LIFE PINNARCA

LIFE NAT/ES/001265

DELIVERABLE DA.1

PLANNING CORRECTION MEASURES: EBRO DELTA

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1 EXECUTIVE SUMMARY

The pen shell (*Pinna nobilis* Linnaeus, 1758) is a bivalve mollusk endemic to the Mediterranean and currently cataloged in critical danger of extinction (BOE 10/17/2018) and included in the Spanish Catalog of Threatened Species (CEEA) (Order TEC / 596 / 2019, of April 8) and in the IUCN red book of threatened species (Kersting et al., 2019) within the category of 'Critically Endangered' due to the disease caused by the protozoan *Haplosporidium pinnae* (Catanese et al., 2018), which causes mortalities close to 100% of the population (Vázquez-Luís et al., 2017). Since the fall of 2016, the entire Mediterranean Sea has been progressively impacted, from the southern Spanish coast to the Sea of Marmara in Turkey, which has been one of the last regions to become infected (Cinar et al., 2021). At present, there are only two pen shell populations left in the Spanish territory: the Ebro Delta (Catalonia), -particularly in Alfacs Bay, since the Fangar population was practically eliminated by the passage of the Gloria storm in January 2020 - and in the Mar Menor (Murcia) where individuals are also strongly threatened by water quality and increasing eutrophication events.

In Alfacs Bay, the only assessment of population size was conducted in 2011-2012 and drawn an overall estimate of ca. 90,000 individuals (Prado et al., 2014). The first outbreaks of the disease began in July 2018, concurring with particularly high temperatures (> 28 ° C). Later, a bacterial pathogen from the Mycobacteria group, was also identified as potentially responsible for mass mortality events of pen shells on the Italian coasts (Carella et al., 2019), and its presence was also confirmed in the Alfacs Bay together with *H. pinnae*. Although its pathological effect is uncertain, it is hypothesized that it may have a role in observed mortality rates (Prado et al., 2021). Both pathogens seem to be affected by the salinity gradient, establishing an approximate protection range for the pen shell at values less than 36.5 and greater than 39 ppt (Cabanellas-Reboredo et al., 2018) that was also confirmed in Alfacs Bay (Prado et al., 2021). Furthermore, given that the shallow distribution limit of the species occurs at very shallow depths (from ca. 20 cm to 2 m, depending on the area), there is a high rate of collision by boat propellers and foil surf keels, which also affects partially to their survival rate (Prado et al., 2014; 2021). Despite this complicated situation of risk of pathogen infection and impact due to the different uses and activities carried out in the territory, the population constitutes one of the last fortresses of the pen shell in the Mediterranean, and, therefore, its conservation is a priority goal for the recovery of the species.

This report describes - in a preliminary way - the physicochemical (FC) conditions (temperature, salinity, oxygen, and pH) of different areas of the Bay of Alfacs and Fangar, during the period comprised between April and December 2021, with the aim of establishing areas with different degrees of risk of infection and determine to what extent these areas can ensure the long-term survival of the species. Besides, we explored FC conditions in the adjacent area of the Trinidad Salt Pans in December 2021 aimed at assessing their feasibility to host the species. We

also provide some management recommendations that could be locally implemented to protect pen shells.

2. LIST OF STAHEHOLDERS

Given the critically endangered status of fan mussels, all the projects involving the species count with the support of local administrations as well as wildlife conservation institutions such as the Barcelona Zoo Foundation. Here below we provide a detailed list of IRTA local partners in the Ebro Delta:

Name	**Position**	**e-mail**	**Contact phone**	**Task/Observations**
Ricard Casanovas	Head of the Fauna and Flora Service at the Generalitat de Catalunya, Departament de Territori i Sostenibilitat, Direcció General de polítiques Ambientals i Medi Natural	arcasur@gencat.cat	(+34) 627482005	Responsible of conservation strategies for fauna and flora in Catalonia.
Josep Maria Olmo	Invertebrate technician at the Fauna and Flora Service at the Generalitat de Catalunya	josep.olmo@gencat.cat	(+34) 639238176	Responsible of endangered invertebrate species in Catalonia. Processing of permissions to manipulate *P. nobilis* in Catalonia.
Miguel Ángel López	Technician in Forestal Catalana S.A, a public company of the Generalitat de Catalunya	mangel.lopez@gencat.cat	(+34) 695560282	Fieldwork technician.
Francesc Vidal	Head of the Ebro Delta Natural Park	fvidale@gencat.cat	(+34) 675786118	Responsible of management actions in the Ebro Delta Natural Parc.
Antoni Curcó	Technician at the Ebro Delta Natural Park	acurcom@gencat.cat		Processing of permissions to manipulate *P. nobilis* in the Ebro Delta.
Natividad Franch	Technician at the Ebro Delta Natural Park	nfranchv@gencat.cat		Technician at the Ebro Delta Natural Park

Name	Position	e-mail	Contact phone	Task/Observations
Francisco J Artigas	Head of the Environmental Office in Ebro Territori	xavier.artigas@gencat.cat	(+34) 97770 7320	Responsible of local environmental policies.
Antoni Espanya	Head of the Provincial Coastal Service in Tarrgona	aespanya@miteco.es	(+34) 627481604	Responsible of coastal concessions and shoreline maintenance.
Ferrán Valero	Technician at the Provincial Coastal Service in Tarrgona	fvalero@miteco.es	(+34) 609186252	Processing of permissions for coastal concessions.
Pablo Cermeño	Technician at the Zoo of Barcelona Foundation	pcermeno@bsmsa.cat	(+34) 617626869	Fieldwork technician.
Xavier Gilabert	Head of the rural agent corps in the Montsià district	axgilva@gencat.cat	(+34) 977706261	Provides field support, when possible, with local marine agents.
Guillermo Álvarez	Head of the Catalonian Federation of Underwater Activities (FECDAS)	g.alvarez.lerma@gmail.com	(+34) 601191509	Provides support in volunteers activities
Miquel Pontes	Coordinator of VIMAR (Marine Life) association	miquelpontes@gmail.com	(+34) 687284347	Provides support in volunteers activities
Verónica López	Student's coordinator at the Institute of Prof. Aquaculture and Environmental Studies	vlopez@iepaac.cat	(+34) 620649895	Provides support in volunteers activities
Clara Franch	Volunteers coordinator Ebro Delta Natural Parc	voluntarispnde@gmail.com	(+34) 661289575	Provides support in volunteers activities

3 RECENT ACTIONS IN EBRO DELTA

3.1 Rescue of 100 individuals of *Pinna nobilis* (multiple partners)

This project funded by the Spanish Ministry of Agriculture, Fisheries and Food (MAPAMA) with the support of LIFE Intemares was an attempt to save animals from the spread of the disease in remaining areas and the first attempt conducted to maintain a large number of individuals under captivity conditions. It included 100 individuals from the Ebro Delta, which were maintained in an open water system at the IRTA.

The project started in November 2017 and ended in December 2019. Initial biopsies were conducted to assess the possible presence of *H. pinnae*, but results were all negative. Yet, the project experienced significant difficulties associated to the lack of former information on adequate stabling conditions (i.e., food quality and doses, number of individuals per tank, and maintenance temperature) which increased the stress of individuals and resulted in mortality due to other pathologies. By the end of the project, only 4 individuals were alive and could be returned to the field.

PCR and histological analyses of death animals confirmed the absence of *H. pinnae*, and only 3 individuals in April 2019 were positive for *Mycobacteria* sp. Given these negative results, microbiological analyzes were performed on three ailing individuals and the bacterium *Vibrio mediterranei* was identified as a potential agent of mortality (Prado et al., 2020a). This Vibrio is widely distributed in the marine environment, including sediments, water, and other benthic species (Tarazona et al., 2014). In addition, the species has been specifically indicated as a pathogen in other marine species including coral *Oculina patagonica*, *Artemia salina*, and some bivalves (Torres et al., 2018). *V. mediterranei* is favored by temperature increases and optimal growth around 25 ° C (Vattakaven et al., 2006), which occurred during the summer due to stabling in an open water system. In further experiments with juvenile *P. nobilis*, it was shown that the bacterium is also present in ca. 60% of individuals in Alfacs Bay and can cause mortality at very low doses (Andree et al., 2021), as it is possibly favored by stress conditions. For these reasons, after a peak of mortality in August 2018 and the identification of *V. mediterranei* in early September 2018, it was decided to transfer the individuals to temperature-controlled tanks during summer months. In addition, different treatments of antibiotics (mostly based on florfenicol, which provided the best results) and vitamins were initiated in order to minimize the infection and the bacterial load in the individuals. However, all these treatments have had limited efficacy, and the disease continued spreading at a lower rate (Prado et al., 2020a).

3.2 Verkami crowdfunding (Not one less fan mussel!) and initial surveillance of disease spread (IRTA, FECDAS, VIMAR, and UB)

Given the entrance of the disease in the Alfacs Bay, the Catalonian government funded a small project to monthly monitor the spread of the disease in the Ebro Delta (also including the Fangar Bay). Besides, a Verkami project was launch by the Catalonian Federation of Subaquatic Activities (FECDAS) to conduct a census of the known population in the Fangar Bay, translocate individuals exposed to desiccation in shallow waters, and conduct a preliminary evaluation of an area of the Northern coast of the Alfacs Bay where some individuals have been previously seen.

Combined results of both projects showed that the salinity gradient of Alfacs Bay (37.4–35.7) was associated to cumulative mortality (100% near the mouth, 43% in middle regions, and 13% in inner regions), thus hindering the spread of pathogens. Young specimens showed to be more tolerant to disease than large adults but become vulnerable over time. In Fangar Bay, lower salinities (30.5–33.5 ppt) prevented the disease but individuals were highly vulnerable to Storm Gloria which caused 60% mortality in 3 weeks, and ~100% in 6 weeks due to extreme salinity reductions to ca. 11 ppt coupled with a large presence of suspended solids (Prado et al., 2021). A total of 351 individuals were translocated to adjacent deeper waters, with an 85.5 % survival 6 moths later, which was associated to the local presence of the parasite and some marginal infection of individuals over time.

3.3 Pinna Spat project (IMEDMAR and IRTA)

The overall aim of this project funded by the Biodiversity Foundation of the Spanish ministry for ecological transition and demographic challenge was to close the life cycle of *P. nobilis*. The project is divided in two parts, one targeting the maturation of individuals in captivity conditions and another targeting the study of the reproductive period of natural populations in the Alfacs Bay, to obtain gametes that together with those obtained in captivity could lead to the production of viable seed. The first part of the project has now been accomplished and fan mussel gametes can be now obtained from captive animals thanks to improved maintenance techniques and temperature cycles (Hernandis et al., in prep). We have also gained knowledge about the period of local natural spawning in the Alfacs Bay, which appears to be constricted to the month of May, possibly due to high temperatures in shallow population areas during most of the summer (up to 30 °C or more that significantly differ from optimal spawning temperatures at ca. 20 30 °C). However, all attempts to develop larval cultures have, so far, failed, due to high mortalities during larval stages of trochophore and D-veliger, which have not been yet solved.

3.4 Recupera Pinna project (IRTA, University of Alicante, and IMEDMAR)

This project also funded by the Biodiversity Foundation is aimed at evaluating the current status of the populations in the Ebro Delta and Mar Menor, and to gain further understanding on the factors controlling the survival of individuals in these areas. The project also targets the translocation of animals exposed to desiccation that still remain in middle regions of the Alfacs Bay to adjacent deeper waters. Thanks to this project, we have now considerable information about infection risks in different areas of the Alfacs and Fangar Bay, which are detailed in the following sections (monitored areas in Ebro Delta Bays and evaluation of risk areas for parasite infection). Study areas are thought to be representative of wider areas of the bays, there is missing information on the overall number of remaining individuals, which would require a modelling approach using distance sampling methodology and will be conducted during the Pinnarca LIFE project for comparisons with estimates prior to mass mortality events (ca. over 90,000 individuals according to Prado et al., 2014). The different translocation areas of Recupera Pinna are also monitored to assess the possible advance of the disease.

4 MONITORED AREAS AND CANDIDATE ZONES

4.1 Monitored areas in Ebro Delta Bays

The evaluated areas correspond with those still hosting *Pinna nobilis* or in which individuals have been observed in the recent past, and therefore could be suitable for the survival of the species.

In Fangar Bay, a first monitoring area was established at the tip of the peninsula (Fangar zone 1), in which there was a population of more than 500 individuals until January 2020, when it was annihilated by the Gloria storm due to strong salinity reductions and high amounts of suspended solids (Prado et al., 2021). A second monitoring area (Fangar zone 2) was established in an area adjacent to the mussel rafts, in which pen shell sightings have been made in the past but there was no environmental data to assess current suitability for hosting the species (Fig. 1A).

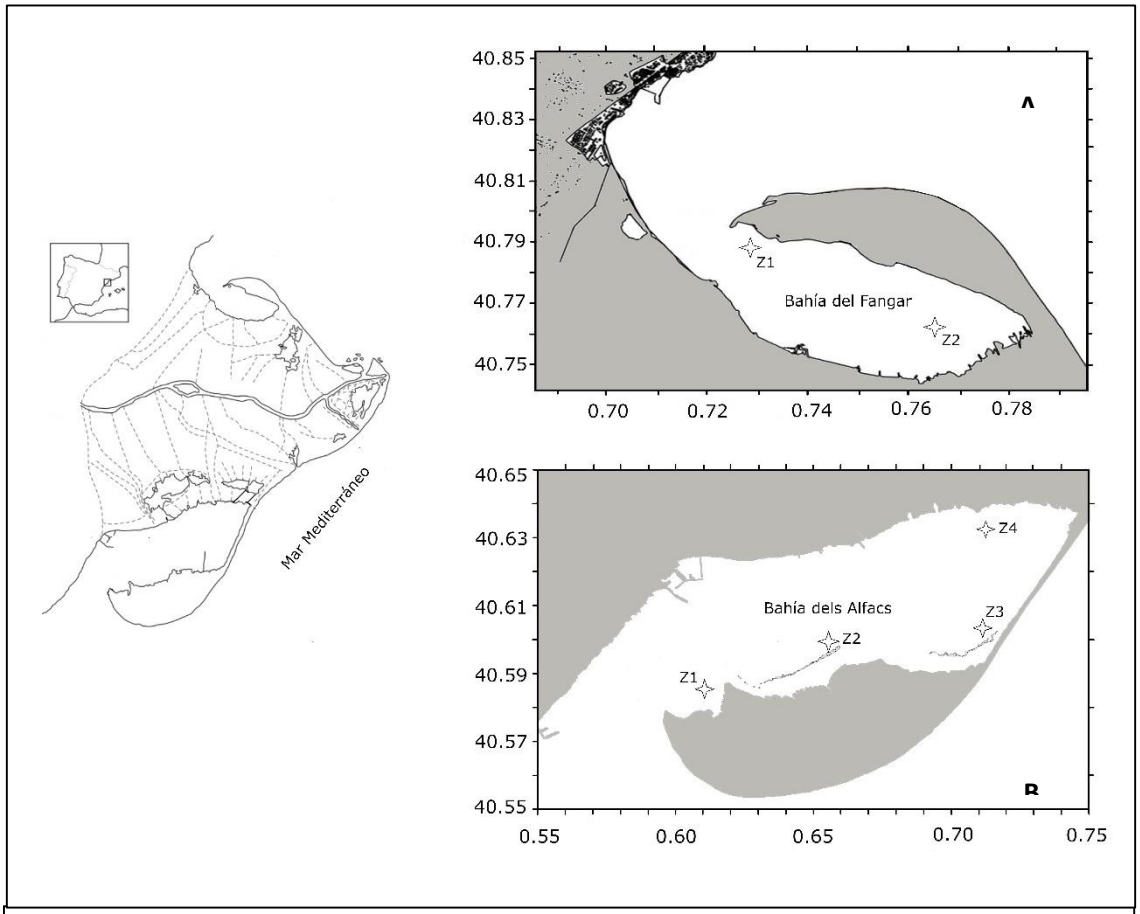

Figure 1. Location of the different areas evaluated in the Fangar and Alfacs Bays between April and December 2021.

Figure 1. Location of the different areas evaluated in the Fangar and Alfacs Bays between April and December 2021.

Four monitoring zones were established in Alfacs Bay. The first of them, located in the outermost part of the Banya peninsula, near the connection with the open sea (Alfacs zone 1), was subject to massive mortalities of specimens in summer 2018 (Prado et al., 2021). A second monitoring zone was established in the middle region of the peninsula (Alfacs zone 2) in which there are still many surviving individuals, but the presence of *H. pinnae* had been detected (Prado et al., 2021). A third location was established in the final section of the Trabucador sandbar (Alfacs zone 3), in which there are also numerous individuals, and had not been evaluated previously. A final monitoring zone (Alfacs zone 4) was established in the vicinity of the Torre de Sant Joan, on the north coast of the bay, in which a preliminary evaluation in an area of ca. 0.6 Ha had been carried out during the crowdfunding campaign *'Not one less pen shell'* in the summer of 2020, and the presence of several hundred apparently healthy individuals were identified (Fig. 1B). Among the different areas evaluated, this area is located right in front of two agricultural water drainage channels from the rice fields that could exert greater protection against pathogens.

4.2 Candidate zones in the Banya Peninsula

The Trinitat saltworks are a privately run salt exploitation that occupies ca. half of the Banya peninsula and contains numerous salt pans that are maintained at stable salinity conditions (Fig. 2). Since the commercial activity is conducted within a protected natural space of the Ebro Delta Natural Parc, the company Ingeniería Forestal S.A. (INFOSA) is committed to the values of biodiversity conservation, mostly waterbirds and endangered salt-tolerant fish, such as the Iberian toothcarp, *Aphanius iberus*, and has also offered their facilities (Fig. 2) to the project for hosting pen shells, shall the conditions be adequate for the species.

In a preliminary meeting with the company and in situ evaluation of salinity conditions by IRTA, it was noted that most of the salt pans have salinities above 50 ppt, which is beyond the ca. 45 ppt superior limit of tolerance inferred from environments hosting fan mussels at those values, such as the Mar Menor lagoon. Only the first salt pan receiving water from the Alfacs Bay features a gradient that ranges between 40 and 50 ppt that could be adequate in some areas closer to the entrance. Yet, this is an extremely shallow area (ca. 20-30 cm depth) and seems inadequate for hosting pen shells. Besides, given the shallow depth and the high isolation degree, temperature ranges are expected to be equal to ambient temperatures, which may locally vary between 0 in some winter nights and 40 ºC at mid-day during summer months, and therefore, is outside the tolerance of the species.

Nonetheless, the seawater entrance channel into the salt pans located in an adjacent saltmarsh was found to be at a salinity of 40-41 ppts, possibly due to more restricted water circulation and greater window of time for evaporation. The area also featured greater depth (ca. 80 cm), which could buffer the effect of ambient temperature but need to be further investigated for seasonal variation. Other shallow saltmarsh areas adjacent to the salt pans but outside INFOSA facilities (Fig. 2) will be also explored for further evaluation of their physicochemical conditions and adequacy to host the species.

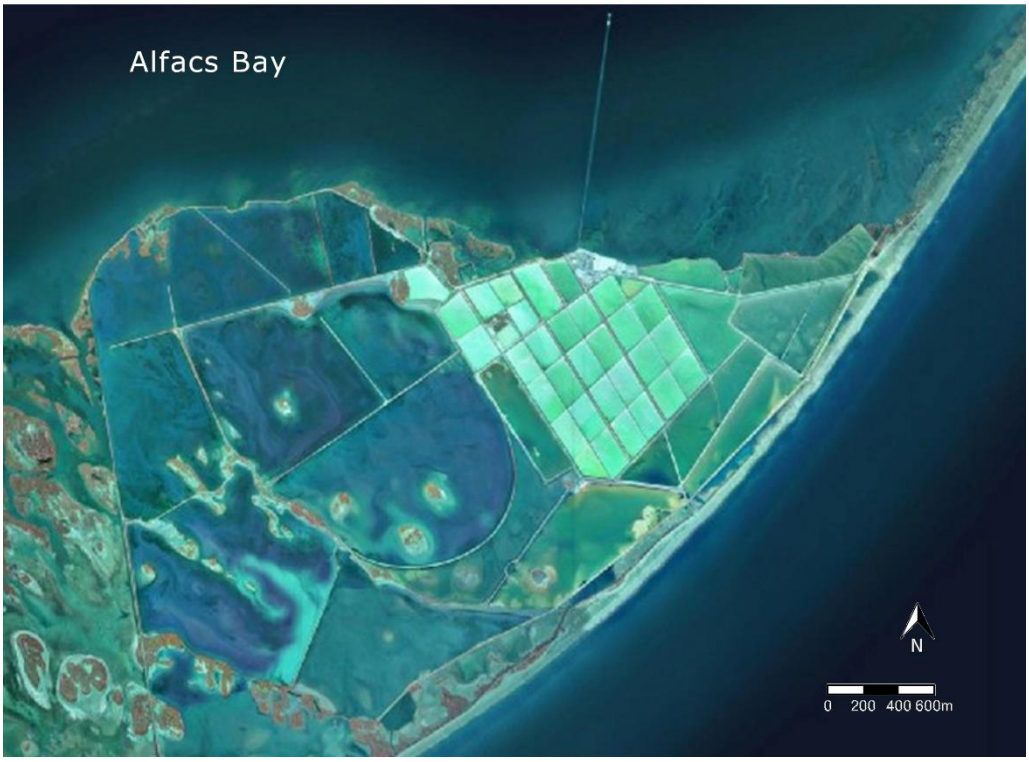

Figure 2. Aerial view of the Trinitat salt pans in the inner part of Banya Peninsula showing the different water pools. The white square indicates the candidate area for evaluation.

5 EVALUATION OF RISK AREAS FOR PATHOGEN INFECTION

Initially, an attempt was made to continuously evaluate salinity and temperature variables -considered as the most relevant for pen shell survival in the context of the current pandemic- by installing 6 data loggers (HOBO® U24 Conductivity Logger model (U24-002-C)). Unfortunately, the bays of the Ebro Delta are richer in nutrients than the open waters of the Mediterranean, and the units were highly epiphytic or covered with suspended sediment within a few days of their installation, -even with the use of protective covers- thus causing an alteration of the readings from the moment in which the sensor was affected and preventing a correct final calibration (two calibrations, initial and final are necessary for a correct data collection). Consequently, there was a large discrepancy (of up to several salinity units) between the data collected with the Hobos and those obtained with the YSI 660 multiparameter probe coupled to an MDS 650 data logger used during the calibration process.

Given that the problematics with the Hobos was observed in February and March 2021 when the temperature was not yet limiting for the growth of macroalgae, it was concluded that this methodology was not suitable in the areas of study. This problem conditioned the collection of FC variables, which we aimed to perform monthly (data collection and cleaning of the data loggers) to weekly, thus quadrupling the sampling effort. This effort - carried out weekly with

the YSI probe - is the same as that that conducted in other monitoring campaigns within the bays financed by the public administration to determine the environmental conditions in culture areas of the Pacific oyster and the Mediterranean mussel (http://www.marinemonitoring.org/) and was considered more rigorous in terms of data quality, despite the obvious limitation in the number of temporal point measurements.

The use of the multiparametric probe also allowed the taking of other FC variables not initially considered, such as oxygen saturation in the water (mg · L^{-1}), and pH; although values obtained across zones are within normal ranges and are not identified as a relevant factor for the definition of risk areas (see Table 1).

	Fangar Bay				Alfacs Bay							
Month	**O_2-Z1**	**pH-Z1**	**O_2-Z2**	**pH-Z2**	**O_2-Z1**	**pH-Z1**	**O_2-Z2**	**pH-Z2**	**O_2-Z3**	**pH-Z3**	**O_2-Z4**	**pH-Z4**
April 21	7.25	8.09	7.21	8.15	7.2	8.2	7.7	8.2	7.95	8.09	11.7	8.2
May 21	7.50	8.13	7.37	8.12	7.4	8.0	7.8	8.08	8.07	8.12	11.8	8.12
Jun 21	7.07	8.13	6.63	8.17	6.8	8.2	7.6	8.24	7.63	8.26	7.66	8.26
Jul 21	6.34	8.04	5.69	8.04	6.6	8.2	6.2	8.23	6.91	8.27	6.59	8.25
Aug 21	5.89	8.08	5.42	8.03	5.5	8.1	5.5	8.21	6.24	8.17	5.43	8.08
Sept 21	6.83	8.11	6.34	8.03	6.2	8.2	7.3	8.37	7.15	8.22	6.25	8.34
Oct	7.1	8.0	7.7	8.0	6.5	8.1	8.0	8.3	8.2	0.81	6.8	8.3
Nov	7.4	8.3	8.6	23.5	7.5	8.2	7.3	8.3	8.2	1.55	7.3	8.3
Dec	8.3	8.4	8.08	8.24	7.87	8.3	8.06	8.26	8.37	8,24	8.38	8.25

Table. 1. Mean monthly values of O_2 (mg · L^{-1}) and pH recorded between April and December 2021 in the different areas of the Fangar and Alfacs Bays.

On the contrary, salinity and temperature measurements allowed to establish a classification of risk areas that included both the risk of pen shell infection by *H. pinnae* and/ or *Mycobacterium* sp., and the environmental conditions that the species need for long-term survival.

For the Trinitat saltworks, salinity conditions are stable and therefore were measured only once in December 2021 to assess their potential use for hosting pen shells. A Master-S/Millox refractometer (Atago) was used instead of the YSI probe since salinity conditions in most salt pans were foreseen to be above the common range for marine waters and would have required two different probes calibrated for measuring at different ranges.

A) Fangar Zone 1: Low infection risk, suboptimal environmental conditions for the species.

The area is considered to feature a low risk of infection due to low salinity values, persistently below the infection threshold at 36.5 ppt during the entire study period (both

monthly averages and maximum values; see Fig. 3A). Minimum recorded values are close to 28 ppt, which, despite being tolerated by pen shells, are far from the conditions that could be considered optimal for the species. However, these are apparently short-term declines, since recorded monthly averages are at least 30.5 ppt.

Regarding temperature, throughout the evaluated period (April to December) temperatures above 13-13.5 ºC are recorded, from which the parasite is capable of infecting (Fig. 3B). Currently the area host 4 healthy individuals that resisted the pass of the Gloria storm, so protection against infection is presumed to be exclusively based on local salinity conditions.

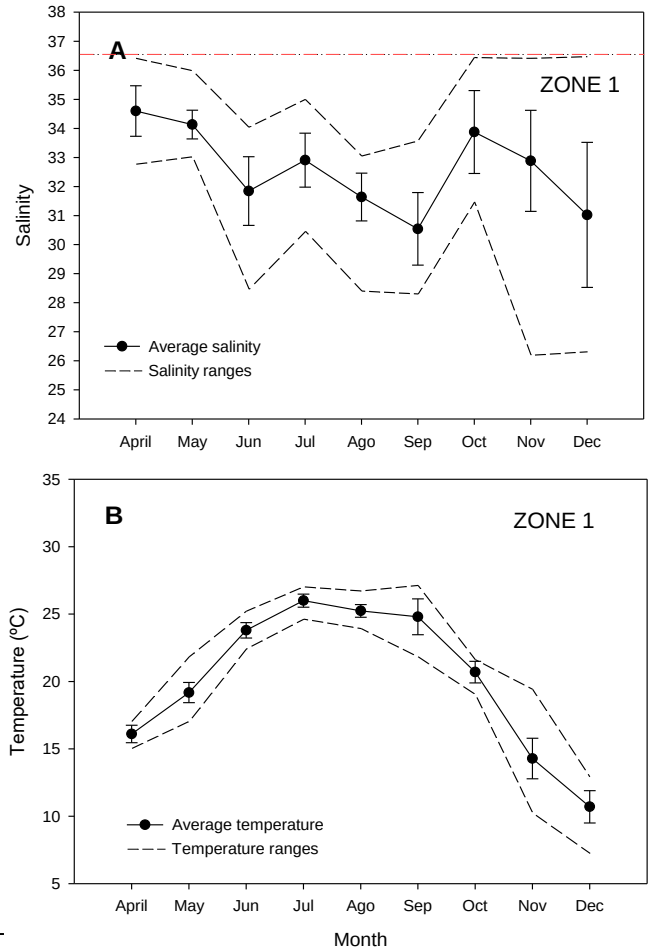

Figure 3. Monthly average values of A) salinity and B) temperature in zone 1 of Fangar Bay (tip of the Fangar peninsula). In Fig. 2A the dashed red line indicates the parasite infection threshold at 36.5 ppt. Dotted lines indicate maximum and minimum monthly values. Error bars are SE.

B) Fangar Zone 2: Low infection risk, current environmental conditions for species of questionable viability.

This area also presents a low risk of infection due to salinity values consistently below 36.5 ppt during the study period. However, monthly averages of only 27.8 ppt were observed in September, with large fluctuations that can reach levels of 24.7 ppt (Fig. 4A). Although the

species is capable of tolerating salinity drops down to 19 ppt (Hernandis et al., 2018), the recurrence and duration of these episodes requires further evaluation.

Temperature conditions were very similar to those of zone 1 and, therefore, the absence of the disease in the zone during the evaluated period is also attributed to salinity (Fig. 4B).

C

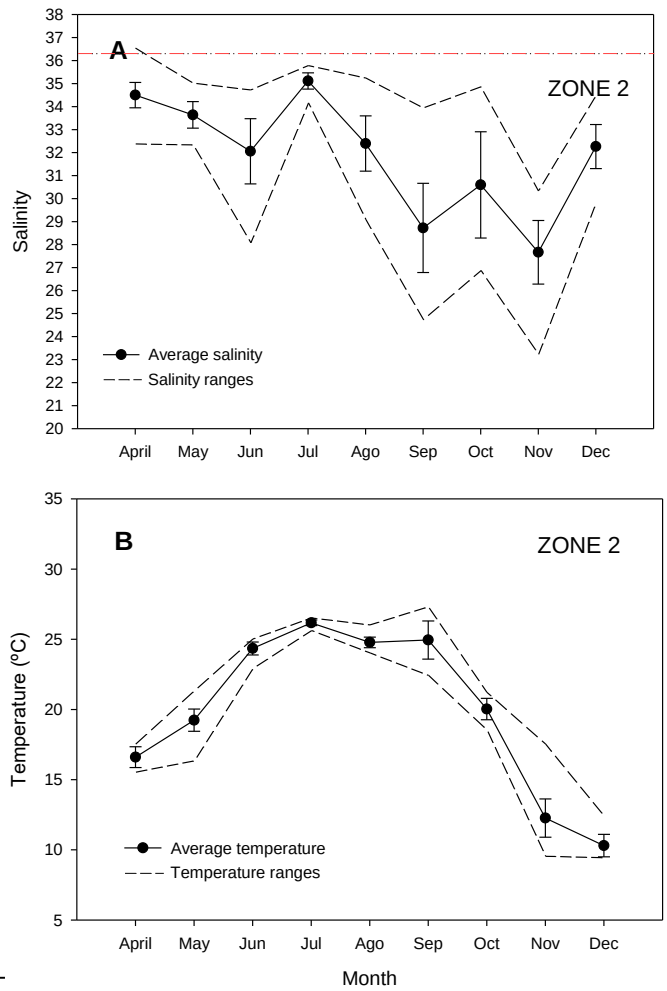

Figure 4. Monthly average values of A) Salinity and B) temperature in zone 2 of the Fangar Bay (adjacent to mussel farming areas). Legend symbols as in Fig. 3.

C) Alfacs Zona 1: Very high risk of infection, optimal environmental conditions for the species.

The area was infected by *H. pinnae* with the presence also of *Mycobacterium* sp. during the summer of 2018. Since then, the number of surviving specimens, mostly young adults from the 2017 recruitment, has not stopped decreasing. Currently IRTA has identified 6 surviving individuals (5 young adults and 1 older adult) that are subjected to periodical monitoring. The area is located in the part closest to the mouth of the bay, and features salinity values higher than 36.5 ppt between May and August, with a peak in July, the month in which the initial infection was detected in 2018 (Fig. 5A) (Prado et al., 2021).

Temperature conditions are similar to those observed in the Fangar Bay, with values above 13-13.5 ºC throughout the study period, which cannot act as a protective barrier against the disease (Fig. 5B).

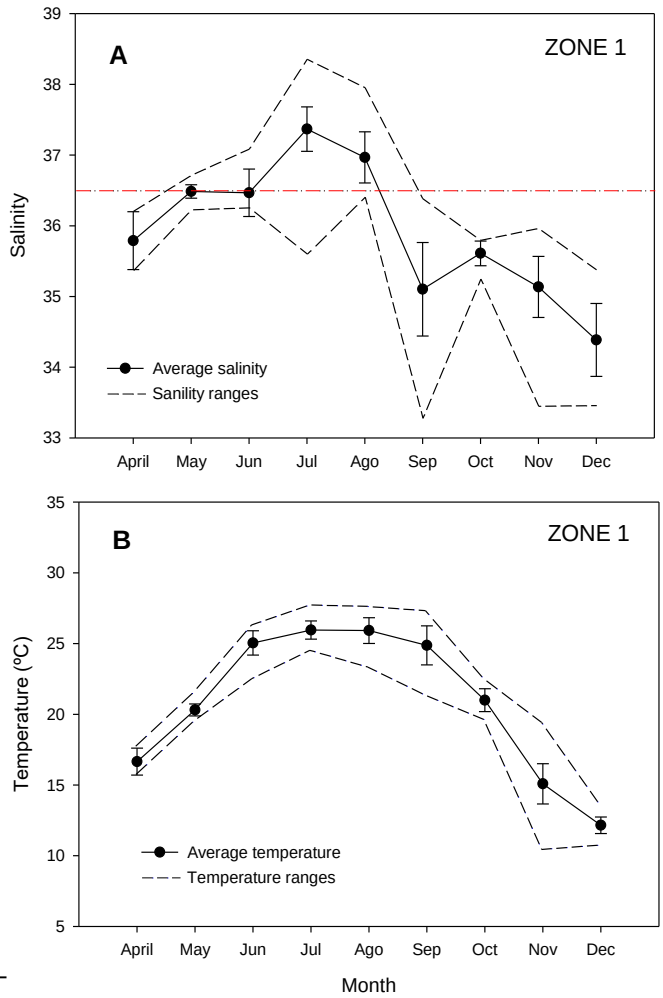

Figure 5. Average monthly values of A) salinity and B) temperature in zone 1 of Alfacs Bay, near the connection with the open sea. Legend symbols as in Fig. 3.

D) Alfacs Zone 2: High risk of infection, optimal environmental conditions for the species.

Zone 2 is located in the middle region of the Banya Peninsula and host a large number of individuals. During censuses conducted in the area between May and June 2021, a total of 1,440 living individuals were counted in only 4.1 Ha. However, the presence of *H. pinnae* was already detected in the area by Prado et al., (2021). During the second half of July 2021, salinity values higher than 36.5 ppt were detected, thus increasing the risk of infection, and spread of the disease (Fig. 6A). In fact, recent observations (end of September 2021) have evidenced high mortality in this zone (12.3 to 37.5%) but with areas of up to 56.7 to 65.2%, presumably associated with the above-mentioned increase in salinity and other factors causing patchiness in disease spread. In a subsequent survey at the end of December, mortality was much reduced (0 to 4.5%), although higher rates (11.1 to 17.3%) were still found in the same areas featuring higher mortality at the end of the summer. Recent results for tissue samples from three ailing

individuals collected during the mortality event evidenced the presence of both *H. pinnae* (1 individual) and *Mycobacterium* sp. (2 individuals) in the area. It is expected that salinity and temperature conditions during the winter will confer protection against the disease, but the possible recurrence of these episodes during the following summers can compromise the survival of the species in this area in the short or medium term, since cumulative mortalities decrease the reproductive potential and the resilience of populations.

No significant changes in temperature conditions are observed with respect to zone 1 (Fig. 6B).

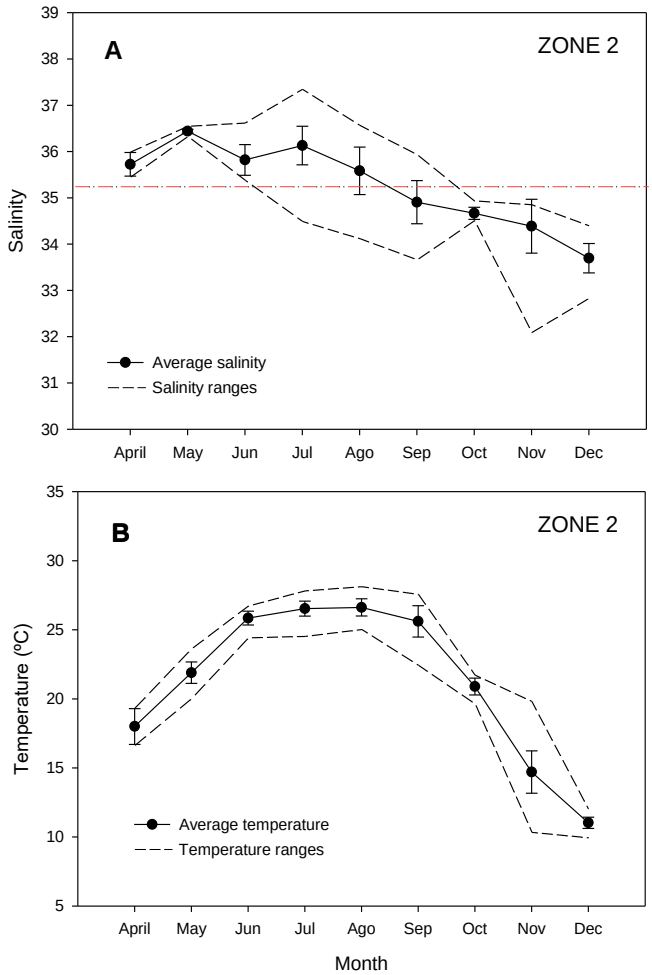

Figure 6. Average monthly values of A) salinity and B) temperature in zone 2 of Alfacs Bay, in the central region of the Banya Peninsula. Legend symbols as in Fig. 3.

E) Alfacs Zone 3: Medium-high risk of infection, optimal environmental conditions for the species.

Zone 3 is located in the far end of the Trabucador bar and also host a large number of living individuals, although less than zone 2. During censuses carried out from February to April 2021, a total of 853 living individuals were counted in 5,7 Ha., this being the first evaluation of the area. Some mortality was also observed during the summer, although lower than that of area 2. Local salinity conditions were also above the critical threshold of 36.5 ppt, although for shorter periods (one week in July and one in August), which may have slowed the spread of the

disease (ca. 15 to 17% mortality in late September, and 4% in December), although the risk remains considerable (Fig. 7A).

No significant changes in temperature conditions are observed with respect to the other areas of the bay (Fig. 7B).

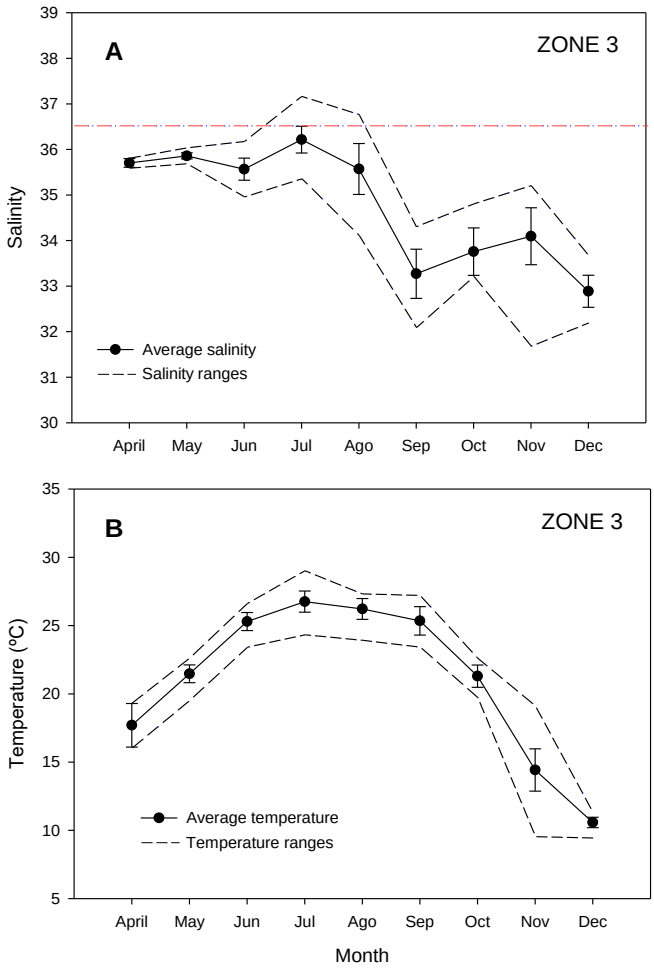

Figure 7. Average monthly values of A) salinity and B) temperature in zone 3 of Alfacs Bay, located in the far end of the Trabucador bar. Legend symbols as in Fig. 3.

F) Alfacs Zone 4: Low risk of infection. A priori optimal environmental conditions for the species, in the absence of data on possible effects of organic loads and sediments from irrigation channels, as well as that potential agrochemical compounds.

Zone 4 is the only one located on the north coast of the bay, although the area occupied by the species must be studied in more detail. The proximity to agricultural drainage channels and the silty consistency of the sediments appears to limit the development of populations, although at the same time they confer enhanced protection against the parasite compared to other areas of the bay. During the censuses carried out in a total of 4.2 Ha of this area, the total number of live individuals counted was 404, without evidence of disease mortality after the summer. In fact, salinity conditions throughout the study period were consistently below 36.5

ppt. However, high variability was observed in June and July 2021 (Fig. 8A), associated to intermittent freshwater discharges during this period and the higher proximity to the drainage area.

There was neither a significant difference in temperature conditions compared to other bay areas (Fig. 8B).

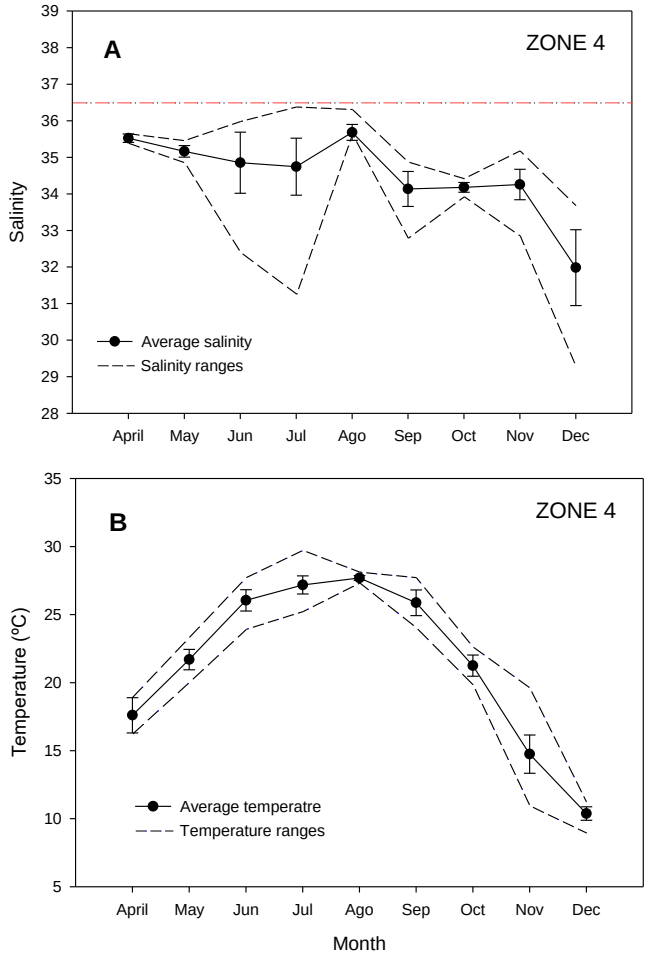

Figure 8. Monthly averages of A) salinity and B) temperature in zone 4 of Alfacs Bay, located on the north coast, close to the St. Joan Tower. Legend symbols as in Fig. 3.

6 EVALUATION OF OTHER LOCAL RISKS

Aside the risk of pathogen infection, pen shells populations in the Ebro Delta are threatened by other factors of anthropogenic nature.

The most pressing, is the severe thinning of the Trabucador sand bar, which separates the Alfacs Bay from the open sea and allows the maintenance of the estuarine conditions. Decreased salinities are the only factor capable of slowing down the spread of the pathogens across the remaining population and are also responsible for the unique biodiversity and productive conditions of the bay, allowing extensive bivalve aquaculture. The combined action of climate change, sea level rise, and lack arrival of riverine sediments due to trapping in Ebro River reservoirs has contributed to the weakening of the sand bar, which is often subjected to breakage during heavy storms such as the Gloria and the Filomena Storms in January 2020 and 2021, respectively (Pintó i Fusalba et al., 2020). After the Gloria Storm, the bay remained connected to the open sea until May 2020, when the Spanish Coastal Service reallocated sand from accumulation areas into breakage areas. On the one hand, this 5-month opening period appear to have allowed the direct entrance of the parasite to pen shell habitats of the Trabucador (zone 3 Alfacs Bay), as suggested by recent research in the area showing higher aggregation patterns in death than in living individuals (Prado et al., submitted). On the other, since temperature conditions constrict the local reproductive period of the species to the month of May, the opening also provided a way out for pen shell larvae, as evidenced by the later finding of infected juvenile pen shells in the outer sea by fishing trawlers (P. Prado, personal communication).

Currently, an action plan for the preservation of the Ebro Delta is being evaluated by the Spanish government (https://www.miteco.gob.es/es/costas/participacion-publica/planparalaprotecciondeldeltadelebro_jmg_signedfe_tcm30-522381.pdf). However, the timing for the implementation of conservation measures is uncertain, while the risk for pen shell loss in case of another breakage would be immediate. Infosa, the company conducting saltwork exploitation, is responsible for rutinary maintenance of the sand track that connects the Trabucador with the Banya Peninsula but cannot implement a deep reconstruction of the sand bar (neither legally nor economically) if destructed by severe weather conditions, which is in the hands of the Spanish coastal service. To date, the lack of the necessary environmental impact assessments for extractions permits (a ca., 3-year period required to obtain the license), have constricted the works conducted to repair the sand bar, which have merely consisted in the reallocation of sand from accumulation areas into breakage areas. However, this is only a short-term solution until the next severe weather event, thus posing pen shells on a razor edge.

Other local risks in the Alfacs Bay include the use of shallow pen shell habitats by many boating and aquatic activities. Given the extremely shallow distribution of pen shells in many areas of the bay -ranging from ca. 20 to 130 cm-, the chances of collision with boat propellers in highly increased, resulting in direct shell breakage and/or knock down of individuals, that end up dying due to burial during storms and swells. Besides, the activity also causes scarring of the seagrass bed of *Cymodocea nodosa*, one of the main local habitats. More recently, another sailing activity, foil surf, featuring a keel of ca. 60 cm have also emerged, causing additional impacts. Part of the problem is the absolute lack of indications about the presence of shallow seagrass areas and the presence of pen shells. A project aimed at beaconing certain areas with

higher densities of pen shells has been presented by the Catalonian government and would be implemented by the Ebro Delta Natural Parc, but it has not been yet resolved by the Spanish government. At the same time, the implementation of local surveillance by rural agents and/ or SEPRONA is also needed. During the summer of 2021, a group of six potentially resistant pen shells in a reservoir area was destroyed by despoilers (some were broken and some lost) and the other six remaining individuals had to be moved into an unmarked area, unnoticeable to passing boaters.

7 CONCLUSIONS

- The two areas evaluated in Fangar Bay show a low risk of infection by the disease caused by *H. pinnae* and / or *Mycobacterium* sp. due to their low levels of salinity (monthly averages between ca. 29 and 35 ppt). However, some of the observed salinity values may pose a certain stress for the species, particularly in zone 2, in which there are currently no specimens and salinities of only 24.7 ppt are reached. Although the survival of pen shells has been documented at salinities of 19 ppt for short periods of time -in the order of a few days- (Hernandis et al., 2019), the classification of the area as optimal for the survival of the species requires further valuation. On the other hand, due to its small size and shallow depth, the bay is especially vulnerable to extreme temporary events (e.g., recent Gloria and Filomena storms), which also condition the long-term survival of the species.

- In zone 1 of Alfacs Bay, the proximity to the open sea favors high salinity values that are within the favorable range for infection and transmission of the disease, particularly during July and August. Mortality rates in this area are estimated to be around 99.9%, with only 4 individuals (young adults from the 2017 recruitment) surviving as of October 2021.

- Zone 2 of Alfacs Bay, located in the middle region of the Banya Peninsula, has suffered a drip of individuals since the disease entered the bay in July 2018 (Prado et al., 2021a) and it appears to be exposed to more critical mortality rates during the summer of 2021, because of the sustained rise in salinity above 36.5 ppt during the last two weeks of July. During the winter mortalities appear to decrease significantly, but whether they will increase again the following summers remains to be seen, and the area is therefore considered to be at high risk for the spread of the disease.

- Zone 3 of the Alfacs Bay has not been evaluated until 2021. Under the integrity of the Trabucador sand Bar, it seems to follow an infectious dynamic similar to zone 2, although milder, which is associated to shorter duration of salinity periods above the infection threshold, and higher proximity to agricultural drainage channels. As in the previous area, the samplings carried out over the next spring and summer will be critical to determine the progression or stabilization of the disease, but the risk for the species is considered between medium and high, due to the proximity of the values of salinity at the threshold of infection, particularly during July and August. Yet, if the Trabucador sand bar breaks during the winter period and is not properly repaired (as foreseen due to the absence of

permits to conduct the works), the risk of infection will be greatly increased and may further compromise the remaining population in zone 2.

- Zone 4, located on the north coast, is the only one in Alfacs Bay that can be considered to have a low risk of infection due to its proximity to the drainage channels, which allow maintaining salinities below 36.5 ppt. Yet, the area is exposed to important contributions of organic matter and possible agrochemicals that constrict the growth of the population. Most of the individuals in the area are small adults, which suggests that there is a limited life expectancy due to environmental conditions, which is supported by the considerably lower local abundance of individuals compared to other bay areas. In a work carried out by Prado et al., (2020b), the authors describe the presence of ~20% of simultaneous hermaphrodites, a condition not described in other populations of pen shell (the species is sequential hermaphrodite) and that could be attributed to induced stress due to the presence of endocrine disruptors of agricultural origin present in irrigation canals, lagoons, and bays of the Ebro Delta (Brossa et al., 2005).

- In general, for the different areas of the bay, the months of greatest risk of infection are concentrated in the period between July and September, in which the highest salinities are observed. In contrast, November to March would be the months with higher risk of breakage of the Trabucador sand bar.

8 RECOMENDATIONS

- The Trabucador sand bar is a necessary element for maintaining the estuarine conditions and the continuity of the populations in the Alfacs Bay and need to be preserved intact. In particular, the possible overlapping of sand bar breakage (due to lack of repair) and high temperatures should be avoided at all costs. It is necessary to start implementing a maintenance plan for the Trabucador sand bar.

- Given the proximity and high number of drainage channels from the rice fields that flow into the bay, in order to avoid the spread of the disease, it would be advisable to carry out preventive management, through specific and controlled contributions of fresh water that would keep the salinity gradient below of 36.5 ppt in July and August.

- Agricultural waters that are discharged into the bays, particularly in the canals that are immediately in front of pen shell populations (zone 4 Alfacs) should first pass through a green filter to reduce the contribution of sediments and other harmful elements for pen shells such as possible agrochemicals. Without proper salinity management, this area could constitute the last stronghold for pen shells in Alfacs Bay, and therefore any possible environmental impact that could negatively affect the survival of the species should be minimized.

- In regards of boating and sailing activities, as well as despoiler events, several approaches are recommended. First, some specific activities such as foil surf should be banned in the bay and conducted instead in the open sea, where no pen shells are present. Second, beaconing shallow areas coupled with dissemination campaigns to the general public is necessary to raise awareness about pen shell collisions and the importance of preserving the species for future generations. Third, further surveillance by local authorities is also necessary to enforce good practices across minorities that are not properly reached through dissemination activities.

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