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

2 **from extinction?**

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Abstract

Anthropogenic drivers and global warming are altering the occurrence of infectious marine diseases, some of which produce mass mortalities with considerable ecosystemic and economic costs. The Mediterranean Sea is considered a laboratory to examine global processes, and the fan mussel *Pinna nobilis* a sentinel species within it. Since September 2016, fan mussels suffer a die-off, very likely provoked by the protozoan *Haplosporidium pinnae*. Population dynamic surveys, rescue programmes, larvae collector installation and protection of infected adults from predators, have increased knowledge about the factors conditioning the spread of the die-off; previous model simulations indicate that water temperature and salinity seem to be related to the manifestation of the disease, which at the end are strongly influenced by climate change and anthropogenic actions. The absence of natural recruitment implies that fan mussel populations are not recovering, but the survival of populations living in paralic environments provides an opportunity to study the disease and its conditioning factors. The fan mussel disease outbreak provides a case example for how climate change may mediate host-protozoan dynamics and poses several questions: are we witnessing the potential extinction of a sentinel species? Can we avoid it by applying active measures? If so, which measures will be more effective? How many other more overlooked species might experience a massive and unnoticed die-off before it is too late to implement any preservation action? This is especially relevant because the loss of keystone species can drive to community effects that influence marine ecosystem processes.

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

1. Introduction

Anthropogenic impacts and global warming are driving shifts in the dynamics of infectious marine disease outbreaks (Tracy et al., 2019). Ectothermic hosts with parasites with environmental transmission stages that can persist outside the host are among the most vulnerable species to global warming (Altizer et al, 2013). Reduction in biodiversity, translocation of species and alteration of natural habitat and community composition due to human action and climate change, facilitate the occurrence of pandemic infectious marine diseases, with considerable economic and ecosystemic costs (Lafferty, et al., 2015; Harvell et al., 2019). However, both, evaluating whether disease is increasing or decreasing in the ocean, and quantifying anthropogenic drivers of infectious marine disease, is challenging owing to the lack

83   of baseline data and of long-term records (Tracy et al., 2019). On this regard, sentinel species

84   such as the Mediterranean endemic fan mussel *Pinna nobilis* (Basso et al, 2015) could provide

85   information on what is to come to global ecology in the context of the present environment

86   overexploitation. Globally, several authors have pointed out that the Mediterranean Sea could

87   be used as a natural laboratory to study geodynamic and paleoclimatic processes on different

88   scales and as a miniature model of the world's oceans (Krijgsman, 2002; Lejeusne et al, 2010).

89   Fan mussel populations have been recently devastated by a die-off that is very likely to be

90   associated with the protozoan *Haplosporidium pinnae* (Catanese et al., 2018). The first reports

91   of the die-off occurred in September 2016 in southeastern Spain (Vázquez-Luis et al., 2017), and

92   it has since spread throughout all Spanish Mediterranean coasts, reaching France, Italy, Greece,

93   Cyprus and other Mediterranean countries, in less than two years (IUCN 2018a). To date, data

94   indicate that the protozoan species is specific to *P. nobilis*, leaving other invertebrates

95   unaffected, including the congeneric species *P. rudis* (Catanese et al., 2018). Prevalence and

96   subsequent mortality virtually reach 100% in infected populations. Previously reported deaths

97   caused by protozoa that affect commercial bivalve species such as *Crassostrea virginica* or

98   *Ostrea edulis* can be massive but do not usually reach 100% (Culloty and Mulcahy, 2007; Bower,

99   2014). Although commercial exploitation of these species has collapsed in some areas, their

100   extinction was never considered a possibility. After many decades, the selection of cohorts that

101   are resistant to parasites (naturally and artificially) has improved the aquaculture of these

102   bivalves (Dégremont et al., 2015). Growing them in low-salinity deltaic water has also been

103   effective for attaining commercially profitable production, although this strategy is

104   compromised during dry years (Soniat et al., 2008). Likewise, previous reported bivalve die-offs

105   in the Mediterranean Sea affecting *Spondylus gaederopus* (Meinesz, 1983; Kersting et al., 2006)

106   and *Arca noae* (Botari et al., 2017) showed reduced mortality percentages and/or extensions

107   compared with observed die-offs of fan mussels. Although dramatic, these die-offs did not

108   suppose a short-term risk of extinction for the affected species.

109   When in early 2017 a Haplosporidian protozoan was found to be the most likely causal agent of

110   the fan mussel die-off, a group of experts following up the event predicted a high risk that the

111   disease would be spread by marine currents throughout the Spanish Mediterranean coasts and

112   beyond its borders in the following months and years, potentially unimpeded due to the absence

113   of marine barriers (Vázquez-Luis et al., 2017). This observation, together with the characteristic

114   biology of fan mussels, could, eventually, cause the ecological, if not total, extinction of the

115   species, which would represent an unprecedented process in the Mediterranean Sea. As a

116   consequence, Spanish authorities changed the status of *P. nobilis* from "endangered" to

117   "endangered with extinction" along the Spanish coasts (Orden TEC/596/2019, de 8 de abril). The

118   International Union for Conservation of Nature (IUCN) also raised the attention of neighbouring

119   countries regarding the imminent arrival of the parasite to their coasts, which could be

120   considered for each country along the Mediterranean (IUCN, 2018b). Recently, a compilation of

121   recommended actions has been released by IUCN (IUCN, 2019). A follow-up of as many as

122   possible of the remaining Spanish populations was initiated, while a rescue programme to

123   maintain individuals in captivity before die-off caused by parasite arrival, was proposed by the

124   Spanish experts and started as early as 2017. The follow-up has helped to better understand the

125   spread of the disease, with surface currents being the main factor influencing local dispersion,

126   whereas disease expression seems to be closely related to temperatures above 13.5°C and to a

127   salinity range between 36.5–39.7 psu (Cabanellas-Revoredo et al., 2019). Rescued individuals

128   have provided valuable advances in the study of the Haplosporidian parasite (Catanese et al.,

129   2018), helping to prepare new tools for the quick identification of its presence in samples (qPCR)

130   (López-Sanmartín et al. 2019), to conduct breeding studies, and in general, to try to better

131   understand the aetiology of disease and its global implications. Additionally, a few individuals in

132   affected populations have shown some resilience, remaining sick for weeks, or even months in

133   indoor facilities before dying, some of which were found being eaten alive by opportunistic

134   predators. It was hypothesized that these individuals could potentially resist the parasite, but

135   the weakness caused by prolonged disease made them extremely vulnerable to predators,

136   which killed them before they could recover from the infection. Consequently, an attempt to

137   protect some sick fan mussels from predators was also undertaken.

138   In the present study, compiled field and ecological data regarding the spread of the die-off in

139   Spain and neighbouring countries between October 2016 and September 2018 are presented.

140   Additional results on the rescue initiative and predator exclusion actions are shown and

141   recommendations provided to improve results for future actions, based on all the acquired

142   knowledge. This information will serve as a baseline for the implementation of protection

143   programmes and to increase the success of active initiatives to save the species from extinction.

144   It will also help to raise awareness and prepare scientists and managers for the future predicted

145   increment of marine invertebrate die-offs provoked by emerging waterborne diseases (Harvell

146   et al., 2002; Fey et al., 2015).

147   The fan mussel already endured some depletions during the last decades of the twentieth

148   century due to the overexploitation and destruction of its main habitat (*Posidonia oceanica*

149   meadows), pollution and indiscriminate boat anchoring (Basso et al., 2015, Vázquez-Luis et al.

150   2015). The species has long been collected for its decorative value (the shell can reach more

151   than one-metre-long), food and bait (Butler et al., 1993). Considerable pressure still exists upon

152   some populations of fan mussels in the Eastern Mediterranean, as its flesh is considered a

153 delicacy (Katsanevakis et al., 2011, 2008). The byssus threads were considered sea-silk or sea-
154 gold by the antique Roman and Greek civilizations due to their soft consistency and iridescent
155 colouration, which were highly appreciated and impossible to imitate in ancient times (Maeder,
156 2017). The creation of marine protected areas and Natura 2000 sites, as well as the
157 implementation of European laws and Directives (Annex II of the Barcelona Convention (SPA/BD
158 Protocol 1995) and Annex IV of the EU Habitats Directive (EU Habitats Directive 2007)),
159 contributed to a notable recovery of the population in the Mediterranean Sea until late 2016.
160
161 **2. MATERIALS AND METHODS**
162
163 2.1 Follow-up of the die-off
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165 After the first report released by Vázquez-Luis et al. (2017), new stations and areas were added
166 to control the spread of mortality. In total, the evolution of mortality in 58 sites along the Spanish
167 and French Mediterranean coasts is reported in the present manuscript (Fig. 1, Table A1). For all
168 locations, the sampling effort was distributed using control areas of different extensions
169 (minimum 1 unit of 100 m^2) as the epicentre of the surveys, which usually corresponded to
170 stations that had been periodically surveyed before the onset of the die-off. Adjacent areas were
171 surveyed using extensive explorative sampling (García-March and Vicente, 2006) for comparison
172 to control areas. Citizen scientific data (local diving clubs, professional and amateur divers) was
173 added to increase the number of observations (besides the 58 stations) and the extension of the
174 surveyed areas. These data, mainly focused on the presence of alive/dead fan mussels, were
175 always controlled by experts, either directly, by visiting the area, or indirectly, by using digital
176 imaging, before being scientifically validated (Cabanellas-Reboredo et al., 2019). Given the
177 variability of data sources and sampling periods, data were provided as monthly percentages of
178 living individuals, including all individuals who were alive on the date of the observation, despite
179 some showing symptoms of sickness. Monitoring of the mortality spread included only
180 populations that still had living individuals in October 2016 (i.e., many populations from
181 Andalucía and most of Murcia, except Mar Menor Lagoon, were excluded). Monthly water
182 temperatures for monitored locations were obtained from different sources, including data
183 collected in situ monthly or bimonthly with a multiparametric probe, every hour with in situ data
184 loggers, averages extracted from daily satellite readings provided by the NOAA
185 (www.seatemperature.org) and local meteorological stations.
186
187 2.2 Relationship between water temperature and survivorship

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

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197   2.3 Recruitment and larvae collectors

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199   To monitor recruitment and spat survival of fan mussels after the die-off, juvenile collectors
200   were installed between June 2017 and November 2017 in 11 stations along Spanish
201   Mediterranean coasts (Fig. 1). Collectors were based on the designs of García-March and Vicente
202   (2006), Cabanellas-Reboredo et al. (2009) and Kersting and García-March (2017), using plastic
203   mesh-bags with either fishing rod or two plastic mesh-bags inside. The lines of collectors were
204   placed at 1 or 1.5-m intervals between depths of 0.5 m and 20 m, depending on the area: in
205   Delta del Ebro, collectors were installed only at a depth of 0.5 m, and in the remaining locations,
206   the shallowest collector was deployed at a depth of 3 m.

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

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

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218   2.4 Rescue of individuals

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220   2.4.1 Extraction of individuals

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222   In February 2017, 16 adult fan mussels were collected from the "Marina Real de Valencia" in the

223   Port of Valencia. Eight individuals were hosted at the IMEDMAR-UCV and the other 8 at the

224   Oceanogràfic Aquarium of Valencia. Given the fast spread of the protozoan along Spanish coasts

225   during spring-summer 2017, another 100 and 115 individuals were collected from the Alfacs Bay

226   (Ebro Delta, Tarragona) and Port Lligat (Cap de Creus, Girona), respectively, in November 2017

227   and transported to indoor facilities. The 100 individuals from Delta del Ebro were hosted at the

228   Institute of Agrifood Research and Technology (IRTA), and the 115 individuals from Port Lligat

229   were separated into 4 groups: 50 were hosted at the Institute for Agricultural and Fisheries

230   Research and Training (IFAPA) Agua del Pino, 50 at the Spanish Oceanographic Institute (IEO) -

231   Murcia, 5 at the Oceanogràfic Aquarium of Valencia and 10 at the marine station of IMEDMAR-

232   UCV in Calpe. The institutions were selected by their experience in the maintenance of marine

233   bivalves and/or *P. nobilis* in indoor facilities, and by their availability to host the fan mussels. The

234   number of individuals per institution depended on the space available to host them under the

235   appropriate conditions. To ensure that all collected fan mussels were adults with reproductive

236   capacity, only specimens with a shell length larger than 40 cm were extracted. For the extraction

237   of individuals from La Marina Real de Valencia and Port Lligat, a hole was dug in the sediment

238   close to the ventral part of the shell to liberate the byssus. For the extraction of individuals from

239   the Alfacs Bay, an electric water pump was used on selected pen shells dwelling on unvegetated

240   patches at a low water depth (0.5-0.7 m). Pressurized water was pumped into the ventral part

241   of the individuals to remove the mud and sand covering the byssus. Each individual was

242   maintained with water until later transportation and only exposed to air for a few seconds for

243   placement within transport tanks.

244

245   2.4.2 Transport

246

247   A truck was equipped with 3 tanks each filled with 300 l of seawater. Constant water aeration

248   was achieved with a portable air compressor. The water temperature was maintained at 14°C,

249   due to a similar ambient temperature during winter or late autumn, when the collection of

250   individuals was performed. During transport, fan mussels were placed within individual mesh

251   containers (5 to 10 individuals per tank) with the antero-posterior axis resting horizontally and

252   either the dorsal or ventral part facing upwards. Although distances from the collection site to

253   the hosting institution ranged from 4 km to 1270 km, all trips were conducted within a single

254   day, switching drivers when necessary.

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256   2.4.4 Maintenance in indoor facilities

257

258   The fan mussels were placed in 400 to 5.000-l tanks in groups of 4 to 12 individuals. Closed

259   circuits were used for the small groups hosted at the Oceanogràfic Aquarium and IMEDMAR-

260   UCV (8 + 8 individuals from la Marina Real and 5 + 10 individuals from Port Lligat, respectively,

261   for each institution). Semi-closed circuits were used for the 50 individuals hosted in the IEO-

262   Murcia (water was renewed twice a week) and IFAPA (water was renewed every two days). Open

263   circuits were used for the 100 fan mussels hosted at the IRTA. In all systems, water was passed

264   through 1-μm filters and exposed to UV radiation (>100 mWs^{-1}cm^{-1}) before passing through the

265   system with the individuals, following the recommendations for *Haplosporidium nelsoni* and

266   *Perkinsus marinus* by Ford et al. (2001). For the reception of individuals at hosting institutions,

267   the water temperature within the tanks was targeted to be above the minimum comfort limit

268   of 16°C (Trigos et al., 2015). However, due to the large volume of water used in the open circuits,

269   water temperature could only be raised up to 13.9 and 15°C at IRTA and IFAPA, respectively. In

270   smaller closed and semi-closed circuits at the IEO-Murcia, Oceanogràfic and IMEDMAR-UCV,

271   water temperature during reception was maintained at 17°C. All individuals were laid vertically

272   with the anterior end of the shell pointing downwards, but unburied and without sediment.

273   Various supports, such as concrete blocks where the individuals were inserted in holes, or PVC

274   tubes where the individuals leaned on one side, were used to maintain the individuals in the

275   vertical position. Individuals were also arranged to avoid the proximity of inhalant and exhalant

276   chambers between contiguous specimens.

277   To feed fan mussels, an initial daily mixture of phytoplankton gel (easyreefs®), autoclaved

278   muddy detritus with high organic matter (OM) content and supplements (lyophilized easy SPS®)

279   was supplied in the following concentrations per average individual of 55 cm:

280

281   2.4-7.2 ml of *Isochrysis galbana* (0.0120-0.036 g OM/ml)

282   2.4-7.2 ml of *Phaeodactylum tricornutum* (0.0121-0.0363 g OM/ml)

283   1.5-4.5 ml of *Tetraselmis chuii* (0.0172-0.0516 g OM/ml)

284   0.3 g of muddy detritus (10% OM content)

285   0.1 g of easy SPS (83.88% OM content)

286

287   Modifications of the quantity of the various components of the diet were also implemented a

288   posteriori, according to the specific settings of the tanks at each institution, food acceptance

289   and pseudofaeces production of fan mussels. The food was provided diluted in water as follows:

290   continuously (IFAPA), in a single dose (Oceanogràfic Aquarium), two doses (IRTA and IEO-

291   MURCIA), or four doses (IMEDMAR-UCV) daily.

292   Epibionts living on the shells were cleaned one month after reception of the individuals to

293   minimize the stress of captivity. Shells were bruised maintaining the fan mussels within trays

294   filled with seawater and then returned to the tanks.

295

296   2.5 Predator exclusion cages

297

298   A total of 35 individuals from the Port lligat (Cap de Creus) population were caged in May (30

299   cages) and June (5 more cages) 2018 -one and two months after detecting the local die-off. Four

300   areas of circa 500 m^2 were selected, with 8 or 9 individuals in each protected by cages (Figure

301   A1). Monthly surveys were conducted until the last of the individuals in the cages died. Every

302   month, the cages were opened and cleaned of epibionts and possible predators that could have

303   entered as juveniles and grown inside the cage.

304   The cages consisted of a cylinder of 5 mm mesh, 30 cm in diameter and 50 cm in length, with

305   closure of the upper part with a 2-mm mesh. The lower part of the flexible mesh cylinder was

306   fitted with a draw cord closure surrounding the shell of the fan mussel, at the point of protrusion

307   from the sediment, to avoid the entry of predators. The cage was opened and closed at the top

308   with another draw cord closure (Figure A2). Three steel pegs with a length of 1 m were used to

309   attach the cage to the sea floor and provide better adjustment of the structure to the flat shape

310   of the fan mussels.

311

312   **3. Results**

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314   3.1 Follow-up of the die-off

315

316   Table A1 shows the living individuals found in the monitored stations from October 2016 to

317   September 2018. The die-off spread northwards and eastwards with time from southeastern

318   Spain, showing mortality peaks in the hotter months. It is noteworthy that the sampling

319   periodicity was variable among locations and that sometimes surveys were not performed

320   during the hottest months. Several critical points were observed, such as the spread to

321   northeastern Spain (Castellón and Cataluña) in late 2017, the start of mortality in France in mid-

322   2018 and the onset of mortality in Delta del Ebro in the summer of 2018, but constrained to the

323   outermost stations closer to the open sea. In the Columbretes Islands and Balearic Islands,

324   surveys were also performed of deep populations down to a depth of 45 m. In all stations

325   surveyed, where adult specimens had previously been observed, only empty shells of dead

326   individuals were found (equivalent to 0% survival). Unfortunately, no information was available

on the status of deeper populations, the fate of which is unknown to date. By September 2018, only 6 fan mussels were found alive in open waters of Spanish coasts. Excluding the populations from the inner sites of the Delta del Ebro and Mar Menor lagoon, as well as 2 individuals from the Columbretes Islands and 4 individuals from the Balearic Islands, all surveys resulted in the identification of 0% live individuals.

3.2 Relationship between water temperature and survivorship

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

3.3 Recruitment and larvae collectors

Only the collectors located in Castellón and Alicante hosted fan mussel juveniles in October 2017. Additionally, 51 juveniles were found among hundreds of *P. rudis* juveniles recruited in the ropes of the Vilajoiosa fish farm (Alicante) -these juveniles were found thanks to citizen scientific collaborations. Considering *P. nobilis* growth rates (Kersting & García-March 2017) and that by February 2018, those juveniles had a size >20 mm, the spat were most likely recruited between July and December 2017. Fifty-two juveniles were also found in a shallow bank at a depth of <0.2 m in Alfacs Bay in Delta del Ebro in January 2018. In September 2018, another 30 fan mussel juveniles were recently found settled in Calpe (Alicante) attached to an exclusion cage structure suspended at a depth of 15 m, designed to protect from predators *P. rudis* juveniles recruited the previous season. The juveniles from the Columbretes Islands and Calpe,

362   collected in October 2017, and from Vilajoiosa fish farm, which were transported to aquaria,

363   only grew a few millimetres in the tanks and died. In fact, the longest life span in the aquarium

364   did not exceed 6 months. The 30 individuals from Calpe collected in September 2018 were

365   placed in situ in the same cages where *P. rudis* juveniles were hosted (29 individuals), and one

366   individual was maintained in the tanks. In November 2018, all *P. nobilis* placed in situ were dead

367   (but no *P. rudis* in the same cage, which all remained alive), and the individuals maintained in

368   the tanks was still alive but showed no growth.

369   Thirty samples of recently deceased *P. nobilis* juveniles from the tanks were analysed for the

370   presence of *H. pinnae* using specific PCR (Catanese et al., 2018). Twenty-five of them were

371   positive, and the DNA quality of the remaining 5 individuals was poor and, thus, provided

372   unreliable results.

373   Juvenile fan mussels have not been found anywhere else apart from the collectors, the ropes of

374   the fish farm and the predator exclusion cages, in any of the surveys carried out to search for

375   live *P. nobilis*.

376

377   3.4 Rescue of individuals

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379   3.4.1 Individuals from la Marina Real of Valencia

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381   The 16 individuals collected from la Marina real of Valencia survived during the transport.

382   Temperature was maintained between 17 and 20°C. Of the 8 individuals maintained at the

383   IMEDMAR-UCV, one individual died in May 2017, another two in July 2017 and the remaining 5

384   in August 2017, seven months after collection. All individuals who died showed symptoms of

385   being affected by the parasite: mantle retraction, lack of response to stimuli and no growth.

386   Samples of the dead individuals were positive to the presence of *H. pinnae* (note that the species

387   was yet unknown by then). Of the 8 individuals maintained at the Oceanogràfic Aquarium, one

388   individual died in April 2017, another two in May 2017 and another one in August 2017. Samples

389   of the dead individuals were positive for the presence of *H. pinnae*. The four remaining

390   individuals, all living in the same tank, showed some symptoms (slight mantle retraction) in

391   August 2017, but they subsequently recovered, extended their mantle again and survived for 18

392   months until August 2018, at which time all four died. One of the individuals closed the gap in

393   July 2018 and remained in that position until August 2018, at which time it opened the gap,

394   retracted its mantle and died. The other three individuals also retracted their mantle, opened

395   the gap and died in few days. Samples collected after death were positive for infection by the

396   parasite.

397

398   3.4.2 Individuals from Port Lligat and Alfacs Bay

399

400   All individuals survived the transport. Only one specimen from Portlligat, transported to IFAPA
401   –Agua del Pino (the longest trip of 1270 km), showed signs of stress upon arrival. In two days,
402   this fan mussel had retracted its mantle and opened the ventral part of the gap. When no
403   response to mechanical stress was observed, it was sacrificed. Analysis of the tissues confirmed
404   that it was infected by the parasite. This was the first evidence that the donor population from
405   Port Lligat was already infected when the rescue was undertaken in November 2017. In contrast,
406   25 random individuals among the 100 collected in Alfacs Bay and stabled at IRTA facilities were
407   subjected to mantle biopsies, all of which were negative for the parasite, demonstrating that
408   the population was free of *H. pinnae* at the time of rescue.

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

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

424

425   3.5 Predator exclusion cages

426

427   In June 2018, 10 individuals protected by the cages were found dead. Five cages were reused
428   and installed again with the only 5 individuals remaining alive close to the sites where the cages
429   were installed the previous month. In July 2018, only 7 individuals remained alive in the cages,
430   and no additional individuals were observed alive around the monitoring stations. In August
431   2018, only dead individuals were found inside or outside the cages, leading to the conclusion

432　that the disease affected all individuals irrespective of the effect of predators. Further surveys

433　in subsequent months confirmed the absence of live individuals in Port Lligat.

434

435

436　**4. Discussion**

437

438　The data gathered during the two years following the start of the die-off showed that it is causing

439　the ecological extinction of *Pinna nobilis*. The pathogen is most likely waterborne and spreads

440　with currents (Vázquez-Luis et al., 2017; Cabanellas-Reboredo et al., 2019). Direct observations

441　have shown the fast spread in only two years of the die-off from southeastern Spain to eastern

442　and northeastern Spain and to other countries such as France, Italy, Malta, Greece and Cyprus

443　(IUCN 2018a; Katsanevakis et al., 2019; Panarese et al., 2019). The presence of uni- and

444　multinucleate cells capable of direct infection, together with the occurrence of resistant spores,

445　confer *H. pinnae* a strong infective and spreading capacity and a strong resilience to changes in

446　environmental conditions (Catanese et al., 2018). Thus, the parasite could remain alive in

447　reservoirs such as unknown hosts or sediment and infect new settlers similarly to *Bonamia*

448　*ostreae* (Culloty and Mulcahy, 2007), thus constraining the repopulation of habitats after local

449　extinction. To date, juvenile settlement has been missing in the affected areas, despite recruits

450　found in seed collectors in the 2017 and 2018 recruitment seasons. In the Spanish

451　Mediterranean, the species has been cornered in the Mar Menor coastal lagoon and the Ebro

452　Delta (Alfacs and Fangar Bays), although the disease had already arrived at Alfacs Bay. Moreover,

453　these locations are heavily anthropogenic habitats, subjected to a number of impacts that make

454　them highly vulnerable and prone to sudden environmental changes (Nasi et al., 2018; Vidovic

455　et al., 2016). These impacts could contribute to compromising the survival of local communities

456　(e.g., Mar Menor lagoon; García-Ayllon, 2018; Quintana et al., 2018) and lead to the near total

457　population extinction of fan mussels throughout the Spanish Mediterranean Sea. It should be

458　noted that, similarly, populations in French lagoons (along the Occitanie coast) have not been

459　impacted to date, also suggesting that, in a few years, lagoons may well be the only areas

460　throughout the Mediterranean Sea where fan mussels will remain. The factors explaining why

461　fan mussels can survive the parasitosis longer in these areas are unknown, but a change in these

462　hypothetical factors would terminate the only reservoir refuge known to date for fan mussels,

463　with fatal and catastrophic consequences for the species. Natural recolonization of fan mussels

464　in these environments, when conditions again become optimal, will be impracticable due to the

465　barrier of open waters with the presence of the parasite. The few living individuals in open

466　waters may be expected to be unable to reproduce due to their low numbers and isolation,

467   making them dismissible as source of seeds to repopulate decimated populations.
468   Consequently, it is reasonable to assume that *P. nobilis* could be facing total extinction over the
469   medium term unless manipulative actions are undertaken, e.g., artificial repopulation of
470   habitats free of parasite using individuals of surviving populations and repopulation with
471   resistant individuals. This action would be favoured by the features of *P. nobilis* of low inter-
472   population differentiation due to high connectivity and low isolation of populations. Therefore,
473   the hypothesis of small isolated populations is inconsistent for this species (Wesselman et al.,
474   2018). Records of marine invertebrate extinctions are extremely rare (Carlton, 1993; Carlton et
475   al., 1999; CIESM, 2013) and have not been previously documented in a consistent manner.
476   Furthermore, recent die-off events have been mostly related to positive thermal anomalies and
477   prolonged exposure to warmer summer conditions, coupled with water column stability and
478   reduced food resources (Coma et al., 2009; Rivetti et al., 2014). Die-offs due to disease are
479   almost never included among the causes of extinction, with a few exceptions, such as Dulvy et
480   al. (2003). In this regard, the eelgrass Limpet *Lottia alveus* went extinct in the 1930s due to the
481   catastrophic decline of its main habitat, the eelgrass *Zostera marina* from Labrador to New York
482   in the western Atlantic Ocean. In this case, the limpet was unable to adapt to the eelgrass
483   brackish water refuge and became extinct (Carlton et al., 1991). The spread and virulence of the
484   infection by *H. pinnae* and the geographical range of fan mussels, which are endemic to the
485   Mediterranean Sea, provide similarly pessimistic predictions for fan mussels. The capacity to live
486   in paralic environments currently seems to be the only ecological opportunity left to fan mussels
487   to avoid the same fate as the eelgrass limpet. Two questions arise, however. Are we witnessing
488   the potential extinction of a sentinel species? Can we avoid it by applying active measures? If
489   so, which measures will be more effective?

490

491   4.1 Relationship between water temperature and survivorship

492

493   A null hypothesis was postulated according to the observations of mortality after the
494   development of the rescue programme, which seemed to be related to the increased water
495   temperature in the tanks with respect to the water temperature in situ. The (null) hypothesis
496   was that water temperature would have little if any effect on the parasite, and the die-off would
497   start in Port LLigart independently of the water temperature. Consequently, reducing the water
498   temperature in the tanks would have little if any effect on reducing fan mussel mortality.
499   Alternatively, water temperature and fan mussel mortality would be correlated, and if water
500   temperature was the determinant of infection and the dispersion capacity of the parasite, the

501   die-off would start in Port Lligat as soon as (and only when) the water temperature rose from

502   the winter minimum, and the mortality in the tanks would be reduced if the water temperature

503   was kept at temperatures similar to the winter minimum in the Mediterranean Sea (between

504   12°C and 14°C). Most remarkable was the observation of the start of mortality in Port Lligat

505   when temperatures rose from 12.3°C to 14°C (Fig. 2), indicating the existence of a threshold

506   temperature for activation of the parasite situated in between these two temperatures. This

507   observation coincides with the model calculations by Cabanellas et al. (2019) and suggests that

508   refuge temperatures for *P. nobilis* are indeed below 14°C. The importance of temperature in the

509   survivorship of fan mussels was reinforced by the higher survivorship in the tanks where a low

510   water temperature was maintained for a longer time. In IFAPA, where the temperature was

511   maintained between 14.1°C and 15.5°C in the December 2017 - March 2018 period, mortality

512   exhibited a lower trend than in the other stations and spiked in April, immediately after the

513   increase to 15.5°C in March. In Oceanogràfic, total mortality of the individuals occurred after

514   maintaining a constant water temperature at 17°C. This result is interpreted as the existence of

515   a range of temperatures between circa 13.5°C and 15°C, at which *H. pinnae* seems to be

516   activated with moderated virulence, but maximum virulence capacity is observed at higher

517   temperatures >15°C. It should be noted that in the tanks, once the temperature was reduced

518   below 14°C, sick individuals could remain alive for several months (in contrast to what occurred

519   when a high water temperature was maintained as in Oceanogràfic). Therefore, mortality of sick

520   individuals who were infected during periods of warm temperatures could occur when the water

521   temperature was cold (<14°C). The Haplosporidian needed a longer time to kill fan mussels at

522   low temperatures, a trend that was clearly observed by the gentle change in slopes of

523   survivorship of IEO, IMEDMAR and IFAPA after reducing the water temperatures. Mortality of

524   sick individuals still occurred for several months at low temperatures before stopping because

525   the reduction of water temperatures did not automatically halt mortality (Fig. 2).

526   Infections of *Ostrea edulis* with the protozoan *Perkinsus marinus* are also seasonal, peaking in

527   summer and fall and decreasing in winter and spring. Parasite latency could occur during the

528   colder winter months and reactivate with the rising temperatures in spring (McCollough et al.,

529   2009). Likewise, infections of *Haplosporidium nelsoni* acquired in late summer by *Crassostrea*

530   *virginica* may persist over the winter and cause mortality in April/May of the following year (Ford

531   and Haskin, 1982), as observed with *H. pinnae* in the Port Lligat population.

532   Regarding the individuals rescued from Alfacs Bay, which were unaffected by *H. pinnae*, the

533   13.5-14°C threshold affecting their survival was not observed. The mortality sustained by these

534   individuals was, however, due to a bacterial (*V. mediterranei*) infection related to warm summer

535   temperatures when water in the tanks rose above 22°C and led to a spike of mortality above

536   25°C. Rodriguez et al. (2017, 2018) isolated *V. mediterranei* strains in two sick fan mussels

537   collected in Almería (Spain) in October 2016 and observed 100% mortality in experimental

538   infections of turbots (*Scophthalmus maximus*) and manila clam (*Ruditapes philippinarum*) at

539   25°C and 24°C, respectively, using these strains. These temperature values are very similar to

540   those observed during the peak mortalities at IRTA. Considering that *V. mediterranei* is a

541   cosmopolitan species (Tarazona et al., 2014) and that its virulence is dependent on hot summer

542   temperatures ≥24°C, the bacteria seem to be opportunistic, affecting some weak individuals,

543   but not responsible for the die-offs in open waters. A *Mycobacterium* has recently been

544   associated with the die-offs observed in the Tyrrhenian Sea (Carella et al., 2019), which suggests

545   that the importance of bacterial infections and the synergistic effects among different

546   pathogens increasing the virulence of the disease should be studied in future research.

547   Conversely, despite the arrival of *H. pinnae* in Alfacs Bay in July 2018, it killed mostly individuals

548   in the external part of the bay, closer to its mouth, suggesting the presence of some ecological

549   barrier constraining its capacity for dispersion or survival in this environment.

550

551   4.2 Recruitment and larvae collectors

552

553   The presence of juveniles from the summer 2017 reproductive cycle in the larval collections

554   from Castellón and Alicante, the fish farm in Villajoiosa and in Alfacs Bay, suggest that 2017 was

555   a good year for fan mussel reproduction. The origin of these larvae is unknown. Apparently, four

556   main sources were available in summer 2017: Columbretes Islands, Delta del Ebro, continental

557   France and Corsica (Wesselmann et al., 2018). Juveniles and larvae of *Ostrea edulis* may be

558   infected by *Bonamia ostreae*, but mortalities mainly affect oysters older than 2 years (Arzul et

559   al., 2011). *Crassostrea virginica* juvenile oysters exposed to *Perkinsus marinus*, however, are

560   rapidly infected and die (McCollough et al., 2009). The presence of fan mussel juveniles in

561   collectors and the fish farm indicates that at least they can survive for a few months in open

562   waters. However, if fan mussel juveniles could survive for up to two years, like those of *O. edulis*

563   infected by *B. ostreae*, at least some of the spat that settled in summer 2016, the size of which

564   should be approximately 20 cm by summer 2018 (García-March et al., 2007; García-March et al.,

565   2011; Kersting & García-March 2017), should have been spotted already. Data gathered to date,

566   however, show that the affected areas are not being repopulated naturally by *P. nobilis*. The

567   confirmed infection of the *Pinna nobilis* juveniles maintained in tanks in the present study, as

568   well as the lack of observations of new juveniles in situ to date, indicate that the juveniles are

569   also being infected by the protozoan and that the response is more similar to that of *C. virginica*

570   infected by *Perkinsus marinus*. This observation differs from the dynamics of infectious diseases

571 of other marine invertebrates, such as the sea urchin *Diadema antillarum* in the Caribbean.

572 Shortly after the onset of the die-off suffered by this species in 1983-1984, natural recruitment

573 was observed, probably from larvae that were already present in the plankton upon arrival of

574 the disease. During the subsequent 30 years, episodic low-level recruitment events of

575 individuals were observed. These new recruits, however, subsequently succumbed to mortality,

576 which was interpreted as the consequence of an unknown factor of post-settlement mortality.

577 The populations of *D. antillarum* have recovered to 12% of those before the die-off because

578 recruitment in populations after the post-mortality period was persistently limited for decades,

579 despite a potentially slowly increasing larval supply (Lessios, 2016). In comparison to the

580 absence of natural recruitment observed in fan mussel populations after mortality, a similar

581 outcome after 30 years seems to be optimistic scenario. Considering the extra constraints

582 encountered by fan mussels to repopulate affected areas, we may be the witnesses of its

583 potential extinction. The recovery of fan mussel populations, if possible, will, in any case, be a

584 long-term process lapsing for many decades. Previously reported die-off events of

585 Mediterranean invertebrates were usually caused by warming seawater temperatures and

586 anomalous low circulation patterns during summer, both in deeper (Garrabou et al., 2009, 2001)

587 and shallow habitats (Kersting et al. 2013), and have not affected *P. nobilis*. These events,

588 however, were usually punctual and did not kill the entire populations, so densities could be

589 recovered through recruitment after a few years (Cerrano et al., 2005). The present die-off

590 experienced by fan mussels is more similar to those observed for *O. edulis* and *C. gigas* and the

591 eelgrass Limpet *Lottia alveus* (Carlton et al., 1991). Ultimately, natural populations of *Pinna*

592 *nobilis* in the Mediterranean Sea are likely to disappear in the next few years, with the exception,

593 perhaps, of lagoonal population. Such a pandemic is very unusual in marine organisms and raises

594 questions concerning actions that should be undertaken in the very near future, before all open

595 sea population go extinct. These actions should be planned considering the emergency, because

596 the disease has eliminated fan mussel populations from Spanish coasts and spread to most of

597 the Mediterranean Sea in just a couple of years.

598

599 4.3 Rescue of individuals

600

601 The rescue programmes have shown that fan mussels are resilient to extraction and transport,

602 even for long distances, as described in the present study.

603

604 4.3.1 Individuals from la Marina Real of Valencia

605

606 Maintenance temperatures (above 14°C), probably accelerated the rapid death of the fan
607 mussels collected from la Marina Real of Valencia, especially at the IMEDMAR-UCV installations.
608 These fan mussels were going to be used for in situ reproduction experimentation, and warm
609 temperatures are necessary to facilitate gonad maturation. The first exploratory survey carried
610 out on this population in December 2016 showed no mortality. Two months later, however,
611 when the collection of individuals was undertaken, 40% mortality was already observed. It is
612 worth noting that by then, the pathogen was still unidentified and that the relationship between
613 water temperature and disease virulence was unsuspected. In contrast, it was hypothesized that
614 maintaining individuals within their comfort temperature range (between 16°C and 24°C) (Trigos
615 et al., 2015) could help improve their capacity to fight the disease under captivity conditions.
616 The higher water temperature maintained at the IMEDMAR-UCV facilities probably accelerated
617 the mortality of the fan mussels, which were all dead 7 months after collection.

618 The four individuals who survived in good condition for 18 months in the Oceanogràfic Aquarium
619 were maintained at a constant temperature of 17°C. These individuals should have died if
620 infected because that temperature was sufficiently high to cause the rapid death of infected
621 individuals. It is possible that these fan mussels had some kind of resistance and were
622 maintaining the protozoan in a chronic condition and that an uncontrolled weakening of their
623 immune system could have led to their final death. Alternatively, the individuals were "luckily"
624 free of the protozoan when collected, and remained uninfected until July 2018, when the tank
625 could have been unintentionally exposed to the protozoan, provoking rapid death of the fan
626 mussels. Regardless of the causes, as the individuals came from an affected population and
627 survived for 18 months, prolonging the life of fan mussels rescued from affected areas is
628 possible.

629

630 4.3.2 Individuals from Port Lligat and Alfacs Bay

631

632 In September 2018, 12 individuals (11%) among those rescued from Port Lligat were still alive,
633 when the donor population had already disappeared and no individuals could be found in situ
634 since one month prior. The main factor constraining the mortality in tanks was the declining
635 water temperature below 14°C. Again, rescuing individuals prolonged their life, confirming that
636 it is possible to save fan mussels originating from infected populations. It is worth noting a priori
637 that only those individuals who have not been infected are expected to survive, which means
638 that survivorship will depend on the prevalence of the infection at the time of collection. The
639 survival was much higher for individuals from Alfacs Bay, which was expected because the
640 disease had not arrived in this region when the individuals were collected. However, the

641 mortality observed throughout the stabling period was produced by the bacterium (*V.*
642 *mediterranei*), which is also known to infect other species of bivalves (Tarazona et al., 2014).
643 With the new knowledge of the occurrence of *V. mediterranei* in *P. nobilis* maintained in
644 captivity, samples analysed for the presence of *H. pinnae* should be re-checked for bacteria to
645 evaluate the possibility of cohabitation of other pathogens in fan mussels.

646

647 4.4 Predator exclusion cages

648

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

657

658 4.5 Potential actions and recommendations

659

660 Based on the information gathered over the previous two years, a series of actions and
661 recommendations for improvement of the survival of fan mussels can be undertaken. These
662 actions will allow extra time and hence a greater probability of survival to *P. nobilis* as a species,
663 but whether their implementation will avoid fan mussel potential extinction or not is currently
664 unpredictable, because extinction depends on many uncontrolled (and some yet unknown)
665 variables. One imminent action should be the protection of the remaining lagoon populations
666 by eliminating or minimizing activities that could create a threat to fan mussels living within.
667 Protecting individuals in cages seems to be inefficient unless large numbers of specimens are
668 protected, and even so, the success of using more cages would need to be re-evaluated.
669 Alternatively, rescuing individuals seems to be a good option even if they originate from an
670 affected population. Another important issue is to understand why individuals living in paralic
671 environments and coastal lagoons are not affected by the die-off (e.g., Mar Menor Lagoon) or
672 are less severely affected (Alfacs Bay). It is not clear if it is a question of temperature and/or
673 salinity, or of some chemical condition occurring in these environments, preventing direct (by
674 killing it) or indirect (by killing its potential vector) entry of the parasite. Although both Mar
675 Menor lagoon and Ebro Delta do not show extreme salinity variations compared with those

676 occurring in the Caloosahatchee estuary in Florida (La Peyre et al., 2003), salinities are
677 hiperhaline and hipohaline, respectively, compared with the Mediterranean Sea. *H. pinnae*
678 could be unadapted to these variations in water temperature and/or salinity (Cabanellas et al.,
679 2019). Alternatively, fan mussels surviving in these environments could possess some kind of
680 resistance. Studying the environmental conditions of the lagoons and the genetic differences
681 between individuals living inside and outside coastal lagoons and deltas could help to better
682 understand this issue. Additionally, gathering more information about other fan mussel
683 populations surviving in paralic environments in affected areas would improve understanding of
684 the factor(s) conditioning their survival, by comparison of coincident environmental features
685 among them. Studies of reproduction in vitro are fundamental to close the life cycle of *P. nobilis*.
686 Once reproduction in vitro is mastered, it could be applied to resistant individuals to grow
687 resistant spat to repopulate devastated fan mussel populations. Finally, extensive searches for
688 resistant individuals in already affected populations is of paramount importance, since these
689 individuals could provide the seeds for future open water repopulations with artificially
690 produced juveniles.

691 Regarding the specific recommended action of the rescue of individuals, it is advisable to extract
692 fan mussels prior to the arrival of the parasite. Otherwise, small groups of no more than 5 to 10
693 individuals should be collected to enable the selection of only those that seem healthy, i.e., total
694 mantle extension and a quick closing response of the valves in response to external stimuli. After
695 placing them in cold water, preferably 12.5°C, individuals should be checked daily for mantle
696 extension during the following weeks to retain only those that show total mantle extension. It
697 is recommended to place no more than 5-6 individuals per tank in independent systems with
698 acclimatizers. If any individual is infected, it may infect others in the same tank, particularly if
699 the water temperature rises above 14°C. Hence, it is very important to maintain low
700 temperatures and quickly isolate any individual showing any symptom of the disease. It is
701 advisable to obtain mantle samples of all individuals, conserve them in absolute ethanol, and
702 analyse them for the presence of the parasite by PCR (Catanese et al., 2018; López-Sanmartín et
703 al. 2019). *Perkinsus marinus* and *H. nelsoni*, infecting *Crassostrea virginica*, undergo reduced
704 transmission and prevalence at winter temperatures from 10-15°C -although *P. marinus* can
705 survive at temperatures as low as 4°C (Soniat et al., 2008). Repeated freshets can also reduce *P.*
706 *marinus* infections in *C. virginica* to low levels, resulting in an overall low prevalence, low oyster
707 mortality and good growth (La Peyre et al., 2003). *Pinna nobilis* can survive in paralic
708 environments subjected to important seasonal salinity and temperature oscillations. Although
709 there is a knowledge gap regarding its tolerance limits, the habitat distribution suggests it is a
710 euryhaline, eurytherm species. Considering that the extreme salinity of Delta del Ebro and Mar

Menor is one of the possible environmental variables preventing infection of fan mussels by the parasite, it would be worth determining whether fan mussels can survive low-temperature (<8°C) and extreme salinity (<15 psu or >45 psu) treatments. Based on the data published by Cabanellas-Reboredo et al. (2019), it is reasonable to suspect that *H. pinnae* transmission and replication may be constrained at least by low temperatures and probably also by extreme salinities, as observed for other similar protozoans. A combined treatment with low temperatures and extreme salinities could be a good option to maintain individuals during the initial weeks of quarantine. This strategy could reduce transmission among individuals, while healthy and sick individuals are separate. Furthermore, it would be worth examining whether the prevalence of the parasite in infected individuals could be reduced with these kinds of treatments.

In conclusion, one additional global view is provided. The Mediterranean Sea has been considered a laboratory to examine global processes (Krijgsman, 2002; Lejeusne et al., 2010) using *P. nobilis* as a model species (Basso et al., 2015). When the fan mussel die-off started, many research teams were already studying the species because of its ecological and social interest. As previously suggested by Dulvy et al. (2003), marine extinctions may be underestimated because of low detection power and a long-term lapse (on average 53 years) between the last sighting of an organism and the reporting of the extinction. If a species of interest that is closely monitored, such as *P. nobilis,* has been shown to experience such a sudden and fatal die-off event, practically eliminating its populations in two years, how many other more overlooked species might experience a massive and unnoticed die-off before it is too late to implement any preservation action? In the present context of anthropogenic impacts and climate change, the case of fan mussels shows that the virulence and speed of mass mortality events may escalate. Rapid management responses to infectious mass mortality events driven by climate change are going to be necessary, and researchers and stakeholders will have to be ready to act collaboratively in order to respond to these threats. Furthermore, the present mass mortality event shows that the effects of infectious diseases have important implications in the future redistribution of species and biodiversity. This would justify their consideration in prediction models (e.g. Jones and Cheung, 2014), because their effects are global and occur in very short time periods, compared even to the speed of climate change, and may have considerable structural effects in the biological community (Harvell et al, 2019).

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7. Compliance with Ethical Standards

- 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.

780   -   All research in the paper not carried out by the authors is fully acknowledged in the
781   manuscript.
782   -   All sources of funding are acknowledged in the manuscript, and the authors declare no
783   direct financial benefits that could result from publication.
784   -   All appropriate ethics, permissions to manipulate animals and other approvals were
785   obtained for the submitted research.
786

787 **8. Competing interests statement**

788

789 There are no competing interests.

790

791 **9. References**

792

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1009   Fig. 1. Distribution map of the stations monitored for population dynamics and installation of
1010   larvae collectors. Circles indicate population dynamic stations, triangles indicate stations of
1011   larvae collectors and squares indicate stations with both population dynamic and larvae
1012   collector stations. 1, Algeciras. 2, Caleta Maro. 3, Marina del Este. 4, Melicena. 5, Agua Amarga.
1013   6, Isla de San Andrés. 7, Loza del Payo. 8, El Calón. 9, Terreros. 10, Mar Menor. 11, Illa de Tabarca.
1014   12, Villajoyosa. 13, Serra Gelada. 14, Calpe. 15, Illa Portixol. 16, Cap de Sant Antoni. 17, Illetes.
1015   18, Cala Gelada. 19, Rates (20 m). 20, Santa María (10 m). 21, Santa María (20 m). 22, Morobuti
1016   (30 m). 23, Cap Pinar. 24, Magaluf. 25, Portals. 26, Sant Elm. 27, Cala Ratjada. 28, Cala Gat. 29,
1017   Pollença. 30, Illa de l'aire. 31, Benibecar. 32, La Mola. 33, Sa Farola. 34, Son Saura. 35, Fornells-
1018   Cabra Salada. 36, Cala Mica. 37, Marina Reial. 38, Illes Columbretes. 39, Prat de Cabanes. 40,
1019   Serra d'Irta. 41, Bahía dels Alfacs. 42, El Fangar. 43, Canyelles. 44, Tossa de Mar. 45, Illes Medes.
1020   46, Montjoi. 47, Joncols. 48, Port Lligat. 49, Peyrefite. 50, Etang de Leucate. 51, Etang de Ayrolles.
1021   52, Agde. 53, Etang de Thau. 54, Frontignan. 55, Les Aresquiers.

1022   Fig. 2. Survivorship curves and water temperature in Ebro Delta and IRTA (DeltaIrta) (top panel),
1023   Port Lligat (medium panel) and the tanks in IEO, IFAPA, Oceanogràfic and IMEDMAR-UCV
1024   (bottom panel). When two lines of a similar type occur in a panel, thick lines represent the water
1025   temperature and thin lines the survivorship for the same tanks. In the top panel, AlfacsZ1C1
1026   (Alfacs circle 1, zone 1) and AlfacsC1C2 (Alfacs circle 1, zone 2) share the same water
1027   temperature (AlfacsT). Note that water temperature in Port-Lligat (medium panel) corresponds
1028   to the closest monitoring station located in open waters of Startit (one mile off the Medas
1029   Islands). Port-Lligat is an enclosed small bay with in situ recorded water temperature during the
1030   rescue programme conducted in November 2017 below 13°C. Therefore, the registered water
1031   temperature at Startit was higher than the directly observed water temperature in Port Lligat in
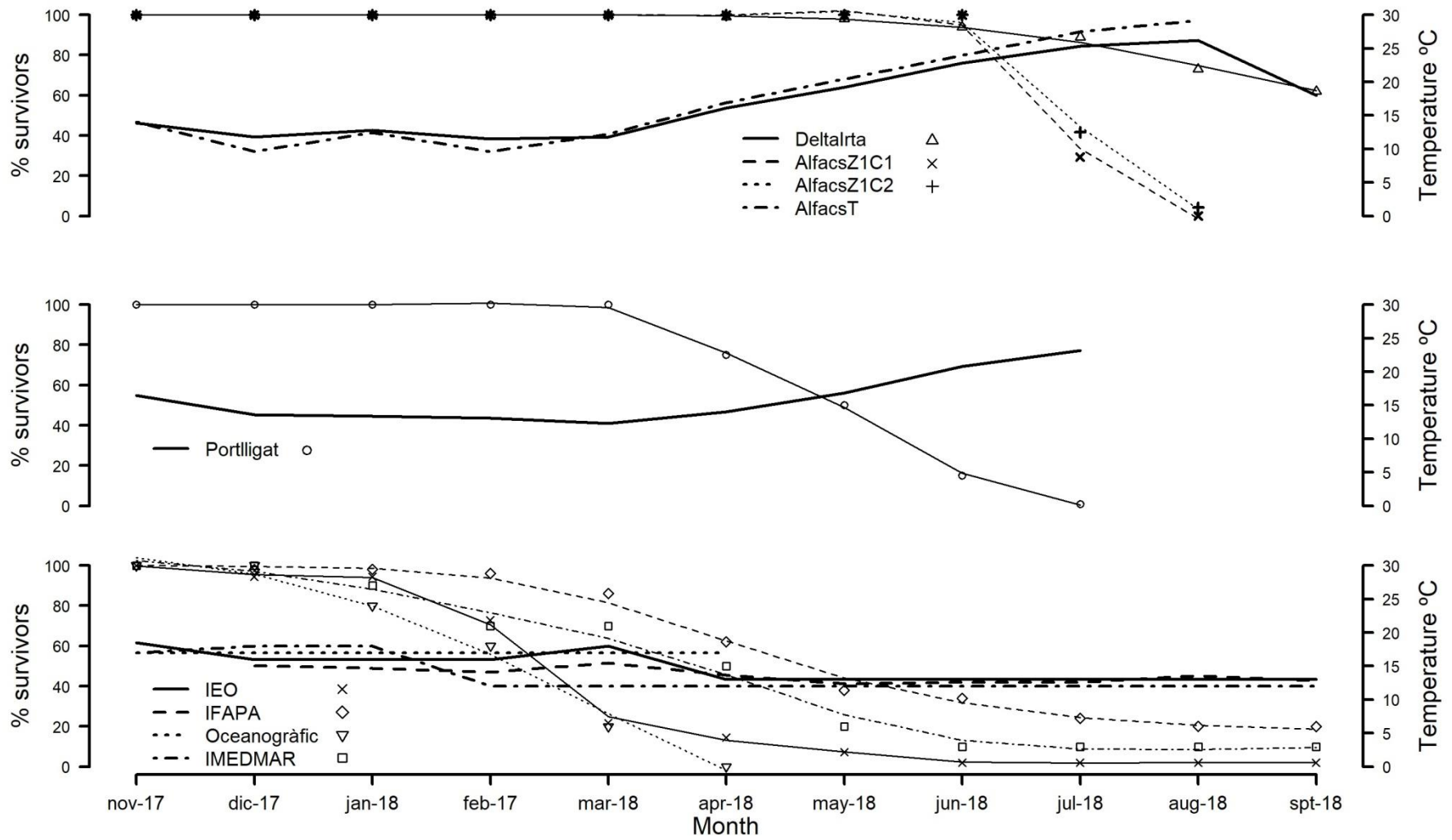1032   November 2017.

1033
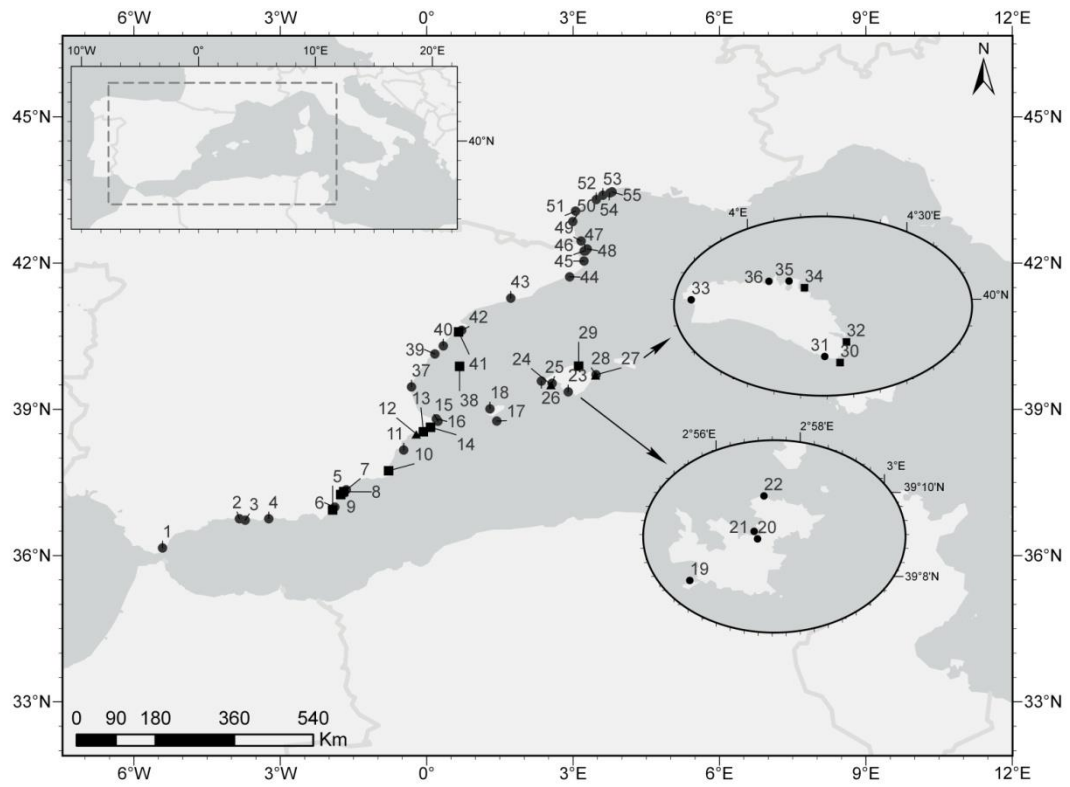
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1036   Fig. 1.

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1038   Fig. 2