Marine Environmental Research 197 (2024) 106428

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Sink-source connectivity for restocking of *Pinna nobilis* in the western Mediterranean Sea

A. Feria-Rodríguez [a,*], D. March [b,c], B. Mourre [d], I.E. Hendriks [e], M. Vázquez-Luis [a]

[a] Instituto Español de Oceanografía (IEO-CSIC). Centro Oceanográfico de Baleares, 07015, Palma de Mallorca, Spain
[b] Unidad de Zoología Marina, Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universitat de València 46100, Paterna, Spain
[c] Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, TR10 9FE, Penryn, United Kingdom
[d] SOCIB, Balearic Islands Coastal Observing and Forecasting System, Parc Bit, Ed., 07121 Palma, Spain
[e] Oceanography and Global Change Department, Mediterranean Institute for Advanced Studies (CSIC-UIB), 07190, Esporles, Spain

ARTICLE INFO

Keywords:
Pinna nobilis
Repopulation
Conservation
Dispersal
Genetic resistance
Mass mortality event
Bivalve

ABSTRACT

The critically endangered endemic bivalve *Pinna nobilis* from the Mediterranean Sea suffered a sudden population decline after a mass mortality event in early autumn 2016. Conservation efforts aimed at preventing extinction included safeguarding resistant individuals and implementing a breeding plan to contribute to the repopulation of the species. This study utilized a model combining Lagrangian dispersion and connectivity analyses to pinpoint optimal restocking sites in the Western Mediterranean. Our approach allowed to identify locations capable of sustaining and generating larvae for broader repopulation in key areas of the Western Mediterranean Sea prior to the mass mortality event. Six important repopulation locations from Murcia, Valencia and Balearic Islands were selected for reintroduction efforts. The results obtained in this study show how the network could be self-sufficient and able to self-replenish itself of recruits. Overall, our work can be used to direct the reintroduction of resistant animals in the Western Mediterranean Sea.

1. Introduction

Marine systems are currently confronting a crisis caused by anthropogenic activity, entailing a threat for marine biodiversity. Habitat loss or degradation are the main causes of loss of marine biodiversity (Gray, 1997; Airoldi et al., 2008; McCauley et al., 2015). Therefore, it is mandatory to apply strategies to conserve marine ecosystems and their services, as a key tool to regenerate and maintain them. Historically logistical challenges associated with collecting information of marine ecosystems have resulted in sparse information for marine organisms and ecosystems conservation (Swearer et al., 2019). Although both terrestrial and marine environments have similar requirements (i.e. the need to protect genetic variability, particularly within dominant or keystone species, as stated by Whitham et al., 2003); they also exhibit important physical and biological differences (Avery, 2003) that are important to take into account when developing conservation strategies. Whereas terrestrial species tend to be less connected between each other; the marine environment is an open system, and the restriction of taxa to a geographical area is less common, as well as the occurrence of endemism (Avery, 2003).

Population connectivity is defined as the "exchange of individuals among marine populations, diurnally, seasonally or during their life cycle" (Treml et al., 2008; Beger et al., 2010). The location of a population is the result of the interactions of abiotic factors with the biotic properties of the species. Dispersal of marine species serves different ecological purposes, from reproductive to colonization of new habitats, and can occur in different life stages. A large proportion of marine organisms have an early pelagic phase during which propagules/larvae disperse, sometimes covering considerable distances depending on pelagic larval duration (Crisp and Spencer Davies, 1976; Abelson and Denny, 1997; Avery, 2003).

Treml et al. (2015) described four stages of population connectivity, with three stages of dispersal; (1) Initiation of emigration, (2) Transport and Movement and (3) Settlement in natal or non-natal habitat; and a (4) stage of Recruitment. These stages are affected by biotic and abiotic factors that determine the success of recruitment and the evolution of populations. Biotic factors such as larvae quantity and quality, behavior, mortality, and pelagic larval duration are the most affecting throughout all stages. The last two parameters (PLD and larval mortality) are considered the most influential for population growth (Treml et al.,

* Corresponding author.
E-mail address: aferia@imedea.uib-csic.es (A. Feria-Rodríguez).

https://doi.org/10.1016/j.marenvres.2024.106428
Received 21 December 2023; Received in revised form 23 February 2024; Accepted 26 February 2024
Available online 11 March 2024

2015). Mortality rates affect settlement rates (Treml et al., 2015), but is not a parameter commonly used when assessing larval dispersal, due to the lack of empirical knowledge of mortality (Swearer et al., 2019). PLD, on the other hand, can be considered the most well studied biological trait influencing larval dispersal (Swearer et al., 2019), and provides information of the distance that larvae traverse (Treml et al., 2015), which helps to define pathways for population connectivity useful to assess the recovery of the networks.

Propagule dispersal is highly influenced by the stochastic nature of the physical environment (Siegel et al., 2008; Ospina-Álvarez et al., 2020). For benthic organisms with a pelagic phase, the dispersal and settlement of larvae depends mainly on oceanic (hydrodynamic) forces (i.e., direction and speed of ocean currents, eddies, salinity, temperature) that limit the distribution and settlement of larvae (Scheltema, 1986; Treml et al., 2008). Direct measurements to quantify larval dispersal are extremely difficult and therefore indirect approaches are mostly employed. Gaylord and Gaines (2000) proved that simple oceanographic features determine dispersal pathways, and showed how ocean currents induce the connectivity among benthic populations. Coupling biological parameters of larval dispersal (spread, supply, larval duration) with oceanic hydrodynamic data allows to create models that can describe and predict patterns for larval dispersal that can be applied for a variety of species (Treml et al., 2008; Bode et al., 2019). One of the main biotic factors that influence larval dispersal is the pelagic larval duration (PLD) (Treml et al., 2008), defined as the period of larval development of a species spent in the water column, where they are susceptible to physical mixing and advection (Sponaugle et al., 2002).

Therefore, biophysical models and graph-theoretic approaches are valuable tools to explore patterns of connectivity in marine ecosystems (Rognstad et al., 2018; Pastor et al., 2023), identify areas that are optimal to focus management and conservation strategies efforts (Treml et al., 2015), and can be used to address species-specific restoration (King et al., 2023). One of the most used approaches to characterize larvae transport is Lagrangian modelling: a technique that incorporates passive and active drift in a two or three-dimensional flow model (McDonald and Nelson, 2021).

Marine ecosystems are deteriorating globally due to several stressors with direct and indirect anthropogenic origin, causing the decline of biodiversity in marine communities globally. Coastal areas, even though highly diverse, are more exposed to habitat loss due to the high human activity they are exposed to (Gray, 1997). Consequently, species that inhabit shallow waters have suffered local and regional extinctions (Gray, 1997). One notable and recent example is highlighted by the noble pen shell *Pinna nobilis* (Linnaeus, 1758), an endemic bivalve from the Mediterranean Sea (García-March, 2005; Katsanevakis, 2005). In early autumn of 2016, an abnormally high mortality of *P. nobilis* individuals was detected almost simultaneously at several points in the Spanish Mediterranean coastline, with the phenomenon recognized as a mass mortality event (MME) (Vázquez-Luis et al., 2017). This MME was caused by the parasite protozoa *Haplosporidium pinnae* (Catanese et al., 2018), and from 2016/17 to 2018 it spread rapidly among populations from the Western Mediterranean Sea (Kersting et al., 2020; Cabanellas-Reboredo et al., 2019; García-March et al., 2020), removing up to 100% of the *P. nobilis* populations along the Mediterranean Sea (Katsanevakis et al., 2022). Consequently, *P. nobilis* was changed from the "Vulnerable" category to "Critically Endangered" in the Spanish legislation, with a serious extinction risk (Orden TEC/1078/2018) and was included as a "Critically Endangered" species on the IUCN Red List in 2019 (Kersting et al., 2020). Monitoring of populations subsequent to the MME have allowed the identification of few individuals with specific gene expression signature showing no symptoms of the disease nor the proliferation of the parasite in them (Salis et al., 2022). Moreover, these individuals have survived several years after the arrival of the MME, so that they can be considered in some way resistant to the pathogen. Under these premises, the existence of disease-resistant individuals of *P. nobilis* can be considered.

Noble pen shell is the largest bivalve of the Mediterranean, reaching up to 120 cm (García-March, 2005; Basso et al., 2015). It has a long-life span that can reach up to 45 years (Rouanet et al., 2015), and is one of the largest and long-living bivalves worldwide (Katsanevakis, 2005). The species inhabits mostly soft-bottom areas with seagrass meadows, mainly *Posidonia oceanica* and *Cymodocea nodosa*, macroalgae *Caulerpa prolifera* meadows or bare sand areas at depths between 0.5 and 60 m (Katsanevakis, 2005; Basso et al., 2015), where it is partially buried and fixed to the foundation.

The life cycle of *P. nobilis* is an example of the classical bivalve development (Trigos et al., 2018). As described by García-March (2005), the fecundation processes occur between March and September. The pen shell is of hermaphrodite reproduction, with an asynchronous gamete differentiation and maturation, external fecundation, and a settlement time of 10 to up to 30 days (Basso et al., 2015; Deudero et al., 2017; Trigos et al., 2018; Kersting et al., 2020). *P. nobilis* has a dispersal phase with pelagic larvae followed by eventual settlement and metamorphosis (Vicente, 1986; Trigos et al., 2018; Acarli, 2021). However, the larval stages and the pelagic larval duration (PLD) are far from being fully described. The first published studies pointed at a duration of the larval phase of 10 days (Butler et al., 1993). However, more recent publications suggest a PLD that could last at least up to one month (Trigos et al., 2018; Deudero et al., 2017; Wesselmann et al., 2018; Kersting et al., 2020).

The ecological role of *P. nobilis* is key for the ecosystems it inhabits (Cabanellas-Reboredo et al., 2019), as a filter feeder (Acarli, 2021), it feeds on both organic and inorganic suspended particles. It has been estimated that a single *P. nobilis* individual can filter more than 2000 L per day (Hernandis et al., 2023); consequently, pen shells contribute to water clarity (Trigos et al., 2014; Acarli, 2021). In addition, due to its large size pen shells often host numerous benthic species on their outer shell increasing biodiversity and complexity (i.e bryozoans, other mollusks, sponges, etc) (García-March, 2005; Guallart and Templado, 2012; Banach-Esteve and Vázquez-Luis, 2015).

In the current situation of pen shell, safeguarding resistant individuals is crucial, as they can play a vital role in introducing new breeding stock. It is necessary to have an extensive breeding plan to be able to close the captive breeding cycle of *Pinna nobilis*; in parallel with the breeding plan, it is necessary to identify optimal sites for restocking. The use of larval dispersal models improves the selection of reintroduction areas, reduce the amount of introduction sites for tests and increase settlement succession. Therefore, the main objective of the present work is the identification of the key repopulation locations in the Spanish Mediterranean coastline that provide optimal dispersal pathways across populations by employing larval dispersal models and connectivity metrics creating a population network of *P. nobilis* able to function and sustain itself successfully. The identification of areas with high densities of *P. nobilis* in some places of the Mediterranean Sea (Basso et al., 2015; Wesselmann et al., 2018) before the MME could pinpoint habitat suitability.

2. Materials & Methods

2.1. Study area and data collection

The dispersal of noble pen shell larvae was studied as a passive process using a biophysical model to estimate the connectivity between sites across the Spanish Mediterranean coast. The populations of *Pinna nobilis* on the Spanish coasts before the MME were well known at many places (Basso et al., 2015; Rodríguez et al., 2017). The repopulation locations for the present study were selected based on the knowledge of abundance of individuals of noble pen shell before 2016 (before the MME), and the level of protection of the area, given the demonstrated and observed evidence of anthropogenic impacts on pen shell populations. In total, 13 locations were selected (Fig. 1; Table 1).

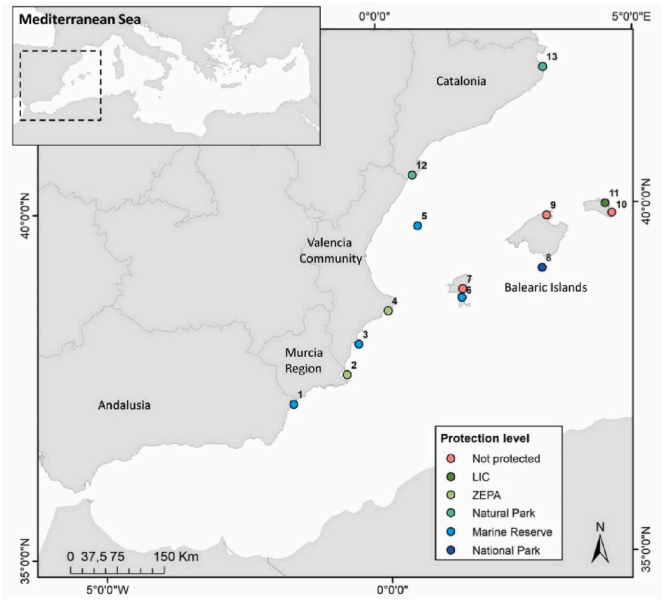

Fig. 1. Map of the Western Mediterranean Sea with the location of selected repopulation locations (further information is provided in Table 1).

Table 1
Repopulation locations selected, their location, average abundance of *P. nobilis* prior to the MME and their protection level. ID: refers to repopulation location in number, CCAA: Autonomous community, Repopulation location name: name of repopulation point, Protection level: Marine Reserve, National Park, Natural Park, ZEPA (Special protection areas for birds) & LIC (places of community Importance).

ID	CCAA	Repopulation location name	Protection level
1	Andalusia	El Calón	Marine Reserve
2	Murcia Region	Isla Grosa	ZEPA
3	Valencia Community	Tabarca	Marine Reserve
4	Valencia Community	Calpe	ZEPA
5	Valencia Community	Columbretes	Marine Reserve
6	Balearic Islands	Formentera, Espalmador Llevant	Marine Reserve
7	Balearic Islands	Ibiza, Talamanca	–
8	Balearic Islands	Cabrera, Santa María	National Park
9	Balearic Islands	Pollensa	–
10	Balearic Islands	Menorca, Mahón	–
11	Balearic Islands	Menorca, Addaia	LIC
12	Catalonia	Alfacs	Natural Park
13	Catalonia	Medas	Natural Park

2.2. Larval dispersal model

After the potential repopulation locations were selected, larval dispersal patterns along the Spanish Mediterranean coast were studied using a biophysical model that simulated the dispersal of Lagrangian particles emitted from each potential repopulation point. This technique incorporates passive and active drift in a three-dimensional or two-dimensional flow (McDonald and Nelson, 2021) to establish the transfer and dispersal of larvae along nodes, which correspond to the previously selected repopulation locations. The present study simulated larval dispersal using surface currents obtained from a high-resolution interannual simulation of the Western Mediterranean Operational forecasting system (WMOP; Juza et al., 2016; Mourre et al., 2018). The hydrodynamic simulation, which provided daily average currents with a 2 km spatial resolution over the Western Mediterranean Sea, was evaluated in Mourre et al. (2018); Aguiar et al. (2020), showing a realistic representation of the mean currents and eddy kinetic energy associated with the mesoscale activity. Moreover, the daily-averaged wave-induced currents estimated from the Copernicus Marine Service Mediterranean Sea wave model (Korres et al., 2019) were added to the WMOP wind- and density-driven currents to also include the contribution of the wave-induced surface drift. The resulting oceanic currents were combined with the PLD of the larvae of *P. nobilis*. The model tracked the movement of 1000 passive particles (larvae) emitted from every repopulation location every 24 h from May 2nd to September 30th of 2009–2017, with both years included. The initial positions were adjusted to the closest model ocean grid points. The trajectories were computed using the TRACMASS Lagrangian code (Jönsson et al., 2015), including a diffusion term (coefficient of 50 m^2/s) to represent the effect of small unresolved scales. This approach has been used successfully for the study of larvae or marine litter dispersion (e.g., Kersting et al., 2020; Compa et al., 2020; Torrado et al., 2021). Here, we defined a larval duration of 25 days, since most recent studies reported PLDs between 20 and 30 days (Deudero et al., 2017; Wesselmann et al., 2018; Trigos et al., 2018). However, since the understanding of the pelagic larval duration of *Pinna nobilis* is still vague, additional PLD scenarios (10, 15, 20 and 30 days) were evaluated in order to account for such uncertainty.

2.3. Connectivity analysis

Subsequent to dispersal simulations, the percentage of particles that arrive to a suitable habitat was calculated for each PLD scenario (settling particles). This ratio of particles was considered as larvae susceptible to successfully settle in the repopulation locations. The connectivity matrix was established to fix the distance measured in days between repopulation locations. The connections were generated based on the number of larvae exchanged from a node i to a node j divided by the initial number of larvae in node i, and weak connections were omitted if the dispersal probability was below a threshold of 0.05%. This matrix allows to obtain connectivity metrics that describe the role of the repopulation location in the network (Table 2).

Network analysis was carried out using the methodology described by Ognyanova (2016), using Rstudio (version 4.2.2) to establish a network with the potential repopulation locations and representing each connectivity metric individually.

Network analysis was studied at three different levels: with (i) metrics that give information about each individual repopulation location (they report the characteristics of the repopulation location itself), (ii) metrics that give information about the role of the repopulation location with respect to the rest of the repopulation locations; and (iii) properties of the whole network. Therefore, the functioning of individual repopulation locations was first described using: (1) Self-recruitment (proportion of locally produced larvae settling in a repopulation location compared to larvae originating from other repopulation locations) and (2) Local retention (settlement success in a repopulation location relative to larval production).

The next stage was to determine the dynamics of a network through the properties the repopulation locations present for the community and to analyze how the repopulation locations connect between them: (3) Normalized Edge Strength (connection between repopulation locations, their directionality and how these pathways are used in terms of larval transport), (4) Betweenness centrality (metric that allows to identify repopulation locations that are crucial to the network in terms of connections with other repopulation locations, that is, repopulation locations acting as bridges between others); and (5) Eigenvector centrality, (metric that measures the influence of a repopulation location in the network, identifying repopulation locations with a high production and recruitment connected to other repopulation locations with similar properties).

Lastly, two metrics were used to describe the functioning of the repopulation locations as a network: (6) Cut-point (repopulation locations that when depleted divide the network in sub-communities); and (7) Community detection (clustering repopulation locations that presented high strength edges into sub networks.

Table 2
Connectivity metrics, definition and interpretation including references.

Metric	Definition	Interpretation	References
Local Retention (LR)	Ratio of larval recruitment in a node to local egg production (Lett et al., 2015).	Does not depend on egg production, and therefore it is independent of temporal changes in adult population size.	Ospina-Álvarez et al., 2020; Lett et al., 2015; Botsford et al., 2009.
Self-recruitment (SR)	Ratio of locally produced larvae settlement to settlement of all origins at a site	Dependent on egg production in the different nodes. Therefore, it is affected by temporal variations in population size. It also depends on connectivity among patches.	Hogan et al., 2012; Lett et al., 2015; Ospina-Alvarez, et al., 2020.
Out-degree	Number of connections linking to downstream neighbors. It refers to the number of edges (larval dispersal vectors) that arise from a given node.	Nodes with high Out-degree value are source areas. They supply the metapopulation with larvae and juveniles.	Pulliam, 1988; Treml, et al., 2008; Ospina-Álvarez, et al., 2020
In-Degree	Number of edges from upstream sources that get to a given node.	Nodes with high In-degree value are nursery nodes and important for conservation. These nodes are sink habitats.	Bonacich, 1987; Pulliam, 1988; Lipcius et al., 2008; Treml et al., 2008; Ospina-Álvarez, et al., 2020
Betweenness	Proportion of shortest paths between all node pairs that pass through the node of interest.	Betweenness highlights the most used routes and represent important dispersal pathways in a metapopulation. Values range from 0 to 1, where 0 means the centrality of all nodes is equal, and 1 that a node is key for the network in terms of dispersal of information.	Freeman, 1977; Barrat et al., 2004; Treml et al., 2008; Ospina-Álvarez et al., 2020
Eigenvector centrality	Parameter of centrality in which the centrality of a node is the result of the sum of its connections to others, weighted by their centralities.	It measures the influence of a node in a network. The values range from 0 to 1. Nodes with high eigenvector centralities mean high-productivity and high-recruitment nodes connected to other high-productivity and high-recruitment nodes.	Bonacich, 1987; Ospina-Álvarez, et al., 2020
Cut-point	Nodes that, when eliminated, break the network in subsections.	Cut nodes are considered important for the network's functioning and have relevance in conservation efforts	Treml, et al. (2008)
Community detection	Group of nodes with similar properties.	Nodes that form communities have common properties among them or display a similar role for the network.	Brandes, et al., 2008; Ospina-Álvarez, et al., 2020

3. Results

The highest density of particles concentrates in the geographical center of the network on a whole network scale (Fig. 2). The percentage settling particles provides information for success of arrival of larvae to the repopulation locations and can be related to settlement. The results for settling particles showed increasing as larval duration prolongs (Tables S1, S2, S3 and S4). When PLD was 25 days, most of the repopulation locations showed values higher than 50% for percent of settling particles (Table 3). Repopulation location 2 (Isla Grosa) showed a maximum value of settling particles with 82.57% (Table 3), which is the highest value of settling particles in the network, whereas the lowest value of 28.44% was found in repopulation location 10 (Mahón).

Betweenness values obtained for the repopulation locations were all below 0.10, with an average value of 0.02 ± 0.03. The highest value of betweenness appeared in repopulation location 5 (Columbretes), with of 0.09, which is located in the geographic center of the network (Fig. 4).

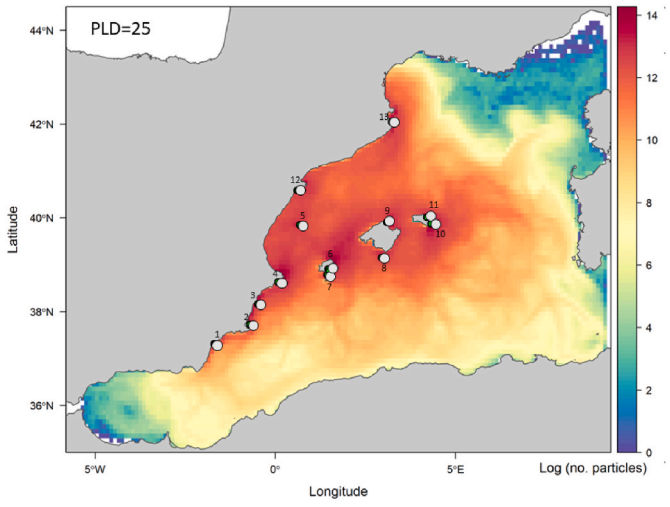

Fig. 2. Density map of larval dispersal for PLD of 25 days; and percentage of larvae that arrive to each repopulation location (% Settling). The different colors represent the density of particles (Log[particles]).

The lowest values of betweenness were found in repopulation locations with scarce edges connecting them with the rest of the network (Fig. 4). For the rest of the scenarios, betweenness values remained below 0.10 except when PLD is 30 days (Fig. S4), where it reached a maximum value of 0.19 in repopulation location 13 (Medas), which presented betweenness values of 0 for the rest of the larval durations.

Eigenvector values for repopulation locations were close to 0 but increase with larval duration (Tables S1, S2, S3 and S4) with an average value of 0.10 ± 0.30 when PLD is 25 days. The only repopulation location without an eigenvector value close to 0 was repopulation location 2 (Isla Grosa), where the eigenvector value was of 1 (maximum) for all PLDs scenarios. Also, for all PLDs, highest values of Eigenvector were found in repopulation locations found in the Valencian Community, Murcia and Andalusia, and lowest values were found in the repopulation locations from Mallorca and Menorca, implying low influence in the network dynamics (Tables S1, S2, S3 and S4). When PLD was of 25 days, eigenvector centrality did not surpass 0.001 value for most repopulation locations excepting 1, 2 3, 4 and 5 (El Calón, Isla Grosa, Tabarca, Calpe and Columbretes, respectively; Fig. 5). Out of these, Isla Grosa presented an eigenvector centrality value of 1 (maximum to this metric), the highest value for the whole network, whereas repopulation location 10 (Mahón) showed the lowest value, of $1.04{\cdot}10^{-9}$. Therefore, Isla Grosa was the repopulation location with higher productivity and successful connections, which can also be observed looking at the edge strengths that Isla Grosa presents (Figs. 3–5).

Edge strengths for the repopulation locations, that give information about the characteristics of the repopulation location in comparison to the rest of repopulation locations; had increased path numbers with larval duration, but also the importance of the edges is increased when PLD was longer (Tables S1, S2, S3 and S4). When a PLD of 25 days was used (Fig. 6) Columbretes presents In-Strengths coming from southern repopulation locations (El Calón, Tabarca, Calpe) and from Alfacs and a strong edge from Talamanca, acting as a sink repopulation location for the larvae from the mentioned points. In terms of Out-Strength, it also presented a strong path for larvae that arrive to Medas, acting as a source (Pulliam, 1988), repopulation location for it. Furthermore, several edges connected the eastern repopulation locations (repopulation locations in Mallorca and Menorca), and stronger repopulation locations connected them with the repopulation locations located in Formentera and Ibiza. At a network level, the study of connectivity metrics in the 13

Table 3
Summary table for the metrics and properties employed to select the fittest repopulation locations for PLD 25 days.

PLD 25 days										
ID	Locality	CCAA	Protection level	% Settling	Local retention	Self recruitment	Betwenness	Eigenvector centrality	Cut-point	Comunities
1	El Calón	Andalucía	Marine Reserve	75.29	0.13	0.69	0.02	0.04		C
2	Isla Grosa	Murcia	ZEPA	82.57	0.35	0.91	0.02	1.00		C
3	Tabarca	C. Valenciana	Marine Reserve	73.12	0.26	0.97	0.04	0.18		C
4	Calpe	C. Valenciana	ZEPA	48.98	0.12	0.90	0.02	0.01		C
5	Columbretes	C. Valenciana	Marine Reserve	54.01	0.17	0.98	0.09	0.00	Cut-Point	D
6	Espalmador Llevant	I. Baleares	Marine Reserve	38.55	0.04	0.60	0.00	0.00		A
7	Talamanca	I. Baleares		43.81	0.06	0.77	0.00	0.00		A
8	Cabrera	I. Baleares	National Park	49.19	0.14	0.91	0.06	0.00		B
9	Pollensa	I. Baleares		60.05	0.09	0.91	0.00	0.00		B
10	Mahón	I. Baleares		28.44	0.10	0.67	0.01	0.00		B
11	Addaia	I. Baleares	LIC	40.15	0.24	0.86	0.00	0.00		B
12	Alfacs	Cataluña	Natural Park	65.42	0.07	0.86	0.00	0.00		D
13	Medas	Cataluña	Natural Park	33.75	0.06	1.00	0.00	0.00		E

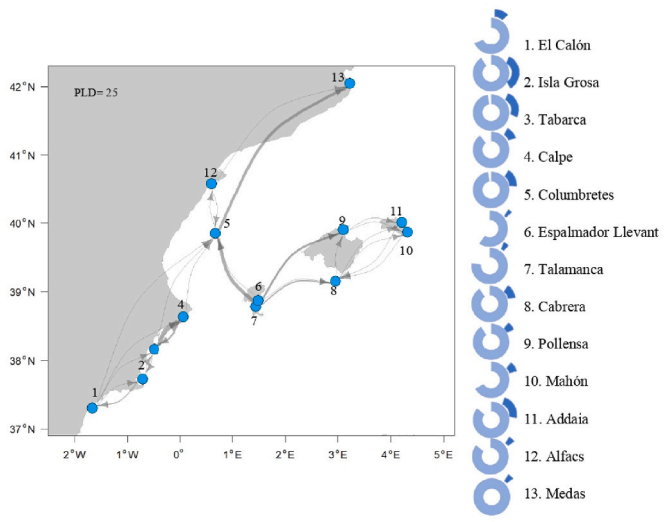

Fig. 3. Self-recruitment (SR, light blue) and Local-retention (LR, dark blue) represented as crown-charts above every repopulation location in a PLD of 25. Scenario of connectivity network is symbolized as arrows representing the normalized edge strength: the wider the arrow, the stronger the edge between repopulation locations, meaning higher amount of larvae transfer.

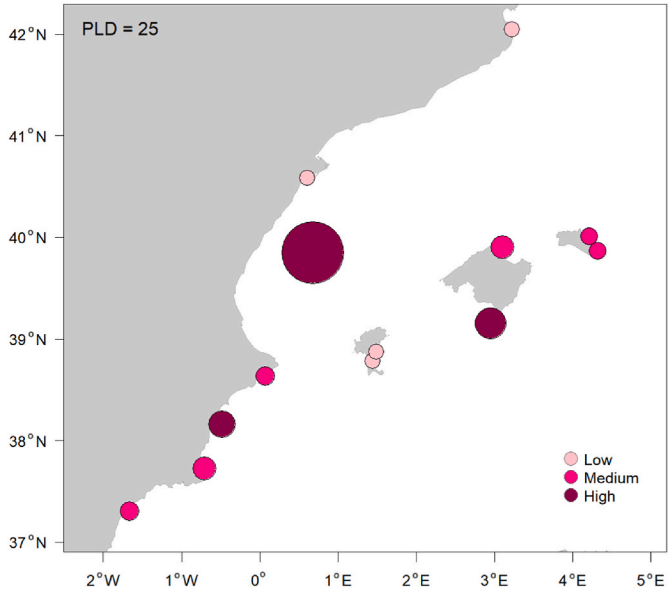

Fig. 4. Betweenness of each repopulation locations and normalized edge strength of the connection of each repopulation location in the network when PLD is of 25 days. Betweenness values are represented with assorted colors and size of the repopulation locations classified in terciles: light pink corresponds to the repopulation locations with lowest betweenness values; pink groups repopulation locations with intermediate betweenness values and deep pink repopulation locations present the highest betweenness values of the network. The size of the repopulation location also is related to betweenness values: wider repopulation locations correspond to higher betweenness values.

repopulation locations in the western Mediterranean Sea showed that abundance of cut-nodes decreases as PLD enlarges and disappearing this property for any repopulation location when larval duration is 30 days (Tables S1, S2, S3 and S4). When PLD is 25 days, the only cut-node that exist in the network is Columbretes (Fig. 6).

Finally, the network is divided into sub-networks using community detection, showing fewer yet larger communities as larval duration lengthens (Fig. 5). The communities formed at the PLD of 20 days scenario remain fixed for the rest of scenarios with higher PLDs. Finally, Repopulation location 13 (Medas) does not form communities with the rest of repopulation locations in any PLD (Tables S1, S2, S3 and S4). At a PLD of 25 days (Fig. 6), the repopulation locations of El Calon, Isla Grosa, Tabarca and Columbretes form a southern community; Calpe and Alfacs another one and Medas remains separated. In the Balearic archipelago, Menorca, Mallorca, and Cabrera (Mahón, Addaia, Pollensa and Cabrera) form a cluster and Ibiza and Formentera (Talamanca and Espalmador-Llevant) another one.

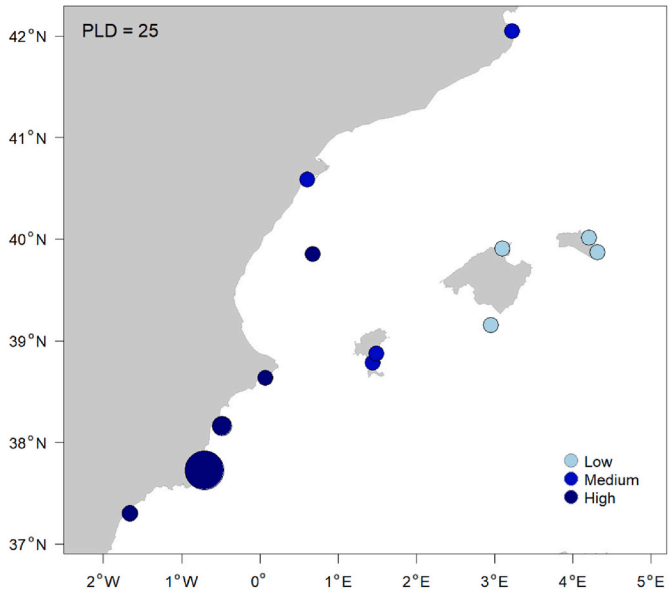

Fig. 5. Eigenvector of each repopulation location and normalized edge strength of the connection of each repopulation location in the network when PLD is of 25 days. Eigenvector values are represented with distinct colors and size of the repopulation locations classified in terciles: light blue corresponds to the repopulation locations with lowest eigenvector values; blue groups repopulation locations with intermediate eigenvector values, and deep blue repopulation locations present the highest eigenvalues values of the network. The width of the repopulation location also is related to eigenvector values: wider repopulation locations correspond to higher eigenvector values.

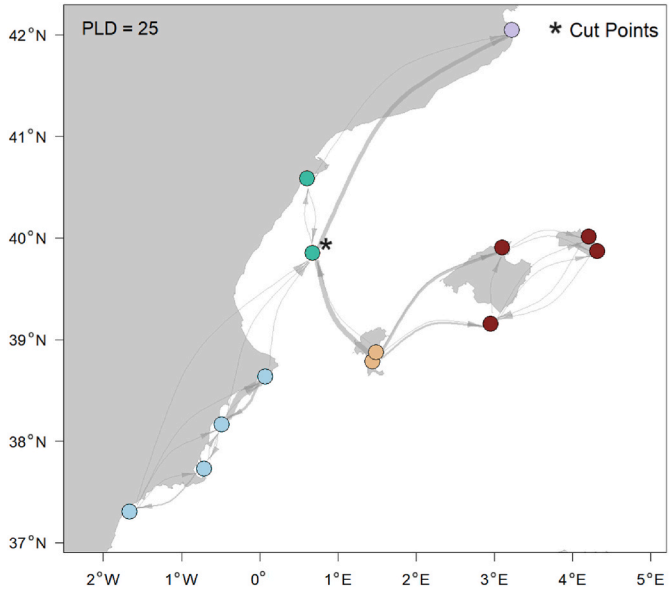

Fig. 6. Sub-communities of repopulation locations that exist when PLD is of 25 days. Repopulation locations are represented in different colors symbolizing the sub-communities formed by strong connections. Arrows represent normalized edge strength.

4. Discussion

This study addresses the restoration of *P. nobilis* using a biophysical model based on Lagrangian particle tracking to describe the connectivity among repopulation locations with a high density of *P. nobilis* prior to the MME to characterize optimal repopulation locations. The model used different larval durations and showed that all the pathways between repopulation locations successfully connect every repopulation location from the network. Under the criteria of the connectivity metrics and the level of protection of the locations, six repopulation locations were selected to begin with repopulation efforts and promote the settlement of individuals in the rest of locations in the Western Mediterranean Sea.

Jahnke and Jonsson (2022) asserted that predictions from biophysical models expound genetic patterns. However, the present results serve as the first stage to prioritize optimal areas for repopulation. Though, this study focusses on the restocking of previously known populations, rather than detect new or unknown repopulation locations, and they need to undergo validation through studies that allow to adjust the model to reality more precisely, and to assure that the assumptions that are made adjust to the real framework of the species populations.

Connectivity outcomes are influenced by both the geographical location of repopulation sites and the surrounding hydrodynamic environment (Treml et al., 2015). When studying larval dispersal and the percentage of larvae reaching the repopulation locations, those with the highest percentage of settling particles are affected by the main current along the northern Mediterranean Spanish coast, which flows southward (Northern Current), resulting in a greater influx of particles to the southern coasts of the peninsula. The area between the Northern Current and the northeastward current branch forming the Balearic Current (Millot, 1999), could then also lead to a local accumulation of particles. This enables the identification of repopulation locations with the highest number of larvae arriving to them. LR is an estimate of the demographic independence of populations permitting to evaluate self-persistence (Hogan et al., 2012; Burgess et al., 2014; Lett et al., 2015). SR does provide additional information for LR, and its empirical validation has lower requirements than LR. These two metrics allowed us to estimate settlement in the different repopulation locations and select those that showed higher values in a "best case scenario", in which all larvae reaching a repopulation location settle. Interestingly, low values of LR combined with high values of SR could indicate that a repopulation location presents a high settlement of locally produced larvae, acting as sink areas. This is the case of repopulation location 13 (Medas).

The present study supports the conclusion that there are repopulation locations that possess optimal characteristics to start *P. nobilis* repopulation efforts. The results obtained support the criteria applied to select potential repopulation locations as those selected present high densities of noble pen shell, and also contribute to understand the mechanisms of population formation in said sites. Highest densities of *P. nobilis* prior to the MME were found in sites that according to the results obtained in the present study show highest values for the connectivity metrics applied.

Isla Grosa (Murcia) and Tabarca (Valencian C.) both present some of the highest values found for all metrics studied and show strong connections with other repopulation locations (Table 3). González-Wangüemert et al. (2004) asserted larval dispersal of *Diplodus sargus* from Murcia, describing the pathways that larvae followed using a genetic approach. The results of the study showed how larvae travelled from Cabo the Palos to Almería and Tabarca. These pathways do coincide with the edges obtained in the present study, as Isla Grosa presents larval dispersal pathways that connects the repopulation locations with the repopulation locations located in El Calón (Almería) and Tabarca (Valencian C.). In addition, the edges obtained showed Tabarca as a location where larvae from south and northern repopulation locations settle. These results are consistent with previous genetic studies that showed allelic diversity in the mentioned repopulation point, corresponding to alleles from individuals of nearby locations (Wesselmann et al., 2018).

Columbretes (Valencian C.) showed pathways that indicate it serves as a sink location for larvae coming from surrounding locations, both from the Peninsula and the Balearic Archipelago. These results could indicate that Columbretes serves as a reservoir of larvae that could favor genetic diversity. Similar results were obtained when modeling larval

dispersal using larval collectors to describe the movements of larvae throughout the years (Kersting, et al., 2020).

These repopulation locations present high settlement both from locally and outer produced larvae, but also act as sources of larvae for surrounding repopulation locations, making them essential for the repopulation of both their subcommunity and the network. In the Balearic archipelago, the repopulation locations present lower values for the metrics when compared to the repopulation locations from the peninsula (Table 3). Still, repopulation efforts must be done on the island to maintain the network that existed prior to the MME. Cabrera (Mallorca) acts as a sink area but also emits larvae to other repopulation locations, located in the north. Nebot-Colomer et al. (2022)studied the genetic structure of the populations of *P. nobilis* before the MME. The study indicated that the replenishment of the population in Cabrera was not solely dependent on SR, but it was possible thanks to the supply of larvae from other populations. These results are consistent with the results obtained in the present study, which indicated that pathways transport larvae from the rest of the islands. These results suggest that Cabrera would act as a bank for genetic diversity. These characteristics along with the high level of protection of this repopulation location (categorized as a no-take area), makes Cabrera a key repopulation location for the restoration of *P. nobilis* in the Spanish Mediterranean Sea (Vázquez-Luis et al., 2014).

The repopulation location in Espalmador-Llevant (Formentera) also presents a commonly used pathway that transports larvae to Columbretes, along the repopulation locations in Mallorca and Menorca (Table 3). These pathways were previously described by Gonzalez-Wangüemert et al. (2019) for *P. nobilis* using a genetic approach. In Menorca, both repopulation locations act as sink habitats for larvae coming from Mallorca, and as source of larvae for Cabrera, making them equally important for the network. However, Addaia presents higher settlement rates than Mahón, and is included in a protected area as a LIC. Finally, Addaia receives larvae from Medas (Cataluña), Cabrera and Pollensa (Mallorca), which was confirmed by genetic modelling by Kersting et al. (2020), making it a better suitor for repopulation. Quantifying connectivity patterns using connectivity metrics is important to establish repopulation locations with the aim to recover a metapopulation. In a population network, edges represent the probability of connection or flow of larvae between repopulation locations (Dale and Fortin, 2010). Therefore, when considering repopulation efforts these pathways must be considered, as they are successful and commonly used routes for larvae dispersal in the community. Betweenness values highlight "stepping-stones" that are key for the functioning of the network (Treml et al., 2008; Gamoyo et al., 2019) and its conservation. The results for betweenness seem to indicate that the repopulation locations are central given the low values obtained for the metric. However, the SD obtained also implies that there is variation for the values of betweenness of each repopulation location, which makes sense since six of the locations present a betweenness value of zero. This could mean that, for the network, pathways between repopulation locations do not necessarily require the presence of intermediate repopulation locations to successfully transport larvae among them. Still, since in a real scenario, various pathways could be used for larval dispersal (Newman, 2005); the highest values must be considered when establishing the repopulation locations to begin reintroduction of resistant individuals. Repopulation locations located centrally seem to present certain importance in terms of potentially connecting repopulation locations when the diffusion of particles from repopulation locations is reduced (Ross et al., 2017). These repopulation locations also present numerous and/or strong edges with other repopulation locations. In these terms, repopulation locations 3, 5 & 8 (Tabarca, Columbretes & Cabrera, respectively) present the highest values of betweenness of the network and are located centrally in the network. In the scenario of a PLD of 30 days, highest betweenness value goes over to the repopulation location 13 (Medas), located in the north of the network, which could be explained by the pathways that form in that scenario, connecting Menorca and Pitiusas Islands (Ibiza & Formentera) with Medas. These results could indicate that the before mentioned points have a subtle influence on the transport of larvae from different areas of the network. However, considering other metrics with more informative values for restocking purposes is recommended. The eigenvector values show that most repopulation locations present equal influence for the network, excepting repopulation location 2 (Isla Grosa), with maximum eigenvector value. Therefore, under the criteria of this metric, Isla Grosa, acts as a critical repopulation location for the studied network.

Network properties allowed us to identify communities and cut-points repopulation locations. Cut-node analysis identified Columbretes as the only repopulation location that would split the network into sub-networks if it would disappear when PLD is 25 days, this may be due to its geographically central position with regard to the rest of repopulation locations. The number of repopulations locations that present this property reduces as larval duration lengthens, until a PLD of 30 days, in which scenario, there are no locations with the cut-point property. In this scenario, the repopulation points are strongly connected. For conservation and reintroduction purposes, identifying repopulation locations with this property is crucial since they are critical to populations connectivity (Treml et al., 2008). Moreover, when analyzing the communities formed by strong edges in addition to connectivity metrics, it becomes possible to identify repopulation locations that are critical to maintain sub-communities and that also connect them (Ospina-Álvarez et al., 2020). This information could be of interest to study in scenarios were the network gets severed, as it allows to define sub-networks that may be auto sufficient even when the network suffers degradation.

The present work assumed equal larval production in every repopulation point, which is unlikely to be realistic. Moreover, the study overlooks several aspects such as biological barriers (i.e health, larvae abundance, resilience, swimming behavior; Scheltema 1986; Treml et al., 2008) and environmental factors (i.e effects of temperature, food availability, acidification) that are of interest to assess the rate of successful recruitment and settlement of larvae (Philippart et al., 2014). In addition, for *P. nobilis* the lack of certainty regarding the duration of the PLD made mandatory to study connectivity in different scenarios. The quantity and structure of habitats exert the most significant influence on larval emission, settlement, and subsequent recruitment processes. Future studies in this species could be oriented on the duration of the larval phase to fit the models to the real-world scenario. However, results obtained for all of them fix the same repopulation locations to begin with reintroduction efforts. Abiotic factors affecting connectivity vary in the different stages, during dispersal stage; being currents and matrix affect the most (Swearer et al., 2019).

Successfully describing larval stages and both biotic and environmental factors affecting such is highly difficult given the similarities found in larval stages of bivalve species (Hendriks et al., 2005). Still, addressing them is desirable in order to adjust the model to the requirements and traits of the species. In future studies, it is highly recommended to delve into biological and environmental traits to assess the settlement and dispersal of larvae.

For *Pinna nobilis*, restoration strategies must consider the disease caused by *H. pinnae*. The extreme virulence of the pathogen, alongside with the slow growth rate of the pen shell, and the scarce number of surviving individuals in their natural habitat (Cabanellas-Reboredo et al., 2019) makes highly difficult to establish successful conservation efforts. However, the current scenario faced by the species may be discouraging. The virulence of the pathogen and the high mortality rate caused by the disease gravely compromises the recovery of the species. For that reason, it is crucial to ensure effective restocking efforts using resistant individuals to obtain generations able to survive the pathogen and sustain the populations (Acarli, 2021).

Immunity of mollusk has been a subject of interest for researchers due to their health and financial importance. For these animals, the innate immunity system represents the only line of host defense against

pathogens with anti-microbial peptides and hemocyte phagocytosis constitute key for mollusk survival to different pathogens (Zannella et al., 2017). Hence, the genetic basis underlying disease tolerance can be crucial for assessing the recovery of the species. For other bivalves, such as *Crassostrea gigas,* disease-resistance for specific pathogens was found associated with single nucleotide polymorphism affecting in several genes underlying traits related to the immune response (Yang et al., 2022). For *P. nobilis*, Salis et al. (2022) identified innate immune receptors overexpressed in surviving individuals, implying the existence of disease-resistance in the species, and some toll-like receptors seems to be involved in the immune response against the pathogen (Coupé et al., 2023). However, few disease-resistant individuals have been identified to date, limiting the ability to understand the mechanisms of tolerance or resistance of the pathogen.

Few is known of the pathogenicity of *H. pinnae* in terms of infection at early or larval stages of noble pen shell. This matter ads up to the recently described co-infection phenomena of pen shell individuals by *H. pinnae, Mycobacterium* spp., and opportunistic *Vibrio* spp. *Mycobacterium* spp. have been demonstrated to present cumulative negative effects for infected by *H. pinnae* individuals causing acute immune responses (Lattos et al., 2021) that could aggravate the mortality events observed in the last years. *Vibrio* spp. was also detected in individuals of *P. nobilis* affected by the parasite (Lattos et al., 2021). This issue entails difficulty to ensure effective restocking efforts, which is why the present study encourages to look into infection during larval stages in the future.

We propose Isla Grosa, Tabarca, Columbretes, Espalmador-Llevant, Cabrera and Addaia as a starting point for the reintroduction efforts for *P. nobilis* populations. These repopulation locations include different levels and categories of protected areas, which assure protection of the introduced resistant individuals. Moreover, repopulation locations located in the geographic periphery of the network: El Calon (Andalusia) and Medas (Catalunya) are interesting to study in a broader scenario where population areas in Algeria and France, respectively, are considered as they might present connections with networks connecting the Spanish littoral with the rest of the Mediterranean basin.

5. Conclusions

To conclude, this study shows how important repopulation locations remain crucial in different PLD scenarios and highlight the importance of dispersal pathways to maintain a self-sufficient network. Next steps to assess the repopulation of noble pen shell in the Spanish coast should consider the genetic variability prior to the MME, larval behavior and mortality rates of larvae, habitat quality and availability in order to be added to the model obtained in this work. Isla Grosa, Tabarca, Columbretes, Espalmador-Llevant, Cabrera and Addaia are proposed as the starting Repopulation locations to begin the reintroduction of resistant individuals of *P nobilis,* as we consider these locations facilitate the later rehabilitation of other populations, creating a self-sufficient and resistant network.

CRediT authorship contribution statement

A. Feria-Rodríguez: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **D. March:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **B. Mourre:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **I.E. Hendriks:** Writing – review & editing, Visualization, Validation. **M. Vázquez-Luis:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Data availability

Data will be made available on request.

Acknowledgements

This work was partially supported by the European Union's LIFE program through the project LIFE PINNARCA (NAT/ES/001265). DM acknowledges support from the CIDEGENT program of the Generalitat Valenciana (CIDEGENT/2021/058).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2024.106428.

References

Abelson, A., Denny, M., 1997. Settlement of marine organisms in flow. Annu. Rev. Ecol. Systemat. 28 (1), 317–339.
Acarli, S., 2021. Population, aquaculture and transplantation applications of critically endangered species *Pinna nobilis* (Linnaeus 1758) in the Mediterranean Sea. Marine Science and Technology Bulletin 10 (4), 350–369.
Aguiar, E., Mourre, B., Juza, M., Reyes, E., Hernández-Lasheras, J., Cutolo, E., Mason, E., Tintoré, J., 2020. Multi-platform model assessment in the Western Mediterranean Sea: impact of downscaling on the surface circulation and mesoscale activity. Ocean Dynamics 70, 273–288.
Airoldi, L., Balata, D., Beck, M.W., 2008. The gray zone: relationships between habitat loss and marine diversity and their applications in conservation. J. Exp. Mar. Biol. Ecol. 366 (1–2), 8–15.
Avery, R.P., 2003. Marine and Terrestrial Conservation Planning-How Different Are They? Royal Zoological Society of New South Wales.
Banach-Esteve, G., Vázquez-Luis, M., 2015. Temporal trends in sessile epibionts of the endemic bivalve *Pinna nobilis*: variability in *Lophocladia lallemandii* colonization. Thalassas: An International Journal of Marine Sciences 31 (2), 19–29.
Barrat, A., Barthelemy, M., Pastor-Satorras, R., Vespignani, A., 2004. The architecture of complex weighted networks. Proc. Natl. Acad. Sci. USA 101 (11), 3747–3752.
Basso, L., Vázquez-Luis, M., García-March, J.R., Deudero, S., Alvarez, E., Vicente, N., Duarte, C.M., Hendriks, I.E., 2015. The pen shell, *Pinna nobilis*: a review of population status and recommended research priorities in the Mediterranean Sea. Adv. Mar. Biol. 71, 109–160.
Beger, M., Grantham, H.S., Pressey, R.L., Wilson, K.A., Peterson, E.L., Dorfman, D., Mumby, P.J., Lourival, R., Brumbaugh, D.R., Possingham, H.P., 2010. Conservation planning for connectivity across marine, freshwater, and terrestrial realms. Biol. Conserv. 143 (3), 565–575.
Bode, M., Leis, J.M., Mason, L.B., Williamson, D.H., Harrison, H.B., Choukroun, S., et al., 2019. Successful validation of a larval dispersal model using genetic parentage data. PLoS Biol. 17 (7), e3000380 https://doi.org/10.1371/journal.pbio.3000380.
Bonacich, P., 1987. Power and centrality: a family of measures. Am. J. Sociol. 92, 1170–1182.
Botsford, L.W., White, J.W., Coffroth, M.A., Paris, C.B., Planes, S., Shearer, T.L., Thorrold, S.R., Jones, G., 2009. Connectivity and resilience of coral reef networks in marine protected areas: matching empirical efforts to predictive needs. Coral Reefs 28, 327–337.
Brandes, U., Delling, D., Gaertler, M., Gorke, R., Hoefer, M., Nikoloski, Z., Wagner, D., 2007. On modularity clustering. IEEE Trans. Knowl. Data Eng. 20 (2), 172–188.
Burgess, Scott C., Nickols, Kerry J., Griesemer, Chris D., Barnett, Lewis A.K., Dedrick, Allison G., Satterthwaite, Erin V., Yamane, Lauren, Morgan, Steven G., White, J. Wilson, Botsford, Louis W., 2014. Beyond connectivity: how empirical methods can quantify population persistence to improve marine protected-area design. Ecol. Appl. 24 (2), 257–270.
Butler, A., Vicente, N., de Gaulejac, B., 1993. Ecology of the pterioid bivalves Pinna bicolor Gmelin. Mar Life 3 (1–2), 37–45.
Cabanellas-Reboredo, M., Vázquez-Luis, M., Mourre, B., Álvarez, E., Deudero, S., Amores, Á., Addis, P., Ballesteros, E., Barrajón, A., Coppa, S., García-March, J.R., Giacobbe, S., Giménez, F., Hadjioannou, L., Jiménez-Guitérrez, S.V., Katsanevakis, S., Kersting, D., Mačić, V., Mavrič, B., Paolo, F., Planes, S., Prado, P., Sánchez, J., Tena-Meidaldea, J., De Vaugelas, J., Vicente, N., Zohra, F., Zupan, I., Hendriks, I.E., 2019. Tracking a mass mortality outbreak of pen shell Pinna nobilis populations: a collaborative effort of scientists and citizens. Sci. Rep. 9 (1), 13355.
Catanese, G., Grau, A., Valencia, J.M., Garcia-March, J.R., Vázquez-Luis, M., Alvarez, E., Deudero, S., Darriba, S., Carballal, M.J., Villalba, A., 2018. Haplosporidium pinnae sp. nov., a haplosporidan parasite associated with mass mortalities of the fan mussel, Pinna nobilis, in the Western Mediterranean Sea. Journal of invertebrate pathology 157, 9–24.
Compa, M., Alomar, C., Mourre, B., March, D., Tintoré, J., Deudero, S., 2020. Nearshore spatio-temporal sea surface trawls of plastic debris in the Balearic Islands. Mar. Environ. Res. 158, 104945.
Coupé, S., Giantsis, I.A., Vázquez Luis, M., Scarpa, F., Foulquié, M., Prévot, J.M., Casu, M., Lattos, A., Michaelidis, B., Sanna, D., García-March, J.R., Tena-Medialdea, J., Vicente, N., Bunet, R., 2023. The characterization of toll-like receptor

repertoire in Pinna nobilis after mass mortality events suggests adaptive introgression. Ecol. Evol. 13 (8), e10383.
Crisp, D.J., Spencer Davies, P., 1976. The role of the pelagic larva. Perspectives in experimental biology 1, 145–155.
Dale, M.R.T., Fortin, M.J., 2010. From graphs to spatial graphs. Annual Review of Ecology, Evolution, and Systematics 41, 21–38.
Deudero, S., Grau, A., Vázquez-Luis, M., Álvarez, E., Alomar, C., Hendriks, I.E., 2017. Reproductive investment of the pen shell *Pinna nobilis* Linnaeus, 1758 in Cabrera National Park (Spain). Mediterr. Mar. Sci. 18, 271–284.
Freeman, L.C., 1977. A Set of Measures of Centrality Based on Betweenness. Sociometry, pp. 35–41.
Gamoyo, M., Obura, D., Reason, C.J., 2019. Estimating connectivity through larval dispersal in the Western Indian Ocean. J. Geophys. Res.: Biogeosciences 124 (8), 2446–2459.
García-March, J.R., 2005. *Aportaciones al conocimiento de la Biología de Pinna nobilis Linneo, 1758 (Mollusca: Bivalvia) en el litoral mediterráneo Ibérico*. Universitat de Valencia (Spain).
García-March, J.R., Hernandis, S., Vázquez-Luis, M., Prado, P., Deudero, S., Vicente, N., Tena-Medialdea, J., 2020. Age and growth of the endangered fan mussel *Pinna nobilis* in the western Mediterranean Sea. Mar. Environ. Res. 153, 104795.
Gaylord, B., Gaines, S.D., 2000. Temperature or transport? Range limits in marine species mediated solely by flow. The American Nauralist 155, 769–789.
González-Wangüemert, M., Pérez-Ruzafa, Á., Marcos, C., García-Charton, J.A., 2004. Genetic differentiation of Diplodus sargus (Pisces: Sparidae) populations in the south-west mediterranean. Biol. J. Linn. Soc. 82 (2), 249–261.
González-Wangüemert, M., Basso, L., Balau, A., Costa, J., Renault, L., Serrão, E.A., Duarte, C.M., Hendriks, I.E., 2019. Gene pool and connectivity patterns of *Pinna nobilis* in the Balearic islands (Spain, western Mediterranean Sea): implications for its conservation through restocking. Aquat. Conserv. Mar. Freshw. Ecosyst. 29 (2), 175–188.
Gray, J.S., 1997. Marine biodiversity: patterns, threats and conservation needs. Biodivers. Conserv. 6 (1), 153–175.
Guallart, J., Templado, J., 2012. Pinna nobilis. En: VV.AA., Bases ecológicas preliminares para la conservación de las especies de interés comunitario en España: Invertebrados. Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid, p. 81.
Hendriks, I.E., Van Duren, L.A., Herman, P.M., 2005. Image analysis techniques: a tool for the identification of bivalve larvae? J. Sea Res. 54 (2), 151–162.
Hernandis, S., Ibarrola, I., Tena-Medialdea, J., Albentosa, M., Prado, P., Vázquez-Luis, M., García-March, J.R., 2023. Physiological responses of the fan mussel *Pinna nobilis* to temperature: ecological and captivity implications. Mediterr. Mar. Sci.
Hogan, J.D., Thiessen, R.J., Sale, P.F., Heath, D.D., 2012. Local retention, dispersal and fluctuating connectivity among populations of a coral reef fish. Oecologia 168, 61–71.
Jahnke, M., Jonsson, P.R., 2022. Biophysical models of dispersal contribute to seascape genetic analyses. Philosophical Transactions of the Royal Society B 377 (1846), 20210024.
Jönsson, B., Döös, K., Kjellsson, J., 2015. TRACMASS: Lagrangian Trajectory Code. Zenodo, Geneva. https://doi.org/10.5281/zenodo.34157.
Juza, M., Mourre, B., Renault, L., Gómara, S., Sebastián, K., Lora, S., Beltran, J.P., Frontera, B., Garau, B., Troupin, C., Torner, M., Heslop, E., Casas, B., Escudier, R., Vizoso, G., Tintoré, J., 2016. SOCIB operational ocean forecasting system and multi-platform validation in the Western Mediterranean Sea. Journal of Operational Oceanography 9 (Suppl. 1), s155–s166.
Katsanevakis, S., 2005. Population ecology of the endangered fan mussel *Pinna nobilis* in a marine lake. Endanger. Species Res. 1, 51–59.
Katsanevakis, S., Carella, F., Çınar, M.E., Cizmek, H., Jimenez, C., Kersting, D.K., Moreno, D., 2022. The fan mussel *Pinna nobilis* on the brink of extinction in the Mediterranean. Imperiled: The Encyclopedia of Conservation 1–3.
Kersting, D.K., Vázquez-Luis, M., Mourre, B., Belkhamssa, F.Z., Álvarez, E., Bakran-Petricioli, T., Barberá, C., Barrajón, A., Cortés, E., Deudero, S., García-March, J.R., Giacobbe, S., Giménez-Casalduero, F., González, L., Jiménez-Gutiérrez, S., Kipson, S., Llorente, J., Moreno, D., Prado, P., Pujol, J.A., Sánchez, J., Spinelli, A., Valencia, J.M., Vicente, N., Hendriks, I.E., 2020. Recruitment disruption and the role of unaffected populations for potential recovery after the *Pinna nobilis* mass mortality event. Front. Mar. Sci. 882.
King, S., Saint-Amand, A., Walker, B.K., Hanert, E., Figueiredo, J., 2023. Larval dispersal patterns and connectivity of *Acropora* on Florida's Coral Reef and its implications for restoration. Front. Mar. Sci. 9, 1038463.
Korres, G., Ravdas, M., Zacharioudaki, A., 2019. Mediterranean Sea waves hindcast (CMEMS MED-Waves). Copernicus Monitoring Environment Marine Service (CMEMS). https://doi.org/10.25423/CMCC/MEDSEA_HINDCAST_WAV_006_012.
Lattos, A., Feidantsis, K., Georgoulis, I., Giantsis, I.A., Karagiannis, D., Theodorou, J.A., Theodorou, J.A., Staikou, A., Michaelidis, B., 2021. Pathophysiological responses of Pinna nobilis individuals enlightens the etiology of mass mortality situation in the mediterranean populations. Cells 10 (11), 2838.
Lett, C., Nguyen-Huu, T., Cuif, M., Saenz-Agudelo, P., Kaplan, D.M., 2015. Linking local retention, self-recruitment, and persistence in marine networks. Ecology 96 (8), 2236–2244.
Lipcius, R.N., Eggleston, D.B., Schreiber, S.J., Seitz, R.D., Shen, J., Sisson, M., Stockhausen, W.T., Wang, H.V., 2008. Importance of network connectivity to restocking and restoration of marine species. Rev. Fish. Sci. 16 (1–3), 101–110.
McCauley, D.J., Pinsky, M.L., Palumbi, S.R., Estes, J.A., Joyce, F.H., Warner, R.R., 2015. Marine defaunation: Animal loss in the global ocean. Science 347 (6219), 1255641–1255641.
McDonald, R.R., Nelson, J.M., 2021. A Lagrangian particle-tracking approach to modelling larval drift in rivers. Journal of Ecohydraulics 6 (1), 17–35.
Millot, C., 1999. Circulation in the western Mediterranean Sea. J. Mar. Syst. 20 (1–4), 423–442.
Mourre, B., Aguiar, E., Juza, M., Hernandez-Lasheras, J., Reyes, E., Heslop, E., et al., 2018. Assessment of high-resolution regional ocean prediction systems using muli-platform observations: illustrations in the Western Mediterranean Sea. In: Chassignet, E., Pascual, A., Tintoré, J. (Eds.), New Frontiers in Operational Oceanography, GODAE Ocean View. J. Verron, pp. 663–694. https://doi.org/10.17125/gov2018.ch24.
Nebot-Colomer, E., Vázquez-Luis, M., Boissin, E., Peyran, C., Deudero, S., Planes, S., 2022. Inferred family structure and dispersal patterns of a critically endangered species, Pinna nobilis, using molecular analyses: Implications for conservation. Endangered Species Research 49, 87–102.
Newman, M.E., 2005. A measure of betweenness centrality based on random walks. Soc. Network. 27 (1), 39–54.
Ognyanova, K., 2016. Network analysis and Visualization with R and igraph. In: NetSciX 2016 School of Code Workshop. https://kateto.net/netscix2016.html.
Ospina-Álvarez, A., de Juan, S., Alós, J., Basterretxea, G., Alonso-Fernández, A., Follana-Berná, G., Palmer, M., Catalán, I.A., 2020. MPA network design based on graph theory and emergent properties of larval dispersal. Mar. Ecol. Prog. Ser. 650, 309–326.
Pastor, A., Catalán, I.A., Terrados, J., Mourre, B., Ospina-Alvarez, A., 2023. Connectivity-based approach to guide conservation and restoration of seagrass Posidonia oceanica in the NW Mediterranean. Biol. Conserv. 285, 110248 https://doi.org/10.1016/j.biocon.2023.110248.
Philippart, C.J., Van Bleijswijk, J.D., Kromkamp, J.C., Zuur, A.F., Herman, P.M., 2014. Reproductive phenology of coastal marine bivalves in a seasonal environment. J. Plankton Res. 36 (6), 1512–1527.
Pulliam, H.R., 1988. Sources, sinks, and population regulation. Am. Nat. 132 (5), 652–661.
Rodríguez, J., Alvarez, E.P., Halcón, R., Araujo, R., Arroyo, M., BallesTeros, M., Doménech, A., Belando, M., EsteLler, J., Bello, G., Castañer, V., Catanese, G., Deudero, S., Fernández, M.C., Garcia-March, J., Garcia, R.M., Moliner, B., Grau, A., Hendriks, I.E., Weitzman, B. (2017). Molusco del Año 2017: "La Nacra Pinna nobilis", in NOTICIARIO DE LA SOCIEDAD ESPAÑOLA DE MALACOLOGIA Nº 67-2017.
Rognstad, R.L., Wethey, D.S., Oliver, H., Hilbish, T.J., 2018. Connectivity modeling and graph theory analysis predict recolonization in transient populations. J. Mar. Syst. 183, 13–22.
Ross, R.E., Nimmo-Smith, W.A.M., Howell, K.L., 2017. Towards 'ecological coherence': assessing larval dispersal within a network of existing. Marine Protected Areas. Deep Sea Research Part i: Oceanographic Research Papers 126, 128–138.
Rouanet, E., Trigos, S., Vicente, N., 2015. From youth to death of old age: the 50-year story of a *Pinna nobilis* fan mussel population at Port-Cros Island (Port-Cros National Park, Provence, Mediterranean Sea). Scientific Reports of the Port-Cros National Park 29, 209–222.
Salis, P., Peyran, C., Morage, T., de Bernard, S., Nourikyan, J., Coupé, S., Bunet, R., Planes, S., 2022. RNA-Seq comparative study reveals molecular effectors linked to the resistance of Pinna nobilis to Haplosporidium pinnae parasite. Sci. Rep. 12 (1), 21229.
Scheltema, R.S., 1986. Long-distance dispersal by planktonic larvae of shoal-water benthic invertebrates among central Pacific islands. Bull. Mar. Sci. 39, 241–256.
Siegel, D.A., Mitarai, S., Costello, C.J., Gaines, S.D., Kendall, B.E., Warner, R.R., Winters, K.B., 2008. The stochastic nature of larval connectivity among nearshore marine populations. Proceedings of the National Academy of Sciences of the United States of America 105, 8974–8979.
Orden TEC/1078/2018, de 28 de septiembre, por la que se declara la situación crítica de Cistus heterophyllus subsp. carthaginensis, Lanius minor, Margaritifera auricularia, Marmaronetta angustirostris, Mustela lutreola, Pinna nobilis y Tetrao urogallus cantabricus en España, y se declaran de interés general las obras y proyectos encaminados a la recuperación de dichos taxones.
Sponaugle, S., Cowen, R.K., Shanks, A., Morgan, S.G., Leis, J.M., Pineda, J., Boehlert, G. W., Kingsford, M.J., Lindeman, K., Grimes, C., Munro, J.L., 2002. Predicting self–recruitment in marine populations: biophysical correlates and mechanisms. Bull. Mar. Sci. 70, 341, 37.
Swearer, S.E., Treml, E.A., Shima, J.S., 2019. A review of biophysical models of marine larval dispersal. Oceanogr. Mar. Biol.
Torrado, H., Mourre, B., Raventos, N., Carreras, C., Tintoré, J., Pascual, M., McPherson, E., 2021. Impact of individual early life traits in larval dispersal: a multispecies approach using backtracking models. Prog. Oceanogr. 192, 102518.
Treml, E.A., Halpin, P.N., Urban, D.L., Pratson, L.F., 2008. Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. Landsc. Ecol. 23, 19–36.
Treml, E.A., Ford, J.R., Black, K.P., Swearer, S.E., 2015. Identifying the key biophysical drivers, connectivity outcomes, and network consequences of larval dispersal in the sea. Movement Ecology 3, 1–16.
Trigos, S., García-March, J.R., Vicente, N., Tena, J., Torres, J., 2014. Utilization of muddy detritus as organic matter source by the fan mussel *Pinna nobilis*. Mediterr. Mar. Sci. 15 (3), 667, 67.
Trigos, S., Vicente, N., Prado, P., Espinós, F.J., 2018. Adult spawning and early larval development of the endangered bivalve *Pinna nobilis*. Aquaculture 483, 102–110.
Vázquez-Luis, M., March, D., Álvarez, E., Álvarez-Berastegui, D., Deudero, S., 2014. Spatial distribution modelling of the endangered bivalve *Pinna nobilis* in a Marine Protected Area. Mediterr. Mar. Sci. 626–634.
Vázquez-Luis, M., Álvarez, E., Barrajón, A., García-March, J.R., Grau, A., Hendriks, I.E., Jiménez, S., Kersting, D., Moreno, D., Pérez, M., Ruiz, J.M., Sánchez, J., Villalba, A.,

Deudero, S., 2017. SOS Pinna nobilis: a mass mortality event in western Mediterranean Sea. Front. Mar. Sci. 4, 220.
Vicente, N., 1986. La grande Nacre de Méditerranée. Parc national de Port-Cros ([plaquette de présentation]. 4 volets).
Wesselmann, M., González-Wangüemert, M., Serrão, E.A., Engelen, A.H., Renault, L., García-March, J.R., Duarte, C.M., Hendriks, I.E., 2018. Genetic and oceanographic tools reveal high population connectivity and diversity in the endangered pen shell *Pinna nobilis*. Sci. Rep. 8 (1), 4770.
Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweitzer, J.A., Shuster, S. M., Wimp, G.M., Fischer, D.G., Bailey, J.K., Lindroth, R.L., Woolbright, S., Kuske, C. R., 2003. Community and ecosystem genetics: a consequence of the extended phenotype. Ecology 84 (3), 559–573.
Yang, B., Zhai, S., Zhang, F., Wang, H., Ren, L., Li, Y., Li, Q., Liu, S., 2022. Genome-wide association study toward efficient selection breeding of resistance to *Vibrio alginolyticus* in Pacific oyster, Crassostrea gigas. Aquaculture 548, 737592.
Zannella, C., Mosca, F., Mariani, F., Franci, G., Folliero, V., Galdiero, M., Tiscar, P.G., Galdiero, M., 2017. Microbial diseases of bivalve mollusks: infections, immunology and antimicrobial defense. Mar. Drugs 15 (6), 182.