Population trends of the fan mussel *Pinna nobilis* from Portofino MPA (Ligurian Sea, Western Mediterranean Sea) before and after a mass mortality event and a catastrophic storm

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Abstract

Two *Pinna nobilis* populations thriving inside the borders of the Portofino Marine Protected Area (MPA) (Ligurian Sea, western Mediterranean Sea) were monitored before (2012) and after (September 2018) a dire mass mortality event that, since September 2016, spread through the whole Mediterranean Sea. In Portofino MPA, recorded mortality rates reached values of 91.29% and 43.94% in the two populations. The presence of a *Haplosporidium* protozoan parasite, considered to be the main cause of the mortality episodes, was confirmed from histological evidence: sporocysts and plasmodia were observed in all the tubes of the digestive glands of the collected specimens. Moreover, a catastrophic storm hit the Ligurian coasts at the end of October 2018, causing considerable damages both below and above the surface; a new survey conducted in November 2018 showed the complete annihilation of the two studied populations, as a probable combination of the continued parasite infections and the mechanical impacts caused by the storm. Finally, in June 2020 the sites were monitored again looking for traces of recovery, but no new specimens were recorded, indicating that *P. nobilis* became virtually absent from the MPA.

Keywords: Mass mortality, climate change, storm, MPA, Pinna nobilis

1. Introduction

The fan mussel *Pinna nobilis* Linnaeus, 1758 is the largest bivalve endemic of the Mediterranean Sea, reaching a maximum size of 120 cm (Zavodnik et al. 1991). It can be found at depths ranging from 0.5 to 60 m (Butler et al. 1993), mainly on seagrass meadows constituted by *Posidonia oceanica* (L.) Delile (Garcia-March et al. 2002). In the past decades, the reckless collection of this mollusc (for food, byssus, ornamental purposes and the occasional presence of aragonitic pearls of no commercial value), the incidental killing due to fishing activities and anchoring (Richardson et al. 2004; Centoducati et al. 2007; Hendriks et al. 2013; Deudero et al. 2015), the loss of eggs, larvae and adults due to the effects of chemical pollutants and the regression of the seagrass beds due to anthropogenic activities contributed to accelerating the decline of this formerly abundant species (Shahidul & Masaru 2004). Nowadays, *P. nobilis* is legally protected under Annex II of the Barcelona Convention (SPA/BD Protocol 1995); moreover, according to the European Council Directive 92/43/EEC on the conservation of natural habitats and wild fauna and flora, it is under strict protection (Annex IV), and all forms of deliberate capture or killing of fan mussels are prohibited (EEC 1992). Because of its relevance from a conservationist and ecological point of view, *P. nobilis* is often regularly or occasionally monitored by most of the Mediterranean Marine Protected Areas (MPAs).

Since the end of September 2016, a mass mortality event involving *P. nobilis* started to spread from the Spanish Mediterranean coasts (Western Mediterranean Sea), affecting specimens of all sizes, depth ranges and habitat types (Vázquez-Luis et al. 2017). Mortality rate soon (March to June 2017) reached 100% in many localities along the Iberian Peninsula, while others (Ebro delta,
Medes Island and Cadaqués) seemed to be unaffected; overall, an early mortality rate of 90% of the Iberian population was estimated (Vázquez-Luis et al. 2017). A highly species-specific newly described protozoan parasite, *Haplosporidium pinnae*, has been reported as the likely cause of the fan mussel mortality (Darriba 2017; Vázquez-Luis et al. 2017; Catanese et al. 2018; García-March et al. 2020). Lately, in 2017, other mass mortality events were recorded from Corse (France) and Italy (Catanese et al. 2018; citizen science at http://www.observadoresdelmar.es), and even from the Eastern Mediterranean basin, with an average mortality of more than 93% of the Lesvos Island (Aegean Sea) population (Katsanevakis et al. 2019) and a general mortality rate of 31% in the Greek seas (Zotou et al. 2020). The spreading of the parasite in the western Mediterranean basin has been related to the action of surface currents (Cabanellas-Reboredo et al. 2019; García-March et al. 2020). In the southern Tyrrhenian Sea, Carella et al. (2019) found evidence of a mycobacterial disease associated with the mortality episodes, suggesting that mass mortality events may be due to a more complex scenario, involving alternatively *H. pinnae*, the Mycobacteria, and/or other joint causes.

During summer 2018, some recreational SCUBA divers reported an unusual number of dead fan mussels inside the borders of the Portofino Marine Protected Area (MPA) (Central Ligurian Sea, NW Mediterranean) (Portofino MPA managing authority, pers. comm.). In addition, on October 29, 2018, the Ligurian coasts (and in particular the area of the MPA) faced an extraordinary catastrophic natural event: a prolonged action of strong SE winds, exceeding 150 km/h, produced waves up to 10 m high: numerous damages to natural and anthropic structures were recorded all along the coasts, and the impact of the storm on the benthic communities of the Portofino Promontory was evident (Betti et al. 2020; Oprandi et al. 2020). Aim of the present study was to follow the changes in the *P. nobilis* population inside the borders of the Portofino MPA, before and after two catastrophic events, such as the mass mortality episode and the exceptional storm.

2. Materials & methods

2.1. Study area

The Portofino MPA, in the eastern portion of the Ligurian Sea (NW Mediterranean Sea) (Figure 1), was established in 1999, and regulates all human activities along the coasts of the Portofino Promontory (Figure 1). With an extension of 346 hectares is among the smallest MPAs in Italy and includes the municipalities of Camogli, Portofino and Santa Margherita Ligure. In addition to being a NATURA 2000 site (SCI IT1332674), since 2005 it has been qualified as a Specially Protected Area of Mediterranean Interest (SPAMI) (UNEP 2005). The MPA consist of three zones characterized by different levels of protection: a small central “no entry-no take” zone, a wide general protection zone developed along the southern front of the promontory, and two partial protection zones (called “C sectors”) located at the eastern and western sides of the promontory. While the southern front is constituted by rocky cliffs, the two sides protected by the C sectors are characterized by non-cohesive bottoms covered in wide *Posidonia oceanica* meadows (Diviacco & Coppo 2006; Cappanera et al. 2012).

![Figure 1. Map of the Mediterranean Sea (inlet) indicating the Portofino MPA area (red square) and the position of the sampling areas.](image-url)
2.2. Pinna nobilis census and sampling

In 2012 two sites, one for each of the C sectors, were chosen for a monitoring activity of the Pinna nobilis populations inside the Portofino MPA: San Rocco (WGS coordinates 44°20′16″ N; 009°09′18″ E) on the western side of the MPA, and Cervara (44°19′00″ N; 009°12′43″ E) on the eastern one (Figure 1). The visual census was conducted by SCUBA diving: two divers investigated a circular area of a diameter of 20 m (for a total explored surface of 314 m²) for each site, counting the encountered fan mussels and noting the health status (alive or dead). The centres of the explored circles were placed about 50 m from the shoreline, on an 8 m depth seafloor. The total investigated depth range was comprised between 1 and 13 m.

During September 2018, around 2 years after the first records of mortality episodes in Spain and immediately following some unusual reports of dead specimens inside the Portofino MPA, the P. nobilis density in each of the 2 chosen study area was investigated again by visual census performed by scuba divers. Observations in each site were conducted along a belt transect (100 m long x 4 m wide, for a total explored area of 400 m²), according to Siletic and Peharda (2003), stretched out perpendicularly to the coastline, starting from 1 m depth; the final depths were approximately 13 m, therefore the investigated areas were considered comparable to the ones explored in 2012. For each encountered specimen, the health status was noted. The same monitoring activity was performed again, in the same area and following the same protocol, during November 2018, just after the catastrophic storm that hit the Ligurian coasts on October 29.

Finally, a last survey was conducted, with the same methodology, in June 2020, to examining a possible recovery of the populations.

2.3. Histological procedures

Contextually, it was decided to investigate the presence of the parasite Haplosporidium pinnae in the fan mussels of the Portofino MPA. Therefore, on October 24 2018, two specimens were collected by SCUBA diving from Cervara site. Specimen 1 was collected at 8.2 m depth; its total shell height was 40 cm and weighed 380 g. When inspected in situ, it did not readily react to tactile stimulation, thus it was considered “sick”. Specimen 2 was collected at 7 m depth; its total shell height was 33 cm and weighed 356 g. When inspected it reacted quickly and was considered “healthy”.

The collected specimens were placed in a cooler container and immediately transferred to the laboratory for dissection. Digestive glands and gills from both the specimens were dissected, fixed in 4% paraformaldehyde phosphate-buffered solution (PBS, pH 7.4), washed in PBS and stored in ethanol (70% in distilled water) at 4°C. The samples were paraffin-embedded, sectioned at 5 μm thickness and stained with hematoxylin-eosin (Bio-Optica, Italy). Sections were examined using Leica DMRB light microscope, and images were acquired with a Leica CCD camera DFC420C (Leica, Switzerland). The histological sections of digestive glands were obtained transversally sectioning the major central duct.

2.4. Environmental conditions

The coastal area of the Portofino Promontory is part of “Long Term Ecological Research” (LTER) net (www.lteritalia.it). Physical features of the water column are monitored fortnightly since 2000 in Lighthouse Cape station (80 m depth, Figure 1), through an Idronaut 316 plus CTD. Moreover, previous data are available for sea surface temperature (SST) in the area (Licandro & Ibanez 2000). Monthly SST of 2018, year of the first recorded mortality episodes in the Portofino MPA, was compared with the average 1985–2017 monthly SST, calculating the standardised anomaly. Moreover, sea-water temperature CTD profiles in the second half of October 2018 were mediated at 1 dbar and the profile on 25th October 2018 (the closest to the day of the storm) was compared to the averaged profile using the available late October data since 2000 to show the water column stratification.

3. Results

3.1. Pinna nobilis density and mortality

The 2012 Pinna nobilis survey conducted in the San Rocco (western C sector) and Cervara (eastern C sector) sites recorded densities of 2.87 and 2.23 living specimens 100 m⁻², respectively. In addition, a density of 0.63 dead specimens 100 m⁻² was recorded in San Rocco, while no dead fan mussels were found in Cervara.

The survey performed on September 2018 showed the effects of the mass mortality event, as only 0.25 living specimen 100 m⁻² of Pinna nobilis was observed in San Rocco (indicating a reduction of 91.29% of the population), and 1.25 living specimen 100 m⁻² (showing a reduction of 43.94% of the previous population) was observed in Cervara (Figure 2(a)). In addition, 0.75 and 1 dead specimen 100 m⁻² were observed in San Rocco and Cervara, respectively; all the dead animals were still found in a vertical position, partially emerging from the seafloor (Figure 2(b)).

Two months later, after the impact of the storm, in both areas no living P. nobilis were found in the
transects. One and two dead specimens 100 m$^{-2}$ were found in San Rocco and Cervara, respectively; the observed valves were completely eradicated from the seafloor (Figure 2(c)). In June 2020, 1.5 year after the storm, still no specimens, neither alive nor dead, were observed in the transects.

3.2. Histological evidence

Parasitic haplosporidan-like protozoans, as described in Catanese et al. (2018), were observed in the epithelium of the gland tubules of both specimens. Sporocysts and plasmodia were in virtually all the tubules, suggesting a widespread presence of the parasite. Conversely, in gills, sporocysts and plasmodia were not observed, and the presence of the parasite was neither clearly detectable in specimen 1 nor 2 (Figure 3).

3.3. Environmental conditions

SST monthly anomaly of 2018 (Figure 4(a)) showed winter values close or below the 1985–2017 average. Conversely, a long period of positive values started from April 2018 until the end of 2018, with the exception of the June value; particularly elevated values occurred in August and September. In Autumn 2018, the heat reached relevant depths (on October 25th temperature higher than 21°C was recorded up to nearly 50 m depth), being higher than the average profile of the end of October since 2000 (Figure 4(b)). In the 0–13 m layer, an average temperature of 21.30 ± 0.01°C was recorded on October 25 2018, definitely higher than the 2000–2017 late October average temperature of 20.18 ± 0.74°C.

4. Discussion

Before the impact of the mass mortality event that spread through the whole Mediterranean Sea since September 2016, fan mussels were well established in the Posidonia oceanica meadows of the Portofino MPA (2.87 and 2.23 living specimens 100 m$^{-2}$ in San Rocco and Cervara, respectively), with average densities in accordance with those measured in other well-developed populations in the north-western Mediterranean, e.g., 1 specimen 100 m$^{-2}$ in Port-Cros MPA (Vicente et al. 1980; Moreteau & Vicente 1982) and 1.5 specimen 100 m$^{-2}$ in the Columbretes Islands Marine Reserve (Spain) (García-March & Kersting 2006). The Portofino populations were quite healthy, since around 82%
of the specimens observed in San Rocco and 100% of the specimens recorded in Cervara were alive.

During 2018, the health status of the *Pinnia nobilis* population completely changed. In September, around 2 years after the first records of mass mortality events along the Spanish coasts, the mortality rates in the Portofino MPA were extremely high: in San Rocco the percentage of dead specimens (91.29%) was comparable to the one observed along the Iberian coasts and the Aegean Sea during the spread of the infection, while Cervara (43.94%) appeared to be at that moment less impacted.

The mortality event could be reconducted to the effects of the protozoan *Haplosporidium pinnae*: sporocysts and plasmodia observed in virtually all the tubules suggested a widespread presence of the parasite in the fan mussel bodies. Despite the low number of analysed specimens, which does not allow deep considerations, the presence of the haplosporidan-like protozoans in both the healthy and the weak specimens can indicate an on-going infection that, although still asymptomatic in some individuals, potentially had already reached all the organisms in that confined area. The parasite was probably transmitted by westerly populations (from Spain and France), since mass mortality events from southern Italy appeared to be due to a mycobacterial infection (Carella et al. 2019) and the northwards and eastwards dispersion of the parasite due to surface currents was already observed along the Spanish coasts (García-March et al. 2020).

The higher than average seawater temperatures in the summer – autumn 2018 may have stressed the fan mussels, triggering or enhancing parasite manifestation, which is favoured by temperature above 13.5°C, that was reached in the area from mid-April onwards, and a range of salinity between 36.5 and 39.7 psu (Cabanellas-Regoredo et al. 2019; García-March et al. 2020), that is typical of the area (Misić et al. 2011; Coppari et al. 2020).

The catastrophic storm event that hit the Ligurian coasts on October 29 2018 caused many damages to benthic organisms of the Portofino MPA, such as the eradication of many *Paramuricea clavata* (Risso, 1826) colonies living at 20 m depth along the Lighthouse Cape (Betti et al. 2020) and the reduction of the *P. oceanica* meadows (Oprandi et al. 2020), and it probably caused eradications and mechanical damages to the *P. nobilis* populations inside the MPA. Nevertheless, the complete disappearance of the fan mussels from San Rocco, an area well sheltered from the strong SE winds that caused

![Figure 3. Histological sections of *P. nobilis*, hematoxylin-eosin. Digestive glands of Specimen 1 (a) and 2 (b); sporocytes (arrows) and multinucleated plasmodia (arrowheads) are visible in the tubules. Gills of specimen 1 (c) and 2 (d); some eosinophilic cells, mast cell-like, can be seen (white arrows). Scale bars: 20 µm.](image-url)
the harms, suggests that the storm might have accelerated the process already underway in the Portofino MPA, but the mass mortality event was probably leading to the complete disappearing of *P. nobilis* from the two monitored areas. In June 2020, 1.5 years after the recorded complete disappearance of *P. nobilis* from the investigated areas, still no traces of recovery were observed, indicating that at the present state the fan mussel is virtually extinct from the MPA. In the past, various mass mortality events have seriously affected other bivalve populations in the Mediterranean Sea, but they never led to a short-term risk of extinction; nevertheless, in the case of *P. nobilis* the possibility of an ecological or even total extinction over medium term is considered likely, unless manipulative actions are soon

Figure 4. (a) monthly SST standardized anomaly in 2018; (b) Temperature (°C) profile in Lighthouse Cape station on 25th October 2018 (in red) compared to the average profiles of the second half of October from 2000 to 2017 (in black; horizontal bars denote standard deviations).
activated (García-March et al. 2020). This study adds new information on the alarming state of this critically endangered mollusc along the Italian coasts, confirming the necessity to quickly undertake serious measures to avoid the extinction of such an ecologically important species.

Disclosure statement

No potential conflict of interest was reported by the authors.

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