frontiers | Research Topics

True limpets as living resources: Biology, ecology, exploitation and sustainability

Edited by
Carlos Alberto Pestana Andrade, Stephen John Hawkins,
Alan Hodgson, Tomoyuki Nakano and Diego Castejón Bueno

Published in
Frontiers in Marine Science

ISSN 1664-8714
ISBN 978-2-8325-4395-5
DOI 10.3389/978-2-8325-4395-5

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

True limpets as living resources: Biology, ecology, exploitation and sustainability

Topic editors
Carlos Alberto Pestana Andrade — Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Portugal
Stephen John Hawkins — University of Southampton, United Kingdom
Alan Hodgson — Rhodes University, South Africa
Tomoyuki Nakano — Kyoto University, Japan
Diego Castejón Bueno — Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Portugal

Citation
Andrade, C. A. P., Hawkins, S. J., Hodgson, A., Nakano, T., Bueno, D. C., eds. (2024). *True limpets as living resources: Biology, ecology, exploitation and sustainability*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4395-5

Table of contents

frontiers | Frontiers in Marine Science

TYPE Editorial
PUBLISHED 18 January 2024
DOI 10.3389/fmars.2024.1364518

OPEN ACCESS

EDITED AND REVIEWED BY
Stephen J. Newman,
Western Australian Fisheries and Marine Research Laboratories, Australia

*CORRESPONDENCE
Diego Castejón
✉ diego.castejon.dcb@gmail.com

RECEIVED 02 January 2024
ACCEPTED 10 January 2024
PUBLISHED 18 January 2024

CITATION
Castejón D, Hodgson A, Nakano T, Hawkins SJ and Andrade CAP (2024) Editorial: True limpets as living resources - biology, ecology, exploitation and sustainability. *Front. Mar. Sci.* 11:1364518. doi: 10.3389/fmars.2024.1364518

Editorial: True limpets as living resources - biology, ecology, exploitation and sustainability

Diego Castejón[1,2]*, Alan Hodgson[3], Tomoyuki Nakano[4], Stephen John Hawkins[5] and Carlos Alberto Pestana Andrade[6]

[1]Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Funchal, Portugal, [2]Interdisciplinary Center for Marine and Environmental Research, University of Porto, Matosinhos, Portugal, [3]Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa, [4]Seto Marine Biological Laboratory, Kyoto University, Wakayama, Japan, [5]School of Ocean and Earth Science, Faculty of Environmental and Life Sciences, University of Southampton, Southampton, United Kingdom, [6]MARE – Marine and Environmental Sciences Centre | ARNET – Aquatic Research Network, Regional Agency for the Development of Research, Technology and Innovation (ARDITI), Funchal, Portugal

KEYWORDS

limpets taxonomy, limpets biology, limpets ecology, limpets exploitation, patellogastropoda

Editorial on the Research Topic
True limpets as living resources - biology, ecology, exploitation and sustainability

Introduction

True limpets (Patellogastropoda Lindberg, 1986) constitute a globally distributed and prominent group of marine gastropods, inhabiting environments ranging from rocky seashores to abyssal depths. Their scientific significance lies in their evolutionary status as basal gastropods, adaptive strategies for survival in challenging conditions, and their role as a dominant group influencing biological communities on rocky substrata (Lindberg, 2008; Henriques et al., 2017). Limpets play a pivotal role as keystone grazers influencing the macro-algal vegetation on rocky shores (Hawkins and Hartnoll, 1983; Jenkins et al., 2005; Coleman et al., 2006), exerting seasonal top-down control on microbial films (Thompson et al., 2004) and are crucial to local algal patchiness (Johnson et al., 1997; Burrows and Hawkins, 1998; Johnson et al., 1998). Limpets also have been exploited since the Pleistocene (Marean et al., 2007). Currently, limpets support various human activities such as food consumption, ornamentation, and as fishing bait (Firth, 2021). In this context, over exploitation coupled with other anthropogenic disturbances has led to increased sustainability risks for numerous limpet populations (Martins et al., 1987; Espinosa and Rivera-Ingraham, 2017; Carballo et al., 2023). Recent advances in methodologies related to the aquaculture (Castejón et al., 2022; Ferranti et al., 2022) and settlement (Castejón et al., 2023a; Castejón et al., 2023b) of different limpet species present an opportunity to improve our understanding of their biology and ecology. This progress is encouraging for the conservation of these species and the potential establishment of restocking programs.

The Research Topic titled "*True Limpets as Living Resources: Biology, Ecology, Exploitation, and Sustainability*" aims to provide a comprehensive understanding of this unique animal group, covering diverse aspects such as ecology, taxonomy, conservation, and sustainability. The collaborative efforts of researchers from Africa, America, Asia, and Europe are integrated into ten Original Research articles and one Systematic Review.

Methods and recent advances in limpet conservation

The critically endangered "ferruginous limpet" *Patella ferruginea* takes center stage in four publications. Ostalé-Valriberas et al. assess the species status in Ceuta (close to the Strait of Gibraltar), revealing a significant population unit and support for the concept of "Artificial Marine Micro-Reserves" as a viable conservation measure aligned with IUCN guidelines. Cascales-Soler et al. review the literature on *P. ferruginea*, concluding that Marine Protected Areas (MPAs) facilitate the growth of larger individuals without influencing population mean density. Conservation efforts extend to technical applications revealing associated risks and optimization practices; with Espinosa et al. developing translocation methods using Artificial Inert Mobile Substrata, and García-Gómez et al. exploring an alternative translocation strategy based on extracting individuals from their natural environment.

Taxonomy and identification challenges

Given the inherent challenges in limpet taxonomy and identification, three publications focus on this aspect. Hollister et al. leverage advances in computer vision to aid shell morphology-based limpet identification. Molecular tools contribute to understanding the evolutionary history and inter-relationship of different limpet taxa. This approach was used to study the biogeography of three *Nacella* species in South America by González-Wevar et al., as well as the internal phylogeny of the Patellogastropoda through including the mitochondrial genome of new Acmaeidae and Lottiidae species by Putri et al.

Ecological insights

Limpet ecology, a timeless subject, is explored in three publications. Branch et al. investigate interactions between three invasive species and the limpet *Scutellastra granularis* in South Africa, revealing nuanced impacts dependent on the ecology of the species involved, environmental conditions, and interspecies interactions. Seabra et al. delve into the recruitment of co-occurring patellids and a siphonariid in Southwest Portugal, highlighting the importance of rock pools and coralline algae as nursery grounds. Vasconcelos et al. provide insights into the fecundity of fishery species in Madeira Island, utilizing histological approaches to describe their reproductive cycle, potentially enhancing adult conservation strategies. Sousa et al. contribute a survey on mollusc consumption in Madeira Island, emphasizing the predominant importance of limpets.

Conclusion

In summary, the diverse and comprehensive research presented in this Research Topic sheds light on the multifaceted aspects of true limpets, encompassing their biology, ecology, exploitation, and potential pathways towards sustainability. The collaborative efforts of researchers from various continents contribute significantly to our understanding of these marine gastropods, providing crucial insights for informed conservation and management strategies in the face of escalating threats to limpet populations.

Author contributions

DC: Conceptualization, Writing – original draft, Writing – review & editing. AH: Writing – review & editing. TN: Writing – review & editing. SH: Writing – original draft, Writing – review & editing. CA: Conceptualization, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Burrows, M. T., and Hawkins, S. J. (1998). Modelling patch dynamics on rocky shores using deterministic cellular automata. *Mar. Ecol. Prog. Ser.* 167, 1–13. doi: 10.3354/meps167001

Carballo, J. L., Bautista-Guerrero, E., Benjamín, Y., Carballo, L. R., and García-Gómez, J. C. (2023). Population status of the world's largest limpet, *Scutellastra mexicana* (Mollusca: Patellidae), on María Madre Island, Mexico: Possible last bastion of a species facing global extinction. *Aquat. Conservation: Mar. Freshw. Ecosyst.* 33, 1049–1060. doi: 10.1002/aqc.3978

Castejón, D., García, L., and Andrade, C. (2023a). Crustose coralline algal factors determining the success of limpet (Patellogastropoda: Patellidae) settlement: species, exposure time, area and soluble cues. *Mar. Biol.* 170, 171. doi: 10.1007/s00227-023-04321-1

Castejón, D., García, L., Cañizares, J. M., De Girolamo, M., Nunes, C., Isidro, E., et al. (2022). Methodologies for patellid limpets' Aquaculture: from broodstock management to juveniles. *Front. Mar. Sci.* 9, 884262. doi: 10.3389/fmars.2022.884262

Castejón, D., García, L., Nogueira, N., and Andrade, C. (2023b). Response of true limpet *Patella aspera* (Patellogastropoda) pediveliger larvae to a range of settlement cues. *Mar. Freshw. Res.* 74, 1296–1313. doi: 10.1071/MF22270

Coleman, R. A., Underwood, A. J., Benedetti-Cecchi, L., Åberg, P., Arenas, F., Arrontes, J., et al. (2006). A continental scale evaluation of the role of limpet grazing on rocky shores. *Oecologia* 147, 556–564. doi: 10.1007/s00442-005-0296-9

Espinosa, F., and Rivera-Ingraham, G. A. (2017). "Chapter Three - Biological conservation of giant limpets: the implications of large size," in *Advances in Marine Biology*. Ed. B. E. Curry (United Kingdom & United States: Academic Press), 105–155.

Ferranti, M. P., Guallart, J., Fanciulli, G., Panzalis, P. A., and Chiantore, M. (2022). Advancements towards restoration of the endangered limpet *Patella ferruginea* Gmelin 1791 through controlled reproduction. *Aquaculture Res.* 53, 782–798. doi: 10.1111/are.15614

Firth, L. B. (2021). What have limpets ever done for us?: On the past and present provisioning and cultural services of limpets. *Int. Rev. Environ. History* 7, 5–45. doi: 10.22459/IREH.07.02.2021.01

Hawkins, S. J., and Hartnoll, R. G. (1983). Grazing of intertidal algae by marine invertebrates. *Oceanography Mar. Biol. Annu. Rev.* 21, 195–282.

Henriques, P., Delgado, J., Sousa, R., and Ray, S. (2017). "Patellid limpets: an overview of the biology and conservation of keystone species of the rocky shores," in *Organismal and Molecular Malacology* (London: IntechOpen Limited), 71–95.

Jenkins, S. R., Coleman, R. A., Della Santina, P., Hawkins, S. J., Burrows, M. T., and Hartnoll, R. G. (2005). Regional scale differences in the determinism of grazing effects in the rocky intertidal. *Mar. Ecol. Prog. Ser.* 287, 77–86. doi: 10.3354/meps287077

Johnson, M. P., Burrows, M. T., Hartnoll, R. G., and Hawkins, S. J. (1997). Spatial structure on moderately exposed rocky shores: patch scales and the interactions between limpets and algae. *Mar. Ecol. Prog. Ser.* 160, 209–215. doi: 10.3354/meps160209

Johnson, M. P., Burrows, M. T., and Hawkins, S. J. (1998). Individual based simulations of the direct and indirect effects of limpets on a rocky shore Fucus mosaic. *Mar. Ecol. Prog. Ser.* 169, 179–188. doi: 10.3354/meps169179

Lindberg, D. (2008). "Patellogastropoda, neritimorpha, and cocculinoidea," in *Phylogeny and evolution of the Mollusca*. Eds. D. R. Ponder and D. R. Lindberg. United States: University of California Press, 271–296.

Marean, C. W., Bar-Matthews, M., Bernatchez, J., Fisher, E., Goldberg, P., Herries, A. I. R., et al. (2007). Early human use of marine resources and pigment in South Africa during the Middle Pleistocene. *Nature* 449, 905–908. doi: 10.1038/nature06204

Martins, H., Santos, R., and Hawkins, S. (1987). Exploitation of limpets (*Patella* spp.) in the Azores with a preliminary analysis of the stocks. *ICES Rep.* 53, 1–17.

Thompson, R. C., Norton, T. A., and Hawkins, S. J. (2004). Physical stress and biological control regulate the producer-consumer balance in intertidal biofilms. *Ecology* 85, 1372–1382. doi: 10.1890/03-0279

Frontiers in Marine Science

TYPE Original Research
PUBLISHED 29 March 2023
DOI 10.3389/fmars.2023.1100194

EDITED BY
Stephen John Hawkins,
University of Southampton,
United Kingdom

REVIEWED BY
Gustavo Martins,
University of the Azores, Portugal
Tomas Vega Fernandez,
Anton Dohrn Zoological Station Naples,
Italy

*CORRESPONDENCE
Free Espinosa
free@us.es

SPECIALTY SECTION
This article was submitted to
Marine Fisheries, Aquaculture and Living
Resources,
a section of the journal
Frontiers in Marine Science

RECEIVED 16 November 2022
ACCEPTED 16 March 2023
PUBLISHED 29 March 2023

CITATION
Espinosa F, Maestre M, García-Gómez JC, Cotaina-Castro MI, Pitarch-Moreno C, Paramio JM, Fort-Santa María P and García-Estévez N (2023) Joining technology and biology to solve conservation problems through translocation in the endangered limpet *Patella ferruginea*. *Front. Mar. Sci.* 10:1100194.
doi: 10.3389/fmars.2023.1100194

Joining technology and biology to solve conservation problems through translocation in the endangered limpet *Patella ferruginea*

Free Espinosa[1]*, Manuel Maestre[1], José Carlos García-Gómez[1], María Isabel Cotaina-Castro[2], Carmen Pitarch-Moreno[2], Juan Manuel Paramio[2], Patricia Fort-Santa María[3] and Natalia García-Estévez[3]

[1]Laboratorio de Biología Marina, Departamento de Zoología, Facultad Biología, Universidad de Sevilla, Sevilla, Spain, [2]División de Medio Ambiente y Prevención de Riesgos Laborales, Departamento de Infraestructuras, Autoridad Portuaria de Melilla, Melilla, Spain, [3]Departamento de Medio Ambiente. Acciona Ingeniería, Madrid, Spain

The Anthropocene era is characterized by a biodiversity crisis, where many species are pushing to extinction, causing alteration of the stability in the ecosystems and loss of their services. In this context, the translocation of endangered species is a powerful resource in conservation as long as its goals and appropriateness are clearly stated and it matches unambiguous criteria. *Patella ferruginea* is one of the most endangered marine species in the Mediterranean, and several translocations have been made with limited results in terms of survival. A methodology that mimics the natural topography of the substrate in the donor population based on Artificial Inert Mobile Substrates (AIMS, 40x20x10 cm) is proposed for restocking purposes. Three different treatments were tested: on substrate, slope, and cantilever depending on the position in which the AIMS were deployed. A total of 660 AIMS were installed within the donor area. The installation of such AIMS did not negatively affect either donor or receiving populations. A total of 188 specimens were translocated in three different attempts in 2018, 2019, and 2020, and no mortality was observed during the translocation process that lasted 24 h. Survival through the medium and long-term for the translocated specimens ranged from 80.6 to 91.5% after one month, 55.5 to 80.9% after eight months, and 48.4 to 76.6% after one year, with survival in the control population being 91.6% after four months and 87.5% after one year. Overall, recruits showed higher survival values than adults, whereas substrate treatment hosted more specimens (both recruits and adults) than slope and cantilever ones, while recruits were more abundant on cantilever treatment. The methodology has been proven to be useful for restocking and reintroduction purposes between donor and receiving areas.

KEYWORDS

Patella ferruginea, translocation, conservation, endangered species, limpets

Introduction

A large mass extinction debt exists today due to habitat disruption by humans within a new era called Anthropocene (Spalding and Hull, 2021). Species perform critical functions in ecosystem dynamics and provide resilience under ever-changing environments (Tilman et al., 2014; Reyers et al., 2018). Therefore, biodiversity erosion induces alteration of the stability in the ecosystems, loss of ecosystem services, and affects human life and socioeconomic systems. In this regard, habitat destruction and overexploitation have been the most relevant threats to biodiversity, being drivers to push so many species to extinction thresholds (Primack and Ros, 2002).

A key tool to offset this biodiversity crisis caused by human impacts on populations and ecosystems has been species translocations (see Pérez et al., 2012 and references therein). However, even though it could be a powerful resource in conservation biology, is not free of criticism due to its use without clear goals, lack of comprehensive analysis of root causes of population decline (Meffe, 1992), and unambiguous criteria to assess if a given project is going to be successful and its implementation is appropriate (Pérez et al., 2012). Translocations involve considerable risks and, consequently, must rely on the best practice guidelines for further policy application (Swan et al., 2016).

Translocations have been conducted elsewhere and have focused on a wide range of species but mainly on terrestrial vertebrates and birds (Seddon et al., 2005, Seddon et al., 2007), with marine species scarcely mentioned in conservation translocation literature reviews (Swan et al., 2016). Moreover, most marine translocations have focused on ecosystem restoration and catch augmentation for economic purposes, with the translocations focused on single species of conservation concern for preventing their extinction being very scarce (Swan et al., 2016). Therefore, there is a paucity of knowledge about the techniques, success rates, and outcomes in the translocation of marine invertebrates in particular. For instance, out of 174 translocation projects in Spain, none were focused on marine species (Pérez et al., 2012). In general, the term translocation includes three different concepts: introduction (the release of specimens outside of its historically known native range), reintroduction (the release of specimens within its historically known native range from which the species was extirpated), and restocking (the release of specimens within an area where the species is found in order to rebuild its stock).

Patella ferruginea (Mediterranean ribbed limpet) is considered the most endangered marine invertebrate in the Mediterranean along with the noble pen shell *Pinna nobilis* (Ramos, 1998; Vázquez-Luis et al., 2017). Its distributional range originally encompassed the entire Western Mediterranean basin but has become restricted to a few areas, being almost extinct in continental Europe (Laborel-Deguen and Laborel, 1991a; Espinosa et al., 2013). It is a giant limpet that lives within the upper intertidal and shows homing behavior, reaching 10 cm in length with remarkable ribs and a star-shaped shell (Espinosa and Rivera-Ingraham, 2017). Due to its conspicuous shell, intertidal habitat, and great size, the species has been harvested by humans since the Palaeolithic Age (Fa et al., 2016; Espinosa and Rivera-Ingraham, 2017). Both human harvesting and habitat destruction by coastal development have been the main drivers to put the species at risk of extinction (Espinosa et al., 2018). Currently, there are only dense and well-preserved populations in scattered points along the Northern African coast: Ceuta, Melilla, and Chafarinas Islands (Spanish enclaves); Rachgoun, Habibas, and Plane Islands (Western Algeria); and Zembra Island (Tunisia). Smaller populations can be also found in Corsica and Sardinia (Espinosa et al., 2013). Out of these places, only isolated specimens have been recently discovered on the Ligurian coast in mainland Italy (Ferranti et al., 2019) or in Egadi and Pantelleria islands (Espinosa et al., 2013). Focusing on the Iberian Peninsula, the species is only found in the Southernmost part from Palos Cape to the Strait of Gibraltar but in low density and showing a fragmented distribution (Espinosa et al., 2013; https://www.juntadeandalucia.es/medioambiente/portal/landing-page-documento/-/asset_publisher/jXKpcWryrKar/content/informes-regionales-sobre-gesti-c3-b3n-sostenible-del-medio-marino-andaluz-2008-2018-/20151). Within this area, the most important population is settled in the Algeciras Bay where hundreds to a few thousands of scattered specimens have been reported (Fernández-Casado et al., 2017). Furthermore, García-Gómez et al. (2011; 2015) pointed out that some artificial substrates hosted important populations of this species (and other endangered species as well), and those sites with limited access could be considered for management and conservation of endangered species as a new figure of protection named Artificial Marine Micro Reserves (AMMRs). The inner part of Gibraltar Bay hosted several specimens settled in the breakwater of 'La Línea' harbor where the access has been restricted by fences, being a location with the potential to develop a reproductive population in this context. It is worth noting that the species is a broadcast spawner, showing protandrous hermaphroditism, and fecundity is highly dependent on size (Espinosa et al., 2006a). Consequently, a minimum number of closely adjacent specimens, with different size classes are required for successful fertilization.

The increasing interest in the conservation of this species has prompted previous translocations for conservation purposes or derived from engineering works in which repair of existing breakwaters or deployment of new ones have threatened local populations (Laborel-Deguen and Laborel, 1991b; Espinosa et al., 2008; APM (Autoridad Portuaria de Melilla), 2014; Fa et al., 2018; Zarrouk et al., 2018). Unfortunately, high mortality rates have often been observed, although the translocations made with the specimens attached to their own substrate have been proven most successful (Fa et al., 2018). Nevertheless, this approach based on moving entire boulders in a breakwater where the limpets are settled seems difficult to use for many individuals or over long distances. Consequently, its applicability in reinforcement or reintroduction of this or similar endangered species would be limited. Moreover, substrate heterogeneity has been shown as a main driver that influences recruitment in limpets (Martins et al., 2010; Espinosa et al., 2011), and, therefore, it should be taken into consideration in any further translocation procedure. Furthermore, Potet et al. (2021) indicated that the surface for recruitment of the European flat oyster (*Ostrea edulis*) must be irregular at the scale of mm.

The goals and criteria to assess the outcomes and appropriateness of translocation must be clearly established. In this sense, Pérez et al. (2012) pointed out that any translocation must be properly justified in terms of necessity, risk evaluation, and technical suitability, providing ten major criteria to be considered prior to undertaking any intervention. The present translocation experiment complies with these requirements, and therefore, the likelihood of success was maximized, and the utility of the study was properly justified from a conservation perspective (see Table 1 for details).

Our aim was to provide a valid protocol to translocate specimens of *Patella ferruginea* to re-stock depleted or repopulated areas by mimicking the original habitat of the donor population. The specific objective was to test the influence of position and impact on the natural substrate on the efficacy of deployment of Artificial Inert Mobile Substrates (AIMS) in translocation,

Material and methods

Study area

The donor population was located in the autonomous city of Melilla, a Spanish enclave in North Africa, where a dense population estimated at 32,821 adults (>3 cm) was found (Guallart et al., 2013). According to these authors, most of the population (68.1%) was on the exterior breakwater of the Melilla's harbor where the specimens were collected for translocation and the mean density of adults (per linear length of the breakwater) was 17.6 ind./m with maximum values up to 66.5 ind./m. Melilla Harbor is exposed to strong wave action and has a low risk of marine spills, being considered moderately pristine in terms of environmental quality due to the efficiency of management measures in place,

TABLE 1 Matching criteria for translocations (adapted from Pérez et al., 2012 based on IUCN guidelines and already published literature).

Level	Criteria	Matching for *Patella ferruginea*	References
1st Necessity of the translocation	1.- Is the species or population under threat?	YES. The species is listed as endangered in several regional, national, and international laws or agreements.	BOE, 1999; Barea-Azcón et al. (2008); Espinosa and Rivera-Ingraham (2017)
	2.- Have the threatening factors been removed or controlled, or were they absent in the release area?	YES. Threatening factors are mainly human harvesting and pollution. The release area is a breakwater of private property fenced and under video surveillance with presence of native specimens. Therefore, human harvesting is controlled, and physicochemical requirements are met.	Espinosa et al. (2013) https://www.europapress.es/esandalucia/cadiz/noticia-junta-colaborara-apba-preservar-invertebrado-marino-peligro-extincion-20150518123416.html
	3.- Are translocations the best tool to mitigate conservation conflicts?	YES. *In situ* conservation actions are already implemented in the receiving area but the population is still small comparing with other populations within its distributional area.	https://www.juntadeandalucia.es/medioambiente/portal/landing-page-documento/-/asset_publisher/jXKpcWryrKar/content/informes-regionales-sobre-gesti-c3-b3n-sostenible-del-medio-marino-andaluz-2008-2018-/20151
2nd Risk evaluation	4.- Are risks for the target species acceptable?	YES. The expected number of translocated specimens (recruits +adults) were 500 at the beginning of the project (188 after), i.e., only 0.5% of the total estimated adult population in the donor site. Genetic relatedness exists.	Guallart et al. (2013); Casu et al. (2011) https://www.liferemopaf.org/web-informe-final-30062021/
	5.- Are risks for other species or ecosystem acceptable?	YES. The species had not long been extirpated from the receiving site, and it is not neither a top predator nor outcompeted sympatric species according with previous studies about competitive interactions.	Rees (2001); Espinosa et al. (2006b); Ricciardi and Simberloff (2009)
	6.- Are the possible effects of the translocation acceptable to local people?	YES. The target species would not be able to jeopardize human lives in any way within the receiving area.	https://www.liferemopaf.org/web-informe-final-30062021/
3rd Technical and logistical suitability	7.- Does the project maximize the likelihood of establishing a viable population?	YES. The fact that a small native population already exists indicates that the requirements for the species are met. Moreover, the translocated specimens will be allocated within a short fringe for facilitating the reproduction.	Denny and Shibata (1989); Fernández-Casado et al. (2017); Espinosa and Rivera-Ingraham (2017)
	8.- Does the project include clear goals and monitoring?	YES. Goals are clearly stated at the beginning of the project whereas a standardized monitoring is considered.	https://www.liferemopaf.org/
	9.- Do enough economic and human resources exist?	YES. Three different entities are involved covering technical and scientific topics with the economic support of the European Commission through LIFE projects.	https://www.liferemopaf.org/
	10.- Do scientific, governmental, and stakeholder groups support the translocation?	YES. The project has the scientific support by the University of Sevilla (Spain) and stakeholders such as environmental administrations and harbour authorities support the project.	https://www.liferemopaf.org/

which comprises environmental management, eco-efficiency, and environmental quality (see Fernández-Macho, 2016; González-Laxe et al., 2017).

In contrast, the population to be re-stocked was located in the exterior breakwater of the harbor in La Línea in the inner part of the Algeciras Bay (Figure 1), where a scattered population has been reported with a mean density below 1 ind./m (https://www.juntadeandalucia.es/medioambiente/portal/landing-page-documento/-/asset_publisher/jXKpcWryrKar/content/informes-regionales-sobre-gesti-c3-b3n-sostenible-del-medio-marino-andaluz-2008-2018-/20151). This population was within an enclosed area under surveillance with restriction to public access (Fernández-Casado et al., 2017), being appropriate to undertake restocking for conservational purposes as has been noted previously. The area nearby the restocked population showed slight pollution levels (see Dissanayake and Bamber, 2010; Rojo-Nieto et al., 2011; Rojo-Nieto and Perales, 2015), with moderate wave exposure.

Artificial inert mobile substrates design

The specimens were transplanted using artificial plates designed to mimic the topography of the substrate where the donor population was settled. For it, the surface of the rock was recorded using different 360° cameras and the images were digitalized. The electronic file was used to create an ABS – FDM (Acrylonitrile Butadiene Styrene - Fused Deposition Modeling) mold using 3D printing technology by the Materialise® company that was subsequently allocated on conventional concrete (type HA-35/F/12/IIIc+Qb) with internal reinforced steel to print a 'negative' that recreated the microtopography of the original substrate. The plates could be easily screwed and unscrewed from the rocks, having a dimension of 40 x 20 x 10 cm (length, width, and height), and showed resistance and durability for 25 years according to the calculations made considering the forces from the wave action in the area (Acciona Ingeniería, unpublished data), covering the life expectancy of the species (see Espinosa et al., 2008).

To test the effect of the AIMS position on Mediterranean ribbed limpet recruitment rate as well as their impact on the intertidal zone, three different treatments were used: Onto the substrate (OS), in which the AIMS were directly fixed on the rock surface so that continuity of hard substrate was maximized, while their impact on the natural substrate through occupation was high; Sloped (S), in which the AIMS were fixed to the rocky substrate at an angle to the natural substrate so that the impact of the manipulation on the natural substrate was largely reduced, while habitat continuity was still substantial; and Cantilever (C), where the AIMS were fixed above the rock surface without direct contact with it, so that the impact of the experiment on the natural substrate was reduced to a minimum at the cost of very reduced hard substrate continuity for limpets (Figure 2). Therefore, it would be expected that colonization

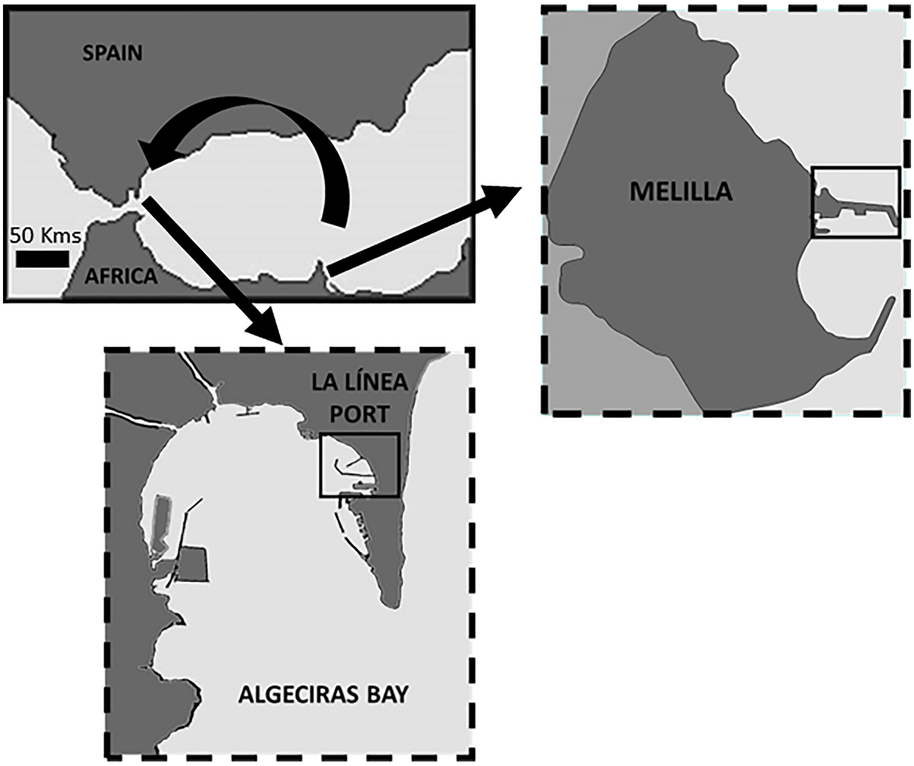


FIGURE 1
Map of the study area showing the donor and receptor sites located in Melilla and La Línea ports, respectively.

and the impact within the intertidal habitat (both of major conservation concern) followed the pattern OS>S>C, according to the decreasing contact with the surrounding environment.

Temporal monitoring of limpet populations

To test if the installation of the AIMS could affect either donor or receiving populations, monitoring was conducted between September 2017 and June 2021. In the donor population, a total of seven 10 m transects were set up along the breakwater and were considered as 'Controls'. Moreover, additional seven transects were interspersed with controls and there, a total of six AIMS were placed per transect (two for each type of position: OS, S, and C), being those transects considered as 'AIMS'. Within each transect, all the adult specimens (>3 cms) were counted and measured using a caliper, recording the longitudinal axis of the shell approximately every four months (Espinosa et al., 2008). Similarly, in the receiving population, three control and three AIMS transects were also set up and monitored since the restocking was performed. The AIMS were deployed in fewer numbers than in the donor population due to its smaller area. Data were recorded similarly (every four months) for the receiving population and the donor population groups, although there was a gap in the first months of 2020 due to logistical constraints derived from the Covid-19 pandemic.

Apart from the status of both populations in donor and receiving areas, a community assessment was carried out within the intertidal habitat where *Patella ferruginea* was located. Five sites were randomly distributed within the donor and receiving populations and five quadrats of 25 x 25 cm were photographed per site either at low or high tidal levels (+25 and +75 cm above zero tidal level, respectively). Each photograph was analyzed digitally by adding a grid of 25 subquadrants to obtain the coverage of each species in % (Guerra-García et al., 2006).

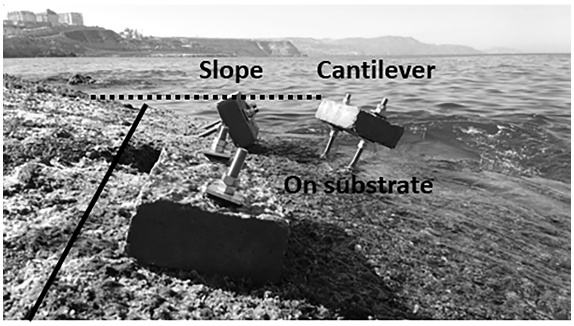


FIGURE 2
Artificial Inert Mobile Substrates (AIMS) used for translocating individuals of *Patella ferruginea*. The three different treatments considered are shown: on substrate, slope, and cantilever. The solid line indicates the lower limit of the habitat of *Patella ferruginea* just above the macroalgal turf (note that deployed AIMS were installed out of the habitat of *Patella ferruginea*). The dashed line indicates the corresponding tidal level of the AIMS surface that relied on the tidal height where the habitat of *Patella ferruginea* is found.

In addition to biological data, several environmental parameters (pH, temperature, salinity, and turbidity) were recorded *in situ* by taking water samples in the vicinity of the transects. All the parameters were measured using a multiparameter probe CRISON MM40© and turbidimeter WTW 335IR©.

Translocation methodology

A total of 660 AIMS were deployed in the donor population several months prior to the recruitment period of the species (late Autumn-early Winter) to allow the development of the typical microfilm on which the species feeds (Burgos-Rubio et al., 2015). Due to logistical constraints in terms of fabrication, transporting, and deploying in the donor population area within the precise tidal level, two different installation events were undertaken. A pilot attempt was done in July-August 2017 when a total of 150 AIMS were deployed, whereas the other 510 AIMS were deployed between June-September 2018. Three different translocation events were undertaken in October 2018, 2019, and September 2020. The AIMS were monitored annually in early September and the specimens settled in them were registered for subsequent translocation. Once the AIMS with recruits and adults were identified, they were unscrewed at low tide, the limpets measured in length, and marked using epoxy putty (Espinosa et al., 2008). Such extracted AIMS were replaced with new ones after the first and second translocations, so the total number of AIMS in the donor population remained constant at 660. The AIMS were then introduced into a plastic container with a rubber base to avoid vibration, since it has been indicated that vibration could detach limpets (Fa et al., 2018). Finally, they were covered with towels soaked with marine water, introduced in a refrigerated truck at 16° C, and transported to the receiving zone. All the AIMS were screwed on the receiving zone at low tide within the next 24h.

Limpets were monitored 24h and 48h after transplantation, weekly during the first month, monthly during the first four months, and every four months afterward. The position of the limpets was recorded to see if they had left the AIMS to settle on the rock and all the transplanted specimens were also measured in length at the different monitoring times to obtain growth rate values.

Statistical analyses

A repeated-measures ANOVA (RM-ANOVA) was carried out to test whether the mean size or density of adults significantly varied through time using all transects (intrasubject factor 'Time' and intersubject factor 'Treatment' with two levels: Control *vs.* AIMS). Mauchly's test of sphericity was used to test the assumption that variance in the differences between all possible pairs of groups is equal. The Huynh-Feldt correction was used when an absence of sphericity was detected.

Possible differences in survival among the three transplantations (2018, 2019, and 2020) through time as well as among recruits and adults were tested using a Chi-squared test. A one-way ANOVA test was used to test differences in growth rates.

A matrix of the community structure based on species composition and their coverages was employed to test if the intertidal community changed through the monitored time using the SIMPROF test. The analysis was run on a triangular similarity matrix derived from the values of the Bray-Curtis similarity on fourth root transformed coverage data. A similar analysis was performed with environmental variables using Euclidean distance instead of Bray-Curtis similarity.

Univariate analyses (Chi-squared test, ANOVA, and RM-ANOVA) were performed using SPSS© software, and multivariate analyses (SIMPROF test) were conducted using PRIMER v.6 +PERMANOVA package (Clarke and Gorley, 2006).

Results

Monitoring in donor and receiving populations

During the two years of monitoring (from September 2017 to September 2019) mean size values (± SE) of the donor population in Melilla port slightly decreased in both control and AIMS transects (from 4.63 ± 0.11 to 4.33 ± 0.06 cm and from 4.95 ± 0.08 to 4.42 ± 0.04 cm, respectively) (Figure 3A). RM-ANOVA indicated significant differences over time in mean size values (Table 2), but not between treatments (control *vs.* AIMS). Moreover, density values fluctuated similarly for both control and AIMS transects (Figure 3A) Nevertheless, the AIMS transects showed an increase from 22.3 to 27.03 ind./m after two years, whereas the control ones remained with similar values after that period (19.21 to 18.57 ind./m), showing fluctuations over the monitored time. In fact, the RM-ANOVA also showed significant differences in density through time (Table 2) but not between treatments (control *vs.* AIMS).

Regarding the receiving population in La Línea port, an increase in mean size values was recorded for control and AIMS transects (from 5.39 to 6.15 cm and from 5.54 to 5.5 cm, respectively) (Figure 3B). The RM-ANOVA also indicated significant differences over time in mean size values (Table 3) but not between treatments (control *vs.* AIMS). Similarly, density values showed an increase from 0.63 to 1.8 ind./m within the AIMS transects and from 0.53 to 1.03 ind./m within the control ones (Figure 3B). The results of the RM-ANOVA were like those recorded for size values (Table 3) or even for the donor population: differences over time but not between the considered treatments.

The intertidal assemblages in the donor (Melilla port) and receiving populations (La Línea port) did not show differences in structure composition over the monitored time according to the SIMPROF test. Furthermore, environmental variables showed similar values throughout the monitoring period, excepting the usual seasonal changes in temperature, and recording low levels of turbidity (0.2-1.65 ntu in Melilla and 0.7-1.6 ntu in La Línea) (Figures 4A, B).

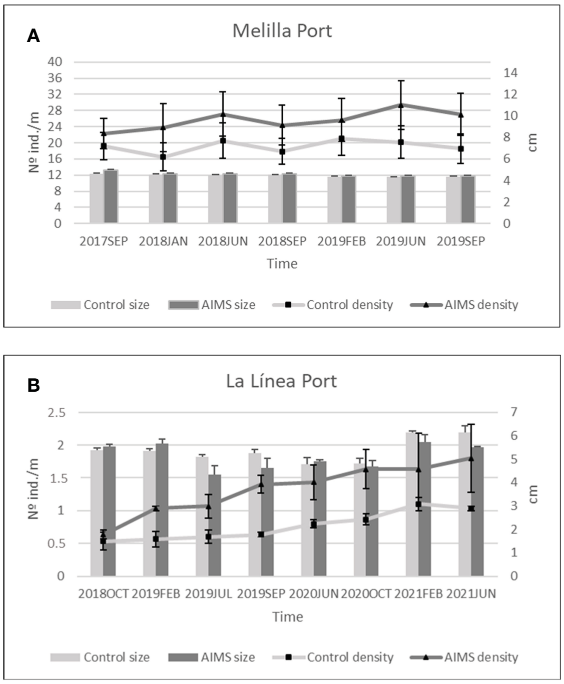


FIGURE 3
Density and size mean values (± SE) during the monitoring period in the Control and AIMS transects. **(A)**: Melilla; **(B)**: La Línea.

Translocation experiments

A total of 188 specimens were translocated in three different translocation events: 31, 47, and 110 respectively (Figure 5). The number of recruits (<3 cm) was higher than adults for the first translocation, but adults outnumbered recruits globally. Besides, the number of AIMS translocated was 20, 40, and 65 for each of the translocation events performed for a total of 125 AIMS translocated to the receiving population during the whole study (Figure 5). The percentage of AIMS with specimens of *Patella ferruginea* settled was 3%, 6%, and 10% for each translocation event, with the total recruited being almost 19% of such experimental structures deployed in the donor population. Regarding the capacity for settlement of *P. ferruginea* individuals on the experimental structures (AIMS), the mean number of individuals per AIMS was similar among the three translocation events (1.55, 1.18, and 1.69 individuals/AIMS, respectively), but the mean number per square meter showed a marked increase from 1.04 to 3.7 ind./m^2 similar to that observed for the mean number of settled individuals in the whole set of AIMS (Figure 6).

Considering the different treatments used (AIMS deployed directly on substrate, sloped, or cantilever) the treatment that supported a greater number of *Patella ferruginea* individuals was onto the substrate, followed by slope and cantilever, and this pattern was constant for each of the three translocations (Figures 7A–D). The results indicated that the distribution of individuals among the three treatments clearly differed from a random one (Chi-square test: 60.1; df: 2; p<0.001). Nevertheless, recruits showed slight differences among translocations (Figures 7A–C), with a greater number on cantilever compared with slope AIMS on the second translocation. Moreover, the distribution of recruits and adults among treatments showed significant differences (Chi-square test: 26.9; df: 2; p<0.001).

During the translocation procedure (from extracting in the donor population, transportation, and reallocation in the receiving population within 24 hours) no specimen died in any of

TABLE 2 Results of a repeated-measured analysis of variance (RM-ANOVA) on the mean size and density of adults (>3 cm) of the monitored transects in the donor population (Melilla).

		Mean size				Density[b]		
Source of variation	df	MS	F	P	df	MS	F	P
	Within-subject variation							
Time[a]	6	0.36	31.23	**<0.001**	4.02	62.62	4.05	**<0.01**
Time x Treatment	6	0.03	2.45	0.069	4.02	23.86	1.54	0.22
Residuals	72	0.01			48.33	15.44		
	Between-subject variation							
Treatment	1	0.38	1.91	0.19		1050.12	1.08	0.32
Residuals	12	0.20				969.39		

[a] Levels of the factor Time correspond with those on Figure 3A.

[b] Huyn-Feldt correction has been used in the case of departure from sphericity for within-subject factor (Time), since ϵ value was lower than 0.75.

Treatment: control *vs* AIMS.

Bold values indicate significant results.

TABLE 3 Results of a repeated-measured analysis of variance (RM-ANOVA) on the mean size and density of adults (>3 cm) of the monitored transects in the receiving population (La Línea).

		Mean size[b]				Density[b]		
Source of variation	df	MS	F	P[b]	df	MS	F	P[b]
	Within-subject variation							
Time[a]	7	1.46	12.87	**<0.001**	2.09	2.045	9.00	**<0.01**
Time x Treatment	7	0.33	2.91	0.08	2.09	0.279	1.23	0.34
Residuals	28	0.11			8.39	0.227		
	Between-subject variation							
Treatment	1	0.26	0.71	0.45		2.34	2.94	0.16
Residuals	4	0.37				0.79		

[a] Levels of the factor Time correspond with those on Figure 3B.

[b] Huyn-Feldt correction has been used in the case of departure from sphericity for within-subject factor (Time), since ϵ value was lower than 0.75.

Treatment: control *vs* AIMS.

Bold values indicate significant results.

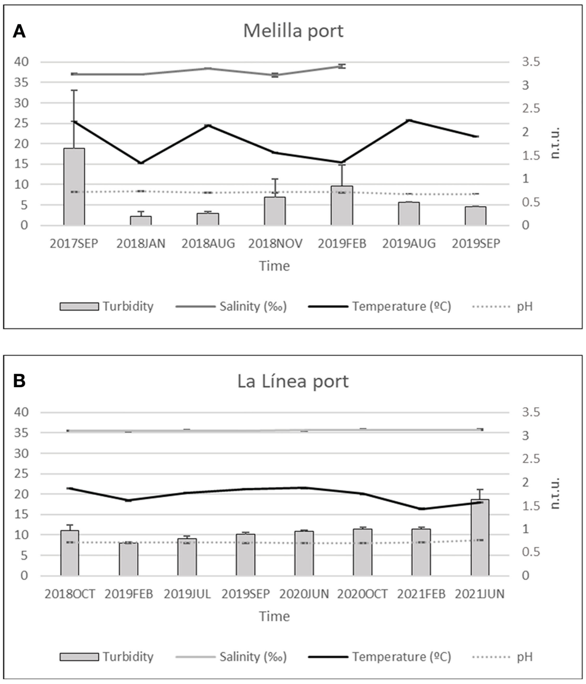


FIGURE 4
Physicochemical variables during the monitoring period. **(A)** Melilla; **(B)** La Línea. Turbidity is represented by the right Y axis (ntu units), whereas salinity, temperature, and pH are all represented by the left Y axis. Salinity values for August and September 2019 in Melilla were not available due to an error with the probe.

the three different attempts. However, the survival of translocated specimens was progressively reduced through time, with differences among the three translocations (Figures 8A–C). Considering all translocations, the mean survival of the three translocations was 87.1% after one month, 75% after four months, and 68% after eight months (Figure 8D). Interestingly, differences were observed between the survival of adults and recruits for all the translocations, with it being greater for recruits than for adults, but significant differences were only recorded in the first translocation (Chi-square test: 1457.9; df: 9; p<0.05). When the three translocations were compared in terms of survival values they showed similar results for the first four months after the translocation, but the differences increased later (Figure 9), ranging from 55.5 to 80.9% after eight months, 48.4 to 76.6% after one year, and 41.9 to 66% after 20 months. Nevertheless, significant differences were only recorded between the second and the third translocations (Chi-square test: 13.4; df: 2; p<0.05). Survival of control individuals in the receiving population showed higher values, being 91.6% after four months, 87.5% after one year, and 83.3% after two years and at 32 months, the end of the monitored period, whereas these values fell to 75%, 62.5%, 41.9%, and 32.3%, respectively, for translocated individuals (Figure 9). The mean observed survival values of the three translocations differed from the expected control values (Chi-square test: 123.7; df: 8; p<0.001).

Growth of translocated individuals was consistently higher for recruits, but also showed differences for the time passed from translocation for the first and second attempts (Figure 10). In this sense, the growth rate was higher in the first month after translocation but decreased over time. The values ranged from 2.09 to 4.19 mm/month for recruits, 0.5 to 1.85 mm/month for adults, and 0.65 to 2.69 mm/month for the total of individuals translocated (recruits+adults). It is worth noting the great increase in the size of some individuals: number 7 during the first translocation increased in size from 2.2 cm to 8.1 cm in 31 months, and numbers 21 and 27 increased in size from 1 cm to 7.7 in the same period. In contrast, the number 36 during the second translocation only increased in size from 6.7 cm to 7.6 cm in 19 months and many others showed similar slow growth. Moreover, high variability in growth rates was recorded among individuals of similar size. In this sense, during the third translocation, specimen number 57 increased from 1.7 cm to 5.5 cm in eight months, whereas number 63 only increased from 1.6 cm to 2.3 cm in the same period.

Discussion

Translocations of individuals for conservation purposes have played a central role in conserving endangered species and routinely yielded the intended benefits without producing unintended harm (Novak et al., 2021). From 1,014 taxa reviewed for 125 years, only a single case of biodiversity loss was reported by these authors in conservation-based governance translocations. Nevertheless, other authors, such as Strayer (2022), have challenged this optimistic view querying the validity of assessments of the costs and benefits of

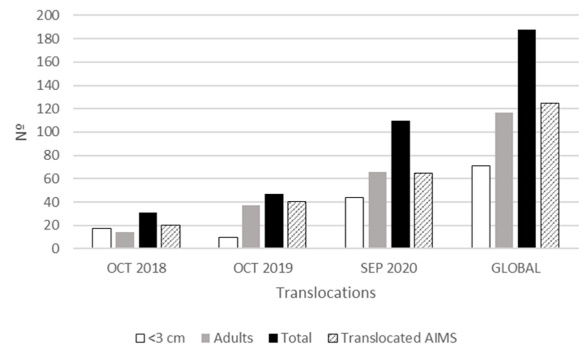


FIGURE 5
Number of individuals (juveniles, adults, and total) translocated per translocation event and at the end of the study (whole). The number of AIMS translocated is also shown.

translocations. The viewpoint is that well-planned translocations could generate ecosystem benefits and should be weighed against the cost of inaction in conservation strategies.

The implemented translocation methodology proved to be feasible in terms of logistical constraints and achievements reached. First of all, the method proposed has been proven not to affect the donor or receiving population according to the results of the monitoring program carried out. The AIMS deployed did not affect the donor or receiving population accordingly with the temporal trends in terms of density and sizes. According to Kleiman et al. (1994), in any translocation, the survival of a wild population of an endangered species must never be jeopardized by such a conservation tool. There were differences in survival between translocation events, with the values of the 2019 translocation being very similar to the control population after one year (76.6% *vs.* 87.5%), whereas the first translocation, done in 2018, only achieved survival of 48.4% after one year. Different values of survival have been already obtained in previous studies, as well as relevant differences in growth rates, sex ratios, or food intake among specimens and populations (Rivera-Ingraham et al., 2011a; Espinosa and Rivera-Ingraham, 2017; Espinosa et al., 2021), indicating high intraspecific variability in life history traits that would be able to affect the specimen's adaptation to different environmental conditions. The same findings had been already reported for other limpets elsewhere (Branch, 1981; Espinosa and Rivera-Ingraham, 2017). Nevertheless, the reported differences can also be a result of a mismatch among different researchers regarding the identification of the relevant temporal and spatial scales of measurement of survival rates in the focused species.

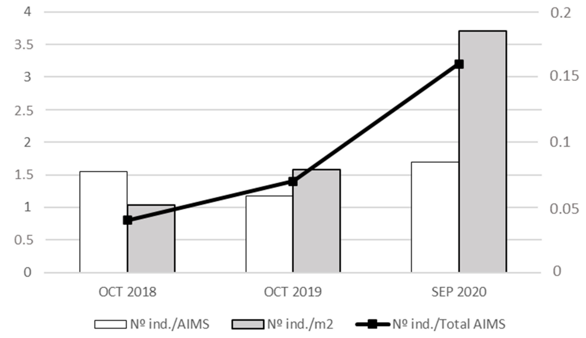


FIGURE 6
Density values per translocation event. No. individuals/AIMS: mean number of individuals per each of the translocated AIMS. No. ind./m^2: mean number of individuals per square meter, considering the whole set of deployed AIMS, which were the available surface for settlement (660 AIMS = 29.7 m^2). No. ind./Total AIMS: mean number of individuals settled considering the whole set of deployed AIMS (660). No. ind./AIMS and No. ind./m^2 are represented by the left Y axis, whereas No. ind./Total AIMS is represented by the right Y axis.

Notwithstanding the lack of broadly accepted criteria for assessing translocation success (Seddon, 1999; Fischer and Lindenmayer, 2000; Moehrenschlager et al., 2013; Robert et al., 2015), survival of released specimens is a main indicator (Swan et al., 2016). In this sense, assessments of success depend on time, and it is controversial because some authors report that the outcomes of long-term monitoring have much higher failure rates (Dalrymple et al., 2011), whereas others pointed out that the highest proportion of failures occurred within the first four years (Bubac et al., 2019). In fact, according to the review by these authors, most studies that conducted post-released monitoring lasted between 1-4 years, which is the range of monitoring of the present study. For *Patella ferruginea*, several translocations can be found in the literature. Summarizing, two different approaches have already been implemented: one in which the specimens were directly dislodged from the rock surface and reattached into a new one, and a second approach in which specimens were translocated with the substrate itself. The former has reported high mortality values in the first days or even hours after the translocation, due to the difficulty of adapting the shell edge to a new topography, provoking water loss and desiccation or increasing the chance of predation. In this sense, Laborel-Deguen and Laborel (1991b) translocated a total of 222 specimens in Corsica recording a survival of 50% after the first day, 25% after one year, and 10% after two years. Similarly, Espinosa et al. (2008) translocated 420 specimens in Ceuta (Strait of Gibraltar) reporting a survival of 50% after a few days, approximately 20% after one year, and 10% after two years, but survival in control specimens was also low: approximately 60% after one year and 30% after two years. Guallart et al. (2014) translocated ten specimens that were stored in aquariums after a captive breeding project to Hormigas Islands (Southern Spain), reporting a survival of 60% after 24 hours and 10% after two years. More recently, Zarrouk et al. (2018) used cages to protect the translocated specimens in Zembra Islands (Tunisia) and the survival was clearly improved (approximately 60% for caged specimens *vs.* 25% for non-caged specimens after almost two years). The survival rate of those specimens protected by cages was like the mean survival rate recorded in the present study after one year (approximately 60% in both studies), but higher after two years (60% *vs.* 41%). Survival of control specimens was slightly higher than 80% after one and two years in that study, similar to the survival obtained in the present study for the control specimens. Cages were removed after 43 days when the specimens had already fixed their new home scars and were not maintained the whole monitoring period. Consequently, the differences in survival between both studies cannot be attributed to this fact and were probably due to the different environmental

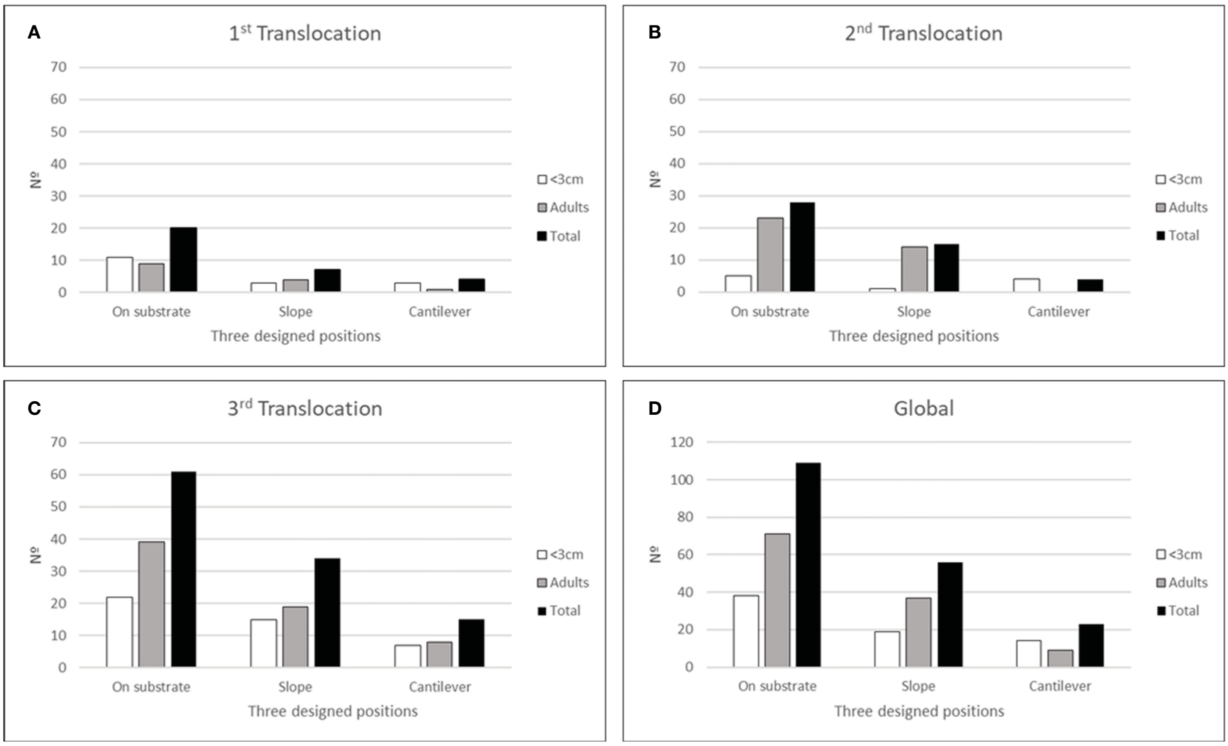


FIGURE 7
Number of individuals (<3cm, adults and total) settled on the three different treatments used (on substrate, slope, and cantilever). **(A)** first translocation; **(B)** second translocation; **(C)** third translocation; **(D)** the whole number of individuals after the three translocation events.

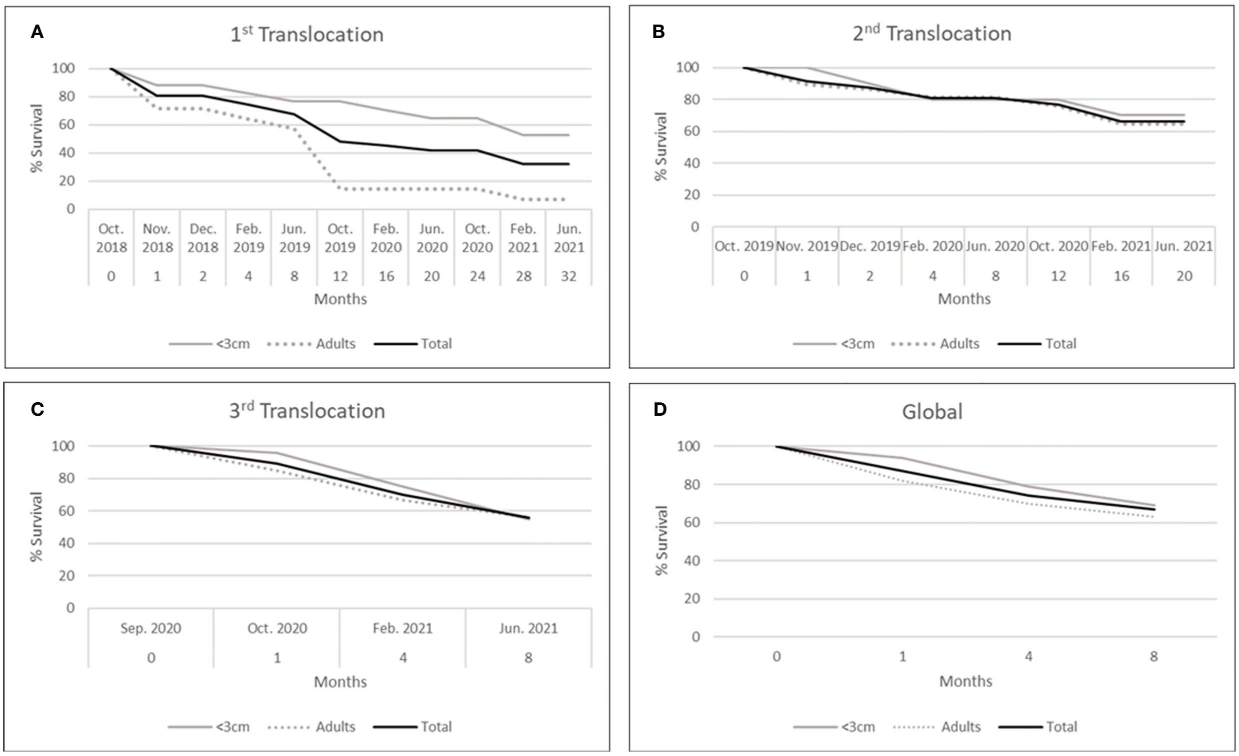


FIGURE 8
Survival percentage for each translocation done and size class (<3 cm: recruits, >3 cm: adults). **(A)**: first translocation (2018); **(B)**: second translocation (2019); **(C)**: third translocation (2020); **(D)**: mean values of the three translocations.

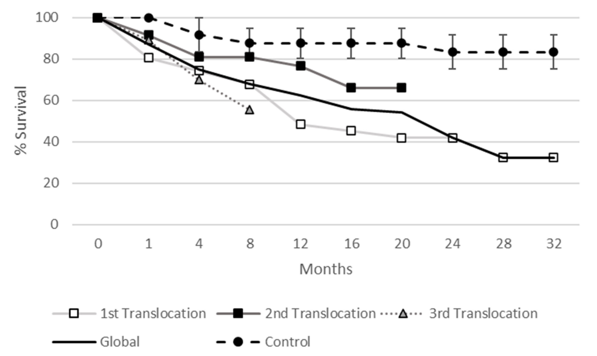


FIGURE 9
Survival percentage for each translocation and the mean of the three performed (total) over the whole monitoring period. The survival percentage of the control individuals in the receiving population located in La Línea port is also shown for comparison. Three 10 m transects were monitored and the mean ± standard error of their survival values is shown for the control threshold.

and ecological conditions between donor and receiving sites that could trigger long-term mortality when the translocation is done to a faraway receiving area. In this sense, Zarrouk et al. (2018) translocated the specimens to a very close area (5 km between donor and receiving sites), but in that study, the authors also performed a long-distance translocation (185 km) in which the survival was very low (69% after two days and 18% after approximately one and two years of monitoring). Considering the second type of translocations reported in the literature (limpets translocated with their substrate), great differences exist between studies. For instance, due to maintenance works, a total of 301 specimens were translocated attached to the boulder rocks of a breakwater from a small harbor within the Chafarinas Islands (North Africa). Survival was only 37% after three months, even though the distance between donor and receiving sites was in the range of tens of meters (APM (Autoridad Portuaria de Melilla), 2014). However, also due to breakwater repair works, Fa et al. (2018) performed a similar translocation procedure to a very close receiving area in Gibraltar (Southern Iberian Peninsula), reporting survival of 87% after ten months for 97 specimens

Transplantation of other limpet species, such as *Patella vulgata* has been reported as a very difficult process indeed (Jenkins and Hartnoll, 2001), similar to the results obtained for *P. ferruginea* according to the information already mentioned. In this sense, home scars confer mechanical advantage primarily in shear resistance (Smith, 1992). Therefore, on one hand, the translocations performed better when the specimens were moved with the substrate in which they had already fixed their home scars, such as in the present study, and on the other hand, short-term translocations (<10 km) also performed better than long-term ones (>100 km). The ultimate reasons would be the importance of establishing the home scar for intertidal limpets, gaining an advantage for facing environmental stress and predation, whereas changes in the physicochemical composition of the water, different availability of trophic resources (in terms of quality and quantity), changes in competition intensity, and predation risk would be affecting the long-term survival for the translocated specimens. Despite being a long-distance translocation, the results of the present study, in terms of survival, are among the best outcomes reported in the literature, not only in the short term (100% of survival during translocation and almost 90% after one month) but also in the long term (62.5% after one year but 75% if the natural mortality is considered in the receiving site).

In addition, recruitment in the donor area and survival in the receiving area were not negatively affected by environmental factors in terms of great fluctuations, considering the stability of physicochemical parameters in the water column together with the maintenance of the ecological structure within intertidal assemblages. In this sense, profound changes in physicochemical parameters such as an increase in turbidity or a decrease in pH values are commonly associated with polluted waters within the study area (Estacio et al., 1997), whereas the ecological composition of the intertidal assemblages in the area has been proved to be very useful for monitoring studies and reflects their common history (Fa et al., 2002; Guerra-García et al., 2006). The decrease in mean size in the donor population during the monitoring period was not, consequently, attributable to any negative environmental issue. On the contrary, it could be explained by the fact that the density increased through the monitoring period mainly due to good recruitment events (both % of colonized AIMS and the density of specimens per m^2 within them increased yearly, being a proxy for recruitment in the area), and the new recruits were progressively censused when they became adults, reducing the mean size of the whole population.

The success of recruitment on the AIMS increased from the first to the third translocation and it could be related to the maturity of the intertidal assemblages that were developed over time on the experimental structures and the presence of conspecifics of *Patella ferruginea* already settled nearby. Moreover, chemical cues either from conspecifics or from intertidal assemblages have been proposed as attractors for recruitment in limpets and particularly in *P. ferruginea* (see Rivera-Ingraham et al., 2011b, Rivera-Ingraham et al., 2015). Therefore, AIMS should be deployed at least one or two years before any translocation attempt is undertaken for maximizing the recruitment. In addition, recruitment has been greater on the AIMS deployed directly over the substrate, collecting either adults or recruits and it would be preferable for restocking since a greater number of specimens could be collected. However, provided the physical impact on the intertidal habitat and the greater mortality shown by adults the use of cantilever disposition could be interesting for preventing potential impacts on the intertidal habitat (in some areas of special concern) and for selecting recruits instead of adults. Bottari et al. (2017) also found greater mortality in translocated adults of the endangered mollusk *Pinna nobilis* compared with juveniles. Even though some authors have advocated against the translocation of adults especially during the reproductive season to avoid additional stress (Guallart, 2014), from a restocking perspective in conservation biology it could be very useful to increase genetic diversity in the receiving population, at least for those species that, in previous translocation experiences, have registered low adult mortality during the first critical months of adaptation to their new environment. Such specimens would release their gametes just after

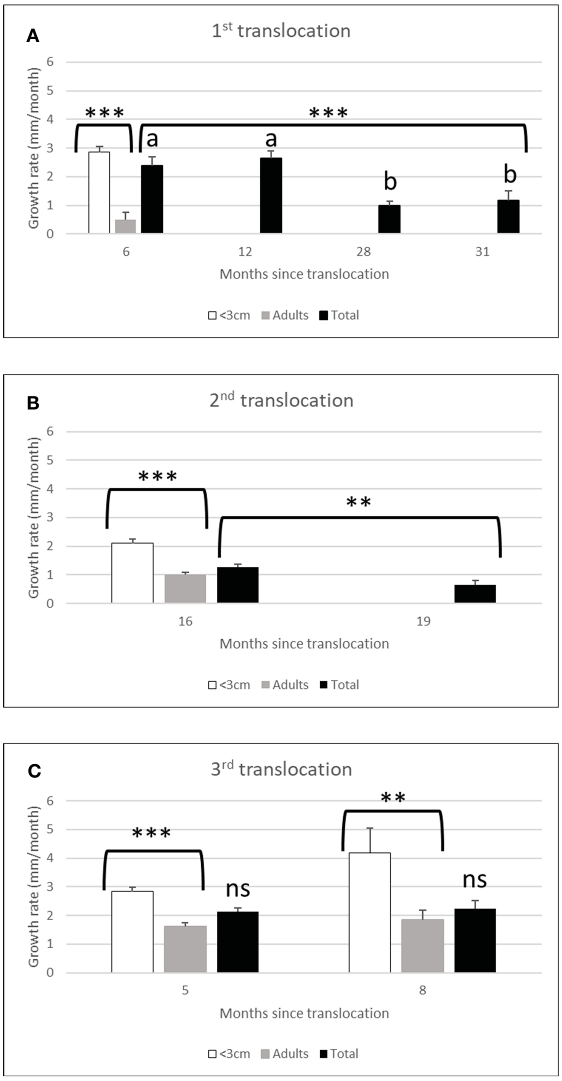


FIGURE 10
Mean growth rates (± SE) for recruits (<3cm), adults, and the total individuals translocated for each of the translocation events. **(A)**: first translocation (Oct. 2018). ***p<0.001 (ANOVA test). Letters indicate homogeneous groups according to *a posteriori* SNK test; **(B)**: second translocation (Oct. 2019). ***p<0.001, **p<0.01 (ANOVA test); and **(C)** third translocation (Sep. 2020). ***p<0.001, **p<0.01, ns, not significant (ANOVA test). Data have been calculated by rating the increase in size (mm) by the months that have passed between time intervals in which size measurements were recorded. The individuals were considered adults if their size was greater than 3 cm at the beginning of the calculated period.

translocation and even if they died after, the translocation would be successful from a reproductive perspective. In this sense, Coates et al. (2013) indicated that restoring abalone populations (*Haliotis corrugata*) by releasing juveniles could limit the success because they are more susceptible to predation, show propensities for dispersal, and those reared in captive conditions may not exhibit some behaviors that promote survival. Furthermore, these authors stated that creating aggregations by transplanting adults may be an alternative to releasing juveniles due to the higher reproductive output. Delgado et al. (2004) pointed out that reproductive specimens of the queen conch (*Strombus gigas*) that were translocated spawned three months after, highlighting that it could be the key to expediting the recovery of this species in the Florida Keys. Similarly, Romero-Barón (2017) indicated that adult colonies of coral should be translocated to accelerate the recruitment process because those colonies would release larvae in the short term. Consequently, the mixed approach used in the present study could be more beneficial from a conservation perspective, although further studies would be required. Furthermore, the donor population from Melilla is adapted to warmer conditions than those found in the Strait of Gibraltar (see temperature map of the Mediterranean in Coll et al., 2010), and the introduction of alleles adapted to such conditions would enhance the resilience of the population in the receiving area against the impact of SST increase derived from climate change. In this sense, Garrabou et al. (2022) pointed out that mass mortality events in several taxa of invertebrates within the Mediterranean will be usual because of marine heatwaves, with the increase in genetic diversity being even more relevant.

The differences observed in growth rates between recruits and adults are concordant with similar findings in previous studies (see Espinosa et al., 2008) since the species shows a decrease in growth rate through the life span. Moreover, the decrease observed in growth rates through the monitoring period is an expected outcome, because the specimens reduce their growth rates progressively.

Moreover, the AIMS used have shown resistance against the intense shear forces generated by waves greater than 15 m, which were recorded within the donor area through the study (REMOPAF, 2021), nevertheless, the whole set of the AIMS remained fixed. That, coupled with its great versatility in terms of unscrewing from donor sites, transportation, and screwing on receiving sites, makes them a suitable procedure in translocation attempts. It is important to highlight that the AIMS could be reutilized when the translocated specimens had already fixed their home scars on the receiving substrate, being a feasible method for persistent restocking over time. In fact, Seddon et al. (2007) pointed out that technical aspects were being promoted in translocations for improving biological outcomes. Mimicking the natural substrate by using 3D printing technology has recently been proposed in marine restoration with promising results (see Evans et al., 2021), with the translocation procedure being used at the forefront of the knowledge in this field.

Many important populations of *Patella ferruginea* can be found settled on artificial substrates such as breakwaters or ripraps of harbor facilities or elsewhere (Espinosa et al., 2008; Guallart et al., 2013; CAGPDS, 2020). Therefore, artificial substrates could play a key role in conservation, and Artificial Marine Micro Reserves (AMMRs) have been proposed by García-Gómez et al. (2011) as a new tool in marine conservation Accordingly, discrete portions of breakwaters with dense populations of endangered species, such as *P. ferruginea*, could be fenced and monitored. Human harvesting should be effectively excluded within such areas since it has proven to be the main threat to the endangered Mediterranean ribbed limpet, and many other limpets (Branch and Odendaal, 2003; Espinosa and Rivera-Ingraham, 2017). These AMMRs should be integrated into a network of interconnected sites, constituting an Artificial Marine Micro Reserves Network (AMMRNs) (see García-Gómez et al., 2015). Finally, this type of figure would be within the concept of 'Other Effective Area-Based Conservation Measures' (OECMs) recently proposed by the IUCN (IUCN-WCPA Task Force on OECMs, 2019), as has been properly discussed by Ostalé-Valriberas et al. (2022). In this context, some of the AMMRs could be donor populations with a permanent system of AIMS deployed that would be translocated periodically to appropriate receiving sites. Therefore, the great number of AIMS that should be deployed for recruiting enough specimens would be located within artificial substrates, with the impact over the intertidal habitat (mainly visual) not being a major concern.

Furthermore, Pérez et al. (2012) pointed out that only 4% of translocations reviewed fully complied with translocation criteria, independently of the outcomes obtained for the target species. Considering that the present study complies with all the criteria already proposed by these authors, and the outcomes obtained, it could be considered not only adequately designed but also recommended for future translocations. In this sense, species of endangered intertidal or even shallow subtidal species within the Mediterranean basin, such as the limpet *Patella candei* from Macaronesia, the vermetid *Dendropoma* spp., or the orange coral *Astroides calycularis*, could potentially benefit (they are already included within endangered species list, see https://www.miteco.gob.es/es/biodiversidad/temas/conservacion-de-especies/especies-proteccion-especial/ce-proteccion-listado-situacion.aspx).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

FE contributed to the conception, design, acquisition, analysis and interpretation of data. MM contributed to the conception, design, acquisition, analysis and interpretation of data. JG-G contributed to the conception, design and interpretation of data. MC-C contributed to the acquisition of data. CP-M contributed to the conception, design and acquisition of data. JP contributed to the conception and design. PF-SM contributed to the design, acquisition and analysis of data. NG-E contributed to the conception and design. All authors contributed to the article and approved the submitted version.

Funding

This study has been financially supported (60% of total eligible costs) by the European Commission through the LIFE project ReMoPaf (Recovery of the endangered Mollusc *Patella ferruginea*) with code LIFE15/NAT/ES/000987. The authors express their gratitude to 'Autoridad Portuaria Bahía de Algeciras' for providing support and collaboration during the project.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

APM (Autoridad Portuaria de Melilla) (2014). "Informe de seguimiento de los ejemplares de patella ferruginea afectados en el proyecto "Rehabilitación del embarcadero de chafarinas"," in *Informe técnico (no publicado) para la dirección general de sostenibilidad de la Costa y del mar, ministerio de agricultura* (Madrid, España: Alimentación y Medio Ambiente), 55.

Barea-Azcón, J. M., Ballesteros-Duperón, E., and Moreno, D. (2008). Libro rojo de los invertebrados de andalucía. *Consejería. Medio. Ambiente. Junta. Andalucía. Sevilla.* 4.

BOE (1999). *Boletín oficial del estado. 22 junio 1999, n° 148* (España: Ministerio de la Presidencia), 23921–23922.

Bottari, T., Spinelli, A., Busalacchi, B., Rinelli, P., and Giacobbe, S. (2017). Transplant trials of the fan mussel *Pinna nobilis* inside the coastal lagoon of capo peloro (Central Mediterranean, Italy). *J. Shellfish. Res.* 36 (1), 3–8. doi: 10.2983/035.036.0102

Branch, G. M. (1981). The biology of limpets. *Oceanogr. Mar. Biol.: an. Annu. Rev.* 19, 235–379.

Branch, G. M., and Odendaal, F. (2003). The effects of marine protected areas on the population dynamics of a south African limpet, *Cymbula oculus*, relative to the influence of wave action. *Biol. Conserv.* 114 (2), 255–269. doi: 10.1016/S0006-3207(03)00045-4

Bubac, C. M., Johnson, A. C., Fox, J. A., and Cullingham, C. I. (2019). Conservation translocations and post-release monitoring: Identifying trends in failures, biases, and challenges from around the world. *Biol. Conserv.* 238, 108239. doi: 10.1016/j.biocon.2019.108239

Burgos-Rubio, V., de la Rosa, J., Altamirano, M., and Espinosa, F. (2015). The role of patellid limpets as omnivorous grazers: a new insight into intertidal ecology. *Mar. Biol.* 162 (10), 2093–2106. doi: 10.1007/s00227-015-2739-0

CAGPDS (2020). *Programa de gestión sostenible del medio marino andaluz. informe regional 2020* (Junta de Andalucía: Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible (CAGPDS).

Casu, M., Rivera-Ingraham, G. A., Cossu, P., Lai, T., Sanna, D., Dedola, G. L., et al. (2011). Patterns of spatial genetic structuring in the endangered limpet patella ferruginea: implications for the conservation of a Mediterranean endemic. *Genetica* 139 (10), 1293–1308. doi: 10.1007/s10709-012-9631-3

Clarke, K. R., and Gorley, R. N. (2006). Primer. *PRIMER-e. Plymouth* 866.

Coates, J. H., Hovel, K. A., Butler, J. L., Klimley, A. P., and Morgan, S. G. (2013). Movement and home range of pink abalone *Haliotis corrugata*: implications for restoration and population recovery. *Mar. Ecol. Prog. Ser.* 486, 189–201. doi: 10.3354/meps10365

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., Aguzzi, J., et al. (2010). The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PloS One* 5 (8), e11842. doi: 10.1371/journal.pone.0011842

Dalrymple, S. E., Stewart, G. B., and Pullin, A. S. (2011) *Are re-introductions an effective way of mitigating against plant extinctions? collaboration for environmental evidence review 07–008 (SR32)*. Available at: www.environmentalevidence.org/SR32.html.

Delgado, G. A., Bartels, C. T., Glazer, R. A., Brown-Peterson, N. J., and McCarthy, K. J. (2004). Translocation as a strategy to rehabilitate the queen conch (*Strombus gigas*) population in the Florida keys. *Fishery. Bull.* 102 (2), 278.

Denny, M. W., and Shibata, M. F. (1989). Consequences of surf-zone turbulence for settlement and external fertilization. *Am. Nat.* 134, 859–889. doi: 10.1086/285018

Dissanayake, A., and Bamber, S. D. (2010). Monitoring PAH contamination in the field (South west Iberian peninsula): Biomonitoring using fluorescence spectrophotometry and physiological assessments in the shore crab carcinus maenas (L.)(Crustacea: Decapoda). *Mar. Environ. Res.* 70 (1), 65–72. doi: 10.1016/j.marenvres.2010.03.003

Espinosa, F., González, A. R., Maestre, M. J., Fa, D., Guerra-García, J. M., and García-Gómez, J. C. (2008). Responses of the endangered limpet *Patella ferruginea* to reintroduction under different environmental conditions: survival, growth rates and life-history. *Ital. J. Zool.* 75 (4), 371–384. doi: 10.1080/11250000801887740

Espinosa, F., Guerra-García, J. M., Fa, D., and García-Gómez, J. C. (2006b). Effects of competition on an endangered limpet patella ferruginea (Gastropoda: Patellidae): Implications for conservation. *J. Exp. Mar. Biol. Ecol.* 330 (2), 482–492. doi: 10.1016/j.jembe.2005.09.020

Espinosa, F., Guerra-Garcia, J. M., FA, D., and Garcia-Gomez, J. C. (2006a). Aspects of reproduction and their implications for the conservation of the endangered limpet, *Patella ferruginea. Invertebrate. Reprod. Dev.* 49 (1-2), 85–92. doi: 10.1080/07924259.2006.9652197

Espinosa, F., Pavón-Paneque, A., and García-Gómez, J. C. (2021). Disentangling the impact of artificial substrata on the trophic ecology of the highly endangered marine invertebrate *Patella ferruginea* gmelin 1791. *Molluscan. Res.* 41 (1), 41–45. doi: 10.1080/13235818.2021.1882925

Espinosa, F., and Rivera-Ingraham, G. A. (2017). Biological conservation of giant limpets: the implications of large size. *Adv. Mar. Biol.* 76, 105–155. doi: 10.1016/bs.amb.2016.10.002

Espinosa, F., Rivera-Ingraham, G., and García-Gómez, J. C. (2011). Influence of habitat structure and nature of substratum on limpet recruitment: Conservation implications for endangered species. *Estuarine. Coast. Shelf. Sci.* 94 (2), 164–171. doi: 10.1016/j.ecss.2011.06.005

Espinosa, F., Rivera-Ingraham, G. A., Maestre, M., González, A. R., Bazairi, H., and García-Gómez, J. C. (2013). Updated global distribution of the threatened marine limpet *Patella ferruginea* (Gastropoda: Patellidae): An example of biodiversity loss in the Mediterranean. *Oryx* 48, 266–275. doi: 10.1017/S0030605312000580

Espinosa, F., Rivera-Ingraham, G. A., Ostalé-Valriberas, E., and García-Gómez, J. C. (2018). Predicting the fate of the most endangered marine invertebrate of the Mediterranean: The power of long-term monitoring in conservation biology. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 28 (6), 1283–1293. doi: 10.1002/aqc.2944

Estacio, F. J., García-Adiego, E. M., Fa, D. A., García-Gómez, J. C., Daza, J. L., Hortas, F., et al. (1997). Ecological analysis in a polluted area of algeciras bay (Southern spain): external 'versus' internal outfalls and environmental implications. *Mar. pollut. Bull.* 34 (10), 780–793. doi: 10.1016/S0025-326X(97)00046-5

Evans, A. J., Lawrence, P. J., Natanzi, A. S., Moore, P. J., Davies, A. J., Crowe, T. P., et al. (2021). Replicating natural topography on marine artificial structures–a novel approach to eco-engineering. *Ecol. Eng.* 160, 106144. doi: 10.1016/j.ecoleng.2020.106144

Fa, D. A., Finlayson, J. C., Finlayson, G., Giles-Pacheco, F., Rodríguez-Vidal, J., and Gutiérrez-López, J. M. (2016). Marine mollusc exploitation as evidenced by the gorham's cave (Gibraltar) excavations 1998–2005: The middle–upper Palaeolithic transition. *Quaternary. Int.* 407, 16–28. doi: 10.1016/j.quaint.2015.11.148

Fa, D. A., Finlayson, C., García-Adiego, E., Sánchez-Moyano, J. E., and García-Gómez, J. C. (2002). Influence of some environmental factors on the structure and distribution of the rocky shore macrobenthic communities in the bay of Gibraltar: Preliminary results. *Almoraima* 28, 73–88.

Fa, D. A., Finlayson, G., Sempere-Valverde, J., and García-Gómez, J. C. (2018). 'Impossible're-introduction of the endangered limpet *Patella ferruginea* gmelin 1791? progress in resolving translocation mortality. *Endangered. Species. Res.* 37, 219–232. doi: 10.3354/esr00921

Fernández-Casado, M., Arroyo, M. C., and Vivas, M. S. (2017). Caracterización de las poblaciones de la lapa ferruginosa (*Patella ferruginea* gmelin 1971) de la costa norte del estrecho de gibraltar". *Almoraima. Rev. Estudios. Campogibraltareños.* 47, 181–202.

Fernández-Macho, J. (2016). Risk assessment for marine spills along European coastlines. *Mar. pollut. Bull.* 113 (1-2), 200–210. doi: 10.1016/j.marpolbul.2016.09.015

Ferranti, M. P., Monteggia, D., Asnaghi, V., Dagnino, A., Gaino, F., Moretto, P., et al. (2019). Distribution of the Mediterranean ribbed limpet *Patella ferruginea* gmelin 1791 along the ligurian coast and implications for conservation actions. *Mediterr. Mar. Sci.* 20 (3), 496–501. doi: 10.12681/mms.20209

Fischer, J., and Lindenmayer, D. B. (2000). An assessment of the published results of animal relocations. *Biol. Conserv.* 96 (1), 1–11. doi: 10.1016/S0006-3207(00)00048-3

García-Gómez, J. C., Guerra-García, J. M., Espinosa, F., Maestre, M. J., Rivera-Ingraham, G., Fa, D., et al. (2015). Artificial marine micro-reserves networks (AMMRNs): an innovative approach to conserve marine littoral biodiversity and protect endangered species. *Mar. Ecol.* 36 (3), 259–277. doi: 10.1111/maec.12167

García-Gómez, J. C., López-Fé, C. M., Espinosa, F., Guerra-García, J. M., and Rivera-Ingraham, G. A. (2011). Marine artificial micro-reserves: A possibility for the conservation of endangered species living on artificial substrata. *Mar. Ecol.* 32 (1), 6–14. doi: 10.1111/j.1439-0485.2010.00409.x

Garrabou, J., Gómez-Gras, D., Medrano, A., Cerrano, C., Ponti, M., Schlegel, R., et al. (2022). Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. *Global Change Biol.* 28, 5708–5725 doi: 10.1111/gcb.16301

González-Laxe, F., Martín-Bermúdez, F., Martín-Palmero, F., and Novo-Corti, I. (2017). Assessment of port sustainability through synthetic indexes. application to the Spanish case. *Mar. pollut. Bull.* 119 (1), 220–225. doi: 10.1016/j.marpolbul.2017.03.064

Guallart, J. (2014). *Condicionantes de la biología de la lapa ferruginosa (Patella ferruginea) en vistas a potenciales traslados de ejemplares* (Ministerio de Agricultura, Alimentación y Medio Ambiente, España: Informe Técnico (no publicado), 98.

Guallart, J., Luque, Á.A., Acevedo, I., and Calvo, M. (2013). Distribución y censo actualizado de la lapa ferrugínea (*Patella ferruginea* gmelin 1791) en el litoral de melilla (Mediterráneo suroccidental). *Iberus* 31 (1), 21–51.

Guallart, J., Templado, J., Acevedo, I., Calvo, M., Machordom, A., Luque, A. A., et al. (2014). *Informe de la reintroducción de ejemplares de la lapa patella ferruginea (catalogada "en peligro de extinción") en la isla hormiga (Murcia)* (Museo Nacional de Ciencias Naturales-CSIC: Informe técnico), 15.

Guerra-García, J. M., Maestre, M. J., González, A. R., and García-Gómez, J. C. (2006). Assessing a quick monitoring method using rocky intertidal communities as a bioindicator: a multivariate approach in algeciras bay. *Environ. Monit. Assess.* 116 (1), 345–361. doi: 10.1007/s10661-006-7572-y

IUCN-WCPA Task Force on OECMs (2019). *Recognising and reporting other effective area-based conservation measures* (Gland, Switzerland: IUCN).

Jenkins, S. R., and Hartnoll, R. G. (2001). Food supply, grazing activity and growth rate in the limpet *Patella vulgata* l.: a comparison between exposed and sheltered shores. *J. Exp. Mar. Biol. Ecol.* 258 (1), 123–139. doi: 10.1016/S0022-0981(01)00211-8

Kleiman, D. G., Price, M. R. S., and Beck, B. B. (1994). "Criteria for reintroductions," in *Creative conservation: interactive management of wild and captive animals*. Eds. P. J. S. Olney, G. M. Mace and A. T. C. Feistner (London, UK: Chapman & Hall).

Laborel-Deguen, F., and Laborel, J. (1991a). "Statut de patella ferruginea gmelin en méditerranée," in *Les Espèces marines à protéger en méditerranée*. Eds. C. F. Boudouresque, M. Avon and V. Gravez (Marseille: GIS Posidonie Publishers), 91–103.

Laborel-Deguen, F., and Laborel, J. (1991b). "Une tentative de reintroduction de patella ferruginea gmel. (Gastropoda) dans le parc national de port-cros (Var, France)," in *Les Espèces marines à protéger en méditerranée*. Eds. C. F. Boudouresque, M. Avon and V. Gravez (Marseille: GIS Posidonie Publishers), 129–132.

Martins, G. M., Thompson, R. C., Neto, A. I., Hawkins, S. J., and Jenkins, S. R. (2010). Enhancing stocks of the exploited limpet *Patella candei* d'Orbigny *via* modifications in coastal engineering. *Biol. Conserv.* 143 (1), 203–211. doi: 10.1016/j.biocon.2009.10.004

Meffe, G. K. (1992). Techno-arrogance and halfway technologies: salmon hatcheries on the pacific coast of north America. *Conserv. Biol.* 6, 350–354. doi: 10.1046/j.1523-1739.1992.06030350.x

Moehrenschlager, A., Shier, D. M., Moorhouse, T. P., and Stanley Price, M. R. (2013). Righting past wrongs and ensuring the future: challenges and opportunities for effective reintroductions amidst a biodiversity crisis. *Key. Topics. Conserv. Biol.* 2, 405–429. doi: 10.1002/9781118520178.ch22

Novak, B. J., Phelan, R., and Weber, M. (2021). US Conservation translocations: Over a century of intended consequences. *Conserv. Sci. Pract.* 3 (4), e394. doi: 10.1111/csp2.394

Ostalé-Valriberas, E., Sempere-Valverde, J., Pavón-Paneque, A., Coppa, S., Espinosa, F., and García-Gómez, J. C. (2022). 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. *Mar. Policy* 136, 104917. doi: 10.1016/j.marpol.2021.104917

Pérez, I., Anadón, J. D., Díaz, M., Nicola, G. G., Tella, J. L., and Giménez, A. (2012). What is wrong with current translocations? a review and a decision-making proposal. *Front. Ecol. Environ.* 10, 494–501. doi: 10.1890/110175

Potet, M., Fabien, A., Chaudemanche, S., Sebaibi, N., Guillet, T., Gachelin, S., et al. (2021). Which concrete substrate suits you? *Ostrea edulis* larval preferences and implications for shellfish restoration in Europe. *Ecol. Eng.* 162, 106159. doi: 10.1016/j.ecoleng.2021.106159

Primack, R. B., and Ros, J. (2002). *Introducción a la biología de la conservación* (Barcelona, Spain: Grupo Planeta (GBS)).

Ramos, M. A. (1998). Implementing the habitats directive for mollusc species in Spain. *J. Conchol. Special. Publ.* 2, 125–132.

Rees, P. A. (2001). Is there a legal obligation to reintroduce animal species into their former habitats? *Oryx* 35, 216–223. doi: 10.1046/j.1365-3008.2001.00178.x

REMOPAF (2021). "Recovery of endangered mollusc patella ferruginea population by artificial inert mobile substrates in Mediterranean Sea," in Madrid, Acciona Ingeniería *Final report. LIFE project n° LIFE15 NAT/ES/000987*, 102.

Reyers, B., Folke, C., Moore, M. L., Biggs, R., and Galaz, V. (2018). Social-ecological systems insights for navigating the dynamics of the anthropocene. *Annu. Rev. Environ. Resour.* 43, 267–289. doi: 10.1146/annurev-environ-110615-085349

Ricciardi, A., and Simberloff, D. (2009). Assisted colonization is not a viable conservation strategy. *Trends Ecol. Evol.* 24, 248–253. doi: 10.1016/j.tree.2008.12.006

Rivera-Ingraham, G. A., Espinosa, F., and García-Gómez, J. C. (2011a). Environmentally mediated sex change in the endangered limpet patella ferruginea (Gastropoda: Patellidae). *J. Molluscan. Stud.* 77 (3), 226–231. doi: 10.1093/mollus/eyr007

Rivera-Ingraham, G. A., Espinosa, F., and Garcia-Gomez, J. C. (2011b). Effect of γ-amino butyric acid on limpet populations: towards the future management and conservation of endangered patellid species. *J. Chem. Ecol.* 37 (1), 1–9. doi: 10.1007/s10886-010-9884-1

Rivera-Ingraham, G. A., Espinosa, F., and Krock, B. (2015). Presence of gamma-aminobutyric acid (GABA) in the pedal mucus of the critically endangered species patella ferruginea. *J. Chem. Ecol.* 41 (5), 501–504. doi: 10.1007/s10886-015-0579-5

Robert, A., Colas, B., Guigon, I., Kerbiriou, C., Mihoub, J. B., Saint-Jalme, M., et al. (2015). Defining reintroduction success using IUCN criteria for threatened species: a demographic assessment. *Anim. Conserv.* 18, 397–406. doi: 10.1111/acv.12188

Rojo-Nieto, E., Garrido-Pérez, C., Anfuso-Melfi, G., Lopez-Aguayo, F., Sales-Marquez, D., and Perales-Vargas-Machuca, J. A. (2011). The zoning of semi-enclosed bodies of water according to the sediment pollution: The bay of algeciras as a case example. *Estuaries. costas.* 34, 1129–1139. doi: 10.1007/s12237-011-9389-3

Rojo-Nieto, E., and Perales, J. A. (2015). Estimating baseline toxicity of PAHs from marine chronically polluted sediments and bioaccumulation in target organs of fish hypothetically exposed to them: a new tool in risk assessment. *Environ. Sci.: Processes. Impacts.* 17 (7), 1331–1339. doi: 10.1039/c5em00111k

Romero-Barón, M. F. (2017). La restauración de arrecifes de coral; una alternativa de conservación en el sistema arrecifal mesoamericano (SAM) (Université de Sherbrooke: Tesis Profesional en Ecología Internacional).

Seddon, P. J. (1999). Persistence without intervention: assessing success in wildlife reintroductions. *Trends Ecol. Evol.* 14, 503–503. doi: 10.1016/S0169-5347(99)01720-6

Seddon, P. J., Armstrong, D. P., and Maloney, R. F. (2007). Developing the science of reintroduction biology. *Conserv. Biol.* 21, 303–312. doi: 10.1111/j.1523-1739.2006.00627.x

Seddon, P. J., Soorae, P. S., and Launay, F. (2005). Taxonomic bias in reintroduction projects. *Anim. Conserv.* 8, 51–158. doi: 10.1017/S1367943004001799

Smith, A. M. (1992). Alternation between attachment mechanisms by limpets in the field. *J. Exp. Mar. Biol. Ecol.* 160, 205–220. doi: 10.1016/0022-0981(92)90238-6

Spalding, C., and Hull, P. M. (2021). Towards quantifying the mass extinction debt of the anthropocene. *Proc. Biol. Sci.* 288, 20202332. doi: 10.1130/abs/2021AM-369486

Strayer, D. L. (2022). Comment: Novak et al.(2021) overestimated the successes of species translocations and minimized their risks. *Conserv. Sci. Pract.* 4 (7). doi: 10.1111/csp2.12694

Swan, K. D., McPherson, J. M., Seddon, P. J., and Moehrenschlager, A. (2016). Managing marine biodiversity: the rising diversity and prevalence of marine conservation translocations. *Conserv. Lett.* 9 (4), 239–251. doi: 10.1111/conl.12217

Tilman, D., Isbell, F., and Cowles., J. M. (2014). Biodiversity and ecosystem functioning. *Annu. Rev. Ecol. Evol. Syst.* 45, 471–493. doi: 10.1146/annurev-ecolsys-120213-091917

Vázquez-Luis, M., Álvarez, E., Barrajón, A., García-March, J. R., Grau, A., Hendriks, I. E., et al. (2017). SOS Pinna nobilis: a mass mortality event in western Mediterranean Sea. *Front. Mar. Sci.* 4, 220. doi: 10.3389/fmars.2017.00220

Zarrouk, A., Romdhane, M. S., and Espinosa, F. (2018). Reintroduction of the highly endangered mollusk *Patella ferruginea* gmelin 1791 in an MPA: A novel approach to achieve high survival rates. *Estuarine. Coast. Shelf. Sci.* 202, 259–269. doi: 10.1016/j.ecss.2018.01.014

frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 20 April 2023
DOI 10.3389/fmars.2023.1166937

Advances in the management and translocation methodology of the endangered mollusk *Patella ferruginea* in artificial habitats of port infrastructures: implications for its conservation

José Carlos García-Gómez[1,2]*, Amparo Cid-Iturbe[1],
Enrique Ostalé-Valriberas[1]*, Rocío Espada[1], José Luis Carballo[1],
Darren Andrew Fa[2], Carlos García-Olaya[3]
and Rocio Blanca-Sújar[1]

[1]Laboratorio de Biología Marina de la Universidad de Sevilla (LBM-US)/Área de Investigación I+D+i del Acuario de Sevilla/Estación de Biología Marina del Estrecho (Ceuta), Universidad de Sevilla, Seville, Spain, [2]Natural Sciences and Environment Research Hub, University of Gibraltar, Gibraltar, United Kingdom, [3]Área de Investigación Biológica I+D+i del Acuario de Sevilla, Sevilla, Spain

OPEN ACCESS

EDITED BY
Alan Hodgson,
Rhodes University, South Africa

REVIEWED BY
Phillip Fenberg,
University of Southampton,
United Kingdom
Rafael Sarda,
Spanish National Research Council (CSIC),
Spain

*CORRESPONDENCE
Enrique Ostalé-Valriberas
✉ enriqueostalevalriberas@gmail.com
José Carlos García-Gómez
✉ jcgarcia@us.es

SPECIALTY SECTION
This article was submitted to
Marine Fisheries, Aquaculture and Living
Resources,
a section of the journal
Frontiers in Marine Science

RECEIVED 15 February 2023
ACCEPTED 28 March 2023
PUBLISHED 20 April 2023

CITATION
García-Gómez JC, Cid-Iturbe A,
Ostalé-Valriberas E, Espada R, Carballo JL,
Fa DA, García-Olaya C and Blanca-Sújar R
(2023) Advances in the management and
translocation methodology of the
endangered mollusk *Patella ferruginea* in
artificial habitats of port infrastructures:
implications for its conservation.
Front. Mar. Sci. 10:1166937.
doi: 10.3389/fmars.2023.1166937

The limpet (*Patella ferruginea*), endemic to the western Mediterranean, is the most endangered intertidal mollusk listed in the Habitats Directive (EU). The translocation methodology of this species is a scientific challenge faced by those port infrastructures – where there are established breeding subpopulations - that may be subject to emergency works, restoration, or extension of breakwaters. This study presents the results of an innovative two-phase pilot project (P1A/B and P2), involving the legally authorized translocation of individuals of this species obtained in three relocation experiments in the Marina of Puerto José Banús (Málaga) in July 2021 (phase 1: P1A and B) and May 2022 (phase 2: P2) (study period: July 2021-October 2022). The specimens translocated in both phases were from the same port except for those of P1B, which came from the Marbella Marina as an exceptional case. These translocations were undertaken on concrete breakwater tetrapod units. We show that the somewhat forced, slow and unregulated extraction of specimens to be translocated during the first experiment of the P1A pilot project was the main cause of mortality, as it generated an important exhaustion of the fixation musculature, which resulted in a low survival (66%) after the first month of transfer, whereas during the same period of time. In the subsequent experiments (where the problem was methodologically corrected, a fast extraction technique -less than 5 seconds-, regulated and without injuries), in P1B was 100%, in P2 with cage was 93.3%, and, in P2 without cage it was 83.3%, which reflected an improvement in translocation methodology. The results of this research are compared according to the descriptors of success and failure of each protocol in order to observe the differences and thus enable their replicability.

KEYWORDS

translocation adults, survival, advances methodology, conservation, endangered *Patellogastropoda*

Introduction

Patella ferruginea Gmelin, 1791 (commonly known as the ferruginean or Mediterranean ribbed limpet), is considered the most endangered endemic marine invertebrate in the Western Mediterranean (Laborel-Deguen and Laborel, 1991a; Ramos, 1998; Espinosa et al., 2014), also by the European Council Directive 92/43/EEC (Council Directive, 1992). It is, therefore, a protected species specifically listed as such in different administrative provisions or international conventions: National Catalogue of Threatened Species (in danger of extinction), Annex IV of the Habitats Directive, Annex II of the Barcelona Convention and Annex II of the Bern Convention. In Spain, this led to the publication of the National Strategy for the conservation of this species (MMAMRM, 2008), under the auspices of the Ministry of the Environment and Rural and Marine Affairs.

Due to the high accessibility of its intertidal habitat, this species has undergone significant regression (Raffaelli and Hawkins, 1996; Haedrich and Barnes, 1997; Rochet and Trenkel, 2003), especially through human exploitation (Aversano, 1986; Guerra-García et al., 2004a; Moreno, 2004), not only for use as fishing bait, due to its muscular foot (Pombo and Escofet, 1996), but also for its ornamental interest. An example of this concerns *Lottia gigantea* G. B. Sowerby, 1834 (Fenberg and Roy, 2012) or *Scutellastra mexicana* (Broderip and Sowerby, 1829), the latter being the largest limpet in the world (Dance, 1967; Keen, 1971) and the only known species of patellid in the Eastern Pacific (Carballo et al., 2020). In this sense, *P. ferruginea* is the largest limpet species in the Mediterranean. Finally, populations of *P. ferruginea* have also been affected by the reduction of habitat quality and alteration by coastal works, the increase of contaminants (Espinosa et al., 2014; Espinosa and Rivera-Ingraham, 2017) along the coast and changes in sea surface temperature (Freitas et al., 2023). The habitat of this species, the upper midlittoral fringe of the intertidal zone, is highly sensitive and vulnerable to anthropogenic influence. In this zone, limpets have been considered keystone species (Henriques et al., 2017; Marra et al., 2017), and, as Raffaelli and Hawkins (1996) point out, they maintain a mosaic of open spaces that allow the coexistence of many species, thus contributing to increase marine biodiversity. Whilst the keystone species attribute has not been sufficiently argued for *P. ferruginea*, its potential as a bioindicator taxon of clean and renewed waters has been described and investigated (Espinosa et al., 2007; García-Gómez, 2015), so it can be used as a sentinel species indicator of anthropogenic environmental disturbances or changes. For these reasons, in most ports and marinas of the Alboran Sea (with the particular exception of the ports of Ceuta, Melilla and Gibraltar, whose internal waters are subject to significant renewal), *P. ferruginea* is common or abundant in the outer docks, being absent -or of accidental presence- in the inner zone (where other limpet species can live).

Distribution and status of populations

The species was widely distributed throughout the western Mediterranean during the Pleistocene (Caton-Thompson, 1946; Laborel-Deguen and Laborel, 1991a) and its presence was abundantly recorded on the coasts of North Africa and Europe until the end of the 19th century. But it was at the beginning of the 20th century, when a clear regression of the species began (Laborel-Deguen and Laborel, 1991a; Templado, 2001). Currently, the species has almost completely disappeared from the western Mediterranean coasts of Europe, from its northern and eastern areas, being relegated to the northern and western coasts of Corsica (Laborel-Deguen and Laborel, 1991a; Sardinia (Porcheddu and Milella, 1991; Doneddu and Manunza, 1992; Cristo et al, 2007; Cristo and Caronni, 2008), Pantellaria and Egadi Islands (Laborel-Deguen and Laborel, 1991a) and Tuscany (Italian peninsula) (Curini–Galletti, 1979; Biagi and Poli, 1986).

The Alboran Sea is the area where the species is most abundant, maintaining high homogeneity and low genetic diversity (Espinosa and Ozawa, 2006; Casu et al., 2011). It is located on the island of Alboran (Paracuellos et al., 2003; Templado et al., 2006), with populations firmly established on Iberian south-Mediterranean coast from Almería to Tarifa (Arroyo et al., 2011), including Gibraltar (Fa et al., 2018) being their western distribution and the strict limit of distribution of the south-Mediterranean coast (García-Gómez and Magariño, 2010), being especially concentrated in external areas of harbors or marina breakwaters (e.g., Motril, Marbella, José Banús, La Duquesa, Sotogrande, La Línea-Alcaidesa, Gibraltar, Algeciras, Tarifa); and North Africa coasts: Ceuta (Guerra-García et al., 2004b; Espinosa, 2006; Espinosa et al., 2009; Rivera–Ingraham et al., 2011a) and Melilla (González-García et al., 2006; González et al., 2015), the Chafarinas

Islands (Guallart, 2006), the Algerian islands of Rachgoun (Frenkiel, 1975) and Habibas (Boumaza and Semroud, 2001; Espinosa, 2009), reaching Cape Bon (Espinosa, 2006) and Zembra Island (Tunisia) (Boudouresque and Laborel–Deguen, 1986).

This species of limpet is sequential protandric hermaphrodite with external fertilization (Espinosa et al., 2006; Rivera-Ingraham et al., 2011b), whose reproductive period is between September and December (Frenkiel, 1975). The larvae phase is development with a lecithotrophic larvae that act as limiting factor in the ability of a larva to reach assume the "desperate larva hypothesis" postulates that lecithotrophic larvae become less discriminating in their settlement requirements over time, due to depletion of energy reserves (Botello and Krug, 2006).

Patella ferruginea in coastal infrastructures, unresolved conflict

In addition, inhabiting in natural rocky habitats, *P. ferruginea* is found on artificial structures, usually on walls, breakwaters and rocks or blocks of breakwaters linked to port infrastructures in the Alboran Sea. In Europe it is the only endangered species that forms important groups of breeding individuals associated with coastal infrastructure breakwaters, even on the smooth, external surfaces of concrete walls linked to such breakwaters (García-Gómez et al., 2011; García-Gómez et al., 2015; Maestre Delgado et al., 2018).

The main conflict in the management of this species in artificial habitats comes from the need to relocate specimens from breakwaters to other places, as a consequence of reconstruction works on breakwaters - either due to severe storms or longer-term deterioration and/or necessary improvements in port infrastructures or their enlargement. In this regard, in Spain, the National Conservation Strategy for this species (MMAMRM, 2008), "discourages the transfer of specimens, except for scientific research or conservation of the species duly justified". This is because of high mortality when translocating individuals. This position has led to the generation of "an unresolved problem" to relevant Port and Environmental Authorities (the latter competent in the granting of authorizations) by directly affecting future works involving the restoration or modification of the layout of seawalls containing the species and, in the context of natural habitats, by making it impossible to reintroduce adults of the species in geographical areas where it used to exist and has now become locally extinct. However, the Strategy advocates the "establishment of appropriate measures to avoid damage to the usual port activities", which requires the development of applied research, with concomitant implications, to help to solve the identified problems.

History of translocations of specimens

Previous experiments of translocations by direct extraction of specimens from their source substrata or on plates designed with 3D technology have been carried out: in Corsica (Laborel-Deguen and Laborel, 1991b), Ceuta, Strait of Gibraltar (Espinosa et al., 2008), Islas Hormigas, southern Spain (Guallart et al., 2014), Chafarinas Islands (APM, 2014), Gibraltar (Fa et al., 2018), Zembra Islands, Tunisia (Zarrouk et al., 2018) and between Melilla (North Africa) and La Línea (Algeciras Bay, southern Iberian Peninsula) (LIFE 15/NAT/ES/000987-REMoPaF Project, 2017-2021). In the discussion the results obtained are compared, although except for those from Gibraltar of translocation of specimens on their substrata (without removing from their home scar) that had very satisfactory survival results (Fa et al., 2018), the others have obtained poor or mediocre results. This supports the furtherance of research aimed at improving the methodology of translocations, a workable solution to which is increasingly urgent due to the problems generated by increasingly frequent and more complex coastal engineering.

Objectives

The main objective of this work was to test a new method of translocation of specimens of *P. ferruginea* in an artificial habitat composed of concrete tetrapods of 6 to 10 tons to assess its suitability for specimen transfer. The method focused on the rapid and manual extraction of specimens (in relaxation phase during the rising tide), transport (in attachment plates and zip bags without fixation) and subsequent relocation of the specimens, in order to obtain higher survival than those obtained in previous experiments and different substrata. This new technique will allow future translocations - when duly justified - to be both viable and sustainable which will also have a direct application for future reintroductions of adult specimens to natural habitats where the species is now absent but where it is known to have previously existed.

Methodology

Study area, physical characteristics of the receptor breakwater and precautions derived from the operational risk

The two ports of Marbella (south of the Iberian Peninsula, Spain) where the pilot project - authorized by the 'Ministro de Agricultura, Pesca y Desarrollo Rural (CAPyDR) del Gobierno de la Junta de Andalucía - has been carried out are Puerto José Banús (PJB) and Puerto Deportivo de Marbella (PDM). The PJB has been the only receptor of all the specimens translocated from the outer breakwater of the same port (a hostile environment due to its spatial configuration and exposure to waves), as well as the specimens from the PDM (very close to PJB), which has only acted as a donor of a small number of specimens in the pilot project (Figure 1). The PJB breakwater is subject to frequent and intense waves (generated by the wind and amplified by the drastic decrease in depth due to the accumulated sedimentation in the outer area near the breakwater). This presented important health and safety problems in the processes of extraction and relocation of specimens due to the final slope of the breakwater, the size of the tetrapods and the smoothness of their slippery arms, as well as the

FIGURE 1
(A) Alboran Sea and location of the study area. **(B)** Location of PJB (1) and PDM (2), as well as the different substrata of their breakwaters of both phases (P1A and B; P2) of the pilot project: B1, key substratum of concrete tetrapods of the study, used as both donor and receptor zones; B2, riprap substratum of the exclusively donor zone, whose receptor zone was located in the tetrapods of B1.

different current vortices generated when the waves break over them according to their orientation. These reasons sometimes made it necessary to designate team members exclusively to watch the waves (sometimes generated suddenly, next to the breakwater) and, on occasion, the use of safety lines, helmets and vests to access where the limpets were located.

Authorized specimens, phases of the pilot-project (P1A and B, and P2) and material used

In 2020, the subpopulation of *P. ferruginea* was censused in PJB, estimated at 1,153 specimens of different sizes (of which 818 were adults, over 30 mm) (CAGPyDS-JA, 2021), along its seawall (1,067 meters long). The pilot project authorized by the Competent Environmental Authority (CAPyDR-JA) for translocation of specimens established on concrete tetrapods was strictly limited to 110 specimens in two phases (P1A, 50 specimens; and P2, 60 specimens). In addition, within the scope of P1 and exceptionally - due to an emergency work situation inside the PDM (14 km from the PJB) - the transfer of 3 adult specimens (P1B) from dolomitic quarry rocks (PDM) to concrete tetrapods in the PJB was authorized, an operation carried out at the end of P1A. This was a special trial because of its significant added value, since the transferred specimens involved transportation between ports, medium and substratum (from natural dolomitic quarry rock to artificial concrete tetrapods), and especially because it allowed modifications to improve P1A before starting P2. This last phase (P2) started in May 2022, with the aim of applying the methodological advances detected in P1A and P1B, as a consequence of the detailed observation of unexpected early mortalities. The translocated specimens (manually extracted from their substrata in the same harbor breakwater of PJB (P1A and P2), have represented 9.5% (110 out of 1,153) of the total contingent found along the exterior arm of the harbor.

The plates used for an express transfer and their subsequent displacement to the receptor area were standardized, made of cement and with a rough, non-slip surface measuring 20 x 20 cm. They were used preliminarily to avoid stressing the limpets (as soon as they were extracted) so that they would use the plates as an intermediate substratum to be installed in a very short time (30 minutes maximum) into pre-established sites, anchored by means of metal bolts previously placed in the receptor area, only requiring to be screwed in. The cages - after previous resistance tests of a previous plastic prototype - were designed in stainless metal mesh (50 X 40 cm), specifically flexible (to mold to the curved arms of the tetrapods and withstand the strong waves), and of low height (to avoid the possible overturning of the transferred limpets and their loss), with flanges secured by flexible aluminum plates on which the cages were screwed to the arms of the tetrapods after drilling them (with a concrete drill bit and a Stanley Fatmax 1250 watt hammer drill) and the use of multifunctional grooved expansion plugs (Fisher type). The cages had an opening to introduce the limpets, which had a metallic fold that acted as a door and allowed its closure with plastic flanges. The cages were sized to contain the limpets for only 3-4 weeks (after which they were disassembled), a critical adaptation period in which, according to pre-existing experiments reviewed in the discussion, the highest mortalities are usually recorded. Rounded-edge spatulas, soft plastic bags (zip type) and plastic coolers were used for the collection, storage and transfer of translocation specimens. To determine mortality and survival within size classes, millimetric precision calipers were used to measure specimens.

Figure 2 shows the zoning of the receptor areas in PJB of the specimens transferred in phases 1 and 2 of the transfer, with their respective control zones - Receptor Control (CR), Donor Control (CD) and Non-Donor Control (CND) for the first phase (P1), and Control (C2) for the second phase (P2). The receptor area for the specimens transferred in the first phase (P1) was established at the east end of the breakwater for TP1A and at the west end of the breakwater for TP1B (3 limpets from PDM). For the second phase (P2), two receptor zones were delimited, one for the translocation of limpets without cages (TP2WCa) and the other for those translocated with cages (TP2Ca), each of them being the donor zone of the other. The limpets translocated in TP1A come from the CD zone and those in TP1B, from PDM, not represented in Figure 2 since it is a separate site to the PJB. The control zones for the P1 phase of translocation are: the CD, the CR (shared with the TP1A zone of translocated limpets), and the non-donor zone CND located at the western end of the PJB breakwater. For the P2 phase, the control zone C2 and adjacent to the CR, which overlaps with the receptor zone TP2WCa, was chosen.

The choice of control zones (untranslocated resident limpets) shared with receptor zones (see Figure 2) was made because it was considered that a comparison of survival percentages between both zones in the same stretch of breakwater and under identical environmental conditions could allow a better deduction of some

GPS position

	Begininig	**Ending**
TP1A and CR:	36.29'18.10"N 4°56'53.00"W	36°29'15.23"N 4°56'55.47"W
TP1B:	36.28'59.40"N 4°57'16.73"W	36.28'59.30"N 4°57'15.83"W
CND:	36.28'58.78"N 4°57'11.73"W	36.28'58.53"N 4°57'15.04"W
CD:	36.29'7.03"N 4°57'2.64"W	36.29'3.22"N 4°57'5.83"W
TP2Ca:	36.29'11.20"N 4°56'59.00"W	36.29'7.43"N 4°57'2.28"W
TP2WCa:	36.29'14.00"N 4°56'56.60"W	36.29'12.00"N 4°56'58.40"W
C2:	36.29'15.17"N 4°56'55.57"W	36.29'10.81"N 4°56'59.24"W

FIGURE 2
Location of receptor, control, and donor areas in PJB. TP1A and TP1B: receptor areas for limpets translocated in phases P1A and P1B of the pilot project. Receptor areas for limpets translocated in phase P2, TP2Ca: limpets relocated in fixed metal cages; TP2WCa: limpets relocated without cages. The 4 control zones considered are delimited in dashed lines, CR, receptor control; CD, donor control; CND, non-donor control; C2, phase 2 control.

of the causes that could explain the disappearance of specimens (e.g. extracted by bathers or illegal shell fishermen). One of the difficulties of this type of study lies in the fact that most of the data supporting the mortality are based on missing specimens and not on specimens found dead, whose detailed examination, including necropsy, could help to determine the cause of death or at least rule out other possible causes such as those indicated in the example mentioned above.

Physical and biological parameters

To better understand the oceanographic and atmospheric environmental conditions, sea surface temperature, prevailing wind (at 10 meters height), wave height and chlorophyll a (Chl-a) in the water column were obtained from specialized web databases.

Temperature by annual station was obtained in degrees Celsius (MODIS-Aqua MODISA_L3m_SST_8d_4k vR 2019 dataset) from NASA's GIOVANNI tool (https://giovanni.gsfc.nasa.gov/giovanni/) at 11 microns and 4 km resolution. This tool allows researchers to visualize and perform small statistical analyses (Acker and Leptoukh, 2007).

The wind data were obtained from the SIMAR point 2025077 https://www.puertos.es/es-es/oceanografia/Paginas/portus.aspx. (official website of Puertos del estado del Ministerio de Transportes, Movilidad y Agenda Urbana de España). HARMONIE-AROME is the meso-scale model used, non-hydrostatic, with high spatial resolution. The data consulted have been obtained at 10 meters above sea level, with a resolution of approximately 2.5 km and a 48 h prediction horizon. Wind speed (extracted by months, for the full year 2021 and up to June 2022, in order to standardize data) is expressed in m/s and wind direction in degrees. Seasonal means and standard deviations were calculated and plotted in bar histograms.

The wave data obtained from the same source as the wind (WAM and WaveWatch models), complemented by the wind fields of the HARMONIE-AROME model. The first two are third-generation spectral models that solve the energy balance equation without making any *a priori* assumptions about the shape of the wave spectrum. The data have a resolution of 700 m. From the SIMAR point 2025077 we extracted and classified by months, the significant wave height data that approximately represents the mean height of the highest third of waves (significant height, Hs), data extracted from the Puertos del Estado website (complete year 2021 and 2022 until June, using this time scale to make the data uniform). Seasonal means and standard deviation were calculated (winter, spring, summer, autumn 2021 and winter and spring 2022). The data were plotted in a bar histogram with their associated standard errors. The wave height varied from wave to wave, and again the mean height of the highest third of waves (Hs) was plotted.

Chl-a concentration data were extracted from the MODIS-Aqua Level-3 sensor available provided by OBPG (NASA Ocean Biology Processing Group, 2018) at 4 km resolution from the NASA Ocean Color website (http://oceancolor.gsfc.nasa.gov/).

Statistical analysis

To compare the results of survival of translocated limpets and establish if there are statistically significant differences between these results and those obtained for "control" individuals, we used the Log-rank hypothesis test (Mantel, 1966; Peto and Peto, 1972), also known as the Mantel-Cox test, which allows us to compare survival distributions of two samples. This test is based on the $\chi 2$ model, in which the events (death) in each group are compared. It provides a p value (probability that the differences are due to chance), in addition to the median or mean survival. To compare the survival of *P. ferruginea* individuals over time, we used the Kaplan-Meier estimator, also called the limit product estimator, which is a nonparametric statistical method that takes into account each of the times contributed by each individual studied (Jager et al., 2008; Stel et al., 2011). Statistical analyses were performed using SPSS-25 software.

Extraction, transfer, and relocation of specimens

The appropriate transfer period was established between March and May (2021 for P1 and 2022 for P2), since between June and August (both inclusive) there could be days of high temperature (which can cause desiccation at low tide since the specimens have not been able to develop their new scar), and also between September and December, since this is the reproductive period of the species (Frenkiel, 1975). The months from December to February are not recommended due to the frequency of bad weather in winter, especially storms in the area. Prior to the definitive establishment of the methodological transfer protocol, tests were carried out on the mobilization of tetrapods and the cutting of their arms, as well as on a prototype plastic cage with metal fixation, all of which were rejected because of their practical and/or technical infeasibility and, in the case of the cages, because they could not withstand the strong waves (Figure 3: 1, 2 and 6).

Before extraction

Prior to the start of the translocation operation, the donor (extraction) and receptor (relocation) zones were defined. In the donor zone, suitable individuals of *P. ferruginea* were identified. In the receptor area, relocation points were previously decided for each individual to be relocated, close to the resident limpets, choosing relocation surfaces as similar as possible in terms of structure, slope and roughness, but avoiding those that were very exposed to direct waves, since during the first weeks after relocation limpets are particularly vulnerable to them. In each of the areas, a series of preliminary works were carried out; A) Donor area: each individual limpet to be transferred was identified with a stamped epoxy resin mark with a numerical identification code, photographed and

FIGURE 3
1 and 2: Preliminary tests of concrete tetrapod transfers and cutting of tetrapod arm sections with diamond head cutter, which reflected their technical infeasibility; 3: limpet marked with epoxy resin stamped with specific code; 4: limpet placed on a small previously defaunated surface (descaled from barnacle *Chthamalus stellatus*) to facilitate its new healing and sealing; 5: opening holes in the arm of a tetrapod, with a 1,200 watt hammer drill; 6: discarded prototype plastic cage and aluminum attachment strips, initially tested at P2, to test its wave resistance; 7: all-metal, stainless steel, wave-resistant cage design successfully used at P2; 8 and 9: intermediate attachment plates for extracted limpets, discarded at the beginning of P1A, were replaced with soft plastic "zip-lock" bags (see text); 10: time of extraction of specimens in heavy surf at the PJB breakwater.

measured (length and width), and the substratum where it was located was characterized. Annotation of its location in a vertical, inclined or horizontal area, rough or smooth, with or without biofouling (e.g. barnacles). B) Receptor area: the choice of suitable relocation sites was based on being as similar as possible to those of its native (donor) area. The upper midlittoral fringe of the species' intertidal zone was previously identified by the resident limpets-control or, if these are not nearby, by the surface of the *P. ferruginea*-specific fringe, typically with frequent presence of the lichen *Verrucaria amphibia* Clemente, 1814 and almost permanent presence of the barnacle *Chthamalus stellatus* (Poli, 1791), and comprised between the lower edge of the supralittoral *Patella rustica* L., 1758 belt and by the upper edge of the lower midlittoral shore zone determined by the red alga *Ellisolandia elongata* (J. Ellis and Solander) Hind and Saunders (2013), (Figure 4).

During extraction (donor site)

The extraction of specimens was carried out during the rising tide, high tide, or the beginning of the ebb tide, since it is at this time of emersion that limpets move out of their home scar. During the initial P1A exercise, it was observed that many limpets resisted, resulting in a rather forced extraction, leading to exhaustion of the peripheral muscles of the foot, subsequent weakness in their fixation in the receptor area and ultimately an increased risk of failure to attach or being dislodged by the swell. This was corrected on P1B and P2 by performing a rapid extraction (<5 seconds) that did not allow the animals to react and offer resistance. Extraction was always carried out through the posterior half of the shell and by "lever effect", pushing the spatula - by the handle - upwards, so that the point of support was the edge of the shell and not soft areas of the body, which could be lethal for the animal, even in the medium term. Once the animals had been extracted by this method, it was checked and recorded whether they had suffered any damage to the foot, since this can also hinder their rapid attachment in the receptor area and, therefore, possible detachment and loss of the animal. If the animals remained too strongly attached for too long during the rising or high tide, since it was impossible to remove them with spatulas, it was planned to do so during low tide together with a small fragment of their substratum to which they were attached, by rapidly fragmenting the concrete or rock with a hammer drill (of at least 1,200 watts) and chisel bit, with additional support from a rotaflex and concrete cutting disc, if necessary. In the receptor area, the fragment of substratum extracted with each animal would be fixed in the receptor area with epoxy marine cement (see discussion).

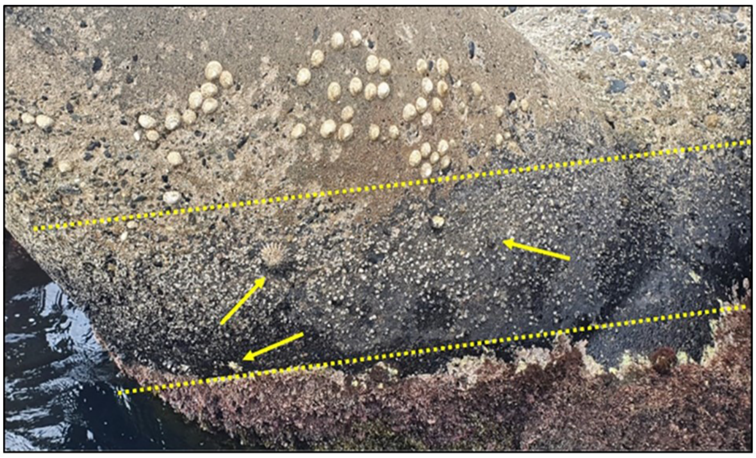


FIGURE 4
The dashed parallel lines delimit the strict strip of the upper midlittoral of the intertidal zone where *P. ferruginea* lives in the Strait of Gibraltar and nearby Mediterranean areas of the Alboran Sea. The upper line is the lower limit of the limpet *P. rustica* (in the image) and the lower line is the upper limit of the red alga *Ellisolandia elongata*, both very well defined in the image.

During transfer

The transfer from the donor to the receptor area was initially carried out (in P1A) on small concrete plates with a granulated surface, with the aim of obtaining a second settlement on these that would minimize the stress of the animal during its transfer to the receptor area (with previously installed bolts), from where the animals could in their own time voluntarily leave the plates and establish their new home scar. However, this was discarded when it was proven *in situ* that the need for a second transfer to a new substratum actually increased stress to the animal. Instead, soft plastic ziplock bags (flooded with sea water and half-opened to allow water exchange), each containing one animal and transported in the dark in simple plastic coolers, proved to be the best method, avoiding the need for a second settlement of the animals. In most cases, the animals were transported within 30 minutes of extraction, although an interval of up to 3 hours was envisaged. All limpets from P1B and P2 were transferred in this way, which, together with the above-mentioned process of rapid extraction without injury, represented an important advance in the success of the transfers (see results and discussion).

During relocation (receptor zone)

In the receptor zone, the animals were deposited on their new surfaces, also during rising tide, high tide, or the beginning of the ebb tide, taking into consideration that, if they were extracted by surprise, quickly, there would be no muscular exhaustion and they would attach immediately, even if the swell was intense. This would also be an early indication, should the animal not be found during follow-up monitoring, that its absence might be due to other factors not associated with muscle exhaustion. Each transferred specimen was relocated, especially the adults (>3cm), taking into account the proximity of other resident control-adults, since there is evidence that chemical traces possibly reduce the stress of the transferred specimens and maximize the inter-sex reproductive potential (the smaller the distance between males and females, the greater the potential). This tendency for adults to aggregate has been observed previously in littoral stretches where there are few specimens (personal obs.). However, to avoid exceeding the carrying capacity of the system and, for a given area of the intertidal, on rocks or isolated artificial structures, it was ensured that the density of transferred specimens, together with residents, did not exceed the maximum observed, for similar sizes, in other sections of the same receptor area with a higher density of resident limpets.

Temporary monitoring

The specimens transferred in July 2021, from P1A and P1B were monitored for 16 months and those from P2, transferred in May 2022 for 5 months. After the relocation of all specimens, the periodicity of monitoring was as follows: every day for the first two weeks; every 2-3 days for the next two weeks; once a week for the second month; once every two weeks for the third and fourth month; and once a month thereafter, unless significant losses were noticed.

Results

For the study period and experimental area, wind speed and wave height data are shown in Figure 5.

A particularly severe storm which took place in April 2022, with correspondingly large wave heights and corresponding material damage caused to the coast, only one of the translocated limpets was found to have disappeared shortly after, with a second loss 15 days later, apparently unrelated to the storm event (Figure 6). This would

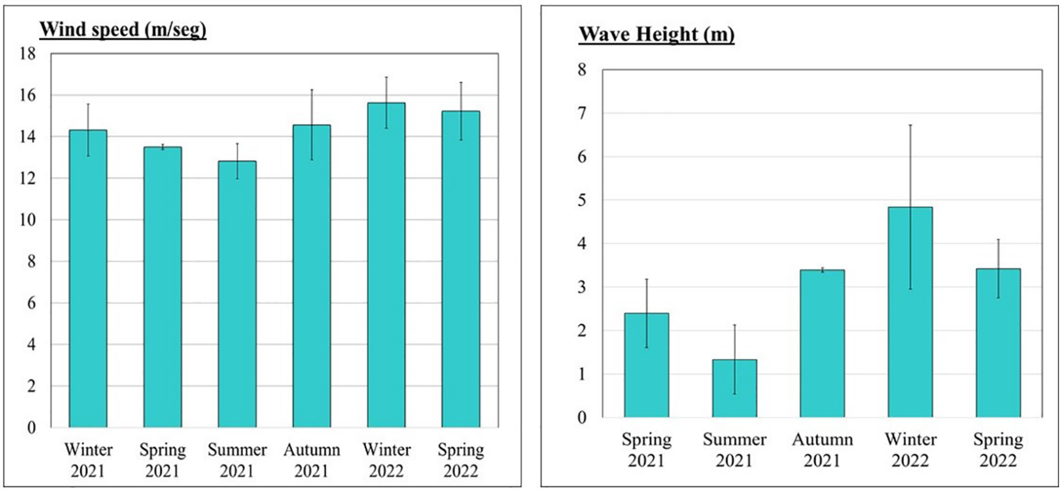


FIGURE 5
Wind speed (left) and wave height (right) data in the study area during the project timeframe.

indicate that, despite the severity of the storm, the species studied showed a great adaptive capacity to the mechanical impact of the waves, and that tenacity and survival in translocated limpets in P1A were more strongly influenced by the condition of the animal (muscular exhaustion and/or physical damage caused by forced physical extraction), than by the strength of wave action experienced.

Satellite data for temperature and Chlorophyll-a are shown in Figure 7. Chlorophyll-a data for the springs of 2021 and 2022 show appreciable differences, with mean values being appreciably higher in spring 2022. Nonetheless, neither of these parameters were found to influence mortality in both control and receptor areas, given the highly positive outcomes in P1B and P2, once the methodological lessons learnt from P1A had been incorporated to the translocation techniques.

Phases P1A and P1B (16 months of monitoring)

The P1A phase revealed the methodological errors of forced manual extraction, causing moderate to severe exhaustion of the foot muscles, resulting in difficulty of adherence in the receptor area and loss of individuals on swell days during the first days or weeks. Figure 8 shows the survival obtained over a time interval of 16 months, showing that the P1A phase obtained a 36% survival percentage while in P1B this was 100%, higher than that of the CR control zone with a survival percentage of 92%.

There are significant differences between treatments ($\chi^2 = 112.37$; $p < 0.001$). The mean life expectancy of the transferred

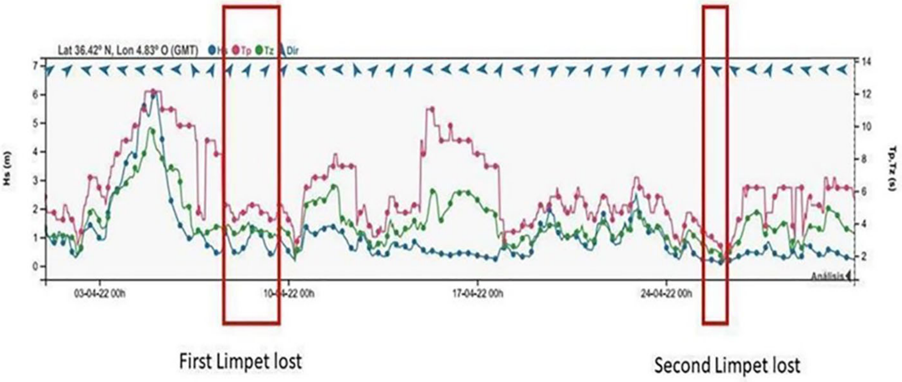


FIGURE 6
Maximum swell recorded during the study period (first week of April 2022), after which there was only the immediate loss of a single relocated limpet and a subsequent one 15 days later.

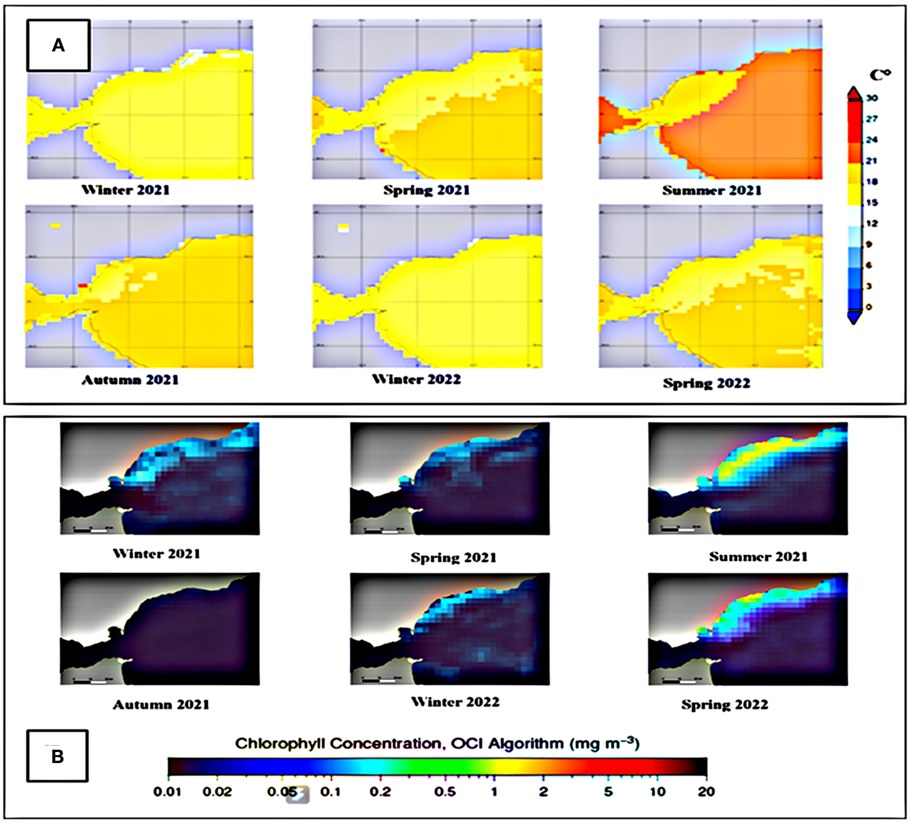


FIGURE 7
Average temperature **(A)** and chlorophyll **(B)** data from autumn 2021 to spring 2022.

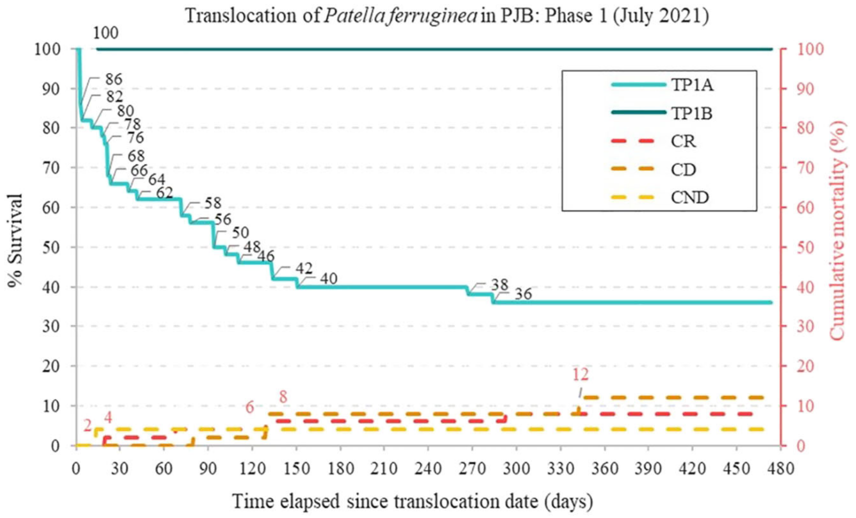


FIGURE 8
Translocations P1A and P1B with control areas in Puerto José Banús. Change in survival (%) of limpets in the translocation experiments in TP1A (Translocation P1A) and TP1B (Translocation P1B) and cumulative mortality (%) in the secondary axis of the control areas (CR, receptor control; CD, donor control; CND, non-donor control) from 15 July 2021 to 31 October 2022 (16 months). Number of limpets surveyed: NTP1A = 50; NTP1B = 3; NCR = 50; NCD = 50; NCND = 50.

individuals (TP1A) was 207.66 days and that of those established in the control areas (CD, CND, and CR) was 451.76 days (See Tables 1–3 of data analysis in Supplementary Material). Kaplan-Meier survival function data are shown in Figure 9A. Among the controls set for the P1A phase, there was no significant difference in the survival of individuals after 470 days ($\chi^2 = 4.75$; $p < 0.93$).

In phase P1B, executed at the end of phase P1A and improving the methodology used in the latter, a 100% survival was obtained, despite being the only phase of the pilot project which presented greater risks to success, given that the transfer was executed between two ports (PDM and PJB) and different types and nature of substrata: from dolomitic quarry rocks (PDM) to concrete tetrapods (PJB). The excellent results obtained in this phase following the methodological improvements outlined and after monitoring over the first 3 months led to the approval of phase P2 by the administrative authority.

Phase P2 (5 months of monitoring)

In phase P2, compared to P1A in the first 5 months, a much higher survival was obtained (Figure 10A), confirming the success of the methodological approach applied in phase P1B. There are no significant differences between the different treatments, after 170 days of follow-up from the transfer of *P. ferruginea* individuals with respect to transferred individuals compared to those in the control area ($\chi^2 = 1.49$; $p = 0.473$), showing that, in statistical terms, mortality is similar between transferred individuals and those in the control areas. Regarding the transferred individuals, although some more deaths were detected in translocations without cages (only 3 individuals), there were no significant differences, which suggests - although this should be confirmed with new experiments with a higher N - that in the future it should be possible to dispense with the installation of cages, with the economic and time savings

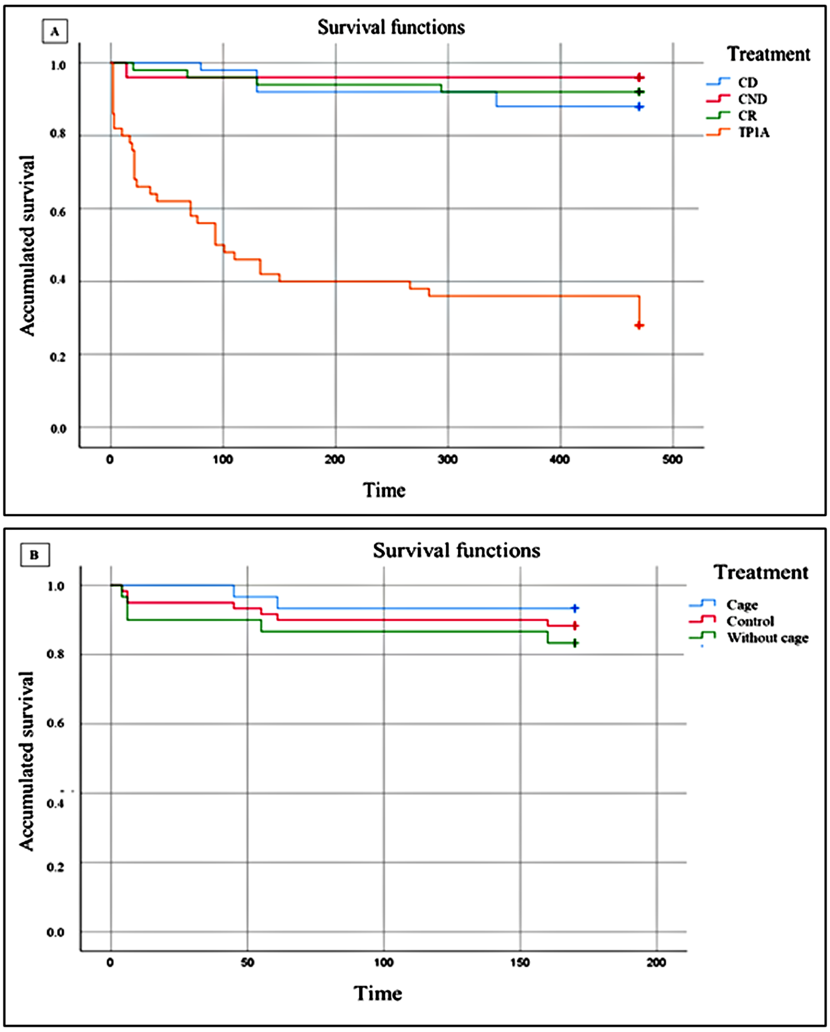


FIGURE 9
Cumulative survival on a linear scale (Kaplan-Meier function). **(A)**: phase P1 (CD, Control donor; CND, Control non-donor; CR, Control receiver; TP1A, Translocation phase 1A); **(B)**: phase P2 (P2WCa: with cage, P2Ca: without cage; C2: control.

that this would entail (see Tables 4–6, of data analysis in Supplementary Material).

The survival function (Kaplan-Meier) data for the results illustrated in Figure 10, from P2, are shown in Figure 9B.

Size frequency according to mortality-survival of transferred and control specimens

The results of the relative size frequencies of both missing and surviving specimens in receptor areas (translocated specimens) and in control areas (non-translocated resident specimens) are shown in Table 1.

Considering the mean size of the translocated specimens (TP1A: 64.5 ± 1.36 mm; TP1B: 65 ± 0.69mm; TP2Ca: 58 ± 0.87mm; TP2WCa: 57 ± 1.23mm) and of the control specimens (CR: 52 ± 1.33mm; CD: 68 ± 1.4mm; CND: 60.5 ± 0.95mm; C2: 51 ± 1.05mm), the majority of specimens lost in the TP1A (46.88%, N= 15), TP2Ca (100%, N=2) and TP2WCa (40%, N=2) transfer phases, and in the CD and CND control areas (50%, N=3, and 100%, N=2, respectively) were between 50-69 mm in length, except for 75% (N=3) of the CR control specimens lost that were between 30-49 mm in length (see Table 1).

Regarding the surviving specimens transferred, most of them had a length between 50-69 mm: 61.11% (N=11) of TP1A, 100% (N=3) of TP1B, 78.57% (N=22) of TP2Ca, 72% (N=18) of TP2WCa; and control specimens CR with 54.35% (N=25), CND with 72.92% (N=35) and C2 with 50% (N=30), except control zone CD, where 54.55% (N=24) of the survivors had a length of 70-89 mm.

Summary of survival results

The overall results obtained are summarized in Table 2 and Figure 10B. The highest survivorships were obtained in P1B (TP1B

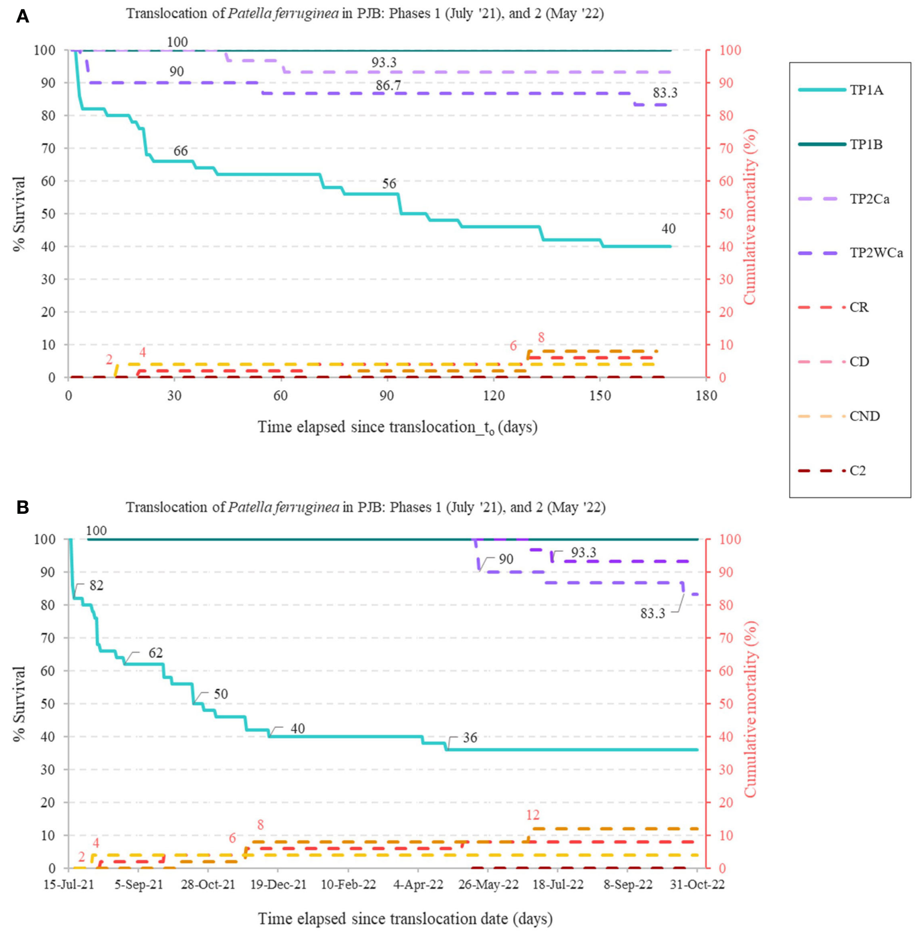


FIGURE 10
(A), Integrated survival results for P1A (TP1A: Translocation phase 1A), P1B (TP1B: Translocation phase 1B) and P2 in specimens transferred with relocation with cage (TP2Ca) and without cage (TP2WCa), during the first 5.5 months of the translocation, this time being the maximum monitoring time of P2 in this study (from 15 May 2022 to 31 October 2022); and **(B)**, shows the integrated survival results for P1 of 16 months (from 15 July 2021 to 31 October 2022) together with P2 since May 2023. The secondary axis represents the cumulative mortality of the control areas (CR, receiver control; CD, donor control; CND, non-donor control; C2, phase 2 control -0% in this case-). Number of limpets studied: NTP1A = 50; NTP1B = 3; NTP2Ca = 30; NTP2WCa = 30; NCR = 50; NCD = 50; NCND = 50; NC2 = 60.

TABLE 1 Relative size frequency (%) of missing and surviving specimens in PJB phase 1B; TP2Ca, Translocation phase 2 with cage; TP2WCa, Translocation phase 2 without cage) and their controls (CR, receiver control; CD, donor control; CND, non-donor control; C2, phase 2 control).

	TP1A	TP1B	TP2Ca	TP2WCa	CR	CD	CND	C2
$N_{\text{Transfered individuals}}$	50	3	30	30	50	50	50	60
Average size ± SD (mm)	64.5 ± 1.36	65 ± 0.69	58 ± 0.87	57 ± 1.23	52 ± 1.33	68 ± 1.4	60.5 ± 0.95	51 ± 1.05
Size frequency (mm)								
Missing (%)								
10.0-29.9	3125.00	0	0	14.29	0	0	0	0
30.0-49.9	15625.00	0	0	14.29	75	33.33	0	0
50.0-69.9	46875.00	0	100	40	0	50	100	0
70.0-89.9	34.38	0	0	20	25	16.67	0	0
N_M	32	0	2	5	4	6	2	0
Survivors (%)								
10.0-29.9	0	0	0	8	8.7	0	0	0
30.0-49.9	5.56	0	17.86	20	32.61	13.64	14.58	43.33
50.0-69.9	61.11	100	78.57	72	54.35	31.82	72.92	50
70.0-89.9	33.33	0	3.57	0	4.35	54.55	12.5	6.67
N_S	18	3	28	25	46	44	48	60

N_0, transferred number of original individuals; N_M, number of missing individuals; N_S, number of survival individuals.

100%; 16 months of monitoring) and P2 with and without cages (TP2Ca: 93.3% and TP2WCa: 83.3%; 5.5 months of monitoring), due to the methodological improvements applied, derived from the first pilot experiment P1A, with a very poor result (survival at 16 months). Even in the P2 variants (caged and uncaged, TP2Ca and TP2WCa respectively) the results were highly satisfactory, since during the first 5 months of monitoring (established as critical ones as verified in the P1A trial, where survival dropped drastically by about 40%), in the two P2 variants, the survival has at least doubled for the same period. Moreover, translocated limpets from P1B and P2 (with cage) exceeded the survival of resident limpets from both the control-donor CD (88%) and control-receptor CR (92%), which was surprising for mechanically extracted limpets not translocated with their own substrata.

Synthesis of failure-success methodological descriptors, essential as a basic protocol for further translocation trials

The results of the different methodologies applied in phase P1A and phases P1B and P2 are shown in Table 3, so that they can be replicated in further experiments.

Additional information to consider for future translocation trials

Rapid extraction depends on the limpets exhibiting a minimum of activity at rising tide, which is essential to leave sufficient room to introduce the extraction spatula. This does not always happen and, sometimes, a full tide takes place without the limpets leaving their home scars. This poses a serious problem for the viability of this type of action if the number of limpets to be removed is high and the time to carry out the action defined. For this reason, once the pilot project described here was completed, based on subsequent confirmatory tests and methodological extension of other projects currently underway (where this problem has been encountered), the extraction of specimens on fragments of substratum where the limpets were fixed was tested at low tide, using a hammer drill (at least 1250 watts of power) with sharp and flat chisels and/or rotaflex (at least 800 watts of power). Small pieces of substrata (personal observations) were extracted with the animals still attached, and these were translocated to receptor areas where the substratum pieces were fixed with epoxy marine cement. It was found that the ultra-fast vibration of the hammer chisel and rotaflex blade for a very short time (less than 5 minutes for substratum removal) did not affect the limpets and they remained attached once moved, until they voluntarily left their footprint at the recipient site with apparently no apparent sign of stress (personal observations).

Discussion

Advances in translocation methodology: Towards a sustainable resolution of future translocation actions

Given that, as explained above, the breakwaters of port infrastructures provide an advantageous artificial habitat for *P. ferruginea* to establish in high densities, this becomes a problem

TABLE 2 Survival of *P. ferruginea* specimens relocated at the PJB in phase 1A (TP1A, Translocation phase 1A) and phase B (TP1B, Translocation phase 1B) from July 2021 to 31 October 2022, approximately 16 months, including controls (CR, receiver control; CD, donor control; CND, non-donor control), plus translocations in phase 2, with cage (TP2Ca) and without cage (TP2WCa) plus an added control for phase 2 (C2), from 15 May 2022 to October 2022, almost 6 months later.

	TP1A	TP1B	CR	CD	CND	TP2Ca	TP2WCa	C2
Initial number of individuals	50	3	50	50	50	30	30	60
Number of missing limpets	32	0	4	8	2	2	5	0
Mortality (%)	64	0	8	12	4	7.7	16.7	0
Survival (%)	36	100	92	88	96	93.3	83.3	100

when the legitimate owners of such infrastructures must carry out emergency works or plan other works that may be authorized in which a relocation of specimens is required. So far, the history of direct and individual removal of specimens of the protected species has not been satisfactory, given the high mortalities recorded as shown in Table 4. Recent experiments on translocations of specimens on Artificial Inert Mobile Substrata (AIMS), carried out with 3D printing, have been conducted in Melilla (donor area) and La Línea (Algeciras Bay) (188 specimens, 15% survival, at two years; survival was higher in juveniles, <30mm, than in adults, >30mm) (see LIFE projects ReLife and REMoPaF; https://www.liferemopaf.org).

In previous experimental trials, only one translocation experiment carried out in Gibraltar moving the limpets together with their home rocks without prior removal of specimens has been successful, (97 specimens, 87% survival at 10 months) (Fa et al., 2018), although a later experiment in the Chafarinas Islands, also using the same method, did not obtain good results (301 specimens, 37% survival, 3 months) (APM, 2014), despite the fact that the transfer of blocks was at a very short distance from the source area

TABLE 3 Methodological results of failure (P1A) and success (P1B and P2), to be considered in further replication on translocation experiments of *P. ferruginea*.

COMPARISON OF TRANSLOCATION METHODOLOGIES				
	Failure descriptors	Success descriptors		
	P1A	P1B	P2Ca	P2WCa
5 months survival	66%	100%	93.3%	88.3%
16 months survival	36%	100%	–	–
Extraction				
	During rising tide, high tide or the beginning of the ebb tide			
	Extraction from any part of the shell with spatulas with non-rounded edges	Removal from the back half of the shell with rounded-edge screwdrivers		
	Muscle exhaustion or damage by distal support in the soft parts of the animal.	Minimal muscle exhaustion or damage by distal support on the substratum.		
	Slow (more than 5s) with persistence	Quick (<5s) without persistence		
Transfer				
	1. With small concrete slabs with a granulated surface (discarded)*	Soft plastic ziplock bags transported individually in the dark in plastic coolers		
	2. Soft plastic ziplock bags transported individually in the dark in plastic coolers			
Relocation				
	Receiving site previously located along the receiving control zone			
	Manually: Extreme difficulty in attaching the transferred animals	Manually: Immediate and strong fixation of the majority of the transferred animals		
	Translocation on the same tide (no more than 2-3 hours to carry out the transfer from the extraction)			
	Without Cage	Without Cage	With Cage	Without Cage

Statistical analysis determined that there were no significant differences between P2 survivals (88.3% survival for all 60 individuals).
Phase 1 (P1A in PJB; NP1A = 50), phase B (P1B from Marina Marbella to PJB; NP1B = 3) from July 2021 to October 2022, and Phase 2 (P2 in PJB; N2 = 60), from May 2022 to October 2022.

TABLE 4 Main data recollected from previous direct and individual removals of specimens of *Patella ferruginea* which proved to be unsuccessful.

Previous relocation experiments of *Patella ferruginea*				
		Survival (%)		
Site	$N_{translocated}$	Short-term	Long-term	Reference
Corsica, France	222	50-25	10	Laborel-Deguen and Laborel, 1991b
Ceuta, Spain	420	50-20	10	Espinosa et al, 2008
Hormigas Islands, Spain	10	60	10	Guallart et al., 2014
Zembra, Tunishia				Zarrouk et al., 2018
with cage	60	-	58	
without cage	20	-	25	
La Galite Island, Tunishia	110	-	18	
Chafarinas Islands, Spain	301	37	-	APM, 2014

of the blocks. However, while the latter may be environmentally sustainable (which is debatable), it is not economically sustainable, given the high costs of the operations due to the logistics and resources required. These difficulties, already complex when dealing with boulders between 100 and 2000 kg, become further compounded when dealing with concrete tetrapods of 6 to 10 Tn- the case we are dealing with - which are almost impossible to reinsert into breakwaters with other tetrapods at the same tidal height and orientation as in their original location, to ensure that the limpets are spatially positioned in the strict upper midlittoral strip that is typical of the species.

The somewhat forced, slow and unregulated extraction of specimens to be translocated during the first experiment of the P1A pilot project presented here, proved to be the main cause of mortality, as it generated an important exhaustion of the foot musculature, which resulted in a relatively low survival (66%) after the first month following translocation. In contrast, in the subsequent experimental trials of this study (where the problem was methodologically corrected, implementing a fast extraction technique - less than 5 seconds - regulated and without injuries), survivorship in P1B and in P2 (both with cages), was 100%, and in P2 (without cages) it was over 90%, reflecting the clear and substantial benefits of the methodological improvements previously outlined. This was confirmed after 5 months, where in P1A a 36% survival was recorded, while for that same period in P2 survivorships of 93.3% (with cages) and 83.3% (without cages) were obtained; and in P1B, a 100% survival was obtained over 16 months of monitoring of this phase, prior to P2. These results could have direct application in future reintroduction projects of adult specimens in natural habitats of the Mediterranean where the species is already extinct or where its populations are at imminent risk of extinction.

Coastal infrastructures, marine biodiversity and *P. ferruginea*, towards their best integrated management

Bulleri and Chapman (2010) consider it crucial to increase our understanding of the ecological functioning of marine habitats created by urban infrastructure and to incorporate ecological criteria into coastal engineering to preserve biodiversity and Naylor et al. (2017) have proposed the new conservation strategy "integrated greening of grey infrastructure (IGGI)" aimed at enhancing the biodiversity of hard infrastructure that cannot be replaced by green solutions. This is currently reinforced by the emerging "Working with Nature (WwN)" philosophy (Vikolainen et al., 2014; Airoldi et al., 2021; Ruijgrok, 2021; Taljaard et al., 2021) adopted by institutions and companies owning coastal infrastructures. The increase of marinas in recent years to meet the growing demand for water sports (Di Franco et al., 2011), and of commercial harbors - or harbor extensions - due to increasing pressure from shipping (Hanson and Nicholls, 2020), as well as the prospects of global warming, sea level rise and related weather phenomena (such as increasingly frequent and extreme storms) (see Doney et al., 2012; Hanson and Nicholls, 2020), together paint a picture of increasing coastal infrastructures that make it necessary to promote more sustainable designs and materials, as well as greater integration with the local nature and the environment, through ecological research on artificial substrata and eco-engineering, which contribute to generating artificial structures and habitats that enhance biodiversity.

The breakwaters of the ports in the Alboran Sea are artificial habitats of great ecological interest, not only because they provide shelter or allow the growth of protected species, but also because among them there may be dense breeding aggregations of endangered species that must be properly monitored, protected and managed as *Dendropoma lebeche* or *Astroides calycularis* among others. (Ostalé-Valriberas et al., 2022). Therefore, *P. ferruginea*, having dense settlements in artificial habitats of port infrastructures subject to improvement and/or expansion works (in addition to being exposed to other impacts derived from them, related to water quality), requires a delicate integrated management plan to ensure its long-term survival as a species. To this end, it is essential to ensure that this species can be used as a reference to encourage and promote port infrastructures to be increasingly environmentally sustainable, since they often lead to environmental impacts widely identified in the scientific literature, e.g., by subsidiary pollution derived from the isolation of water bodies and the related anthropogenic pressure (Estacio et al., 1997; Seitz et al., 2006; Birch et al., 2008; Masiá et al., 2021), by influencing local change in coastal currents, sediment transport and increased coastal erosion (El-Asmar and White, 2002; Gittman et al., 2015; Critchley and Bishop, 2019; Vona et al., 2020), or by direct or indirect effects on part of the local biota decreasing its biodiversity (Connell and Glasby, 1999; Chapman, 2003; Guerra-García and García-Gómez, 2004; Moschella et al., 2005), in addition to constituting a risk factor for the introduction and dispersal of exotic species with invasive potential (Bulleri and Airoldi, 2005; Glasby et al., 2007).

Protected species established in coastal infrastructures can be very useful in monitoring ecosystem quality in this type of artificial environment. In this respect, *P. ferruginea* is a bioindicator species of environmental quality in coastal and harbor waters (Espinosa et al., 2006), and, like other indicator species established in harbor breakwaters, it can contribute to the control and valorization of the "ecological potential" contemplated in the European Marine Strategy Framework Directive (MSFD) (Directive 2008/56/EC) for "heavily modified water bodies" (harbor waters). For *P. ferruginea* this is an additional relevant aspect to ensure its protection and conservation and to revalue its ecological and environmental role as a "sentinel" species for negative environmental changes that may occur in the coastal system.

On the other hand, since entrance to ports is usually restricted, guarded and access to the base of the breakwaters is dangerous, these tend to contain large specimens of protected species, which contracts with the strong extractive pressure the same species will undergo in easily and publicly accessible natural coastal habitats in the surrounding areas. For this reason, and taking *P. ferruginea* as the main reference, Artificial Marine Micro-Reserves have been proposed in some exhaustively studied ports (García-Gómez et al., 2011; García-Gómez et al., 2015; Firth et al., 2016; Ostalé-Valriberas et al., 2022; Sempere-Valverde et al., 2023).

During the development of this work it has been found that the areas surrounding the PJB and the PDM -as well as those of other nearby ports with important densities of *P. ferruginea*- benefit of 'reserve effect' where the port breakwaters not only recruit larvae from the wild (a phenomenon accentuated by the high spatial heterogeneity of the artificial substrata of the dikes, which benefits the establishment of larvae) but that these recruits then thrive thanks to the restricted access usually in force, leading to the generation of reproductive clusters or subpopulations, with medium and large adults, which in turn enhance genetic connectivity. In this regard, Martins et al. (2010) and Espinosa et al. (2011) indicated that limpet recruitment is influenced by substratum heterogeneity. On the contrary, it is often the case that along the natural rocky areas surrounding the aforementioned ports and those of other ports of the Western Mediterranean coast the species is usually not present, or its presence is accidental and maintained through immigration (except in unfrequented, difficult to access, or protected coastal areas), due to the accessibility of its habitat and anthropogenic pressure. For *P. ferruginea*, this confirms the importance of the artificial habitats of port infrastructures in maintaining important densities of adult (breeding) specimens of the species, as well as their genetic connectivity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on animals in accordance with the local legislation and institutional requirements.

Author contributions

JG-G: conceptualization of the work and development the methodology. EO-V, JG-G: application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data. EO-V, JG-G, AC-I: data analysis. JG-G, CG-O: participation in the sampled, extraction and relocation of specimens. AC-I, EO-V, RE, JLC: participation in the sampled, relocation of specimens. AC-I, RB-S: monitoring program. CG-O: designs of the two cage prototypes used and, installation and dismantling. JG-G, EO-V, AC-I, RE: writing review & editing. JG-G, EO-V, AC-I, DAF: final revision of the manuscript. JG-G: Management and coordination responsibility for the research activity planning and execution. JG-G: acquisition of the financial support for the project leading to this publication. All authors contributed to the article and approved the submitted version.

Acknowledgments

To the competent environmental authority, the Consejería de Sostenibilidad, Medio Ambiente y Economía Azul de la Junta de Andalucía, for granting the required authorization to carry out both phases of the pilot project whose results are presented here: and to Puerto José Banús, for having financed this study within the scope of the projects developed by the LBM of the US related to the ferruginean limpet in the Alboran Sea to contribute to its better knowledge, management and conservation. We also thank Juan Núñez Insausti for all the logistic support provided during the course of the pilot project, and Curro Sánchez, Estefanía Martín, Antonio Martín, Altai Pavón, Alfonso (PJB), Curro (PJB), Isabel Cárdenas, Liliana Olaya and their collaboration in the sampling campaigns and/or in some aspects related to the elaboration of the manuscript. Additionally, we express our deep gratitude to the technicians of the Consejería de Agricultura, Pesca y Desarrollo Rural, Eugenio Montes and Manuel Grondona for their valuable collaboration and advice on many different specialized topics related to the subject matter. We also thank MACVALNERA and TECNOAMBIENTE for the technical recommendations and technical tests carried out on the concrete tetrapods.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1166937/full#supplementary-material

References

Acker, J., and Leptoukh, G. (2007). Online analysis enhances use of NASA earth science data. *EOS Trans. AGU* 88 (2), 14–17. doi: 10.1029/2007EO020003

Airoldi, L., Beck, M. W., Firth, L. B., Bugnot, A. B., Steinberg, P. D., and Dafforn, K. A. (2021). Emerging solution to return nature to the urban ocean. *Ann. Rev. Mar. Sci.* 13 (1), 445–477. doi: 10.1146/annurev-marine-032020-020015

APM (2014). *Informe anual del traslado del plan de seguimiento y vigilancia ambiental. rehabilitación del embarcadero del puerto de chafarinas* (Puerto de Melilla: Ministerio de Fomento).

Arroyo, C., Moreno, D. M., Mínguez, A. B., de la Linde, A., Menéndez, J. M. R., de la Rosa, J. D. L., et al. (2011). Trabajos de seguimiento de la lapa ferruginosa *Patella ferruginea* gmelin 1791 en andalucía en el marco de la estrategia nacional de conservación de la especie. *Mediterránea: Serie Estudios Biológicos* 22), 12–49. doi: 10.14198/MDTRRA2011.ESP.02

Aversano, F. R. (1986). Esperimento di insediamento artificiale di *Patella ferruginea* gmelin 1791 nelle acque del golfo di arzachena (Sardegna settentrionale). *Boll. Malacol.* 22, 169–170.

Biagi, V., and Poli, D. (1986). Considerazioni su una popolazione di *Patella ferruginea* gmelin 1971 per le acque del promontorio di piombino. *Boll. Malacol.* 22, 171–174.

Birch, G. F., McCready, S., Long, E. R., Taylor, S. S., and Spyrakis, G. (2008). Contaminant chemistry and toxicity of sediments in Sydney harbour, Australia: spatial extent and chemistry-toxicity relationships. *Mar. Ecol. Prog. Ser.* 363, 71–88. doi: 10.3354/meps07445

Botello, G., and Krug, P. J. (2006). 'Desperate larvae' revisited: Age, energy and experience affect sensitivity to settlement cues in larvae of the gastropod alderia sp. *Mar. Ecol. Progr. Ser.* 312, 149–159. doi: 10.3354/meps312149

Boudouresque, C. F., and Laborel–Deguen, F. (1986). *Patella ferruginea, in le benthos marin de l'ile de zembra (Parc national, tunisie).* Eds. C. F. Boudouresque, J. G. Harmelin and A.J. de Grissac (Marseille: GIS Posidonie Publishers), 105–110.

Boumaza, S., and Semroud, S. (2001). Inventaire de la population de *Patella ferruginea* gmelin 1791 des iles habibas (ouest algerien). *Rapport du Congres la Commission Internationale pour l'Exploration Scientifique la Mer Mediterranée* 36, 361.

Broderip, W. J. , and Sowerby, I. G. B. (1829). Observations on new or interesting Mollusca contained, for the most part, in the museum of the zoological society. *Zoological J.* 4, 359–379.

Bulleri, F., and Airoldi, L. (2005). Artificial marine structures facilitate the spread of a non-indigenous green alga, *Codium fragile* ssp. *tomentosoides*, in the north Adriatic Sea. *J. Appl. Ecol.* 42, 1063–1072. doi: 10.1111/j.1365-2664.2005.01096.x

Bulleri, F., and Chapman, M. G. (2010). The introduction of coastal infrastructures as a driver of change in marine environments. *J. Appl. Ecol.* 47, 26–35. doi: 10.1111/j.1365-2664.2009.01751.x

CAGPyDS (JA). (2021). Informe sobre: El Proyecto de ampliación del puerto josé banús (Marbella, málaga)," in *Programa de gestión sostenible del medio Marino andaluz*, 12. Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible. Junta de Andalucía. Sevilla.

Carballo, J. L., Yáñez, B., Bautista-Guerrero, E., García-Gómez, J. C., Espinosa, F., Tortolero-Langarica, J. J., et al. (2020). Decimation of a population of the endangered species *Scutellastra mexicana* (Broderip and sowerby 1829) (Mollusca, Gastropoda) in the marías island (Eastern ocean pacific) biosphere reserve. *Aquat. Conserv.: Mar. Freshw.* 30 (1), 20–30. doi: 10.1002/aqc.3239

Casu, M., Rivera-Ingraham, G. A., Cossu, P., Lai, T., Sanna, D., Dedola, G. L., et al. (2011). Patterns of spatial genetic structuring in the endangered limpet *Patella ferruginea*: implications for the conservation of a Mediterranean endemic. *Genetica* 139, 1293–1308. doi: 10.1007/s10709-012-9631-3

Caton-Thompson, G. (1946). The aterian industry: its place and significance in the paleolithic world. *J.R. Anthropol. Inst. G.B. Irel.* 76, 87–130. doi: 10.2307/2844512

Chapman, M. G. (2003). Paucity of mobile species on constructed seawalls: effects of urbanization on biodiversity. *Mar. Ecol. Progr. Ser.* 264, 21–29. doi: 10.3354/meps264021

Connell, S. D., and Glasby, T. M. (1999). Do urban structures influence local abundance and diversity of subtidal epibiota? a case study from Sydney harbour, Australia. *Mar. Environ. Res.* 47, 373–387. doi: 10.1016/S0141-1136(98)00126-3

Council Directive. (1992). On the conservation of natural habitats and of wild fauna and flora. *OJ L* 206(7), 50. doi: 10.1017/CBO9780511610851.039

Cristo, B., and Caronni, S. (2008). Osservazioni sullo stato di conservazione di *Patella ferruginea* gmelin 1791, nelle vicinanze di capo ceraso (Golfo di olbia, sardegna nord–orientale). *Biol. Mar. Mediterr.* 15, 302–303.

Cristo, B., Caronni, S., and Floris, A. (2007). Osservazioni su *Patella ferruginea* gmelin 1791 (Mollusca, Gastropoda) nel golfo di olbia.). *Biol. Mar. Mediterr.* 14, 344–345.

Critchley, L. P., and Bishop, M. J. (2019). Differences in soft-sediment infaunal communities between shorelines with and without seawalls. *Estuaries Coasts* 42, 1127–1137. doi: 10.1007/s12237-019-00527-z

Curini–Galletti, M. (1979). Ritrovamento di una *Patella ferruginea*. Notiz. C.I.S.M.A. *Roma* 1 (1), 53–54.

Dance, M. S. P. (1967). *Shell collecting. an illustrated history* (London: Faber and Faber).

Di Franco, A., Graziano, M., Franzitta, G., Felline, S., Chemello, R., and Milazzo, M. (2011). Do small marinas drive habitat specific impacts? a case study from Mediterranean Sea. *Mar. pollut. Bull.* 62 (5), 926–933. doi: 10.1016/j.marpolbul.2011.02.053

Doneddu, M., and Manunza, B. (1992). Valutazione dell'impatto antropico relativo alla balneazione estiva su una popolazione di *Patella ferruginea* gmeli Del litorale di aglientu. *Boll. Malacol.* 28, 161–168.

Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English, C. A., et al. (2012). Climate change impacts on marine ecosystems. *Ann. Rev. Mar. Sci.* 4, 11–37. doi: 10.1146/annurev-marine-041911-111611

El-Asmar, H. M., and White, K. (2002). Changes in coastal sediment transport processes due to construction of new damietta harbour, Nile delta, Egypt. *Coast. Eng.* 46 (2), 127–138. doi: 10.1016/S0378-3839(02)00068-6

Espinosa, F. (2006). Caracterización biológica del molusco protegido *Patella ferruginea* gmelin 1791 (Gastropoda: Patellidae): bases para su gestión y conservación (Univ. of Seville).

Espinosa, F. (2009). Populational status of the endangered mollusk *Patella ferruginea* gmelin 1791 (Gastropoda: Patellidae) on Algerian islands (SW Mediterranean). *Anim. Biodivers Conserv.* 32, 19–28. doi: 10.32800/abc.2009.32.0019

Espinosa, F., González, A. R., Maestre, M. J., Fa, D., Guerra-García, J. M., and García-Gómez, J. C. (2008). Responses of the endangered limpet *Patella ferruginea* to reintroduction under different environmental conditions: survival, growth rates and life-history. *Ital. J. Zool.* 75 (4), 371–384. doi: 10.1080/11250000801887740

Espinosa, F., Guerra-García, J. M., FA, D., and García-Gomez, J. C. (2006). Aspects of reproduction and their implications for the conservation of the endangered limpet, *Patella ferruginea*. *Invertebrate Reprod. Dev.* 49 (1-2), 85–92. doi: 10.1080/07924259.2006.9652197

Espinosa, F., Guerra-García, J. M., and García-Gómez, J. C. (2007). Sewage pollution and extinction risk: an endangered limpet as a bioindicator? *Biodiv. Conserv.* 16 (2), 377–397. doi: 10.1007/s10531-005-3014-3

Espinosa, F., and Ozawa, T. (2006). Population genetics of the endangered limpet *Patella ferruginea* (Gastropoda: Patellidae): taxonomic, conservation and evolutionary considerations. *J. Zool. Syst. Evol. Res.* 44 (1), 8–16. doi: 10.1111/j.1439-0469.2005.00348.x

Espinosa, F., and Rivera-Ingraham, G. A. (2017). Biological conservation of giant limpets: the implications of large size. *Adv. Mar. Biol.* 76, 105–155. doi: 10.1016/bs.amb.2016.10.002

Espinosa, F., Rivera–Ingraham, G. A., Fa, D. A., and García–Gómez, J. C. (2009). Effect of human pressure on population size structures of the endangered ferruginean limpet: towards future management measures. *J. Coast. Res.* 25, 857–863. doi: 10.2112/08-1005.1

Espinosa, F., Rivera-Ingraham, G., and García-Gómez, J. C. (2011). Influence of habitat structure and nature of substratum on limpet recruitment: Conservation implications for endangered species. *Estuar. Coast. Shelf Sci.* 94 (2), 164–171. doi: 10.1016/j.ecss.2011.06.005

Espinosa, F., Rivera-Ingraham, G. A., Maestre, M., González, A. R., Bazairi, H., and García-Gómez, J. C. (2014). Updated global distribution of the threatened marine limpet *Patella ferruginea* (Gastropoda: Patellidae): an example of biodiversity loss in the Mediterranean. *Oryx* 48 (2), 266–275. doi: 10.1017/S0030605312000580

Estacio, F. J., García-Adiego, E. M., Fa, D. A., García-Gómez, J. C., Daza, J. L., Hortas, F., et al. (1997). Ecological analysis in a polluted area of algeciras bay (Southern spain): external 'versus' internal outfalls and environmental implications. *Mar. Poll. Bull.* 34 (10), 780–793. doi: 10.1016/S0025-326X(97)00046-5

Fa, D. A., Finlayson, G., Sempere-Valverde, J., and García-Gómez, J. C. (2018). 'Impossible're-introduction of the endangered limpet *Patella ferruginea* gmelin 1791? progress in resolving translocation mortality. *Endanger. Species Res.* 37, 219–232. doi: 10.3354/esr00921

Fenberg, P. B., and Roy, K. (2012). Anthropogenic harvesting pressure and changes in life history: insights from a rocky intertidal limpet. *Am. Nat.* 180 (2), 200–210. doi: 10.1086/666613

Firth, L. B., Knights, A. M., Bridger, D., Evans, A. J., Mieszkowska, N., Moore, P. J., et al. (2016). Ocean sprawl: Challenges and opportunities for biodiversity management in a changing world. *Oceanogr. Mar. Biol.* 54, 193–269. doi: 10.1201/9781315368597-5

Freitas, D., Arenas, F., Vale, C. G., Pinto, I. S., and Borges, D. (2023). Warning of warming limpets: sea temperature effects upon intertidal rocky assemblages. *J. Mar. Biol. Assoc. United Kingdom* 103, e7. doi: 10.1017/S0025315422001096

Frenkiel, L. (1975). Contribution à l'étude des cycles de reproduction des patellidae en algérie. *Pubblicacione la Stazzione Zoologica di Napoli* 39, 153–189.

García-Gómez, J. C. (2015). *A guide on environmental monitoring of rocky seabeds in Mediterranean marine protected areas and surrounding zones* (Tunis: Marine Biology Laboratory, Department of Zoology, Faculty of Biology, University of Seville. R+D+I Biological Research Area, Seville Aquarium. Ed. RAC/ SPA— MedMPAnet Project). Available at: http://www.rac-spa.org/sites/default/files/doc_medmpanet/final_docs_regional/78.guide_suivi_amp_med_en.pdf.

García-Gómez, J. C., Guerra-García, J. M., Espinosa, F., Maestre, M. J., Rivera-Ingraham, G., Fa, D., et al. (2015). Artificial marine micro-reserves networks (AMMRNs): an innovative approach to conserve marine littoral biodiversity and protect endangered species. *Mar. Ecol.* 36, 259–277. doi: 10.1111/maec.12167

García-Gómez, J. C., López-Fé, C. M., Espinosa, F., Guerra-García, J. M., and Rivera-Ingraham, G. A. (2011). Marine artificial micro-reserves: a possibility for the conservation of endangered species living on artificial substrata. *Mar. Ecol.* 32, 6–14. doi: 10.1111/j.1439-0485.2010.00409.x

García-Gómez, J. C., and Magariño, S. (2010). Bucear en el último confín de Europa, la isla de tarifa. instituto de estudios campogibraltareños. *Serie Cienc. Naturales* 35, 350.

Gittman, R. K., Joel, F. F., Popowich, A. M., Keller, D. A., Bruno, J. F., Currin, C. A., et al. (2015). Engineering away our natural defenses: An analysis of shoreline hardening in the US. *Front. Ecol. Environ.* 13, 301–307. doi: 10.1890/150065

Glasby, T. M., Connell, S., Holloway, M. G., and Hewitt, C. L. (2007). Nonindigenous biota on artificial structures: Could habitat creation facilitate biological invasions? *Mar. Biol.* 151, 887–895. doi: 10.1007/s00227-006-0552-5

González, J. A., Paredes, P., Enrique, C., Calzado, P., and Bueno, I. (2015). *Patella ferruginea*, patrimonio del litoral melillense: biología, ecología y conservación. *GEEPP Ediciones*, 157.

González-García, J. A., Bueno del Campo, I., García Peña, H., and Bazairi, H. (2006). "Las poblaciones de *Patella ferruginea* gmeli (Mollusca, Gastropoda, patellidae) en los acantilados de melilla y tres forcas," in *XIV simposio ibérico de estudios de biología Marina*. Eds. M. Ballesteros, C. Palacín and X. Turon(Barcelona), 152–153.

Guallart, J. (2006). *Estado de patella ferruginea en las islas chafarinas y estudios previos para la traslocación de ejemplares* (Madrid: Technical scientific report (unpublished) for Organismo Autónomo de Parque Nacionales, Ministerio de Medio Ambiente).

Guallart, J., Templado, J., Acevedo, I., Calvo, M., Machordom., A., Luque, Á.A., et al. (2014). Informe de la reintroducción de ejemplares de la lapa *Patella ferruginea* (catalogada "en peligro de extinción") en las islas hormigas (Murcia). *Unpublished*, 26.

Guerra-García, J. M., Corzo, J., Espinosa, F., Fa, D. A., and García-Gómez, J. C. (2004a). Extinction risk and harbours as marine reserves. *J. Moll. Stud.* 70, 96–98. doi: 10.1093/mollus/70.1.96

Guerra-García, J. M., Corzo, J., Espinosa, F., and García-Gómez, J. C. (2004b). Assessing habitat use of the endangered marine mollusc *Patella ferruginea* (Gastropoda, patellidae) in northern Africa: preliminary results and implications for conservation. *Biol. Conserv.* 116, 319–326. doi: 10.1016/S0006-3207(03)00201-5

Guerra-García, J. M., and García-Gómez, J. C. (2004). Crustacean assemblages and sediment pollution in an exceptional case study: a harbour with two opposing entrances. *Crustaceana* 77, 353–370. doi: 10.1163/1568540041181538

Haedrich, R. L., and Barnes, S. M. (1997). Changes over time of the size structure in an exploited shelf fish community. *Fish. Res.* 31 (3), 229–239. doi: 10.1016/S0165-7836(97)00023-4

Hanson, S. E., and Nicholls, R. J. (2020). Demand for ports to 2050: Climate policy, growing trade and the impacts of sea-level rise. *Earth's Future* 8, e2020EF001543. doi: 10.1029/2020EF001543

Henriques, P., Delgado, J., Sousa, R., and Ray, S. (2017). Patellid limpets: an overview of the biology and conservation of keystone species of the rocky shores. *Organismal Mol. malacology*, 71–95. doi: 10.5772/67862

Hind, K. R., and Saunders, G. W. (2013). A molecular phylogenetic study of the tribe Corallineae (Corallinales, Rhodophyta) with an assessment of genus-level taxonomic features and descriptions of novel genera. *J. Phycol.* 49 (1), 103–114.

Jager, K. J., Van Dijk, P. C., Zoccali, C., and Dekker, F. W. (2008). The analysis of survival data: the Kaplan–Meier method. *Kidney Int.* 74 (5), 560–565. doi: 10.1038/ki.2008.217

Keen, A. M. (1971). *Sea Shells of tropical West america. marine mollusks from Baja California to Peru. 2nd ed* (Stanford, California: Stanford University Press).

Laborel-Deguen, F., and Laborel, J. (1991a). ""Statut de *Patella ferruginea* gmelin en méditerranée,"," in *Les Espèces marines à protéger en méditerranée*. Eds. C. F. Boudouresque., M. Avon. and V. Gravez (Marseille: GIS Posidonie Publishers), 91–103.

Laborel-Deguen, F., and Laborel, J. (1991b). "Une tentative de réintroduction de *Patella ferruginea* gmel. (Gasteropoda) dans le parc national de port-cros (Var, France)," in *Les Espèces marines à protéger en méditerranée, boudouresque*. Eds. C. F. Boudouresque, M. Avon. and V. Gravez (Marseille, France: GIS Posidonie publ.), 129–132.

LIFE REMoPaF Project (2017-2021). *LIFE-15/NAT/ES/000987-REMoPaF* (UE). Available at: https://www.liferemopaf.org.

Maestre Delgado, M. J., Espinosa, F., Fa, D., Pavón, A., and García-Gómez, J. C. (2018). Estado de la población de *Patella ferruginea* en el área del estrecho de Gibraltar. *Almoraima. Rev. Estudios Campogibraltareños* 49, 115–130).

Mantel, N. (1966). Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother. Rep.* 50, 163–170.

Marra, S., Coppa, S., Camedda, A., Massaro, G., and de Lucia, G. A. (2017). The exploitation of limpets in a Mediterranean marine protected area: assessing the effectiveness of protection in the intertidal zone. *Mediterr. Mar. Sci.* 18 (3), 406–423. doi: 10.12681/mms.2087

Martins, G. M., Thompson, R. C., Neto, A. I., Hawkins, S. J., and Jenkins, S. R. (2010). Enhancing stocks of the exploited limpet patella candei d'Orbigny *via* modifications in coastal engineering. *Biol. Conserv.* 143 (1), 203–211. doi: 10.1016/j.biocon.2009.10.004

Masiá, P., Ardura, A., Gaitan, M., Gerber, S., Rayon-Vina, F., and Garcia-Vazquez, E. (2021). Maritime ports and beach management as sources of coastal macro-, meso-, and microplastic pollution. *Environ. Sci. Poll. Res.* 28, 30722–30731. doi: 10.1007/s11356-021-12821-0.org/10.3354/meps326011

MMAMRM (2008). "Estrategia para la conservación de la lapa ferrugínea (*Patella ferruginea*) en españa," in *Ministerio de medio ambiente y medio rural y Marino (MMAMRM)*, 49, Madrid.

Moreno, D. (2004). "Prosobranquios y heterobranquios. fauna andaluza, in proyecto andalucía. naturaleza XIV, zoología II, los animales esquizocelomados," in *Moluscos, anélidos, sipuncúlidos y equiúridos*. Eds. J. A. Tinaut and F. Pascual (Sevilla: Publicaciones Comunitarias, Grupo Hércules), 79–114.

Moschella, P. S., Abbiati, M., Aberg, P., Airoldi, L., Anderson, J. M., Bacchiocchi, F., et al. (2005). Low-crested coastal defence structures as artificial habitats for marine life: Using ecological criteria in design. *Coast. Eng.* 52 (10–11), 1053–1071. doi: 10.1016/j.coastaleng.2005.09.014

NASA Goddard Space Flight Center, Ocean Ecology Laboratory and Ocean Biology Processing Group (2018). *Moderate-resolution imaging spectroradiometer (MODIS) aqua chlorophyll data* (Greenbelt, MD, USA: NASA OB. DAAC). doi: 10.5067/AQUA/MODIS/L3M/CHL/2018.07/20/2022

Naylor, L. A., MacArthur, M., Hampshire, S., Bostock, K., Coombes, M. A., Hansom, J. D., et al. (2017). Rock armour for birds and their prey: ecological enhancement of coastal engineering. *Proc. Institution Civil Engineers-Maritime Eng.* 170 (2), 67–82. doi: 10.1680/jmaen.2016.28

Ostalé-Valriberas, E., Sempere-Valverde, J., Pavón-Paneque, A., Coppa, S., Espinosa, F., and García-Gómez, J. C. (2022). 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. *Mar. Policy* 136, 104917. doi: 10.1016/j.marpol.2021.104917

Paracuellos, M., Nevado, J. C., Moreno, D., Giménez, A., and Alesina, J. J. (2003). Conservational status and demographic characteristics of *Patella ferrguinea* gmelin

1791 (Mollusca, Gastropoda) on the alboran island (Western Mediterranean). *Anim. Biodiv. Conserv.* 26, 29–37.

Peto, R., and Peto, J. (1972). Asymptotically efficient rank invariant test procedures. *J. R. Stat. Soc Ser. A* 135 (2), 185–198. doi: 10.2307/2344317

Pombo, O. A., and Escofet, A. (1996). Effect of exploitation on the limpet *Lottia gigantea*: a field study in Baja California (USA). *Pac. Sci.* 50, 393–403. Available at: http://hdl.handle.net/10125/2914.

Porcheddu, A., and Milella, I. (1991). "Aperçu sur l'ecologie et sur la distribution de *Patella ferruginea* (L.) gmelin 1791 en mers italiennes," in *Les Espèces marines à protéger en méditerranée*. Eds. C. F. Boudouresque, M. Avon and V. Gravez (Marseille: GIS Posidonie Publishers), 119–128.

Raffaelli, D., and Hawkins, S. (1996). *Intertidal ecology* (London: Chapman and Hall).

Ramos, M. A. (1998). Implementing the habitats directive for mollusc species in Spain. *J. Conchol. Special Publication.* 2, 125–132.

Rivera–Ingraham, G. A., Espinosa, F., and García–Gómez, J. C. (2011a). Conservation status and updated census of *Patella ferruginea* (Gastropoda, patellidae) in ceuta: distribution patterns and new evidence of the effects of environmental parameters on population structure. *Anim. Biodiv. Conserv.* 34 (1), 83–99.

Rivera-Ingraham, G. A., Espinosa, F., and García-Gómez, J. C. (2011b). Environmentally mediated sex change in the endangered limpet *Patella ferruginea* (Gastropoda: Patellidae). *J. Moll. Stud.* 77 (3), 226–231. doi: 10.1093/mollus/eyr007

Rochet, M. J., and Trenkel, V. M. (2003). Which community indicators can measure the impact of fishing? a review and proposals. *Can. J. Fish. Aquat. Sci.* 60, 86–99. doi: 10.1139/f02-164

Ruijgrok, E. (2021). Natural solutions versus technical solutions: How ecosystem benefits can make a difference in public decisions. *Res. Urban. Ser.* 7, 261–282. doi: 10.47982/rius.7.137

Seitz, R. D., Lipcius, R. N., Olmstead, N. H., Seebo, M. S., and Lambert, D. M. (2006). Influence of shallow-water habitats and shoreline development on abundance, biomass, and diversity of benthic prey and predators in Chesapeake bay. *Mar. Ecol. Progr. Ser.* 326, 11–27. doi: 10.3354/meps326011

Sempere-Valverde, J., Guerra-García, J. M., García-Gómez, J. C., and Espinosa, F. (2023). "Coastal urbanization, an issue for marine conservation," in *Coastal habitat conservation*. Ed. F. Espinosa (Laboratorio biología Marina, Universidad de Sevilla, Sevilla, Spain: Elsevier-Academic Press), 41–79.

Stel, V. S., Dekker, F. W., Tripepi, G., Zoccali, C., and Jager, K. J. (2011). Survival analysis I: the Kaplan-Meier method. *Nephron Clin. Pract.* 119 (1), c83–c88. doi: 10.1159/000324758

Taljaard, S., Slinger, J. H., Arabi, S., Weerts, S. P., and Vreugdenhil, H. (2021). The natural environment in port development: A 'green handbrake' or an equal partner? *Ocean Coast. Manage.* 199, 105390. doi: 10.1016/j.ocecoaman.2020.105390

Templado, J. (2001). "*Patella ferruginea* (Gmelin 1791)," in *Los Invertebrados no insectos de la directiva hábitats en españa*. Eds. M. A. Ramos, D. Bragado and J. Fernández (Madrid: Ediciones Serie Técnica, Organismo Autónomo Parques Nacionales, Dirección General de Conservación de la Naturaleza, Ministerio de Medio Ambiente), 41–49.

Templado, J., Calvo, M., Moreno, D., Flores, A., Conde, F., Abad, R., et al. (2006). *Flora y fauna de la reserva Marina de pesca de la isla de alborán* (Madrid, Spain: Secretaría General de Pesca Martítima, MAPA).

Vikolainen, V., Bressers, H., and Lulofs, K. (2014). A shift toward building with nature in the dredging and port development industries: Managerial implications for projects in or near natura 2000 areas. *Environ. Manage.* 54 (1), 3–13. doi: 10.1016/10.1007/s00267-014-0285-z

Vona, I., Gray, M. W., and Nardin, W. (2020). The impact of submerged breakwaters on sediment distribution along marsh boundaries. *Water* 12 (4), 1016. doi: 10.3390/w12041016

Zarrouk, A., Salah Romdhane, M., and Espinosa, F. (2018). Reintroduction of the highly endangered mollusk *Patella ferruginea* gmelin 1791 in an MPA: A novel approach to achieve high survival rates. *Estuar. Coast. Shelf Sci.* 202, 259–269. doi: 10.1016/j.ecss.2018.01.014

frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 24 April 2023
DOI 10.3389/fmars.2023.1149888

OPEN ACCESS

EDITED BY
Oscar Sosa-Nishizaki,
Center for Scientific Research and Higher Education in Ensenada (CICESE), Mexico

REVIEWED BY
Sílvia Lourenço,
Instituto Politécnico de Leiria, Portugal
Levent Bat,
Sinop University, Türkiye

*CORRESPONDENCE
Pedro Ideia
✉ pedro.di.freitas@madeira.gov.pt

SPECIALTY SECTION
This article was submitted to Marine Fisheries, Aquaculture and Living Resources, a section of the journal Frontiers in Marine Science

RECEIVED 23 January 2023
ACCEPTED 03 April 2023
PUBLISHED 24 April 2023

CITATION
Sousa R, Gaspar M, Lucas J, Freitas M and Ideia P (2023) Shellfish consumption preferences in an oceanic archipelago. *Front. Mar. Sci.* 10:1149888.
doi: 10.3389/fmars.2023.1149888

Shellfish consumption preferences in an oceanic archipelago

Ricardo Sousa[1,2,3], Madalena Gaspar[1], Jorge Lucas[1], Mafalda Freitas[1,2,3,4] and Pedro Ideia[1,5*]

[1]Direção Regional do Mar (DRM)/Direção de Serviços de Monitorização, Estudos e Investigação do Mar (DSEIMar), Funchal, Madeira, Portugal, [2]MARE – Marine and Environmental Sciences Centre/ ARNET - Aquatic Research Network, Agência Regional Para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Funchal, Madeira, Portugal, [3]Observatório Oceânico da Madeira, Agência Regional Para o Desenvolvimento da Investigação Tecnologia e Inovação (OOM/ARDITI) – Edifício Madeira Tecnopolo, Funchal, Madeira, Portugal, [4]Faculdade de Ciências da Vida, Universidade da Madeira, Campus Universitário da Penteada, Funchal, Portugal, [5]CQM – Centro de Química da Madeira, Universidade da Madeira, Campus Universitário da Penteada, Funchal, Madeira, Portugal

A survey on shellfish consumption preferences was conducted in the Eastern Atlantic archipelago of Madeira. A total of 402 valid questionnaires were collected from locals older than 15 years old. Gender, age, highest education level, work status and salary were considered in the sample composition. Participants were inquired about frequency, amount and risks associated with consumption, as well as concerns regarding the freshness of seafood. Shellfish appears to be consumed less than once a week, and 79.1% of these consumers prefer limpets. GLM analysis showed that total salary amount was the demographic variable which most affects seafood consumption. The results indicated that the majority of the respondents consume limpets less than once a week, in an amount that varies between a quarter and a half dose. With regard to health concerns, most respondents are conscious of the importance of products' freshness and approximately half of them are aware of the existence of risks associated with consumption. In general, the awareness about freshness and possible risks of consumption is dependent on the level of education. Limpets are preferably consumed during summer, both grilled and cooked, respectively. The present study provides preliminary information on shellfish consumption trend in the archipelago of Madeira. Raising the awareness of seafood consumers in line with a health surveillance network in preparation in Macaronesia could be an important tool for spreading information related to the risks associated to its consumption.

KEYWORDS

seafood consumption, demography, limpets, awareness, Madeira archipelago

1 Introduction

Seafood is broadly recognized as an important component of a healthy diet (Minnens et al., 2020). It is pivotal for body functions and promotes proper brain and nervous system development during gestation and infancy. Additionally, it helps to prevents cardiovascular disease in adults and has anticancer properties (Liao and Chao, 2009). However, concerns about shellfish consumption have risen over the risks associated with contaminants such as toxic metals, dioxins and polychlorinated biphenyls (Minnens et al., 2020). Nevertheless, several studies have shown that the health benefits far outweigh the potential risks for the general population (EFSA, 2012; Hellberg et al., 2012).

Seafood is of high nutritional value and is especially important in oceanic islands, where marine organisms are a more accessible source of protein (Sousa et al., 2019). They provide high-quality proteins rich in essential amino acids, a wide variety of minerals, including iron, calcium, zinc, phosphorus, magnesium and selenium, vitamins A, B, and D and polyunsaturated fatty acids particularly omega-3 (Cardoso et al., 2013; Nesheim et al., 2015; Chen et al., 2022).

Portugal has the second highest fish consumption per capita in the European Union per year (*ca.* 60.62 kg) (European Commission, 2020). Seafood consumption is not only associated with the intrinsic features of fishing resources (appearance, taste, smell and texture), but also with the economic, cultural and heritage characteristics of consumers. These socio-demographic features are in turn related to place of residence, health, wellbeing, tradition and religion (Almeida et al., 2015; Murray et al., 2017; Govzman et al., 2021).

In the archipelago of Madeira, shellfish consumption is part of the region's gastronomic heritage and dates back to the fifteenth century when the Portuguese discovered and colonized the archipelago (Silva and Menezes, 1921). The accessibility to rocky shores and coastal settlement prompted the exploitation of these resources along the coast over the years (Henriques et al., 2017). This activity is of high importance for the region's small-scale fisheries, directly benefitting coastal communities and indirectly providing for the whole community (Sousa et al., 2020). The economic impact of harvesting limpets has gradually increased over the years, marked by annual catches of up to 88 tonnes and yielding a first value of *ca.* 0.5M€ in 2021 (Regional Directorate for Fisheries, 2022).

There are few studies reported in the literature on fish consumption preferences in Portugal. Fish consumption preferences in the archipelago of Madeira were addressed by Hermida and Costa (2020). Lourenço et al. (2021) studied the preferences on the consumption of sea urchins which were stated as valuable seafood to be consumed in restaurants. The present work details the preferences associated with the consumption of limpets in this archipelago.

The present characterization intents to provide readers with a comprehensive outlook on shellfish consumption preferences for locals aged 15 years and older in the archipelago of Madeira. This study was based on a questionnaire survey carried out in Madeira and Porto Santo Islands considering demographic features such as gender, age group, level of education, occupation, preferences and associated consumption risks linked to heavy metals, toxins, food poisoning among others.

2 Materials and methods

2.1 Survey design

The archipelago of Madeira has a resident population of 250744 inhabitants (INE, 2022). As such, and in order to obtain a precision of ± 5% at a 95% confidence level, a minimum of 400 valid questionnaires were performed (Israel, 1992). These were designed to obtain information on the population's seafood consumption preferences and on the communities' awareness of the possible risks associated with consumption.

The survey was carried out randomly in both in-person interviews and online forms in order to reach the study's target population, which included locals aged 15 years and older. In-person interviews and online forms were conducted in the islands of Madeira and Porto Santo between October 2021 and March 2022. Information on the study's purpose and data confidentiality was provided to all participants before starting the questionnaire.

There were 14 questions in total, including both open and closed questions to allow a more comprehensive data collection. The questionnaire also included dependent questions or contingent to the previous one. Questions were divided into three sections: sociodemographic characterization, shellfish consumption and health concerns (Supplementary material).

Sociodemographic characterization considered place of residence, gender, age group, level of education, work status and salary. Work status was organized in three groups: "Employed", "Unemployed" and "Not in the labour force". Options were codified as 0, 1 and 2. Regarding salary amount, options were "<500€", "501-750€", "751-1500" and ">1500€". When the respondents did not answer, the attributed code was 0. Options were codified as 1, 2, 3 and 4.

The main part of the questionnaire was dedicated to shellfish consumption preferences considering the frequency of weekly intake for limpets, and the options "Do not consume", "Less than 1", "1 to 3", "4 to 6" and "More than 6" were included. In order to calculate proportions, options were codified as 0, 1, 2, 3 and 4, respectively. The approximate amount of consumption for each time, in doses, provided the options "Do not consume", "1/4", "1/2", "1" and "More than 1", which were codified as 0, 1, 2, 3 and 4. A dose of limpets includes approximately 20 limpets with an average size of 55 mm, corresponding to an edible portion of approximately 80 g.

The question regarding the preferable place of consumption contemplated the options "Do not consume", "Domestic" and "Restaurants", codified as 0, 1 and 2. In addition, participants were asked about the preferred season for consuming shellfish, ("Do not consume", "Winter", "Spring", "Summer" or "Autumn") and favourite cooking method ("Do not consume", "Raw", "Cooked", "Grilled" or "Other").

In order to infer about respondents' awareness on the potential risks associated with seafood consumption, the question "Do you consider that there is some risk associated with consumption?

("Yes", "No")" was added. If yes should be the response, the participant was then directed to a question regarding the nature of the potential risks ("Allergies", "Food poisoning", "Presence of toxins", "Presence of heavy metals", "Presence of bacteria", "Presence of viruses", "Other").

2.2 Statistical analysis

Statistical analysis was performed using R Statistical Software (version 1.4.1006; R Foundation for Statistical Computing, Vienna, Austria). A Chi-square test was used for comparing variances between and within groups for each variable. For each consumption measure, the influence of each demographic variable was evaluated by applying a General Linear Model (GLM) with a Poisson distribution. Gender, age group, level of education, work status and total salary amount were considered as explanatory variables. For all tests, statistical significance was accepted when $p<0.05$.

3 Results

A total of 441 questionnaires were randomly distributed, 402 of which were considered as valid after a preliminary analysis. The majority of the survey participants (94.2%) was from Madeira archipelago ($\chi 2$ = 327.80; $p<0.05$). Surveys assigned to participants under 15 years old and non-residents in Madeira archipelago were discarded from the study.

3.1 Socio-demographic characterization

A general socio-demographic characterization of the survey participants is presented in Table 1 (n=402), adjacent to a comparison with official data (INE, 2022). The group was characterized by a higher percentage of women (54.2%) (χ^2 = 2.88; $p>0.05$), and a significant majority of participants was aged between 25 and 64 years old (χ^2 = 597.33; $p<0.05$). Regarding education level, there was an unintentional deviation from the official data, with a higher representation of people with both a high school and university education (42.5% and 43.8%, respectively) (χ^2 = 214.42; $p<0.05$).

Considering employment, the majority of participants were employed (79.1%), followed by not in the labour force (16.2%) and unemployed (4.7%) (χ^2 = 420.10; $p<0.05$). Furthermore, it was verified that income significantly differs among groups and that most participants earn between € 751-1500 a month (37.6%) (χ^2 = 96.44; $p<0.05$).

3.2 Seafood consumption

79% of the inquired reported consuming limpets (χ^2 = 15.1; $p<0.05$). Among these, the majority (55%) has never harvested limpets for their own consumption.

When asked about frequency of seafood consumption, 61.9% of participants stated that they consume less than a portion of limpets (χ^2 = 68.8; $p<0.05$) per week (χ^2 = 491.3; $p<0.05$). With regards to consumption preferences, limpets are preferably consumed grilled

TABLE 1 Demographic characteristics of surveys sample, in comparison with official data for Madeira archipelago population.

	Survey sample	Madeira Island population
Gender[a]		
Female	54.2% (n=218)	53.1% (n=133059)
Male	45.8% (n=184)	46.9% (n=117685)
Age group[a]		
15 – 24 years	16.4% (n=66)	11.2% (n=28189)
25 – 64 years	78.9% (n=317)	56.1% (n=140557)
65 years and over	4.7% (n=19)	20.0% (n=50060)
Level of education[a]		
Did not complete basic education	4.2% (n=17)	14.6% (n=36485)
Basic education	9.5% (n=38)	50.3% (n=126148)
High school	42.5% (n=171)	20.7% (n=52015)
University education	43.8% (n=176)	14.4% (n=36096)
Work status[a]		
Employed	79.1% (n=318)	40.6% (n=100642)
Unemployed	4.7% (n=19)	5.5% (n=13810)
Not in the labour force	16.2% (n=65)	54.4% (n=136292)

[a]– Census INE 2021

($\chi2$ = 198.6; $p<0.05$) in restaurants ($\chi2$ = 34.6; $p<0.05$) during summer ($\chi2$ = 799.8; $p<0.05$) (Figure 1 and Table 2).

The majority of both male and female consume between a quarter and a half dose ($p<0.05$) of limpets at a frequency inferior to once a week ($p<0.05$). Consumption is preferred at restaurants (females χ^2 = 6.600; $p<0.05$ and males χ^2 = 0.509; p=0.467), grilled ($p<0.05$) and during summer ($p<0.05$). No significant differences were observed between genders ($p>0.05$) for all questions, with the exception of consumption frequency (χ^2 = 12.750; $p<0.05$).

Results show that the age group between 25-64 years old presented the highest proportion of consumers. Analysis also revealed that most participants consume limpets less than once a week, regardless of the age group (χ^2 = 8.942; p=0.347). Contrarily to participants older than 24 years old, young adults demonstrated a preference for consuming limpets at home (χ^2 = 1.143; p=0.285).

Concerning education level, participants prefer to consume limpets less than once a week ($p<0.05$). However, this trend was not statistically significant for those without a basic education (χ^2 = 1.529; p=0.465). Responders with a basic education or less ($p<0.05$), as well as unemployed (despite with no statistical significance: χ^2 = 1.667; p=0.197), prefer to consume limpets at home. It was also found that an increase in salary leads to a preference for consumption in restaurants (χ^2 = 12.820; $p<0.05$).

Total salary amount is the demographic feature with more influence in the results in all consumption measures (Table 3).

3.3 Health concerns

Approximately 80% of the inquired have concerns about the freshness of the consumed seafood (χ^2 = 229.9; $p<0.05$), and 62.2% consider that there is some risk associated with consuming limpets (χ^2 = 23.9; $p<0.05$). From those that acknowledge risk, 48% pointed out food poisoning as the major menace. It was also considered that seafood consumption could promote the development of allergies, the bioaccumulation of heavy metals and illnesses caused by the presence of toxins, bacteria and virus (χ^2 = 285.9; $p<0.05$).

Regarding the risks associated with allergies and heavy metals, analysis among groups showed statistically significant differences between genders (χ^2 = 6.013; $p<0.05$ and χ^2 = 4.304; $p<0.05$, respectively). Concerning food poisoning and the presence of

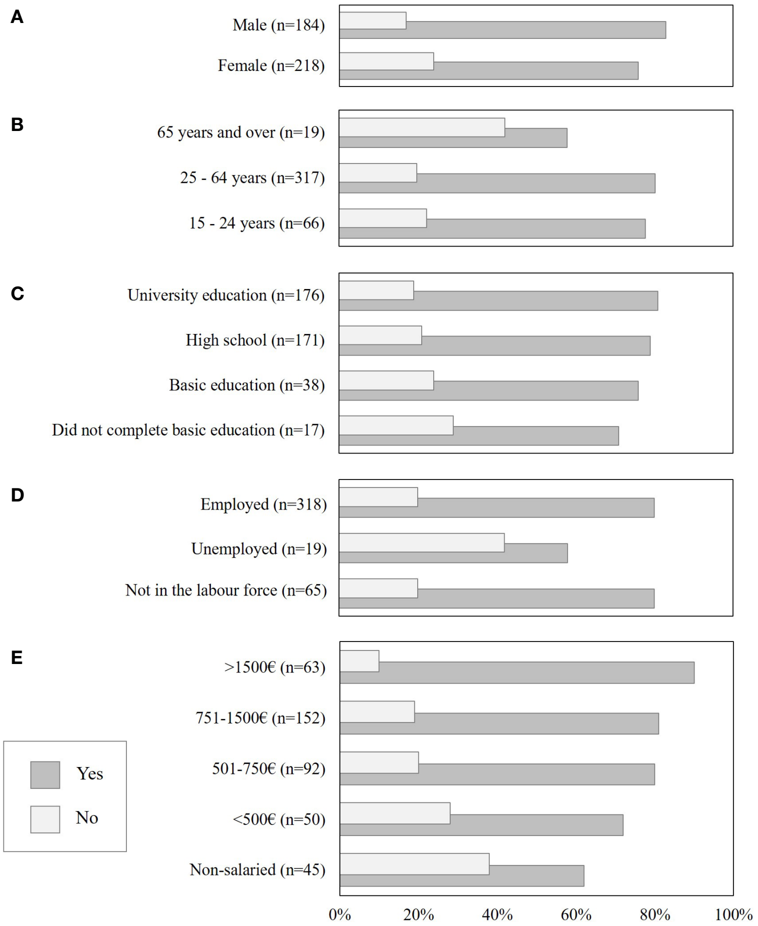


FIGURE 1
Seafood consumption in Madeira Island by gender **(A)**, age group **(B)**, level of education **(C)**, work status **(D)** and salary **(E)**.

TABLE 2 Preferences of consumers regarding frequency, amount, place, season and form of seafood consumption in Madeira archipelago.

	Consumption frequency					Consumption amount				Place		Season				Form of consumption			
	Do not consume	Less than 1/week	1 to 3/ week	4 to 6/ week	More than 6/week	1/4 of a dose	1/2 of a dose	1 dose	More than 1 dose	Domestic	Restaurant	Winter	Spring	Summer	Autumn	Raw	Cooked	Grilled	Other
Valids (n=402)	🟡	🔵	🟠	🟠	🟠	🟡	🟡	🟠	🟠	🟡	🟢	🟠	🟠	🔵	🔴	🟠	🟠	🔵	🔴
Gender																			
Female (n=218)	🟡	🔵	🟠	🟠	🟠	🟡	🟡	🟠	🟠	🟡	🟢	🔴	🟠	🔵	🔴	🟠	🟠	🔵	🔴
Male (n=184)	🟠	🔵	🟠	🟠	🟠	🟡	🟡	🟠	🟠	🟡	🟢	🟠	🟠	🔵	🔴	🟠	🟠	🔵	🔴
Age group																			
15 - 24 years (n=66)	🟡	🔵	🟠	🔴	🔴	🟡	🟢	🟠	🟠	🟢	🟡	🟠	🔴	🔵	🟠	🟠	🟠	🔵	🔴
25 - 64 years (n=317)	🟠	🔵	🟠	🟠	🟠	🟠	🟡	🟡	🟠	🟡	🟢	🟠	🟠	🔵	🔴	🟠	🟠	🔵	🔴
65 years and over (n=19)	🟢	🟢	🟠	🟠	🟠	🟢	🟡	🟠	🟠	🟠	🟢	🔴	🔴	🟢	🔴	🔴	🔴	🟢	🔴
Level of education																			
Did not complete basic education (n=17)	🟡	🟢	🟡	🔴	🔴	🟠	🟠	🟡	🟠	🟢	🟠	🟠	🔴	🔵	🔴	🔴	🔴	🔵	🔴
Basic education (n=38)	🟡	🟢	🟡	🟠	🟠	🟠	🟡	🟡	🟠	🟢	🟡	🟠	🟠	🔵	🔴	🟠	🟠	🔵	🔴
High school (n=171)	🟡	🔵	🟠	🟠	🟠	🟡	🟠	🟠	🟠	🟡	🟢	🟠	🟠	🔵	🟠	🟠	🟠	🔵	🔴
University education (n=176)	🟠	🔵	🟠	🟠	🟠	🟡	🟡	🟠	🟠	🟡	🟢	🟠	🟠	🔵	🔴	🟠	🟠	🔵	🔴
Work status																			
Employed (n=318)	🟠	🔵	🟠	🟠	🟠	🟡	🟡	🟠	🟠	🟡	🟢	🟠	🟠	🔵	🔴	🟠	🟠	🔵	🔴
Unemployed (n=19)	🟡	🟢	🟠	🔴	🔴	🟡	🟠	🟠	🟠	🟢	🟡	🔴	🟠	🟢	🔴	🔴	🟠	🟢	🔴
Not in the labour force (n= 65)	🟡	🔵	🟠	🔴	🔴	🟡	🟡	🟠	🟠	🟡	🟢	🟠	🟠	🔵	🟠	🟠	🟠	🔵	🔴
Total salary amount																			
Non-salaried (n=45)	🟡	🔵	🟠	🔴	🔴	🟡	🟠	🟠	🟠	🟡	🟡	🔴	🟠	🟢	🟠	🔴	🟠	🟢	🔴
<500€ (n=50)	🟡	🟢	🟠	🔴	🔴	🟡	🟠	🟠	🟠	🟡	🟡	🟠	🟠	🔵	🔴	🟠	🟠	🔵	🔴
501-750€ (n=92)	🟠	🟢	🟠	🟠	🟠	🟡	🟡	🟡	🟠	🟢	🟡	🟠	🟠	🔵	🔴	🟠	🟠	🔵	🔴
751-1500€ (n=152)	🟠	🟢	🟠	🟠	🟠	🟡	🟡	🟡	🟠	🟡	🟢	🟠	🟠	🔵	🔴	🟠	🟠	🔵	🔴
>1500€ (n=63)	🟠	🔵	🟠	🟠	🟠	🟢	🟡	🟠	🟠	🟡	🟢	🟠	🔴	🔵	🔴	🟠	🟠	🔵	🔴

🔴 0; 🟠 1-20; 🟡 21-40; 🟢 41-60; 🔵 61-80 ⚫ 81-100.

toxins, no statistically significant differences were shown (p>0.05) (Table 4).

Age group, level of education and total salary amount were the demographic variables responsible for the statistically significant differences observed regarding concerns about bacteria and viruses (p<0.05).

4 Discussion

The present study provides the first outlook on shellfish consumption preferences and the associated health concerns in the archipelago of Madeira. The questionnaire survey yielded a total of 402 valid questionnaires corresponding to a representative sample of the inhabitants of the archipelago (Israel, 1992). Participant related socio-demographic features such as place of residence, gender, age group, level of education, work status and salary were analysed. Questionnaires from non-residents and locals under 15 years old were discarded from the analysis as they were residual.

Seafood consumers in the archipelago of Madeira appear to be predominantly between 35 and 64 years old, with high educational qualifications, employed and more affluent. However, gender does not seem to directly influence the population's shellfish consumption. This is in line with results reported by Govzman et al. (2021), in a review from several works related to the determinants of consuming seafood.

The current analysis also demonstrated that the majority of local people (80%) usually consume limpets. From these, approximately half has already harvested limpets for their own consumption. In fact, traditional harvesting in the archipelago is allowed up to 3 kg/day/person. This high percentage may be linked to the cultural heritage of oceanic regions, generally characterized by a lack of animal protein. Once known as "the poor people's food' or 'famine food', limpets are nowadays a highly prized product, appreciated by both locals and tourists (Firth, 2021).

Additionally, results suggested that there is a local awareness about the degree of freshness of seafood and the potential risks associated with its consumption. According to the survey, approximately 80% of participants recognize the importance of seafood's freshness, 62% of which are mindful of the existence of risks associated with the consumption of limpets. Regarding the latter, food poisoning was elected as the greater potential threat by 48% of the inquired.

Despite being aware of the risks, participants did not express the expected concern in relation to the problem of heavy metals in limpets, regardless of their education level. This is particularly important since these molluscs tend to bioaccumulate metals from both geogenic and anthropogenic origins (*e.g.* cadmium) (Pedro et al., 2021).

The tolerable weekly intake (TWI) currently accepted as appropriate for cadmium in view of a safe seafood consumption is 2.5 µg/kg (ww) (EFSA, 2011). The maximum cadmium content in limpets, legislated by the Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, is 1 mg of cadmium per 1 kg of seafood wet weight. Thus, an adult weighing approximately 60 kg (FAO/WHO, 2004) can consume up to 150 g of limpets edible parts, with the maximum legislated cadmium content. Nonetheless, the cadmium content in limpets depends on different factors, including geographic location (Collado et al., 2006; Reinecke et al., 2012), season (Reinecke et al., 2012), type of substrate on which it feeds (Mbandzi et al., 2021) and size of the limpet (Cubadda et al., 2001). Therefore, the tolerable weekly quantity of limpet soft tissue should not be considered linearly, since the cadmium content in certain batches may overwrite the maximum legislated content reaching, in some cases, 3 times this maximum content (Pedro et al., 2021).

Since traditional harvesting is duly regulated and allows the harvesting of 3 kg/day/person without the need for a license, it becomes impractical to control the heavy metal content in these batches. However, since only a small portion of the inquired consume limpets more than once a week (*ca.* 17%) and considering that the amount consumed each time is always less than one dose (*ca.* 6%), consumption of limpets should not pose a threat to health of these consumers. Even so, there is an urgent need to raise awareness on this topic.

TABLE 3 Results of GLM models for seafood consumption preferences.

Consumption measure	Model fit	Demographic variables				
	Deviance explained	Gender	Age group	Level of education	Work status	Total salary amount
Consumption frequency	33%	**0.012**	0.253	0.085	0.354	**0.02**
Consumption amount	25%	0.395	0.643	**0.023**	0.461	**0.007**
Place	58%	0.083	0.067	**2.00E-06**	0.661	**1.42E-04**
Season	27%	**0.007**	0.097	0.857	0.526	**0.005**
Form	34%	**0.032**	0.951	0.995	**0.008**	**0.001**

Significance of each variable is given as the p-value of the Chi-square test for the GLM models and the p-value of the F test for the linear model, respectively.
Bold values means statistically significant (p<0.05).

TABLE 4 Consumers concerns regarding possible risks associated to seafood consumption in Madeira Island.

	Allergies	Food poisoning	Toxins	Heavy metals	Bacteria	Viruses	Other
Total (n=402)	🟡	🟡	🟠	🟠	🟠	🟠	🟠
Gender							
Female (n=218)	🟡	🟡	🟠	🟠	🟠	🟠	🔴
Male (n=184)	🟠	🟡	🟠	🟠	🟠	🟠	🟠
Age group							
15 - 24 years (n=66)	🟠	🟠	🟠	🟠	🟠	🟠	🔴
25 - 64 years (n=317)	🟡	🟡	🟠	🟠	🟠	🟠	🟠
65 years and over (n=19)	🟠	🟡	🟡	🟡	🟠	🟠	🟠
Level of education							
Did not complete basic education (n=17)	🟠	🟡	🟠	🟡	🟠	🟠	🟠
Basic education (n=38)	🟠	🟡	🟠	🟠	🟠	🟠	🔴
High school (n=171)	🟡	🟡	🟠	🟠	🟠	🟠	🟠
University education (n=176)	🟡	🟡	🟡	🟠	🟠	🟠	🔴
Work							
Employed (n=318)	🟡	🟡	🟠	🟠	🟠	🟠	🔴
Unemployed (n=19)	🟠	🟡	🟠	🟠	🟠	🟠	🔴
Not in the labour force (n= 65)	🟡	🟡	🟠	🟠	🟠	🟠	🟠
Total salary amount							
Non-salaried (n=45)	🟡	🟡	🟠	🟠	🟠	🟠	🔴
<500€ (n=50)	🟡	🟡	🟠	🟠	🟠	🟠	🟠
501-750€ (n=92)	🟡	🟡	🟠	🟠	🟠	🟠	🔴
751-1500€ (n=152)	🟡	🟡	🟠	🟠	🟠	🟠	🟠
>1500€ (n=63)	🟠	🟡	🟡	🟡	🟠	🟠	🔴

🔴 0; 🟠 1-20; 🟡 21-40; 🟢 41-60; 🟩 61-80 ⚫ 81-100.

Based on this study findings, future intervention strategies may take place in order to grow awareness about the real risks associated with the consumption of gastropod molluscs.

5 Conclusions

Limpets have been a delicacy consumed in oceanic archipelagos for several decades. Once consumed to combat hunger and lack of nutrients, nowadays it is considered a gourmet dish in such regions, highly sought by both locals and visitors.

The present study had the goal to identify preferences of limpet's consumption in Madeira Island. For so, a survey was conducted to evaluate several consumption measures, namely frequency, amount, season and risks associated. A relationship between these data and the demographic characteristics of the respondents was performed.

Some of the results showed that seafood consumers resident in Madeira Island are predominantly between 35 and 64 years old, presenting educational qualifications and better financial background. It was also evident a local awareness regarding the degree of freshness of seafood and the potential risks associated with its consumption. However, it was possible to state that work has to be done in the field of information related to the potential risks due to the accumulation of heavy metals in gastropod molluscs, in special cadmium from anthropogenic and geogenic origins. Moreover, the total salary amount was the sociodemographic characteristic which had more influence in the seafood consumption preferences.

The present study also made it possible to identify the existence of misinformation regarding health risks associated with limpets' consumption. Thus, the creation of a health surveillance network for fishery products must include actions to raise awareness of these aspects among the population.

This work adds new data on seafood consumption preferences in Madeira Island.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RS, MF and PI contributed to conception and design of the survey. RS, MG and JL carried out field data collection. RS and PI organized and the data and performed statistical analysis. RS, MG and PI wrote the sections of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by RASPA project (co-financed by EU Interreg 14-20 MAC program) and the Regional Government of Madeira. This study had the support of FCT through the strategic project UIDB/04292/2020 awarded to MARE and through project LA/P/0069/2020 granted to the Associate Laboratory ARNET. PI was the recipient of a PhD Grant under the project M1420-09-5369-FSE-000001 by ARDITI-Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação.

Acknowledgments

We thank Dr Sandra Brito, Dr Filipa Duarte, Dr Roger Corominola, for their collaboration in the collection of data for this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1149888/full#supplementary-material

References

Almeida, C., Altintzoglou, T., Cabral, H., and Vaz, S. (2015). Does seafood knowledge relate to more sustainable consumption? *Br. Food J.* 117, 894–914. doi: 10.1108/BFJ-04-2014-0156

Cardoso, C., Lourenço, H., Costa, S., Gonçalves, S., and Nunes, M. L. (2013). Survey into the seafood consumption preferences and patterns in the portuguese population. gender and regional variability. *Appetite* 64, 20–31. doi: 10.1016/j.appet.2012.12.022

Chen, J., Jayachandran, M., Bai, W., and Xu, B. (2022). A critical review on the health benefits of fish consumption and its bioactive constituents. *Food Chem.* 369, 130874. doi: 10.1016/j.foodchem.2021.130874

Collado, C., Ramírez, R., Bergasa, O., Hernández-Brito, J. J., Gelado-Caballero, M. D., and Haroun, R. J. (2006). "Heavy metals (Cd, Cu, Pb and zn) in two species of limpets (Patella rustica and patella candei crenata) in the canary islands, Spain," in *Water pollution VIII: Modelling, monitoring and management* (Bologna, Italy: WIT Press), 45–53. doi: 10.2495/WP060051

Cubadda, F., Conti, M. E., and Campanella, L. (2001). Size-dependent concentrations of trace metals in four Mediterranean gastropods. *Chemosphere* 45, 561–569. doi: 10.1016/S0045-6535(01)00013-3

EFSA (2011). Statement on tolerable weekly intake for cadmium. *EFSA J.* 9, 1-19. doi: 10.2903/j.efsa.2011.1975

EFSA (2012). Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA J.* 10, 1- 241. doi: 10.2903/j.efsa.2012.2985

European Commission (2020). *The EU fish market: 2020 edition* (LU:Brussles). doi: 10.2771/664425

FAO/WHO (2004). *Evaluation of certain food additives and contaminants: sixty-first report of the joint FAO/WHO expert committee on food additives* (Geneva: WHO).

Firth, L. B. (2021). What have limpets ever done for us? on the past and present provisioning and cultural services of limpets. *Int. Rev. Environ. Hist.* 7, 5–45. doi: 10.22459/IREH.07.02.2021.01

Govzman, S., Looby, S., Wang, X., Butler, F., Gibney, E. R., and Timon, C. M. (2021). A systematic review of the determinants of seafood consumption. *Br. J. Nutr.* 126, 66–80. doi: 10.1017/S0007114520003773

Hellberg, R. S., DeWitt, C. A. M., and Morrissey, M. T. (2012). Risk-benefit analysis of seafood consumption: A review. *Compr. Rev. Food Sci. Food Saf.* 11, 490–517. doi: 10.1111/j.1541-4337.2012.00200.x

Henriques, P., Delgado, J., and Sousa, R. (2017). "Patellid limpets: An overview of the biology and conservation of keystone species of the rocky shores," in *Organismal and molecular malacology*. Ed. S. Ray Rijeka - Croatia doi: 10.5772/67862

Hermida, M., and Costa, S. (2020). Between tradition and taste: Fish consumption habits in a small Portuguese archipelago. *J. Aquat. Food Prod.* 29(4), 1–15. doi: 10.1080/10498850.2020.1734892

INE (2022) *Censos 2021 - resultados definitivos*. Available at: https://bit.ly/3CSFb2X.

Israel, G. D. (1992). *Determining sample size. fact sheet PEOD-6, a series of the program evaluation and organizational development, Florida cooperative extension service* (Gainesville (Florida: Institute of Food and Agricultural Sciences, University of Florida).

Liao, I. C., and Chao, N.-H. (2009). Aquaculture and food crisis: opportunities and constraints. *Asia Pac. J. Clin. Nutr.* 18, 564–569.

Lourenço, S., Mendes, S., Raposo, A., Santos, P. M., Gomes, A. S., Ganhão, R., et al. (2021). Motivation and preferences of Portuguese consumers' towards sea urchin roe. *Int. J. Gastron Food Sci.* 24, 1 - 9. doi: 10.1016/j.ijgfs.2021.100312

Mbandzi, N., Nakin, M., Saibu, G., and Oyedeji, A. (2021). Heavy metal profiles in limpets and algae on the Eastern cape coast of south Africa. *Afr. J. Mar. Sci.* 43, 293–308. doi: 10.2989/1814232X.2021.1948918

Minnens, F., Marques, A., Domingo, J. L., and Verbeke, W. (2020). Consumers' acceptance of an online tool with personalized health risk-benefit communication about seafood consumption. *Food Chem. Toxicol.* 144, 111573. doi: 10.1016/j.fct.2020.111573

Murray, G., Wolff, K., and Patterson, M. (2017). Why eat fish? factors influencing seafood consumer choices in British Columbia, Canada. *Ocean Coast. Manage.* 144, 16–22. doi: 10.1016/j.ocecoaman.2017.04.007

Nesheim, M. C., Oria, M., and Yih, P. T. (2015) *A framework for assessing effects of the food system* (Institute of Medicine, Washington (DC: National Academies Press (US). Available at: http://www.ncbi.nlm.nih.gov/books/NBK305181/ (Accessed January 17, 2023).

Pedro, S., Cardoso, C., Caetano, J., Mourato, M., and Lourenço, H. M. (2021). Chemical and microbiological contamination in limpets (Patella aspera) of the Portuguese coast. *Food Control* 119, 107492. doi: 10.1016/j.foodcont.2020.107492

Reinecke, A. J., Mdzeke, N. P., and Reinecke, S. A. (2012). Spatial and temporal variation in cadmium body loads of four intertidal invertebrates from false bay, south Africa. *Afr. Zool.* 47, 12–25. doi: 10.1080/15627020.2012.11407518

Silva, F. A., and Menezes, C. A. (1921). *Elucidário madeirense* (Funchal, Madeira, Portugal: Tipografia Esperança, Funchal).

Sousa, R., Riera, R., Vasconcelos, J., Gouveia, L., Rita Pinto, A., Delgado, J., et al. (2020). "Artisanal harvest of shellfish in the northeastern Atlantic: The example of limpet and topshell fisheries in the archipelago of Madeira," in *Invertebrates - ecophysiology and management*. Eds. S. Ray, G. Diarte-Plata and R. Escamilla-Montes Rijeka - Croatia. doi: 10.5772/intechopen.85728

Sousa, R., Vasconcelos, J., Henriques, P., Pinto, A. R., Delgado, J., and Riera, R. (2019). Long-term population status of two harvested intertidal grazers (*Patella aspera* and *Patella candei*), before, (1996–2006) and after, (2007–2017) the implementation of management measures. *J. Sea Res.* 144, 33–38. doi: 10.1016/j.seares.2018.11.002

Frontiers in Marine Science

TYPE Original Research
PUBLISHED 26 April 2023
DOI 10.3389/fmars.2023.1134991

EDITED BY
Khor Waiho,
University of Malaysia Terengganu, Malaysia

REVIEWED BY
Yingying Ye,
Zhejiang Ocean University, China
Leila Belén Guzmán,
Instituto de Biología Subtropical, Argentina
Jun Liu,
Institute of Deep-Sea Science and Engineering, Chinese Academy of Sciences (CAS), China

*CORRESPONDENCE
Joong-Ki Park
jkpark@ewha.ac.kr

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Marine Fisheries, Aquaculture and Living Resources, a section of the journal Frontiers in Marine Science

RECEIVED 31 December 2022
ACCEPTED 20 March 2023
PUBLISHED 26 April 2023

CITATION
Putri ET, Lee D, Kwak H, Kim Y, Nakano T and Park J-K (2023) Complete mitochondrial genomes of the "Acmaeidae" limpets provide new insights into the internal phylogeny of the Patellogastropoda (Mollusca: Gastropoda). *Front. Mar. Sci.* 10:1134991. doi: 10.3389/fmars.2023.1134991

Complete mitochondrial genomes of the "Acmaeidae" limpets provide new insights into the internal phylogeny of the Patellogastropoda (Mollusca: Gastropoda)

Eggy Triana Putri[1†], Damin Lee[1†], Haena Kwak[1], Yukyung Kim[1], Tomoyuki Nakano[2] and Joong-Ki Park[1,3*]

[1]Division of EcoScience, Ewha Womans University, Seoul, Republic of Korea, [2]Seto Marine Biological Laboratory, Kyoto University, Nishimuro, Wakayama, Japan, [3]Natural History Museum, Ewha Womans University, Seoul, Republic of Korea

The subclass Patellogastropoda (called "true limpets") is one of the most primitive groups of the Gastropoda and contains approximately 350 species worldwide. Within this subclass, internal phylogeny among family members, including relationships of the "Acmaeidae" with other patellogastropod families, remains incompletely clarified. Here, we newly determined two complete mitochondrial genome sequences of "Acmaeidae" (*Acmaea mitra* and *Niveotectura pallida*) and one sequence from Lottiidae species (*Discurria insessa*) and combined them with mitochondrial genome sequences of 20 other published limpet species for phylogenetic analysis of the sequence dataset (nucleotides and amino acids) of 13 protein-coding genes using maximum likelihood and Bayesian inference methods. The resulting phylogenetic trees showed monophyly of Patellogastropoda species that were subsequently subdivided into two clades [clade I (Nacellidae, Pectinodontidae, Acmaeidae, and Patellidae) and clade II (Eoacmaeidae and Lottiidae)]. The sister relationship between the Acmaeidae and Pectinodontidae species revealed by phylogenetic analysis was also supported by sharing their similar gene arrangement patterns, which differ substantially from those of clade II members including the Lottiidae species. The polyphyletic relationship between Acmaeidae (grouped with Pectinodontidae as a sister taxon in clade I) and Lottiidae species (grouped with Eoacmaeidae in clade II) corroborates that they are phylogenetically distinct from each other. This mitochondrial genome phylogeny contradicts previous morphology-based hypotheses, yet highlights that Acmaeidae and Pectinodontidae are the most closely related. Further in-depth analysis of the complete mitochondrial genome sequences based on a broad range of samples including those from relatively unstudied and/or underrepresented taxa is required to fully understand the mitochondrial genome evolution and a more comprehensive phylogeny among the major groups of the Patellogastropoda.

KEYWORDS

mitochondrial genome, Acmaeidae, Pectinodontidae, Lottiidae, Patellogastropoda phylogeny

1 Introduction

Acmaeidae is a family of Patellogastropoda (called "true limpets") that represents one of the most primitive groups of the Gastropoda and contains approximately 350 species worldwide. They are found in lower intertidal to subtidal zones and constitute macrobenthic communities on coastal rocky shores, playing an important role in littoral ecosystems (Branch, 1985). This family is characterized by having a foliated shell with a fibrillar prismatic shell microstructure, one feather-shaped ctenidium, and a single pair of tricuspid radular teeth (Lindberg, 1998). Over the last century, external shell morphology has been the feature most widely used for both species-level taxonomy and investigating the internal phylogeny of the Patellogastropoda. However, morphological traits of limpet species are highly variable with different morphs from the ontogenic or ecophenotypic origin, resulting in substantial taxonomic confusion and disagreement on their phylogenetic relationships (Nakano and Ozawa, 2007). Soft body anatomy including radula characters has also been considered phylogenetically informative and widely used to investigate the relationships among the Patellogastropoda families in the past decades (Lindberg, 1998; Sasaki, 1998). Nevertheless, the taxonomic confusion associated with the absence of key characters for defining the family boundaries, and convergent evolution and ecophenotypic plasticity in their morphological features has resulted in different classification systems of the Patellogastropoda over the last decades (Sasaki et al., 2006; Nakano and Sasaki, 2011).

Against this background, phylogenetic relationships including the taxonomic status of the "Acmaeidae" with the Patellogastropoda have remained unresolved from previous studies that employed different sources of taxonomic information, such as shell characters, internal morphology, and molecular sequence data [Dall, 1876; Lindberg, 1998; Sasaki, 1998 (morphology); Harasewych and McArthur, 2000; Nakano and Ozawa, 2007 (molecular data)]. The Acmaeidae was traditionally divided into two subfamilies, Acameinae and Pectinodontinae, based on morphological perspectives (Lindberg, 1998), but these two subfamilies (*sensu* Lindberg, 1998) were not found as a monophyletic group by subsequent molecular analysis of three mitochondrial genes (mtDNA 12S rDNA, 16S rDNA, and *cox1*; Nakano and Ozawa, 2007). Moreover, despite this molecular study reporting that the Acmaeidae species (*Acmaea mitra*, *Niveotectura pallida*, and *Erginus sybaritica*) were grouped with Lottiidae species, their relationships were not concordant with other studies using the morphology or using different molecular markers, i.e., nuclear vs. mitochondrial DNA: The Acmaeidae species were included as a subgroup of Lottiidae based on their morphological similarity with lottiid species (e.g., shell microstructure, radula characters, and presence of a ctenidium) (Lindberg, 1998), but they were revived as a separate family (Nakano and Sasaki, 2011) from the Lottiidae. These phylogenetic uncertainties of the "Acmaeidae" necessitate further study using different molecular evidence to elucidate their relationships with other Patellogastropoda families.

During recent decades, comparative analysis of the complete mitochondrial genome information (i.e., nucleotide sequence and gene order data) has been widely used for investigating the phylogenetic relationships in many molluscan groups including Patellogastropoda groups where interrelationships were not fully resolved (Uribe et al., 2019; Feng et al., 2020; Xu et al., 2022). In this study, we aimed to (1) resolve phylogenetic uncertainty of the Acmaeidae and (2) extend our knowledge of mitochondrial genome diversity among major groups of the Patellogastropoda. For this, we determined two complete genome sequences of "Acmaeidae" (*Acmaea mitra* and *Niveotectura pallida*), the first representatives from the Acmaeidae, and one complete genome sequence of Lottiidae (*Discurria insessa*), combined them with the mitochondrial genome sequences of 20 other published limpet species plus 12 non-patellogastropod species (including 2 chiton species), and performed phylogenetic analysis based on nucleotide sequence information and gene order data.

2 Materials and methods

2.1 DNA extraction

Genomic DNA from three limpet species (*A. mitra, N. pallida*, and *D. insessa*) was extracted from muscle tissues of an ethanol-fixed single individual using E.Z.N.A. mollusk DNA kit (Omega Biotek, Norcross, GA, USA), in accordance with the manufacturer's instructions, and subjected to next-generation sequencing (NGS) library construction and sequencing.

2.2 Library preparation and next-generation sequencing

NGS sequencing libraries were prepared using Illumina TruSeq DNA library preparation kit and sequenced with a 151 bp paired-end run of the Illumina HiSeq X sequencing platform (Illumina, San Diego, CA, USA).

2.3 Mitochondrial genome assembly and gene annotation

Low-quality reads and adaptor sequences were trimmed using Trimmomatic v0.39 (Bolger et al., 2014). The clean reads of *A. mitra* (clean reads: 76,270,083), *D. insessa* (clean reads: 73,627,056), and *N. pallida* (clean reads: 14,140,656) were *de novo* assembled into circular DNAs using NOVOPlasty V.4.2 (Dierckxsens et al., 2017) and their genes were annotated on the MITOS webserver (Bernt et al., 2013). The protein-coding genes (PCGs) and ribosomal RNA (rRNA) genes were also confirmed by comparing them with those of other Patellogastropoda species using Geneious$^{®}$ v.11.0.11. The tRNA genes for each mtDNA sequence were identified using the tRNAscan-SE program (Lowe and Eddy, 1997) or by manually inspecting potential secondary structures and anticodon sequences.

2.4 Phylogenetic analyses

We performed phylogenetic analyses for 29 mitochondrial genome sequences from 23 Patellogastropoda species (including three newly sequenced in this study) and 4 non-Patellogastropoda (Heterobranchia and Vetigastropoda) species, as well as 2 chiton species as outgroups (Table 1). Nucleotide sequences of 13 protein-coding genes were individually translated to amino acid sequences and multiple-aligned by 'reverse translation' of the aligned protein sequences using RevTrans 1.4 webserver (Wernersson and Pedersen, 2003), and concatenated using MEGA v10.2.6 (Kumar et al., 2018). The best-fit substitution models for nucleotide (NT) and amino acid (AA) sequences for each of the 13 PCGs were identified (see Table 2) using jModelTest (NT; Darriba et al., 2012) and ProtTest 3.4.2 (AA; Darriba et al., 2011) on the CIPRES webserver (Miller et al., 2010). Phylogenetic relationships were reconstructed using Bayesian inference (BI) with MrBayes 3.2.6 (Ronquist et al., 2012) and maximum likelihood (ML) with RAxML V.8.2.9 (Stamatakis, 2014) on the CIPRES portal (Miller et al., 2010). A Markov chain Monte Carlo (MCMC) analysis was performed using two independent runs of 1×10^6 generations with four chains, sampling every 100 generations for the BI tree. Bayesian posterior probability (BPP) values were estimated after discarding the initial 2×10^3 trees that reached a stationary stage of the parameters as burn-in. The bootstrap analysis was also performed with 1,000 replicates for the resulting

2.5 Phylogenetic analysis using gene order information

Pairwise comparisons of the gene order among Patellogastropoda species were conducted to infer the parsimonious rearrangement scenarios (e.g., transpositions, reverse transpositions, reversals and tandem-duplication-random-loss events) using CREx (Bernt et al., 2007) based on common intervals. The MLGO web server was used to determine the phylogeny among Patellogastropoda species based on gene order information (PCGs, rRNAs, and tRNAs) (Hu et al., 2014).

2.6 Divergence time estimation

Divergence times among Patellogastropoda species were estimated based on nucleotide dataset of 13 protein-coding genes using an uncorrected lognormal relaxed clock model with a Yule speciation process as a tree prior in BEAST v2.6.7 (Bouckaert et al., 2019). A calibrated Yule speciation model was used as a tree prior, and the best partition schemes and the best evolution models were estimated in Partition Finder 2 based on BIC. We set the time calibration on three points based on Nacellidae (47.8–56 MYA, million years ago) from Nakano & Ozawa (2007), Patellogastropoda (336–339.4 MYA), and Neoloricata (460-466 MYA) data from the Paleobiology Database (https://paleobiodb.org/). We ran Markov chain for three times, each running for $10x10^7$ generations, and sampling every 1,000 generations. The convergence of chains were confirmed with Tracer v1.7.2 (Rambaut et al., 2018) and the first 10% of samples were discarded as burn-in. The results were combined with Log combiner and Tree annotator of the BEAST package. The maximum clade credibility (MCC) tree was visualized in FigTree 1.4.4 (Rambaut, 2018).

3 Results

3.1 Characteristics of mitochondrial genomes of the Acmaeidae

The mitochondrial genomes of the two Acmaeidae species are 17,903 bp (*A. mitra*), 18,670 bp (*N. pallida*) and *D. insessa* mtDNA is 18,225 bp in size, each containing 37 genes (13 PCGs, 22 tRNA genes, and 2 rRNA genes). The *D. insessa* mtDNA has a duplicated copy of *trnM*, a universal feature of Lottiidae species that were determined thus far. All tRNA genes from these three newly determined mitochondrial genomes have a typical clover-leaf secondary structure, with the exception of one tRNA in *N. pallida* (*trnS1*) and three tRNAs in *D. insessa* (*trnS1*, *trnS2*, and *trnE*). Both *trnS1* and *trnS2* lack a DHU stem, whereas the TΨC arm is absent in *trnE*. This result is consistent with an earlier study that DHU stems are missing in *trnS1* and *trnS2* in *Lottia goshimai* and *Nipponacmea fuscoviridis* (Feng et al., 2020). Information on the genome organization, overall nucleotide composition, start/termination codons, and codon usage of 13 PCGs for the three species is presented in the supplementary data (Supplementary Tables 1–9). Nucleotide compositions of the three newly sequenced species were 26% A, 36.9% T, 24.2% G, and 12.9% C for *A. mitra*; 28% A, 37.8% T, 22.3% G, and 11.91% C for *N. pallida*; and 30.1% A, 30.3% T, 17.5% G, and 22.1% C for *D. insessa* (see Supplementary Tables 2, 5, 8). In their entire genome sequences, there is a strong bias toward A and T, with the A + T content ranging from 60.4% (*D. insessa*) to 65.8% (*N. pallida*), which is similar to previously reported findings in other Patellogastropoda species (Feng et al., 2020).

3.2 Phylogenetic relationships of the Acmaeidae within the Patellogastropoda

Phylogenetic analyses of nucleotide (NT) and amino acid (AA) sequences using ML and BI methods found an identical tree topology (Figure 1): The resulting trees show that the Patellogastropoda species formed a monophyletic grouping that was strongly supported [76% BP in ML and 1.0 BPP in BI (NT dataset) and 84% BP in ML and 0.99 BPP in BI (AA dataset)], relative to other gastropod groups (e.g., Heterobranchia,

TABLE 1 Complete mitochondrial genomes used for phylogenetic analysis in this study.

Family	Species	Length (bp)	GenBank accession no.	Reference
Acmaeidae	*Acmaea mitra**	17,903	OQ133456	This study
	*Niveotectura pallida**	18,670	OQ133458	This study
Eoacmaeidae	*Eoacmaea* sp.	5,801 (partial sequence)	MH916652.1	Uribe et al., 2019
Lottiidae	*Discurria insessa**	18,225	OQ133457	This study
	Lottia cassis	18,797	MZ130258	Xu et al., 2022
	Lottia digitalis	26,835	DQ238599.1	Simison et al., 2006
	Lottia goshimai	17,849	MZ766128	Xu et al., 2022
	Nipponacmea fuscoviridis	18,720	MK395167.1	Feng et al., 2020
	Patelloida conulus	17,030	MZ048283	Xu et al., 2022
	Patelloida ryukyuensis	16,319	MZ130310	Xu et al., 2022
	Patelloida saccharina lanx	19,427	MW735838	Xu et al., 2022
Nacellidae	*Cellana grata*	16,181	MW722939	Xu et al., 2022
	Cellana nigrolineata	16,148	MW727704	Xu et al., 2022
	Cellana radiata	16,194	MH916651.1	Uribe et al., 2019
	Cellana toreuma	16260	MW716504	Xu et al., 2022
	Nacella clypeater	16,742	KT990124.1	Gaitán-Espitia et al., 2019
	Nacella concinna	16,761	KT990126.1	Gaitán-Espitia et al., 2019
	Nacella magellanica	16,663	KT990125.1	Gaitán-Espitia et al., 2019
Patellidae	*Patella ferruginea*	14,400	MH916654.1	Uribe et al., 2019
	Patella vulgata	14,808	MH916653.1	Uribe et al., 2019
	Scutellastra flexuosa	15,233	MW735839	Xu et al., 2022
Pectinodontidae	*Bathyacmaea lactea*	18,446	MW309841.1	Liu et al., 2021
	Bathyacmaea nipponica	16,792	MF095859.1	Sun et al., 2019
Aplysiidae	*Aplysia californica*	14,117	AY569552.1	Knudsen et al., 2006
Fissurellidae	*Diodora graeca*	17,209	KT207825.2	Uribe et al., 2016a
	Fissurella volcano	17,575	JN790612.1	Unpublished data
Hydrocenidae	*Georissa bangueyensis*	15,267	KU342664.1	Uribe et al., 2016b
Neritidae	*Nerita melanotragus*	15,261	GU810158.1	Castro and Colgan, 2010
Onchidiidae	*Onchidella celtica*	14,150	AY345048.2	Grande et al., 2004
Peltospiridae	*Chrysomallon squamiferum*	15,388	AP013032.1	Nakagawa et al., 2014
	Gigantopelta aegis	16,097	MW442948.1	Lan et al., 2021
Pomatiopsidae	*Tricula hortensis*	15,179	EU440735.1	Unpublished data
Vermetidae	*Ceraesignum maximum*	15,578	HM174253.1	Rawlings et al., 2010
Acanthochitonidae	*Cryptochiton stelleri*	15,082	KJ569363.1	Irisarri et al., 2014
Ischnochitonidae	*Lepidozona coreanica*	16,572	NC_046935.1	Unpublished data

*: newly determined in this study.

TABLE 2 The best-fit models estimated from each of the 13 protein-coding genes of 23 patellogastropod and 6 other gastropod species used for phylogenetic analysis.

Gene	Nucleotide	Amino acid
atp6	GTR+I+G	LG+I+G+F
atp8	TIM3+I+G	HIVb+I+G+F
cytb	TVM+I+G	CpREV+I+G+F
cox1	GTR+I+G	LG+I+G+F
cox2	GTR+I+G	LG+I+G+F
cox3	GTR+I+G	CpREV+G+F
nad1	GTR+I+G	MtArt+I+G+F
nad2	GTR+I+G	LG+I+G+F
nad3	GTR+I+G	MtArt+I+G+F
nad4	TVM+I+G	MtREV+I+G+F
nad4L	TVM+I+G	MtArt+I+G+F
nad5	TVM+I+G	JTT+I+G+F
nad6	GTR+I+G	MtREV+G+F

Vetigastropoda), and were subsequently subdivided into two groups (clades I and II) with high branch support. Inclusion of other gastropod groups (e.g., Neritimorpha, Caenogastropoda, and Neomphalina) in the analysis did not change the internal relationships among Patellogastropoda species (Supplementary Figure 1). Within the Patellogastropoda, clade I is represented by four monophyletic limpet families (Nacellidae, Pectinodontidae, Acmaeidae, and Patellidae), whereas clade II comprises the two remaining families: Eoacmaeidae and Lottiidae. Compared with clade II, clade I was more strongly supported [92% BP in ML and 1.00 BPP in BI (NT dataset) and 97% BP in ML and 1.00 BPP in BI (AA dataset)]. Within clade I, the Patellidae was positioned basal to the group of families Acmaeidae, Pectinodontidae, and Nacellidae, in which the former two families formed a robust sister relationship. The two Acmaeidae species (*A. mitra* and *N. pallida*) were grouped with Pectinodontidae species (*Bathyacmaea lactea* and *B. nipponica*) receiving high supporting values from the NT dataset (93% BP in ML and 1.00 BPP in BI; Figure 1), but relatively moderate support in ML analysis for the AA dataset (72% BP in ML; see high branch support of 1.00 BPP in BI). Within clade II, a partial sequence of *Eoacmaea* sp. representing the family Eoacmaeidae formed a sister relationship with the strongly supported monophyletic Lottiidae including *D. insessa* (newly sequenced) [70% BP in ML and 1.00 BPP in BI (NT dataset); 80% BP in ML and 0.99 BPP in BI (AA dataset)]. Within Lottiidae, the monophyletic *Patelloida* species was sister to the group of *Lottia*, *Discurria*, and *Nipponacmea* species with high branch support (100% BP in ML and 1.00 BP in BI). Phylogenetic relationships among the Patellogastropoda species inferred from inclusion of other gastropod groups in phylogenetic analysis (e.g., Neritimorpha, Caenogastropoda, and Neomphalina; Supplementary Figures 1, 2) were identical to those depicted in Figure 1.

3.3 Gene arrangement patterns among major Patellogastropoda groups

Comparison of gene order information among Patellogastropoda species indicates that the gene arrangement patterns among clade I members are relatively conserved, compared to the clade II members in which gene rearrangements are very substantial (Figure 2). Excluding tRNA genes that are highly variable in their position even between closely related species, the gene arrangement pattern of 13 PCGs and two rRNA genes among the group of three families Nacellidae, Pectinidontidae, and Acmaeidae is identical, but radically differs from that of their sister group Patellidae. By contrast, an extensive gene order rearrangement was observed among clade II members, even between congeneric species belonging to the genera *Patelloida* and *Lottia*. Phylogenetic tree from parsimony analysis based on gene order data (Supplementary Figure 3) is generally consistent with relationships among the family members inferred from phylogenetic analysis of the protein-coding gene sequence data (see Discussion section for the details of their relationships).

3.4 Estimated divergence times among major Patellogastropoda groups

Divergence time estimation using mitochondrial 13 PCGs inferred the origin of Patellogastropoda was dated 337.20 MYA (95% HPD interval 332.60–341.81 MYA) in the middle Carboniferous period (Figure 3). Subsequent divergence among major family groups belonging to clade I and clade II was estimated to begin in the Permian-Triassic boundary (clade I; 257.67 MYA; 185.20–325.34) and in the early Permian period (clade II; 295.84 MYA; 95% HPD interval 255.72–329.43 MYA), respectively. Within clade I, the family Patellidae started branching off first from the common ancestor of the remaining families (Nacellidae, Pectinodontidae, and Acmaeidae) in the Permian-Triassic boundary, followed by lineage splitting of the Acmaeidae from the Pectinodontidae in the Jurassic-Cretaceus boundary (132.62 MYA; 95% HPD interval 65.84–206.46 MYA).

4 Discussion

The Acmaeidae is one of the most taxonomically debated Patellogastropoda families, particularly in terms of their taxonomic status and phylogenetic relationships with other groups. Nonetheless, to date there has been only minimal study of this group using molecular data (Nakano and Sasaki, 2011). The relationships inferred in many previous studies did not agree with each other, according to the data sources used in the analysis, such as morphology vs. molecular data, or even between molecular markers (i.e., nuclear vs. mitochondrial DNA). Members of the family Acmaeidae were originally assigned to many lottiid genera and species (e.g., Pilsbry, 1891). However, this scheme was substantially revised based on the shell structure (Lindberg, 1986). After this revision, the genus *Acmaea* was restricted to the type

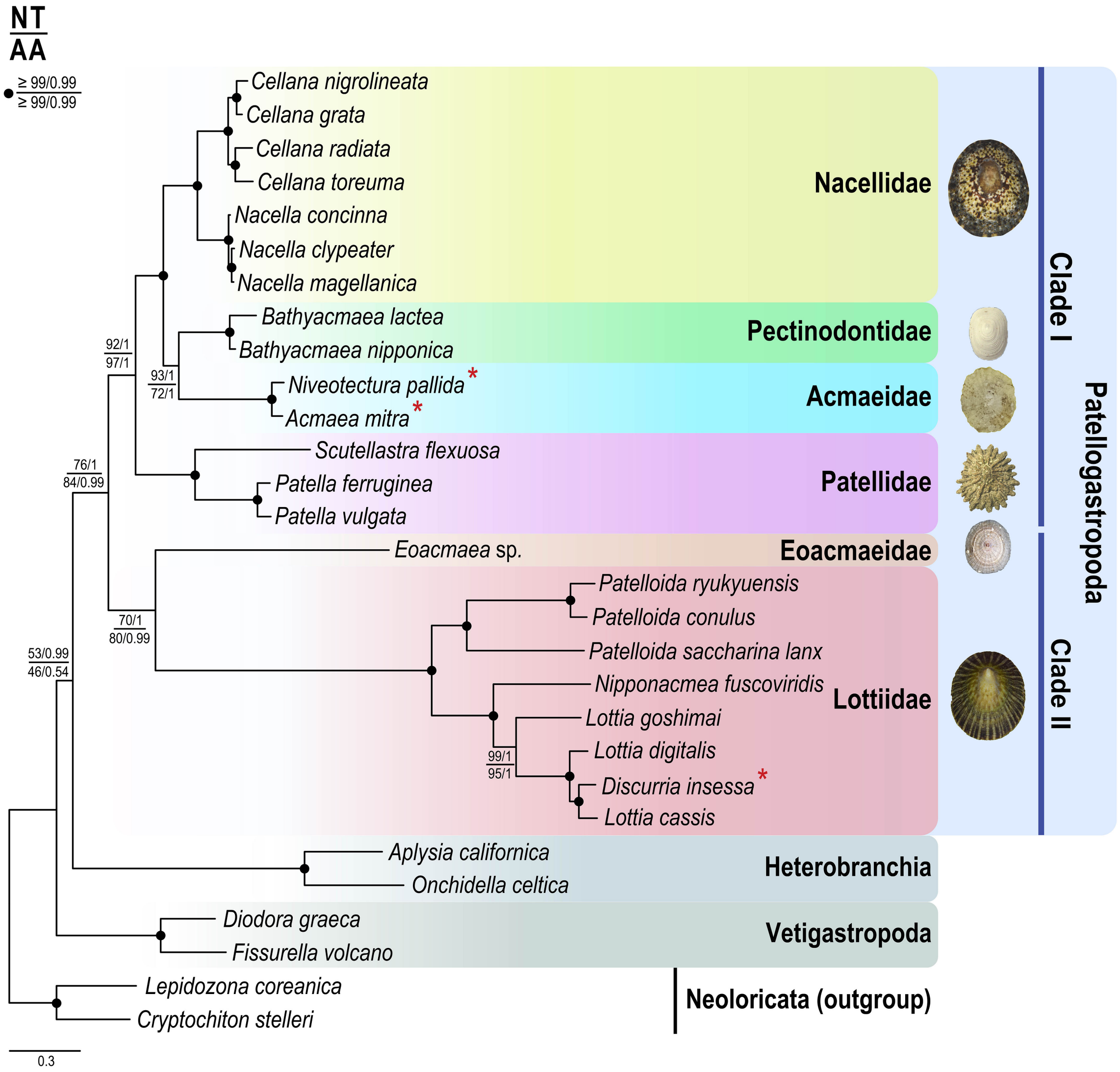


FIGURE 1
Phylogenetic relationships among Patellogastropoda species inferred from maximum likelihood and Bayesian methods for concatenated nucleotide and amino acid sequences of 13 protein-coding genes of the complete mitochondrial genomes. Node supporting values are indicated as BP (bootstrap percentage)/BP (Bayesian posterior probability) in ML and BI, respectively. An asterisk (*) denotes mtDNA sequences newly determined in this study.

species *Acmaea mitra*, and the rest of the species formerly belonging to Acmaeidae were transferred to the Lottiidae (Lindberg, 1986). The mitochondrial genome phylogeny reconstructed in this study provides new and invaluable insights into the internal phylogeny of the Patellogastropoda. The monophyletic grouping of Acmaeidae + Pectinodontidae species contradicts the previous analysis of partial gene fragments of mtDNA (Nakano and Ozawa, 2007), in which the "Acmaeinae" was nested within the Lottiidae, whereas the "Pectinodontinae" was clustered with Lepetidae species. These inferred relationships provided a basis of taxonomic treatment in which the "Pectinodontinae" was elevated to the familial rank (Nakano and Ozawa, 2007). Nakano and Sasaki (2011) later corrected their earlier erroneous interpretation derived from sequence contamination of *Acmaea mitra* by placing it as a junior synonym of Lottiidae. The Pectinodontidae still retained its familial status thereafter, awaiting taxonomic clarification from further analysis. In this regard, our mitochondrial genome tree highlights that Acmaeidae and Pectinodontidae are the most closely related, and their close affinity lends strong support to the previous morphology-based classification in which the pectinodontid species were treated as a subfamily of the family Acmaeidae (Lindberg, 1998). Nevertheless, further extensive studies based on re-evaluation of their morphological characters including shell and soft body anatomy will be necessary to clarify the taxonomic status of the pectinodontid species as a subgroup or confirm them as a taxonomically valid, separate family from the Acmaeidae.

Historically, phylogenetic relationships of the Acmaeidae with other groups have not yet clearly defined (Nakano and Sasaki, 2011). Based on morphological similarity [e.g., shell microstructure, radula characters (Lindberg, 1998), and presence of a left ctenidium (Sasaki, 1998)] and molecular analysis [12S and 16S mtDNA sequence data (Nakano and Ozawa, 2004)], Acmaeidae was once

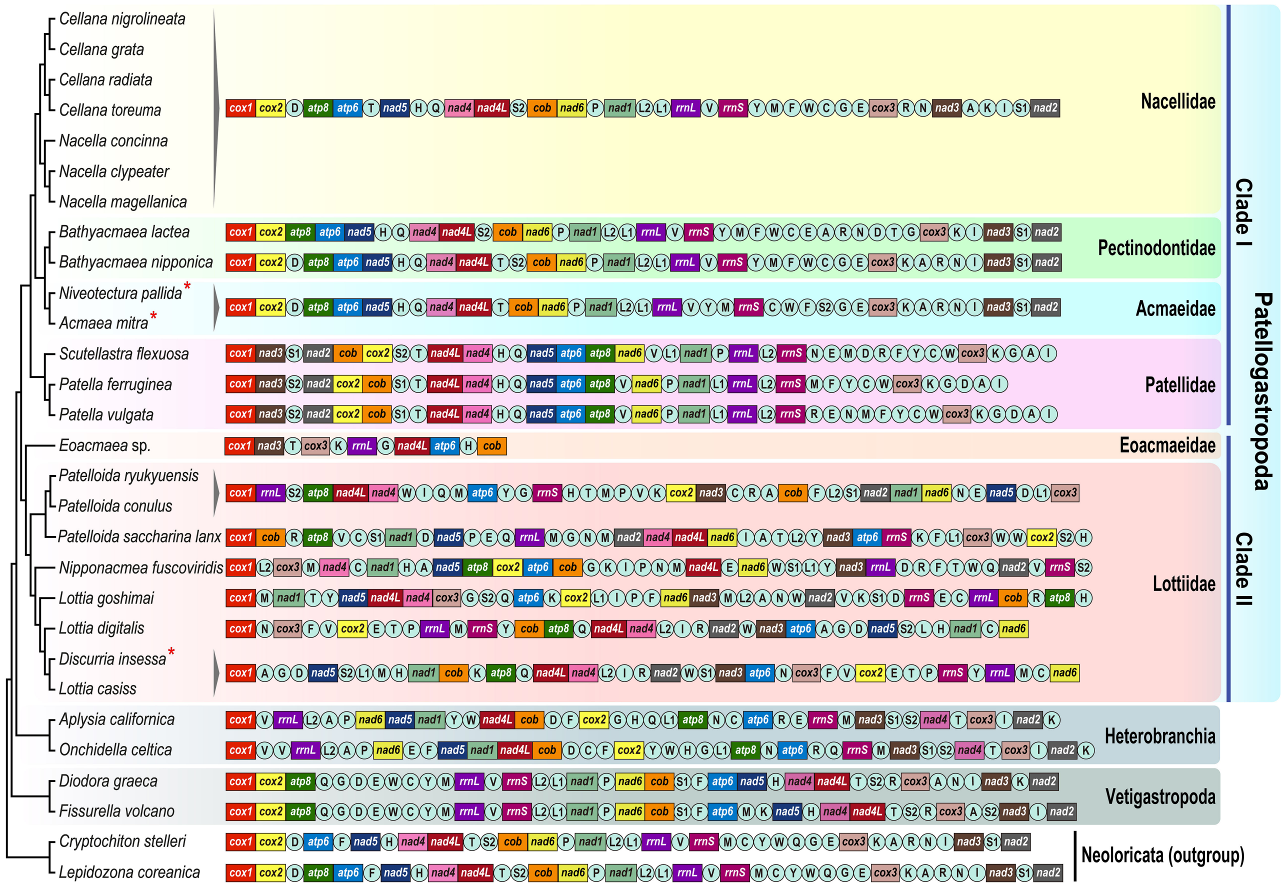


FIGURE 2
Linearized mitochondrial gene arrangement of Patellogastropoda and other gastropod (Heterobranachia and Vetigastropoda) species lay alongside the phylogenetic tree (see Figure 1 for their detailed relationships). The tRNA genes are labeled by single-letter abbreviations of the amino acid code. An asterisk (*) denotes mtDNA sequences newly determined in this study.

considered more closely related to Lottiidae than to Patellidae, Nacellidae, and Lepetidae, and was included as a subgroup of Lottiidae. In contrast, later analysis of mtDNA *cox1* and histone H3 sequence data revealed that they were distinct from Lottiidae and were subsequently revived as a separate family from Lottiidae (Nakano and Sasaki, 2011). Polyphyletic relationships between Acmaeidae and Lottiidae species discovered from this mitochondrial genome phylogeny provide strong taxonomic justification of their independent family status. Moreover, the clade I monophyly (Patellidae, Nacellidae, Pectinodontidae + Acmaeidae) that is separated from clade II (Eoacmaeidae + Lottiidae) indicates that Acmaeidae is a member of the superfamily Patelloidea along with other clade I family members, not belongs to the superfamily Lottioidea. Note that the time-calibrated phylogenetic tree (Figure 3) indicates that the divergence of the Acmaeidae from the Pectinodontidae is estimated to begin in the Jurassic-Cretaceus boundary (132.62 MYA), being much more recent event than the clade I/clade II separation (in the middle Carboniferous; 337.20 MYA) and the Lottiidae/Eoacmaeidae splitting (in the Carboniferous-Permian boundary; 295.84 MYA).

In many metazoan groups including molluscan species, comparison of the gene arrangement of the complete mitochondrial genome has been widely used and sharing similar synteny pattern observed among certain taxonomic groups can often be interpreted as additional evidence of their phylogenetic affinity (Lee et al., 2019). Likewise, gene order comparison across the limpet species provides additional insights into the phylogenetic relationships and mitochondrial genome evolution among the family members of the Patellogastropoda. The gene arrangement patterns of the two Acmaeidae species (*A. mitra* and *N. pallida*) showed the same gene order. Excluding the tRNAs that are often highly mobile even between closely related species, the arrangement patterns of PCGs and rRNA genes of the clade I members are highly conserved showing very similar synteny blocks within each family member (Figure 2). Notably, the three families Acmaeidae, Pectinodontidae, and Nacellidae display the identical gene arrangement in PCGs and rRNA genes, lending additional support to their phylogenetic relatedness. By contrast, the gene order of the Lottiidae species belonging to clade II is highly variable according to the genus and even between congeneric species (see *Lottia* species), as previously reported (Xu et al., 2022). Moreover, the gene order of Lottiidae species shows substantially different patterns from those of clade I members (see Figure 2 for more details). Inferred relationships among Patellogastropoda species

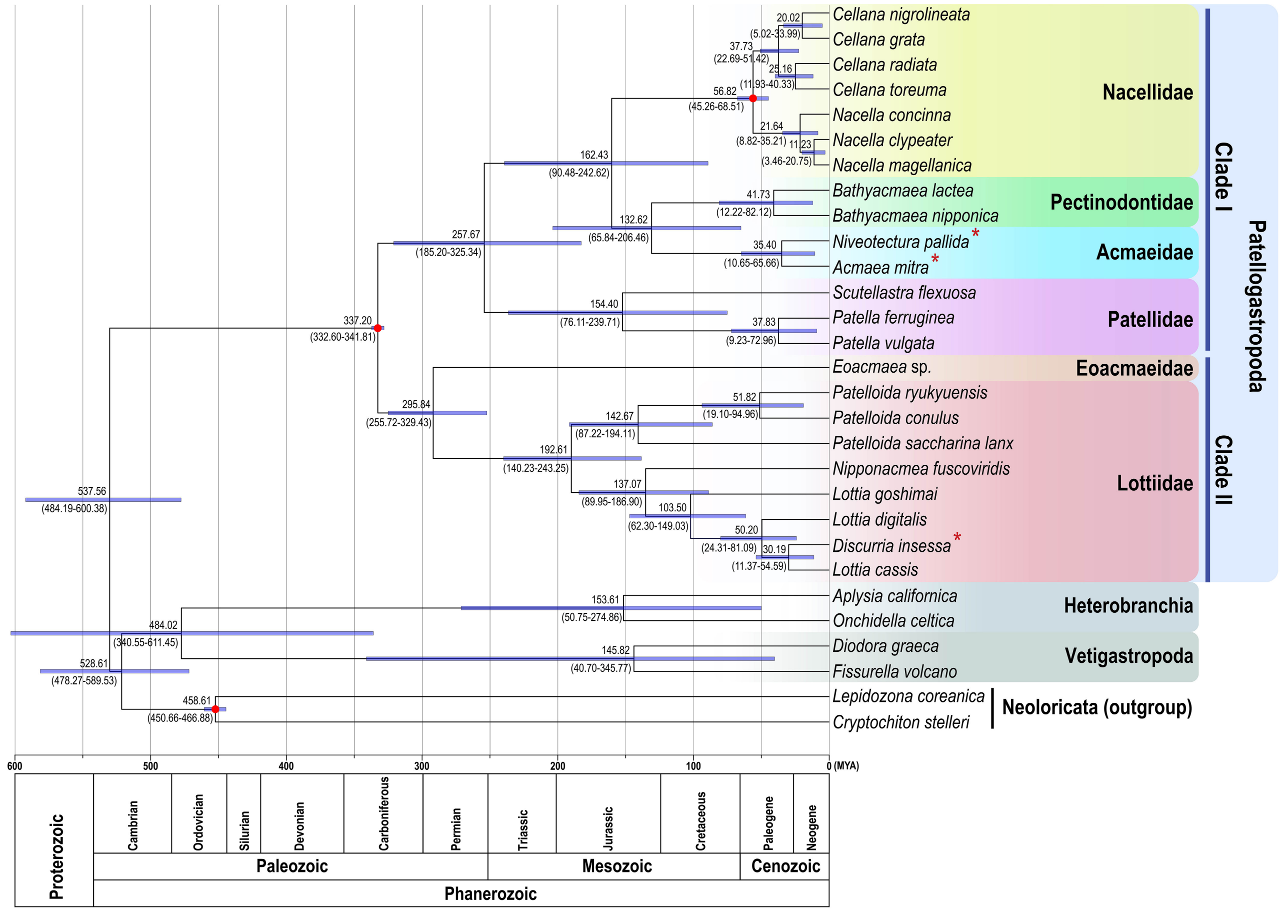


FIGURE 3
Time-calibrated phylogenetic chronogram showing the mean age estimates (with 95% HPD intervals) based on 13 protein coding genes of patellogastropod mitochondrial genomes. Newly determined mtDNA sequences in this study are marked with an asterisk (*), and calibration constraints are represented by red dots on the corresponding nodes.

from gene order analysis (Supplementary Figure 3) agreed with tree topology from the sequence analysis (Figure 1 and Supplementary Figures 1, 2) in that Patellogastropoda species were subdivided into two clades (clade I and clade II) and inferred relationships among clade I family members. One exception is that the two Pectinodontidae species (*B. lactea* and *B. nipponica*) were not grouped as monophyletic in their gene order tree: the former was clustered with Nacellidae species, whereas the latter was grouped with Acmaeidae species (Supplementary Figure 3). This discrepancy results from *B. lactea* species shows an intermediate pattern between the Nacellidae and Acmaeidae in some tRNA synteny blocks. Within clade II, most of branches leading to the Lottiidae species were unresolved and showed extremely long branches that are ascribed to their extensive gene rearrangement events between the Lottiidae species. In addition, it is also interesting to note that the Acmaeidae and one Pectinodontidae species (*B. nipponica*) retain the same consecutive tRNA block of "tRNA KARNI" (*trnK*, *trnA*, *trnR*, *trnN*, and *trnI*), which has long been considered an ancestral state across the major clades of the Gastropoda including other subclasses (Uribe et al., 2016b; Gaitán-Espitia et al., 2019) and outgroup (chiton) species (Irisarri et al., 2014). At present, we are unable to precisely address the evolutionary history of gene arrangement patterns across the patellogastropods. To this end, in-depth analysis of complete mitochondrial genome sequences based on a full breadth of taxon coverage from relatively unstudied and/or underrepresented taxa is required to fully understand the mitochondrial genome evolution and a more comprehensive phylogeny among the major groups of the Patellogastropoda.

Data availability statement

The data presented in the study are deposited on GenBank (accession numbers: OQ133456, OQ133457 and OQ133458).

Author contributions

J-KP designed and led the study. EP, DL, HK, YK collected samples, conducted the laboratory work, and performed phylogenetic analysis of the sequence data. EP, TN, and J-KP wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the management of Marine Fishery Bio-resources Center (2023) funded by the National Marine Biodiversity Institute of Korea (MABIK) and the Basic Science Research Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant no. 2020R1A2C2005393).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1134991/full#supplementary-material

References

Bernt, M., Merkle, D., Ramsch, K., Fritzsch, G., Perseke, M., Bernhard, D., et al. (2007). CREx: Inferring genomic rearrangements based on common intervals. *Bioinformatics* 23 (21), 2957–2958. doi: 10.1093/bioinformatics/btm468

Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., et al. (2013). MITOS: Improved *de novo* metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol.* 69 (2), 313–319. doi: 10.1016/j.ympev.2012.08.023

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: A flexible trimmer for illumina sequence data. *Bioinformatics* 30 (15), 2114–2120. doi: 10.1093/bioinformatics/btu170

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., et al. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PloS Comput. Biol.* 15 (4), 1–28. doi: 10.1371/journal.pcbi.1006650

Branch, G. M. (1985). "'Limpets: their role in littoral and sublittoral community dynamics.'," in *The ecology of rocky coasts* (London: Hodder and Stoughton Educational), 97–116.

Castro, L. R., and Colgan, D. J. (2010). The phylogenetic position of neritimorpha based on the mitochondrial genome of nerita melanotragus (Mollusca: Gastropoda). *Mol. Phylogenet. Evol.* 57 (2), 918–923. doi: 10.1016/j.ympev.2010.08.030

Dall, W. H. (1876). On the extrusion of the seminal products in limpets, with some remarks on the phyllogeny of the docoglossa. *Acad. Nat. Sci.* 28, 34–35.

Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. (2011). ProtTest 3: Fast selection of best-fit models of protein evolution. *Bioinformatics* 27 (8), 1164–1165. doi: 10.1093/bioinformatics/btr088

Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. (2012). JModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* 9 (8), 772. doi: 10.1038/nmeth.2109

Dierckxsens, N., Mardulyn, P., and Smits, G. (2017). NOVOPlasty: *De novo* assembly of organelle genomes from whole genome data. *Nucleic Acids Res.* 45 (4), 1–9. doi: 10.1093/nar/gkw955

Feng, J. T., Guo, Y. H., Yan, C. R., Ye, Y. Y., Li, J. J., Guo, B. Y., et al. (2020). Comparative analysis of the complete mitochondrial genomes in two limpets from lottiidae (Gastropoda: Patellogastropoda): rare irregular gene rearrangement within Gastropoda. *Sci. Rep.* 10 (1), 19277. doi: 10.1038/s41598-020-76410-w

Gaitán-Espitia, J. D., González-Wevar, C. A., Poulin, E., and Cardenas, L. (2019). Antarctic And sub-Antarctic nacella limpets reveal novel evolutionary characteristics of mitochondrial genomes in patellogastropoda. *Mol. Phylogenet. Evol.* 131, 1–7. doi: 10.1016/j.ympev.2018.10.036

Grande, C., Templado, J., Cervera, J. L., and Zardoya, R. (2004). Molecular phylogeny of euthyneura (Mollasca: Gastropoda). *Mol. Biol. Evol.* 21 (2), 303–313. doi: 10.1093/molbev/msh016

Harasewych, M. G., and McArthur, A. G. (2000). A molecular phylogeny of the patellogastropoda (Mollusca: Gastropoda). *Mar. Biol.* 137 (2), 183–194. doi: 10.1007/s002270000332

Hu, F., Lin, Y., and Tang, J. (2014). MLGO: Phylogeny reconstruction and ancestral inference from gene-order data. *BMC Bioinf.* 15 (1), 1–6. doi: 10.1186/s12859-014-0354-6

Irisarri, I., Eernisse, D. J., and Zardoya, R. (2014). Molecular phylogeny of acanthochitonina (Mollusca: Polyplacophora: Chitonida): three new mitochondrial genomes, rearranged gene orders and systematics. *J. Nat. Hist.* 48 (45–48), 2825–2853. doi: 10.1080/00222933.2014.963721

Knudsen, B., Kohn, A. B., Nahir, B., McFadden, C. S., and Moroz, L. L. (2006). Complete DNA sequence of the mitochondrial genome of the sea-slug, aplysia californica: Conservation of the gene order in euthyneura. *Mol. Phylogenet. Evol.* 38 (2), 459–469. doi: 10.1016/j.ympev.2005.08.017

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35 (6), 1547–1549. doi: 10.1093/molbev/msy096

Lan, Y., Sun, J., Chen, C., Sun, Y., Zhou, Y., Yang, Y., et al. (2021). Hologenome analysis reveals dual symbiosis in the deep-sea hydrothermal vent snail gigantopelta aegis. *Nat. Commun.* 12 (1), 1–15. doi: 10.1038/s41467-021-21450-7

Lee, Y., Kwak, H., Shin, J., Kim, S. C., Kim, T., and Park, J. K. (2019). A mitochondrial genome phylogeny of mytilidae (Bivalvia: Mytilida). *Mol. Phylogenet. Evol.* 139, 106533. doi: 10.1016/j.ympev.2019.106533

Lindberg, D. R. (1986). Name changes in the "Acmaeidae. *Veliger* 29 (2), 142–148.

Lindberg, D. R. (1998). "Subclass eogastropoda and order patellogastropoda," in *Mollusca: The southern synthesis, fauna of Australia* (Melbourne: CSIRO).

Liu, Y., Li, Y., Wang, M., Bao, Z., and Wang, S. (2021). The complete mitochondrial genome and phylogenetic analysis of the deep-sea limpet bathyacmaea lactea. *Mitochondrial DNA Part B: Resour.* 6 (7), 2090–2091. doi: 10.1080/23802359.2021.1923422

Lowe, T. M., and Eddy, S. R. (1997). tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25 (5), 955–964. doi: 10.1093/nar/25.5.955

Miller, M. A., Pfeiffer, W., and Schwartz, T. (2010). Creating the CIPRES science gateway for inference of large phylogenetic trees (Accessed 2010 Gateway Computing Environments Workshop, GCE 2010).

Nakagawa, S., Shimamura, S., Takaki, Y., Suzuki, Y., Murakami, S. I., Watanabe, T., et al. (2014). Allying with armored snails: The complete genome of gammaproteobacterial endosymbiont. *ISME J.* 8 (1), 40–51. doi: 10.1038/ismej.2013.131

Nakano, T., and Ozawa, T. (2004). Phylogeny and historical biogeography of limpets of the order patellogastropoda based on mitochondrial DNA sequences. *J. Molluscan Stud.* 70 (1), 31–41. doi: 10.1093/mollus/70.1.31

Nakano, T., and Ozawa, T. (2007). Worldwide phylogeography of limpets of the order patellogastropoda: Molecular, morphological and palaeontological evidence. *J. Molluscan Stud.* 73 (1), 79–99. doi: 10.1093/mollus/eym001

Nakano, T., and Sasaki, T. (2011). Recent advances in molecular phylogeny, systematics and evolution of patellogastropod limpets. *J. Molluscan Stud.* 77 (3), 203–217. doi: 10.1093/mollus/eyr016

Pilsbry, H. (1891). "Acmaeidae, lepetidae, patellidae, titiscaniidae," in *Manual of conchology; structural and systematic* Vol. 13 . Ed. H. A. Pilsbry (Philadelphia: Academy of Natural Sciences).

Rambaut, A. (2018) *'FigTree v. 1.4.4'.* Available at: http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., and Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using tracer 1.7. *Syst. Biol.* 67 (5), 901–904. doi: 10.1093/sysbio/syy032

Rawlings, T. A., MacInnis, M. J., Bieler, R., Boore, J. L., and Collins, T. M. (2010). Sessile snails, dynamic genomes: Gene rearrangements within the mitochondrial genome of a family of caenogastropod molluscs. *BMC Genomics* 11 (1), 440. doi: 10.1186/1471-2164-11-440

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., et al. (2012). Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61 (3), 539–542. doi: 10.1093/sysbio/sys029

Sasaki, T. (1998). Comparative anatomy and phylogeny of the recent archaeogastropoda (Mollusca: Gastropoda). *Bulletin* 38, 1–223.

Sasaki, T., Okutani, T., and Fujikura, K. (2006). Anatomy of bathyacmaea secunda okutani, fujikura & sasaki 1993 (Patellogastropoda: Acmaeidae). *J. Molluscan Stud.* 72 (3), 295–309. doi: 10.1093/mollus/eyl007

Simison, W. B., Lindberg, D. R., and Boore, J. L. (2006). Rolling circle amplification of metazoan mitochondrial genomes. *Mol. Phylogenet. Evol.* 39 (2), 562–567. doi: 10.1016/j.ympev.2005.11.006

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30 (9), 1312–1313. doi: 10.1093/bioinformatics/btu033

Sun, J., Liu, Y., Xu, T., Zhang, Y., Chen, C., Qiu, J. W., et al. (2019). The mitochondrial genome of the deep-sea limpet bathyacmaea nipponica (Patellogastropoda: Pectinodontidae). *Mitochondrial DNA Part B: Resour.* 4 (2), 3175–3176. doi: 10.1080/23802359.2019.1668732

Uribe, J. E., Colgan, D., Castro, L. R., Kano, Y., and Zardoya, R. (2016b). Phylogenetic relationships among superfamilies of neritimorpha (Mollusca: Gastropoda). *Mol. Phylogenet. Evol.* 104, 21–31. doi: 10.1016/j.ympev.2016.07.021

Uribe, J. E., Irisarri, I., Templado, J., and Zardoya, R. (2019). New patellogastropod mitogenomes help counteracting long-branch attraction in the deep phylogeny of gastropod mollusks. *Mol. Phylogenet. Evol.* 133, 12–23. doi: 10.1016/j.ympev.2018.12.019

Uribe, J. E., Kano, Y., Templado, J., and Zardoya, R. (2016a). Mitogenomics of vetigastropoda: Insights into the evolution of pallial symmetry. *Zool. Scr.* 45 (2), 145–159. doi: 10.1111/zsc.12146

Wernersson, R., and Pedersen, A. G. (2003). RevTrans: Multiple alignment of coding DNA from aligned amino acid sequences. *Nucleic Acids Res.* 31 (13), 3537–3539. doi: 10.1093/nar/gkg609

Xu, T., Qi, L., Kong, L., and Li, Q. (2022). Mitogenomics reveals phylogenetic relationships of patellogastropoda (Mollusca, Gastropoda) and dynamic gene rearrangements. *Zool. Scr.* 51 (2), 147–160. doi: 10.1111/zsc.12524

Frontiers in Marine Science

TYPE Systematic Review
PUBLISHED 19 May 2023
DOI 10.3389/fmars.2023.1158470

Reviewing the knowledge on the genus *Patella* in the Mediterranean Sea: testing the effect of protection on the mean abundance and size of *Patella ferruginea*

Ana Cascales-Soler, Alfonso Angel Ramos-Espla and Aitor Forcada*

Department of Marine Sciences and Applied Biology, University of Alicante, Alicante, Spain

OPEN ACCESS

EDITED BY
Diego Castejón Bueno,
Centro Interdisciplinar de Investigação Marinha e Ambiental da Madeira (CIIMAR-Madeira), Portugal

REVIEWED BY
Free Espinosa Torre,
Sevilla University, Spain
Salvatore Giacobbe,
University of Messina, Italy
José Carlos García-Gómez,
University of Seville, Spain

*CORRESPONDENCE
Aitor Forcada
forcada@ua.es

RECEIVED 03 February 2023
ACCEPTED 02 May 2023
PUBLISHED 19 May 2023

CITATION
Cascales-Soler A, Ramos-Espla AA and Forcada A (2023) Reviewing the knowledge on the genus *Patella* in the Mediterranean Sea: testing the effect of protection on the mean abundance and size of *Patella ferruginea*.
Front. Mar. Sci. 10:1158470.
doi: 10.3389/fmars.2023.1158470

Increasing human pressure has strongly impacted the littoral environment, altering the habitats and population parameters of some species. The Mediterranean Sea is one of the regions that have been highly affected by these events. In response to these events, marine protected areas (MPAs) have emerged as one of the main conservation tools for marine habitats and species. In this regard, harvesting of limpets (*Patella* spp.) is a good example of the impact of human activity on the coast. Limpets are mollusks that are collected as food or fishing bait, and their exploitation causes an immediate decrease in the density and size structure of individuals. In the Mediterranean Sea, the genus *Patella* is represented by five species: *Patella rustica*, *P. caerulea*, *P. ulyssiponensis*, *P. ferruginea*, and *P. depressa*. To verify the effectiveness of the MPAs, a deep review of 75 studies on *Patella* spp. in the Mediterranean Sea was conducted to analyze the spatial and temporal distribution of studies. Data extracted from these articles were used to assess the effect of protection on the density and size of *P. ferruginea*. Regarding spatial distribution, the studies reviewed were performed at 67 sites in 13 countries, of which 23 were in MPAs and 44 in non-protected or control areas. The findings of this study show that *P. ferruginea* is the species most studied, because it is one of the most threatened marine invertebrates in the Mediterranean Sea. This explains the temporal distribution of the studies, which coincides with the establishment of the conservation status and the concern about the population status of *P. ferruginea.* Analysis of the effect of protection on the density and mean size of *P. ferruginea* individuals revealed no significant difference in mean density between the control sites and MPAs. However, mean size between individuals was significantly higher in MPAs than control sites. This pattern may be related to intra- and interspecific competition between individuals.

KEYWORDS

***Patella* spp., Mediterranean Sea, density, size, marine protected areas**

1 Introduction

Human settlements have long been attracted to coastal areas, which provide a wealth of resources, commercial opportunities, and leisure activities (McGranahan et al., 2007). Coastal areas and marine ecosystems are being pressured by population development, resulting in accelerated habitat loss and degradation (Burak et al., 2004), mainly because of stressors such as industrial expansion, overfishing, and tourism (Stanchev et al., 2015).

Tourism has become a growing trend in coastal regions worldwide. Globally, marine and coastal tourism is one of the major economic activities (Smith, 2000; Zahedi, 2008). Therefore, their impact on the coastal environment is significant and extremely difficult to manage (Davenport and Davenport, 2006). Overexploitation, pollution, coastal destruction, biological invasion by non-indigenous species, and climate change have major environmental impact on marine ecosystems (Jackson et al., 2001; Probert, 2017). Shellfishing, if not properly managed, can lead to serious alterations in the ecosystem, such as biodiversity loss, serial depletion, habitat degradation, and changes in the food chain (Murawski, 2000; Scheffer et al., 2005).

The Mediterranean region is not an exception and suffers the consequences of human activities and climate change. For many years, the coastal areas of the Mediterranean region have attracted attention for their environment and culture (Burak et al., 2004). Due to its peculiar characteristics and history, the Mediterranean Sea can be considered a miniature ocean (Bianchi and Morri, 2000), whose reduced extent and volume help that even minor modifications can cause major changes in the climate and functioning of the Mediterranean, which makes it a region potentially vulnerable to climate change and human activities (Lionello et al., 2006; Garrabou et al., 2022). In fact, it is considered one of the most prominent “hot spots” in future projections of climate change (Giorgi, 2006).

The problems caused by factors such as human activity and climate change have led to initiatives for the protection of marine and coastal ecosystems. This is how the term “marine protected area” or “MPA” was adopted by Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008. According to this law, MPAs are natural spaces whose objective is to protect ecosystems, communities, and biological or geological elements of the marine environment that, depending on their rarity, fragility, or importance, deserves special protection. In total, 1,062 MPAs are distributed throughout the coast of the Mediterranean Sea (by 2019, according to Claudet et al., 2020). The main objective of MPAs is the conservation of marine life and its intrinsic processes (Ballantine and Langlois, 2008). By protecting these areas from damage and degradation, MPAs facilitate the recovery of disturbed habitats, especially those that are vulnerable and highly diverse (Selig and Bruno, 2010). Numerous studies have shown that the main direct effects of protection of marine areas are positive, such that biological variables (e.g., biomass, density of individuals, species richness, or body size) are increased within the limits of the MPA (Claudet et al., 2008; Lester et al., 2009). Despite all these positive effects, the effectiveness of MPAs is questioned. This depends on various factors, such as the design of the MPA and the levels of protection established (Halpern, 2003). In addition, MPAs may provide insufficient protection on their own, because they are not isolated from environmental impacts (Allison et al., 1998). For this reason, the effectiveness of MPAs depends on the protection provided outside their boundaries (Agardy, 1994).

Harvesting has been a major threat to the conservation of intertidal species worldwide (Kingsford et al., 1991; Keough et al., 1993; Thompson et al., 2002). Mollusk species have evolved survival strategies, adapting their life cycles, reproduction rates, behavioral mechanisms, and so forth, to survive the challenges of their physical and biotic environment (Hockey, 1994). Human predation through shellfishing is a relatively recent phenomenon in evolutionary terms that has not been able to predict (Mannino and Thomas, 2002). *Patella* is one of the main genera that are affected by shellfishing in the Mediterranean Sea. These limpets are collected as food or bait for fishing, and their exploitation causes an immediate decrease in density, change in size structure of individuals, an imbalance in the sex ratio, and a decrease in reproduction (Paracuellos et al., 2003; Rivera-Ingraham et al., 2011). These actions lead to the decline of the species, as well as alter the ecological succession that occurs in this habitat, because this herbivore plays an important ecological role in community structuring, regulating the recruitment of macroalgae through consumption (Guerra and Gaudencio, 1986). Therefore, these limpets act as key species providing space for many others, thereby enhancing biodiversity (Power et al., 1996; Raffaelli and Hawkins, 1996).

In the Mediterranean Sea, the genus *Patella* is represented by four species: *P. rustica* Linnaeus, 1758; *P. caerulea* Linnaeus, 1758; *P. ulyssiponensis* Gmelin, 1791; and *P. ferruginea* Gmelin, 1791 (Mauro et al., 2003), to which *P. depressa* Pennant, 1777—recorded in the Alboran Sea—must be added (Gofas et al., 2011). Although all species can inhabit intertidal rocky areas, they avoid overlapping their distribution by placing themselves at different vertical levels on rocks: *P. rustica* is usually located at the supralittoral level, above the upper mesolittoral occupied by *P. ferruginea*; *P. ulyssiponensis* is usually located in the lower mesolittoral; and *P. caerulea* can be found in both intertidal and upper infralittoral habitats (Guallart and Templado, 2012). Of the five species, *P. ferruginea* should be highlighted, because it is one of the most threatened marine invertebrates according to Directive 92/43/EEC of the European Council, as well as in “danger of extinction” according to the Barcelona Convention (UNEP/MAP-SPA/RAC, 2018). The state of this species is mainly due to human activities on the Mediterranean coast and its excessive collection.

Although marine protection figures tend to encompass as many habitats as possible within their boundaries, not all are given the same importance. An intertidal rocky area is one of these habitats, whose visibility is usually lower, but importance in the ecosystem is very high. Rocky shores are likely to be more vulnerable than other marine habitats due to their susceptibility to terrestrial and marine disturbances (Schmitt and Osenberg, 1996). The creation of MPAs covering intertidal rocky areas aims to remedy and prevent the decline of threatened species of intertidal mollusks, thus improving their population density, average size, and population viability

(Keough et al., 1993). In fact, the effects of protection or harvesting on limpets are a global concern (Branch and Odendaal, 2003; Lasiak, 2006; Sagarin et al., 2007; Espinosa et al., 2009; Martins et al., 2017; Sousa et al., 2019a; Sousa et al., 2019b; Sousa et al., 2020; Baliwe et al., 2022).

To verify the effectiveness of Mediterranean MPAs on limpets and analyze the spatial and temporal distribution of studies, a deep review of studies on the genus *Patella* in the Mediterranean was performed. With the data extracted from these articles, the effect of protection was assessed on the density and size of *P. ferruginea*. In addition, correlations between the density and size of individuals were evaluated.

2 Materials and methods

2.1 Literature review

A bibliographic review of existing studies (scientific papers, "gray literature") on the genus *Patella* in the Mediterranean Sea until October 2022 was performed following the scheme of Moher et al. (2009). A recompilation of articles was made through a search in all databases of the Web of Science (WoS) entering the keywords: "*Patella* + Mediterranean + density" (n = 68), "*Patella* + Mediterranean + size" (n = 110), "limpet + Mediterranean + density" (n = 79), and "limpet + Mediterranean + size" (n = 108) in the TOPIC field. Additionally, for each one of the species of the genus *Patella* present in the Mediterranean Sea, a specific search entering species name + Mediterranean was performed (n = 334). Because some works not published in the journals included in WoS (including the gray bibliography) may contain interesting evidence for this review of the genus *Patella*, additional studies were identified through a Google Scholar search. The information has been supplemented with data from authors' colleagues and includes theses, technical reports, working papers, and so forth. These searches produced n = 410 articles after duplicate removal. After a first review of all the studies collected, studies without information on the density or size of *Patella* were discarded. The final number of studies selected for analysis was n = 75. The information collected from each study included year of publication, site and country of study, level of protection (differentiating between MPAs and non-protected or control areas), species studied, and information about density and size in as raw format as possible (at least the mean values of these variables were extracted). The sites identified as artificial habitats (port infrastructures) with prohibited access (frequently protected with metal fences) were considered MPAs, because they act as Artificial Marine Micro-Reserves (AMMR) for these species (Espinosa et al., 2009; García-Gómez et al., 2011; García-Gómez et al., 2015; Ostalé-Valriberas et al., 2022).

In this review, information about the density and size was found for the following species: *P. rustica*, *P. caerulea*, *P. ulyssiponensis*, and *P. ferruginea*, considering as *Patella* spp. those information that could not be identified at the species level. No studies were found on the density and size of *P. depressa*. The complete list of studies compiled can be found in Supplementary Data.

2.2 Data analyses

The temporal distribution of *Patella* studies was analyzed representing the number of studies performed per year, considering the species included in each study. To assess the spatial distribution of *Patella* studies, all study sites recorded in the review were georeferenced, and the total number of studies performed at each site was calculated, considering whether the studies analyzed the density, size, or both density and size of each species. Using these data, two maps were generated using QGIS software (QGIS Development Team, 2022), showing the spatial distribution of studies on density and size separately.

Because most data compiled in the review were from *P. ferruginea*, the evaluation focused on the effect of protection on density and size of this species. For each study site with data on *P. ferruginea*, the protection status (MPA or control site) was considered, and mean density and mean size were calculated to identify a pattern of protection effect related to a country. Additionally, the effect of protection on the density and size of *P. ferruginea* at the Mediterranean level was analyzed, considering data as raw as possible for all MPAs and control areas. Due to the unbalanced nature of the analysis, and the lack of normality of the data, univariate PERMANOVA (Anderson, 2001) tests were performed over the untransformed density and size of *P. ferruginea* to detect the effect of protection, comparing data from MPAs with control areas obtained from the previous review. For density analysis, n = 154 and n = 118, and for size analysis, n = 337 and n = 2,350 were considered in MPAs and control areas, respectively. Euclidean distance matrix and 9,999 permutations were used. Finally, the relationship between the density and mean size of *P. ferruginea* was evaluated using Pearson's correlation index for overall data (n = 143) and for data from MPA (n = 105) and control areas (n = 38) separately. Analyses were performed using R statistical computing software (R Core Team, 2020) and the R's statistical software packages ggplot2 (Wickham, 2016) and Vegan (Oksanen et al., 2019).

3 Results

In this research, a total of 75 studies have been found and reviewed (Supplementary Data), in which four species of the genus *Patella* have been investigated. *P. ferruginea* was the most studied species, appearing in 56 studies. *P. caerulea* and *P. rustica* appeared in 15 and 13 studies, respectively, and *P. ulyssiponensis* in 1. Reports on the density or size of *P. depressa* in the Mediterranean Sea were lacking. Species were not identified in six of the studies reviewed, considered *Patella* spp.

3.1 Temporal and spatial distribution of studies on *Patella* in the Mediterranean

Regarding the temporal distribution of studies on the genus *Patella* (Figure 1), the first studies on the size and density of

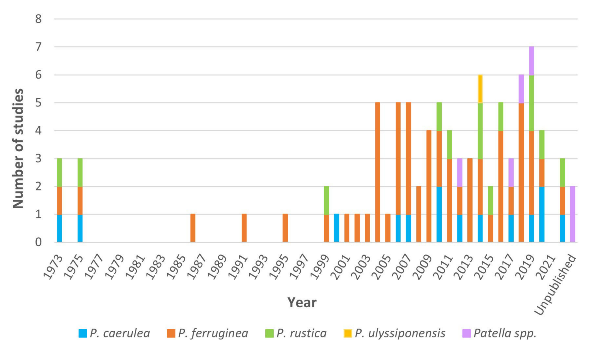


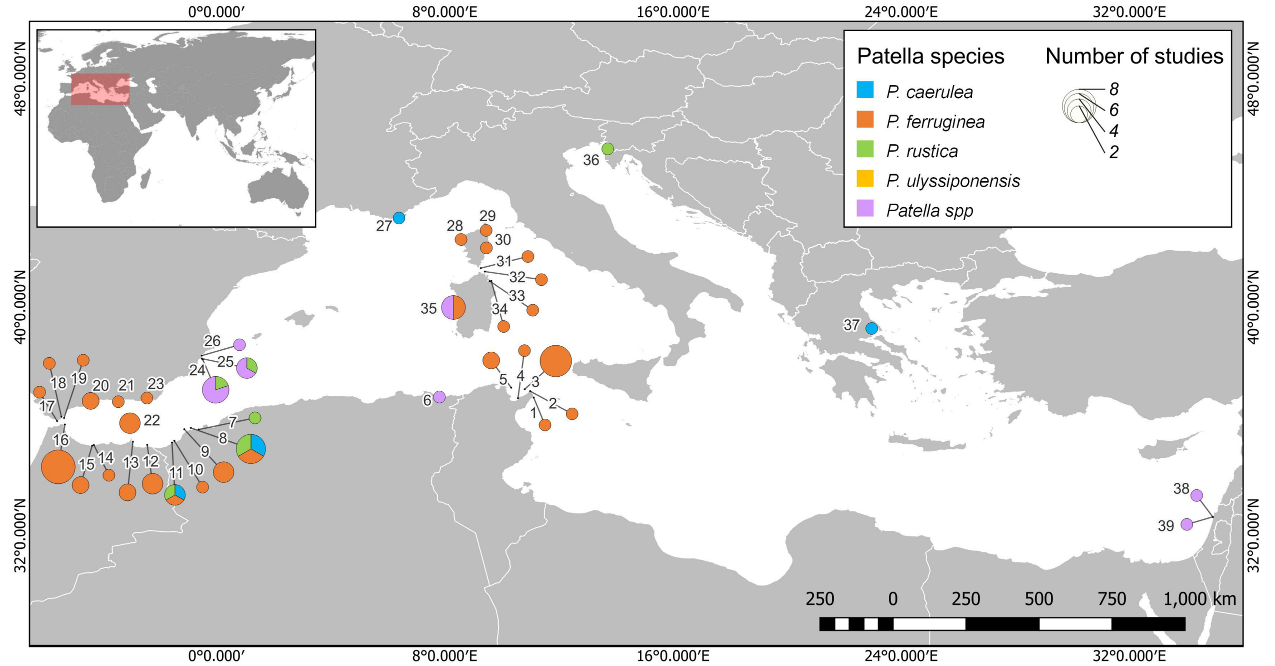
FIGURE 1
The total number of studies per year on *P. caerulea*, *P. ferruginea*, *P. rustica*, *P. ulyssiponensis*, and *Patella* spp. The bar of "unpublished" includes studies with no date of publication.

individuals were performed in 1973 and 1986, respectively. Between 1975 and 1999, studies on this genus were lacking; however, an increasing trend was seen in the number of studies from the early 2000s, reaching the highest number in 2019 (seven studies), followed by 2014 and 2018, with six studies. In general, all species appeared in the studies during all periods, with the exception of *P. ulyssiponensis*, which was included in a study in 2014.

In terms of spatial distribution, *Patella* was investigated at 67 sites of the Mediterranean Sea, covering 13 countries. From these sites, 23 were MPAs and 44 were control areas. The countries that contributed the highest number of studies on *Patella* included Spain (27), Algeria (12), Tunisia (12), and Italy (10).

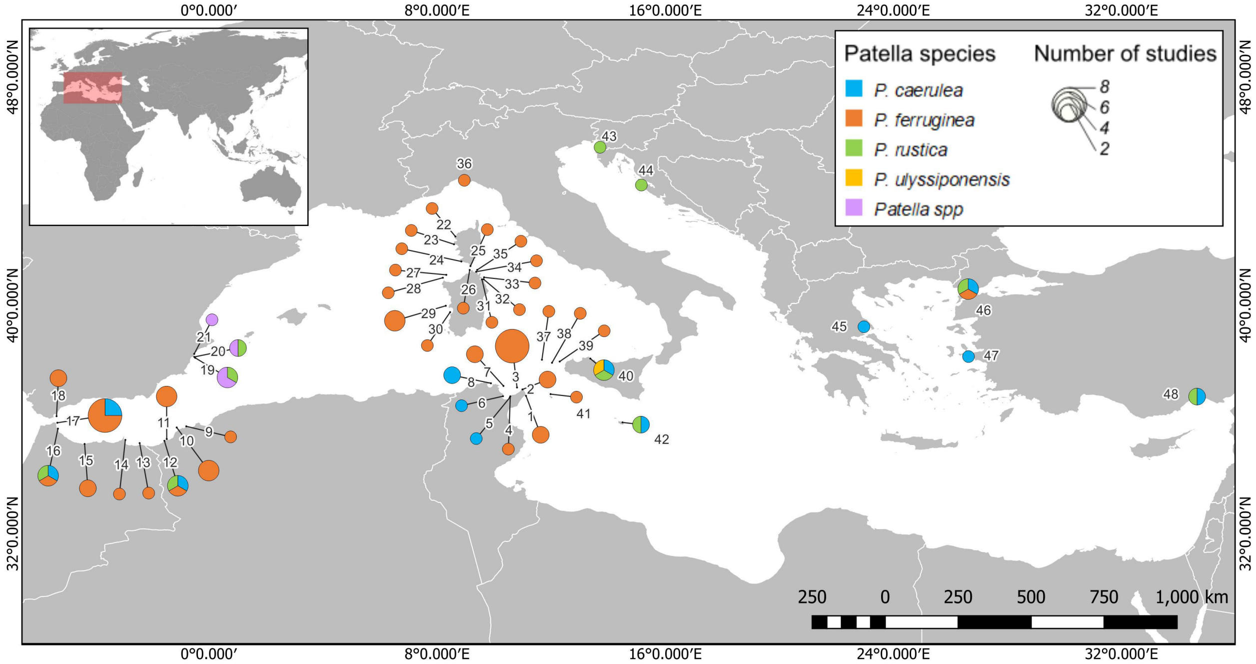
Focusing on studies that included data on the density of *Patella*, 39 sites were registered distributed in 10 countries (Figure 2). Most of these sites are located in Spain, Morocco, Algeria, and Tunisia, as well as Corsica (France) and Sardinia (Italy). The sites that contributed the highest number of studies included Ceuta (Spain) and Zembra Island (Tunisia), with eight studies each, followed by Paloma Island (Algeria) with six studies, Tabarca Island (Spain) with five studies, and Mal di Ventre (Sardinia) with four studies. The density of *P. ferruginea* was studied the most at sites along the Mediterranean Sea (Figure 2). Despite the large number of sites found in the review, more than one species of *Patella* was studied in only six studies. Notably, studies on the density of *P. caerulea*, *P. ferruginea*, and *P. rustica* were conducted on Paloma Island and the West Coast of Algeria.

Regarding studies that included data on the size of *Patella* individuals, 48 sites were identified in the Mediterranean, distributed in 12 countries (Figure 3). The places where the studies are more concentrated include Corsica (France), Sardinia



- 1.Kelibia
- 2.El Haouaria
- 3.Zembra Islands
- 4.Korbous
- 5.Plane island
- 6.Gulf of Annaba
- 7.Oran
- 8.Paloma Island
- 9.Habibas Islands
- 10.Rachgoun
- 11.West Coast of Algeria
- 12.Chafarinas Islands
- 13.Melilla
- 14.Peñón de Vélez de la Gomera
- 15.Cala Iris of Al Hoceima
- 16.Ceuta
- 17.El Estrecho Natural Park
- 18.Algeciras Bay
- 19.Gibraltar
- 20.Malaga
- 21.Granada
- 22.Alboran Island
- 23.Almería
- 24.Tabarca Island
- 25.Santa Pola
- 26.Aguamarga Beach (Alicante)
- 27.Marseille and Cannes Bay
- 28.Scandola Nature Reserve (Corsica)
- 29.Bastia
- 30.Corsica
- 31.Lavezzi Islands
- 32.Magdalena Archipelago
- 33.Gulf of Olbia (Sardinia)
- 34.Cape Ceraso (Sardinia)
- 35.Mal di Ventre (Sardinia)
- 36.Koper Bay
- 37.Pagasitikos Gulf
- 38.Dor Habonim Reserve
- 39.Nahsholim Beach

FIGURE 2
Distribution of studies that include data on densities of *Patella* individuals along the Mediterranean Sea. Each color represents one of the *Patella* species, and the diameter of the pie chart indicates the number of studies on density at each site. A list with the names of all the sites registered in the review can be seen below the map.



- 1.Kelibia
- 2.El Haouaria
- 3.Zembra Islands
- 4.Korbous
- 5.Sidi Rais Beach
- 6.La Goulette
- 7.Plane island
- 8.Channel ofBizerte
- 9.Capo Falcon
- 10.Habibas Islands
- 11.Rachgoun
- 12.West Coast of Algeria
- 13.Chafarinas Islands
- 14.Melilla
- 15.Cala Iris of Al Hoceima
- 16.Rincón, M'Diq
- 17.Ceuta
- 18.Gibraltar
- 19.Tabarca Island
- 20.Santa Pola
- 21.Aguamarga Beach (Alicante)
- 22.Galeria (Corsica)
- 23.Cargese (Sardinia)
- 24.Titian (Corsica)
- 25.Bonifacio (Corsica)
- 26.Capo Testa (Sardinia)
- 27.Asinara Island (Sardinia)
- 28.Capo Falcone (Sardinia)
- 29.Mal di Ventre (Sardinia)
- 30.Capo San Marco (Sardinia)
- 31.Gulf of Olbia (Sardinia)
- 32.Cape Ceraso (Sardinia)
- 33.Cala Moresca (Sardinia)
- 34.Porto Rafael (Sardinia)
- 35.Magdalena Archipelago
- 36.Genoa
- 37.Grotta Preseppe (Sicily)
- 38.Punta Mugnona, Aegadian Islands
- 39.Punta Sottile (Sicily)
- 40.Palermo (Sicily)
- 41.Punta Rosso di Nica
- 42.Manoel Island
- 43.Koper Bay
- 44.Zaton
- 45.Pagasitikos Gulf
- 46.Saros Bay
- 47.Urla
- 48.Mersin

FIGURE 3
Distribution of studies that include data on the size of *Patella* individuals along the Mediterranean Sea. Each color represents one of the *Patella* species, and the diameter of the pie chart indicates the number of studies on size at each site. A list with the names of all the sites registered in the review can be seen below the map.

(Italy), and Tunisia. In most cases, only one study on the size of individuals was performed at each site; however, Ceuta (Spain) and Zembra Island (Tunisia) hosted eight studies each. Although *P. ferruginea* was the most notable species in studies on the size of individuals along most of the sites, *P. caerulea* was also studied at several sites, mainly located in the central and eastern Mediterranean. The size of more than one species of *Patella* was studied at only eight sites.

3.2 Effect of protection on *P. ferruginea*

Mean density values of *P. ferruginea* for each site recorded in the review are shown in Figure 4. Although most countries had MPA sites that were studied, the number of control sites studied was greater; specifically, there were included in the studies reviewed 14 MPAs and 17 control sites. Regarding the mean density of individuals, a wide variety of values is appreciated, generally between 0.1 and 10 individuals/m. The mean density of individuals was slightly higher in MPAs than control areas only in Spain, France, and Italy. The highest mean density was observed at the control sites of Plane Island (Tunisia) (15.920 ± 9.790 individuals/m) (s.e., standard error) and Melilla (Spain) (14.154 ± 5.766 individuals/m) and in the MPAs of Chafarinas Island (Spain) (8.865 ± 1.668 individuals/m) and Melilla (Spain) (10.000 individuals/m).

Considering all the available data, although the mean density of *P. ferruginea* individuals was slightly higher in the control areas (4.192 ± 0.662 individuals/m) than MPAs (3.950 ± 0.434 individuals/m) (Figure 5), statistical analysis of the effect of protection on density with PERMANOVA revealed no significant differences between MPA and control densities (pseudo$F_{1,270}$ = 0.101, $P > 0.05$).

The mean size of *P. ferruginea* individuals (Figure 6) was studied in a greater number of control sites than MPAs. This variable was measured at 20 and 17 sites, respectively. In fact, in three of the eight countries that hosted studies on *P. ferruginea* size, MPAs were not included in the sites studied. The mean size of *P. ferruginea* generally ranged between 2 and 6 cm at most sites. This variable showed greater values for MPAs than control sites in Tunisia, Algeria, Spain, and Italy; however, the opposite trend was seen in Morocco. The MPAs with the highest mean sizes of *P. ferruginea* were located in Habibas Islands (Algeria) (6.193 ± 0.115 cm) and Asinara Island (Italy) (6.140 ± 2.030 cm). In the control sites, the highest mean size values were recorded in Rincón/M'Diq (Morocco) (6.080 cm; size range from 3.8 to 8.9 cm) and Genoa (Italy) (5.367 ± 1.124 cm).

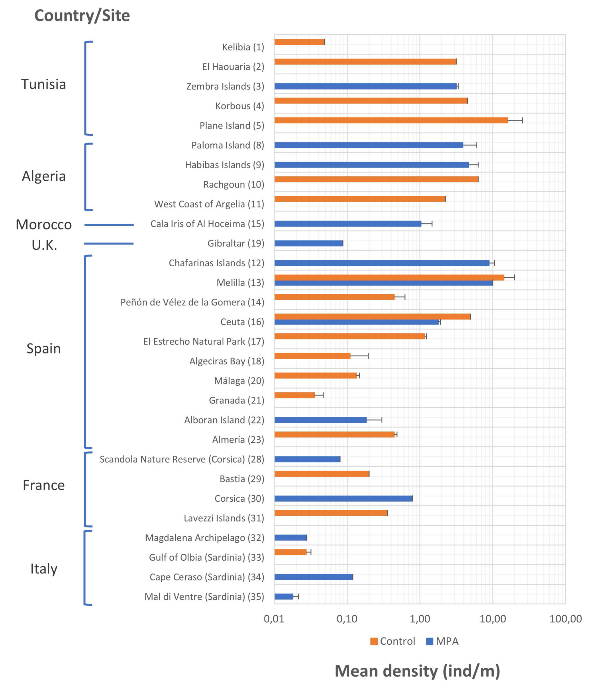


FIGURE 4
Mean densities and standard error (s.e.) of *Patella ferruginea* (individuals/m) according to the study site and level of protection. In bars where s.e. is not indicated means that it was not available in the studies or could not be calculated. The numerical code on the right side of the site name corresponds to the list of sites represented in the distribution map (Figure 2). Data are represented on a logarithmic scale.

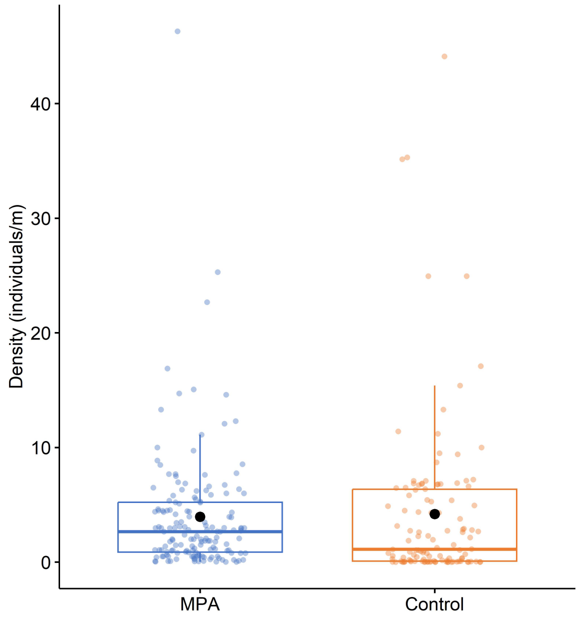


FIGURE 5
Boxplot of the density of *Patella ferruginea* (individuals/m) in MPAs and control areas. Within each box, central lines denote median values, boxes extend from the 25th to 75th percentile, and whiskers denote values within 1.5 interquartile range of the 25th and 75th percentiles. Dots above or below the whiskers are outliers. Black dots show mean values.

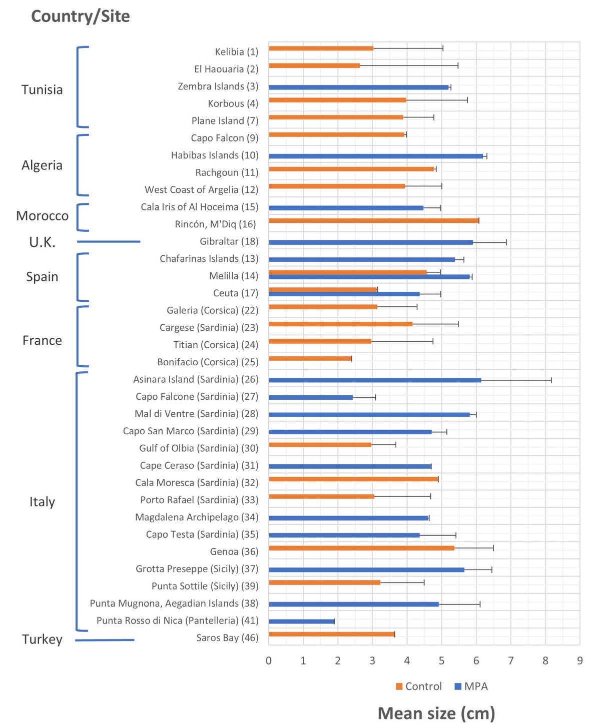


FIGURE 6
Mean size and standard error (s.e.) of *Patella ferruginea* individuals (cm) according to the study site and level of protection. Bars where s.e. is not indicated means that it was not available in the studies or could not be calculated. The numerical code on the right side of the site name corresponds to the list of sites represented in the distribution map (Figure 3).

Comparing all available data on the size of *P. ferruginea* individuals in MPAs and control sites (Figure 7) revealed that although big individuals were observed at control sites (some reaching > 8 cm), mean size was clearly higher in MPAs (5.778 ± 0.080 cm) than control areas (3.531 ± 0.031 cm). This pattern was statistically confirmed after analyzing the effect of protection on mean size with PERMANOVA, which showed that the mean size of *P. ferruginea* was significantly larger in MPAs than control areas ($\text{pseudoF}_{1,2685} = 676.180$, $P < 0.001$).

After extracting data from the reviewed studies for sites where both the density and size of *P. ferruginea* individuals were sampled (18 studies), the relationship between these two variables was evaluated. Although a slight negative correlation was seen between the density and mean size of *P. ferruginea* individuals in both MPAs and control sites, Pearson's correlation index was not statistically significant in any case (Figure 8).

4 Discussion

The findings of this study reveal that among the five species of the genus *Patella* present in the Mediterranean, *P. ferruginea* is the most studied. This is not surprising, because *P. ferruginea* is one of the most threatened marine invertebrates in the Mediterranean Sea and is listed as "endangered" according to the Barcelona

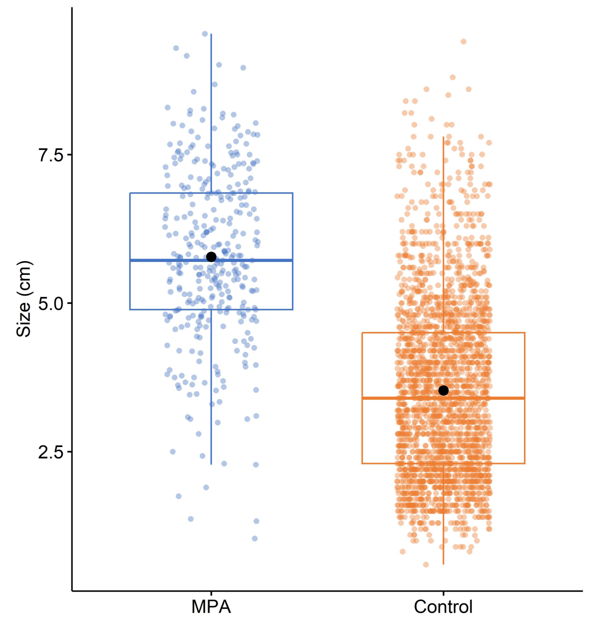


FIGURE 7
Boxplot for the size of *Patella ferruginea* (cm) in MPAs and control areas. Within each box, central lines denote median values, boxes extend from the 25th to 75th percentile, and whiskers denote values within 1.5 interquartile range of the 25th and 75th percentiles. Dots above or below the whiskers are outliers. Black dots show mean values.

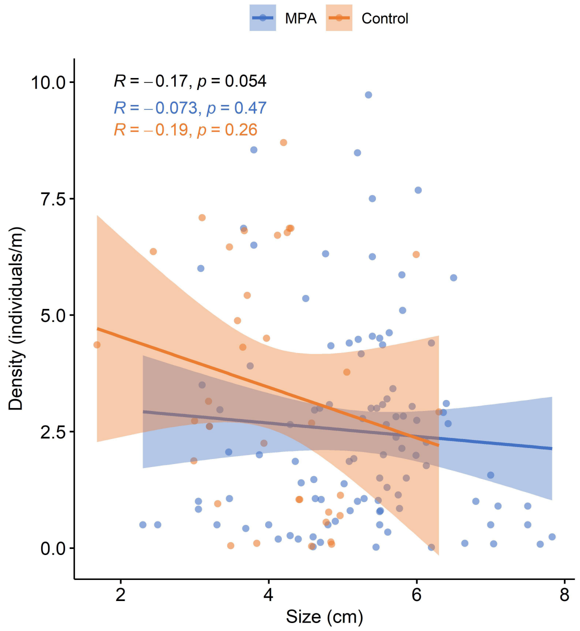


FIGURE 8
Mean density as a function of mean size of *Patella ferruginea* individuals in MPAs and control areas. Fitted lines from linear regression are presented with 95% confidence interval for MPAs (blue) and control areas (orange). R: Pearson's correlation index for MPA data (blue), control area data (orange), and all data (black).

Convention (UNEP/MAP-SPA/RAC, 2018). However, although *P. ferruginea* is on the list of European regulations and several Mediterranean countries, it has not been evaluated by the IUCN Red List. Their endangered status can be attributed to human activities on the coast and excessive exploitation as food or fishing bait, as well as for ornamental purposes (Rivera-Ingraham, 2010; Tlig-Zouari et al., 2010). Studies on the density and size of the other four Mediterranean species are few. However, many studies have performed molecular and phylogenetic analyses on these species (Mauro et al., 2003; Casu et al., 2010; Prusina et al., 2014).

Regarding of studies on the density and size of species of the genus *Patella*, 67 sites were investigated, which most are located in the western Mediterranean Sea. Because *P. ferruginea* is the most vulnerable member of this genus and had almost completely disappeared from the European coasts (Templado and Moreno, 1997; Guallart et al., 2013), studies were performed in areas where the species persisted due to conservation concerns. The known distribution of *P. ferruginea* is practically limited to the coasts of North Africa—from the Strait of Gibraltar (Ceuta) to the Zembra Islands (Tunisia)—some parts of southern Spain (coasts of Málaga, Granada, and Almería), the Alboran Island, and the coasts of Corsica and Sardinia (Laborel-Deguen and Laborel, 1991; Templado et al., 2004; Moreno and Arroyo, 2008; Guallart and Templado, 2012). The hot spots of *P. ferruginea* must be protected as a priority owing to the general regression of its geographic distribution (Templado, 2001; Guerra-García et al., 2004; Templado et al., 2004; Espinosa, 2009; Espinosa et al., 2014).

Regarding the temporal distribution of studies on *Patella* (Figure 3), an increase was seen in the number of publications since the early 2000s, because most of the studies focused on *P. ferruginea*, which was listed as an endangered species in Spain in 1999 (BOE, 1999) and considered "threatened" by the EU Habitat Directive (Annex IV) and the Barcelona Convention (Annex II). Thus, the concern for *P. ferruginea* has increased because of its population decline.

Many studies have compared the densities of individuals in MPAs and control zones, confirming the presence of a positive effect on the density and size of limpets in protected areas. The effects of protection on limpets were observed in South Africa for *Helcion concolor* (Branch, 1975), *Cymbula oculus* (Branch and Odendaal, 2003; Baliwe et al., 2022), *C. granatina*, and *Scutellastra argenvillei* (Baliwe et al., 2022); in the Madeira archipelago for *Patella aspera* and *P. candei* (Martins et al., 2017; Sousa et al., 2019a; Sousa et al., 2019b; Sousa et al., 2020), in the Canary Islands for *Patella ulyssiponensis* (López et al., 2012); in Chile for *Fissurella crassa* and *F. limbata* (Durán and Castilla, 1989; Godoy and Moreno, 1989); and in the USA for *Lottia gigantea* (Sagarin et al., 2007; Fenberg and Roy, 2012). However, in this study, the mean density of *P. ferruginea* individuals was slightly higher in the control sites than in MPAs; the statistical analyses revealed no significant differences between the levels of protection, which is consistent with the results of Espinosa et al. (2009) for this species in the Mediterranean. In fact, intra- and interspecific competition effects, related to increase in limpet size and the consequent decrease in population density, were seen, because the distance between nearest neighbors increased as the sum of their sizes increased (Black, 1979; Espinosa et al., 2006; Espinosa et al., 2009). In this study, the populations with the highest average densities were in unprotected areas (Plane Island and Melilla). However, other factors may affect the density of individuals regardless of protection, such as the effect of hydrodynamism, because many studies have shown that limpet species tend to be more abundant in exposed rocks (Laborel-Deguen and Laborel, 1991; Marra et al., 2018). As key species of rocky ecosystems (Menge, 2000), limpets depend on the biota that surrounds them, such as the availability of food or the presence of natural predators, thus altering population densities.

The effect of protection on mean size showed different results than those observed for mean density; in this case, mean size was significantly greater in MPAs than control areas. Generally, larger individuals are the main target of illegal catches (Sagarin et al., 2007; Espinosa, 2009); therefore, in unprotected areas, where they can be easily caught, limpets had a lower mean size. Human pressure can shift the population structure toward higher frequencies of smaller individuals in unprotected areas (Kido and Murray, 2003), reducing the proportion of larger individuals (mostly females), reducing gonadal production, and lowering reproductive success, which is the main cause for population vulnerability (Ostalé-Valriberas et al., 2022). Therefore, if with the implementation of MPAs, big individuals could be preserved, as seems to happen for *P. ferruginea* in the Mediterranean, can be obtained by indirect effects regarding the reproductive output of these broadcast spawners.

Therefore, the major factor allowing for the coexistence of size classes is probably the effect of very high-intraspecific competition within large sizes, which reduces densities below levels at which small limpets can be eliminated (Boaventura et al., 2003). However, intraspecific competition depends on the species, because it is related to behavior and spatial distribution (Espinosa and Rivera-Ingraham, 2017). In *P. ferruginea*, intraspecific competition appears to be lower than in other species (Espinosa et al., 2006). By contrast, the recruitment of *Patella* is lower in sheltered areas (Fernández et al., 2016), which must be compensated with higher survival and growth rates, because lower densities allow for a lower degree of intraspecific competition. Moreover, some experimental studies support the hypothesis that greater growth in sheltered areas is specifically more due to lower density per unit grazeable area, meaning higher food availability (Jenkins and Hartnoll, 2001). However, as the study has shown, harvesting seems to be the main factor affecting the size classes of *Patella* spp., being significantly higher in MPAs (Espinosa et al., 2008), although the surveillance factor is fundamental (Coppa et al., 2016; Marra et al., 2018).

From the findings of this study, it can be concluded that of the five species of the genus *Patella* present in the Mediterranean, the most studied is *P. ferruginea*. This explains the temporal distribution of the studies, because the increase in studies occurred in the early 2000s, which coincides with the establishment of the conservation status and the concern about the population status of *P. ferruginea*. The analysis of the effects of protection on the density and mean size of *P. ferruginea* individuals revealed no significant differences in mean density between the control sites and MPAs. However, the mean size of individuals was significantly higher in MPAs than control sites. This pattern may be related to the intra- and interspecific competition between individuals.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

AF and AC-S contributed to the conception and design of the study. AC-S and AR-E have collaborated in the bibliographic review. AC-S organized the database. AF and AC-S performed the statistical analysis. AC-S wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

Many thanks are due to the editor and to the reviewers for their comments and constructive criticism of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1158470/full#supplementary-material

References

Agardy, M. T. (1994). Advances in marine conservation: the role of marine protected areas. *Trends Ecol. Evol.* 9 (7), 267–270. doi: 10.1016/0169-5347(94)90297-6

Allison, G. W., Lubchenco, J., and Carr, M. H. (1998). Marine reserves are necessary but not sufficient for marine conservation. *Ecol. Appl.* 8 (1), 79–92. doi: 10.2307/2641365

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x

Baliwe, N. G., Pfaff, M. C., and Branch, G. M. (2022). Assessing the effects of no-take zones in a marine protected area spanning two ecoregions and rock substrate types. *Front. Mar. Sci.* 9, 893260. doi: 10.3389/fmars.2022.893260

Ballantine, W. J., and Langlois, T. J. (2008). Marine reserves: the need for systems. *Challenges to Mar. Ecosyst.* 202, 35–44. doi: 10.1007/978-1-4020-8808-7_3

Bianchi, C. N., and Morri, C. (2000). Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Mar. pollut. Bull.* 40 (5), 367–376. doi: 10.1016/S0025-326X(00)00027-8

Black, R. (1979). Competition between intertidal limpets: an intrusive niche on a steep resource gradient. *J. Anim. Ecol.* 48, 401–411. doi: 10.2307/4169

Boaventura, D., Da Fonseca, L. C., and Hawkins, S. J. (2003). Size matters: competition within populations of the limpet *Patella depressa*. *J. Anim. Ecol.* 72 (3), 435–446. doi: 10.1046/j.1365-2656.2003.00713.x

BOE (1999). *Orden de 9 de junio de 1999 por la que se incluyen en el catálogo nacional de especies amenazadas determinadas especies de cetáceos, de invertebrados marinos y de flora y por la que otras especies se excluyen o cambian de categoría* (Madrid, Spain: Boletín Oficial del Estado).

Branch, G. M. (1975). Notes on the ecology of *Patella concolor* and *Cellana capensis*, and the effects of human consumption of limpet populations. *Zoologica Africana* 10, 75–85. doi: 10.1080/00445096.1975.11447494

Branch, G. M., and Odendaal, F. (2003). The effects of marine protected areas on the population dynamics of a south African limpet, *Cymbula oculus*, relative to the influence of wave action. *Biol. Conserv.* 114, 255–269. doi: 10.1016/S0006-3207(03)00045-4

Burak, S., Dogan, E., and Gazioglu, C. (2004). Impact of urbanization and tourism on coastal environment. *Ocean Coast. Manage.* 47 (9-10), 515–527. doi: 10.1016/j.ocecoaman.2004.07.007

Casu, M., Sanna, D., Cristo, B., Lai, T., Dedola, G. L., and Curini-Galletti, M. (2010). COI sequencing as tool for the taxonomic attribution of patella spp. (Gastropoda): the case of morphologically undistinguishable juveniles settled on a patella ferruginea adult. *J. Mar. Biol. Assoc. United Kingdom* 90 (7), 1449–1454. doi: 10.1017/S0025315409991603

Claudet, J., Loiseau, C., Sostres, M., and Zupan, M. (2020). Underprotected marine protected areas in a global biodiversity hotspot. *One Earth* 2 (4), 380–384. doi: 10.1016/j.oneear.2020.03.008

Claudet, J., Osenberg, C. W., Benedetti-Cecchi, L., Domenici, P., García-Charton, J. A., Pérez-Ruzafa, Á., et al. (2008). Marine reserves: size and age do matter. *Ecol. Lett.* 11 (5), 481–489. doi: 10.1111/j.1461-0248.2008.01166.x

Coppa, S., De Lucia, G. A., Massaro, G., Camedda, A., Marra, S., Magni, P., et al. (2016). Is the establishment of MPAs enough to preserve endangered intertidal species? the case of *Patella ferruginea* in mal di ventre island (W Sardinia, Italy). *Aquat. Conservation: Mar. Freshw. Ecosyst.* 26 (4), 623–638. doi: 10.1002/aqc.2579

Davenport, J., and Davenport, J. L. (2006). The impact of tourism and personal leisure transport on coastal environments: a review. *Estuarine Coast. Shelf Sci.* 67 (1-2), 280–292. doi: 10.1016/j.ecss.2005.11.026

Durán, L. R., and Castilla, J. C. (1989). Variation and persistence of the middle rocky intertidal community of central Chile, with and without human harvesting. *Mar. Biol.* 103, 555–562. doi: 10.1007/BF00399588

Espinosa, F. (2009). Populational status of the endangered mollusc *Patella ferruginea* gmelin 1791 (Gastropoda, patellidae) on Algerian islands (SW Mediterranean). *Anim. Biodiversity Conserv.* 32 (1), 19–28. doi: 10.32800/abc.2009.32.0019

Espinosa, F., Guerra-García, J. M., Fa, D., and García-Gómez, J. C. (2006). Effects of competition on an endangered limpet *Patella ferruginea* (Gastropoda: patellidae): implications for conservation. *J. Exp. Mar. Biol. Ecol.* 330 (2), 482–492. doi: 10.1016/j.jembe.2005.09.020

Espinosa, F., and Rivera-Ingraham, G. A. (2017). Biological conservation of giant limpets: the implications of large size. *Adv. Mar. Biol.* 76, 105–155. doi: 10.1016/bs.amb.2016.10.002

Espinosa, F., Rivera-Ingraham, G. A., Fa, D., and García-Gómez, J. C. (2009). Effect of human pressure on population size structures of the endangered ferruginean limpet: toward future management measures. *J. Coast. Res.* 25 (4), 857–863. doi: 10.2112/08-1005.1

Espinosa, F., Rivera-Ingraham, G., and García-Gómez, J. C. (2008). Seasonal activity and foraging behaviour of the endangered limpet patella ferruginea. *Ethology Ecol. Evol.* 20 (2), 173–181. doi: 10.1080/08927014.2008.9522537

Espinosa, F., Rivera-Ingraham, G. A., Maestre, M., González, A. R., Bazairi, H., and García-Gómez, J. C. (2014). Updated global distribution of the threatened marine limpet *Patella ferruginea* (Gastropoda: patellidae): an example of biodiversity loss in the Mediterranean. *Oryx* 48 (2), 266–275. doi: 10.1017/S0030605312000580

Fenberg, P. B., and Roy, K. (2012). Anthropogenic harvesting pressure and changes in life history: insights from a rocky intertidal limpet. *Am. Nat.* 180, 200–210. doi: 10.1086/666613

Fernández, N., Alborés, I., and Aceña-Matarranz, S. (2016). Spatial variability of the reproductive cycle and physiological condition of patella spp. (Mollusca Gastropoda) in the NW of the Iberian peninsula: implications for exploitation. *Fisheries Res.* 179, 76–85. doi: 10.1016/j.fishres.2016.02.010

García-Gómez, J. C., Guerra-García, J. M., Espinosa, F., Maestre, M. J., Rivera-Ingraham, G., Fa, D., et al. (2015). Artificial marine micro-reserves networks (AMMRNs): an innovative approach to conserve marine littoral biodiversity and protect endangered species. *Mar. Ecol.* 36 (3), 259–277. doi: 10.1111/maec.12167

García-Gómez, J. C., López-Fé, C. M., Espinosa, F., Guerra-García, J. M., and Rivera-Ingraham, G. A. (2011). Marine artificial micro-reserves: a possibility for the conservation of endangered species living on artificial substrata. *Mar. Ecol.* 32 (1), 6–14. doi: 10.1111/j.1439-0485.2010.00409.x

Garrabou, J., Gómez-Gras, D., Medrano, A., Cerrano, C., Ponti, M., Schlegel, R., et al. (2022). Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. *Global Change Biol.* 28 (19), 5708–5725. doi: 10.1111/gcb.16301

Giorgi, F. (2006). Climate change hot-spots. *Geophysical Res. Lett.* 33 (8), L08707. doi: 10.1029/2006GL025734

Godoy, C., and Moreno, C. A. (1989). Indirect effects of human exclusion from the rocky intertidal in southern Chile: a case of cross-linkage between herbivores. *Oikos* 54, 101–106. doi: 10.2307/3565902

Gofas, S., Salas, C., and Moreno, D. (2011). *Moluscos marinos de andalucía (Vol. 1)* (Málaga: Universidad de Málaga, Servicio de Publicaciones e Intercambio Científico), 342.

Guallart, J., Luque, Á.A., Acevedo, I., and Calvo, M. (2013). Distribución y censo actualizado de la lapa ferrugínea (*Patella ferruginea* gmelin 1791) en el litoral de melilla (Mediterráneo suroccidental). *Iberus* 31 (1), 21–51.

Guallart, J., and Templado, J. (2012). *Patella ferruginea. bases ecológicas preliminares para la conservación de las especies de interés comunitario en españa: invertebrados* (Madrid: Ministerio de Agricultura, Alimentación y Medio Ambiente), 86.

Guerra, M. T., and Gaudencio, M. J. (1986). Aspects of the ecology of patella spp. on the Portuguese coast. *Hydrobiologia* 142, 57–69. doi: 10.1007/BF00026747

Guerra-García, J. M., Corzo, J., Espinosa, F., and García-Gómez, J. C. (2004). Assessing habitat use of the endangered marine mollusc *Patella ferruginea* (Gastropoda, patellidae) in northern Africa: preliminary results and implications for conservation. *Biol. Conserv.* 116 (3), 319–326. doi: 10.1016/S0006-3207(03)00201-5

Halpern, B. S. (2003). The impact of marine reserves: do reserves work and does reserve size matter? *Ecol. Appl.* 13 (1), 117–137. doi: 10.1890/1051-0761(2003)013[0117:TIOMRD]2.0.CO;2

Hockey, P. A. R. (1994). "Man as a component of the littoral predator spectrum: a conceptual overview," in *Rocky shores: exploitation in Chile and south Africa*. Ed. W. R. Siegfriedd (Berlin, Heidelberg: Springer). vol 103, 17–31.

Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., et al. (2001). Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293 (5530), 629–637. doi: 10.1126/science.1059199

Jenkins, S. R., and Hartnoll, R. G. (2001). Food supply, grazing activity and growth rate in the limpet *Patella vulgata* l.: a comparison between exposed and sheltered shores. *J. Exp. Mar. Biol. Ecol.* 258 (1), 123–139. doi: 10.1016/s0022-0981(01)00211-8

Keough, M. J., Quinn, G. P., and King, A. (1993). Correlations between human collecting and intertidal mollusc populations on rocky shores. *Conserv. Biol.* 7 (2), 378–390. doi: 10.1046/j.1523-1739.1993.07020378.x

Kido, J. S., and Murray, S. N. (2003). Variation in owl limpet *Lottia gigantea* population structures, growth rates, and gonadal production on southern California rocky shores. *Mar. Ecol. Prog. Ser.* 257, 111–124. doi: 10.3354/meps257111

Kingsford, M. J., Underwood, A. J., and Kennelly, S. J. (1991). Humans as predators on rocky reefs in new south Wales, Australia. *Mar. Ecol. Prog. Ser.* 72, 1–14. doi: 10.3354/meps072001

Laborel-Deguen, F., and Laborel, J. (1991), 91–103. Statut de Patella ferruginea Gmelin en Méditerranée. Les Espèces marines à protéger en Méditerranée, GIS Posidonie.

Lasiak, T. (2006). Spatial variation in density and biomass of patellid limpets inside and outside a marine protected area. *J. Molluscan Stud.* 72, 137–142. doi: 10.1093/mollus/eyi058

Lester, S. E., Halpern, B. S., Grorud-Colvert, K., Lubchenco, J., Ruttenberg, B. I., Gaines, S. D., et al. (2009). Biological effects within no-take marine reserves: a global synthesis. *Mar. Ecol. Prog. Ser.* 384, 33–46. doi: 10.3354/meps08029

Lionello, P., Malanotte-Rizzoli, P., Boscolo, R., Alpert, P., Artale, V., Li, L., et al. (2006). The Mediterranean climate: an overview of the main characteristics and issues. *Developments Earth Environ. Sci.* 4, 1–26. doi: 10.1016/S1571-9197(06)80003-0

López, C., Poladura, A., Hernández, J. C., Martín, L., Concepción, L., Sangil, C., et al. (2012). Contrasting effects of protection from harvesting in populations of two limpet species in a recently established marine protected area. *Scientia Marina* 76, 799–807. doi: 10.3989/scimar.03601.15C

Mannino, M. A., and Thomas, K. D. (2002). Depletion of a resource? the impact of prehistoric human foraging on intertidal mollusc communities and its significance for human settlement, mobility and dispersal. *World Archaeology* 33 (3), 452–474. doi: 10.1080/00438240120107477

Marra, S., Coppa, S., Camedda, A., Massaro, G., and de Lucia, G. A. (2018). The exploitation of limpets in a Mediterranean marine protected area: assessing the effectiveness of protection in the intertidal zone. *Mediterr. Mar. Sci.* 18 (3), 406–423. doi: 10.12681/mms.2087

Martins, G. M., Borges, C. D. G., Vale, M., Ribeiro, P. A., Ferraz, R. R., Martins, H. R., et al. (2017). Exploitation promotes earlier sex change in a protandrous patellid limpet, *Patella aspera* rödin. *Ecol. Evol.* 7, 3616–3622. doi: 10.1002/ece3.2925

Mauro, A., Arculeo, M., and Parrinello, N. (2003). Morphological and molecular tools in identifying the Mediterranean limpets *Patella caerulea, patella aspera* and *Patella rustica*. *J. Exp. Mar. Biol. Ecol.* 295 (2), 131–143. doi: 10.1016/S0022-0981(03)00291-0

McGranahan, G., Balk, D., and Anderson, B. (2007). The rising tide: assessing the risks of climate change and human settlements in low elevation coastal zones. *Environ. Urbanization* 19 (1), 17–37. doi: 10.1177/0956247807076960

Menge, B. A. (2000). Top-down and bottom-up community regulation in marine rocky intertidal habitats. *J. Exp. Mar. Biol. Ecol.* 250 (1-2), 257–289. doi: 10.1016/S0022-0981(00)00200-8

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G.The PRISMA Group (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PloS Med.* 6, e1000097. doi: 10.1371/journal.pmed.1000097

Moreno, D., and Arroyo, M. C. (2008). "Patella ferruginea gmeli," in *Libro rojo de los invertebrados de andalucía*, vol. I . Eds. J. M. Barea-Azcón, E. Ballesteros-Duperón and D. Moreno (Junta de Andalucía, Sevilla: Consejería de Medio Ambiente), 308–319.

Murawski, S. A. (2000). Definitions of overfishing from an ecosystem perspective. *ICES J. Mar. Sci.* 57 (3), 649–658. doi: 10.1006/jmsc.2000.0738

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019) *Vegan: community ecology package. r package version 2.5-6*. Available at: https://CRAN.R-project.org/package=vegan.

Ostalé-Valriberas, E., Sempere-Valverde, J., Pavón-Paneque, A., Coppa, S., Espinosa, F., and García-Gómez, J. C. (2022). 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. *Mar. Policy* 136, 104917. doi: 10.1016/j.marpol.2021.104917

Paracuellos, M., Nevado, J. C., Moreno, D., Giménez, A., and Alesina, J. J. (2003). Conservational status and demographic characteristics of *Patella ferruginea* gmelin 1791 (Mollusca, gastropoda) on the alboran island (Western Mediterranean). *Anim. Biodiversity Conserv.* 26 (2), 29–37.

Power, M. E., Tilman, D., Estes, J. A., Menge, B. A., Bond, W. J., Mills, L. S., et al. (1996). Challenges in the quest for keystones: identifying keystone species is difficult but essential to understanding how loss of species will affect ecosystems. *BioScience* 46 (8), 609–620. doi: 10.2307/1312990

Probert, P. K. (2017). *Marine conservation* (Cambridge, United Kingdom: Cambridge University Press).

Prusina, I., Ezgeta-Balić, D., Ljubimir, S., Dobroslavić, T., and Glamuzina, B. (2014). On the reproduction of the Mediterranean keystone limpet *Patella rustica*: histological overview. *J. Mar. Biol. Assoc. United Kingdom* 94 (8), 1651–1660. doi: 10.1017/S0025315414000976

QGIS Development Team (2022). *QGIS geographic information system* (QGIS Association). Available at: http://www.qgis.org.

Raffaelli, D., and Hawkins, S. (1996). *Intertidal ecology* (London: Chapman and Hall).

R Core Team (2020). *R: a language and environment for statistical computing* (Vienna, Austria: R Foundation for Statistical Computing). Available at: https://www.R-project.org.

Rivera-Ingraham, G. A. (2010). *Biología de la conservación de especies de patélidos en el umbral atlántico-mediterráneo* (Sevilla, Spain: University of Seville). [dissertation/PhD thesis.

Rivera-Ingraham, G. A., Espinosa, F., and García-Gómez, J. C. (2011). Environmentally mediated sex change in the endangered limpet patella ferruginea (Gastropoda: patellidae). *J. Molluscan Stud.* 77 (3), 226–231. doi: 10.1093/mollus/eyr007

Sagarin, R. D., Ambrose, R. F., Becker, B. J., Engle, J. M., Kido, J., Lee, S. F., et al. (2007). Ecological impacts on the limpet lottia gigantea populations: human pressure over a broad scale on island and mainland intertidal zones. *Mar. Biol.* 150, 399–413. doi: 10.1007/s00227-006-0341-1

Scheffer, M., Carpenter, S., and de Young, B. (2005). Cascading effects of overfishing marine systems. *Trends Ecol. Evol.* 20 (11), 579–581. doi: 10.1016/j.tree.2005.08.018

Schmitt, R. J., and Osenberg, C. W. (1996). *Detecting ecological impacts: concept and applications in coastal habitats* (San Diego: Academic Press).

Selig, E. R., and Bruno, J. F. (2010). *A global analysis of the effectiveness of marine protected areas in preventing coral loss* (5(2: PLoS One).

Smith, H. D. (2000). The industrialisation of the world ocean. *Ocean Coast. Manage.* 43 (1), 11–28. doi: 10.1016/S0964-5691(00)00028-4

Sousa, R., Henriques, P., Vasconcelos, J., Pinto, A. R., Delgado, J., and Riera, R. (2020). The protection effects of marine protected areas on exploited molluscs from an oceanic archipelago. *Aquat. Conservation: Mar. Freshw. Ecosyst.* 30, 717–729. doi: 10.1002/aqc.3285

Sousa, R., Vasconcelos, J., Henriques, P., Pinto, A. R., Delgado, J., and Riera, R. (2019a). Long-term population status of two harvested intertidal grazers (*Patella aspera* and *Patella candei*), before, (1996-2006) and after, (2007-2017) the implementation of management measures. *J. sea Res.* 144, 33–38. doi: 10.1016/j.seares.2018.11.002

Sousa, R., Vasconcelos, J., Riera, R., Pinto, A. R., Delgado, J., and Henriques, P. (2019b). Potential impact of harvesting management measures on the reproductive parameters of the limpets *Patella aspera* and *Patella candei* from Madeira island. *Estuarine Coast. Shelf Sci.* 226, 106264. doi: 10.1016/j.ecss.2019.106264

Stanchev, H., Stancheva, M., and Young, R. (2015). Implications of population and tourism development growth for Bulgarian coastal zone. *J. Coast. Conserv.* 19 (1), 59–72. doi: 10.1007/s11852-014-0360-x

Templado, J. (2001). "Patella ferruginea (Gmelin 1791)," in *Los Invertebrados no insectos de la directiva hábitats en españa. ediciones serie técnica, organismo autónomo parques nacionales, dirección general de conservación de la naturaleza*. Eds. M. A. Ramos, D. Bragado and J. Fernández (Madrid: Ministerio de Medio Ambiente), 41–49.

Templado, J., Calvo, M., Garvía, A., Luque, A., Maldonado, M., and Moro, L. (2004). *Guía de invertebrados y peces marinos protegidos por la legislación nacional e internacional* (58-59: M° Medio Ambiente-CSIC).

Templado, J., and Moreno, D. (1997). La lapa ferrugínea. *Biológica: conocer y conservar la naturaleza* 6, 80–81.

Thompson, R. C., Crowe, T. P., and Hawkins, S. J. (2002). Rocky intertidal communities: past environmental changes, present status and predictions for the next 25 years. *Environ. Conserv.* 29 (2), 168–191. doi: 10.1017/S0376892902000115

Tlig-Zouari, S., Rabaoui, L., Fguiri, H., and Ben Hassine, O. K. (2010). Status, habitat and distribution of the endangered limpet *Patella ferruginea* along the northern and eastern Tunisian coastline: results and implications for conservation. *Cahiers Biologie Mar.* 51 (1), 75.

UNEP/MAP-SPA/RAC (2018). *Annex II: list of endangered or threatened species* (Tunis Cedex, Tunisia: SAP/ RAC, SPA-BD Protocol).

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis* (New York: Springer-Verlag).

Zahedi, S. (2008). Tourism impact on coastal environment. *WIT Trans. Built Environ.* 99, 45–57. doi: 10.2495/CENV080051

Frontiers in Marine Science

TYPE Original Research
PUBLISHED 22 May 2023
DOI 10.3389/fmars.2023.1173629

Check for updates

OPEN ACCESS

EDITED BY
Diego Castejón Bueno,
Centro Interdisciplinar de Investigação Marinha e Ambiental da Madeira (CIIMAR-Madeira), Portugal

REVIEWED BY
Alan Hodgson,
Rhodes University, South Africa
Sher Khan Panhwar,
University of Karachi, Pakistan

*CORRESPONDENCE
Joana Vasconcelos
joana.vasconcelos@staff.uma.pt;
joanapatricia.reis@ulpgc.es

[†] PRESENT ADDRESS
Joana Vasconcelos,
Grupo en Biodiversidad y Conservación (BIOCON), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria,
Marine Scientific and Technological Park,
Telde, Spain

RECEIVED 24 February 2023
ACCEPTED 02 May 2023
PUBLISHED 22 May 2023

CITATION
Vasconcelos J, Sousa R, Ferro J, Pinto AR, Freitas M and Riera R (2023) Fecundity, an overlooked life-history trait for coastal management of commercial molluscs? *Front. Mar. Sci.* 10:1173629.
doi: 10.3389/fmars.2023.1173629

Fecundity, an overlooked life-history trait for coastal management of commercial molluscs?

Joana Vasconcelos[1,2,3*†], Ricardo Sousa[3,4], João Ferro[5], Ana Rita Pinto[4], Mafalda Freitas[3,4] and Rodrigo Riera[2,6]

[1]Faculdade de Ciências da Vida, Universidade da Madeira, Campus Universitário da Penteada, Funchal, Portugal, [2]Grupo en Biodiversidad y Conservación (BIOCON), Instituto Universitario de Investigación en Acuicultura Sostenible y Ecosistemas Marinos (IU-ECOAQUA), Universidad de Las Palmas de Gran Canaria, Marine Scientific and Technological Park, Telde, Spain, [3]MARE - Marine and Environmental Sciences Centre/Aquatic Research Network (ARNET), Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Funchal, Portugal, [4]Direção de Serviços de Monitorização, Estudos e Investigação do Mar, Direção Regional do Mar, Funchal, Portugal, [5]Faculdade de Ciências Exatas e da Engenharia, Universidade da Madeira, Campus Universitário da Penteada, Funchal, Portugal, [6]Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Concepción, Chile

Gonadal development in limpets is well-known. However, the integration of fecundity data on coastal management actions remains overlooked. Here, we use fecundity of two exploited limpets (*Patella ordinaria* and *P. aspera*) to demonstrate its potential as a conservation tool to preserve the harvested populations of both species. From October 2021 to June 2022, 158 females of *P. ordinaria* and 70 of *P. aspera* were collected from the coastal rocky shores of the archipelago of Madeira (NE Atlantic Ocean). The fecundity in both species appears to be determinate, and estimated batch fecundity 365,638 ± 204,462 oocytes for *P. ordinaria* and 73,029 ± 43,496 oocytes for *P. aspera*. The presence of spawning individuals of both species until May is of significant relevance to their management and conservation. A harvest-ban (November-March) is currently implemented in the coastal governance actions of *P. ordinaria* and *P. aspera*. Based on the fecundity results, small adjustments can be made to further improve the conservation of adult individuals, which are important for the continuity of future generations. So far, fecundity data constitute an overlooked life trait of key importance to preserve populations of exploited species, primarily those with limited mobility such as semi-sessile littoral limpets.

KEYWORDS

reproduction, commercial species, conservation, limpet, intertidal, North-eastern Atlantic Ocean

1 Introduction

The extensive human footprint has underpinned drastic changes to ecosystems all over the globe (Steffen et al., 2015; Venter et al., 2016). Marine, freshwater and terrestrial realms have been, and still are, subject to habitat loss and fragmentation (Dubois et al., 2018; Jones et al., 2018; Ellis et al., 2021). Conservation actions have been developed to halt this destructive trend and also to preserve scarcely-touched areas on our planet (Geldmann et al., 2013; Ward et al., 2022), such as the spatial management with the creation of no-take marine reserves. The main aim of a high portion of protected areas is to preserve certain endangered species, e.g. large mammals in Africa (Pacifici et al., 2020), or commercially valuable species, e.g. large-sized demersal fish (Hackradt et al., 2014). The establishment of these protected areas is mainly based on conventional biodiversity measures such as, abundance of individuals, densities per surface area, and also species richness in biodiversity hotspots (Blowes et al., 2020; Davies et al., 2021). Their assessment is typically based on management actions that follow up the previous parameters used to declare these spaces as protected areas (Dudley and Stolton, 2008). Other parameters remain overlooked such as, functional traits, phylogenetic diversity or those related to fecundity or reproductive potential (see exceptions Coleman et al., 2015; Guilhaumon et al., 2015). The integration of information on fecundity and reproductive potential would be an asset for conservation assessment studies. However, most of the studies are limited to the harvest control measures catch and bag limits, gear restrictions, closed seasons and minimum size limit (Worm et al., 2009; Gwinn et al., 2015; Liu et al., 2016). The latter has been used as a threshold to separate reproductively active adults and pre-adults or juveniles (e.g. Lavin et al., 2021), as it occurs in a wide range of commercial marine species, i.e. fish, crustaceans and molluscs (Ellis and Cowan, 2001; Alós et al., 2014; Sousa et al., 2019b). Fecundity studies may provide a more accurate picture of the reproductive cycle of a species throughout the year. There are management actions that target this temporal variability on the reproductive potential of the species such as, the implementation of seasons; yet, they are mostly based on maturity stages data classified according only to the progression of the gonads volume in the haemocoel, and only focused on the months where reproductive individuals are reported (de Mitcheson et al., 2020). An exhaustive analysis of fecundity data is expected to identify nuances and subtle variations of gonad development among individuals that may result in accurate management actions that ensure the continuity of the species under protection (Lambert, 2008).

Chronic fishing and harvesting activities have substantial effects on the reproductive parameters of the targeted species (Walsh et al., 2006; Wright and Trippel, 2009; Pellowe and Leslie, 2020). Reproductive dynamics are thought to suffer shifts due to long-term exploitation (Murphy et al., 1994; Sousa et al., 2019b; Pellowe and Leslie, 2020). In harvested molluscs, such as intertidal and shallow subtidal limpets, several reproductive parameters have been used as a proxy to establish the exploitation status of their populations (Riera et al., 2016; Sousa et al., 2019a). The impact of harvesting on littoral limpets from Madeira has been extensively studied in recent years (e.g., Sousa et al., 2019a; Sousa et al., 2019b; Sousa et al., 2020a; Sousa et al., 2020b). Reproductive parameters, including size and age at first maturity, have traditionally been used to assess the level of exploitation of *P. ordinaria* and *P. aspera* in this region. The populations of these two exploited species have been drastically reduced, with a more pronounced decrease in accessible sites (Sousa et al., 2019a). The persistent exploitation of this coastal resource has underpinned changes in life history traits, reproductive output, genetic diversity, among others (Henriques et al., 2017). These shifts are still occurring despite the implementation of management actions such as a closed season during the reproductive season (November-March) (Portaria n. 151/2022; Sousa et al., 2019a) and a minimum harvesting size of 40 mm shell length (Sousa et al., 2019b). This size limit management action was implemented to prevent the harvest of a portion of the spawning stock to maintain replenishment rates (as size at first maturity for *P. ordinaria* and *P. aspera* are 37.5 and 37.4 mm SL, respectively; Sousa et al., 2019b). This leaves a portion of the spawning stock vulnerable to fishing pressure, diminishing the reproductive output (Liu et al., 2016; Lavin et al., 2021) that increases hyperallometrically with size and age (Hixon et al., 2014; Barneche et al., 2018; Vasconcelos et al., 2023). This size-selective harvesting is worrisome, especially for the protandrous hermaphrodite *P. aspera*. In this species, smaller and younger limpets reach sexual maturation as males, with a fraction of them changing to females afterwards (Orton et al., 1956; Espinosa et al., 2006; Espinosa et al., 2009; Martins et al., 2017; Sousa et al., 2019b). This increase of females in larger size classes as the result of sex change, make them extremely vulnerable and a target to harvest (Espinosa et al., 2006), with overfishing affecting the more fecund females and thus reducing the reproductive output of populations (Espinosa et al., 2009). In this sense, we hypothesize that the potential fecundity of *P. aspera* is lower when compared with *P. ordinaria*, as a consequence of *P. aspera* being a protandrous hermaphrodite. Also, asynchronous spawning seasons may be expected between the northern and southern populations of both species. Preliminary results on the fecundity of one of these species were published recently (Vasconcelos et al., 2023). While studying *P. ordinaria* sampled along the Madeiran archipelago during the months of spawning, the authors found differences in the fecundity of northern and southern populations of this species, which may be indicative of this asynchrony.

We here used these two limpets as a model of study to explore the potential of fecundity data to be integrated into coastal management actions. The main goals of the present study are to: (i) analyze at a microscopic level, the gametogenic stages of *P. ordinaria* and *P. aspera* females, (ii) study the four main criteria applied for fecundity type determination for both patellid limpets, and (iii) compare the number and size of vitellogenic oocytes for the northern and southern populations of Madeira. The integration of the former parameters may result in management actions that would potentially be the cornerstone of the sustainable exploitation of these molluscs in the medium and long-term.

2 Material and methods

2.1 Data sampling

For this study, 158 specimens of *Patella ordinaria* (49.34 ± 6.86 mm total shell length, SL) and 70 *P. aspera* (45.65 ± 6.83 mm SL), two species that coexist in the lower intertidal to the subtidal from 0 to 6 m deep (Côrte-Real et al., 1996; Sousa et al., 2017; Sousa et al., 2020b), were randomly collected between October 2021 and June 2022 in four coastal settlements in the Madeira archipelago, two on the North coast (Porto Moniz and São Vicente) and two on the South coast (Funchal and Porto Santo) (Table 1; Figure 1). Due to the fact that these species are winter breeders (Henriques et al., 2012; Sousa et al., 2017), there were some limitations in the monthly (e.g., December) and site (as the North coast of Porto Santo Island) sampling of the reproductive stock, mainly due to the sea adverse conditions, particularly on the north coast of the archipelago (Castejón et al., 2022; Vasconcelos et al., 2023).

In the laboratory, fresh individuals were sorted by species, measured for total shell length (SL, 0.01 mm accuracy) and weighted for total body wet weight (TW, 0.01 g accuracy). A small cut was made in the mantle at the posterior part of the foot (Cañizares et al., 2021) the gonads gently removed and weighted (GW, to the nearest 0.01 g). Sex was determined according to gonad pigmentation Orton et al. (1956), and female gonads preserved as a whole in Roti-Histofix ECO PLUS for histological analysis (Vasconcelos et al., 2023).

2.2 Histological analysis and sexual cycle

Histological sections were assembled from small portions of gonad dehydrated with ethanol at different concentrations (70, 90 and 95%) and embedded in Technovit 7100 resin (Vasconcelos et al., 2017). The homogeneity of oocyte distribution within ovaries was checked from histological sections (5 μm) made on the anterior, middle, and posterior regions of the ovary. As the middle area was representative of the entire gonad, sections were made on this part of the ovary, stained with methylene blue and digitized using a visual image analysis system (Leica DM2700 P microscope coupled to the Leica DFC 450 C camera and the software Leica Application Suite X – LAS 3.7, Leica Microsystems). Classification of oogenesis stages and oocytes into previtellogenic (PO), vitellogenic (VO) and atresic oocytes (AO) (Supplementary Figure 1) were based on Prusina et al. (2014) (adopted and modified from McCarthy et al., 2008; Belkhodja et al., 2011): inactive; early development; late active; ripe; atresic; spawning; and spent.

The gonadosomatic index (GSI) was calculated according to the equation:

$$GSI = 100 * \frac{GW}{TW} \qquad (1)$$

where GW is the gonad weight (in g) and TW the total body wet weight (in g).

2.3 Fecundity analysis

Fecundity of *P. ordinaria* and *P. aspera*, was assessed based on: (i) the presence or absence of an hiatus between PO and VO; (ii) the number of VO during the spawning period; (iii) average size of VO throughout the spawning period; and (iv) the incidence of atresia throughout the spawning period (Hunter, 1992; Greer Walker et al., 1994; Murua et al., 2003). For the first three criteria, 95 *P. ordinaria* (62 in the maturity stage ripe and 33 at the beginning of spawning) and 30 *P. aspera* (27 in ripe and 3 at the beginning of spawning) were analyzed. For the analysis of the first criterion, histological

TABLE 1 Number (N) of individuals and the average total length (TL, mm) and respective standard deviation (SD) of *Patella ordinaria* and *P. aspera* from the archipelago of Madeira (NE Atlantic Ocean) sampled between October 2021 and June 2022.

			Patella ordinaria			*Patella aspera*	
		N	Mean ± SD	Min. - Max.	N	Mean ± SD	Min. - Max.
	October	2	52.45 ± 1.88	51.12 - 53.78	13	44.95 ± 8.59	28.14 - 65.64
	November	11	46.22 ± 1.98	42.87 - 49.22	14	37.34 ± 3.65	32.35 - 42.61
	January	18	42.93 ± 3.95	34.52 - 49.77	–	–	–
Month	February	16	54.02 ± 5.09	41.61 - 62.91	15	49.50 ± 5.43	43.36 - 60.97
	March	33	47.58 ± 9.64	24.92 - 61.37	14	48.60 ± 4.47	40.77 - 56.90
	May	56	49.80 ± 5.48	41.53 - 64.93	14	47.55 ± 3.02	43.60 - 52.70
	June	22	53.91 ± 3.68	46.28 - 61.22	–	–	–
	Total	158	49.34 ± 6.86	24.92 - 64.93	70	45.65 ± 6.83	28.14 - 65.64
Island orientation	North	123	49.12 ± 5.79	34.52 - 64.93	43	42.44 ± 6.15	32.35 - 52.70
	South	35	47.85 ± 9.43	24.92 - 61.37	27	46.84 ± 6.90	28.14 - 65.64

Min Minimum, Max Maximum.
For *P. aspera*, there are no samples for the months January and June (-).

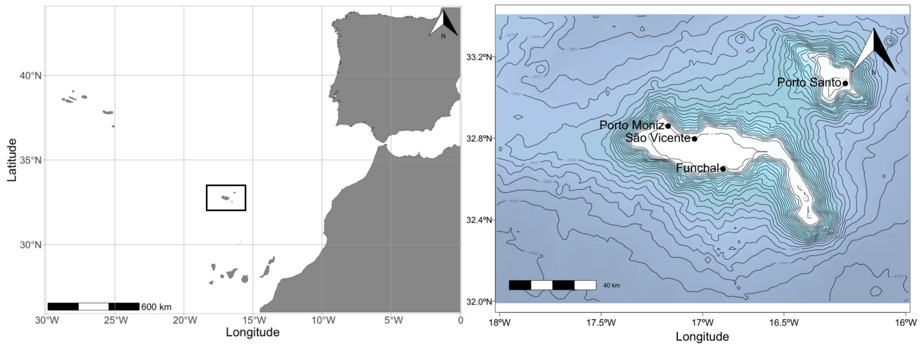


FIGURE 1
Map showing the sampling areas (Funchal, Porto Moniz, Porto Santo and São Vicente) of *Patella ordinaria* and *P. aspera* collected from October 2021 to June 2022 in the Madeira archipelago (NE Atlantic Ocean).

sections were used, while for the second and third criteria, the gravitometric method was applied (Hunter et al., 1989) following the protocol used previously for *P. ordinaria* (Vasconcelos et al., 2023). The number of oocytes per individual was registered and the diameter measured on 2,766 PO (1,815 *P. ordinaria* and 951 *P. aspera*) and 5,365 VO (3,614 *P. ordinaria* and 1,751 *P. aspera*) using the ImageJ software v1.53r (http://imagej.nih.gov/ij/) (Schneider et al., 2012). For the fourth criteria, the relative intensity of atresia, defined as the percentage of atretic vitellogenic oocytes in relation to total number of normal oocytes present in the ovary (Hunter and Macewicz, 1985), was estimated by examining histological sections of 104 gonads of *P. ordinaria* (16 in late active, 60 in ripe and 28 in spawning stage) and 48 of *P. aspera* (19 in late active, 26 in ripe and 3 in the spawning stage). Gonads in the atresic state of maturation were not considered as this could cause a miscalculation because the vast majority of oocytes are in atresia. The prevalence of atresia (defined as the proportion of females with atresia stage oocytes in the total number of females) was also investigated.

Batch fecundity measurements were based on the direct gravimetric method. From the histological examination, 46 females in the ripe stage (25 of *P. ordinaria* and 16 of *P. aspera*) were selected and the number of VO counted throughout the spawning season. Counts performed per specimen may be underestimated due to the loss of some oocytes during the gravimetric process. This problem was encountered by Guallart et al. (2020) and Vasconcelos et al. (2023).

2.4 Data analysis

Differences in the average number and size of oocytes across months were tested. As ANOVA assumptions were not met, the non-parametric Kruskal-Wallis rank sum test (*stats* R package, R Core Team, 2022) was used to investigate trends and differences in oocyte size and number across months. Kruskal-Wallis test does not require sample sizes to be the same in all groups. The effect size for Kruskal-Wallis test was computed as the eta squared based on the H-statistic (η^2, *rstatix* R package, Tomczak and Tomczak, 2014; Kassambara, 2022b). The eta squared estimate ranges from 0 to 1, where values closer to 1 indicate a higher proportion of variance in the dependent variable explained by the independent variable. Values from 0.01 to < 0.06 represent a small effect, 0.06 to < 0.14 moderate effect and ≥ 0.14 a large effect (Tomczak and Tomczak, 2014; Kassambara, 2022b). The *post-hoc* Pairwise Wilcoxon Test was applied to calculate pairwise comparisons across months with corrections for multiple testing (*stats* R package, R Core Team, 2022). The method selected for adjusting p values was the "BH" (Benjamini and Hochberg, 1995), a more powerful method than the family-wise error rate. The relative intensity of atresia throughout months was compared with the Kruskal-Wallis rank sum test (*stats* R package, R Core Team, 2022). The correlation between limpet size and the absolute batch fecundity was investigated using the Pearson correlation test (*ggpubr* R package, Kassambara, 2022a).

3 Results

3.1 Histological analysis and sexual cycle

The frequency distribution of different gametogenic stages observed throughout the study period is illustrated in Figure 2. Mature limpets were represented in all sampling months. Gametogenesis occurred prior to October, with 50% of the females in the Late active and 50% in Ripe stages in *P. ordinaria* whilst only 39% in the Early active and Late Active stage in *P. aspera*. Ripe gonads were more common from October to May in *P. ordinaria* and from February to March in *P. aspera*. During ripening, an average of 24% of female gonads of *P. ordinaria* were in the atresic stage, with most of oocytes undergoing atresia. In *P. aspera*, the atresic stage was observed in October, November and February with an average of 19%.

The gonadosomatic index increased from October-November to March for females of *P. aspera* and *P. ordinaria* respectively (Figure 3). Monthly values revealed an increase from November to March for both species (Kruskal-Wallis test (K-W); *P. ordinaria*: $H = 107.98$, $p < 0.001$, $\eta^2_H = 0.687$; *P. aspera*: $H = 48.484$, $p < 0.001$, $\eta^2_H = 0.684$; Figure 3), supporting the maturity frequency

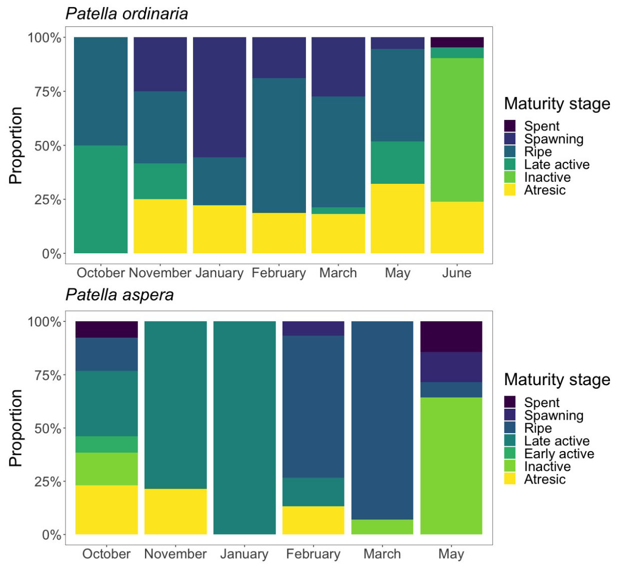


FIGURE 2
Monthly variation of different gametogenic stages in *Patella ordinaria* and *P. aspera* collected from October 2021 to June 2022 in the Madeira archipelago (NE Atlantic Ocean).

distributions based on the microscopic analysis of females gonads (Figure 2). The maximum GSI values were found in March for both species, where females of *P. ordinaria* attained 21.9% (10.9 ± 6.32, mean ± SD) and the females of *P. aspera* 9.12% (5.14 ± 2.65).

3.2 Fecundity estimates

The homogeneity of variance and the normality of the distribution of the number and size of PO and VO were tested for *P. ordinaria* and *P. aspera*. Regarding the number (Levene's test: W = 1.1629, $p > 0.05$) and size (W = 1.7854, $p > 0.05$) of PO in *P. ordinaria*, we can assume the homogeneity of variances across months. Number (W = 8.2877, $p < 0.001$) and size (W = 6.5546, $p < 0.001$) of VO across months did not exhibit homogeneous variance. For *P. aspera*, the number (Levene's test: W =7.1013, $p < 0.001$) and size (W = 3.1174, $p < 0.05$) of PO and the number of VO (W = 3.6633, $p < 0.05$) did not exhibit homogeneous variance across months. The size of VO (W = 2.1727, p = 0.05466) exhibits homogeneity of the variances across months. The size and number of PO and VO departed significantly from normality (Shapiro-Wilk test: $p < 0.001$) in both species.

Fecundity was analyzed under the four above-mentioned criteria. The analysis of the oocytes size frequency distribution showed a distinct interruption between PO and VO in most of the sampled months for both *Patella* species (Figure 4). A progressing dominant cohort in the oocyte size frequency distribution can be observed during the sampling period. The average size of PO varied between 45 ± 18 μm (mean ± SD; 10 - 98 μm) for *P. ordinaria* and 47 ± 17 μm (15 - 100) for *P. aspera*. The size of VO ranged from 80 – 248 μm (149 μm ± 23) for *P. ordinaria* and from 83 – 216 μm (136 μm ± 30) for *P. aspera* (Figure 4). For *P. ordinaria*, the average number of PO decreased significantly from November till March

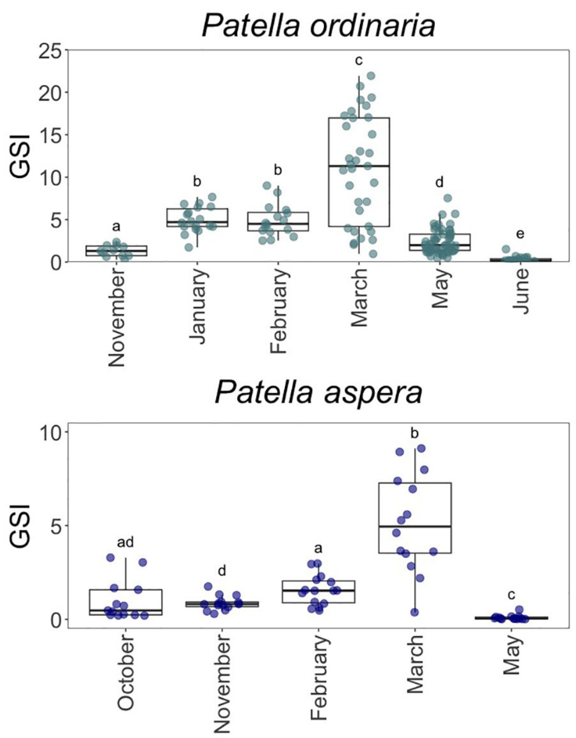


FIGURE 3
Gonadosomatic index (GSI) for *Patella ordinaria* and *P. aspera* sampled from October 2021 to June 2022 in the archipelago of Madeira (NE Atlantic Ocean). Boxplot showing the minimum and maximum values in the data, the 25th percentile, the median and the 75th percentile. Different letters indicate statistically significant differences between months ($p < 0.05$).

(Kruskal-Wallis test (K-W): H = 28.132, $p < 0.05$, $\eta^2_H = 0.228$; Figure 5A). By contrast, for the same period there was an increase in the number of VO (K-W: H = 40.799, $p < 0.05$, $\eta^2_H = 0.359$; Figure 5B). For *P. aspera*, the Kruskal-Wallis test showed significant differences in the number of PO (H = 26.802, $p < 0.05$, $\eta^2_H = 0.519$; Figure 5E) and VO (H = 30.138, $p < 0.05$, $\eta^2_H = 0.599$; Figure 5F). Yet, there was no trend in the number of PO (Figure 5E) such as the increase seen in VO from October to March (Figure 5F). The average size of PO of both *P. ordinaria* (H = 8.2708, $p > 0.05$, $\eta^2_H = 0.00862$; Figure 5C) and *P. aspera* (H = 8.9925, $p > 0.05$, $\eta^2_H = 0.00576$; Figure 5G) did not vary significantly across months. The size of VO of *P. ordinaria* decreased significantly from November till June (H = 141.89, $p < 0.05$, $\eta^2_H = 0.0320$; Figure 5D). During the same period, the average size of VO of *P. aspera* increased significantly (H = 189.3, $p < 0.05$, $\eta^2_H = 0.112$; Figure 5H).

The number and size of PO and VO was analyzed per maturity stage (Figure 6). For the VO, an increase in their number from Late active to Ripe stage, followed by a decrease during spawning stage was observed for both *P. ordinaria* (H = 30.774, $p < 0.001$, $\eta^2_H = 0.285$; Figure 6B) and *P. aspera* (H= 20.94, $p < 0.001$, $\eta^2_H = 0.421$; Figure 6F). Regarding their size, there was an increased from Late active to the Spawning stage in both *P. ordinaria* (H = 338.23, $p < 0.001$, $\eta^2_H = 0.102$; Figure 6D) and *P. aspera* (H = 172.83, $p < 0.001$, $\eta^2_H = 0.103$; Figure 6H). PO attained their lowest number in the Ripe stage (*P. ordinaria*: H = 26.406, $p < 0.001$, $\eta^2_H = 0.242$,

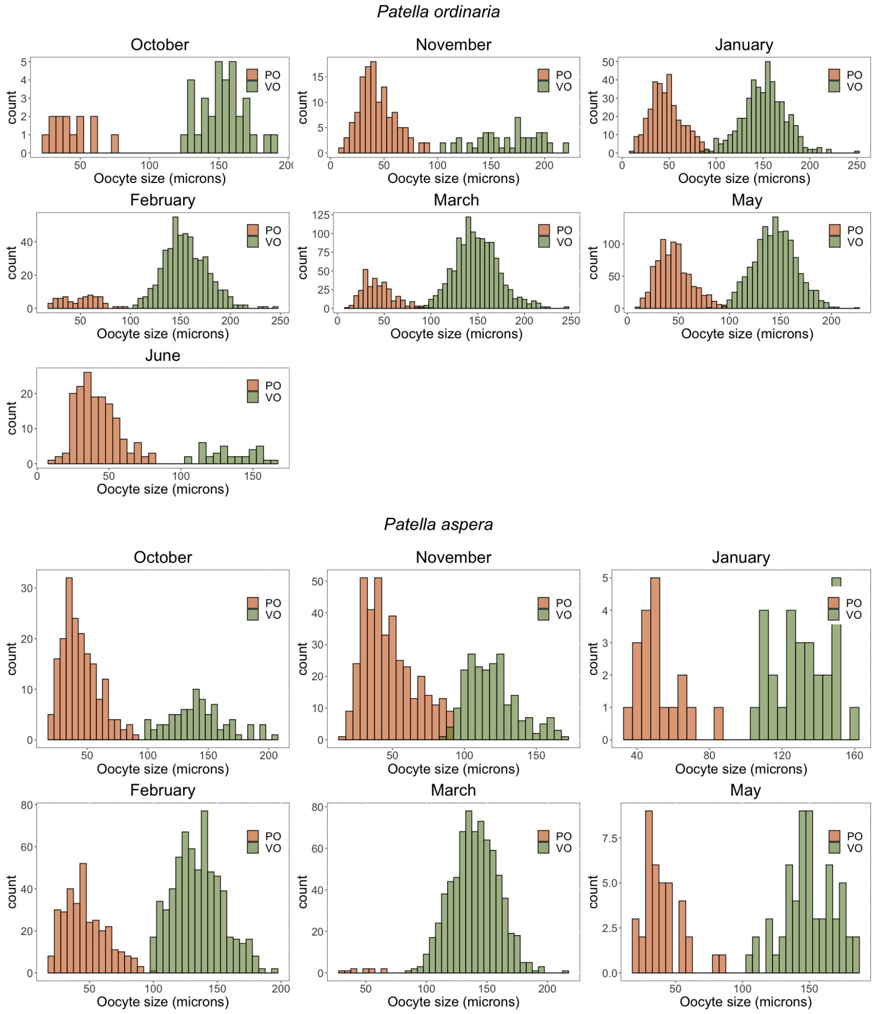


FIGURE 4
Oocyte size frequency distribution in the limpets *Patella ordinaria* and *P. aspera* sampled from October 2021 to June 2022 in the archipelago of Madeira (NE Atlantic Ocean). PO Previtellogenic oocytes, VO vitellogenic oocytes.

Figure 6A; *P. aspera*: $H = 17.625$, $p < 0.001$, $\eta^2_H = 0.347$; Figure 6E), associated with an increase in their size from Late active to the Ripe stage (*P. ordinaria*: $H = 14.86$, $p < 0.001$, $\eta^2_H = 0.00965$; Figure 6C; *P. aspera*: $H = 6.4793$, $p < 0.05$, $\eta^2_H = 0.00786$; Figure 6G) followed by a decrease in spawning stage in *P. ordinaria*.

As no clear trends were observed in the number and size of PO and VO of both species (Figure 5), samples were spatially separated into northern and southern populations. For the northern populations of both species, the average number of PO decreased from November to May (*P. ordinaria*: $H = 14.143$, $p < 0.05$, $\eta^2_H = 0.192$; Figure 7A; *P. aspera*: $H = 8.5234$, $p < 0.05$, $\eta^2_H = 0.502$; Figure 7E). However, no trends in their size were observed (Figures 7C, G). For *P. ordinaria*, the average number of VO increase significantly from November to May ($H = 25.093$, $p < 0.05$, $\eta^2_H = 0.381$; Figure 7B) associated with a decrease in their size ($H = 87.476$, $p < 0.05$, $\eta^2_H = 0.0506$; Figure 7D). For *P. aspera*, the opposite was observed. The number of VO decreased significantly ($H = 6.9579$, $p < 0.05$, $\eta^2_H = 0.381$; Figure 7F), with the increase in their size ($H = 86.021$, $p < 0.05$, $\eta^2_H = 0.322$; Figure 7H). The southern populations of *P. ordinaria* and *P. aspera* were analyzed for number and size of VO (Supplementary Figure 2). From the analysis of the only available months of sampling in the southern part of the archipelago (October and March), there is an increasing trend in the number of VO, although significant only for *P. aspera* (*P.ordinaria*: $H = 1.8968$, $p > 0.05$, $\eta^2_H = 0.0332$; *P. aspera*: $H = 10.531$, $p < 0.05$, $\eta^2_H = 0.561$). No trend was observed in the average size of VO (*P. ordinaria*: $H = 1.0521$, $p > 0.05$, $\eta^2_H = 0.0000461$; *P. aspera*: $H = 1.4769$, $p > 0.05$, $\eta^2_H = 0.000619$).

There was an increase from 9.5 ± 2.12% (mean ± SD) in November to 24.4 ±15.4% in May in the relative intensity of the atresia for *P. ordinaria*, although at the limit of significance ($H = 12.154$, $p < 0.05$, $\eta^2_H = 0.0746$; Figure 8). For *P. aspera*, no significant differences were found in the relative intensity of atresia across months ($H = 8.6772$, $p > 0.05$, $\eta^2_H = 0.0876$; Figure 8), although atresia varied between 10.3 ± 1.53% in May to 44 ± 4.24% in January (Figure 8). The prevalence of atresia, i.e. the proportion of females with oocytes in atresia, was high for both species (*P. ordinaria* = 97%; *P. aspera* = 98%).

Lastly, batch fecundity, estimated based on females in the ripe stage, ranged between 80,006 and 774,504 oocytes for females of *P.*

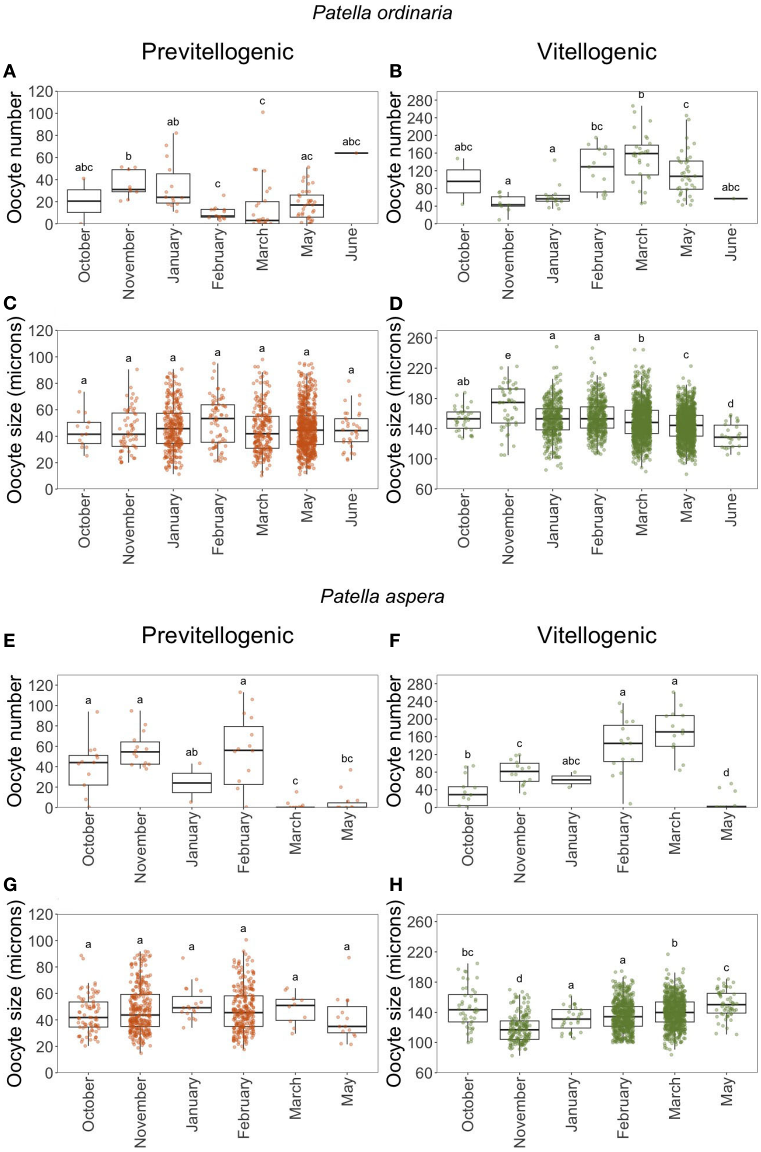


FIGURE 5
Monthly variation in the number **(A, B, E, F)** and size **(C, D, G, H)** of previtellogenic and vitellogenic oocytes of *Patella ordinaria* and *P. aspera* sampled in the archipelago of Madeira (NE Atlantic Ocean). Boxplot shows the minimum and maximum values in the data, the 25*th* percentile, the median and the 75*th* percentile. Different letters indicate statistically significant differences between months (p < 0.05).

ordinaria with 45.72 mm SL and 61.37 mm SL, respectively (354,572 ±189,883, mean ± SD). For *P. aspera*, batch fecundity was on average 77,404 ± 43,910 oocytes, ranging from 22,371 (44.71 mm SL) to 177,202 oocytes (53.38 mm SL). A significant positive correlation was found between the number of oocytes and the shell size of *P. ordinaria* (Pearson's correlation: R = 0.884, $p < 0.001$; Supplementary Figure 3). This relationship was not that clear in *P. aspera* (R = 0.272, $p > 0.05$; Supplementary Figure 3).

4 Discussion

Monitoring species' life histories, particularly their reproductive strategy, is crucial for their effective and long-term management. However, much of the available information is based on the macroscopic identification of the maturity stages, which can lead to errors (Neves et al., 2022). Until recently, all works published on the reproductive biology of the limpets *P. ordinaria* and *P. aspera* to determine their exploitation status in Madeira archipelago were based on the macroscopic analysis of gonads (Henriques et al., 2012; Sousa et al., 2017; Sousa et al., 2019b; Sousa et al., 2020a). In this analysis, maturity stages are classified according only to the progression of gonad volume in the haemocoel, going from either the rudimentary virgin stage or resting after discharge of gametes (inactive or neuter) to fully developed occupying the entire haemocoel (stage V) (Orton et al., 1956). Only two recent works refer to preliminary data on the fecundity of *P. aspera* (assays on females fecundity to develop methodologies for the culture of both limpet species, Castejón et al., 2022) and *P. ordinaria* (Vasconcelos et al., 2023). In this regard, the present work fills the existing gaps by

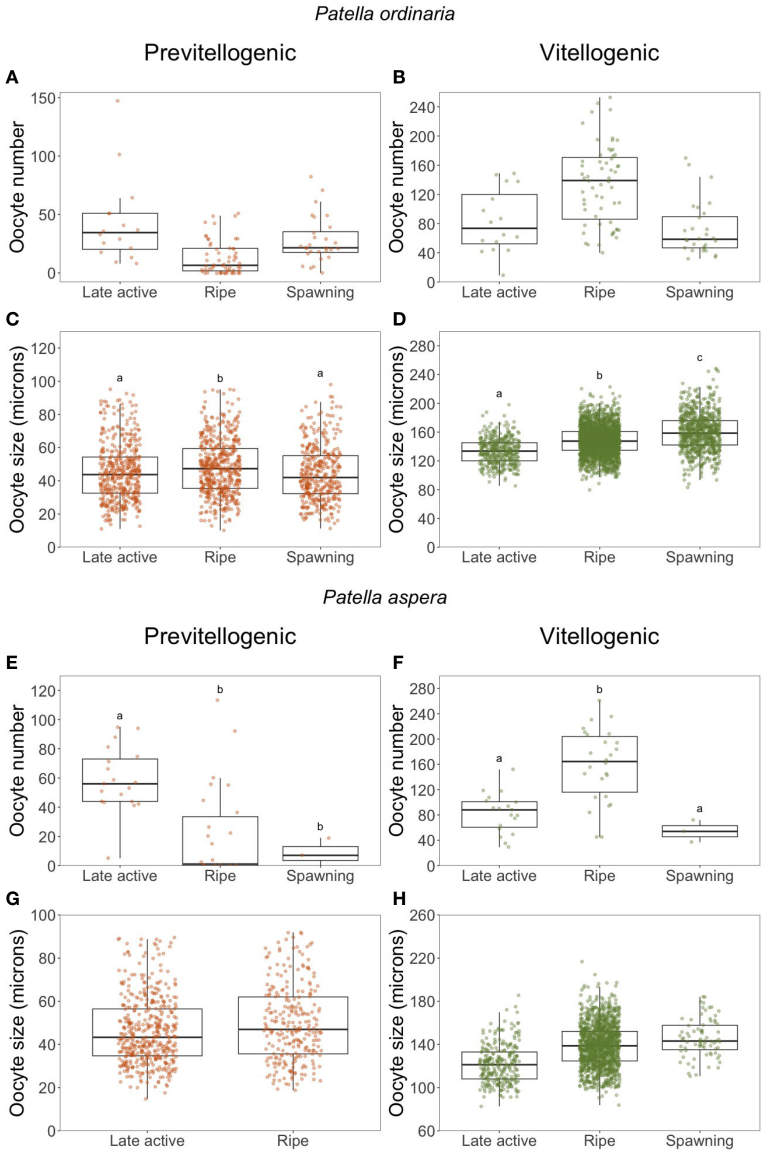


FIGURE 6
Number **(A, B, E, F)** and size **(C, D, G, H)** of previtellogenic and vitellogenic oocytes per maturity stages of *Patella ordinaria* and *P. aspera* sampled in the archipelago of Madeira (NE Atlantic Ocean). Boxplot showing the minimum and maximum values in the data, the 25th percentile, the median and the 75th percentile. Different letters indicate statistically significant differences between maturity stages ($p < 0.05$).

characterizing the fecundity of both limpet species based on microscopic analysis of female gonads to support its management in Madeira archipelago. Reproduction of both patellid limpets has been considered to occur during winter, from November to April (Henriques et al., 2012; Sousa et al., 2017), with spawning most likely to occur from January till April (Pinto et al., 2010; Henriques et al., 2012; Sousa et al., 2017) and with maximum GSI values reported in January and February for *P. aspera* and *P. ordinaria*, respectively (Pinto et al., 2010; Henriques et al., 2012; Sousa et al., 2017). When using the microscopic analysis of females' gonad in the present work, adults in the maturity stages of ripe and spawning were observed until May, despite the decrease in the GSI values after the peak in March. This proves the importance of the microscopic analysis in the determination of the spawning and resting periods.

All lines of evidence to assess fecundity were analyzed for the limpets *P. ordinaria* and *P. aspera*. During the spawning season, a hiatus and a trend can be observed in the oocyte size frequency, with two recognizable cohorts of oocytes, a smaller one of PO that progresses across the sampling period to a larger one that represents the VO. This is indicative of a disruption in the oocyte recruitment with the fecundity well set before the onset of spawning (Hunter, 1992). This bimodal distribution has been previously observed for *P. ordinaria* from Madeira archipelago (Vasconcelos et al., 2023), *S. granularis* from the SE coast of South Africa (Vat, 2000) and *P. rustica* from the SE Adriatic (Prusina et al., 2014). When analyzing the number of oocytes across months and maturity stages, the average number of PO decreased while the VO increased for both limpet species. Also, an expected decrease in the number of VO at

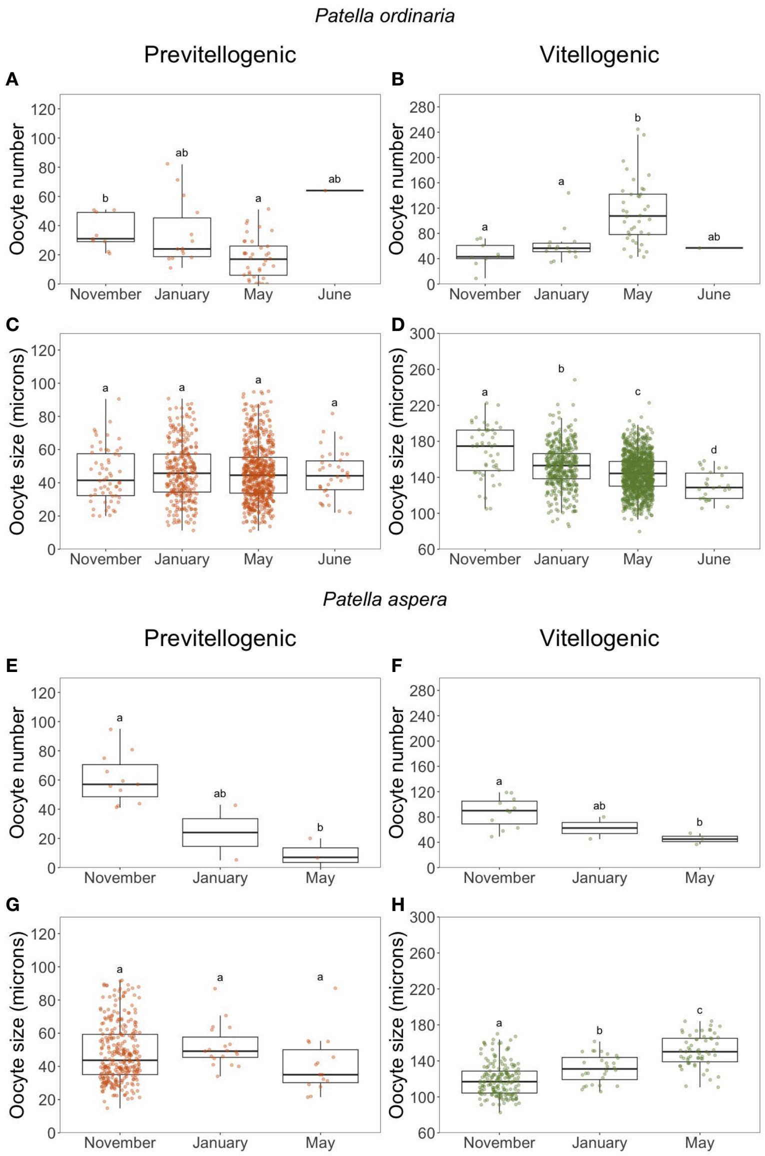


FIGURE 7
Monthly variation in the number **(A, B, E, F)** and size **(C, D, G, H)** of previtellogenic and vitellogenic oocytes of *Patella ordinaria* and *P. aspera* sampled in the north coast of the archipelago of Madeira (NE Atlantic Ocean). Boxplot shows the minimum and maximum values in the data, the 25^{th} percentile, the median and the 75^{th} percentile. Different letters indicate statistically significant differences between months ($p < 0.05$).

the end of the spawning season, more precisely in May, was detected for both species, corroborating the presence of the hiatus between PO and VO and that no new VO are recruited to replace those that have been shed during spawning. In a previous work on the fecundity of *P. ordinaria* from the same region (Vasconcelos et al., 2023), this predicted decrease could not be observed, most likely due to the sampling period that lasted only till March. And the mean diameter of VO increased significantly over the spawning period, though more evident for *P. aspera*, corroborating the presence of a dominant size class of oocytes that progresses across months. Given the increase in the average size of VO and the decrease in their number, we can suggest that this is the case of determined fecundity. For indeterminate fecundity, the size of VO might remain constant or decrease (Murua et al., 2003). The evidences indicates that spawning, though to a lesser extent, may occur until May in both species, two months later than the end of the harvest-ban season (November-March). For *P. ordinaria* (9.5% - 24.4%) and *P. aspera* (10.3% - 44%), the low levels of the relative intensity of atresia detected across months, may be also indicative of determinate spawners (Hunter, 1992). The higher value found in January for *P. aspera* (44%) is a consequence of the lower number of individuals analyzed. Low levels of atresia were reported previously for *P. ordinaria* of Madeira archipelago (7.9% - 20.8% Vasconcelos et al., 2023).

Fecundity parameters also showed some variability between the northern and southern populations for *P. ordinaria* and *P. aspera*. Unfortunately, due to the absence of a greater number of samples throughout the spawning season, the expected trends regarding the number and size of VO were not as evident as when analyzed along the stages of maturation (Figure 6). For the northern populations of

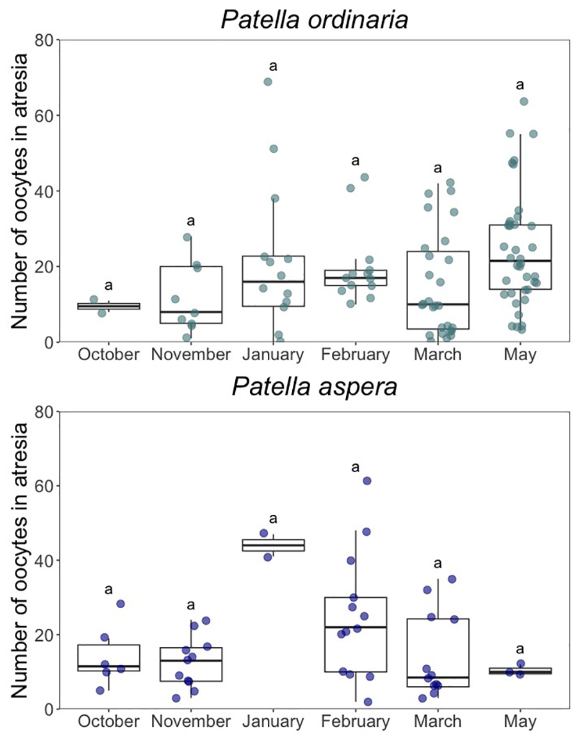


FIGURE 8
Relative intensity of the atresia estimated for *Patella ordinaria* and *P. aspera* sampled in the archipelago of Madeira (NE Atlantic Ocean) across the spawning season. Boxplot showing the minimum and maximum values in the data, the 25th percentile, the median and the 75th percentile. Different letters indicate statistically significant differences between months ($p < 0.05$).

P. ordinaria, there was an increase in the number of VO till May, followed by a decrease. For *P. aspera* an increase in the size of VO was observed from November to May. The fact that the samples are too separated in time, i.e., without specimens between January and May, it is hardly difficult to infer whether there is an increase or decrease in the average size of VO of *P. ordinaria* and in the number of VO of *P. aspera*. However, for *P. ordinaria*, a large number of vitellogenic oocytes and an average GSI of 2.40% was still detected in May, giving the suspicion that this population extends its reproductive cycle beyond the currently established closed season. The harsher hydrodynamic and higher speed wind, typical conditions of the north coast of Madeira, induce spawning (Orton et al., 1956). It is also very likely the extension of the breeding season, when compared to the populations inhabiting the south coasts of the archipelago. Though the environmental conditions are the same for *P. aspera*, after the closed season the largest individuals are the target of exploitation leading to a reduction of females, as a fraction of males changes to females after reaching sexual maturation. Hence, the removal of larger animals will largely target females (Martins et al., 2017) that may still be spawning, greatly affecting the reproductive potential of this species. Regarding southern populations (Supplementary Figure 2), as only the months of October and March are available, it is difficult to infer trends throughout the spawning season. However, for both limpets, there was an expected decrease in the number of PO and a consequent increase in the number of VO, though with no significant increase in their average size.

The estimated fecundity (354,572 ± 189,883) was similar to that previously obtained for *P. ordinaria* in the same area (average of 385,613 194,902 oocytes; 46 - 59 mm SL Vasconcelos et al., 2023) but much higher than the one estimated in an assay for gametes and larval production for *P. ordinaria* aquaculture which averaged 186,000 oocytes (37 - 57 mm SL, Castejón et al., 2022). The fecundity in *P. aspera* females was on average 77,404 ± 43,910 oocytes, very similar to the one estimated in the same assay for *P. aspera* aquaculture which averaged 59,000 oocytes (34 - 53 mm SL, Castejón et al., 2022). Although the size range is similar for both species, the lower average size of *P. aspera* explains, in part, the clear differences in batch fecundity with *P. ordinaria*, as fecundity increases hyperallometrically with size. Moreover, the size-selective harvesting targets mainly females of the protandric hermaphrodite species *P. aspera*. These differences are of particular concern and should be taken in consideration when creating possible new management measures for limpet harvesting. *P. ferruginea*, currently considered at risk of extinction (Guallart et al., 2020), shows greater fecundity for shell sizes smaller than those sampled in the present work. For instance, while female fecundity of *P. ferruginea* with 40.0 mm SL is around 189,200 oocytes (Chafarinas Islands; Guallart et al., 2020), *P. ordinaria* from Madeira presents around 80,006 (present study) to 92,098 oocytes (Vasconcelos et al., 2023) in females with 46 mm SL. For *P. ordinaria*, Castejon et al. (2022) estimated an even lower fecundity for specimens with 37 mm SL, which was around 12,000 oocytes. When considering the maximum sizes analyzed, *P. vulgata* from the SW of England (500,000 eggs with 52 mm SL; Ballantine, 1961), and *Cellana ornata* from southern New Zealand (200,000 to 360,000 eggs for females > 40 mm SL; Dunmore and Schiel, 2000), showed fecundities lower than those estimated for *P. ordinaria*, but still higher than the ones verified for *P. aspera* (177,202 oocytes for 53.38 mm SL). For females of *P. ferruginea* with sizes higher than 80 mm SL, the number of oocytes ranged from 2.3 to 5.0 million eggs (Espinosa et al., 2006; Guallart et al., 2020). Since individuals of *P. ordinaria* and *P. aspera* larger than 60 mm SL are more common in the marine protected areas (MPAs) of the Madeira archipelago (with maximum shell lengths of 79.63 mm SL for *P. ordinaria* and 84.22 mm SL for *P. aspera*; Sousa et al., 2020a), and that all specimens used were captured in areas of considerable anthropogenic exploitation (capture only ceases during the closed season between November and March), we could not access or find evidence that these species can attain a level of fecundity as high as that reported for *P. ferruginea*. Moreover, as egg production is sensitive to selective pressures (Ramirez Llodra, 2002), this may explain the lower levels observed especially for *P. aspera*, the most popular limpet species consumed by the Madeiran population. Like the taxa *P. ferruginea* (Espinosa et al., 2006; Guallart et al., 2020), *Cellana ornata* (Dunmore and Schiel, 2000) and *C. sandwicensis* (Mau et al., 2018), *P. ordinaria* from Madeira exhibits size-dependent fecundity. Whilst this relationship was not that clear for *P. aspera* (present study and Castejón et al., 2022), limpets at MPAs may exhibit higher batch fecundity.

Fecundity is one of the major cornerstones of population biology (Bradshaw and McMahon, 2008). Temporal variations in fecundity are influenced by a wide range of factors such as, age, body size relationships, population density, mate choice, and environmental variability. Reproductive and fecundity data have traditionally been overlooked in fisheries management regardless of their importance to leverage the reproductive potential of large adult fish (Marshall et al., 2021). The integration of size-fecundity relationships into stock assessment models have been shown to be of utmost importance to achieve sustainable management goals, and their potential to establish spatio-temporal closures (STCs) and harvest slots (HSs) (Marshall et al., 2021). These limpets have allometric growth (Henriques et al., 2012; Sousa et al., 2017); hence, identifying and protecting size-age groups with maximum reproductive potential has been previously shown as a pivotal step to halt overfishing through managing growth of individuals and recruitment of stocks (Brown-Peterson et al., 2011). In the present study, the four criteria analyzed suggest that both exploited limpets may have determinate fecundity. It also showed the presence of spawning adults beyond the closed season (November-March), during the months of April and May. Furthermore, a spatial variability was observed between northern and southern populations, where the former ones showed vitellogenic oocytes till May in *P. ordinaria* whilst *P. aspera* showed a size increase of the vitellogenic oocytes from November to May. This means that some adults may be still spawning in May for both species, and hence to get an effective conservation of adult specimens and to ensure offspring of next generations, a more prolonged closed season till May is urgently needed. As evidenced by this study, fecundity data constitute a tool of key importance to preserve populations of exploited species, primarily those with limited mobility such as semi-sessile limpets.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JV: Conceptualization, data acquisition, data curation, methodology, formal analysis, validation, critical analysis, resources, project administration, supervision, writing—original draft preparation, and writing—review and editing. RS: biological sampling, methodology, validation, critical analysis and revision of the paper. JF: data acquisition, data curation and interpretation. AP: biological sampling, validation. MF: resources, revision of the paper. RR: data interpretation, critical analysis, project administration, supervision, writing—original draft preparation, writing—review and editing. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the Portuguese national funds through Fundação para a Ciência e a Tecnologia I.P. (FCT) within the project “Metacommunity dynamics of complex life cycle species in exploited ecosystems” (LAPACOM) grant number EXPL/MAT-APL/0262/2021, MARE grant number UIDB/04292/2020, Associate Laboratory ARNET grant number LA/P/0069/2020, Interreg MAC within the project RASPA grant number MAC2/1.1a/305, the National Data Collection Framework, and the University of Las Palmas of Grancanaria (ULPGC). JV was supported by the ‘Grants for the Viera y Clavijo training program for researchers’ of the Government of the Canary Islands (VIERA Y CLAVIJO-2022-CIENCIAS-1).

Acknowledgments

We would like to express our gratitude to Dr. Manfred Kaufmann (University of Madeira) and Dr. Pedro Neves (OOM/ARDITI) and Dr. Cláudia Ribeiro (IFCN) for kindly facilitating their image analysis systems. We extend our gratitude to DRM, for providing all the facilities and material needed for the development of this work, and to the students José Mateus and Ricardo Mota, for having participated in the various stages of fecundity determination during their 1-month “Summer internships for University Students” funded by the Regional Youth Directorate of the Government of Madeira.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1173629/full#supplementary-material

References

Alós, J., Palmer, M., Catalan, I. A., Alonso-Fernández, A., Basterretxea, G., Jordi, A., et al. (2014). Selective exploitation of spatially structured coastal fish populations by recreational anglers may lead to evolutionary downsizing of adults. *Mar. Ecol. Prog. Ser.* 503, 219–233. doi: 10.3354/meps10745

Ballantine, W. J. (1961). *The population dynamics of patella vulgata and other limpets* (Queen Mary University of London).

Barneche, D. R., Robertson, D. R., White, C. R., and Marshall, D. J. (2018). Fish reproductive-energy output increases disproportionately with body size. *Science* 360, 642–644. doi: 10.1126/science.aao6868

Belkhodja, H., Jaafoura, M. H., Missaoui, H., and Romdhane, M. S. (2011). Histological investigation of the reproductive cycle of the limpet *Patella caerulea* linnaeu. *Cahiers biol. Mar.* 52, 279–290. doi: 10.21411/CBM.A.C8C14E76

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc.: Ser. B (Methodological)* 57, 289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x

Blowes, S. A., Chase, J. M., Di Franco, A., Frid, O., Gotelli, N. J., Guidetti, P., et al. (2020). Mediterranean Marine protected areas have higher biodiversity *via* increased evenness, not abundance. *J. Appl. Ecol.* 57, 578–589. doi: 10.1111/1365-2664.13549

Bradshaw, C., and McMahon, C. (2008). "Fecundity," in *Encyclopedia of ecology, five-volume set*.

Brown-Peterson, N. J., Wyanski, D. M., Saborido-Rey, F., Macewicz, B. J., and Lowerre-Barbieri, S. K. (2011). A standardized terminology for describing reproductive development in fishes. *Mar. Coast. Fish.* 3, 52–70. doi: 10.1080/19425120.2011.555724

Cañizares, J. M., Castejón, D., Haroun, R., Nogueira, N., and Andrade, C. A. (2021). Enhancing oocyte maturation and fertilisation in the black-foot limpet *Patella candei* d' Orbigny 1840 (patellidae, mollusca). *Aquacul. Rep.* 21, 100856. doi: 10.1016/j.aqrep.2021.100856

Castejón, D., García, L., Cañizares, J. M., De Girolamo, M., Nunes, C., Isidro, E., et al. (2022). Methodologies for patellid limpets' aquaculture: from broodstock management to juveniles. *Front. Mar. Sci.* 9, 884262. doi: 10.3389/fmars.2022.884262

Coleman, M., Bates, A., Stuart-Smith, R., Malcolm, H., Harasti, D., Jordan, A., et al. (2015). Functional traits reveal early responses in marine reserves following protection from fishing. *Diversity Distrib.* 21, 876–887. doi: 10.1111/ddi.12309

Côrte-Real, H., Hawkins, S., and Thorpe, J. (1996). An interpretation of the taxonomic relationship between the limpets *Patella rustica* and *P. piperata*. *J. Mar. Biol. Assoc. United Kingdom* 76, 717–732. doi: 10.1017/S0025315400031416

Davies, B. F., Holmes, L., Rees, A., Attrill, M. J., Cartwright, A. Y., and Sheehan, E. V. (2021). Ecosystem approach to fisheries management works–how switching from mobile to static fishing gear improves populations of fished and non-fished species inside a marine-protected area. *J. Appl. Ecol.* 58, 2463–2478. doi: 10.1111/1365-2664.13986

de Mitcheson, Y. J. S., Linardich, C., Barreiros, J. P., Ralph, G. M., Aguilar-Perera, A., Afonso, P., et al. (2020). Valuable but vulnerable: over-fishing and under-management continue to threaten groupers so what now? *Mar. Policy* 116, 103909. doi: 10.1016/j.marpol.2020.103909

Dubois, N., Saulnier-Talbot, É., Mills, K., Gell, P., Battarbee, R., Bennion, H., et al. (2018). First human impacts and responses of aquatic systems: a review of palaeolimnological records from around the world. *Anthropocene Rev.* 5, 28–68. doi: 10.1177/2053019617740365

Dudley, N., and Stolton, S. (2008). *Defining protected areas: an international conference in almeria, spain* (Gland: IUCN).

Dunmore, R., and Schiel, D. (2000). Reproduction in the intertidal limpet *Cellana ornata* in southern new zealand. *New Z. J. Mar. Freshw. Res.* 34, 653–660. doi: 10.1080/00288330.2000.9516966

Ellis, S. L., and Cowan, D. F. (2001). Volunteer-based monitoring of juvenile american lobster, *Homarus americanus*. *Mar. Freshw. Res.* 52, 1103–1112. doi: 10.1071/MF01194

Ellis, E. C., Gauthier, N., Klein Goldewijk, K., Bliege Bird, R., Boivin, N., Díaz, S., et al. (2021). People have shaped most of terrestrial nature for at least 12,000 years. *Proc. Natl. Acad. Sci.* 118, e2023483118. doi: 10.1073/pnas.2023483118

Espinosa, F., Guerra-Garcia, J. M., FA, D., and Garcia-Gomez, J. C. (2006). Aspects of reproduction and their implications for the conservation of the endangered limpet, *Patella ferruginea*. *Invertebrate Reprod. Dev.* 49, 85–92. doi: 10.1080/07924259.2006.9652197

Espinosa, F., Rivera-Ingraham, G., and García-Gómez, J. C. (2009). Gonochorism or protandrous hermaphroditism? evidence of sex change in the endangered limpet *Patella ferruginea*. *Mar. Biodiversity Records* 2, e153. doi: 10.1017/S1755267209990790

Geldmann, J., Barnes, M., Coad, L., Craigie, I. D., Hockings, M., and Burgess, N. D. (2013). Effectiveness of terrestrial protected areas in reducing habitat loss and population declines. *Biol. Conserv.* 161, 230–238. doi: 10.1016/j.biocon.2013.02.018

Greer Walker, M., Witthames, P., and Bautista De Los Santos, I. (1994). Is the fecundity of the atlantic mackerel (*Scomber scombrus*: scombridae) determinate? *Sarsia* 79, 13–26. doi: 10.1080/00364827.1994.10413543

Guallart, J., Peña, J. B., Pérez-Larruscaín, J., Luque, Á.A., and Templado, J. (2020). Filling gaps: closing the life cycle of the endangered mediterranean limpet patella ferruginea gmelin 1791 (gastropoda, patellidae). *Mediterr. Mar. Sci.* 21, 400–419. doi: 10.12681/mms.22508

Guilhaumon, F., Albouy, C., Claudet, J., Velez, L., Ben Rais Lasram, F., Tomasini, J.-A., et al. (2015). Representing taxonomic, phylogenetic and functional diversity: new challenges for mediterranean marine-protected areas. *Diversity Distrib.* 21, 175–187. doi: 10.1111/ddi.12280

Gwinn, D. C., Allen, M. S., Johnston, F. D., Brown, P., Todd, C. R., and Arlinghaus, R. (2015). Rethinking length-based fisheries regulations: the value of protecting old and large fish with harvest slots. *Fish Fish.* 16, 259–281. doi: 10.1111/faf.12053

Hackradt, C. W., García-Charton, J. A., Harmelin-Vivien, M., Perez-Ruzafa, A., Le Direach, L., Bayle-Sempere, J., et al. (2014). Response of rocky reef top predators (serranidae: epinephelinae) in and around marine protected areas in the western mediterranean sea. *PloS One* 9, e98206. doi: 10.1371/journal.pone.0098206

Henriques, P., Delgado, J., Sousa, R., and Ray, S. (2017). Patellid limpets: an overview of the biology and conservation of keystone species of the rocky shores. *Organismal Mol. Malacol.*, 71–95. doi: 10.5772/67862

Henriques, P., Sousa, R., Pinto, A., Delgado, J., Faria, G., Alves, A., et al. (2012). Life history traits of the exploited limpet *Patella candei* (mollusca: patellogastropoda) of the north-eastern atlantic. *J. Mar. Biol. Assoc. United Kingdom* 92, 1379–1387. doi: 10.1017/S0025315411001068

Hixon, M. A., Johnson, D. W., and Sogard, S. M. (2014). Boffffs: on the importance of conserving old-growth age structure in fishery populations. *ICES J. Mar. Sci.* 71, 2171–2185. doi: 10.1093/icesjms/fst200

Hunter, J. R. (1992). Fecundity, spawning, and maturity of female dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fish. Bull.(Wash. DC)* 90, 101–128.

Hunter, J. R., and Macewicz, B. J. (1985). Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax*. *Fishery Bull.* 83, 119–136.

Hunter, J., Macewicz, B. J., and Kimbrell, C. A. (1989). Fecundity and other aspects of the reproduction of sablefish, *Anoplopoma fimbria*, in central california waters. *CalCOFI Rep.* 30, 61–72.

Jones, K. R., Klein, C. J., Halpern, B. S., Venter, O., Grantham, H., Kuempel, C. D., et al. (2018). The location and protection status of earth's diminishing marine wilderness. *Curr. Biol.* 28, 2506–2512. doi: 10.1016/j.cub.2018.06.010

Kassambara, A. (2022a). *Ggpubr: 'ggplot2' based publication ready plots*.

Kassambara, A. (2022b). *Rstatix: pipe-friendly framework for basic statistical tests*.

Lambert, Y. (2008). Why should we closely monitor fecundity in marine fish populations? *J. Northwest Atlantic fishery Sci.* 41, 93–106. doi: 10.2960/J.v41.m628

Lavin, C. P., Jones, G. P., Williamson, D. H., and Harrison, H. B. (2021). Minimum size limits and the reproductive value of numerous, young, mature female fish. *Proc. R. Soc. B* 288, 20202714. doi: 10.1098/rspb.2020.2714

Liu, O. R., Thomas, L. R., Clemence, M., Fujita, R., Kritzer, J. P., McDonald, G., et al. (2016). An evaluation of harvest control methods for fishery management. *Rev. Fish. Sci. Aquacul.* 24, 244–263. doi: 10.1080/23308249.2016.1161002

Marshall, D. J., Bode, M., Mangel, M., Arlinghaus, R., and Dick, E. (2021). Reproductive hyperallometry and managing the world's fisheries. *Proc. Natl. Acad. Sci.* 118, e2100695118. doi: 10.1073/pnas.2100695118

Martins, G. M., Borges, C. D., Vale, M., Ribeiro, P. A., Ferraz, R. R., Martins, H. R., et al. (2017). Exploitation promotes earlier sex change in a protandrous patellid limpet, *Patella aspera* rödin. *Ecol. Evol.* 7, 3616–3622. doi: 10.1002/ece3.2925

Mau, A., Bingham, J.-P., Soller, F., and Jha, R. (2018). Maturation, spawning, and larval development in captive yellowfoot limpets (*Cellana sandwicensis*). *Invertebrate Reprod. Dev.* 62, 239–247. doi: 10.1080/07924259.2018.1505670

McCarthy, M., Woosnam, P., and Culloty, S. (2008). Histological investigation of the reproductive cycles of the limpets *Patella vulgata* and *Patella ulyssiponensis*. *Mar. Biol.* 153, 871–877. doi: 10.1007/s00227-007-0859-x

Murphy, E., Rodhouse, P., and Nolan, C. (1994). Modelling the selective effects of fishing on reproductive potential and population structure of squid. *ICES J. Mar. Sci.* 51, 299–313. doi: 10.1006/jmsc.1994.1031

Murua, H., Kraus, G., Saborido-Rey, F., Witthames, P., Thorsen, A., and Junquera, S. (2003). Procedures to estimate fecundity of marine fish species from field samples in relation to reproductive strategy. *J. Northwest Atlantic fishery Sci.* 33, 33–54. doi: 10.2960/J.v33.a3

Neves, A., Sequeira, V., Vieira, A. R., Silva, E., Silva F, D. A., Mendes, S., et al. (2022). Reproduction of the blue jack mackerel, *Trachurus picturatus*, in western portugal: microscopic gonad analysis reveals indeterminate fecundity and skipped spawning patterns. *Zool. Stud.* 61, 41. doi: 10.6620/ZS.2022.61-41

Orton, J., Southward, A., and Dodd, J. (1956). Studies on the biology of limpets: ii. the breeding of *Patella vulgata* l. in britain. *J. Mar. Biol. Assoc. United Kingdom* 35, 149–176. doi: 10.1017/S0025315400009036

Pacifici, M., Di Marco, M., and Watson, J. E. (2020). Protected areas are now the last strongholds for many imperiled mammal species. *Conserv. Lett.* 13, e12748. doi: 10.1111/conl.12748

Pellowe, K. E., and Leslie, H. M. (2020). Size-selective fishing leads to trade-offs between fishery productivity and reproductive capacity. *Ecosphere* 11, e03071. doi: 10.1002/ecs2.3071

Pinto, A. R., Alves, A., Faria, G., Reis, R., and Delgado, J. (2010). "Reproductive biology of *Patella candei* d'Orbigny 1840 in madeira archipelago," in *XVI Simposio Iberico de Estudios de Biologia Marina* (Alicante: Departamento de Ciencias del Mar y Biología Aplicada de la Universidad de Alicante).

Prusina, I., Ezgeta-Balić, D., Ljubimir, S., Dobroslavić, T., and Glamuzina, B. (2014). On the reproduction of the mediterranean keystone limpet *Patella rustica*: histological overview. *J. Mar. Biol. Assoc. United Kingdom* 94, 1651–1660. doi: 10.1017/S0025315414000976

Ramirez Llodra, E. (2002). Fecundity and life-history strategies in marine invertebrates. *Adv. Mar. Biol.* 43, 87—–170. doi: 10.1016/S0065-2881(02)43004-0

R Core Team (2022). *R: a language and environment for statistical computing* (Vienna, Austria: R Foundation for Statistical Computing).

Riera, R., Pérez, Ó., Álvarez, O., Simón, D., Díaz, D., Monterroso, Ó., et al. (2016). Clear regression of harvested intertidal mollusks. a 20-year, (1994–2014) comparative study. *Mar. Environ. Res.* 113, 56–61. doi: 10.1016/j.marenvres.2015.11.003

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). Nih image to imagej: 25 years of image analysis. *Nat. Methods* 9, 671–675. doi: 10.1038/nmeth.2089

Sousa, R., Delgado, J., Pinto, A. R., and Henriques, P. (2017). Growth and reproduction of the north-eastern atlantic keystone species *Patella aspera* (mollusca: patellogastropoda). *Helgoland Mar. Res.* 71, 1–13. doi: 10.1186/s10152-017-0488-9

Sousa, R., Henriques, P., Vasconcelos, J., Pinto, A. R., Delgado, J., and Riera, R. (2020a). The protection effects of marine protected areas on exploited molluscs from an oceanic archipelago. *Aquat. Conservat.: Mar. Freshw. Ecosyst.* 30, 717–729. doi: 10.1002/aqc.3285

Sousa, R., Riera, R., Vasconcelos, J., Gouveia, L., Pinto, A. R., Delgado, J., et al. (2020b). *Artisanal harvest of shellfish in the northeastern atlantic: the example of limpet and topshell fisheries in the archipelago of Madeira*. In Ray, S., Diarte-Plata, G., and Escamilla-Montes, R. *Invertebrates: ecophysiology and management* (Rijeka: IntechOpen), 147–164.

Sousa, R., Vasconcelos, J., Henriques, P., Pinto, A. R., Delgado, J., and Riera, R. (2019a). Long-term population status of two harvested intertidal grazers (*Patella aspera* and *Patella candei*), before, (1996–2006) and after, (2007–2017) the implementation of management measures. *J. Sea Res.* 144, 33–38. doi: 10.1016/j.seares.2018.11.002

Sousa, R., Vasconcelos, J., Riera, R., Pinto, A. R., Delgado, J., and Henriques, P. (2019b). Potential impact of harvesting management measures on the reproductive parameters of the limpets *Patella aspera* and *Patella candei* from madeira island. *Estuar. Coast. Shelf Sci.* 226, 106264. doi: 10.1016/j.ecss.2019.106264

Steffen, W., Broadgate, W., Deutsch, L., Gaffney, O., and Ludwig, C. (2015). The trajectory of the anthropocene: the great acceleration. *Anthropocene Rev.* 2, 81–98. doi: 10.1177/2053019614564785

Tomczak, M., and Tomczak, E. (2014). The need to report effect size estimates revisited. an overview of some recommended measures of effect size. *Trends Sport Sci.* 21, 19–25.

Vasconcelos, J., Faria, G., Freitas, R., and Gordo, L. S. (2017). Fecundity regulation strategy of the blue jack mackerel, *Trachurus picturatus* (bowdich 1825), off madeira island (ne atlantic). *Fish. Res.* 190, 150–156. doi: 10.1016/j.fishres.2017.02.009

Vasconcelos, J., Sousa, R., Ferro, J., Pinto, A. R., and Riera, R. (2023). Fecundity strategy of the highly exploited limpet *Patella ordinaria*, from an oceanic archipelago. *Reg. Stud. Mar. Sci.* 60, 102849. doi: 10.1016/j.rsma.2023.102849

Vat, L. S. (2000). *The growth and reproduction of patella granularis (Mollusca: patellogastropoda) on the south-east coast of south Africa* (Rhodes University).

Venter, O., Sanderson, E. W., Magrach, A., Allan, J. R., Beher, J., Jones, K. R., et al. (2016). Sixteen years of change in the global terrestrial human footprint and implications for biodiversity conservation. *Nat. Commun.* 7, 1–11. doi: 10.1038/ncomms12558

Walsh, M. R., Munch, S. B., Chiba, S., and Conover, D. O. (2006). Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. *Ecol. Lett.* 9, 142–148. doi: 10.1111/j.1461-0248.2005.00858.x

Ward, D., Melbourne-Thomas, J., Pecl, G. T., Evans, K. ,. M., McCormack, P. C., et al. (2022). Safeguarding marine life: conservation of biodiversity and ecosystems. *Rev. Fish Biol. Fish.* 32, 65–100. doi: 10.1007/s11160-022-09700-3

Worm, B., Hilborn, R., Baum, J. K., Branch, T. A., Collie, J. S., Costello, C., et al. (2009). Rebuilding global fisheries. *Science* 325, 578–585. doi: 10.1126/science.1173146

Wright, P. J., and Trippel, E. A. (2009). Fishery-induced demographic changes in the timing of spawning: consequences for reproductive success. *Fish Fish.* 10, 283–304. doi: 10.1111/j.1467-2979.2008.00322.x

frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 29 May 2023
DOI 10.3389/fmars.2023.1154755

Check for updates

OPEN ACCESS

EDITED BY
Tomoyuki Nakano,
Kyoto University, Japan

REVIEWED BY
Douglas Eernisse,
California State University, Fullerton,
United States
Lijun He,
East China Normal University, China

*CORRESPONDENCE
Claudio A. González-Wevar
claudio.gonzalez@uach.cl

RECEIVED 31 January 2023
ACCEPTED 08 May 2023
PUBLISHED 29 May 2023

CITATION
González-Wevar CA, de Aranzamendi MC, Segovia NI, Rosenfeld S, Maturana CS, Molina CR, Brickle P, Gardenal CN, Bastida R and Poulin E (2023) Genetic footprints of Quaternary glacial cycles over the patterns of population diversity and structure in three *Nacella* (Patellogastropoda: Nacellidae) species across the Magellan province in southern South America.
Front. Mar. Sci. 10:1154755.
doi: 10.3389/fmars.2023.1154755

Genetic footprints of Quaternary glacial cycles over the patterns of population diversity and structure in three *Nacella* (Patellogastropoda: Nacellidae) species across the Magellan province in southern South America

Claudio A. González-Wevar[1,2,3*], María Carla de Aranzamendi[4,5], Nicolás I. Segovia[2,6,7], Sebastián Rosenfeld[2,8,9,10], Claudia S. Maturana[2,9], Cristian Ríos Molina[1,2], Paul Brickle[11,12], Cristina N. Gardenal[5], Ricardo Bastida[13] and Elie Poulin[2,14]

[1]Instituto de Ciencias Marinas y Limnológicas (ICML), Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile, [2]Instituto Milenio Biodiversidad de Ecosistemas Antárticos y Subantárticos (BASE), Ñuñoa, Santiago, Chile, [3]Centro Fondap de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Universidad Austral de Chile, Valdivia, Chile, [4]Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Cátedra de Ecología Marina, Córdoba, Argentina, [5]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Diversidad y Ecología Animal (IDEA), Ecosistemas Marinos y Polares (ECOMARES), Córdoba, Argentina, [6]Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile, [7]Instituto Milenio en Socio-ecología Costera (SECOS), Coquimbo, Chile, [8]Laboratorio de Ecosistemas Marinos Antárticos y Subantárticos, Universidad de Magallanes, Punta Arenas, Chile, [9]Cape Horn International Center (CHIC), Universidad de Magallanes, Punta Arenas, Chile, [10]Centro de Investigación Gaia-Antártica, Universidad de Magallanes, Punta Arenas, Chile, [11]South Atlantic Environmental Research Institute, Falkland Islands, Port Stanley, United Kingdom, [12]School of Biological Sciences (Zoology), University of Aberdeen, Aberdeen, United Kingdom, [13]Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales (FCEyN), Universidad Nacional de Mar del Plata (UNMdP)-CONICET, CC1260, Mar del Plata, Argentina, [14]Laboratorio de Ecología Molecular (LEM), Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

Quaternary glaciations severely altered landscape/seascape at high latitudes and had major consequences on species geographical ranges, population sizes, genetic differentiation and speciation rates. The Magellan province in southern South America, constitutes an interesting area to evaluate the effect of glaciations over near-shore marine benthic organisms. Existing data have showed clear signatures of population genetic bottlenecks during glacial maxima followed by recent recolonizations and expansions during the interglacial. Here, we present new population-based analyses in species of the patellogastropod genus *Nacella* (*N. deaurata*, *N. magellanica*, and *N. mytilina*). The species *N. magellanica* and *N. deaurata* inhabit rocky ecosystems while *N. mytilina* lives closely associated with kelps. The analyzed species exhibit narrow bathymetric ranges and consequently should have been severely affected by recurrent glacial cycles. We performed phylogeographic and demographic analyses in *Nacella* species including different localities across their respective distributions in the Magellan province including the Falkland/Malvinas Islands (F/M). Genetic analyses showed that all *Nacella* species exhibited low levels of genetic diversity, the presence of single dominant broadly distributed haplotypes, lack of correlation between geographical and genetic distance, and recent demographic growths, which are evidence supporting rapid postglacial expansions. Such results may have been facilitated by larval and/or rafting-mediated dispersal following the Cape Horn Current System. The three species showed strong and significant differentiation between F/M and southern Pacific margin (SPM) populations including localities across the Strait of Magellan and Cape Horn. Haplotype genealogies and mismatch analyses recognized older and more complex demographic histories in the F/M than in South America. Different glaciological histories between SPM and F/M may be responsible of the marked phylogeographic structure in the analyzed species of *Nacella*. Alternatively, as previously proposed, the F/M represent a glacial refugium for *Nacella* species (sink area), as well as a secondary contact zone where endemic haplotypes are found together with recently arrived South American ones. Historical and contemporary processes, contrasting glacial histories between the analyzed areas, as well as life history traits of the analyzed organisms are main factors explaining current phylogeographic patterns of shallow Magellan marine benthic organisms.

KEYWORDS

quaternary, last glacial maximum, population genetics, phylogeography, Nacella, Magellan Province

Introduction

Ice advances and retreats during the glacial episodes of the Quaternary, and particularly the Last Glacial Maximum (LGM) around 20,000 years ago, generated major landscape/seascape shifts and drastic reduction of available habitats at higher latitudes (Hewitt, 2000; Hewitt, 2004; Fraser et al., 2012). Concomitantly, glacial events affected the distribution and the geographical ranges of species, as well as the demography and populations sizes (Provan and Bennett, 2008; Marko et al., 2010; Allcock and Strugnell, 2012; González-Wevar et al., 2012; González-Wevar et al., 2013; Riesgo et al., 2015; González-Wevar et al., 2018; González-Wevar et al., 2021). Paleontological and palynological evidence from the Northern Hemisphere together with biogeographic and population-based studies provided the empirical basis for the Expansion-Contraction (EC) model of Quaternary biogeography proposed by Provan and Bennett (2008); a relative simple but useful model to describe the geographical response of population and species to past glacial and interglacial periods, which allows one to test demographic and phylogeographic hypotheses (Bennett et al., 1991; Webb and Bartlein, 1992; Williams et al., 1998; Hewitt, 2004; Maggs et al., 2008; Allcock and Strugnell, 2012).

Under a basic EC model, species contracted their distributions into lower latitude glacial refugia throughout glacial maxima while, during interglacials, they expanded their distributions to areas that were previously glaciated (Provan and Bennett, 2008; Marko et al., 2010; Fraser et al., 2012; González-Wevar et al., 2012; González-Wevar et al., 2018; González-Wevar et al., 2021). Accordingly, lower latitude non-glaciated refugial areas are expected to harbor older populations with higher levels of genetic diversity compared to those areas that were heavily impacted by ice and/or recently recolonized ones that are associated with recent postglacial demographic expansions (Marko, 2004; Maggs et al., 2008; Marko et al., 2010; González-Wevar et al., 2013; Fernández Iriarte et al., 2020).

During the LGM, the Pacific margin of the Magellan province in southern South America, from Chiloe Island (42°S) to Cape Horn (56°S), was almost completely covered by the Patagonian Ice Sheet (McCulloch et al., 2000; Hulton et al., 2002; Hein et al., 2010; Rabassa et al., 2011). The availability of shallow sheltered hard-substrate habitats was limited or even absent and hence prevented the survival of most of the near-shore marine species in this Magellan area. During interglacial periods, formerly ice-impacted areas were recolonized following the deglaciation process through dispersal from refugial populations. Recurring rounds of extinctions and recolonization may have enhanced the diversification of near-shore marine invertebrates across this province by the availability of vacant habitats and/or through the survival in multiple refugia which, allowed allopatric divergence (Valdovinos et al., 2003; Kiel and Nielsen, 2010; González-Wevar et al., 2011; González-Wevar et al., 2017). Contrarily to the glacial history recorded across the Pacific Magellan margin, glacial periods of the Quaternary would have affected to a much lesser extent the marine ecosystems across the Atlantic margin of this province (Glasser and Jansson, 2008) and the Falkland/Malvinas Islands (Clapperton and Sugden, 1976; Wilson et al., 2002; Wilson et al., 2008).

Several phylogeographic and population-based studies have been performed in distinct groups of Magellan near-shore marine (Fraser et al., 2010; de Aranzamendi et al., 2011; Ceballos et al., 2012; González-Wevar et al., 2012; de Aranzamendi et al., 2014; Nuñez et al., 2015; Ceballos et al., 2016; González-Wevar et al., 2016a; González-Wevar et al., 2018; Fernández Iriarte et al., 2020) and freshwater (Ruzzante et al., 2006; Zemlak et al., 2008; González-Wevar et al., 2015a; González-Wevar et al., 2015b) organisms. Studies in marine organisms have determined that most of the analyzed taxa exhibited low levels of genetic diversity, absence of genetic structure and strong signals of recent demographic growth, supporting the hypothesis of recent postglacial expansions (Fraser et al., 2010; Macaya and Zuccarello, 2010; de Aranzamendi et al., 2011; González-Wevar et al., 2012; de Aranzamendi et al., 2014; González-Wevar et al., 2017; Pardo-Gandarillas et al., 2018). Such studies have provided evidence of Quaternary glacial refugia on the East side of the Andes, along the Patagonia steppe (Ruzzante et al., 2006; Zemlak et al., 2008), refugia on the West side of the Andes both within (Xu et al., 2009; Nuñez et al., 2011), and outside the limit of the glaciers (Ruzzante et al., 2006; González-Wevar et al., 2015a; González-Wevar et al., 2015b). Moreover, species with high dispersive potential such as the buoyant macroalgae *Durvillaea antarctica* and *Macrocystis pyrifera* recolonized the Magellan province following the LGM from geographically distant regions (Macaya and Zuccarello, 2010; Fraser et al., 2012). These studies demonstrate the key role of long-distance dispersal mediated by rafting in the biogeography of sub-Antarctic marine near-shore benthic organisms (Waters, 2008; Fraser et al., 2009; Leese et al., 2010; Nikula et al., 2010; Moon et al., 2017; González-Wevar et al., 2018; González-Wevar et al., 2021). Even when many near-shore marine species are broadly distributed across the whole Magellan province, few genetic studies have been conducted across both the Pacific (PM) and the Atlantic (AM) margins of southern South America (Ceballos et al., 2016; González-Wevar et al., 2012; Trovant et al., 2015; González-Wevar et al., 2016a; González-Wevar et al., 2017; Fernández Iriarte et al., 2020). Moreover, just a couple of population-based studies have included populations from the Falkland/Malvinas Islands (F/M) (González-Wevar et al., 2012; González-Wevar et al., 2016a; González-Wevar et al., 2017; Fernández Iriarte et al., 2020; González-Wevar et al., 2021).

True limpets of the genus *Nacella* (Patellogastropoda: Nacellidae) are dominant invertebrates of Antarctic and sub-Antarctic intertidal and subtidal rocky ecosystems. *Nacella* species are gonochoric with external fertilization with a larval lifespan that can extend for more than two months (Bowden et al., 2009; Peck et al., 2016). Currently, the genus includes 12 nominal species that are distributed in different provinces of the Southern Ocean such as maritime Antarctica, southern South America and sub-Antarctic islands (South Georgia, Marion, Kerguelen, Heard, Macquarie and Campbell) (Powell, 1973; Valdovinos and Rüth, 2005; González-Wevar et al., 2019). Biogeographical analyses in *Nacella* identified the role of recent long-distance dispersal processes associated with the colonization of geographically isolated sub-Antarctic islands (González-Wevar et al., 2016b; González-Wevar et al., 2017). Moreover, the evolution of *Nacella* includes a recent Quaternary radiation in the Magellan province where four different species (*N. deaurata*, *N. flammea*, *N. magellanica* and *N. mytilina*) diversified (González-Wevar et al., 2011; González-Wevar et al., 2017, González-Wevar et al., 2019). The species *N. magellanica* exhibits the broadest distribution with populations along PM between Chiloé Island (42°S) and Diego Ramírez Archipelago (56°S) (González-Wevar et al., 2019). In AM *N. magellanica* is found from the Río Negro Province (41°S) to Isla de los Estados (55°S) and is also abundant in F/M (González-Wevar et al., 2019). The species *N. deaurata* and *N. mytilina* exhibit much narrower distributions from Guarello Island (50°S) to Diego Ramírez Archipelago (56°S) in PM while in AM they are mainly restricted to Tierra del Fuego and F/M (González-Wevar et al., 2019). Population-based studies in *N. magellanica* recorded a single genetic unit across PM (González-Wevar et al., 2012) and low levels of genetic structure across 2900 km of coast in AM (de Aranzamendi et al., 2014). Also, significant genetic differences have been identified among three main groups PM, AM, and F/M (González-Wevar et al., 2012). Similarly, the species *N. mytilina* showed absence of genetic differentiation across PM and strong phylogeographic signal between PM and F/M (González-Wevar et al., 2016a).

This study aims to evaluate the impacts of Quaternary glaciations over near-shore marine invertebrates across their distributions in southern South America using as model organisms three species of *Nacella* (*N. magellanica*, *N. mytilina*, and *N. deaurata*). For this, we performed phylogeographic and population-based analyses using fragments of the mitochondrial cytochrome c subunit I (COI) using previously published data sets in *N. magellanica* (de Aranzamendi et al., 2009; de Aranzamendi et al., 2011; González-Wevar et al., 2012; de Aranzamendi et al., 2014) and *N. mytilina* (González-Wevar et al., 2016a) and *N. deaurata* (de Aranzamendi et al., 2009; de Aranzamendi et al., 2011) as well new data for *N. magellanica* and *N. deaurata*. We hypothesize that Quaternary glacial cycles have differently impacted PM, AM and F/M populations of *N. magellanica* because the extent of habitat eradication would have varied in these areas. Populations across PM are expected to harbor genetic footprints of recent demographic expansions while non-glaciated areas of AM and F/M Islands are expected to show higher levels of genetic diversity because of more stable demographic histories. In those *Nacella* species with the narrowest geographical distributions (*N. mytilina* and *N. deaurata*), specifically those restricted to the southern tip of South America and F/M, we expect to find similar phylogeographic signatures; each should have a low level of genetic diversity structure in a southern South American population (SPM = Strait of Magellan and Cape Horn) and strong genetic structure between this and a population from F/M. Through this study we aim to further understand how key near-shore marine benthic elements survived Quaternary climate shifts in the southern tip of South America in areas that were differentially impacted by ice advances and retreats.

Material and methods

Samplings, DNA extraction and COI amplifications

Individuals of different species of *Nacella* (*N. magellanica, N. mytilina, and N. deaurata*) were collected from intertidal and subtidal rocky shore areas between 2010 and 2018 across the Magellan province in three main areas: the Pacific margin (PM), the Atlantic margin (AM) and the Falkland/Malvinas Islands (F/M) (Figure 1; Table 1). We analyzed a total of 18 localities of *N. magellanica* across the species' distribution including populations across PM, AM, and F/M. In the case of *Nacella* species with more restricted distributions such as *N. deaurata* and *N. mytilina* we analyzed 8 and 13 localities, respectively, across these species' distributions in PM including populations from the Strait of Magellan, Cape Horn, and F/M. *Nacella* specimens were identified following Powell, 1973, Valdovinos and Rüth (2005) and González-Wevar et al. (2011, 2019). All the individuals were fixed in ethanol (95%) and DNAs preparations were done using a standard salt-extraction methodology following Aljanabi and Martinez (1997). A partial fragment of the mitochondrial cytochrome c subunit I gene (COI) was amplified using universal primers LCO1490 and HCO2198 (Folmer et al., 1994) following

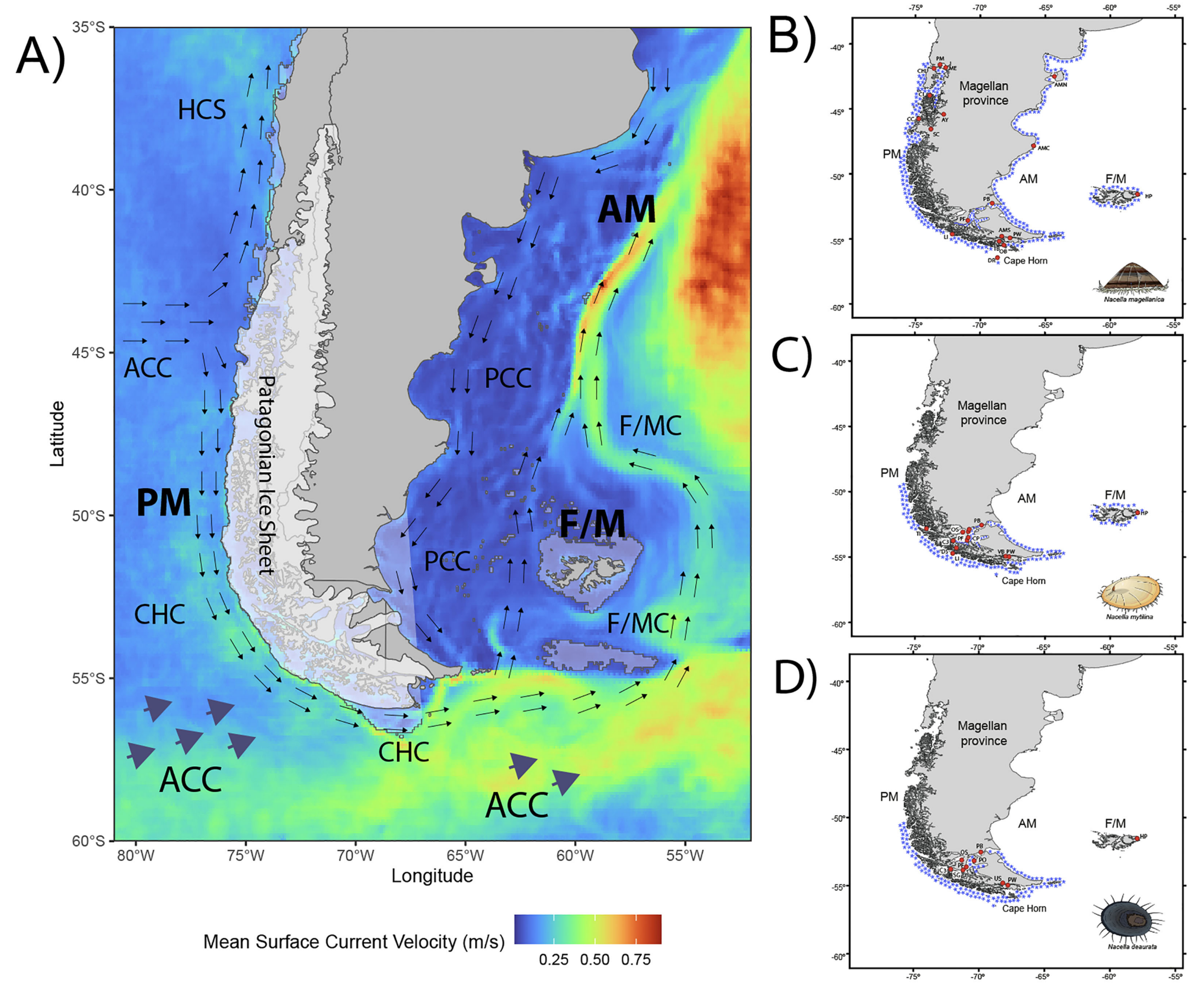


FIGURE 1
(A) The Magellan province in southern South America showing main oceanographic circulation patterns and directionality, the extension of continental ice and the position of the coastlines during the Last Glacial Maximum (LGM). Current world coastlines shapefile and DEM raster for LGM bathymetry simulation (-150 m) from Natural Earth (public domain). Sampling localities and distribution of **(B)** *Nacella magellanica*, **(C)** *Nacella mytilina* and **(D)** *Nacella deaurata* along the Pacific Magellan margin (PM), the Atlantic Magellan margin (AM) and the Falkland/Malvinas (F/M) Islands. Where: ACC, Antarctic Circumpolar Current; HCS, Humboldt Current System; CHC, Cape Horn Current; F/MC, Falkland/Malvinas Current; PCC, Patagonian Coastal Current. Sampling localities; PM, Puerto Montt; ME, Metri; CHI, Chiloé; CI, Concoto Island; AY, Aysén; CC, Costa Channel; SC, Serrano Channel; LI, London Island; PF, Port Famine; PB, Possession Bay; OB, Orange Bay; TB, Tekenika Bay; PW, Puerto Williams; DR, Diego Ramírez Island; AMN, Atlantic margin north; AMC, Atlantic margin center; AMS, Atlantic margin south; HP, Hookers Point; TI, Tamar Island; DS, Duntze Sound; C3, Carlos III Island; CP, Carrera Point; OS, Otway Sound; CH, Chabunco; LB, Laredo Bay; VB, Virginia Bay; PB, Paula Bay; SG, San Gregorio Bay; US, Ushuaia.

González-Wevar et al. (2010) and de Aranzamendi et al. (2009). PCR amplification products were purified and sequenced in both directions at Macrogen Inc. (Seoul, South Korea). Forward and reverse sequences were assembled and edited independently for each *Nacella* species using GENEIOUS (http://www.geneious.com). Multiple sequence alignments were performed using MUSCLE (Edgar, 2004). Nucleotide base compositions were calculated in MEGA 11 (Tamura et al., 2022). Wright's mitochondrial codon usage was computed using the Effective Number of Codons (ENC) in DnaSP v5 (Librado and Rozas, 2009). New *Nacella deaurata* COI sequences are available at GenBank under the following Accession Numbers: OQ587619 - OQ587817. Additionally, we included previously published sequences under the GenBank Accession Numbers: EU870927–EU870985, HQ880439–HQ880550; JX262742–JX262797 for *N. magellanica*; EU870986 – EU0870999; HQ880551–HQ880573 for *N. deaurata* and KX600474 - KX600492 for *N. mytilina* (de Aranzamendi et al., 2009; de Aranzamendi et al., 2011; González-Wevar et al., 2012; González-Wevar et al., 2016a).

Genetic diversity and population structure in Magellan *Nacella* species

We performed a DNA saturation analysis following Xia and Xie (2001) to evaluate how saturation of transitions accumulates in relation to nucleotide divergence in each *Nacella* species COI data set. Moreover, we estimated levels of population mtDNA polymorphism in the three species of *Nacella* through standard diversity indices including number of haplotypes (*k*), haplotype diversity (*H*), and the number of segregating sites (*S*). We also determined the average number of pairwise differences and the nucleotide diversity across the species distributions using DnaSP (Table 1).

We determined patterns of population differentiation for each *Nacella* species following Pons and Petit (1996) using haplotype frequencies (G_{ST}) and mean pairwise differences (N_{ST}) in Arlequin v.3 (Excoffier et al., 2005). The statistical significances of average genetic pairwise differences were calculated using permutation tests (20,000 iterations) and the adjustment for multiple testing was done through False Discovery Rate (FDR) correction (Narum, 2006). Moreover, for each *Nacella* species we estimated levels of genetic differentiation using the nearest-neighbor statistic (S_{nn}) which measures how often nearest neighbor (in sequence space) sequences are from the same locality in geographic space (Hudson, 2000). The statistical significance of S_{nn} was determined through permutation tests (20,000 iterations) for each *Nacella* species.

We used two different clustering methods to determine the spatial genetic structure of the analyzed *Nacella* species. First, we determined the number and the composition of panmictic groups and the spatial boundaries using a Bayesian model computed in GENELAND v.2.0.0 (Guillot et al., 2005) in the R environment (Ihaka and Gentleman, 1996). Through a Markov Chain Monte Carlo (MCMC) this analysis estimates the best clustering of samples considering genetic and geographic information. Geneland analyses were run using 50 x 10^6 MCMC iterations sampled each 1,000 steps. Assembled scores were graphed against generations in Tracer v.1.5. (Rambaut et al., 2018) to identify stationarity and the number of generations to be discarded as burn-in. A maximum number of clusters for each species (*N. magellanica* K = 19, *N. deaurata* K = 9 and *N. mytilina* K = 14) were run to estimate the model parameters and posterior probabilities of group membership. Second, we determined the spatial genetic structure for each *Nacella* species. We used Spatial Analysis of Molecular Variance (SAMOVA) (Dupanloup et al., 2002) to estimate the number and composition of groups that were most differentiated based on our sequence data

TABLE 1 Main patterns of genetic diversity and neutrality test in the recognized genetic clusters of A) *Nacella magellanica*, B) *Nacella mytilina* and C) *Nacella deaurata*.

N. magellanica	N	k	H	S	Π	π	Tajima's D	Fu's F_S
NPM	183	25	0.640	24	1.099	0.00174	-2.08*	-24.31***
SPM/AM	336	69	0.797	60	1.988	0.00316	-2.31**	-97.59***
F/M	46	19	0.802	26	3.779	0.00600	-1.20	-6.935***
TOTAL	565	97	0.779	79	2.331	0.00307	-2.31**	-146.19***
N. mytilina	N	k	H	S	Π	π	Tajima's D	Fu's F_S
SPM/AM	276	13	0.435	12	0.599	0.0008	-1.62*	-9.64*
F/M	24	8	0.822	19	4.18	0.0060	-0.64	0.68
TOTAL	300	19	0.481	26	0.996	0.0014	-2.11**	-13.49***
N. deaurata	N	k	H	S	Π	π	Tajima's D	Fu's F_S
SPM/AM	208	37	0.813	36	2.970	0.0043	-1.51	-22.324***
F/M	33	14	0.706	19	2.500	0.0036	-1.58	-6.156**
TOTAL	241	50	0.855	46	4.179	0.0060	-1.37	-30.54***

n, number of sampled specimens; k, number of haplotypes detected; S, polymorphic sites; H, haplotype diversity; Π, average number of nucleotide difference; π, nucleotide diversity *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Where: NPM, northern Pacific margin; SPM, southern Pacific margin (Strait of Magellan and Cape Horn); AM, Atlantic margin; and F/M, Falkland/Malvinas Islands.

set. This analysis allowed us to partition the genetic variance into i) within populations, ii) among populations within groups and iii) among groups.

Demographic analyses in Magellan *Nacella* species

Haplotype genealogical relationships in the analyzed *Nacella* species were reconstructed through median-joining and maximum parsimony networks in Network 10 (Forster et al., 2001) and Hapview (Salzburger et al., 2011), respectively. Moreover, we performed neutrality statistical tests (Tajima's D and Fu's FS) using DnaSP for the whole COI data set, for each species, to estimate whether sequences deviate from mutation-drift equilibrium. Population demographic histories were estimated comparing the distribution of pairwise differences between haplotypes (mismatch distribution) for each *Nacella* species under the sudden expansion growth model of Rogers and Harpending (1992). This analysis rests in the estimation of three parameters: i) τ = the date of growth/decline measured in units of mutational time ($\tau = 2\mu t$) where t = time in years and μ = mutational rate per sequence per year, ii) initial theta $\theta i = 2 Ni\mu$ before the population growth/decline) and iii) final theta $\theta t = 2 Nt\mu$ after population growth/decline. The demographic expansion parameters were estimated using the nonlinear least square approach implemented in Arlequin (Schneider and Excoffier, 1999). The goodness of fit between the observed and expected mismatch distributions was tested using a parametric bootstrap approach that uses the sum of squared deviations as a statistic test implemented in Arlequin. Moreover, we reconstructed past population dynamics through time using a Bayesian skyline plot method in BEAST (Drummond and Rambaut, 2007; Drummond et al., 2012). A strict-clock model was selected as the best fit for COI data in *Nacella* species and we used a specific population-level mutational rate (1% per million years) previously estimated for COI in Nacellidae limpets (González-Wevar et al., 2010; González-Wevar et al., 2012; González-Wevar et al., 2017). We performed independent Bayesian MCMC runs for each *Nacella* species recognized genetic groups using the GTR + G model, previously estimated using MrModeltest v.2.3 (http://www.abc-se/~nylander). Two independent runs for each *Nacella* species' groups were made for 250 x 10^6 generations (sampled every 10000 step), discarding a 10% of the trees as burn-in. The convergence of each run was determined using Tracer (Rambaut et al., 2018) ensuring a minimum of 1000 effective sampling for each statistic (ESS). The results of the multiple runs were combined using LogCombiner (Drummond and Rambaut, 2007). The median and corresponding credibility intervals of the Bayesian skylines plots were depicted with Tracer.

Gene flow and connectivity

We compared different models of gene flow between the recorded genetic groups (PM, AM, and F/M) in the analyzed *Nacella* species to test for distinctive scenarios using the software MIGRATE v.3.5 (Beerli and Felsenstein, 2001). We defined candidate models for each species constraining the directionality of gene flow between the recorded groups. The analyses were performed using a HKY + I + F substitution model and transition/transversion ratio of 10.56432 (*N. magellanica*), 6.4682 (*N. deaurata*) and 10.8857 (*N. mytilina*) as previously estimated by ModelFinder in the program IQ-TREE v.2.2.0 (Kalyaanamoorthy et al., 2017). The specific substitution rate for the selected marker was set to constant following the recommendation of the software developer. Analysis consisted of one long chain with 500,000 recorded parameter steps, a sampling interval of 100 and a burn-in of 10%, running multiplate replicates (10 independent chains). We used a heated scheme (1.0, 1.5, 3.0, 1000000.0) to calculate the marginal likelihoods for model comparisons. Moreover, we used a thermodynamic integration approximation (T.I.) for the log-equivalent Bayes Factor (LBF) considering that this analysis results in LBFs with high repeatability and little variation following Beerli and Palczewski (2010). Higher T.I. values are associated with a better fit of the model than lower ones. The associated probability of each model in relation to others was measured following Kaas and Raftery (1995).

Results

Genetic diversity in *Nacella* species

The COI data sets for each analyzed *Nacella* species are summarized in Table 1. No insertion/deletion or stop codons were found in the data sets and sequences were not saturated at any position. Population analyses in *Nacella* species included specimens of *N. deaurata* (N = 241), *N. magellanica* (N = 565) and *N. mytilina* (N = 300) (Figure 1; Table 2, Tables S1–S3). The COI set in *N. magellanica* included 630 nucleotide positions coding for 210 amino acids while the COI data sets in *N. deaurata* and *N. mytilina* included 687 positions coding for 229 amino acids. In *N. magellanica* we found two amino acids substitutions at positions 111 (V for I) and 201 (M for L) using the invertebrate mitochondrial table. In the case of *N. deaurata* we detected three amino acids substitutions at positions 79 (P to R), 186 (L to F), and 197 (M to T) while a single amino acid substitution was found in *N. mytilina* at position 182 (A to V). Medium levels of genetic diversity characterized populations of *N. magellanica* with 79 polymorphic sites (12.5%) and 44 of them (55.69%) were parsimoniously informative. Low levels of genetic diversity characterized populations of *N. deaurata*/*N. mytilina* with 46/26 polymorphic sites (6.69%/3.78%) and 25/16 of them (54.34%/61.53%) were parsimoniously informative, respectively. Sequences in Magellan *Nacella* species were A-T rich (60.8% in all the three species) compared to the main G – C content and no evidence of codon bias was detected (ENC *N. magellanica* = 35.89, ENC *N. mytilina* = 39.28, and ENC *N. deaurata* = 35.19).

The haplotype diversity (*H*) in *N. magellanica* varied between 0.863 (Atlantic North) and 0.370 (Costa Channel) (Table S1). The number of polymorphic sites (*S*) in *N. magellanica* varied between 5

TABLE 2 Pairwise G_{ST} values, based on haplotypic frequencies, (below diagonal) and average number of nucleotide differences between localities N_{ST} (above diagonal) in the analyzed *Nacella* species where A) *Nacella magellanica*, B) *Nacella deaurata* and C) *Nacella mytilina* 20200 iterations, significative values (FDR corrected) are marked in bold.

A)

Locality	ME	PM	CHI	CI	AYS	CC	SE	LI	PF	PB	OB	TB	PW	DR	AN	AC	AS	F/M
Metri (ME)	****	0.011	0.000	0.026	0.000	0.000	0.000	0.009	0.000	0.004	0.026	0.002	0.039	0.020	0.029	0.008	0.001	**0.524**
Puerto Montt (PM)	0.001	****	0.000	0.000	0.002	0.011	0.000	0.021	0.033	0.034	0.036	0.028	**0.060**	0.021	**0.066**	0.031	0.005	**0.554**
Chiloé (CHI)	0.000	0.005	****	0.013	0.000	0.000	0.000	0.000	0.000	0.005	0.013	0.000	0.033	0.004	0.029	0.000	0.000	**0.517**
Concoto Island (CI)	0.034	0.020	0.000	****	0.000	0.024	0.016	0.011	0.049	0.060	0.029	0.022	**0.061**	0.002	**0.075**	0.028	0.006	**0.531**
Aysen (AYS)	0.018	0.037	0.000	0.000	****	0.000	0.003	0.000	0.006	0.030	0.019	0.002	0.039	0.000	0.042	0.011	0.000	**0.536**
Costa Channel (CC)	**0.063**	**0.078**	0.008	0.000	0.000	****	0.001	0.001	0.000	0.014	0.024	0.005	0.039	0.005	0.031	0.007	0.000	**0.531**
Serrano Channel (SE)	0.000	0.000	0.000	0.014	0.008	**0.052**	****	0.012	0.006	0.000	0.031	0.013	0.037	0.026	0.033	0.008	0.000	**0.517**
London Island (LI)	0.029	0.026	0.001	0.000	0.008	0.035	0.018	****	0.000	0.039	0.000	0.000	0.012	0.000	0.040	0.002	0.000	**0.519**
Port Famine (PF)	0.000	0.000	0.000	0.040	0.028	**0.071**	0.000	0.017	****	0.008	0.025	0.000	0.005	0.019	0.024	0.012	0.000	**0.511**
Possession Bay (PB)	0.001	0.000	0.021	**0.063**	**0.069**	**0.116**	0.000	0.057	0.000	****	0.040	0.024	0.033	**0.053**	0.004	0.014	0.019	**0.507**
Orange Bay (OB)	0.009	0.008	0.014	**0.055**	**0.060**	**0.106**	0.009	0.019	0.004	0.009	****	0.000	0.006	0.000	0.023	0.000	0.001	**0.489**
Tekenika Bay (TB)	0.000	0.002	0.003	**0.048**	0.048	**0.096**	0.000	0.011	0.000	0.004	0.000	****	0.000	0.000	0.024	0.000	0.000	**0.497**
Puerto Williams (PW)	0.006	0.006	0.000	0.004	0.002	0.035	0.000	0.000	0.000	0.021	0.000	0.000	****	0.000	0.000	0.024	0.000	**0.472**
Diego Ramírez (DR)	0.0133	0.022	0.000	0.000	0.000	0.006	0.003	0.000	0.011	**0.049**	0.027	0.019	0.000	****	0.050	0.010	0.000	**0.519**
Atlantic North (AN)	**0.051**	**0.049**	**0.077**	**0.127**	**0.127**	**0.173**	**0.051**	**0.094**	**0.036**	0.022	0.020	0.028	**0.061**	**0.099**	****	0.003	0.034	**0.525**
Atlantic Center (AC)	0.017	0.019	0.031	**0.073**	**0.073**	**0.112**	0.017	0.049	0.011	0.005	0.000	0.000	0.022	**0.049**	0.029	****	0.010	**0.527**
Atlantic South (AS)	0.000	0.000	0.000	0.023	0.031	**0.071**	0.000	0.007	0.000	0.003	0.000	0.000	0.000	0.008	0.042	0.010	****	**0.495**
Falkland/Malvinas (F/M)	**0.178**	**0.162**	**0.217**	**0.268**	**0.277**	**0.328**	**0.181**	**0.226**	**0.165**	**0.151**	**0.148**	**0.146**	**0.198**	**0.238**	**0.139**	**0.141**	**0.157**	****

B)

Locality	T.I.	S.D.	L.I.	C.III	C.B.	S.A.	CH	L.B.	P.S.	V.B.	F/M
Tamar Island (TI)	****	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	**0.128**
Duntze Sound (SD)	0.000	****	0.000	0.017	0.001	0.000	0.015	0.028	0.005	0.000	**0.084**
London Island (LI)	0.000	0.057	****	0.000	0.000	0.000	0.030	0.000	0.000	0.000	**0.164**
Carlos III Island (C3)	0.000	0.057	0.000	****	0.000	0.000	0.063	0.000	0.000	0.000	**0.159**
Carrera Bay (CB)	0.000	0.001	0.000	0.000	****	0.000	0.052	0.000	0.000	0.000	**0.136**

(Continued)

TABLE 2 Continued

B)

Locality	T.I.	S.D.	L.I.	C.III	C.B.	S.A.	CH	L.B.	P.S.	V.B.	F/M
Santa Ana (SA)	0.000	0.000	0.000	0.000	0.000	****	0.052	0.000	0.000	0.000	**0.157**
Chabunco (CH)	0.009	0.000	0.092	0.093	0.044	0.036	****	0.060	0.065	0.058	**0.182**
Laredo Bay (LB)	0.000	0.000	0.005	0.000	0.000	0.000	0.037	****	0.000	0.000	**0.172**
Possession Bay (PS)	0.000	0.010	0.000	0.000	0.000	0.000	0.058	0.000	****	0.000	**0.147**
Virginia Bay (VB)	0.000	0.029	0.000	0.000	0.000	0.000	0.067	0.000	0.000	****	**0.160**
Falkland/Malvinas (F/M)	**0.149**	**0.049**	**0.251**	**0.235**	**0.162**	**0.158**	**0.123**	**0.146**	**0.173**	**0.202**	****

C)

Locality	C3	SG	PF	OS	PO	PW	US	F/M
Carlos III Island (C3)	****	0.000	0.000	0.000	0.000	0.000	0.000	**0.651**
San Gregorio (SG)	0.000	****	0.000	0.000	0.000	0.000	0.000	**0.656**
Port Famine (PF)	0.000	0.000	****	0.000	0.000	0.000	0.000	**0.677**
Otway Sound (OS)	0.005	0.016	0.000	****	0.000	0.000	0.000	**0.679**
Porvenir (PO)	0.000	0.000	0.000	0.000	****	0.000	0.000	**0.660**
Puerto Williams (PW)	0.000	0.000	0.019	0.029	0.000	****	0.000	**0.644**
Ushuaia (US)	0.000	0.000	0.000	0.000	0.000	0.001	****	**0.658**
Falkland/Malvinas (F/M)	**0.216**	**0.224**	**0.270**	**0.303**	**0.231**	**0.189**	**0.247**	****

(Costa Channel) and 27 (Atlantic Center). The average number of nucleotide differences (Π) and the mean nucleotide diversity (π) in *N. magellanica* were low in most of the localities across PM and AM while the diversity of these indices was comparatively higher in the F/M (Table S1). The haplotype diversity (*H*) in *N. deaurata* varied between 0.706 (Hookers Point, F/M) and 0.907 (Puerto Williams) (Table S2). The number of polymorphic sites (*S*) in *N. deaurata* varied between 10 (Ushuaia) and 22 (Puerto Williams). The average number of nucleotide differences and the mean nucleotide diversity (✓/π) in *N. deaurata* varied between 2.500/0.00364 (Hookers Point, F/M)) and 3.211/0.00467 (Puerto Williams) (Table S2). In the case of *N. mytilina* the haplotype diversity (*H*) varied between 0.284 (Carlos III Island) and 0.822 (Hookers Point, F/M) (Table S3). The number of polymorphic sites (*S*) in *N. mytilina* varied between 3 (several localities) and 19 (Hookers Point, F/M). Similarly, the number of haplotypes (k) in *N. mytilina* varied between 4 (several localities) and 8 (Hookers Point, F/M). The average number of nucleotide differences (✓) and the mean nucleotide diversity (π) in *N. mytilina* were low in most of the localities across the southern Pacific margin (Strait of Magellan and Cape Horn) while the diversity of these indices was comparatively higher in F/M (Table S3).

Genetic structure in *Nacella* species across the Magellan province

Mean general values of differentiation estimated in the analyzed *Nacella* species were low, especially considering average values of G_{ST} and N_{ST} (Table 2). Most part of the population-level differences were detected in the three species of *Nacella* when comparing F/M against the rest of the populations from South America. Nevertheless, *N. magellanica* also showed significant levels of differentiation between northern PM populations (NPM) and those from the Strait of Magellan, Cape Horn (SPM) and AM (Table 2). In the case of *N. deaurata* and *N. mytilina* general levels of differentiation across their distributions in the Strait of Magellan and Cape Horn were very low and pairwise populations comparisons did not reveal significant structure in these taxa across these areas. The nearest neighbor statistic (S_{nn}) in *Nacella* species (S_{nn} *N. magellanica* = 0.1656; S_{nn} *N. deaurata* = 0.236 and S_{nn} *N. mytilina* = 0.09) showed low but significant levels of phylogeographic signal ($p < 0.0001$) for the whole COI data set. However, when S_{nn} analyses were performed considering the main pattern of genetic differentiation recorded for each species, this statistic became very high (S_{nn} *N. magellanica* = 0.95, S_{nn} *N. deaurata* = 0.976 and S_{nn} *N. mytilina* = 0.9) showing the high degree of phylogeographic signal between South American and F/M populations in the three species of *Nacella* analyzed here. The patterns of genetic structure found in each *Nacella* species was supported by the model based on Bayesian clustering algorithm which detected three main clusters in *N. magellanica* and two main clusters in *N. deaurata* and *N. mytilina*, respectively (Figure 2). The first cluster in *N. magellanica* included northern localities across PM (NPM), the second one encompassed southern localities of PM (SPM = Strait of Magellan and Cape Horn) together with AM sites and the third one the F/M locality (Figure 2A). In the case of *N. deaurata* (Figure 2B) and *N. mytilina* (Figure 2C), the first cluster included all the localities across SPM while the second cluster the F/M population, respectively. Values of cluster membership were high for all localities (c.a. P = 0.9). Similarly, SAMOVA analyses in *N. magellanica* recorded three maximally differentiated groups (NPM, SPM/AM, and F/M) accounting for 32.98% of the total variance (Table 3). In *N. deaurata* and *N. mytilina* SAMOVA analyses recorded two maximally differentiated groups (SPM and F/M) accounting for 65.01% (*N. deaurata*) and 43.09% (*N. mytilina*) of the total variance (Table 3).

Demographic inference in Magellan *Nacella* species

Network reconstructions: Parsimony network of *N. magellanica* depicts 79 distinct haplotypes (Figures 3A; S1A) with a typical star-like topology and a short genealogy. The central haplotype (H01) was the most frequent (47.88%) and broadly distributed across the species range, which extends throughout the Magellan province. The most derived haplotype in *N. magellanica* is related to H01 with a maximum branch length of 9 mutational steps (Figure 3A). A second haplotype (H02) was present in 9.9% of the individuals across the species distribution. Several haplotypes with intermediate frequencies were present in different localities across PM and AM (Figure 3A). The remaining haplotypes occurred at low frequencies and 61 singletons were identified in this species. Several individuals (N = 20) from the F/M shared a dominant haplotype (H03) surrounded by 12 endemic F/M private haplotypes. This dominant F/M haplotype (H03) is separated from the dominant H01 by six mutational steps.

Maximum parsimony network of *N. mytilina* included a total of 19 different haplotypes and exhibited a typical star-like topology and a very short genealogy (Figures 3B; S1B). A dominant haplotype (HA) was most frequent one (71%) and was found at all localities (Figure 3B) and the most derived one is related to it with a maximum branch length of 8 mutational steps (HD) (Figure 3B). Another haplotype (HB) of intermediate frequency (10%), was also found in all the analyzed populations (Figure 3B). Several haplotypes were recorded in more than three individuals belonging to different localities across SPM. We identified 10 singletons in *N. mytilina*, six across SPM and four in F/M (Figure 3B). Several F/M haplotypes are closely related and even shared with the diversity recorded at SPM. Two haplotypes of medium frequency (HC and HD) were only found at F/M and are separated from the dominant H01 by several mutational steps.

Maximum-joining network of *N. deaurata* included a total of 50 different haplotypes and exhibited a short genealogy with several haplotypes of intermediate frequency (Figures 3C, S1C). A dominant haplotype (HI) showed a frequency of 33.19% and was only recorded at SPM localities from the Strait of Magellan and Cape Horn (Figure 3C). Three medium frequency haplotypes (HII – HIV) were recorded in *N. deaurata*, two of them (HII and HIV) were also found only in SPM localities while HIII was also found in a single individual from F/M. The most derived haplotype from F/

M is related to it with a maximum branch length of 11 mutational steps. Several haplotypes were recorded in more than three individuals belonging to different localities across SPM. We identified 35 singletons in *N. deaurata*, 25 across PM localities and 10 in F/M (Figure 3C). Four haplotypes in *N. deaurata* from F/M were closely related and even shared with the diversity recorded in SPM. Nevertheless, a dominant F/M haplotype found in 18 specimens (HV) was separated by eight mutational steps from HI. Again, this dominant F/M haplotype was surrounded by several endemic ones. In contrast to the other analyzed *Nacella* species, the dominant haplotype found in *N. deaurata* (HI) was not documented in the F/M.

As expected for a star-like genealogy, global Tajima's D and Fu's F neutrality tests were both negative and significant for all the analyzed Magellan *Nacella* species (Table 1). The distribution of pairwise differences varied among the analyzed *Nacella* species. The

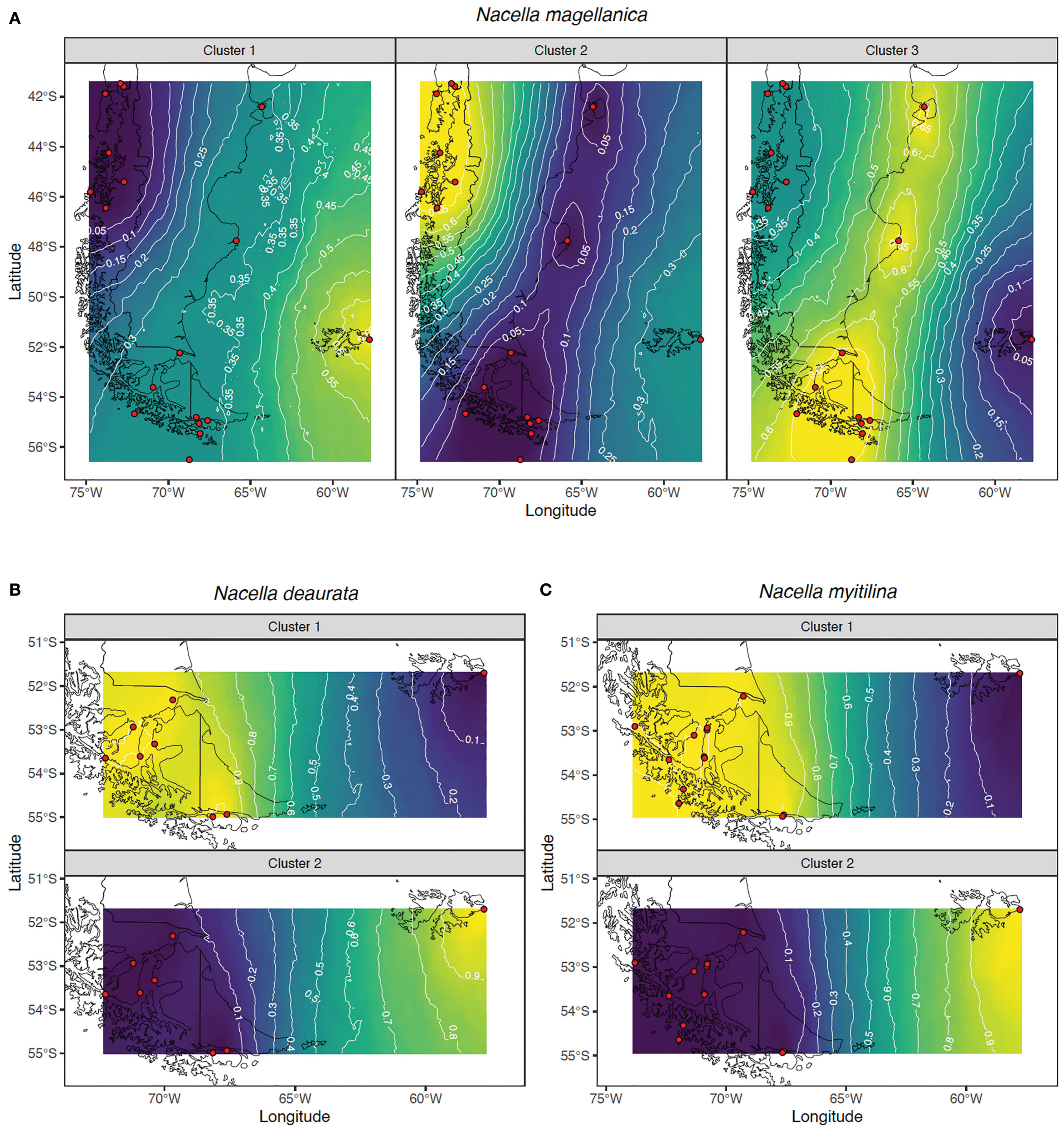


FIGURE 2
Spatial output from Geneland using all **(A)** *N. magellanica*, **(B)** *N. deaurata* and **(C)** *N. mytilina* populations. Red circles indicate the relative positions of the sampling localities. Darker and lighter shadings are proportional to posterior probabilities of membership to a particular cluster where lighter (yellow areas) show the highest probabilities of cluster. Posterior probabilities of membership were plotted to the shapefiles of the Magellan coastline available in the database GEOdas (NOAA) and filtered using GEOdas Coastline Extractor v.1.1.3.1 (https://www.ngdc.noaa.gov/mgg/geodas/geodas.html).

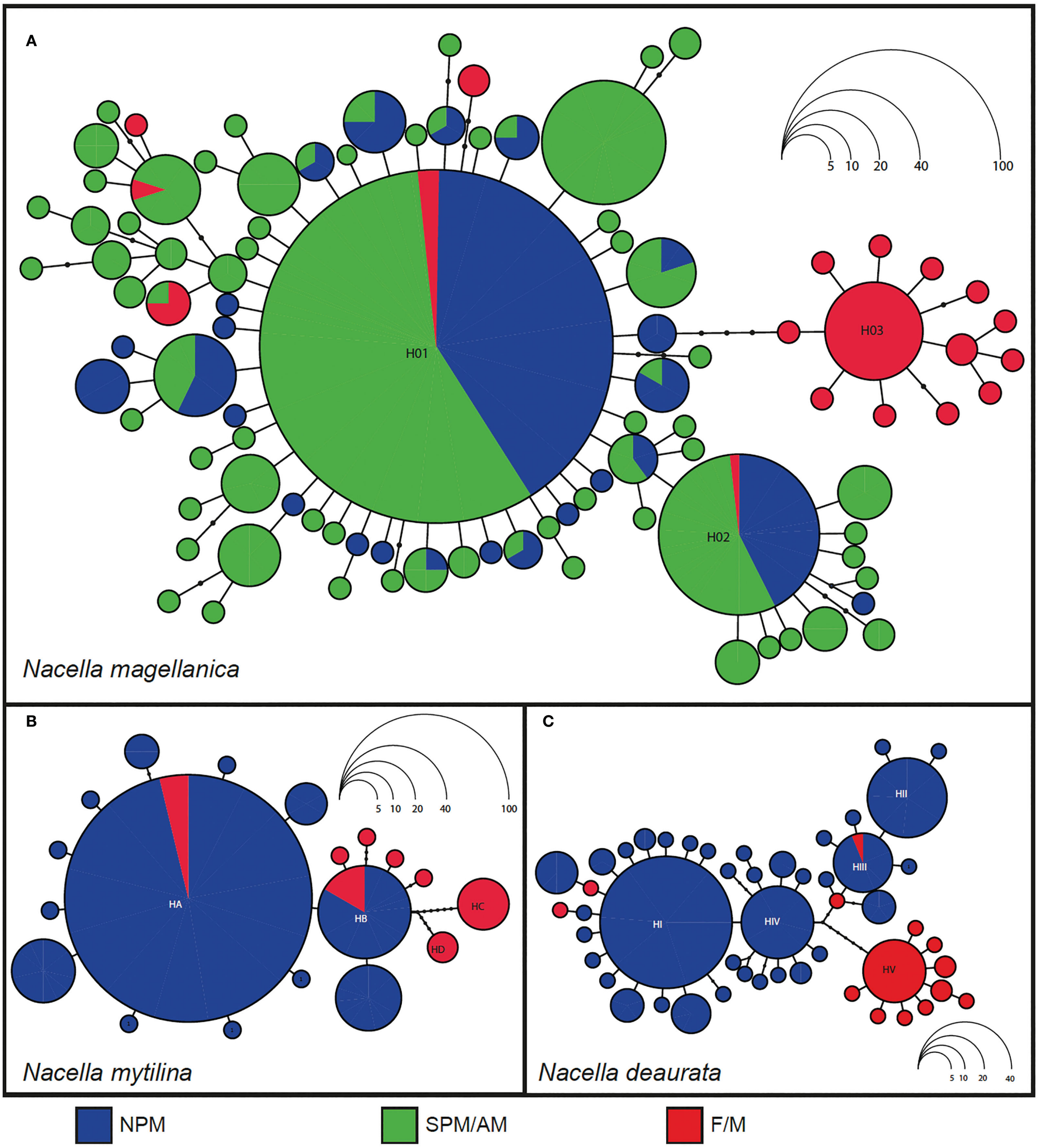


FIGURE 3
Parsimony mtDNA (COI) haplotype networks in **(A)** *Nacella magellanica*, **(B)** *Nacella mytilina*, and **(C)** *Nacella deaurata* across their distributions in the Magellan Province. Each haplotype is represented by a colored circle indicating the main genetic cluster where it was collected (for each species). Circles sizes are proportional to the frequency of the haplotype in the whole sampling effort. The size of the haplotypes circles **(B, C)** is proportional to the their frequency. Where NPM, northern Pacific margin; SPM, southern Pacific margin; AM, Atlantic margin; F/M, Falkland/Malvinas Islands.

species *N. magellanica* showed a typical unimodal curve while *N. mytilina* showed an L-shaped one. In contrast, *N. deaurata* exhibited a multimodal distribution (Figure 4).

Demographic reconstructions: Bayesian skyline plot analyses recorded differences in the times of the most recent common ancestor (trmca) and populations expansions between F/M and South American populations in *Nacella deaurata* and *Nacella magellanica* (Figure 5). Considering the number of endemic F/M haplotypes of *N. mytilina* (n = 7), BSP analysis in this population was not conducted. Population expansions of *N. magellanica* and *N. deaurata* at the F/M occurred around 15,000 and 14,000 years ago, respectively. Population expansions in *N. deaurata* and *N. mytilina* in southern SPM occurred around 9,000 and 7,000 years ago. In the case of *N. magellanica*, the tmrca of SPM occurred around 18,000 years ago while SAM populations expanded around 15,000 years ago. Results on populations sizes estimations per species/groups are presented in Supplementary Table 4.

Gene flow in Magellan *Nacella* species

Gene flow analyses using different migration models detected evidence of asymmetrical gene flow from SPM to the F/M in the species *N. deaurata* and *N. mytilina*. We chose a stepping-stone model for *N. magellanica* including either symmetric migration from northern PM localities (NPM) towards SPM/AM (Cluster 2) or asymmetrical migration from SPM/AM towards F/M (Table 4).

Discussion

Reconstructions of how antarctic and sub-antarctic species or ecosystems responded to Quaternary glacial cycles can help understand the evolution and biogeography of this biota during the last million years. These reconstructions also provide key information concerning their potential responses to future climate change (Fraser et al., 2010; Fraser et al., 2012; González-Wevar et al., 2012; González-Wevar et al., 2013; Poulin et al., 2014; Riesgo et al., 2015; González-Wevar et al., 2016a; Moon et al., 2017; Halanych and Mahon, 2018; González-Wevar et al., 2021). Major landscape and seascape changes along the Magellan fjords and channels of southern South America resulted in the sporadic eradication of the associated fauna across these ecosystems (Valdovinos et al., 2003; Zattara and Premoli, 2005; Kiel and Nielsen, 2010). During the last two decades, molecular-based studies have assumed an essential role for unravelling the relationship between Quaternary climate shifts and the distribution and demography of species and populations (Hewitt, 2004; Maggs et al., 2008; Provan and Bennett, 2008; Marko et al., 2010). In this study we performed population-based mtDNA genetic analyses in three Magellan near-shore patellogastropods species of the genus *Nacella* (*N. deaurata*, *N. magellanica* and *N. mytilina*) to understand the role of Quaternary climatic oscillations over their patterns of genetic diversity and structure. As a general pattern, the three species of *Nacella* analyzed here showed classical signals of the Expansion-Contraction Quaternary biogeography model with low levels of genetic diversity, absence of genetic structure across broad geographical distances and strong signals of recent post-glacial demographic expansion across their respective distributions. Such results have also been found in other groups of Magellan marine near-shore organisms including macroalgae (Fraser et al., 2010; Macaya and Zuccarello, 2010; Billard et al., 2015), mollusks (Nuñez et al., 2015; González-Wevar et al., 2017; González-Wevar et al., 2018; González-Wevar et al., 2019; Pardo-Gandarillas et al., 2018; Fernández Iriarte et al., 2020; González-Wevar et al., 2021) and fishes (Ceballos et al., 2016; Segovia et al., 2022). Moreover, similar demographic patterns have also been recorded in higher latitude

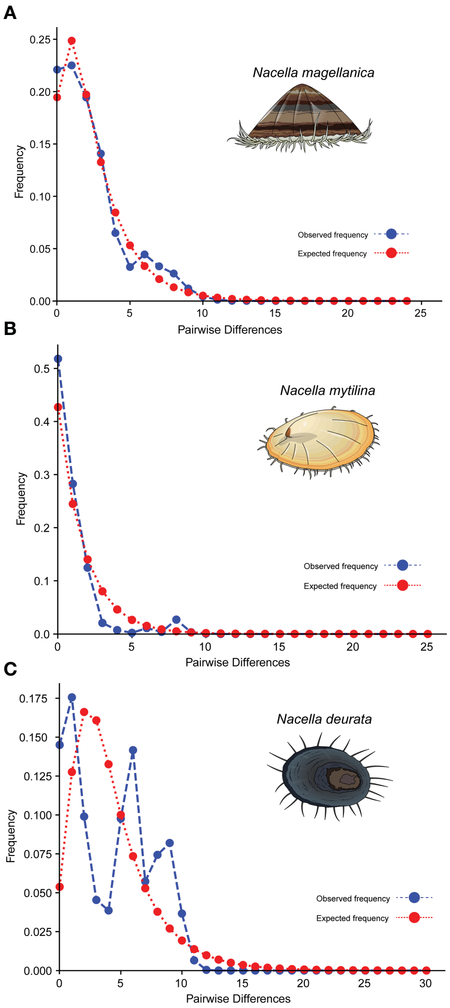


FIGURE 4
Distribution of pairwise differences for the mtDNA COI gene in **(A)** *Nacella magellanica*, **(B)** *Nacella mytilina* and **(C)** *Nacella deaurata*. X-axis = Pairwise differences and y-axis = frequency.

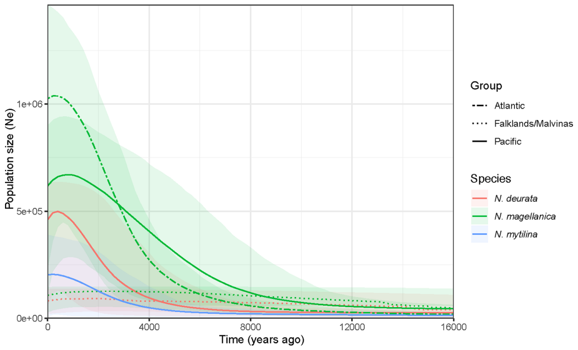


FIGURE 5
Historical demographic trajectories of the effective population sizes (N_e) constructed using a Bayesian skyline plot approach based on COI haplotypes of *N. magellanica* (green), *N. mytilina* (red) and *N. deaurata* (red) along their distribution in the Magellan province (southern South America and the Falkland/Malvinas Islands). The y-axis is the product of effective population size (N_e) and generation length in a log scale while the x-axis is the time before present. The median estimate are shown for each of the genetic groups recorded in the analyzed species.

TABLE 3 Spatial analysis of molecular variance (SAMOVA) in A) *Nacella magellanica*, B) *N. mytilina*, and C) *N. deaurata* depicting the percentage of variation explained among the recognized groups, among populations within groups and within populations. Where FSC = differentiation within populations among groups and FCT = Differentiation among groups (**<p 0.01, *** p<0.001).

A)				
Source of variation	d.f.	Sum of squares	Variance components	% of variation
Among groups	2	139.375	0.45410 Va	32.98
Among populations within groups	15	15.646	0.00415 Vb	0.30
Within populations	547	502.411	0.91848 Vc	66.71
Total	564	657.432	1.37674	

Fixation Indices
F_{SC}: 0.00450***
F_{CT}: 0.32984***

B)				
Source of variation	d.f.	Sum of squares	Variance components	% of variation
Among groups	1	14.645	0.32492 Va	43.09
Among populations within groups	11	2.455	0.00881 Vb	0.30
Within populations	305	133.567	0.43792 Vc	56.71
Total	317	150.667	0.75404	

Fixation Indices
FSC: 0.02052***
FCT: 0.43091***

C)				
Source of variation	d.f.	Sum of squares	Variance components	% of variation
Among groups	1	154.011	2.69012 Va	65.01
Among populations within groups	6	5.058	0.02124 Vb	0.51
Within populations	233	342.382	1.46944 Vc	35.99
Total	240	501.448	4.13832	

Fixation Indices
FSC: 0.01466***
FCT: 0.65005***

marine invertebrates from Antarctica (Wilson et al., 2009; Allcock and Strugnell, 2012; González-Wevar et al., 2013), the sub-Antarctic (Fraser et al., 2009; Moon et al., 2017; González-Wevar et al., 2019; González-Wevar et al., 2021), New Zealand (Goldstien et al., 2006), Europe (Hewitt, 2000; Hewitt, 2004; Maggs et al., 2008; Villamor et al., 2014) and North America (Marko et al., 2010). Levels of genetic diversity in Magellan *Nacella* species ranged from medium (*N. magellanica*) to low (*N. deaurata* and *N. mytilina*) compared to temperate (Nakano and Ozawa, 2005; Goldstien et al., 2006; Sá Pinto et al., 2007) and tropical (Bird et al., 2007) patellogastropods but higher (in most of the cases) than in the Antarctic relative *N. concinna* (González-Wevar et al., 2013; de Aranzamendi et al., 2022). While levels of genetic polymorphism recorded in *N. mytilina* are lower than those found in the antarctic *N. concinna*, they are comparable to those in other sub-antarctic nacellid limpets from New Zealand (Reisser et al., 2011) and Marion Island. As proposed by González-Wevar et al. (2016a) exceptionally low levels of mtDNA genetic diversity in this species could be associated with its strong habitat dependency on macroalgae. Post-glacial recolonization of *N. mytilina* populations would have required the previous settlement of macroalgae. Accordingly, the post-glacial expansion in *N. mytilina* would have been hindered or delayed compared to the other Magellan species analyzed here. The glacial history of the region together with the larval-mediated dispersal potential of the analyzed species are strongly associated to their respective patterns of genetic diversity and structure. In concert with the dispersal potential, we found evidence of rapid postglacial recolonization processes across a very complex landscape likely related to deglaciation processes across the species' distributions. As estimated trough BSP analyses, phylogeographic patterns in sub-Antarctic Magellan species of *Nacella* are probably related to genetic drift processes driven by demographic bottlenecks during glacial maxima, and particularly to the Last Glacial Maximum. After the LGM, *Nacella* populations

TABLE 4 Gene flow model selection for the analyzed *Nacella* species considering their respective genetic clusters across the Magellan province.

A. Nacella magellanica				
Model	Diagram	Bezier Approximation	Model	Model
Full	NPM ⟺ SPM/AM ⟺ F/M ⟺ NPM	-3165.82	$1.13503e^{-18}$	6
Stepping-Stone (Symmetric)	NPM ⟺ SPM/AM ⟺ F/M	-3152.64	$6.01187e^{-13}$	4
Stepping Stone (SA Sym + F/M PM Asym)	NPM ⟺ SPM/AM ⟹ F/M	-3124.51	0.9901784	1
Stepping Stone (SA Sym + F/M AM Asym)	NPM ⟺ SPM/AM ⟸ F/M	-3129.15	0.0095628	2
Steppng Stone (Asym to PM)	NPM ⟹ SPM/AM ⟹ F/M	-3132.76	0.0002586	3
Stepping Stone (Asym to AM)	NPM ⟸ SPM/AM ⟸ F/M	.3156,50	1.2665e-^{14}	5
B. *Nacella deaurata*				
Model	Diagram	Bezier Approximation	Model	Model
Bidirectional	SPM ⟺ F/M	-1896.75	$2.7061e^{-6}$	2
Asymmetric (SPM to F/M)	SPM ⟹ F/M	1883.93	0.999997	1
Asymmetric (F/M to SPM)	SPM ⟸ F/M	1904.93	$7.5825e^{-10}$	3
B. *Nacella mytilina*				
Model	Diagram	Bezier Approximation	Model	Model
Bidirectional	SPM ⟺ F/M	-1632.82	$2.4748e^{-14}$	2
Asymmetric (SPM to F/M)	SPM ⟹ F/M	-1600.82	1	1
Asymmetric (F/M to SPM)	SPM ⟸ F/M	-1644.42	$1.1608e^{-19}$	3

Where NPM, Northern Pacific margin; SPM, Southern Pacific margin; F/M, Falkland/Malvinas Islands.

suffered postglacial expansions following the deglaciation processes across their respective distributions. Our BSP demographic estimations in *Nacella* are in basic agreement with the timing of the LGM extent and the onset of the deglaciation process in the Magellan province, a process that occurred synchronously across the region (Hein et al., 2010). The final advances of ice in northern areas of Pacific Patagonia are dated about 18,000 years ago (Heusser et al., 1998; Heusser et al., 1999; Moreno et al., 1999) and the warming began 17,500 years ago (Moreno and León, 2003). The final advances of ice in the Strait of Magellan occurred around 17,000 years ago (McCulloch et al., 2005) while a fast-warming period occurred between 14,000 and 10,000 years ago (De Pol-Holz et al., 2006).

Contrary to our expectations, populations of *N. magellanica* along temperate areas of the Atlantic (AM), an area considered as less impacted by ice during glacial periods, did not exhibit higher genetic diversity than those populations located inside areas that were heavily impacted by ice across PM and they seem to represent a continuity of the diversity found in the SPM (Strait of Magellan and Cape Horn). A plausible explanation for these results in the Atlantic margin relies in the fact that during the LGM the level of the sea was between 120 and 140 m below its present level (Fleming et al., 1998). During glacial maxima a large portion of the Atlantic Continental Shelf (ACS) was exposed with the development of major plains dominated by depositional environments and sandy environments (Rabassa et al., 2005; Ponce et al., 2011; Rabassa et al., 2011; Violante et al., 2014). As proposed for the intertidal pulmonate limpet *Siphonaria lessonii* (Fernández Iriarte et al., 2020) and for the mussel *Perumytilus purpuratus* (Trovant et al., 2015), suitable hard substrate habitat for *N. magellanica* during glacial maxima would have been strongly reduced south of 46°S in Tierra del Fuego. Accordingly, refuges for *N. magellanica* would have been more available in the southern tip of the southwest Atlantic, from where the species could have recolonized lower latitudes following the deglaciation process. As proposed by Fernández Iriarte et al. (2020) phylogeographical patterns in broadly distributed near-shore Magellan species are not essentially related to Quaternary glacial continental ice expansions and contractions but also to the availability of habitats for the species.

Each of the analyzed *Nacella* species are characterized by star-like genealogies with the presence of a dominant and widely distributed haplotype surrounded by several unique, low- and medium-frequency ones. Such demographic patterns are consistent on the one hand of the strong demographic impact of glacial processes resulting in the reduction of their populations sizes through repetitive bottleneck events during the coolest periods of the Quaternary. Moreover, broadly distributed haplotypes recorded in Magellan *Nacella* species are also consistent with the hypothesis of recent range expansion and high levels of migration. In this context, the absence of structure across large geographical areas, the presence of dominant haplotypes, and the absence of correlation between geographic and genetic distances support the hypothesis of recent expansions of these patellogastropods, probably mediated by their planktonic dispersal as larvae reflecting the general oceanographic circulation patterns of the study area (Escribano et al., 2003; Acha et al., 2004; Alemany et al., 2009; Bustos et al., 2011). Finally, significant negative Tajima's D and Fu's FS indices are the result of an excess of low frequency haplotypes, commonly explained by recent demographic processes, as evidenced here in the analyzed *Nacella* species through Bayesian skyline plots reconstructions.

Main patterns of genetic structure recorded across the Magellan province in marine organisms includes a) absence or moderate genetic differentiation between South American populations and the Falkland/Malvinas Islands (M/F) (Fernández Iriarte et al., 2020; González-Wevar et al., 2021; González-Wevar et al., 2022; Segovia et al., 2022), b) strong phylogeographic signaling between PM and F/M (Macaya and Zuccarello, 2010; González-Wevar et al., 2012; González-Wevar et al., 2016a; González-Wevar et al., 2018) and c) the presence of different species in the F/M (Leese et al., 2008; González-Wevar et al., 2019; González-Wevar et al., 2022). All the *Nacella* species analyzed here showed strong phylogeographic structure between SPM/AM and F/M supporting previous studies in near-shore Magellan marine organisms. As proposed by Leese et al. (2008), even when the F/M marine biota has often been considered as part of the Magellan inventory, molecular analyses are showing that F/M populations may be strongly differentiated populations and/or even reproductive isolated units. Moreover, F/M populations of *N. mytilina* and *N. magellanica* showed higher levels of genetic diversity than PM and AM populations but this pattern was not detected in *N. deaurata*. As previously proposed in *Nacella*, the marked differences in diversity and structure between SPM and F/M populations may be associated with the different glaciological trajectories of these areas during the coldest of the Quaternary glaciations (González-Wevar et al., 2012; González-Wevar et al., 2016a; Fernández Iriarte et al., 2020). The SPM was almost completely covered by ice during the LGM and hence the shallow marine ecosystems would have been severely reduced across this area. Thus, marked decline in genetic diversity associated with repeated rounds of bottleneck events and/or founder effects is expected as recorded in several marine near-shore species (González-Wevar et al., 2011; González-Wevar et al., 2012; González-Wevar et al., 2015b; González-Wevar et al., 2016b; González-Wevar et al., 2018; de Aranzamendi et al., 2014; Güller et al., 2015; González-Wevar et al., 2021; González-Wevar et al., 2022; Nuñez et al., 2011; Nuñez et al., 2015; Segovia et al., 2022). In contrast, in the F/M there is little evidence of glacial ice accumulation apart from small cirques and short glacially eroded valleys (Clapperton and Sugden, 1976; Clapperton, 1994; Hodgson et al., 2014). In fact, cosmogenic isotopes surface exposure dates suggest an absence of widespread glaciations at altitude during the LGM and there is no evidence for glaciers extending offshore in these islands (Clark et al., 1998; Wilson et al., 2002; Wilson et al., 2008). Under such glaciological scenarios, our results in *Nacella* species could be explained through contrasting demographical histories between populations from areas that were dramatically affected by continental ice across PM and those populations located in non-glaciated areas such as F/M. Quaternary genetic models include the prediction that glacial refugia should exhibit a longer demographic history with higher levels of genetic diversity (Provan and Bennett, 2008). Nevertheless, the higher genetic diversity and longer demographic histories recorded in *Nacella* populations from the F/M may also be a consequence of the asymmetric gene flow from South America towards the F/M. Evidence of these interpretation is provided by the presence of endemic F/M haplotypes that survived the LGM *in situ* and are found together with recently arrived South American ones. Following this explanation, the F/M could be considered as a glacial refugium for the species analyzed here, comprised of a sink area together with a secondary contact zone (González-Wevar et al., 2012; González-Wevar et al., 2016a).

In marine ecosystems, dispersal plays a key role in providing connectivity of populations, preventing speciation, species persistence despite temporal/local extinctions, and recruitment to potentially favorable environments (Ayre et al., 2009). For benthic organisms with low autonomous mobility the dispersal potential is usually related to the earliest life history stages and the processes that influence developmental modes, types of larvae, larval development, and lifespan (Strathmanns, 1985; Palumbi, 1994; Poulin et al., 2002; Marko, 2004; Cowen and Sponaugle, 2009). Accordingly, in these patellogastropods the duration of the dispersive stages is expected to correlate with the dispersal potential and with the patterns of genetic structure (Paulay et al., 2006; Miller and Ayre, 2008; Ayre et al., 2009; Puritz et al., 2017). Absence of genetic structure across the PM has been found in different marine organisms with dispersal potential (Toro et al., 2004; Ceballos et al., 2012; González-Wevar et al., 2012; González-Wevar et al., 2016a, b; Hüne et al., 2015; González-Wevar et al., 2018; Fernández Iriarte et al., 2020; González-Wevar et al., 2021; Segovia et al., 2022). Currently, there is no information concerning the larval duration in Magellan *Nacella* species but their developments should be similar to the Antarctic relative *N. concinna* (Bowden et al., 2009; Peck et al., 2016) and extends for at least six weeks. A main asymmetrical dispersal pattern from South America to F/M found in the *Nacella* species analyzed here is probably associated with the oceanographical circulation patterns in the Magellan province, as well as by the reproductive biology and the ecology of these species. The Cape Horn Current (CHC), a southward branch of the West Wind Drift, flows around Cape Horn and is responsible for the main oceanographic regime in this province. After surrounding the southern tip of South America, the CHC is divided into two minor branches, one flows east towards South Georgia and the second one flows northward on both sides of the F/M (Knox, 1960; Brattström and Johanssen, 1983; Bastida et al., 1992; Acha et al., 2004). Accordingly, gene flow mediated by larval dispersal, and/or rafting in the case of *N. mytilina*, may be favored by the main oceanographic conditions in southern South America.

Conclusions

The results of our mtDNA-based phylogeographic analyses for three Magellan Nacella species have provided evidence that historical and contemporary processes have played key roles in explaining the current patterns of genetic diversity, population structure and connectivity by larval dispersal. The main phylogeographic footprints revealed for these Magellan *Nacella* species include low levels of genetic diversity, the presence of broadly distributed dominant haplotypes and recent post-glacial demographic expansions. Together, these provide further evidence for the Expansion-Contraction (EC) model of Quaternary biogeography proposed by Provan and Bennett (2008). At the same time, the phylogeographic structure patterns revealed marked genetic differences between South American populations and those from the Falkland/Malvinas. Even in the presence of asymmetric levels of gene flow from South America towards the Falkland/Malvinas Islands, the migration rate is not enough to homogenize populations and they constitute different genetic units in all the *Nacella* species analyzed here. Contrary to our expectations, *N. magellanica* populations from the Atlantic margin, an area that was less impacted by ice during glacial maxima, did not exhibit higher levels of genetic diversity than heavily ice impacted populations from the Pacific margin. The phylogeographic patterns appear best explained by the interplay of ecological factors including habitat availability, restriction to shallow depths, and obligate planktonic dispersal facilitated by strong but directional currents.

Data availability statement

New COI sequences in Magellan Nacella species have been deposited in GenBank under the following accession numbers: OQ587619–OQ587817. Additionally, we included previously published sequences under the GenBank Accession Numbers: EU870927–EU870985, HQ880439–HQ880550; JX262742–JX262797 for N. magellanica; EU870986–EU0870999; HQ880551–HQ880573 for N. deaurata and KX600474–KX600492 for N. mytilina (de Aranzamendi et al., 2009; de Aranzamendi et al., 2011; González-Wevar et al., 2012; González-Wevar et al., 2016a).

Author contributions

CG-W, MA, EP, CG, RB, CRM, and NS conceived the idea of the study and designed the study. CG-W, SR, EP, PB, MA collected *Nacella* specimens from different localities across their distribution in the Magellan province. CG-W, SR, CSM, and MA performed molecular experiments. CG-W, NS, SR, CSM, CRM conduced phylogeographic analyses and wrote the manuscript. CG-W, EP, NS, CRM, MA, and SR contributed intellectually to the interpretation and discussion of results. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by Fondecyt Regular Project 1210787, ANID – Millennium Science Initiative Program – ICN2021_002, and Program FONDAP (15150003) to CG-W. Fondecyt Regular Project 1151336 to EP and CG-W and Fondecyt Postdoctoral 3210063 to CSM. Program PR-06-CRN-18 (Universidad de Magallanes) to SR.

Acknowledgments

Many thanks to Angie Díaz, Jaime Ojeda, Mathias Hüne, Javier Naretto, and Karin Gérard, for field assistance and samplings in southern South America. *Nacella* specimens were captured under the Chilean legislation (Technical Memorandum P.INV N° 258/2021 SUBPESCA). We are mostly grateful to the collaboration of the South Atlantic Environmental Research Institute (SAERI) and the Shallow Marine Surveys Group of the collection of *Nacella* specimens in the Falkland Islands.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1154755/full#supplementary-material

References

Acha, E. M., Mianzan, H. W., Guerrero, R. A., Favero, M., and Bava, J. (2004). Marine fronts at the continental shelves of austral south America. *J. Mar. Syst.* 44, 83–105. doi: 10.1016/j.jmarsys.2003.09.005

Alemany, D., Acha, E. M., and Iribarne, O. (2009). The relationship between marine fronts and fish diversity in the Patagonian shelf Large marine ecosystem. *J. Biogeogr.* 36, 2111–2124. doi: 10.1111/j.1365-2699.2009.02148.x

Aljanabi, S. M., and Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res.* 25, 4692–4693. doi: 10.1093/nar/25.22.4692

Allcock, A. L., and Strugnell, J. M. (2012). Southern ocean diversity: new paradigms from molecular ecology. *Trends Ecol. Evol.* 27, 520–528. doi: 10.1016/j.tree.2012.05.009

Ayre, D. J., Minchinton, T. E., and Perrin, C. (2009). Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? *Mol. Ecol.* 18, 1887–1903. doi: 10.1111/j.1365-294X.2009.04127.x

Bastida, R., Roux, A., and Martínez, D. E. (1992). Benthic communities of the Argentine continental shelf. *Oceanol. Acta* 15, 687–698.

Beerli, P., and Felsenstein, J. (2001). Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4563–4568. doi: 10.1073/pnas.081068098

Beerli, P., and Palczewski, M. (2010). Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics* 185, 313–326. doi: 10.1534/genetics.109.112532

Bennett, K. D., Tzedakis, K., and Willis, J. (1991). Quaternary refugia of north European trees. *J. Biogeogr.* 18, 103–115. doi: 10.2307/2845248

Billard, E., Reyes, J., Mansilla, A., Faugeron, S., and Guillemin, M.-L. (2015). Deep genetic divergence between austral populations of the red alga gigartina skottsbergii reveals a cryptic species of endemic to the Antarctic continent. *Polar Biol.* 38, 2021–2034. doi: 10.1007/s00300-015-1762-4

Bird, C. E., Holland, B. S., Bowen, B. W., and Toonen, R. J. (2007). Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. *Mol. Ecol.* 16, 3173–3186. doi: 10.1111/j.1365-294X.2007.03385.x

Bowden, D. A., Clarke, A., and Peck, L. S. (2009). Seasonal variation in the diversity and abundance of pelagic larvae of Antarctic marine invertebrates. *Mar. Biol.* 156, 2033–2047. doi: 10.1007/s00227-009-1235-9

Brattström, H., and Johanssen, A. (1983). Ecological and regional zoogeography of the marine benthic fauna of Chile. *Sarsia* 68, 289–339. doi: 10.1080/00364827.1983.10420583

Bustos, C. A., Landaeta, M. F., and Balbontín, F. (2011). Ichthyoplankton spatial distribution and its relation with water column stratification in fjords of southern Chile (46°48'- 50°09'S) in austral spring 1996 and 2008. *Cont. Shelf Res.* 31, 293–303. doi: 10.1016/j.csr.2010.03.013

Ceballos, S. G., Lessa, E. P., Licandeo, R., and Fernández, D. A. (2016). Genetic relationships between Atlantic and pacific populations of the notothenioid fish *Eleginops maclovinus*: the footprints of quaternary glaciations in Patagonia. *Heredity* 116, 372–377. doi: 10.1038/hdy.2015.106

Ceballos, S. G., Lessa, E. P., Victorio, M. F., and Fernández, D. A. (2012). Phylogeography of the sub-Antarctic notothenioid fish *Eleginops maclovinus*: evidence of population expansion. *Mar. Biol.* 159, 499–505. doi: 10.1007/s00227-011-1830-4

Clapperton, C. M. (1994). The quaternary glaciation of Chile: a review. *Rev. Chil. Hist. Nat.* 67, 369–383.

Clapperton, C. M., and Sugden, D. E. (1976). The maximum extent of glaciers in part of west Falkland. *J. Glaciol.* 17, 73–77. doi: 10.3189/S0022143000030732

Clark, R., Huber, U. M., and Wilson, P. (1998). Late pleistocene sediments and environmental change at plaza creek, Falkland islands, south Atlantic. *J. Quat. Sci.* 13, 95–105. doi: 10.1002/(SICI)1099-1417(199803/04)13:2<95::AID-JQS351>3.0.CO;2-G

Cowen, R. K., and Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Ann. Rev. Mar. Sci.* 1, 443–446. doi: 10.1146/annurev.marine.010908.163757

de Aranzamendi, M. C., Bastida, R., and Gardenal, C. N. (2014). Genetic population structure in *Nacella magellanica*: evidence of rapid range expansion throughout the entire species distribution on the Atlantic coast. *J. Exp. Mar. Biol. Ecol.* 460, 53–61. doi: 10.1016/j.jembe.2014.06.008

de Aranzamendi, M. C., Bastidas., R., and Gardenal, C. (2011). Different evolutionary histories in two sympatric limpets of the genus *Nacella* (Patellogastropoda) in the south-western Atlantic coast. *Mar. Biol.* 158, 2405–2418. doi: 10.1007/s00227-011-1742-3

de Aranzamendi, M. C., Gardenal, C. N., Martin, J. P., and Bastida, R. (2009). Limpets of genus *Nacella* (Patellogastropoda) from the southwestern Atlantic: species identification based on molecular data. *J. Mollusc. Stud.* 75, 241–251. doi: 10.1093/mollus/eyp025

de Aranzamendi, M. C., Martínez, J. J., Held, C., and Sahade, R. (2022). Parallel shape divergence between ecotypes of the limpet nacella concinna along the Antarctic peninsula: a new model species for parallel evolution. *Zoology* 150, 125983. doi: 10.1016/j.zool.2021.125983

De Pol-Holz, R., Ulloa, O., Dezileau, L., Kaiser, J., Lamy, F., and Hebbeln, D. (2006). Melting of the Patagonian ice sheet and deglacial perturbations of the nitrogen cycle in the eastern south pacific. *Geophys. Res. Lett.* 33, L04704. doi: 10.1029/2005GL024477

Drummond, A. J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214. doi: 10.1186/1471-2148-7-214

Drummond, A. J., Suchard, M. A., Xie, D., and Rambaut, A. (2012). Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973. doi: 10.1093/molbev/mss075

Dupanloup, I., Schneider, S., and Excoffier, L. (2002). A simulated annealing approach to define the genetic structure of populations. *Mol. Ecol.* 11, 2571–2581. doi: 10.1046/j.1365-294x.2002.01650.x

Edgar, R. C. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinf.* 5, 113. doi: 10.1186/1471-2105-5-113

Escribano, R., Fernández, M., and Aranis, A. (2003). Physical-chemical processes and patterns of diversity of the Chilean eastern boundary pelagic and benthic marine ecosystems: an overview. *Gayana* 67, 190–205. doi: 10.4067/S0717-65382003000200008

Excoffier, L., Laval, G., and Schneider, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* 1, 37–50. doi: 10.1177/117693430500100003

Fernández Iriarte, P. J., González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Hüne, M., Fainburg, L., et al. (2020). Quaternary ice sheets and sea level regression drove divergence in a marine gastropod along Eastern and Western coasts of south America. *Sci. Rep.* 10, 844. doi: 10.1038/s41598-020-57543-4

Fleming, K., Johnston, P., Zwartz, D., Yokoyama, Y., Lambeck, K., and Chappell, J. (1998). Refining the eustatic sea-level curve since the last glacial maximum using far– and intermediate–field sites. *Earth Planet. Sci. Lett.* 163, 327–342. doi: 10.1016/S0012-821X(98)00198-8

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA Primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biotech.* 3, 294–299.

Forster, P., Torroni, A., Renfrew, C., and Röhl, A. (2001). Phylogenetic star contraction applied to Asian and papuan mtDNA evolution. *Mol. Biol. Evol.* 18, 1864–1881. doi: 10.1093/oxfordjournals.molbev.a003728

Fraser, C. I., Nikula, R., Ruzzante, D. E., and Waters, J. M. (2012). Poleward bound: biological impacts of southern hemisphere glaciation. *Trends Ecol. Evol.* 27, 462–471. doi: 10.1016/j.tree.2012.04.011

Fraser, C. I., Nikula, R., Spencer, H. G., and Waters, J. M. (2009). Kelp genes reveal effects of subantarctic sea during the last glacial maximum. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3249–3253. doi: 10.1073/pnas.0810635106

Fraser, C. I., Thiel, M., Spencer, H. G., and Waters, J. M. (2010). Contemporary habitat discontinuity and historic glacial ice drive genetic divergence in Chilean kelp. *BMC Evol. Biol.* 10, 203. doi: 10.1186/1471-2148-10-203

Glasser, N., and Jansson, K. (2008). The glacial map of southern south America. *J. Maps* 4, 175–196. doi: 10.4113/jom.2008.1020

Goldstien, S. J., Schiel, D. R., and Gemmel, N. J. (2006). Comparative phylogeography of coastal limpets across a marine disjunction in new Zealand. *Mol. Ecol.* 15, 3259–3268. doi: 10.1111/j.1365-294X.2006.02977.x

González-Wevar, C. A., Chown, S. L., Morley, S., Coria, N., Saucéde, T., and Poulin, E. (2016b). Out of Antarctica: quaternary colonization of sub-Antarctic Marion island by the limpet genus *Nacella* (Patellogastropoda: nacellidae). *Polar Biol.* 39, 77–89. doi: 10.1007/s00300-014-1620-9

González-Wevar, C. A., Hüne, M., Cañete, J. I., Mansilla, A., Nakano, T., and Poulin, E. (2012). Towards a model of postglacial biogeography in shallow marine species along the Patagonian province: lessons from the limpet *Nacella magellanica* (Gmelin 1791). *BMC Evol. Biol.* 12, 139. doi: 10.1186/1471-2148-12-139

González-Wevar, C. A., Hüne, M., Segovia, N. I., Nakano, T., Spencer, H. G., Chown, S. L., et al. (2017). Following the Antarctic circumpolar current: patterns and processes in the biogeography of the limpet *Nacella* (Mollusca: patellogastropoda) across the southern ocean. *J. Biogeogr.* 44, 861–874. doi: 10.1111/jbi.12908

González-Wevar, C. A., Nakano, T., Cañete, J. I., and Poulin, E. (2010). Molecular phylogeny and historical biogeography of *Nacella* (Patellogastropoda: nacellidae) in the southern ocean. *Mol. Phylogenet. Evol.* 56, 115–124. doi: 10.1016/j.ympev.2010.02.001

González-Wevar, C. A., Nakano, T., Cañete, J. I., and Poulin, E. (2011). Concerted genetic, morphological and ecological diversification in *Nacella* limpets in the magellanic province. *Mol. Ecol.* 20, 1936–1951. doi: 10.1111/j.1365-294X.2011.05065.x

González-Wevar, C. A., Rosenfeld, S., Segovia, N. I., Hüne, M., Gérard, K., Ojeda, J., et al. (2016a). Genetics, gene flow, and glaciation: the case of the south American limpet *Nacella mytilina*. *PloS One* 11, e0161963. doi: 10.1371/journal.pone.0161963

González-Wevar, C. A., Salinas, P., Hüne, M., Segovia, N. I., Vargas-Chacoff, L., Astorga, M., et al. (2015b). Phylogeography in *Galaxias maculatus* (Jenyns 1848) along two biogeographical provinces in the Chilean coast. *PloS One* 10 (7), e0131289. doi: 10.1371/journal.pone.0131289

González-Wevar, C., Salinas, P., Hüne, M., Segovia, N., Vargas-Chacoff, L., Oda, E., et al. (2015a). Contrasting genetic structure and diversity of *Galaxias maculatus* (Jenyns 1848) along the Chilean coast: stock identification for fishery management. *J. Hered.* 106, 439–447. doi: 10.1093/jhered/esv005

González-Wevar, C. A., Saucède, T., Morley, T., Chown, S. L., and Poulin, E. (2013). Extinction and recolonization of maritime Antarctica in the limpet *Nacella concinna* (Strebel 1908) during the last glacial cycle: toward a model of quaternary biogeography in shallow Antarctic invertebrates. *Mol. Ecol.* 22, 5221–5236. doi: 10.1111/mec.12465

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Maturana, C. S., Jeldres, V., Pinochet, R., et al. (2022). Seven snail species hidden in one: biogeographic diversity in an apparently widespread periwinkle in the southern ocean. *J. Biogeogr* 49, 1521–1534. doi: 10.1111/jbi.14453

González-Wevar, C. A., Gérard, K., Rosenfeld, S., Saucède, T., Naretto, J., Díaz, A., et al. (2019). Cryptic speciation in Southern Ocean Aequiyoldia eightsii (Jay, 1839): Mio-Pliocene trans-Drake Passage separation and diversification. *Prog. Oceanogr* 174, 44–54. doi: 10.1016/j.pocean.2018.09.004

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Noll, D., Maturana, C. S., Hüne, M., et al. (2021). Contrasting biogeographical patterns in *Margarella* (Gastropoda: calliostomatidae: margarellinae) across the Antarctic polar front. *Mol. Phylogenet. Evol.* 156, 107039. doi: 10.1016/j.ympev.2020.107039

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Ojeda, J., Hüne, M., Naretto, J., et al. (2018). Unexpected absence of island endemics: long-distance dispersal in higher latitude sub-Antarctic *Siphonaria* (Gastropoda: euthyneura) species. *J. Biogeogr.* 45, 874–884. doi: 10.1111/jbi.13174

Guillot, G., Mortier, F., and Estoup, A. (2005). GENELAND: a computer package for landscape genetics. *Mol. Ecol. Notes* 5, 712–715. doi: 10.1111/j.1471-8286.2005.01031.x

Güller, M., Zelaya, D. G., and Ituarte, C. (2015). How many siphonaria species (Gastropoda: euthyneura) live in southern south America? *J. Mollusc. Stud.* 82, 80–96. doi: 10.1093/mollus/eyv036

Hüne, M., González-Wevar, C. A., Poulin, E., Mansilla, A., Fernández, D. A., and Barrera-Oro, E. (2015). Low levels of genetic divergence between *Harpagifer* fish species (Perciformes: notothenioidei) suggests a quaternary colonization of Patagonia from the Antarctic peninsula. *Polar Biol.* 38, 607–617. doi: 10.1007/s00300-014-1623-6

Halanych, K. M., and Mahon, A. R. (2018). Challenging the dogma concerning biogeographic patterns of Antarctica and the southern ocean. *Annu. Rev. Ecol. Evol. Syst.* 49, 355–378. doi: 10.1146/annurev-ecolsys-121415-032139

Hein, A. S., Hulton, N. R. J., Dunai, T. J., Sugden, D. E., Kaplan, M. R., and Xu, S. (2010). The chronology of the last glacial maximum and deglacial events in central Argentine Patagonia. *Quat. Sci. Rev.* 29, 1212–1227. doi: 10.1016/j.quascirev.2010.01.020

Heusser, C. J. (1998). Deglacial paleoclimate of the American sector of the southern ocean: late glacial–Holocene records from the latitude of canal beagle (55°S), Argentine tierra del fuego. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 141, 277–301. doi: 10.1016/S0031-0182(98)00053-4

Heusser, C. J., Heusser, L. E., and Lowell, T. V. (1999). Paleoecology of the southern chilean lake district-isla grande de chiloe during the middle-late llanquihue glaciation and deglaciation. *Geogr. Ann. A* 81, 231–284. doi: 10.1111/j.0435-3676.1999.00058.x

Hewitt, G. (2000). The genetic legacy of the quaternary ice ages. *Nature* 405, 907–913. doi: 10.1038/35016000

Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the quaternary. *Phil. Trans. R. Soc Lond. B* 359, 183–195. doi: 10.1098/rstb.2003.1388

Hodgson, D. A., Graham, A. G. C., Roberts, S. J., Bentley, M. J., Cofaigh, C., Verleyen, E., et al. (2014). Terrestrial and submarine evidence for the extent and timing of the last glacial maximum and the onset of deglaciation on the maritime-Antarctic and sub-Antarctic islands. *Quat. Sci. Rev.* 100, 137–158. doi: 10.1016/j.quascirev.2013.12.001

Hudson, R. R. (2000). A new statistic for detecting genetic differentiation. *Genetics* 155, 2011–2014. doi: 10.1093/genetics/155.4.2011

Hulton, N. R. J., Purves, R. S., McCulloch, D. M., Sugden, D. E., and Bentley, M. J. (2002). The last glacial maximum and deglaciation in southern south America. *Quat. Sci. Rev.* 21, 233–241. doi: 10.1016/S0277-3791(01)00103-2

Ihaka, R., and Gentleman, R. (1996). R: a language for data analysis and graphics. *J. Comp. Graph. Stat.* 5, 299–314. doi: 10.2307/1390807

Kaas, R. E., and Raftery, A. E. (1995). Bayes factors. *J. Am. Stat. Assoc.* 90, 773–795. doi: 10.2307/2291091

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., and Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589. doi: 10.1038/nmeth.4285

Kiel, S., and Nielsen, S. N. (2010). Quaternary origin of the inverse latitudinal diversity gradient among southern Chilean mollusks. *Geology* 38, 955–958. doi: 10.1130/G31282.1

Knox, G. A. (1960). Littoral ecology and biogeography of the southern ocean. *Proc. R. Soc Lond.* 152, 577–624. doi: 10.1098/rspb.1960.0068

Leese, F., Agrawal, S., and Held, C. (2010). Long-distance dispersal hopping without dispersal stages: transportation across major zoogeographic barriers in a southern ocean isopod. *Naturwissenshaften* 97, 583–594. doi: 10.1007/s00114-010-0674-y

Leese, F., Kop, A., Wägele, J.-W., and Held, C. (2008). Cryptic speciation in a benthic isopod from Patagonian and Falkland island waters and the impact of glaciations on its population structure. *Front. Zool.* 5, 19. doi: 10.1186/1742-9994-5-19

Librado, P., and Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452. doi: 10.1093/bioinformatics/btp187

Macaya, E. C., and Zuccarello, G. C. (2010). Genetic structure of the giant kelp *Macrocystis pyrifera* along the southeastern pacific. *Mar. Ecol–Prog. Ser.* 420, 103–112. doi: 10.3354/meps08893

Maggs, C. A., Castilho, R., Foltz, D., Henzler, C., Jolly, M. T., Kelly, J., et al. (2008). Evaluating signatures of glacial refugia for north Atlantic benthic marine taxa. *Ecology* 89, S108–S122. doi: 10.1890/08-0257.1

Marko, P. B. (2004). 'What's larvae got to do with it?' disparate patterns of post-glacial population structure in two benthic marine gastropod with identical dispersal potential. *Mol. Ecol.* 13, 597–611. doi: 10.1046/j.1365-294X.2004.02096.x

Marko, P. B., Hoffman, J. M., Emme, S. A., McGovern, T. M., Keever, C. C., and Cox, N. (2010). The 'Expansion–contraction' model of pleistocene biogeography: rocky shores suffer a sea change? *Mol. Ecol.* 19, 146–167. doi: 10.1111/j.1365-294X.2009.04417.x

McCulloch, R. R., Bentley, M. J., Purves, R. S., Hulton, N. R. J., Sugden, D. E., and Clapperton, C. M. (2000). Climatic inferences from glacial and paleoecological evidence at the last glacial termination, southern south America. *J. Quat. Sci.* 15, 409–417. doi: 10.1002/1099-1417(200005)15:4<409::AID-JQS539>3.0.CO;2-%23

McCulloch, R. R., Fogwill, C. J., Sugden, D. E., Bentley, M. J., and Kubik, P. W. (2005). Chronology of the last glaciation in central strait of Magellan and bahía inútil, southernmost south America. *Geogr. Ann. A* 87, 1–24. doi: 10.1111/j.0435-3676.2005.00260.x

Miller, K. J., and Ayre, D. J. (2008). Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. *J. Anim. Ecol.* 77, 713–724. doi: 10.1111/j.1365-2656.2008.01387.x

Moon, K. L., Chown, S. L., and Fraser, C. I. (2017). Reconsidering connectivity in the sub-Antarctic. *Biol. Rev.* 92, 2164–2181. doi: 10.1111/brv.12327

Moreno, P. I., and León, A. L. (2003). Abrupt vegetation changes during the last glacial to Holocene transition in mid-latitude south America. *J. Quat. Sci.* 18, 787–800. doi: 10.1002/jqs.801

Moreno, P. I., Lowell, T. V., Jacobson, G. L.Jr., and Denton, G. H. (1999). Abrupt vegetation and climate changes during the last termination in the Chilean lake district: a case study from canal de la puntilla (41°S). *Geogr. Ann. A* 81, 285–311. doi: 10.1111/j.0435-3676.1999.00059.x

Nakano, T., and Ozawa, T. (2005). Systematic revision of *Patelloida pygmaea* (Dunker 1860) (Gastropoda: lottiidae), with a description of a new species. *J. Molluscan Stud.* 71, 357–370. doi: 10.1093/mollus/eyi039

Narum, S. R. (2006). Beyond bonferroni: less conservative analyses for conservation genetics. *Cons. Genet.* 7, 783–787. doi: 10.1007/s10592-005-9056-y

Nikula, R., Fraser, C. I., Spencer, H. G., and Waters, J. M. (2010). Circumpolar dispersal by rafting in two subantarctic kelp-dwelling crustaceans. *Mar. Ecol-Prog. Ser.* 405, 221–230. doi: 10.3354/meps08523

Nuñez, J. D., Fernández Irirarte, P. J., Ocampo, E. H., Iudica, M., and Cledón, M. (2015). Deep phylogeographic divergence among populations of limpet *Siphonaria lessoni* on the east and west coasts of south America. *Mar. Biol.* 162, 595–605. doi: 10.1007/s00227-014-2607-3

Nuñez, J. J., Wood, N. K., Rabanal, F. E., Fontanella, F. M., and Sites, J. J.W. (2011). Amphibian phylogeography in the antipodes: refugia and postglacial colonization explain mitochondrial haplotype distribution in the Patagonian frog *Eupsophus calcaratus* (Cycloramphidae). *Mol. Phylogenet. Evol.* 58, 343–352. doi: 10.1016/j.ympev.2010.11.026

Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annu. Rev. Ecol. Syst.* 25, 547–572. doi: 10.1146/annurev.es.25.110194.002555

Pardo-Gandarillas, M. C., Ibañez, C. M., Torres, F. I., Sanhueza, V., Fabres, A., Escobar-Dodero, J., et al. (2018). Phylogeography and species distribution modelling reveal the effects of the pleistocene ice ages on an intertidal limpet from the south-eastern pacific. *J. Biogeogr.* 45, 1751–1767. doi: 10.1111/jbi.13362

Paulay, G., and Meyer, C. (2006). Dispersal and divergence across the greatest ocean region: Do larvae matter? *Int. Comp. Biol.* 46, 269–281. doi: 10.1093/icb/icj027

Peck, L. S., Heiser, S., and Clark, M. (2016). Very slow embryonic and larval development in the Antarctic limpet *Nacella polaris*. *Polar Biol* 39, 2273–2280. doi: 10.1007/s00300-016-1894-1

Ponce, J. F., Rabassa, J., Coronato, A., and Borromei, A. M. (2011). Palaeogeographical evolution of the Atlantic coast of pampa and Patagonia from the last glacial maximum to the middle Holocene. *Biol. J. Linn. Soc* 103, 363–379. doi: 10.1111/j.1095-8312.2011.01653.x

Pons, O., and Petit, R. J. (1996). Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics* 144, 1237–1245. doi: 10.1093/genetics/144.3.1237

Poulin, E., González-Wevar, C., Díaz, A., Gérard, K., and Hüne, M. (2014). Divergence between Antarctic and south American marine invertebrates: what molecular biology tells us about Scotia arc geodynamics and the intensification of the Antarctic circumpolar current. *Global Planet. Change* 123, 392–399. doi: 10.1016/j.gloplacha.2014.07.017

Poulin, E., Palma, A. T., and Féral, J.-P. (2002). Evolutionary versus ecological success in Antarctic benthic invertebrates. *Trends Ecol. Evol.* 17, 218–222. doi: 10.1016/S0169-5347(02)02493-X

Powell, A. W. B. (1973). *The patellid limpets of the world (Patellidae)* (New Zealand: Auckland Institute and Museum Auckland), 1–132.

Provan, J., and Bennett, K. D. (2008). Phylogeographic insights into cryptic glacial refugia. *Trends Ecol. Evol.* 23, 564–571. doi: 10.1016/j.tree.2008.06.010

Puritz, J. B., Keever, C. C., Addison, J. A., Barbosa, S. S., Byrne, M., Hart, M. W., et al. (2017). Life-history predicts past and present population connectivity in two sympatric sea stars. *Ecol. Evol.* 7, 3916–3930. doi: 10.1002/ece3.2938

Rabassa, J., Coronato, A., and Martínez, O. (2011). Late Cenozoic glaciations in Patagonia and tierra del fuego: an updated review. *Biol. J. Linn. Soc* 103, 316–335. doi: 10.1111/j.1095-8312.2011.01681.x

Rabassa, J., Coronato, A. M., and Salemme, M. (2005). Chronology of the late Cenozoic Patagonian glaciations and their correlation with biostratigraphic units of the pampean region (Argentina). *J. South Am. Earth. Sci.* 20, 81–103. doi: 10.1016/j.jsames.2005.07.004

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., and Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using tracer. *Syst. Biol.* 67, 901–904. doi: 10.1093/sysbio/syy032

Reisser, C. M. O., Wood, A. R., Bell, J. J., and Gardner, J. P. A. (2011). Connectivity, small islands and large distances: the *Cellana strigilis* limpet complex in the southern ocean. *Mol. Ecol.* 20, 3399–3413. doi: 10.1111/j.1365-294X.2011.05185.x

Riesgo, A., Taboada, S., and Avila, C. (2015). Evolutionary patterns in Antarctic marine invertebrates: an update on molecular studies. *Mar. Genomics* 23, 1–13. doi: 10.1016/j.margen.2015.07.005

Rogers, A. R., and Harpending, H. C. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol.* 9, 552–569. doi: 10.1093/oxfordjournals.molbev.a04727

Ruzzante, D. E., Walde, S. J., Cussac, V. E., Dalebout, M. L., Seibert, J., Ortubay, S., et al. (2006). Phylogeography of the percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciation, and volcanism. *Mol. Ecol.* 15, 2949–2968. doi: 10.1111/j.1365-294X.2006.03010.x

Salzburger, W., Ewing, G. B., and Von Haessler, A. (2011). The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Mol. Ecol.* 20, 1952–1963. doi: 10.1111/j.1365-294X.2011.05066.x

Sá Pinto, A., Branco, M., Sayanda, D., and Alexandrino, P. (2007). Patterns of colonization, evolution and gene flow in species of the genus *Patella* in the macaronesian islands. *Mol. Ecol.* 17, 519–532. doi: 10.1111/j.1365-294X.2007.03563.x

Schneider, S., and Excoffier, L. (1999). Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites. application to human mitochondrial DNA. *Genetics* 152, 1079–1089. doi: 10.1093/genetics/152.3.1079

Segovia, N. I., González-Wevar, C. A., Naretto, J., Rosenfeld, S., Brickle, P., Hüne, M., et al. (2022). The right tool for the right question: contrasting biogeographic patterns in the notothenioid fish harpagifer spp. along the Magellan province. *Proc. R. Soc* 289, 20212738. doi: 10.1098/rspb.2021.2738

Strathmanns, R. R. (1985). Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Ann. Rev. Ecol. Syst.* 16, 339–361. doi: 10.1146/annurev.es.16.110185.002011

Tamura, K., Stecher, G., and Kumar, S. (2022). MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38, 3022–3027. doi: 10.1093/molbev/msab120

Toro, J. E., Ojeda, J. A., and Vergara, A. M. (2004). The genetic structure of *Mytilus chilensis* (Hupe 1854) populations along the Chilean coast based on RAPDs analysis. *Aquat. Res.* 35, 1466–1471. doi: 10.1111/j.1365-2109.2004.01172.x

Trovant, B., Orenzanz (Lobo), J. M., Ruzzante, D. E., Stotz, W., and Basso, N. G. (2015). Scorched mussels (BIVALVIA: MYTILIDAE: BRACHIDONTINAE) from the temperate coasts of south America: phylogenetic relationships, trans-pacific connections and the footprints of quaternary glaciations. *Mol. Phylogenet. Evol.* 82, 60–74. doi: 10.1016/j.ympev.2014.10.002

Valdovinos, C., Navarrete, S. A., and Marquet, P. A. (2003). Mollusk species diversity in the southeastern pacific: why are there more species towards the pole? *Ecography* 26, 139–144. doi: 10.1034/j.1600-0587.2003.03349.x

Valdovinos, C., and Rüth, M. (2005). Nacellidae limpets of southern south America: taxonomy and distribution. *Rev. Chil. Hist. Nat.* 78, 497–517. doi: 10.4067/S0716-078X2005000300011

Villamor, A., Costantini, F., and Abbiati, M. (2014). Genetic structuring across marine biogeographic boundaries in rocky shore invertebrates. *PloS One* 9, e101135. doi: 10.1371/journal.pone.0101135

Violante, R. A., Paterlini, C. M., Marcolini, S. I., Costa, I. P., Cavalloto, J. L., Laprida, C., et al. (2014). "The Argentine continental shelf: morphology, sediments, processes and evolution since the last glacial maximum. in: continental shelves of the world: their evolution during the last glacio-eustatic cycle," in *The geological society of London*. Eds. F. L. Chiocci and A. R. Chivas (UK: Geological Society of London), 55–68. doi: 10.1144/M41.6

Waters, J. M. (2008). Driven by the West wind drift? a synthesis of southern temperate marine biogeography, with new directions for dispersalism. *J. Biogeogr.* 35, 417–427. doi: 10.1111/j.1365-2699.2007.01724.x

Webb, T.III, and Bartlein, P. J. (1992). Global changes during the last 3 million years: climatic controls and biotic responses. *Annu. Rev. Ecol. Evol. Syst.* 23, 141–173. doi: 10.1146/annurev.es.23.110192.001041

Williams, J. W., Summers, R. L., and Webb, III, T. (1998). Applying plant functional types to construct biome maps from eastern north American pollen data. *Quat. Sci. Rev.* 17, 607–627. doi: 10.1016/S0277-3791(98)00014-6

Wilson, P., Bentley, M. J., Schnabel, C., Clark, R., and Xu, S. (2008). Stone run (block stream) formation in the Falkland islands over several cold stages, deduced from cosmogenic isotope (^{10}Be and ^{26}Al) surface exposure dating. *J. Quat. Sci.* 23, 461–473. doi: 10.1002/jqs.1156

Wilson, P., Clark, R., Birnie, J., and Moore, D. M. (2002). Late pleistocene and Holocene landscape evolution and environmental change in the lake sulivan area, Falkland islands, south Atlantic. *Quat. Sci. Rev.* 21, 1821–1840. doi: 10.1016/S0277-3791(02)00008-2

Wilson, N. G., Schrödl, M., and Halanych, K. M. (2009). Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, nudibranchia). *Mol. Ecol.* 18, 965–984. doi: 10.1111/j.1365-294X.2008.04071.x

Xia, X., and Xie, Z. (2001). DAMBE: software package for data analysis in molecular biology and evolution. *J. Hered.* 92, 371–373. doi: 10.1093/jhered/92.4.371

Xu, J., Pérez-Losada, M., Jara, C. G., and Crandall, K. A. (2009). Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufiin* Chilean Patagonia. *Mol. Ecol.* 18, 904–918. doi: 10.1111/j.1365-294X.2008.04070.x

Zattara, E. E., and Premoli, C. (2005). Genetic structuring in Andean landlocked populations of galaxias maculatus: effects of biogeographic history. *J. Biogeogr.* 32, 5–14. doi: 10.1111/j.1365-2699.2004.01164.x

Zemlak, T. S., Habit, E. M., Walde, S. J., Battini, M. A., Adams, E. D. M., and Ruzzante, D. E. (2008). Across the southern Andes on fin: glacial refugia, drainage reversals and a secondary contact zone revealed by the phylogeographical signal of *Galaxias platei* in Patagonia. *Mol. Ecol.* 17, 5049–5061. doi: 10.1111/j.1365-294X.2008.03987.x

frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 30 May 2023
DOI 10.3389/fmars.2023.1190456

OPEN ACCESS

EDITED BY
Stephen John Hawkins,
University of Southampton,
United Kingdom

REVIEWED BY
David Schiel,
University of Canterbury, New Zealand
Natacha Nogueira,
Direção Regional do Ambiente e
Alterações Climáticas, Governo Regional
da Madeira, Portugal

*CORRESPONDENCE
George M. Branch
✉ mbranch@mweb.co.za

RECEIVED 20 March 2023
ACCEPTED 11 May 2023
PUBLISHED 30 May 2023

CITATION
Branch GM, Steffani N, Pfaff MC, Baliwe NG and Zeeman Z (2023) Complex interplays between limpets and alien species in South Africa: multispecies interactions, zonation and size effects.
Front. Mar. Sci. 10:1190456.
doi: 10.3389/fmars.2023.1190456

Complex interplays between limpets and alien species in South Africa: multispecies interactions, zonation and size effects

George M. Branch[1]*, Nina Steffani[1], Maya C. Pfaff[1], Ndiviwe G. Baliwe[1,2] and Zannè Zeeman[1]

[1]Department of Biological Sciences and Marine Research Institute, University of Cape Town, Cape Town, South Africa, [2]Department of Forestry, Fisheries and Environment, Cape Town, South Africa

Integrating observations and experiments, we address the progressive effects of three alien species, the mussels *Mytilus galloprovincialis* and *Semimytilus patagonicus*, and the barnacle *Balanus glandula*, on limpet species in South Africa. We describe four aspects: (1) Interactions among algae, the limpet *Scutellastra granularis* and *M. galloprovincialis*. (2) The influences of *B. glandula* on *S. granularis* and on the periwinkle *Afrolittorina knysnaensis*. (3) Transformation of the zonation of *S. granularis* by the successive arrival of these three aliens. (4) Assessment of how effective the published predictors of the effects of *M. galloprovincialis* have been when applied to *S. patagonicus*. We conclude: (a) *Scutellastra granularis* improves mussel survival and condition by regulating algae that would otherwise overgrow and smother the mussels. (b) *Balanus glandula* has largely occupied the upper half of rocky shores, and at high densities depletes or eliminates suitable bare-rock habitat for *S. granularis*. However, it has positive effects on another gastropod, *A. knysnaensis.* (c) *Mytilus galloprovincialis* dominates the midshore and has positive effects on the recruitment of *S. granularis*, but negatively influences adults of this limpet, so that its size composition, density, reproductive output and zonation are all altered by this mussel. (d) *Semimytilus patagonicus* presents different challenges to those created by *M. galloprovincialis*, settling at much greater densities, lower down the shore, and reaching smaller maximum sizes. Rather than generating a favourable habitat for epizootic *S. granularis* recruits and juveniles, it almost completely excludes that limpet. We conclude that the influences of alien species are not readily predictable, depending on the nature of the invader and recipient species, environmental conditions and complex interactions among species. Collectively, the three alien species now cover almost all zones on wave-exposed rocky shores, completing the 'zonation squeeze' on limpets, but are less influential on wave-sheltered shores and in years when recruitment is low.

KEYWORDS

barnacles, competition, facilitation, invasive, mussels, patellids, algae, rocky shores

1 Introduction

This paper deals with three of the alien species that have occupied the shores of southern Africa, namely *Mytilus galloprovincialis* (Mediterranean mussel), *Semimytilus patagonicus* (bisexual mussel) and *Balanus glandula* (Pacific barnacle), and their interactions with the indigenous limpets, particularly *Scutellastra granularis*, as well as the effects of *B. glandula* on the indigenous periwinkle *Afrolittorina knysnaensis*.

All of these alien species have become dominant on southern African rocky shores and have spread substantially from their points of origin. *Mytilus galloprovincialis* arrived in the late 1970s (Grant and Cherry, 1985) and now covers over 2000 km of the coast from northern Namibia to the south-east coast of South Africa. *Balanus glandula* was first formally recorded in South Africa in 2008 but probably arrived as early as 1992 (Jenkins et al., 2008; Laird and Griffiths, 2008; Simon-Blecher et al., 2008). It currently occurs over 500 km of the southern west coast. *Semimytilus patagonicus* (until recently known as *S. algosus*) originated from Chile, arrived in Namibia as early as 1930 and abruptly spread from there to South Africa in 2009, where it now occurs over almost the entire west coast and has penetrated to a limited extent on the south coast (De Greef et al., 2013; Alexander et al., 2015a; Ma et al., 2020a; Ma et al., 2020b; Zeeman et al., 2020).

The sequential arrival of these species at Marcus Island in Saldanha Bay on the West Coast is documented by Robinson et al. (2007) and Sadchatheeswaran et al. (2015; 2018), who showed that (a) after its arrival, *M. galloprovincialis* formed deep beds and increased habitat complexity, consequently augmenting community diversity; (b) *S. patagonicus* failed to have these effects, and (c) in zones where *B. glandula* later replaced *M. galloprovincialis*, the simpler monolayered structure of this barnacle substantially reduced complexity and diversity.

The interactions between *M. galloprovincialis* and the indigenous brown mussel *Perna perna* on the south coast of South Africa have attracted particular attention (Erlandsson et al., 2006; Zardi et al., 2006; Hanekom, 2007; Nicastro et al., 2007; Zardi et al., 2007; Nicastro et al., 2008; von der Meden et al., 2008; Nicastro et al., 2010a; Nicastro et al., 2010b; von der Meden et al., 2010; Lathlean et al., 2016; synthesized in Branch and Branch, 2018). In brief, survival of *M. galloprovincialis* is greater higher on the shore, and that of *P. perna* lower on the shore, hence their partial segregation and continued coexistence.

Interactions between *M. galloprovincialis* and the limpet *S. granularis* have also been explored (Griffiths et al., 1992; Hockey and van Erkom Schurink, 1992; Branch et al., 2010). In short, dense beds of the mussel provide a haven for recruits of the limpet, boosting its numbers; but adults of the limpet are crowded out when they become too large to occupy mussel shells (Supplementary Figure S1). The demographics and normal zonation pattern of the limpet have consequently been transformed. Before the arrival of *M. galloprovincialis*, the limpet was one of a group of limpets that Branch (1975; 1976) termed 'migratory', in the sense that they settle low on the shore and then progressively migrate upshore as their size and tolerance to physical to physical stresses increases. Arrival of *M. galloprovincialis* has overturned that, as we describe in this paper.

Alien species are one of the major threats to the integrity of marine systems, increasingly transforming and homogenizing the nature of ecosystems world-wide (Thompson et al., 2002; Robinson et al., 2007; Pyšek et al., 2020), being 'a potent driver of change with no sign of saturation' (Ros et al., 2023, p81). They are amply living up to the prognosis for the future made in 2008 that by 2025 their rate of spread would have radically increased (Branch et al., 2008a). In South Africa alone, the rate at which alien species have been recorded has escalated exponentially from just one in 1950 to 95 by 2023 (Robinson et al., 2005; Griffiths et al., 2009; 2010; Mead et al., 2011a; Mead et al., 2011b; Robinson et al., 2016; Robinson et al., 2020; Van Wilgen et al., 2023).

Jennsen (2015) expresses a pessimistic view: 'You have loosed a chaotic, unstable element into the Mosaic. They will destroy everything.' The effects of alien species are, however, not all unmitigatedly negative. Some, such as the ascidian *Pyura praepucialis* in Chile increase habitat complexity and enhance biodiversity as a result (Castilla et al., 2004). Arrival of the Mediterranean or blue mussel *Mytilus galloprovincialis* in South Africa has boosted the food supplies of the African Black Oystercatcher *Haematopus moquini*, improving its conservation status by trebling of the rate at which pairs of birds raise two chicks rather than one (Hockey and van Erkom Schurink, 1992; Coleman and Hockey, 2008).

A central focus of our paper is that alien species have complex and unpredictable effects on the structure and functioning of South African rocky shores. Part of the reason is that their effects of are moderated by a range of factors, including geographic distribution (Ma et al., 2023), zonation (Rius and McQuaid, 2006), wave action (Steffani and Branch, 2003a; Steffani and Branch, 2003b; Hampton and Griffiths, 2007; Branch et al., 2010), responses to predators (Alexander et al., 2015a), resistance to desiccation (Nicastro et al., 2010a), attachment strength (Zeeman et al., 2018), the effects of upwelling (Xavier et al., 2007), and the relative sizes of organisms (Branch and Steffani, 2004).

A second focus of the paper is the interplay of patellid limpets with alien species. Patellids are dominant grazers and can influence alien species either directly by removing them or indirectly by controlling other species such as algae that may influence them. Firth (2021, p30) describes limpets as 'the champion grazers of the rocky intertidal zone'. From the pioneering experimental removals of *Patella* spp. in Great Britain (Jones, 1946; Lodge, 1948) that demonstrated limpet grazing and not wave action was responsible for keeping algal growth in check, through multiple other experiments, removal of limpets has consistently resulted in algal proliferation (Southward, 1964; Branch, 1981; Hawkins and Hartnoll, 1983; Branch, 1985; Farrell, 1988; Dye, 1995; Jenkins et al., 1999; Lindegarth et al., 2001; Arrontes et al., 2004; Phillips and Hutchison, 2008; Maneveldt et al., 2009; Tejada-Martinez et al., 2016). A common pattern is that removal of limpets is followed sequentially by development of a film of diatoms and sporelings, then emphemeral algae such as *Ulva* spp., and finally slower-growing corticated algae (Hawkins, 1981).

Added to this, limpets can act is 'bulldozers', reducing the abundance of small settlers of species such as barnacles (Menge et al., 2010; Ellrich et al., 2020). The situation is even more complex when other members of the community indirectly influence the outcome of interactions. Barnacles may aid settlement and survival of algae, but algal settlement can increase the mortality rate of the barnacles and enhance recruitment of limpets that then deplete the algae (Hartnoll and Hawkins, 1985). Cycles may arise in which the abundances of algae and limpets alternate over roughly 10-year periods (Little et al., 2017).

Considering the multiplicity of factors influencing the effects of both alien species and grazers, it is not simple to forecast their precise interactions, which can range from competitive exclusion to facilitation (Miyamoto and Noda, 2004).

This paper addresses four aspects of interaction between alien species and limpets on South African shores. The first concerns a three-way interplay between *M. galloprovincialis*, algae and *S. granularis*. Grazing by the limpet on shells of the mussel may control algal growth, but the benefits (or otherwise) of this for the mussel are not easy to predict, as grazing on the mussel shells weakens them (Day et al., 2000) and may offset any benefits. Second, the influence of *B. glandula* on the abundances of both *S. granularis* and the periwinkle *Afrolittorina knysnaensis* is explored. The latter was selected because of previous correlative evidence of the influence of *B. glandula* on it (Laird and Griffiths, 2008), which we verify experimentally. Third, the influences of *M. galloprovincialis*, *S. patagonicus* and *B. glandula* on the zonation of *S. granularis* are described. Finally, we turn to predictions previously made about why *M. galloprovincialis* has successfully dominated large portions of the coast of southern Africa (Branch and Steffani, 2004) and, drawing on both our results and information in the literature, we consider the applicability of these predictions when applied to the later arrival and spread of *S. patagonicus*.

We address four specific hypotheses:

1. Grazing by the limpet *S. granularis* will benefit *M. galloprovincialis* by preventing algal overgrowth. Conversely, the mussel will support high densities of *S. granularis*, but only small individuals.
2. Dense settlements of the alien barnacle *B. glandula* will exclude *S. granularis* but will enhance densities of the periwinkle *A. knysnaensis*.
3. The 'normal' progressively upshore migration of *S. granularis* to establish an increasing gradient of size and a decreasing gradient of density will be interrupted by the arrival of *M. galloprovincialis* and further altered by *S. patagonicus* and *B. glandula*.
4. Factors that successfully predicted the invasive capabilities of *M. galloprovincialis* will not be the same as those applicable to *S. patagonicus*.

2 Materials and methods

2.1 Interactions among limpets, mussels and algae

Manipulative experiments were conducted at two sites just south of Groenriviermond on the west coast of South Africa (Figure 1), approximately 450 km north of Cape Town: Island Point (30°54.93´S; 17°36.17´E) and Nina's Site (30°54.88´S; 17°36.28´E), from January to June 2000, after which the plots were destructively sampled, and measurement of the variables taken as described below. Steffani and Branch (2003a) class both sites as exposed, experiencing average wave forces of 10.0-12.0 × 10^3 N m^{-2}, and they fall in an area of active upwelling with high productivity (Andrews and Hutchings, 1980), and high algal, limpet and mussel biomass (Bustamante et al., 1995b). Tidal range was 1.9 m.

Five replicate plots per treatment of 30×30 cm were established at each site (five replicates × four treatments × two sites). These were installed at the mid-tide level (tidal height 0.9 m), where *M. galloprovincialis* comprised 97% of all mussels present, and the limpet *S. granularis* constituted 95% of all limpets present. Plots that were designed to exclude limpets were surrounded by 5-cm-wide band of lead oxide antifouling paint, to control ingress or egress of limpets. Undisturbed control plots and three treatments were employed, as visualized in Figure 2A: (1) Control plots that contained both mussels and limpets (+M+L). (2) Limpet removal plots from which all limpets were removed (+M–L) and exclusion maintained by ongoing monthly removals. (3) Mussel removal plots (–M+L) in which mussels were removed, but any limpets that entered were allowed to remain there. (4) Removal of both mussels and limpets (–M–L). Monitoring took place monthly, when plots were inspected and the required treatments maintained; but only data for the terminal sampling are presented, partly to avoid temporal pseudo-replication, but also because that was when the plots were sampled destructively to allow full assessment.

The outcomes of these treatments were assessed by measuring: (a) algal % cover and composition; (b) mussel condition and percentage survival relative to original densities at the start of the experiment; (c) limpet sizes, densities, reproductive output for the populations (wet g m^{-2}), and life-time reproductive output per individual (wet g indivual^{-1}). For all variables except condition and reproductive output, scoring was done *in situ* and (where feasible) a second estimate obtained using photographs of plots. The greater of these two values was accepted as being the most accurate. Population reproductive output was calculated from the counts and size composition of limpets measured per plot, converting sizes to ages using regressions taken from data in Branch (1975), and ages to wet-mass reproductive output per annum per plot, based on conversions derived from data in Branch (1974a). Life-time outputs per individual were calculated for standard individuals occupying mussels or rock, taking into account average survivorship per year, and outputs per annum summed over a lifespan, also based on survivorship and size-related annual reproductive outputs in

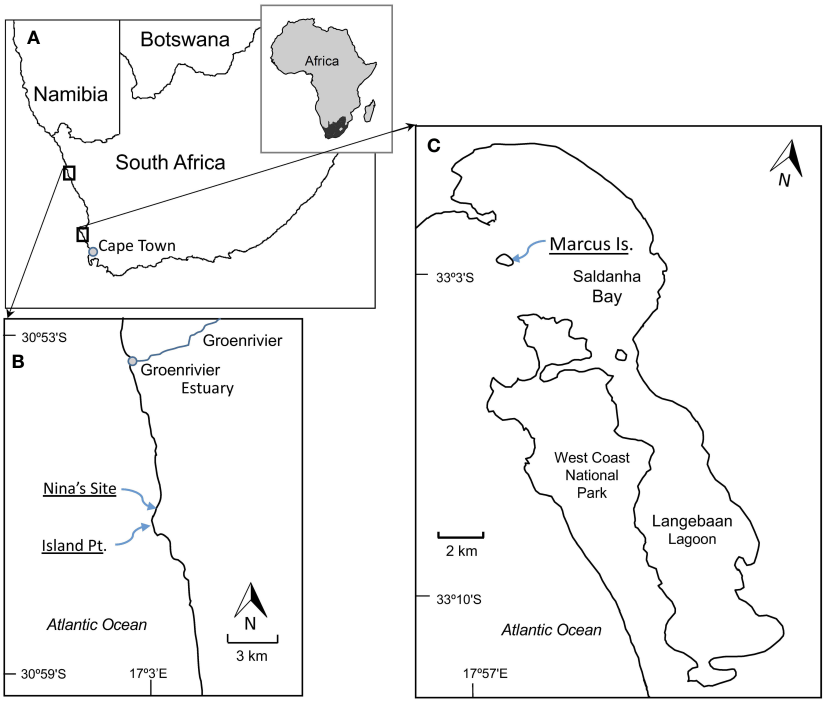


FIGURE 1
Maps of Africa and South Africa **(A)** and details **(B, C)** of the study sites (names underlined).

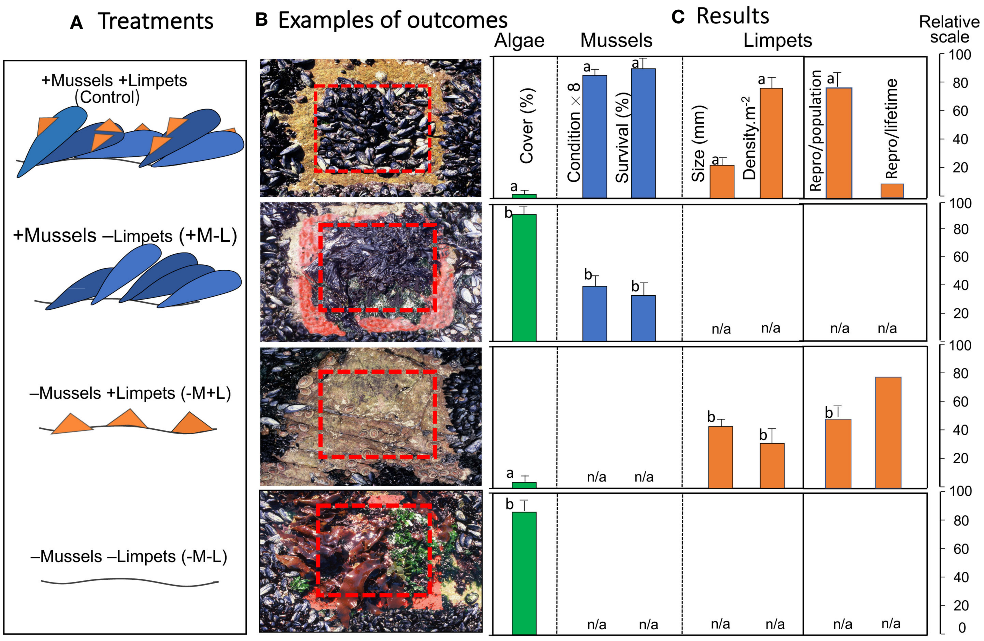


FIGURE 2
(A) Design of a field experiment to assess the interplay between the alien mussel *M. galloprovincialis*, the limpet *S. granularis* and algae. **(B)** Visual examples of outcomes. **(C)** Results showing the proliferation of algae in absence of limpets, better condition and survival of mussels in presence of limpets, and a drastic decline in lifetime reproductive output of (smaller) limpets that live on mussels versus rock, despite their greater densities. Data are mean values +1SD, n = 10; except for reproductive output per lifetime, which is a single calculated value. The data are standardised to the same scale of 0-100 in units specified for each variable, but values for condition have been multiplied by a factor of eight to improve visibility. Repro/population = the reproductive output (g wet mass m^{-2} yr^{-1}) of the population, calculated from replicate plots. Repro/lifetime = the reproductive outputs (g wet mass) per individual calculated over its lifetime, assuming individuals remain on shells or on rocks. No error term is attached to this value as it constitutes a single calculation. n/a, not applicable due to the absence or one or more taxa from the treatment. Lower case letters above bars differ if treatment were significantly different.

Branch (1974a; 1974b). These two measures are distinctly different and not necessarily correlated. The population reproductive output estimates the output per annum per unit area for each plot, so that a mean (and SD) value can be calculated for the population as a whole. The life-time reproduction is a single composite measure of what an individual (standard) limpet will achieve over its estimated lifespan. The former measure is relevant to population dynamics; the latter to the evolutionary reproductive success of individuals that remain on mussels or live on rocks. Both can be calculated for limpets on either mussels or rocks, making the assumption that individuals will remain there throughout their lives.

Mussel condition was determined from subsamples of 20 mussels (40-50 mm shell length) per plot taken at the end of the experiment and was measured as (dry) body mass divided by shell mass × 100 (Steffani and Branch, 2003a). For mussel survival, only mussels classed as adult, i.e., > 35 mm shell length, were counted, as those smaller than that were too difficult to score accurately at the start of the experiment. For the same reason, for limpet densities, only individuals > 10mm were counted.

To test for possible ‘cage’ (i.e., paint) effects on limpet presence or absence and algal growth, procedural controls with paint applied just at the corners of plots were established. We hypothesized entry of limpets would not be precluded by the paint, and that if limpets did enter the plots, they would control algal growth, but that if they did not, algal growth would proliferate to the same extent as in fully enclosed plots excluding limpets.

2.2 Barnacles, limpets and periwinkles

Interactions between *B. glandula* and *S. granularis*, and between *B. glandula* and the littorinid *Afrolittorina knysnaensis* were assessed at Marcus Island [33°02.59´S; 17°58.26´E; Figure 1)], starting April 2012, shortly after the barnacle was detected there, during a period when it covered extensive portions of the mid to high shore at tidal heights of 0.99 to 1.39 m above spring low-tide levels (SLT). Tidal range was 1.96 m and wave force rated as exposed, averaging 11.3×10^3 N m^{-2} (Sadchatheeswaran et al., 2015).

Interactions were assessed in two ways: (a) by first determining correlations between the numbers of barnacles and either limpets or littorinids, which provided information on natural patterns of relative abundance, and (b) by using manipulative experiments that followed immediately after the correlative data had been collected, to experimentally explore the responses of limpets or littorinids to barnacle cover, providing an independent cause-and-effect test of the relationship between barnacles and these species.

Correlations between the abundances *B. glandula* (% cover) and *S. granularis* and *A. knysnaensis* (counts) were determined from 50 randomly placed 14.1 × 14.1 cm quadrats, in the mid shore for *S. granularis*, and high shore for *A. knysnaensis*. Densities were converted to numbers m^{-2} for presentation.

For the manipulative experiment, six replicates of three treatments were established in plots of 20 × 20 cm at these two respective shore heights, in each of two areas separated by 50 m. Treatments comprised (a) control plots of in which *B. glandula* covered 95-100% of the substratum (Supplementary Figure S2), which were left unaltered (+*Balanus*), (b) removal plots of the same size in which 50% of the barnacles were removed, and (c) all barnacles were removed by scraping to bedrock (-*Balanus*). Numbers of any *S. granularis* that occupied plots were recorded after 1 month, and sizes estimated from either the total sample, or from a subsample of the first 50 individuals encountered per plot if numbers exceeded this. Subsampling was employed only after test sampling showed that 50 individuals were sufficient to secure a mean measure within 3% accuracy of that from total samples of up to 90 individuals.

2.3 Effects of alien species on zonation of *S. granularis*

To test the extent to which the arrival of *M. galloprovincialis*, *S. patagonicus and B. glandula* has altered the previously described progressive ‘migratory’ up-shore movement of *S. granularis* (Branch, 1975; see also Table 1, and hypothesis 3 in introduction), data on the densities and sizes of *S. granularis* were obtained from Marcus Island in April 1970 before the detection of any alien species; in April 2001 after *M. galloprovincialis* had become established and was at its peak abundance, and in April 2012 when *M. galloprovincialis* had diminished but *S. patagonicus* and *B. glandula* were established. On five replicate vertical transects up the shore and within quadrats of 1.0 × 0.5 m, five zones were surveyed, designated as Infratidal (0.0-0.1 m above SLT), Low (0.25-0.30 m), Mid (0.85-0.95 m), High (1.10-0.26 m) and Top (1.30-0.1.56 m). Densities were converted to numbers m^{-2}.

2.4 Predictors of the invasive success of *S. patagonicus*

To assess the features that could be used to predict successful invasions of respectively *M. galloprovincialis* and *S. patagonicus*, we integrated our results with published material (mainly Branch and Steffani, 2004; Zeeman et al., 2018, but also papers cited in Table 2), based on a literature search of journal articles in google and scholar.google.com for key words *Semimytilus algosus, Semimytilus patagonicus*, Mytilidae, mytilids, mussels.

2.5 Statistical analyses

For interactions among limpets, mussels and algae at Groenrivier, a two-way analysis of variance (ANOVA) with treatments nested in sites showed that differences between sites were non-significant for all variables: $ANOVA_{1,4}$, $p > 0.25$, so the data for the two sites were pooled (Quinn and Keough, 2002). In the case of algal cover, for which four treatment levels could be compared, the assumptions of normality and equality of variance for ANOVA could not be met even after transformations, so a non-parametric Kruskal-Wallis test was applied, followed by Dunn *post-hoc* comparisons among treatments. For the remaining variables

TABLE 1 (A) Summary of features characterising migratory and non-migratory limpets, derived from Branch (1975) and Lindberg (2007), and (B) how these have changed (bold entries) or not changed for *Scutellastra granularis* after the arrival of first *Mytilus galloprovincialis*, and later *Semimytilus patagonicus* and *Balanus glandula*.

(A)	Features of Migratory species	Features of Non-migratory species
	1. Settle low on shore, then shift upshore. Density drops but size increases upshore	1. Settles low on shore, remain there for life. Densities and sizes unrelated to zonation
	2. None are territorial	2. Many are territorial
	3. None 'garden' algal patches	3. Many 'garden' algal patches
	4. Diets are generalized and diverse	4. Diets specialised and narrow
	5. Growth often fast, gonad output high, longevity low	5. Growth usually slow, gonad output low, longevity high
	6. No differentiation between juveniles and adults	6. Strong differentiation between juveniles and adults
(B)	Features of *S. granularis* zonation in 2001, post-*Mytilus* arrival	Features of *S. granularis* in 2012 post-*Semimytilus* & *Balanus* arrival
	1. Settles mid- to high shore in *Mytilus* beds, shifts up and down with age	**1. Settles mid shore in *Mytilus* beds, shifts upshore; excluded from the low shore**
	2. Not territorial	2. Not territorial
	3. Does not 'garden' algal patches	3. Does not 'garden' algal patches
	4. Diet generalised and diverse	4. Diet generalised and diverse
	5. Growth slower in *Mytilus* beds, reproduction and longevity reduced there	**5. Growth slower in mussels; reproduction, density and longevity very low in *Semimytilus* zones**
	6. Strong differentiation between juveniles on *Mytilus* and adults on bare rock	**6. Juveniles on *Mytilus*; excluded from low shore by *Semimytilus*, Adults in top-shore**

where only two treatment levels existed (mussel condition/survival, limpet densities/sizes, population reproductive output), the assumptions of normality (assessed with normal probability plots) and of equal variances (accepted if ratios of variance did not differ by more than a factor of five; Quinn and Keough, 2002) were met without transformation, and Students t-tests applied. Life-time individual reproductive outputs were calculated for idealized individuals, thus yielding single values for limpets on mussels versus rocks, so no statistical tests of their difference were possible.

For the field-sampled relationship between *B. glandula* on the densities of respectively *S. granularis* and *A. knysnaensis*, Pearson's correlation coefficients were determined. For the manipulative experiment, differences between the two areas were again non-significant ($\text{ANOVA}_{1,5}$ $P > 0.25$ in all cases) so the data were pooled for analysis. Densities of *S. granularis* could not be normalized and the variances remained unequal even after transformation because there were multiple zero values for the +*Balanus* treatment. We therefore applied Kruskal-Wallis tests followed by Dunn *post-hoc* comparisons among treatments. For *Afrolittorina knysnaensis* abundance, assumptions of equality of variance and normality (assessed as above) were met and the data analyzed without transformation. For sizes, normality and equality of variance were achieved by log-log transformations and one-way ANOVAs were applied to those data, followed by Tukey *post-hoc* tests of any significantly different data.

To test for differences in densities and for sizes of *S. granularis* amongst the five zones, the years (1970, 2001 and 2012) and their interaction, a two-way ANOVA was run with years and zones as fixed factors, using the aov function in R (R Core Team, 2019). For density, the data were log (x+1) transformed to meet assumptions of normality and equality of variance. The data for sizes did not require transformation, and were averaged for each quadrat (i.e., within each zone and transect) to achieve a balanced ANOVA design. No limpets were present in three of the five replicate samples in the infratidal zone and in these cases missing size data were substituted with dummy values without altering the mean and variance of the model cell. Tukey HSD was used for posthoc comparisons.

3 Results

3.1 Interactions among limpets, mussels and algae

There was no significant 'cage' (i.e., paint) effect. Limpets entered cage control plots in 7/10 cases, and inhibited algal growth, the abundance of which remined low and did not differ significantly from the respective +M+L and –M+L treatments (t-tests, df =8, p > 0.05 in all treatments). For the remaining control plots (3/10), limpets failed to enter, algae flourished and reached levels not significantly different from +M–L and –M–L treatments (t-tests, df =8, p > 0.05 in all treatments). In short, limpets were not inhibited from entering by partial 'cages', and algal growth depended on the presence or absence of limpets, not on the extent of the paint enclosure.

In the experimental plots that were fully enclosed with paint, the limpet *S. granularis* never entered plots from which they had been removed. In plots containing limpets, their densities remained within 85-100% of initial densities. Thus, the –L and +L conditions were effectively maintained.

In plots with the limpet *S. granularis* present (+M+L and -M+L), minimal amounts of algae became established, whereas plots from which the limpet was excluded (+M-L and -M-L) developed a prolific cover of close to 100% (Figure 2). The differences were significant (Kruskal-Wallis H = 29.95, df = 36; p < 0.0001), and Dunn *post-hoc* tests showed that significant differences (p < 0.05) were restricted to the two +L treatments versus the two -L treatments.

In plots that contained mussels, body condition and survivorship of mussels were both significantly lower in –L treatments, in which the mussels were covered by a dense algal growth, than in +L plots (Figure 2), with the differences being significant (t-tests = 20.18 and 28.58 respectively, df = 18, p < 0.0001 in both cases).

Limpet sizes were significantly smaller, and limpet densities significantly greater, in plots that contained mussels than in those lacking mussels (Figure 2; t-tests = 8.28 and 9.79 respectively, df =18, p < 0.0001 in both cases). Limpet reproductive output calculated per unit area for the population on mussels was about 1.33 times that of limpets on rocks in areas without mussels, the difference being significant (t-test = 2.58, df = 18, p = 0.018). Conversely, life-time reproductive output of those on rocks was about 7.9 times greater than that of limpets on mussels (Figure 2).

3.2 Barnacles, limpets and periwinkles

In the mid shore to high shore at Marcus Island, densities of *S. granularis* were significantly negatively correlated with those of the alien barnacle *B. glandula*, whereas those of the littorinid *Afrolittorina knysnaensis* were significantly positively correlated with *B. glandula* (Figure 3).

In the experimental plots in which the barnacles were left untouched, thinned by 50% or removed altogether (Figure 4A), the densities and sizes of *S. granularis* that became established were significantly different among all three treatments (Respectively Kruskal-Wallis H = 21.210, df = 2, n = 26, P < 0.0001, Dunn *post-hoc* tests P < 0.05 among all three treatment for sizes; and ANOVA $F_{2,27}$ = 66.807, P < 0.0001 and Tukey posthoc tests P < 0.05 among all treatments for densities).

For *A. knysnaensis* (Figure 4B), densities in the three treatments differed significantly (Kruskal-Wallis H = 25.865, df = 2, n = 30, P < 0.0001; Dunn *post-hoc* test P < 0.05 among all three treatments). Sizes did not, however, differ among treatments (ANOVA F = 0.059, df = 2,27, P = 0.94).

3.3 Effects of alien species on zonation of *S. granularis*

The densities of *S. granularis* on Marcus Island were significantly affected by an interaction between zones and years (ANOVA, F = 34.52, DF = 8,60, P <0.0001). *Post-hoc* Tukey tests showed that this was because of a shift from a progressive decrease in limpet density up the shore in 1970, to (a) a concentration in the mid and high shore, where the limpet increased from around 20 m^{-2} in 1970 to about 65 m^{-2} in 2001 when *M. galloprovincialis* had become dominant, and (b) declines in abundance of *S. granularis* in 2012 to about 2 m^{-2} in the infratidal and low-shore zones (Figure 5A) with the arrival and establishment of *S. patagonicus*, and to around 5-10 m^{-2} in the high zone associated with declines in *M. galloprovincialis* abundance and the range of its zonation, and the establishment of *B. glandula* in the high shore.

The sizes (shell lengths) of *S. granularis* (Figure 5B) also changed among years and zones with a significant interaction (ANOVA, F = 226.1, DF = 8,60, P <0.0001), which affected interpretation of the effects of year and zone. The interaction arose mainly from a reversal in of the 1970 pattern of an upshore increase in size, to one with smallest sizes in the mid and high shore, and then a restoration in 2012 to close to the original 1970 pattern of zonation. Tukey *post-hoc* tests showed this was attributable to (a) declines in the sizes of limpets in the mid to top-shore in 2001, associated with the occupation of *M. galloprovincialis* shells by limpet recruits and juveniles in the mid and high shore, and their

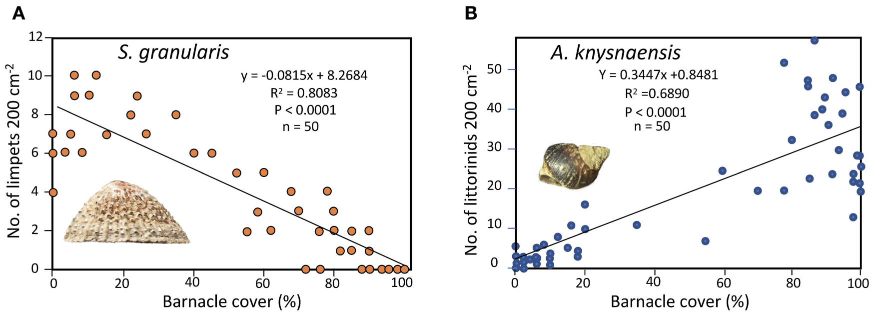


FIGURE 3
Densities of **(A)** the limpet *Scutellastra granularis* and **(B)** the littorinid *Afrolittorina knysnaensis* relative to the percentage cover of the alien barnacle *Balanus glandula* at Marcus Island, South Africa.

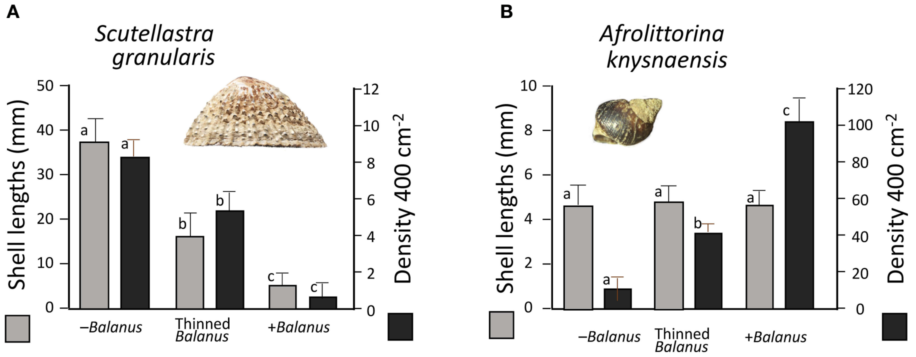


FIGURE 4
The sizes and densities of **(A)** *Scutellastra granularis* and **(B)** *A. knysnaensis* at Marcus Island, in relation to the three treatments of −Balanus (complete removal of barnacles), Thinned Balanus (removal of 50%) and +Balanus (natural density of barnacles close to 100%). Values are means +1SD; lowercase letters differ for treatments that were significantly different on *post-hoc* tests.

migration from there into both the infratidal and the top-shore zones, (b) radical reductions of sizes of the limpet in 2012 in the infratidal and lowshore, coinciding with the arrival of *S. patagonicus*, and (c) increases in size in the mid- to high shore with a reduction in the cover of *M. galloprovincialis* and arrival of *B. glandula* in the mid to high shore. Sizes in the top-shore, which was above the zonation range of any of the alien species, remained the highest or the second-highest values across all three periods.

In the context of classifying limpets as either migratory or non-migratory, Table 1A summarises the characteristics of these two groups, and Table 1B lists how the zonation patterns of *S. granularis* have changed from before to after the arrival of alien species.

3.4 Predictors of the invasive success of *S. patagonicus*

Table 2 synthesises nine potential predictors of invasion success. All proved applicable to *M. galloprovincialis*; but only seven were relevant for *S. patagonicus*, as we amplify in the discussion.

4 Discussion

As McQuaid and Arenas (2009, pp 316-317) note: ‘the effects of invasive species are highly dependent on their ecological context …

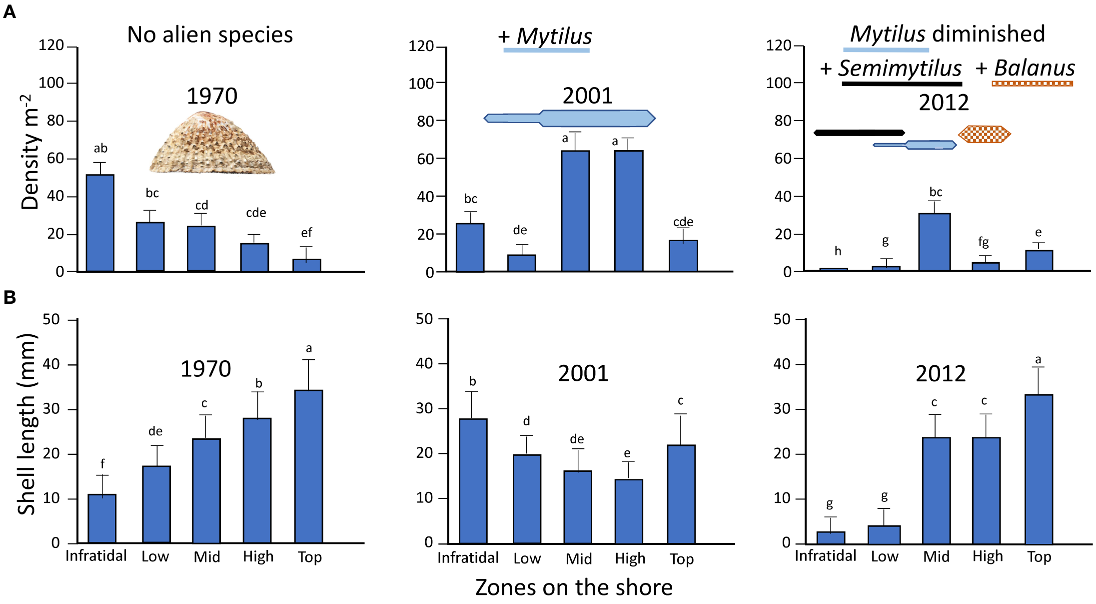


FIGURE 5
Densities **(A)** and sizes **(B)** of the limpet *S. granularis* in relation to zonation, in 1970 prior to arrival of alien species, in 2001 after arrival of *M. galloprovincialis* and in 2012 when *M. galloprovincialis* had diminished and *S. patagonicus* and *B. glandula* had arrived. Kite diagrams non-quantitatively show the relative zonation and abundance of alien species, coded by species. Values are means +1SD. Lower-case letters are shared if values are not significantly different.

TABLE 2 Efficacy of applying the characteristics of *Mytilus galloprovincialis* to predict its invasive capabilities and effects as an alien species, compared to their efficacy when applied to *Semimytilus patagonicus.*

Predictors of invasion success by features of the species	Applicability to *Mytilus*		Applicability to *Semimytilus*	
1. History of invasiveness	✓	Mediterranean and European NE Atlantic origin: now world-wide in temperate seas[1]	✗	Native to Ecuador, Peru, Chile. Arrival in Namibia 1931. Abrupt arrival and rapid spread in South Africa 2009[12]
2. Physiological superiority	✓	Rapid growth, desiccation-tolerant[2]; fast uptake of food[3]	✗	Slow growth, desiccation intolerant; moderate rate of food intake[14]
3. Reproduction, recruitment and dispersal	✓	High reproductive output, high settlement, fast dispersal[4]	✓✓	High reproductive output; exceptionally high recruitment; rapid spread[13]
4. Better performance at strong wave action	✓	Faster individual & population growth, greater abundance and greater settlement at strong but not extreme wave action[5]	✓	Densities and sizes greatest at localities with strong wave action[15]
5. Provide more food for predators	✓	Abundances of oystercatchers and whelks increased since arrival of mussel[6]	✓	Consumed by gulls[16]; but unlikely to affect gull numbers as they are increasing due to use of human sources of food[17]
6. Sizes of mussel and limpets affect interactions	✓	Large enough and sufficiently longlived to support small limpets but not large individuals or species[7]	✓	Too small and short-lived to support epibiotic limpets[18]
7. Zonation affects interaction with native species	✓	Dominates mid to high-shore zones; often excluded from lowshore by wave action due to weak attachment[8]	✓	Dominates low on the shore; excluded from higher zones by intolerance of physical stress[19]
8. Escape from predation	✓	Predation rates incapable of controlling mussel due to high recruitment rate[9]; individual whelks, lobsters and starfish prefer indigenous mussels, but conditioning changes the lobsters' preference to alien species, and in combination lobsters and whelks alter prey preferences[10]	✓	Heavily predated upon by gulls & oystercatchers; but predators incapable of limiting mussel populations[20]
9. Freedom from parasites	✓	Low rate of parasitism compared with *Perna*[11]	✓	Parasite load nil to extremely low[21]

[1] Branch and Steffani, 2004; Zardi et al., 2018; Lins et al., 2021.

[2] van Erkom Schurink and Griffiths, 1993; Branch and Branch, 2018; Zeeman et al., 2018.

[3] Alexander et al., 2015b.

[4] van Erkom Schurink and Griffiths, 1991; Reaugh-Flower et al., 2011; Branch and Branch, 2018; Zeeman et al., 2018.

[5] Steffani and Branch, 2003a; Steffani and Branch, 2003b; Steffani and Branch, 2003c; Branch and Steffani, 2004; Hammond and Griffiths, 2003; Rius and McQuaid, 2006; Branch et al., 2008b; Pfaff et al., 2011; Hoffmann et al., 2012; Skein et al., 2018a

[6] Hockey and van Erkom Schurink, 1992; Branch and Steffani, 2004.

[7] Griffiths et al., 1992; Hockey and van Erkom Schurink, 1992; Branch and Steffani, 2004.

[8] Bownes and McQuaid, 2006; Bownes and McQuaid, 2010; Rius and McQuaid, 2006; Rius and McQuaid, 2009; Zardi et al., 2007; Hoffmann et al., 2012.

[9] Branch and Steffani, 2004; Reaugh-Flower et al., 2011; unpublished experiments manipulating whelk densities.

[10] Alexander et al., 2015b; Robinson et al., 2015; Skein et al., 2018b; Skein et al., 2020; Alexander et al., 2022.

[11] Calvo-Ugarteburu and McQuaid, 1998a; Calvo-Ugarteburu and McQuaid, 1998b.

[12] De Greef et al., 2013; Ma et al., 2020a; Ma et al., 2020b; Zeeman et al., 2020.

[13] Branch and Branch, 2018; Zeeman et al., 2018.

[14] De Greef et al., 2013; Zeeman et al., 2020.

[15] De Greef et al., 2013; Zeeman et al., 2020.

[16] Personal observations.

[17] Steele, 1992.

[18] Current paper.

[19] De Greef et al., 2013; Zeeman, 2016; Skein et al., 2018a.

[20] Calculation based on per capita consumption and abundance.

[21] GMB and ZZ personal observations.

(Derived from Branch and Steffani, 2004, with two additional features.).

Tick = applicable; double tick = strongly so, and crosses = inapplicable.

difficult to predict … idiosyncratic and context-dependent'. Indeed, that was true in our results, with differences emerging even between the two invasive mussels, despite their being members of the same family, Mytilidae. Nevertheless, key principles emerged about the factors influencing the effects of the alien species:

1. The interactions were between organisms with very different trophic needs and lifestyles: sessile phototrophic algae, mobile herbivorous grazers, and sessile filter feeders. Space was the common resource over which competition took place. Space is an absolute requirement (Branch, 1984) and if it is insufficient, exclusion of subordinate competitors is likely. However, offsetting this, intertidal organisms can provide secondary space for epibionts even if they dominate primary space.
2. Size matters. Occupation of secondary space on shells depends on the relative size of the host and the epibiont.
3. Abundance powerfully affects competitive capability, and is influenced by settlement and recruitment rates, which can vary temporally and spatially, often with considerable vagaries (Underwood and Fairweather, 1989).
4. Ecological traits have proved valid predictors of the competitive advantages and invasive capabilities of *M. galloprovincialis* (Branch and Steffani, 2004). These include tolerance to thermal stress and desiccation, which can set differential vertical zonation patterns and potentially lead to spatial segregation that ameliorates competition. Differential adaptations to wave action and upwelling may determine horizontal differences in abundance, again moderating competition. In the concluding section of this discussion, we revisit the conclusions of Branch and Steffani (2004) to inquire how many of the successful predictions made about the invasive capability of *M. galloprovincialis* are applicable to *S. patagonicus*.

We explore these perspectives below, in the context of the four experimental and comparative cases we examined.

4.1 Interactions among limpets, mussels and algae

The capacity of limpets to control algal growth is amply demonstrated by a plethora of limpet removal experiments (see references in the introduction). In addition, evidence arises from marine protects areas (MPAs) in which protection from harvesting has increased limpet abundances and consequently decreased algal abundance (Micheli et al., 2005; Martins et al., 2010; Baliwe et al., 2022). In inaccessible areas such as islands, limpet numbers are often greater than in accessible mainland localities, increasing food supplies to predators such as oystercatchers which, in turn, reduce the numbers of limpets and confine them to vertical surfaces out of reach (Hockey and Branch, 1983; Branch, 1985; Branch et al., 1987; Lindberg et al., 1998). The effects of limpets on macroalgae cascade downwards to encrusting algae, which benefit because they would otherwise be overgrown (Steneck, 1982), and their speed of lateral growth – and, hence, ability to occupy space – is increased by grazing (Vanmari and Maneveldt, 2019). The ripple effects of grazers such as limpets thus extend up and down interaction chains.

Whereas the influence of *S. granularis* on macroalgal growth was predictable, the effects of algae on mussels were not. Others have reported that overgrowth by algae has detrimental effects on mussels, reducing growth and reproductive output (Dittman and Robles, 1991), density (Albrecht and Reise, 1994), productivity (Metri et al., 2002) and survivorship (O'Connor et al., 2006).

Our research on the three-way interaction between limpets, mussels and algae showed conclusively that *M. galloprovincialis* benefitted from the presence of limpets on their shells, improving their body condition and survival. The nearest parallel to our study is one by Miyamoto and Noda (2004), who tested the effects of grazers (two *Lottia* spp. and two littorinids) on algae growing on mussels. Unlike our study, they concluded that grazers had only minimal effects on the growth of algae. Grazing effects on host mussels are thus not universal.

Because *M. galloprovincialis* is relatively large, its shells provide secondary habitat for limpets, but limpets may use this only if they are sufficiently small. Miyamoto and Noda (2004) recorded densities of small (6-14 mm) *Lottia* spp. were 20 times greater on the mussel *Septifer virgatus* than on bare rock. Small (mostly juvenile, 3-25 mm) *S. granularis* reached much higher densities on mussels that on bare rock; but larger individuals (adults 25-65 mm) were excluded from the mussel beds and limited to bare rock, as previously reported (Griffiths et al., 1992; Hockey and van Erkom Schurink, 1992; Branch et al., 2008b).

While densities of *S. granularis* rise significantly on mussel beds, mean sizes are reduced. Individuals of < 0.8 g wet somatic mass (ca. 10-24 mm shell length) are pre-reproductive. At larger sizes, reproductive output per annum increases linearly with somatic mass and exponentially with shell length (Branch, 1974a; Branch, 1974b). Our results (Figure 2) show that taking into account densities and sizes and translating these to reproductive output yields two interesting facts. Firstly, reproductive output for the *population* (g yr^{-1} m^{-2}) is about 30% greater in mussel beds than that for limpets living on bare rock, with the greater densities of limpets in mussels more than offsetting their smaller sizes there. Secondly, and in contrast, from an evolutionary, life-time perspective, each *individual* limpet will achieve a much greater output – around seven times – if it spends its life on rock rather than in mussels, because it will achieve a much greater maximum size and greater longevity. This approximation is of course simplistic in that it assumes a given limpet will spend its whole life on mussels or on rock: but it is not unrealistic, given that the extensive cover of *M. galloprovincialis* in many parts of the west coast of South Africa (Supplementary Figure S1) leaves them no option but to occupy mussel shells, and the fact that prior to the arrival of this mussel (and in areas where it is sparse or absent), *S. granularis* spends its entire life on bare rock (Griffiths et al., 1992; Hockey and van Erkom Schurink, 1992).

Mytilus galloprovincialis has much worse effects on another co-occurring indigenous South African limpet, *Scutellastra argenvillei*, which is much larger (up to 95 mm) and matures sexually at about 47 mm. Dense mussel beds reduce its density, survivorship and homing fidelity (Ruiz Sebastián et al., 2002), confining it to small patches (Supplementary Figure S3), and no *S. argenvillei* ever reaches sexual maturity on mussel shells (Steffani and Branch, 2005).

Wave action does, however, moderate the effects of *M. galloprovincialis* on *S. argenvillei*. Abundance of *M. galloprovincialis* peaks at strong (but not extreme) levels of wave action, where food supply for the mussel is optimal (Bustamante and Branch, 1996); and the densities, sizes and reproductive potential of *S. argenvillei* are substantially reduced under those conditions. In contrast, at moderate levels of wave action the mussel settles and grows at lower rates, and the two species co-exist with minimal effects on each other (Steffani and Branch, 2003b; Steffani and Branch, 2003c; Branch and Steffani, 2004). Both the size of *S. argenvillei* and the intensity of wave action thus influence the outcome.

Various other factors influence the magnitude, speed and even direction of such interactions among mussels, barnacles, algae and limpet including geographic differences related to physical stress (Arrontes et al., 2004; Jenkins et al., 2005; Coleman et al., 2006; Crowe et al., 2011) and differences in mode of feeding. Not all limpets are conventional roam-and-scrape feeders. Some defend algal patches or garden specific algae (Stimson, 1973; Branch, 1975; Branch et al., 1992; McQuaid and Froneman, 1993), or trap drift algae (Bustamante et al., 1995a; Bustamante and Branch, 1996), and their effects on algal growth will be substantially less. The effects of limpet grazing on algae also depend on algal productivity. In New Zealand, limpet grazing has substantial effects at low-nutrient sites but not at upwelling sites (Guerry and Menge, 2017). Our study at Groenriver was, however, conducted in an area of active upwelling (Andrews and Hutchings, 1980) and high algal productivity (Bustamante et al., 1995b), yet the effects of limpet exclusion were decisive.

4.2 Barnacles, limpets and periwinkles

Our results show that the barnacle *B. glandula* has negative effects on *S. granularis* but positive effects on littorinids. There are many other examples of negative barnacle-limpet interactions (Dayton, 1971; Branch, 1976; Hawkins, 1983; Dungan, 1986; Santini et al., 2019; Ellrich et al., 2020), but in New South Wales, Creese (1982) showed that a small species of limpet, *Patelloida latistrigata*, benefits from the presence of barnacles that provide a refuge from competition with a larger species of limpet, *Cellana tramoserica*, which is excluded because it cannot move over and feed effectively on barnacles. Again, size matters.

The effects of *B. glandula* on *S. granularis* are particularly severe for two reasons. First, indigenous barnacles are generally scarce on the west coast of South Africa (Boland, 1997). In particular, there is a large gap in distribution of the native high-shore *Chthamalus dentatus* between Namibia and the south coast of South Africa (with genetic differences between the two populations of *C. dentatus* suggesting they are separate species; Motro et al., 2023). This gap is now filled by *B. glandula*. Second, *B. glandula* predominates in the high shore where there was previously a void in occupation of the shore by barnacles. Our demonstrations that the barnacle *B. glandula* has negative effects on *S. granularis* but positive effects on littorinids, support previous findings by respectively Griffiths et al. (1992) and Laird and Griffiths (2008).

4.3 Effects of alien species on zonation of *S. granularis*

The successive advents of *M. galloprovincialis*, *S. patagonicus* and *B. glandula* have radically altered the zonation patterns of *S. granularis*. Prior to their arrival, *S. granularis* was recognized as a typical member of a group of 'migratory' limpets that Branch (1975; 1976) distinguished on the grounds that they settle low on the shore and then migrate progressively upwards as they age, in contrast to 'non-migratory' species that remain in the same zone as they settle throughout their lives. Table 1A summarises the features of migratory and non-migratory southern African species of patellid limpets. Hobday (1995) has demonstrated for the limpet *Lottia digitalis* that this zonation pattern is maintained by migration. Arrival of *M. galloprovincialis* changed those patterns (Table 1B). Most notably, recruits and juveniles of *S. granularis* became concentrated in beds of *M. galloprovincialis* and then shifted both up and down the shore with an increase in size (and age). The advent of *S. patagonicus* and *B. glandula* brought further changes. Low on the shore where *S. patagonicus* predominates, *S. granularis* is virtually excluded and no individuals over 8 mm recorded were recorded there. Upwards migration of *S. granularis* from the (diminished) recruitment beds of *M. galloprovincialis* into the top zone did restore a semblance of the size-zonation pattern originally present, but high densities of *B. glandula* in the high zones largely exclude the limpet. Both *S. patagonicus* and *B. glandula* constitute a poor substratum for the limpet: the former because of its small size and short life, which make it an unsuitable host, and the latter because its rough texture makes attachment and feeding problematic – both difficulties that Creese (1982) suggests large limpets will experience among barnacles.

4.4 Predictors of the invasive success of *S. patagonicus*

Ros et al. (2023) bemoan that little research has compared whether the ecosystem impacts of alien species are related to their life-history traits. Based on comparisons of such traits among South African mussels, Branch and Steffani (2004) advanced seven predictors of whether the characteristics of *M. galloprovincialis* influence its capacity to invade. With subsequent research, including the results reported in this paper, it is possible to extend the list of predictors for *M. galloprovincialis* to nine (Table 2), and to then use those traits to explore the extent to which they have proven valid predictors of the invasions recorded for *S. patagonicus*.

The almost world-wide invasion of temperate waters by *M. galloprovincialis* made it an obvious predictor that it would invade South Africa (Table 2, item 1). It was not, however a useful predictor of the arrival of *S. patagonicus*, which was first described from material in Argentina (d´Orbigny, 1842, p 647), but has not been recorded there since (Signorelli and Pastorino, 2021), apart from transient records of it on fishing-boat hulls in 2013 (Bigatti et al., 2014). Thus, there was no evidence of it invading other parts of the world before its arrival in Namibia in 1931. Its subsequent spread in that country, abrupt and dense settlement in South Africa in 2009 and recent rapid range expansion there (Zeeman et al., 2018; Ma et al., 2020a; Ma et al., 2020b) were therefore not events predictable by any history of invasions elsewhere. Likewise, the physiological superiority exhibited by *M. galloprovincialis* in terms of its rapid growth, desiccation tolerance, shell strength and food uptake rate (Alexander et al., 2015b; Zeeman et al., 2018) associated with its invasion capabilities was not evident in *S. patagonicus* (Table 2, item 2).

The most successful predictor of the capacity of *S. patagonicus* to invade and spread in Namibia and South Africa was its astounding rate of recruitment (Table 2, item 3), reaching around 2-5 million recruits m^{-2} in the low shore, orders of magnitude greater than that of any of the indigenous mussels (Reaugh-Flower et al., 2011). The remaining predictors of invasion capability (Table 2, items 4-9) serve as well for *M. galloprovincialis* as for *S. patagonicus*, both of which reach greatest densities (and maximal effects) on shores with strong wave action, and provide additional food for predators, although none of the known predators provide any predator-driven resistance to invasion by *S. patagonicus* (Robinson et al., 2015; Skein et al., 2020). The brief lifespan, high densities and particularly the small size of *S. patagonicus* are firm predictors of its almost complete exclusion of limpets. This accords with other limpet-mussel interactions in South Africa. For example, the relatively tiny (20-30-mm) *Scutellastra aphanes* lives almost its entire live on shells of *Perna perna* (Robson, 1986) whereas the large (80-100 mm) *S. argenvillei* is largely excluded by dense beds of *M. galloprovincialis* (Steffani and Branch, 2003a; Steffani and Branch, 2003b; Steffani and Branch, 2003c; Branch and Steffani, 2004).

Skein et al. (2021) have broadened the consideration of characteristics that influence the invasion success of alien species to include features of both prey and predator. For prey, they list gregarious behaviour, high fecundity, high recruitment, dispersal potential and refuge from predation; for predators, preference for alien over native species, high feeding rate and high abundance feature. All the features listed for prey are applicable to *M. galloprovincialis*, *S. patagonicus* and *B. glandula*. High reproductive capacity and high to exceptionally high settlement and recruitment are important features for *M. galloprovincialis* and *S. patagonicus*, and Skein et al. (2021) make the point that this may overwhelm any predatory biotic resistance to alien species. The same is true for *B. glandula* (Robinson et al., 2015). In short, although there are several know predators of the two alien mussels, none seem positioned to provide effective biotic resistance to them. Both *M. galloprovincialis* (Calvo-Ugarteburu and McQuaid, 1998a; Calvo-Ugarteburu and McQuaid, 1998b) and *S. patagonicus* (personal observations) are relatively free of parasites, which is likely to increase their invasive capabilities.

Four factors are, however, likely to ameliorate the effects of the alien mussels and barnacle: wave action, upwelling, zonation and recruitment. All three alien species predominate on wave-exposed shores and are less abundant at upwelling headlands than downstream bays (Pfaff et al., 2011). Their recruitment is diminished on sheltered shores and at upwelling headlands and, correlated with this, adult abundances are reduced (Hoffmann et al., 2012), reducing their competitive capability.

The three alien species are also differentially zoned: *B. glandula* is concentrated in the high shore (Hoffmann et al., 2012; Supplementary Figure S2), *M. galloprovincialis* predominates in the mid to high shore, although it can extend to cover the low shore (Hoffmann et al., 2012; De Greef et al., 2013) and *S. algosus* is strictly a low-shore species (De Greef et al., 2013) that extends well into the subtidal zone (Skein et al., 2018a). However, in combination, *S. patagonicus*, *M. galloprovincialis* and *B. glandula* now exert a 'zonation squeeze' that covers all but the highest (top) zone on the shore (Sadchatheeswaran et al., 2015; Supplementary Figure S4).

Finally, recruitment of many marine organisms is patchy, both spatially and temporally (Underwood and Fairweather, 1989). For mussels and barnacles, concentration of settlement and recruitment at bays downstream of upwelling headland and on wave-exposed shore focuses their effects there (Pfaff et al., 2011). More importantly, periodic 'sweepstake' settlements take place, with periods of several years between which settlement and recruitment are very low to absent (Reaugh-Flower et al., 2011). Maximal effects are felt when settlement is dense, but between such events mussel and barnacle populations diminish in vertical extent and abundance, as we recorded for *M. galloprovincialis* between 2001 and 2012.

5 Conclusions

Returning to the hypotheses outlined in the introduction:

(1) Grazing by the limpet *S granularis* did benefit *M. galloprovincialis* by preventing algal overgrowth, improving survivorship and condition of the mussel. In turn, the mussel supported high densities of small individuals of *S. granularis*; but excluded large ones.
(2) Dense settlements of the alien barnacle *B. glandula* in the high shore excluded *S. granularis* but enhanced densities of the periwinkle *A. knysnaensis*.
(3) The previously described progressively upshore migration of *S. granularis* was disrupted by the arrival of *M. galloprovincialis*, which concentrated recruits of the limpet in the mid to high shore; and further altered by *S. patagonicus* and *B. glandula*, which largely excluded the limpet from respectively the low- and high-shore zones.
(4) Factors that predicted the invasive success of *M. galloprovincialis* were largely applicable to *S. patagonicus*, with the most important departures being (a) the absence of an invasive history that would have forecast its arrival in Namibia, and thence South Africa; and (b) the slow growth, intolerance of physical stresses and relatively low rate of food uptake in *S. patagonicus* that are at odds with its successful invasions and spread. Pivotal to its success is its astonishingly high rate of recruitment.

Factors mitigating the negative effects of the alien species were (a) differences in their zonation, response to wave action and upwelling that partially separate species; (b) differences among species in their relative sizes, which determined whether the alien species could provide a secondary substratum for native species; and (c) fluctuations in recruitment that grant respite between periods of heavy settlement of alien species. Generalities did, thus emerge, but each alien was sufficiently different from the others to be unique in its interactions. As Silverberg (2015) says: 'The main thing about aliens is that they are alien. They feel no responsibility for fulfilling any of your expectations'.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

GB conceived and supervised the research, undertook data collection, analysis and writing. NS was responsible for the work on limpet-mussel-algae interaction including the experiments, data collection and revision of that section of the paper. MP and NB were mainly involved with work on changing zonation patterns, including data gathering, analysis and revision of the text. ZZ was responsible for work on *Semimytilus* and helped revise that section of the paper. All authors contributed to the article and approved the submitted version.

Funding

Funding was received from the Andrew Mellon Foundation; the University of Cape Town and the South African National Research Foundation.

Acknowledgments

SANParks granted access and permission to work on Marcus Island. Janine Dunlop provided assistance with references and Claudio Velasquez, Margo Branch, Saachi Sadchatheeswaran, Tammy Robinson contributed to fieldwork. Guido Patorino updated our information on the status of *Semimytilus* in South America. All are thanked with appreciation. This paper is dedicated to Professors Alan Southward (1928-2007) and Brian Allanson (1928-2022), both of whom were 'impatient of administrators' and 'devoted much time to encouraging young scientists' and who were also PhD examiners for the first author eons ago.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1190456/full#supplementary-material

References

Albrecht, A., and Reise, K. (1994). Effects of *Fucus vesiculosus* covering intertidal mussel beds in the Wadden Sea. *Helgol. Wiss. Meeresunters* 48, 243256. doi: 10.1007/bf02367039

Alexander, M. E., Adams, R., Dick, J. T. A., and Robinson, T. B. (2015b). Forecasting invasions: resource use by mussels informs invasion patterns along the South African coast. *Mar. Biol.* 162, 2493–2500. doi: 10.1007/s00227-015-2742-5

Alexander, M. E., Raven, H. J., and Robinson, T. B. (2015a). Foraging decisions if a native whelk, *Trochia cingulata* Linnaeus, and the effects of invasive mussels on prey choice. *J. Exp. Mar. Biol. Ecol.* 470, 26–33. doi: 10.1016/j.jembe.2015.04.023

Alexander, M. A., Skein, L., and Robinson, T. B. (2022). Rapid learning in a native predator shifts diet preferences towards invasive prey. *Biol. Lett.* 18, 20210655. doi: 10.1098/rbl.2021.0655

Andrews, W. R. H., and Hutchings, L. (1980). Upwelling in the Southern Benguela Current. *Prog. Oceanogr.* 9, 1–81. doi: 10.1016/0079-6611(80)90015-4

Arrontes, J., Arena, F., Fernandez, C., Rico, J. M., Oliveros, J., Martinez, B., et al. (2004). Effects of grazing by limpets on mid-shore species assemblages in northern Spain. *Mar. Ecol. Prog. Ser.* 277, 117–133. doi: 10.3354/meps277117

Baliwe, N. G., Pfaff, M. C., and Branch, G. M. (2022). Assessing the effects of no-take zones in a marine protected area spanning two ecoregions and rock substrate types. *Front. Mar. Sci.* 9. doi: 10.3389/fmars.2022.893260

Bigatti, G., Signorelli, J. H., and Schwindt, E. (2014). Potential invasion of the Atlantic coast of South America by *Semimytilus algosus* (Gould 1850). *BioInvasions Rec.* 3, 241–246. doi: 10.3391/bir.2014.3.4.05

Boland, J. M. (1997). Is the geographic pattern in the abundance of South African barnacles due to pre-recruitment or post-recruitment factors? *S. Afr. J. Mar. Sci.* 18, 63–73. doi: 10.2989/025776197784161036

Bownes, S. J., and McQuaid, C. D. (2006). Will the invasive mussel *Mytilus galloprovincialis* Lamarck replace the indigenous *Perna perna* L. on the South coast of South Africa? *J. Exp. Mar. Biol. Ecol.* 338, 14–151. doi: 10.1016/j.jembe.2006.07.006

Bownes, S. J., and McQuaid, C. D. (2010). Mechanisms of habitat segregation between and invasive and an indigenous mussel: settlement, post-settlement mortality and recruitment. *Mar. Biol.* 156, 991–1006. doi: 10.1007/s00227-009-1143-z

Branch, G. M. (1974a). The ecology of *Patella* Linnaeus from the Cape Peninsula, South Africa. 2. Reproductive cycles. *Trans. R. Soc S. Afr.* 41, 111–160. doi: 10.1080/00359197409520068

Branch, G. M. (1974b). The ecology of *Patella* Linnaeus from the Cape Peninsula, South Africa. 3. Growth rates. *Trans. R. Soc S. Afr.* 41, 161–193. doi: 10.1080/00359197409520069

Branch, G. M. (1975). Mechanisms reducing intraspecific competition in *Patella* spp.: migration, differentiation and territorial behaviour. *J. Anim. Ecol.* 44, 575–600. doi: 10.2307/3612

Branch, G. M. (1976). Interspecific competition experienced by South African *Patella* spp. *J. Anim. Ecol.* 4, 507–529. doi: 10.2307/3888

Branch, G. M. (1981). The biology of limpets: physical factors, energy flow, and ecological interactions. *Oceanogr. Mar. Biol. Ann. Rev.* 19, 235–380.

Branch, G. M. (1984). Competition between marine organisms: ecological and evolutionary implications. *Oceanogr. Mar. Biol. Ann. Rev.* 22, 429–593. doi: 10.1201/9781482267242-14

Branch, G. M. (1985). "Limpets: their role in littoral and sublittoral community dynamics," in *The ecology of rocky coasts*. Eds. P. G. Moore and R. Seed (London: Hodder and Stoughton), 97–116.

Branch, G. M., Barkai, A., Hockey, P. A. R., and Hutchings, L. (1987). Biological interactions: causes or effects of variability in the Benguela ecosystem? *S. Afr. J. Mar. Sci.* 5, 425–445. doi: 10.2989/025776187784522144

Branch, G. M., and Branch, M. L. (2018). *Living shores – interacting with southern Africa's marine ecosystems* (Cape Town: Struik Nature).

Branch, G. M., Harris, J. M., Parkins, C., Bustamante, R. H., and Eekhout, S. (1992). "Algal 'gardening' by grazers: a comparison of the ecological effects of territorial fish and limpets," in *Plant-animal interactions in the marine benthos*. Special Volume, No. 46. Eds. D. M. John, S. J. Hawkins and J. H. Price (Oxford: Systematics Association Clarendon Press), 405–423.

Branch, G. M., Odendaal, F., and Robinson, T. B. (2008b). Long-term monitoring of the arrival, expansion and effects of the alien mussel *Mytilus galloprovincialis* relative to wave action. *Mar. Ecol. Prog. Ser.* 370, 171–183. doi: 10.3354/meps07626

Branch, G. M., Odendaal, F., and Robinson, T. B. (2010). Competition and facilitation between the alien mussel *Mytilus galloprovincialis* and indigenous species: moderation by wave action. *J. Exp. Mar. Biol. Ecol.* 383, 65–78. doi: 10.3354/meps07626

Branch, G. M., and Steffani, C. N. (2004). Can we predict the effects of alien species? a case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). *J. Exp. Mar. Biol. Ecol.* 300, 189–215. doi: 10.1016/j.jembe.2003.12.007

Branch, G. M., Thomson, R. C., Crowe, T. P., Castilla, J. C., Langmead, O., and Hawkins, S. J. (2008a). "Rocky intertidal shores: prognosis for the future," in *Aquatic ecosystems; trends and global prospects*. Ed. N. Polunin (Cambridge: Cambridge University Press), 209–225.

Bustamante, R. H., and Branch, G. M. (1996). The dependence of intertidal consumers on kelp-derived organic matter on the west coast of South Africa. *J. Exp. Mar. Biol. Ecol.* 196, 1–28. doi: 10.1016/0022-0981(95)00093-3

Bustamante, R. H., Branch, G. M., and Eekhout, S. (1995a). Maintenance of an exceptional intertidal grazer biomass in South Africa: subsidy by subtidal kelps. *Ecology* 76, 2314–2329. doi: 10.1016/0022-0981(95)00093-3

Bustamante, R. H., Branch, G. M., Eekhout, S., Robertson, B., Zoutendyk, P., Schleyer, M., et al. (1995b). Gradients of intertidal productivity around the coast of South Africa and their relationships with consumer biomass. *Oecologia* 102, 189–201. doi: 10.1007/BF00333251

Calvo-Ugarteburu, G., and McQuaid, C. D. (1998a). Parasitism and invasive species: effects of digenetic trematodes on mussels. *Mar. Ecol. Prog. Ser.* 169, 149–163. doi: 10.3354/meps169149

Calvo-Ugarteburu, G., and McQuaid, C. D. (1998b). Parasitism and introduced species: epidemiology of trematodes in the intertidal mussels *Perna perna* and *Mytilus galloprovincialis*. *J. Exp. Mar. Biol. Ecol.* 220, 47–65. doi: 10.1016/s0022-0981(97)00083-x

Castilla, J. C., Lagos, N. A., and Cerda, M. (2004). Marine ecosystem engineering by the alien ascidian *Pyura praeputialis* on a mid-intertidal rocky shore. *Mar. Ecol. Prog. Ser.* 268, 119–130. doi: 10.3354/meps268119

Coleman, R., and Hockey, P. A. R. (2008). Effects of an alien invertebrate species and wave action on prey selection by African black oystercatchers (*Haematopus moquini*). *Aust. Ecol.* 33, 232–240. doi: 10.1111/j.1442-9993.2008.01864.x

Coleman, R. A., Underwood, A. J., Benedetti-Cecchi, L., Åberg, P., Arenas, F., Arrontes, J., et al. (2006). A continental scale evaluation of the role of limpet grazing on rocky shores. *Oecologia* 147, 556–564. doi: 10.1007/s00442-005-0296-9

Creese, R. G. (1982). Distribution and abundance of the acmaeid limpet *Patelloida latistrigata*, and its interaction with barnacles. *Oecologia* 52, 85–96. doi: 10.1007/bf00389010

Crowe, T. P., Frost, N. J., and Hawkins, S. J. (2011). Interactive effects of losing key grazers and ecosystem engineers vary with environmental context. *Mar. Ecol. Prog. Ser.* 430, 223–234. doi: 10.3354/meps09023

d´Orbigny, A. D. (1842). *Voyage dans l'Amerèrique mèridionale (le Brazil, la rèpublique orientale de l'Uruguay, la rèpublique Argentine, la patagonie, la rèpublique du chili, la rèpublique Bolivia, la rèpublique pèrou), exècute pendant le annès 1826, 1827, 1928. 1832 et 1833*. Eds. P. Bertrand and V. Levrault (Paris: Tome cinquème, 3e partie: Mollusques).

Day, E., Branch, G. M., and Viljoen, C. (2000). How costly is molluscan shell erosion? A comparison of two limpets with contrasting shell structures. *J. Exp. Mar. Biol. Ecol.* 243, 185–208. doi: 10.1016/s0022-0981(99)00120-3

Dayton, P. K. (1971). Competition, disturbance, and community organisation: the provision and subsequent utilisation of space in a rocky intertidal community. *Ecol. Monogr.* 41, 351–389. doi: 10.2307/1948498

De Greef, K., Griffiths, C. L., and Zeeman, Z. (2013). Déjà vu? a second *Mytilus* mussel *Semimytilus algosus*, invades South Africa's west coast. *Afr. J. Mar. Sci.* 35, 307–313. doi: 10.2989/1814232X.2013.829789

Dittman, D., and Robles, C. (1991). The effects of algal epiphytes on the mussel *Mytilus californianus*. *Ecology* 72, 286–296. doi: 10.2307/1938922

Dungan, M. L. (1986). Three-way interactions: barnacles, limpets, and algae in a Sonoran Desert rocky intertidal zone. *Am. Nat.* 127, 292–316. doi: 10.1086/284486

Dye, A. H. (1995). The effects of excluding limpets from the lower balanoid zone of rocky shores in Transkei, South Africa. *S. Afr. J. Mar. Sci.* 15, 9–15. doi: 10.2989/025776195784156313

Ellrich, J. A., Yorisue, T., and Momota, K. (2020). Limpet disturbance effects on barnacle recruitment are related to recruitment density but not recruit size. *Peer J* 8, e9190. doi: 10.7717/peerj.9190

Erlandsson, J., Pal, P., and McQuaid, C. D. (2006). Re-colonisation rate differs between co-existing indigenous and invasive intertidal musselsfollowing major disturbance. *Mar. Ecol. Prog. Ser.* 320, 169–176. doi: 10.3354/meps320169

Farrell, T. M. (1988). Community stability: effects of limpet removal and reintroduction in a rocky intertidal community. *Oecologia* 75, 190–197. doi: 10.1007/bf00378596

Firth, L. (2021). What have limpets ever done for us? On the past and present provisioning and cultural services of limpets. *Int. Rev. Environ. History* 7, 5–45. doi: 10.22459/ireh.07.02.2021.01

Grant, W. S., and Cherry, M. I. (1985). *Mytilus galloprovincialis* Lmk. in southern Africa. *J. Exp. Mar. Biol. Ecol.* 90, 179–191. doi: 10.1016/0022-0981(85)90119-4

Griffiths, C. L., Hockey, P. A., Van Erkom Schurink, C., and Le Roux, P. J. (1992). Marine invasive aliens on South African shores: implications for community structure and trophic functioning. *S. Afr. J. Mar. Sci.* 12, 713–722. doi: 10.2989/02577619209504736

Griffiths, C. L., Mead, A., and Robinson, T. B. (2010). A brief history of marine bio-invasions in South Africa. *Afr. Zool.* 44, 241–247. doi: 10.3377/004.044.0212

Griffiths, C. L., Robinson, T. B., and Mead, A. (2009). "The status and distribution of marine alien species in South Africa," in *Biological invasions in marine ecosystems*, vol. 204. Eds. G. Rilov and J. A. Crooks (Heidelberg: Springer-Verlag), 393–408.

Guerry, A. D., and Menge, B. A. (2017). Grazer impacts on algal community structure vary with the coastal upwelling regime. *J. Exp. Mar. Biol. Ecol.* 48, 10–23. doi: 10.1016/j.jembe.2016.12.011

Hammond, W., and Griffiths, C. L. (2003). Influence of wave exposure on South African mussel beds and their associated infaunal communities. *Mar. Biol.* 144, 547–552. doi: 10.1007/s00227-003-1210-9

Hampton, S. L., and Griffiths, C. L. (2007). Why *Carcinus maenas* cannot get a grip on South Africa's wave-exposed shores. *Afr. J. Mar. Sci.* 29, 123–126. doi: 10.2989/AJMS.2007.29.1.11.76

Hanekom, N. (2007). Invasion of an indigenous *Perna perna* mussel bed on the south coast of South Africa by an alien mussel *Mytilus galloprovincialis* and its effect on the associated fauna. *Biol. Inv.* 10, 233–244. doi: 10.1007/s10530-007-9125-x

Hartnoll, R. G., and Hawkins, S. J. (1985). Patchiness and fluctuations on moderately exposed shores. *Ophelia* 24, 53–63. doi: 10.1080/00785236.1985.10426619

Hawkins, S. G. (1981). The influence of *Patella* grazing on the fucoid/barnacle mosaic on moderately exposed rocky shores. *Kieler Meerersforsch. Sonderh.* 5, 537–543.

Hawkins, S. J. (1983). Interactions of *Patella* and macroalgae with settling *Semibalanus balanoides* (L.). *J. Exp. Mar. Biol. Ecol.* 71, 55–72. doi: 10.1016/0022-0981(83)90104-1

Hawkins, S. J., and Hartnoll, R. G. (1983). Grazing of intertidal algae by marine invertebrates. *Oceanogr. Mar. Biol. Ann. Rev.* 21, 195–282.

Hobday, A. (1995). Body-size variation exhibited by an intertidal limpet: influence of wave exposure, tidal height and migratory behavior. *J. Exp. Mar. Biol. Ecol.* 18, 29–45. doi: 10.1016/0022-0981(95)00009-g

Hockey, P. A. R., and Branch, G. M. (1983). Oystercatchers and limpets: impact and implications a preliminary assessment. *Ardea* 72, 199–206.

Hockey, P. A. R., and van Erkom Schurink, C. (1992). The invasive biology of the mussel *Mytilus galloprovincialis* on the southern African coast. *Trans. R. Soc S. Afr.* 48, 123–139. doi: 10.1080/00359199209520258

Hoffmann, V., Pfaff, M. C., and Branch, G. M. (2012). Spatio-temporal patterns of larval supply and settlement of intertidal invertebrates reflect a combination of passive transport and larval behaviour. *J. Exp. Mar. Biol. Ecol.* 418–419, 83–90. doi: 10.1016/j.jembe.2012.03.008

Jenkins, S. R., Coleman, R. A., Santina, P. D., Hawkins, S. J., Burrows, M. T., and Hartnoll, R. G. (2005). Regional scale differences in the determinism of grazing effects in the rocky intertidal. *Mar. Ecol. Prog. Ser.* 287, 77–86. doi: 10.3354/meps287077

Jenkins, S. R., Hawkins, S. J., and Norton, T. A. (1999). Direct and indirect effects of a macroagal canopy and limpet grazing in structuring a sheltered inter-tidal community. *Mar. Ecol. Prog. Ser.* 188, 81–92. doi: 10.3354/meps188081

Jenkins, S. R., Laird, M. C., and Griffiths, C. L. (2008). Present distribution and abundance of the introduced barnacle *Balanus glandula* Darwin in South Africa. *Afr. J. Mar. Sci.* 30, 93–100. doi: 10.2989/ajms.2008.30.1.9.459

Jennsen, G. S. (2015). *Sidespace: aurora renegrades. Book one* (Hauppauge, New York: Hypernova Publishing).

Jones, N. S. (1946). Browsing of *Patella*. *Nature* 158, 557. doi: 10.1038/158557b0

Laird, M. C., and Griffiths, C. L. (2008). Present distribution and abundance of the introduced barnacle *Balanus glandula* Darwin in South Africa. *Afr. J. Mar. Sci.* 30, 93–100. doi: 10.2989/ajms.2008.30.1.9.459

Lathlean, J. A., Seuront, L., McQuaid, C. D., Ng, T. P. T., Zardi, G. I., and Nicastro, K. R. (2016). Cheating the locals: invasive mussels steal and benefit from the cooling effects of indigenous mussels. *PloS One* 11 (3), e0152556. doi: 10.1371/journal.pone.0152556

Lindberg, D. R. (2007). Reproduction, ecology, and evolution of the Indo-Pacific limpet *Scutellatra flexuosa*. *Bull. Mar. Sci.* 81, 2019–2234.

Lindberg, D. R., Estes, J. A., and Warheit., K. I. (1998). Human influences on trophic cascades along rocky shores. *Ecol. Applic.* 8, 880–890. doi: 10.1890/1051-0761(1998)008[0880:hiotca]2.0.co;2

Lindegarth, M., Åberg, P., Cervin, G., and Nilsson, P. G. (2001). Effects of grazing on the structure of mid-shore, intertidal assemblages on moderately exposed rocky shores of the Swedish west coast. *Mar. Ecol. Prog. Ser.* 212, 29–38. doi: 10.3354/meps212029

Lins, D. M., Zbawicka, M., Wenne, R., Pocwirz-Kotus, A., Molina, J. R. A., Alves, L. P., et al. (2021). Ecology and genetics of *Mytilus galloprovincialis*: threat to bivalve aquaculture in southern Brazil. *Aquaculture* 540, 73653. doi: 10.1016/j.aquaculture.2021.736753

Little, C., Trowbridge, C. D., Pilling, G. M., Stirling, P., Morritt, D., and Williams, G. A. (2017). Long-term fluctuations in intertidal communities in an Irish sea-lough: limpet-fucoid cycles. *Estuar. Coast. Shelf Sci.* 196, 70–82. doi: 10.1016/j.ecss.2017.06.036

Lodge, S. M. (1948). Algal growth in the absence of *Patella* on an experimental strip of foreshore, Port St. Mary, Isle of Man. *Proc. Trans. Liverpool Biol. Soc* 56, 78–85.

Ma, K. C. K., McQuaid, C. D., Pulfrich, A., and Robinson, T. B. (2020b). Status of a decennial marine invasion by the biexual mussel *Semimytilus algosus* (Gould 1850) in South Africa. *Afr. J. Mar. Sci.* 42, 507–515. doi: 10.2989/1814232x.2020.1820376

Ma, K. C. K., Monsinjon, J. R., Froneman, P. W., and McQuaid, C. V. D. (2023). Thermal stress gradient causes increasingly negative effects towards the range limit of an invasive mussel. *Sci. Tot. Env.* 865, 161184. doi: 10.1016/j.scitotenv.2022.161184

Ma, K. C. K., Zardi, G. I., McQuaid, C. D., and Nicastro, K. R. (2020a). Historical and contemporary range expansion of an invasive mussel, *Semimytilus algosus*, in Angola and Namibia despite data scarcity in an infrequently surveyed region. *PloS One* 15 (9), e0239167. doi: 10.1371/journal/pone.0239167

Maneveldt, G. W., Eager, R. C., and Bassier, A. (2009). Effects of long-term exclusion of the limpet *Cymbula oculus* (Born) on the distribution of intertidal organisms on a rocky shore. *Afr. J. Mar. Sci.* 31, 171–179. doi: 10.2989/ajms.2009.31.2.5.877

Martins, G. M., Thompson, R. C., Neto, A. I., Hawkins, S. J., and Jenkins, S. R. (2010). Exploitation of intertidal grazers as a driver of community divergence. *J. Appl. Ecol.* 47, 1282–1289. doi: 10.1111/j.1365-2664.2010.01876.x

McQuaid, C. D., and Arenas, F. (2009). "Biological invasions: insights from marine benthic communities," in *Marine hard bottom communities*, vol. 206. Ed. M. Whal (Berlin Heidelberg: Springer-Verlag), 309–320. doi: 10.1007/b76710_22

McQuaid, C. D., and Froneman, P. W. (1993). Mutualism between the territorial intertidal limpet *Patella longicosta* and the crustose alga *Ralfsia verrucosa*. *Oecologia* 96, 128–133. doi: 10.1007/bf00318040

Mead, A., Carlton, J. T., Griffiths, C. L., and Rius, M. (2011a). Introduced and cryptic marine and estuarine species in South Africa. *J. Nat. Hist.* 45, 2463–2524. doi: 10.1080/00222933.2011.595836

Mead, A., Carlton, J. T., Griffiths, C. L., and Rius, M. (2011b). Revealing the scale of marine bio-invasions in developing regions: a South African re-assessment. *Biol. Inv.* 13, 1991–2008. doi: 10.1007/s10530-011-0016-9

Menge, B. A., Foley, M. M., Pamplin, J., Murphy, G., and Pennington, C. (2010). Supply-side ecology, barnacle recruitment, and rocky intertidal community dynamics: do settlement surface and limpet disturbance matter? *J. Exp. Mar. Biol. Ecol.* 392, 160–175. doi: 10.1016/j.jembe.2010.04.032

Metri, R., da Rocha, R. M., and Marenzi, A. (2002). Epibiosis reduction on productivity in a mussel culture of *Perna* (Linné, 1758). *Braz. Arch. Bio. Technol.* 45, 325–331. doi: 10.1590/s1516-89132002000300011

Micheli, F., Benedetti-Cecchi, L., Gambaccini, S., Bertocci, I., Borsini, C., Osio, G. C., et al. (2005). Cascading human impacts, marine protected areas, and the structure of Mediterranean reef assemblages. *Ecol. Monogr.* 75, 81–102. doi: 10.1890/03-4058

Miyamoto, Y., and Noda, T. (2004). Effects of mussels on competitively inferior species: competitive exclusion to facilitation. *Mar. Ecol. Prog. Ser.* 276, 293–298. doi: 10.3354/meps276293

Motro, U., Simon-Blecher, N., Frumin, S., and Achituv, Y. (2023). Brewed in the African pot: the phylogeography of the toothed barnacle *Chthamalus dentatus* (Chthamaloidea: Chthamalidae). *Mar. Biol. Res.* doi: 10.1080/17451000.2023.2193899

Nicastro, K. R., Zardi, G. I., and McQuaid, C. D. (2007). Behavioural response of invasive *Mytilus galloprovincialis* and indigenous *Perna perna* mussels exposed to risk of predation. *Mar. Ecol. Prog. Ser.* 336, 169–175. doi: 10.3354/meps336169

Nicastro, K. R., Zardi, G. I., and McQuaid, C. D. (2008). Movement behaviour and mortality in invasive and indigenous mussels: resilience and resistance strategies at different spatial scales. *Mar. Ecol. Prog. Ser.* 372, 119–126. doi: 10.3354/meps07671

Nicastro, K. R., Zardi, G. I., and McQuaid, C. D. (2010a). The role of gaping behaviour in habitat partitioning between coexisting intertidal mussels. *BMC Ecol.* 10, 17. doi: 10.1186/1472-6785-10-17

Nicastro, K. R., Zardi, G. I., and McQuaid, C. D. (2010b). Differential reproductive investment, attachment strength and mortality of invasive and indigenous mussels across heterogeneous environments. *Biol. Inv.* 12, 2165–2177. doi: 10.1007/s10530-009-9619-9

O'Connor, N. E., Crowe, T. P., and McGrath, D. (2006). Effects of epibiotic algae on survival, biomass and recruitment of mussels, *Mytilus* L. (Bivalvia: Mollusca). *J. Exp. Mar. Biol. Ecol.* 328, 265–276. doi: 10.1016/j.jembe.2005.07.013

Pfaff, M. C., Branch, G. M., Wieters, E. A., Branch, R. A., and Broitman, B. R. (2011). Upwelling intensity and wave exposure determine recruitment of intertidal mussels and barnacles in the Southern Benguela upwelling region. *Mar. Ecol. Prog. Ser.* 425, 141–152. doi: 10.3354/meps09003

Phillips, N. E., and Hutchison, E. (2008). Grazer effects on algal assemblages and mussel recruitment in two different mid-intertidal communities in the Cook Strait, New Zealand. *N. Z. J. Mar. Freshw. Res.* 42, 297–306. doi: 10.1080/00288330809509957

Pyšek, P., Hulme, P. E., Simberloff, D., Bacher, S., Blackburn, T. M., Carlton, J. T., et al. (2020). Scientists' warning on invasive alien species. *Biol. Rev.* 95, 1511–1534. doi: 10.1111/brv.12627

Quinn, G. P., and Keough, M. J. (2002). *Experimental design and data analysis for biologists* (Cambridge: Cambridge University Press).

R Core Team. (2019). *R: a language and environment for statistical computing* (Vienna, Austria: R Foundation for Statistical Computing). Available at: https://www.R-project.org/.

Reaugh-Flower, K., Branch, G. M., Harris, J. M., McQuaid, C. D., Currie, B., Dye, A., et al. (2011). Scale-dependent patterns and processes of intertidal mussel recruitment around southern Africa. *Mar. Ecol. Prog. Ser.* 434, 101–119. doi: 10.3354/meps09169

Rius, M., and McQuaid, C. D. (2006). Wave action and competitive interactions between the invasive mussel *Mytilus galloprovincialis* and the indigenous *Perna perna* in South Africa. *Mar. Biol.* 150, 69–78. doi: 10.1007/s00227-006-0322-4

Rius, M., and McQuaid, C. D. (2009). Facilitation and competition between invasive and indigenous mussels over a gradient of physical stress. *Basic Appl. Ecol.* 10, 607–613. doi: 10.1016/j.baae.2009.03.008

Robinson, T. B., Alexander, M. E., Simon, C. A., Griffiths, C. L., Peters, K., Sibanda, S., et al. (2016). Lost in translation? standardising the terminology used in marine invasion biology and updating South African alien species lists. *Afr. J. Mar. Sci.* 38, 129–140. doi: 10.2989/1814232X.2016.1163292

Robinson, T. B., Branch, G. M., Griffiths, C. L., Govender, A., and Hockey, P. A. R. (2007). Changes in South African rocky intertidal invertebrate community structure associated with the invasion of the mussel *Mytilus galloprovincialis*. *Mar. Ecol. Prog. Ser.* 340, 163–171. doi: 10.2989/18142320509504088

Robinson, T. B., Griffiths, C. L., McQuaid, C., and Rius, M. (2005). Marine alien species of South Africa-status and impacts. *Afr. J. Mar. Sci.* 27, 297–306. doi: 10.2989/18142320509504088

Robinson, T. B., Peters, K., and Brooker, B. (2020). "Coastal invasions: the South African context," in *Biological invasions in South Africa*, vol. 14. Eds. B. W. Van Wilgen, J. Measey, D. M. Richardson and J. R. Wilson (Berlin/Heidelberg, Germany: Springer), 229–249. doi: 10.1007/978-3-030-32394-3_9

Robinson, T. B., Pope, H. R., Hawken, L., and Binneman, C. (2015). Predation-driven biotic resistance fails to restrict the spread of a sessile rocky shore invader. *Mar. Ecol. Prog. Ser.* 522, 169–179. doi: 10.3354/meps11167

Robson, G. (1986). A new species of South African limpet, *Patella aphanes*, (Mollusca: Gastropoda: Patellidae), with a discussion of *P. obtecta*. *Durban Museum Novitates* 13 (22), 305–320.

Ros, M., Ashton, G. V., Cabezas, M. P., Cacabelos, E., Canning-Clode, J., Carlton, J. T., et al. (2023). "Marine bioinvasions in the anthropocene: challenges and opportunities," in *Coastal habitat conservation*. Ed. F. Espinosa (London: Academic Press), 81–110.

Ruiz Sebastián, C., Steffani, C. N., and Branch, G. M. (2002). Homing and movement patterns of a South African limpet *Scutellastra argenvillei* in an area invaded by an alien mussel *Mytilus galloprovincialis*. *Mar. Ecol. Prog. Ser.* 243, 111–122. doi: 10.3354/meps243111

Sadchatheeswaran, S., Branch, G. M., and Robinson, T. B. (2015). Changes in habitat complexity resulting from sequential invasions of a rocky shore: implications for community structure. *Biol. Invasions* 17, 1799–1816. doi: 10.1007/s10530-014-0837-4

Sadchatheeswaran, S., Branch, G. M., and Robinson, T. B. (2018). Impacts of alien 'ecosystem engineers' overwhelm interannual and seasonal shifts in rocky-shore community composition on Marcus Island, South Africa. *Afr. J. Mar. Sci.* 40, 137–147. doi: 10.2989/1814232X.2018.1462729

Santini, G., Thompson, R. C., Jenkins, S. R., Chelazzi, G., Hartnoll, R. G., Kay, S. M., et al. (2019). Barnacle cover modifies foraging behabiour of the intertidal limpet *Patella vulgata*. *J. Mar. Biol. Ass. U. K.* 99, 1779–1786. doi: 10.1017/s0025315419000778

Signorelli, J., and Pastorino, G. (2021). *Semimytilus patagonicus* (d´Orbigny 1842) (Bivalvia: Mytilidae) revealed as the valid name with priority over *Semimytilus algosus* (Gould 1850). *Arch. Molluskenkd.* 150 (1), 55–63. doi: 10.1127/arch.moll/150/055-063

Silverberg, R. (2015). "Hannibal's elephants," in *Dark city lights: New York short stories*. Ed. L. Block (Three Rooms Press: New York).

Simon-Blecher, N., Granevitze, Z., and Achituv, Y. (2008). *Balanus glandula*: from North-West America to the west coast of South Africa. *Afr. J. Mar. Sci.* 30, 85–92. doi: 10.2989/ajms.2008.30.1.8.458

Skein, L., Alexander, M., and Robinson, T. (2018b). Contrasting invasion patterns in intertidal and subtidal mussel communities. *Afr. Zool.* 53, 1–6. doi: 10.1080/15627020.2018.1448420

Skein, L., Alexander, M. E., and Robinson, T. B. (2020). Co-occurring predators increase biotic resistance against invasive prey. *Mar. Env. Res.* 157, 104929. doi: 10.1016/j.marenvres.2020.104929

Skein, L., Alexander, M. E., and Robinson, T. B. (2021). Characteristics of native predators are more important than those of alien prey in determining the success of biotic resistance in marine systems. *Aquat. Ecol.* 55, 97–106. doi: 10.1007/s10452-020-09814-5

Skein, L., Robinson, T. B., and Alexander, M. E. (2018a). Impacts of mussel invasions on the prey preferences of two native predators. *Behav. Ecol.* 29, 353–359. doi: 10.1093/beheco/arx172

Southward, A. J. (1964). *Limpet grazing and the control of vegetation on rocky shores* (Blackwell Scientific: Oxford).

Steele, W. K. (1992). Diet of Hartlaub's gull *Larus hartlaubii* and the kelp gull *L. dominicanus* in the southwestern Cape Province, South Africa. *Ostrich* 63, 68–82. doi: 10.1080/00306525.1992.9633952

Steffani, C. N., and Branch, G. M. (2003a). Growth rate, condition, and shell shape of *Mytilus galloprovincialis*: responses to wave exposure. *Mar. Ecol. Prog. Ser.* 246, 197–209. doi: 10.3354/meps246197

Steffani, C. N., and Branch, G. M. (2003b). Spatial comparisons of populations of an indigenous limpet *Scutellastra argenvillei* and an alien mussel *Mytilus galloprovincialis* along a gradient of wave energy. *Afr. J. Mar. Sci.* 25, 195–212. doi: 10.2989/18142320309504010

Steffani, C. N., and Branch, G. M. (2003c). Temporal changes in an interaction between an indigenous limpet *Scutellastra argenvillei* and an alien mussel *Mytilus galloprovincialis*: effects of wave exposure. *Afr. J. Mar. Sci.* 25, 213–229. doi: 10.2989/18142320309504011

Steffani, C. N., and Branch, G. M. (2005). Mechanisms and consequences of competition between an alien mussel, *Mytilus galloprovincialis*, and an indigenous limpet, *Scutellastra argenvillei*. *J. Exp. Mar. Biol. Ecol.* 317, 127–142. doi: 10.3354/meps246197

Steneck, R. S. (1982). A limpet-coralline alga association: adaptations and defenses between a selective herbivore and its prey. *Ecology* 63, 507–522. doi: 10.2307/1938967

Stimson, J. (1973). The role of territory in the ecology of the intertidal limpet *Lottia gigantea* (Gray). *Ecology* 54, 1020–1030. doi: 10.2307/1935568

Tejada-Martinez, D., López, D. N., Bonta, C. C., Sepúlveda, R. D., and Valdivia, N. (2016). Positive and negative effects of mesograzers on early-colonizing species in an intertidal rocky-shore community. *Ecol. Evol.* 6, 5761–5770. doi: 10.1002/ece3.2323

Thompson, R. C., Crowe, T. P., and Hawkins, S. J. (2002). Rocky intertidal communities: past environmental changes, present status and predictions for the next 25 years. *Environ. Conserv.* 29, 168–191. doi: 10.1017/s0376892902000115

Underwood, A. J., and Fairweather, P. G. (1989). Supply-side ecology and benthic marine assemblages. *TREE* 4, 16–20. doi: 10.1016/0169-5347(89)90008-6

van Erkom Schurink, C., and Griffiths, C. L. (1991). A comparison of reproductive cycles and reproductive output in four mussel species. *Mar. Ecol. Prog. Ser.* 76, 123–134. doi: 10.3354/meps076123

van Erkom Schurink, C., and Griffiths, C. L. (1993). Factors affecting the rates of growth in four South African mussel species. *Aquaculture* 109, 257–273. doi: 10.1016/0044-8486(93)90168-x

Vanmari, D., and Maneveldt, G. W. (2019). Mechanisms of interference and exploitation competition in a guild of encrusting algae along a South African rocky shore. *Afr. J. Mar. Sci.* 41 (4), 353–359. doi: 10.2989/1814232X.2019.1666738

Van Wilgen, N. J., Faulkner, K. T., Robinson., T. B., South, J., Beckett, H., and Janion-Scheepers, C. (2023). "Climate change and biological invasions in South Africa," in *Invasive species and climate change*, vol. 14. Ed. L. H. Ziska (Oxfordshire, UK: CAB International), 158–202.

von der Meden, C. E. O., Porri, F., Erlandsson, J., and McQuaid, C. D. (2008). Coastline topography and the distribution of indigenous and invasive mussels. *Mar. Ecol. Prog. Ser.* 372, 135–145. doi: 10.3354/meps07731

von der Meden, C. E. O., Porri, F., McQuaid, C. D., Faulkner, K., and Robey, J. (2010). Fine-scale ontogenetic shifts in settlement behaviour of mussels: changing responses to biofilm and conspecific settler presence in *Mytilus galloprovincialis* and *Perna perna*. *Mar. Ecol. Prog. Ser.* 411, 161–171. doi: 10.3354/meps08642

Xavier, B. M., Branch, G. M., and Wieters, E. (2007). Abundance, growth and recruitment of *Mytilus galloprovincialis* on the west coast of South Africa in relation to upwelling. *Mar. Ecol. Prog. Ser.* 346, 189–201. doi: 10.3354/meps07007

Zardi, G. I., McQuaid, C. D., Jacinto, R., Lourenço, C. R., Serrão, E. A., and Nicastro, K. R. (2018). Re-assessing the origins of the invasive mussel *Mytilus galloprovincialis* in southern Africa. *Mar. Freshw. Res.* 69, 607–613. doi: 10.1071/mf17132

Zardi, G. I., McQuaid, C. D., and Nicastro, K. R. (2007). Balancing survival and reproduction: seasonality of wave action, attachment strength and reproductive output in indigenous *Perna perna* and invasive *Mytilus galloprovincialis* mussels. *Mar. Ecol. Prog. Ser.* 334, 155–183. doi: 10.3354/meps334155

Zardi, G. I., Nicastro, A. R., McQuaid, C. D., Rius, M., and Porri, F. (2006). Hydrodynamic stress and habitat partitioning between indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussels: constraints of an evolutionary strategy. *Mar. Biol.* 150, 79–88. doi: 10.1007/s00227-006-0328-y

Zeeman, S. C. F. (2016). *Genetics and ecosystem effects of the invasive Semimytilus algosus, on the west coast of South Africa* (South Africa: University of Cape Town).

Zeeman, Z., Branch, G. M., and Pillay, D. (2018). Comparisons of life-history patterns of the alien invasive *Semimytilus algosus* and three other mussel species on the west coast of South Africa. *Mar. Ecol. Prog. Ser.* 607, 113–127. doi: 10.3354/meps12794

Zeeman, Z., Branch, G. M., Pillay, D., and van der Heyden, S. (2020). Origin and genetic diversity of the invasive mussel *Semimytilus algosus* in South Africa, relative to source populations in Chile and Namibia. *Biol. Inv.* 22, 2309–2323. doi: 10.1007/s10530-020-02257-x

frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 12 July 2023
DOI 10.3389/fmars.2023.1127630

OPEN ACCESS

EDITED BY
Alan Hodgson,
Rhodes University, South Africa

REVIEWED BY
Francesco Tiralongo,
University of Catania, Italy
Mariachiara Chiantore,
University of Genoa, Italy

*CORRESPONDENCE
E. Ostalé-Valriberas
✉ enriqueostalevalriberas@gmail.com

RECEIVED 19 December 2022
ACCEPTED 08 June 2023
PUBLISHED 12 July 2023

CITATION
Ostalé-Valriberas E, Sabino-Lorenzo Á, Ali-Ahmed A, Pavón-Paneque A, Sempere-Valverde J, Espinosa F and García-Gómez JC (2023) When the population of an endangered marine mollusc (*Patella ferruginea*) increases almost three-fold in ten years. Reality or fiction? *Front. Mar. Sci.* 10:1127630. doi: 10.3389/fmars.2023.1127630

When the population of an endangered marine mollusc (*Patella ferruginea*) increases almost three-fold in ten years. Reality or fiction?

E. Ostalé-Valriberas*, Á. Sabino-Lorenzo, A. Ali-Ahmed, A. Pavón-Paneque, J. Sempere-Valverde, F. Espinosa and J. C. García-Gómez

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), Universidad de Sevilla, Seville, Spain

The critically endangered species *Patella ferruginea* (Gastropoda, Patellidae), endemic to the western Mediterranean, has breeding populations in both natural and artificial habitats, the latter of which are generally linked to port infrastructures. Over the past decade, the temporal change of this species' population has been monitored (structure and density) using exhaustive censuses along Ceuta's coast (Strait of Gibraltar), one of the few stronghold populations within the entire Mediterranean basin. This study focuses on the population dynamics of *P. ferruginea* in Ceuta and the environmental factors that affect the structure of this population, such as wave exposure, coastline heterogeneity, substratum roughness, substratum lithology, and chlorophyll-a concentration. Different potential negative interactions were also considered: angling, shell fishing, bathing in the intertidal, bathing near the intertidal, recreational boating and temporary migrant campsites nearby. The results have shown in the period 2011-2021, the estimated size of *P. ferruginea* population has increased by 200 %, from 55,902 to 168,463 individuals (of which 131,776 are adults). The subpopulation with the greatest increase in these years was the one settled on dolomitic rip-raps inside the Ceuta's harbor, with an increase of 1,288%. The results of the present study indicate that Ceuta hosts the main population of this endangered species through its distributional range (Western Mediterranean), being a source population on the Southern Iberian Peninsula that its preservation must be prioritized. Statistical modelling has shown that the adult density of *P. ferruginea* is positively influenced by coastal heterogeneity, habitat area and substratum roughness, but negatively by vertical inclination, concentration of chlorophyll-a, and anthropogenic impact. These results also support the concept of ¨Artificial Marine Micro-Reserves¨ as a new area-based conservation measure according with the IUCN guidelines, as these will contribute to setting up a network of these source populations that promote genetic flow among populations, with eventual recolonization throughout its original distribution.

KEYWORDS

***Patella ferruginea*, patellogastropoda, conservation, extinction risk, population structure, artificial marine micro-reserves, artificial substratum, source population**

1 Introduction

Coastal areas host half of the world's cities and more than half of the world's population, with population density near the coast three times higher than the global average (Creel, 2003; Small and Nicholls, 2003; Airoldi and Beck, 2007). Shoreline urbanization combined with the increase of tourist, recreational and commercial activities, has resulted in the introduction and proliferation of artificial structures in marine coastal habitats worldwide (Bulleri and Chapman, 2010; Firth et al., 2016). The human impact associated with these structures has been reported to affect biological processes on all spatial and temporal scales (Airoldi et al., 2005). Among them, fragmentation of natural habitats by urbanization processes can cause a reduction in population size and connectivity of species, and thus increase their risk of extinction in the face of certain permanent disturbances (Aguilera et al., 2020; Chase et al., 2020).

Limpets are considered keystone species in rocky coastal ecosystems as they maintain the composition and structure of the biological community and, when they disappear from their habitats, cause a cascading effect on them (Raffaelli and Hawkins, 1996). Therefore, limpets may be the most important group of grazers in temperate rocky intertidal zones, because their feeding activity limits the growth of macroalgae and controls barnacle numbers (Branch, 1981).

Among limpets, the larger species are frequently the most ecologically relevant, being defined as 'giant limpets' (shell length greater than 100 mm). With this classification a total of 14 species in the world can be found, three of them catalogued as threatened (Espinosa and Rivera-Ingraham, 2017). *Patella ferruginea* Gmelin, 1791 (Gastropoda: Patellidae) belongs to this classification, as it achieves a maximum recorded shell length of 115 mm (Supplementary Figure 1-S). It is endemic to the Western Mediterranean Sea and it is considered the most endangered marine invertebrate (Ramos, 1998), together with *Pinna nobilis*, within the European coastline (Vázquez-Luis et al., 2017; Lattos et al., 2021).

Like other limpet species, *P. ferruginea* is a broadcast spawner with external fertilization so that the urban development of coastal areas has dramatically fragmented its habitat (Airoldi et al., 2005; Firth et al., 2016). Moreover, the conspicuous star-shaped shell and large sizes that *P. ferruginea* can reach has made this species the target of human collection since the Quaternary (Espinosa, 2006; Espinosa et al., 2009; Rivera-Ingraham et al., 2011a; Guallart et al., 2013a).

On account of these anthropogenic impacts, its distribution range has progressively been contracted, and *P. ferruginea* has nearly disappeared from European continental coasts, except for the small and fragmented populations present in Andalusia Southern Spain, Liguria (Italy) and Southern France (Cottalorda et al., 2004; Espinosa et al., 2014b; Luque et al., 2018; Ferranti et al., 2019; CMAOT, 2020). The remaining most notable populations are found in scattered North African gated communities: Ceuta, Melilla, Chafarinas Islands, Habibas Islands, and the island of Zembra (Espinosa et al., 2014b; Luque et al., 2018). In this regard, it has recently been identified that possibly the population located in Ceuta (Strait of Gibraltar, North Africa) plays the role of source population in the area, maintaining nearby sink populations in the south of the Iberian Peninsula (Espinosa et al., 2018) according to the "source-sink population model" (see Pulliam, 1988; Pulliam, 1996).

This study focuses on the population dynamics of *P. ferruginea* in Ceuta and the environmental factors that shape the population size structure. Consequently, the objectives of the present study are i) to determine the current status of this *P. ferruginea* population, ii) track the any change in the population in the last 10 years, iii) to confirm the result of the ¨population viability analysis¨ (PVA) prediction method performed by Espinosa et al. (2018) of the *P. ferruginea* population in Ceuta and iv) explore the environmental factors affecting the populations of *P. ferruginea*.

2 Materials and methods

2.1 Study area

The Strait of Gibraltar is a very important geological region, bordering by the Mediterranean, Lusitanian and Mauritanian biogeographic provinces, so it is a zone in which marine flora and fauna from the Mediterranean and Atlantic overlap (Coll et al., 2010). Likewise, it is one of the world's busiest hubs for maritime traffic, as it is a place where many commercial routes converge and where important harbors are present (Algeciras, Gibraltar and Tangier Med), which makes it an area with high risk of disturbances, impacts and environmental disasters (Piniella and Walliser, 2013: Nachite et al., 2020).

The harbor of Ceuta, a Spanish city in North Africa, has an unusual structure, because it is located between two bays (North and South) connected to both by the Royal Moat (Figure 1A). This defensive construction, built by the Portuguese during the 16th century, increases water movement and exchange within the structure (shorting the residence time of the water mass), when compared to other conventional harbors, maintaining moderate oxygen levels in the water and sediment (Guerra-García et al., 2004a; Guerra-Garcia and García-Gómez, 2005; Sánchez-Badorrey et al., 2021).

2.2 Sampling methods

2.2.1 *Patella ferruginea* census

A census on *P. ferruginea* population in Ceuta was carried out during 2019 and 2020 (14 months). To estimate the status and evolution of the *P. ferruginea* population, the sampling methodology repeated the one carried out by Rivera-Ingraham et al. (2011b) in 2010 in the same area. The complete coastline was divided into 18 sectors; accounting for the type of substratum (natural, dolomitic rip-rap and concrete cubes) and its geographic orientation (see sectors at Figure 1B).

To know the ¨population size structure¨ of *P. ferruginea's* population, the 20% of the entire rocky midlittoral, where the species occurs, was sampled at each sector. Ten meter linear transects were proportionally distributed within each sector separated by 40 m. The shell length of the specimens found

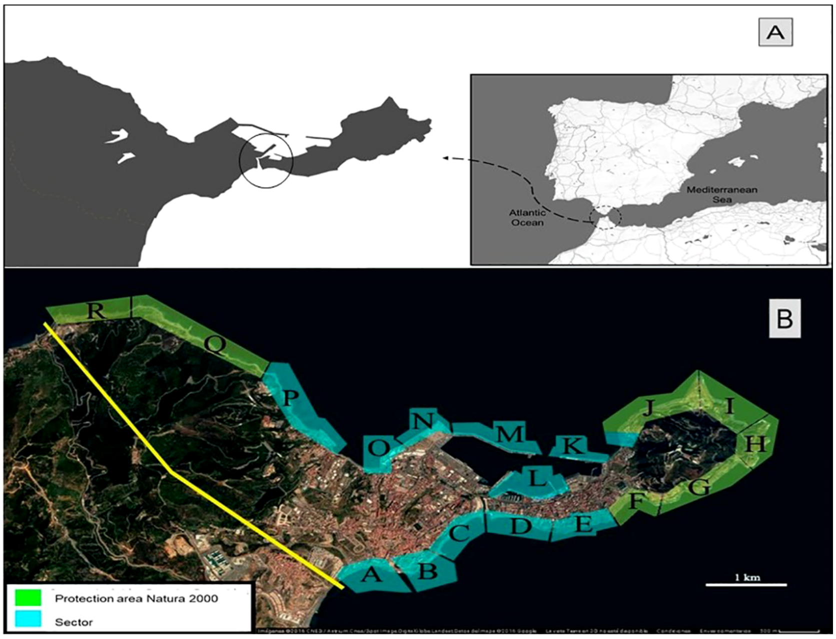


FIGURE 1
(A) Location of Ceuta, inside the black circle can be found the royal Moat; **(B)** Sectorization of the coastline to estimate the population size distribution of *P. ferruginea*. Source: Google Earth.

within the intertidal at each transect were measured to the nearest millimetre using a calliper (Guerra-García et al., 2004b; Espinosa, 2009b; Rivera-Ingraham et al., 2011b). Finally, 4,560 m of rocky midlittoral coastline were sampled out of a total of 23,617 m of rocky coastline present in the city of Ceuta (Table 1).

To estimate the total number of individuals in each sector and thus of the population, the results were extrapolated from the total length (m^{-1}) of *P. ferruginea* habitat in the sector. In the present study, recruits were defined as individuals smaller than 30 mm and adults greater than or equal to 30 mm (Espinosa et al., 2006) and the "class size" was categorize for every 10 mm of shell length (e.g., class size 3 pertains to individuals from 21mm to 30mm).

To track any change of the population structure in the last 10 years, it has been considered that in the preceding work performed by Rivera-Ingraham et al. (2011b). In the said study, the total length of rocky shoreline where the species could potentially be present was calculated by using 1:9,000 scale maps obtained using Google Earth©. Therefore, the estimations from the previous census have been recalculated, according to the used estimate of coastline length in the present work (10 m resolution), in order to make both estimations comparable and to be able to evaluate the percent increase or decrease of the population in each sector.

The percentage change in the number of individuals (%) between both studies was calculated using the equation:

$$Percentage\ \ change\ \ (\%) = (\frac{estimation\ \ 2020}{estimation\ \ 2010}\ )\ x\ \ 100) - 100$$

2.2.2 Environmental factors

Once the sampling of *P. ferruginea* was completed, the environmental factors were studied. All environmental factors were studied in the same transect that were randomly selected in each sector (three transects per sector, 54 transects in total) in July of 2022.

Substratum lithology of each sector was characterized by collecting three rock chips from each sector, which were powdered in the laboratory using a ball mill with stainless steel balls as grinding media. Each rock chip was obtained by hammer and chisel. The elemental composition and calcination percentage of each sample was quantified by X- ray fluorescence (XRF) using an AXIOS spectrometer. Mineralogic absorption spectra were detected by X-ray diffraction (XRD) using a powder diffractometer (Bruker D8 Advance) equipped with a high temperature chamber (Anton Paar XRK 900) and a fast response/high sensitivity detector (Bruker Vantec 1) with radial Soller slits (full methodology can be accessed in Valverde et al., 2015). Afterwards, XRF and XRD data were interpreted to quantify the crystallinity and lithology composition of each sample using DRIFAC.EVA.4.1 software.

To assess the influence of microalgal abundance on *P. ferruginea* demographic, the chlorophyll-a concentration on the substratum surface was estimated in the same rock samples used to study lithology. The resulting slurry was treated with 20 ml of 100% ethanol to extract the pigments. Then, samples were frozen and maintained in darkness until analysis (minimum of 48 h) according with the protocol used by Kido and Murray (2003). Samples were filtered through a Whatman GF/C filter and measurements of absorbance were carried out with a spectrophotometer (Pharmacia Biotech Novaspec II).

Given the large extension of the study area, some environmental factors like chlorophyll a were collected once, being a snapshot of the environment. Moreover, for the Chl-a we took the 54 samples in two consecutive days of July 2020 using a zodiac and sampling in

TABLE 1 Comparison of the mean density, adult mean density and the total estimate individuals between both studies about *P. ferruginea* population of Ceuta (change from 2010 to 2020).

Sector	Rivera-Ingramham et al., 2011b							Present study						Percentage change in the estimated number of the limpet, present study respect to Rivera-Ingramham et al., 2011b
	Total habitat length (m)	Sampled length (m)	Total individuals registered (ind.)	Density (ind./m)	Adult mean density (ind./m)	Total estimate individuals	Total estimate individuals according the total habitat length of the present study	Total habitat length (m)	Sampled length (m)	Total individuals registered (Ind.)	Density (ind./m)	Adult mean density (ind./m)	Total estimate individuals	
A	300	100	195	1.95	1.72	585	1,043.25	535	110	115	1.05	0.87	559.32	-46.39
B	311	311	2,260	7.27	5.83	2,260	5,922.51	815	170	672	3.95	3.38	3,221.65	-45.60
C	712	712	3,518	4.94	3.25	3,518	3,952.81	800	160	808	5.05	3.88	4,040.00	2.21
D	350	350	2,262	6.46	4.28	2,262	4,200.86	650	130	788	6.06	5.30	3,940.00	-6.21
E	1142	100	104	1.04	1.03	1,187	1,352.00	1,300	260	1,286	4.95	4.68	6,430.00	375.59
F	350	100	106	1.06	0.86	371	848.00	800	160	874	5.46	5.03	4,370.00	415.33
G	1,710	100	297	2.97	2.22	5,079	6,237.00	2,100	420	2,126	5.06	4.39	10,630.00	70.43
H	2,201	258	479	1.86	1.79	4,086	3,434.69	1,850	370	3,263	8.82	7.89	16,315.00	375.01
I	1,271	100	440	4.40	3.56	5,592	10,340.00	2,350	470	4,921	10.47	8.81	24,605.00	137.96
J	959	100	19	0.19	0.15	182	313.50	1,650	330	277	0.84	0.77	1,385.00	341.79
K	555	100	709	7.09	4.28	3,934	4,254.00	600	120	2,435	20.29	15.35	12,175.00	186.20
L+L'	3,754	3,754	4,111	1.10	0.66	4,111	2,792.50	2,550	510	7,755	15,21	9.27	38,775.00	1,288.54
M	1,115	50	244	4.88	3.70	5,441	5,616.88	1,151	230	3,065	13.33	9.01	15,338.33	173.08
N	1,156	30	23	0.77	0.10	886	881.67	1,150	230	1,536	6.68	4.79	7,680.00	771.08
O	316	100	273	2.73	1.71	863	682.50	250	50	661	13.22	9.04	3,305.00	384.25
P	1,217	163	351	2.15	0.21	2,987	2,963.04	1,376	270	1,964	7.27	5.21	10,009.13	237.80
Q	1,139	100	42	0.42	0.37	477	667.80	1,590	320	785	2.45	2.00	3,900.47	484.08
R	900	100	19	0.19	0.18	171	399.00	2,100	420	357	0.85	0.76	1,785.00	347.37
Total	19,458	6,628	15,452	2.86	1.99	43,992	55,902.01	23,617	4,560	33,687	7.28	5.58	168,463.89	201.36

the same hour of the day. The chlorophyll-a concentration was calculated according to the formula of Thompson et al. (1999):

$$[Chlorophyll - a] = \frac{12.2\ \ x\ \ A665\ \ x\ \ v}{(d\ \ x\ \ V)}$$

Where, 12.2 = constant for ethanol, A6655 = net absorbance of solution at 665 nm, v = final volume of solution (20 ml), d = path length of cell (1.6 cm), V = surface area of sample ($100cm^2$). Chlorophyll-a concentration is expressed in µg cm^{-2}.

The wave exposure was quantified for each sector based on a fetch model index developed by Howes et al. (1994) (see Terrón-Sigler et al., 2018). Fetch models have been successfully used to predict marine community patterns (e.g. Ros et al., 2016) by providing quantitative estimates of wave exposure using a combination of two indices: maximum fetch (Fi) and modified effective fetch (Fe). Maximum fetch is defined as the maximum fetch distance in km measured from the point of interest. When a vector does not find and obstacle (i.e. open ocean), a value of 1,000 km is conventionally used. Effective fetch (Fe) is calculated from the equation:

$$Fe = \frac{[\sum\ (cos\ \ \Theta i) \times\ \ Fi]}{\sum\ \ cos\ \ \Theta i\,.\,Fe}$$

Where Θi is the angle between the shore-normal and the directions 0°, 45° to the left ad 45° to the right, and Fi is the fetch distance in km along the relevant vector. Combining the values obtained for each index, the wave exposure class of each sector was determined based on the classification proposed by Howes et al. (1994). The different Fi values (-45°, 0° and 45°) were calculated for each transect studied using Google Earth©.

The heterogeneity of the coast (macroscale roughness) was calculated by lying a 10 m flexible measuring tape along the upper mid-littoral, which was allocated following as closely as possible the contours of the bare substratum along the transect. Using the same methodology, three replicates were recorded on each transect randomly selected along the height of the rocky midlittoral (habitat of *P. ferruginea*). This information was used to obtain was used to obtain the area of *P. ferruginea* habitat in each sector: profile of longitudinal x profile of height (m).

Regarding microscale roughness, three 25 cm profile gauges per transect with 0.5 mm pins were pushed onto the bare rock within the same transects already selected for macroscale roughness to record substrata surface heterogeneity (Frost et al., 2005). The resulting profiles were photographed, and the images were digitally processed with Adobe© Photoshop to obtain two-coloured images. The length of the contour of the profile was obtained with ImageJ software. In both cases (macro- and microscale), substratum roughness was calculated as in Rivera-Ingraham et al., 2011b; Rivera-Ingraham et al., 2011c and Sedano et al., 2020 using the equation by Blanchard and Bourget (1999): $Roughness\ = \frac{Tr}{Ts}$. Where Tr is the contour measured between two points and Ts the linear distance between those points.

The inclination of the substratum was taken recording three replicates within the already selected transects using a digital clinometer (see Rivera-Ingraham et al., 2011c).

The impact of human activity in the limpet's habitat (intertidal zone) was sampled in July 2021, since it is the month of the year when more people are present within the littoral (personal observations from Ostalé-Valriberas). In the present study, the variable "impact of human activity" was restricted to the direct interaction of the citizen with the rocky intertidal. Each sector was visited twice by boat to quantify the different interactions. Sampling was conducted on two weekends in July due to the large number of citizens around the shoreline on these days. The first weekend sampled was from 11 a.m. to 2 p.m. in the morning and the second from 6 p.m. to 9 p.m., each sector was observed for half an hour each day. In each sampling session, all the sectors were visited. Each interaction was scored from 1 to 3 to categorise the different impacts on *P. ferruginea* habitat. The difference interactions registered were angling (x3), shell fishing (x3), bathing in the intertidal (x2), bathing near the intertidal (x1), recreational boat nearby (x1) and irregular camp (x1) (the concept ¨irregular immigrant camp¨ derives from the exceptional migratory problem of 17th May of 2021 in which the local administration assistance was overwhelmed, see investigation of Casey and Bautista (2021). To estimate the used index for the GLM, the total of each interaction was multiplied by the impact value on the habitat (e.g. 9 people bathing in the intertidal (x2) gives a value of 18). In the methodology for quantifying human activity, value 1 is "low impact" and 3 is "high impact".

A constrained ordination approach to assess how well the biological data relate to different abiotic variables that characterize the different sectors was carried out. The parameters considered were heterogeneity of the coast (macroscale roughness), microscale roughness, area of *P. ferruginea* habitat, concentration of chlorophyll-a, inclination of the substratum, wave exposure (fetch) of the littoral, impact of human activity and rock elemental composition.

2.3 Statistical analyses

2.3.1 *Patella ferruginea* census

Among the sectors, a PERMANOVA analysis was carried out between the different size classes to differentiate the population size structure of *P. ferruginea* distribution. Before the analyses, a square root transformation along with the Bray Curtis similarity index was applied to different size classes. Differences among sectors were established using nNMDS (non-metric multidimensional scaling) and cluster analysis.

The change of the *P. ferruginea* population in the last 10 years was determined by comparing the adult density between the present study and preceding work performed by Rivera-Ingraham et al. (2011b). To obtain this result, Euclidean distance matrices were calculated and tested using univariate PERMANOVA.

PERMANOVA analyses were performed using the PRIMER v.6 +PERMANOVA package (Clarke and Gorley, 2005; Anderson et al., 2008).

2.3.2 Environmental parameters

The effect in the *P. ferruginea* population's structure (adult density and adult mean size) of different environmental parameters were modelled using generalized linear models (GLMs; McCullagh and Nelder, 1989). Models were carried out using normal distributions and identity link functions to determine which of the parameters gave the best fit to the data (see Cayuela, 2010). The environmental parameters added to the model were the percentage of calcium oxide in the substratum (CaO %), chlorophyll-a concentration, wave exposure (fetch), roughness of substratum (macroscale and microscale), area of *P. ferruginea* habitat, inclination of the littoral and anthropogenic impact. Prior to the analysis, environmental variables were tested for multicollinearity, assuming collinearity when variance inflation factor (VIF) values were much greater than 1. Model selection was carried out based on Akaike's information criterion for small sample sizes (AICc) (Burnham and Anderson, 2002). Variability explained by the best models was computed using deviance values (D^2), comparing residual deviance with the deviance of a null model (null deviance). Among all significant models, the minimal Akaike value indicated the best model that explained the greatest proportion of variance under the restriction that all the predictors in the model must be significant (see Cayuela, 2010; Ros et al., 2015).

The density of recruits (individuals< 30 mm) was not considered in the GLM because their presence is highly fluctuating throughout the year (recruits of this species settle on the intertidal rock between December and March) (see Espinosa, 2006), and it was impossible to sample all sectors at the same time in those months.

Generalized Lineal Models were carried out using SPSS 25.0© (IBM, New York, NY, USA) (Pallant, 2020).

3 Results

3.1 Present status of *Patella ferruginea* in Ceuta

In the 20% of the *P. ferruginea* habitat (4,560 m), it was registered 33,687 individuals so that it was estimate that the population of Ceuta have 168,464 individuals distributed in a total of 23,617 m of rocky coastline (Table 1). From the whole population, it was estimated that 131,775.87 were adult individuals (≥ 30 mm). PERMANOVA analysis showed that there were significant differences between the population structure (different size classes) of the different sectors (Table 2).

Individuals of *P. ferruginea* were present in all sampled sectors (Figure 2), with the greater mean density of individuals and adults occurring in the dolomitic rip-rap inside the harbour (sector L), with a mean density of 32.2 ind./m and adult mean density of 19.57 ind/m (Figure 3). The greatest values per transect were achieved in this sector (54.84 ind./m and 30.84 ind./m of adults), although the concrete seawalls in the same area (subsector L') had one of the lowest values. The natural habitat with greater *P. ferruginea*

TABLE 2 One-way PERMANOVA results for *P. ferruginea* size classes on different studied sectors.

	Source of variation	Df	MS	Pseudo-F	P (perm)
Size clases (cm)	Sector	18	10100	15.93	< 0,0001
	Transect (sector)	452	633.98		
	Total	470			

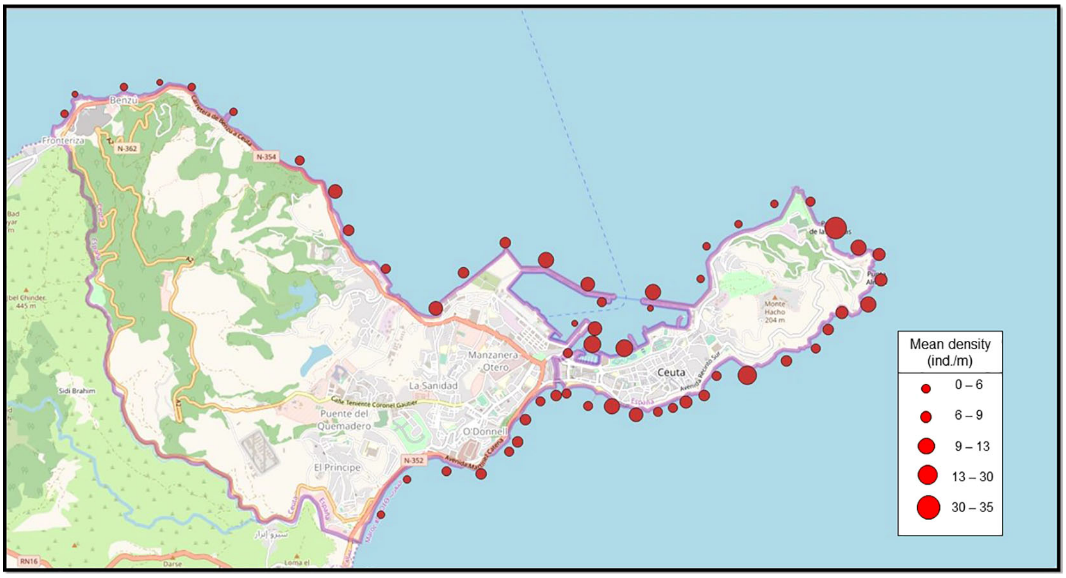


FIGURE 2
General distribution of *P. ferruginea* in Ceuta. Circle diameter corresponds to the density of individuals. Google Earth.

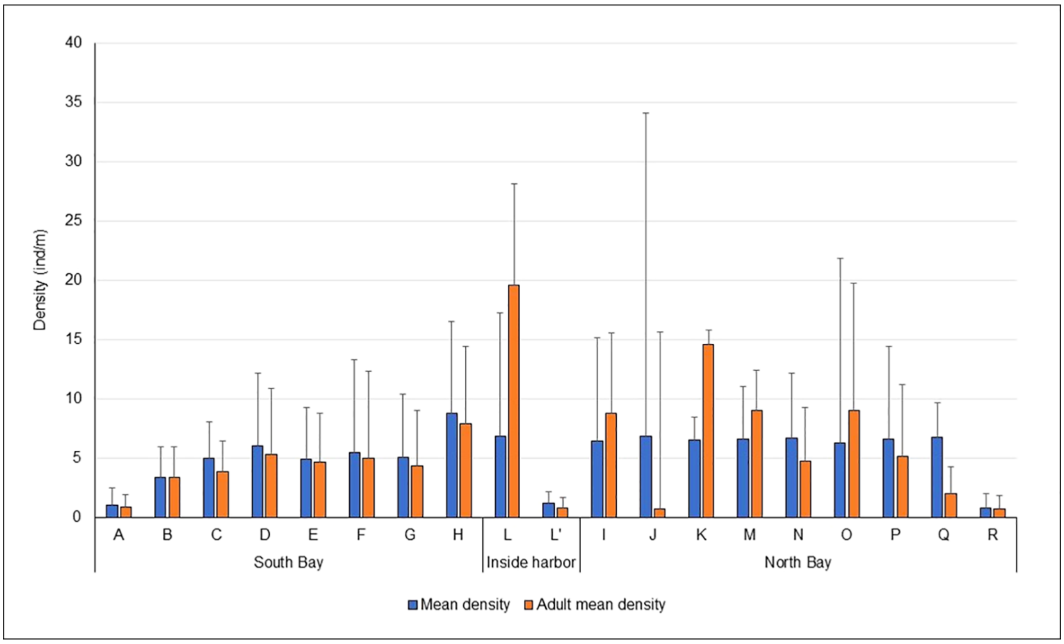


FIGURE 3
Mean density and adult mean density of *P. ferruginea* for each of the sectors and standard deviation.

densities was Acantilados de la Sirena (sector H) with 8.82 ind./m and 7.89 ind./m of adults.

Greater average shell length was registered in sector E, in which mean size was 584 mm and the adult mean size 603 mm (Figure 4). The biggest individual measured had a maximum shell length of 113 mm (Supplementary Figure 1-S). The average shell size of Ceuta's population was 447 mm and the mean adult size 508 mm.

Multivariate analyses revealed differences in the structure of *P. ferruginea* population (abundances within size classes) among sectors (Figures 5–7). The combination of NMDS and Cluster analyses showed the segregation of sector L from other sectors. The subpopulations most similar to sector L were K, M and O, all these sectors were composed of rip-raps made with quarry rocks (Supplementary Table 4-S). On the contrary, coinciding with the less dense sectors, were the sectors R, J, A and L' (Table 1; Figures 2, 3).

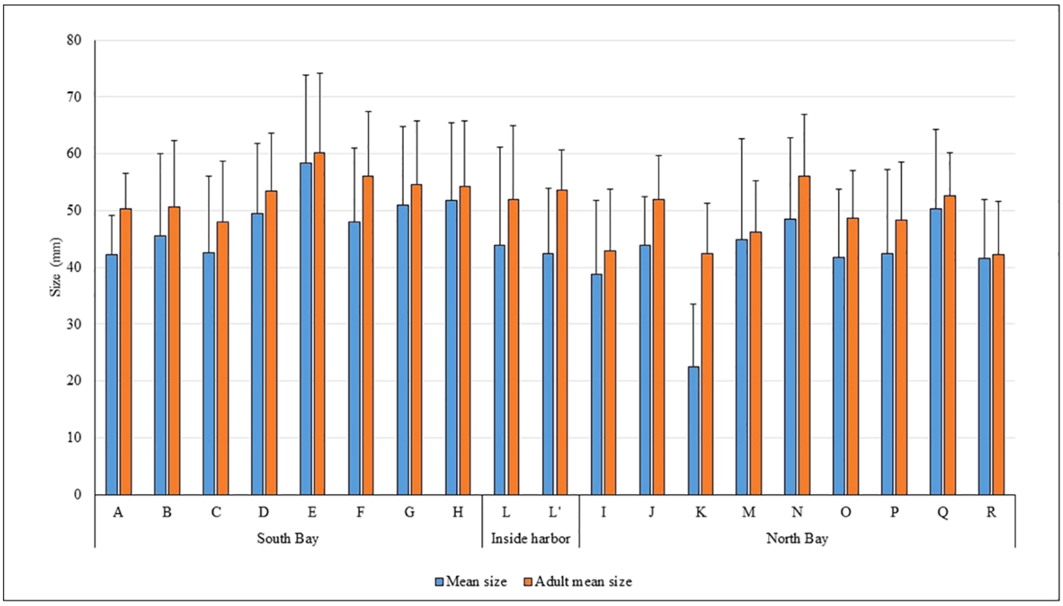


FIGURE 4
Mean size and adult mean size of *P. ferruginea* for each of the sectors and standard deviation.

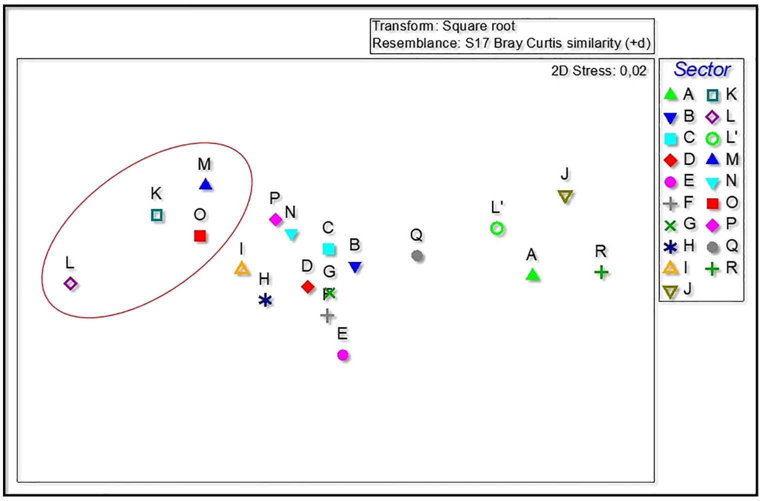


FIGURE 5
nMDS computed using the abundances of each size class and showing PCO centroids for each of the sectors. Red circle: sectors with dolomitic rip-rap of the commercial harbor where the access is restricted with a great heterogeneity substratum (macro and micro scale).

3.2 Change in the population structure of the *Patella ferruginea* population in the last 10 years

Recalculating the total estimated individuals from the study of Rivera-Ingraham et al. (2011b) according to the total habitat length of the present study, the population has increased from 55,902 to 168,463 individuals. This means that the population has increased by an average of 201.36% compared to the existing population 10 years ago (Table 1).

The subpopulation with the higher increase in these years was the one inhabiting dolomitic rip-rap inside the harbor (sector L), with an increase of 1,288%. On the opposite side, the subpopulations with higher decrease were those on sectors A and B with a -46.39% and -45.60% respectively (Table 1).

Univariate PERMANOVA showed that the adult mean density (ind./m) of the present study was significantly higher than the Rivera-Ingraham et al. (2011b) study (Pseudo-F = 14.68; $p < 0.001$).

3.3 Effects of environmental parameters on population structure

According to an omnibus test, only the variable adult density was significant. The VIF values show that the variables used to evaluate their influence on adult density were not correlated with each other.

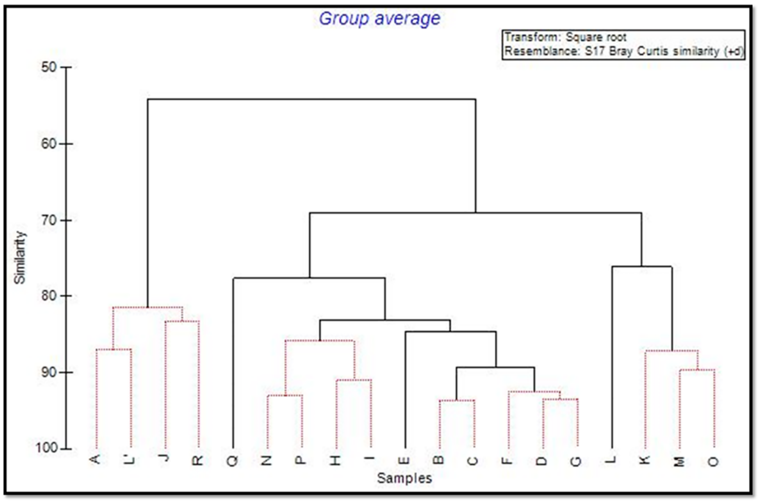


FIGURE 6
Cluster analysis computed using the mean abundances of the different size classes of *P. ferruginea* for each of the sectors considered. Discontinuous lines indicate significantly different groups of sectors segregated by SIMPROF analysis.

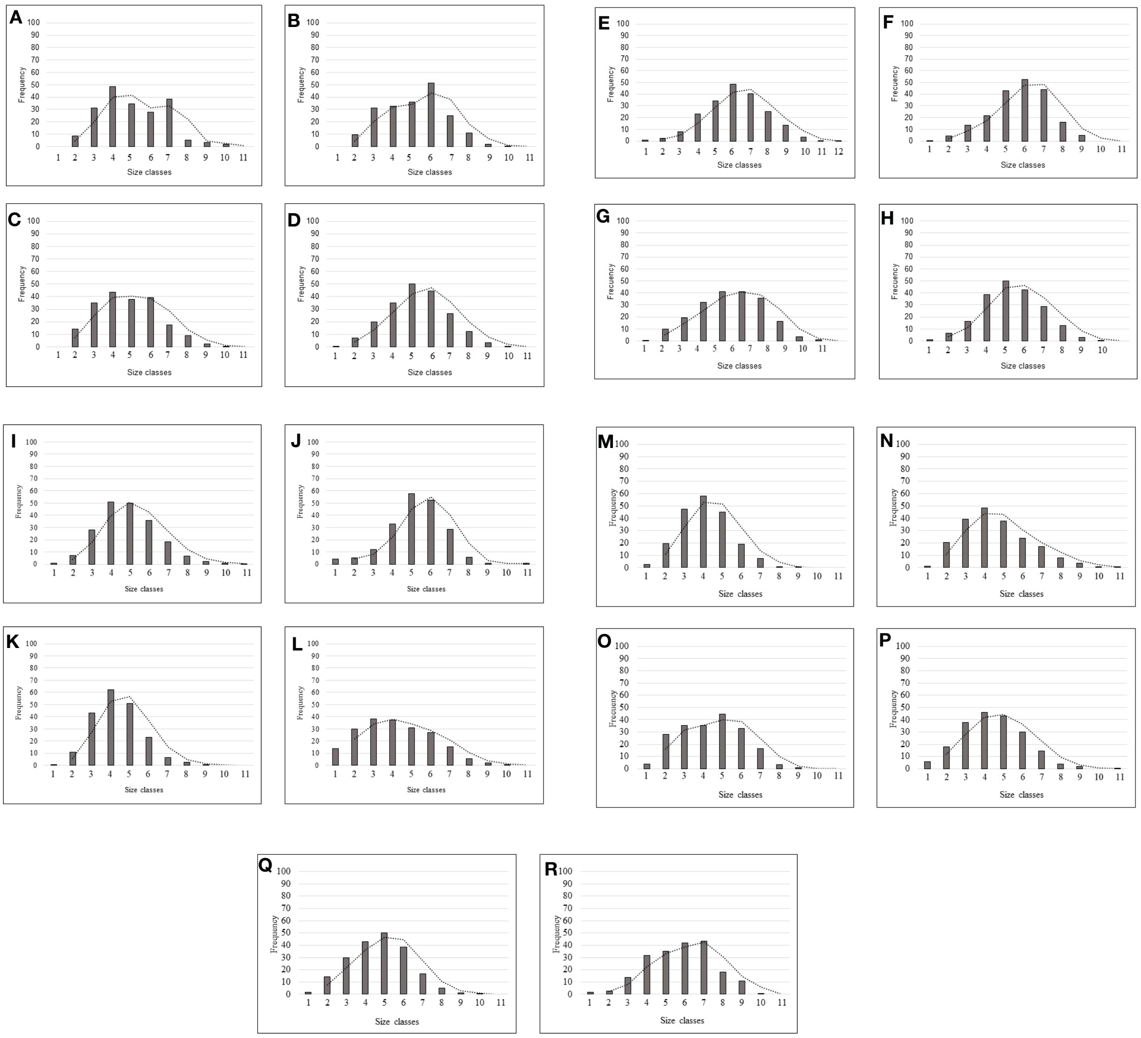


FIGURE 7
Size class frequencies of *P. ferruginea* for each of the sectors considered to know the population structure (trend line). Letters indicate the sampled sectors (see Figure 1B).

The model demonstrated that the adult density is significantly influenced by heterogeneity (macroscale), microscale roughness, inclination of the littoral, chlorophyll-a concentration and anthropogenic impact. According to AICc values, the best model was the combination of these five parameters, reporting the 85.9% of the variability explained (Table 3). Adult density showed a positive correlation with the macroscale and microscale variables, while the variables inclination, chlorophyll-a concentration and anthropogenic impact showed a negative correlation.

Analyzing the results obtained for the microalgae (Chl-a concentration) in each sector, it is observed that the highest values (marked in green in Table 1-S) correspond to the natural

TABLE 3 Model selection results explaining the environmental parameters that have influence in the adult density and adult mean size.

	df	AICc	β	Exp(β)	Δi	LogLik	D^2(%)
Adult density Normal distribution Link function: Logit							
Macro* + Micro* + Incli** + Chl* + Anthro**	12	38.82	2.35/0.24/-0.06/ -0.8/-0.38	10.52/1.27/0.94/ 0.45/0.962	0	-6.81	85.9
Macro** + Incli** + Chla* + Anthro**	13	39.08	3/-0.75/-0.66/-0.38	20.24/0.93/0.51/0.96	0.26	-9.72	80.5

Only significant models according to an omnibus test are shown. Model averaged coefficients (β) of explanatory variables in the significant models; Exp(β): exponential. Akaike's information criterion corrected for small sample size (AICc) and AICc difference between the AICc of each model and the AICc of the best fitted model (Δi) were used for comparison. Variability explained by the best models is shown using deviance (D^2). The model with the lowest Δi is the best AICc model. Variables considered were: Macro, macroscale; Micro, microscale; Area, surface; Incli, inclination; Chl-a, concentration of chlorophyll-a; Anthro, anthropogenic impact; Fetch, wave exposure; CaO, calcium oxide. $^{**}P < 0.001$. $^{*}P < 0.05$.

substratum (sectors: R, B, D and G) and the lowest (marked in red in Table 1-S) correspond to the artificial substratum (sectors: N, O, K and L) (see Table 1 and Figure 2).

The adult density did not show any significant correlation with the variables area of habitat, wave exposure (fetch) and percentage of calcium oxide in the substratum (CaO %). The results of the environmental factors used in the GLM are shown as Supplementary Material (Table 1-S). The results of impact human activity (Supplementary Table 2-S) and rock elemental composition (elemental composition, Supplementary Table 3-S); mineralogy composition, Supplementary Table 4-S) were show like Supplementary Material.

4 Discussion

The results have shown that from 2011 to 2021 the population or *P. ferruginea* increased by about 200%. Currently, the population numbers 168,464 individuals, of which 131,776 are adults. In about a decade, the population has grown from 55,902 to 168,464 individuals. Recent studies have shown that there has been recolonization and repopulation of *P. ferruginea* in some localities of the Andalusia, Melilla, Alboran and Chafarinas island (Casado et al., 2017; Maestre et al., 2018; CMAOT, 2020). Therefore, Ceuta's population could be an important contributor to the recolonization of these areas, especially when considering that nearly 80% of its individuals are adults. Nevertheless, the situation of *P. ferruginea* is still critical because the individuals settled in Andalucía are the last stronghold of continental Europe, depending heavily on recruits from North African populations (CMAOT, 2020). These results show that the area of Ceuta is a ¨stronghold¨ for this endangered species and support the hypothesis that this population has the role of a ¨source population¨ of new recruits for other nearby ¨sink populations¨ in the metapopulation of the Alboran Sea supporting the hypothesis proposed by Espinosa et al., 2018.

These conclusions can be supported by different oceanographic and biological studies carried out in this biogeographic area. In this sense, it is known that all *P. ferruginea* populations from the Alboran Sea and North Africa up to Tunisia belong to the same genetic cluster (Casu et al., 2011). Dispersal studies showed that the directionality of the surface currents of the Alboran Sea, especially those near the Strait of Gibraltar in its first loop go from south to north (therefore, the potential larval input should go from south to north and not the other way around, at least from the coast of Ceuta) (see Periáñez, 2007). Likewise, dispersal studies of *P. ferruginea* larvae in the Alboran Sea show simulated regional trajectories for virtual larvae released from Ceuta with strong prevailing westerly wind at high and low spring tide conditions with a Pelagic Larval Duration of 14 days during January 2016 can cross and complete the first loop of the Alboran Sea (Stephen Warr unpublished data). The settlement ability of larvae of this species is reached 3-4 days after fertilization, depending on water temperature, and crawling and swimming pediveliger larvae have a phase of 7-32 days (probably up to 40 days) until metamorphosis to juvenile occurs (Ferranti et al., 2022), which would argue that the population studied in Ceuta should be classified as a "source population" according to the "source-sink population model" (see Pulliam, 1988; Pulliam, 1996).

Although the sampling effort was larger than previous studies, in light of the results a review of the existing literature, reveals that the population in Ceuta is the largest within the species' natural distribution (see Table 4).

The mean density of the population has increased from 2.33 ind/m in 2010 (Rivera-Ingraham et al., 2011b) to 7.39 ind/m in 2020. This is in agreement with the output of the population viability analysis (PVA) model carried out by Espinosa et al. (2018), and based on monitoring during a ten-year period. This model indicates the unlikeliness of the population in Ceuta facing extinction within the next 50 years. This confirms the effectiveness of the control and temporary monitoring programs of the population proposed in Spain's National Conservation Strategy for *P. ferruginea*, established in 2008 by the Spanish Ministry of Environment (MMAMRM, 2008).

The increase of Ceuta's population could be due to two different factors. Firstly, there are some littoral zones with difficult or restricted access, such as sectors H and I, which are a natural cliffs, and sectors K, L and M, which are access-restricted areas in the commercial harbor of artificial structure type riprap (great heterogeneity, macroscale and microscale). These sectors would be therefore protected from anthropogenic impact, which has been identified as the main factor promoting the decline of the species (Ostalé-Valriberas et al., 2022 and references therein). Secondly, there has been an improvement in social awareness of the conservation status of this endangered species, promoted by dissemination activities performed by NGOs and the environmental administration, and increased enforcement by local authorities. In this sense, many projects and news have been published in social media publications (Mar, 2020), giving notoriety to *P. ferruginea* and turning it into a flagship species. In this sense, flagship species attract funding, increase awareness, and promote the conservation of habitats, acting as umbrella species (Smith and Sutton, 2008; Home et al., 2009).

An analysis of the evolution of the population of *P. ferruginea* over the last two decades in a biggest area distribution, the Alboran Sea, shows an increase in the number of individuals (Maestre et al., 2018; CMAOT, 2022). The control by the local authorities (environmental administration and security forces) in the application of the strict legislation that protects the species (community, national and regional regulations), in addition to the increase in the awareness of the local population through environmental education, together with the advances in research on the species give an impression of good results.

Statistical modeling has shown that *P. ferruginea* adult density increases with coastal heterogeneity (macroscale in meters) and substratum roughness (microscale in cm), and decreases with slope, chlorophyll-a concentration and anthropogenic impact. Among the different environmental factors, the coastal heterogeneity (m) has the most impact on the adult density. A one-point increase in the macroscale value favors adult density by 10.52 while a one-point increase in the microscale value (cm) favors it only by 1.27. Therefore, coastal and substratum heterogeneity at multiple scales must be taken into consideration for designing sites for further

TABLE 4 Current known distribution of the *P. ferruginea* specie in the Western Mediterranean sea.

Country	Area	Source	Year of sampling	Mean density (ind/m)	Estimated total population
Spain	Andalucía	CMAOT, 2019	2019	0.63 ind/m*	17,032**
	Ceuta	Present study	2019-2020	7.28-5.56* ind/m	131,775**
	Melilla	Guallart et al., 2013b	2010	2.82 ind/m*	32,821**
	Chafarinas	Guallart and Templado, 2016	2000	4.36 ind/m*	42,230**
	Alborán	CMAOT, 2019	2018		115**
Morocco	MPA of Al Hoceima (Cala Iris Islet)	Bazairi et al., 2012	2012	1.22 ind/m	576
	MPA of Al Hoceima (El Jebha)	Bazairi et al., 2012	2012	3.66 ind/m	
	Cape Three Forks	Espinosa et al., 2014a	2012	from 0.36 to 0.83 ind./m	
Algeria	Plane Island	Kallouche et al., 2020	2019	1.78 ind/m	3,993
	Habiba Island	Espinosa, 2009b	2008	4.8 – 3.23* ind/m	50,400
	Rachgoun Island	Taibi et al., 2014		3-10 ind/m^2	
Tunisia	Zembra	Zarrouk et al., 2016	2015	2.83 ind/m^{2}*	40,404**
	North-East Tunisian coast	Tlig-Zouari et al., 2010	2006	0.025-4.5 ind/m^2	
Italy	Sinis Peninsula MPA (Sardinia)	Coppa et al., 2012	2009	0.02 ind/m	196
	Gulf of Olbia (Sardinia)	Cristo et al., 2007	2007	0.023 ind/m	69
	Maddalena Archipelago (Sardinia)	Cossu et al., 2006	2005	0.028 ind/m	736
	Mal di Ventre Island & Cape San Marco (central-western Sardinia)	Marra et al., 2016	2013-2014	0.009 & 0.004 ind/m	
	Lingurian coasts	Ferranti et al., 2019	2017-2018	0.005 ind/m*	32
France	Port Cros	Cottalorda et al., 2004		<0.01 ind/m	2
	Cap Corse/Bastia harbour (Corsica)	Vela and Leoni, 2007		0.2 ind/m^2	

*, Adult mean density (ind/m); **, Estimated adult population.

protection (Marine protected areas or Area-based conservation measures such as Artificial marine micro-reserves), to establish critical areas for its conservation or for future translocation procedures for reinforcement and/or reintroduction, or due to destruction of the original habitat (see MMAMRM, 2008; García-Gómez et al., 2011; García-Gómez et al., 2015; IUCN-WCPA, 2019; Agung et al., 2022; Ostalé-Valriberas et al., 2022).

The vertical slope of the coastline negatively influences the density of *P. ferruginea* adults. Within vertical substrata such as cliffs or seawalls, a smaller surface (area) of settlement for planktonic larvae is available, which explains the result.

A practical example of these results has been shown in the different abundances between the subpopulation settled inside the harbor (sectors L and L'). Yet still sharing the same environment, the subsector of dolomitic rip-rap (L) and the vertical concrete wall (L') show the most different results according to the population structure (number of individuals in each size class) (see Figures 5, 6).

Analyzing the results obtained for the Chl-a concentration in each sector, it is observed that there are other environmental factors that more strongly affect the species.

According to the results of previous studies, the accessibility and visitation of the coastal areas negatively affect limpet populations of *P. ferruginea*, biasing the populations structure *via* harvesting of the greatest sizes (mostly females) and therefore affecting the adult density (see Figure 7; Table 2-S) (Espinosa et al., 2009; Ostalé-Valriberas et al., 2022). In fact, the results of the present study show that a one-point decrease in the value of accessibility increases the adult density by 0.96.

5 Conservational implications

The results indicate that Ceuta is the main population of this endangered species in its entire range, being a source population on the Southern Iberian Peninsula that should be conserved as a priority. According to the Spanish National Conservation Strategy, sectors H, I, K, L and M have the highest number of adults and should therefore be categorized and managed as Critical Areas (MMAMRM, 2008; see Supplementary Figure 2-S). It could benefit many species of conservation concern also settled in the same habitat ("umbrella effect"), as demonstrated by Ostalé-Valriberas et al. (2022).

Previous studies have shown that 90% of the populations of a metapopulation are usually "sink populations" and only 10% are "source populations" (Pulliam, 1988), so this population is of great ecological relevance for this species.

The results also support the concept of "Artificial Marine Micro-Reserves" as a new area-based management tool for marine conservation, establishing a network of these source populations that promote gene flow between populations with eventual recolonization of their original distribution (García-Gómez et al., 2011; Ostalé-Valriberas et al., 2022) (Supplementary Figure 2-S).

Finally, for future translocation procedures, the reported results could serve as a guide to select the most suitable receptor sites. Such areas should have high values of macro- or microscale heterogeneity and, similarly, should avoid vertical substrata and areas with anthropogenic impact.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

EO-V, JS-V, FE, G-GJ conceptualization of the work and development the methodology. EO-V, FE application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data. EO-V, AL, AA-A, JS-V, AP-P: Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection. EO-V writhing the original draft, visualization work and supervision. AP-P, JS-V, FE, G-GJ writing -review & editing. EO-V, AL Management and coordination responsibility for the research activity planning and execution. EO-V, G-GJ acquisition of the financial support for the project leading to this publication. All authors contributed to the article and approved the submitted version.

Funding

The present study was financed with the Cooperation Agreement between two public administrations, the Sociedad, Obras, Infraestructuras y Medio Ambiente de Ceuta S.A.U. (OBIMASA) and the Fundación de Investigación de la Universidad de Sevilla (FIUS). We are grateful to Ana Garciá and Francisco Javier Martínez-Medina for their work in the Cooperation Agreement. JS-V was supported by a FPI Grant (PRE2018-086266) from Ministerio de Ciencia, Innovación y Universidades (Project CGL 2017-82739-P) co-financed by ERDF European Union and Agencia Estatal de Investigación, Gobierno de España.

Acknowledgments

The authors would like to express their gratitude to our colleague Celeste Dominguez-Monge from the Laboratorio de Biología Marina of the University of Sevilla who helped us in the sampling process and in the mineral analysis. We are also particularly grateful for the assistance given by the diving center WaterGame Gran Azul, especially by Javier Ibañez and José Manuel Ávila. We also thank the editor of Frontiers in Marine Science, Professor Alan Hodgson, and the two reviewers for their constructive comments, which considerably improved the first version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1127630/full#supplementary-material

References

Aguilera, M. A., Tapia, J., Gallardo, C., Núñez, P., and Varas-Belemmi, K. (2020). Loss of coastal ecosystem spatial connectivity and services by urbanization: natural-to-urban integration for bay management. *J. Environ. Manage.* 276, 111297. doi: 10.1016/j.jenvman.2020.111297

Agung, M. F., Adhuri, D. S., Ferse, S. C., Sualia, I., Andradi-Brown, D. A., Campbell, S. J., et al. (2022). Marine conservation beyond MPAs: towards the recognition of other effective area-based conservation measures (OECMs) in Indonesia. *Mar. Policy* 137, 104939. doi: 10.1016/j.marpol.2021.104939

Airoldi, L., Abbiati, M., Beck, M. W., Hawkins, S. J., Jonsson, P. R., Martin, D., et al. (2005). An ecological perspective on the deployment and design of low-crested and other hard coastal defence structures. *Coast. Eng.* 52, 1073–1087. doi: 10.1016/j.coastaleng.2005.09.007

Airoldi, L., and Beck, M. W. (2007). Loss, status and trends for coastal marine habitats in Europe. *Oceanography Mar. Biology: Annu. Rev.* 45, 345–405.

Anderson, M. J., Gorley, R. N., and Clarke, R. K. (2008). *PERMANOVA+ para PRIMER: guía para el programa y métodos estadísticos* (Plymouth, UK: PRIMER-E, Massey University), 264.

Bazairi, H., Benhissoune, S., Ben Haj, S., Benhoussa, A., Ouerghi, A., and Idrissi, H. R. (2012). "New and updated quantitative data on the Mediterranean endangered limpet patella ferruginea gmelin 1791 along the Moroccan coast," in *XVII Iberian symposium on marine biology studies*, vol. 19. (Revista de Investigación Marina), 609.

Blanchard, D., and Bourget, E. (1999). Scales of coastal heterogeneity: influence on intertidal community structure. *Mar. Ecol. Prog. Ser.* 179, 163–173. doi: 10.3354/meps179163

Branch, G. M. (1981). The biology of limpets. *Oceanography Mar. Biology: Annu. Rev.* 19, 235–379.

Bulleri, F., and Chapman, M. G. (2010). The introduction of coastal infrastructure as a driver of change in marine environments. *J. Appl. Ecol.* 47 (1), 26–35. doi: 10.1111/j.1365-2664.2009.01751.x

Burnham, K. P., and Anderson, D. R. (2002). Model selection and multimodel inference. *A practical information-theoretic approach (2nd ed.).* (New York, NY: Springer).

Casado, M. F., del Carmen Arroyo, M., and Navarro, M. S. V. (2017). Caracterización de las poblaciones de la lapa ferruginosa (*Patella ferruginea* gmelin 1971) de la costa norte del estrecho de Gibraltar. *Almoraima: Rev. estudios campogibraltareños* 47), 181–202.

Casey, N., and Bautista, J. (2021) *A wave of the hand sets off Spain-Morocco migrant fight. the new York times.* Available at: https://www.google.com/amp/s/www.nytimes.com/2021/06/02/world/europe/spain-ceuta-migrants-morocco.amp.html.

Casu, M., Rivera-Ingraham, G. A., Cossu, P., Lai, T., Sanna, D., Dedola, G. L., et al. (2011). Patterns of spatial genetic structuring in the endangered limpet *Patella ferruginea*: implications for the conservation of a Mediterranean endemic. *Genetica* 139, 1293–1308. doi: 10.1007/s10709-012-9631-3

Cayuela, L. (2010) *Modelos lineales generalizados (GLM).* Available at: https://es.scribd.com/document/353511291/3-Modelos-lineales-generalizados-CAYUELA-pdf/.

Chase, J. M., Blowes, S. A., Knight, T. M., Gerstner, K., and May, F. (2020). Ecosystem decay exacerbates biodiversity loss with habitat loss. *Nature* 584 (7820), 238–243. doi: 10.1038/s41586-020-2531-2

Clarke, K. R., and Gorley, R. N. (2005). *PRIMER: plymouth routines in multivariate ecological research* (Plymouth: PRIMER-E Ltd.).

CMAOT (2019). *Programa de gestión sostenible del medio Marino andaluz. informe final de resultados 2018* (Sevilla, SPAIN: Consejería de Medio Ambiente y Ordenación del Territorio (CMAOT).

CMAOT (2020). *Programa de gestión sostenible del medio Marino andaluz. informe final de resultados 2019* (Sevilla, SPAIN: Consejería de Medio Ambiente y Ordenación del Territorio (CMAOT).

CMAOT (2022). *Programa de gestión sostenible del medio Marino andaluz. informe final de resultados 2021* (Sevilla, SPAIN: Consejería de Medio Ambiente y Ordenación del Territorio (CMAOT).

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., Aguzzi, J., et al. (2010). The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PloS One* 5 (8), e11842. doi: 10.1371/journal.pone.0011842

Coppa, S., De Lucia, G. A., Massaro, G., and Magni, P. (2012). "Density and distribution of patella ferruginea in a marine protected area," in *Constraint analysis for population conservation*, vol. 13. (Western Sardinia, Italy: Mediterranean Marine Science), 108–117.

Cossu, A., Deluca, M., and Guelfi, C. (2006). *Distribuzione spaziale dei popolamenti a lithophyllum byssoides, a patella ferruginea e della frangia a cystoseira sp. nell'arcipelago di la maddalena* Vol. 13 (Sardegna-Italia: Biologia Marina Mediterra nea), 84–85.

Cottalorda, J. M., Meinesz, A., Thibaut, T., and Chiaverini, D. (2004). Représentation cartographique de l'abondance de quelques algues et invertébrés sur le littoral des îlots du rascass et de la gabinière (Parc national de port-cros, var; France). *Sci. Rep. Port-Cros Natl. Park* 20, 195–209.

Creel, L. (2003). *Ripple effects: population and coastal regions* (Washington, DC: Population reference bureau), 1–7.

Cristo, B., Caronni, S., and Floris, A. (2007). Osservazioni su *Patella ferruginea* gmelin 1791 (Mollusca, Gastropoda) nel golfo di olbia. *Biol. Marina Mediterr.* 14 (2), 344–345.

Espinosa, F. (2006). Caracterización biológica del molusco protegido Patella ferruginea Gmelin, 1791 (Gastropoda: Patellidae): Bases para su gestión conservación (PhD thesis). University of Sevilla, Spain, pp55.

Espinosa, F. (2009b). Populational status of the endangered mollusc *Patella ferruginea* gmelin 1791 (Gastropoda, patellidae) on Algerian islands (SW Mediterranean). *Anim. Biodiversity Conserv.* 32 (1), 19–28. doi: 10.32800/abc.2009.32.0019

Espinosa, F., Guerra-Garcia, J. M., , FA, , D., and Garcia-Gomez, J. C. (2006). Aspects of reproduction and their implications for the conservation of the endangered limpet, Patella ferruginea. *Invertebrate Reproduction & Development* 49(1-2), 85–92.

Espinosa, F., Navarro-Barranco, C., González, A. R., Maestre, M., García-Gómez, J. C., Benhoussa, A., et al. (2014a). A combined approach to assessing the conservation status of cap des trois fourches as a potential MPA: is there a shortage of MPAs in the southern Mediterranean? doi: 10.12681/mms.775

Espinosa, F., and Rivera-Ingraham, G. A. (2017). Biological conservation of giant limpets: the implications of large size. *Adv. Mar. Biol.* 76, 105–155. doi: 10.1016/bs.amb.2016.10.002

Espinosa, F., Rivera-Ingraham, G. A., Fa, D., and García-Gómez, J. C. (2009). Effect of human pressure on population size structures of the endangered ferruginean limpet: toward future management measures. *J. Coast. Res.* 25 (4), 857–863. doi: 10.2112/08-1005.1

Espinosa, F., Rivera-Ingraham, G. A., Maestre, M., González, A. R., Bazairi, H., and García-Gómez, J. C. (2014b). Updated global distribution of the threatened marine limpet *Patella ferruginea* (Gastropoda: patellidae): an example of biodiversity loss in the Mediterranean. *Oryx* 48 (2), 266–275. doi: 10.1017/S0030605312000580

Espinosa, F., Rivera-Ingraham, G. A., Ostalé-Valriberas, E., and García-Gómez, J. C. (2018). Predicting the fate of the most endangered marine invertebrate of the Mediterranean: the power of long-term monitoring in conservation biology. *Aquat. Conservation: Mar. Freshw. Ecosyst.* 28 (6), 1283–1293. doi: 10.1002/aqc.2944

Ferranti, M. P., Guallart, J., Fanciulli, G., Panzalis, P. A., and Chiantore, M. (2022). Advancements towards restoration of the endangered limpet *Patella ferruginea* gmelin 1791Through controlled reproduction. *Aquaculture Res.* 53 (3), 782–798. doi: 10.1111/are.15614

Ferranti, M. P., Monteggia, D., Asnaghi, V., Dagnino, A., Gaino, F., Moretto, P., et al. (2019). Distribution of the Mediterranean ribbed limpet *Patella ferruginea* gmelin 1791 along the ligurian coast and implications for conservation actions. *Mediterr. Mar. Sci.* 20 (3), 496–501. doi: 10.12681/mms.20209

Firth, L. B., Knights, A. M., Bridger, D., Evans, A. J., Mieszkowska, N., Moore, P. J., et al. (2016). Ocean sprawl: challenges and opportunities for biodiversity management in a changing world. *Oceanography Mar. Biology: an Annu. Rev.* 54, 189–262.

Frost, N. J., Burrows, M. T., Johnson, M. P., Hanley, M. E., and Hawkins, S. J. (2005). Measuring surface complexity in ecological studies. *Limnology Oceanography: Methods* 3 (4), 203–210. doi: 10.4319/lom.2005.3.203

García-Gómez, J. C., Guerra-García, J. M., Espinosa, F., Maestre, M. J., Rivera-Ingraham, G., Fa, D., et al. (2015). Artificial marine micro-reserves networks (AMMRNs): an innovative approach to conserve marine littoral biodiversity and protect endangered species. *Mar. Ecol.* 36 (3), 259–277. doi: 10.1111/maec.12167

García-Gómez, J. C., López-Fé, C. M., Espinosa, F., Guerra-García, J. M., and Rivera-Ingraham, G. A. (2011). Marine artificial micro-reserves: a possibility for the conservation of endangered species living on artificial substrata. *Mar. Ecol.* 32 (1), 6–14. doi: 10.1111/j.1439-0485.2010.00409.x

Guallart, J., Forner, J. B. P., and Larruscain, J. P. (2013). Primeras imágenes de una forma juvenil de la lapa ferruginosa. *Quercus* 325), 52–53.

Guallart, J., Luque, A. A., Acevedo, I., and Calvo, M. (2013b). Distribution and updated census of the ferrugineous limpet (*Patella ferruginea* gmelin 1791) in the littoral of melilla (SW Mediterranean). *Iberus Sociedad Española Malacología* 31 (1), 21–51.

Guallart, J., and Templado, J. (2016). Distribución, abundancia y selección del hábitat de *Patella ferruginea* (Mollusca, Gastropoda) en las islas chafarinas (Mediterráneo suroccidental). *Iberus* 34 (2), 127–162.

Guerra-García, J. M., Corzo, J., Espinosa, F., Fa, D., and García-Gómez, J. C. (2004a). Extinction risk and harbours as marine reserves? *J. Molluscan Stud.* 70 (1), 96–98. doi: 10.1093/mollus/70.1.96

Guerra-García, J. M., Corzo, J., Espinosa, F., and García-Gómez, J. C. (2004b). Assessing habitat use of the endangered marine mollusc *Patella ferruginea* (Gastropoda, patellidae) in northern Africa: preliminary results and implications for conservation. *Biol. Conserv.* 116 (3), 319–326. doi: 10.1016/S0006-3207(03)00201-5

Guerra-Garcia, J. M., and García-Gómez, J. C. (2005). Oxygen levels versus chemical pollutants: do they have similar influence on macrofaunal assemblages? a case study in a harbour with two opposing entrances. *Environ. pollut.* 135 (2), 281–291. doi: 10.1016/j.envpol.2004.10.004

Home, R., Keller, C., Nagel, P., Bauer, N., and Hunziker, M. (2009). Selection criteria for flagship species by conservation organizations. *Environ. Conserv.* 36 (2), 139–148. doi: 10.1017/S0376892909990051

Howes, D., Harper, J., and Owens, E. (1994). Physical shore-zone mapping system for British Columbia. Report prepared by Environmental Emergency Services, Ministry of Environment. (Victoria, BC), Coastal and Ocean Resources Inc.(Sidney, BC), and Owens Coastal Consultants (Bainbridge, WA).

IUCN-WCPA (2019). *Guidelines for recognising and reporting other effective area-based conservation measures* (Gland, Switzerland: IUCN).

Kallouche, M. E. M., Benaissa, N., Rouane-Hacene, O., Soufi, M. E. A., Bouderbala, M., Bouras, D., et al. (2020). Date endangered status of *Patella ferruginea* in a limpet assemblage hotspot (Plane island: south West alboran, Mediterranean sea): local distribution factors and species abundance. *J. Mar. Island Cultures* 9, 1–21. doi: 10.21463/jmic.2020.09.2.10

Kido, J. S., and Murray, S. N. (2003). Variation in owl limpet *Lottia gigantea* population structures, growth rates, and gonadal production on southern California rocky shores. *Mar. Ecol. Prog. Ser.* 257, 111–124. doi: 10.3354/meps257111

Lattos, A., Bitchava, K., Giantsis, I. A., Theodorou, J. A., Batargias, C., and Michaelidis, B. (2021). The implication of vibrio bacteria in the winter mortalities of the critically endangered *Pinna nobilis*. *Microorganisms* 9 (5), 922. doi: 10.3390/microorganisms9050922

Luque, Á.A., Guallart, J., González, J. T., and Pola, M. (2018). Recopilación y análisis de la información científica disponible sobre *Patella ferruginea*. *Sociedad Española Malacología*, 74.

Maestre, M. J., Espinosa, F., Fa, D. A., Pavón, A., and García-Gómez, J. C. (2018). Estado de la población de *Patella ferruginea* en el área del estrecho de Gibraltar. *Almoraima: Rev. estudios campogibraltareños* 49), 115–130.

Mar (2020) *La lapa que da la lata. ecologistas en acción*. Available at: https://www.ecologistasenaccion.org/134671/la-lapa-que-da-la-lata/.

Marra, S., de Lucia, G. A., Camedda, A., Espinosa, F., and Coppa, S. (2016). New records of the distribution and conservation status of the endangered limpet *Patella ferruginea* in Sardinia (Italy, W Mediterranean). *Aquat. Conservation: Mar. Freshw. Ecosyst.* 26 (3), 607–612. doi: 10.1002/aqc.2615

McCullagh, P., and Nelder, J. A. (1989). *Generalized linear models. 2nd ed* (New York: Chapman and Hall).

MMAMRM (2008). "Estrategia de conservación de la lapa ferrugínea (Patella ferruginea) en españa," in *Conferencia sectorial de medio ambiente, ministerio de medio ambiente, medio rural y Marino*(Madrid, Spain), 56.

Nachite, D., Domínguez, N. D. E., El M'rini, A., and Anfuso, G. (2020). Environmental sensitivity index maps in a high maritime transit area: the Moroccan coast of the Gibraltar strait study case. *J. Afr. Earth Sci.* 163, 103750. doi: 10.1016/j.jafrearsci.2020.103750

Ostalé-Valriberas, E., Sempere-Valverde, J., Pavón-Paneque, A., Coppa, S., Espinosa, F., and García-Gómez, J. C. (2022). 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. *Mar. Policy* 136, 104917. doi: 10.1016/j.marpol.2021.104917

Pallant, J. (2020). *SPSS Survival manual: a step by step guide to data analysis using IBM SPSS* (Routledge).

Periáñez, R. (2007). Chemical and oil spill rapid response modelling in the strait of Gibraltar–alborán Sea. *Ecol. Model.* 207 (2-4), 210–222. doi: 10.1016/j.ecolmodel.2007.04.033

Piniella, F., and Walliser, J. (2013). Maritime safety in the strait of Gibraltar: taxonomy and evolution of emergencies rate in 2000-2004 period. *J. Maritime Res.* 10 (1), 25–30.

Pulliam, H. R. (1988). Sources, sinks, and population regulation. *Am. Nat.* 132 (5), 652–661. doi: 10.1086/284880

Pulliam, H. R. (1996). *Sources and sinks: empirical evidence and population consequences* (Population dynamics in ecological space and time).

Raffaelli, D., and Hawkins, S. (1996). *Intertidal ecology* (London: Chapman and Hall).

Ramos, M. A. (1998). Implementing the habitats directive for mollusc species in Spain. *J. Conchology Special Publ.* 2, 125–132.

Rivera-Ingraham, G. A., Espinosa, F., and García-Gómez, J. C. (2011a). Environmentally mediated sex change in the endangered limpet *Patella ferruginea* (Gastropoda: patellidae). *J. Molluscan Stud.* 77 (3), 226–231. doi: 10.1093/mollus/eyr007

Rivera-Ingraham, G. A., Espinosa, F., and García-Gómez, J. C. (2011b). Conservation status and updated census of *Patella ferruginea* (Gastropoda, patellidae) in ceuta: distribution patterns and new evidence of the effects of environmental parameters on population structure. *Anim. Biodiversity Conserv.* 34 (1), 83–99. doi: 10.32800/abc.2011.34.0083

Rivera-Ingraham, G. A., Espinosa, F., and Garcia-Gomez, J. C. (2011c). Ecological considerations and niche differentiation between juvenile and adult black limpets (*Cymbula nigra*). *J. Mar. Biol. Assoc. United Kingdom* 91 (1), 191–198. doi: 10.1017/S0025315410000159

Ros, M., Lacerda, M. B., Vázquez-Luis, M., Masunari, S., and Guerra-García, J. M. (2016). Studying exotics in their native range: can introduced fouling amphipods expand beyond artificial habitats? *Biol. invasions* 18 (10), 2983–3000. doi: 10.1007/s10530-016-1191-5

Ros, M., Vazquez-Luis, M., and Guerra-Garcia, J. M. (2015). Environmental factors modulating the extent of impact in coastal invasions: the case of a widespread invasive caprellid (Crustacea: amphipoda) in the Iberian peninsula. *Mar. pollut. Bull.* 98 (1-2), 247–258. doi: 10.1016/j.marpolbul.2015.06.041

Sánchez-Badorrey, E., García-García, J. A., Ortego-Jurado, R., and García Anguita, F. J. (2021). *Variabilidad climática, renovación y calidad de las aguas del puerto de ceuta y su entorno* (Instituto de Estudios Ceutíes), 115–118.

Sedano, F., Navarro-Barranco, C., Guerra-García, J. M., and Espinosa, F. (2020). Understanding the effects of coastal defence structures on marine biota: the role of substrate composition and roughness in structuring sessile, macro-and meiofaunal communities. *Mar. pollut. Bull.* 157, 111334. doi: 10.1016/j.marpolbul.2020.111334

Small, C., and Nicholls, R. J. (2003). A global analysis of human settlement in coastal zones. *J. Coast. Res.* 19 (3), 584–599.

Smith, A. M., and Sutton, S. G. (2008). The role of a flagship species in the formation of conservation intentions. *Hum. Dimensions Wildlife* 13 (2), 127–140. doi: 10.1080/10871200701883408

Taibi, A., Oubaziz, B., Ghermaoui, M., Kaddour Hocine, A., and Bendimerad, M. E. (2014). Etude de la biométrie de la patelle géante *Patella ferruginea* à l'île de rachgoun. *Proc. BEL* 3, 26–28.

Terrón-Sigler, A., León-Muez, D., Peñalver-Duque, P., and Torre, F. E. (2018). A method for increasing the survival of shallow-water populations of the endemic coral *Astroides calycularis*. *J. Mar. Biol. Assoc. United Kingdom* 98 (4), 811–818. doi: 10.1017/S0025315416001806

Thompson, R. C., Tobin, M. L., Hawkins, S. J., and Norton, T. A. (1999). Problems in extraction and spectrophotometric determination of chlorophyll-a from epilithic microbial biofilms: towards a standard method. *J. Mar. Biol. Assoc. United Kingdom* 79 (3), 551–558. doi: 10.1017/S0025315498000678

Tlig-Zouari, S., Rabaoui, L., Fguiri, H., and Ben Hassine, O. K. (2010). Status, habitat and distribution of the endangered limpet *Patella ferruginea* along the northern and eastern Tunisian coastline: results and implications for conservation. *Cahiers Biologie Mar.* 51 (1), 75.

Valverde, J. M., Perejon, A., Medina, S., and Perez-Maqueda, L. A. (2015). Thermal decomposition of dolomite under CO 2: insights from TGA and *in situ* XRD analysis. *Phys. Chem. Chem. Phys.* 17 (44), 30162–30176. doi: 10.1039/C5CP05596B

Vázquez-Luis, M., Álvarez, E., Barrajon, A., Garcia-March, J. R., Grau, A., Hendriks, I. E., et al. (2017). SOS *Pinna nobilis*: a mass mortality event in western Mediterranean Sea. *Front. Mar. Sci.* 4, 220. doi: 10.3389/fmars.2017.00220

Vela, A., and Leoni, V. (2007). Etude des espèces de l'étage médio-littorale sur la jetée du port de commerce de bastia-recensement des effectifs de *Patella ferruginea*. *Contrat sentinelle collectivité territorial Corse* 18 p. doi: 10.12681/mms.20209

Zarrouk, A., Romdhane, M. S., and Espinosa, F. (2016). Usefulness of marine protected areas as tools for preserving the highly endangered limpet, *Patella ferruginea*, in the Mediterranean Sea. *Mar. Biol. Res.* 12 (9), 917–931. doi: 10.1080/17451000.2016.1213396

Frontiers in Marine Science

TYPE Original Research
PUBLISHED 27 July 2023
DOI 10.3389/fmars.2023.1167818

OPEN ACCESS

EDITED BY
Tomoyuki Nakano,
Kyoto University, Japan

REVIEWED BY
Marshall Weisler,
The University of Queensland, Australia
Yunwei Dong,
Ocean University of China, China

*CORRESPONDENCE
Jack D. Hollister
jdh2n21@soton.ac.uk

RECEIVED 16 February 2023
ACCEPTED 07 July 2023
PUBLISHED 27 July 2023

CITATION
Hollister JD, Cai X, Horton T, Price BW, Zarzyczny KM and Fenberg PB (2023) Using computer vision to identify limpets from their shells: a case study using four species from the Baja California peninsula. *Front. Mar. Sci.* 10:1167818.
doi: 10.3389/fmars.2023.1167818

Using computer vision to identify limpets from their shells: a case study using four species from the Baja California peninsula

Jack D. Hollister[1,2,3]*, Xiaohao Cai[1], Tammy Horton[2,3], Benjamin W. Price[3], Karolina M. Zarzyczny[1,3] and Phillip B. Fenberg[1,3]

[1]School of Ocean and Earth Sciences, University of Southampton, Southampton, United Kingdom, [2]Ocean BioGeosciences, National Oceanography Centre, Southampton, United Kingdom, [3]Natural History Museum, London, United Kingdom

The shell morphology of limpets can be cryptic and highly variable, within and between species. Therefore, the visual identification of species can be troublesome even for experts. Here, we demonstrate the capability of computer vision models as a new method to assist with identifications. We investigate the ability of computers to distinguish between four species and two genera of limpets from the Baja California peninsula (Mexico) from digital images of shells from both dorsal and ventral orientations. Overall, the models performed marginally better (97.9%) than experts (97.5%) when predicting the same set of images and did so 240x faster. Moreover, we utilised a heatmap system to both verify that models are focussing on the specimens and to view which features on the specimens the models used to distinguish between species and genera. We then enlisted the expertise of limpet ecologists specialised in identification of species from the Baja peninsula to comment on whether the heatmaps are indeed focusing on specific morphological features per species/genus. They confirm that in their opinion, the majority of the heatmaps appear to be highlighting areas and features of morphological importance for distinguishing between groups. Our findings reveal that the cutting-edge technology of computer vision holds tremendous potential in enhancing species identification techniques used by taxonomists and ecologists. Not only does it provide a complementary approach to traditional methods, but it also opens new avenues for exploring the biology and ecology of limpets in greater detail.

KEYWORDS

Baja California, computer vision, convolutional neural network, heatmap, limpets, rocky intertidal, taxonomy

Introduction

Limpets are abundant, diverse, and ecologically important members of rocky shore communities (Kordas et al., 2017; Firth, 2021). In addition, some limpet species are important culturally and as food sources for modern and pre-historic human societies (Fenberg and Roy, 2008; Fenberg and Roy, 2012; Firth, 2021; Weisler and Rogers, 2021). Yet, despite their ubiquity, limpet species can sometimes be difficult to tell apart in the field (Simison and Lindberg, 2003; Burdi, 2015), at archaeological sites (Rogers and Weisler, 2020a) and in museum collections (Kuo and Sanford, 2013) owing to their highly variable shell morphologies and colour patterns (Nakano and Spencer, 2007). Even within species, shell features can vary according to substrate, size (age), population, and geographic region, sometimes resulting in distinct shell morphologies (Williams, 2017) and shapes (Rogers and Weisler, 2020b) To further complicate matters, shell erosion and encrusting symbionts can also impede visual identification. As a result, taxonomists frequently rely on using internal anatomical features, such as radular structure, as distinguishing characters (Simison and Lindberg, 1999). In more recent decades, molecular methods have revealed new limpet species, confirmed/rejected species validity, and clarified nomenclatural confusion among morphologically similar species (Simison and Lindberg, 2003; Crummett and Eernisse, 2007). Nevertheless, the use of internal anatomical or molecular characters for distinguishing similar looking and highly variable species can be time consuming and resource limiting, while offering little advance in species-level identifications using the most easily accessible external features – their shells.

Recent developments in computer-based image recognition and detection may be harnessed to develop accurate, fast, and cost-effective means to distinguish between limpet species from their shells. In addition, these emerging technologies can also provide insight into the morphological characteristics that can be used to distinguish between similar looking species (Pinho et al., 2022). The aim of this paper is to evaluate the feasibility of these new computer-based methods for distinguishing between limpet species and genera using digital images of their shells.

Computer vision (CV) is currently pushed forward by deep learning (DL) and artificial intelligence (AI) and focuses on the development of algorithms and techniques for computers to process, understand and analyse visual data inputs. This can involve tasks such as image and video recognition (the recognition of specified subjects within images and video), object detection (the recognition and location of subjects within an image and video) and scene understanding (the recognition of a subject within a 3D environment with respect to its relationship to other subjects). CV involves the understanding of pixel patterns and their respective colour values. Furthermore, CV systems have the capability to operate for prolonged periods, handle very large datasets, and produce results at very fast speeds (Wilson et al., 2022), which are unachievable and/or unfeasible for humans.

Recently, CV has been adopted by the life sciences as a method to visually identify and group organisms together based on their morphology (Wäldchen and Mäder, 2018; Greeff et al., 2022; Hollister et al., 2022), and has been recognised as an emerging tool for ecology, evolution, and taxonomic research (Høye et al., 2021; Lürig, 2022). The accelerated use of CV in the natural sciences has coincided with the massive digitisation efforts of natural history museums (Popov et al., 2021; Wilson et al., 2022), where tens of millions of digital images of specimens and collection data are now available for researchers worldwide. For example, Wilson et al. (2022) applied CV models to >180,000 specimens of digitised natural history specimens of butterflies, resulting in highly accurate sex identifications and body size measurements over a short timescale (one week), showcasing the emerging power of CV for the natural sciences.

The evaluation of CV methods for identifying similar looking species has not been well studied to date with mixed results from the few studies that have. For example, CV models achieved accuracy scores of ~ 50% for identifying species of British carabid beetles (Hansen et al., 2020). But more recently, some researchers have achieved highly accurate results (upwards of 97%) for identifying species of cryptic lizards (Pinho et al., 2022), suggesting that CV models are either getting more accurate and/or that the results can be taxon specific. Regardless, even if highly accurate CV models are achieved, on their own, they do not give researchers any information about how specimens of different species can be distinguished from each other. Similarly, while DNA barcoding can allow for the species-level identification of specimens, traditional morphological taxonomy is required to find distinguishing features between species (Tautz et al., 2003). For CV to be practically useful for identification purposes, they must not only be trained on specimens with known species-level identification (which can be achieved through DNA barcoding and/or expert identification), but newly developed methods need to be integrated to the workflow to provide insights to the decisions made by CV models. In other words, we need to overcome the "black box" problem (Savage, 2022).

DL based systems are often viewed as "black boxes" with internal processes too complicated for comprehension, which can lead to the development of biased models that generate incorrect or biased results, leading to distrust in their results (Sham et al., 2022). To address these issues, a significant number of researchers are working to improve various aspects of AI. Fortunately, CV has made significant strides in this area, as evidenced by the development of explainable AI (XAI) in the form of heatmaps. Heatmaps come in many forms and can be used with a variety of applications. Within convolutional neural networks (CNN), heatmaps are often used as a visualisation tool that can be generated to show which features are learned during the training processes and which parts of an input image were used to make predictions (Selvaraju et al., 2016).

Ecologists and taxonomists are now beginning to realise the potential of integrating heatmaps into their CV models to help classify morphologically similar or cryptic species and to highlight morphologically important characters. Recently, researchers applied CV models and heatmaps for species identification problems of a cryptic group of lizards (Pinho et al., 2022). The researchers found that the heatmaps from their CV models were focussing on areas of the body that were morphologically variable between species (while also noting that future research should focus on the interpretation of heatmap results). Although still in its infancy, we believe that the use of CV and heatmaps will provide insightful, cost effective, and rapid means for the identification of limpet species using shell features. If found to be robust, similar techniques could be used to tell apart cryptic species, populations, and perhaps even shell differences caused by microhabitat or phylogeographic factors.

In this paper, we apply CV models and heatmaps to four limpet species from the rocky intertidal of the Baja California peninsula, Mexico: *Lottia strigatella*, (Carpenter, 1864), *Lottia conus* (Test, 1945), *Fissurella volcano* (Reeve, 1849), and *Fissurella rubropicta* (Pilsbry, 1890). Each species overlaps in range and occupies the rocky shore habitat in the high to mid-intertidal zone. We focus on these species because of their diverse shell morphologies and colour patterns on their dorsal and ventral sides. For example, *Lottia conus* has a variety of dorsal shell patterns that can be described as "wavy", "ribbed", "speckled", or "mixed" (Burdi, 2015; Ross, 2022).

The "true limpets", which include the *Lottia* species, are in the subclass Pattelogastropoda, whereas the *Fissurella* species (keyhole limpets) are members of the distantly related subclass, Vetigastropoda. We include *Fissurella* in this analysis because they are ecologically and functionally similar to the true limpets and they live in the same rocky shore habitat. But importantly, the shells of the *Fissurella* species are easily distinguishable by eye from the *Lottia* species due to the distinctive keyhole found only in the Fissurelidae family. Therefore, we expect the heatmaps to also focus on this shell difference when making predictions on which genus a specimen belongs (*Lottia* versus *Fissurella*) and have high accuracy scores. If true, it will give us confidence that the models are focussing on important morphological differences for distinguishing between taxa.

Species level identifications within both genera are more difficult, and therefore, more challenging for both human and CV-based methods of identification. For example, authors have observed multiple cases of misidentifications of *F. volcano* with *F. rubropicta* (and vice versa) in museum collections and *L. strigatella* and *L. conus* each have their own history of taxonomic confusion (Simison and Lindberg, 2003; Burdi, 2015). The *Lottia* species can sometimes be difficult to tell apart as they are both relatively small, have highly variable shell patterns, and live within the same microhabitat (on top of rocks or as epibionts on other shells in the high to mid-intertidal). By applying CV and heatmaps to digital images of the shells of these species, our broader aim is to help solve these classification problems while also identifying shell characteristics that researchers can use to distinguish species, both in the field and in museum collections. To this end, we have trained CV models on specimens with confirmed species identifications (using DNA barcoding) and calculated model accuracies for making correct predictions. We compare the results of the CV models with expert identifications of the same specimens (without prior knowledge of the model results). We then used expert opinion to determine if the heatmaps focused on important or unique morphological features that may be useful for identification purposes. Finally, we asked the experts to view incorrect predictions and provide interpretations as to why these were made.

Methodology

Field sampling and DNA barcoding

Four species were selected for this investigation: *Lottia conus*, *Lottia strigatella*, *Fissurella volcano* and *Fissurella rubropicta*. These species are co-distributed in the mid to high rocky intertidal zone along the Pacific coast of the Baja California peninsula (Mexico). Specimens were sampled from the field at sites spanning the peninsula, from ~23-30°N. Limpet specimens were fixed in 70% ethanol in the field and transferred to absolute ethanol in the laboratory. To confirm species identification, DNA was extracted from foot tissue using the DNeasy Blood and Tissue Kit following the manufactures instructions (Qiagen). For all species, we amplified a ~630bp fragment of a section of mitochondrial Cytochrome Oxidase Subunit I (COI) gene and sequenced on an ABI 3730 DNA Analyser at the Natural History Museum, London (UK). Total specimen numbers: *L. strigatella = 158*, *L. conus = 120*, *F. volcano = 82*, and *F. rubropicta = 70*. Pairwise sequence distances within each group were calculated and a neighbour joining tree was performed in MEGA (Tamura et al., 2021) to confirm the monophyly of each species. Pairwise distances within each group are small and range from 0.05 (*Lottia conus*) to 0.00 (*Fissurella rubropicta*) and monophyly of each species was confirmed. Further, we used BLAST searches to match sequences to species on the NCBI database. *Fissurella volcano* and *Lottia strigatella* are on the NCBI database and our sequences matched with a percent identity of >97%. *Lottia conus* sequences were matched (>95%) to sequences obtained from Dawson et al., 2014. There are no published COI sequences of *Fissurella rubropicta*, but the very low pairwise distances between specimens within this group (see above) and its clear divergence from *F. volcano* sequences (77%) gives us high confidence of the identity of this species for our models. Correctly labelled data are essential for creating accurate and un-biased training datasets and to assess the accuracy of model results (Rädsch et al., 2023). We use DNA barcoding, but if available, researchers could also use expert identification from taxonomists to confirm species identity (or a combined approach). Further details of the molecular methods of each species and genbank accession numbers are in Zarzyczny et al. (under minor revision)[1].

Dataset construction

High-resolution images of the dorsal and ventral sides of the shells of each specimen were captured using an Olympus SZX10 microscope. To optimize image quality, a focal step function was implemented, and a black velvet backdrop was used to minimize background interference. Images were taken in a room with controlled lighting to allow for uniformity. In total, six image sets

---

[1] Zarzyczny, K. M., Hellberg, M. E., Lugli, E. B., Maclean, M., García, D. P., Rius, M., et al. (under minor revision). Tropicalisation and phylogeographic structure of rocky shore gastropods on the Baja California Peninsula

were created. Four models examined species vs species differences and two examined genus vs genus differences. These were as follows: Dorsal *L. conus* vs *L. strigatella*; ventral *L. conus* vs *L. strigatella*; dorsal *F. rubropicta* vs *F. volcano*; ventral *F. rubropicta* vs *F.volcano*; dorsal *Lottia* vs *Fissurella*; and ventral *Lottia* vs *Fissurella*. Therefore, each model was made up of two classes. The models were designed to learn features from visual data inputs (the images) through a computational training process, resulting in predictions based on the learned features.

The images in each class were divided into three groups: training, validation, and test. The training images were used to train the model, the validation images were used for self-verifying and updating model weights during the training process, and the test images were reserved for the final evaluation of the model performances. Due to the limited number of specimens available, models may struggle to train effectively due to a lack of data to identify unique features. To address this issue, we employed image augmentation, a technique that generates artificial images based on the original stock. This has been shown to improve model performance when faced with such situations by creating a larger stock of images, but where the desired features remain unique and non-repeated (Perez and Wang, 2017; Xu et al., 2023). To preserve the integrity of the specimens' morphology, we chose augmentations that did not alter their colour or shape. We utilized a range of random flips (vertical and horizontal), two rotations functions (a fixed 90^0 clockwise or anticlockwise and a separate clockwise or anticlockwise rotation, up to a maximum of 89^0), and a zoom out (decreases the size by a maximum of 10%). Each of these were set with an 80% probability of being selected and programmed to not create duplications. Before any augmentation was applied, 20 images from each class (i.e., 40 images in total per model) were randomly selected from each image set and set aside as the test set. The test set must remain neutral, un-augmented and unseen by the model. 20 images from each class were randomly chosen and used as the validation set. These 20 validation images were augmented to a combined total of 400 images. The remaining images in each class were used for training and were augmented to a combined total of 3000 images. Overall, each model would contain 6000 training images, 800 validation images and 40 test images.

Computer vision model

We used a high-specification workstation equipped with an NVIDIA GPU with TensorFlow and Python programming. The image classification technique, which consisted of a CNN, was deemed the most appropriate for this scenario. The VGG16 CNN algorithm (Simonyan and Zisserman, 2014) with custom top layers and transfer learning using the ImageNet dataset, was employed. Models were tuned using KerasTuner to find optimum hyperparameter and learning rate (Joshi et al., 2021). They were initially trained for three epochs. Afterwards, all models were fine-tuned by unlocking previously trained layers starting from layer 11, and each model had a final learning rate set lower than its original (initial learning rate/10) and continuously trained until the model validation accuracy plateaued. The time taken for the model development (training and validation) and testing phases for each model were noted for comparison with the expert classification (see below).

Model and expert identifications: evaluation and comparisons

The experts visually identified the same test sets of images for each model but with the species labels removed and with no prior knowledge of model results. They also kept track of how long it took to go through the dataset. Expert accuracy scores were then compared to the confirmed species identifications based on the barcoding results. They were subsequently compared to the prediction accuracy scores for each CV model. Further, the incorrect predictions for specimens for each method (expert versus CV model identification) were compared to look for any congruent patterns (e.g., do both methods misidentify the same specimens?).

The accuracies for both methods of identification (model versus expert) were calculated as the proportion of the correct predictions out of the total number of possible predictions. Accuracies are therefore scored between 0 – 100%, with 100% being a perfect score. The model and expert predictions were further evaluated using a bootstrap analysis to create a 95% confidence interval on the accuracy scores (resampling single specimen predictions with replacement 10,000 times). Overlap in 95% CI was used to judge if there were significant differences between expert and model predictions. Differences in the time taken to make predictions between the expert and the models were also noted. In addition, for each specimen that was incorrectly identified, the experts made a *post-hoc* judgement as to why they thought an incorrect identification was made and whether the models and experts made the same mistakes.

Heatmap evaluation

The Gradient-weighted Class Activation Mapping (GradCam) system (Selvaraju et al., 2016) was selected to create heatmaps for each specimen image in the test datasets. GradCam is a technique used in CV to understand which parts of an image influenced a DL model decision. It works by analysing DL model activations and gradients to create a heatmap that highlights the important regions in the image. This heatmap helps us see what the model focused on when making its prediction. The heatmap images of each specimen in the test datasets were then shown to the experts to help evaluate which features of the shell, if any, were used to make predictions. Both experts are limpet ecologists (PBF and KMZ) that use visual cues to determine species identification of Baja Peninsula limpets, often using digital images of shells taken in the field.

To further evaluate the use of heatmaps to distinguish between classes, we compared the intensity values between each class per model. When the heatmaps are produced, a value is assigned to each pixel depending on how strongly a particular pixel contributes to the classification decision made by the model. The higher the intensity score of a pixel, the more significant its contribution to the predicted class. These values are then summed up to produce the overall heatmap intensity value per specimen. Although the value itself cannot tell us what part of the shell is being used for prediction, significant differences in overall heatmap intensity values between classes might be evidence that the models are using different features of each class to make predictions. Comparisons in the mean difference of heatmap intensity values between each class per model were evaluated using two sample Wilcoxon tests (due to violations of normality for some models).

Results

Final model and expert accuracies

The models and experts produced highly accurate results (Table 1). Overall, the models only incorrectly predicted five images (out of 240), for an overall accuracy score of 97.9%. The experts also performed well overall, with only six images incorrectly predicted (out of the same 240 images), for an overall accuracy score of 97.5%. Both produced a 100% correct prediction rate using the test sets from models 1, 2 and 6. The experts' worst performance was with the test set from model 4 with an accuracy score of 92.5%. The models' worst performance were models 3 and 5, with an accuracy score of 95%. The 95% confidence intervals overlap for all models, suggesting a non-significant difference between model and expert identification of limpet shells. The experts performed the predictions on all test images in 59 minutes while the models predicted the test images in ~30 seconds in total.

Heatmaps and expert interpretation

After the heatmaps were shown to the experts, they confirmed the following: Across all six models, all the heatmaps were focussed on the specimens (except for one image within model 1 within the *Lottia* class). Across all six models, all heatmaps appeared to be focussed on specific areas of the shells (except for the same one image in model 1). Heatmaps often focused on a single area of the shells while others focused on multiple features. These features were often common across all images within each respective class (e.g., for the *Fissurella* class in the genus models 1&2, the focus was always on the keyhole). To review which features were highlighted most frequently, we tallied the responses within the comments made by the experts. For example, if a shell feature/area was focused on in all images from a single class, it would equal 20/20.

Expert opinion: Model 1

For the *Fissurella* images (Figures 1A–D), 20/20 heatmaps focused on the keyhole. For the *Lottia* images (Figures 1E–H), 19/20 heatmaps focused on patterns around the shell margin. One heatmap focused its attention around the outside of the shell rather than on it but was still correctly predicted as *Lottia*. It was noted that the specimens within the *Lottia* class had a high degree of variable shell patterns and morphology.

Expert opinion: Model 2

For the *Fissurella* images (Figures 2A–D), 20/20 heatmaps focus on the keyhole. For the *Lottia* images (Figures 2E–H) 19/20 focused on the areas within the muscle scar and not on the shell margins, while 1/20 focused on the muscle scar to the shell margin.

Expert opinion: Model 3

For the *L. conus* images (Figures 3A–D), 20/20 heatmaps focus on the ribbing pattern on the shell, but not on the apex. For the *L. strigatella* images (Figures 3E–H) 17/20 heatmaps focused on the apex, while 3/10 focussed on patterns around the apex.

TABLE 1 Accuracy scores of all trained models with 95% confidence intervals in brackets.

Model	Expert Accuracy	Computer model Accuracy
Model 1: Dorsal *Fissurella* vs *Lottia*	100% [100, 100]	100% [100, 100]
Model 2: Ventral *Fissurella* vs *Lottia*	100% [100, 100]	100% [100, 100]
Model 3: Dorsal *L. conus* vs *L. strigatella*	95% [87.5, 100]	95% [87.5, 100]
Model 4: Ventral *L. conus* vs *L. strigatella*	92.5% [82.5, 100]	97.5% [92.5, 100]
Model 5: Dorsal *F. rubropicta* vs *F. volcano*	97.5% [92.5, 100]	95% [87.5, 100]
Model 6: Ventral *F. rubropicta* vs *F. volcano*	100% [100, 100]	100% [100, 100]

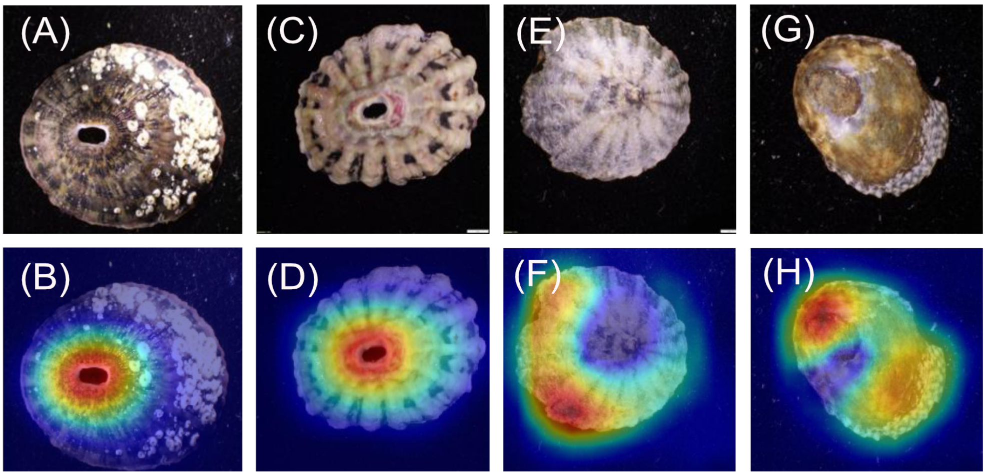


FIGURE 1
Model 1: Dorsal *Fissurella* **(A–D)** vs *Lottia* **(E–H)**.

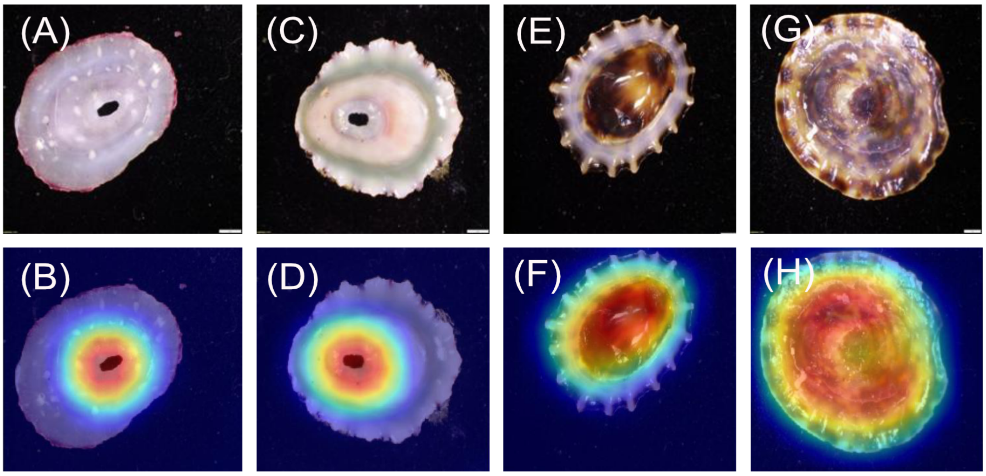


FIGURE 2
Model 2: Ventral *Fissurella* **(A–D)** vs *Lottia* **(E–H)**.

Expert opinion: Model 4

For the *L. conus* images (Figures 4A–D), 19/20 heatmaps focus on the area between the muscle scar and the shell margin. 1/20 focused on a very small portion of the shell margin, however, this shell was noted as containing no pattern and was predicted incorrectly (Figure 5). For the *L. strigatella* images (Figures 4E–H), 20/20 heatmaps focused on areas of the shell margin which is often bordered by a dark or mottled band. Additionally, 2/20 also focused on the centre of the interior portion of the shell within the muscle scar.

Expert opinion: Model 5

For the *F. rubropicta* images (Figures 6A–D), 20/20 heatmaps focus on the ribbing pattern on the shell, but not on the keyhole. It was noted that some of the shells were highly eroded but the heatmap still focused on any remaining ribbing patterns. For the *F. volcano* images (Figures 6E–H) 20/20 heatmaps focused directly on the keyhole. It was noted that the keyhole shape between the two species is different.; *F. rubropicta* is more lemniscate while *F.volcano* is ellipsed.

Expert opinion: Model 6

For the *F. rubropicta* images (Figures 7A–D), 20/20 heatmaps focus on the area between the muscle scar and callus (which usually contains a deep red colour) but not on the shell margin. For the *F. volcano* images (Figures 7E–H) 18/20 heatmaps focused on the margin. 2/20 focused on the margin and on the interior of the shell.

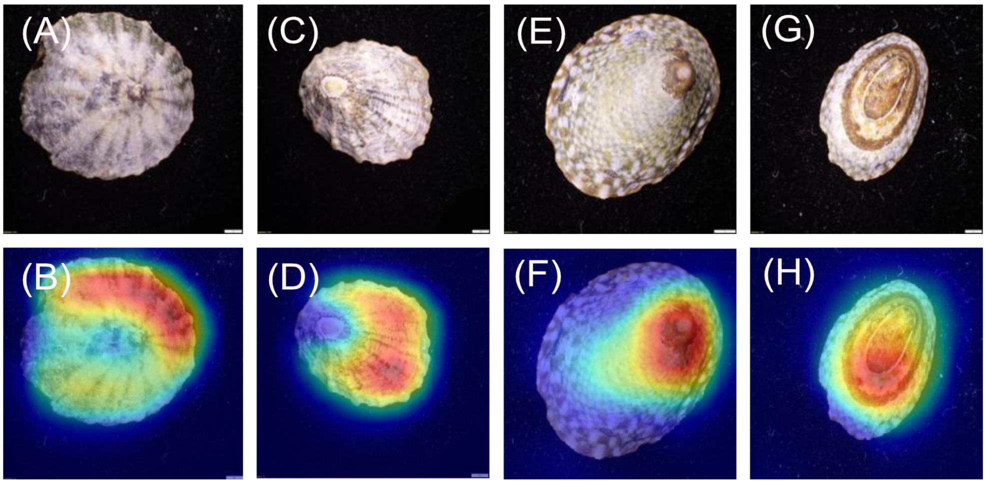


FIGURE 3
Model 3: Dorsal *Lottia conus* **(A–D)** vs *Lottia strigatella* **(E–H)**.

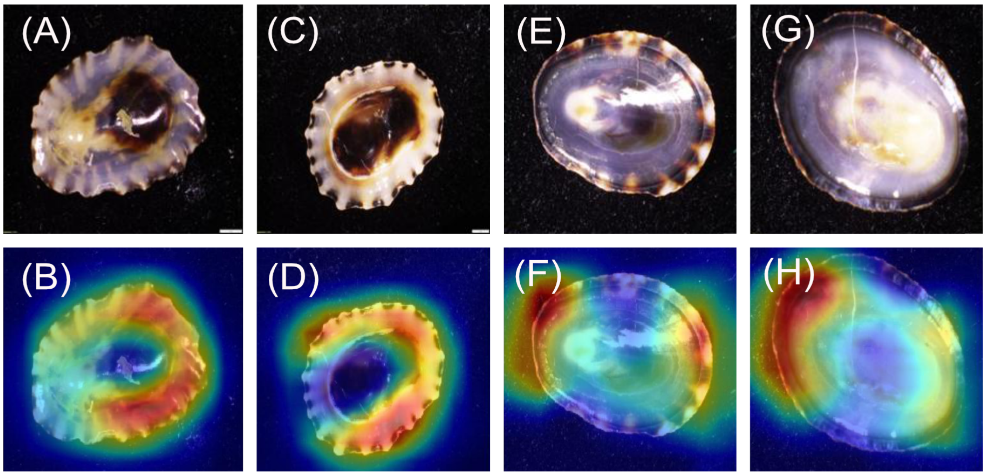


FIGURE 4
Model 4: Ventral *Lottia conus* **(A–D)** vs *Lottia strigatella* **(E–H)**.

Incorrect model predictions and expert interpretation

All incorrect model predictions and all incorrect expert predictions (Figure 5) were shown to the experts who were asked to provide an opinion on what morphological features may have caused the misidentification.

Expert opinion: Incorrect model predictions

The *F. volcano* specimen in Figure 5A was incorrectly predicted by both the model (model 5) and the experts (both incorrectly predicted it as *F. rubropicta*). Experts determined that this specimen has ribbing patterns normally associated with *F. rubropicta*. Experts were convinced they were correct but after visual inspection of the ventral side and a correct prediction by the ventral model (6), they concluded that this may just be an outlier individual with dorsal characteristics of both species of *Fissurella*. In image B, the *F. rubropicta* specimen was incorrectly predicted by model 5 as *F. volcano*. Experts determined that this specimen displayed features that they would expect from *F. volcano* as it has less defined ridging. Image C, an *L. conus* specimen was incorrectly predicted by model 3 as *L. strigatella*. This same specimen was also incorrectly predicted by the experts. Upon subsequent inspection, the experts determined that the morphological features of this specimen are not typically associated with *L. conus* such as not having a banding pattern and the shell pattern is more stippled, which they often attribute to *L. strigatella*. Image D, an *L. strigatella* specimen was incorrectly predicted by model 3 as *L. conus.* The experts determined that the shell is highly eroded and very little morphological information can be used to make a prediction. Image E, a *L. conus* specimen was incorrectly predicted by model 4 as *L. strigatella*. Experts

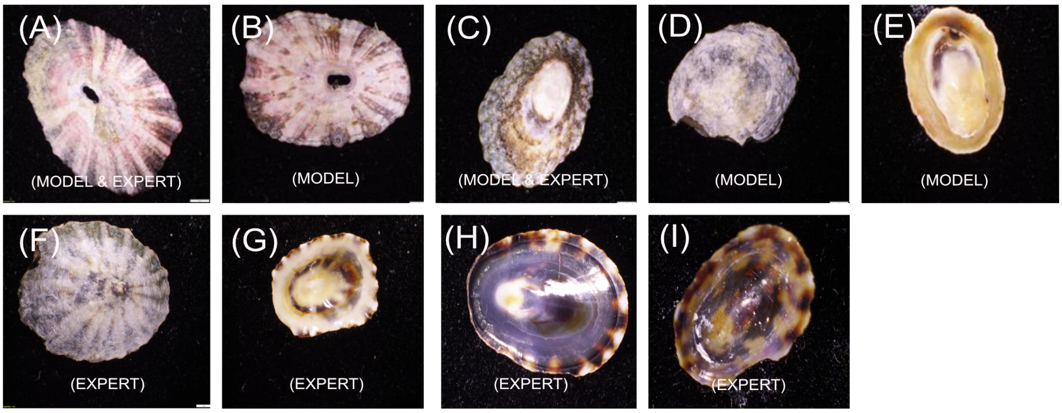


FIGURE 5
All incorrect model and expert image predictions.

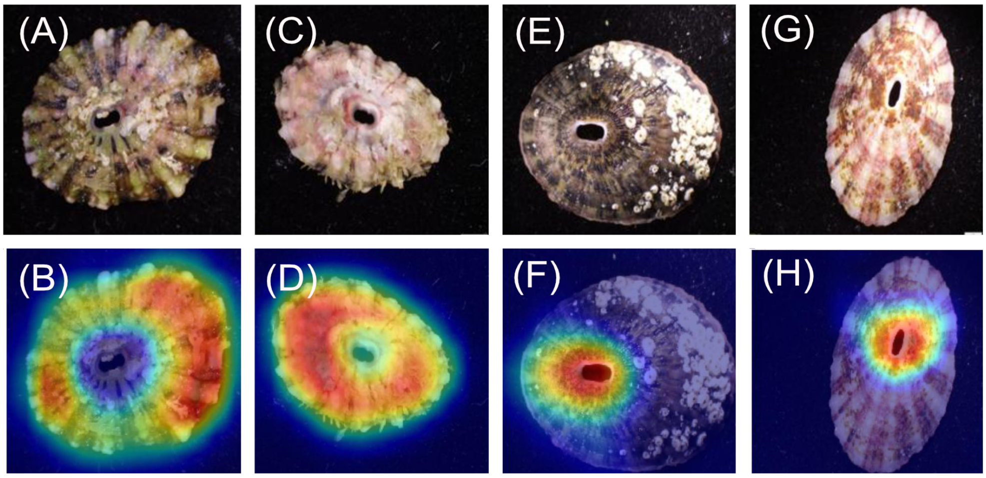


FIGURE 6
Model 5: Dorsal *Fissurella rubropicta* **(A–D)** vs *Fissurella volcano* **(E–H)**.

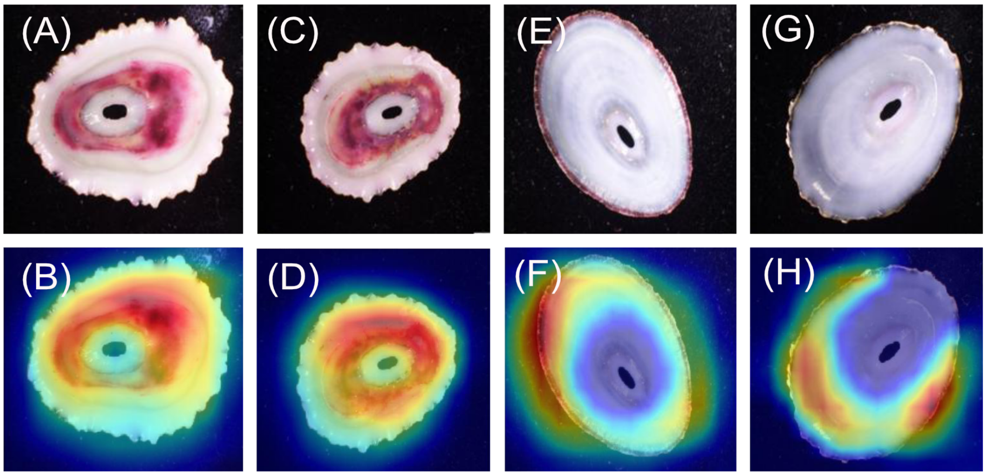


FIGURE 7
Model 6: Ventral *Fissurella rubropicta* **(A–D)** vs *Fissurella volcano* **(E–H)**.

determined that it also has very little pattern and largely monochromatic, making it difficult to identify.

Expert opinion: Incorrect expert predictions

Images A and C were incorrectly predicted by both the models and the experts, with reasonings outlined above. Image F is a dorsal view of an *L. conus* specimen that was incorrectly predicted by the experts as *L. strigatella.* On reflection, experts commented that they can see some clear *L. conus* morphological features (clear banding pattern) and were unsure how they incorrectly predicted the specimen initially. Image G is a ventral view of an *L. conus* specimen that was incorrectly predicted by the experts as *L. strigatella.* Again, on reflection, experts determined that they could see *L. conus* features (ribbed margin) and were unsure how they incorrectly predicted the specimen. Image H is a ventral view of an *L. strigatella* specimen that was incorrectly predicted by the experts as *L. conus.* On reflection experts determined that the banding pattern around the margin is a feature they would usually associate with *L. conus*, making this specimen a difficult one to predict (but was correctly predicted by the model). Image I is a ventral view of a *L. strigatella* specimen and was incorrectly predicted by the experts as *L. conus.* Experts determined that the pattern on this specimen is unusual and is displaying a tortoiseshell pattern that they could attribute to both *Lottia* species (the model predicted this specimen correctly).

Heatmap intensity values

For the heatmap intensity values, all models showed a significant difference ($P<0.05$; two sample Wilcoxon tests) in the mean values between each class (Figure 8). For both genus models (1&2), the *Fissurella* values are much lower and with a smaller range of values than the *Lottia* values. For the species comparisons (Figure 8), both the dorsal and ventral views for the *Lottia* models (3&4) are higher, on average for *L. strigatella* than *L. conus*. Likewise, the values for *F. rubropicta* are on average higher than *F. volcano* (5&6).

Discussion

Computer vision-based limpet identification

The use of CV to help distinguish between species is starting to gain traction amongst ecologists and taxonomists (Wäldchen and Mäder, 2018; Greeff et al., 2022; Hollister et al., 2022). However, few have attempted to pair CV models with heatmaps to help visually distinguish between species with high morphological variability. Limpets, including those species used in this study, can have multiple colour morphs and shell patterns due to several different ecological and life history factors, including substrate type, age, and patterns of shell erosion (Bird, 2011; Williams, 2017). It is therefore not uncommon for field ecologists and museum curators/taxonomists to make mistakes in species identification. To help assist identification, our CV models performed very well and the heatmaps largely focus on shell areas that are morphologically informative between genera and species.

When considering the genera, the models achieved 100% predicted accuracy for the dorsal and ventral orientations (models 1 and 2 respectively). Previous research has shown that higher taxonomic levels tend to score greater than lower levels with computer-based classification problems (Hansen et al., 2020). This is most likely due to having more unique images and having

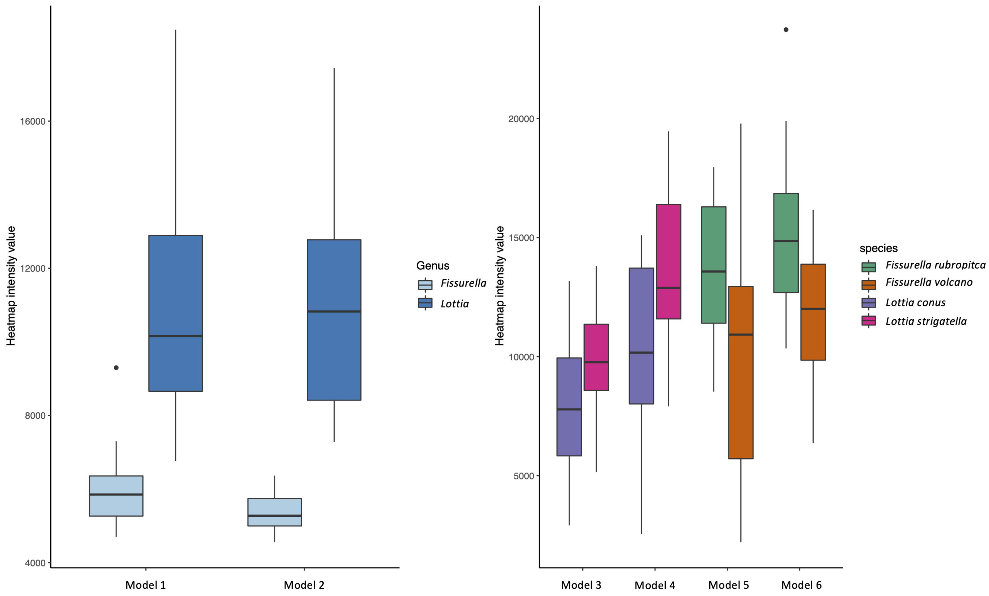


FIGURE 8
Boxplots showing heatmap intensity values for all models.

a larger selection of features to associate to each respective class, both of which are shown to improve the performance of CV models (Shorten and Khoshgoftaar, 2019). This follows general taxonomic identification procedures, where higher taxonomic levels are more easily distinguished (Hennig, 1966). It is important to recognise that all *Fissurella* species have a distinctive keyhole in their shells, whereas true limpets (including *Lottia*) do not. This is a very clear method of distinguishing the two by eye and the high genus level accuracies evidence this through perfect model performance. This is further supported by the heatmap analysis which clearly shows that the models are focussing on the keyhole of all *Fissurella* specimens within both models. When viewing the *Lottia* specimens, the heatmaps are looking at different areas of the specimens, which is reflective of the varied morphology of *Lottia*. When viewing the heatmap intensity values (Figure 8), the *Fissurella* class have a much lower mean and spread of values, while the *Lottia* class has a much higher mean and spread of values. This shows that the models utilise much less visual information to determine the *Fissurella* class while requiring a lot more information to determine the *Lottia* class. The experts commented that the keyhole, or a lack of, would be their defining feature to classify either class.

The species vs species models achieved more variable, but still highly accurate results. The ventral oriented models performed better than the dorsal oriented models across both species' groups. The *Lottia* ventral model (model 4) performed slightly worse (achieving a prediction accuracy of 97.5%) than the *Fissurella* ventral model (model 6), which achieved a prediction accuracy score of 100%. However, the *Fissurella* dorsal model (model 5) and the *Lottia* dorsal model (model 3) performed equally well (95%). We believe this slight difference in performance between the ventral and dorsal orientations lies in the fact that the dorsal sides will incorporate many factors that can alter appearance, such as erosion and encrusting symbionts that can cover the shell, all of which would hinder the accuracy of CV models. However, the ventral side remains hidden and protected from physical elements. Thus, the ventral side may provide a clearer picture of the differences between species and therefore provide maximum identification opportunities for the CV models. Although this option is not preferable for field identification as the body tissue would need to be removed from shells. Dry shell collections of museum specimens or those collected for other purposes (e.g., population genetics) however, could benefit from the use CV on the ventral and dorsal shell for identification. Again, the heatmap intensity values for the species-based models showed significant differences between each class per model. This suggests that the models found morphological features or areas of similar importance within each class when making their respective predictions. We believe this type of assessment could help researchers interpret decisions made by CV models. For instance, if a prediction does not fit into a known boundary of heatmap intensities for a given class, then it could either be ruled as incorrect or could, at the very least, warrant further investigation, either by revisiting visually by an expert or by molecular means. The heatmap intensity values for the incorrectly predicted specimens (n=5) tend to be lower than the values for the correctly predicted specimens (n=235).

Expert identification and comparison to model performance

Experts performed marginally worse (by one specimen) than the model predictions when considering all images used for the test datasets (n=240). The experts achieved 100% on the genus-based models (models 1 and 2) and the ventral *F. rubropicta* vs *F. volcano* (model 6) which is equal to the model performance. The experts performed marginally better on model 5 by 2.5%, performed equally on model 3 and performed worse on model 4. These small differences however are not significant (Table 1). What is striking is the difference in time it takes for the experts and models to make their predictions. It took the experts on average 10 minutes to identify each test set (59 minutes in total) while each model could process their respective test images in less than 6 seconds (30 seconds in total). The experts combined years of limpet-based experience in the study region totals over 22 years, having viewed countless specimens to achieve their personal knowledge base. In contrast, each model used no more than 350 unique images and was created in less than 5 minutes of training time. Therefore, the sheer speed at which CV models can make accurate predictions is one of its primary advantages.

The more unique images that are available for training, then the better the performance of the finished model (Shorten and Khoshgoftaar, 2019). However, at the time of the project, a limited number of specimens were available to create the models, so it is highly likely that if more unique images were available (e.g., from confirmed museum specimens) then we believe that subsequent models could perform even better than those achieved within this project. Interestingly, when viewing the incorrect predictions by themselves, the experts felt that some of their incorrect predictions were a result of human error. A typical downside of the human condition is that performance can decrease due to fatigue or many other cognitive and physical conditions (Mallis et al., 2004), which computers do not suffer. Thus, in the future, we envision that many thousands of specimens can be fed into similar models for identification purposes (e.g., for bulk field collected specimens or un-catalogued museum accessions), alongside confirmation and quality control from expert taxonomists and molecular ecologists.

Heatmap production and expert interpretation

The heatmaps were found to almost always focus upon the specimens, regardless of the model or class. This is a good indicator that the models trained effectively despite the relatively low number of unique images. After the heatmaps were shown to the experts, it was agreed that almost all were focussing on parts of the shell considered to be morphologically important. Occasionally, models focussed on a singular feature, whilst other times they would focus on multiple features. Neither outcome could be considered incorrect. When visually identifying specimens, a human would use a variety of features to make a final decision. However, with cryptic or highly variable species (e.g., some limpet species), the number of defining features is likely to be limited and/or variable among specimens. For instance, the dorsal orientation of *L. conus* vs *L. strigatella* can appear similar (model 3), with only a couple of shell characteristics that can be used to distinguish them by eye. In addition, the dorsal side of both shells can be highly eroded making species identification more difficult when only viewed dorsally (e.g., as they are *in situ*). Regardless, the heatmaps for model 3 appeared to find consistent morphological differences between the species. The *L. conus* heatmaps mainly focussed on the shell patterns around the apex and looking at shell patterns, while the *L. strigatella* heatmaps mainly focussed on apex itself. The apex on the dorsal shell of *L. strigatella* is often highly eroded (Keen, 1971), more so than on *L. conus*. The apex is the oldest part of the limpet shell, and therefore it is often the most eroded. It is therefore possible that the pattern of shell erosion on the apex is different between the two *Lottia* species, which may reveal differences in their internal shell structures or microhabitats (Day et al., 2000), but this has not yet been studied in these species.

Again, there are consistent differences between the *Lottia* species on the ventral sides of their shells. The heatmaps mainly focussed on the area between the muscle scar and the margin areas of the ventral sides of *L. conus* shells. Whereas on *L. strigatella*, focussed on the margin perimeter which often contains a dark band.

The dorsal orientations of *F. rubropicta* vs *F. volcano* (model 5) heatmaps displayed consistent differences. The *F. rubropicta* heatmaps consistently focussed on the area around the keyhole/callus, but not on it, while the *F. volcano* images consistently focussed on the keyhole. The *F. rubropicta* specimens have more pronounced ribbing on their shells, which the heatmaps appear to focus on. Whereas *F. volcano* shells are smooth with black/reddish rays. These shell differences may be related to their microhabitat differences: *F. volcano* are usually found underneath rocks and in sheltered crevices (Morris et al., 1980) while *F. rubropicta* are exposed and found on top of rocks (PBF and KMZ personal observations). The smooth shells of *F. volcano* are more suited to life underneath rocks and in crevices, whereas the heavy ribbing of *F. rubropicta* likely helps reduce water loss (due to higher surface area) during long periods of aerial exposure. Again, the ventral orientation of *F. rubropicta* vs *F. volcano* (model 6) heatmaps displayed consistent differences. The *F. rubropicta* consistently focused on the area within the muscle scar and around the callus, while the *F. volcano* consistently focused on the margin which usually contains a dark band.

Future considerations

For CV models to be robust, images of accurately identified specimens are required for training purposes. To do this, we relied on DNA barcoding to confirm the species level identifications of the training dataset and to evaluate the accuracy of the test dataset identifications from both the CV models and experts. All specimens were therefore already identified to species level prior to developing the CV models. However, molecular work can be expensive and time consuming. To reduce costs and time, the workflow could be adjusted where only the training dataset are barcoded, and then a smaller sub-sample of specimens in the test dataset could be barcoded to statistically assess the accuracy of the models. Ultimately however, the more specimens that are available for training purposes, the more accurate the model results. If large datasets of confirmed and standardized training images are made publicly available for the known species in a study region, then future researchers could use them to supplement their own training datasets. In particular, we need more training images of the dorsal side of limpet shells, as they are primarily used for field identifications.

More research is also needed to help interpret the utility of heatmaps for understanding ecological questions related to limpet shell morphology (Bird, 2011; Hamilton et al., 2020). With more robust training datasets per species from multiple populations, age/size ranges, and habitat types, we may be able use heatmaps to help decipher if and how shell morphology varies intra-specifically over local to regional scales. For example, the intensity and location of heatmaps may differ based on factors such as: microhabitat, population, size/age, and region. We can then use this information to shed new light on how and why limpet shells have such high morphological variability (Giesel, 1970).

Conclusion

This project demonstrates the effectiveness of using CV in identifying limpets based on images of their shells. Despite the variable shell morphologies and colour patterns within and between species, the CV models were able to classify them to genus (100%) and species level (95% - 100%) with high accuracy and quickly, even with small datasets. The use of heatmaps confirmed that the models were focusing on the limpet shells, and when reviewed by expert taxonomists, they agreed that the heatmaps highlighted significant and unique morphological features for each genus and species.

Typically, DL models are considered as 'black box' systems due to their complex decision-making processes and the 'impossibility' of truly understanding how these types of systems come to their final conclusions. However, the use of heatmaps offers a means to understand how CV makes its decisions. The results show that the models can differentiate between visually similar species or those with high morphological variability, and that they utilize unique morphological features to distinguish them. In the future, we envision this type of system being used by taxonomists as a tool to assist them in identifying important or new morphological features to help distinguish between visually similar and cryptic species. Additionally, similar methods could assist with field identification of limpets and potentially replace the need to collect numerous specimens purely for identification purposes. Computer models, once trained, require far less computation power to perform identifications, and most can be uploaded and used from a modern mobile phone.

It is important to consider the strengths and limitations of CV models for identification purposes. No single method is perfect, but combining the strengths of CV, molecular methods, and human expertise will allow us to gain new insights for taxonomy and ecology. Not only for limpets, but for all of biodiversity.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Animal Welfare and Ethical Review Body - ERGO II 63575.

Author contributions

JH, took images of limpets, wrote model code, ran CV models, produced heatmaps, wrote bulk of paper, consulted with morphology analysis. PF, collected samples from field, assisted heavily with writing of paper, gave expert opinion on morphology analysis. KZ, collection samples from field, identified using molecular methods, gave expert opinion of morphology analysis, comment on and edited final drafts. BP, commented on and edited final drafts. TH, commented on and edited final drafts. XC, commented on CV methods, commented on and edited final drafts. All authors contributed to the article and approved the submitted version.

Acknowledgments

We would like to thank Moira Maclean and David Paz García for their assistance with fieldwork and specimen collection. We would like to thank the University of Southampton, the National Oceanography centre, and the Natural History Museum for their assistance with additional equipment needs. JDH and KMZ would like to thank NERC and the INSPIRE doctoral training programme for funding portions of this research. PBF acknowledges funding from NERC grant: NE/X011518/1. We would like to thank Sanson Poon from the Natural History Museum for his consultations for appropriate statistical evaluations for this paper.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Bird, C. E. (2011). Morphological and behavioral evidence for adaptive diversification of sympatric Hawaiian limpets (*Cellana* spp.). *Integr. Comp. Biol.* 51, 466–473. doi: 10.1093/icb/icr050

Burdi, C. (2015). *A test of diagnostic shell differences of the limpets Lottia conus and Lottia scabra identified with PCR-based assay*. Masters dissertation. (California State University, Fullerton).

Carpenter, P. P. (1864). XXXII.—Diagnoses of new forms of mollusks collected at Cape St. Lucas by Mr. J. Xantus. *Ann. Magazine Natural History* 13, 311–315. doi: 10.1080/00222936408681615

Crummett, L. T., and Eernisse, D. J. (2007). Genetic evidence for the cryptic species pair, *Lottia digitalis* and *Lottia austrodigitalis* and microhabitat partitioning in sympatry. *Mar. Biol.* 152, 1–13. doi: 10.1007/s00227-007-0621-4

Dawson, M. N., Hays, C. G., Grosberg, R. K., and Raimondi, P. T. (2014). Dispersal potential and population genetic structure in the marine intertidal of the eastern North Pacific. *Ecol. Monogr.* 84, 435–456. doi: 10.1890/13-0871.1

Day, E. G., Branch, G. M., and Viljoen, C. (2000). How costly is molluscan shell erosion? A comparison of two patellid limpets with contrasting shell structures. *J. Exp. Mar. Biol. Ecol.* 243, 185–208. doi: 10.1016/S0022-0981(99)00120-3

Fenberg, P. B., and Roy, K. (2008). Ecological and evolutionary consequences of size-selective harvesting: how much do we know? *Mol. Ecol.* 17, 209–220. doi: 10.1111/j.1365-294X.2007.03522.x

Fenberg, P. B., and Roy, K. (2012). Anthropogenic harvesting pressure and changes in life history: insights from a rocky intertidal limpet. *Am. Nat.* 180, 200–210. doi: 10.1086/666613

Firth, L. B. (2021). What have limpets ever done for us?: On the past and present provisioning and cultural services of limpets. *Int. Rev. Environ. History* 7, 5–45. doi: 10.22459/IREH.07.02.2021.01

Giesel, J. T. (1970). On the maintenance of a shell pattern and behavior polymorphism in *Acmaea digitalis*, a limpet. *Evolution* 24, 98–119. doi: 10.1111/j.1558-5646.1970.tb01743.x

Greeff, M., Caspers, M., Kalkman, V., Willemse, L., Sunderland, B. D., Bánki, O., et al. (2022). Sharing taxonomic expertise between natural history collections using image recognition. *Res. Ideas Outcomes* 8, e79187. doi: 10.3897/rio.8.e79187

Hamilton, A. M., Selwyn, J. D., Hamner, R. M., Johnson, H. K., Brown, T., Springer, S. K., et al. (2020). Biogeography of shell morphology in over-exploited shellfish reveals adaptive trade-offs on human-inhabited islands and incipient selectively driven lineage bifurcation. *J. Biogeogr.* 47, 1494–1509. doi: 10.1111/jbi.13845

Hansen, O. L., Svenning, J. C., Olsen, K., Dupont, S., Garner, B. H., Iosifidis, A., et al. (2020). Species-level image classification with convolutional neural network enables insect identification from habitus images. *Ecol. Evol.* 10, 737–747. doi: 10.1002/ece3.5921

Hennig, W. (1966). *Phylogenetic Systematics* (Champaign: University of Illinois Press).

Hollister, J., Vega, R., and Azhar, M. H. B. (2022). Automatic identification of non-biting midges (Chironomidae) using object detection and deep learning techniques. *ICPRAM* 1, 256–263. doi: 10.5220/0010822800003122

Høye, T. T., Ärje, J., Bjerge, K., Hansen, O. L., Iosifidis, A., Leese, F., et al. (2021). Deep learning and computer vision will transform entomology. *Proc. Natl. Acad. Sci.* 118, e2002545117. doi: 10.1073/pnas.2002545117

Joshi, S., Owens, J. A., Shah, S., and Munasinghe, T. (2021). Analysis of preprocessing techniques, Keras tuner, and transfer learning on cloud street image data. *2021 IEEE International Conference on Big Data (Big Data)*, Orlando, FL, USA, 4165–4168.

Keen, A. M. (1971). *Sea Shells of Tropical West America: Marine Mollusks from Baja California to Peru* (Stanford: Stanford University Press).

Kordas, R. L., Donohue, I., and Harley, C. D. G. (2017). Herbivory enables marine communities to resist warming. *Sci. Adv.* 3, e1701349. doi: 10.1126/sciadv.1701349

Kuo, E. S., and Sanford, E. (2013). Northern Distribution of the Seaweed Limpet *Lottia insessa* (Mollusca: Gastropoda) along the Pacific Coast. *Pacific Sci.* 67, 303–313. doi: 10.2984/67.2.12

Lürig, M. D. (2022). Phenopype: a phenotyping pipeline for Python. *Methods Ecol. Evol.* 13, 569–576. doi: 10.1111/2041-210X.13771

Mallis, M. M., Mejdal, S., Nguyen, T. T., and Dinges, D. F. (2004). Summary of the key features of seven biomathematical models of human fatigue and performance. *Aviation Space Environ. Med.* 75, 4–14.

Morris, R. H., Abbott, D. P., and Haderlie, E. C. (1980). *Intertidal invertebrates of California* (Stanford: Stanford University Press).

Nakano, T., and Spencer, H. G. (2007). Simultaneous polyphenism and cryptic species in an intertidal limpet from New Zealand. *Mol. Phylogenet. Evol.* 45, 470–479. doi: 10.1016/j.ympev.2007.07.020

Perez, L., and Wang, J. (2017). The effectiveness of data augmentation in image classification using deep learning. *arXiv*:1712.04621v1. doi: 10.48550/arXiv.1712.04621

Pilsbry, H. (1890). *Manual of conchology, structural and systematic, with illustrations of the species. 1(12): Stomatellidae, Scissurellidae, Pleurotomariidae, Haliotidae, Scutellinidae, Addisoniidae, Cocculinidae, Fissurellidae* (Philadelphia: Conchological Section, Academy of Natural Sciences).

Pinho, C., Kaliontzopoulou, A., Ferreira, C. A., and Gama, J. (2022). Identification of morphologically cryptic species with computer vision models: wall lizards (Squamata: Lacertidae: *Podarcis*) as a case study. *Zoological J. Linn. Soc.* 198, 184–201. doi: 10.1093/zoolinnean/zlac087

Popov, D., Roychoudhury, P., Hardy, H., Livermore, L., and Norris, K. (2021). The value of digitising natural history collections. *Res. Ideas Outcomes* 7, e78844. doi: 10.3897/rio.7.e78844

Rädsch, T., Reinke, A., Weru, V., Tizabi, M. D., Schreck, N., Kavur, A. E., et al. (2023). Labelling instructions matter in biomedical image analysis. *Nat. Mach. Intell.* 5, 273–283. doi: 10.1038/s42256-023-00625-5

Reeve, L. (1849). “Monograph of the genus Fissurella,” in *Conchologia Iconica, or, illustrations of the shells of molluscous animals* (London: Reeve & Co).

Rogers, A. J., and Weisler, M. I. (2020a). Assessing the efficacy of genus-level data in archaeomalacology: A case study of the Hawaiian limpet (*Cellana* spp.), Moloka ‘i, Hawaiian islands. *J. Island Coast. Archaeol.* 15, 28–56. doi: 10.1080/15564894.2018.1481467

Rogers, A. J., and Weisler, M. I. (2020b). Limpet (*Cellana* spp.) shape is correlated with basalt or eolianite coastlines: Insights into prehistoric marine shellfish foraging and mobility in the Hawaiian Islands. *J. Archaeological Sci.: Rep.* 34, 102561. doi: 10.1016/j.jasrep.2020.102561

Ross, E. (2022). *Phylogeography of the cryptic intertidal gastropod Lottia conus along the Pacific coast from Southern California to Central Mexico*. Masters Dissertation. (University of Southampton).

Savage, N. (2022). Breaking into the black box of artificial intelligence. *Nature*. doi: 10.1038/d41586-022-00858-1

Selvaraju, R., Cogswell, M., Das, A., Vedantam, R., Parikh, D., and Batra, D. (2016). "Grad-CAM: Visual Explanations from Deep Networks via Gradient-Based Localization,", *2017 IEEE International Conference on Computer Vision (ICCV)*, (Venice, Italy) 618–626. doi: 10.1007/s11263-019-01228-7

Sham, A. H., Aktas, K., Rizhinashvili, D., Kuklianov, D., Alisinanoglu, F., Ofodile, I., et al. (2022). Ethical AI in facial expression analysis: racial bias. *Signal Image Video Process.* 17, 1–8. doi: 10.1007/s11760-022-02246-8

Shorten, C., and Khoshgoftaar, T. M. (2019). A survey on image data augmentation for deep learning. *J. Big Data* 6, 1–48. doi: 10.1186/s40537-019-0197-0

Simison, W. B., and Lindberg, D. R. (1999). Morphological and molecular resolution of a putative cryptic species complex: a case study of *Notoacmea fascicularis* (Menke 1851)(Gastropoda: Patellogastropoda). *J. Molluscan Stud.* 65, 99–109. doi: 10.1093/mollus/65.1.99

Simison, W. B., and Lindberg, D. R. (2003). On the identity of *Lottia strigatella* (Carpenter 1864)(Patellogastropoda: Lottiidae). *Veliger* 46, 1–19.

Simonyan, K., and Zisserman, A. (2014). Very deep convolutional networks for large-scale image recognition. *arXiv*:1409:1556. doi: 10.48550/arXiv.1409.1556

Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38, 3022–3027. doi: 10.1093/molbev/msab120

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H., and Vogler, A. P. (2003). A plea for DNA taxonomy. *Trends Ecol. Evol.* 18, 70–74. doi: 10.1016/S0169-5347(02)00041-1

Test, A. (1945). Description of new species of *Acmaea*. *Nautilus* 58, 92–96.

Wäldchen, J., and Mäder, P. (2018). Machine learning for image based species identification. *Methods Ecol. Evol.* 9, 2216–2225. doi: 10.1111/2041-210X.13075

Weisler, M. I., and Rogers, A. J. (2021). Ritual use of limpets in late Hawaiian prehistory. *J. Field Archaeol.* 46, 52–61. doi: 10.1080/00934690.2020.1835267

Williams, S. T. (2017). Molluscan shell colour. *Biol. Rev.* 92, 1039–1058. doi: 10.1111/brv.12268

Wilson, R. J., De Siqueira, A. F., Brooks, S. J., Price, B. W., Simon, L. M., van der Walt, S. J., et al. (2022). Applying computer vision to digitised natural history collections for climate change research: Temperature-size responses in British butterflies. *Methods Ecol. Evol.* 14, 372–384. doi: 10.1111/2041-210X.13844

Xu, M., Yoon, S., Fuentes, A., and Park, D. S. (2023). A comprehensive survey of image augmentation techniques for deep learning. *Pattern Recognit.* 137, 109347. doi: 10.1016/j.patcog.2023.109347

frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 04 December 2023
DOI 10.3389/fmars.2023.1206159

Check for updates

OPEN ACCESS

EDITED BY
Stephen J. Newman,
Western Australian Fisheries and Marine Research Laboratories, Australia

REVIEWED BY
Paul Brewin,
South Atlantic Environmental Research Institute, Falkland Islands
Yunwei Dong,
Ocean University of China, China

*CORRESPONDENCE
Maria Inês Seabra
✉ iseabra@uevora.pt

†These authors share last authorship

RECEIVED 24 April 2023
ACCEPTED 13 October 2023
PUBLISHED 04 December 2023

CITATION
Seabra MI, Penteado N, Cruz T and Hawkins SJ (2023) Variability and connectivity in populations of different limpet species across rockpool-generated mosaic landscapes.
Front. Mar. Sci. 10:1206159.
doi: 10.3389/fmars.2023.1206159

Variability and connectivity in populations of different limpet species across rockpool-generated mosaic landscapes

Maria Inês Seabra[1]*, Nélia Penteado[1], Teresa Cruz[1,2]† and Stephen J. Hawkins[3,4,5]†

[1]Marine and Environmental Sciences Centre (MARE)/Aquatic Research Network (ARNET), Laboratório de Ciências do Mar, Universidade de Évora, Sines, Portugal, [2]Departamento de Biologia, Universidade de Évora, Évora, Portugal, [3]The Marine Biological Association of the United Kingdom, The Laboratory, Plymouth, United Kingdom, [4]School of Ocean and Earth Science, University of Southampton, Southampton, United Kingdom, [5]School of Biological and Marine Sciences, University of Plymouth, Plymouth, United Kingdom

Limpets are keystone grazers on rocky shores. We studied spatial patterns of four co-occurring patellids (*Patella ulyssiponensis*, *Patella depressa*, *Patella rustica*, *Patella vulgata*) and one siphonariid (*Siphonaria pectinata*) across rockpools and their surrounds. Our approach considered each rockpool as a concentric system of three micro-habitats where limpets were censused (species, counts, and size of individuals): inside rockpool, edge zone, and surrounding open-rock. The whole intertidal zone was sampled on six rocky shores with contrasting landscape variability (amount of open-rock and other micro-habitats) in Southwest Portugal. Additionally, open-rock surfaces far away (>25cm) from rockpools were surveyed on the mid-shore. Three groups of predictors ("physical, habitat-composition, and connectivity variables") were assessed for each rockpool system. Limpets of various sizes of the three most common species—*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*—were found to coexist inside and outside rockpools on all shores, with rockpools extending their vertical distribution upwards. *Patella vulgata* was rare; *P. rustica* was absent from rockpools and their edges. Intra-specific connectivity (correlations in density of the same species between adjacent micro-habitats) showed a decreasing gradient with distance from the pool, being sharper for *P. ulyssiponensis* (a largely rockpool-resident) than for *P. depressa* and *S. pectinata* (suggesting their emergence from rockpools to edges and surrounding open-rock). Abundance and size structure of both *P. depressa* and *S. pectinata* were similar on mid-shore open-rock surrounding rockpools and far from rockpools. Landscape variability explained distribution of *P. depressa* (shores with less open-rock had more and smaller limpets). Habitat-composition (assemblages and substrata) was more important than physical predictors in explaining among-rockpool variability of each limpet species. Densities of *P. ulyssiponensis* and *P. depressa* inside rockpools were positively associated with cover of coralline algae and negatively associated with sand cover. Less explanatory power was found in predictive models of *S. pectinata*, suggesting a more opportunistic nature. There was limited evidence of inter- or intra-specific competition of the three species in rockpools and open-rock. Competition between *P. ulyssiponensis* and *P. depressa* was most likely in rockpool edges. Rockpool-generated mosaic landscapes are linked by intra-specific connectivity of limpet populations and modulated by interactions among different limpet species.

KEYWORDS

limpet populations, rockpools/tidepools, emergent substrata, habitat connectivity, patellids, siphonariids, mosaic landscapes, co-existence

1 Introduction

Spatial patterns of keystone species and their coexistence are fundamental for understanding organization of benthic communities and informing marine conservation (Tanner et al., 1994; Ortiz et al., 2017). Given the ecological importance of limpets on rocky shores (Branch, 1981; Hawkins and Hartnoll, 1983; Branch et al., 1985; Fretter and Graham, 1994; Hodgson, 1999; Coleman et al., 2006), their spatial distribution has been extensively investigated on several scales worldwide (e.g., Creese, 1980; Williams and Morritt, 1995; Lima et al., 2006; Vermeij, 2017; Broitman et al., 2018). Coexisting limpets may be from the same (e.g., Aguilera et al., 2013; Freitas et al., 2023a; Freitas et al., 2023b) or different (e.g., Creese and Underwood, 1982; Aguilera et al., 2019) phylogenetically related groups, with similar or distinct traits (e.g., Davies, 1970; Wolcott, 1973; Aguilera and Navarrete, 2012). Many studies have documented distribution patterns of sympatric patellogastropods in South Africa (e.g., Branch, 1971; Branch, 1975; Branch, 1976; Branch and Marsh, 1978; Carneiro, 2021) and the North-East Atlantic (e.g., Boaventura et al., 2002a; Boaventura et al., 2002b; Firth and Crowe, 2008; Casal et al., 2018; Oróstica et al., 2020; Freitas et al., 2023a; Freitas et al., 2023b). On North-East Atlantic shores, patellid limpets have long-been recognized as keystone grazers (e.g., Hawkins and Hartnoll, 1983; Jenkins et al., 2005; Coleman et al., 2006; Henriques et al., 2017). Patellids can co-occur with *Siphonaria pectinata* on Atlantic and Mediterranean rocky shores in South Europe and North Africa (Rivera-Ingraham et al., 2011; Rubal et al., 2013; Vasconcelos et al., 2021; Slama et al., 2022).

Limpet species can co-occur in two contrasting intertidal micro-habitats: freely draining emersed surfaces (open-rock) and depressions retaining water during low tide (rockpools or tidepools) (Firth and Crowe, 2008; Firth et al., 2009; Noël et al., 2009; Seabra et al., 2020). On open-rock, patterns of distribution, abundance, and size structure of populations of individual species of limpets have been traditionally described vertically with tidal level (e.g., Orton, 1929; Lewis, 1954; Blackmore, 1969), horizontally with different wave exposure (e.g., Evans, 1947; Evans, 1957; Thompson, 1979; Thompson, 1980; Ocaña, 2003; Silva et al., 2003), or on larger geographic scales (e.g. Lewis, 1986; Rubal et al., 2015). Local-scale micro-habitat variation also can determine distribution patterns of limpets living outside rockpools, such as mussel clumps (Lewis and Bowman, 1975; Silva et al., 2003), fucoid and barnacle patches (Hartnoll and Hawkins, 1985; Hawkins et al., 1992; Johnson et al., 1997; Burrows and Hawkins, 1998; Jenkins et al., 1999; Moore et al., 2007; Marzinelli et al., 2012), vertical surfaces, overhangs or crevices (Wolcott, 1973; Garrity, 1984; Williams and Morritt, 1995; Gray and Hodgson, 1998; Aguilera and Navarrete, 2011), sunny versus shaded rock (Lima et al., 2016; Seabra et al., 2016), or substrata associated with crypsis by polymorphic species (e.g., Giesel, 1970; Sorensen and Lindberg, 1991). Such studies have demonstrated the role of landscape variability (the mosaic of micro-habitats available on each shore) for the underlying processes determining limpet survival or growth, with consequences for spatial heterogeneity of assemblages (Hawkins et al., 2019). Inside rockpools, high densities of early life-history stages of patellids or siphonariids consistently occur (e.g., Lewis and Bowman, 1975; Bowman, 1981; Ostalé-Valriberas et al., 2018). The role of rockpools as nurseries for coexisting limpets has been experimentally demonstrated across different regions (Delany et al., 1998; Seabra et al., 2020). Rockpools extend the upper limits of *Patella ulyssiponensis* (Evans, 1947; Thompson, 1979; Firth and Crowe, 2008), possibly due to its association with crustose-coralline algae (hereafter CCA) (Delany et al., 2002; Seabra et al., 2019; Gomes et al., 2022). While this pattern has been described for other low-shore organisms that are susceptible to desiccation (Goss-Custard et al., 1979; Kooistra et al., 1989; Araújo et al., 2006), unequivocal quantitative evidence is still lacking for other limpets (but see Menconi et al., 1999 for crevices influencing vertical distribution of Mediterranean patellids).

Connectivity is likely to occur between limpet populations inhabiting rockpools versus open-rock. These are clearly distinct environments, especially physically when the tide is out and in their algal communities (Metaxas and Scheibling, 1993; Araújo et al., 2006). However, bi-directional movements of individual limpets across the two micro-habitats happen (Delany et al., 2002; Noël et al., 2009). As limpet intra-specific competition occurs inside rockpools (Firth et al., 2009; Firth and Crowe, 2010), coupled with the importance of these micro-habitats as limpet nurseries (Seabra et al., 2020), rockpools may act as sources of limpet emigration to surrounding open-rock areas. Conversely, as limpets with home scars outside rockpools enter rockpools when foraging at high tide (Noël et al., 2009), rockpools could act as feeding grounds or refuges from desiccation or other stresses, attracting limpets from surrounding open-rock. Limpets also establish home scars along the edge of rockpools (Delany et al., 1998). Rockpool edges are interfaces straddling neighboring micro-habitats with different physical and biological characteristics (e.g., insolation, evaporation, algal and microbial food): permanently submerged inside-pool areas and tidally emersed outside-pool areas slightly away from pools. Patterns of limpet population structure have not been previously described for this pool to open-rock transition nor the wider mosaics of micro-habitats created by the presence of pools, likely to be important at emerging landscape scales.

Large variability at small spatial scales occurs among different rockpools due to their physical and biological characteristics, with consequences for biodiversity and ecosystem functioning (Metaxas et al., 1994; Griffin et al., 2010). The influence of rockpool characteristics on species richness, community structure and functioning has been examined (e.g., shore height, Kooistra et al., 1989; surface area, Underwood and Skilleter, 1996; depth, Martins et al., 2007; volume, Wolfe and Harlin, 1988; slope of pool substratum, Firth et al., 2014; presence of sea urchin grazers, Benedetti-Cecchi and Cinelli, 1995; abundance of molluscan grazers, Masterson et al., 2008; topographic-complexity, Schaefer et al., 2023). Moreover, several variables have been identified as important predictors of abundance and dynamics of invertebrate populations in rockpools or in surrounding rock (e.g., geographic location, Souza and Matthews-Cascon, 2019; shore height, Johnson, 2001; rockpool substrata and biota, Carvalho et al., 2021; distance to rockpool edge, Noël et al., 2009; presence of coexisting species of limpets in the same micro-habitat, Firth and Crowe, 2008). However, the potential effects of other rockpool characteristics (e.g., shape, distance to low-water mark) or proxies of connectivity among populations of mobile invertebrates across rockpool edges (e.g., densities of coexisting species of limpets in adjacent micro-habitats) have not been studied yet.

We studied the four limpets of the genus *Patella* (*P. ulyssiponensis*, *P. depressa*, *P. rustica*, and *P. vulgata*) and one siphonariid limpet (*S. pectinata*) that coexist on the continental Portuguese coast. Patterns of distribution, abundance, and/or size structure of these species and underlying driving mechanisms have been examined for open-rock within shore levels, mostly for adults (Boaventura et al., 2002a; Boaventura et al., 2002b; Boaventura et al., 2003; Rubal et al., 2013; Lima et al., 2016; Seabra et al., 2016). The distribution and abundance of juvenile limpets, especially their association with rockpools or patchy micro-habitats at different shore levels plus temporal and spatial variation in recruitment, have also been investigated (Guerra and Gaudencio, 1986; Silva et al., 2003; Seabra et al., 2019; Seabra et al., 2020). Preliminary observations suggest much small-scale variability in spatial patterning of patellid and siphonariid populations across rockpools and their surrounds. Here, we take an integrative view along the continuous gradient of shore height of the intertidal zone, focusing on micro-habitats both inside and outside rockpools and their interfaces creating mosaic landscapes at each tidal level (hereafter “rockpool-generated landscapes”).

Our overall aim was to examine the spatial patterns of co-occurrence of intertidal limpets in rockpool-generated landscapes describing variability of limpet populations within each micro-habitat and connectivity among them. Each species was quantified (number and size of individuals) in the following micro-habitats: inside-rockpools (POOL), on the narrow edge-zone around the rim of rockpools (EDGE), and on open-rock surrounding rockpools (NEAR). Surveys were made over the whole intertidal gradient on six natural rocky shores in the southwest of continental Portugal (Figure 1), to assess diversity, distribution, abundance, and size structure of limpets throughout the distinct micro-habitats and among shores. Additionally focusing only on the mid-shore, areas of open-rock at a minimum distance of 25 cm from any rockpool (FAR) were also surveyed on the same shores, to measure the density of all limpet species-size classes occurring within these areas. Three main objectives were addressed (Table 1). First, (1) we described the distribution of all limpet species present in each micro-habitat on the six shores, and we examined the spatial variation in abundance and size of the most common limpet species at different horizontal scales. Specifically, (1a) we tested how the density and size structure of each of the three most abundant species (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*) within each of three micro-habitats (POOL, EDGE, and NEAR) varied among shores and within each shore. For these three species, (1b) we also tested if the total density of the same species was correlated between adjacent micro-habitats to assess their intra-specific connectivity across rockpool-generated landscapes. For the two most common species on mid-shore open-rock (*P. depressa* and *S. pectinata*), (1c) we compared density and size structure between open-rock surfaces surrounding mid-shore rockpools (MidNEAR) and further away from rockpools (FAR), and assessed whether the pattern of proximity to rockpools (MidNEAR versus FAR) varied among shores of differing landscape. Landscape variability among shores was described in terms of contrasting heterogeneity in the presence of intertidal micro-habitats, by measuring the relative occurrence of open-rock, rockpools, crevices, overhangs, and channels, in mid-shore areas within each shore. Second, (2a) we described any differences with shore height in abundance of each limpet species within each micro-habitat and shore, and (2b) we compared the vertical distribution patterns of the most common species (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*) between areas inside versus outside rockpools, specifically testing the following hypothesis: inside rockpools (POOL), each species occurs further upshore than its occurrence on the open-rock (NEAR). Third, (3) we described the small-scale (among rockpools) variability in density and size-class structure of individual species (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*) within each micro-habitat (POOL, EDGE, and NEAR), and we investigated which predictive variables explain most of these spatial patterns. For every

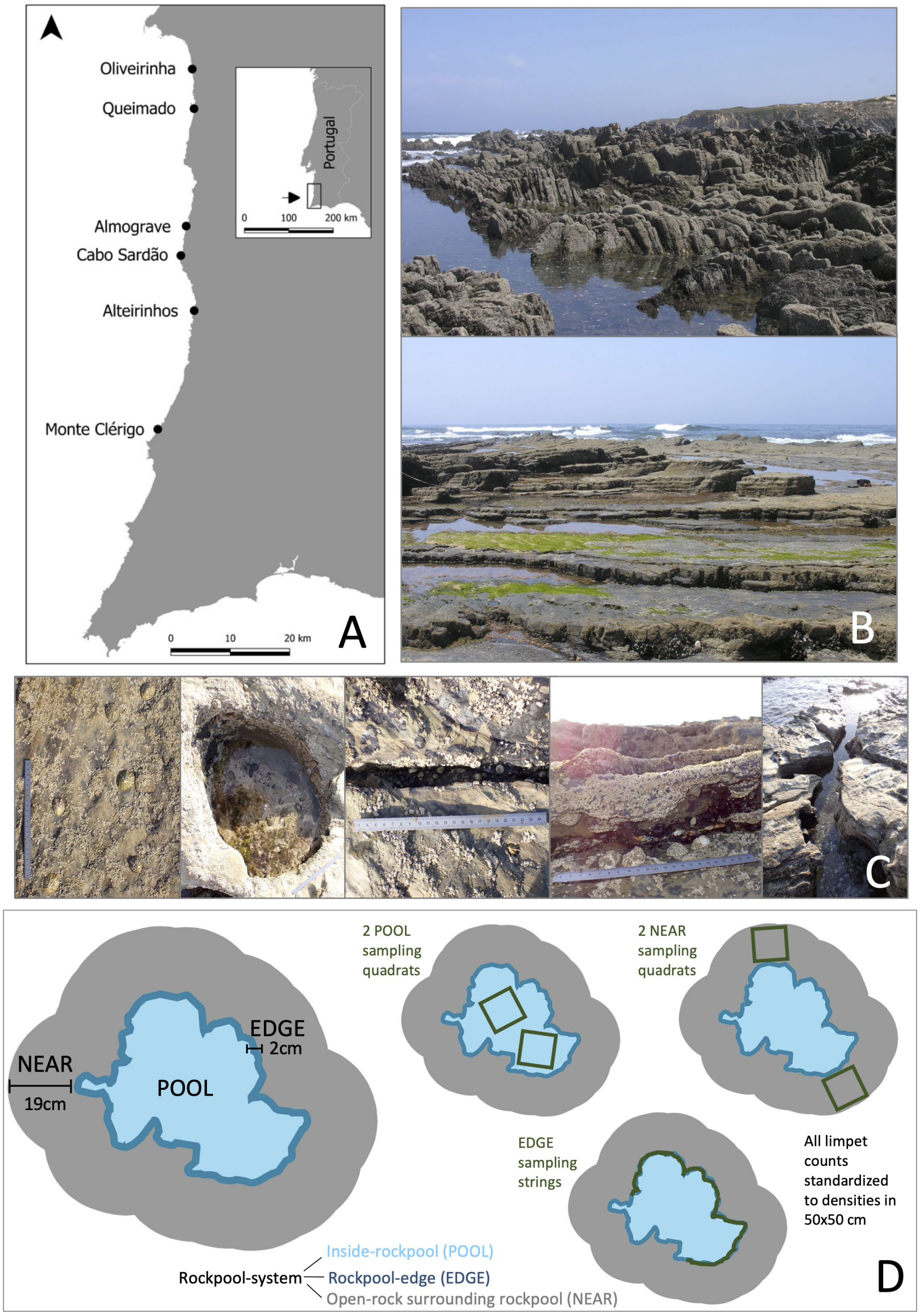


FIGURE 1
(A) Map of the study region and the six sampled shores; **(B)** shores varied in geomorphology, namely, in the cleavage orientation of shale strata, from flatter-layered platforms (Oliveiriinha, Queimado, and Monte Clérigo—example of Monte Clérigo on photograph below) to steeper bedrock (Almograve and Cabo Sardão; intermediate orientation in Alteirinhos—example of Almograve on photograph above); **(C)** five micro-habitat categories considered for landscape variability assessment (open-rock, rockpool, crevice, overhang, and channel); **(D)** diagram representing a rockpool system, with its three concentric micro-habitats (POOL, EDGE, and NEAR) and respective sampling units (POOL and NEAR quadrats, EDGE strings).

species and micro-habitat, we assessed the influence of the following groups of predictors: i) metrics describing size, shape, topography, and position of rockpools and surrounding rock (hereafter "physical variables"); ii) both inorganic (e.g., rock and sand) and biotic (algae and invertebrates) variables describing composition of the substratum coverage and benthic assemblages (hereafter, "habitat-composition variables"—cover of major substrata types and sessile taxa, plus density of other limpet species and molluscan grazers in the same micro-habitat); and iii) "connectivity variables" describing inter-specific influences on

TABLE 1 Summary-table outlining the general objectives of the present study, specific research questions or hypotheses, corresponding response and predictive variables, and figures and/or tables of *Results* (including Supplementary Material).

General objective	Specific objective (question or hypothesis)	Response variables	Predictive variables	Figures and tables of Results
1—Horizontal variation in the abundance and size structure of limpets (*Section 2.4.1* of Statistical analyses; *Section 3.1* of Results)	1a) How do the abundance and size of each limpet species in three micro-habitats (rockpools, rockpool edges, open-rock surrounding rockpools) vary on the six shores? (see Figure 1) Are there differences in spatial variation (among shores and within each shore) in the density and size structure of each of the three most abundant limpet species within each micro-habitat?	9 response matrices analyzed separately (3 limpet species × 3 micro-habitats); 3 limpet species: *Patella ulyssiponensis*, *P. depressa*, *Siphonaria pectinata*; 3 micro-habitats: POOL, EDGE, and NEAR. Each response matrix formed by five response variables: standardized densities (to 50 × 50 cm) of 5 size-classes of one species recorded within one micro-habitat.	2 factors: Shore; Stretch within shore	Figures 2–4; Table 2 (nine two-way PERMANOVAs). Description for the least abundant species (*P. vulgata* and *P. rustica*): In text (*Section 3.1*) and in Supplementary Figures S1, S2).
	1b) Is the total density of the same species correlated between adjacent micro-habitats (intra-specific connectivity)?	3 pairs of variables (3 groups of adjacent micro-habitats—POOL vs. EDGE; EDGE vs. NEAR; POOL vs. NEAR) per limpet species; 3 limpet species: *Patella ulyssiponensis, P. depressa, Siphonaria pectinata*. Each pair of variables: the total density of a single species recorded in two adjacent micro-habitats.		In text of *Section 3.1* (three Spearman correlations per species)
	1c) Are there differences between open-rock surfaces surrounding rockpools and further away from rockpools in the density and size structure of each of the two most common limpet species on the mid-shore open-rock? Do these patterns of proximity to rockpools vary among shores of differing landscape?	2 response matrices analyzed separately (2 limpet species—Patella *depressa* and *Siphonaria pectinata*). Each response matrix formed by five response variables: standardized densities (to 50 × 50 cm) of 5 size-classes of one species recorded on mid-shore open-rock surfaces of two treatments (MidNEAR—surrounding rockpools; FAR—further away from rockpools).	3 factors: Proximity to rockpools, Shore, and Stretch within shore. Shores differed in landscape variability (relative availability of open-rock and topographic micro-habitats, see Figure 1).	Figure 5; Table 3 (two three-way PERMANOVAs). Estimated values for landscape variability assessment: Supplementary Figure S3.
2—Vertical distribution of limpet species inside and outside pools (*Section 2.4.2* of Statistical analyses; *Section 3.2* of Results)	2a) How does the abundance of each limpet species in three micro-habitats (rockpools, rockpool edges, open-rock surrounding rockpools) vary with shore height on each shore?	15 response matrices (5 limpet species × 3 micro-habitats; 5 limpet species: *Patella ulyssiponensis*, *P. depressa*, *P. vulgata*, *P. rustica*, *Siphonaria pectinata*; 3 micro-habitats (POOL, EDGE, NEAR). Each response matrix: the total density of one species recorded within one micro-habitat along the whole gradient of shore height of each shore.	Shore; shore height levels	Figure 6
	2b) We predict that inside rockpools each of the most common limpet species occurs further upshore than its occurrence on the open-rock.	4 pairs of variables (the median, mean, highest, and lowest shore height of species occurrence on each stretch of coast) per limpet species; 3 limpets species (*Patella ulyssiponensis*, *P. depressa*, *Siphonaria pectinata*). Each pair of variables: the shore height of a single species recorded inside rockpools and on the open-rock.		Figure 7; Table 4 (four Binomial tests per species)
3—Patterns among highly variable rockpool systems (*Section 2.4.2* of Statistical analyses; *Section 3.3* of Results)	What is the relationship between the spatial variability of individual limpet species within each micro-habitat and several predictive variables? What are the most important predictors to explain the small-scale (among rockpool systems) variability in spatial patterns of the three most abundant limpet species within each micro-habitat?	9 response matrices analyzed separately (3 limpet species × 3 micro-habitats); 3 limpet species: *Patella ulyssiponensis*, *P. depressa*, *Siphonaria pectinata*; 3 micro-habitats: POOL, EDGE, and NEAR. Each response matrix formed by five response variables: standardized densities (to 50 × 50 cm) of 5 size classes of one species recorded within one micro-habitat.	Predictors assessed for all 323 rockpool systems and classified in three groups: physical, habitat-composition, and connectivity variables (description and assessment in text of *Sections 1*, *2.3*, and *2.4.2*; detailed definition and measurement in Supplementary Table S1; full list of predictors used to build each model in Supplementary Table S2)	Figures 8–10; Table 5 (nine DistLM models). Estimated values for habitat-composition and physical predictors: Supplementary Figures S4, S5 (percentage cover within POOL and within NEAR), Supplementary Figure S6 (counts of trochids and littorinids within POOL and within NEAR) and Supplementary Figure S7 (physical variables).

connectivity (i.e., density of other limpet species in adjacent micro-habitats).

2 Materials and methods

2.1 Study species

On the Portuguese coast, the warm-temperate *P. depressa* on the mid-shore and *P. ulyssiponensis* lower down are the most abundant limpet species on open-rock (Guerra and Gaudencio, 1986; Boaventura et al., 2002b), with *P. ulyssiponensis* occupying pools at higher shore levels (Guerra and Gaudencio, 1986). The less common cold-temperate *P. vulgata* occurs between low and high shore levels (Cabral and Simões, 2007), mostly found on the lower-mid shore (Boaventura et al., 2002b), frequently in shade or local shelter (Lima et al., 2016). Warm-temperate *P. rustica* is mostly restricted to steep high-shore levels on exposed shores (Boaventura et al., 2002b; Lima et al., 2006). In SW Portugal, *S. pectinata* occurs on open-rock and pools across all tidal levels (CIEMAR, 2018; Seabra et al., 2020), being particularly abundant in wave-sheltered sites (CIEMAR, 2018), with much small-scale variation in abundance on mid-shore open-rock (Rubal et al., 2013).

2.2 Study region and shores

Surveys were made in Southwest Portugal (Figure 1A), within the "*Parque Natural do Sudoeste Alentejano e Costa Vicentina*," a marine park where the rocky littoral is characterized by high biodiversity (Horta e Costa et al., 2018), and many rockpools. Intertidal limpets (*Patella* spp. and *S. pectinata*) are abundant (e.g., Boaventura et al., 2002b); harvesting of patellids is mostly permitted and spatially widespread across the marine park, being considered a regularly exercised but low-intensity activity in terms of frequency and harvest yields with low (*P. depressa* and *P. vulgata*) to medium (*P. ulyssiponensis*) social–economic importance relative to other rocky-intertidal species (Castro et al., 2020 and references therein). Six natural wave-exposed shores were selected from easily accessible locations with extensive reefs along 60 km of coastline (Figure 1A): i) Oliveirinha (37°53′12.38″N, 8°47′47.97″W), ii) Queimado (37°49′35.95″N, 8°47′33.60″W), iii) Almograve (37°38′54.42″N, 8°48′21.96″W), iv) Cabo Sardão (37°36′13.09″N, 8°48′56.91″W), v) Alteirinhos (37°31′12.40″N, 8°47′22.93″W, and vi) Monte Clérigo (37°20′23.34"N, 8°51′25.88"W). All shores were of the dominant rock type on this coast (shale sedimentary/schist metamorphic rock, Boaventura et al., 2002b). Shores differed in cleavage orientation of strata (Figure 1B), from flat-layered platforms (Oliveirinha, Queimado and Monte Clérigo—hereafter "flatter shores") to steep bedrock (Almograve and Cabo Sardão—hereafter "steeper shores"); Alteirinhos has an intermediate/mixed pattern of flat and steep surfaces. These geomorphological differences drive landscape variability of these shores in terms of heterogeneity of intertidal micro-habitats. The tidal regime is semidiurnal with 3.5 m maximum amplitude.

2.3 Field surveys

2.3.1 Survey design and sampling procedures (landscape variability, limpets, and habitat-composition variables)

Surveys were made during low tides from August to October 2009, on days without rainfall. On each shore, two stretches of 10 m were surveyed. In each stretch, a 10-m chain was deployed along eight transects running sea parallel, haphazardly spread at different distances from low-water mark along the whole shore. In each transect, the micro-habitats present at 21 points spaced at 50-cm intervals along the chain were recorded, by assigning one of the following categories to each point: open-rock, rockpool, channel, crevice, or overhang (Figure 1C). The percentage frequency of occurrence was calculated for each category on each transect. The mean percentages of micro-habitat occurrence over all transects sampled on the mid-shore of the same stretch were then assessed as a measure of landscape variability. From the whole set of rockpools intersected by transects, a minimum of 25 rockpools per stretch were randomly selected and individually marked in the field for sampling (numbered removable epoxy tag on adjacent rock). We sampled 323 rockpools (Oliveirinha, 52; Queimado, 51; Almograve, 57; Cabo Sardão, 56; Alteirinhos, 53; Monte Clérigo, 54).

Each rockpool was treated as a system of three concentric micro-habitats (Figure 1D): i) inside-rockpool (POOL); ii) edge of rockpool (EDGE); and iii) open-rock surrounding the edge (NEAR). POOL was sampled using two equal-sized quadrats deployed on the bottom of each rockpool, encompassing inside-pool surfaces located at least 2cm away from the pool waterline. POOL quadrat-size varied with rockpool size; so that a minimum of 50% of the total surface area of rockpool-bottom was covered by the two sampling quadrats. For each rockpool, the size of the POOL quadrat was selected from eight differently sized quadrats, made from ~1.3-cm grid of plastic wire mesh, with the following areas (respective quadrat-size in number of grid units): 40 cm^2 (5×5), 161 cm^2 (10×10), 361 cm^2 (15×15), 640 cm^2 (20×20), 1,005 cm^2 (25×25), 1,444 cm^2 (30×30), 2,581 cm^2 (40×40), and 4,032 cm^2 (50×50). EDGE was defined as the thin rim comprising a 2-cm wide band surrounding the pool waterline, including a 1-cm wide marginal inside-pool surface and a 1-cm wide marginal outside-pool surface. EDGE was sampled with strings of 25×2 cm length deployed along the pool waterline, using enough strings to cover a minimum of 50% of the perimeter of each rockpool. NEAR was defined as the adjacent outside-pool area of open-rock, bounded by a halo-line positioned at approximately 2 cm away from the pool waterline and extended to a constant width of 19 cm. NEAR was sampled by two quadrats of 361 cm^2 (19×19 cm) randomly deployed within open-rock surfaces surrounding each rockpool. All quadrats and strings were made of flexible material to fit the topography of sampled surfaces. For each variable assessed within POOL or NEAR, a replicate consisted of the mean obtained from the two sampling quadrats of the same micro-habitat of every rockpool system. For variables assessed within EDGE, a replicate consisted of the sum obtained from all strings sampled along the edge of every rockpool. Additionally, mid-shore surfaces of open-rock located at least 25 cm away from any rockpool (FAR) were sampled by six replicate quadrats of 361 cm^2 per stretch. For comparison with FAR replicates, a subset of six NEAR replicates per stretch, all located around mid-shore rockpools, was selected during surveys (hereafter “MidNEAR replicates”).

Species identification, allocation to size classes (maximum shell length—MSL, measured with calipers), and counts were made for all limpets within the four micro-habitats (POOL, EDGE, NEAR, and FAR). Five size classes were considered for both *Patella* spp. (MSL, ≤ 1 cm; 1–2 cm, 2–3 cm, 3–4 cm, and ≥ 4 cm) and *S. pectinata* (MSL, ≤ 0.5 cm, 0.5–1 cm, 1–2 cm, 2–3 cm, and 3–4 cm). Every individual limpet within the sampling areas was recorded for the respective species-size class, micro-habitat, and individual rockpool system. There were two exceptions: a) juvenile limpets (MSL ≤ 1 cm for patellids and MSL ≤ 0.5 cm for *S. pectinata*, see Seabra et al., 2020) within POOL were sub-sampled using two quadrats of 40 cm^2; and b) limpets with MSL ≤ 0.5 cm were not sampled within EDGE due to the transitional nature of this micro-habitat. All limpet counts were then standardized to densities in 50×50 cm.

Within both POOL and NEAR of every rockpool system, we assessed the following habitat-composition variables (details in Supplementary Table S1): i) the percentage cover of 15 space-occupying categories (rock, sand, Lichinaceae, Verrucariaceae, cyanophytes, crustose non-coralline algae, CCA, articulated coralline algae, seaweed, Porifera, sea anemones, barnacles, mussels, sea urchins, and other sessile invertebrates), i.e., substratum types and functional groups of sessile organisms and ii) counts of two groups of non-limpet mobile grazer gastropods, i.e., trochids (including *Steromphala umbilicalis*, *S. pennanti*, *Phorcus sauciatus*, and *P. lineatus*) and littorinids (*Melarhaphe neritoides*), both standardized to densities in 50×50 cm. All taxa were visually identified in the field to the lowest possible taxonomic resolution and then lumped into functional groups of sessile or mobile organisms.

2.3.2 Physical characteristics of rockpools

A set of 19 physical variables was assessed for each of the 323 sampled rockpools, in the field or through image analysis (details in Supplementary Table S1). Each variable corresponds to a quantitative or qualitative metric associated with size (perimeter, surface area, and volume), shape (circularity, roundness, aspect ratio, and curved or straight edges), topography (maximum and mean depth, POOL and NEAR slope, POOL and NEAR topographic complexity, “confinement”), or position (straight and contoured distance to the nearest rockpool, shore height, distance to low-water mark, and “barriers”) of rockpools and/or surrounding rock. “Confinement” was the height of the rocks surrounding the pool (1, pool flushes with surrounding rock; 2, low walls, <3 cm; 3, medium-height walls, 3–6 cm; 4, high walls, 6–10 cm; 5, very-high walls, >10 cm and often with obtuse angles). “Barriers” was presence or absence of outcrops of rock to seaward at <1 m from the pool. Although a few variables applied only to a single micro-habitat (volume, maximum, and mean depth: POOL-only; slope and topographic complexity: separately assessed inside pools and on surrounding rock), most were physical descriptors of each rockpool system across POOL, EDGE, and NEAR (shape and position variables applying to the three micro-habitats; perimeter and surface area of a rockpool are proportional to the ones of its surrounds).

2.4 Statistical analyses

Prior to multivariate analyses described in *Sections 2.4.1* and *2.4.3*, each response matrix—formed by five response variables, i.e., standardized densities (to 50×50 cm) of the five size classes of a single limpet species recorded on all replicates of a single micro-habitat (hereafter “population size structure and density” or “size-class densities” of an individual species within a micro-habitat)—was transformed (as a pre-treatment of the analysis for a given species-by-micro-habitat combination): i) fourth-root transformation applied to response matrices inside rockpools (POOL); ii) square-root transformation applied to response matrices of the other micro-habitats (EDGE or NEAR or MidNEAR vs. FAR). All multivariate analyses were based on Euclidean distances calculated from transformed size-class densities. All the procedures for multivariate analyses were implemented in PERMANOVA+ for PRIMER package (Anderson et al., 2008), an add-on to PRIMER v7 (Clarke and Gorley, 2015).

2.4.1 Horizontal variation in the abundance and size structure of limpets

Two-way non-parametric multivariate analyses of variance (PERMANOVA, Anderson, 2001) were made separately for each of the three species of limpets (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*), within each of the three micro-habitats (POOL, EDGE, and NEAR), to test for differences in limpet populations among the six shores and among the two stretches within each shore (shore—fixed factor with six levels; stretch—random factor with two levels, nested in shore; sample size varied between 25 and 30 rockpools per stretch).

To assess intra-specific connectivity, correlations of the total density of the same species between pairs of micro-habitats (POOL and EDGE; EDGE and NEAR; and POOL and NEAR) were made separately for the three species (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*) using Spearman's coefficient (n = 323 rockpool systems).

Three-way PERMANOVAs were performed separately for each of two species (*P. depressa* and *S. pectinata*) to test for differences in limpet populations living on mid-shore open-rock between areas close to and further away from pools, and among shores and stretches (proximity to rockpools—fixed factor with two levels: MidNEAR and FAR; Shore—fixed factor with six levels; Stretch—random factor with two levels, nested in Shore; n = 6). In the case of *P. depressa*, for which a significant interaction was found between Proximity and Shore, non-metric multidimensional scaling (MDS) was then done separately for each level of factor Proximity (MidNEAR, FAR), to visualize the distance among stretches of all shores and correlation vectors of the response variables (size-class densities of *P. depressa*). In duplicate MDS plots, vectors of predictors of landscape variability were overlaid (i.e., mean percentages of occurrence of five micro-habitat categories within stretches of every shore).

PERMANOVA tests employed permutation of residuals under a reduced model using 999 permutations and type III (partial) sum of squares. Where differences were detected by PERMANOVA, pair-wise tests determined which levels of each factor differed. The similarity percentages breakdown (*SIMPER*) procedure (Clarke, 1993) was applied to identify which response variables were the major contributors to the differences between groups detected by pair-wise tests (size classes most responsible for multivariate distances between significantly different factor levels).

2.4.2 Vertical distribution of limpets inside and outside rockpools

Binomial tests (Sokal and Rohlf, 1995) assessed if upward extension occurred in three species of limpets (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*) inside rockpools (POOL), compared to outside rockpools (NEAR). Data were assembled separately for each species, through the following steps: i) every replicate where the presence of a given species was found was selected from the 323 replicates of both POOL and NEAR; ii) for each of the two micro-habitats, the shore-height records of all selected replicates were considered; iii) from these records, the median, mean, maximum, and minimum values of shore height within each micro-habitat (four pairs of variables per species) were calculated for each sampled stretch of coast (12 stretches, corresponding to two stretches within each of six shores). The general null hypothesis that the median, mean, highest, or lowest shore height of a limpet species occurrence is similar between the two micro-habitats was analyzed by individual binomial tests. In each binomial test, 12 POOL and 12 NEAR values were compared.

2.4.3 Patterns among highly variable rockpool systems

Distance-based linear models (DistLM) were used to examine the relationship between the small-scale variability of individual limpet species inside pools, at the edge of pools or in the surrounding open-rock (size-class densities of a target species within a micro-habitat) and several predictors assessed for each rockpool system (physical, habitat-composition, and connectivity variables). Predictive variables were initially assembled for each target species within each micro-habitat (full list of predictive variables used to build the model for each response matrix in Supplementary Table S2). The total density of every other co-occurring species of limpets were included as follows: i) habitat-composition variables if co-existing with the target species in the same micro-habitat or ii) connectivity variables if estimated in an adjacent micro-habitat. Connectivity variables were coded (e.g., PU_adjacent_Pool) by the abbreviation of species name (*P. ulyssiponensis*—PU; *P. depressa*—PD; *S. pectinata*—SP) followed by "_adjacent_" and the micro-habitat code (Pool; Edge; Near). In the two cases where significant differences were detected among shores by the previously described two-way PERMANOVA tests (*Section 2.4.1*), specifically for *P. depressa* within NEAR and for *S. pectinata* within EDGE, the factor shore was included as an additional predictive variable for DistLM.

After preliminary procedures of elimination and transformation of predictive variables, their selection was made with BEST procedure and based on AIC criterion (methodological details given in Supplementary Text). After obtaining the "BEST final model" for each response matrix, we further selected the "top predictors" as those that contributed most to explain total variation and for which the inclusion in this model added a minimum of 1% in R^2. Finally, distance-based redundancy analysis (db-RDA) plots were produced to identify the response variables (size classes) that best described the variability of each target species within each micro-habitat, and their association with the "top predictors." Correlation vectors of response variables and "top predictors" were overlaid in duplicate db-RDA plots for comparison.

A second DistLM analysis was ran for each species within each micro-habitat, with the "top-predictors" of the "BEST final model" (obtained by the first DistLM analyses) classified in three indicator groups of predictors: physical, habitat-composition, and connectivity variables. We assessed the contribution of each of these groups to the overall explanation of final models.

3 Results

A total of 15,619 limpets were recorded. Within each micro-habitat, the numbers sampled and percentage of each species were as follows: 7,603 limpets within pools (POOL: 54% *P. ulyssiponensis*, 32% *P. depressa*, 14% *S. pectinata*, 0.1% *P. vulgata*, and 0.6% of non-identified juvenile patellids); 3,472 limpets around pool edges (EDGE: 58% *P. depressa*, 35% *P. ulyssiponensis*, 6% *S. pectinata*, and 1% *P. vulgata*); 3,771 limpets on open-rock surfaces near to pools (NEAR: 79% *P. depressa*, 12% *P. ulyssiponensis*, 6% *S. pectinata*, 2% *P. rustica*, and 1% *P. vulgata*) and 773 limpets on mid-shore open-rock surfaces further away from pools (FAR: 95% *P. depressa*, 4% *S. pectinata*, and 1% *P. rustica*). *Patella ulyssiponensis*, *P. depressa*, and *S. pectinata* were present across POOL, EDGE, and NEAR on all shores (Figures 2–4), while the two other patellids were rare (Supplementary Figures S1, S2).

3.1 Horizontal variation in the abundance and size structure of limpets

3.1.1 *Patella ulyssiponensis*

On every shore, there was a consistent pattern of decreasing abundance of this species from pools to further away: the total mean density of *P. ulyssiponensis* was 10 times higher in POOL compared to EDGE, four times higher in EDGE compared to NEAR, and null within FAR (Figure 2). Consistently on all shores, nearly 90% of the total mean density within pools corresponded to PU ≤ 1 cm (Figure 2, POOL). Within the surrounding micro-

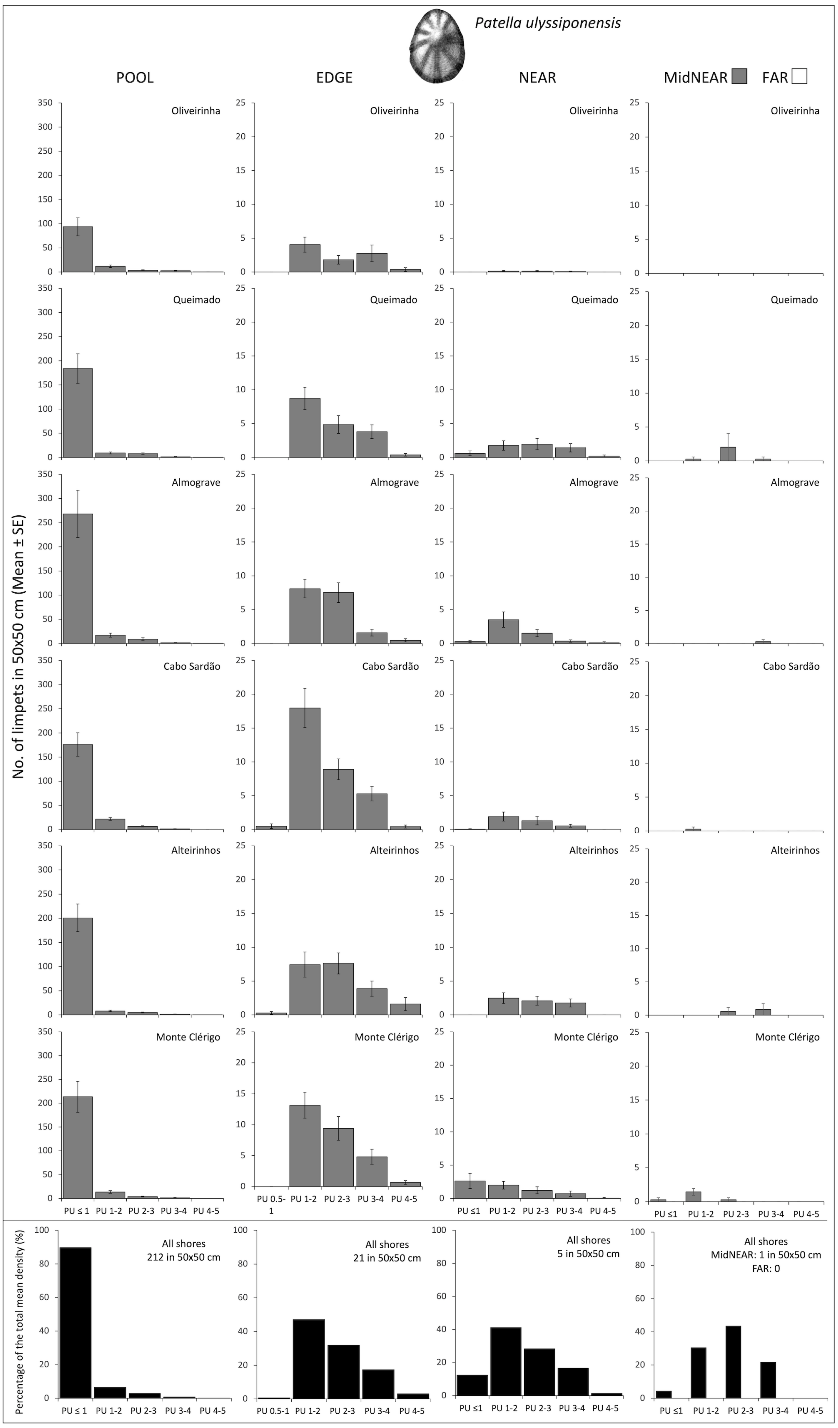


FIGURE 2

Densities and size-class structure of *Patella ulyssiponensis* on six shores within POOL (inside rockpools), EDGE (edge of rockpools), and NEAR (open-rock surrounding rockpools) micro-habitats (three left columns); and within areas of mid-shore open-rock at two proximity categories from rockpools (MidNEAR and FAR, far right column). For POOL, EDGE, and NEAR, the sample size in each shore was the following: 52 in Oliveirinha, 51 in Queimado, 57 in Almograve, 56 in Cabo Sardão, 53 in Alteirinhos, and 54 in Monte Clérigo. For MidNEAR and FAR, n = 12 on each shore. Gray bars are mean (± SE) number of limpets of five size classes per 50×50 cm. Note different y-axis scale between graphs of POOL vs. others. Black bars (bottom row) are percentages of every size class relatively to the total density of this species (value given top right of each graph) within each micro-habitat and across all shores. NB *P. ulyssiponensis* was absent in FAR.

habitats, the modal size class was PU 1–2 cm on most shores (Figure 2, EDGE and NEAR). This species was present on mid-shore open-rock areas near pools on five shores in very low abundances (the highest total mean density of 3 per 50 × 50 cm was recorded in Queimado) with variable size structure among shores (Figure 2, MidNEAR).

Population size structure and density within pools did not differ among shores or stretches within each shore (Table 2, PU within

TABLE 2 Two-way PERMANOVA on the spatial variation (among shores and stretches) of size-class densities of a species (*Patella ulyssiponensis*—PU, *P. depressa*—PD and *Siphonaria pectinata*—SP) within POOL (inside rockpools), EDGE (in the edge of rockpools) and NEAR (on the open-rock surrounding rockpools) micro-habitats.

PU within POOL		df	MS	Pseudo-F	p-value	permutations
	Sh	5	16.02	2.49	ns	998
	St(Sh)	6	1.12	1.12	ns	998
	Residual	311	5.7			
PU within EDGE		df	MS	Pseudo-F	p-value	permutations
	Sh	5	52.72	1.28	ns	999
	St(Sh)	6	42.57	3.37	**	997
	Residual	311	12.59			
	Pair-wise	Stretch A ≠ Stretch B in Alteirinhos and Monte Clérigo.				
PU within NEAR		df	MS	Pseudo-F	p-value	permutations
	Sh	5	10.34	0.64	ns	998
	St(Sh)	6	16.09	4.08	**	998
	Residual	311	3.94			
	Pair-wise	Stretch A ≠ Stretch B in four shores (all except Oliveirinha and Cabo Sardão)				
PD within POOL		df	MS	Pseudo-F	p-value	permutations
	Sh	5	5.34	0.35	ns	999
	St(Sh)	6	14.89	3.13	**	999
	Residual	311	4.75			
	Pair-wise	Stretch A ≠ Stretch B in Alteirinhos and Monte Clérigo.				
PD within EDGE		df	MS	Pseudo-F	p-value	permutations
	Sh	5	112.63	1.08	ns	999
	St(Sh)	6	103.99	4.85	**	998
	Residual	311	21.43			
	Pair-Wise	Stretch A ≠ Stretch B in four shores (all except Oliveirinha and Almograve)				
PD within NEAR		df	MS	Pseudo-F	p-value	permutations
	Sh	5	157.82	3.83	**	999
	St(Sh)	6	41.15	3.15	**	999
	Residual	311	13.05			
	Pair-wise	Cabo Sardão ≠ Oliveirinha, Queimado and Alteirinhos Stretch A ≠ Stretch B in Oliveirinha and Almograve				
SP within POOL		df	MS	Pseudo-F	p-value	permutations
	Sh	5	9.51	1.59	ns	997
	St(Sh)	6	5.98	1.71	ns	999
	Residual	311	3.48			
SP within EDGE		df	MS	Pseudo-F	p-value	permutations
	Sh	5	20.59	5.18	*	999
	St(Sh)	6	3.97	0.9	ns	997
	Residual	311	4.4			
	Pair-wise	Monte Clérigo ≠ Almograve, Cabo Sardão and Alteirinhos				

(Continued)

TABLE 2 Continued

PU within POOL		df	MS	Pseudo-F	p-value	permutations
SP within NEAR		df	MS	Pseudo-F	p-value	permutations
	Sh	5	12.77	1.72	ns	998
	St(Sh)	6	7.39	4.41	**	998
	Residual	311	1.67			
	Pair-wise	Stretch A ≠ Stretch B in Monte Clérigo and Queimado				

Shore: Sh (fixed factor with 6 levels—Oliveirinha, Queimado, Almograve, Cabo Sardão, Alteirinhos and Monte Clérigo); Stretch: St(Sh) (random factor with 2 levels and nested within shore—stretches A and B); n varied between 25 and 30 rockpools per stretch. Fourth-root transformed data for POOL; square-root transformed data for EDGE and NEAR. Analyses based on Euclidean distances. **p < 0.01; *p < 0.05; ns, non-significant. Pairwise "≠": factor levels with significant differences.

POOL). Within the surrounding micro-habitats, differences were only found between stretches (Table 2, PU within EDGE and NEAR). Correlations of the total density of *P. ulyssiponensis* between adjacent micro-habitats were all positively significant between POOL and EDGE (r_s = 0.495, p<0.001), between EDGE and NEAR (r_s = 0.423, p<0.001), and between POOL and NEAR (r_s = 0.136, p=0.014).

3.1.2 *Patella depressa*

On every shore, the abundance of this species was consistently higher inside rockpools compared to the surrounding micro-habitats: the total mean density of *P. depressa* in POOL was three times higher than in EDGE and five times higher than in NEAR (Figure 3). Inside pools and consistently on all shores, more than 90% of the total mean density corresponded to juveniles (PD ≤ 1cm) (Figure 3, POOL). The modal size class along pool edges was PD 2–3 cm on all shores except Cabo Sardão, where the size structure was dominated by PD 1–2 cm (Figure 3, EDGE). On the open-rock surfaces, the modal size class was either PD 1–2 cm or PD 2–3 cm, the former consistently on the two steeper shores (Almograve and Cabo Sardão) and the latter consistently on the three flatter shores (Figure 3, NEAR, MidNEAR, and FAR).

Population size structure and density inside and around the edge of pools did not differ among shores but were significantly different among stretches within shores (Table 2, PD within POOL and EDGE). On the open-rock near pools, there were significant differences in population size structure and density among shores and among stretches; pair-wise tests detected differences between Cabo Sardão and three other shores (Oliveirinha, Queimado, and Alteirinhos), and between the two stretches of Alteirinhos and Monte Clérigo (Table 2, PD within NEAR). Differences among shores were mostly explained by higher densities of the two smallest size classes (mainly PD 1–2 but also PD ≤ 1) in Cabo Sardão than in the other shores (SIMPER, Figure 3). Cabo Sardão was the shore where the highest abundance was recorded for this species within NEAR (total mean density of 52 limpets per 50×50 cm) (Figure 3). Correlations of the total density of *P. depressa* between adjacent micro-habitats were positively significant between POOL and EDGE (r_s = 0.610, p<0.001) and between EDGE and NEAR (r_s = 0.120, p=0.031), but non-significant between POOL and NEAR (r_s = 0.076, p=0.173).

Regarding population size structure and density of *P. depressa* on mid-shore open-rock surfaces at different proximity to rockpools, a significant interaction was found between factors Proximity and Shore, and significant differences were found among stretches at Oliveirinha and Alteirinhos (Table 3). Pair-wise tests on Proximity detected no differences between mid-shores surfaces close to and far from pools on any shore (Table 3, MidNEAR = FAR). Pair-wise tests on Shore revealed a different among-shore pattern for the two categories of Proximity. For mid-shores surfaces near to pools, the most evident pattern of variation was between Cabo Sardão and all shores other than Almograve (Table 3, MidNEAR); this was due to a higher density of PD 1–2 cm and PD ≤ 1 cm in Cabo Sardão (SIMPER, Figures 3, 5A); Cabo Sardão was the shore with the lowest relative proportion of open-rock (Figure 5B) (20% for the average of the two stretches, Supplementary Figure S3). For mid-shores surfaces far from pools, significant differences were found between the three flatter shores and the group of two steeper and one intermediate shores (Table 3, FAR); this reflected a higher density of PD 1–2 cm and PD ≤ 1 cm in the group formed by steeper and intermediate shores (Almograve, Cabo Sardão, and Alteirinhos) (SIMPER, Figures 3, 5C); these shores had more crevices and less open-rock (Figure 5D), their relative proportions of crevices (33%) and open-rock (23%) being four times higher and two times lower on average compared to the ones recorded in flatter shores (8% and 49%, respectively) (Supplementary Figure S3).

3.1.3 *Patella vulgata*

This species (Supplementary Figure S1) did not occur at Queimado, and only one individual was recorded at Oliveirinha. It was consistently found in very low densities across the other four shores—the highest total mean density of three limpets per 50×50 cm was recorded inside pools in Alteirinhos. The presence of *P. vulgata* was most consistently found: (i) along pool edges, compared to the other micro-habitats and (ii) in Cabo Sardão, compared to the other shores. Inside pools (POOL), *P. vulgata* individuals were either juveniles (PV ≤ 1cm) or small-sized (PV 1–2 cm). Within the surrounding micro-habitats (EDGE and NEAR), the commonest size classes were PV 1–2 cm and PV 2–3 cm. Within open-rock far from pools (FAR), only PV ≤ 1cm or PV 2–3 cm were recorded.

3.1.4 *Patella rustica*

This species (Supplementary Figure S2) was absent from POOL and EDGE and did not occur at Oliveirinha or Queimado, being consistently present on four shores within NEAR in very low

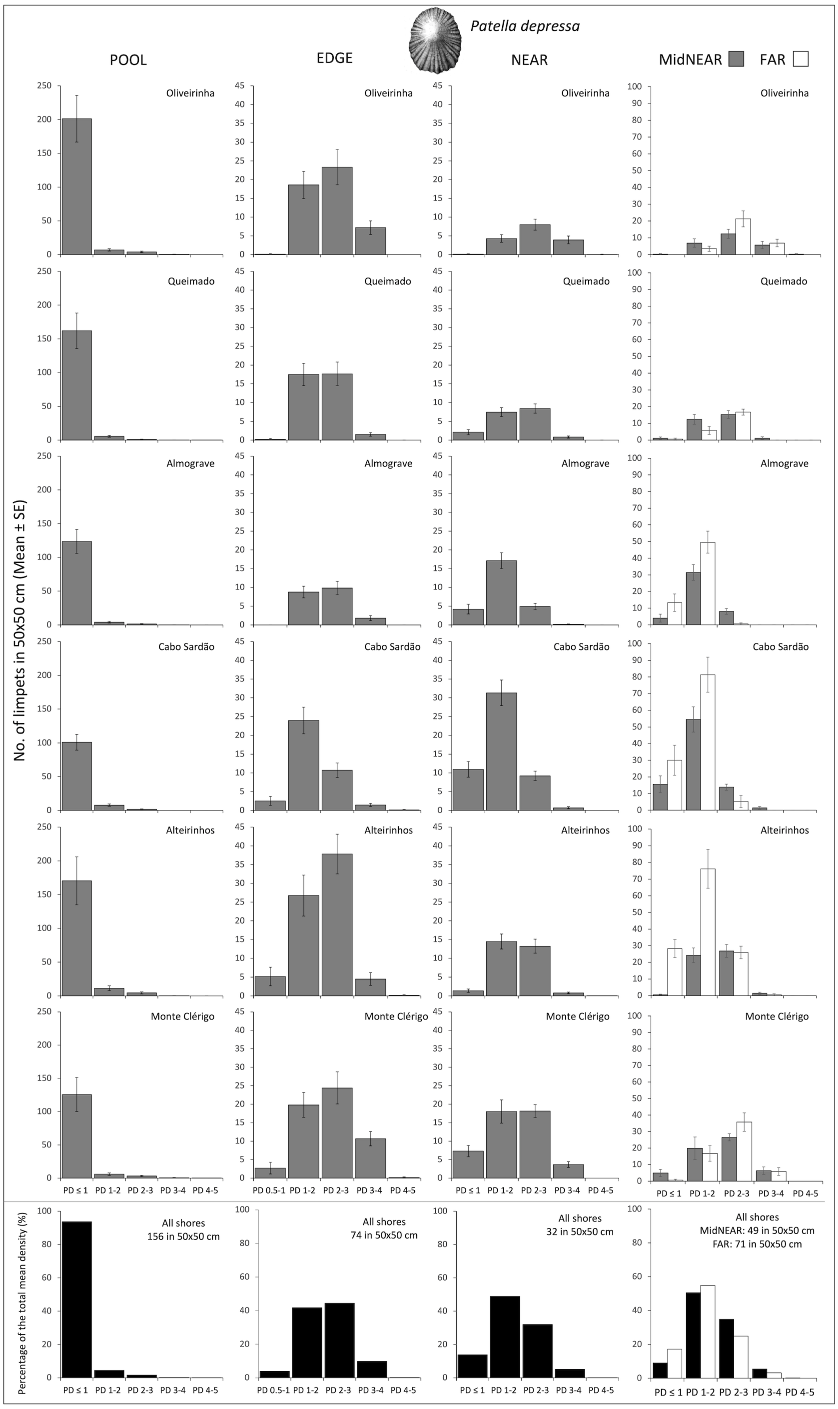


FIGURE 3
Densities and size-class structure of *Patella depressa* on six shores within POOL (inside rockpools), EDGE (edge of rockpools), and NEAR (open-rock surrounding rockpools) micro-habitats (three left columns); and within areas of mid-shore open-rock at two proximity categories from rockpools (MidNEAR and FAR, far right column). Gray and white bars are mean (± SE) number of limpets of five size classes per 50×50 cm. Note different y-axis scale: between graphs of POOL vs. others, and between graphs of MidNEAR and FAR vs. others. Sample size indicated in caption of Figure 1. Black and white bars (bottom row) are percentages of every size class relatively to the total density of this species (value given top right of each graph) within each micro-habitat and across all shores.

densities—the highest total mean density of four limpets per 50×50 cm was recorded in Almograve—and variable size-structure—the modal size class was either PR 1–2 cm or PR 2–3 cm, depending on the shore. Only a few sampled individuals occurred on the mid-shore open-rock of the two steeper shores: Almograve (both within MidNEAR and FAR) and Cabo Sardão (only within FAR).

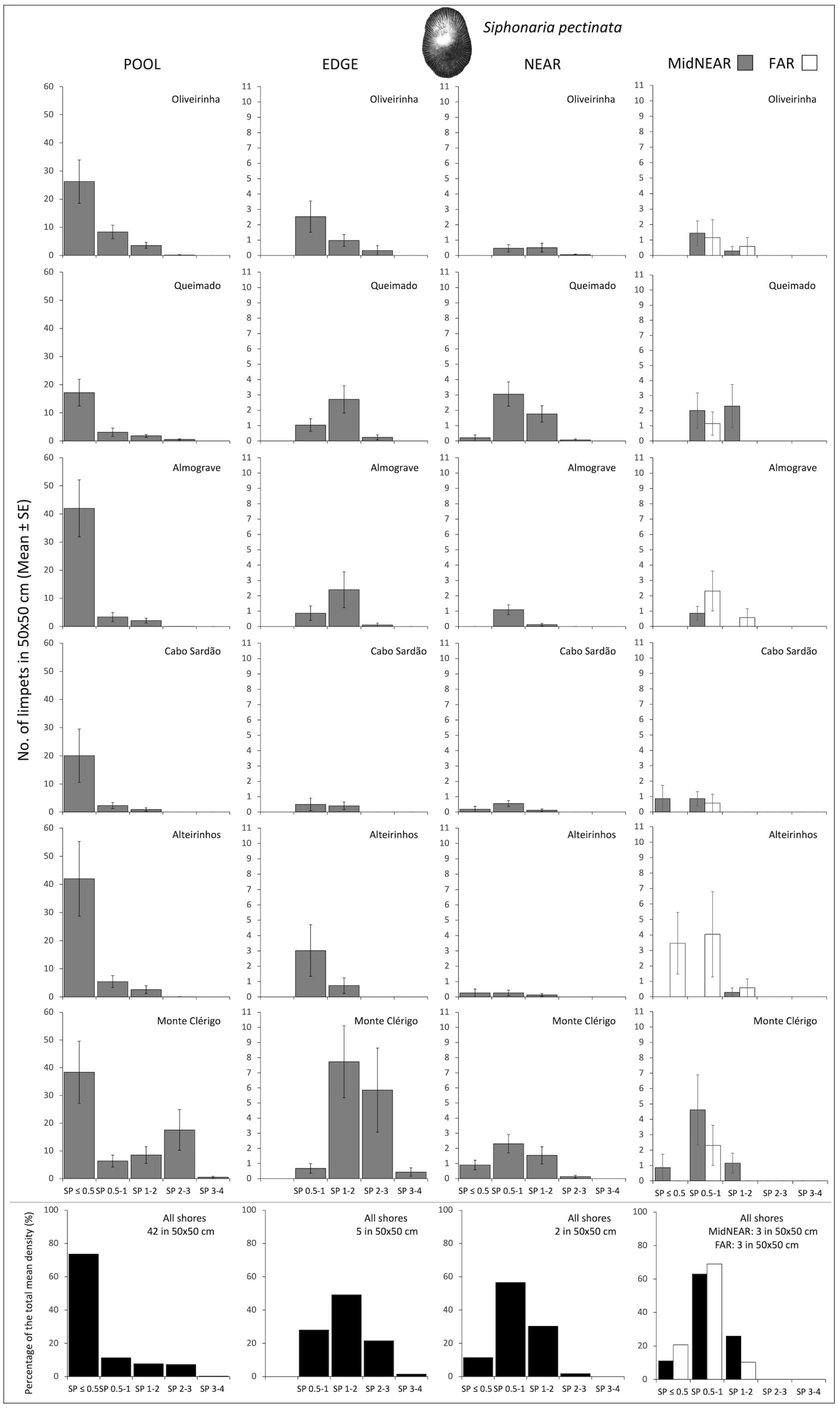


FIGURE 4
Densities and size-class structure of *Siphonaria pectinata* on six shores within POOL (inside rockpools), EDGE (edge of rockpools), and NEAR (open-rock surrounding rockpools) micro-habitats (three left columns); and within areas of mid-shore open-rock at two proximity categories from rockpools (MidNEAR and FAR, far right column). Gray and white bars are mean (± SE) number of limpets of five size classes per 50×50 cm. Note different y-axis scale between graphs of POOL vs. others; sample size indicated in caption of Figure 1. Black and white bars (bottom row) are percentages of every size class relatively to the total density of this species (value given top right of each graph) within each micro-habitat and across all shores.

3.1.5 *Siphonaria pectinata*

On every shore, this species was consistently more abundant inside rockpools compared to the surrounding micro-habitats: on average, the total mean density of *S. pectinata* in POOL was eight times higher than in EDGE and 19 times higher than in NEAR (Figure 4). Inside pools, the size structure of *S. pectinata* was dominated by juveniles (SP ≤ 0.5cm) on all shores (74% on average), although a comparatively lower proportion of juveniles (54%) and exceptionally higher densities of the three larger size classes were found in Monte Clérigo (Figure 4). Within pool

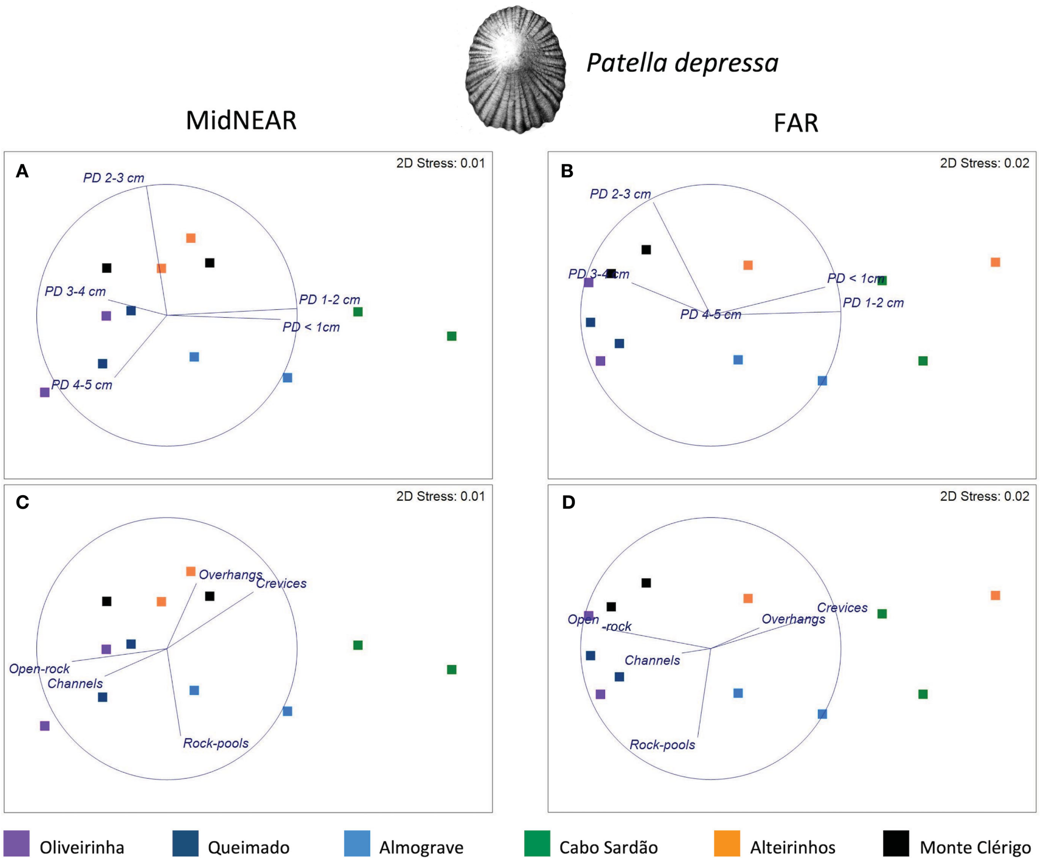


FIGURE 5
MDS plots for *Patella depressa* within areas of mid-shore open-rock at two proximity categories from rockpools: MidNEAR **(A, B)** and FAR **(C, D)**. Overlaid vectors of the response variables, i.e., densities of five size classes **(A, C)**; overlaid vectors of predictors of landscape variability, i.e., proportions of open-rock and topographic micro-habitats assessed on the mid-shore of two coastal stretches on every shore **(B, D)**.

surrounding areas, the modal size class depended on the shore, being either SP 0.5–1 cm, SP 1–2 cm, or equal proportions of these two size classes (Figure 4, EDGE, NEAR, and MidNEAR). Away from pools, the modal size class was SP 0.5–1 cm on all shores (Figure 4, FAR). Monte Clérigo had the highest abundance of *S. pectinata* within all micro-habitats (POOL, EDGE, and NEAR) and mid-shore categories (MidNEAR and FAR) (Figure 4). However, no significant differences were found among shores or stretches within each shore in population size structure and density within pools (Table 2, SP within POOL). Within pool edges, differences in population size structure and density were significant among shores and not among stretches, with Monte Clérigo differing significantly from Almograve, Cabo Sardão, and Alteirinhos (Table 2, SP within EDGE). Differences between Monte Clérigo and the other shores were mostly explained by higher densities of SP 1–2 cm and SP 2–3 cm within rockpool edges of Monte Clérigo (SIMPER, Figure 4). Moreover, the highest total mean density within EDGE (15 individuals per 50×50 cm) was at Monte Clérigo (Figure 4). On open-rock near pools, only differences among stretches were significant (Table 2, SP within NEAR). Correlations of the total density of *S. pectinata* between adjacent micro-habitats were positively significant between POOL and EDGE (r_s = 0.455, p<0.001) and between EDGE and NEAR (r_s = 0.214, p<0.001) but non-significant between POOL and NEAR (r_s = 0.066, p=0.236). Population size structure and density of *S. pectinata* on mid-shore open-rock did not differ with respect to proximity to rockpools, or among shores or stretches (Table 3).

3.2 Vertical distribution of limpet species inside and outside pools

The complete range of shore levels was not present on all shores: it was not possible to sample areas at <1.5 m above C.D. at Almograve; the two highest shore levels (>2.5 m above C.D) were absent at Monte Clérigo (Figure 6). Moreover, the total number of rockpool systems sampled at the five shore levels differed among shores (N.B. sample sizes in Figure 6 caption).

3.2.1 *Patella ulyssiponensis*

The highest abundance inside rockpools was consistently recorded at the second lowest interval of shore height (1.5–2 m above C.D.), decreasing progressively at higher shore levels. This was observed on all shores except Monte Clérigo, where a slightly

TABLE 3 Three-way PERMANOVA comparing size-class densities of *P. depressa* (PD) and *Siphonaria pectinata* (SP) between mid-shore open-rock areas located at two proximity categories to rockpools, i.e., adjacent to rockpools (MidNEAR) and located at least 25 cm away from any rockpool (FAR), and also among shores and stretches within shores.

PD MidNEAR vs. FAR	df	MS	Pseudo-F	p-value	Permutations
Pr	1	64.03	4.53	*	997
Sh	5	286.62	9.18	**	951
St(Sh)	6	31.19	3.11	**	999
Pr × Sh	5	63.48	4.49	**	999
Pr × St(Sh)	6	14.11	1.41	ns	997
Residual	120	10.01			
Pair-wise	Within each shore: MidNear = FAR for all shores Within MidNear: Cabo Sardão ≠ all shores except Almograve, and Almograve ≠ Monte Clérigo				
	Within FAR: (*Oliveirinha* = Queimado = Monte Clérigo) ≠ (Cabo Sardão, Almograve and Alteirinhos)				
	Stretch A ≠ Stretch B in Oliveirinha and Alteirinhos				
SP MidNEAR vs. FAR	**df**	**MS**	**Pseudo-F**	**p-value**	**Permutations**
Pr	1	8.32	0.23	ns	999
Sh	5	24.24	0.68	ns	234
St(Sh)	6	35.8	1.53	ns	999
Pr × Sh	5	36.5	1.04	ns	999
Pr × St(Sh)	6	35.13	1.5	ns	999
Residual	120	23.34			

PD, *Patella depressa*; SP, *Siphonaria pectinata*; Proximity, Pr (fixed factor with 2 levels, MidNear and FAR); Shore, Sh (fixed factor orthogonal with Pr and with 6 levels – Oliveirinha, Queimado, Almograve, Cabo Sardão, Alteirinhos and Monte Clérigo); Stretch, St(Sh) (random factor with 2 levels and nested within Shore— stretches A and B); n = 6 per stretch; Analyses based on Euclidean distances of square-root transformed data. **$p < 0.01$; *$p < 0.05$; ns: non-significant. Pairwise "≠" or "=": factor levels with or without significant differences, respectively.

higher abundance was found on the upper mid shore (2–2.5 m) (Figure 6, POOL). The highest abundance of this species in the open-rock surrounding rockpools was always recorded at the lowest shore level available on each of the six shores, declining upshore to complete absence (in Oliveirinha and Queimado), or sharply on the other four shores (Figure 6, NEAR). A similar pattern was found for rockpool edges, but with a less-abrupt decrease in abundance with increasing shore height (Figure 6, EDGE).

3.2.2 *Patella depressa*

Within every micro-habitat, this species was generally most abundant at mid-shore levels; the exception was Monte Clérigo, where abundances of *P. depressa* within all the three micro-habitats were similar across all three shore levels (Figure 6). On the other five shores within pools and their edges, very low densities or complete absence were found at the lowest shore level, with most individuals occurring from 1.5 to 3 m above C.D. at Oliveirinha and Queimado and higher than 2 m above C.D. at Almograve, Cabo Sardão, and Alteirinhos (Figure 6, POOL and EDGE). Outside rockpools, *P. depressa* was almost absent for the two higher shore levels at Oliveirinha and Queimado, while it was present across all shore levels at Almograve, Cabo Sardão, and Alteirinhos; the highest abundances were found at the 1.5–2 m level on three shores (Oliveirinha, Queimado, and Alteirinhos) and from 2 to 3 m above C.D. at Almograve and Cabo Sardão (Figure 6, NEAR).

3.2.3 *Patella vulgata*

There were no clear vertical patterns in this low-density range-edge species; where present, it occurred mostly on pool edges at various heights on different shores (Figure 6).

3.2.4 *Patella rustica*

This species was most abundant at the highest shore levels, being usually found on the steeper shores (Almograve and Cabo Sardão) (Figure 6).

3.2.5 *Siphonaria pectinata*

The vertical distribution patterns of this species were the most variable among shores; the highest abundances within pools were found on the mid-shore and/or upper shore (Figure 6, POOL). On all shores except Monte Clérigo, a consistent absence or low abundance both inside and in the edge of pools was found at the two lower shore levels (Figure 6, POOL and EDGE). Inside pools, it was found at high shore levels, being most abundant at 2.5–3 m at Oliveirinha, Almograve, Cabo Sardão, and Alteirinhos; the distribution inside pools at Queimado was vertically discontinuous as mostly restricted to two separate levels (2–2.5 m and >3 m), with maximum abundance at the highest level (Figure 6, POOL). On pool edges, it was most abundant at mid-tidal heights in Oliveirinha and Queimado (1.5–3 m above C.D.), while only being found at higher levels (>2.5 m above C.D.) in Almograve, Cabo

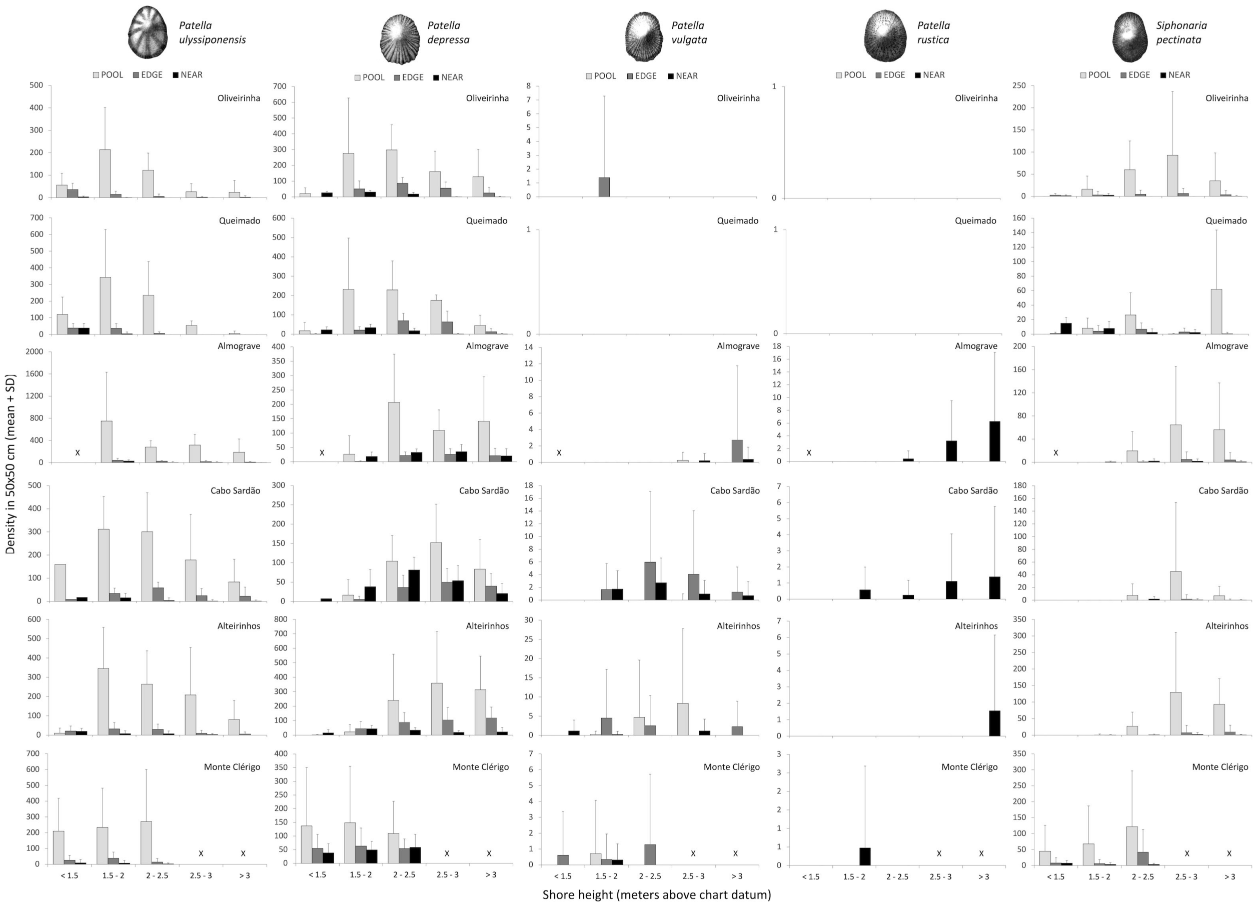


FIGURE 6
Total density of five limpet species along the vertical intertidal gradient of six shores within POOL (inside rockpools, light grey), EDGE (edge of rockpools, dark grey), and NEAR (open-rock surrounding rockpools, black) micro-habitats. Values are mean (± SD) number of limpets per 50×50 cm at five intervals of shore height; note different y-axis scale among shores and among species. Variable sample size among intervals of shore height on different shores—for the intervals <1.5, 1.5–2, 2–25, 2.5–3 > 3 m above chart datum, respectively: 3, 18, 11, 6, and 14 in Oliveirinha; 6, 15, 18, 3, and 9 in Queimado; 0, 6, 8, 16, and 27 in Almograve; 1, 6, 14, 25, and 10 in Cabo Sardão; 6, 16, 10, 12, and 9 in Alteirinhos; 20, 22, 12, 0, and 0 in Monte Clérigo. The symbol “X” means not available shore level.

Sardão, and Alteirinhos (Figure 6, EDGE). Outside rockpools, it was mostly found on the lower shore levels in Oliveirinha and Queimado, and at mid-tidal levels (from 2 to 3 m above C.D.) at Almograve, Cabo Sardão, and Alteirinhos (Figure 6, NEAR). In Monte Clérigo, the highest abundances within both pools and pool edges were found at the highest level, whereas the highest abundance on open-rock was at the lowest level (Figure 6).

Our hypothesis, that inside rockpools (POOL) each of the most common species (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*) would occur higher up the shore than on open-rock (NEAR), was generally supported (Figure 7; Table 4). This pattern was consistent across most of the 12 sampled coastal stretches, both when considering extreme (minimum and maximum) or central-location (median and mean) values of shore height (Table 4; Figures 7A). There was an exception: the lowest shore height where the presence of *P. ulyssiponensis* was recorded in each stretch did not differ between pools and open-rock (Table 4). Compared to open-rock, the median shore height inside rockpools was extended upwards in 0.6 m for *P. ulyssiponensis*, 0.2 m for *P. depressa*, and 0.7 m for *S. pectinata* (Figures 7B).

3.3 Patterns among highly variable rockpool systems

The different sets of predictors assessed for all 323 rockpool systems and used in DistLM analyses reflected the great variability among rockpools present on all shores, encompassing cover of different substrata or sessile organisms (Supplementary Figures S4, S5), density of trochids and littorinids (Supplementary Figure S6), physical characteristics (Supplementary Figure S7), and total density of each co-occurring limpet species (Figures 2–4).

3.3.1 *Patella ulyssiponensis*

The models retrieved by DistLM analyses for *P. ulyssiponensis* inside rockpools (PU POOL) and on the open-rock surrounding rockpools (PU NEAR) explained identical proportions of the variability among replicates (44%), higher than the model regarding this species in the edge of rockpools (PU EDGE, 34%) (Table 5).

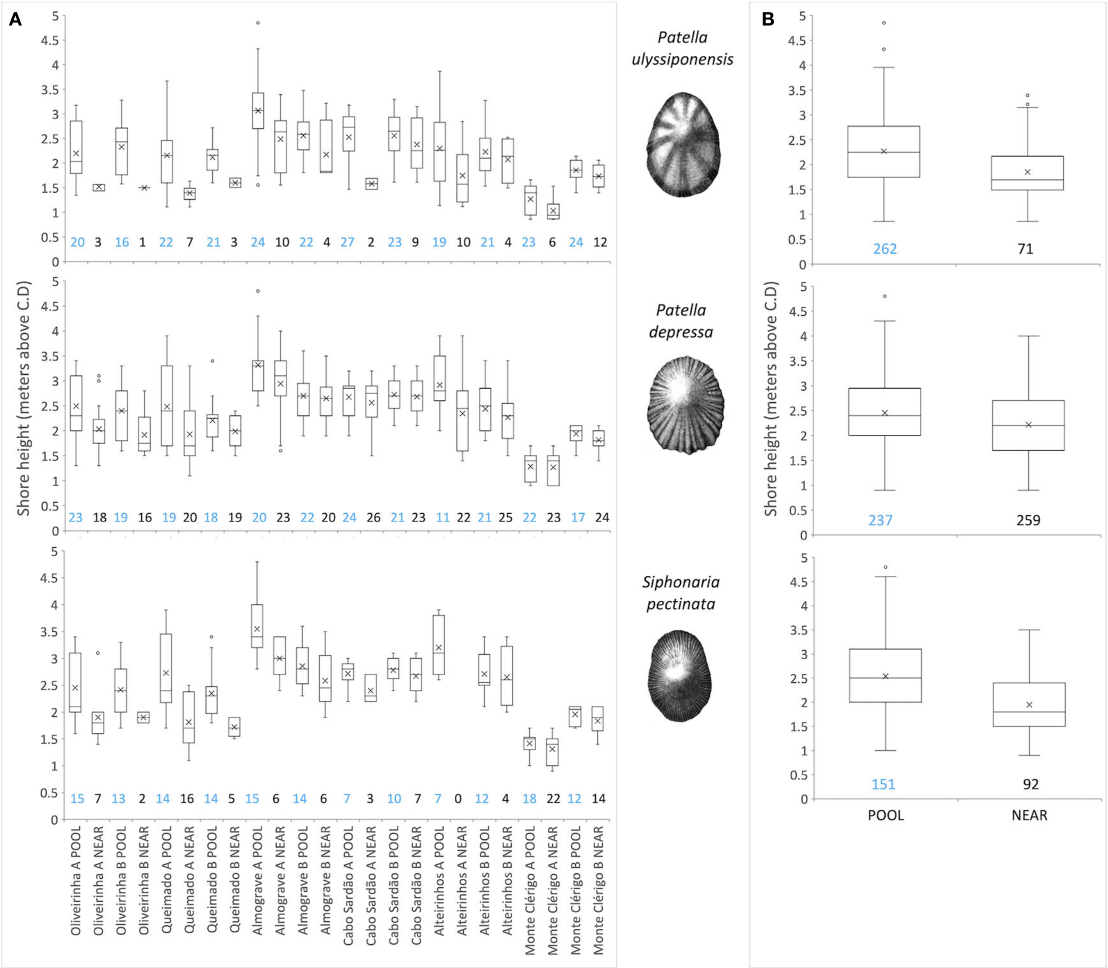


FIGURE 7
Vertical distribution of three species of limpets inside (POOL) and outside (NEAR) rockpools. Values are shore-height records (meters above chart datum) of all rockpools (POOL) or open-rock surfaces (NEAR) where each species was present (from a total of 323 sampled rockpools and surrounding open-rock). **(A)** Data for each micro-habitat on each of 12 coastal stretches (stretch A and B sampled within each of six shores). **(B)** Data for each micro-habitat across all shores and stretches. Boxplots show interquartile range (IQR—the first quartile Q1 to the third quartile Q3; box), median (horizontal line), mean (cross symbol), "minimum" (Q1 – 1.5 × IQR) and "maximum"(Q3 + 1.5 × IQR) (whiskers) and outliers (dots) of standardized shore height. Numbers below boxplots correspond to the respective number of rockpools (blue, POOL) and open-rock surfaces (black, NEAR).

In both PU POOL and PU NEAR models, habitat-composition variables were the most important group of "top-predictors" for structuring *P. ulyssiponensis* size-class densities (40% and 39%, respectively, Table 5). Two habitat-composition variables estimated as percentage cover were selected in common for these models (Table 5): articulated coralline algae (the first selected variable in both models, contributed alone to explain 13% and 30% of the variation inside and outside rockpools, respectively) and CCA (explained 5% and 2% of the variation inside and outside rockpools, respectively). Both were positively associated with overall abundance of this species within both micro-habitats: articulated coralline algae was mostly related with density of *P. ulyssiponensis* juveniles (PU ≤ 1cm) inside rockpools and with densities of *P. ulyssiponensis* adults (all size classes except PU ≤ 1 cm) outside rockpools; whereas CCA was mostly related with *P. ulyssiponensis* adults inside and outside rockpools (Figures 8A, C). Other habitat-composition predictors were selected exclusively for the PU POOL model: the cover of sand (also including detritus and cobbles in minor proportions, Supplementary Table S2) was negatively related with densities of *P. ulyssiponensis* adults, whereas both the cover of mussels and sea urchins were positively related with densities of *P. ulyssiponensis*, particularly juveniles (Figure 8A). Both the cover of sea anemones and barnacles were selected exclusively for the PU NEAR model (Table 5), both positively related with density of *P. ulyssiponensis* juveniles outside rockpools and responsible for the segregation of a few samples (squares in the bottom part of the db-RDA plot, Figure 8C).

The group of physical variables was also important for PU POOL and PU NEAR (adding another 5% of explanation to the models), while connectivity variables were not selected for these models (Table 5). Shore height was selected as a "top predictor" for both models (Table 5), with a general negative association found between this predictor and *P. ulyssiponensis* abundance inside and outside rockpools, particularly negatively related with density of

TABLE 4 Binomial tests comparing the vertical distribution of three species of intertidal limpets between micro-habitats inside (POOL) and outside (NEAR) rockpools.

	Shore-height comparisons between POOL and NEAR			
Species	Median	Mean	Maximum	Minimum
PU	**0.003** (11/12)	**0.0002** (12/12)	**0.003** (11/12)	0.09 (5/6)
PD	**0.001** (10/10)	**0.0002** (12/12)	**0.01** (7/7)	**0.004** (8/8)
SP	**0.02** (8/9)	**0.0005** (11/11)	**0.004** (8/8)	**0.01** (9/10)

PU, *Patella ulyssiponensis*; PD, *Patella depressa*; SP, *Siphonaria pectinata*.

Comparisons of the median, mean, maximum, and minimum of shore height (meters above Chart datum) where the presence of each species was recorded within each micro-habitat; differences in these values between POOL and NEAR were calculated for every sampled stretch of coast (12 stretches, corresponding to two stretches within each of six shores). Values are the probability (significant p-values in bold) and the ratio of the number of cases with positive POOL-NEAR differences to the total number of cases for which differences were different than zero (in parenthesis).

juveniles inside rockpools and with densities of adults outside rockpools (Figures 8A, C). Outside rockpools, distance to low-water mark was also negatively related with densities of *P. ulyssiponensis* adults (Figure 8C).

In the PU EDGE model, the most prominent predictor was a connectivity variable, which alone explained 22% of the model variation: the total density of *P. depressa* on the surrounding open-rock (PD_adjacent_Near) (Table 5). This variable was positively associated with densities of *P. ulyssiponensis* of the smallest size classes (PU 0.5–1 cm, PU 1–2 cm) around rockpool edges (Figure 8, middle). The other variables responsible for the spatial pattern of *P. ulyssiponensis* at rockpool edges were distance to low-water mark (higher shore heights had lower overall abundance of *P. ulyssiponensis*), shore height, and the abundance of the congener *P. depressa* (PD) (both negatively associated with the abundance of larger *P. ulyssiponensis*) (Table 5; Figure 8B).

3.3.2 *Patella depressa*

Both DistLM models produced for *P. depressa* inside rockpools (PD POOL) and on the open-rock surrounding rockpools (PD NEAR) explained 47% of variation among rockpool systems, while the model regarding the EDGE explained 26% of the variability (Table 5).

The group of habitat-composition predictors was the most important for explaining *P. depressa* size-class densities on both PD POOL and PD NEAR models (43% and 25%, respectively); the cover of CCA and mussels contributed to more than 20% of the variation inside and outside rockpools respectively (Table 5). These predictors greatly influenced spatial structuring within each micro-habitat (samples along the horizontal axis db-RDA1), with CCA positively associated with overall abundance of *P. depressa* inside rockpools and mussels positively associated with overall abundance of *P. depressa* outside rockpools (Figures 9A, C). The other habitat-composition variables that also explained variation inside rockpools were cover of

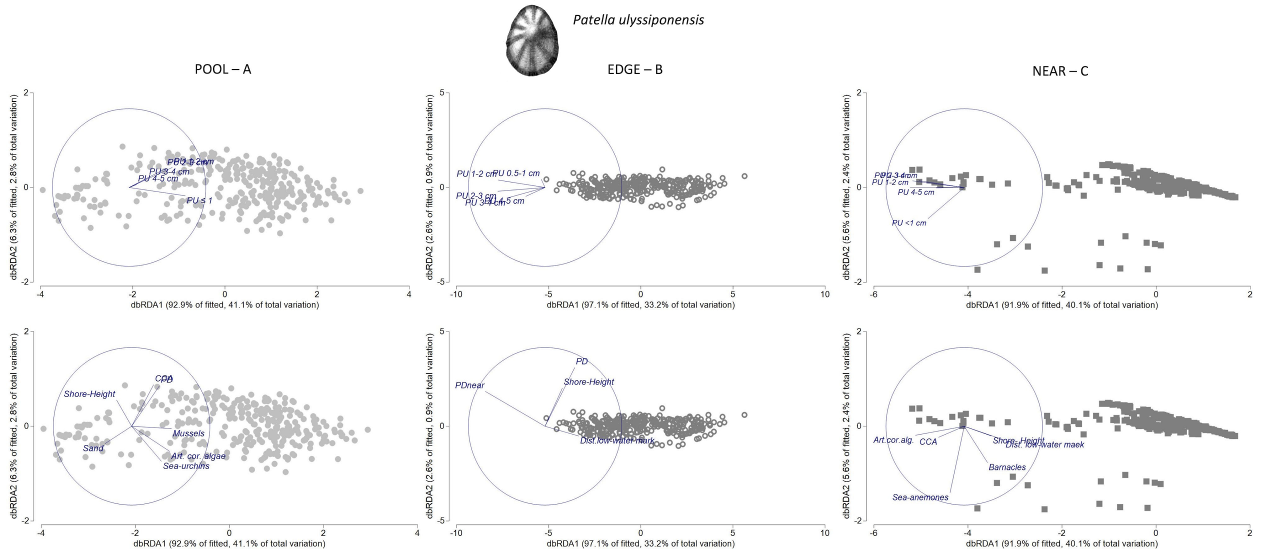


FIGURE 8
Distance-based redundancy analysis (*db-RDA*) plots for the spatial variability in abundance and size structure of *Patella ulyssiponensis* within each of three neighboring micro-habitats: **(A)** POOL (rockpools, gray circles); **(B)** EDGE (edge of rockpools, open circles); **(C)** NEAR (open-rock adjacent to rockpools, gray squares), (n = 323 samples). Vectors on top plots are response variables (size-class densities); vectors on bottom plots are “top predictors” selected by distance-based linear models (DistLM, see Table 5). On the plot in the top left, the superimposed vector labels correspond to: PU 1–2 and PU 2–3. On the plot in the top middle, the superimposed vector labels correspond to: PU 2–3 and PU 3–4.

TABLE 5 Distance based linear models (DistLM) for three species of limpets (*P. ulyssiponensis, PU*; *P. depressa*, PD; *S. pectinata*, *SP*) within three neighboring micro-habitats (POOL, EDGE, and NEAR).

	“BEST model”	Model with “top variables”	Selected variables by order of selection (% of R^2 for cumulative groups of variables)
PU POOL	16 variables: 50% in R^2; AIC: 379.85	**7** variables: **44%** in R^2; AIC: 398.64	1v: **Articulated coralline algae** (13.9%) 2v: 1v + **Sand** (27.3%) 3v: 2v + **CCA** (32.2%) 4v: 3v + **Mussels** (37.9%) 5v: 4v + **Shore height** (40.6%) 6v: 3v + **Sea urchins** + **PD** (42.9%) 7v: 6v + **Shore height** (44.2%) Variables that explained <1% in R^2: Distance to low-water mark, Sea anemones, Perimeter, Other algae, POOL_Topographic-complexity, Circularity, PV_adjacent_Edge, Other sessile invertebrates, Littorinids
	BEST model for 3 indicators of “top variables”		1st group: Habitat-composition (40%) 2nd group: Habitat-composition + Physical (44%)
PU EDGE	7 variables: 36% in R^2; AIC: 717.08	**4** variables: **34%** in R^2; AIC: 721.14	1v: **PD_adjacent_Near** (21.5%) 2v: 1v + **PD** (30.5%) 3v: 2v + **Distance to low-water mark** (32.8%) 4v: 3v + **Shore height** (34.2%) Variables that explained <1% in R^2: Perimeter, PV_adjacent_Near, PD_adjacent_Pool
	BEST model for 3 indicators of “top variables”		1st group: Connectivity (22%) 2nd group: Connectivity + Physical (31%) 3rd group: Connectivity + Physical + Habitat-composition (34%)
PU NEAR	13 variables: 48% in R^2; AIC: 287.5	6 variables: **44%** in R^2; AIC: 296.7	1v: **Articulated coralline algae** (30.0%) 2v: 1v + PD_adjacent_Edge (34.1%) 3v: 2v+ **Sea-anemones** (36.7%) 4v: 1v + **Sea-anemones** + **Distance to low-water mark** + **Barnacles** (39.6%) 5v: 4v + **Shore height** (41.5%) 6v: 5v + **CCA** (43.6%) Variables that explained <1% in R^2: Porifera, PD_adjacent_Edge, Mussels, Surface-area, Perimeter, Mussels, Seaweed, Crustose non-coralline algae
	BEST model for 3 indicators of “top variables”		1st group: Habitat-composition (39%) 2nd group: Habitat-composition + Physical (44%)
PD POOL	14 variables; 50% in R^2; AIC: 322.65	**8** variables: **47%** in R^2; AIC: 327.75	1v: **CCA** (24.5%) 2v: 1v + **Sea urchins** (32.0%) 3v: 2v + **PU** (37.3%) 4v: 3v + **Distance to low-water mark** (40.9%) 5v: 4v + **SP** (42.9%) 6v: 5v + **Articulated coralline algae** (45.0%) 7v: 6v + **Sand** (46.1%) 8v: 7v + **POOL Slope** (47.1%) Variables that explained <1% in R^2: Seaweed, Circularity, PU_Adjacent_Edge, Surface area, Volume, Crustose non-coralline algae, Straight-distance to nearest rockpool
	BEST model for 3 indicators of “top variables”		1st group: Habitat-composition (43%) 2nd group: Habitat-composition + Physical (47%)
PD EDGE	9 variables; 27% in R^2; AIC: 954.78	**7** variables: **26%** in R^2; AIC: 955.22	1v: **PU_adjacent_NEAR** (11.0%) 2v: 1v + **Distance to low-water mark** (13.6%) 3v: 2v + **PU_adjacent_Pool** (18.3%) 4v: 3v + **Shore height** (20.0%) 5v: 4v + **PU** (21.8%) 6v: 5v + **Confinement** (24.8%) 7v: 6v + **Circularity** (26.0%) Variables that explained <1% in R^2: PV Pool, PR Near
	BEST model for 3 indicators of “top variables”		1st group: Connectivity (12%) 2nd group: Connectivity + Physical (24%) 3rd group: Connectivity + Physical + Habitat-composition (26%)
PD NEAR	14 variables: 52% in R^2; AIC: 695.33	**6** variables: **47%** in R^2; AIC: 712.35	1v: **Mussels** (20.9%) 2v: 1v + **Shore** (30.8%) 3v: 1v + **Shore height** + **PU_adjacent_Edge** (33.1%) 4v: 3v + **PU** (38.6%) 5v: 4v + **Shore** (44.3%)

(Continued)

TABLE 5 Continued

	"BEST model"	Model with "top variables"	Selected variables by order of selection (% of R^2 for cumulative groups of variables)
			6v: 5v + **Verrucariaceae** (46.5%) Variables that explained <1% in R^2: Distance to low-water mark, Rock, PR, Other sessile invertebrates, Sea-anemones, SP, CCA, Articulated coralline algae, Barnacles
	BEST model for 3 indicators of "top variables" + Shore		1st group: Habitat-composition (25%) 2nd group: Habitat-composition + Shore (37%) 3rd group: Habitat-composition + Shore + Physical (42%) 4th group: Habitat-composition + Shore + Physical + Connectivity (47%)
SP POOL	9 variables 28% in R^2; AIC: 333.74	**6** variables: **26%** in R^2; AIC: 335.95	1v: **PD** (11.6%) 2v: 1v + **Articulated coralline algae** (16.6%) 3v: 2v + **Confinement** (22.3%) 4v: 3v + **Distance to low-water mark** (23.9%) 5v: 4v + **Shore height** (25.3%) 6v: 5v + **Roundness** (26.3%) Variables that explained <1% in R^2: CCA, Seaweed, Barnacles
	BEST model for 3 indicators of "top variables"		1st group: Habitat-composition (17%) 2nd group: Habitat-composition + Physical (26%)
SP EDGE	6 variables 23% in R^2; AIC: 442.59	**5** variables: **22%** in R^2; AIC: 442.59	1v: **Confinement** (10.9%) 2v: 1v + Distance to low-water mark (14.6%) 3v: 2v + **Shore** (18.7%) 4v: 1v + **Shore** + **PU_adjacent_Near** + **PD_adjacent_Near** (20.8%) 5v: 4v + **PD_adjacent_Pool** (21.9%) Variables that explained <1% in R^2: Contoured-distance to nearest rockpool
	BEST model for 3 indicators of "top variables" + Shore		1st group: Physical (10%) 2nd group: Physical + Connectivity (16%) 3rd group: Physical + Connectivity + Shore (22%)
SP NEAR	5 variables: **15%** in R^2; AIC: 175.68	**5** variables: **15%** in R^2; AIC: 175.68	1v: **Crustose non-coralline algae** (6.3%) 2v: 1v + **Other sessile invertebrates** (9.2%) 3v: 2v + **Shore height** (12.1%) 4v: 3v + **PD** (13.5%) 5v: 4v + **NEAR Slope** (14.5%)
	BEST model for 3 indicators of "top variables"		1st group: Habitat-composition (11%) 2nd group: Habitat-composition + Physical (15%)

PU, *Patella ulyssiponensis*; PD, *Patella depressa*; SP, *Siphonaria pectinata*; nv, a model with n number of variables.
Green text refers to habitat-composition, brown text to physical, and blue text to connectivity variables.
Habitat-composition variables: related with benthic assemblages and substrata, including other limpet species co-existing in the same micro-habitat. Physical variables: related with physical characteristics of rockpools and surrounding rock. Connectivity variables: the total density of a co-occurring limpet species in an adjacent micro-habitat; coded by the abbreviation of species name followed by "_adjacent_" and the micro-habitat code (Pool; Edge; Near).
Selection with BEST procedure and AIC criterion. Selected "top predictors" are shown in bold—predictive-variables selected in the "BEST model" as the ones that most contributed to explain total variation and for which inclusion in the model added a minimum of 1% in R^2. Results for a second DistLM model—considering indicator groups of "top-predictors" related to the objectives (habitat-composition, physical, and connectivity predictors, and shore, see Supplementary Tables S1 and S2)—are presented in the last line of each species/micro-habitat. Note that the habitat-composition group of EDGE models did not include cover and density of other species besides limpets.

sea urchins—negative relationship with juveniles (PD ≤ 1cm); total density of *P. ulyssiponensis* within the same micro-habitat (PU) and cover of articulated coralline algae—negative relationships with the largest size class (PD 4–5 cm); total density of *S. pectinata* within the same micro-habitat (SP)—positive relation with *P. depressa* adults; and cover of sand—negative relation with *P. depressa* adults (Table 5; Figure 9A). Outside rockpools, besides the above-mentioned positive association with mussels, Verrucariaceae cover was negatively associated with density of small-sized *P. depressa* (PD 1–2 cm), and the total density of *P. ulyssiponensis* within the same microhabitat (PU) was negatively associated with density of large-sized *P. depressa* (PD 2–3 cm) (Figure 9C).

The group of physical predictors was also important for the spatial patterns of both PD POOL and PD NEAR (adding 4% and 5% of explanation to these models, respectively). Distance to low-water mark (included on both models) and/or shore height (included on PD NEAR model) were the physical variables most important for explaining the variation in *P. depressa* among samples within each micro-habitat (Table 5). Inside rockpools, distance to the low-water mark was positively associated with densities of mid- and large-sized *P. depressa* (PD 2–3 cm, and PD 3–4cm) (Figure 9, top). Outside rockpools, shore height was negatively associated with density of PD 3–4 cm (Figure 9C). An additional physical variable, the slope of the rockpool bottom, was negatively associated with PD ≤ 1cm inside rockpools (Figure 9A).

While no connectivity variable was selected for the PD POOL model (Table 5), the total density of *P. ulyssiponensis* in rockpool edges (PU_adjacent_Edge) was selected for the PD NEAR model, being positively associated with density of PD 1–2 cm outside rockpools (Figure 9, bottom). Furthermore, shore was included as a factor in the PD NEAR model (contributing to 12% of total

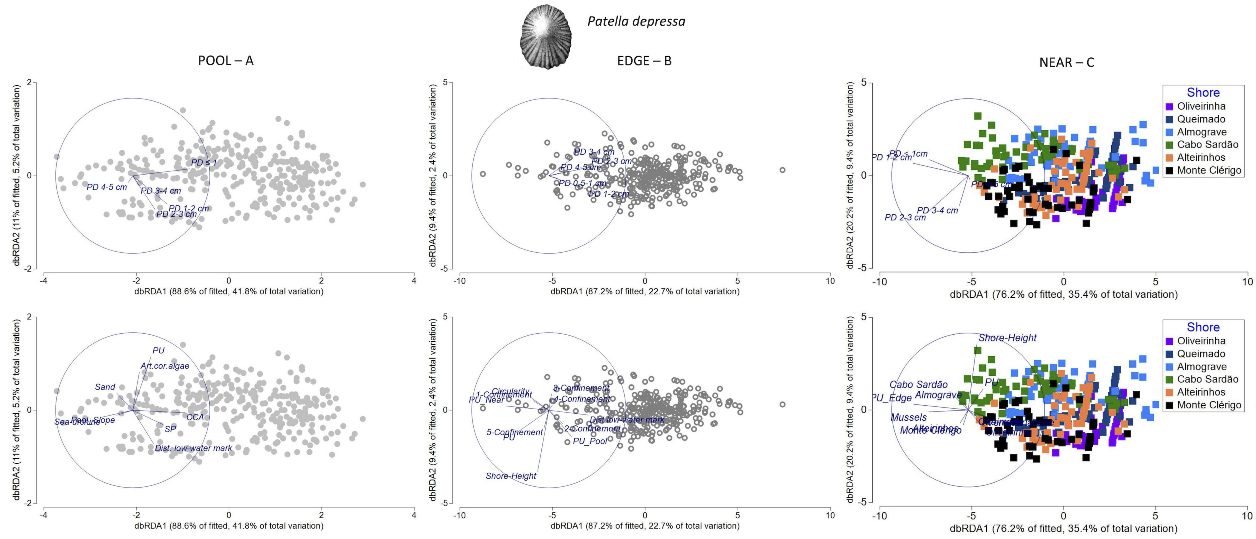


FIGURE 9

Distance-based redundancy analysis (*db-RDA*) plots for the spatial variability in abundance and size structure of *Patella depressa* within each of three neighboring micro-habitats: **(A)** POOL (rockpools, gray circles); **(B)** EDGE (edge of rockpools, open circles); and **(C)** NEAR (open-rock adjacent to rockpools, gray squares), (n = 323 samples). Vectors on top plots are response variables (size-class densities); vectors on bottom plots are "top predictors" selected by distance-based linear models (DistLM, see Table 5). On the plot of the bottom right corner, the superimposed vector labels correspond to: Alteirinhos and Monte Clérigo (at the left); Queimado and Verrucariaceae and Oliveirinha (at the right, coincident with several sample symbols).

explanation, Table 5), with the following positive associations: i) Cabo Sardão and Almograve with densities of the smallest size classes (PD ≤ 1 cm and PD 1–2 cm); ii) Oliveirinha and Queimado with density of the largest size class (PD 4–5 cm); and iii) Alteirinhos and Monte Clérigo with densities of mid and large-sized *P. depressa* (PD 2–3 cm and PD 3–4 cm) (Figure 9C).

For *P. depressa* present around rockpool edges (PD EDGE), the structuring among samples was mainly driven by association with two connectivity variables acting in opposite directions (accounting for 12% of explanation, Table 5): a negative relationship with the total density of *P. ulyssiponensis* on the surrounding open-rock (PU_adjacent_Near) and a positive relationship with the total density of *P. ulyssiponensis* inside rockpools (PU_adjacent_Pool) (Figure 9B). The second group of predictors selected for the PD EDGE model was the one of physical variables, namely, distance to low-water mark (positive relation with overall abundance of *P. depressa*), shore height, and circularity (negative association with abundance of *P. depressa* in general) and confinement (Figure 9B). It is worth noting the negative relationship between very-high confinement (category 5, the most-recessed pools) and density of large-sized *P. depressa* (PD 3–4 cm) (Figure 9, middle). Finally, a habitat-composition variable was also selected for this PD EDGE model: abundance of *P. ulyssiponensis* within the same micro-habitat (PU)— negatively related with large-sized *P. depressa* (Table 5; Figure 9B).

3.3.3 *Siphonaria pectinata*

The DistLM models for *S. pectinata* inside rockpools (SP POOL) and in the edge of rockpools (SP EDGE) explained similar proportions of the variability (26% and 22%, respectively), higher than the model for this species on the open-rock surrounding rockpools (SP NEAR—15%).

In SP POOL and SP NEAR models, habitat-composition predictors were most important for the spatial patterns of *S. pectinata* (17% and 11%, respectively) (Table 5). The first selected predictor in the SP POOL model was the total density of *P. depressa* in the same micro-habitat (PD) (Table 5), for which a positive relation with density of *S. pectinata* juveniles (SP ≤ 0.5cm) was suggested (Figure 10A). The other habitat-composition predictor included in the SP POOL model was the cover of articulated coralline algae (Table 5), for which a negative relationship was suggested with the density of the middle size class (SP 1–2 cm) (Figure 10A). For the SP NEAR model, three different habitat-composition predictors were included: cover of crustose non-coralline algae was positively related with densities of small-sized *S. pectinata* (SP ≤ 0.5 cm and SP 0.5–1 cm); the variable "Other sessile invertebrates" was negatively associated with densities of larger *S. pectinata* (SP 1–2 cm and SP 2–3 cm); and the total density of *P. depressa* in the same micro-habitat (PD) was positively associated with densities of small-sized *S. pectinata* (SP ≤ 0.5 cm and SP 0.5–1 cm) (Table 5; Figure 10C).

The group of physical predictors also contributed to the SP POOL and SP NEAR models (adding another 9% and 4% of explanation, respectively). Common to both models was the selection of shore height, which was positively associated with *S. pectinata* abundance inside rockpools, namely, with juveniles within POOL (SP ≤ 0.5cm), and negatively associated with *S. pectinata* abundance outside rockpools, namely, with mid- and large-sized *S. pectinata* within NEAR (SP 1–2 cm and SP 2–3 cm) (Table 5; Figures 10A, C). The other physical predictors selected in

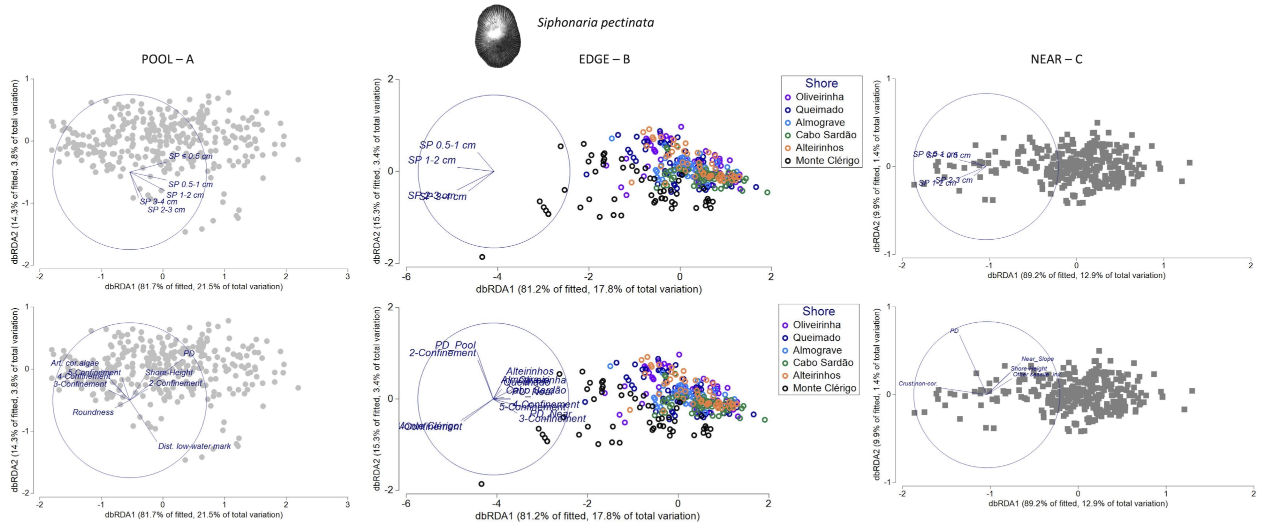


FIGURE 10

Distance-based redundancy analysis (*db-RDA*) ordination plots for the spatial variability in abundance and size structure of *Siphonaria pectinata* within each of three neighboring micro-habitats: **(A)** POOL (rockpools, gray circles), **(B)** EDGE (edge of rockpools, colored circles), and **(C)** NEAR (open-rock adjacent to rockpools, gray squares), (n = 323 samples). Vectors on top plots are response variables (size-class densities); vectors on bottom plots are "top predictors" selected by distance-based linear models (DistLM, see Table 5). On the plot in the top middle, the superimposed vector labels correspond to: SP 2–3 and SP 3–4. On the plot in the bottom middle, the superimposed vector labels correspond to: Monte Clérigo and 1-Confinement (at the left); Oliveirinha, Queimado, and Almograve (at the right); Cabo Sardão and PU Near (at the right); 4-Confinement and 5-Confinement (at the right). On the plot in the top right, the superimposed vector labels correspond to: SP < 1 and SP 0.5–1.

SP POOL model were the following: confinement—particularly the negative relationship between high confinement and the largest size classes (SP 2–3 cm and SP 3–4 cm); distance to low-water mark—positive association with these largest size classes; and roundness—negative relation with juveniles (SP ≤ 0.5cm) (Table 5; Figure 10A). The other physical variable selected in SP NEAR model was Slope NEAR, showing a negative relationship with mid- and large-sized SP (SP 1–2 cm and SP 2–3 cm) (Table 5; Figure 10C).

In both the SP POOL and SP NEAR models, no connectivity variables were selected (Table 5). In contrast, three connectivity variables were selected for the SP EDGE model (responsible for 6% of total explanation): *P. ulyssiponensis* within open-rock surfaces surrounding rockpools (PU_adjacent_Near)—negative association with mid-sized SP (SP 1–2 cm); *P. depressa* within open-rock surfaces surrounding rockpools (PD_adjacent_Near)—negative association with small-sized *S. pectinata* (SP 0.5–1 cm); and *P. depressa* inside rockpools (PD_adjacent_Pool)—positive association with small-sized *S. pectinata* (Table 5; Figure 10B). However, in the SP EDGE model, the variable that stood out was confinement, a physical predictor that explained 11% of the variation (Table 5), the most evident pattern being the positive associations between low confinement (category 2) and small-sized *S. pectinata* (SP 0.5–1 cm) and between very-low confinement (category 1) and the largest size classes (SP 2–3 cm and SP 3–4 cm) (Figure 10B). Besides the connectivity variables and confinement, the factor shore was selected in this model (Table 5), for which the most evident pattern was the positive association between the largest size classes (SP 2–3 cm and SP 3–4 cm) and Monte Clérigo (Figure 10B). This shore had the highest percentage of very-low confinement (category 1): 24% of rockpools (Supplementary Figure S7).

In summary, the cover of articulated coralline algae was a mutual habitat-composition "top predictor" in models of the three species inside rockpools and of *P. ulyssiponensis* on the open-rock, whereas the cover of mussels and crustose non-coralline algae were the most relevant "top predictors," respectively, for *P. depressa* and *S. pectinata* on the open-rock (Table 5).

4 Discussion

4.1 Summary of main findings

Coexistence of mobile species that share common habitats may arise from differences in the spatial patterns of different species at small scales. This can be caused by overlap in populations of individual species with different centers of distribution or with life-history-driven time windows of occupancy within the same habitat, and by connectivity of any species across adjacent habitats. Our most important results (relative to the specific objectives) were i) Limpets of various sizes of the three most common species—*P. ulyssiponensis*, *P. depressa* and *S. pectinata*—coexist on all shores, both inside and outside rockpools, and along a large part of the vertical tidal gradient within rockpools, thus sharing much of the available rocky-intertidal space in SW Portugal (objectives 1a and 2); ii) low abundance of the cold-water species *P. vulgata* scattered across micro-habitats on shores in SW Portugal, being more common at pool-edges and the wider landscape at Cabo Sardão, which might act as possible refuges for this range-edge species (objective 1a); iii) absence of *P. rustica* from rockpools and their edges, reflecting occupancy of open-rock and steeper shores (objective 1a); iv) a decreasing gradient of intra-specific connectivity from rockpools to their edges and surrounding open-rock was found for the three most common species (significant positive correlations in the abundance of the same species between pairs of adjacent micro-habitats), being steeper in *P. ulyssiponensis* than in *P. depressa* and *S. pectinata*, possibly due to *P. ulyssiponensis* being largely resident in rockpools and to gradual emergence (migration outward from rockpool-nursery areas) of the other two species onto open-rock (objective 1b); v) mid-shore populations of *P. depressa* and *S. pectinata* did not differ between open-rock near to and far away from rockpools, which can be considered a single habitat for these species (objective 1c); vi) landscape variability within shores, and hence the micro-habitat mosaics available, likely influenced among-shore patterns of abundance and size of *P. depressa* on mid-shore open-rock, with shores with less open-rock and more crevices associated with more numerous and smaller limpets (objective 1c); vii) habitat-composition of the substratum and benthic assemblages was generally a more important proximate factor than physical predictors in explaining variability of each limpet species both within rockpools and open-rock (3); viii) inter-specific influence on connectivity explained a great part of the variability of the three main species within rockpool edges, emphasizing the importance of this interface micro-habitat for limpet migration and for interactions between different limpet species (both positive and negative relationships were found between the spatial pattern of each individual limpet species at rockpool edges and the abundance of other limpet species in adjacent micro-habitats) (objective 3).

4.2 Diversity, dominance, and rare species

Five species of intertidal limpets comprising four patellids and one siphonariid co-occur in SW Portugal: *P. ulyssiponensis* was the dominant species within POOL; *P. depressa* was dominant within EDGE, NEAR, and FAR; *S. pectinata* was the third ranked species in abundance across all micro-habitats; *P. vulgata* infrequently occurred mostly within EDGE and NEAR; and *P. rustica* was exclusively present on NEAR and FAR. Rockpools did not seem to act as nursery grounds for the two least abundant species (*P. vulgata* and *P. rustica*), although a few *P. vulgata* juveniles were present within rockpools at Alteirinhos and Monte Clérigo. This agrees with a previous study (including Oliveirinha; Seabra et al., 2020), where limpet recruitment was measured within pools regularly cleared of limpets for 3 years: only 22 P. *vulgata* (all juveniles) and no *P. rustica* were recorded out of a total of 13,791 recruits (unpublished data). In Ireland, *P. vulgata* was described to occur in pools "in very small numbers" (Firth and Crowe, 2008: 15 juveniles in 30×30 quadrats in several shores and levels), but still in much higher (~20 times) densities than the ones reported here. Vertical distribution of these rare species (*P. vulgata* across mid-high levels and *P. rustica* mostly at high shore, as described in *Section 2.1*) and low densities of *P. vulgata* on open-rock were generally consistent with previous studies in our region (e.g., Rubal et al., 2013). The abundances of *P. vulgata* and *P. rustica* were consistently higher on shores with steeper cleavage of schist strata (Almograve and Cabo Sardão), where higher mean values of NEAR Slope and NEAR Topographic index (Supplementary Figure S7) and a higher frequency of crevices were found (Supplementary Figure S3). This agreed with previously described positive associations of these species with the following habitat features: *P. vulgata* with topographically irregular or damp surfaces (Cabral and Simões, 2007) and shady open-rock at mid-high shore (Seabra et al., 2011; Lima et al., 2016; Seabra et al., 2016), and *P. rustica* with steep open-rock surfaces of exposed shores (Lima et al., 2006) and crevices (Menconi et al., 1999). Further studies dedicated to these two species should be made on the SW Portuguese coast, given its biogeographic importance for both species (close to the southern limit of *P. vulgata*; not far from the northern limit of *P. rustica* and included in the region where it is most abundant in Portugal—e.g., Boaventura et al., 2002b). The role of micro-habitats surrounding rockpools, steeper surfaces, or a potentially colder regime in Cabo Sardão (a Cape, likely with upwelling-enriched waters) as potential thermal refuges for southern-edge populations of *P. vulgata* in this coast deserve further investigation.

4.3 Vertical distribution and among-shore patterns

When considering the micro-habitat inside-rockpools, the three most common limpets are present along most of the intertidal gradient, being abundant at many shore heights on all shores. The vertical distribution patterns on the open-rock were as expected from past work (e.g., Boaventura et al., 2002b), showing zonation of the three species at different tidal levels (low-shore *P. ulyssiponensis*, mainly mid-shore *P. depressa*, *S. pectinata* scattered and non-consistent zonation pattern across shores). The hypothesis of extension of the upper distribution limit in areas inside pools compared to contiguous areas outside pools was supported for the three common species. The presence of *P. ulyssiponensis* in rockpools at conspicuously higher shore levels than the low shore (where it is commonly found on the open-rock) has long been described (e.g., Evans, 1947; Firth and Crowe, 2008). The upper limits of subtidal and lower-intertidal organisms are generally pushed upwards by rockpools, as shown by many macroalgae (e.g., Femino and Mathieson, 1980; Araújo et al., 2006), seagrasses (Dethier, 1984) and also by the limpet *Acmaea mitra* and other invertebrates in Canada (Green, 1971). There was no such extension in the lower distribution limit (minimum value of shore height) of *P. ulyssiponensis*, suggesting equal habitat suitability between rockpools and open-rock at low shore (Firth and Crowe, 2008). For *P. depressa* and *S. pectinata*, the lower distribution limit also varied between micro-habitats. Within the open-rock, spatial and temporal variations in both vertical distribution limits of *P. depressa* in Portugal was previously related to sea-water temperature (Nobre et al., 2019). Here, the highest vertical extension was found for *S. pectinata* (difference in the median shore height between inside and outside rockpools, Figure 7). This species can be particularly abundant upon open-rock and in rockpools located at both extremes of the vertical gradient (e.g., low-shore open-rock vs. splash pools) (Seabra et al., 2020; this study). Amelioration against abiotic stress due to permanent submersion has been the main explanation for rockpools supporting more diverse assemblages compared to open-rock (Metaxas et al., 1994; Firth et al., 2014), and for the role of rockpools as nurseries for limpets due to sensitivity of their early-life stages to desiccation (e.g., Lewis and Bowman, 1975; Seabra et al., 2020). A recent study on the metabolic stress responses of *Cellana toreuma* in China demonstrated that limpets (size not specified) on rockpools suffer less cold stress than those living on open-rock during low tide in winter (Sun et al., 2023). Any environmental differences at low tide from pools to open-rock become greater at higher tidal levels (e.g., Huggett and Griffiths, 1986) increasing the ecological importance of pools.

Differences in density and size structure of limpets among shores were not found for *P. ulyssiponensis* within any of the three micro-habitats of rockpool systems, but were found for *P. depressa* within NEAR and *S. pectinata* within EDGE. The general pattern of higher abundance and lower individual size of *P. depressa* was found on the NEAR micro-habitat of steeper shores compared to flatter shores. This pattern could be a consequence of lower growth rates (probably due to the less available open-rock space and consequent limited foraging activity) and/or higher recruitment (possibly due to the higher abundance of crevices) onto open-rock of steeper shores. Among-shore variation detected for *S. pectinata* was due to a higher abundance, namely, of larger size classes, within pool edges in Monte Clérigo. This was the most sheltered and extensive gently sloping shore, with the shallowest, less topographically complex, and least-confined rockpools, likely to warm quickly (the lowest values of maximum and mean depth, POOL topographic index and slope, and confinement, Supplementary Figure S7). Such conditions are associated with high abundance (Rubal et al., 2013; Slama et al., 2021) and high growth rate (Ocaña, 2003) of *S. pectinata*. However, as Monte Clérigo is the most southern shore, this pattern might also reflect geographic affinity of *S. pectinata*. For all species and micro-habitats, sporadic or frequent variation was found between stretches within each shore, which could be related to smaller-scale variation in physical and biological processes.

4.4 Horizontal distribution and intra-specific connectivity across micro-habitats

On all shores, there were many more limpets of the three common species inside rockpools than in the surrounding open-rock: *P. ulyssiponensis* was 44 times, *S. pectinata* was 19 times, and *P. depressa* was 5 times more abundant within POOL compared to NEAR. This pattern was mostly due to newly settled and juvenile limpets, confirming that rockpools were undoubtfully important nurseries for these three species in this region (Seabra et al., 2020). Regarding only the adults (patellids of MSL > 1cm and siphonariids of MSL > 0.5cm), on all shores, there were five times more *P. ulyssiponensis* adults and six times more *S. pectinata* adults inside than outside rockpools (POOL versus NEAR, on average), but there were three times more *P. depressa* adults outside than inside rockpools (NEAR versus POOL, on average). This suggests that rockpools provide important adult habitats for *P. ulyssiponensis* and *S. pectinata*, whereas they serve primarily as nurseries for *P. depressa*. Moreover, *P. depressa* was also the species for which the gradient in abundance between adjacent micro-habitats was less sharp, namely, between POOL and EDGE (EDGE being a continuum from POOL for *P. depressa*, since its abundance was only three times higher inside rockpools than their edges; the same ratio was 10 and 8, respectively for *P. ulyssiponensis* and *S. pectinata*). This horizontal pattern suggested a gradual transition through migration out of pools (similarly to *P. vulgata* in Ireland, Delany et al., 1998), perhaps moderated by intra-age class interactions (Boaventura et al., 2003). Furthermore, whereas the abundance of *P. ulyssiponensis* within EDGE was consistently higher compared to NEAR on all shores (four times on average), this was only found on four and five of the six shores, respectively, for *P. depressa* and *S. pectinata*. Therefore, pool edges were mostly an intermediate zone from inside to outside rockpools, possibly acting as a frontier for the largely pool-resident *P. ulyssiponensis* above the low shore and a potential a transition route for migratory species (*P. depressa* and *S. pectinata*). The ontogenic distinction between residence within pools until adulthood (*P. ulyssiponensis*) vs. emigration of old juveniles of other patellids was previously described (e.g., Delany et al., 1998).

Considering intra-specific connectivity at the scale of the rockpool system, the strength of correlations in the abundance of the same species between pairs of adjacent micro-habitats lessened with distance from the pool. This pattern was found in common for all species, suggesting emergence of vagrant juveniles of the three species from their settlement sites within rockpools to drier open-rock only suitable for older/larger limpets (e.g., Bowman, 1981; Bowman, 1985). However, the positive relationship between rockpools and open-rock was only direct for *P. ulyssiponensis* (the only species with a significant correlation between POOL and NEAR). A direct connection from POOL to NEAR was not found for *P. depressa* and *S. pectinata*, although a continuum of positive relationships was found between rockpools and their edges and between edges and their surrounding open-rock. For these two species compared to *P. ulyssiponensis*, a more gradual connection was suggested between rockpools vs. open-rock at this horizontal small scale. *Patella ulyssiponensis* abundance consists mostly of rockpool populations, with spill-over to open-rock only possible on the low shore. This might be due to similarity in habitat suitability between rockpools and open-rock at low shore or to intra-specific interactions (Firth and Crowe, 2010).

The two most abundant species at the mid-shore open-rock (*P. depressa* and *S. pectinata*) did not differ with proximity to rockpools (MidNEAR and FAR areas). Therefore, at this tidal level, the open-rock surrounding rockpools can be considered as part of the open-rock habitat in general. Once emerged from mid-shore rockpools and their edges, limpets of these species likely form unique populations living across the mid-shore open-rock. Landscape variability was associated with among-shore differences in population structure of *P. depressa* on the mid-shore. This pattern could be interpreted as intra-specific connectivity with nurseries at this horizontal scale: shores with less open-rock and more crevices might have more abundant and smaller-sized limpets due to a higher connection to nursery areas (less-fragmented mosaics of patchy micro-habitats for recently settled limpets) across the mid-shore landscape. Mid-shore crevices were identified as important juvenile micro-habitats for *P. depressa* and *S. pectinata* in SW Portugal (Seabra et al., 2020). However, landscape variability did not seem important for *S. pectinata* on the mid-shore. Our results on overall patterns of intra-specific connectivity suggested migration outward from nursery areas. Further studies are needed on this process and on survival of early-life stages and ontogenetic shifts in traits (e.g., tolerance of emersion, Bowman, 1981) of these species across micro-habitats.

4.5 Patterns among highly variable rockpool-systems

The interpretation of DistLM models and relationships highlighted by db-RDA must be made with caution, as based on associations between response and predictive variables without investigating the causes of variation with manipulative experiments. Our study prompts future experimental work on the variables identified to be most relevant for the spatial patterns of each species within each micro-habitat. For all three species, the group of habitat-composition variables explained most of the variation in response matrices of the three species within both rockpools and open-rock. Previous studies showed the proximate importance of biotic predictors in determining molluscan patterns (e.g., Gonçalves et al., 2023). Nevertheless, physical factors can ultimately shape assemblage composition in pools (Huggett and Griffiths, 1986; Legrand et al., 2018), which is characterized by high small-scale variability (Araújo et al., 2006; Rubal et al., 2011; Bertocci et al., 2012). In general, the percentage of explained variation in EDGE models was lower than that in POOL and NEAR models, probably due to the smaller number of habitat-composition variables assessed within EDGE, (as the percentage cover of sessile taxa and the density of mobile grazers other than limpets were not measured on pool edges). A much greater percentage of the variation was explained for the two patellids (44% in *P. ulyssiponensis*, 47% in *P. depressa*, in both POOL and NEAR models) compared to *S. pectinata* (26% in POOL and 15% in NEAR). This lower explanatory power of *S. pectinata* models and the lack of rigid vertical distribution (see above) was probably related to an opportunistic nature. For instance, their presence in extremely shallow and very-low-confined splash-zone rockpools (where extreme physical–chemical conditions are likely) was consistent with distribution patterns of other siphonariid species (Hodgson, 1999). Hodgson (1999) also described several traits of siphonariids responsible for their success in occupancy of environmentally harsh micro-habitats, including efficient respiration in air and water, optimized foraging activity, unpalatability to predators, facultative metabolic rate depression, and possible anaerobiosis under unfavorable conditions. Their ability to re-hydrate more rapidly compared to patellogastropods, coupled with greater tolerances to salinity fluctuations (Hodgson, 1999), may also enable a wider range of conditions to be occupied by *S. pectinata*.

Inter-specific influences on connectivity were remarkably important for all three species in characterizing their variability within the transition environment (pool edges), but not within POOL and NEAR micro-habitats (connectivity variables selected only in the *P. depressa* NEAR model). On the contrary, abundances of other limpet species in the same micro-habitat were selected in most models, suggesting both positive facilitation and negative interactions between different limpet species (see below).

Inside pools, the relationships that seem to be established between different species are mostly positive, namely between patellids (the exception was a negative relationship between the abundance of *P. ulyssiponensis* and the largest size-class of *P. depressa*), and between *P. depressa* and *S. pectinata* (POOL dbRDA plots with predictor vectors). This agrees with Firth and Crowe (2010), who did not find evidence of inter-specific competition of *P. ulyssiponensis* and *P. vulgata* inside rockpools. There was also no evidence of intra-specific competition inside pools, as the different size classes of the same species also suggest positive relationships (POOL dbRDA plots with limpet-size vectors). This is not in agreement with Firth and Crowe (2010), who found evidence of intra-specific competition of *P. ulyssiponensis* inside rockpools. Abundances of both patellids inside rockpools were positively related with cover of CCA and articulated coralline algae and negatively related with sand cover. Observation of these patellids within “Lithothamnia-lined pools” and coralline turfs (Evans, 1947; Kooistra et al., 1989; Benedetti-Cecchi and Cinelli, 1996) and their grazing on these algal groups (Kooistra et al., 1989; Delany et al., 2002) has long been described. While siphonariids are known to be tolerant to sand inundation (Hodgson, 1999), negative effects of sediment have been found on grazing activity and survival of patellids (e.g., Airoldi and Hawkins, 2007). Contrasting patterns between the two patellids were observed inside pools: higher *P. ulyssiponensis* density occurred at lower shore, along with more sea urchins cover; *P. depressa* density was higher with distance from low-water mark and less sea urchins. Similar contrasting associations with *Paracentrotus lividus* within low-shore rockpools were found for juveniles of the two species in this region (Seabra et al., 2019; Seabra et al., 2020). The highest abundances of *S. pectinata* within pools and at pool edges were associated with the lowest category of confinement and the shore with more pools of this category (Monte Clérigo). For *S. pectinata* inside pools, a positive relationship with distance to low-water mark was found (similarly with *P. depressa*), and a higher abundance of *S. pectinata* was related to lower coverage of articulated coralline algae (contrarily with *P. depressa*). Indeed, *S. pectinata* in SW Portugal is frequently found at high-shore rockpools dominated by bare rock (juveniles, Seabra et al., 2020; adults, personal observations).

Outside pools (NEAR), apart from the negative relationship between the abundance of *P. ulyssiponensis* and large-sized (2–3 cm) *P. depressa*, there were no other negative relationships established between the two patellids and with S. pectinata, suggesting limited evidence of inter-specific competition. This agrees with experimental evidence of weak inter-specific competition between *P. vulgata* and *P. depressa* on open-rock in Portugal (Boaventura et al., 2002a). A negative relationship with shore height was observed in this micro-habitat for the three species, possibly reflecting greater recruitment, survival, and growth lower down (Guerra and Gaudencio, 1986). The other highlighted relationships outside rockpools were the following positive associations: i) *P. ulyssiponensis* with cover of both articulated coralline algae and CCA; ii) *P. depressa* with mussel cover and the factor shore; and iii) *S. pectinata* with crustose non-coralline algae. Adults of *S. pectinata* living outside rockpools in SW Portugal are very conspicuous within *Ralfsia verrucosa* and *Nemoderma tingitanum* patches at low and lower-mid shore open-rock (personal observations). This association might suggest promotion of settlement, growth, and/or survival of these algae by *S. pectinata* as described for *S. sirius* and *Ralfsia* crusts (Iwasaki, 1993 *in* Hodgson, 1999) or mutualism as found between *P. longicosta* and *Ralfsia verrucosa* (McQuaid and Froneman, 1993).

The influence of CCA, namely, "Lithothamia" *sensu* Hawkins and Jones (1992), on the recruitment of North-East Atlantic patellids both inside and outside pools has been described (Bowman, 1981; Delany et al., 2002; Seabra et al., 2019; Castejón et al., 2021). *Patella ulyssiponensis* among *Corallina* spp. on the open-rock, and patellid juveniles among mussels inside and outside pools were previously observed in Portugal (Guerra and Gaudencio, 1986). Articulated coralline algae are an important constituent of the flora of rockpools and lower-shore open-rock (Evans, 1957; Boaventura et al., 2002c; Firth and Crowe, 2008), often forming a turf full of small mussels at the thalli base (personal observations). Thus, the association with these algae inside and outside pools might be due to an association with mussels. *Mytilus galloprovincialis* patches retaining moisture on the open-rock of Oliveirinha was the unique CCA-absent habitat with abundant *P. ulyssiponensis* juveniles (Seabra et al., 2019), also important for *P. depressa* juveniles (Seabra et al., 2020).

In EDGE models, reciprocal negative relationships were established between the two patellids, and the abundance of one species outside pools was negatively related to the abundance of its congener at pool edges. A positive relationship was also established between the abundance of *S. pectinata* on pool-edges and the abundance of *P. depressa* within pools. Moreover, the greater abundances of *P. depressa* and *P. ulyssiponensis* outside pools were negatively related to *S. pectinata* on pool edges. As EDGE was an interface, most of the other relevant predictors were common to the ones selected for inside-pool and open-rock models, such as shore height that indicated a negative relationship with the abundance of both patellids at pool edges or the very-low confinement associated with a greater abundance of *S. pectinata* at pool edges.

4.6 Concluding remarks

Limpets of the three most common species in SW Portugal co-occur in connected populations across a landscape of rockpools, pool edges, and open-rock micro-habitats. Rockpools create a mosaic of micro-habitats that differ most when the tide is out (Huggett and Griffiths, 1986), likely sharpening ecological edge effects with the surrounding rock; pools can also create patchiness when the tide is in due to larval settlement (Castejón et al., 2021) or to foraging activity (Noël et al., 2009). Various species of limpets use these micro-habitats differentially, due to individual preferences on habitat-composition and physiological optima that can change ontogenetically (Davies, 1970; Delany et al., 1998; Delany et al., 2002). The three most common species in SW Portugal use rockpools as nursery grounds (Seabra et al., 2020). Subsequently, largely permanent residence into pools (*P. ulyssiponensis*—except on the low shore) or gradual emergence onto open-rock (*P. depressa* and *S. pectinata*) likely happen. Thus, different species have differential occupancy across highly localized horizontal gradients and edge transitions of rockpool systems, which are superimposed on their vertical and among-shore patterns at broader scales. Shore topography ultimately creates landscape variability, which provides nursery areas (Seabra et al., 2020) or refuges (Sun et al., 2023) for limpets, allows coexistence of different limpet species (Firth and Crowe, 2010), and boosts biodiversity (Firth et al., 2014), thereby contributing to ecosystem functioning (Griffin et al., 2010). Our study suggests that rockpool-generated mosaics are strongly linked by intra-specific connectivity of limpet populations with patterns modulated by interactions between different limpet species at rockpool edges. Much further experimental work is required to explore the underlying causes of the described patterns and associations with habitat-composition predictors revealed in our study, including the effects of limpets on rockpool communities (as their role as keystone species has not been studied for rockpool micro-habitats in Portugal). The importance of rockpools for patellids harvested in SW Portugal may have implications for regional monitoring programs in intertidal systems, assessing effectiveness of protection in special areas of the marine park where fishing is totally or partially forbidden and other human activities are regulated (Castro et al., 2021). More attention should be given to the role of rockpools in sustaining limpet populations in the context of anthropogenic disturbance including harvesting, as part of biodiversity surveillance and conservation of temperate reefs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on animals in accordance with the local and EU legislation, and institutional requirements. Limpets are not included in the listing of animals under article 2nd of the Directive n.° 2010/63/UE, of the European Parliament and Conseil from 22nd September 2010. The work was descriptive and not involving sacrifice or stress to the animals, as sampling was a completely descriptive and non-destructive survey.

Author contributions

MS, TC, and SH conceived the idea and designed the study. MS was responsible for data collection and visualization and wrote the first draft of the manuscript. NP was involved in field surveys, data curation, and manuscript preparation. TC and MS analyzed the data. TC and SH participated in data interpretation and manuscript writing also providing supervision. All authors read and commented on the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study had the support of FCT through the strategic project UIDB/04292/2020 awarded to MARE and through project LA/P/0069/2020 granted to the Associate Laboratory ARNET and the doctoral grant awarded to MIS (SFRH/BD/16693/2004). SJH was funded by the Marine Biological Association of the UK (Dept 40).

Acknowledgments

We would like to express our gratitude to the scientific illustrator Ivan Gromicho, for the drawings of limpets presented in Figures 2–10. We thank volunteer students (Verónica Sanita, Ricardo Branca, Tiago Neves, and Francisco Calado) that help on sampling of physical variables. We also thank to the two reviewers for their constructive comments, which considerably improved the first version of the manuscript. Professor M. J. Anderson provided advice on statistics during the manuscript revision. During revison, Dr. João J. Castro provided advice on issues related to regional conservation and management.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1206159/full#supplementary-material

SUPPLEMENTARY FIGURE S1

Densities and size-class structure of *Patella vulgata* on six shores within POOL (inside-rockpools), EDGE (edge of rockpools) and NEAR (open-rock adjacent to rockpools) micro-habitats (three left columns); and within areas of mid-shore open-rock at two proximity categories from rockpools (MidNEAR and FAR, far right column). Grey and white bars are mean (± SE) number of limpets of five size-classes per 50x50 cm; Note different y-axis scale between graphs of POOL vs. others; number of replicates indicated in caption of Figure 2. Black and white bars (bottom row) are percentages of every size-class relatively to the total density of this species (value given top right of each graph) within each micro-habitat and across all shores.

SUPPLEMENTARY FIGURE S2

Densities and size-class structure of *Patella rustica* on six shores within POOL (inside-rockpools), EDGE (edge of rockpools) and NEAR (open-rock adjacent to rockpools) micro-habitats (three left columns); and within areas of mid-shore open-rock at two proximity categories from rockpools (MidNEAR and FAR, far right column). Grey and white bars are mean (± SE) number of limpets of five size-classes per 50x50 cm; number of replicates indicated in caption of Figure 2. Black and white bars (bottom row) are percentages of every size-class relatively to the total density of this species (value given top right of each graph) within each micro-habitat and across all shores.

SUPPLEMENTARY FIGURE S3

Mean proportion of occurrence of emersed open-rock, rockpools, crevices, overhangs, and channels on the mid-shore of twelve coastal stretches (two random stretches, **(A, B)**, within each of six shores). Number of replicates within each stretch varied between 2 and 4 transects parallel to the sea.

SUPPLEMENTARY FIGURE S4

Percentage cover of space-occupying categories within the POOL micro-habitat. Values are mean % cover (± SE) of each substratum type or functional group of sessile organisms recorded inside-rockpools on six shores. The number of replicates in each shore was the following: 52 in Oliveirinha, 51 in Queimado, 57 in Almograve, 56 in Cabo Sardão, 53 in Alteirinhos and 54 in Monte Clérigo.

SUPPLEMENTARY FIGURE S5

Percentage cover of space-occupying categories (substratum types and functional groups of sessile organisms) within NEAR micro-habitat. Values are mean (± SE) cover estimates assessed on open-rock surfaces of six shores using the following semi-quantitative index (and respective percent cover intervals): 0 (not found); 1 (< 1%); 2 (1-5%); 3 (5-10%); 4 (10-25%); 5 (25-50%); 6 (50-75%); 7 (75-90%); 8 (90-95%); 9 (> 95%). The number of replicates in each shore was the following: 52 in Oliveirinha, 51 in Queimado, 57 in Almograve, 56 in Cabo Sardão, 53 in Alteirinhos and 54 in Monte Clérigo.

SUPPLEMENTARY FIGURE S6

Densities (mean ± SE) of the total number of trochids (including the species *Steromphala umbilicalis*, *Steromphala pennanti*, *Phorcus sauciatus*, *Phorcus lineatus*) and the total number of littorinids *Melarhaphe neritoides* within POOL and NEAR micro-habitats. The number of replicates in each shore was the following: 52 in Oliveirinha, 51 in Queimado, 57 in Almograve, 56 in Cabo Sardão, 53 in Alteirinhos and 54 in Monte Clérigo.

SUPPLEMENTARY FIGURE S7

Physical variables of rockpool-systems on six shores of SW Portugal (see Table 1 for description of each variable). The number of replicates in each shore was the following: 52 in Oliveirinha, 51 in Queimado, 57 in Almograve, 56 in Cabo Sardão, 53 in Alteirinhos and 54 in Monte Clérigo. For clarity, outliers were not shown for four variables: NEAR topographic complexity, surface-area, volume and roundness. Physical variables were used as predictors in DistLM models for which the response-variables were size-class densities of a limpet species present within each of three neighboring micro-habitats of rockpool-systems: inside-rockpools (POOL), rockpool-edges (EDGES), and surrounding emersed open-rock (NEAR).

SUPPLEMENTARY TABLE S1

Definition and measurement details of all predictive-variables of three groups (physical, habitat-composition and connectivity) assessed for every sampled rockpool (n = 323). Under the column "Micro-habitat", "only" is indicated for variables that refer exclusively to one micro-habitat, whereas "All" identifies those variables that were considered as physical descriptors of each rockpool-system across POOL, EDGE, and NEAR micro-habitats.

SUPPLEMENTARY TABLE S2

List of all predictive-variables used to build the distance-based linear model (DistLM) for each of the three species (Patella ulyssiponensis – PU; P.

depressa – PD; Siphonaria pectinata – SP) within each of the three micro-habitats (POOL; EDGE; NEAR). "Transf." refers to transformation applied as pre-treatment before DistLM analyses; "sqrt" refers to square root transformation; * refers to a variable (maximum-depth) that was eliminated due to collinearity with another variable (mean-depth).

SUPPLEMENTARY TEXT

Methodological details of distance-based linear models (DistLM) to examine the relationship between the spatial structure of individual limpet species within each micro-habitat and several predictors (physical, habitat-composition, and connectivity variables).

References

Aguilera, M. A., and Navarrete, S. A. (2011). Distribution and activity patterns in an intertidal grazer assemblage: influence of temporal and spatial organization on interspecific associations. *Mar. Ecol. Prog. Ser.* 431, 119–136. doi: 10.3354/meps09100

Aguilera, M. A., and Navarrete, S. A. (2012). Inter-specific competition for shelters in territorial and gregarious intertidal grazers: consequences for individual behaviour. *PloS One* 7 (9), e46205. doi: 10.1371/journal.pone.0046205

Aguilera, M. A., Valdivia, N., and Broitman, B. R. (2013). Spatial niche differentiation and coexistence at the edge: co-occurrence distribution patterns in *Scurria* limpets. *Mar. Ecol. Prog. Ser.* 483, 185–198. doi: 10.3354/meps10293

Aguilera, M. A., Weiß, M., and Thiel, M. (2019). Similarity in predator-specific anti-predator behavior in ecologically distinct limpet species, *Scurria viridula* (Lottiidae) and *Fissurella latimarginata* (Fissurellidae*). Mar. Biol.* 166, 1–13. doi: 10.1007/s00227-019-3485-5

Airoldi, L., and Hawkins, S. J. (2007). Negative effects of sediment deposition on grazing activity and survival of the limpet *Patella vulgata*. *Mar. Ecol. Prog. Ser.* 332, 235–240. doi: 10.3354/meps332235

Anderson, M. J. (2001). Permutation tests for univariate or multivariate analysis of variance and regression. *Can. J. Fish. Aquat.* 58 (3), 626–639. doi: 10.1139/f01-00

Anderson, M., Gorley, R. N., and Clarke, K. (2008). *PERMANOVA+ for primer: Guide to software and statistical methods* (Plymouth, UK: PRIMER-E).

Araújo, R., Sousa-Pinto, I., Bárbara, I., and Quintino, V. (2006). Macroalgal communities of intertidal rock pools in the northwest coast of Portugal. *Acta Oecol.* 30 (2), 192–202. doi: 10.1016/j.actao.2006.04.002

Benedetti-Cecchi, L., and Cinelli, F. (1995). Habitat heterogeneity, sea urchin grazing and the distribution of algae in littoral rock pools on the west coast of Italy (western Mediterranean). *Mar. Ecol. Prog. Ser.* 126, 203–212. doi: 10.3354/meps126203

Benedetti-Cecchi, L., and Cinelli, F. (1996). Patterns of disturbance and recovery in littoral rock pools: nonhierarchical competition and spatial variability in secondary succession. *Mar. Ecol. Prog. Ser.* 135, 145–161. doi: 10.3354/meps135145

Bertocci, I., Araújo, R., Incera, M., Arenas, F., Pereira, R., Abreu,, et al. (2012). Benthic assemblages of rock pools in northern Portugal: seasonal and between-pool variability. *Sci. Mar.* 76 (4), 781–789. doi: 10.3989/scimar.03669.21A

Blackmore, D. T. (1969). Studies of *Patella vulgata* L. Growth, reproduction and zonal distribution. *J. Exp. Mar. Biol. Ecol.* 3 (2), 200–213. doi: 10.1016/0022-0981(69)90018-5

Boaventura, D., Alexander, M., Della Santina, P., Smith, N. D., Ré, P., da Fonseca, L. C., et al. (2002c). The effects of grazing on the distribution and composition of low-shore algal communities on the central coast of Portugal and on the southern coast of Britain. *J. Exp. Mar. Biol. Ecol.* 267 (2), 185–206. doi: 10.1016/S0022-0981(01)00372-0

Boaventura, D., da Fonseca, L. C., and Hawkins, S. J. (2002a). Analysis of competitive interactions between the limpets *Patella depressa* Pennant and *Patella vulgata* L. on the northern coast of Portugal. *J. Exp. Mar. Biol. Ecol.* 271 (2), 171–188. doi: 10.1016/S0022-0981(02)00044-8

Boaventura, D., Da Fonseca, L. C., and Hawkins, S. J. (2003). Size matters: competition within populations of the limpet *Patella depressa*. *J. Anim. Ecol.* 72 (3), 435–446. doi: 10.1046/j.1365-2656.2003.00713.x

Boaventura, D., Ré, P., Da Fonseca, L. C., and Hawkins, S. J. (2002b). Intertidal rocky shore communities of the continental Portuguese coast: Analysis of distribution patterns. *Mar. Ecol.* 23, 69–90. doi: 10.1046/j.1439-0485.2002.02758.x

Bowman, R. S. (1981). The morphology of *Patella* spp. juveniles in Britain, and some phylogenetic inferences. *J. Mar. Biolog. Assoc. U. K.* 61 (3), 647–666. doi: 10.1017/S0025315400048104

Bowman, R. S. (1985). "The biology of the limpet Patella vulgata L. in the British Isles: spawning time as a factor determining recruitment success," in *The Ecology of Rocky Coasts: essays presented to JR Lewis, D. Sc.* Eds. P. G. Moore and R. Seed (London: Hodder and Stoughton), 178–193.

Branch, G. M. (1971). The ecology of *Patella* Linnaeus from the cape Peninsula, South Africa I. Zonation, movements and feeding. *Afr. Zool.* 6 (1), 1–38. doi: 10.1080/00445096.1971.11447402

Branch, G. M. (1975). Mechanisms reducing intra-specific competition in Patella spp.: migration, differentiation and territorial behaviour. *J. Anim. Ecol.* 44 (2), 575–600. doi: 10.2307/3612

Branch, G. M. (1976). Inter-specific competition experienced by South African *Patella* species. *J. Anim. Ecol.* 45 (2), 507–529. doi: 10.2307/3888

Branch, G. M. (1981). The biology of limpets: physical factors, energy flow, and ecological interactions. *Ann. Rev. Mar. Sci.* 19, 235–380. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCALZOOLINEINRA82X0007825.

Branch, G. M., and Marsh, A. C. (1978). Tenacity and shell shape in six *Patella* species: adaptive features. *J. Exp. Mar. Biol. Ecol.* 34 (2), 111–130. doi: 10.1016/0022-0981(78)90035-7

Branch, G. M., Trueman, E. R., and Clarke, M. R. (1985). "Limpets: evolution and adaptation," in *The Mollusca Vol. 10*. Eds. E. R. Trueman and M. R. Clarke (FL: Academic Press, Inc), 187–220.

Broitman, B. R., Aguilera, M. A., Lagos, N. A., and Lardies, M. A. (2018). Phenotypic plasticity at the edge: Contrasting population-level responses at the overlap of the leading and rear edges of the geographical distribution of two *Scurria* limpets. *J. Biogeogr.* 45 (10), 2314–2325. doi: 10.1111/jbi.13406

Burrows, M. T., and Hawkins, S. J. (1998). Modelling patch dynamics on rocky shores using deterministic cellular automata. *Mar. Ecol. Prog. Ser.* 167, 1–13. doi: 10.3354/meps167001

Cabral, J. P., and Simões, J. (2007). The southern limit of distribution of *Patella vulgata*. *Iberus* 25, 57–75. doi: 10.5281/zenodo.4532807

Carneiro, A. P. (2021). *Diversity and Distribuition of Patellid Limpets Along the Southwestern African Coast (Benguela Current)* (Faro (PT: Universidade do Algarve).

Carvalho, J., Mendonça, V., Vinagre, C., and Silva, A. (2021). Environmental factors impacting the abundance and distribution of amphipods in intertidal rock pools. *J. SeaRes.* 172, 102035. doi: 10.1016/j.seares.2021.102035

Casal, G., Aceña-Matarranz, S., Fernández-Márquez, D., and Fernández, N. (2018). Distribution and abundance patterns of three coexisting species of *Patella* (Mollusca Gastropoda) in the intertidal areas of the NW Iberian Peninsula: Implications for management. *Fish. Res.* 198, 86–98. doi: 10.1016/j.fishres.2017.10.011

Castejón, D., Nogueira, N., and Andrade, C. A. (2021). Limpet larvae (*Patella aspera* Röding 1798), obtained by gonad dissection and fecundation *in vitro*, settled and metamorphosed on crustose coralline algae. *J. Mar. Biolog. Assoc. U. K.* 101 (7), 991–1002. doi: 10.1017/S0025315421000916

Castro, J. J., Quintella, B. R., Afonso, C. M. L., Almeida, P. R., Andrade, M., Antunes,, et al. (2021). *Relatório final de execução, modelo de monitorização e medidas de gestão. Relatório, projeto MARSW Sistemas de Informação e Monitorização da Biodiversidade Marinha das Áreas Classificadas do Sudoeste Alentejano e Costa Vicentina* (Sines: Universidade de Évora, Faculdade de Ciências da Universidade de Lisboa, MARE – Centro de Ciências do Mar e do Ambiente, CCMAR – Centro de Ciências do Mar e Universidade do Algarve).

Castro, J. J., Quintella, B. R., Almeida, P. R., Antunes, A. C., Antunes, M., Bentes, L., et al. (2020). *Caracterização da pesca na área marinha do Parque Natural do Sudoeste Alentejano e Costa Vicentina. Relatório, projeto MARSW – Sistemas de Informação e Monitorização da Biodiversidade Marinha das Áreas Classificadas do Sudoeste Alentejano e Costa Vicentina* (Sines: Universidade de Évora, Faculdade de Ciências da Universidade de Lisboa, MARE – Centro de Ciências do Mar e do Ambiente, CCMAR – Centro de Ciências do Mar e Universidade do Algarve).

CIEMAR (2018). *Substrato duro intertidal. Monitorização de Ambientes Marinhos do Porto de Sines - MAPSi 2015/2017. Relatório final* (Sines: Laboratório de Ciências do Mar da Universidade de Évora).

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Austral Ecol.* 18 (1), 117–143. doi: 10.1111/j.1442-9993.1993.tb00438.x

Clarke, K. R., and Gorley, R. N. (2015). *Getting started with PRIMER v7* Vol. 20 (Plymouth: PRIMER-E: Plymouth, Plymouth Marine Laboratory), 1.

Coleman, R. A., Underwood, A. J., Benedetti-Cecchi, L., Aberg, P., Arenas, F., Arrontes, J., et al. (2006). A continental scale evaluation of the role of limpet grazing on rocky shores. *Oecologia* 147, 556–564. doi: 10.1007/s00442-005-0296-9

Creese, R. G. (1980). An analysis of distribution and abundance of populations of the high-shore limpet, Notoacmea petterdi (Tenison-Woods). *Oecologia* 45, 252–260. doi: 10.1007/BF00346466

Creese, R. G., and Underwood, A. J. (1982). Analysis of inter-and intra-specific competition amongst intertidal limpets with different methods of feeding. *Oecologia* 53, 337–346. doi: 10.1007/BF00389010

Davies, P. S. (1970). Physiological ecology of *Patella* IV. Environmental and limpet body temperatures. *J. Mar. Biolog. Assoc. U. K.* 50 (4), 1069–1077. doi: 10.1017/S0025315400005956

Delany, J., Myers, A. A., and McGrath, D. (1998). Recruitment, immigration and population structure of two coexisting limpet species in mid-shore tidepools, on the west coast of Ireland. *J. Exp. Mar. Biol. Ecol.* 221 (2), 221–230. doi: 10.2307/1942457

Delany, J., Myers, A. A., and McGrath, D. (2002). "A comparison of the interactions of the limpets Patella vulgata and Patella ulyssiponensis with crustose coralline algae," in *New survey of Clare Island: v. 3: marine intertidal ecology*. Ed. A. Myers (Dublin: The Royal Irish Academy) 3, 79–90.

Dethier, M. N. (1984). Disturbance and recovery in intertidal pools: maintenance of mosaic patterns. *Ecol. Monogr.* 54 (1), 99–118. doi: 10.2307/1942457

Evans, R. G. (1947). "Studies on the Biology of British Limpets," in *Proceedings of the Zoological Society of London* (Oxford, UK: Blackwell Publishing Ltd), 411–423. doi: 10.1111/j.1096-3642.1953.tb00179.x

Evans, R. G. (1957). The intertidal ecology of some localities on the Atlantic coast of France. *J. Ecol.* 45 (1), 245–271. doi: 10.2307/2257088

Femino, R. J., and Mathieson, A. C. (1980). Investigations of New England marine algae IV: The ecology and seasonal succession of tide pool algae at Bald Head Cliff, York, Maine, USA. *Bot. Mar.* 23, 319–332. Available at: http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCAL8010470072.

Firth, L. B., and Crowe, T. P. (2008). Large-scale coexistence and small-scale segregation of key species on rocky shores. *Hydrobiologia* 614, 233–241. doi: 10.1007/s10750-008-9509-7

Firth, L. B., and Crowe, T. P. (2010). Competition and habitat suitability: small-scale segregation underpins large-scale coexistence of key species on temperate rocky shores. *Oecologia* 162, 163–174. doi: 10.1007/s00442-009-1441-7

Firth, L. B., Crowe, T. P., Moore, P., Thompson, R. C., and Hawkins, S. J. (2009). Predicting impacts of climate-induced range expansion: an experimental framework and a test involving key grazers on temperate rocky shores. *Glob. Change Biol.* 15 (6), 1413–1422. doi: 10.1111/j.1365-2486.2009.01863.x

Firth, L. B., Schofield, M., White, F. J., Skov, M. W., and Hawkins, S. J. (2014). Biodiversity in intertidal rock pools: Informing engineering criteria for artificial habitat enhancement in the built environment. *Mar. Environ. Res.* 102, 122–130. doi: 10.1016/j.marenvres.2014.03.016

Freitas, D., Arenas, F., Vale, C. G., Pinto, I. S., and Borges, D. (2023a). Warning of warming limpets: sea temperature effects upon intertidal rocky assemblages. *J. Mar. Biolog. Assoc. U. K.* 103, e7. doi: 10.1017/S0025315422001096

Freitas, D., Borges, D., Arenas, F., Pinto, I. S., and Vale, C. G. (2023b). Forecasting distributional shifts of Patella spp. in the Northeast Atlantic Ocean, under climate change. *Mar. Environ. Res.* 186, 105945. doi: 10.1016/j.marenvres.2023.105945

Fretter, V., and Graham, A. (1994). *British prosobranch molluscs, their functional anatomy and ecology* (London, UK: The Ray Society). doi: 10.1017/S0025315400090299

Garrity, S. D. (1984). Some adaptations of gastropods to physical stress on a tropical rocky shore. *Ecology* 65 (2), 559–574. doi: 10.2307/1941418

Giesel, J. T. (1970). On the maintenance of a shell pattern and behavior polymorphism in Acmaea digitalis, a limpet. *Evolution* 24 (1), 98–119. doi: 10.2307/2406717

Gomes, N., Antunes, C., and Costa, D. D. A. (2022). Insights into the Migration Routes and Historical Dispersion of Species Surviving the Messinian Crisis: The Case of *Patella ulyssiponensis* and Epizoic Rhodolith *Lithophyllum hibernicum*. *Hydrobiology* 1 (1), 10–38. doi: 10.3390/hydrobiology1010003

Gonçalves, I., Vinagre, C., and Silva, A. (2023). Patterns of rockpool molluscs in differing shore exposures. *Mar. Biol.* 170 (6), 77. doi: 10.1007/s00227-023-04223-2

Goss-Custard, S., Jones, J., Kitching, J. A., and Norton, T. A. (1979). "Tide pools of Carrigathorna and Barloge Creek," in *Philosophical Transactions of the Royal Society of London*, vol. 287. (London, UK: B, Biological Sciences), 1–44. doi: 10.1098/rstb.1979.0051

Gray, D. R., and Hodgson, A. N. (1998). Foraging and homing behaviour in the high-shore, crevice-dwelling limpet *Helcion pectunculus* (Prosobranchia: Patellidae). *Mar. Biol.* 132, 283–294. doi: 10.1007/s002270050394

Green, J. M. (1971). Local distribution of *Oligocottus maculosus* Girard and other tidepool cottids of the west coast of Vancouver Island, British Columbia. *Can. J. Zool.* 49 (8), 1111–1128. doi: 10.1139/z71-172

Griffin, J. N., Laure, M. L. N., Crowe, T. P., Burrows, M. T., Hawkins, S. J., Thompson, R. C., et al. (2010). Consumer effects on ecosystem functioning in rock pools: roles of species richness and composition. *Mar. Ecol. Prog. Ser.* 420, 45–56. doi: 10.3354/meps08844

Guerra, M. T., and Gaudencio, M. J. (1986). Aspects of the ecology of *Patella* spp. on the Portuguese coast. *Hydrobiologia* 142, 57–69. doi: 10.1007/BF00026747

Hartnoll, R. G., and Hawkins, S. J. (1985). Patchiness and fluctuations on moderately exposed rocky shores. *Ophelia* 24 (1), 53–63. doi: 10.1080/00785236.1985.10426619

Hawkins, S. J., and Hartnoll, R. G. (1983). Grazing of intertidal algae by marine invertebrates. *Oceanogr. Mar. Biol.* 21, 195–282.

Hawkins, S. J., John, D. M., and Price, J. H. (1992). Plant-animal interactions on hard substrata in the northeast Atlantic. In: Eds. D. M. John, S. J. Hawkins and J. H. Price *Plant-animal interactions in the marine benthos* (Systematics Association). (Oxford: Oxford University Press), 1–32.

Hawkins, S. J., and Jones, H. D. (1992). *Marine Conservation Society Marine Field Course Guide 1* (London: Immel).

Hawkins, S. J., Pack, K. E., Firth, L. B., Mieskowska, N., Evans, A. J., Martins, G. M., et al. (2019). "The intertidal zone of the North-East Atlantic region: pattern and process," in *Interactions in the marine benthos: global patterns and processes*. Eds. S. J. Hawkins, K. Bohn, L. B. Firth and G. A. Williams (Cambridge: Cambridge University Press), 7–46. doi: 10.1017/9781108235792.003

Henriques, P., Delgado, J., Sousa, R., and Ray, S. (2017). "Patellid limpets: an overview of the biology and conservation of keystone species of the rocky shores," in *Organismal and molecular malacology* (London: IntechOpen Limited), 71–95. doi: 10.5772/67862

Hodgson, G. (1999). The biology of siphonariid limpets (Gastropoda: Pulmonata). *Oceanogr. Mar. Biol.* 37, 245–314.

Horta e Costa, B., Sousa, I., Belackova, A., Henriques, N. S., Rangel, M., Oliveira,, et al. (2018). *Relatório de Síntese da Biodiversidade Marinha da área marinha do Parque Natural do Sudoeste Alentejano e Costa Vicentina* (Faro: Relatório técnico do Projecto MARSW).

Huggett, J., and Griffiths, C. L. (1986). Some relationships between elevation, physico-chemical variables and biota of intertidal rock pools. *Mar. Ecol. Prog. Ser.* 29, 189–197. doi: 10.3354/MEPS029189

Iwasaki, K. (1993). "Individual variation, social structure, community organization and hierarchical views in the marine context" in *Mutualism and community organization: behavioural, theoretical and food-web approaches* H. Kawanabe, et al (eds). (Oxford: Oxford University Press), 13–36. (not seen).

Jenkins, S. R., Coleman, R. A., Della Santina, P., Hawkins, S. J., Burrows, M. T., and Hartnoll, R. G. (2005). Regional scale differences in the determinism of grazing effects in the rocky intertidal. *Mar. Ecol. Prog. Ser.* 287, 77–86. doi: 10.3354/meps287077

Jenkins, S. R., Norton, T. A., and Hawkins, S. J. (1999). Interactions between canopy forming algae in the eulittoral zone of sheltered rocky shores on the Isle of Man. *J. Mar. Biolog. Assoc. U. K.* 79 (2), 341–349. doi: 10.3354/meps08729

Johnson, M. P. (2001). Metapopulation dynamics of *Tigriopus brevicornis* (Harpacticoida) in intertidal rock pools. *Mar. Ecol. Prog. Ser.* 211, 215–224. doi: 10.3354/meps211215

Johnson, M. P., Burrows, M. T., Hartnoll, R. G., and Hawkins, S. J. (1997). Spatial structure on moderately exposed rocky shores: patch scales and the interactions between limpets and algae. *Mar. Ecol. Prog. Ser.* 160, 209–215. doi: 10.3354/meps160209

Kooistra, W. H. C. F., Joosten, A. M. T., and Van den Hoek, C. (1989). Zonation patterns in intertidal pools and their possible causes: A multivariate approach. *Bot. Mar.* 32 (2), 9–26. doi: 10.1515/botm.1989.32.1.9

Legrand, E., Riera, P., Pouliquen, L., Bohner, O., Cariou, T., and Martin, S. (2018). Ecological characterization of intertidal rockpools: Seasonal and diurnal monitoring of physico-chemical parameters. *Reg. Stud. Mar. Sci.* 17, 1–10. doi: 10.1016/j.rsma.2017.11.003

Lewis, J. R. (1954). Observations on a high-level population of limpets. *J. Anim. Ecol.* 23 (1), 85–100. doi: 10.2307/1661

Lewis, J. R. (1986). Latitudinal trends in reproduction, recruitment and population characteristics of some rocky littoral molluscs and cirripedes. *Hydrobiologia* 142, 1–13. doi: 10.1007/BF00026742

Lewis, J. R., and Bowman, R. S. (1975). Local habitat-induced variations in the population dynamics of *Patella vulgata* L. *J. Exp. Mar. Biol. Ecol.* 17 (2), 165–203. doi: 10.1016/0022-0981(75)90029-5

Lima, F. P., Gomes, F., Seabra, R., Wethey, D. S., Seabra, M. I., Cruz, T., et al. (2016). Loss of thermal refugia near equatorial range limits. *Glob. Change Biol.* 22 (1), 254–263. doi: 10.1111/gcb.13115

Lima, F. P., Queiroz, N., Ribeiro, P. A., Hawkins, S. J., and Santos, A. M. (2006). Recent changes in the distribution of a marine gastropod, *Patella rustica* Linnaeus 1758 and their relationship to unusual climatic events. *J. Biogeogr.* 33 (5), 812–822. doi: 10.1111/j.1365-2699.2006.01457.x

Martins, G. M., Hawkins, S. J., Thompson, R. C., and Jenkins, S. R. (2007). Community structure and functioning in intertidal rock pools: effects of pool size and shore-height at different successional stages. *Mar. Ecol. Prog. Ser.* 329, 43–55. doi: 10.3354/meps329043

Marzinelli, E. M., Underwood, A. J., and Coleman, R. A. (2012). Modified habitats change ecological processes affecting a non-indigenous epibiont. *Mar. Ecol. Prog. Ser.* 446, 119–129. doi: 10.3354/meps09472

Masterson, P., Arenas, F. A., Thompson, R. C., and Jenkins, S. R. (2008). Interaction of top down and bottom up factors in intertidal rockpools: effects on early successional macroalgal community composition, abundance and productivity. *J. Exp. Mar. Biol. Ecol.* 363 (1-2), 12–20. doi: 10.1016/j.jembe.2008.06.001c

McQuaid, C. D., and Froneman, P. W. (1993). Mutualism between the territorial intertidal limpet *Patella longicosta* and the crustose alga *Ralfsia verrucosa*. *Oecologia* 96, 128–133. doi: 10.1007/BF00318040

Menconi, M., Benedetti-Cecchi, L., and Cinelli, F. (1999). Spatial and temporal variability in the distribution of algae and invertebrates on rocky shores in the northwest Mediterranean. *J. Exp. Mar. Biol. Ecol.* 233 (1), 1–23. doi: 10.3354/meps215079

Metaxas, A., Hunt, H. L., and Scheibling, R. E. (1994). Spatial and temporal variability of macrobenthic communities in tidepools on a rocky shore in Nova-Scotia, Canada. *Mar. Ecol. Prog. Ser.* 105, 89–103. doi: 10.3354/meps105089

Metaxas, A., and Scheibling, R. E. (1993). Community structure and organization of tidepools. *Mar. Ecol. Prog. Ser.* 98, 187–198. doi: 10.3354/meps098187

Moore, P., Hawkins, S. J., and Thompson, R. C. (2007). Role of biological habitat amelioration in altering the relative responses of congeneric species to climate change. *Mar. Ecol. Prog. Ser.* 334, 11–19. doi: 10.3354/meps334011

Nobre, D., Seabra, M. I., and Cruz, T. (2019). Patterns of the vertical distribution limits of the limpet *Patella depressa* on the Portuguese coast. *Front. Mar. Sci. Conf. Abstract.: IMMR'18. | Int. Meeting. Mar. Res. 2018.* doi: 10.3389/conf.FMARS.2018.06.00051

Noël, L., Hawkins, S. J., Jenkins, S. R., and Thompson, R. C. (2009). Grazing dynamics in intertidal rockpool: connectivity in microhabitats. *J. Exp. Mar. Biol. Ecol.* 370 (1-2), 9–17. doi: 10.1016/j.jembe.2008.11.005

Ocaña, T. M. (2003). Growth, mortality and longevity in two populations of *Siphonaria pectinata* (Pulmonata) at Gibraltar. *J. Molluscan. Stud.* 69 (2), 162–164. doi: 10.1093/mollus/69.2.162

Oróstica, M. H., Hawkins, S. J., Broitman, B. R., and Jenkins, S. R. (2020). Performance of a warm-water limpet species towards its poleward range edge compared to a colder-water congener. *Mar. Ecol. Prog. Ser.* 656, 207–225. doi: 10.3354/meps13461

Ortiz, M., Hermosillo-Nuñez, B., González, J., Rodríguez-Zaragoza, F., Gómez, I., and Jordán, F. (2017). Quantifying keystone species complexes: Ecosystem-based conservation management in the King George Island (Antarctic Peninsula). *Ecol. Indic.* 81, 453–460. doi: 10.1016/j.ecolind.2017.06.016

Orton, J. H. (1929). Observations on *Patella vulgata*. Part III. Habitat and habits. *J. Mar. Biolog. Assoc. U. K.* 16 (1), 277–288. doi: 10.1017/S0025315400029805

Ostalé-Valriberas, E., Sempere-Valverde, J., Coppa, S., García-Gómez, J. C., and Espinosa, F. (2018). Creation of micro-habitats (tidepools) in ripraps with climax communities as a way to mitigate negative effects of artificial substrate on marine biodiversity. *Ecol. Eng.* 120, 522–531. doi: 10.1016/j.ecoleng.2018.06.023

Rivera-Ingraham, G. A., Espinosa, F., and García-Gómez, J. C. (2011). Effect of γ-amino butyric acid on limpet populations: towards the future management and conservation of endangered patellid species. *J. Chem. Ecol.* 37, 1–9. doi: 10.1007/s10886-010-9884-1

Rubal, M., Veiga, P., Cacabelos, E., Moreira, J., and Sousa-Pinto, I. (2013). Increasing sea surface temperature and range shifts of intertidal gastropods along the Iberian Peninsula. *J. Sea. Res.* 77, 1–10. doi: 10.1016/j.seares.2012.12.003

Rubal, M., Veiga, P., Maldonado, C., Torres, C., and Moreira, J. (2015). Population attributes and traits of *Siphonaria pectinata* (Mollusca: Siphonariidae) in range-edge and non range-edge populations at its Eastern Atlantic northern distribution boundary. *J. Exp. Mar. Biol. Ecol.* 471, 41–47. doi: 10.1016/j.jembe.2015.05.015

Rubal, M., Veiga, P., Vieira, R., and Sousa-Pinto, I. (2011). Seasonal patterns of tidepool macroalgal assemblages in the North of Portugal. Consistence between species and functional group approaches. *J. Sea. Res.* 66 (3), 187–194. doi: 10.1016/j.seares.2011.07.003

Schaefer, N., Mayer-Pinto, M., Johnston, E. L., and Dafforn, K. A. (2023). Understanding the role of microhabitats in intertidal rock pools to guide future eco-engineering designs. *Mar. Biol.* 170 (4), 44. doi: 10.1007/s00227-023-04196-2

Seabra, M. I., Cruz, T., Fernandes, J. N., Silva, T., and Hawkins, S. J. (2019). Recruitment of the limpet *Patella ulyssiponensis* and its relationship with crustose coralline algae: patterns of juvenile distribution and larval settlement. *J. Mar. Biolog. Assoc. U. K.* 99 (8), 1787–1796. doi: 10.1017/S0025315419000869

Seabra, M. I., Hawkins, S. J., Espírito-Santo, C., Castro, J. J., and Cruz, T. (2020). Rockpools as nurseries for co-existing limpets: Spatial and temporal patterns of limpet recruitment. *Reg. Stud. Mar. Sci.* 37, 101339. doi: 10.1016/j.rsma.2020.101339

Seabra, R., Wethey, D. S., Santos, A. M., Gomes, F., and Lima, F. P. (2016). Equatorial range limits of an intertidal ectotherm are more linked to water than air temperature. *Glob. Change Biol.* 22 (10), 3320–3331. doi: 10.1111/gcb.13321

Seabra, R., Wethey, D. S., Santos, A. M., and Lima, F. P. (2011). Side matters: microhabitat influence on intertidal heat stress over a large geographical scale. *J. Exp. Mar. Biol. Ecol.* 400 (1-2), 200–208. doi: 10.1016/j.jembe.2011.02.010

Silva, A., Boaventura, D., and Ré, P. (2003). Population structure, recruitment and distribution patterns of *Patella depressa* Pennant 1777 on the central Portuguese coast. *Bol. Inst. Esp. Oceanogr.* 19 (1/4), 461.

Slama, T., Abidli, S., El Menif, N. T., and Lahbib, Y. (2022). Grazer gastropods as alternative species for monitoring butyltins contamination in harbors. *J. Sea. Res.* 190, 102287. doi: 10.1016/j.seares.2022.102287

Slama, T., Nouet, J., Chassefière, E., El Menif, N. T., and Lahbib, Y. (2021). Monitoring of coastal pollution using shell alterations in the false limpet *Siphonaria pectinata*. *Mar. pollut. Bull.* 173, 113063. doi: 10.1016/j.marpolbul.2021.113063

Sokal, R. R., and Rohlf, F. J. (1995). *Biometry: The Principles and Practice of Statistics in Biological Research. 3rd Edition* (New York: W.H. Freeman and Co.).

Sorensen, F. E., and Lindberg, D. R. (1991). Preferential predation by American black oystercatchers on transitional ecophenotypes of the limpet *Lottia pelta* (Rathke). *J. Exp. Mar. Biol. Ecol.* 154 (1), 123–136. doi: 10.1016/0022-0981(91)90078-B

Souza, S. M., and Matthews-Cascon, H. (2019). Molluscan assemblages in rock pools on sandstone reefs: local and between pools variability. *J. Shellfish. Res.* 38 (1), 201–208. doi: 10.2983/035.038.0119

Sun, Y. X., Hu, L. S., and Dong, Y. W. (2023). Micro-habitat-specific diurnal metabolomic responses of the intertidal limpet *Cellana toreuma* to winter low temperature. *iScience* 26, 3. doi: 10.1016/j.isci.2023.106128

Tanner, J. E., Hughes, T. P., and Connell, J. H. (1994). Species coexistence, keystone species, and succession: a sensitivity analysis. *Ecology* 75 (8), 2204–2219. doi: 10.2307/1940877

Thompson, G. B. (1979). Distribution and population dynamics of the limpet *Patella aspera* (Lamarck) in Bantry Bay. *J. Exp. Mar. Biol. Ecol.* 40 (2), 115–135. doi: 10.1016/0022-0981(79)90039-X

Thompson, G. B. (1980). Distribution and population dynamics of the limpet *Patella vulgata* L. @ in Bantry Bay. *J. Exp. Mar. Biol. Ecol.* 45 (2), 173–217. doi: 10.1016/0022-0981(80)90058-1

Underwood, A. J., and Skilleter, G. A. (1996). Effects of patch-size on the structure of assemblages in rock pools. *J. Exp. Mar. Biol. Ecol.* 197 (1), 63–90. doi: 10.1016/0022-0981(95)00145-X

Vasconcelos, P., Janeiro, F., Pereira, F., Moura, P., Carvalho, A. N., and Gaspar, M. B. (2021). Shell shape, morphometrics and relative growth of four sympatric limpet species from the Algarve coast (southern Portugal). *J. Mar. Biolog. Assoc. U. K.* 101 (5), 791–800. doi: 10.1017/S0025315421000734

Vermeij, G. J. (2017). The limpet form in gastropods: evolution, distribution, and implications for the comparative study of history. *Biol. J. Linn. Soc Lond.* 120 (1), 22–37. doi: 10.1111/bij.12883

Williams, G. A., and Morritt, D. (1995). Habitat partitioning and thermal tolerance in a tropical limpet, *Cellana grata*. *Mar. Ecol. Prog. Ser.* 124, 89–103. doi: 10.3354/meps124089

Wolcott, T. G. (1973). Physiological ecology and intertidal zonation in limpets (Acmaea): a critical look at" limiting factors". *Biol. Bull.* 145 (2), 389–422. doi: 10.2307/1540048

Wolfe, J., and Harlin, M. (1988). Tidepools in Southern Rhode Island, U.S.A. II. Species diversity and similarity analysis of macroalgal communities. *Bot. Mar.* 31 (6), 537–546. doi: 10.1515/botm.1988.31.6.537

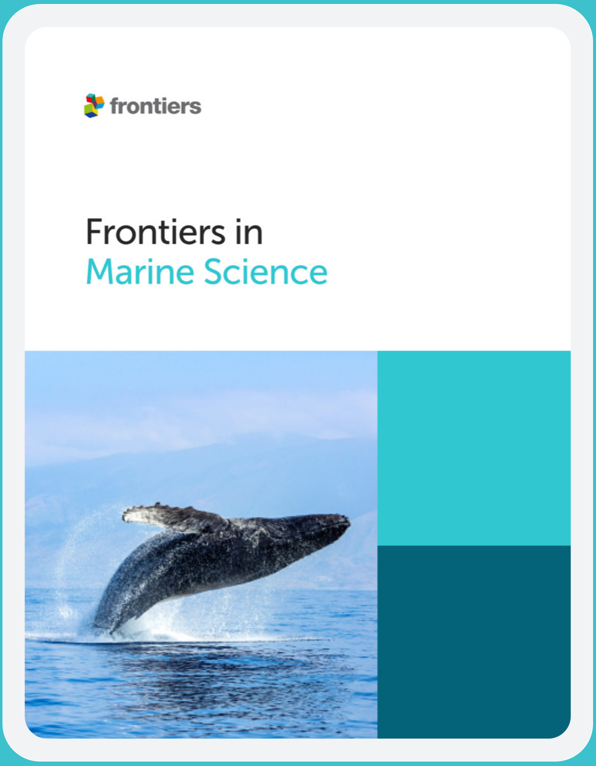
Frontiers in
Marine Science

Explores ocean-based solutions for emerging global challenges

The third most-cited marine and freshwater biology journal, advancing our understanding of marine systems and addressing global challenges including overfishing, pollution, and climate change.

Discover the latest Research Topics

See more →



frontiers

Frontiers in
Marine Science

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact

frontiers | Research Topics