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Engineering effect of *Pinna nobilis* shells on benthic communities[☆]

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Summary Within the framework of the possibility of using the Mediterranean pen shell *Pinna nobilis* in restoration and conservation plans of benthic habitats, an in situ experiment was conducted using empty *P. nobilis* shells. The latter were transplanted in a bare soft-bottomed area and their associated fauna were followed along 120 days and compared at different temporal points and with the assemblages living in the surrounding soft-sediment area. Compared to soft-sediment communities, an evidently increasing succession of species richness, abundance, and diversity descriptors (Shannon-Wiener H' and Pielou's evenness J') was observed with the community inhabiting empty *Pinna* shells. Among the forty-five (45) species found in association with the transplanted empty shells, seventeen (17) were found constantly in the three temporal points; the other twenty-eight (28) species appeared in the samples collected in the second and/ or third sampling time. While motile and sessile species associated to *Pinna* shells showed an increasing pattern of appearance and abundance along the experiment time, those of soft

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sediment remained almost constant. The comparison between *Pinna* shells and soft-sediment associated communities showed that the species richness was slightly different between the two different sample types (49 for soft sediment versus 45 for empty *Pinna* shells); however the total abundance was found more important with empty *Pinna* shells. The results obtained herein argue in favor of the important engineering effect of *P. nobilis* in soft benthic habitats and therefore for the necessity of its conservation.

1. Introduction

In marine systems, it is well known that some animal species play an important ecological role contributing to the complexity of benthic communities. Within this context, some sessile benthic species are very important since they interact with many other associated species. It is for example the case of pen shells which have been studied as micro-ecosystems providing habitat for many sessile species (Kay and Keough, 1981; Keough, 1984; Zavodnik, 1963). Some previous works have demonstrated that the macrofauna diversity and structure in soft-sediment habitats are affected by the presence of pen shell species in particular *Atrina* spp. and *Pinna* spp. (Cummings et al., 1998; Hewitt et al., 2002; Munguia, 2004; Warwick et al., 1997).

Due to their benefits on the ecosystem, pen shells are considered, like other benthic organisms, as ecosystem engineers (Jones et al., 1994; Passarelli et al., 2014). In fact, the presence of these benthic key species can modify the physico-chemical and biological properties of the local environment (Braeckman et al., 2010) and also provide, through their physical presence, a substratum for various epibionts. However, depending on the species, the engineering effect on the community is not always positive in terms of diversity and abundance (Passarelli et al., 2014). Although it is argued by some researchers that ecosystem engineers can be used for restoration and conservation purposes (Byers et al., 2006), the restoration process of native populations, communities and ecosystem processes is difficult to establish because of the complexity of ecological interaction of the ecosystem (Byers et al., 2006; Geist and Galatowitsch, 1999; Jungwirth et al., 2002).

In the Mediterranean Sea, the endemic pen shell *Pinna nobilis* (Linnaeus, 1758) is a good example of an ecosystem engineer species offering an adequate substratum to many benthic invertebrate species to which they are associated. This ecological role of *P. nobilis* has attracted the interest of some scientists reporting the richness and structure complexity of its associated fauna (Addis et al., 2004; Corriero and Pronzato, 1987; Cosentino and Giacobbe, 2007; Rabaoui et al., 2009; Šiletić and Peharda, 2003). Thus, *P. nobilis* can be a good candidate to enhance the biodiversity and abundance of benthic communities in degraded habitats. Transplantation of the species in disturbed areas can attract other sessile and motile invertebrate species, which may result in a positive effect in terms of local benthic biodiversity. To our knowledge, no experimental studies have been carried out up to now in this regard. We attempt herein to make for the first time an in situ experiment of *P. nobilis* shell transplantation in a bare soft-bottomed area, in Elketef, South-eastern Tunisia, in order to check the effect of the physical presence of the species on the local benthic diversity and better understand the colonization process of empty pinnids highlighting their role in enriching and complicating the structure of benthic communities in soft-sediment habitats. Therefore the objectives of this study are (i) to follow temporally the colonization pattern of empty *P. nobilis* shells transplanted by epibionts, (ii) to describe the temporal variations of diversity of the associated macrofaunal assemblages and (iii) to highlight the engineering effect of *P. nobilis* disproving or confirming the possibility of its use for restoration of benthic habitats.

2. Material and methods

2.1. Study area and experimental design

The study was conducted in Elketef, in the southern part of the gulf of Gabes. The experiment area (33°11′45″N, 11°28′21″E) was selected between the fishing port of Elketef and the southern edge of Elbibane lagoon. It extends almost 700 m from the shoreline and it is of less than 2-m depth (Fig. 1). The substratum is sandy mixed with gravel and barely covered with algae. Empty shells of *P. nobilis* used herein were obtained from a previous study on the growth of the species in Tunisia (Rabaoui et al., 2011). All the shells were cleaned before and therefore they were free of fouling. The experiment consisted in transplanting thirty-six empty shells along three transects, of 300 m length each, fixed parallel to the shoreline. In each transect, twelve empty *P. nobilis* shells were transplanted, in a line along the transect, at three levels: four shells in the beginning, four shells in the middle and four shells at the end of the transect. The three levels of a transect were 100 m equidistant, and the same distance was also kept between the three transects. The transplantation of empty shells was done in April 2011 and lasted 120 days. Although *P. nobilis* shell size was not reported to be a factor on which depends the epibiontic fauna of the species (Cosentino and Giacobbe, 2007; Rabaoui et al., 2009), the shells used for this experiment were selected to have a total antero-posterior length ranging between 24 and 31 cm, i.e. the "average size" category according to a previous classification done by Rabaoui et al. (2009). Samples of empty *Pinna* shells were collected at three time intervals: after 30, 60, and 120 days from the starting date of the experiment. At each sampling time,
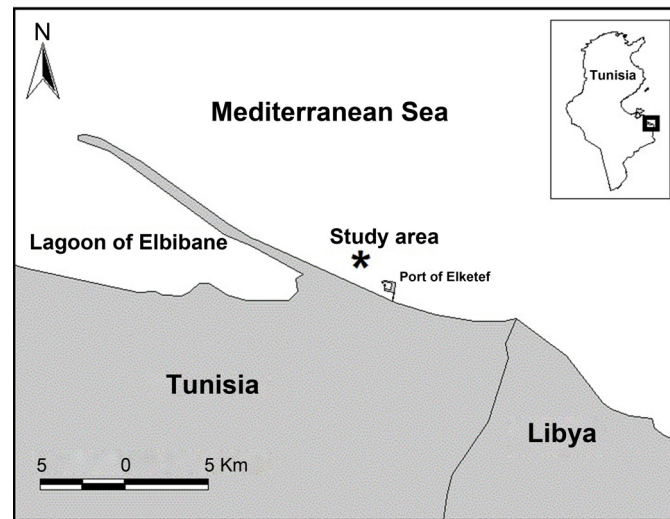
Figure 1 Location of the study area (33°11′45″N, 11°28′21″E). The experiment zone is located between the southern edge of Elbibane lagoon and the harbor of Elketef, South-eastern Tunisia.

three samples, of four *Pinna* empty shells each, were taken separately (i.e. each four transplanted shells correspond to one sample). In parallel, three samples of soft-sediment macro-invertebrates were collected using a 0.25 m² quadrat. Each soft-sediment sample was taken at 5-m distance parallel to the transects and along which empty *Pinna* shells were transplanted and collected. After collection, the sampled empty shells were taken separately in plastic bags to the laboratory. The species found on the shells were collected by scraping off and washing out the valvar surface of each *Pinna* shell sample over a 0.5 mm sieve. Prior to this, the non-countable species mainly sponges and bryozoans, found either on the external or internal valves of the shells were sub-sampled (for later identification) and each colony was considered as one individual (Ben Ismail et al., 2012; Hayward, 1974). Soft-sediment macro-invertebrate samples were also sieved over a 0.5 mm sieve and the species found (in both empty *Pinna* shell and soft-sediment samples) were separated into the major taxonomic groups, preserved in 5% buffered formalin solution and classified at the highest possible level.

2.2. Statistical analysis

Diversity indices of number of species S, abundance of species N, Shannon-Wiener's diversity H' ($\log_e$) and Pielou's evenness J' were calculated for each shell sample and each soft-sediment sample in each of the three sampling times. P1-1 to P1-3, P2-1 to P2-3, P3-1 to P3-3 and S1-1 to S1-3, S2-1 to S2-3, S3-1 to S3-3 were the names used to designate the empty shell and soft-sediment samples collected in the first, second and third sampling surveys respectively (P1, P2, P3 and S1, S2, S3 were used to designate the average values of diversity indices in the total shell and soft-sediment samples collected in the first, second and third sampling surveys respectively). The differences of the diversity indices used between the empty shells samples at each of the three sampling times were tested using Kruskal-Wallis test (KW). The statistical analyses were performed using MS Excel, SPSS, and PRIMER E-6 packages.

3. Results

3.1. Temporal variations of species composition

A total of 684 specimens belonging to 60 macro-invertebrate species were collected in empty *Pinna* shell and soft-sediment samples. 45 species counting 383 individuals were encountered in *P. nobilis* shells; while in soft-sediment samples, 49 species were found corresponding to a total of 301 individuals.

The examination of species richness in the two types of samples showed that molluscs, annelids (mainly sessile species), and cnidarians were the best represented groups in *Pinna* shell samples (Fig. 2A and B). However, in soft-sediment samples, the most diverse groups were molluscs, annelids, and crustaceans (Fig. 2C and D). The temporal monitoring of the associated fauna of empty *Pinna* shells showed that after 30 days from the starting of the transplantation experiment, the species richness was mainly represented by molluscs and cnidarians, followed by bryozoans and ascidians and thereafter by annelids and sponges. No crustaceans or echinoderms were encountered in these latter samples. The communities sampled, 60 days after the shell transplantation, showed also a prevalence of molluscs and cnidarians; however annelids and crustaceans occupied the second position, followed by sponges, bryozoans, echinoderms and ascidians. In the case of samples collected at the third sampling time i.e. 120 days after the starting of the transplantation experiment, a remarkable shift in the fauna structure was observed. In fact, the faunistic assemblages of these samples were dominated by molluscs, annelids and crustaceans, followed by cnidarians and ascidians and thereafter by sponges, bryozoans and echinoderms (Fig. 2A). Considering the global shell samples (combining the three samples obtained at each sampling survey) collected at the three sampling times, their species richness examination showed that the structure of the associated fauna followed the same patterns described with the separate shell samples (Fig. 2B). While the number of species associated to empty
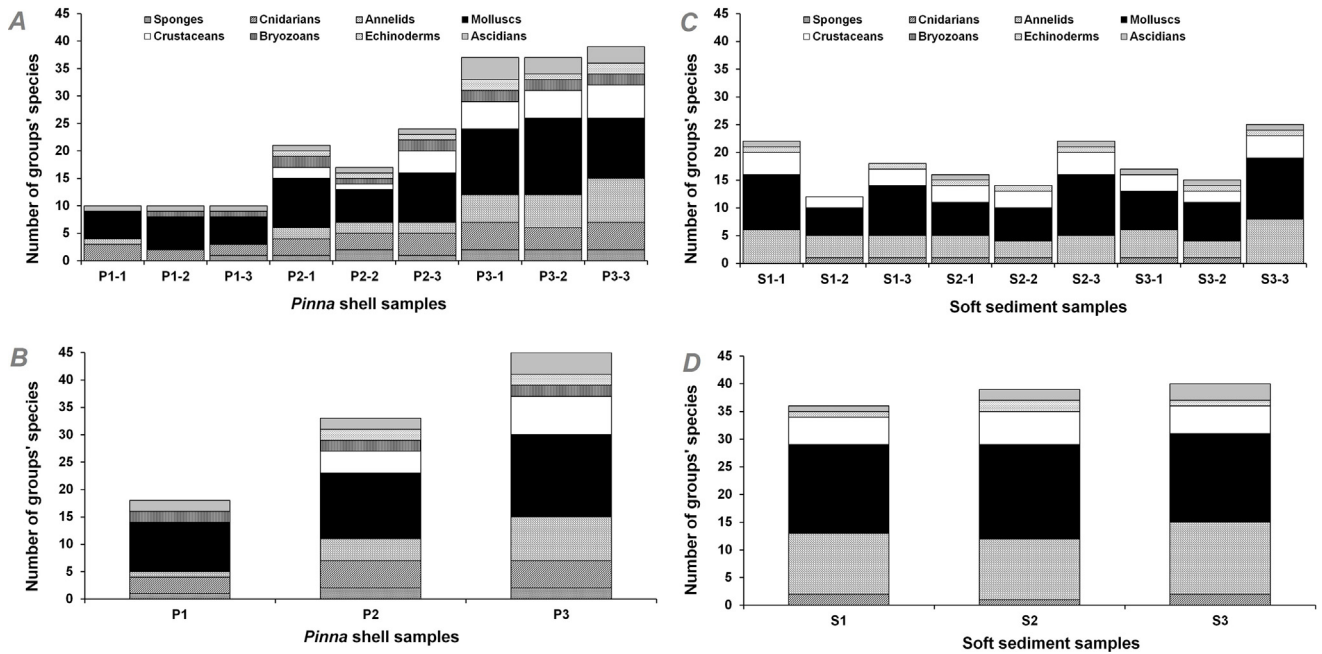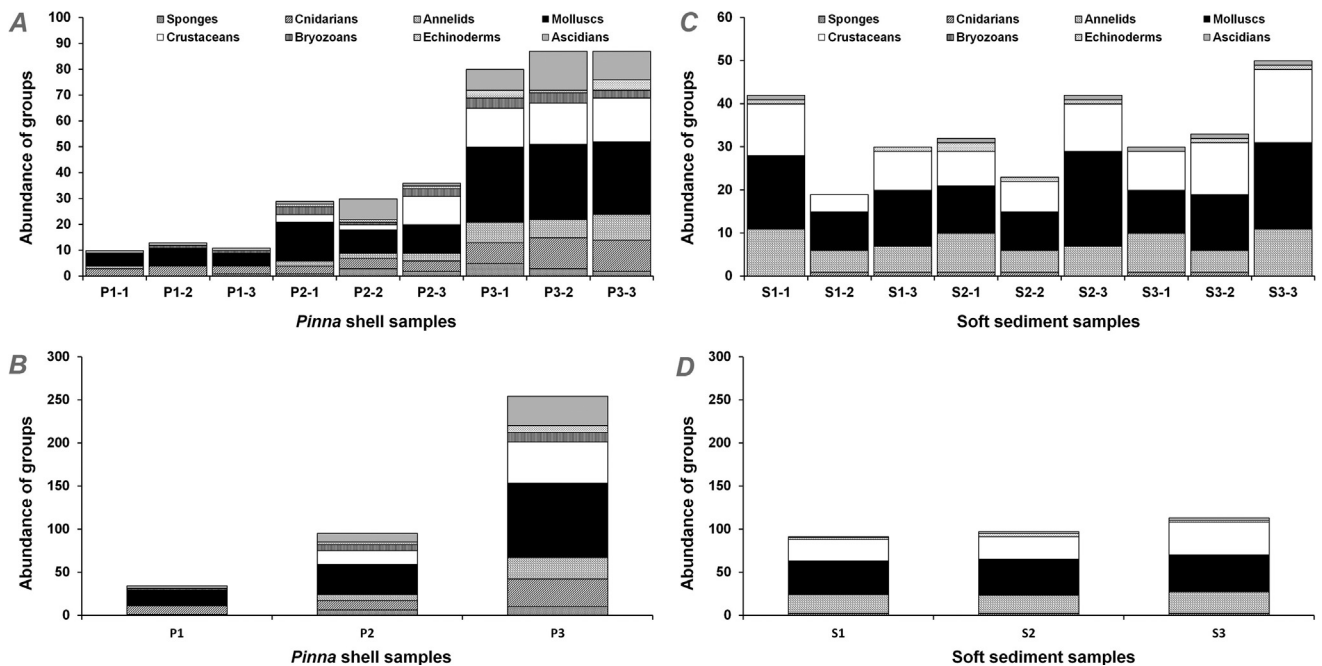
Figure 2 Variations of the species number of each taxonomic group in the separate and total *Pinna* shell (A and B) and soft-sediment samples (C and D) collected at each of the three sampling times. P1-1 to P3-3, P1 to P3, S1-1 to S3-3 and S1 to S3 correspond to the separate and total empty shell and soft-sediment samples collected in the first, second and third sampling times respectively.

Pinna shells showed an increasing pattern along the experiment period, that of soft-sediment samples remained almost constant showing very slight variations between samples and sampling times (Fig. 2).

Considering the abundance of individuals, the most abundant groups in both *Pinna* shell and soft-sediment samples were found to be molluscs, crustaceans and annelids (Fig. 3A—D). The *Pinna* shell samples collected at the first

sampling time were dominated by molluscs and cnidarians, followed by ascidians, bryozoans and thereafter by sponges and annelids. For the second sampling survey, the associated assemblages were mainly formed by molluscs and crustaceans, followed by cnidarians or ascidians (depending on the samples examined). The remaining zoological groups occupied different successive positions depending on the samples. At the third sampling time, the macro-invertebrate

Figure 3 Variations of the abundance of each taxonomic group in the separate and total *Pinna* shell (A and B) and soft-sediment samples (C and D) collected at each of the three sampling times. P1-1 to P3-3, P1 to P3, S1-1 to S3-1 and S1 to S3 correspond to the separate and total empty shell and soft-sediment samples collected in the first, second and third sampling times respectively.

communities were found to be dominated by molluscs and crustaceans. The following order of dominance was occupied either by cnidarians or annelids or ascidians, followed by the other taxa, depending on the examined samples (Fig. 3A). Considering the global samples, their corresponding macro-invertebrate assemblages were found to have the same variations patterns observed with the separate samples. Molluscs and cnidarians prevailed in the community sampled, 30 days after the starting of the transplantation experiment, followed by ascidians, bryozoans and sponges. However in the two other sampling surveys, prevalence was for molluscs and crustaceans, followed by cnidarians and ascidians or vice versa depending on the analyzed samples; the other taxa were found to occupy various ranking positions among the samples analyzed (Fig. 3B). Similarly to species richness, the abundance of communities associated to *Pinna* shell samples showed also an evident increasing pattern along the experiment period (Fig. 3A and B) compared to the soft-sediment samples where the abundance showed only very small variations between samples and sampling times (Fig. 3C and D).

3.2. Temporal variations of motile and sessile fauna

Most of the collected species were commonly found in *Pinna* shell and soft-sediments samples. The species which were not found in soft sediment (11 species) were mainly sessile which need a hard substratum to live: 2 sponges, 2 cnidarians, 1 annelid, 3 molluscs, 1 crustacean and 2 bryozoans (Fig. 4A and B). As for the species which were not found in *Pinna* shell samples (15 species), they were mainly represented by species of soft-sediment affinity, in particular annelids (8 species) and molluscs (7 species) (Fig. 4A and B). Among the forty-five (45) invertebrate species found in association with *Pinna* shell samples, the presence of seventeen (17) species was found to be constant in all the samples collected at the three sampling times. Sixteen (16) species appeared in the samples collected in the second sampling time and were also present in the samples collected at the third sampling time (i.e. 120 days after starting the transplantation experiment). The number of species which appeared only in the samples collected at the third sampling time was estimated as eleven (11). Only one species, *Pyra dura*, was encountered in the first and third sampling survey and it was absent in the samples collected during the second sampling time (Fig. 4A). In all, the number of sessile and motile species was found to be almost similar (23 sessile species versus 22 motile species). The number of sessile species increased from 12 species found in the first sampling survey to 21 and 23 species recorded in the second and third sampling times respectively. Motile species showed also an increasing pattern of appearance passing from 6 in the first sampling time to 12 and then 22 in the other two sampling surveys (Fig. 5A). The temporal variations in the abundance of sessile and motile species associated to *Pinna* shells are described in Fig. 5B. The number of sessile individuals passed from 24 in the samples of the first sampling survey to 71 and then to 184 individuals in the samples collected in the second and third sampling times of the experiment. The abundance of motile individuals also increased from 10 (in

the first sampling time) to 24 and then to 70 (in the second and third sampling times) (Fig. 5A and B). It is worth noting that the percentage cover of colonial species (sponges and bryozoans), assessed visually, showed a general increasing pattern along the experiment period. Also, the presence of certain epibiontic algae was observed on the empty shells collected during the three sampling surveys. These algae species were particularly represented by *Caulerpa*, *Enteromorpha* and *Padina* genus. The comparison of the number and abundance of motile and sessile species between *Pinna* shell and soft-sediment samples showed a prevalence of sessile species in the former type of sample and a prevalence of motile taxa in the latter. Also both motile and sessile species showed an increasing pattern of number and abundance with *Pinna* shell samples along the experiment period. However, the number and abundance of sessile and motile species found in soft-sediment samples showed very slight temporal variations, but remain almost constant (Fig. 5C and D).

3.3. Temporal variations of diversity indices *S*, *N*, *H'* and *J'*

Examining the diversity indices of the communities associated to empty *Pinna* shells samples, some differences were found. The species richness (*S*), computed in the samples taken after 30 days, was estimated at 10 (average = 10.00 ± 0.00). After 60 days, the species richness was found to vary from 17 to 24 (average = 20.67 ± 3.51), while after 120 days, the number of species ranged between 37 and 38 (average = 37 ± 0.58) (Table 1). The abundance (*N*) showed also an increasing pattern along the experiment period. It varied from 10 to 13 (average = 11.00 ± 1.53), from 29 to 36 (average = 32 ± 3.79), and from 80 to 87 (average = 85.00 ± 4.04) during the first, second, and third sampling times respectively (Table 1). As for Shannon-Wiener diversity (*H'*) estimated with empty shell samples, it varied between 2.20 and 2.30 (average = 2.26 ± 0.05), between 2.55 and 3.00 (average = 2.83 ± 0.24), and between 3.24 and 3.36 (average = 3.30 ± 0.06) respectively after 30, 60, and 120 days (Table 1). Hence, Pielou's evenness index (*J'*) varied accordingly. This index was found to vary from 0.96 to 1 (average = 0.98 ± 0.02) in the first sampling time, while in the two other sampling times, it varied between 0.90 and 0.96 (average = 0.94 ± 0.03) and between 0.90 and 0.92 (average 0.91 ± 0.01) (Table 1). The average values of the latter diversity indices, estimated with the global samples collected at the three sampling times, showed an increasing pattern along the experiment duration, except for *J'* which was found to decrease slightly between the first and third sampling times (Table 1), probably due to the ascending pattern of some species' dominance, in particular sessile ones which showed an increasing pattern of abundance along the experiment. Kruskal-Wallis test showed significant differences between the *Pinna* shell samples collected along the experiment with *S* (KW = Chi-Square = 7.513; *p*-value <0.05), *N* (KW = Chi-Square = 7.261; *p*-value <0.05) and *H'* (KW = Chi-Square = 7.200; *p*-value <0.05). No significant difference was highlighted with the other ecological index i.e. *J'* (KW = Chi-Square = 5.067; *p*-value >0.05). Comparing the four diversity indices estimated with *Pinna* shell and
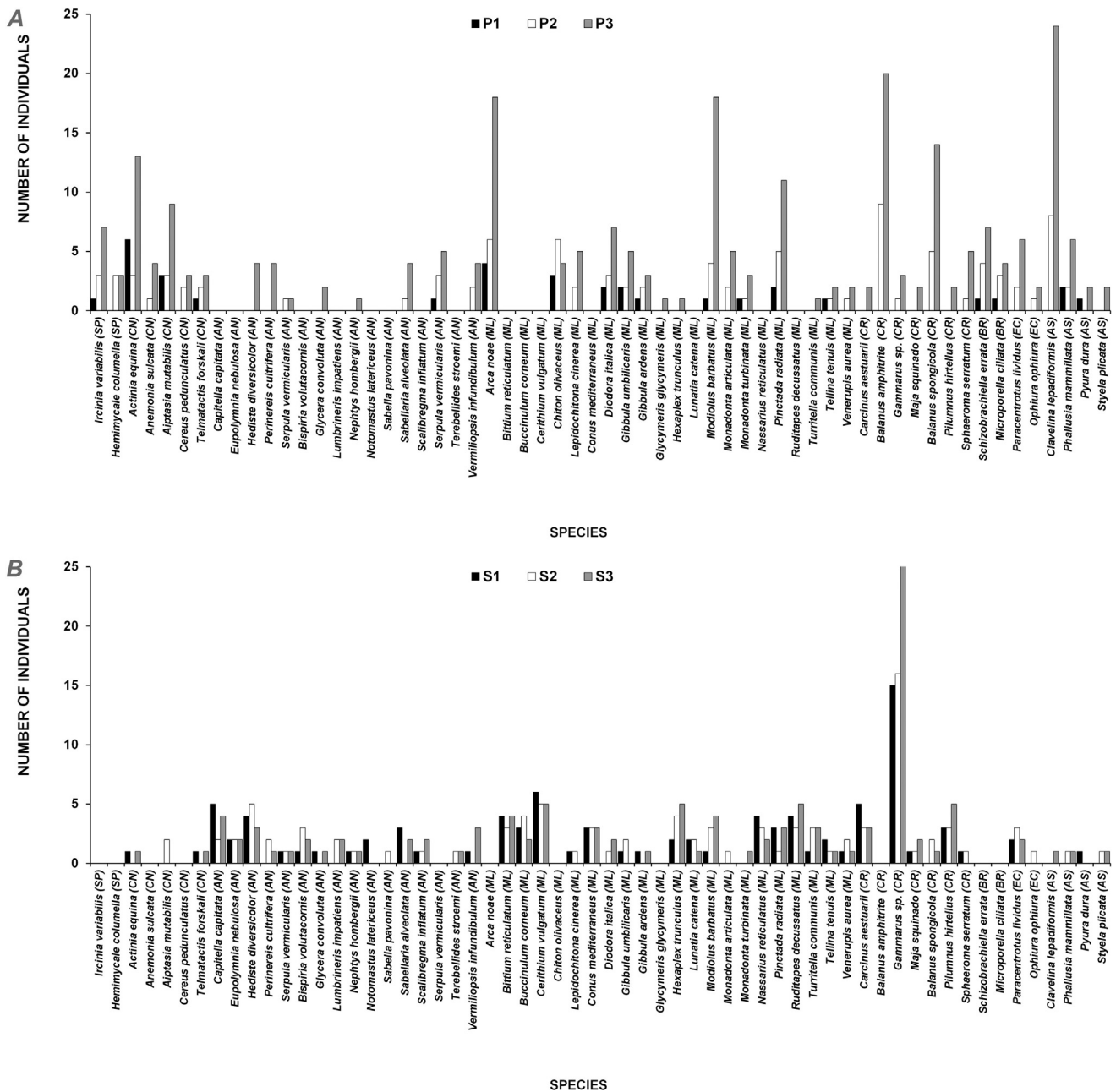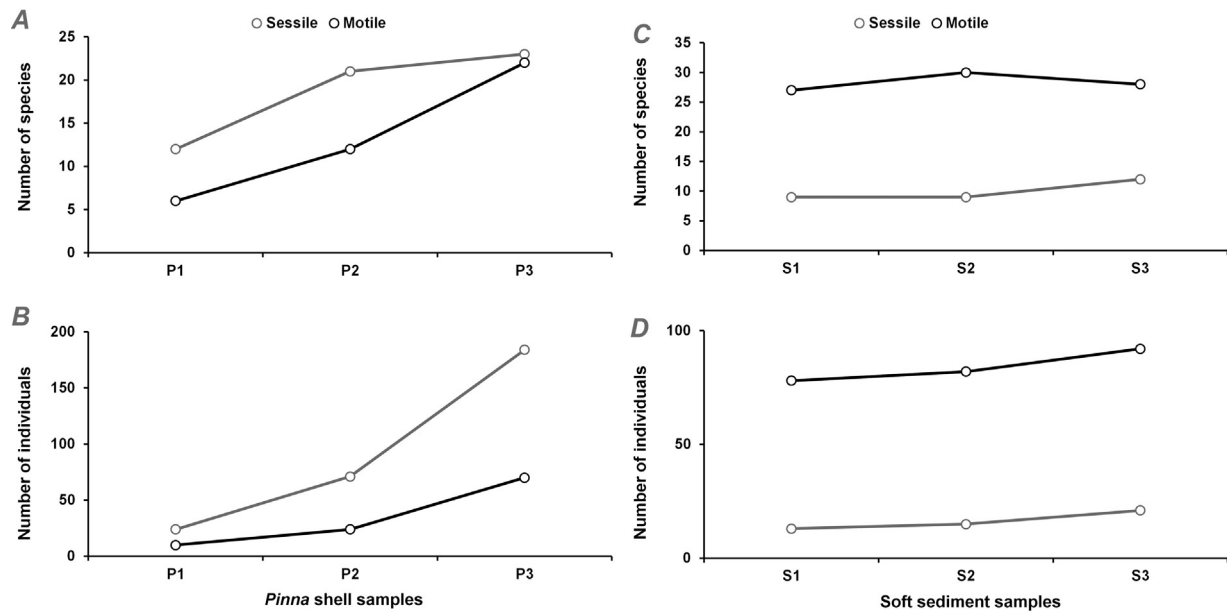
Figure 4 Number of individuals of the species recorded in the total *Pinna* shell (P1 to P3; A) and soft-sediment samples (S1 to S3; B) collected at each of the three sampling times. SP, sponge; CN, cnidarian; AN, annelid; ML, mollusc; CR, crustacean; BR, bryozoan; EC, echinoderm; AS, ascidian.

soft-sediment samples along the experimental period showed a general increasing pattern with *Pinna* samples from the beginning to the end of the experiment. With soft-sediment samples, the diversity indices varied slightly between separate samples with sampling times remaining almost constant along the experiment time (Table 2).

4. Discussion

It appeared from this experiment that the transplanted *P. nobilis* shells, through their physical presence, have enhanced the local biodiversity by offering an adequate substratum suitable for many hard-substrata species which do not exist normally in soft habitats. Unfortunately, it was not possible to conduct the experiment for longer period, but based on the results obtained herein, the effect of the empty pinnids was found to be positive on the local community, through increasing the abundance, number of species and diversity indices (Figs. 2, 3, 5 and Tables 1, 2). Many authors have also reported the positive ecological role of the pen shell (Addis et al., 2004; Corriero and Pronzato, 1987; Cosentino and Giacobbe, 2007; Rabaoui et al., 2009; Šiletić and Peharda, 2003; Zavodnik, 1963). However the high diversity indices noted in these latter works can be probably due to not only the physical presence of the living pinnids but also to

Figure 5 Number and abundance of sessile and motile species collected in the total *Pinna* shell (P1 to P3; A and B) and soft-sediment samples (S1 to S3; C and D) collected at each of the three sampling times.

their biological properties (i.e. providing local currents through the valve movement or providing food resources for some species; living pinnids are usually inhabited by some endobiontic decapod species) or even to the physical properties of the local habitat itself (nature of the substratum, vegetal cover, seagrass). Although transplanted empty *Pinna* shells were used, the experiment conducted herein enabled to show the engineering effect of the physical presence of *P. nobilis* in soft-benthic habitats and allowed to describe how the succession of colonizing the pinnid shells occurs which is an important issue in the concept of using pen shells in restoration plans of benthic soft-sediment habitats affected by anthropogenic factors.

Most of the common species that are encountered in association with empty shells transplanted, during the three sampling times, showed an increasing pattern of abundance, however only rare species showed a variable abundance (or even remain constant). These latter rare species were mainly represented by motile annelids, crustaceans and molluscs (Fig. 4A) and their rarefaction was responsible on the general (slight) decreasing pattern of Pielou's evenness index (*J*′) (Table 1). Munguia (2004) also noted, in his work, the rarefaction of many species along his experiment and he explained that the rarefaction of species may allow local diversity to be independent of the regional species pool size. It is worth noting that most of the species inhabiting empty

Table 1 Variations of species richness (*S*), total abundance of individuals (*N*), Shannon-Wiener diversity (*H*′) and Pielou's evenness index (*J*′) estimated in the separate and total *Pinna* shell (average ± standard deviation (SD)) collected during the three sampling times. P1-1 to P3-3 and P1 to P3 correspond to the separate and total empty shell samples collected in the first, second and third sampling times respectively.

Pinna shell samples	S	N	*J*′	*H*′
P1-1	10	10	1.00	2.30
P1-2	10	13	0.96	2.20
P1-3	10	11	0.99	2.27
P1 (average ± SD)	10.00 ± 00	11.33 ± 1.53	0.98 ± 0.02	2.26 ± 0.05
P2-1	21	29	0.96	2.94
P2-2	17	30	0.90	2.55
P2-3	24	36	0.94	3.00
P2 (average ± SD)	20.67 ± 3.51	31.67 ± 3.79	0.94 ± 0.03	2.83 ± 0.24
P3-1	37	80	0.92	3.31
P3-2	37	87	0.90	3.24
P3-3	38	87	0.92	3.36
P3 (average ± SD)	37.33 ± 0.58	84.67 ± 4.04	0.91 ± 0.01	3.30 ± 0.06

Table 2 Variations of species richness (*S*), total abundance of individuals (*N*), Shannon-Wiener diversity (*H'*) and Pielou's evenness index (*J'*) estimated in the separate and total soft-sediment samples (average ± standard deviation (SD)) collected during the three sampling times. S1-1 to S3-3 and S1 to S3 correspond to the separate and soft-sediment samples collected in the first, second and third sampling times respectively.

Soft-sediment samples	*S*	*N*	*J'*	*H'*
S1-1	22	42	0.94	2.91
S1-2	12	19	0.96	2.38
S1-3	18	30	0.92	2.67
S1 (average ± SD)	17.33 ± 5.03	30.33 ± 11.50	0.94 ± 0.02	2.65 ± 0.26
S2-1	16	32	0.94	2.60
S2-2	14	23	0.95	2.51
S2-3	22	42	0.95	2.93
S2 (average ± SD)	17.33 ± 4.16	32.33 ± 9.50	0.95 ± 0.01	2.68 ± 0.22
S3-1	17	30	0.96	2.72
S3-2	15	33	0.88	2.38
S3-3	25	50	0.90	2.89
S3 (average ± SD)	19.00 ± 5.29	37.66 ± 10.79	0.91 ± 0.04	2.66 ± 0.26

shells appeared either in the second or third sampling times (only few appeared once, either in the second or third sampling time), and most of these latter species are motile which belong to the groups of annelids, crustaceans and molluscs. This may be due to the fact that their late appearance may be due to the first appearance of sessile/colonial species, mainly sponges, and also algae. Besides, the number and abundance of sessile and motile species were found to increase similarly along the experiment time (Fig. 5A and B), which can be probably explained by the fact that motile species are dependent on some sessile species, in particular colonial species like sponges for example. They may use the sessile species to hide from predators or for nutrient reasons. Many other factors including the spawning periods, migration and availability of species in the surrounding areas may also explain the different appearance times of motile and sessile species. Within this context, Wahl (2009) reported that a successful settlement and growth of epibionts depend on the properties of the basibiont's surface and also on the presence of previous settlers. Railkin (2004) reported that some multicellular colonizers are able to produce fouling-modulating metabolites affecting thus the survival and the settlement behavior of conspecifics or other species. Besides some colonizers in particular barnacles and tube-building polychaetes can modify the three-dimensional structure of the substratum offering different scales of rugosity (Bourget and Harvey, 1998; Wahl, 2009; Wahl and Hoppe, 2002), which facilitates the settlement and recruitment of other organisms through the structures they provide (Thieltges and Buschbaum, 2007; Wahl, 2009). Within this context, some authors showed that mussel larvae have a preference of settling on filamentous structure, enhanced probably by algal exudates and certain biofilms (Davis and Moreno, 1995; Dobretsov, 1999). There are numerous examples of settlement inducers of epibionts (Dreanno et al., 2006; Hadfield and Paul, 2001).

Although many motile species were encountered in the associated fauna of empty *Pinna* shells and can be common

with the fauna of soft sediments surrounding the pinnids, it is evident that the pen shell *P. nobilis* has an important contribution to the richness of the wider ecosystem. However, it is worth noting that the experiment conducted herein used dead pinnids i.e. empty shells and it is known that living basibionts can interact with their epibionts through the possible trophic resources provided for some epibionts and the local currents generated by the valves movements of *P. nobilis* that can enhance the feeding activity of some associated species. Hence, the results of a similar experiment using living pinnids may be different to those found herein. In fact, many beneficial effects can be provided by the basibiont to the epibionts including the exuded nutrients from the host, favorable hydrodynamics and irradiation and associational resistance (Wahl, 1989, 1997, 2009). For *P. nobilis*, the shell space was reported to enhance the settlement of a typical epibenthic community of hard substrata (Rabaoui et al., 2009; Zavodnik, 1963).

Based on the results described herein, the empty shells of *P. nobilis* were found to affect the diversity and structure of surrounding habitat community. The role of pen shells in affecting the benthic diversity in the habitats where they live was also reported in other areas (Cummings et al., 1998; Kay and Keough, 1981; Keough, 1984; Munguia, 2004; Srivastava et al., 2004; Warwick et al., 1997). Within this context, the congeneric species *Atrina zelandica* was reported to play an important role in coastal ecosystems by enriching the surrounding seafloor sediments in carbon and nitrogen, and consequently the species number and abundance of associated macrofaunal assemblages (Norkko et al., 2001). The latter species were found to be a habitat facilitator, especially when the suspended sediment concentration exerted by the surrounding habitat is low, and consequently it has an effect on the diversity of benthic community (Norkko et al., 2006). The experiment developed in this study does not allow defining the facilitation mechanisms that this mollusc (alive) may exert in soft-sediment habitats to affect the diversity and structure of surrounding

habitat community and hence deeper experimental designs are worth adopting in further studies to answer this question.

Acknowledgments

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