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EMPTY SHELLS OF *PINNA NOBILIS* (MOLLUSCA: BIVALVIA) AS HABITAT FOR A DIVERSE CRUSTACEAN COMMUNITY

CONCHIGLIE VUOTE DI PINNA NOBILIS *(MOLLUSCA: BIVALVIA) COME HABITAT PER UNA COMUNITÀ DIVERSIFICATA DI CROSTACEI*

Abstract – *A preliminary structural and functional analysis of the crustacean community associated to the empty shells of* Pinna nobilis *(Linnaeus, 1758) has been investigated to assess the persistence of the bio-engineering role of the mediterranean endemic fan mussel following the mass mortality event. Dead specimens of* P. nobilis *were sampled from two different sites in the Gulf of Trieste (Miramare and San Pietro) by scuba diving following a specially developed protocol aimed to collect exclusively organisms living in and on the shells. A structural and functional diverse crustacean community has been found in the empty shells, witnessing the large ecological relevance of* P. nobilis *as ecosystem engineer in sedimentary environments, able to increase spatial heterogeneity and complexity and to promote local biodiversity.*

Keywords: *ecosystem engineer, epibenthic community, structural diversity, functional traits*

Introduction – In sedimentary environments, solitary larger epibenthic bivalves including the Mediterranean endemic species *Pinna nobilis* (Linnaeus, 1758), known also as fan mussel, emerge as crucial ecosystem engineers. By creating new suitable and available hard 3D-substrate for other species and by contributing to sediment stabilisation through byssal threads, they promote the increasing of local biodiversity (Jones *et al.,*1994; Bouma *et al.,* 2009; Meadows *et al.,* 2012; Ysebaert *et al.,* 2019). However, the fan mussel has recently experienced a severe mass mortality event throughout the region reaching even the 100% of mortality in the northern Adriatic between 2019 and 2021. Nevertheless, the large empty shells, in many locations, still remain intact and firmly anchored in the sediment, continuning to serve as a colonising surface for a diverse epibenthic community. This suggests that the bio-engeneering role of *P. nobilis* persists despite the death of the animal. To better understand and highlight this role, the associated macrofaunal community has been investigated. Similarly to sedentary polychaetes and sessile molluscs, marine arthropods, including amphipods, decapods and cirripeds represent an ecologically relevant component of the macrofouling community in marine environment. Furthermore, although the commensalism between living *P. nobilis* and the two crustacean species *Pontonia pinnophylax* (Otto, 1821) and *Nepinnotheres pinnotheres* (Linnaeus, 1758) is well documented (Rabaoui *et al.,* 2008), little is still known about the crustacean assemblages associated with the empty shells. Therefore, the main aim of this study is providing a first structural and functional description of the crustacean community hosted by the fan mussel dead shells from the sedimentary bottom of the northern Adriatic sea.

Materials and methods – Seven dead specimens of *P. nobilis* have been sampled from two different sites in the Gulf of Trieste (northern Adriatic): three from Miramare (MIR) the inshore site (less than 100 m from the coast, 5 m maximum depth) and four from

S. Pietro (SP) (9 km from the coast, 13-15 m depth) located close to the biogenic outcrop and SIC "Trezze San Pietro and Bardelli" (Fig.1).

Fig.1- Map of the study area and sampling sites selected for the collection of *Pinna nobilis* empty shells. Blue symbol indicates the inshore site "Miramare" (MIR) and the red symbol indicate the offshore site "S. Pietro" (SP).
Mappa dell'area di studio e dei siti di campionamento scelti per la raccolta delle conchiglie vuote di Pinna nobilis. *Il simbolo blu indica il sito vicino alla costa "Miramare" (MIR) e il simbolo rosso indica il sito più a largo "S. Pietro" (SP).*

The collection was performed by scuba diving following a specially designed protocol aiming to sample all macrofauna living exclusively in and on the shells. The protocol consisted of three main phases: 1) selection of the empty shells still intact and vertically anchored to the sediment, 2) approaching in the opposite direction of light for avoiding shadow projection on the selected shells followed by wrapping with a nylon bag with 0.5 mm of mesh size and 3) sealing the sample and transfering to the surface and to the laboratory. Once in the laboratory shell surfaces of the samples has been scratched and rinsed on a 0.5 mm sieve to collect the associated macrofauna. Afterthat, macrofauna has been carefully sorted under a stereomicroscope and crustaceans were separated from the other taxonomic categories (e.g., molluscs, polychaetes, etc.) and preserved in a 80% ethanol solution. Crustaceans were then morphologically identified at the lowest possible taxonomic level using identification keys and relevant available literature and, finally, counted. Univariate indices of diversity like species richness (S), number of individuals (N), Margalef's richness index (d), Pielou's evennes index (J') and Shannon-Wiener diversity index (H'($\log_2$)) were also calculated. To assess differences based on location, a one-way Kruskall-Wallis ANOVA was performed. Furthermore, to assess differences in crustacean community structure, an agglomerative hierarchical cluster analysis (group-average linkage) was performed on the Bray-Curtis similarity resemblance matrix from square root transformed species abundance data. The SIMilarity PROFile analysis routine (SIMPROF) was applied to clustering outputs to objectively identify valid clusters. To test differences in community composition between sites, PERMANOVA analysis was also conducted. Then, to better visualise the distribution patterns of the assemblage the nMDS was also performed. For the functional analysis, Biological Trait Analysis (BTA) was performed by selecting two continuous traits (e.g. max adult size and longevity) and three categorical traits (e.g. mobility, feeding and living habit). For each trait some categories has been chosen according to Clare *et al.* (2022). Maximum adult size included the following categories: under 5 mm, between 5-10 mm, between 10-30 mm and over 30 mm. Adult longevity was categorised as less than 1 year, between 1-3 years and between 3-10 years. Mobility included the following categories: sessile, semi-motile and motile. Feeding habit included suspension feeder, surface deposit feeder, scavenger, predator and parasite.

Lastly, living habit comprised the epi-endobenthic species, those living in crevices-hole-under stones, burrow-tube dweller and free living species. Each species was assigned to specific category by using available information in primary literature. The minimum and maximum affinity for each category was expressed with a binary code (0-1) and a species-trait matrix weighted by abundance was created. PERMANOVA was also performed to test changes in functional patterns according to the site. Finally, functional patterns of the crustacean community were then plotted in a PCO (Principal coordinates analysis), performed on the Gower resemblance matrix.

Results – A total of 55 crustacean taxa, of which 49 recognised at species level belonging to seven different orders (Amphipoda, Decapoda, Isopoda, Tanaidacea, Balanomorpha, Cumacea and Leptostraca), were identified in seven dead specimens of *P. nobilis*. Overall, Amphipoda counted the most species (26), followed by Decapoda with 11 species and Isopoda with 7 species. Medium-high values of diversity indices were observed in both sites, but the K-W ANOVA analysis revealed no significant differences. In general, all diversity indices were higher in S.Pietro than in Miramare, except for the number of individuals (N), which was higher at the inshore site than at the offshore one (Fig.2a). This was due to the high colonisation of the cirriped species *Balanus trigonus,* which contributed with an average of 85.67 ± 50.83 individuals in Miramare. In contrast, in terms of community structure, crustacean communities resulted significantly different in the two sites. The nMDS (Fig.2b) shows the sampled *P. nobilis* shells grouped by site which is highlighted by the SIMPROF analysis ($p < 0.05$), overlaid on the plot. The differences were mainly due to the higher dominance of decapod species in Miramare than in S. Pietro (on average 61.00 ± 24.25 individuals and 18.25 ± 17.29, respectively) and the higher dominance of both amphipods and isopods in S.Pietro (79.25 ± 28.91 and 33.75 ± 25.25, respectively) than in Miramare (48.67 ± 42.34 and 4.66 ± 4.16, respectively).

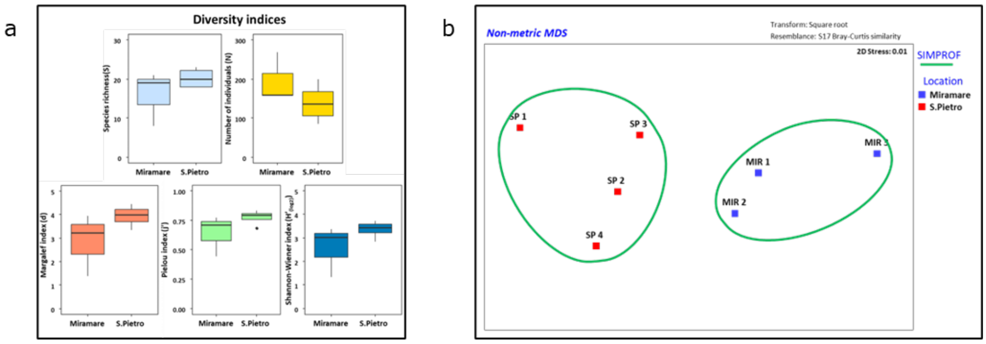

Fig. 2 - (a) Box plot of diversity indices in the sampled locations: Miramare (MIR) and S. Pietro (SP); (b) nMDS ordination plot based on crustacean abundance values at the sampled sites: Miramare (MIR) and S. Pietro (SP).

(a) Box plot degli indici di diversità nelle stazioni campionate: Miramare (MIR) e S.Pietro (SP); (b) Ordinamento nMDS basato sui valori di abbondanza dei crostacei trovati nei due siti di campionamento: Miramare (MIR) e S. Pietro (SP).

Regardless of functional analysis, the main functional traits which occurred in the crustacean community were maximum adult size, feeding habits and mobility (Fig.3a). Most of the crustacean community was characterised by organisms with a maximum adult size ranging from 5 to 10 mm, suspension feeders, motile and semi motile. Furthermore, PERMANOVA analysis on functional traits occurrency revealed significant differences between the two sites ($p< 0.05$), as a reflection of the structure of the community. In the PCO the total explained variation of the multivariate ordination was 90.7% (PCO axis1= 53.3% and PCOaxis2= 37.4%) (Fig.3b). Sampling distribution in the ordination differentiated the two sites Miramare (MIR) and S. Pietro (SP), with samples from Miramare predominantly ordered on the left of the graph, while samples from the offshore site were grouped at the top right of the graph. Miramare (MIR) were mainly characterised by sessile organisms, attached to the substrate, with 5-10 mm adult maximum size, scavengers and predator, whereas S. Pietro (SP) showed a crustacean community characterised mostly by free living, motile and semi-motile species, tending to live in the sediment, creating burrows or tubes and feeding on surface deposit or as parasite (traits expressed mostly by amphipods and isopods).

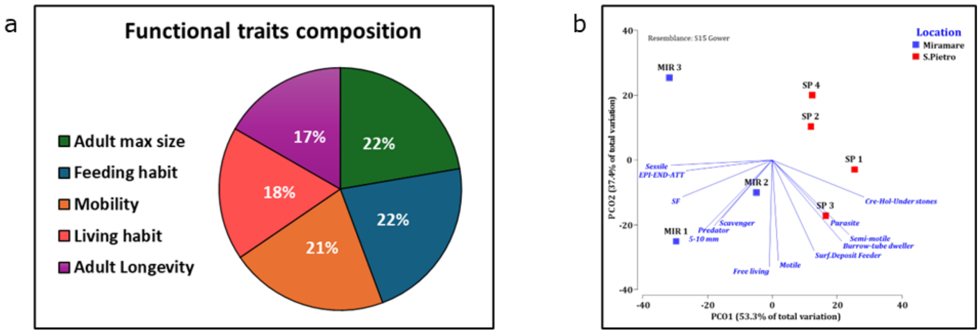

Fig. 3 - (a) Functional traits composition occurred in the Crustacean community; (b) Canonical Analysis of Principal Coordinates of Crustacean values abundances in the two different sites (Miramare and S. Pietro). The Main functional traits which contributed to the variation overlaid on the plot.
(a) Composizione dei tratti funzionali espressi dalla comunità di crostacei (b) Analisi canonica delle coordinate principali sui valori di abbondanza dei crostacei nei due differenti siti (Miramare e S. Pietro). I principali tratti funzionali che contribuivano alla variabilità tra i due siti sono stati sovraimposti al grafico.

Conclusions –The study provided the first structural and functional description of the crustacean community hosted by empty *Pinna nobilis* shells from the northern Adriatic Sea. The medium to high diversity index values, along with the structural and functional variability observed in the crustacean community, indicate and demonstrate the positive effect of empty shells, comparable to that of living specimens, in creating and maintaining new ecological niches that promote local biodiversity, as previously observed by Iannucci *et al.* (2023). This effect is particularly evident and remarkable in monotonous soft-bottom environments, where the presence of *P. nobilis* shells significantly increases spatial heterogeneity.

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