

# LIFE PINNARCA

LIFE NAT/ES/001265



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PLANNING CORRECTION MEASURES: SPAIN

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# 1 REPORT ON NATURAL RECRUITMENT OF PINNA NOBILIS POPULATIONS EVALUATED WITH LARVAL COLLECTORS INCLUDING PAST RECRUITMENT EVENTS

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## 1.1 Background

To assess the impact of the MME on *Pinna nobilis*' larval recruitment, a network of larval collector stations was implemented over several thousands of kilometers along the Western Mediterranean coasts during the three years after the onset of the MME.

Artificial recruitment (i.e., by means of larval collectors) has proved to be a useful tool for assessing recruitment potential in *P. nobilis* (Cabanellas-Reboredo et al. 2009, Alomar et al. 2015, Kersting & García-March 2017, Wesselmann et al. 2018), providing insights into larval supply and recruitment previous to the exposure to pressures like predation or dislodgement (Kersting & García-March 2017). *Pinna nobilis* larval collectors were specifically designed and used for the first time by De Gaulejac et al. (2003). Later on, several studies have used larval collectors to study different aspects of *P. nobilis* recruitment, adapting this design to the specific conditions of study sites and experimental settings (Cabanellas-Reboredo et al. 2009, Kersting & García-March 2017, Wesselmann et al. 2018). In Cabanellas-Reboredo et al. (2009) and in Kersting & García-March (2017) larval collectors were used to assess the larval settlement period of *P. nobilis* in the Balearic and Columbretes islands, respectively. Both studies established the peak of the settlement period between August and September. In Kersting & García-March (2017) larval collectors were used as well to assess recruitment of the species in the long-term, showing the high interannual variability in recruitment rates of this species over a 9-year period and suggesting a positive correlation between water temperature and recruitment rates. This study also evidenced the feasibility of using larval collectors to obtain *P. nobilis* spat to be placed afterwards in protected cages submerged *in situ*, where recruits can be grown to be used later for restocking or restoring actions in impacted populations. Larval collectors have been used as well to assess genetic connectivity in *P. nobilis*, showing the existence of source and sink populations and the connectivity potential of the species (Wesselmann et al. 2018).

Because of the current status of the species and the general importance of recruitment in the recovery of impacted populations, an extensive effort (both spatial and human) has been undertaken to tackle *P. nobilis* recruitment in larval collectors over several thousands of kilometers along the Western Mediterranean coasts, the region first impacted by the MME (Vázquez-Luis et al. 2017), and in the Adriatic Sea, where the MME was first recorded in the final year of this study (2019) but most populations still remained unaffected (Kersting et al. 2019, Čižmek et al. 2020, S. Kipson unpubl. data). As a result, Kersting et al. 2020 aimed to provide an overall interannual picture of *P. nobilis* recruitment during three years after the 2016 MME, in order to assess potential larval connectivity and the role of recruitment in the future recovery of the species in the area; here we present a summary of these results.

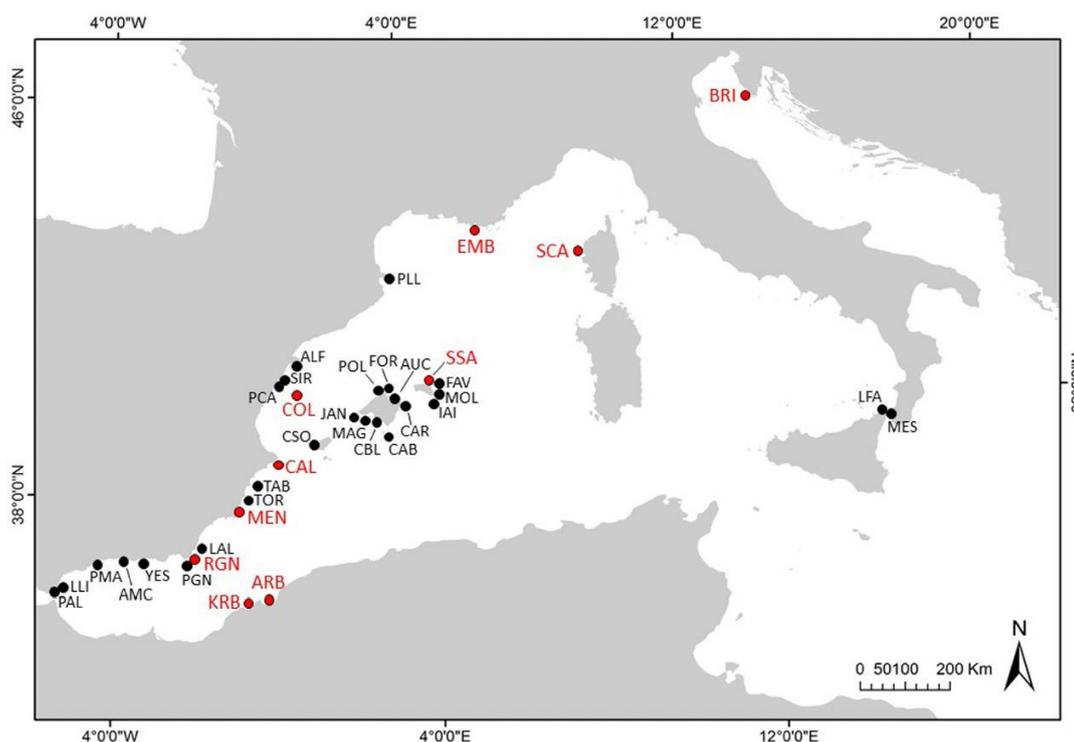
## 1.2 Current network and study sites

Here we report on larval collectors installed in 37 sites distributed along the coast of the Western Mediterranean Sea including north Africa, and in the northern Adriatic Sea (Fig. 1) during the reproductive seasons of 2017-2019. Data contribution and collaborators are listed in Table 1. The sites were selected according to the occurrence of *P. nobilis* populations previous to the mortality outbreak, with many of them subjected to periodical monitoring. However, most of the populations (with the exception of Scandola, Embiez Islands, Alfacs Bay, Mar Menor, Arzew and Kristel bays, and Brijuni MPA) were affected by the MME by the time the first collectors were installed at the beginning of the summer of 2017. The



impact of the MME on each location has been assessed in previous studies (Vázquez-Luis et al. 2017, Vicente et al. 2018, Cabanellas-Reboredo et al. 2019) or during the installation of collectors in the present study.

Time series on *P. nobilis* recruitment in larval collectors previous to this study were available for some of the selected sites: the Columbretes Islands (Kersting & García-March 2017), Embiez Islands (Vicente et al. 2017, 2018, Vicente 2020), Calpe (J. R. García-March unpubl. data), Cabrera (M. Vázquez-Luis unpubl. data) and for Pollença and Magaluf (I.E. Hendriks unpubl. data).



**Figure 1.** Larval collector sites in the Western Mediterranean and Adriatic sea. In red, sites where recruitment was recorded in, at least, one season (from Kersting et al. 2020). Full name of sites in Table 2.

**Table 1.** Institutes and collaborators involved in data collection (from Kersting et al. 2020)

<b>Kersting DK</b>	Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, IRBIO, Universitat de Barcelona, Barcelona, Spain
<b>Vázquez-Luis M, Álvarez E, Deudero S</b>	Instituto Español de Oceanografía (IEO). Centro Oceanográfico de Baleares, Palma de Mallorca, Spain.
<b>Mourre B</b>	Balearic Islands Coastal Observing and Forecasting System (SOCIB), Palma de Mallorca, Spain.

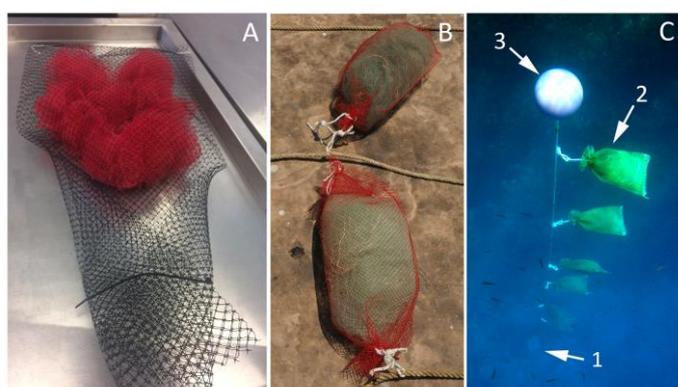
<b>Belkhamssa FZ</b>	Laboratoire Protection, Valorisation et Gestion des Ressources Marines et Littorales & Systématique Moléculaire/Département des Sciences de la Mer et de l'Aquaculture (LPVGRML), Faculté des Sciences de la Nature et de la Vie, Université Abdelhamid Ibn Badis de Mostaganem, Algeria.
<b>Bakran-Petricioli T, Kipson S</b>	Department of Biology, Faculty of Science, Zagreb University, Zagreb, Croatia.
<b>Barberá C</b>	Centro de Investigación Marina (CIMAR)-Universitat d'Alacant, Santa Pola, Spain.
<b>Barrajón A, Moreno D</b>	Programa de Gestión Sostenible del Medio Marino, Agencia de Medio Ambiente y Agua de Andalucía, Almería, Spain.
<b>Cortés E</b>	Acuario de la Universidad de Murcia, Murcia, Spain.
<b>García-March JR</b>	Instituto de Investigación en Medio Ambiente y Ciencia Marina (IMEDMAR-UCV), Universidad Católica de Valencia, Valencia, Spain.
<b>Giacobbe S</b>	Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, ChiBioFarAm, Università degli Studi di Messina, Italy.
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<b>Jiménez-Gutiérrez S</b>	Instituto de Ecología Litoral. El Campello, Spain
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<b>Sánchez J</b>	SUBMON: Awareness, Study and Conservation of the Marine Environment, Barcelona, Spain
<b>Spinelli A</b>	Oceanographic, City of Arts and Sciences, Department of Biology, Valencia, Spain.
<b>Valencia JM</b>	LIMIA Govern de les Illes Balears, Palma de Mallorca, Spain. / INAGEA (INIA-CAIB-UIB), Palma de Mallorca, Spain.

<b>Vicente N</b>	Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE) Aix-Marseille Université, Marseille, France. / Institut Océanographique Paul Ricard, île des Embiez, France.
<b>Hendriks IE</b>	Global Change Research Group, Mediterranean Institute for Advanced Studies (CSIC-UIB), Esporles, Spain.

### 1.3 Current larval collector design

The adopted design of larval collectors consisted of a series of plastic mesh bags containing entangled nylon filament or onion bags (see De Gaulejac et al. 2003, Cabanellas-Reboredo et al. 2009, Kersting & García-March 2017, Vicente 2020, for more details). The bags were attached to a main rope that was fixed to a concrete mooring and kept vertical by a submerged buoy, covering a 0.5-20 m depth range (Fig. 2).

Larval collectors were moored annually in early summer (June) and removed in mid-autumn (November) from 2017 to 2019 (see Table 1 for details), thus covering the main reproduction and settlement period of the species (Cabanellas-Reboredo et al. 2009, Deudero et al. 2017, Kersting & García-March 2017). Once removed, the bags were immediately opened, and all *P. nobilis* recruits were carefully collected from the nylon mesh following the procedure in Kersting & García-March (2017). Observation of *P. nobilis* recruits was undertaken with the naked eye, allowing the detection of recruits of sizes down to 0.3 cm antero-posterior length. Recruits extracted from the collectors were either installed in aquaria (García-March et al. 2020, Vicente 2020) or in growth cages in the field following Kersting & García-March (2017). It must be noted that at small sizes distinguishing *P. nobilis* from *P. rudis* juveniles can be difficult. In bigger juveniles, *P. rudis* can be easily distinguished by the lower number of radiating ribs (4-5 ribs) and scales, which are also bigger and sturdier than in *P. nobilis* (Fig. 3). This issue is easily solved by keeping the juveniles in growth cages or in aquaria, where they grow to a size that allows identification. In addition, genetical analyses were used to contrast identification in 5 juveniles. Nevertheless, even if small, a certain identification error must be assumed, especially if juveniles die before growing to the needed size.



**Figure 2.** Larval collectors. Plastic mesh bags containing onion bags (A) and entangled nylon filament (B). (C) Components of a larval collector:

concrete mooring (1), bags (2) and submerged buoy (3). From Kersting et al. 2020.

## 1.4 Identification of source sites through Lagrangian trajectory modelling

A high-resolution regional hydrodynamic model WMOP (Juza et al. 2016, Mourre et al. 2018) was used to simulate backward trajectories from the observation sites where recruitment was recorded, with the objective to identify the potential origin of larvae transported by ocean currents over the basin during the recruitment period. The WMOP model, developed at the Balearic Islands Coastal Observing and Forecasting System (SOCIB, [www.socib.es](http://www.socib.es)), provides daily predictions of the Western Mediterranean circulation from the Strait of Gibraltar to Corsica and Sardinia with a 2-km spatial resolution. The WMOP surface currents result from the effects of 3-dimensional basin-to-coastal-scale ocean processes driven by atmospheric forcing (wind, evaporation-precipitation and heat fluxes), river inflows and open boundary inputs over a realistic bathymetry. The model, based on the ROMS modelling system (Shchepetkin & McWilliams 2005), is nested in the larger scale Mediterranean model from the Copernicus Marine Service (Clementi et al. 2017). The model air-sea fluxes are computed through bulk formulae applied to the high-resolution atmospheric fields provided by the Spanish Meteorological Agency (AEMET) Hirlam (for years 2017 and 2018) and Harmonie (for year 2019) models. The climatological runoffs of the six major rivers of the modelling domain are implemented as point sources of low saline waters. Further details of the forecasting system and model evaluations can be found in Juza et al. (2016), Mourre et al. (2018) and Aguiar et al. (2020). Operational data assimilation of sea level, sea surface temperature, Argo profiles and Ibiza Channel High-Frequency radar was implemented in November 2018, using the method described in Hernandez-Lasheras & Mourre (2018).

Moreover, the daily average wave-induced drift provided by the Copernicus Marine Service Mediterranean Sea waves model (Korres et al. 2019a, 2019b) was added to the WMOP currents to represent the contribution of surface ocean waves.

The TRACMASS algorithm (Jönsson et al. 2015) was used to generate Lagrangian trajectories over a 1-month period to approximate larval period duration (Deudero et al. 2017, Kersting & García-March 2017, Trigos et al. 2018). Virtual particles were released once a week during the recruitment period from July 1<sup>st</sup> to September 15<sup>th</sup> (Cabanelas-Reboredo et al. 2009, Kersting & García-March 2017) from all sites and for all years where recruitment was recorded, with the exception of Mar Menor because of its low exchange with open waters (i.e., semi enclosed coastal lagoon). In the case of Arzew and Kristel bays in Algeria, a single location (Arzew) was used given the proximity of the two sites. Cluster of 1000 particles were released at each location, and modelled trajectories were the result of advection by the WMOP surface currents and wave-induced drifts plus a diffusive term accounting for the effect of model uncertainties and unresolved processes.



**Figure 3.** *Pinna nobilis* (left) and *P. rudis* (right) juveniles at a size of 5-6

cm (antero-posterior length). Notice how the number of radiating ribs and the morphology of the scales clearly differs in both species (from Kersting et al. 2020).

**Table 2.** Larval collector sites, with recruitment data from 2017-2019. Year of MME noted for each site (from Kersting et al. 2020).

Site	Country	Recruits 2017	Recruits 2018	Recruits 2019	MME	Collectors (n)	Bags (n)	Depth (m)
<b>Brijuni MPA</b>	BRI Croatia	n. i.	n. i.	<b>72</b>	no	3	3	6–10
Strait of Messina	MES Italy	Disappeared	Disappeared	Disappeared	2017	4	3	6–7
Lake Faro	LFA Italy	0	Disappeared	Disappeared	2017	4	2	3–4
<b>Reserve Naturelle de Scandola</b>	SCA France	<b>18</b>	n. i.	<b>3</b>	2018	3	10	4–18
<b>Embiez Islands</b>	EMB France	n. i.	<b>12</b>	0	2019	3	10	4–18
Port Lligat	PLL Spain	n. i.	Disappeared	n. i.	2018	1	3	3
<b>Son Saura</b>	SSA Spain	0	<b>1</b>	Disappeared	2017	1	3	5
Favàritx	FAV Spain	0	n. i.	n. i.	2017	1	3	5
La Mola	MOL Spain	0	0	Disappeared	2017	1	3	5
Illa de l'Aire	IAI Spain	0	0	Disappeared	2017	1	3	5
Reserva Marina de Llevant-Cala Ratjada	CAR Spain	0	0	n. i.	2016	3	2	10–12
Parque Nacional del Archipiélago de Cabrera	CAB Spain	0	0	0 (1)	2016	1	3	5
Cala Blava	CBL Spain	0	n. i.	n. i.	2016	1	3	5
Magaluf	MAG Spain	0	0	0	2016	3	2	6
Jaulas Andratx	JAN Spain	0	0	0	2016	3	5	0.5–3
Pollença	POL Spain	0	0	0	2016	3	2	5
Cap Formentor	FOR Spain	0	n. i.	n. i.	2016	1	3	3–5
Aucanada	AUC Spain	0	n. i.	n. i.	2016	1	3	5
Badia dels Alfacs (Delta del Ebro)	ALF Spain	0	0	0	2018	10–15	1	0.5–2
Serra d'Irta	SIR Spain	n. i.	n. i.	Disappeared	2017	1	3	5
Prat de Cabanes	PCA Spain	n. i.	n. i.	Disappeared	2017	1	3	5
<b>Reserva Marina de las Islas Columbretes</b>	COL Spain	<b>187</b>	<b>5</b>	<b>2</b>	2017	1	6	5–13
Caló de s'Oli	CSO Spain	0	n. i.	n. i.	2016	1	3	5
<b>Calpe</b>	CAL Spain	<b>9</b>	0 (30)	0 (2)	2016	3	12	5–18
Reserva Marina de la Isla de Tabarca	TAB Spain	0	0	0	2016	2	4	2–11
Torreveija	TOR Spain	0	n. i.	n. i.	2016	2	2	5
<b>Mar Menor</b>	MEN Spain	<b>1</b>	0	n. i.	no	2–5	2	1–3
SAC, SPAMI Fondos Marinos Levante Almeriense	LAL Spain	0 (2)	0	n. i.	2016	1–3	1–3	13–20
<b>Reserva Marina Cabo de Gata–Níjar</b>	RGN Spain	<b>3 (6)</b>	0	n. i.	2016	3	2	10–20
Parque Natural Cabo de Gata–Níjar	PGN Spain	0 (1)	0	n. i.	2016	1	1–3	15–18
Los Yesos	YES Spain	n. i.	0	n. i.	2016	1	3	6.5
Paraje Natural Acantilados de Maro-Cerro Gordo	AMC Spain	n. i.	0	n. i.	2016	1	3	9
Puerto pesquero de Málaga	PMA Spain	n. i.	0	n. i.	2016	1	3	9.5
La Línea	LLI Spain	n. i.	0	n. i.	2017	2	3	5
Puerto de Algeciras	PAL Spain	n. i.	0	n. i.	2017	1	3	20
<b>Arzew bay</b>	ARB Algeria	n. i.	<b>60 (13)</b>	<b>102 (15)</b>	no	1	10	5–10
<b>Kristel bay</b>	KRB Algeria	n. i.	<b>37 (8)</b>	<b>30 (12)</b>	no	1	10	5–10

In bold, sites where recruitment has been recorded.  
n.i.: not installed.  
In brackets, *P. rudis* recruits when occurred.

## 1.5 Current knowledge on larval recruitment of *Pinna nobilis* after the MME

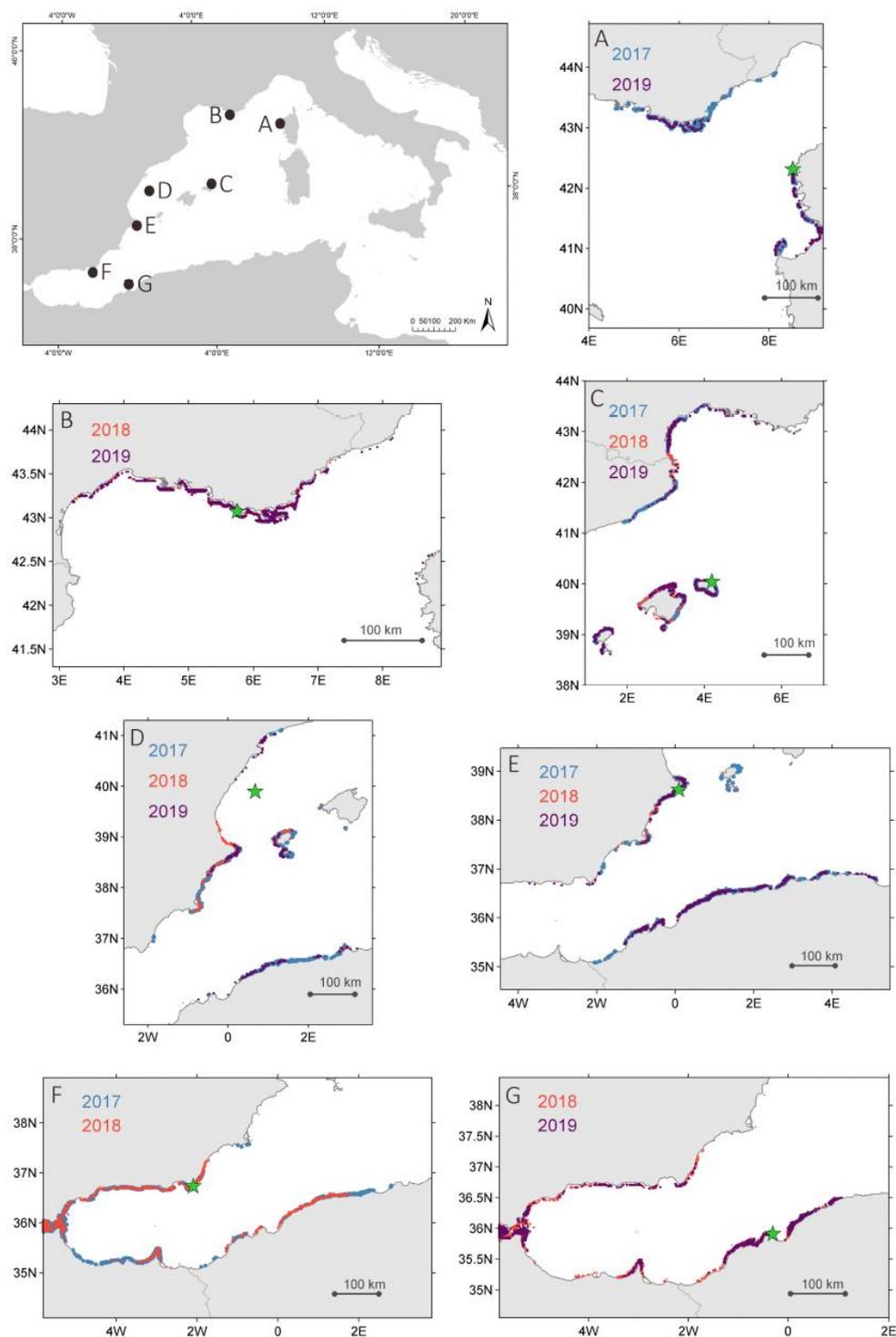
*Pinna nobilis* recruits were found in the collectors installed at 10 sites (Scandola, Embiez Islands, Son Saura, Columbretes Islands, Calpe, Mar Menor, Cabo de Gata, Arzew and Kristel bays, and Brijuni); while no recruits were retrieved from the collectors in the remaining sites (Table 2). All sites had been impacted by *H. pinnae*, either before or during the study, apart from the sites in Algeria, Croatia and Mar Menor (Spain) (Table 2). Only one of the MME-affected sites, Columbretes Islands, recorded recruitment in the larval collectors during the three reproductive seasons (2017-2019). Besides this site, all other sites where a large number of recruits was recorded, were located in unaffected or partially unaffected regions. The



congeneric species *P. rudis*, unaffected by the mortality, recruited in several sites during the study (Table 2). Time series of *P. nobilis* recruitment in larval collectors previous to this study (i.e., Columbretes Islands, Embiez Islands, Calpe, Cabrera, Pollença and Magaluf) showed that, although with the inherent interannual variability, recruitment in larval collectors at these sites was recorded annually before the MME.

Because larval collectors are installed in shallow waters, they are highly exposed to storms and intentional or accidental removal; collectors at 8 sites were lost due to these causes during at least 1 recruitment season (Table 2). All extracted recruits from MME affected sites that were kept in growth cages died presumably because *H. pinnae* during the first months, as did the ones taken to aquaria according to García-March et al. (2020).





**Figure 4.** Results of the Lagrangian trajectory model showing the potential origin of *P. nobilis* larvae for each site and year (from Kersting et al 2020), (A) Scandola, (B) Embiez, (C) Son Saura, (D) Columbretes, (E) Calpe, (F) Cabo de Gata, (G) Arzew. Sites are represented on the maps by the green star.

## 1.6 Pinpointing source populations for larval transport

The Lagrangian trajectory model from the sites where recruitment was recorded shows the potential geographical origin of larvae during the main reproduction and recruitment period of the species (Fig. 4). After disregarding the sites where *P. nobilis* had disappeared because of the MME (i.e., most of the coast of Spanish mainland coast and the Balearic Islands), three main regions harbouring unaffected populations remain as potential larval sources in the Western Mediterranean: French Mediterranean coast, Delta del Ebro region and the north African coasts (mainly Algeria). The northern sites showing recruitment (i.e., Son Saura, Embiez Islands and Scandola) received larvae potentially from the French coasts, while southern locations (i.e., Calpe, Cabo de Gata, Arzew and Kristel bays) were nourished by the north African coasts. Columbretes Islands represented an in-between situation, receiving larvae both from the south (north Africa) and the north (Delta del Ebro region) (Fig. 4). According to the model, Mar Menor, which hosts unaffected populations, could be also considered as an exporting site. However, its limited water exchange with the open sea will probably hinder its potential role as a significant larval donor.

## 1.7 Summary from larval recruitment during 2017-2019, first three years after the MME

Collapse of regional larval recruitment in many marine invertebrate species has been associated to the loss of adults after catastrophic events, seriously hindering recoveries (Miner et al. 2006, Miller et al. 2009, Lessios 2016, Hughes et al. 2019). As expected, the high mortality rates recorded during the MME have impacted *P. nobilis* reproduction and are therefore impairing recruitment. In general, the recruitment during the first three years after the MME show a disruption of *P. nobilis* larval recruitment over a vast geographical area in the Western Mediterranean Sea.

There were, however, some exceptions to this generalized absence of *P. nobilis* recruitment. Among the sites already affected by the MME, recruitment was recorded every year (2017-2019) in the Columbretes Islands, despite the fact that pen shell populations deceased in this area during the summer of 2017 (Cabanellas-Reboredo et al. 2019, Kersting et al. 2019). Moreover, although to a lesser extent, recruits were also recorded as well during at least one season in Scandola (Corsica, France), Son Saura (Spain), Calpe (Spain), Mar Menor (Spain) and Cabo de Gata (Spain) (Table 2). Larval recruitment was abundant in 2018 and 2019 in other Mediterranean regions like the northern Adriatic Sea (Croatia) or some sites in the southern Mediterranean like Algeria, where the mortality is still arriving (Kersting et al. 2019, Čížmek et al. 2020) and many *P. nobilis* populations remained unaffected. Recruitment recorded in these sites was similar to that recorded in the Columbretes Islands before the MME (Kersting & García-March 2017). The same applies to Scandola and Embiez Islands (France), where larval recruitment was recorded during the years preceding the mortality that impacted these sites in 2018 and 2019, respectively (Vicente et al. 2020).

## 1.8 An outlook for the future

Long-term follow-ups of recoveries after disease-triggered die offs of marine invertebrates have shown that recovery can be slow and geographically heterogeneous (Miner et al. 2006, Miller et al. 2009). Of course, in the case presented here it is understood that populations' recoveries through recruitment will only occur if at least some of the settled individuals show resistance to the disease, which represents an important additional obstacle. All juveniles settled in the collectors and installed in growth cages within the MME-affected sites died before the first year of age, presumably because of *H. pinnae* infection. However, if resistant juveniles could establish, recovered sites could eventually export larvae to other locations and trigger a stepping stone recovery process through larval export and connectivity.



Nevertheless, potential recoveries of mortality-impacted populations will presumably differ among sites and will be highly dependent on their geographical location relative to unaffected populations and the oceanographic current patterns in the area. According to Sanna et al. (2013), *P. nobilis* populations seem to share a common origin and a recent eastward expansion across the Mediterranean, likely facilitated by marine currents. Therefore, a similar mechanism could mediate in the recovery and, therefore, new expansion of the species after the MME if resistant recruits should occur.

In the best scenario, i.e., with resistant juveniles, successful recoveries through recruitment could take a long time and it must be regarded that natural recruitment is also limited by other factors such as predation, especially in protected sites, where predators are abundant (Kersting & García-March 2017). In fact, in predator-rich environments, a refuge size of 45 cm has been estimated for the species, which would represent an age of about 8 years (Kersting & García-March 2017). Therefore, it is of great importance to start assessing recruitment in the field and recruit's survival, especially in those sites where recruits have been observed in the collectors.

## 1.9 Future plans

The larval collector network has been kept for the years after those reported here, with remarkable less recruitment after 2019 and an increase in the appearance of occasional recruits of *Pinna rudis*. These results need to be compiled and analyzed, and the continuation of recurrent sites with yearly data needs to be prioritized. Also important is to continue the standardization of the design, in order to be able to compare the number of larvae, as now the settlement surface differs between stuffed bags with nylon fishing threads and the design with 13m of onion mesh in aquaculture bags. As it seems to be hard to generalize designs in areas with different environmental conditions an alternative is to deploy both designs in one site and evaluate the recruitment in both in order to be able to “translate” between designs. The difference in environmental conditions also calls for site-specific deployment times, with the need in shallow sites (warming up faster) for deployment in April, especially if these sites lack in water exchange like in lagoons like the Mar Menor. In more open sites with water exchange and water depths >5m deployment can be like currently scheduled in June, with intermediate deployment times (May) for sites with limitations in water exchange or depths. Recovery of the collectors should be done before end October, and can be earlier for sites with earlier deployment with a maximum of 6 months deployment. Longer deployment of recruitment bags will increase the changes of a recruit disappearing through predation or might cause deformation of the shell due to growth limitations within the mesh. In summary:

- Standardization of designs taking into account environmental parameters
- Evaluation of performance of different designs through simultaneous deployment
- Adaptation of deployment dates based on suspected increase in temperature (due to depth / water exchange)
- Continuation of current deployment sites
- Expansion of the network targeting a wider cover of the region

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