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Artificial marine micro-reserves as a new ecosystem-based management tool for marine conservation: The case of *Patella ferruginea* (Gastropoda, Patellidae), one of the most endangered marine invertebrates of the Mediterranean

E. Ostalé-Valriberas [a,*], J. Sempere-Valverde [a], A. Pavón-Paneque [a], S. Coppa [b], F. Espinosa [a], J. C. García-Gómez [a]

[a] *Laboratorio de Biología Marina de la Universidad de Sevilla (LBMUS)/Área de Investigación I+D+i del Acuario de Sevilla/Estación de Biología Marina del Estrecho (Ceuta), Seville, Spain*

[b] *Consiglio Nazionale delle Ricerche, Istituto per lo studio degli impatti Antropici e Sostenibilità in ambiente marino (CNR-IAS), Oristano, Italy*

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ABSTRACT

During the Anthropocene, species are becoming extinct at unprecedented rates, a trend that will be difficult to reverse, even if we ignore the possibility of a considerable extinction debt. Among the different factors that affect the natural environment, fragmentation of ecosystems by urbanization processes can cause a reduction in species population sizes, thus enhancing their risk of extinction. Nevertheless, some species can maintain stable populations in these urbanized ecosystems. This is the case of the intertidal mollusc *Patella ferruginea* (Gmelin, 1791), a broadcast spawner, and a sequential protandrous hermaphrodite limpet, whose populations have been historically decimated due to human harvesting. In this study, we analyse the benefits of a new marine conservation tool called ̈Artificial Marine Micro-Reserves ̈ (AMMR) in *P. ferruginea*, one of the most endangered marine invertebrates of the Mediterranean Sea. The results showed that accessibility is the main factor concerning the conservation status of this species, with no-entry areas where populations achieve balanced sex-ratios and high reproductive outputs. The present study was conducted in Ceuta (North Africa, Gibraltar Area), and among its varying results, it shows that in the same body of water inside the port, the proportion of females of *P. ferruginea* in the area without accessibility (high protection) was 4.68 and 43.54 times higher than in the medium and low accessibility (non-protected areas), respectively. Therefore, the effective protection of these artificial areas has a positive effect on population size structures, as the female's percentage in the population is crucial for fostering the creation of genetic bridges for the recolonization of natural habitats. Furthermore, a potential 'umbrella effect' can be derived from the implementation of the proposed AMMR in other protected species, bioindicators, or commercially exploited species detected in artificial structures. In this sense, the creation of artificial marine microreserve networks (AMMRNs) in coastal defense structures is in line with the interdisciplinary approach of Ecosystem-Based Management (EMB), given that this methodology balances ecological, social and governmental principles for achieving humane sustainable development.

1. Introduction

Urban sprawl is expanding into the marine realm with the construction of artificial structures. In areas of Europe, the United States or North America, Australia, and Asia, more than 50% of the shoreline has been altered by hard engineering (man-made coastal defenses) such as breakwaters, ripraps, seawalls, pontoons, groins, etc. to protect against erosion and wave action and for recreational purposes [16,43]. The human impact associated with these structures has been reported to affect biological processes on all spatial and temporal scales [3]. These impacts have negative effects on marine ecosystems in general on local (1–10 km) and regional scales (over hundreds of kilometers) [10,22,44]. Among them, fragmentation of natural habitats by urbanization processes can cause a reduction in species population size and connectivity,

* Corresponding author.
E-mail address: enriqueostalevalriberas@gmail.com (E. Ostalé-Valriberas).

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and thus increase their risk of extinction in the face of certain permanent disturbances [12,1]. In order to handle these impacts, the field of coastal ecological engineering aims to integrate ecology, economy, and society's need to design artificial marine structures that maintain the greatest biodiversity in relation to the natural environment [7]. Essentially, it represents the incorporation of ecological considerations into the design of artificial marine structures [32]. Such an approach is urgently needed, given that anthropic demands on coastlines are expected to increase in the future [3].

Recent studies have shown that organisms that provide important ecosystem services and even protected species can benefit from artificial structures [33,88]. Some of these protected species have substantial populations inhabiting artificial habitats. This is the case of the endangered limpet *Patella ferruginea* (Gmelin, 1791), whose densest populations exist in the breakwaters of the Strait of Gibraltar and Alboran Sea harbors [27,46,49,48]. In addition to this species, many sessile or vagile organisms of conservational importance can also be found in artificial structures (see Table 1).

In the case of *P. ferruginea*, the presence of a strictly protected species on artificial structures has led to a problem for many administrations within the western Mediterranean, as these structures require frequent restoration, modification, or reconstruction [29,49].

The concept of micro-reserve has been developed in eastern Spain for rare endemic plants with small distribution zones, for which the usual conservation areas such as parks or reserves are unsuitable [58,60]. Microreserves are a very useful tool for the conservation of reduced populations of nonmobile organisms such as plants, and should be equally applicable to coastal sessile biota, which are very similar to land plants in terms of dispersion and substrate occupation [40,59]. The terrestrial micro-reserves of the Community of Valencia are located in natural areas, (some of them moderately human-disturbed) but not in artificial places (Figura de protección de especies silvestres denominada microrreserva vegetal, 1994; [17]). In this regard, García-Gómez et al. [40,41] proposed a new form of environmental protection, the 'Artificial Marine Micro-Reserve' (AMMR), defined as an artificial coastal construction that, by mutual agreement between owners and authorities, is protected due to the environmental value of species or ecosystems it hosts.

According to García-Gómez et al. [40], the criteria to be met by a marine artificial habitat for its protection under an AMMR are the following:

1. It has hosted at least one endangered species, whose individuals are settled naturally. In addition to this, both the recruitment and growth (adult) of these individuals should be present to generate a reproductively viable population. The AMMR could be applied to an entire coastal structure or parts thereof (for example, a section of breakwater), but always presents different age classes and reproductive individuals. The creation of AMMRs should encourage a network to promote genetic bridges.

2. The proposal for the creation of an AMMR must be limited to already build installations, where endangered species have already naturally settled. The positive results of AMMRs must not be used as a reasoning for building a new artificial structure.

3. An AMMR cannot be assigned without the agreement of its owner, public or private. The agreement can be temporary and must be compatible with the normal use of the installation. The AMMR must be permanently armoured with fences and video monitoring, which is not very expensive, since harbors are already guarded by their respective administration.

4. AMMRs must be assigned to a coastline with a good environmental quality of marine waters. In the case it is located inside a port, it ought to be implemented to areas that fall within the quality requirements of the Water Framework Directive 2000/60/EC (hereafter WFD; [21]) and must include an emergency contingency plan for oil spills (i.e. sea booms, oil skimmer, etc.) or different environmental impacts.

5. The statement of AMMRs should not pose a problem in itself. In

Table 1
Summary of organisms of conservation importance associated with artificial structures.

Group, species	Legislation (Nº annexe)	Source
Algae		
Cystoseira amentacea	Bern Convention (I); Barcelona Convention (II)	Susini et al.,[90]
Cystoseira barbata	Barcelona Convention (II)	Perkol-Finkel et al.,[74];[31]; Ferrario et al.,[30]
Lithophyllum byssoides	Barcelona Convention (II)	García-Gómez et al.,[41]; Present study
Gymnogongrus crenulatus	Barcelona Convention (II)	Present study
Porifera		
Spongia agaricina	Bern Convention (III)	García-Gómez et al.,[41]
Spongia offinalis	Bern Convention (III)	de Voogd[95]
Tethya aurantium/ Tethya sp. *plur.*	Barcelona Convention (II)	García-Gómez et al.,[41]
Cnidaria		
Astroides calycularis	Bern Convention (II), Barcelona Convention (II)	García-Gómez et al.,[41]; present study
Lophelia pertusa	Habitats; Barcelona Convention (II)	Gass & Roberts[42]
Mollusca		
Charonia lampas	Bern Convention (II); Barcelona Convention (II)	García-Gómez et al.,[41]; present study
Cymbula safiana	Bern Convention (II); Barcelona Convention (II)	Rivera–Ingraham et al.,[80]; García-Gómez et al.,[41]; present study
Dendropoma cristatum	Bern Convention (II); Barcelona Convention (II)	García-Gómez et al.,[41]; present study
Lithophaga lithophaga	CITES, Bern Convention (II); Barcelona Convention (II)	Devescovi & IVEŠA[19]; García-Gómez et al.,[41]
Luria lurida	Bern Convention (II); Barcelona Convention (II)	García-Gómez et al.,[41]
Patella ferruginea	Habitats; Bern Convention (II); Barcelona Convention (II)	Guerra-Garcıa et al.,[48]; Espinosa et al.,[25,28,27]; García-Gómez et al.,[41]; Martins et al.,[67]; present study
Pinna nobilis	Habitats; Barcelona Convention (II)	García-Gómez et al.,[41]
Pinna rudis	Bern Convention (II); Barcelona Convention (II)	García-Gómez et al.,[41]; present study
Crustacea		
Homarus gammarus	Bern Convention (III)	Langhamer & Wilhelmsson[61]; García-Gómez et al.,[41]
Maja squinado	Bern Convention (III)	García-Gómez et al.,[41]; present study
Palinurus elephas	IUCN (VU), Bern Convention (III)	García-Gómez et al.,[41]
Megabalanus azoricus	OSPAR	Southward[89]
Echinodermata		
Centrostephanus longispinus	Bern Convention (II), Barcelona Convention (II)	García-Gómez et al.,[41]; present study
Echinus esculentus	IUCN (NT)	Moore[68]
Ophidiaster ophidianus	Bern Convention (II)	García-Gómez et al.,[41]; present study
Paracentrotus lividus	Bern Convention (III)	García-Gómez et al.,[41]; present study

Note: Species were selected if protected under international and/or national legislation. CITES = Convention on International Trade in Endangered Species of Wild Fauna and Flora; IUCN = International Union for Conservation of Nature Red List; NT = Near Threatened; VU = Vulnerable; Bern Convention on the conservation of European wildlife and natural habitats; Barcelona Convention for the Protection of the Mediterranean Sea Against Pollution; Habitats = EU

Habitats Directive on the conservation of natural habitats and of wild fauna and flora; OSPAR = Oslo and Paris Conventions.

contrast, it would be an opportunity to protect and monitor endangered and sensitive species together with the competent environmental authority. It can also contribute to control and improve the quality of coastal water effectively and play a significant role in achieving the objectives of the WFD [35,36]. In addition, it should be managed for integration into society and landscape. It must include a management plan that promotes interest in the marine environment and its biota in the local population, as well as encourages environmental education.

The creation of artificial marine microreserve networks (AMMRNs), as a new conservation tool, is in line with the interdisciplinary approach of Ecosystem-Based Management (EMB). This methodology balances ecological, social and governmental principles on appropriate temporal and spatial scales in a distinct geographical area to achieve the use of sustainable resources (Long, 2015), hence the AMMR would be a conservation tool for the port sector for a possible action guide. In addition, AMMRs fit conceptually within new management approaches such as 'novel ecosystems' (Hobbs et al., [53]) or Locally Managed Marine Area ([54,56]) which have already been developed.

Therefore, the AMMR is a new form of protection that has been proposed but not yet implemented. The present study focuses on providing empirical data on its viability. Consequently, the objectives of the present study are (i) to determine whether the artificial coastal defense structures of Ceuta (Strait of Gibraltar) meet the requisites mentioned above for implementing an AMMR and to identify which species could benefit from such protection, and (ii) what effect is expected on the population of the endangered mollusc *Patella ferruginea* as a model species benefitting from this novel approach.

2. Materials and methods

2.1. Study area

The Strait of Gibraltar is one of the marine areas with the greatest biological diversity of the European coastline, hosting typical species from the temperate and subtropical regions of the European and African Atlantic and the Mediterranean, as well as numerous endemic species [14,37]. On the other hand, it is also an area of confluence of many

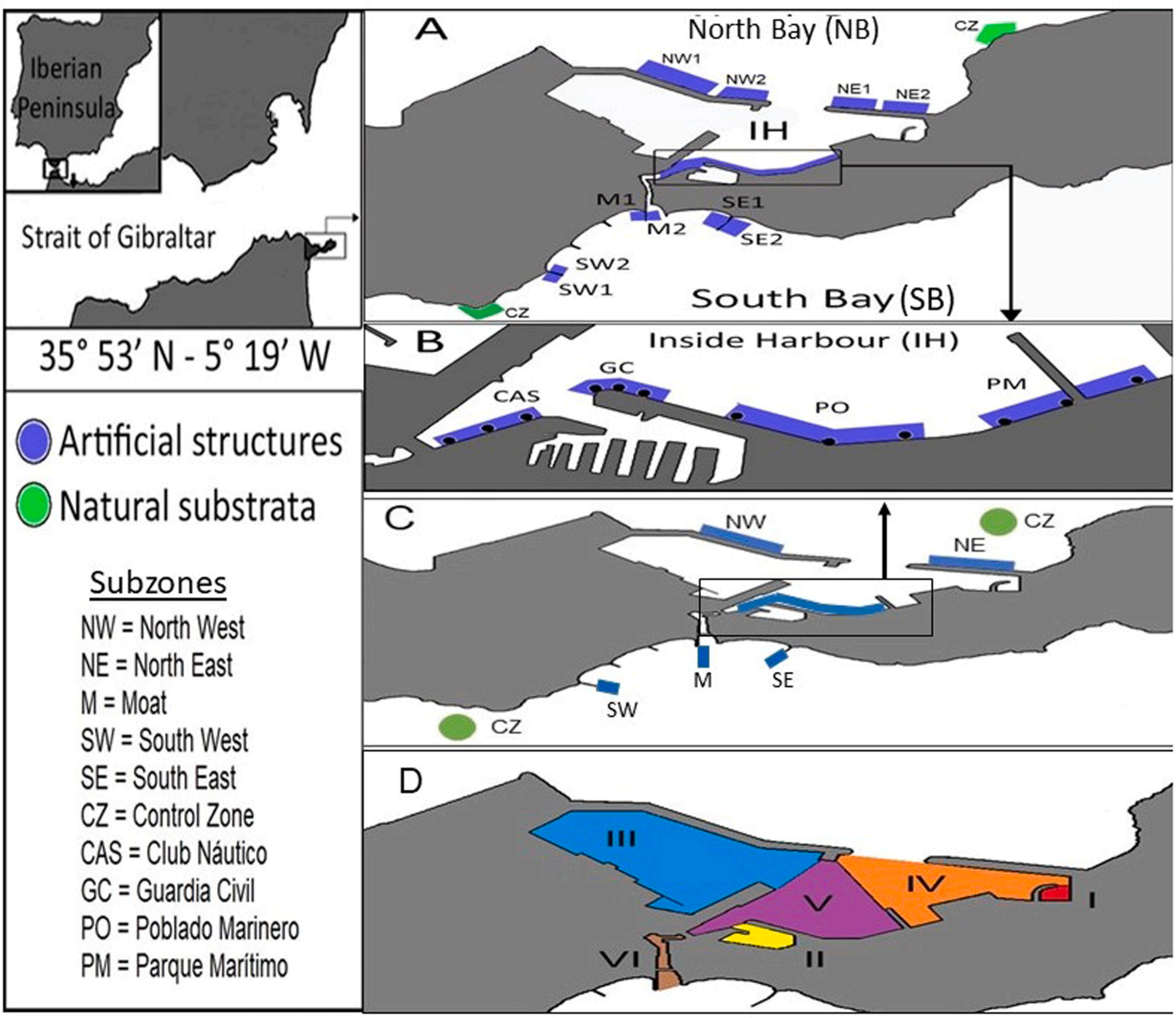

Fig. 1. Study area: A: intertidal sampling zones on Ceuta's riprap structures (North Bay, South Bay, Inside Harbor) and nearby natural substrata (Natural Zone); B: within the zone Inside Harbor, four subzones were considered to the intertidal and subtidal study; C: subtidal sampling subzones; D: different water bodies inside the harbor of Ceuta.
Figure modified from the work of Sánchez-Badorrey & García Anguita [84].

commercial routes, being one of the most transited areas worldwide and shelter for important ports (Algeciras, Gibraltar and Tangier Med), which makes it a habitat with a high risk of disturbances, impacts or eventual environmental disasters ([76,79]). All of these features make the Gibraltar Strait an ideal area for conducting studies on the environmental management of endangered species under anthropogenic pressure and their habitat.

The port of Ceuta, a Spanish city in North Africa, has an unusual structure; it is located between two bays and connected to both by the Royal Channel (Fig. 1A). This channel increases water movement and exchange within the structure (residence time), when compared to other conventional harbors, maintaining moderate oxygen levels in the water and sediment ([47]; Elena Sánchez et al., in press 2021). Consequently, its community is rich in species, as oxygen is not a limiting factor, but pollution levels are relatively high and species' composition differs compared to unpolluted areas [23,47].

2.2. Community study

According to available published research, the most endangered marine species inhabiting the artificial structures of Ceuta is the mollusc *Patella ferruginea,* so the sampling methodology was designed to map its population [27,40,41,83]. Furthermore, the intertidal and subtidal assemblages of the different artificial structures and the near natural substrate were studied to complete knowledge of the habitat.

2.2.1. Intertidal study

The intertidal data were collected in summer 2013. Without prior exploration, in artificial structures (dolomitic riprap) and natural adjacent areas, 4 different zones were set up (NB, IH, SB, CZ) with subzones (N = 12) located within them (Fig. 1). For each subzone, three 10-m-long transects were selected, at the same distance, using Google Earth©. The intertidal benthic community was sampled using a photographic methodology at low midlittoral (+0.25 m from zero tidal level) and high midlittoral (+0.75 m from zero tidal level). Within each transect, five 25 × 25 cm quadrats were photographed on vertical surfaces and five on horizontal surfaces at each level (2 heights × 2 orientations × 5 replicates = 20 images per transect). Percent species coverage was obtained by superimposing a digital 10 × 10 grid over each image with Adobe PhotoShop CS6© software and counting species presence/absence in each subquadrat. This system aided in estimation accuracy [18,20] and optimized the working time [34,63].

In addition to the photographic quantitative sampling, the presence of any species of interest in all intertidal zones was semiquantitatively recorded for each transect using a 0–3 scale: 0) Absence, (1) the species was found at least in one meter of the transect, (2) the species was found in 2–7 m of the transect, and (3) the species was found in more than 7 m of the transect. The species of interest were considered according to the legal status and [41]. These were classified into four categories: somehow protected (Pr), bioindicators (Bi), invasive or nonindigenous species (In), and regulated harvesting species (Re). In addition, in each transect the number of *P. ferruginea* specimens were counted.

2.2.2. Subtidal study

In summer 2014, a subtidal study was conducted in the same zones as the intertidal study (Fig. 1). A subtidal benthic community was sampled at 2 and 4 m depths by means of a photographic methodology. The photographs were taken from vertical illuminated surfaces using an Olympus Tough Tg-3 camera and INON S-2000 flash. In each subzone, three transects of 10 m in length were sampled with five photographs (25 × 25 cm quadrats) taken by transect. The percentage of species coverage was obtained similarly to the intertidal study.

In addition to the photographic samplings, the different microhabitats (horizontal surface, caves and rock ledge) of the substrate were explored in search of any species of interest within the same transects. Inspection was done from the surface to a six-meter depth. In the North Bay, due to its greater depth, a six-meter range above the bottom was also explored (located at 20 m).

2.2.3. Patella ferruginea census

After conducting the intertidal community study in 2013, in summer 2014 the artificial structures inside Ceuta s harbor were intensively sampled (see Fig. 1-A-B).

In this extensive sampling, the population structure of *P. ferruginea* was compared among four different subzones: Club Náutico (CAS), Guardia Civil (GC), Poblado Marinero (PO) and Parque Marítimo (PM). Twelve 10 m transects were allocated per subzone, encompassing 480 m of the 1,130 m of total riprap shore length. The different transects were systematically located on the riprap shoreline in each subzone. The *P. ferruginea* individuals found were measured with caliper to the nearest mm by taking records on shell length (widest diameter of the shell), width (narrowest diameter of the shell) and height (elevation of the shell from the base to the apex) [48,73]. Therefore, it was possible to obtain the mean volume of *P. ferruginea* using the equation for a cone (V = (π·r2·h)/3). To estimate the total number of individuals inside the harbor, the results were extrapolated from the total length of the riprap.

Inside the harbor, there were three areas with different levels of accessibility to the riprap shoreline: accessible (low protection or non-protected areas: subzone PO), fenced (medium protection: subzones PM and CAS) and under custody (high protection or no entry/no take area: subzone GC). To evaluate the effect of human pressure on *P. ferruginea,* we selected ten transects at each level of accessibility in the same water body within the harbor (water body V in Fig. 1-C). The different water bodies within the Ceuta harbor were defined by [84] and [85].

Raw data on fecundity and sex ratio within the harbor were used to estimate proportions of males and females and the fecundity (number of oocytes produced by spawning season) of the female population ([25]a; [81]).

2.3. Statistical analyses

A Principal Coordinate Ordination (PCO) was carried out to visualize the contribution of sample distribution and variables (species coverage) to intertidal and subtidal community structure (Anderson and Walsh, [4]).

All statistical analyses were carried out by testing a nested design with three factors: Zone (fixed), Subzone (fixed, nested in "Zone") and Site (random, nested in "Subzone"). For both intertidal and subtidal studies, percent benthic species coverage data were square root transformed and used to calculate Bray-Curtis similarity matrixes. These matrixes were analyzed using multivariate PERMANOVA. Additionally, the intertidal and subtidal species' richness (S) and Shannon diversity (H′) were individually used to calculate Euclidean distance matrixes, which were tested using univariate PERMANOVA. Finally, univariate PERMANOVAs were carried out in the same way for the number and percentage coverage of *Patella ferruginea* in the intertidal region. PERMANOVAs were performed using 9999 permutations. A nonparametric approach was selected for univariate tests because of the data being unbalanced (see Fig. 1). To this end, PERMDISP analyses were also carried out for each PERMANOVA analysis to test possible heterocedasticity and Behrens–Fisher distribution problems [5]. When PERMANOVA and/or PERMDISP detected significant differences, the source of the difference was identified using pairwise comparisons.

Dealing with the study focused on the *P. ferruginea* subpopulation inside the harbor, two one-way ANOVAs were carried out to test differences in adult density (ind/m) and number of oocytes (according to the % of female) produced in the different subzones of the harbor (four levels: CAS, GC, PO and PM; fixed). Previously, data were tested for homocedasticity with Levene's test. Post-hoc differences were tested using the Student-Newman-Keuls (SNK) test. ANOVA is robust to non-normality (Underwood, [92]) and therefore deviations from normality were not considered a reason to reject the parametric procedure. To evaluate the effect of accessibility to the shoreline on a population of *P. ferruginea,* one-way ANOVAs were carried out to test differences in mean size (mm), mean adult size (mm), mean volume (mm^3), mean adult volume (mm^3), overall density (ind/m), adult density (ind/m), percentage of recruitment (%) and number of oocytes (according to the % of female) produced at different levels of accessibility to the riprap shoreline already explained (accessible: Low, fenced: Medium and under custody: High; fixed). Data sets were first examined for heterogeneity of variance using the Levene test after square root data transformation. Differences among means were then examined using the Student-Newman-Keuls (SNK) test.

PERMANOVA analyses were performed using the PRIMER v.6 +PERMANOVA package [13,5]. ANOVA analyses were performed using IBM SPSS 22 [72].

3. Results

3.1. Subtidal study

In the intertidal macrobenthic community, 22 species of flora and 24 benthic vagile and sessile invertebrates were identified. Among these, six species were protected, five were bioindicator species, one was an invasive or nonindigenous species, and two were species for which harvesting was regulated (supplementary material Table 1).

The structure of the intertidal community was different between zones (Table 2 and Fig. 2). The species richness (S) and Shannon diversity index (H′) were higher in the Control Zone (CZ) and North Bay (NB) than inside the Harbor (IH) and South Bay (SB) (Table 2 and Fig. 3).

The coverage results for the endangered species *P. ferruginea* showed that the subpopulation within IH and NB was significantly higher than the other zones (Table 2 and Fig. 3). The species census shows that the subpopulation of IH was significantly higher (Table 2) and the subzone with a larger number of individuals was Parque Marítimo (Fig. 3).

Regarding bioindicators, it should be noted that *Lithophyllum byssoides* showed a higher percentage coverage on the artificial substrate in the NB while *Astroides calycularis* and *Actinia equina* were more abundant on the artificial substrate of the SB. *Fucus spiralis* was detected only on the natural substrate (CZ; Fig. 4).

3.2. Subtidal study

The macrobenthic community of the subtidal was constituted by forty flora species and seventy-six fauna species. Among the flora, one protected species, six bioindicators, and three exotic species were detected. Among the fauna, five protected species, five bioindicators and one regulated harvesting species were detected (supplementary material Table 2). Since 2016, in the subtidal of the biographical area studied, a new invasive macroalgae *Rugulopteryx okamurae* [38,39,87] has been settled.

The structure of the subtidal community was different among the four zones, although some zones were similar to each other (Table 3 and Fig. 5). At the univariate level, the species richness (S) was higher in the Control Zone (CZ) and North Bay (NB) than in Inside Harbor (IH) and South Bay (SB) (Table 3). Shannon Diversity Index (H′) was lower in South Bay (SB) than in the other zones.

3.3. Patella ferruginea census

In the specific study of the *P. ferruginea* subpopulation within the harbor, 7,204 individuals were recorded (mean overall density 15 ± 12 ind/m and adult density 9.5 ± 6.77 ind/m). In this zone, the total number of estimated individuals was 16,914.

The adult density (ind/m) was significantly higher in Poblado Marinero and Parque Marítimo subzones ($p < 0.01$; $F = 14{,}93$; SNK test: PO = PM > GC = CAS (Fig. 6).

The number of oocytes, obtained according to the percentage of female individuals, was significantly higher in the Parque Marítimo ($p < 0.05$; F= 4,62; SNK test: PM > (GC=CAS) > PO) (Fig. 6).

The results of the different levels of accessibility to the riprap shoreline showed that the mean volume of *P. ferruginea* was significantly higher in the High than in the Low protection zone (Table 4).

Compared to the low and medium protection zone, the high protection zone showed large individuals in their size frequency distribution, with the subzone presenting the highest number of oocytes (Table 4 and Fig. 7).

The percentage of female individuals in the high protected zone were 4.68 times higher than in the medium protected zone and 43.54 times higher than in the low protected zone (Table 5).

4. Discussion

The results have shown that the zones IH and NB of the artificial coastal defense structures of Ceuta (Strait of Gibraltar) comply with the criteria of the conservation tool "Artificial Marine Micro-Reserves" (AMMRs) [40,41] because there were several intertidal and subtidal protected species settled naturally in these artificial structures. Among

Table 2
Results of the multivariate and univariate PERMANOVA (intertidal study); NB: North Bay; SB: South Bay; IH: inside harbor and CZ: control zone.

All species	Source of variation	Df	MS	Pseudo-F	P (perm)	Pairwise comparisons
	Zone	3	34,030	6.72	**< 0.001**	NB, IH, SB, CZ
	Subzone (zone)	12	5291.2	3.18	**< 0.001**	
	Site (subzone (zone)	36	1562.3	2.40	**< 0.001**	
	Residual	188	650.4			
Species Richness (S)	Zone	3	404.2	11.80	**< 0.001**	CZ, NB, SB, IH
	Subzone (zone)	12	35.86	2.09	**< 0.05**	
	Site (subzone(zone))	36	16.11	4.20	**< 0.001**	
	Residual	188	3.83			
Shannon Diversity (H′)	Zone	3	4.27	7.11	**< 0.01**	CZ, NB, SB, IH
	Subzone (zone)	12	0.63	2.19	**< 0.05**	
	Site (subzone(zone))	36	0.27	4.16	**< 0.001**	
	Residual	188	6.4991E-2			
Patella ferruginea (% cover)	Zone	3	100.02	4.31	**< 0.05**	NB, IH, CZ, SB
	Subzone (zone)	12	24.27	3.84	**< 0.001**	
	Site (subzone(zone))	36	5.95	1.87	**< 0.05**	
	Residual	188	3.18			
Patella ferruginea (N ind.)	Zone	3	11,995	4.88	**< 0.05**	IH, NB, SB, CZ
	Subzone (zone)	12	2466.1	4.58	**< 0.001**	
	Residual	32	538.62			

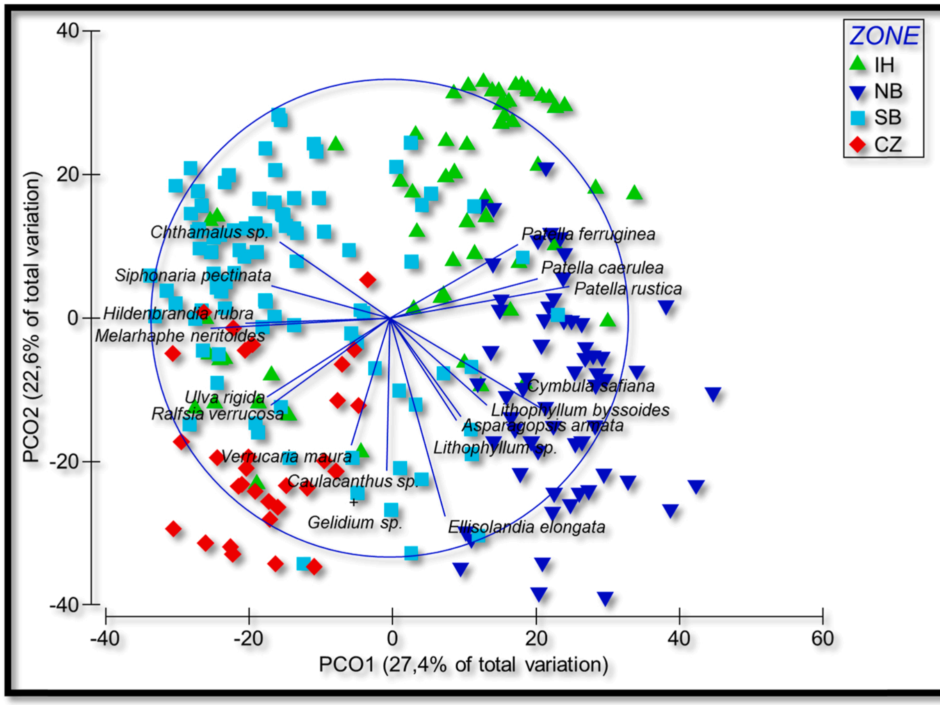

Fig. 2. Principal coordinates analysis (PCO) for the intertidal community structure (all species) from the different studied zones (variables shown have a correlation higher than 0.5 with any of the PCO axis).

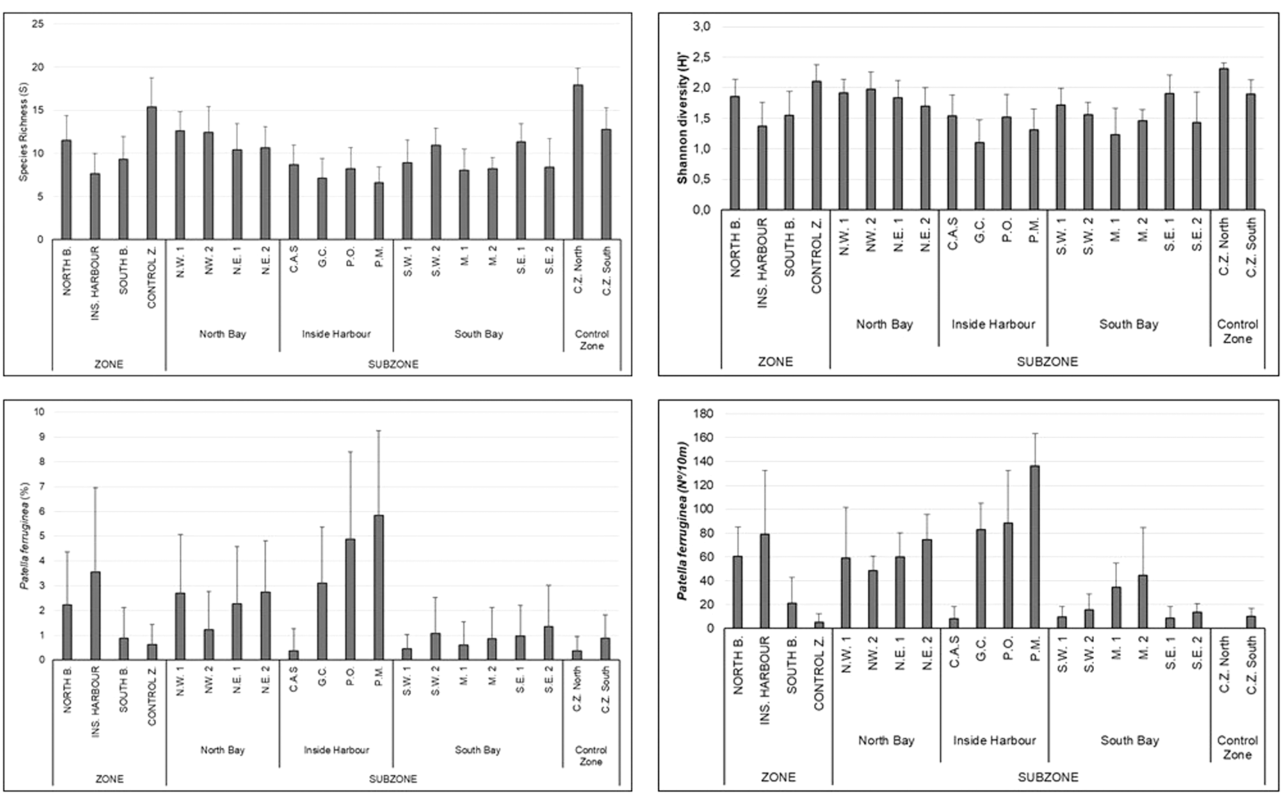

Fig. 3. Results of species richness (S), Shannon diversity (H′), coverage and census of *Patella ferruginea* in the different zones and subzones of the study (mean and standard deviation).

them, the only one with a high risk of extinction was *P. ferruginea*, which is the most endangered marine invertebrate, together with *Pinna nobilis* within the Western Mediterranean basin ([77]; Vázquez-Luis et al., [93]; [62]).

Although this species has been detected in all the studied zones, the highest coverage and individual numbers were found in the artificial substrata rather than in the nearby natural control zones. In particular, the abundance of species was higher in the zones inside the harbor and

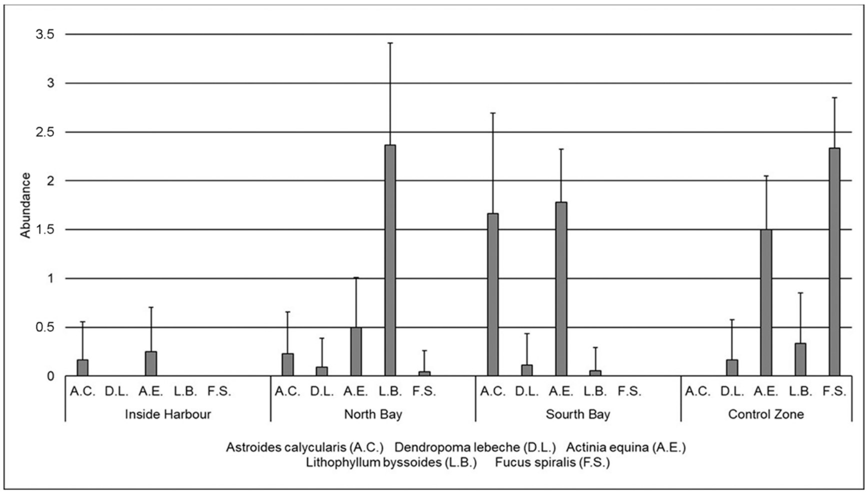

Fig. 4. Results of the semi-quantitative sampling (mean and standard deviation). Species of interest: somewhat protected (Pr) and bioindicator species (Bi). *A. calycularis*: A.C. (Pr and Bi), *D. lebeche*: D.L. (Pr and Bi), *A. equina*: A.E. (Bi), *L. byssoides*: L.B. (Pr and Bi) and *F. spiralis*: F.S. (Bi).

Table 3
Results of multivariate and univariate PERMANOVA (subtidal study). NB: North Bay; SB: South Bay; IH: inside harbor and CZ: control zone.

All species (% cover.)	Source of variation	Df	MS	Pseudo-F	P (perm)	Pairwise comparisons
	Zone	3	38,607	2.86	**< 0.001**	IH, NB, CZ, SB
	Subzone (zone)	7	13,473	2.32	**< 0.001**	
	Site (subzone (zone))	22	5804.9	2.89	**< 0.001**	
	Residual	297	2003.4			
Species Richness (S)	Zone	3	126.54	27.95	**< 0.001**	NB, CZ, IH, SB
	Subzone (zone)	7	4.52	0.63	0.72	
	Site (subzone(zone))	22	7.18	1.28	0.16	
	Residual	297	5.58			
Shannon Diversity (H′)	Zone	3	1.13	8.42	**< 0.05**	IH, NB, CZ, BS
	Subzone (zone)	7	0.13	0.64	0.71	
	Site (subzone(zone))	22	0.21	1.64	**< 0.05**	
	Residual	297	0.12			

North Bay. As required by AMMR criteria, both zones presented a well-structured population, with different age classes and reproductive individuals, which indicates the existence of a self-maintaining population. In this sense, the establishment of a network of such source populations would promote genetic bridges [27,40,41]. Within these zones, the subzones Parque Marítimo (PM) and Dique Levante 2 (NE1) showed the highest mean density (Fig. 3). The implementation of an AMMR on these ripraps located in the North Bay and inside the harbor could enhance the larval supply in Ceuta, which would help to preserve the species and recolonize the local natural substrata. Nevertheless, larval supply must be known to estimate realized connectivity whereas variations in key factors of population demographics such as size structure and egg production should be monitored and reported [71].

In this regard, the positive effects of protection, in the sense of AMMR, have been demonstrated and evaluated in the present study for *P. ferruginea* by comparing three different levels of protection within the same oceanographic conditions. In particular, the impact of human pressure on the female fraction was defined: the percentage of female individuals in the high protected zone was 4.68 times higher than in the medium protected zone and 43.54 times higher than in the zone without protection. Other studies have also shown the anthropogenic influence on size distribution and, subsequently, on the reproductive output of *P. ferruginea* [26] and other limpet species [9]. Moreover, *P. ferruginea* is a sequential protandrous hermaphrodite species, since all individuals first occurred as males and their sex change depended on population density and size [25,78]. According to the sex ratio in the studied area, there was a higher number of females within the protected ripraps and this caused the population to produce a considerably larger number of oocytes in these protected areas. Similar results had previously been advocated by Espinosa et al. [26] in the artificial substrata of this area or by Coppa et al. [15] in the natural substrata of the MPA 'Penisola del Sinis-Isola di Mal di Ventre' from Sardinia. In addition, the results showed that the sex change to female in the zone is between the class size 6–7 cm [25,78]. When it comes to management conservation, the fact that adult density (individuals bigger than 3 cm) has not been correlated with the percentage of females and, therefore, with the number of oocytes should be taken into account (see Fig. 5, Poblado Marinero vs. Parque Marítimo).

On the other hand, although a population of origin can be defined by considering the percentage of females and not by the single parameter of adult density, Hixon et al., [52] highlighted that egg production is not only related to the number of individuals, but also to the relative contribution of the different size classes to the total number of hatchlings. In this sense, although a balanced sex ratio in diecious species is ideal, the case at hand (sequential protandrous hermaphroditism and external fertilization) is different since the number of gametes that can be expelled by females must be taken into consideration according to their size (a single 8-cm female generates a similar number of gametes as ten 6-cm females, the sex ratio was obtained from Espinosa, [24] and Rivera-Ingraham, [78]. Therefore, it is important to consider large female individuals in a population more than the value of the sex ratio itself. Thus, a population located in restricted areas has been observed to have a more balanced sex ratio, while other populations in accessible areas showed a male-biased sex ratio [81] (Fig. 7)). An enhancement of the sex change rate in populations lacking larger specimens combined with a greater recruitment (and more small adults, mostly male) has

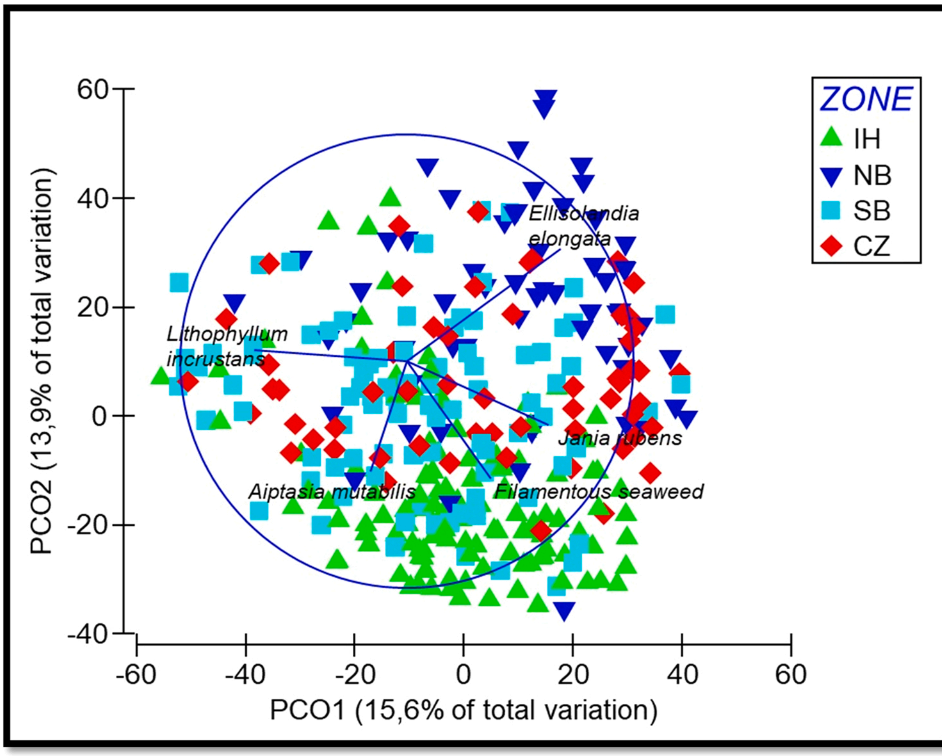

Fig. 5. Principal coordinates analysis (PCO) for the subtidal community structure (all species) from the different studied zones (variables shown have a correlation higher than 0.5 with any of the PCO axis).

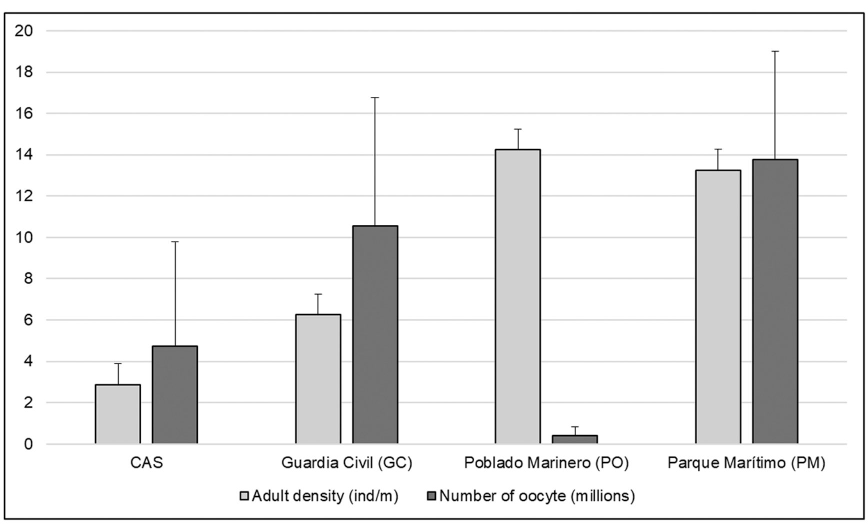

Fig. 6. Adult density *of P. ferruginea* and estimated production of oocytes according to the percentage of female in different subzones inside the harbor (mean and standard deviation).

been proposed as an explanation for this finding [78]. However, it does not mean less efficiency in reproductive terms. The more balanced sex ratio values observed in the present study for no accessible areas could be explained by the fact that recruitment was low and the presence of large specimens (mostly females) was dominant.

For effective species conservation management, it is important to promote genetic bridges that allow genetic flow among populations with eventual recolonization of its original distribution. Ospina-Alvarez et al., [71] pointed out that closeness (i.e. the shortest path to all other nodes in the network) serves to identify those areas that export a huge amount of eggs or larvae to adjacent sites in the context of MPA connectivity. It is particularly relevant for broadcast spawning species with external fertilization and larval phase. For *P. ferruginea*, a permanently armoured network of reserves could be essential to create larval source populations which recolonize their natural geographic distribution as García-Gómez et al. [41] pointed out. Previous studies have highlighted that the Ceuta *P. ferruginea* population may play the role of the source population in the area and maintain nearby populations in the southern Iberian peninsula according to the source-sink population model [27]. According to Groom et al., [45], only an average of 10% of wild populations plays a role as a source, and thus conservation efforts must be focused on these cases. In this sense, the harbor of the nearby city of Melilla supports a similarly high number of *P. ferruginea* compared to the harbor of Ceuta (i.e., mean value of 17 ind./ m, and a maximum of 66

Table 4
Results of ANOVA analysis carried out with the biometric parameters of *P. ferruginea* settled in zones with different levels of protection.

	High	Medium	Low	F	p	Post-Hoc
	Mean ± SD	Mean ± SD	Mean ± SD			
Mean size (mm)	49.26 ± 21.02	32.46 ± 15.58	42.88 ± 19.18	2.05	0.15	
Mean adult size (mm)	49.26 ± 21.03	42.88 ± 19.19	32.46 ± 15.58	2.05	0.15	
Mean volume (mm^3)	15,938.55 ± 19,387.29	10,731.88 ± 10,710.02	3,942.68 ± 3,427.93	2.61	0.13	
Mean adult volume (mm^3)	17,374.29 ± 18,643.35	11,729.24 ± 9,869.49	4,844.38 ± 3,310.62	3.34	$p \leq 0.05$	High = Medium > Medium = Low
Overall density (ind/m)	5.5 ± 4.74	6.73 ± 6.53	7.65 ± 9.71	0.02	0.98	
Adult density (ind/m)	6.75 ± 3.71	3.62 ± 3.22	4.47 ± 5.51	1.44	0.25	
% Recruitment	22.69 ± 22.46	29.73 ± 28.36	26.41 ± 24.70	0.19	0.82	
Number of oocytes (according to the female (%))	12,287,479.39 ± 3,496,116.38	2,871,268.68 ± 2,782,710.73	128,505.53 ± 222,578.11	18.28	$p \leq 0.05$	High > Medium = Low

ind./m). In fact, 68% of this population settled in breakwaters outside of Melilla harbor [46].

As they are inaccessible armored areas, AMMRs would be a good complement to Marine Protected Areas (MPA) by reducing the fragmented distribution of *P. ferruginea*, one of the main limits for the conservation of this species [23,65]. In the case of *P. ferruginea*, evidence of restricted dispersal suggests that a network of marine reserves spaced 10–20 km from one another, as [8] proposed for the Mediterranean, could likely ensure genetic connectivity among populations [86]. In this case, the creation of AMMRNs for *P. ferruginea* could be beneficial for all intertidal and subtidal communities through what is called the 'umbrella species' concept in conservation biology (sensu [69]). The results of the present study indicate that there are many species of conservation interest settled in the intertidal or subtidal habitats of these protected ripraps that could benefit from the 'umbrella effect' of *P. ferruginea* [82].

In this sense, it was also demonstrated that sessile sensitive species are naturally settled in the artificial structures (see Tables 1 and 2 of supplementary material). Therefore, it would be viable to create a monitoring program to effectively control and improve coastal water quality, which would play a significant role in achieving the WFD objectives [21]. This monitoring program could serve to early identify nonnative species establishment, since it is known that harbors facilitate the establishment and spread of exotic species [3,10].

4.1. *Artificial marine micro-reserves, a new management tool for marine conservation*

AMMRs are relevant for the formulation of marine policy because their implementation requires institutional arrangements for the sake of management and regulation of artificial substrates, including conflict resolution between the functionality of such man-made structures and conservation goals. In this sense, harbor facilities can be public or private and the effectivity of AMMRs would rely on the active collaboration between these owners and the environmental responsible of local, regional or national administrations. In addition, an integrated management of such protected areas should be mandatory within a framework of collaborative interactions among stakeholders at great enough geographical scales.

We consider two important proposals for the use of the AMMR as management tools, so that they can serve as support to the coastal natural zones for the periodic control, environmental monitoring and conservation of their biodiversity: (1) That the competent administrations formally declare the AMMR as legal protection figure together with management protocols compatible with the function and maintenance of artificial coastal infrastructures that are declared AMMR; and (2) that the owners of the coastal infrastructures are integrated in the emerging current of "Working with Nature" ([2,91,94]), since the "development-conservation" symbiosis is currently necessary so that the biodiversity associated with coastal infrastructures is not considered as a problem but as an authentic asset that, in addition to projecting a good and desirable image for port institutions or companies, promote - through the AMMR - the establishment of reproductive populations of protected species and, therefore, contribute to the increase of genetic connectivity between them and the populations established in nearby natural areas.

The present management conservation tool is developed with the objective of protecting specially endangered species in a critical situation. The criteria to be met by a marine artificial habitat for its protection under an AMMR are the following [40]:

1. In the coastal structure or parts there of it has hosted one endangered species, whose individuals are settled naturally. Additionally, the recruitment and growth (adult) of these individuals should be present to generate a reproductively viable population. The creation of AMMRs should encourage a network to promote genetic bridges.

2. The proposal for the creation of an AMMR must be limited to already built installations, where endangered species have already

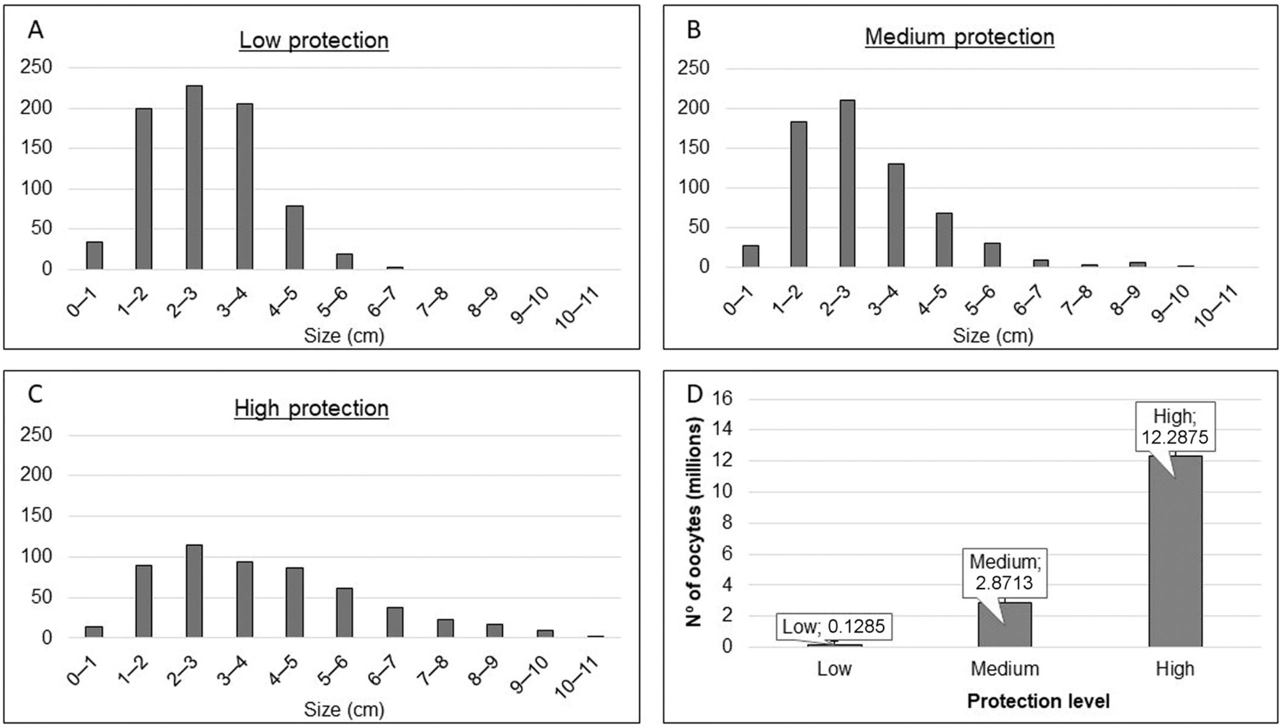

Fig. 7. Size frequency distribution of *P. ferruginea* over different levels of accessibility (A, B and C). Number of oocytes according to the percentage of females settled in the different levels of protection (D).

Table 5
Percentage (%) of recruits, males and females of *P. ferruginea* individuals according to the different levels of protection. The relation between the size class and the sex ratio was obtained from Espinosa, 2006 and Rivera-Ingraham *et al.,* 2011.

	Recruit	Male	Female
High	17.78	76.09	6.13
Medium	29.56	69.13	1.31
Low	29.30	70.56	0.14

naturally settled. The positive results of AMMRs must not be used as a reason for the construction of a new artificial structure.

3. An AMMR cannot be assigned without the agreement of its owner, public or private. Depending on the status of the species, the agreement can be temporary and must be compatible with the normal use of the installation. The AMMR must be permanently armored with fences and video monitoring, which is not very expensive since the harbors are already guarded by their respective responsible.

4. In the case that it is located inside a port, it ought to be implemented to areas that fall within the quality requirements of the WFD and must include an emergency contingency plan for oil spills (i.e. sea booms, oil skimmer, etc.) or different environmental impacts.

The statement of AMMRs should not pose a problem in itself. In contrast, it would be an opportunity to protect and monitor endangered and sensitive species together with the competent environmental authority. In addition, a well management plan can promote to society interest in the marine environment, biota, and port sector ([33]; Janis et al., [55]; O′Neil et al., [70]).

The AMMR conservation tool can be introduced into regional or national legislation for the environmental authority, for example, the Government of Gibraltar declared Marine Conservation Zone some areas of the artificial coastal defense structures [66],

In addition, AMMRNs can fit within the conceptual framework of several sustainable development or conservation approaches, so that it could be a conservation tool for the port sector in a possible action guide. Moreover, AMMRNs can fit within the conceptual framework of Ecosystem Based Management (EBM; see [64]), 'novel ecosystems' (see Hobbs et al., [53]) or more particularly within the concept called Locally Managed Marine Areas (LMMAs). In this sense, Jupiter et al., [56] indicated that "*LMMAs have garnered support because of their adaptability to different contexts and focus on locally identified objectives*". These authors concluded that the management actions undertaken on LMMAs included permanent closures, periodically-harvested closures, restrictions on gear, access or species that are the kind of measures that could be easily implemented within AMMRs in artificial substrata. Furthermore, according to the Aichi target 11 (conserving biodiversity in situ), the IUCN has considered the LMMAs as an example of secondary conservation [54] and, consequently, this approach for a more adaptive and locally managed conservation effort has gained attention. In this sense, AMMRs could play a new role in marine conservation. In fact, the marine conservation tool AMMR recognizes coupled socio-ecological systems with stakeholders involved in an integrated and adaptive management process where decisions reflect societal choice, similarly to EBM (Long, 2015).

Humanity has been modifying the planet in an unmeasurable way for thousands of years. Recently, this influence has been such that a new era has been proposed: the Anthropocene and the Sixth Extinction, as the ongoing extinction event of species in present times [6,51,57]. Species are becoming extinct at unprecedented rates, even if we ignore the possibility of a considerable extinction debt [50,75]. The urgency of the situation leaves no room for 'paralysis by over-analysis' (sensu [11]). It is no longer legitimate to assert that the AMMR concept has not been tested and therefore should not be applied.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.marpol.2021.104917.

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