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Artificial portable plates: A brand new method for assisted colonization of an endangered marine invertebrate, *Patella ferruginea*

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Abstract

1. Limpets play an important role in structuring the intertidal fringe, one of the most heavily impacted marine habitats. A large number of limpet species are listed as Endangered or Vulnerable, including *Patella ferruginea*, one of the most endangered marine invertebrates in the Mediterranean Sea.
2. Relocation attempts have been made as a conservation tool for reintroductions and population reinforcements for *P. ferruginea*. Some relocations were mandatory because of port infrastructure repairs in areas where viable populations of this limpet occurred. However, most relocations attempted to date have experienced high mortality rates, leading to the Spanish National Conservation Strategy not recommending this approach for this species.
3. In this study, 84 individuals of *P. ferruginea* were relocated, using artificial portable plates (APPs) installed on the ripraps of the Port of Ceuta (Western Mediterranean Sea). These plates were divided into two groups and relocated: (i) as a translocation to a nearby site with similar environmental conditions to the donor area (procedural control); and (ii) as a reinforcement translocation to a relatively distant location (30 km, Algeciras Bay) (experimental procedure). The mortality of the translocated individuals in the procedural control after 10 weeks was similar (6.6%) to that expected for natural populations, whereas the mortality rate for individuals in the reinforcement translocation was higher (26.6%).
4. The APPs have been proven to be a feasible method for translocation, as they result in relatively low mortality by preventing the stress and damage caused by limpet detachment from substrata. The constraints caused by reinforcement translocation to a new environment seem to be the cause of the increased mortality, as the translocated limpets changed their home scar at a much higher rate than the control limpets did.

KEYWORDS
conservation, extinction risk, limpets, marine Gastropoda, protected species, reintroduction, Strait of Gibraltar, translocation, transplantation

1 | INTRODUCTION

Marine ecosystems are increasingly threatened by global climate change, interacting with regional and local scale impacts (Firth & Hawkins, 2011; Hawkins, 2012), and restoration efforts are increasing (Geist & Hawkins, 2016), including the translocation of species at risk. In this context, Swan et al. (2016) conducted a comprehensive review of the global literature from 1983 to 2013 on marine conservation translocation, identifying 242 translocated species. Among the translocated invertebrate species (comprising 44% of projects), Swan et al. (2016) found that 71% were Anthozoa, 15% were Bivalvia, 5% were Gastropoda, 2% were Malacostraca, 2% were Porifera, 2% were Echinoidea, 2% were Hydrozoa, and 1% were Polychaeta. As translocation is becoming a widespread tool for conservation, under the current scenario of global environmental change, the development of effective translocation methods is necessary to prevent local extinction and replenish depleted populations. However, experience with translocation in molluscs remains limited to only a few studies, generally with high rates of mortality: 55% after 1 year for clams, 35% after 18 months for abalone, and 100% after 72 days for the fan mussel in shallow waters (Lemasson et al., 2019).

According to commonly accepted definitions in conservation (Fischer & Lindenmayer, 2000; IUCN/SSC, 2013), translocation (or relocation) is the deliberate and human-mediated movement of individuals from one area to another. If this movement occurs within the natural distribution range of a species and the individuals are released into an existing population, it is termed reinforcement, restocking, or supplementation. Furthermore, translocations have been a useful tool around the globe in terms of conservation, management of human–animal conflict, and restocking animal populations, with the use of translocation increasing over the last few decades (Weeks et al., 2011; Gusset, 2012; Pérez et al., 2012; Butt et al., 2021). Although reinforcement (or restocking) is an available method for managing severely depleted populations, it requires thorough evaluation before large-scale implementation (Bell et al., 2006). The effectiveness of translocation is contingent upon various factors, including the suitability and quality of the habitat at the release site, the similarity between the donor and receptor habitats, the number of introduced individuals, and the genetics of the donor and receptor populations. Additionally, the public's perception towards the species in question plays a significant role (Hodder & Bullock, 1997; IUCN/SSC, 2013).

Limpets have long been known to play a key role in the maintenance of the diversity of rocky coastal ecosystems (Hawkins & Hartnoll, 1983; Raffaelli & Hawkins, 1996; Coleman et al., 2006; Hawkins et al., 2019; Mieszkowska et al., 2019), by shaping the composition and structure of the community, and their loss or reduction can have cascading effects on the entire intertidal community. Most limpets inhabit the intertidal zone, which is one of the most highly impacted marine habitats because of its high accessibility (Thompson, Crowe & Hawkins, 2002; Benedetti-Cecchi et al., 2019), with many impacts related to coastal urbanization (Bulleri & Chapman, 2010; Bulleri & Chapman, 2015). With their widespread distribution and limited movement, limpets have been used as bioindicators (Espinosa, Guerra-García & García-Gómez, 2007; García-Gómez et al., 2015; Martins et al., 2017; Reguera, Couceiro & Fernández, 2018; Carballo et al., 2023).

There are 14 species of giant limpets that can grow to more than 10 cm in shell length (Espinosa & Rivera-Ingraham, 2017). These species are more likely to be collected, for possible consumption, ornamentation, and bait purposes (Espinosa & Rivera-Ingraham, 2017; personal observation, A.P.-P. & E.O.-V.), as they provide more biomass and are easier to detect than smaller species. Among these species, *Patella ferruginea* Gmelin, 1791 (Gastropoda: Patellidae) is a Western Mediterranean endemic species in which the geographical distribution has greatly decreased since the 19th century (Laborel-Deguen & Laborel, 1991a; Boudoresque et al., 1996). This species is now at serious risk of extinction and is considered one of the most endangered marine species found on Western Mediterranean rocky shores (Ramos, 1998). It is also included in two other European lists of species for which specific conservation strategies must be implemented (Annex II of the Bern Convention and Annex II of the Barcelona Convention), and there is a Spanish National Strategy for the conservation of *P. ferruginea*, meaning that collection is strictly prohibited (MMAMRM, 2008; Espinosa & Rivera-Ingraham, 2017). However, the species has not yet been evaluated for the International Union for the Conservation of Nature (IUCN) Red List.

The main reasons for the decline of *P. ferruginea* populations are overexploitation, consumption, and habitat loss through coastal urbanization (Espinosa & Rivera-Ingraham, 2017; Ferranti et al., 2021a; Ostalé-Valriberas et al., 2022). It has, however, been demonstrated that the species can establish large populations on artificial structures, such as ripraps or breakwaters of dolomitic rock, with similar or even higher densities than neighbouring natural areas (Rivera-Ingraham, Espinosa & García-Gómez, 2011; Espinosa et al., 2018). At present, the most abundant populations of *P. ferruginea* are found in North Africa: Ceuta, Melilla, and the Chafarinas Islands (Spain); the Tres Forcas Cape; the Habibas and Rachgoun islands (Algeria); and the Cap Bon Peninsula and Zembra Island (Tunisia) (for details, see figure 6 in Espinosa et al., 2013; Doukara, 2019; Ostalé-Valriberas et al., 2023). In Europe, the species is present in low numbers along the northern coast of the Alboran Sea and the islands of Corsica and Sardinia, with scattered specimens throughout the Ligurian coast and the Tuscan archipelago (Coppa et al., 2015; Marra et al., 2016; Ferranti et al., 2019; S. Hawkins, personal communication). This species is long-lived (over 30 years)

(Espinosa et al., 2008), has one breeding season per year in autumn, and reaches sexual maturity at the age of 2 years (Guallart & Acevedo, 2006; Espinosa, Rivera-Ingraham & García-Gómez, 2009). The species has a protandrous hermaphroditic sexual ontogeny, although reverse sex change has also been observed (Guallart et al., 2013). These biological traits have contributed to the decline in *P. ferruginea* populations, as collection usually targets the larger individuals (females). Moreover, a number of genetic studies have revealed that genetic diversity is low and the populations from Corsica and Sardinia have genetic differences to those found in Southern Spain and Northern Africa, which are genetically close (Espinosa & Ozawa, 2006; Casu et al., 2011; Cossu et al., 2017; Bouzaza, Vera & Mezali, 2021).

In the case of *P. ferruginea*, the depletion of its population and its endangered status have prompted attempts at reintroduction or reinforcement since the 1980s (see Zarrouk, Salah Romdhane & Espinosa, 2018). However, the survival rates decreased when individuals were transferred to distant locations (i.e. >100 km) (Laborel-Deguen & Laborel, 1991b; Zarrouk, Salah Romdhane & Espinosa, 2018). Furthermore, the presence of large populations of this species on the breakwaters of various large harbours has given rise to an additional management issue, as these structures unavoidably require maintenance and restoration. To alleviate the impacts of maintenance on such populations, several relocations of individuals from artificial substrates have been carried out as emergency measures to compensate for any losses (Fa et al., 2018). However, mortality rates have been high in several of these relocation attempts (Table 1), and more knowledge is needed to help reach a consensus on the best protocol for the translocation of this species.

The translocation of limpets along with the substrata to which they are attached greatly reduces stress and potential tissue damage during removal (Fa et al., 2018). However, this method is expensive and requires complex logistical procedures, so is not recommended for medium (10–100 km) or long-distance translocations (>100 km). Further attempts have recently been made in the framework of the LIFE Project REMoPaF in the Alboran Sea, which has tested the feasibility of artificial inert mobile substrata (AIMS) as a long-range translocation method (Maestre-Delgado et al., 2019; Espinosa et al., 2023). Moreover, the RE-LIFE project in the Ligurian Sea is testing both direct translocations and the controlled spawning of adults under laboratory conditions (Ferranti et al., 2021b).

The main sources of stress that lead to an increase in mortality during the translocation process are: (i) damage caused by removal; (ii) accumulated stress during transportation; (iii) inability to adapt to the new environment; and (iv) decreased resistance to competition and predation in the new environment (Dickens, Delehanty & Romero, 2010). In this context, the aim of the present study was to develop an affordable translocation protocol for the translocation of *P. ferruginea* individuals from a source population to a depleted population (reinforcement purposes). This involves the translocation of limpets and the substratum with their home scar, which greatly reduces the physiological impact of the operation. The main hypothesis of this study is that this new APP method will considerably reduce the limpet mortality caused by translocation stress, when compared with existing procedures (Table 1). Additionally, the present study weighs up the different contributions to overall mortality from accumulated stress during transportation and the inability to adapt (Dickens, Delehanty & Romero, 2010).

2 | METHODS

The donor population was located in the Port of Ceuta, a Spanish autonomous city on the African coast of the Strait of Gibraltar (Figure 1). The surroundings of this town hosts one of the biggest populations of *P. ferruginea*, with an estimated 44,000 individuals along its 20-km coastline in 2010 (Rivera-Ingraham, Espinosa & García-Gómez, 2011). According to more recent censuses, the population has greatly increased over the last decade, with up to 168,463 individuals in 2020 (Ostalé-Valriberas et al., 2023).

In December 2016, 100 concrete cladding plates (APPs) (Model PIRINEOS ANETO. Leroy Merlin®), measuring 28 × 14 cm and weighing 1,450 g, were screwed onto the breakwaters of Ceuta harbour (Figure 2). The APPs were placed in the high intertidal zone (+75 cm above zero tide level) at both the donor and recipient sites on rocks where individuals of the species were observed in close proximity, to ensure that this was suitable habitat for the species. To be sure that these plates did not negatively impact the donor population (affecting foraging behaviour or movement), a Before–After Control–Impact (BACI) study was carried out, as recommended for human-mediated environmental alterations (Underwood, 1991; Smith, 2002; Conner et al., 2016). For this study, six linear transects of 10 m in length, parallel to the coast and covering the whole intertidal (up to approx. 1.5 m), were established within the donor area (three within the donor area where the plates were installed and three within the same donor area where no plates were fixed). The whole intertidal was censused within each transect, counting all *P. ferruginea* individuals. The surveys were undertaken before (November 2016) and after (February 2018) the installation of the plates to assess the potential impact of the installation of the experimental plates in terms of any decrease in density in the donor population.

The APPs were left for 10–13 months to allow recruits to settle and for adult limpets to migrate their home scars to the APP before translocation. First, a procedural control within the Port of Ceuta was performed in September 2017 (C: movement within the Port of Ceuta) to check whether the procedure caused excessive mortality (>50%, as reported in a previous study in the same area; Espinosa et al., 2008). Subsequently the experimental treatment took place once it was confirmed that the translocation method resulted in low mortality rates, and was completed in January 2018 (Tr: movement from a donor population in Ceuta to a receptor population in La Linea) (Figure 1). For both the procedural control and translocation experiment procedures, all APPs on which *P. ferruginea* individuals had established home scars were unscrewed and replaced with a new plate during low tide. The plates were transported in plastic trays and covered with seawater-soaked cotton towels inside a refrigerated truck held at 18°C. To minimize vibration, there were cushion sheets positioned between the

TABLE 1 Compilation of relocations performed with *Patella ferruginea* and other gastropoda.

References	Relocation methodology	Individuals	Survival rate (%)	Study duration (months)	Distance (km)	Transportation methodology	Mortality relocate./transp.	Observations
Patella ferruginea translocations								
Aversano (1986)	Manual relocation	3	100	24	18	NA	NA	Method not reported
Laborel-Deguen & Laborel (1991b)	Manual relocation	188	12	24	200	Immersed in aquarium	45%	40% mortality (especially <35 mm)
Espinosa et al. (2008)	Manual relocation	420	30–35/18	12/48	<5	Immersed in aquarium with aeration	50%	50% mortality immediately after transplantation, higher mortality in larger individuals
Guallart (2014)	Detachable natural substrata were moved	NA	0	12	1	NA	NA	Not detailed
Guallart (2014)	Manual relocation	10	10	24	400	With wet cloth	40%	40% mortality after first day
APM (2014)	Rocks with limpets were moved	301	37	3	<0.05	Using a crane to move the individuals to the next location	6.70%	Differences between sources
Fa et al. (2018)	Rocks with limpets were moved	97	87	10	<1	On trucks with cushion sheet	Very low	Heavy rocks were moved; lower mortality than control
Zarrouk, Salah Romdhane & Espinosa (2018)	Manual removal, but specimens relocated within cages	94	58	23	6.7	Immersed with continuous water renovation	High	High mortality was registered during transport
Zarrouk, Salah Romdhane & Espinosa (2018)	Manual removal, but specimens relocated within cages	110	18	20	185	Immersed with some water renovation	High	High mortality was registered during transport
García-Gómez et al. (2023)	Fast manual relocation	113	36/93/83	16/5.5/5.5	<2	Immersed in soft plastic ziplock bag		Some treatments with cages
Espinosa et al. (2023)	Artificial inert mobile substrates (AIMS)	188	87/75/68	1/4/8	240	Refrigerate truck with rubber base	0	No mortality during transportation
Present study	Artificial portable plates were moved	30	93.3	2.4	0.5	With wet cotton towels on a refrigerated truck with cushion sheet	0	No mortality during transportation
Present study	Artificial portable plates were moved	45	73.3	2.4	30	With wet cotton towels on a refrigerated truck with cushion sheet	0	No mortality during transportation
Other gastropoda translocation								
Burak & Hopper (2020)	Basalt cobbles were collected with *Idaholanx fresti* on them	19	0	4	8	Immersed in aquarium with aeration and coolers	0	No mortality during transportation and it is a freshwater limpet
Tablado, López Gappa & Magaldi (1994)	Manual relocation in *Siphonaria lessoni*	1,000	1.3	9	<1	Manual transportation	N/A	Different treatments were applied

Note: NA, not available.

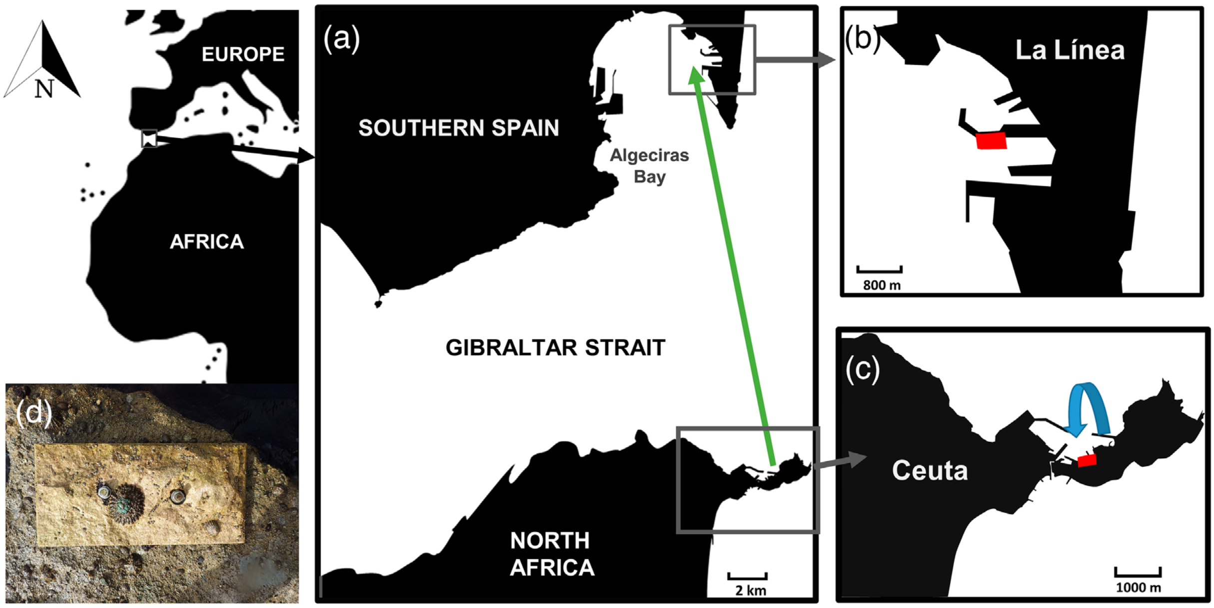

FIGURE 1 Study site and translocation plate. Translocation (arrow in map a). Procedural control (arrow in map c). The receptor zones are in red for translocation (map b) and control (map c). (d) Artificial portable plate (APP) hosting a marked individual of *Patella ferruginea*.

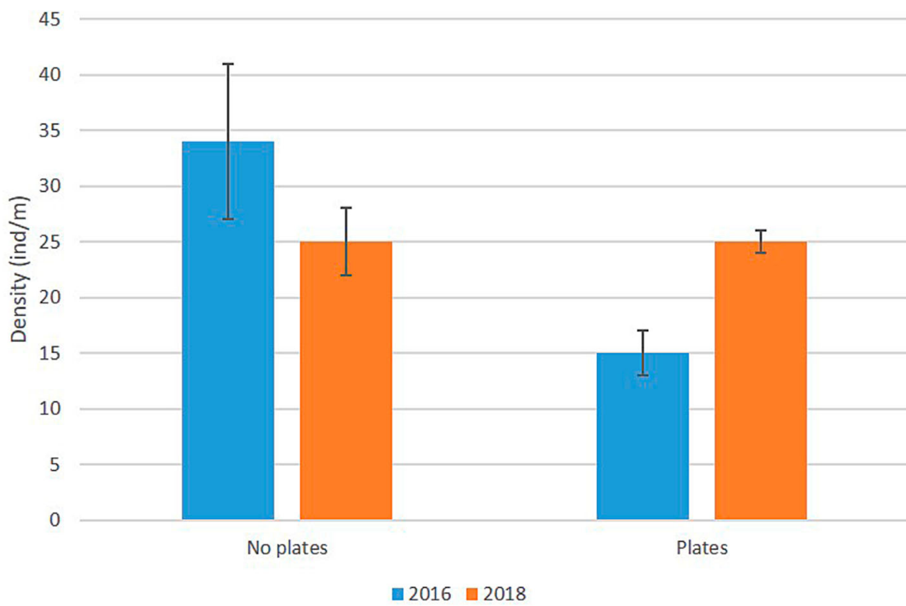

FIGURE 2 BACI (before–after and control–impact) study of the installation of artificial portable plates (APPs). Density of limpets in zones with no plates and with plates installed in the year 2016 and 2018. The error bars indicate standard deviation (SD).

trays and the bed of the truck. For both procedures, the limpets were kept inside the truck for a total transportation time of 24 h. For both procedures, plates were relocated during the low tide of the next day. Both receptor areas were located on fenced breakwaters with restricted access to avoid collection by the local population.

La Alcaidesa is a marina located in La Linea de la Concepción in Algeciras Bay (Figure 1). It was chosen as a receptor site for the translocation because: (i) it is surrounded by fences and is patrolled by the port police and technical personnel from the Bahía de Algeciras Port Authority; (ii) it may be a suitable environment, as there was a previously settled population with some scattered individuals present; and (iii) it is easily accessible by car, which facilitates the transportation of the individuals to the reception area.

To monitor the limpets, each individual was marked and numbered using epoxy resin (Eporai 1127©) in both the procedural control and translocation procedures. The individuals were revisited on the first (24 h), second (48 h), and seventh days after relocation, and then weekly until the fifth week and, finally, again in the 10th week after relocation. The monitoring period was limited to 10 weeks, which comprises the period of peak mortality reported in previous studies after translocation (Espinosa et al., 2008; APM, 2014; Guallart, 2014). On each sampling visit, all marked individuals were counted and measured (length measurements were taken to the nearest mm along the anteroposterior axis using plastic callipers). Missing individuals were recorded, and found individuals were marked again with epoxy resin if necessary during low-tide conditions (extending no more than 2–3 h per tide). Individuals that had abandoned their original home scar to establish a new home scar outside their APP were also recorded.

The topographical heterogeneity of the plates was compared with that of rocks by measuring the heterogeneity of 10 plates and 10 random rocks from the donor breakwater. For each plate and rock, three longitudinal transects of 16 cm were measured using the Wright index (total lineal profile perimeter/linear distance sampled) and fractal dimensions methodology (Wright, 1983; Espinosa, Rivera-Ingraham & García-Gómez, 2011; Selkoe & Toonen, 2011), which consists of consecutive recalculations of the total perimeter (P) of each rock profile using progressively smaller steps (λ). The total profile perimeter (L) for each step size can be expressed as: L = (λ × number of steps taken) + any remaining distance smaller than the λ taken (rest). Steps of 40, 20, 10, and 5 mm were used for the measurement of the fractal dimensions (Espinosa, Rivera-Ingraham & García-Gómez, 2011).

Permissions for undertaking this research were obtained from the environmental agencies of the regional governments in Ceuta (Exp: 9361/2016) and Andalusia (Exp: 2016001005501), in accordance with law 42/2007 in Spain.

2.1 | Statistical analyses

Differences in topographical heterogeneity between the APPs and the natural substrate were tested using the Mann–Whitney *U*-test. Limpet abundance per year and treatment in the BACI experiment transects were tested for normality using a Kolmogorov–Smirnov test, and for homoscedasticity using Levene's test. In the absence of normality or homoscedasticity, these were analysed using nonparametric univariate permutational multivariate analysis of variance (PERMANOVA) on Euclidean distances (Anderson, Gorley & Clarke, 2008). This test was carried out using a two-way orthogonal design with the fixed factors 'year' (two levels: 2016 and 2018) and 'treatment' (two levels: plates and control).

Possible differences in survival between the procedural control and translocated populations 10 weeks after relocation were tested using a chi-square test. An individual's growth, measured as an increase in shell size over the monitoring period, was correlated with the individual's shell size (mm). The correlation trend lines plotted were those with the best R^2 fit. Differences in limpet growth rates between the procedural control and translocated populations were analysed using analysis of covariance (ANCOVA). For this test, limpet shell size during translocation (initial size) was added as a covariate, T-treatment (two levels: procedural control and translocation experiment) was added as a fixed factor, and limpet shell size 10 weeks after each translocation was added as the response variable. These analyses were carried out using SPSS Statistics 22 (IBM, Armonk, NY, USA) and PERMANOVA+ with PRIMER V6 (Primer-e, Auckland, New Zealand) (Clarke & Gorley, 2006; Anderson, Gorley & Clarke, 2008). Finally, a chi-square test of independence was performed to analyse variations among size classes on the percentage of individuals that abandoned their original home scar on the plates after treatment to establish a new home scar outside their plates.

3 | RESULTS

Ten months after the deployment of the 100 original plates at the port of Ceuta, 41 plates and 30 individuals were used for the procedural control (14 ind. <25 mm, 9 ind. 25–50 mm, and 7 ind. >50 mm) and 51 plates and 54 individuals (23 ind. <25 mm, 21 ind. 25–50 mm, and 10 ind. >50 mm) were used for the translocation experiment. In both procedures, some of the plates were empty. The individuals translocated represented less than 0.1% of the limpet population at the donor site. The installation of the plates did not have an impact on the donor population. In fact, the number of individuals showed a greater increase in the sector where the plates were deployed compared with the control sector, where no plates were deployed (BACI study; Figure 2; Table 2). Plates showed similar surface heterogeneity (fractal dimension) when compared with the breakwater rock substrata (plate, mean = 1.0083, SD = 0.0024; rock, mean = 1.0117, SD = 0.0056; Mann–Whitney *U*-test, N1 = 10, N2 = 10, $P = 0.28$).

For both treatments (i.e. procedural control and translocation), there was no mortality during the transportation processes. For the control treatment, only two individuals out of 30 were not found after 10 weeks (6.6% mortality): one disappeared after 24 h (21 mm length limpet) and the other disappeared after 1 week (31 mm length limpet). For the translocation experiment, the mortality rate was greatly influenced by a severe storm with an average wind speed of 50 km/h and wind gusts of over 90 km/h (Datosclima, 2020) that hit the receptor site 4 days after translocation. Five plates with nine specimens were destroyed by the waves and lost, and these plates and specimens were not considered in the analysis, to make the results comparable between the control and the translocation treatments. For the remaining limpets, four out of 45 individuals were not found (8.8%) between days 2 and 9, the period of the storm, and by the 10th week, 12 out of 45 individuals had disappeared (26.6% total mortality), compared with the 6.6% in the procedural control (Figure 3).

A significant difference in mortality was found between both procedures, showing a higher mortality rate in the translocation group than in the procedural control ($\chi^2(10) = 18.31$, $P < 0.05$). For the translocation, the mortality rate was highest for the smallest size class of <25 mm (58% total mortality, C1), whereas it was around 33% for specimens of 25–50 mm (C2) and 8% for the largest specimens of >50 mm (C3) (Figure 3).

Shell growth rates were higher in the translocated experiment than in the procedural control, although differences in growth rates were also related to the individual's initial shell size (Table 3). For both treatments, particularly the translocation experiment, the smaller individuals of *P. ferruginea* had higher growth rates than the larger specimens (Figure 4; Table 3). In accordance with this observation, 33% of the translocated limpets (15 ind.) had changed their home scar by migrating from the APP to the breakwater rock just 9 days after being removed from the donor site (overall, 40% limpets after 10 weeks). In contrast, none of the control limpets changed their home scars during the entire monitoring period. For the translocation, all the surviving limpets that had changed their home scar showed a brighter colour along the edge of the shell, which indicates recent

TABLE 2 PERMANOVA results for the abundance of *Patella ferruginea* (ind./m) in the before–after and control–impact (BACI) experiment, for the factors year (2016 and 2018) and B-treatment (plates and control).

Source	*df*	*MS*	Pseudo *F*	*P* (PERMANOVA)
Year	1	0.12	0.001	0.976
B-treatment	1	536.76	4.784	0.019
Year × B-treatment	1	521.73	4.650	0.032
Residual	20	112.20	–	–

Note: Pairwise (year × B-treatment): 2016, plates < control; 2018, plates = control.

growth, and they were perfectly fitted to the rock surface. A significant difference in the number of limpets that emigrated from the plates was found between treatments for all size classes studied, with the highest emigration rate occurring for the highest size class, C3 (6/9 ind.), followed by the smallest size class, C1 (6/16 ind.), and lastly by the middle size class, C2 (2/20 ind.), compared with the equal emigration rate expected ($\chi^2(2) = 7{,}118 \times 10^{-10}$, $P < 0.05$).

4 | DISCUSSION

Overall, APPs are a feasible method for the short-range relocation of *P. ferruginea* individuals (<10 km), as they greatly reduce the mortality rate recorded in previous studies, during and immediately after relocation, using different approaches (Table 1). This was probably linked to the absence of stress associated with the mechanical detachment of the individuals from their home scar and the subsequent effort of reattachment and shell adjustment to a new substratum (Espinosa et al., 2008), which can be avoided with the APP methodology. Hence, clearly APPs offer a viable approach for population reinforcement, serving as a proactive tool to significantly elevate both the total and the effective population sizes. This strategic approach helps mitigate the imminent threat of genetic or demographic collapse resulting from stochastic effects, by preventing critically low population size thresholds being reached (Seddon, 2010). Finally, the cost of the APP plates and installation materials can be very low (€3.06 per plate in this study), and the transportation expenses are relatively cheap because of the small size of the plates and the option of piling them in trays during transportation. Moreover, the plates showed a similar surface heterogeneity compared with the adjacent rocks, making the APPs a significantly more practical and affordable method than those used in previous studies (Fa et al., 2018).

Excluding the mortality caused directly by the storm, other environmental and ecological factors, such as differing hydrodynamic regimes, food supply, and substrata features, and decreased resistance to competition and predation, may have contributed to the higher mortality rate of the translocated population (Dickens, Delehanty & Romero, 2010). The higher number of translocated limpets changing their home scar and showing an increase in growth rate may indicate an effort to find a more favourable home scar location. Therefore, the translocated population could have faced a transitional phase in which adaptation to the environmental conditions of the receptor site would make the limpets more vulnerable to detachment resulting from extreme weather events or predation. Future studies should focus on reducing the mortality rate after translocation; one way to help achieve this is by taking into account the weather forecast to avoid certain conditions, such as storms and heatwaves, for the week following translocation, as well as by adding cages that protect the translocated limpets against predation and competition in the receptor area, as suggested by Zarrouk, Salah Romdhane & Espinosa (2018). Thus, the incorporation of cages in this methodology, with the utilization of APPs and effective surveillance measures to safeguard the receptor area against collection, holds the potential to enhance translocation success (García-Gómez et al., 2015; Swan et al., 2016).

Patella ferruginea is an endangered species and any action that may cause damage to its populations must be carefully weighed. Translocations result in the loss of individuals from the donor population, and hence exactly where and which individuals are translocated must be chosen wisely to ensure that their removal will have a minimal effect on the donor population, removing only a very small fraction of the population (<1%), similar to the protocol described by Espinosa et al. (2023). In this case, the installation of the APPs and the removal of a fraction of the population did not produce a significant effect on the population of the Port of Ceuta (Figure 2; Table 2). There are a number of studies that show that density-dependent processes can regulate the abundance of limpet populations (Branch, 1981; Hawkins & Hartnoll, 1983; Underwood, 1992; Boaventura, Da Fonseca & Hawkins, 2003). Therefore, removing smaller individuals from a dense donor population could decrease the intraspecific competition and increase the survival rate of the remaining individuals, particularly in the smallest size classes, which have the highest mortality rates in *P. ferruginea* (Espinosa et al., 2008) and other limpet species, such as *Patella vulgata* (Thompson, 1980).

The precarious status of the majority of *P. ferruginea* populations, excepting the populations situated along the North African coast, poses a significant conservation challenge. Given the dependence on population density for the successful external fertilization of the species, a low number of widely scattered individuals could lead to eventual extinction, primarily through the Allee effect (Stephens & Sutherland, 1999). In addition, larval behaviour and the life-history traits of the species can play a critical role in determining dispersal distances (Shanks, 2012). In the case of *P. ferruginea*, the dispersal rates of the pelagic lecithotrophic larvae phase duration

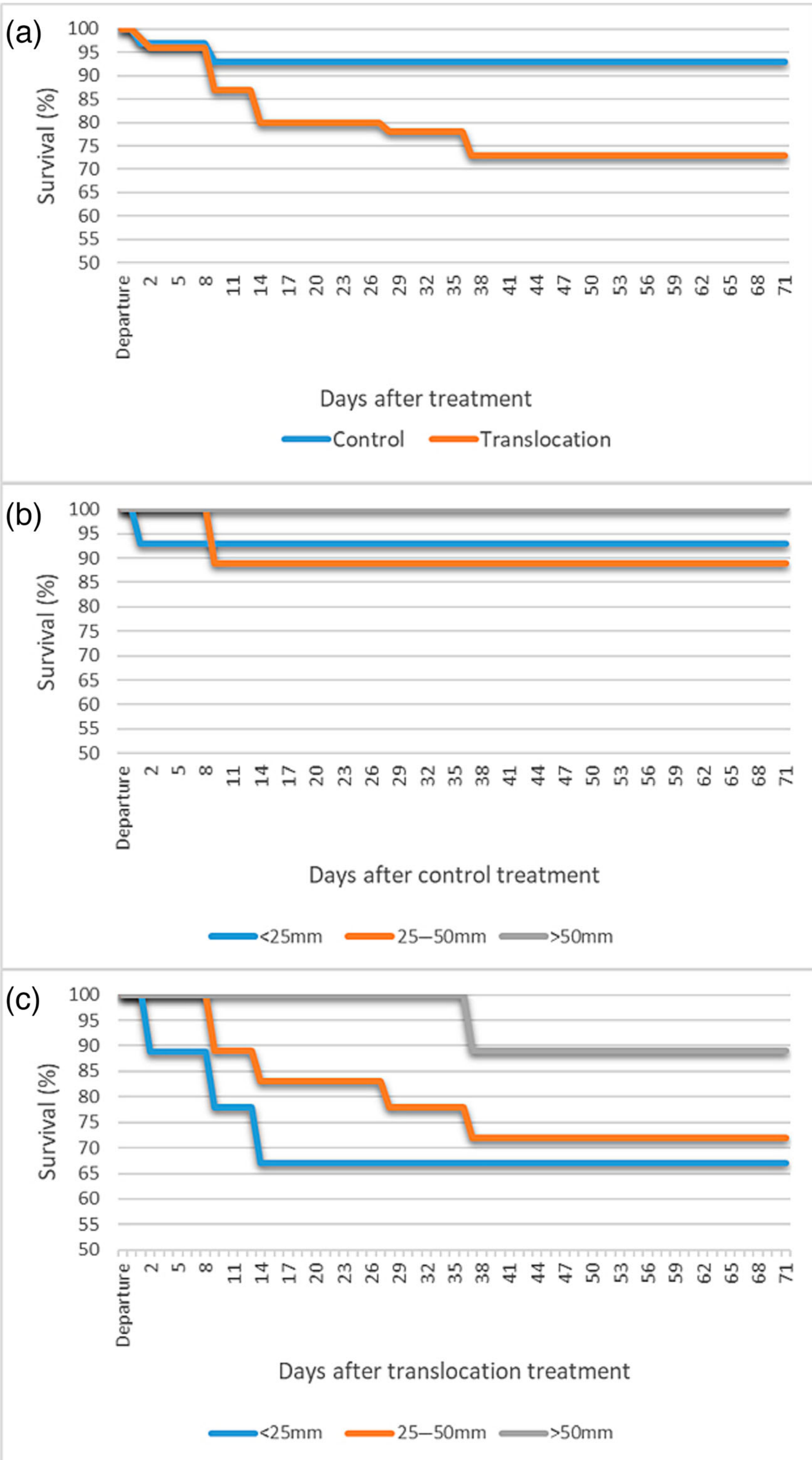

FIGURE 3 Percentage of survival of *Patella ferruginea* individuals during the experiment (a). Procedural control (blue line): 30 individuals, carried out in September 2017. Translocation treatment (orange line): 45 individuals, carried out in January 2018. Percentage of survival of *P. ferruginea* individuals for the control (b) and for the translocation (c) treatments for each size class.

(PLD) are hypothesized to be low, with large variability in settlement ability (lasting from 3 to 32 days after fertilization), depending on temperature and other, as yet undefined, environmental factors (Guallart, Peña & Pérez-Larruscain, 2013; Guallart et al., 2020; Ferranti et al., 2021b). For PLDs greater than 1 day, genetic data have generally overestimated the dispersal distance. It has been assumed that the passive movement of the tide carries the pelagic larvae further away, but in some cases, this does not happen. A mechanism has been suggested that may help to retain larvae in shelf waters through the interaction of diel vertical migration with the two-layer flow structure of upwelling/downwelling circulation, as has been demonstrated for *Carcinus maenas* (crab) and *Chthamalus montagui* (barnacle) larvae (Connolly & Roughgarden, 1998; Queiroga et al., 2007). This wide range in PLD duration is probably associated

TABLE 3 ANCOVA results for the shell growth of *Patella ferruginea*, using the initial shell size after translocation as the covariate and the total shell growth, as total growth (mm) and as percentage increase in relation to total shell length (%), at the end of each translocation (T-treatment) monitoring period as the response variable.

		Total growth (mm)			Percentage increase (%)		
Source	***df***	***MS***	***F***	***P***	***MS***	***F***	***P***
Covariate	1	2.81	4.92	0.030	1,098.3	54.47	0.001
T-treatment	1	29.93	52.41	0.001	645.02	31.99	0.001
Residual	60	0.571	–	–	20.17	–	–

Note: Pairwise comparison for T-treatment: procedural control < translocation experiment.
Abbreviation: MS, mean square sum.

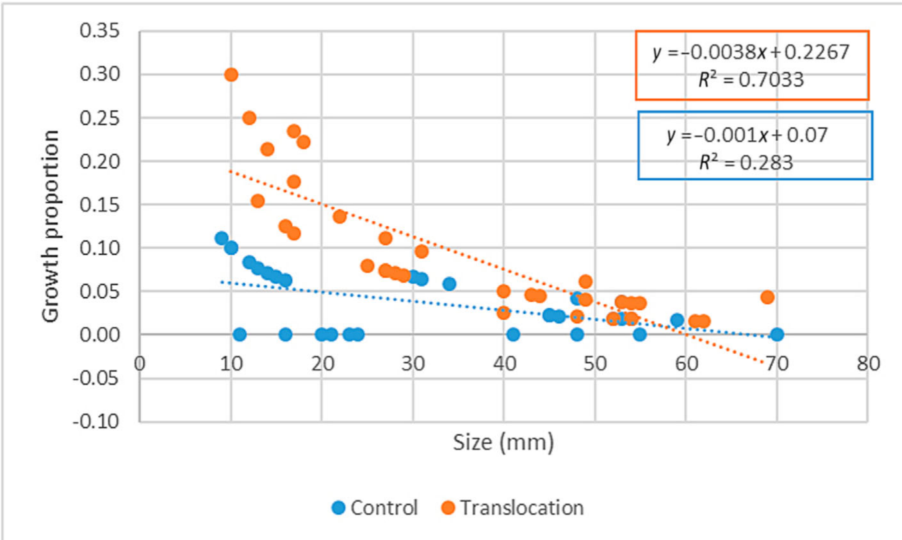

FIGURE 4 Percentage of growth for limpet size: control procedure (blue) and translocation (orange). The inset gives the equations describing the trend lines for each treatment.

with the effect of rare individuals that disperse long distances, increasing allele diversity and lessening genetic differences among populations.

Translocation is a tool focused on conservation, such as the reintroduction of specimens where populations are extinct, and the reinforcement of weak populations, with the purpose of creating or maintaining a viable population of a focal species and promoting the gene exchange between translocated and resident individuals (Chapman,1986; Blowes et al., 2019). The measures of success for translocation are based on abundance (establishment, fecundity, and population size), extent (dispersal capability and demographic distribution), resilience (genetic variation and resistance to perturbation), and persistence (Weeks et al., 2011; Cossu et al., 2017). With the low rate of success achieved in previous translocations, particularly those that used the manual removal of individuals (Table 1), translocations were not recommended in the conservation strategy for *P. ferruginea* in Spain, except for research purposes (MMAMRM, 2008). However, other attempts have shown promising outcomes, such as a study that obtained an 87% survival rate 10 months after the translocation of 97 specimens (Fa et al., 2018). However, these individuals were moved to an area very close to the place from which they originated, which could be considered comparable with the control treatment in the present study, which achieved a 93.4% survival rate after 10 weeks. In this regard, translocations made without detaching the specimens seem to be the best procedure (Fa et al., 2018).

The main factors underlying the mortality associated with translocations are a limiting issue for reintroduction and repopulation programmes, but remain not very well understood. Nevertheless, the mortality associated with translocation using APPs was minimal, as observed in the procedural control, although the stress arising from varying environmental conditions in the translocation experiment resulted in some mortality. These conditions encompass differences in tidal flows between the southern (S) and northern (N) parts of the Strait of Gibraltar (Sammartino et al., 2015), as well as variations in sea surface temperature (SST), upwelling events, and chlorophyll concentration (Stanichny et al., 2005). These authors demonstrate heightened upwelling intensity along the African coast (S) compared with the European coast (N), attributed to greater wind-induced turbulence along the African coast. This increased upwelling intensity induces a more pronounced cooling effect on the SST in the African coastal area. Regarding chlorophyll concentration, the study identifies a time delay between surface cooling and the subsequent elevation in chlorophyll levels. This suggests that chlorophyll enrichment stems from upwelled nutrients rather than from the rise of chlorophyll-rich deeper layers. These disparities in SST, upwelling events, and chlorophyll concentration between the southern (S) and northern (N) sections of the Strait of Gibraltar can impact study outcomes. Variations in currents and the distinct shapes of the southern and northern coastlines result in differences in the movement of upwelled waters and the distribution of thermal signatures on the surface.

Consequently, the analysis and interpretation of results should duly take these regional discrepancies into account.

The growth rate of juveniles (<25 mm) is higher than that of adult *P. ferruginea*. This is the case in most animals with indeterminate growth and is typical for most limpets (Guallart & Acevedo, 2006; Espinosa et al., 2008). In this study, the growth rates followed this pattern, although they were lower for procedural control individuals than for translocated individuals, which is probably associated with the higher rate of changing the home scar found for translocated individuals. This change involves the growth of the shell to adapt to the new home scar and may also have influenced the higher growth rate of translocated individuals (Espinosa et al., 2008).

In the present study, the mortality rate was higher in the two smallest size classes (<50 mm), whereas using a manual removal methodology, Espinosa et al. (2008) reported higher mortality in specimens that were >60 mm, and in another study, juveniles (<30 mm) survived longer than adults (Espinosa et al., 2023). It is suggested that these differences may be linked to local adaptations and variability within the population. Considering that the largest specimens contribute disproportionately more to reproductive events (Espinosa et al., 2006), only a few specimens translocated into a different area could contribute to the next reproductive event and would directly affect local recruitment and genetic diversity. If high survival rates are achieved within the largest size classes, repopulation programmes that focus on the largest individuals could increase the positive impact on the reinforced population.

4.1 | Conservation and future perspectives

Many marine invertebrates that are endangered or harvested rely heavily on a critical density of individuals to ensure successful external fertilization processes and avoid population collapse caused by the Allee effect (Gascoigne & Lipcius, 2004). Assisted colonization to create aggregations by translocating wild specimens has already been proposed in marine benthic molluscs as a conservation measure (Tutschulte, 1976; Catton & Rogers-Bennett, 2013; Coates et al., 2013). Therefore, the use of APPs in assisted colonization could be a useful restoration technique for harvested or endangered sessile or sedentary marine invertebrates. In this sense, the action should be repeated annually until the receptor zone has received more than 100 translocated breeding animals (see Espinosa et al., 2023) from different populations to increase genetic diversity, while avoiding any major impact on a single donor population.

Global change can amplify the risks of local or global extinction from regional and local scale pressures. In the coastal zone, habitat loss and urban sprawl caused by construction creating artificial habitats (Hawkins et al., 2017; Bugnot et al., 2020) can be a major impact, and is likely to accelerate as society adapts to rising and stormier seas, driven by climate change (Airoldi et al., 2005; Firth et al., 2016; Schaefer et al., 2020; Paprotny et al., 2021). O'Connor et al. (2007) demonstrated a strong effect of temperature on planktonic larval duration, being consistent across nearly all species tested, with elevated temperatures resulting in decreased larval lifespan. Although fertilization rates may remain unaffected by elevated pCO_2, embryonic and larval development will be highly sensitive to ocean acidification, with substantial reductions in size and decreased survival of larvae, together with increases in the number of abnormal larvae and increased mortality in the long term (Mawdsley, O'Malley & Ojima, 2009; Doney et al., 2012; Gazeau et al., 2013). The lowering of calcium carbonate saturation states has impacts on various shell-forming marine organisms, from plankton to benthic molluscs, echinoderms, and corals (Doney et al., 2009; Langer et al., 2014). Consequently, future environmental conditions will reduce larval dispersal capability by decreasing larval development and survival, which will affect the connectivity between populations. In this sense, reinforcement or reintroduction attempts would be beneficial to create effective stepping stones to assure the connectivity between populations.

Coastal infrastructure such as ports, breakwaters, and sea defences provide artificial habitat for various species (Moschella et al., 2005) including *P. ferruginea* that have naturally recruited there, reaching the maximum sizes of the species and their maximum reproductive potential. Therefore, such habitats are very suitable zones for the protection, conservation, and management of the species, which enhances the idea of promoting them as artificial marine micro-reserves (AMMRs) that can generate demographic and genetic connectivity networks (AMMRN) among them. Thus the impacts of ocean sprawl are not always negative (Guerra-García et al., 2004; García-Gómez et al., 2011; García-Gómez et al., 2015; Bishop et al., 2017). There are also opportunities to manipulate these habitats to enhance biodiversity in general (Firth et al., 2016; Morris et al., 2019; O'Shaughnessy et al., 2020; Strain et al., 2020; Evans et al., 2021), or to enhance recruitment to populations of target species such as limpets (Martins et al., 2010).

In conclusion, the proposed translocation method aligns with the IUCN guidelines: (i) the population in the reception area is small (Espinosa et al., 2013); (ii) nonetheless, the presence of hundreds of individuals in the reception and surrounding areas indicates that the environment is good enough to sustain populations of the species (Maestre-Delgado et al., 2018); (iii) threats in the reception area are mitigated or eliminated by preventing public access by means of fences, to reduce the harvesting that has been proven as the main driver jeopardizing *P. ferruginea* populations (Espinosa, Rivera-Ingraham, Fa, & García-Gómez, 2009); (iv) the removal of individuals from the donor site does not have negative impacts on the source population, given the available information about its population dynamics, number of individuals, and viability analysis (Espinosa et al., 2018; Ostalé-Valriberas et al., 2023); and (v) the source population and the reinforced population belong to the same genetic cluster (Casu et al., 2011).

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CONFLICT OF INTEREST STATEMENT

All the authors declare that they have no conflicts of interest associated with this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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