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1 **Can we save a marine species affected by a highly infective, highly lethal, waterborne**
2 **disease from extinction?**

3

4 García-March JR^{a*}, Tena-Medialdea J^a, Henandis-Caballero S^{a,b}, Vázquez-Luis M^c, López D^a,
5 Téllez C^a, Prado P^d, Navas JI^e, Bernal J^a, Catanese G^{f,g}, Grau A^{f,g}, López-Sanmartín M^e, Nebot-
6 Colomer E^{a,c,h}, Ortega Aⁱ, Planes S^h, Kersting D^j, Jimenez S^k, Hendriks I^{l,m}, Moreno Dⁿ, Giménez-
7 Casalduero F^o, Pérez M^p, Izquierdo A^o, Sánchez J^q, Vicente N^{r,s}, Sanmarti N^p, Guimerans M^t,
8 Crespo JL^t, Valencia JM^{f,g}, Torres J^a, Barrajon Aⁿ, Álvarez E^c, Peyran C^h, Morage T^h, Deudero S^c.

9

10 ^aInstitute of Environment and Marine Science Research (IMEDMAR-UCV), Universidad Católica de
11 Valencia SVM. C/Explanada del Puerto S/n, 03710, Calpe (Alicante), Spain.

12 ^bEscuela de Doctorado, Universidad Católica de Valencia San Vicente Mártir

13 ^cInstituto Español de Oceanografía (IEO). Centro Oceanográfico de Baleares. Muelle de Poniente s/n,
14 07015. Palma de Mallorca, Spain

15 ^dIRTA-Aquatic Ecosystems, Ctra. Poble Nou Km 5.5, 43540 Sant Carles de la Ràpita (Tarragona), Spain

16 ^eIFAPA Agua del Pino, Ctra. El Rompido-Punta Umbría 3.8, Cartaya, Huelva, Spain

17 ^fLIMIA Govern de les Illes Balears, Av. Gabriel Roca 69, 07158 Port d'Andratx, Mallorca, Spain

18 ^gINAGEA (INIA-CAIB-UIB) Ctra. Valldemossa, km. 7,5 Ed. Edifici Guillem Colom Casasnoves, Palma de
19 Mallorca, Spain

20 ^hPSL Research University: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, 52 Avenue Paul
21 Alduy, 66860 Perpignan Cedex, France

22 ⁱInstituto Español de Oceanografía (IEO). Centro Oceanográfico de Murcia. Carretera de la Zoia s/n,
23 Puerto de Mazarrón. 30860 Murcia

24 ^jWorking Group on Geobiology and Anthropocene Research, Institute of Geological Sciences, Freie
25 Universität Berlin, 12249 Berlin, Germany

26 ^kInstituto de Ecología Litoral. C/ Santa Teresa, 50. 03560, El Campello (Alicante) Spain.

27 ^lGlobal Change Research Group, IMEDEA, Instituto Mediterráneo de Estudios Avanzados, C/Miquel
28 Marqués, 21, 07190, Esporles, Spain

29 ^mBiology department, University of the Balearic Islands (UIB), crta. Valldemossa km 7,5, 07122, Palma de
30 Mallorca, Spain

31 ⁿAgencia del Medio Ambiente y Agua de Andalucía. Junta de Andalucía. C/ Johan G. Gutenberg, 1 - Isla
32 de la Cartuja. 41092 - Sevilla Spain

33 ^oDepartment of marine Science and Applied biology. University of Alicante. Carretera de Sant Vicent del
34 Raspeig s/n, 03690-San Vicente del Raspeig. Alicante (Spain)

35 ^pDepartment of Evolutionary Biology, Ecology and Environmental Sciences. University of Barcelona, Av.
36 Diagonal 643, 08028 Barcelona, Spain

37 ^qSUBMON® - Divulgació, estudi i conservació del medi marí. C/ Rabassa, 49-51, 08024 Barcelona, Spain

38 ^rInstitut Océanographique Paul Ricard, Ile des Embiez, 83140 Six Fours les Plages, France

39 ^sInstitut Méditerranéen de la Biodiversité et de l'Ecologie marine et continentale (IMBE), Aix-, Marseille

40 Université, France

41 ^tFundación Oceanogràfic, Avanqua-Oceanogràfic, Ciutat de les Arts i les Ciències. C/Eduardo

42 Primo Yúfera 1B, 46013 Valencia, Spain

43

44 *corresponding author: jr.garcia@ucv.es

45 contact telephone: +34 617845340

46

47 García-March JR, e-mail: jr.garcia@ucv.es

48 Tena-Medialdea J, e-mail: josetena@ucv.es

49 Henandis-Caballero S, e-mail: sebastia.hernandis@ucv.es

50 Vázquez-Luis M, e-mail: maite.vazquez@ieo.es

51 López D, e-mail: diana.lopez@ucv.es

52 Téllez C, e-mail: clara.tellez@ucv.es

53 Prado P, e-mail: patricia.prado@irta.cat

54 Navas JJ, e-mail: josei.navas@juntadeandalucia.es

55 Bernal J, e-mail: jaimebernal@hotmail.com

56 Catanese G, e-mail: gcatanese@dgpesca.caib.es

57 Grau A, e-mail: amaliagrau@dgpesca.caib.es

58 López-Sanmartín M, e-mail: monserrat.lopez.s@juntadeandalucia.es

59 Nebot-Colomer E, e-mail: eli.nebotcolomer@gmail.com

60 Ortega A, e-mail: aurelio.ortega@ieo.es

61 Planes S, e-mail: planes@univ-perp.fr

62 Kersting D, e-mail: diegokersting@gmail.com

63 Jimenez S, e-mail: s.jimenez@ecologialitoral.com

64 Hendriks I, e-mail: iris@imedea.uib-csic.es

65 Moreno D, e-mail: diego.moreno@juntadeandalucia.es

66 Giménez-Casalduero F, e-mail: francisca.gimenez@ua.es

67 Pérez M, e-mail: maperez@ub.edu

68 Izquierdo A, e-mail: andres.izquierdo@ua.es

69 Sánchez J, e-mail: jordisanchez@submon.org

- 70 Vicente N, e-mail: nardo.vicente@sfr.fr
- 71 Sanmarti N, e-mail: neussanmarti@ub.edu
- 72 Guimerans M, e-mail: mguimerans@oceanografic.org
- 73 Crespo JL, e-mail: jlcrespo@oceanografic.org
- 74 Valencia JM, e-mail: jmvalencia@dgpesca.caib.es
- 75 Torres J, e-mail: javier.torres@ucv.es
- 76 Barrajon A, e-mail: agustin.barrajon@juntadeandalucia.es
- 77 Álvarez E, e-mail: elvira.alvarez@ieo.es
- 78 Peyran C, e-mail: claire.peyran@univ-perp.fr
- 79 Morage T, e-mail: titouan.morage@gmail.com
- 80 Deudero S, e-mail: salud.deudero@ieo.es

1 **Can we save a marine species affected by a highly infective, highly lethal, waterborne disease**
2 **from extinction?**

3
4 **Abstract**

5 Anthropogenic drivers and global warming are altering the occurrence of infectious marine
6 diseases, some of which produce mass mortalities with considerable ecosystemic and economic
7 costs. The Mediterranean Sea is considered a laboratory to examine global processes, and the
8 fan mussel *Pinna nobilis* a sentinel species within it. Since September 2016, fan mussels suffer a
9 die-off, very likely provoked by the protozoan *Haplosporidium pinnae*. Population dynamic
10 surveys, rescue programmes, larvae collector installation and protection of infected adults from
11 predators, have increased knowledge about the factors conditioning the spread of the die-off;
12 previous model simulations indicate that water temperature and salinity seem to be related to
13 the manifestation of the disease, which at the end are strongly influenced by climate change
14 and anthropogenic actions. The absence of natural recruitment implies that fan mussel
15 populations are not recovering, but the survival of populations living in paralic environments
16 provides an opportunity to study the disease and its conditioning factors. The present situation
17 is proposed as an example of what is to come in the global context of climate change and poses
18 several questions: are we the witnesses of the potential extinction of a sentinel species? Can we
19 avoid the potential extinction of this species by applying active measures, and which measures
20 will be more effective? How many other more overlooked species might experience a massive
21 and unnoticed die-off before it is too late to implement any preservation action? For the
22 extinction of community structure species will provoke unpredicted ecological cascade effects
23 with global implications.

24
25 **Key words:** *Pinna nobilis*; *Haplosporidium pinnae*; Mediterranean endemism; mass mortality;
26 protozoan; parasite

27
28 **1. Introduction**

29
30 Anthropogenic drivers and global warming are altering the occurrence of infectious marine
31 diseases, in opposing directions depending on taxon and the nature of the impact Tracy et al.,
32 (2019). Ectothermic hosts with parasites with environmental transmission stages that can
33 persist outside the host are among the most vulnerable species to global warming (Altizer et al,
34 2013). Reduction in biodiversity, translocation of species and alteration of natural habitat and
35 community composition due to human action and climate change, facilitate the occurrence of

36 pandemic infectious marine diseases, with considerable economic and ecosystemic costs
37 (Lafferty, et al., 2015; Harvell et al., 2019). However, both, evaluating whether disease is
38 increasing or decreasing in the ocean, and quantifying anthropogenic drivers of infectious
39 marine disease, is challenging owing to the lack of baseline data and of long-term records (Tracy
40 et al., 2019). On this regard, sentinel species such as the Mediterranean endemic fan mussel
41 *Pinna nobilis* (Basso et al, 2015) could provide information on what is to come to global ecology
42 in the context of the present environment overexploitation. Globally, several authors have
43 pointed out that the Mediterranean Sea could be used as a natural laboratory to study
44 geodynamic and paleoclimatic processes on different scales and as a miniature model of the
45 world's oceans (Krijgsman, 2002; Lejeune et al, 2010).

46 Fan mussel populations have been recently devastated by a die-off that is very likely to be
47 associated with the protozoan *Haplosporidium pinnae* (Catanese et al., 2018). The first reports
48 of the die-off occurred in September 2016 in southeastern Spain (Vázquez-Luis et al., 2017), and
49 it has since spread throughout all Spanish Mediterranean coasts, reaching France, Italy, Greece,
50 Cyprus and other Mediterranean countries, in less than two years (IUCN 2018a). To date, data
51 indicate that the protozoan species is specific to *P. nobilis*, leaving other invertebrates
52 unaffected, including the congeneric species *P. rudis* (Catanese et al., 2018). Prevalence and
53 subsequent mortality virtually reach 100% in infected populations. Previously reported deaths
54 caused by protozoa that affect commercial bivalve species such as *Crassostrea virginica* or
55 *Ostrea edulis* can be massive but do not usually reach 100% (Culloty and Mulcahy, 2007; Bower,
56 2014). Although commercial exploitation of these species has collapsed in some areas, their
57 extinction was never considered a possibility. After many decades, the selection of cohorts that
58 are resistant to parasites (naturally and artificially) has improved the aquaculture of these
59 bivalves (Dégremont et al., 2015). Growing them in low-salinity deltaic water has also been
60 effective for attaining commercially profitable production, although this strategy is
61 compromised during dry years (Soniati et al., 2008). Likewise, previous reported bivalve die-offs
62 in the Mediterranean Sea affecting *Spondylus gaederopus* (Meinesz, 1983; Kersting et al., 2006)
63 and *Arca noae* (Botari et al., 2017) showed reduced mortality percentages and/or extensions
64 compared with observed die-offs of fan mussels. Although dramatic, these die-offs did not
65 suppose a short-term risk of extinction for the affected species.

66 When in early 2017 a Haplosporidian protozoan was found to be the most likely causal agent of
67 the fan mussel die-off, a group of experts following up the event predicted a high risk that the
68 disease would be spread by marine currents throughout the Spanish Mediterranean coasts and
69 beyond its borders in the following months and years, potentially unimpeded due to the absence
70 of marine barriers (Vázquez-Luis et al., 2017). This observation, together with the characteristic

71 biology of fan mussels, could, eventually, cause the ecological, if not total, extinction of the
72 species, which would represent an unprecedented process in the Mediterranean Sea. As a
73 consequence, Spanish authorities changed the status of *P. nobilis* from "endangered" to
74 "endangered with extinction" along the Spanish coasts (Orden TEC/596/2019, de 8 de abril). The
75 International Union for Conservation of Nature (IUCN) also raised the attention of neighbouring
76 countries regarding the imminent arrival of the parasite to their coasts, which could be
77 considered for each country along the Mediterranean (IUCN, 2018b). Recently, a compilation of
78 recommended actions has been released by IUCN (IUCN, 2019). A follow-up of as many as
79 possible of the remaining Spanish populations was initiated, while a rescue programme to
80 maintain individuals in captivity before die-off caused by parasite arrival, was proposed by the
81 Spanish experts and started as early as 2017. The follow-up has helped to better understand the
82 spread of the disease, with surface currents being the main factor influencing local dispersion,
83 whereas disease expression seems to be closely related to temperatures above 13.5°C and to a
84 salinity range between 36.5–39.7 psu (Cabanellas-Revoredo et al., 2019). Rescued individuals
85 have provided valuable advances in the study of the Haplosporidian parasite (Catanese et al.,
86 2018), helping to prepare new tools for the quick identification of its presence in samples (qPCR)
87 (López-Sanmartín et al. 2019), to conduct breeding studies, and in general, to try to better
88 understand the aetiology of disease and its global implications. Additionally, a few individuals in
89 affected populations have shown some resilience, remaining sick for weeks, or even months in
90 indoor facilities before dying, some of which were found being eaten alive by opportunistic
91 predators. It was hypothesized that these individuals could potentially resist the parasite, but
92 the weakness caused by prolonged disease made them extremely vulnerable to predators,
93 which killed them before they could recover from the infection. Consequently, an attempt to
94 protect some sick fan mussels from predators was also undertaken.

95 In the present study, compiled field and ecological data regarding the spread of the die-off in
96 Spain and neighbouring countries between October 2016 and September 2018 are presented.
97 Additional results on the rescue initiative and predator exclusion actions are shown and
98 recommendations provided to improve results for future actions, based on all the acquired
99 knowledge. This information will serve as a baseline for the implementation of protection
100 programmes and to increase the success of active initiatives to save the species from extinction.
101 It will also help to raise awareness and prepare scientists and managers for the future predicted
102 increment of marine invertebrate die-offs provoked by emerging waterborne diseases (Harvell
103 et al., 2002; Fey et al., 2015).

104 The fan mussel already endured some depletions during the last decades of the twentieth
105 century due to the overexploitation and destruction of its main habitat (*Posidonia oceanica*

106 meadows), pollution and indiscriminate boat anchoring (Basso et al., 2015, Vázquez-Luis et al.
107 2015). The species has long been collected for its decorative value (the shell can reach more
108 than one-metre-long), food and bait (Butler et al., 1993). Considerable pressure still exists upon
109 some populations of fan mussels in the Eastern Mediterranean, as its flesh is considered a
110 delicacy (Katsanevakis et al., 2011, 2008). The byssus threads were considered sea-silk or sea-
111 gold by the antic Roman and Greek civilizations due to their soft consistency and iridescent
112 colouration, which were highly appreciated and impossible to imitate in ancient times (Maeder,
113 2017). The creation of marine protected areas and Natura 2000 sites, as well as the
114 implementation of European laws and Directives (Annex II of the Barcelona Convention (SPA/BD
115 Protocol 1995) and Annex IV of the EU Habitats Directive (EU Habitats Directive 2007)),
116 contributed to a notable recovery of the population in the Mediterranean Sea until late 2016.

117

118 **2. MATERIALS AND METHODS**

119

120 **2.1 Follow-up of the die-off**

121

122 After the first report released by Vázquez-Luis et al. (2017), new stations and areas were added
123 to control the spread of mortality. In total, the evolution of mortality in 58 sites along the Spanish
124 and French Mediterranean coasts is reported in the present manuscript (Fig. 1, Table A1). For all
125 locations, the sampling effort was distributed using control areas of different extensions
126 (minimum 1 unit of 100 m²) as the epicentre of the surveys, which usually corresponded to
127 stations that had been periodically surveyed before the onset of the die-off. Adjacent areas were
128 surveyed using extensive explorative sampling (García-March and Vicente, 2006) for comparison
129 to control areas. Citizen scientific data (local diving clubs, professional and amateur divers) was
130 added to increase the number of observations (besides the 58 stations) and the extension of the
131 surveyed areas. These data, mainly focused on the presence of alive/dead fan mussels, were
132 always controlled by experts, either directly, by visiting the area, or indirectly, by using digital
133 imaging, before being scientifically validated (Cabanellas-Reboredo et al., 2019). Given the
134 variability of data sources and sampling periods, data were provided as monthly percentages of
135 living individuals, including all individuals who were alive on the date of the observation, despite
136 some showing symptoms of sickness. Monitoring of the mortality spread included only
137 populations that still had living individuals in October 2016 (i.e., many populations from
138 Andalucía and most of Murcia, except Mar Menor Lagoon, were excluded). Monthly water
139 temperatures for monitored locations were obtained from different sources, including data
140 collected in situ monthly or bimonthly with a multiparametric probe, every hour with in situ data

141 loggers, averages extracted from daily satellite readings provided by the NOAA
142 (www.seatemperature.org) and local meteorological stations.

143

144 2.2 Relationship between water temperature and survivorship

145

146 To evaluate the relationship between water temperature and mortality in the presence of *H.*
147 *pinnae*, data from Delta del Ebro (used as the control group) and Port Lligat (used as the
148 experimental group), both from open waters and tanks, were analysed. These data were
149 selected from the whole dataset (Table A1), because 1) the presence/absence of *H. pinnae* had
150 been confirmed by PCR (numerous positives in Port Lligat individuals, and no positive in samples
151 from Delta del Ebro individuals until July 2018) and, 2) both temperature and survivorship had
152 been recorded monthly between November 2017 and September 2018.

153

154 2.3 Recruitment and larvae collectors

155

156 To monitor recruitment and spat survival of fan mussels after the die-off, juvenile collectors
157 were installed between June 2017 and November 2017 in 11 stations along Spanish
158 Mediterranean coasts (Fig. 1). Collectors were based on the designs of García-March and Vicente
159 (2006), Cabanellas-Reboredo et al. (2009) and Kersting and García-March (2017), using plastic
160 mesh-bags with either fishing rod or two plastic mesh-bags inside. The lines of collectors were
161 placed at 1 or 1.5-m intervals between depths of 0.5 m and 20 m, depending on the area: in
162 Delta del Ebro, collectors were installed only at a depth of 0.5 m, and in the remaining locations,
163 the shallowest collector was deployed at a depth of 3 m.

164 Between October and November 2017, the collectors were extracted and thoroughly examined,
165 and all pinnids that were visible to the naked eye were collected and stored in acclimated and
166 aerated water. All individuals were transported alive to the Institute of Environment and Marine
167 Science Research (IMEDMAR-UCV) facilities within the day of collection. Once at the laboratory,
168 the juveniles were placed in closed circuit water systems and fed the same phytoplankton gel as
169 the adults (see section 2.3.4). Less than 1 ml/day of each species of phytoplankton gel was
170 necessary to feed all juveniles. Water was filtered to 1 μ and exposed to $>100 \text{ mWs}^{-1}\text{cm}^{-1}$
171 ultraviolet radiation.

172 Additional censuses of recruitment in situ were carried out each time an adult survival station
173 was monitored in the present study.

174

175 2.4 Rescue of individuals

176

177 2.4.1 Extraction of individuals

178

179 In February 2017, 16 adult fan mussels were collected from the “Marina Real de Valencia” in the
180 Port of Valencia. Eight individuals were hosted at the IMEDMAR-UCV and the other 8 at the
181 Oceanogràfic Aquarium of Valencia. Given the fast spread of the protozoan along Spanish coasts
182 during spring-summer 2017, another 100 and 115 individuals were collected from the Alfacs Bay
183 (Ebro Delta, Tarragona) and Port Lligat (Cap de Creus, Girona), respectively, in November 2017
184 and transported to indoor facilities. The 100 individuals from Delta del Ebro were hosted at the
185 Institute of Agrifood Research and Technology (IRTA), and the 115 individuals from Port Lligat
186 were separated into 4 groups: 50 were hosted at the Institute for Agricultural and Fisheries
187 Research and Training (IFAPA) Agua del Pino, 50 at the Spanish Oceanographic Institute (IEO) -
188 Murcia, 5 at the Oceanogràfic Aquarium of Valencia and 10 at the marine station of IMEDMAR-
189 UCV in Calpe. The institutions were selected by their experience in the maintenance of marine
190 bivalves and/or *P. nobilis* in indoor facilities, and by their availability to host the fan mussels. The
191 number of individuals per institution depended on the space available to host them under the
192 appropriate conditions. To ensure that all collected fan mussels were adults with reproductive
193 capacity, only specimens with a shell length larger than 40 cm were extracted. For the extraction
194 of individuals from La Marina Real de Valencia and Port Lligat, a hole was dug in the sediment
195 close to the ventral part of the shell to liberate the byssus. For the extraction of individuals from
196 the Alfacs Bay, an electric water pump was used on selected pen shells dwelling on unvegetated
197 patches at a low water depth (0.5-0.7 m). Pressurized water was pumped into the ventral part
198 of the individuals to remove the mud and sand covering the byssus. Each individual was
199 maintained with water until later transportation and only exposed to air for a few seconds for
200 placement within transport tanks.

201

202 2.4.2 Transport

203

204 A truck was equipped with 3 tanks each filled with 300 l of seawater. Constant water aeration
205 was achieved with a portable air compressor. The water temperature was maintained at 14°C,
206 due to a similar ambient temperature during winter or late autumn, when the collection of
207 individuals was performed. During transport, fan mussels were placed within individual mesh
208 containers (5 to 10 individuals per tank) with the antero-posterior axis resting horizontally and
209 either the dorsal or ventral part facing upwards. Although distances from the collection site to

210 the hosting institution ranged from 4 km to 1270 km, all trips were conducted within a single
211 day, switching drivers when necessary.

212

213 2.4.4 Maintenance in indoor facilities

214

215 The fan mussels were placed in 400 to 5.000-l tanks in groups of 4 to 12 individuals. Closed
216 circuits were used for the small groups hosted at the Oceanogràfic Aquarium and IMEDMAR-
217 UCV (8 + 8 individuals from la Marina Real and 5 + 10 individuals from Port Lligat, respectively,
218 for each institution). Semi-closed circuits were used for the 50 individuals hosted in the IEO-
219 Murcia (water was renewed twice a week) and IFAPA (water was renewed every two days). Open
220 circuits were used for the 100 fan mussels hosted at the IRTA. In all systems, water was passed
221 through 1- μ m filters and exposed to UV radiation ($>100 \text{ mWs}^{-1}\text{cm}^{-1}$) before passing through the
222 system with the individuals, following the recommendations for *Haplosporidium nelsoni* and
223 *Perkinsus marinus* by Ford et al. (2001). For the reception of individuals at hosting institutions,
224 the water temperature within the tanks was targeted to be above the minimum comfort limit
225 of 16°C (Trigos et al., 2015). However, due to the large volume of water used in the open circuits,
226 water temperature could only be raised up to 13.9 and 15°C at IRTA and IFAPA, respectively. In
227 smaller closed and semi-closed circuits at the IEO-Murcia, Oceanogràfic and IMEDMAR-UCV,
228 water temperature during reception was maintained at 17°C. All individuals were laid vertically
229 with the anterior end of the shell pointing downwards, but unburied and without sediment.

230 Various supports, such as concrete blocks where the individuals were inserted in holes, or PVC
231 tubes where the individuals leaned on one side, were used to maintain the individuals in the
232 vertical position. Individuals were also arranged to avoid the proximity of inhalant and exhalant
233 chambers between contiguous specimens.

234 To feed fan mussels, an initial daily mixture of phytoplankton gel (easyreefs®), autoclaved
235 muddy detritus with high organic matter (OM) content and supplements (lyophilized easy SPS®)
236 was supplied in the following concentrations per average individual of 55 cm:

237

238 2.4-7.2 ml of *Isochrysis galbana* (0.0120-0.036 g OM/ml)

239 2.4-7.2 ml of *Phaeodactylum tricornutum* (0.0121-0.0363 g OM/ml)

240 1.5-4.5 ml of *Tetraselmis chuii* (0.0172-0.0516 g OM/ml)

241 0.3 g of muddy detritus (10% OM content)

242 0.1 g of easy SPS (83.88% OM content)

243

244 Modifications of the quantity of the various components of the diet were also implemented a
245 posteriori, according to the specific settings of the tanks at each institution, food acceptance
246 and pseudofaeces production of fan mussels. The food was provided diluted in water as follows:
247 continuously (IFAPA), in a single dose (Oceanogràfic Aquarium), two doses (IRTA and IEO-
248 MURCIA), or four doses (IMEDMAR-UCV) daily.

249 Epibionts living on the shells were cleaned one month after reception of the individuals to
250 minimize the stress of captivity. Shells were bruised maintaining the fan mussels within trays
251 filled with seawater and then returned to the tanks.

252

253 2.5 Predator exclusion cages

254

255 A total of 35 individuals from the Port Iligat (Cap de Creus) population were caged in May (30
256 cages) and June (5 more cages) 2018 -one and two months after detecting the local die-off. Four
257 areas of circa 500 m² were selected, with 8 or 9 individuals in each protected by cages (Figure
258 A1). Monthly surveys were conducted until the last of the individuals in the cages died. Every
259 month, the cages were opened and cleaned of epibionts and possible predators that could have
260 entered as juveniles and grown inside the cage.

261 The cages consisted of a cylinder of 5 mm mesh, 30 cm in diameter and 50 cm in length, with
262 closure of the upper part with a 2-mm mesh. The lower part of the flexible mesh cylinder was
263 fitted with a draw cord closure surrounding the shell of the fan mussel, at the point of protrusion
264 from the sediment, to avoid the entry of predators. The cage was opened and closed at the top
265 with another draw cord closure (Figure A2). Three steel pegs with a length of 1 m were used to
266 attach the cage to the sea floor and provide better adjustment of the structure to the flat shape
267 of the fan mussels.

268

269 3. Results

270

271 3.1 Follow-up of the die-off

272

273 Table A1 shows the living individuals found in the monitored stations from October 2016 to
274 September 2018. The die-off spread northwards and eastwards with time from southeastern
275 Spain, showing mortality peaks in the hotter months. It is noteworthy that the sampling
276 periodicity was variable among locations and that sometimes surveys were not performed
277 during the hottest months. Several critical points were observed, such as the spread to
278 northeastern Spain (Castellón and Cataluña) in late 2017, the start of mortality in France in mid-

279 2018 and the onset of mortality in Delta del Ebro in the summer of 2018, but constrained to the
280 outermost stations closer to the open sea. In the Columbretes Islands and Balearic Islands,
281 surveys were also performed of deep populations down to a depth of 45 m. In all stations
282 surveyed, where adult specimens had previously been observed, only empty shells of dead
283 individuals were found (equivalent to 0% survival). Unfortunately, no information was available
284 on the status of deeper populations, the fate of which is unknown to date. By September 2018,
285 only 6 fan mussels were found alive in open waters of Spanish coasts. Excluding the populations
286 from the inner sites of the Delta del Ebro and Mar Menor lagoon, as well as 2 individuals from
287 the Columbretes Islands and 4 individuals from the Balearic Islands, all surveys resulted in the
288 identification of 0% live individuals.

289

290 3.2 Relationship between water temperature and survivorship

291

292 Fig. 2 shows survivorship and temperature data for the stations used in the experiment. In Port
293 Lligat, mortality was first observed in April 2018, when the temperature rose to 14°C from the
294 winter minimum of 12.3°C observed in March 2018 (see Table A1). From that moment, the
295 survivorship curve displayed a highly negative slope, with a total mortality after 5 months from
296 the last observation without mortality. Regarding the tanks in Oceanogràfic, where temperature
297 in the tanks remained constant at 17°C, mortality followed a similar trend to Port Lligat (highly
298 negative slope with total mortality after 5 months from the last observation without mortality).
299 In contrast, at IFAPA, IEO and IMEDMAR, where the water temperature was reduced below 14°C,
300 there was a change in slope of the survivorship curve after reducing the water temperature,
301 maintaining live individuals in the three locations by September 2018. Furthermore, survivorship
302 was lower in the stations where water temperature remained above 15°C for a longer time (i.e.,
303 IEO - IMEDMAR - IFAPA in increasing order of survivorship and decreasing order of temperature);
304 at IFAPA, where water temperature was maintained at or below 15°C most of the time,
305 survivorship was the highest.

306

307 3.3 Recruitment and larvae collectors

308

309 Only the collectors located in Castellón and Alicante hosted fan mussel juveniles in October
310 2017. Additionally, 51 juveniles were found among hundreds of *P. rudis* juveniles recruited in
311 the ropes of the Vilajoiosa fish farm (Alicante) -these juveniles were found thanks to citizen
312 scientific collaborations. Considering *P. nobilis* growth rates (Kersting & García-March 2017) and
313 that by February 2018, those juveniles had a size >20 mm, the spat were most likely recruited

314 between July and December 2017. Fifty-two juveniles were also found in a shallow bank at a
315 depth of <0.2 m in Alfacs Bay in Delta del Ebro in January 2018. In September 2018, another 30
316 fan mussel juveniles were recently found settled in Calpe (Alicante) attached to an exclusion
317 cage structure suspended at a depth of 15 m, designed to protect from predators *P. rudis*
318 juveniles recruited the previous season. The juveniles from the Columbretes Islands and Calpe,
319 collected in October 2017, and from Vilajoiosa fish farm, which were transported to aquaria,
320 only grew a few millimetres in the tanks and died. In fact, the longest life span in the aquarium
321 did not exceed 6 months. The 30 individuals from Calpe collected in September 2018 were
322 placed in situ in the same cages where *P. rudis* juveniles were hosted (29 individuals), and one
323 individual was maintained in the tanks. In November 2018, all *P. nobilis* placed in situ were dead
324 (but no *P. rudis* in the same cage, which all remained alive), and the individuals maintained in
325 the tanks was still alive but showed no growth.

326 Thirty samples of recently deceased *P. nobilis* juveniles from the tanks were analysed for the
327 presence of *H. pinnae* using specific PCR (Catanese et al., 2018). Twenty-five of them were
328 positive, and the DNA quality of the remaining 5 individuals was poor and, thus, provided
329 unreliable results.

330 Juvenile fan mussels have not been found anywhere else apart from the collectors, the ropes of
331 the fish farm and the predator exclusion cages, in any of the surveys carried out to search for
332 live *P. nobilis*.

333

334 3.4 Rescue of individuals

335

336 3.4.1 Individuals from la Marina Real of Valencia

337

338 The 16 individuals collected from la Marina real of Valencia survived during the transport.
339 Temperature was maintained between 17 and 20°C. Of the 8 individuals maintained at the
340 IMEDMAR-UCV, one individual died in May 2017, another two in July 2017 and the remaining 5
341 in August 2017, seven months after collection. All individuals who died showed symptoms of
342 being affected by the parasite: mantle retraction, lack of response to stimuli and no growth.
343 Samples of the dead individuals were positive to the presence of *H. pinnae* (note that the species
344 was yet unknown by then). Of the 8 individuals maintained at the Oceanogràfic Aquarium, one
345 individual died in April 2017, another two in May 2017 and another one in August 2017. Samples
346 of the dead individuals were positive for the presence of *H. pinnae*. The four remaining
347 individuals, all living in the same tank, showed some symptoms (slight mantle retraction) in
348 August 2017, but they subsequently recovered, extended their mantle again and survived for 18

349 months until August 2018, at which time all four died. One of the individuals closed the gap in
350 July 2018 and remained in that position until August 2018, at which time it opened the gap,
351 retracted its mantle and died. The other three individuals also retracted their mantle, opened
352 the gap and died in few days. Samples collected after death were positive for infection by the
353 parasite.

354

355 3.4.2 Individuals from Port Lligat and Alfacs Bay

356

357 All individuals survived the transport. Only one specimen from Portlligat, transported to IFAPA
358 -Agua del Pino (the longest trip of 1270 km), showed signs of stress upon arrival. In two days,
359 this fan mussel had retracted its mantle and opened the ventral part of the gap. When no
360 response to mechanical stress was observed, it was sacrificed. Analysis of the tissues confirmed
361 that it was infected by the parasite. This was the first evidence that the donor population from
362 Port Lligat was already infected when the rescue was undertaken in November 2017. In contrast,
363 25 random individuals among the 100 collected in Alfacs Bay and stabled at IRTA facilities were
364 subjected to mantle biopsies, all of which were negative for the parasite, demonstrating that
365 the population was free of *H. pinnae* at the time of rescue.

366 During the November 2017 - January 2018 period, water temperature was maintained at 11.8°C
367 at IRTA, 15°C at IFAPA, 16°C at IEO-Murcia, 17°C at Oceanogràfic Aquarium and 18°C at
368 IMEDMAR-UCV. In February-March, water temperature was raised to 18°C at IEO-Murcia,
369 reduced to 12°C at IMEDMAR-UCV and maintained at 11.8°C at IRTA and 15.5°C at IFAPA.
370 Beginning in April, temperatures were maintained below 14°C at all sites except IRTA and the
371 Oceanogràfic Aquarium (16°C and 17°C respectively). Temperatures of the tanks in the IRTA
372 installations increased the following months similarly to the Delta del Ebro water temperatures,
373 reaching a maximum of 26.2°C in August 2018 (Table A1, Fig. 2).

374 In January 2018, two months after collecting the individuals, another 11 individuals from Port
375 Lligat died. In April 2018, 63 individuals from Port Lligat were dead. In July 2018, 81 individuals
376 from Port Lligat and 6 from Delta del Ebro had died, and in September 2018, 12 individuals from
377 Port Lligat and 62 from Delta del Ebro remained alive (Table A1). The tests of dead individuals
378 from Port Lligat were positive for the presence of the parasite, but they were negative for the
379 dead individuals from Delta del Ebro. In contrast, the dead individuals in Delta del Ebro were
380 found to be positive for bacterial infection by *Vibrio mediterranei* (Prado et al., 2020).

381

382 3.5 Predator exclusion cages

383

384 In June 2018, 10 individuals protected by the cages were found dead. Five cages were reused
385 and installed again with the only 5 individuals remaining alive close to the sites where the cages
386 were installed the previous month. In July 2018, only 7 individuals remained alive in the cages,
387 and no additional individuals were observed alive around the monitoring stations. In August
388 2018, only dead individuals were found inside or outside the cages, leading to the conclusion
389 that the disease affected all individuals irrespective of the effect of predators. Further surveys
390 in subsequent months confirmed the absence of live individuals in Port Lligat.

391

392

393 **4. Discussion**

394

395 The data gathered during the two years following the start of the die-off showed that it is causing
396 the ecological extinction of *Pinna nobilis*. The pathogen is most likely waterborne and spreads
397 with currents (Vázquez-Luis et al., 2017; Cabanellas-Reboredo et al., 2019). Direct observations
398 have shown the fast spread in only two years of the die-off from southeastern Spain to eastern
399 and northeastern Spain and to other countries such as France, Italy, Malta, Greece and Cyprus
400 (IUCN 2018a; Katsanevakis et al., 2019; Panarese et al., 2019). The presence of uni- and
401 multinucleate cells capable of direct infection, together with the occurrence of resistant spores,
402 confer *H. pinnae* a strong infective and spreading capacity and a strong resilience to changes in
403 environmental conditions (Catanese et al., 2018). Thus, the parasite could remain alive in
404 reservoirs such as unknown hosts or sediment and infect new settlers similarly to *Bonamia*
405 *ostreae* (Culloty and Mulcahy, 2007), thus constraining the repopulation of habitats after local
406 extinction. To date, juvenile settlement has been missing in the affected areas, despite recruits
407 found in seed collectors in the 2017 and 2018 recruitment seasons. In the Spanish
408 Mediterranean, the species has been cornered in the Mar Menor coastal lagoon and the Ebro
409 Delta (Alfacs and Fangar Bays), although the disease had already arrived at Alfacs Bay. Moreover,
410 these locations are heavily anthropogenic habitats, subjected to a number of impacts that make
411 them highly vulnerable and prone to sudden environmental changes (Nasi et al., 2018; Vidovic
412 et al., 2016). These impacts could contribute to compromising the survival of local communities
413 (e.g., Mar Menor lagoon; García-Ayllon, 2018; Quintana et al., 2018) and lead to the near total
414 population extinction of fan mussels throughout the Spanish Mediterranean Sea. It should be
415 noted that, similarly, populations in French lagoons (along the Occitanie coast) have not been
416 impacted to date, also suggesting that, in a few years, lagoons may well be the only areas
417 throughout the Mediterranean Sea where fan mussels will remain. The factors explaining why
418 fan mussels can survive the parasitosis longer in these areas are unknown, but a change in these

419 hypothetical factors would terminate the only reservoir refuge known to date for fan mussels,
420 with fatal and catastrophic consequences for the species. Natural recolonization of fan mussels
421 in these environments, when conditions again become optimal, will be impracticable due to the
422 barrier of open waters with the presence of the parasite. The few living individuals in open
423 waters may be expected to be unable to reproduce due to their low numbers and isolation,
424 making them dismissible as source of seeds to repopulate decimated populations.
425 Consequently, it is reasonable to assume that *P. nobilis* could be facing total extinction over the
426 medium term unless manipulative actions are undertaken, e.g., artificial repopulation of
427 habitats free of parasite using individuals of surviving populations and repopulation with
428 resistant individuals. This action would be favoured by the features of *P. nobilis* of low inter-
429 population differentiation due to high connectivity and low isolation of populations. Therefore,
430 the hypothesis of small isolated populations is inconsistent for this species (Wesselman et al.,
431 2018). Records of marine invertebrate extinctions are extremely rare (Carlton, 1993; Carlton et
432 al., 1999; CIESM, 2013) and have not been previously documented in a consistent manner.
433 Furthermore, recent die-off events have been mostly related to positive thermal anomalies
434 and prolonged exposure to warmer summer conditions, coupled with water column stability
435 and reduced food resources (Coma et al., 2009; Rivetti et al., 2014). Die-offs due to disease are
436 almost never included among the causes of extinction, with a few exceptions, such as Dulvy et
437 al. (2003). In this regard, the eelgrass Limpet *Lottia alveus* went extinct in the 1930s due to the
438 catastrophic decline of its main habitat, the eelgrass *Zostera marina* from Labrador to New York
439 in the western Atlantic Ocean. In this case, the limpet was unable to adapt to the eelgrass
440 brackish water refuge and became extinct (Carlton et al., 1991). The spread and virulence of the
441 infection by *H. pinnae* and the geographical range of fan mussels, which are endemic to the
442 Mediterranean Sea, provide similarly pessimistic predictions for fan mussels. The capacity to live
443 in paralic environments currently seems to be the only ecological opportunity left to fan mussels
444 to avoid the same fate as the eelgrass limpet. Two questions arise, however. Are we the
445 witnesses of the potential extinction of a sentinel species? Can we avoid the potential extinction
446 of this species by applying active measures, and which measures will be more effective?

447

448 4.1 Relationship between water temperature and survivorship

449

450 A null hypothesis was postulated according to the observations of mortality after the
451 development of the rescue programme, which seemed to be related to the increased water
452 temperature in the tanks with respect to the water temperature in situ. The (null) hypothesis

453 was that water temperature would have little if any effect on the parasite, and the die-off would
454 start in Port Lligat independently of the water temperature. Consequently, reducing the water
455 temperature in the tanks would have little if any effect on reducing fan mussel mortality.
456 Alternatively, water temperature and fan mussel mortality would be correlated, and if water
457 temperature was the determinant of infection and the dispersion capacity of the parasite, the
458 die-off would start in Port Lligat as soon as (and only when) the water temperature rose from
459 the winter minimum, and the mortality in the tanks would be reduced if the water temperature
460 was kept at temperatures similar to the winter minimum in the Mediterranean Sea (between
461 12°C and 14°C). Most remarkable was the observation of the start of mortality in Port Lligat
462 when temperatures rose from 12.3°C to 14°C (Fig. 2), indicating the existence of a threshold
463 temperature for activation of the parasite situated in between these two temperatures. This
464 observation coincides with the model calculations by Cabanellas et al. (2019) and suggests that
465 refuge temperatures for *P. nobilis* are indeed below 14°C. The importance of temperature in the
466 survivorship of fan mussels was reinforced by the higher survivorship in the tanks where a low
467 water temperature was maintained for a longer time. In IFAPA, where the temperature was
468 maintained between 14.1°C and 15.5°C in the December 2017 - March 2018 period, mortality
469 exhibited a lower trend than in the other stations and spiked in April, immediately after the
470 increase to 15.5°C in March. In Oceanogràfic, total mortality of the individuals occurred after
471 maintaining a constant water temperature at 17°C. This result is interpreted as the existence of
472 a range of temperatures between circa 13.5°C and 15°C, at which *H. pinnae* seems to be
473 activated with moderated virulence, but maximum virulence capacity is observed at higher
474 temperatures >15°C. It should be noted that in the tanks, once the temperature was reduced
475 below 14°C, sick individuals could remain alive for several months (in contrast to what occurred
476 when a high water temperature was maintained as in Oceanogràfic). Therefore, mortality of sick
477 individuals who were infected during periods of warm temperatures could occur when the water
478 temperature was cold (<14°C). The Haplosporidian needed a longer time to kill fan mussels at
479 low temperatures, a trend that was clearly observed by the gentle change in slopes of
480 survivorship of IEO, IMEDMAR and IFAPA after reducing the water temperatures. Mortality of
481 sick individuals still occurred for several months at low temperatures before stopping because
482 the reduction of water temperatures did not automatically halt mortality (Fig. 2).

483 Infections of *Ostrea edulis* with the protozoan *Perkinsus marinus* are also seasonal, peaking in
484 summer and fall and decreasing in winter and spring. Parasite latency could occur during the
485 colder winter months and reactivate with the rising temperatures in spring (McCollough et al.,
486 2009). Likewise, infections of *Haplosporidium nelsoni* acquired in late summer by *Crassostrea*

487 *virginica* may persist over the winter and cause mortality in April/May of the following year (Ford
488 and Haskin, 1982), as observed with *H. pinnae* in the Port Lligat population.

489 Regarding the individuals rescued from Alfacs Bay, which were unaffected by *H. pinnae*, the
490 13.5-14°C threshold affecting their survival was not observed. The mortality sustained by these
491 individuals was, however, due to a bacterial (*V. mediterranei*) infection related to warm summer
492 temperatures when water in the tanks rose above 22°C and led to a spike of mortality above
493 25°C. Rodriguez et al. (2017, 2018) isolated *V. mediterranei* strains in two sick fan mussels
494 collected in Almería (Spain) in October 2016 and observed 100% mortality in experimental
495 infections of turbot (*Scophthalmus maximus*) and manila clam (*Ruditapes philippinarum*) at
496 25°C and 24°C, respectively, using these strains. These temperature values are very similar to
497 those observed during the peak mortalities at IRTA. Considering that *V. mediterranei* is a
498 cosmopolitan species (Tarazona et al., 2014) and that its virulence is dependent on hot summer
499 temperatures $\geq 24^\circ\text{C}$, the bacteria seem to be opportunistic, affecting some weak individuals,
500 but not responsible for the die-offs in open waters. A mycobacterium bacteria has recently been
501 associated with the die-offs observed in the Tyrrhenian Sea (Carella et al., 2019), which suggests
502 that the importance of bacterial infections and the synergistic effects among different
503 pathogens increasing the virulence of the disease should be studied in future research.
504 Conversely, despite the arrival of *H. pinnae* in Alfacs Bay in July 2018, it killed mostly individuals
505 in the external part of the bay, closer to its mouth, suggesting the presence of some ecological
506 barrier constraining its capacity for dispersion or survival in this environment.

507

508 4.2 Recruitment and larvae collectors

509

510 The presence of juveniles from the summer 2017 reproductive cycle in the larval collections
511 from Castellón and Alicante, the fish farm in Villajoyosa and in Alfacs Bay, suggest that 2017 was
512 a good year for fan mussel reproduction. The origin of these larvae is unknown. Apparently, four
513 main sources were available in summer 2017: Columbretes Islands, Delta del Ebro, continental
514 France and Corsica (Wesselmann et al., 2018). Juveniles and larvae of *Ostrea edulis* may be
515 infected by *Bonamia ostreae*, but mortalities mainly affect oysters older than 2 years (Arzul et
516 al., 2011). *Crassostrea virginica* juvenile oysters exposed to *Perkinsus marinus*, however, are
517 rapidly infected and die (McCollough et al., 2009). The presence of fan mussel juveniles in
518 collectors and the fish farm indicates that at least they can survive for a few months in open
519 waters. However, if fan mussel juveniles could survive for up to two years, like those of *O. edulis*
520 infected by *B. ostreae*, at least some of the spat that settled in summer 2016, the size of which
521 should be approximately 20 cm by summer 2018 (García-March et al., 2007; García-March et al.,

2011; Kersting & García-March 2017), should have been spotted already. Data gathered to date, however, show that the affected areas are not being repopulated naturally by *P. nobilis*. The confirmed infection of the *Pinna nobilis* juveniles maintained in tanks in the present study, as well as the lack of observations of new juveniles in situ to date, indicate that the juveniles are also being infected by the protozoan and that the response is more similar to that of *C. virginica* infected by *Perkinsus marinus*. This observation differs from the dynamics of infectious diseases of other marine invertebrates, such as the sea urchin *Diadema antillarum* in the Caribbean. Shortly after the onset of the die-off suffered by this species in 1983-1984, natural recruitment was observed, probably from larvae that were already present in the plankton upon arrival of the disease. During the subsequent 30 years, episodic low-level recruitment events of individuals were observed. These new recruits, however, subsequently succumbed to mortality, which was interpreted as the consequence of an unknown factor of post-settlement mortality. The populations of *D. antillarum* have recovered to 12% of those before the die-off because recruitment in populations after the post-mortality period was persistently limited for decades, despite a potentially slowly increasing larval supply (Lessios, 2016). In comparison to the absence of natural recruitment observed in fan mussel populations after mortality, a similar outcome after 30 years seems to be optimistic scenario. Considering the extra constraints encountered by fan mussels to repopulate affected areas, we may be the witnesses of its potential extinction. The recovery of fan mussel populations, if possible, will, in any case, be a long-term process lapsing for many decades. Previously reported die-off events of Mediterranean invertebrates were usually caused by warming seawater temperatures and anomalous low circulation patterns during summer, both in deeper (Garrabou et al., 2009, 2001) and shallow habitats (Kersting et al. 2013), and have not affected *P. nobilis*. These events, however, were usually punctual and did not kill the entire populations, so densities could be recovered through recruitment after a few years (Cerrano et al., 2005). The present die-off experienced by fan mussels is more similar to those observed for *O. edulis* and *C. gigas* and the eelgrass Limpet *Lottia alveus* (Carlton et al., 1991). Ultimately, natural populations of *Pinna nobilis* in the Mediterranean Sea are likely to disappear in the next few years, with the exception, perhaps, of lagoonal population. Such a pandemic is very unusual in marine organisms and raises questions concerning actions that should be undertaken in the very near future, before all open sea population go extinct. These actions should be planned considering the emergency, because the disease has eliminated fan mussel populations from Spanish coasts and spread to most of the Mediterranean Sea in just a couple of years.

555

556 4.3 Rescue of individuals

557

558 The rescue programmes have shown that fan mussels are resilient to extraction and transport,
559 even for long distances, as described in the present study.

560

561 4.3.1 Individuals from la Marina Real of Valencia

562

563 Maintenance temperatures (above 14°C), probably accelerated the rapid death of the fan
564 mussels collected from la Marina Real of Valencia, especially at the IMEDMAR-UCV installations.

565 These fan mussels were going to be used for in situ reproduction experimentation, and warm
566 temperatures are necessary to facilitate gonad maturation. The first exploratory survey carried

567 out on this population in December 2016 showed no mortality. Two months later, however,

568 when the collection of individuals was undertaken, 40% mortality was already observed. It is

569 worth noting that by then, the pathogen was still unidentified and that the relationship between

570 water temperature and disease virulence was unsuspected. In contrast, it was hypothesized that

571 maintaining individuals within their comfort temperature range (between 16°C and 24°C) (Trigos

572 et al., 2015) could help improve their capacity to fight the disease under captivity conditions.

573 The higher water temperature maintained at the IMEDMAR-UCV facilities probably accelerated

574 the mortality of the fan mussels, which were all dead 7 months after collection.

575 The four individuals who survived in good condition for 18 months in the Oceanogràfic Aquarium

576 were maintained at a constant temperature of 17°C. These individuals should have died if

577 infected because that temperature was sufficiently high to cause the rapid death of infected

578 individuals. It is possible that these fan mussels had some kind of resistance and were

579 maintaining the protozoan in a chronic condition and that an uncontrolled weakening of their

580 immune system could have led to their final death. Alternatively, the individuals were “luckily”

581 free of the protozoan when collected, and remained uninfected until July 2018, when the tank

582 could have been unintentionally exposed to the protozoan, provoking rapid death of the fan

583 mussels. Regardless of the causes, as the individuals came from an affected population and

584 survived for 18 months, prolonging the life of fan mussels rescued from affected areas is

585 possible.

586

587 4.3.2 Individuals from Port Lligat and Alfacs Bay

588

589 In September 2018, 12 individuals (11%) among those rescued from Port Lligat were still alive,

590 when the donor population had already disappeared and no individuals could be found in situ

591 since one month prior. The main factor constraining the mortality in tanks was the declining

592 water temperature below 14°C. Again, rescuing individuals prolonged their life, confirming that
593 it is possible to save fan mussels originating from infected populations. It is worth noting a priori
594 that only those individuals who have not been infected are expected to survive, which means
595 that survivorship will depend on the prevalence of the infection at the time of collection. The
596 survival was much higher for individuals from Alfacs Bay, which was expected because the
597 disease had not arrived in this region when the individuals were collected. However, the
598 mortality observed throughout the stabling period was produced by the bacterium (*V.*
599 *mediterranei*), which is also known to infect other species of bivalves (Tarazona et al., 2014).
600 With the new knowledge of the occurrence of *V. mediterranei* in *P. nobilis* maintained in
601 captivity, samples analysed for the presence of *H. pinnae* should be re-checked for bacteria to
602 evaluate the possibility of cohabitation of other pathogens in fan mussels.

603

604 4.4 Predator exclusion cages

605

606 All fan mussels used in the experiment died in August 2018. Higher numbers of cages could
607 favour the survival of some individuals –the strongest–, however, installing and maintaining one
608 or two orders of magnitude more cages in situ would be highly demanding for the low level of
609 expected success (only a few individuals would be expected to survive). Considering these
610 results, it seems more reasonable and affordable to rescue individuals than encage them.
611 Comparatively, however, the most positive impact of encaging vs. rescuing individuals is that,
612 because of the cage, a survivor would have stronger resistance to *H. pinnae*, which would make
613 it much more capable of fighting the die-off.

614

615 4.5 Potential actions and recommendations

616

617 Based on the information gathered over the previous two years, a series of actions and
618 recommendations for improvement of the survival of fan mussels can be undertaken. These
619 actions will allow extra time and hence a greater probability of survival to *P. nobilis* as a species,
620 but whether their implementation will avoid fan mussel potential extinction or not is currently
621 unpredictable, because extinction depends on many uncontrolled (and some yet unknown)
622 variables. One imminent action should be the protection of the remaining lagoon populations
623 by eliminating or minimizing activities that could create a threat to fan mussels living within.
624 Protecting individuals in cages seems to be inefficient unless large numbers of specimens are
625 protected, and even so, the success of using more cages would need to be re-evaluated.
626 Alternatively, rescuing individuals seems to be a good option even if they originate from an

627 affected population. Another important issue is to understand why individuals living in paralic
628 environments and coastal lagoons are not affected by the die-off (e.g., Mar Menor Lagoon) or
629 are less severely affected (Alfacas Bay). It is not clear if it is a question of temperature and/or
630 salinity, or of some chemical condition occurring in these environments, preventing direct (by
631 killing it) or indirect (by killing its potential vector) entry of the parasite. Although both Mar
632 Menor lagoon and Ebro Delta do not show extreme salinity variations compared with those
633 occurring in the Caloosahatchee estuary in Florida (La Peyre et al., 2003), salinities are
634 hiperhaline and hipohaline, respectively, compared with the Mediterranean Sea. *H. pinnae*
635 could be unadapted to these variations in water temperature and/or salinity (Cabanellas et al.,
636 2019). Alternatively, fan mussels surviving in these environments could possess some kind of
637 resistance. Studying the environmental conditions of the lagoons and the genetic differences
638 between individuals living inside and outside coastal lagoons and deltas could help to better
639 understand this issue. Additionally, gathering more information about other fan mussel
640 populations surviving in paralic environments in affected areas would improve understanding of
641 the factor(s) conditioning their survival, by comparison of coincident environmental features
642 among them. Studies of reproduction in vitro are fundamental to close the life cycle of *P. nobilis*.
643 Once reproduction in vitro is mastered, it could be applied to resistant individuals to grow
644 resistant spat to repopulate devastated fan mussel populations. Finally, extensive searches for
645 resistant individuals in already affected populations is of paramount importance, since these
646 individuals could provide the seeds for future open water repopulations with artificially
647 produced juveniles.

648 Regarding the specific recommended action of the rescue of individuals, it is advisable to extract
649 fan mussels prior to the arrival of the parasite. Otherwise, small groups of no more than 5 to 10
650 individuals should be collected to enable the selection of only those that seem healthy, i.e., total
651 mantle extension and a quick closing response of the valves in response to external stimuli. After
652 placing them in cold water, preferably 12.5°C, individuals should be checked daily for mantle
653 extension during the following weeks to retain only those that show total mantle extension. It
654 is recommended to place no more than 5-6 individuals per tank in independent systems with
655 acclimatizers. If any individual is infected, it may infect others in the same tank, particularly if
656 the water temperature rises above 14°C. Hence, it is very important to maintain low
657 temperatures and quickly isolate any individual showing any symptom of the disease. It is
658 advisable to obtain mantle samples of all individuals, conserve them in absolute ethanol, and
659 analyse them for the presence of the parasite by PCR (Catanese et al., 2018; López-Sanmartín et
660 al. 2019). *Perkinsus marinus* and *H. nelsoni*, infecting *Crassostrea virginica*, undergo reduced
661 transmission and prevalence at winter temperatures from 10-15°C -although *P. marinus* can

662 survive at temperatures as low as 4°C (Soniati et al., 2008). Repeated freshets can also reduce *P.*
663 *marinus* infections in *C. virginica* to low levels, resulting in an overall low prevalence, low oyster
664 mortality and good growth (La Peyre et al., 2003). *Pinna nobilis* can survive in paralic
665 environments subjected to important seasonal salinity and temperature oscillations. Although
666 there is a knowledge gap regarding its tolerance limits, the habitat distribution suggests it is a
667 euryhaline, eurytherm species. Considering that the extreme salinity of Delta del Ebro and Mar
668 Menor is one of the possible environmental variables preventing infection of fan mussels by the
669 parasite, it would be worth determining whether fan mussels can survive low-temperature
670 (<8°C) and extreme salinity (<15 psu or >45 psu) treatments. Based on the data published by
671 Cabanellas-Reboredo et al. (2019), it is reasonable to suspect that *H. pinnae* transmission and
672 replication may be constrained at least by low temperatures and probably also by extreme
673 salinities, as observed for other similar protozoans. A combined treatment with low
674 temperatures and extreme salinities could be a good option to maintain individuals during the
675 initial weeks of quarantine. This strategy could reduce transmission among individuals, while
676 healthy and sick individuals are separate. Furthermore, it would be worth examining whether
677 the prevalence of the parasite in infected individuals could be reduced with these kinds of
678 treatments.

679 In conclusion, one additional global view is provided. The Mediterranean Sea has been
680 considered a laboratory to examine global processes (Krijgsman, 2002; Lejeusne et al., 2010)
681 using *P. nobilis* as a model species (Basso et al., 2015). When the fan mussel die-off started,
682 many research teams were already studying the species because of its ecological and social
683 interest. As previously suggested by Dulvy et al. (2003), marine extinctions may be
684 underestimated because of low detection power and a long-term lapse (on average 53 years)
685 between the last sighting of an organism and the reporting of the extinction. If a species of
686 interest that is closely monitored, such as *P. nobilis*, has been shown to experience such a sudden
687 and fatal die-off event, practically eliminating its populations in two years, How many other
688 more overlooked species might experience a massive and unnoticed die-off before it is too late
689 to implement any preservation action? In the present context of anthropogenic impacts and
690 climate change, the case of fan mussels shows that the virulence and speed of mass mortality
691 events may escalate in the following years. Quick responses are going to be necessary, and
692 researchers and stakeholders will have to be ready to act collaboratively in order to respond to
693 the new threats. Furthermore, the present pandemic shows that the effects of infectious
694 diseases have important implications in the future redistribution of species and biodiversity,
695 which would justify their consideration in prediction models (e.g. Jones and Cheung, 2014),
696 because their effects are global and occur in very short time periods, compared even to the

697 speed of climate change, and may have considerable structural effects in the biological
698 community (Harvell et al, 2019).

699

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701

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728

729 **7. Compliance with Ethical Standards**

730

731 - The submitted work results from all original research conducted by the authors.

- 732 - All authors agree with the contents of the manuscript and its submission to the journal.
733 - No part of the research has been published in any form elsewhere, unless fully
734 acknowledged in the manuscript.
735 - The manuscript is not being considered for publication elsewhere while it is being
736 considered for publication in Biological Conservation.
737 - All research in the paper not carried out by the authors is fully acknowledged in the
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741 - All appropriate ethics, permissions to manipulate animals and other approvals were
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743

744 **8. Competing interests statement**

745

746 There are no competing interests.

747

748 **9. References**

749

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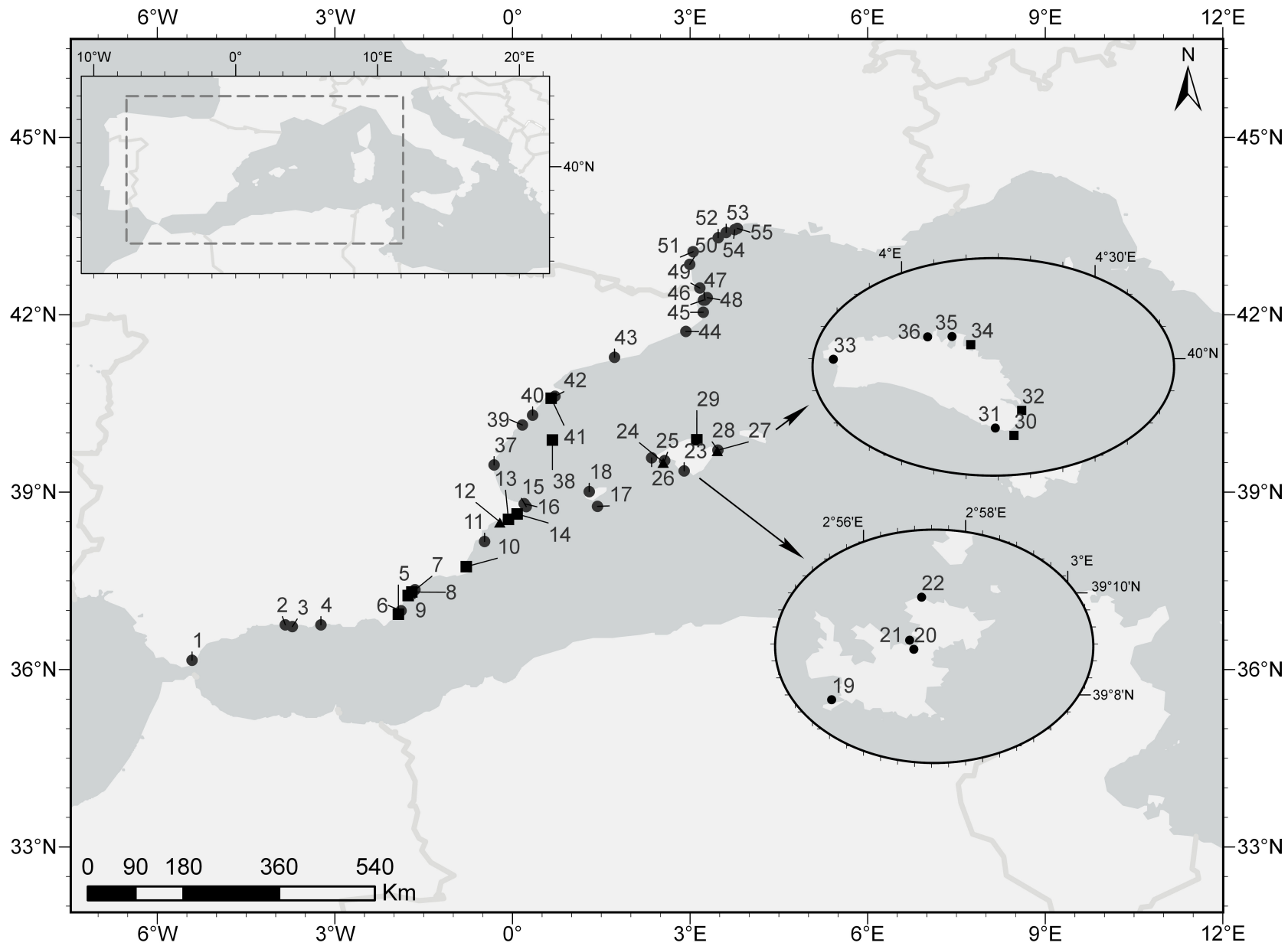
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966 Fig. 1. Distribution map of the stations monitored for population dynamics and installation of
967 larvae collectors. Circles indicate population dynamic stations, triangles indicate stations of
968 larvae collectors and squares indicate stations with both population dynamic and larvae
969 collector stations. 1, Algeciras. 2, Caleta Maro. 3, Marina del Este. 4, Melicena. 5, Agua Amarga.
970 6, Isla de San Andrés. 7, Loza del Payo. 8, El Calón. 9, Terreros. 10, Mar Menor. 11, Illa de Tabarca.
971 12, Villajoyosa. 13, Serra Gelada. 14, Calpe. 15, Illa Portixol. 16, Cap de Sant Antoni. 17, Illetes.
972 18, Cala Gelada. 19, Rates (20 m). 20, Santa María (10 m). 21, Santa María (20 m). 22, Morobuti
973 (30 m). 23, Cap Pinar. 24, Magaluf. 25, Portals. 26, Sant Elm. 27, Cala Ratjada. 28, Cala Gat. 29,
974 Pollença. 30, Illa de l'aire. 31, Benibecar. 32, La Mola. 33, Sa Farola. 34, Son Saura. 35, Fornells-
975 Cabra Salada. 36, Cala Mica. 37, Marina Reial. 38, Illes Columbretes. 39, Prat de Cabanes. 40,
976 Serra d'Irta. 41, Bahía dels Alfacs. 42, El Fangar. 43, Canyelles. 44, Tossa de Mar. 45, Illes Medes.
977 46, Montjoi. 47, Joncols. 48, Port Lligat. 49, Peyrefite. 50, Etang de Leucate. 51, Etang de Ayrolles.
978 52, Agde. 53, Etang de Thau. 54, Frontignan. 55, Les Aresquiers.

979 Fig. 2. Survivorship curves and water temperature in Ebro Delta and IRTA (DeltaIrtá) (top panel),
980 Port Lligat (medium panel) and the tanks in IEO, IFAPA, Oceanogràfic and IMEDMAR-UCV
981 (bottom panel). When two lines of a similar type occur in a panel, thick lines represent the water
982 temperature and thin lines the survivorship for the same tanks. In the top panel, AlfacsZ1C1
983 (Alfacs circle 1, zone 1) and AlfacsC1C2 (Alfacs circle 1, zone 2) share the same water
984 temperature (AlfacsT). Note that water temperature in Port-Lligat (medium panel) corresponds
985 to the closest monitoring station located in open waters of Startit (one mile off the Medas
986 Islands). Port-Lligat is an enclosed small bay with in situ recorded water temperature during the
987 rescue programme conducted in November 2017 below 13°C. Therefore, the registered water
988 temperature at Startit was higher than the directly observed water temperature in Port Lligat in
989 November 2017.



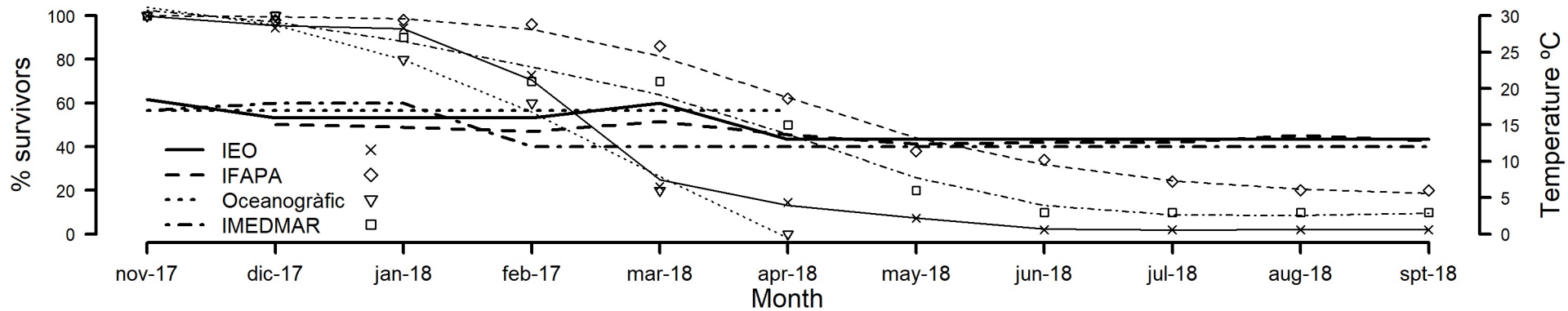
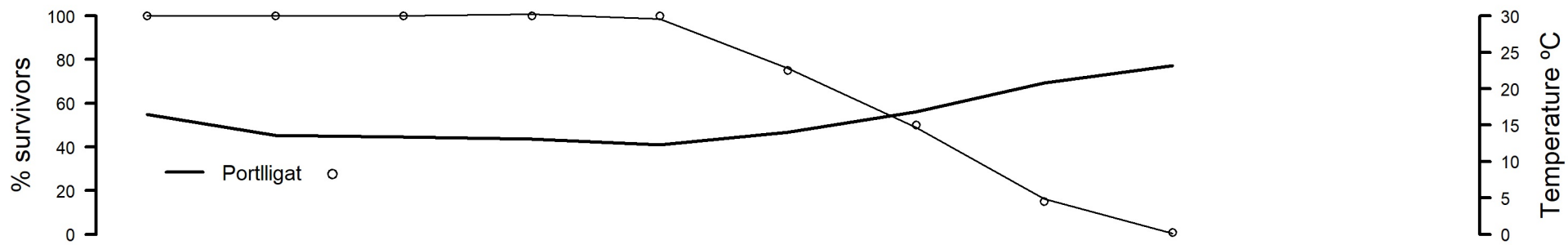
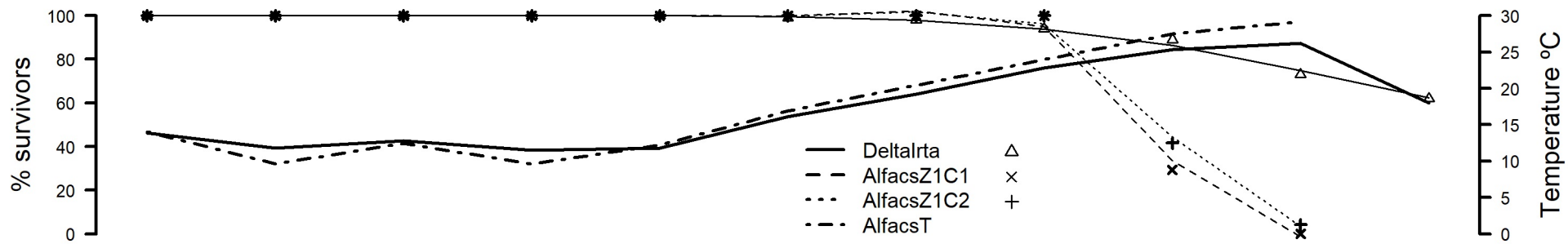




Fig. A1. Location of the stations where the fan mussels were protected with predator exclusion cages. Numbers in brackets indicate how many fan mussels were caged at each site.

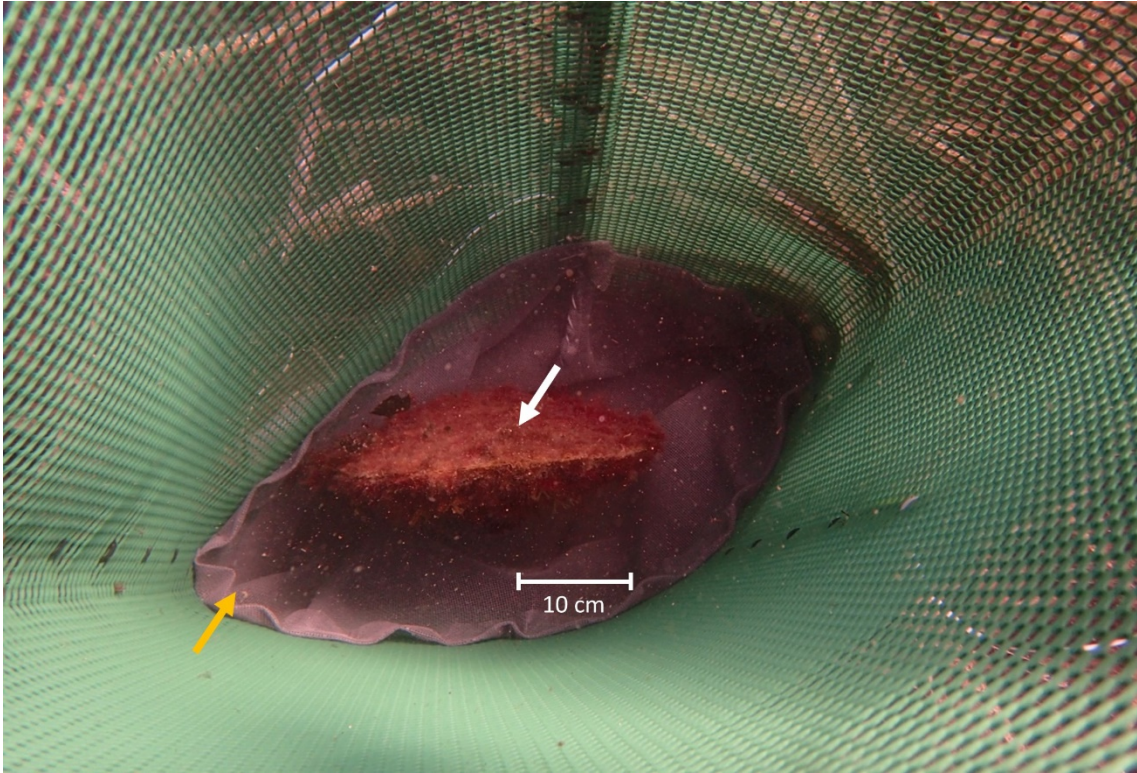


Fig. A2. Example of the interior of a predator exclusion cage used in Port Lligat (zenith view). White arrow shows the fan mussel, whereas the yellow arrow points the bottom cage closure that is wrapped around the shell to avoid the entry of predators through the base of the cage

Conflict of interest

- The submitted work results from all original research conducted by the authors.
- All authors agree with the contents of the manuscript and its submission to the journal.
- No part of the research has been published in any form elsewhere, unless fully acknowledged in the manuscript.
- The manuscript is not being considered for publication elsewhere while it is being considered for publication in Biological Conservation.
- All research in the paper not carried out by the authors is fully acknowledged in the manuscript.
- All sources of funding are acknowledged in the manuscript, and the authors declare no direct financial benefits that could result from publication.
- All appropriate ethics, permissions to manipulate animals and other approvals were obtained for the submitted research.

Role of the funding source

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