REPORTING FORM

FOR *IN SITU* CONSERVATION PROJECTS FUNDED

1 TITLE OF PROJECT	Assessing the condition of the noble pen shell (<i>Pinna nobilis</i> Linnaeus, 1758) in the south Adriatic Sea
2 NAME OF APPLICANT INSTITUTION ADDRESS	Dr Milena Micic, PhD Aquarium Pula d.o.o. Verudela bb, 52100 Pula, Croatia
TEL:	00385 52 381402
FAX:	00385 52 381404
E-MAIL:	milena.micic@aquarium.hr
DATE OF REPORT:	30 th June 2020

WHAT YEAR WAS YOUR PROJECT FUNDED BY EUAC?
2019

3 LOCATION OF PROJECT (REGION & COUNTRY)

Croatia - Dubrovnik - Neretva County, islands of Mljet, south Adriatic Sea

Croatia - Istria County - additional research was carried out due to unexpected changes in the state of the noble pen shell population

4 PROJECT START AND END DATES:

1st July 2019 – 1th June 2020 with additional ongoing research

5 PROJECT CO-ORDINATOR, ADDRESS AND INSTITUTIONAL AFFILIATION

(IF DIFFERENT FROM APPLICANT)

6 PROJECT TYPE

(TICK ANY COMPONENTS THAT APPLY)

- BIOLOGICAL/ECOLOGICAL RESEARCH
- VETERINARY/CONSERVATION MEDICINE
- ANIMAL WELFARE
- CAPTIVE BREEDING
- RE-INTRODUCTION/RE-STOCKING/TRANSLOCATION
- HUMAN-WILDLIFE CONFLICT

- EDUCATION/PUBLIC AWARENESS
 - TRAINING/WORKSHOPS
 - COMMUNITY-BASED/SOCIAL POLICY
 - ECOTOURISM/SUSTAINABLE DEVELOPMENT
 - SUSTAINABLE USE
 - WARDENING/LAW ENFORCEMENT
 - PROTECTED AREAS MANAGEMENT
 - OTHER: _____
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7 FOCAL SPECIES (COMMON AND SCIENTIFIC NAME)

The noble pen shell, *Pinna nobilis* (Linnaeus, 1758)

8 IUCN RED LIST STATUS (OR OTHER THREAT LISTING) OF FOCAL SPECIES

Due to mass mortality events which decimated the noble pen shell population in the entire Mediterranean region, the species has been designated by the IUCN as critically endangered on 18th October 2019.

It is also protected under the EU Habitats Directive, Annex IV (European Council Directive 92/43/EEC) and the Barcelona Convention, Annex II (SPA/BD Protocol 1995). It is a Natura 2000 species, listed under code 1028, and strictly protected by Croatian law by the “Ordinance on Strictly Protected Species NN144/2013”

CITES YES NO

APPENDIX

9 PROJECT BACKGROUND

Until 2016, when the first mass mortality event (MME) was recorded in Spain (Vázquez-Luis et al., 2017), the threats to survival of the noble pen shell were mostly related to human activities, including habitat degradation, illegal trawling, coastal construction, boat anchoring, illegal extraction and pollution (Basso et al., 2015). After 2016, mortality events have been recorded in France, Italy, Tunisia, Turkey and Greece (Catanese et al., 2018, Katsanevakis et al., 2019, Panarese et al., 2019; Cabanellas- Reboredo et al., 2019; Lattos et al., 2020; García-March et al., 2020), with mortality rates often reaching 100%. The reasons for the high mortality of this species continue to be evaluated, and the primary cause (or interrelated causes) remains unknown. Plausible causes of MME include parasitic infection by the recently described haplosporidan parasite (Darriba et al., 2017), later named *Haplosporidium pinnae* (Catanese et al., 2018) and/or bacterial infection with a *Mycobacterium* sp. strain similar to the human mycobacterium *M. sherrissii* (Carella et al., 2019). The infection of *P. nobilis* with *H. pinnae* is associated with a strong inflammatory response, linked with considerable infiltration of the digestive glands by uninucleate cells, plasmodia, and spores, which likely leads to the hosts severe general dysfunction, starvation and death (Catanese et al., 2018). Alternatively, mycobacterial cells found within the molluscs’ immune cells are distributed within the connective tissue surrounding the gonads, mantle and digestive tissue and probably spread via the haematic and lymphatic routes to the entire body, becoming a systemic disease (Carella et al., 2019). Until the summer of 2018, no reports of anomalous mortality affecting this species were recorded in the eastern Mediterranean but, by the end of 2018, indications that massive mortality was occurring in various eastern Mediterranean locations increased (Katsanevakis et al., 2019). The Adriatic Sea, however, seemed unaffected by such MME until spring 2019.

Numerous extractions of the noble pen shell from nature have been performed in Spain. Many institutions from Alicante, Barcelona, Huelva, Murcia and Valencia have removed individuals from their habitat and maintained them in their institutions, in order to create genetic reserves in captivity. Due to infection with *H. pinnae*, *Mycobacterium* sp. and *Vibrio mediterranea* all individuals died (Garcia-March et al., 2020). Aquarium Pula, co-financed by EUAC funds and authorised by the Ministry of Environmental Protection and Energy (MEPE), followed the initiative to save this species by also removing healthy individuals from nature and keeping them in *ex situ* conditions.

10 WAS THE OVERALL PROJECT PURPOSE FULFILLED?

The aim of this project was to ascertain the state of the population of noble pen shells in the south Adriatic, in relation to the MME in the western Mediterranean. Considering that the obtained results from the surveyed area indicated a high rate of mortality (up to 100%), it was decided that, for the purpose of preserving the species from extinction, healthy individuals should be removed from nature and kept in *ex situ* conditions until the disappearance of the disease. It is believed that the only possible solution for saving the species from infection was to keep them in closed aquarium systems with the subsequent induction of spawning. All the actions were carried out with the permission of the Ministry of Environmental Protection and Energy (MEPE). The aquarium Oceanogràfic de València assisted by sharing protocols for the husbandry of the noble pen shell. The project was enabled by funds from EUAC who recognised the need for saving *P.nobilis*.

11 WHAT OBJECTIVES WERE MET?

The overall objective of setting up an expert collaboration with the aim of initiating systematic research of the noble pen shell in the Croatian part of the Adriatic, was achieved.

The following specific objectives were also fulfilled:

1. Basic research on the noble pen shell in the coastal area and shallow bays of the island of Mljet has been performed in June 2019. Many areas with mass mortality events of the noble pen shell were recorded.
2. In October 2019 areas in the south of Istria with still vital populations were found, samples were taken and tested for the presence of the infectious agents (*H. pinnae* and *Mycobacterium* sp.) which was not confirmed. Based on those analyses, Aquarium Pula removed about 30 individuals and placed them in a quarantine basin with the prior permission of the MEPE for all removals.
3. On the basis of the results of this project and new findings, all current knowledge and information about the biology of the species and the way they are maintained in captivity, were gathered. Aquarium Pula is included in a working group on the National level for all decisions regarding future procedures with the aim of preserving the species. A collaboration with the universities in Zagreb, Pula and Gödöllő, Hungary, were initiated together with the Croatian Veterinary Institute and the Ruđer Bošković Institute. Aquarium Pula wrote the “Expert base proposal for *ex situ* husbandry of noble pen shells, with special emphasis on removal from nature, breeding and treatment in case of infection outbreaks”.
4. Aquarium Pula regularly informs the media about the course of events related to the state of the noble pen shell in the sea, but also their maintenance in the aquarium, thus raising public awareness about this important endangered species.

WHAT OBJECTIVES WERE NOT MET?

The area of the southern Adriatic has been partially investigated; coastal areas around the city of Dubrovnik were omitted from the survey since this covered the territory of Istria instead. The zones that were “parasite free” at the time of concluding the project activities also became affected, therefore other solutions have to be applied when planning not only re-population activities but also those for maintaining the already infected individuals.

12 WHAT PROJECT ACTIVITIES WERE UNDERTAKEN?

The present study was conducted by means of underwater visual census (UVC) to monitor the health status of noble pen shells and to detect population hotspots off the island of Mljet, and subsequently in the northern Adriatic Sea, i.e. southern Istria. Unfortunately, noble pen shells from the island of Mljet already showed signs of infection, so we harvested several individuals from the Pula archipelago in order to assess their health status. In September 2019, three locations near Pula (northern Adriatic), were explored. To assess *P. nobilis* infection, 3 of the visually smallest individuals were collected at each site for laboratory analysis. Two individuals were removed from the extreme boundaries of the habitat, while one was taken from the centre of the site. Pieces of tissue were stored in absolute alcohol (tissue to medium ratio of 1: 5) and sent for analysis. Tissue samples were also stored in 10% formaldehyde. Molecular analyses were performed which showed negative results for the presence of *H. pinnae* and *Mycobacterium* sp. All removals were carried out under permit issued by the Ministry of Environmental Protection and Energy (MEPE) in accordance with the guidelines of the Plan for Monitoring and Surveillance of Noble Pen Shell Infections in the Croatian part of the Adriatic.

Further, in February 2020, about 300 individuals from Istrian waters were removed. One individual from each locality was tested for the presence of the parasites *H. pinnae* and *Mycobacterium* sp. and no parasites were found. Unfortunately, these individuals subsequently began to show symptoms of the disease and in May 2020 analysis results for the presence of one or both of the organisms that cause infection, i.e. *H. pinnae* and *Mycobacterium* sp., were positive. There is a possibility that the parasite was not active during the cold season and initial testing showed a false negative result.

Currently in the Aquarium about 20 specimens that have tested negative for infectious agents, and about 100 specimens positive for *H. pinnae*, are kept in separate basins.

The public is continuously informed about cases of mass mortality of the noble pen shell through social networks and newspapers, local and national television, as well as radio media. Citizens are also involved in activities by encouraging them to send information about sightings of noble pen shells (citizen science). Aquarium Pula has made a documentary about the case of *P. nobilis* which shows the importance of conserving this species in the Mediterranean, the premiere of which will be shown at the traditional Pula Film Festival in July 2020.

13 WHAT OUTCOMES WERE ACHIEVED DURING THE COURSE OF THE PROJECT?

1. A system for maintaining individuals in the quarantine of the Aquarium Pula and a system for the production of food for noble pen shells has been established.
2. All knowledge and experience of Aquarium Pula are joined in an Expert framework for *ex situ* breeding of noble pen shells, with special emphasis on collection from nature, breeding and treatment in case of outbreaks of infectious diseases and submitted to MEPE.
3. Long-term cooperation has been established with interest institutions involved in the active protection of the noble pen shells (University of Zagreb, Dubrovnik and Pula, Gödöllő, the Croatian Veterinary Institute and Ruđer Bošković Institute).
4. The Institute for Nature Protection from MEPE established a crisis headquarter for noble pen shells and included Aquarium Pula as the only institution in Croatia with the long-term possibility of *ex situ* maintenance of this species.
5. The public is informed about all activities of protection of noble pen shell and is actively involved in reporting of findings of the species in nature.
6. A short documentary film was made about the event of the extinction of the noble pen shell and the possibilities of its survival with English subtitles.

ARE ANY ONGOING?

The daily care of noble pen shells in the Aquarium is still ongoing. The main purpose is to keep individuals alive in *ex situ* conditions until the infection rate in the sea subsides. In addition to looking for new solutions for treating infected individuals, significant efforts are being made to enable their breeding in quarantine conditions.

DID ANY EXPECTED OUTCOMES FAIL?

No

14 DID LOCAL PEOPLE/COMMUNITIES PARTICIPATE IN THE PROJECT? IF SO, HOW MANY AND WILL CONTINUED CONTACT BE MADE?

Aquarium Pula advocates the need for saving the *P. nobilis* population to citizens, and encourages their involvement in making efforts to save this species. Citizen science actions have been promoted through Aquarium Pula media campaigns which have led to many people calling to report noble pen shell sightings. Further, NGO (scientific or non-scientific) have contacted us to share knowledge and news about the condition of *P. nobilis* populations in Croatian coastal areas.

15 DID THE GOVERNMENT OF THE HOST COUNTRY RECEIVE INFORMATION ON THE PROJECT'S RESULTS?

The government is constantly informed on all procedures related to the noble pen shell *in situ* research and the husbandry of healthy animals in Aquarium Pula. All actions have been previously approved by the Ministry of Environmental Protection and Energy.

16 HOW DID THE RELATIONSHIP WITH OTHER NGOS WORK? WERE THERE ANY ISSUES?

For the purposes of this project, a collaboration with the NGO "Marine Explorers Society – 20000 leagues." has been established, as they work on the detection of mass mortality events of noble pen shells in the middle Adriatic. The collaboration is still ongoing in other activities related to *P. nobilis* conservation (spawning efforts and larval collection).

17 TOTAL PROJECT BUDGET AND EXPENDITURE (IN EUROS)

50.000,00 €

**18 AMOUNT OF MATCHING FUNDS
SPENT: 41.000,00 €****19 AMOUNT SPENT FROM EUAC FUNDS:
9.000,00 €**

20 EXPENDITURE BREAKDOWN (IN EUROS)

TRAVEL	2.000,00 € (Fuel and toll charges for the trip to Mljet and fuel for 10 field trips in Istria county)
SALARIES	
ACCOMMODATION	1.000,00 € (Rent of an apartment for the south Adriatic survey - 7 days, 4 persons)
EQUIPMENT	2.500,00 € (storage boxes, cool-boxes, dissection kit, laboratory accessories, diving equipment)
COMMUNICATION	
MISCELLANEOUS	2.000,00 € (Histological and molecular analysis costs for 10 samples) 1.500,00 € (rental of a boat for the survey off the island of Mljet)
TOTAL	9.000,00 €

21 PUBLICATIONS PRODUCED AS A RESULT OF THE PROJECT

An expert framework for *ex situ* breeding of noble pen shells has been prepared, with special emphasis on collection from nature, breeding and treatment in case of outbreaks of infectious diseases. The framework was submitted to the Institute for Nature Protection of the Ministry of Environmental Protection and Energy.

Aquarium Pula has produced numerous articles that are occasionally published in the media at the national and international level throughout the project.

A short documentary was made about the event of the extinction of the noble pen shell and the possibilities of its survival, with English subtitles.

Link: <https://youtu.be/rHR3olxrUSY>
