Universidade de Évora - Instituto de Investigação e Formação Avançada

Programa de Doutoramento em Biologia

Tese de Doutoramento

Rock-pools and limpets: patchiness and connectivity in temperate intertidal reefs

Maria Inês Gomes Leandro de Seabra

Orientador(es) | Teresa Paula Cruz
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Aos meus pais São e Jorge

“na maré vaza as rochas apareciam cobertas de limos, de búzios, de anémonas, de lapas, de algas e de ouriços. Havia poças de água, rios, caminhos, grutas, arcos, cascatas. Havia pedras de todas as cores e feitios, pequeninas e macias, polidas pelas ondas. E a água do mar era transparente e fria.

(...)

Com muito cuidado, para não fazer barulho, levantou-se e pôs-se a espreitar escondido entre duas pedras. E viu um grande polvo a rir, um caranguejo a rir, um peixe a rir e uma menina muito pequenina a rir também. A menina, que devia medir um palmo de altura, tinha cabelos verdes, olhos roxos e um vestido feito de algas encarnadas. E estavam os quatro numa poça de água muito limpa e transparente, toda rodeada de anémonas. E nadavam e riam.”

Sophia de Mello Breyner Andresen, *A menina do mar*, Ed. *Figueirinhas*

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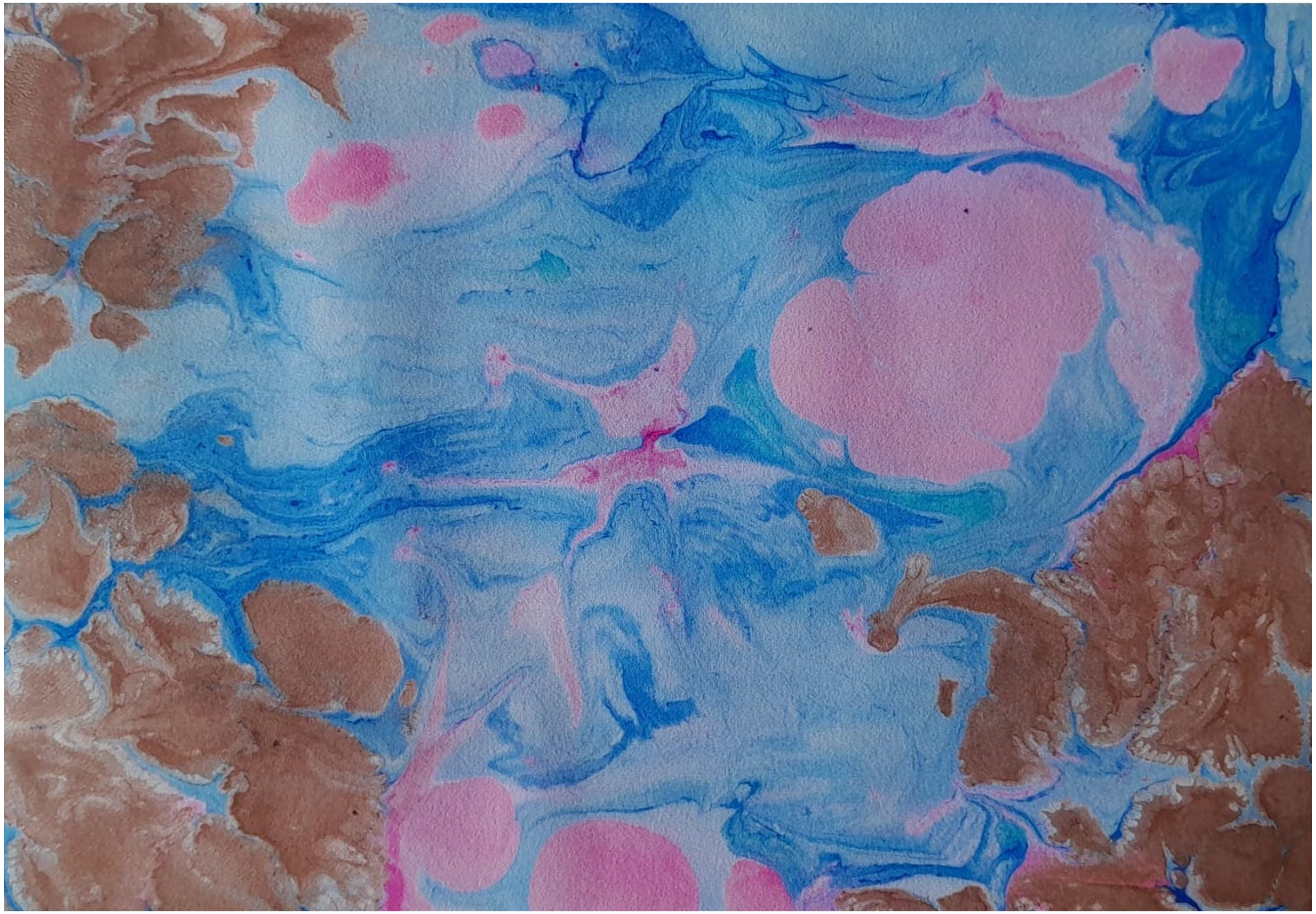

Rock-pool by Clara.

Rock-pool by Alice.

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Abstract

This thesis advanced the knowledge of temperate reef ecology by examining the role of rock-pools as mosaic microhabitats for intertidal limpets. Five limpet species – *Patella ulyssiponensis* (PU)*, P. depressa* (PD)*, P. rustica* (PR), *P. vulgata* (PV), and *Siphonaria pectinata* (SP) – were studied (distribution, density, and size-structure) inside and outside rock-pools along the Portuguese southwest coast. Recruitment and population dynamics of the three most common species (PU, PD, and SP) were examined across distinct microhabitats near Sines (SW Portugal), including natural and artificial habitats inside the Port of Sines.

Juveniles of PU were almost exclusively found in microhabitats dominated by crustose coralline algae (CCA). There was no evidence of CCA-enhanced settlement from an experiment in vitro conditions. CCA are crucial for the early benthic life of PU, likely promoting post-settlement survival.

Juveniles of PD and SP were widely distributed in moist microhabitats, and most abundant in rock-pools. PU, PD, and SP recruited into mid-shore rock-pools year-round. Recruitment was lower inside the Port, where artificial structures proliferate. Rock-pools in SW Portugal are nurseries for both patellid and siphonariid limpets.

Limpets (PU, PD, and SP) of various sizes co-occurred across rock-pools, pool edges, and open-rock; rock-pools extend their vertical distribution upwards. PV occurred most frequently along pool edges; PR was absent from rock-pools and their edges. Patterns of intra-specific density evidenced pool-residency of PU above the low-shore, and gradual migration of PD and SP outwards from rock-pools. Landscape variability (heterogeneity of microhabitats) of shores with different geomorphology influenced the population structure of PD on the mid-shore open-rock. CCA cover predicted the abundance of both patellids inside rock-pools.

Over a decade-long experiment on rock-armour structures in the Port of Sines, drill-cored rock-pools were found to provide limpet nurseries and source-areas for their ontogenic emigration to open-rock. Effects were species-specific (abundance of PD and SP was enhanced by four and nine times, respectively) and context-dependent (shaped by the colonization of pool assemblages). Eco-engineered rock-pools can generate persistent and cross-microhabitat biodiversity benefits.

Resumo

Poças-de-maré e lapas: fragmentação e conectividade em litorais rochosos entremarés de regiões temperadas

Esta tese contribuiu para o conhecimento da ecologia de litorais rochosos de regiões temperadas ao examinar o papel das poças-de-maré como micro-habitats em mosaico para lapas em ambientes entremarés. Cinco espécies de lapas – *Patella ulyssiponensis* (PU), *P. depressa* (PD), *P. rustica* (PR), *P. vulgata* (PV) e *Siphonaria pectinata* (SP) – foram estudadas (distribuição, densidade e estrutura dimensional) dentro e fora de poças-de-maré ao longo da costa sudoeste portuguesa. O recrutamento e a dinâmica populacional foram examinados em microhabitats distintos para as três espécies de lapas mais comuns (PU, PD e SP), na linha de costa perto de Sines (SW Portugal), incluindo habitats naturais e artificiais dentro do Porto de Sines.

Os juvenis de *P. ulyssiponensis* foram encontrados quase exclusivamente em micro-habitats dominados por algas coralináceas incrustantes (CCA). Não houve evidência de assentamento larvar induzido por CCA numa experiência em condições *in vitro*. As CCA são cruciais para o recrutamento de PU, provavelmente promovendo a sobrevivência dos primeiros estádios de vida bentónica após o assentamento larvar.

Os juvenis de *P. depressa* e *S. pectinata* distribuíram-se amplamente em micro-habitats húmidos; sendo mais abundantes em poças-de-maré; PU, PD e SP recrutaram durante todo o ano em poças-de-maré no nível médio de maré; o recrutamento foi menor no interior do Porto, onde proliferam as estruturas artificiais. As poças-de-maré no sudoeste de Portugal são berçários de lapas para patelídeos e sifonariídeos.

A presença de lapas (PU, PD e SP) de vários tamanhos foi observada em poças-de-maré, margens de poças e rocha emersa circundante. As poças-de-maré estenderam a distribuição vertical destas espécies para cotas superiores. PV ocorreu com maior frequência em redor de margens de poças; *P. rustica* não ocorreu em poças-de-maré nem nas suas margens. Os padrões de densidade intra-específica evidenciaram a residência de PU em poças-de-maré acima do nível intertidal inferior e uma migração gradual de PD e SP para fora de poças-de-maré. A variabilidade da paisagem (heterogeneidade de microhabitats) de praias com diferente geomorfologia influenciou a estrutura populacional de PD em rocha emersa do nível intertidal médio de maré. A cobertura por CCA previu a abundância de ambos os patelídeos no interior de poças-de-maré.

Ao longo de uma experiência de uma década em estruturas artificiais no Porto de Sines, descobriu-se que as poças-de-maré criadas artificialmente através da perfuração de blocos fornecem berçários de lapas e áreas de origem para a sua emigração ontogénica para rocha emersa. Os efeitos foram específicos para cada espécie (a abundância de PD e SP foi aumentada em quatro e nove vezes, respetivamente) e dependentes do contexto local (moldados pela colonização de povoamentos bentónicos de poças-de-maré). As poças-de-maré artificiais podem gerar benefícios persistentes para a biodiversidade de uma forma transversal em vários micro-habitats.

Contents

CHAPTER 1: General Introduction

My thesis examines how intertidal rock-pools influence limpet populations, both of patellids and of the co-occurring siphonariid *Siphonaria pectinata* in SW Portugal. Focusing on their early shore-life stages, I have investigated the primacy of rock-pools as nurseries for limpets. The role of rock-pools as conspicuous, diverse, and patchy microhabitats for limpets of various species and sizes has also been investigated. By studying limpets inhabiting rock-pools and the surrounding microhabitats, knowledge has been acquired on the ecological importance of rock-pools as source areas of limpet emigration onto open-rock. This has practical applications for ecological engineering of marine infrastructure in highly modified coastlines.

In this introductory chapter, I have reviewed the literature on main topics and relevant concepts. Firstly, I outline the suitability of rocky-shores as study systems and the ecological importance of limpets. Then, the understudied topic of rock-pools is considered, particularly regarding their uniqueness and consequently high among-rockpool variability. Next, I summarize the uses of rock-pools by several limpet species worldwide. The association between crustose coralline algae (CCA) and limpets across several life-stages, especially its significance as a positive interaction influencing limpet recruitment inside rock-pools, is also highlighted. The role of rock-pools for limpet populations is briefly considered relative to the concepts of nursery habitat, as well as settlement and recruitment. I then introduce the concepts of patchiness and connectivity by clarifying the nature and scales which apply in the scope of this thesis and their distinction from other concepts with similar designations. I have also reviewed the main impacts of coastal artificial structures and the efficacy of eco-engineering interventions at the global level, particularly focusing on the work concerning eco-engineered rock-pools. I briefly introduce the study area, the Southwest Portuguese coast including the Port of Sines. I then introduce the focal species, *Patella ulyssiponensis* the China limpet, *Patella depressa* the Black-footed limpet, *Patella vulgata* the Common limpet, *Patella rustica* the Lusitanian limpet, and *Siphonaria pectinata* the Striped limpet. Finally, I outline the rationale and structure of the thesis in terms of overall aim and specific objectives of chapters.

1. Literature review

1.1. Rocky shores, limpets, and heterogeneity of microhabitats

Rocky shores (also known as intertidal reefs) are windows on the ocean for many people. Worldwide rocky shores support high biodiversity and provide a myriad of benefits for human welfare (Miloslavich et al., 2016). These can be categorized as provisioning (direct food supply), regulating (biological, atmospheric, and climatic regulation, including biofiltration and coastal protection), supporting (primary productivity, nutrient cycling, and ecosystem maintenance) and cultural (recreation and tourism, education, and research) ecosystem services (Vinueza et al., 2014; Elliff and Kikuchi, 2015). Rocky shores are also a sentinel system for detecting global change in the wider ocean, as changes at the seashore reflect those offshore in the plankton, benthos, and fish (Southward, 1980; Southward et al., 1995, 2004; Mieszkowska et al., 2014). Moreover, rocky shores provide accessible observatories for sustained surveillance of species and long-term monitoring of biological responses to warming and higher frequency of extreme events (Southward et al., 1995; Hawkins et al., 2008, 2009; Helmuth et al., 2016; Hawkins et al., 2022, Hesketh and Harley, 2023).

Rocky shores have been extensively used as tractable model systems for experimental ecology, bringing important contributions to general ecological theory (Hawkins et al., 2020a). Our understanding of ecological patterns and processes on rocky shores has been particularly enriched by rigorously designed studies, which gather quantitative data using descriptive or manipulative experimental approaches, thereby prompting continuous development and testing of logical hypotheses (Underwood, 2000; Underwood et al., 2000). Pioneering manipulative experiments on rocky shores have shown that a strongly-interacting species can have a major top-down effect on species diversity patterns and competition (Paine, 1966). Paine (1969a, 1969b) first coined the term “keystone” species, as those that determine the structure and persistence of biological assemblages by exerting a disproportionately large effect relative to their abundance or biomass-dominance. Although this concept has been challenged (e.g. criticism by Mills et al., 1993), then redefined (e.g. Paine et al., 1995; Davic, 2003) and revalidated (e.g. Menge et al., 1994; Power et al., 1996), there is growing evidence that there are grazing or predatory species of clearly demonstrable importance for the overall ecosystem functioning (O’Connor and Crowe, 2005; Cottee-Jones and Whittaker, 2012). Consumer impacts of marine herbivores on reducing the abundance of benthic primary producers were found across the globe, with the highest magnitude in rocky intertidal habitats (Poore et al., 2012). Grazing by rocky-intertidal gastropods has been known to have a profoundly important role in the community structure and functioning on both tropical (Macusi and Deepananda, 2013 and references therein) and temperate (e.g. Lubchenco and Gaines, 1981; Hawkins and Hartnoll, 1983; Aguilera et al., 2013) regions.

"True limpets" (Patellogastropoda) are often recognized as keystone species of temperate intertidal reefs (e.g. Henriques et al., 2017 and references therein; Fraser et al., 2015). They have been the subject of extensive research, given their wide distribution, high relative abundance, diversity, ecological importance, and ease of manipulation (Branch, 1981; Underwood, 1979; Lindberg, 2008). Patellogastropods of the genus *Patella* are ubiquitous grazers in European rocky shores (Southward et al., 1995; Benedetti-Cecchi et al., 2001; Weber and Hawkins, 2005; Freitas et al., 2023). According to Hawkins et al. (2019), the importance of grazing by patellid limpets in controlling algal vegetation has been demonstrated in over 70 years of field experiments in the NE Atlantic (e.g. Jones, 1946, 1948; Hawkins, 1981a, b; Jenkins et al., 2005; Coleman et al., 2006). North-East Atlantic patellids also have been recently considered as ecosystem engineers, promoting both trophic and facilitation cascades on rocky-intertidal communities (Firth et al., 2023). In contrast, siphonariids (Heterobranchia), frequently called "false limpets" as phylogenetically unrelated to the Patellogastropoda (White, 2011), are generally associated with warmer latitudes (Dayrat et al., 2014) and their ecology has been comparatively less studied (but see Hodgson, 1999 for a review).

Intertidal limpets, and rocky-shore ecology in general, have been mostly studied upon open-rock microhabitats (also known as emergent substrata): emersed surfaces on freely-draining rock during low tide. Comparatively little is known about limpets living within permanently-immersed or moist microhabitats such as rock-pools, edge of rock-pools, crevices, overhangs, or vertical surfaces. These areas correspond to specific structural components linked to topographic complexity of the rocky substratum, which positively affect the density and richness of rocky intertidal gastropods (e.g. Beck, 2000). Topographically-complex microhabitats of rocky shores are ultimately formed by physical properties of the substrata namely the lithology and shaped by the local geomorphology (geogenic origin). However, they can be also reshaped by biological processes (biogenic origin), namely those driven by the presence of organisms that secrete calcium carbonate (calcareous algae and reef-building invertebrates such as oysters, mussels and worms) or canopy-forming algae (e.g. kelps and fucoids). Geogenic and biogenic microhabitats create patchiness (due to their fragmented nature) and variability at the intertidal landscape (heterogeneity in the mosaic of microhabitats available within shores), with positive consequences for biodiversity and ecosystem functioning (e.g. Johnson et al., 2003; Firth et al., 2014a).

1.2 Rock-pools

Rock-pools (also known as tidepools or tidal pools) are particularly attractive places for nature exploration and marine life observation. In his book entitled "*Glimpses of ocean life; or, rockpools and the lessons they teach*", the marine zoologist John Harper (1960) states that rock-pools are poetically called "fairy-grots" (p. 153). They are rock cavities that retain seawater during low-tide, forming well-delimited, patchily-distributed, and distinctive features in the intertidal landscape (see Metaxas and Scheibling, 1993 for a review). Rock-pools can be seen as oases of biodiversity amongst usually less diverse open-rock (Firth et al. 2013a, 2014a). Rock-pools are likely an essential component of the mosaic of (micro)habitats of the seashore, due to their ubiquity and inherent fragmented nature. Despite extensive work on rocky shores over the last century and gathering pace of experimental studies in the last five decades, until the last decade surprisingly little was known about the ecology of rock-pools. This was perhaps because the intrinsic variability present among different rock-pools due to their unique characteristics, creating highly-variable patterns at small spatial and temporal scales (e.g. Dethier, 1984; Metaxas et al, 1994; Hunt and Scheibling, 1995; Benedetti-Cecchi, 2000).

Early studies on the ecology of rock-pools were mostly qualitative, focused on classification of typologies of rock-pools (e.g. Goss-Gustard et al., 1979; Wolfe and Harlin, 1988) or on the environmental tolerance of organisms living on rockpools (e.g. Naylor and Slinn, 1958). Quantitative studies followed on patterns of diversity and composition of rock-pool assemblages (e.g. Dethier, 1982; Kooistra, 1989; Astles, 1993; Zhuang, 2006; González-Murcia et al., 2020; Schaefer et al., 2024), their relationships with physical and biological characteristics of rock-pools (e.g. Martins et al., 2007; White et al., 2015), and experiments on the underlying abiotic and biotic processes shaping rock-pool communities (e.g. Dethier, 1984; Benedetti-Cecchi and Cinelli, 1992, 1995, 1996; Benedetti-Cecchi et al., 2005, Masterson et al., 2008). To counter the intrinsic variability of rock-pools, arrays of replicate rock-pools have been drilled out on natural shores for hypotheses-testing on identical systems (van Tamelen, 1996; Underwood and Skilleter, 1996; Nielsen, 2001; Schaefer et al., 2023).

There were some early (Daniel and Boyden, 1975; Morris and Taylor, 1983; Huggett and Griffiths, 1986) and more recent (Legrand et al., 2018; Clarke and Beaumont, 2020) studies on the physical-chemical environment of rock-pools. The physical-chemical environment of rock-pools fluctuates at various spatial and temporal scales (Morris and Taylor, 1983). The degree of fluctuations is regulated by the tidal cycle (Huggett and Griffiths, 1986), interacting with the specific characteristics of each unique rock-pool such as size, shape, tidal elevation, wave action and algal biomass (Astles, 1993; Metaxas and Scheibling, 1993 and references therein). The balance of community photosynthesis and respiration over diurnal and tidal cycles determines gas concentrations, leading to high concentrations of oxygen and high pH during day-time tide out, with high carbon dioxide and hence low pH at night (Morris and Taylor, 1983; Huggett and Griffiths, 1986; Denny and Gaines, 2007).

Rock-pools support distinct and diverse assemblages, depending upon several biotic and abiotic interacting processes. In recent years, these microhabitats have been utilized as convenient mesocosms for work on the relationship between biodiversity and ecosystem functioning (e.g. O'Connor and Crowe, 2005; Griffin et al., 2010). They have been found to support complex food webs (Mendonça et al., 2018; Gauzens et al, 2020; Vinagre and Mendonça, 2023). Due to their constant submersion, rock-pools may provide a more benign habitat for rockpool-dwelling organisms in comparison with the surrounding open-rock. They offer refuges from harsh intertidal conditions to fishes and soft-bodied animals (e.g. Clark, 1968; Newell, 1979; Vinagre et al., 2019), and allow the extension of the upper distribution limits of several species (e.g. Goss-Custard et al., 1979). On the other hand, the upward penetration of rockpool populations of marine invertebrates was found to be limited by energy balance, as exposure to air is energetically less costly than isolation in a rock-pool in temperate regions (Clarke and Griffiths, 1990). In tropical latitudes, rock-pools have been found to be especially stressful microhabitats acting as ecological traps (Vinagre et al., 2018).

To date, ecological studies on rock-pools in mainland Portugal have examined spatial and temporal patterns of benthic assemblages, namely focusing on macroalgae (Araújo et al., 2006; Rubal et al., 2011), algal plus invertebrate assemblages (Bertocci et al., 2012), amphipods (Carvalho et al., 2021), molluscs (Gonçalves et al., 2023), and whole food webs (Mendonça et al., 2018; Vinagre and Mendonça, 2023). Thermal physiology (Vinagre et al., 2013, 2016, 2019; Madeira et al., 2015), as well as effects of loss of canopies (Bertocci et al., 2014) and invasive algal species (Silva et al., 2020, 2021) were studied for rockpool inhabitants in the Portuguese coast. Marine nektonic species in this coast have been found to use rock-pools as feeding grounds (e.g. Dias et al., 2014), and/or as nursery grounds for fishes (Dias et al., 2016; Roma et al., 2018) and shrimp (Vinagre et al., 2015). Therefore, rock-pools possibly influence processes within and beyond their limits; especially in what regards motile animals, that use rock-pools for early life stages or during another part of their life-cycle, either remaining rockpool-resident or migrating to outer populations afterwards.

1.3 Uses of rock-pools by limpets and biotic interactions with CCA

Information on limpets occupying rock-pools was summarised for 60 limpet species that were reported in the literature to occur inside rock-pools or their edges (Supplementary Table S1.1). Listed species include limpets from different taxonomic groups (Subclass Patellogastropoda: Families Nacellidae, Patellidae, Fissurellidae, Lottiidae; Subclass Heterobranchia: Family Siphonariidae), world regions (including tropical and temperate rocky-shores in all continents), and individual sizes (including very small limpets referred as "recruits", plus juveniles and adult life stages).

Limpet species occur and use rock-pools in diverse ways. Rock-pools and moist microhabitats are well known to provide nursery grounds for limpets (e.g. Lewis and Bowman, 1975; Guerra and Gaudêncio, 1986; Delany et al., 1998) or home refugia for their temporary or permanent residence (e.g. Garrity, 1984; Williams and Morritt, 1995; Firth and Williams, 2009). Such refuges reduce thermal stress (from hot or cold conditions) or desiccation, due to water or moisture retention (e.g. Bowman, 1981; Sun et al., 2023). Siphonariids often lay egg masses in rock-pools, from which planktonic developing larvae or directly-developed juveniles hatch (reviews by Chambers and McQuaid, 1994a, b). Some species of limpets use pool edges as a thermally protective microhabitat (Williams and Morritt, 1995; Branch, 1975b) or to establish home scars from which they migrate across the transition between rock-pools and surrounding rock (e.g. Delany et al., 2018; Delany et al., 2002; Noël et al., 2009).

Limpets have been also known to form associations with CCA inside rock-pools (Branch, 1973; Steneck, 1982; Delany et al., 2002; McGrath 1992). In some species, the associations are temporary as in *Patella pellucida* (e.g. McGrath, 1992; McGrath et al., 2005), while they can be maintained across the limpet life span in specialist species such as *Testudinalia testudinalis* in North America or *Patella ulyssiponensis* in the North-east Atlantic and Mediterranean coasts (Steneck, 1982; Delany et al., 2002). These limpet-CCA associations can also occur outside rock-pools, in other intertidal wet/damp areas and subtidal environments. This is the case of the territorial limpet *Scutellastra cochlear*, that is extremely abundant in CCA-dominated low-shore areas in South Africa (Branch, 1975a). Furthermore, smaller recruits of *Patella ferruginea* were found on the lower infralittoral on the fringe of the vermetid gastropod *Dendropoma lebeche* and the CCA *Neogoniolithon brassica-florida*, suggesting localized settlement at this shore level (Guallart et al., 2022).

It has long been known that there is an intimate relationship between CCA and larval settlement of other gastropods, the most classic case being the one of abalone (Morse et al., 1979). This is considered in more detail in the introduction of Chapter 2 published as Seabra et la. (2019). Since that study was published, there has been an explosion in aquaculture-related work on *Patella* species from Madeira, also occurring throughout other Macaronesian Archipelagos (Canaries, Azores) (e.g. Castejón et al., 2021, 2022, 2023, 2024). This work has explored the presence of cues inducing their settlement on CCA, providing evidence of the ability of limpet larvae to detect chemical or biological cues at the CCA surfaces and trigger selective settlement (e.g. Castejón et al., 2021). This includes *Patella aspera*, a sister species of *P. ulyssiponensis* present in continental Europe (Weber and Hawkins, 2005).

1.4 Nursery ground and recruitment to a certain size

Limpets as outlined above (section 1.3) widely use rockpools as nurseries (see also Supplementary Table S.1.1). The nursery ground concept emerged primarily from the fisheries literature: post-larval or juvenile fish recruit to nurseries where they grow rapidly before migrating and joining the adult population (see Cushing, 1986). It applies to places that are essential for the survival of early life stages or that have greater contribution per unit area to the production of individuals that recruit to adult populations (Lefcheck et al., 2019). When settling from the plankton, many species of fish and invertebrates use estuaries and a range of nearshore-structured habitats as nurseries, including marshes, biogenic reefs and rocky reefs. Given the vulnerability of larvae and newly metamorphosed juveniles, settlement from the plankton and subsequent metamorphosis is usually in damp places; with rock-pools being used by several species as nursery microhabitats (Beckley, 1985; Bennett, 1987; Anger, 1995; Krück et al., 2009). Recruitment as a process should be considered as occurring *to* a defined phase in life history of a population (juvenile, adult stage), which can also be operationally defined as to a certain size class after settlement (Rodriguez et al., 1993) – an approach adopted in this thesis.

1.5 Patchiness and connectivity

Given their fragmentation and isolation during low-tide, rock-pools are often referred as “islands” or “enclaves” encompassed by a different environment (Saldanha, 1997). The term patchiness refers to the spatial patterning of fragmented microhabitats (patches) that form a mosaic of small habitat units distributed within a wider matrix (landscape). Patchiness implies heterogeneity, and heterogeneity is essential to biodiversity (Levin et al., 1994; Cacabelos et al., 2019). As explained in section 1.1, patchily distributed microhabitats, namely rock-pools as water-retaining features, are mostly a consequence of variation in the topography of the rocky substrata, but biotic interactions with foundation or habitat-forming species can also create habitat patchiness.

Within a mosaic of small habitat units (either geomorphologically or biologically-created microhabitats), links may occur between these units and the surrounding matrix, or among the different fragmented units across the intertidal seascape (Nagelkerken et al., 2015). These links can be operated by dispersal and migration of individuals of mobile species (Levin et al., 1994), namely ontogenic shifts that connect “seascape nurseries” (Nagelkerken et al., 2015). Due to the probable role of rock-pools as nurseries for limpets, and their subsequent migration to open-rock, this likely applies to connectivity of limpet populations over the intertidal landscape of shores. Cross-habitat connectivity is generally considered at the population level of individual species, but there are implications for inter-specific interactions and coexistence of different species, affecting whole community assemblages.

The term connectivity and associated theory is widespread in the scientific literature, with several concepts differing in meaning and application to different scales and domains in marine sciences. The contrast between connectivity of fluxes of matter (sensus Bishop et al., 2017) versus demographic connectivity (of populations) is important. Demographic connectivity can include genetic connectivity (i.e. panmixia, see Waples and Gaggiotti, 2006), larval connectivity (Pineda et al., 2010) and post-settlement connectivity (i.e post-settlement migration by individuals across the seascape: Weeks, 2017). The related concept of metapopulation network does not apply to this thesis, as it implies limited migration between microhabitat patches (Johnson, 2001).

Mosaics of physically and biologically generated habitats are easily visualized on rocky shores and apply to the spatial patterning of rock-pools. Limpets move across rock-pools and their surrounds; thus, their populations are connected through distinct microhabitats. An analogue terrestrial system that has been widely used as a model of microhabitat network is that of migration of mites across patches of mosses on emergent rocks (e.g. Strong, 1967; Hoyle and Gilbert, 2004; Gonzalez and Chaneton, 2002).

1.6 Artificial habitats and eco-engineering

Whilst many natural rocky shores remain on near pristine coastlines despite being susceptible to a variety of human impacts (Thompson et al., 2002), shores located in urbanized or industrial settings are dominated by hard artificial substrata with low topographic complexity and scarcity of patchy microhabitats (Moschella et al., 2005; Firth et al., 2016a). The extent of artificialized coastlines is increased by port installations (moles, breakwaters, quays, dock basins, hard standing for containers, onward transport infrastructure or roads and rail). Sea-defences often front reclaimed land or are built to combat flood and erosion risk of existing infrastructure and property (e.g. Airoldi et al., 2005; Loke et al., 2019). The deployment of hard artificial substrata along human-modified coastlines has destroyed natural habitats, changed local hydro and sediment dynamics and hence infauna (Martin et al., 2005). These new hard surfaces provide habitat to be colonized by marine benthic life (e.g. Chapman and Bulleri, 2003; Heery et al., 2017), but in comparison with natural rocky habitats support depauperate populations of particular species (e.g. Cacabelos et al., 2016; Moreira et al., 2006; Fauvelot et al., 2009), reduced number of native taxa (e.g. Chapman, 2003; Moschella et al., 2005), different species composition (e.g. Airoldi and Bulleri, 2011; Bulleri et al., 2005) and hence non-natural ecosystem functioning (e.g. Aguilera, 2018, Mayer-Pinto et al., 2018). Much attention has been given to the ecology of coastlines highly modified with maritime infrastructure to understand and mitigate the impacts of so-called "ocean sprawl" (Firth et al., 2016a; Bugnot et al., 2020; Hawkins et al., 2020b).

Ocean sprawl (i.e. the proliferation of built structures in the sea – Duarte et al., 2012) represents a global threat to biodiversity. This is a major challenge for applied ecology in the marine and coastal realms (reviews by Bishop et al., 2017; Dafforn et al., 2015a; Firth, et al., 2016a). This challenge stems from increasing human demographic pressures leading to inevitable land reclamation in worldwide coasts, squeezed further by the need for adaptation to climate change (Bugnot et al., 2021, Chee et al., 2017; Floerl et al., 2021; Lai et al., 2015). It prompts as urgent need for mitigation of habitat loss and biodiversity decline in marine ecosystems (e.g. Aguilera, 2018; Dafforn et al., 2015b; Perkins et al., 2015). Promisingly, ecosystem and societal sustainability of industrial and urban seascapes can be met alongside provisioning of novel or restored habitats, through the integration of emerging "nature-based solutions" into marine research and policy (Airoldi et al., 2021; Riisager-Simonsen et al., 2022; Morris et al., 2023).

Ecological research aiming to minimize ecological impacts of hard artificial structures in the marine environment has been undertaken since the early 2000s, being mostly focused on sea defences (reviews by Moschella et al., 2005; Airoldi et al., 2005; Bulleri and Chapman, 2010, 2015; Dungan et al., 2011; Firth et al., 2013b; Loke et al., 2019). In addition, there has been much interest in recovery, remediation, restoration, and re-conciliation of these coastlines (Chapman et al., 2017). Numerous efforts have been recently trialled to enhance biodiversity and ecosystem functioning on both intertidal and artificial structures (e.g. Chapman and Underwood, 2011; O'Shaughnessy et al., 2020). Biodiversity-enhancement trials have been aimed to increase abundance of target species (e.g. Martins et al., 2010; Perkol-Finkel et al., 2012; Strain et al., 2017), enhance native species richness (e.g. Firth et al., 2014b) or reduce colonization by non-native, invasive/opportunistic or nuisance species (e.g. Dafforn, 2017). Ultimately this leads to more environmentally-sensitive and multifunctional structures designed for ecological co-benefits, i.e. that not only enable their primary purpose (coastal protection) but also other benefits such as biodiversity enhancement (e.g. Evans et al., 2017). The approach has been named 'ecological engineering' or 'integrated blue-greening of grey infrastructure' (see Naylor et al., 2017). In recent years, much research effort has been devoted to eco-engineering, aiming to enhance biodiversity or to target specific outcomes that enable delivery of ecosystem services on marine artificial structures (reviews by Firth et al., 2016a; Morris et al., 2018a; O'Shaughnessy et al., 2020; Evans et al., 2021; Firth et al., 2024).

Global scientific evidence has been recently assembled on the effectiveness of such approaches (reviews by Strain et al., 2018; Evans et al., 2021) and on the remaining knowledge gaps (Firth et al., 2024). They have been put into practice by a set of conservation and management actions (eco-engineering interventions), which include the creation of artificial microhabitats (Evans et al. 2021). Ecological benefits of the created microhabitats are not solely driven by the increase of substrate surface area *per se*, but instead their ameliorative role reducing stress of intertidal organisms (effects on individual ecophysiology: e.g. Chapman and Underwood, 2011, Heath and Moody, 2013; and on behavioural change: e.g. Morris et al., 2018b). As such they should be matched to the niche of target species and to environmental conditions (Bishop et al., 2022). The changes that have been promoted on coastal defences include incorporation of i) materials with more environmentally sensitive properties (e.g. alternative concrete mixes with reduced carbon footprint, see Dennis et al., 2018) and ii) physical features that are originally absent or uncommon from artificial structures (e.g Aguilera et al. 2014). Physical features incorporated into intertidal marine urban structures improve their habitat potential at a range of experimental scales, from the increment of substrate texture (nm-mm scale) (e.g. Coombes et al., 2015) to the addition of (micro)habitats (mm-cm to cm-m scales) such as pits, holes, grooves, crevices, and rock-pools (see O'Shaughnessy et al., 2020; Evans et al., 2021 and references therein). Besides general biodiversity-enhancement effects, the incorporation of physical small-scale features has been particularly found to have a positive and long-lasting effect on the abundance of limpets (Martins et al. 2016; Ostalé-Valriberas et al., 2024).

One common eco-engineering intervention is the incorporation of water-retaining features, eco-engineered rock-pools or artificial tidepools, in the intertidal zone of artificial structures, to enhance topographic complexity and provide refugia from abiotic stress (Browne and Chapman, 2014; Chapman and Blockley, 2009; Evans et al., 2016; Ostalé-Valriberas, 2024; Bone et al., 2024). These novel habitats have been mostly created within wave-sheltered settings (estuaries, straits, marinas, dikes) (Evans et al. 2021). Less frequently, eco-engineered rock-pools have been also added to human-built intertidal structures set within open coastlines, such as a causeway (Galway Bay, Ireland: Firth et al., 2016b), and rock-armouring structures (groynes, breakwaters, and ripraps) (Irish Sea, UK: Firth et al., 2014b; Evans et al., 2016; Alboran Sea, Spain: Ostalé-Valriberas et al., 2018, 2024).

Eco-engineered rock-pools can be either built into construction or retrofitted to existing structures and have been installed using multiple techniques. Two alternative methods for rockpool creation in intertidal artificial structures can be categorized. One implies replacement of a limited number of standard building blocks of artificial structures – this includes pools formed as troughs or depressions in the space left from individually removed blocks (e.g. Chapman and Blockley, 2009; Chapman and Underwood, 2011) or pools included into pre-cast topographically-complex and habitat-enhanced blocks (e.g. the BIOBLOCK: Firth et al., 2014b; commercially-designed pools from ECOncrete and ReefSystems: Perkol-Finkel and Sella, 2016; Merlijn, 2022). The alternative method implies adding or removing material to standard building units (blocks or boulders) of artificial structures: this includes bolt-on pools (e.g. Browne and Chapman, 2011), concrete-attached (Waltham and Sheaves, 2018), mould-in (e.g. Firth et al., 2016b), drill-cored (e.g. Evans et al., 2016), jackhammer-carved (Ostalé-Variberas, 2018) or made from concrete in-filled cores (Firth et al., 2014b).

Eco-engineered rock-pools have been designed with a variety of shapes (rectangular base: e.g. Chapman and Blockley, 2009; cylindrical: e.g. Evans et al., 2016; Vertipools^{TM}: Hall et al., 2019, Bone et al., 2022; stepped-sided: Perkol-Finkel and Sella, 2016; half-flowerpot shaped: e.g. Browne and Chapman 2011; bucket-shaped: Firth et al. 2016b; and irregularly-shaped: Ostalé-Variberas, 2018). Consequently, the substratum composition of eco-engineered rock-pools has been either rock (e.g. Chapman and Blockley, 2009; Chee et al., 2020), standard concrete (e.g. Browne and Chapman, 2011; Hall et al., 2019), or commercial bio-enhancing concrete compositions (e.g. ECOncrete®, Sella et al., 2018). Other materials have been included in a few cases, using covers with pond-liners and limestone gravel (Heath and Moody, 2013), or short flexible coir panels (Morris et al., 2018c).

Eco-engineered rock-pools have been particularly successful in their efficacy in biodiversity-enhancement of intertidal artificial structures (Strain et al., 2018; Evans et al., 2021; Bishop et al. 2022). Most studies addressing the effects of eco-engineered rock-pools are limited in terms of the time (duration of study) and spatial extent (rarely including scales beyond the area of intervention, apart from controls established on adjacent areas). Such periods of study generally lasted for: i) less or equal to 12 months (e.g. Perkol-Finkel and Sella 2016, Ostalé-Valriberas et al., 2018; Morris et al., 2018c); ii) up to 18 months (e.g. Evans et al., 2016); iii) up to 24 months (Firth et al., 2016b, Waltham and Sheaves, 2018; Bone et al., 2024); or iv) the exceptional maxima of three (Chee et al. 2020), five (Hall et al., 2019) and 7.5 (Ostalé-Valriberas et al., 2024) years after the creation of rockpools. Moreover, response variables of these studies mostly regard biodiversity indices and other community-level responses to interventions (Evans et al., 2021). Conversely, effects produced by eco-engineered rock-pools were more rarely examined for responses at the population-level of target species or at the individual-level (Evans et al., 2021). Additionally, the influence of eco-engineered rock-pools on the surrounding substratum of artificial structures has been considered in a few experiments, but generally restricted to surfaces immediately next to the edge of pools within a few cm-radius (MacArthur et al., 2020; Ostalé-Valriberas et al., 2018, 2024). Chapter 5 addresses this knowledge deficit by a 10 year study of artificial pools and their influence on the surrounding emergent rock.

1.7 Study area

The southwest coast of mainland Portugal, located southwards from the prominent headland of the Cape of Sines, is composed by a mixture of rocky shores, cliffs, and sandy beaches, also punctuated by the estuary of the Mira River and by streams (with small estuaries or coastal lagoons) and temporary water lines. This region mostly lies within the natural park of *Parque Natural do Sudoeste Alentejano e Costa Vicentina* (PNSACV), designated since 1995 which extends by ca. 130 km of coastline from São Torpes in Alentejo (immediately south of the city of Sines) to Burgau in Algarve.

Natural rocky shores in this coast are mostly composed by schist/shale and shaped by the underlying geomorphology. Different lithologies are also locally present in some coastal stretches, such as vulcanic rocky massifs (in the Cape of Sines) and localized sandstone platforms (to the south from Sines). Despite proximity to areas impacted by intensive agriculture, fishing activities and tourism, the southwest coast of mainland Portugal is considered as near pristine, claimed by tourist guides as one of the best preserved and least disturbed coastal areas in southern Europe.

In contrast, the coastline immediately south of the Cape of Sines has been highly modified with artificial habitats and land claim due to setting of the Port of Sines. The Port of Sines is an open deep-water seaport. The extensive area of this port accommodates shipping, industrial and logistic activities focusing on oil and gas, plus a fishing port and a marina for recreational boats. Marine artificial structures in the Port of Sines include a complex of breakwaters, seawalls and quays, fronting land reclamation for buildings, hard standing, recreational and amenity use. Rip-rap structures for sea defence are built from concrete blocks or from rock-armour boulders from a local quarry (Monte Chãos).

The southwest coast of mainland Portugal is characterized by frequent upwelling during spring and summer months, triggered by dominant northerly winds, pumping colder subsurface waters to the upper layers along the coast (Relvas et al., 2007). Outside the upwelling season, the presence of a equatorward flow is a well-established characteristic along the mainland Portuguese west coast (Relvas et al., 2009). The tidal regime is semidiurnal (approximately 3.5 metres of maximum amplitude). This coastline is mostly exposed to wave action, with a few low wave-exposed shores, including those immediately south of Cape Sines (e.g. shores inside the Port of Sines). The hydrodynamic regime of the Port of Sines has been characterized by a well-mixed system, marked by intense flushing and water renewal, due to its wide entrances and deep bathymetry that play an important role in shaping coastal circulation at a local scale (Gomes et al., 2020).

The rocky coastline of PNSACV is characterized by high biodiversity (Horta e Costa et al., 2018). In the northern area of PNSACV and in the Port of Sines area, continuous environmental monitoring of physical, chemical, and biological variables has been carried for more than 20 years in several marine environments, namely on rocky shores (e.g. CIEMAR, 2000, 2009, 2021). The intertidal hard-bottom environment has been studied by comparison between artificial structures and nearby rocky shores. In general, there is less taxa richness on intertidal rocky structures inside the port when compared with natural adjacent rocky shores (Cruz and Castro, 2002). Based on these studies, common invertebrates like stalked barnacles and limpets are less abundant inside the Port of Sines.

A general assessment of human exploitation on rocky shores of Alentejo was made at the beginning of this century by Castro (2004). Based on this study, these rocky shores have been considered subject to a frequent and intense human exploitation of living resources, varying with accessibility and with season (e.g. sea-urchins catches happen in spring when gonads are ripe), and affecting several target species for several purposes (subsistence, trading and/or recreation). Main target species were the common octopus (*Octopus vulgaris*), the velvet swimming crab (*Necora puber*), the purple sea urchin (*Paracentrotus lividus*), the stalked barnacle (*Pollicipes pollicipes*), topshells (*Phorcus lineatus, P. sauciatus, Steromphala umbilicalis, G. pennanti*), mussels (*Mytilus galloprovincialis*) and limpets (*Patella ulyssiponensis, P. vulgata,* *P. depressa*) (Castro et al., 2020). The commercial species with the highest economical value on these rocky shores is the stalked barnacle *Pollicipes pollicipes*, which occurs in very-exposed shores (see Cruz et al., 2022, for a review). Limpets are commonly harvested from rocky shores primarily for local consumption, and mostly on informal commercial and/or recreational basis (Castro et al., 2020).

Harvesting of patellids is mostly permitted and widespread across the marine park, being considered as 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 the other exploited rocky-intertidal species (Castro et al., 2020 and references therein). In 2011 (after our surveys on natural shores of SW Portugal, Chapters 2, 3 and 4), different marine protection levels were implemented in different areas of PNSACV, and human exploitation of limpets became forbidden in special protection areas where fisheries and other human activities are prohibited or restricted (Castro et al., 2020). In PNSACV, as in the rest of the Portuguese mainland coast, the minimum catch size of *Patella* limpets is 2 cm of maximum shell length (MSL).

1.8 Focal species

Four species of patellid limpets (*Patella ulyssiponensis, P. depressa, P. rustica*, and *P. vulgata*) and one siphonariid, the striped false limpet *Siphonaria pectinata* occur in the continental Portuguese coast. Patellids and siphonariids have contrasting life-cycles; *Siphonaria pectinata* has internal fertilization followed by egg-mas laying from which a veliger hatches (Figure 1.1). This provides an interesting system to explore co-existence of sympatric species and the importance of life-history traits in niche segregation. Key information on their geographic range, local distribution in SW Portugal, life-history traits, phenology, and risks from human predation, is summarized in Table 1.1, with selected key references.

An illustrative plate (Figure 1.2) of the early life stages of three limpet species (*P. ulyssiponensis, P. depressa* and *S.* pectinata) is shown, with the size definitions considered in recruitment studies of this thesis. The size range of recruits was defined in Chapter 3 as individuals with a maximum post-settlement age of three-months (corresponding to maximum shell length, MSL, of 5 mm), whereas juveniles were defined as individuals with a MSL until 10 mm for patellids and individuals with a MSL until 5 mm for siphonariids.

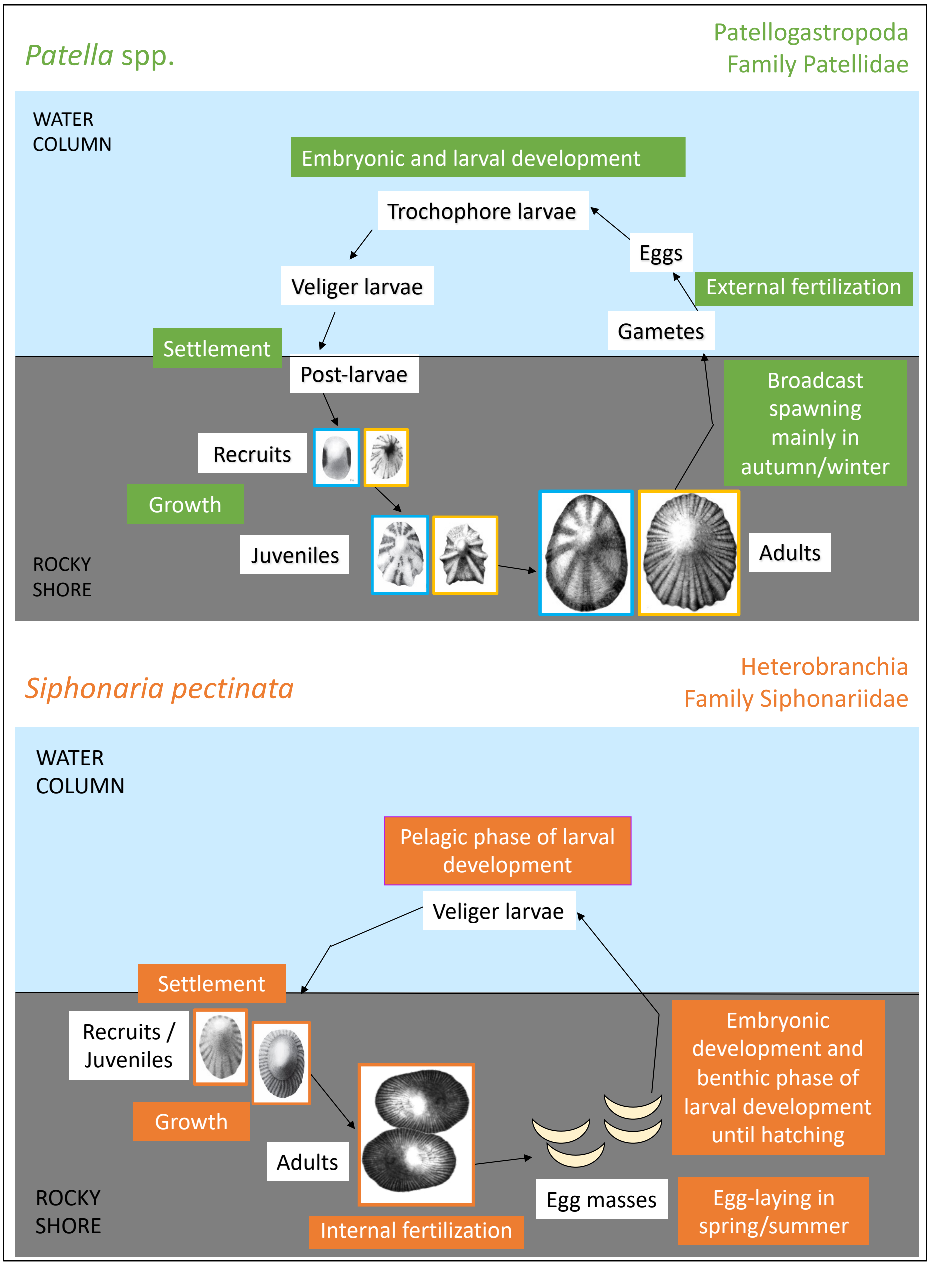
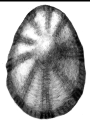
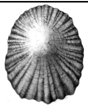


Figure 1.1. Schematic representation of the life cycle of patellids and siphonariids.

Table 1.1 Summary of key information on the five focal species of this thesis.

					
	Patella ulyssiponensis	***Patella depressa***	***Patella vulgata***	***Patella rustica***	***Siphonaria pectinata***
Geographical range	Eastern Atlantic (Mauritania to Bergen in Norway) and Mediterranean, dubious records on the Black Sea; Centre of distribution range in Portugal; Lusitanian (warm-temperate) species.	Eastern Atlantic (Senegal to North Wales); Centre of distribution range in Portugal; Lusitanian (warm-temperate) species.	Eastern Atlantic (Praia da Ingrina in the Algarve to Northern Norway in the Arctic); Equatorialward range edge in Portugal; Boreal (cold-temperate) species.	Eastern Atlantic (Mauritania to Cap Breton in France); Approaching poleward range edge in Portugal; Warm-temperate species.	Eastern Atlantic (Angola to the north border between Portugal and Spain in Moledo, possibly spreading northwards in Galicia) and in Mediterranean (Strait of Gibraltar, the African coastline up to Algeria and the Spanish coastline up to Murcia/Valencia area, spreading eastwards in Tunisia, Greece and Croatia). Range edge in North Portugal; Subtropical species.
Local distribution in SW Portugal	Very abundant on moderately to more exposed shores; Adults on low-shore open-rock and rock-pools covered with CCA above low-intertidal levels; Preliminary observations of juveniles in mid-shore CCA-covered rock-pools.	Shores of all exposures; Adults present from low-water neap tides to the splash zone, mostly abundant on mid-shore open-rock. Preliminary observations of juveniles on rock-pools and moist microhabitats.	Adults occur on all tidal levels, mostly abundant in shade and localized shelter, where juveniles can also occur.	Exposed shores; Adults and juveniles on open-rock at high intertidal levels, mostly on steep walls.	Particularly abundant in wave-sheltered sites; Adults can occur on open-rock across all tidal levels; Egg masses in cracks, crevices and open-rock at mid-tidal levels; High small-scale variation in abundance on mid-shore open-rock. Preliminary observations of juvenile individuals and egg-masses in shallow rock-pools on mid and high levels.
Larval biology	Planktonic trochophore followed by veliger	Planktonic trochophore followed by veliger	Planktonic trochophore followed by veliger	Planktonic trochophore followed by veliger	Lays benthic egg masses from which veliger are released into the water.
Protandry	Yes	Unlikely.	Yes	Yes	Simultaneous hermaphrodite; Pseudo-copula.
Reproductive seasonality in Portugal	Multiple broods in Autumn to early spring; resting period during late spring-summer.	Multiple broods in Autumn to early spring; resting period over late spring-summer.	Simple brood	Simple brood in summer-autumn	Occurrence of egg-ribbons in spring-summer.
Feeding	Deep gauging shovel-like radula; possibly feeding on algal turfs and CCA.	Microbial films and juvenile algae.	Microbial films, macroalgae and algal detritos.	Very long radula; microbial films and juvenile algae	Fine toothed radula feeding on microbial films
Human Predation	Yes, favored species (larger sizes).	Some exploitation.	Some exploitation.	Not commonly exploited (rarely or incidentally).	No exploitation; Anti-predatory toxins.
Key selected references	Christiaens, 1973; Guerra and Gaudêncio, 1986; Boaventura et al., 2002; Castro, 2004; Weber and Hawkins, 2005; Ribeiro, 2008; Ribeiro et al., 2009	Christiaens 1973; Guerra and Gaudêncio, 1986; Boaventura et al., 2002; Castro 2004; Ribeiro, 2008; Ribeiro et al., 2009; Brazão et al., 2003; Smith, 2020.	Boaventura et al., 2002; Cabral and Simões, 2007; Seabra et al., 2011; Lima et al., 2016; Castro, 2004; Ribeiro, 2008; Ribeiro et al., 2009.	Boaventura et al., 2002; Lima et al., 2006; Cabral, 2007; Ribeiro et al., 2009; Sousa et al., 2012; Prusina et al., 2014, 2015.	Ocaña and Emson, 1999; Ocaña and Fa, 2003; Rubal et al., 2013, 2015; Espinosa et al., 2016; Giribet and Kawauchi, 2016; Crocetta, 2016 and references therein; Simone and Seabra, 2017; Slama et al., 2020.

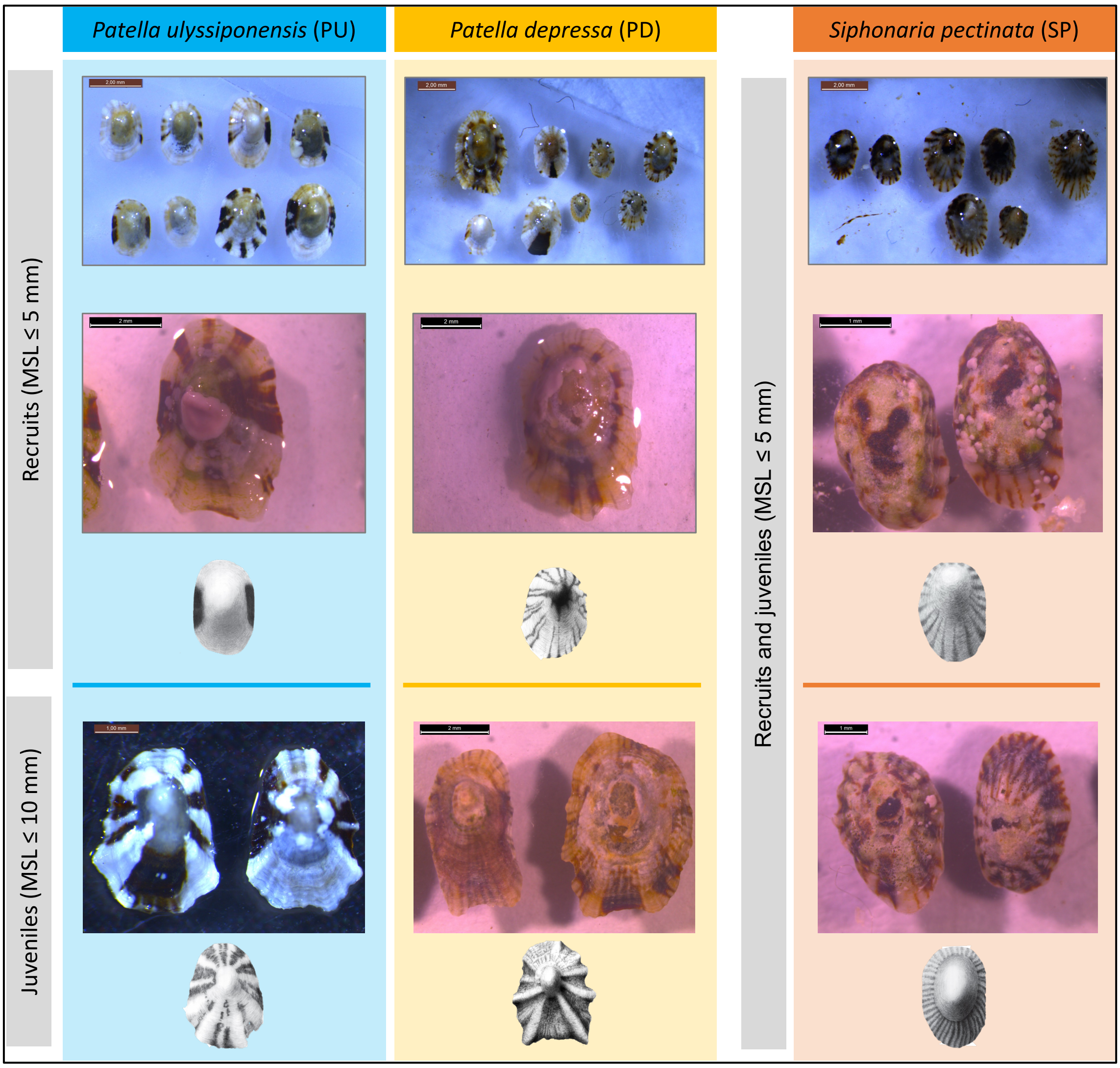


Figure 1.2. Scientific drawings (Author: Ivan Gromicho), photographs and size-ranges of early life stages (recruits and juveniles) of the three limpet species for which recruitment was studied. The second line of photographs are recruits with shells covered by CCA collected in mid-shore rock-pools.

1.9 Rationale and logic of thesis

The rationale behind this thesis is built on the presumed ecological importance of rock-pools (the focal microhabitat) for intertidal limpets (the focal set of species). It is based on two concepts: a) the patchiness of rock-pools (as spatially-confined, fragmented, and isolated microhabitats during low-tide); and b) the connectivity of populations of different limpet species across patches of microhabitat (demographic links throughout rock-pools and their surrounds). The connectivity of populations of mobile animals across patches of habitat that form mosaics on the wider landscape is emphasized, given the spatial patterning of rock-pools and their role as nursery grounds or temporary refuges for limpets.

To our knowledge, before this thesis there has been no study on the ecology of limpets inside rock-pools in the Portuguese coast. The Portuguese mainland coast hosts five limpet species, namely: *Patella ulyssiponensis*, *P. depressa*, *P. vulgata*, *P. rustica* and *Siphonaria pectinata* (Boaventura et al., 2002; see Table 1.1). The pioneering study by Boaventura et al. (2002), conducted in the late 1990s, provided a comprehensive description of the vertical and horizontal patterns of distribution of intertidal communities on the open-rock of rocky shores in the northern, central, and southern regions of Portugal. Boaventura et al. (2002) also examined the spatial variation, with vertical level and horizontal scales (shores and regions), in the abundance of limpets inhabiting the mid-shore open-rock in mainland Portugal.

Patella ulyssiponensis, P. depressa and *Siphonaria pectinata* are the most common species of intertidal limpets in southwest coast of Portugal (Boaventura et al., 2002; Castro, 2004). Preliminary observations on the mid intertidal levels of shores in SW Portugal suggested that *P. depressa* and *S. pectinata* seem to use rock-pools mostly as a nursery ground, with large individuals of *P. depressa* living on the open-rock. In contrast, both small and large-sized individuals of the low-shore species *P. ulyssiponensis* are often found inside mid-shore rock-pools, indicating that they might be largely pool-dwellers throughout their life-span. The use of rock-pools and other microhabitats by limpets within the complete size-range of each species and at different intertidal levels was examined in this thesis, considering the variability present among different rock-pools and among different shores in the study region.

The overall aim of the thesis was to study the structure of limpet populations inside and outside rock-pools in SW Portugal, to understand the role of rock-pools for both the early-life stages and adults of each limpet species across the whole intertidal gradient. The methodological approach included the study of a process (i.e. recruitment to a certain size range after settlement) for the three most common focal species, including their variation in population dynamics across space and time. Larval settlement onto rocky substrata with and without CCA was also studied for one of these species. Spatial patterns of limpet populations of all the studied species were assessed by measuring abundances and size-structure inside rock-pools, around their edges and on the open-rock. For each rock-pool, a system of three concentric microhabitats (inside, edge and surrounding open-rock) was defined to examine the connectivity of limpet populations across these small-scale horizontal gradient. Demographic connectivity of limpets was also assessed by studying limpet populations living on open-rock surfaces at different distance categories from rockpool rims (pool edges). I studied ecological patterns and relationships related to the distribution of limpets at small microhabitat-scales, having consequences at intertidal landscape-scales. Many rocky shores have been described to include key microhabitats for conservation due their uniqueness or ecological importance (Thompson et al., 2002). This might apply to rock-pools and their role as nurseries sustaining populations of intertidal species.

1.10 Thesis structure

Following this General Introduction chapter, this thesis is structured with four chapters of research work in the form of standalone scientific articles (three published papers and one manuscript in latter stages of preparation), and a final integrative chapter providing an Overview and Synthesis.

In Chapter 2 entitled "Recruitment of the limpet *Patella ulyssiponensis* and its relationship with crustose coralline algae: patterns of juvenile distribution and larval settlement", the role of living crustose coralline algae (CCA) for the recruitment of *P. ulyssiponensis* is explored. *Patella ulyssiponensis* has been previously considered a specialist species that can remain in CCA-covered areas across its life-time (see Supplementary Table S1.1). A mechanistic approach focusing on patterns of juvenile distribution and larval settlement was taken. The specific objectives were to assess the potential relationship with the presence of crustose coralline algae (CCA) in the spatial variation in the abundance of juvenile *Patella ulyssiponensis* in a range of microhabitats on three natural shores in the coastline of Sines (SW Portugal) and to test the hypothesis that larval settlement would be higher on chips of rock encrusted by CCA in comparison with chips of bare-rock.

Rock-pools were then examined as a nursery ground for coexisting limpet species with different life history traits (Chapter 3: Rock-pools as nurseries for co-existing limpets: Spatial and temporal patterns of limpet recruitment). The distribution of juveniles of *Patella depressa* and *Siphonaria pectinata* plus the temporal and spatial patterns of recruitment of three limpet species onto rock-pools were studied on three natural shores in the coastline near Sines, SW Portugal. The the specific objectives were to understand the relative importance of rock-pools as habitats for juveniles of *Patella depressa* and *Siphonaria pectinata*, and to describe recruitment patterns of *P. depressa*, *P. ulyssiponensis* and *S. pectinata* onto mid-shore rock-pools. We hypothesized that juveniles of both species would be more abundant in habitats of rock-pools compared to other available intertidal microhabitats and that abundance of recruits into rock-pools would vary temporally according to the yearly reproductive cycle of each species and would be lower inside than outside the Port of Sines.

Patterns of five limpet species across landscape mosaics created by the presence of rock-pools are considered in Chapter 4, entitled "Variability and connectivity in populations of different limpet species across rockpool-generated mosaic landscapes". The overall aim was to examine the spatial patterns of co-occurrence of intertidal limpets in rockpool-generated landscapes: a concentric system of three microhabitats formed by the rock-pool, its edge and its surrounding rock was considered. Horizontal and vertical patterns of abundance of the five focal species were assessed. Variability in the proportion of patchy microhabitats available in shores with different geomorphology was also investigated. Connectivity of limpet populations across adjacent microhabitats was explored descriptively for each of the three most common species. Relationships between each species occurrence and different physical and biological characteristics of rockpool systems were examined, to identify the most important predictors to explain the small-scale (among rockpool systems) variability in spatial patterns of each species within each microhabitat.

In a ten-year study (Chapter 5: Efficacy of eco-engineered rock-pools: long-term enhancement of intertidal limpet populations across artificial habitats), an eco-engineering approach (creation of eco-engineered rock-pools) was used to explore means of boosting density of limpets in artificial habitats (rock-armour structures) of the Port of Sines. Within the created pools, sequences of colonization of pool assemblages over time were described in parallel with counts of limpets of each species recruiting into the pools. Beyond the created pools, population density and size-structure of two mid-shore limpet species (*Patella depressa* and *Siphonaria pectinata*) were censused on the surrounding open-rock of manipulated boulders at two distance categories from the pools and in control boulders (with no pools). The overall aim was to test the general hypothesis of a positive effect of rockpool creation on the abundance of limpets on the open-rock of mid-tidal boulders of rock-armour structures. This was novel in terms of the duration of the study and for considering scales beyond the area of intervention.

The final chapter (Chapter 6: Overview and Synthesis) integrates the findings of the thesis before discussing selected themes and making suggestions for further work.

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CHAPTER 2: Recruitment of the limpet *Patella ulyssiponensis* and its relationship with crustose coralline algae: patterns of juvenile distribution and larval settlement

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2.1 Abstract

Recruitment of the limpet *Patella ulyssiponensis* was investigated in relation to the presence of living crustose coralline algae (CCA) in rocky-shore microhabitats. Juvenile limpets (≤10 mm maximum shell length) were counted in CCA-present and CCA-absent microhabitats, on three shores in SW Portugal during summer 2007 and winter 2009. Furthermore, the settling response of laboratory-reared larvae of *P. ulyssiponensis* to CCA-covered substratum, and bare-rock, was examined. Across the intertidal zone, we found a clear association between the distribution and abundance of juveniles and the presence of CCA. Although the presence of CCA was not an absolute requisite for juvenile occurrence, null juvenile densities were mostly recorded in CCA-absent areas. The highest juvenile densities (maximum of 64 individuals in 15 × 15 cm) were consistently found in CCA-dominated microhabitats, namely steep wave-exposed areas at low-shore and rock-pools. The hypothesis of CCA-enhanced settlement was not supported, as settlement intensities of laboratory-reared larvae were similar between chips of rock encrusted by CCA and chips of bare-rock. From the overall number of settlers onto CCA-encrusted rock chips, 51% were found in tiny pits lacking CCA. This was the first study of the settlement patterns of larvae of the genus *Patella* using naturally occurring rocky substrata. These results are preliminary and should be confirmed with choice-experiments and improved monitoring of the position of settlers. We suggest that CCA plays a role in the recruitment of *P. ulyssiponensis*, potentially promoting survivorship of early benthic stages, but possibly not enhancing settlement.

Key words: Coralline crusts; limpet recruitment; patellid larvae; Patellogastropoda; rocky-shore microhabitats; settlement cues; SW Portugal

2.2 Introduction

Biotic interactions involving crustose coralline algae (CCA) are widely recognized as key ecological processes affecting the patterns of distribution and abundance of associated species (e.g. de Figueiredo et al., 1996; Vermeij, 2005; Maneveldt et al., 2006). These interactions can drive the structure and persistence of communities within marine benthic ecosystems (reviewed by Nelson, 2009).

Recruitment success, and consequent population demographic dynamics, of many benthic invertebrates may be dependent on the availability of CCA-covered substrata (sponges: Whalan et al., 2012; corals: Sebens, 1983; Morse et al., 1988; Tebben et al., 2015; polychaetes: Gee, 1965; asteroids: Barker, 1977; Johnson et al., 1991; sea urchins: Rowley, 1989; Pearce and Scheibling, 1990; chitons: Barnes and Gonor, 1973; Rumrill and Cameron, 1983; abalone: Morse et al., 1979; Morse and Morse, 1984; Roberts et al., 2010; and limpets: Steneck, 1982; McGrath, 1992; Guallart et al., 2017). This relationship is based on field observations of the association of young individuals with CCA (e.g. McGrath, 1992), and/or evidence of the ability of larvae to detect chemical or biological cues at the CCA surfaces and trigger selective settlement (e.g. Morse et al., 1979). The case study of abalone larvae and their highly specific requirement for a unique CCA inducer of settlement and metamorphosis (a small water- soluble peptide similar to the neurotransmitter gamma-aminobutyric acid (GABA)), provides the most complete model for the behavioural, cellular and molecular mechanisms involved in the control of the process of habitat selection during gastropod settlement (reviews by Morse, 1991; Roberts, 2001).

In intertidal limpets, mutual or commensal interactions with CCA or other crustose algae have been documented worldwide, establishing a common link between the abundance of recruits and the presence of algal species (Branch, 1975*a*; Steneck, 1982; Quinn, 1988; McQuaid and Froneman, 1993). In the North-east Atlantic, a taxonomically difficult CCA complex named 'Lithothamnia' (*sensu* Hawkins and Jones, 1992) is frequently the most abundant space-occupier within shallow rock-pools (e.g. Kooistra et al., 1989). These 'Lithothamnia'-lined rock-pools and other rocky-shore microhabitats where CCA are conspicuously present have been considered important nurseries for limpets of the family Patellidae (Lewis and Bowman, 1975; Bowman, 1985; Bowman and Lewis, 1986; McGrath and Foley, 2005).

'Lithothamnia'-induced settlement has long been suggested for patellid larvae, based on the spatial patterns of distribution of the smallest field-detectable recruits (Bowman, 1981; Kooistra et al., 1989; McGrath, 1992). However, settlement cues for patellid limpets remain unknown. This is partly because settlers of these species (only detectable by microscopic examination), have rarely been found in nature (but see Bowman, 1985; Gardner, 1986; McGrath and Foley, 2005). Rearing of larval patellids to settlement and metamorphosis is also very rare (but see Smith, 1935; Dodd, 1957; Wanninger et al., 1999; Ferranti et al., 2018). Laboratory assays with *Patella* embryos and larvae are often referred to as methodologically difficult (Dodd, 1957; Smaldon and Duffus, 1984; Espinosa et al., 2010; Pérez et al., 2016), which has probably impaired the progress of laboratory settlement studies.

The rough limpet, *Patella ulyssiponensis* (Gmelin, 1791), formerly known as *Patella aspera* Röding, 1798 (currently in use only for Macaronesia: see Weber and Hawkins, 2005), is an abundant and conspicuous herbivorous gastropod on rocky shores in the North-east Atlantic (Norway to Mauritania) and Mediterranean. Along with other widely distributed patellids, it has long been considered a 'keystone' species in rocky intertidal community structure and functioning (e.g. Branch, 1981; Hawkins and Hartnoll, 1983; Hawkins et al., 1992; Coleman et al., 2006). It occurs in the low intertidal zone of wave-exposed sites (Thompson, 1979), and at higher shore levels but mainly restricted to 'Lithothamnia'-dominated rock-pools (Guerra and Gaudêncio, 1986; Kooistra et al., 1989; Firth and Crowe, 2008).

CCA is a critically important substratum for the early life stages of *P. ulyssiponensis*. In the British Isles, Bowman (1981, 1985) observed post-settlement phases of *P. ulyssiponensis* (0.2–0.5 mm transparent individuals, larval shell still attached) as well as newly emerged *P. ulyssiponensis* (individuals with an adult calcified shell reaching a maximum shell length – MSL = 1 mm) solely within microhabitats dominated by 'Lithothamnia'. High densities of *P. ulyssiponensis* recruits (MSL < 3 mm) have also been found occupying the 'Lithothamnia' surface or in its close vicinity around the edges of *Mytilus* and *Corallina* clumps, within rock-pools or wet runnels located at lower tidal levels (Bowman, 1981; Guerra and Gaudêncio, 1986). Unlike other congeneric species, whose older individuals (MSL of 6–10 mm) seem to migrate to emersed open-rock (Bowman, 1981; Guerra and Gaudêncio, 1986; Delany et al., 1998), 'Lithothamnia'-dominated rock-pools may provide a microhabitat for *P. ulyssiponensis* until adulthood (Delany et al., 1998; Firth and Crowe, 2008). In addition, adults exploit 'Lithothamnia' as a food source (Hawkins et al., 1989; Delany et al., 2002). This life-long relationship between *P. ulyssiponensis* and 'Lithothamnia' reinforces the hypothesis of enhanced larval settlement by CCA-covered substrata and/or the existence of potential adaptive advantages for the maintenance of this microhabitat association. Adaptation to CCA-dominated microhabitats, which are usually permanently wet or submerged, would benefit *P. ulyssiponensis*, given its low tolerance of desiccation (Davies, 1969) and limited metabolic flexibility (Davies, 1967), compared with other patellids.

Presently, there is no published description of larval development of *P. ulyssiponensis* nor protocols to culture this species *in vitro*. However, Ribeiro (2008) studied the effect of temperature on the survival and development of laboratory-reared larvae of *P. ulyssiponensis*, *Patella depressa* (Pennant, 1977) and *Patella vulgata* (Linnaeus, 1758) and included data on settlement assays. The substrata used in those assays had been previously subjected to manipulation such as hard mechanical brushing or defaunation (Ribeiro, 2008; Ribeiro, personal communication). To our knowledge, our study is the first to address settlement patterns of *P. ulyssiponensis* larvae in relation to unmanipulated (i.e. naturally occurring) rocky substrata, in the presence or absence of CCA.

Here, we examined the spatial variation in recruitment patterns of *P. ulyssiponensis* in SW Portugal and its potential relationship with the presence of CCA in rocky-shore microhabitats. The CCA taxon in this study was the complex 'Lithothamnia', mostly composed of *Lithophyllum incrustans* (Philippi, 1837) in the intertidal zone of the South-west Portuguese coast (Berecibar E., personal communication); hereafter it will be referred to as 'CCA'. We aimed to characterize differences in field patterns of distribution and abundance of juveniles (MSL ≤ 10 mm) of *P. ulyssiponensis* among a series of intertidal microhabitats, with and without CCA. We also report on a laboratory settlement experiment with larvae of *P. ulyssiponensis* exposed to chips of CCA-covered or bare-rock substrata. Specific hypotheses tested were: (1) patterns of juvenile densities with respect to microhabitat would vary depending on the presence or absence of CCA, predicting higher densities in microhabitats where CCA is present; and (2) larval settlement would be higher on chips of rock encrusted by CCA in comparison with chips of bare-rock.

2.3 Materials and methods

2.3.1 Field surveys to assess juvenile distribution

Two surveys, focusing on the search for small limpets (*Patella ulyssiponensis* juveniles – MSL ≤ 10 mm), were undertaken in low water spring tide periods. The first survey was carried out during August–early September 2007 (summer 2007) and the second during February 2009 (winter 2009). These reflect the trough and peak in the yearly reproductive cycle of this species in SW Portugal (Guerra and Gaudêncio, 1986; Castro, 2004). Spring/summer reproductive inactivity is followed by several peaks of gonadal development and spawning from late summer to early spring, and settlement mainly in late autumn to winter/early spring. Surveys were performed across a wide range of intertidal microhabitats present at three rocky shores with diverse geomorphology, located in the region of Sines, south-west coast of continental Portugal (see

Figure 2.1). The study shores (Figure 2.1) were: (i) a natural shore, formed by steeply sloping basaltic massifs, adjacent to the breakwater of the General Cargo Terminal of the Port of Sines, hereafter named Port, (ii) Vale Marim, composed of metamorphic rocks interspersed with shale intrusions, boulders and pebbles and (iii) Oliveirinha, characterized by extensive and gently sloping shale platforms. All shores had wave-exposed platforms extending across the intertidal zone, with a heterogeneous mixture of open-rock, rock-pools, crevices and other patchy microhabitats, where limpets (*Patella* spp. and *Siphonaria pectinata*) are commonly found. The tidal regime is semi-diurnal, with maximum amplitude of ~3.5 m during spring tides.

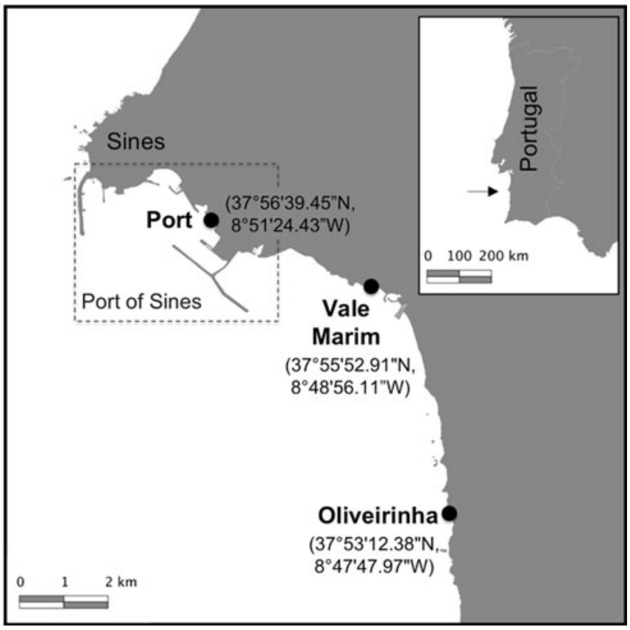

Figure 2.1 Map of the study region and shores sampled.

In each survey, a series of microhabitats (Table 2.1), categorized as 'CCA-present' or 'CCA-absent' (named using subscripts 'p' and 'a', respectively, following the microhabitats' abbreviation names – seeTable 2.1), was searched. Four (replicate) randomly sampled areas were assessed in each survey.

Within each sampling area, all limpets (≤10 mm MSL) were collected using dissecting forceps, needles, magnifying lens and lamps. This field search was complemented with further observations in the laboratory of material removed by destructive sampling in the case of complex three-dimensional or multi-layered microhabitats (i.e. calcareous substrata of the microhabitat 'urchin burrows' (U_p) and thalli/shells of *Lichina*/*Mytilus*/*Fucus* (L_a, M_a, F_a)).

Sample area differed among microhabitats: in the case of U_p, each area consisted of a 15 × 15 cm quadrat within large lower–midshore rock-pools including at least 10 adult *Paracentrotus lividus*, which were removed to examine their surrounding substrata; for microhabitats with well-defined boundaries, such as rock-pools (PM_p, PMS_p, PHL_p, PHR_a) and patches of L_a/M_a/F_a, the total surface area of each replicate was sampled and estimated from scaled digital photographs; along crevices (CM_a, CMS_a, CH_a), replicateswere 50 × 2 cm transects; and finally, for open-rock (OL_p, OL_a, OLM_a, OM_a) and vertical walls (VN_p, VS_a), 50 × 50 cm quadrats were sampled, using sub-sampling when densities of juvenile limpets exceeded 15 individuals per 15 × 15 cm.

All collected limpets were field-preserved in 96% ethanol and stored in the lab at 4°C, to be later identified under a dissecting stereomicroscope following Bowman (1981), and their MSL measured to the nearest 100 µm using a calibrated eyepiece.

All juvenile *P. ulyssiponensis* densities presented here were standardized to a 15 × 15 cm area and their records on sampling areas of all microhabitats were treated as the response variable in analyses for each shore and survey.

Table 2.1 List of intertidal microhabitats surveyed ('x') during searches for juvenile *P. ulyssiponensis* carried out in summer 2007 and winter 2009, at three shores (Port, Vale Marim, Oliveirinha) on the SW coast of Portugal, organized in 'CCA-present' or 'CCA-absent' categories (subscripts 'p' and 'a', respectively, following the microhabitats' acronyms).

		Summer 2007			Winter 2009		
Habitat description	Category	Port	Vale Marim	Oliveirinha	Port	Vale Marim	Oliveirinha
U_p – Urchin burrows – 'Lithothamnia' areas surrounding *Paracentrotus lividus* within medium-depth rock-pools at low-shore	CCA-Present			x			x
PM_p – Rock-pools at mid-shore – dominated by 'Lithothamnia'	CCA-Present	x	x	x	x	x	x
PMS_p – Rock-pools at mid-shore within shale intrusions – dominated by 'Lithothamnia'	CCA-Present		x			x	
PHL_p – Rock-pools at high-shore – dominated by 'Lithothamnia'	CCA-Present	x		x	x		x
VN_p – Vertical north-facing walls – shaded platform ridges, moist bare-rock among mixed assemblages of encrusting algae (*Ralfsia verrucosa, Lithophyllum tortuosum*, 'Lithothamnia', *Codium adhaerens)* and the barnacle *Perforatus perforatus*	CCA-Present			x			x
OL_p – Open-rock at low-shore – the main occurrence zone of *P. ulyssiponensis*, at Port is dominated by 'Lithothamnia'	CCA-Present	x			x		
OL_a – Open-rock at low-shore – the main occurrence zone of *P. ulyssiponensis*, in Vale Marim and Oliveirinha is characterized by foliose algae and algal turf interspersed with *Nemoderma tingitanum* and/or other encrusting algae (e.g. *Codium adhaerens*)	CCA-Absent		x	x			x
OLM_a – Open-rock at lower-mid shore level	CCA-Absent	x	x	x			
OM_a – Open-rock at mid-shore – the main occurrence zone of large-size *P. depressa* limpets and the barnacle *Chthamalus montagui*	CCA-Absent	x	x	x			
PHR_a – Rock-pools at high-shore dominated by bare-rock	CCA-Absent	x	x	x	x	x	x
CM_a – Crevices at mid-shore	CCA-Absent	x	x	x	x	x	x
CMS_a – Crevices at mid-shore within shale intrusions	CCA-Absent		x				
CH_a – Crevices at high-shore	CCA-Absent	x	x	x	x		x
L_a – *Lichina* patches – rock underneath and bordering *L. pygmaea* thalli at the high-shore	CCA-Absent	x	x	x			
VS_a – Vertical south-facing walls – sun-oriented platform ridges, with overhangs and cracks, dominated by bare-rock and the barnacle *Chthamalus montagui*	CCA-Absent			x			x
M_a – *Mytilus* patches – mussel shells and bordering rock within overhangs at the upper mid-shore	CCA-Absent			x			
F_a – *Fucus* patches – rock underneath and bordering *F. spiralis* thalli at the upper mid-shore	CCA-Absent			x			

2.3.2 Larval settlement in laboratory conditions

Larval rearing was carried out in a temperature-conditioned room, using 0.2 µm Millipore-filtered and UV-sterilized natural seawater (FSW). Larval breeding proceedings were generally based on Ribeiro (2008 and references therein), although several methodological aspects were modified after pilot assays to increase the production of larvae.

During one of the peaks of the annual reproductive period of this species in SW Portugal (Castro, 2004), live *P. ulyssiponensis* with MSL > 30 mm were collected during low tide on 13 February 2011, at low-shore open-rock in Oliveirinha. Specimens were carefully removed from the substrata and transported to the laboratory in refrigerated conditions on top of wet stones and kept alive in an aquarium with aerated seawater at 17–18°C. After 2 h, collected specimens were dissected and a set of eight females and five males showing maximal reproductive development stages (stage V defined by Orton et al., 1956) was selected immediately following dissection, to provide gametes.

All artificial fertilization procedures were made in a controlled- temperature room at 18°C. Whole female gonads were carefully dissected out and placed in a bowl with 150 ml of FSW; each ovary was then gently pulled apart and agitated to release oocytes.

The suspension of oocytes was subsequently stirred with a plastic pipette, so that clean oocytes could be collected from the bottom of the bowl and then separated from debris by transferring them to a clean glass dish with FSW. After repeating this washing procedure twice, oocytes were treated with alkaline FSW (made up to pH 9 using NH_4OH) for 10 min, according to Hodgson et al. (2007). Following alkalinization, oocytes were returned to clean FSW to be rinsed twice using the same washing procedure, and then distributed among four beakers with 500 ml of FSW. Whole male gonads were dissected out, placed in a Petri dish containing a few drops of FSW and cut into pieces so that sperm were released. Suspended sperm were then collected and transferred with a plastic pipette into a 50 ml Falcon tube, adding FSW to bring the total volume to 50 ml. The suspension of sperm was mixed and finally, to activate sperm prior to *in vitro* fertilization (Hodgson et al., 2007), it was maintained for 30 min at 4°C. For fertilization, a total of 5 ml of motile sperm (motility verified under the microscope) was estimated to be added to each 500 ml-beaker containing oocytes, to obtain an ideal concentration of 10^6 sperm ml^{-1} (checked using a Neubauer improved hemocytometer chamber), determined for *P. ulyssiponensis* by Hodgson et al. (2007). Therefore, the contact between gametes was made through the sequential addition of 1, 1.5 and 2.5 ml of the sperm suspension in three 10-min separated times, followed by manual and gentle stirring of the beaker volume during 30 min. The fertilization time was considered as the time of the first sperm addition.

Two and a half hours post-fertilization, sperm were washed off by replacing the water with 500 ml of clean FSW and eggs were incubated during a 15-h period for development of trochophore larvae (easily detected by swimming movements near the water surface), followed by a second water change. After the hatching of swimming trochophores, cultures were left undisturbed at a constant temperature of 20°C. Culture vessels were not stirred, since stirring techniques (airlift-droplet system – Strathmann, 1987; standard aeration and magnetic stirring) tested during our pilot assays did not yield a higher number of larvae compared with still water. Cultures were not fed (cf. Dodd, 1957; Ribeiro, 2008; Aquino de Souza et al., 2009), as *Patella* larval development can be lecithotrophic (Wanninger et al., 1999). Water was changed regularly every 12 h until the third day post-fertilization, when larvae reached competency. The stage of competency was detected by observations of larval behaviour and morphology: post-torsional *veligers* swimming close to the bottom surface or moving in fast upward bursts followed by passive sinking; competent larvae were capable of withdrawing into the protoconch and had an operculum, cephalic tentacles and bi-lobed foot. All culture water changes included the passage of the whole volume of each beaker through a stack-filter formed by a 250-µm-mesh sieve (used to catch flocculant waste and debris) placed on top of a funnel, which bottom end was positioned inside a permanently submerged 50-µm-mesh sieve, where eggs or larvae were retained and transferred into a 500 ml beaker with fresh isothermal FSW.

Cultures were sampled after the first two water changes (2.5 and 15 h post-fertilization), so that fertilized eggs and trochophores could be observed and counted under a stereo dissecting microscope. Eggs were counted in three subsamples of 1 ml using a Bogorov Plankton Counting Chamber and trochophores were counted in three subsamples of 2.5 ml transferred into a Petri dish. The total numbers of competent larvae produced were counted, by observing the contents of the 50-µm-mesh sieve after the last filtration of the volume of each culture beaker. Only eggs and larvae with normal development were considered in all counts. Larval survival rate until the developmental stages of trochophore and competency was estimated by dividing the mean number of larvae that had reached that stage in 500 ml by the mean number of eggs ascertained for the same volume.

Around 69 h post-fertilization (on 16 February 2011), a no-choice settlement experiment began, in which competent larvae were introduced to small glass dishes (4 cm tall, 9 cm diameter), each one containing 100 ml of FSW at 20°C and a single type of substratum at the bottom (set-up). The number of competent larvae introduced in each dish was variable (between 35 and 70 larvae, 51 on average). Two contrasting treatments were considered: rock encrusted with live CCA from mid-shore rock-pools (referred as 'CCA-chips', five replicate dishes) and untouched bare-rock from mid-shore open-rock (referred to as 'rock-chips', eight replicate dishes). Both treatments consisted of 3 × 3 × 0.5 cm chips of natural substratum, which were collected in Oliveirinha using a hammer and chisel, a few hours prior to the settlement assay. Chips of substrata were transported to the laboratory within separate containers in damp refrigerated conditions and maintained submersed in aerated seawater without additional manipulation before use. Following the introduction of competent larvae, dishes were monitored under a dissecting microscope, and notes were taken regarding larval behaviour and morphology 24 and 48 h after set-up (four to five days post-fertilization). At these two observation times, all non-swimming larvae were counted within each dish: settlers (onto the chip of substratum) and individuals attached to the glass surface. The percentage of settlers at the end of the 24-h and the 48-h periods relative to the initial number of larvae introduced in each dish was taken as the response variable and compared between the two treatments separately for each time. In the case of the treatment 'CCA-chips', apparent aggregations of settlers were observed in particularly small pits of substratum lacking any CCA cover (each pit measuring a maximum of 1–2 mm^2 and the sum of these pits within every single CCA-chip occupying far less than 5% of the total area of the chip). Therefore, for settlers found on CCA-chips, individual positions were distinguished between two zones, concerning their occurrence within the overall CCA surface or, alternatively, within pits not covered by CCA.

2.3.3 Statistical analyses

Analysis of variance (ANOVA) was used to test for differences in the distribution of juvenile *P. ulyssiponensis* in relation to intertidal microhabitat. Six separate one-factor analyses were performed: one for each shore (Port, Vale Marim, Oliveirinha) in each survey (summer 2007 and winter 2009), using microhabitat as a fixed factor with number of levels variable among comparisons (due to different number of surveyed microhabitats in each shore/survey). Cochran's test was used to test for the assumption of homogeneity of variances and in cases where variances were heterogeneous, data were transformed using ln(x + 1) or ln(x + constant). Student–Newman–Keuls (SNK) procedure was used to make post-hoc comparisons among levels when the term microhabitat was significant. WinGMAV® version 5 for Windows (designed, coded and compiled by A. J. Underwood and M.G. Chapman, Institute of Marine Ecology, University of Sydney, Australia) was used for computations.

The percentages of settlers estimated at each observation time were compared between two groups of dishes, each group containing one of the two types of substrata ('CCA-chips' *vs* 'rock-chips') considered in the settlement experiment. These comparisons were analysed by means of *t*-tests for independent samples with equal variances, considering 11 degrees of freedom, after testing for homogeneity of variances between treatments.

2.4 Results

A total of 3359 juvenile *Patella ulyssiponensis* were field-collected, identified and measured (MSL in mm, mean ± SE: 4.56 ± 0.04; 0.62–10.00 min-max). Abundances varied among microhabitats, shores and surveys, although shores and surveys were not formally compared and are treated separately (Figure 2.2). The presence of juvenile *P. ulyssiponensis* in relation to microhabitat varied between CCA-present and CCA-absent microhabitat types (Figure 2.2). In general, significantly higher densities of juveniles were found in microhabitats classified as 'CCA-present', compared with all the other microhabitats (Figure 2.2; Table 2.2, all SNK tests). Juvenile *P. ulyssiponensis* were most abundant in mid-shore rock-pools covered by CCA (PM_p of all shores in both time periods, and PMS_p in Vale Marim summer 2007) and/or in other CCA-dominated microhabitats, namely the urchin burrows in Oliveirinha (U_p – Figure 2.2; Table 2.2, SNK tests) and the steep low-shore open-rock at Port, where the maximum density of 64 juveniles per 15 × 15 cm was recorded in summer 2007 (OL_p at Port: Figure 2.2; Table 2.2, SNK tests). In addition, no *P. ulyssiponensis* juveniles were found on mid-shore open-rock in any shore (OM_a) or on patches of *Lichina* (L_a) and *Fucus* (F_a) (Figure 2.2, summer 2007). Consistently at all sampled shores and surveys, densities registered at the other microhabitats classified as 'CCA-absent' were either null or very low (the highest mean density of 5.06 juveniles in 15 × 15 cm was recorded in mid-shore crevices within shale intrusions – CMS_a in Vale Marim during summer 2007). The unique exception was the *Mytilus* microhabitat (M_a in Oliveirinha in summer 2007: Figure 2.2; Table 2.2, SNK test), for which the mean density of 12 juveniles in 15 × 15 cm was found to be significantly higher than in all the other CCA-absent microhabitats and similar to two CCA-present microhabitats.

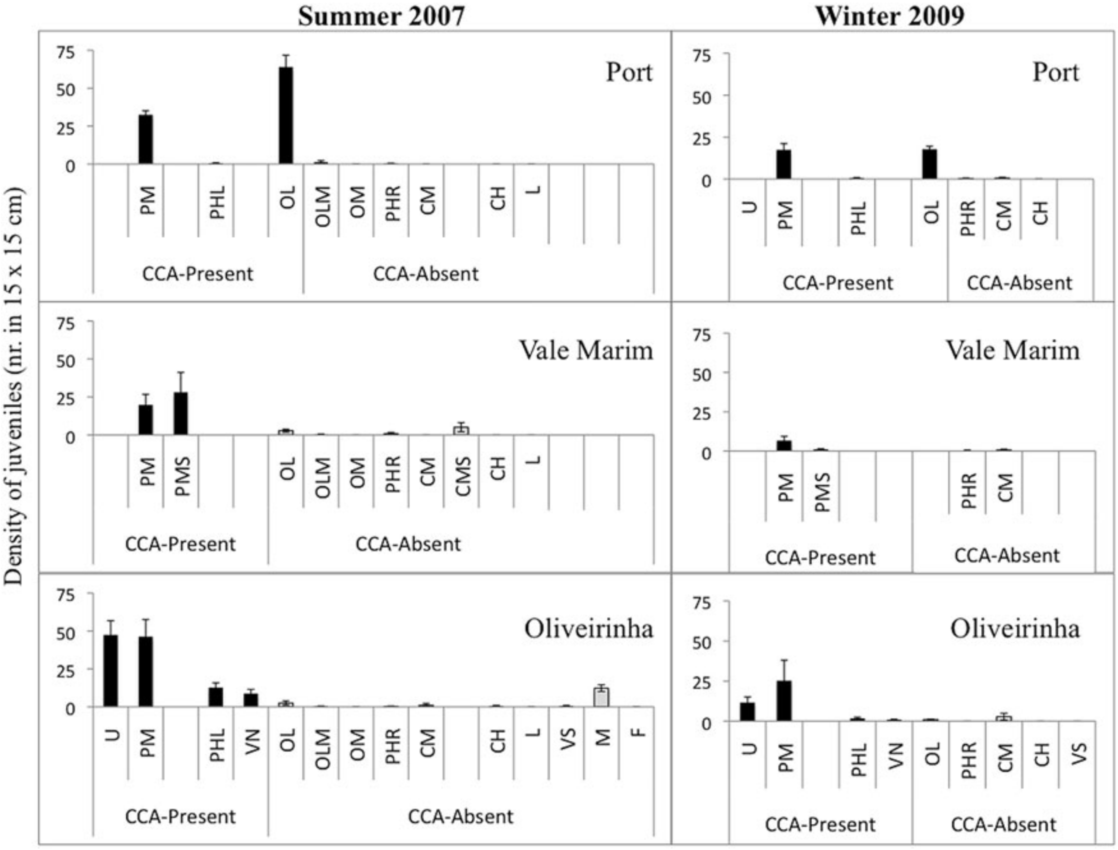

Figure 2.2 Abundance of juvenile *Patella ulyssiponensis* (mean ± SE, N = 4) on CCA-present (black bars) and CCA-absent (grey bars) microhabitats, at three shores (Port, Vale Marim, Oliveirinha) on the SW coast of Portugal, during two surveys (summer 2007 and winter 2009). microhabitat acronyms listed in Table 2.1. Blank spaces displayed at the x-axes correspond to non-sampled microhabitats at a given shore/survey (should not be confounded with records of null/very low densities at sampled microhabitats, for which x-axes categories are labelled).

Table 2.2 Summary of results of analyses of variance to determine the distribution of juvenile *P. ulyssiponensis* along a range of intertidal microhabitats, within three shores (Port, Vale Marim, Oliveirinha) in SW Portugal, in summer 2007 and winter 2009.

Period	Shore	Data	df	MS	*F*	*P*-	SNK
Summer 2007	Port	$\ln(x + \text{constant}_a)$	8	2.79	222.98	***	$OL_p > PM_p >$ others
	Vale Marim	$\ln(x + \text{constant}_b)$	9	5.12	17.49	***	Undefined pattern but $(PMS_p = PM_p) >$ others
	Oliveirinha	$\ln(x + 1)$	13	7.93	30.74	***	$(U_p = PM_p) > (PHL_p = M_a = VN_p) >$ others
Winter 2009	Port	$\ln(x + 1)$	5	7.58	60.76	***	$(OL_p = PM_p) >$ others
	Vale Marim	$\ln(x + 1)$	3	2.15	5.49	*	$PM_p >$ others
	Oliveirinha	$\ln(x + \text{constant}_c)$	8	6.34	10.78	***	$(PM_p = U_p) >$ others

df, degrees of freedom; MS, mean sum of squares; *F*, F-statistic; SNK, Student–Newman–Keuls test.
$\text{constant}_a = 8$; $\text{constant}_b = 1.2$; $\text{constant}_c = 0.5$.
Habitat acronyms listed in Table 2.1.* $P < 0.05$,** $P < 0.01$,*** $P < 0.001$.

Regarding the laboratory larval rearing assay, the survival rate of healthy individuals relative to the initial number of eggs (45,125 ± 4399.67 eggs in 500 ml – mean ± SE, N = 4 beakers), until the trochophore and competent post-torsional *veliger* stages was 24.02 ± 3.02% and 0.34 ± 0.07% (mean ± SE percentages), respectively.

Larvae of *P. ulyssiponensis* settled within all the 13 experimental dishes that were set up during the settlement experiment. From the total number of 673 competent larvae initially introduced, 75 (11.1%) and 115 (17.1%) settlers were recorded on the chips of substrata, as well as 95 (14.1%) and 54 (8%) individuals attached to the glass surfaces, respectively at the end of the 24-h and the 48-h observation periods.

Observed settlers were well-developed pediveliger larvae, with the cephalic region oriented downwards and the foot firmly attached to the substratum surface, either fixed or in some cases showing exploratory behaviour by crawling along the substrata. Nevertheless, observations of metamorphosis (defined as the complete loss of the swimming organ *vellum*) were residual (93.9% of settlers still maintained some cilia after 48 h of exposure to substrata). Morphology of settlers corresponded to the late larval stage described for *Patella vulgata* as the 'crawling larva with velum' (*sensus* fig. I*d*, p. 109 in Lebour, 1931) and measured ~200–220 µm in cross-shell length.

The hypothesis of induction of higher settlement of *P. ulyssiponensis* larvae by CCA was not supported, as at both observation times, the percentage of settlers relative to the initial number of larvae was similar between chips of rock encrusted by CCA and chips of bare-rock (Figure 2.3 – 24 h: *t* = 0.73, *P* = 0.48; 48 h: *t* = 0.75, *P* = 0.47). The maximum percentage of larval settlement onto the chips of substrata was registered on bare-rock (34.8%).

Comparing the numbers of larvae settled onto the chips of substrata with the ones settled onto glass in each treatment and at each observation time, it was found that, from the total number of settled larvae in each set of dishes: at 24 h, most larvae settled onto CCA-chips (66%) while a minority settled onto rock-chips (36%); whereas at 48 h, the majority of larvae settled onto rocky substratum independently of being encrusted or not by CCA (80% on CCA-chips; 62% on rock-chips).

Furthermore, in the set of CCA-chips, 60 and 40% of the total number of settlers, observed at 24 and 48-h respectively, were recorded in pits of substratum not covered by CCA (Figure 2.4). Given the small area of these pits, observed settlers within each pit were positioned very close together.

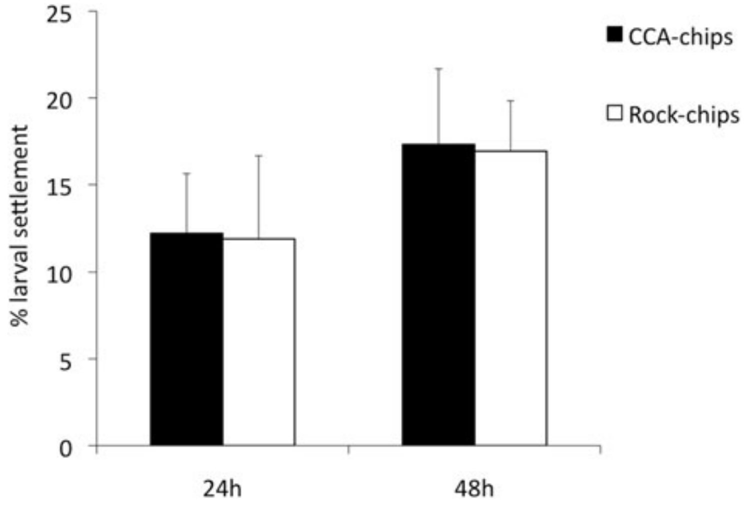

Figure 2.3 Settlement of *Patella ulyssiponensis* (percentage of settlers relative to the initial number of competent larvae; mean ± SE), 24 and 48 h after exposure to substrata, in CCA-chips (N = 5, black bars) and rock-chips (N = 8, white bars).

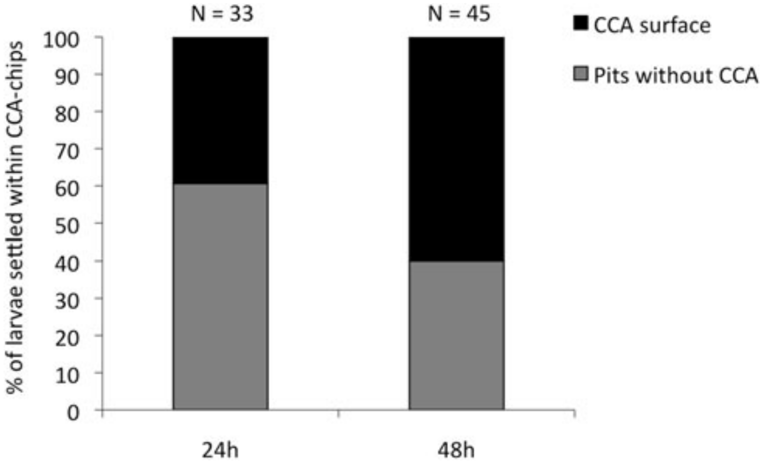

Figure 2.4 Distribution of settlers of *Patella ulyssiponensis* in a set of five CCA-chips, 24 and 48 h after exposure to substrata, with respect to their occurrence within the overall CCA surface (bar portions in black) or within tiny pits of substratum lacking CCA cover occupying a maximum of 5% of the total area of each chip (bar portions in grey).

2.5 Discussion

The focus of our study was the relationship between early life-history stages of *Patella ulyssiponensis* and CCA. Our results demonstrated differing patterns from settlers to juveniles in relation to CCA microhabitat presence. We found that juvenile *P. ulyssiponensis* (MSL < 10 mm) in SW Portugal occur almost exclusively in microhabitats characterized by the dominance of CCA. This species-habitat association was consistently found in all sampled shores and times and it is probably specific for *P. ulyssiponensis*, as it was not observed for other limpet species

(*Patella depressa* and *Siphonaria pectinata*) sampled on the same surveys (Seabra et al., in prep.).

The highest juvenile densities were found at the low-shore open-rock of Port (OL_p), with values nearly 20 times higher, on average, compared with the low-shore open-rock at the other sampled shores on both surveys (OL_a in Vale Marim and Oliveirinha). At Port, low-shore zones occur within particularly steep and wave-exposed platforms, where, in contrast with the other shores, foliose algae are scarce and CCA covers most of the space available (CIEMAR, 2018). Although vertically narrow, this CCA-dominated low-shore area supports an exceptionally large population of *P. ulyssiponensis* (CIEMAR, 2018). Moreover, observations on the shells of live large-sized *P. ulyssi- ponensis* (MSL > 30 mm) in the low-shore at Port indicated that the majority of these individuals were completely overgrown by well-developed rugose forms of CCA, also providing home for *P. ulyssiponensis* juveniles (personal observations). These population patterns parallel those described for the pear limpet *Scutellastra cochlear*. This patellid species dominates a characteristic 'cochlear zone' at the low-shore level of South African shores, where it occurs in clear association with the CCA *Spongites yendoi* (formerly referred as 'lithothamnion': e.g. Branch, 1975*a*). Through territorial gardening, *S. cochlear* excludes most algae and macrofauna (Branch, 1976), limiting the biodiversity in its vicinity to *S. yendoi* and to strips of filamentous red algae surrounding each limpet (Branch, 1975*b*; Plaganyi and Branch, 2000). Density of *S. cochlear* is positively related to wave action, so dense populations formed by uniformly spaced and closely packed individuals occur on wave-exposed shores, with juveniles mostly occurring on the backs of adults due to crowding (Branch, 1975*a*, 1975*b*). Similarly, the relatively greater wave action at Port probably contributes to the dominance of CCA and the high abundance of *P. ulyssiponensis* on the low-shore, by dislodging the less robust erect algae and releasing suitable substrata for limpet establishment and high recruitment rates. As in the *S. cochlear–S. yendoi* interaction, *P. ulyssiponensis* at Port probably feeds mainly on the 'Lithothamnia' present on the rock and on conspecific shells, preventing overgrowth by other algae as settling spores are cleared away through limpet grazing activity.

The other microhabitats where significantly higher juvenile densitiesof *P. ulyssiponensis* were found on all shores consisted mostly of CCA-covered rock-pools distributed across the intertidal zone (U_p in the lower levels, PM_p/PMS_p in the mid shore and/or PHL_p at the upper levels). Similar spatial patterns of association of small size-classes of *P. ulyssiponensis* with rockpool microhabitats dominated by CCA have been reported for the Portuguese coast (Guerra and Gaudêncio, 1986) and the North Atlantic in general (e.g. Bowman, 1981; Delany et al., 1998; Firth and Crowe, 2008).

The recruitment of *P. ulyssiponensis* is probably limited by its low desiccation tolerance (Davies, 1969) and by high susceptibility of recent benthic stages to temperature variation and storm damage (e.g. Thompson, 1979; Bowman and Lewis, 1986). Nevertheless, in our study, juvenile association with CCA-dominated microhabitats was apparently not an outcome of the limpet preference for submerged/damp/wave-protected microhabitats. For instance, during the summertime survey in Oliveirinha, significant differences were found between high-shore rock-pools with and without CCA (PHL_p *vs* PHR_a), which had similar dimensions (respectively, 318 cm^2 *vs* 331 cm^2 of surface area, on average; all <10 cm depth) and were closely located at the same rocky platforms and shore level. Overall, our results on juvenile distribution patterns suggest the importance of the 'Lithothamnia' itself for *P. ulyssiponensis* population recruitment, over other co-varying factors in the intertidal environment. This assumption is in agreement with the findings of a manipulative study carried out by Delany et al. (2002) on Clare Island (Ireland). They tested the effect of the presence of this living algal complex on the recruitment of juveniles and on the persistence of adults of intertidal limpets within rock-pools, separately for *P. ulyssiponensis* and *P. vulgata*. In contrast to *P. vulgata*, juvenile recruitment and adult immigration rates of *P. ulyssiponensis* were significantly reduced in rock-pools where 'Lithothamnia' had been killed by blowtorching the substratum (Delany et al., 2002). Comparable importance has been given to biological interactions over physical features in dictating limpet population characteristics (such as distribution, size-structure, behaviour and feeding habits), in the case of *Patella vulgata* in Britain (Lewis and Bowman, 1975) and several *Patella* species in South Africa (Branch, 1976).

CCA presence is possibly not an obligatory condition of the nursery grounds of *P. ulyssiponensis* in SW Portugal, as juveniles occurred within a few CCA-absent microhabitats. Particularly, within *Mytilus* patches, sampled during our summertime survey in Oliveirinha, the abundance of juveniles was similar to those estimated in two CCA-present microhabitats. This is in agreement with the work of Delany et al. (2002), which was the first record of *P. ulyssiponensis* recruiting in the absence of any living CCA, despite its demonstrated importance for recruitment of this limpet.

Although not strictly essential for juvenile occurrence, our field surveys supported the idea that CCA do play a role in the settlement and/or recruitment of *P. ulyssiponensis* in SW Portugal. Our laboratory study on the settling response of *P. ulyssiponensis* was aimed to test the hypothesis that CCA would have some attribute detectable by *P. ulyssiponensis* larvae and capable of inducing higher rates of settlement, compared with other rocky substrata. Our results showed similar larval settlement between chips of CCA-encrusted rock and chips of bare-rock.

These two types of substratum were chiselled from rock-pools and open-rock at the same tidal level. Therefore, settlement patterns of laboratory-reared larvae did not match the distribution of juveniles across intertidal microhabitats, suggesting that the spatial variation in recruitment of this species is probably not the outcome of a specific CCA-inducement cue triggering larval settlement.

The results of our settlement experiment are similar to previous results obtained in December 2010 (fewer replicates, data not shown). Nonetheless, our present results are preliminary because they should be confirmed by choice-experiments where CCA-chips, and rock-chips, are simultaneously presented to competent larvae. Our results provide estimates of larval settlement onto different natural substrata, and do not allow for inferences about selection or microhabitat preference of *P. ulyssiponensis* larvae in the field. Nevertheless, given 'the choice' between the chip of substratum and the glass surface within each dish, larvae settled mainly onto rocky substratum rather than onto glass, independent of the presence/absence of CCA (at 48 h, 71% of the total number of settled larvae, on average, were recorded onto substratum). Moreover, the fact that, within the area of CCA-chips, clustering of settlers was observed at both 24 and 48 h in very tiny pits lacking CCA-cover, suggests that *P. ulyssiponensis* larvae may have the ability to discriminate between different natural substrata at a very small spatial scale (few mm^2). The occupation of 'CCA-free' pits by 51% of the total number of settlers recorded onto CCA-chips was impressive, especially if one considers the space-limiting nature of the pits. This observation supports our results on the absence of a positive effect of CCA presence on the larval settlement of *P. ullyssiponensis.* However, our results on the distribution of settlers should be confirmed in future experiments with frequent and accurate monitoring of individual positions of settlers (e.g. by time-lapse photography of settlement surfaces), to detect their timing and location at settlement, and to follow their possible movements after settlement.

Little information is reported in the literature regarding settlement and metamorphosis of patellids in controlled conditions. The conditions to promote larval settlement seem to vary among *Patella* species, e.g. presence of a conspecific adult cue for *P. caerulea* (Wanninger et al., 1999; Ferranti et al., 2018) and proximity to the air-water interface for *P. ferruginea* (Guallart et al., 2017). Experimental investigation *in vitro* regarding settlement is hampered by the fact that the actual quantity of settlers and/or metamorphosed juveniles produced in laboratory studies was either very limited (Smith, 1935; Wanninger et al., 1999; Guallart et al., 2017) or not quantified (Dodd, 1957; Ferranti et al., 2018). Additionally, consistent evidence has been recently gathered concerning the need to supply settlers with a food suitable for their benthic mode of feeding (an algal film to be scraped by the radulae, i.e. microalgae and/or macroalgae sporelings and germlings, coating the surfaces available for settlement) to follow the development of metamorphosing specimens in the laboratory. Despite confirming the lecithotrophic nature of pelagic larvae of *Patella caerulea*, Ferranti et al. (2018) have demonstrated that larvae need to be fed as soon as the pediveliger stage is reached. In our study, the algal film naturally present on the surface of chips of substrata was possibly insufficient, given the observed lack of complete metamorphosis and the fact that a high mortality of settlers was observed 72 h after the start of the experiment.

Developing protocols for culturing larvae of *P. ulyssiponensis* was laborious, and not fully established during our pilot assays. In fact, 10 unsuccessful pilot assays were tried before the methods reported here. These observations were in agreement with the study of Espinosa et al. (2010) in which larvae of *Patella ferruginea* did not survive beyond the pre-torsional veliger stage, suggesting a common pattern of high sensitivity of lab-reared larvae at this development stage (also referred to by Smith, 1935) and/or a biological constraint of the genus during the ontogenic phase of torsion. Recently, Pérez et al. (2016) standardized a *Patella* spp. embryo-larval bioassay for use in ecotoxicological studies, which included data on *P. ulyssiponensis*, providing reliable protocols for larval rearing. However, the endpoint established in Pérez et al. (2016) was the percentage of normal trochophore larvae and therefore it would be important to apply the same approach to the latter stages of larval development. In contrast with Hodgson et al. (2007), it is possible to compare our results to those of Pérez et al. (2016) for rearing *P. ulyssiponensis* up to the trochophore stage because they assessed survival in the same way (percentage of normal trochophores relative to the initial number of fertilized eggs) and used the same incubation temperature (18°C). Our fertilization success was low (24%), although within the range of 21.6–46.3% obtained by Pérez et al. (2016) under optimized conditions. When applying these conditions in a very recent study (Pérez et al., 2019), an even lower percentage of normal *P. ulyssiponensis* trochophores (11.56%) was recorded (stated as '%NL' in Table 2.1, p. 154 in Pérez et al., 2019). Taken together these results point to the high variability in successful larval development and the need for further methodological improvement (Pérez et al., 2016).

Clearly, more work is required to better understand the process of settlement in *P. ulyssiponensis*. Quantifying settlement in the field is often precluded by spatial patchiness, temporal unpredictability, and/or scarce numbers – all characteristic of broadcast-spawning intertidal gastropods (Underwood, 2004). Therefore, laboratory studies are probably the best alternative to study this process, notwithstanding the above-mentioned difficulties.

Overall, our study established different patterns of association with CCA in different stages of *P. ulyssiponensis* benthic life. This was due to the contrast found between larvae soon after settlement and juveniles, in terms of microhabitat fidelity. Settlers in our laboratory experiment were relatively similar-sized individuals with a maximum of two days post-settlement. In contrast, juveniles in the field showed greater variation in size and likely in age. Even juvenile patellids of the same size may belong to different age groups, due to substantial variance of individual growth rates depending on microhabitat (Lewis and Bowman, 1975). Therefore post-settlement age of the whole set of sampled juveniles may have spanned from a few months to more than a year (Bowman, 1981). Consequently, juvenile distributions at the time of surveys may not correspond to settlement locations. During the interval between initial settlement and the time at which juveniles are large enough to be visible and identifiable in the field, distribution across microhabitats can change due to differential mortality or movement (e.g. Keough and Downes, 1982; Rodriguez et al., 1993). We propose that the mismatch between juvenile distribution and larval settlement patterns is that recruitment densities of *P. ulyssiponensis* are not driven directly by the larval settling response to the presence of CCA, but instead from differential effects of selective pressures among different microhabitats causing spatial variation in post-settlement mortality. Accordingly, we suggest that the survivorship of *P. ulyssiponensis* settlers would be higher in CCA-dominated microhabitats, due to presence of potentially favourable conditions. Given the deep excavating capacity of the radula of *P. ulyssiponensis* (Hawkins et al., 1989), the algal complex 'Lithothamnia' could be important for survival of early benthic stages of this species, possibly providing a reliable food resource. The next step should be exploring the mechanisms responsible for potentially higher survival in CCA.

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CHAPTER 3: Rock-pools as nurseries for co-existing limpets: Spatial and temporal patterns of limpet recruitment

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3.1 Abstract

Rock-pools are considered to be important microhabitats for early shore-life of intertidal limpets. We investigated recruitment patterns of three co-existing limpet species in the region of Sines (SW Portugal): the patellids *Patella depressa* and *Patella ulyssiponensis*, and the siphonariid *Siphonaria pectinata*. Juvenile limpets (shell length ≤10 mm for *P. depressa* and ≤5 mm for *S. pectinata*) were counted in summer 2007 and winter 2009, in a wide range of intertidal microhabitats, on three natural shores (one shore inside the Port of Sines and two shores outside the Port, 4 and 9 km away). Temporal and among-shore patterns of abundance of recruits (shell length < 5 mm for all species) into mid-shore rock-pools were examined over quarterly periods, from May 2005 to July 2008. Despite a widespread distribution of *P. depressa* and *S. pectinata* juveniles, significantly higher juvenile abundance was mostly found in microhabitats of rock-pools (maxima of 94 and 92 juveniles in 15x15 cm, respectively, both in rock-pools at mid-shore). All species were found to recruit into mid-shore rock-pools year-round. Recruitment of both patellid species followed a similar seasonal pattern, being consistently low during all summer periods (maxima of 64 recruits of *P. depressa* during spring 2008 and 45 recruits of *P. ulyssiponensis* during spring 2007, both in 15x15 cm). High inter-annual variation was found in the intensity of recruitment of *S. pectinata*, with the yearly highest densities of recruits during summer or autumn periods (maximum of 134 recruits in 15x15 cm during summer 2005). Recruitment of all species was highest on the farthest shore from the Port. This study suggests the primacy of rock-pools as nurseries for both patellogastropod and pulmonate limpets and the value of these microhabitats for sustaining limpet populations on this coast, where rocky-shores have been gradually replaced by artificial structures with scarcity of rock-pools.

Keywords: Patellid, Siphonariid, Tidepool, Nursery ground, SW Portugal, Port of Sines

3.2 Introduction

Many animals have distinctive nurseries. The functional significance of these microhabitats for sustaining populations of marine organisms has been widely accepted, despite substantial debate on the challenging concept of 'nursery habitat' within coastal ecosystems (see recent contrasting approaches in Litvin et al., 2018; Lefcheck et al., 2019). Marine nurseries provide food availability and/or refuge to young individuals, protecting them from physical environmental extremes or from intense biological interactions (e.g. predation) that affect juvenile density, growth and survival (Beck et al., 2001; Dahlgren et al., 2006). These areas often correspond to an integrated network of specific microhabitat patches that are functionally interconnected in a dynamic 'nursery seascape' mosaic (Sheaves et al., 2015 and references therein). This is especially the case in marine species possessing a larval stage in their life cycles, such as nektonic fauna (mostly fishes and decapod crustaceans, e.g. Sheridan and Hays, 2003) and mobile benthic invertebrates (e.g. Stoner, 2003). Processes acting in nursery grounds have consequences for the recruitment of juveniles to adult populations in terms of both abundance and distribution. Much attention has been given to these processes in population demography of commercial fish species (e.g. Nash and Geffen, 2000) mainly within wetlands (Lefcheck et al., 2019), but they equally apply to most marine invertebrates (Beck et al., 2001; Nagelkerken et al., 2015), including those inhabiting rocky shores.

Rocky-shore limpets are gastropod grazers embracing taxonomic groups of greatly different evolutionary origins (Branch, 1985), such as the families Patellidae (Patellogastropoda) and Siphonariidae (Heterobranchia), characterized by presence of gills and lungs, respectively. Patellid limpets have been extensively recognized as 'keystone species' in rocky intertidal communities (e.g. Branch, 1981; Hawkins and Hartnoll, 1983; Hawkins et al., 1992). In contrast, the ecological role of siphonariid limpets in intertidal marine ecosystems has received comparatively less attention (but see Hodgson, 1999 for a review). Patellids are broadcast spawners with fully planktonic larval development wherein trochophore larvae develop into pre and post-torsional veligers, before settling and metamorphosing on hard substrata (e.g. Branch, 1981). In contrast, siphonariid limpets are hermaphrodites reproducing by internal fertilization, as in all pulmonates (Branch, 1981; Hodgson, 1999). Their life cycle includes egg-laying within egg capsules surrounded by a protective gelatinous matrix, which hatch into planktotrophic veliger larvae in the majority of species (Chambers and McQuaid, 1994a). Lusitanian (warm-temperate) patellid species occur in North-East Atlantic coastlines, two of them are *Patella depressa* Pennant, 1777 and *Patella ulyssiponensis* Gmelin, 1791. Their population attributes and life history traits have been less commonly assessed than for the boreal (cold-temperate) species *Patella vulgata* Linnaeus, 1758 (but see information on the three congeneric species in: Bowman (1981), Bowman and Lewis (1986), Guerra and Gaudêncio (1986) and Casal et al. (2018)). Siphonariids are globally widespread but mostly found in the southern hemisphere (Hodgson, 1999), namely in lower latitudes as the Indo-Pacific (Dayrat et al., 2014). However, the sub-tropical species *Siphonaria pectinata* (Linnaeus 1758) (striped-false limpet) is present on European coastlines, where its distribution range has been extending eastwards in the Mediterranean (Crocetta, 2016) and northwards on the West Atlantic coast of the Iberian Peninsula (Rubal et al., 2013).

Rock-pools are potentially important microhabitats for the recruitment of intertidal limpets. These microhabitats harbour highly diverse marine communities and have long been considered as refuges from stressful environmental conditions due to smaller fluctuations in physical conditions in comparison with the surrounding emersed open-rock (reviewed by Metaxas and Scheibling, 1993). Their nursery function has been extensively documented for pelagic organisms, such as fishes (e.g. Beckley, 1985; Moring, 1986; Bennett, 1987; Krück et al., 2009; Dias et al., 2016; Lobato et al., 2016) or shrimps (Vinagre et al., 2015). In the case of patellid limpets, newly settled recruits and/or high juvenile densities have been commonly found in the microhabitat of rock-pools (e.g. Lewis and Bowman, 1975; Guerra and Gaudêncio, 1986; Delany et al., 1998; Firth and Crowe, 2008). This pattern has been mainly discussed on the basis of the apparent vulnerability to temperature variations of patellids during early shore-life (see panel settlement studies by Bowman, 1985; Bowman and Lewis, 1986) or as a result of its association with crustose coralline algae that are conspicuously present in shallow rock-pools (McGrath, 1992; Delany et al., 2002; Seabra et al., 2019). In the case of siphonariid limpets, despite a few reports on species that are pool-dwellers as adults (*Kerguelenella lateralis*, Davenport, 1997; *S. maura*, Garrity, 1984; *S. capensis*, Branch and Cherry, 1985; *S. alternata*, Cook, 1979) or that deposit their egg-masses into rock-pools (*S. denticulata*, Creese, 1980; *S. capensis*, Chambers and McQuaid, 1994b; *S. australis*, Russell and Phillips, 2009), the use of rock-pools by recruits/juveniles is largely unknown. Nevertheless, Ostalé-Valriberas et al. (2018) found that experimentally created rock-pools increased the overall abundance of *S. pectinata* on artificial substrata of coastal defence structures in Ceuta, with individuals (non-specified size) and egg masses being mostly found inside the created pools.

Besides rock-pools, other wet and patchy intertidal microhabitats may be presumably important nursery grounds for limpets. Juvenile abundance of *P. vulgata* was found to be associated with damp crevices and mussel patches (Lewis and Bowman, 1975), and experimentally added pits were shown to enhance recruitment and sustain long-term populations of *Patella candei* on a man-made seawall (Martins et al., 2010, 2016). For several siphonariid species, the use of cracks and crevices by adults during spawning has been considered as an adaptive behaviour for egg-mass protection from predation, desiccation and wave action (e.g. Levings and Garrity, 1986; Ocaña and Emson, 1999).

The relative importance of rock-pools as limpet nurseries compared to other potential nursery microhabitats has been rarely assessed (but see Lewis and Bowman, 1975). Moreover, studies on the ecology of early benthic stages of limpets comprising both patellids and siphonariids in the Northern hemisphere are scarce (but see Espinosa et al., 2011; Rivera-Ingraham et al., 2011).

For limpet species living in sympatry, microhabitat partitioning or inter-specific differences in population dynamics can act as co-existence strategies (Firth and Crowe, 2008, 2010; Casal et al., 2018). These mechanisms may arise from differences in spatial or temporal patterns of recruitment, allowing segregation of ecological niches between biologically or ecologically similar species. Identifying the subset of juvenile microhabitats that is most important for the recruitment of co-occurring species and the temporal and site-specific variation in their nursery value is likely to have implications in conservation and management of coastal regions.

Three limpet species are abundant and co-exist throughout the Southwest Portuguese coast: the patellogastropods *P. depressa* and *P. ulyssiponensis*, and the pulmonate *S. pectinata* (Boaventura et al., 2002). In the region of Sines (SW Portugal), the configuration of the shoreline at the leeward side of the Cape of Sines has experienced major anthropogenic changes since the 1970s, due to the establishment of the Port of Sines, a deep-water oceanic port with worldwide maritime importance. Inside the limits of the Port, man-made breakwaters mainly compose the rocky-intertidal environment, where rock-pools are mostly absent. On these breakwaters, a lower density of limpets, compared with nearby natural areas outside the Port, has been consistently reported in the scope of long-term environmental monitoring of intertidal assemblages within the Port (CIEMAR, 2018 and references therein). If rock-pools are important as a limpet nursery microhabitat, it would be expected that the proliferation of coastal defence structures in this urban seascape could negatively affect the recruitment of limpets, not only on artificial structures (due to scarcity of favourable microhabitats), but also on adjacent natural shores inside the Port, due to a potential decrease of overall population fertility and/or larval supply.

Our overall aim was first to understand the relative importance of rock-pools as microhabitats for juvenile limpets, through the assessment of their distribution across the intertidal zone, focusing on *P. depressa* and *S. pectinata*. Having demonstrated the importance of rock-pools as juvenile microhabitats of limpets, the next stage was to describe, for rock-pools alone, seasonal and inter-annual variation in recruitment of three species (*P. depressa*, *P. ulyssiponensis* and *S. pectinata*) and differences among natural rocky-shores. One sampled shore is located within the Port of Sines (hereafter called Port), being presumably impacted by the extensive system of man-made structures, and the other two located at 4 and 9 km away from the port. Our specific hypotheses were: (1) juveniles of *P. depressa* and *S. pectinata* are more abundant in microhabitats of rock-pools compared to other available intertidal microhabitats; (2) the abundance of recruits into rock-pools vary temporally according to the yearly reproductive cycle of each species; and (3) the abundance of recruits into rock-pools is lower on the shore located inside the Port compared to other shores. Additionally, we investigated if there were among-shore differences in the patterns of immigration of adult limpets into rock-pools.

3.3 Materials and methods

3.3.1 Study area

This study was made on the Southwest coast of continental Portugal, where the Port of Sines is located (Figure 3.1). The tidal regime is semidiurnal (approximately 3.5 m of maximum amplitude) and limpets (*Patella* spp. and *S. pectinata*) are abundant on natural rocky shores.

Three natural shores were selected for sampling, at different settings relatively to the Port of Sines (Figure 3.1): (i) the only wave-exposed natural shore remaining inside the Port, adjacent to the General Cargo Terminal, hereafter named ‘‘Port’’ - 37°56’39.45"N, 8°51’24.43"W), (ii) Vale Marim, located less than 1.5 km from the Southern vicinity of the Port of Sines (~4 km away from Port — 37°55’52.91"N, 8°48’56.11"W) and (iii) Oliveirinha, located approximately 6 km of shoreline further South (~9 km away from Port — 37°53’12.38"N, 8°47’47.97"W). A description of the geomorphology of these shores is given in Seabra et al. (2019) (Chapter 2).

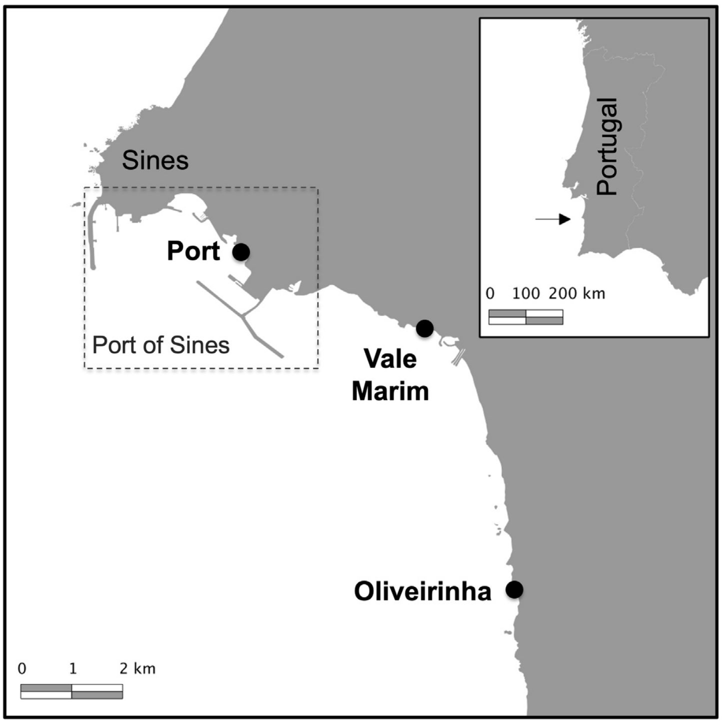

Figure 3.1 Study region, location of the Port of Sines and sampling shores (Port, Vale Marim and Oliveirinha - inside the Port of Sines, 4 and 9 km away, respectively).

3.3.2 Focal species

The focus was on three common and geographically widely distributed limpet species. *Patella depressa* occurs from Senegal to North Wales; whereas *P. ulyssiponensis* ranges from the North African coast as far north as Norway, also present across the Mediterranean (Christiaens, 1973). *S. pectinata*, previously considered as an amphi-Atlantic species (Voss, 1959), is currently confirmed to be restricted to the Eastern Atlantic (Espinosa et al., 2016; Giribet and Kawauchi, 2016), with recently assessed range edges extending from Angola to the north border between Portugal and Spain (Rubal et al., 2015) and in the Mediterranean (Strait of Gibraltar, the African coastline up to Algeria and the Spanish coastline up to Murcia/Valencia area, Tunisia, Greece and Croatia; Crocetta, 2016 and references therein). On the Portuguese coast, *P. depressa* and *P. ulyssiponensis* are the most abundant limpet species inhabiting open-rock areas at mid and low-shore levels, respectively (Guerra and Gaudêncio, 1986; Boaventura et al., 2002); while *S. pectinata* is distributed across all tidal levels (CIEMAR, 2018) with considerable small-scale variation in abundance (Rubal et al., 2013). According to Castro (2004), breeding cycles of both *P. ulyssiponensis* and *P. depressa* in SW Portugal involve a resting period from April to August and presence of mature individuals most of the year, with maximum development and spawning in November and March. The seasonal presence of mature eggs in the gonads of *S. pectinata* is presently unknown for the Portuguese coast but, on natural shores of the region of Sines, egg-ribbons were found from April/May to September/October, their abundance differing between years but with two consistent maxima in June and August (Seabra and Cruz, unpublished data).

3.3.3 Relative importance of rock-pools as microhabitats for juveniles of Patella depressa and Siphonaria pectinata

Two surveys were carried in August–early September 2007 and in February 2009 (hereafter named summer 2007 and winter 2009) at three shores — Port, Vale Marim and Oliveirinha. Surveys searched for limpet juveniles, namely all field-detectable individuals with maximum shell length (MSL) of less or equal to 10 mm in the case of *Patella* spp. (assumed to be under the size reached within their first year; Bowman, 1981) and 5 mm in the case of *S. pectinata* (since size at first sexual maturity was reported to range between 5 and 6 mm; Ocaña and Emson, 1999).

For each survey and on each shore, a series of microhabitats with different physical and/or biological features was considered (Table 3.1). Four random sampling areas were assessed as replicates of each microhabitat. Sampling area differed among microhabitats (Table 3.1). Sampling procedures are described in detail in Seabra et al. (2019). Surveys were undertaken during low water spring tide periods by collecting and field-preserving all juveniles present within each sampling area. Species identification (following Bowman, 1981, in the case of patellids) and individual MSL measurement (to the nearest 0.1 mm) of all juveniles were carried out in the laboratory.

Table 3.1 List of intertidal microhabitats surveyed (‘‘x’’) during searches for juvenile limpets carried out in Summer 2007 and Winter 2009, on three shores (Port, Vale Marim, Oliveirinha — inside the Port of Sines, 4 and 9 km away, respectively) at the SW coast of Portugal.

Habitat type	Summer 2007			Winter 2009			Sampling areas
	Port	Vale Marim	Oliveirinha	Port	Vale Marim	Oliveirinha	
U - Urchin burrows in rock-pools - "Lithothamnia" areas surrounding *Paracentrotus lividus* within medium-depth rock-pools at low-shore			x			x	10 burrows containing large urchins clumped within the limits of a 15 x 15 cm quadrat
PM - Rock-pools at mid-shore - dominated by "Lithothamnia"	x	x	x	x	x	x	Patches with well-defined boundaries, total surface area estimations from scaled digital photographs
PMS - Rock-pools at mid-shore within shale intrusions - dominated by "Lithothamnia"		x			x		
PHL - Rock-pools at high-shore - dominated by "Lithothamnia"	x		x	x		x	
PHR - Rock-pools at high-shore dominated by bare-rock	x	x	x	x	x	x	
CM - Crevices at mid-shore	x	x	x	x	x	x	50 cm-long x 2 cm-wide transects along crevices
CMS - Crevices at mid-shore within shale intrusions		x					
CH - Crevices at high-shore	x	x	x	x		x	
M - *Mytilus* patches - mussel shells and bordering rock within overhangs at the upper mid-shore			x				Patches with well-defined boundaries, total surface area estimations from scaled digital photographs
F - *Fucus* patches - rock underneath and bordering *F. guiryi* thalli at the upper mid-shore			x				
L - *Lichina pygmaea* patches - rock underneath and bordering *L. pygmaea* thalli at the high-shore			x				
VN - Vertical North-facing walls - shaded platform ridges, moist bare-rock among mixed assemblages of encrusting algae (*Ralfsia verrucosa, Lithophyllum tortuosum, "Lithothamnia", Codium adhaerens*) and the barnacle *Perforatus perforatus*			x			x	Random 50x50 cm quadrats
VS - Vertical South-facing walls - sun-oriented platform ridges, with overhangs and cracks, dominated by bare-rock and the barnacle *Chthamalus montagui*			x			x	
OL - Open-rock at low-shore - the main occurrence zone of *P. ulyssiponensis*, in Vale Marim and Oliveirinha is characterized by foliose algae and algal turf interspersed with *Nemoderma tingitanum* and/or other encrusting algae (e.g. *Codium adhaerens*)	x	x	x	x		x	
OLM - Open-rock at lower-mid shore level	x	x	x				
OM - Open-rock at mid-shore - the main occurrence zone of *P. depressa* and the barnacle *Chthamalus montagui*	x	x	x				

Table 3.2 Sampling times, preceding quarterly (seasonal) periods and number of intertidal rock-pools for which limpet recruitment was assessed. Recruitment over three months was monitored in two sets of four rock-pools following initial limpet clearance (first set in May 2005 and second set in August 2006) by limpet re-clearance of substrata within rock-pools at every sampling time.

Sampling time	Quarterly recruitment period (seasonal periods between consecutive sampling times)		Number of sampled pools per shore
August 2005 (6th to 7th)	May-July 2005	Spring 2005	4
November 2005 (1st to 7th)	August-October 2005	Summer 2005	4
31st January to 1st February 2006	November 2005-January 2006	Autumn 2005	4
April 2006 (26th to 27th)	February-April 2006	Winter 2006	4
August 2006 (25th July to 1st)	May-July 2006	Spring 2006	4
October 2006 (19th to 21th)	July-September 2006	Summer 2006	8
January 2007 (12th to 13th)	October-December 2006	Autumn 2006	8
April 2007 (15th to 17th)	January-March 2007	Winter 2007	8
July 2007 (13th to 15th)	April-June 2007	Spring 2007	8
October 2007 (13th)	July-September 2007	Summer 2007	8
January 2008 (7th to 12th)	October-December 2007	Autumn 2007	8
April 2008 (5th to 6th)	January-March 2008	Winter 2008	8
July 2008 (5th to 6th)	April-June 2008	Spring 2008	8

Only data on *P. depressa* and *S. pectinata* are reported here. *Patella ulyssiponensis* was considered in Seabra et al. (2019) (Chapter 2). The juvenile density of each species recorded at each sampling area was standardized to a 15×15 cm area and taken as response variables. For each species, six separate analyses were performed, one for each shore in each survey, with microhabitat considered as a fixed factor with variable number of levels (due to different number of surveyed microhabitats in each shore/survey). To test for homogeneity of variances, Cochran's test was used, and square-root transformation was applied when this assumption was not met. Post-hoc comparisons among levels of factor microhabitat were assessed by Student–Newman–Keuls (SNK) tests. One-way analyses of variance (ANOVA) to factor microhabitat were carried using GMAV5 program (University of Sydney, Underwood and Chapman, 1997).

3.3.4 Temporal and spatial variation in limpet recruitment into rock-pools

3.3.4.1 Temporal recruitment patterns

On each of the three shores (Port, Vale Marim and Oliveirinha), an initial clearance of all visible limpets (to a minimum shell length of 0.4 mm) from a set of rock-pools was made in May 2005 (four pools) and then in August 2006 (other four pools). All rock-pools were situated at mid-shore, within the barnacle (*Chthamalus montagui*) zone, characterized by similar dimensions (surface area of 98–392 cm2, less than 10 cm mean depth) and bottom surfaces dominated by crustose coralline algae (CCA) (i.e. ''Lithothamnia'', *sensu* (Hawkins and Jones, 1992)). Rock-pools initially cleared in May 2005 or in August 2006 were subsequently re-cleared of all limpets on a quarterly basis until July 2008, and the species, number and size of each individual limpet were recorded for every rock-pool at every sampling time. Limpet recruitment into rock-pools was then assessed for every quarterly period prior to all of the 13 sampling times after initial limpet clearance (Table 3.2).

During limpet clearance, all limpets measuring less than 12 mm in MSL were collected with the use of forceps and dissecting needles, field-preserved in 96% ethanol and stored in the lab at 4◦C. They were later identified under a dissecting stereo microscope (following Bowman, 1981, for *Patella*), with their MSL measured to the nearest 0.1 mm using a calibrated eyepiece. All the other were removed from the substratum (with no subsequent replace-larger limpets present in sampled rock-pools (MSL ≥12 mm) ment) using a knife, and their identification and measurement was made in the field, measuring their MSL with callipers (1 mm precision). Pool surface area was estimated through analyses of digital photography. Limpet densities were calculated as numbers per 15×15 cm. Recruitment over three months was defined as the density of individuals measuring less than 5 mm in MSL (hereafter named as ‘‘recruits’’), recorded into rock-pools three months following limpet clearance. This size definition was based on a preliminary analysis of the size-frequency distribution of each species for three selected periods (autumn 2005, autumn 2006 and winter 2008 for both *Patella* species; summers of 2005, 2006 and 2007 for *S. pectinata*; Figures A.1–A.3 in Appendix). Each of these periods was selected for being preceded by a period of distinctive low recruitment of each species (all below 17% of the maximum densities recorded for every species, corresponding to a mean density of six *P. depressa*, four *P. ulyssiponensis* and nine *S. pectinata* recruits per 15×15 cm). This procedure minimized the potential influence of limpet migration into rock-pools on the definition of recruits. For both *Patella* species, the mean percentage of the densities of individuals measuring less than 3 mm (considered as monthly recruits into rock-pools by Delany et al. (1998)) and less than 5 mm in MSL were calculated relatively to the total density of each species on the three above-mentioned periods (Table S3.1 in Appendix). In the case of *S. pectinata*, as no relevant information was available in the literature, we calculated, for each of the above-mentioned periods, the mean percentage of the densities of individuals with MSL less than 2, 3, 4 and 5 mm relatively to the total density of this species (Table S3.2 in Appendix). For every species, percentage values consistently above 80% were only found considering individuals with MSL until 5 mm (Tables A.1 and A.2 in Appendix) and therefore a limit of 5 mm was established in common for the analyses of recruitment of the three species.

Recruitment of each species over time is presented as the mean density of recruits into all sampled rock-pools on the same shore (n = 4 or n = 8, Table 3.2) for each of the 13 quarterly periods.

3.3.4.2 Among-shore recruitment patterns

Recruitment patterns of each species (*P. depressa*, *P. ulyssiponensis* and *S. pectinata*) into rock-pools were examined for differences among shores: Port, Vale Marim and Oliveirinha. The same dataset used to describe temporal recruitment patterns (Section 3.3.4.1) was used, considering the data collected only between October 2006 and July 2008. During this time, eight replicate rock-pools (Table 3.2) were monitored quarterly on each of the three shores. Preliminary analyses indicated that no significant differences were found among shores in terms of dimensions (surface area and volume) and initial percentage cover of CCA of sampled rock-pools (data not shown).

For each species, the densities of recruits (MSL < 5 mm) assessed at eight sampling times in every sampled rock-pool were taken as the response variables and analysed through a one-way non-parametric multivariate analysis of variance (PERMANOVA - (Anderson, 2001)) with shore considered as a fixed factor. Additionally, the quarterly densities of adult immigrants (individuals of all the three species with MSL ≥10 mm) recorded in every sampled rock-pool at eight sampling times (response variables) were compared among shores (one-way PERMANOVA analysis to factor shore). Adult immigration was defined as the presence of limpets above this size (MSL ≥10 mm) within rock-pools three months following limpet clearance, assuming that the arrival of these individuals between consecutive sampling times was due to individual movement from outside the pools, rather than larval settlement and subsequent growth inside the pools. The method used was unrestricted permutation of raw data using 999 permutations. This was done after testing for differences in multivariate dispersion among shores (measured as distances to the centroids) using the PERMDISP routine (Anderson et al., 2008). The same analyses were done on square-root transformed densities when PERMDISP analysis was significant. Where differences were detected by PERMANOVA, pair-wise comparisons between shores were used to determine which shores differed. All these procedures were implemented in PERMANOVA+ for PRIMER package (Anderson et al., 2008). In all analyses, the Euclidean distance was used as the similarity index.

3.4 Results

3.4.1 Relative importance of rock-pools as microhabitats for juveniles of Patella depressa and Siphonaria pectinata

A total of 2484 and 147 juveniles of *P. depressa* were found during summer 2007 and winter 2009, respectively. For *S. pectinata*, 600 and 1499 juveniles were respectively sampled during the same summertime and wintertime surveys. Juveniles of *P. depressa* and *S. pectinata* were present in a wide diversity of intertidal microhabitats but, in general, their abundance differed greatly among microhabitats (Figures 3.2 and 3.3; Table 3.3). Exceptions were found where there was least diversity of sampled microhabitats: during the wintertime survey in the Port and at Vale Marim, in which juvenile densities of both species did not differ with respect to microhabitat (Figures. 3.2 and 3.3; Table 3.3). In most cases, juvenile densities of both species were highest in rock-pools (maxima of 94 and 92 individuals in 15×15 cm on average, respectively for *P. depressa* in the summertime survey and *S. pectinata* in the wintertime survey, both within rock-pools at mid-shore in Oliveirinha). Lowest densities of both species were generally found in Lichina and Fucus patches (L, F), urchin burrows (U) and/or open-rock microhabitats (OM, OLM, OL) (Figures 3.2 and 3.3).

Significant differences among microhabitats were mainly found between two groups of microhabitats, rock-pools at mid-shore dominated by ‘’Lithothamnia’’ (PM) or other microhabitats of rock-pools at mid and high-shore (PMS, PHL, PHR) where recruitment was higher *versus* all other microhabitats or mostly open-rock microhabitats where recruitment was lower (Table 3.3). In the summertime survey at Vale Marim, juvenile density of both species was significantly higher not only in microhabitats of rock-pools but also in mid-shore crevices (CM and CMS; Figures 3.2 and 3.3; Table 3.3). Density of *P. depressa* juveniles was also significantly higher in both rock-pools and crevices at mid-shore (PM and CM) in the wintertime survey in Oliveirinha (Figure 3.2; Table 3.3).

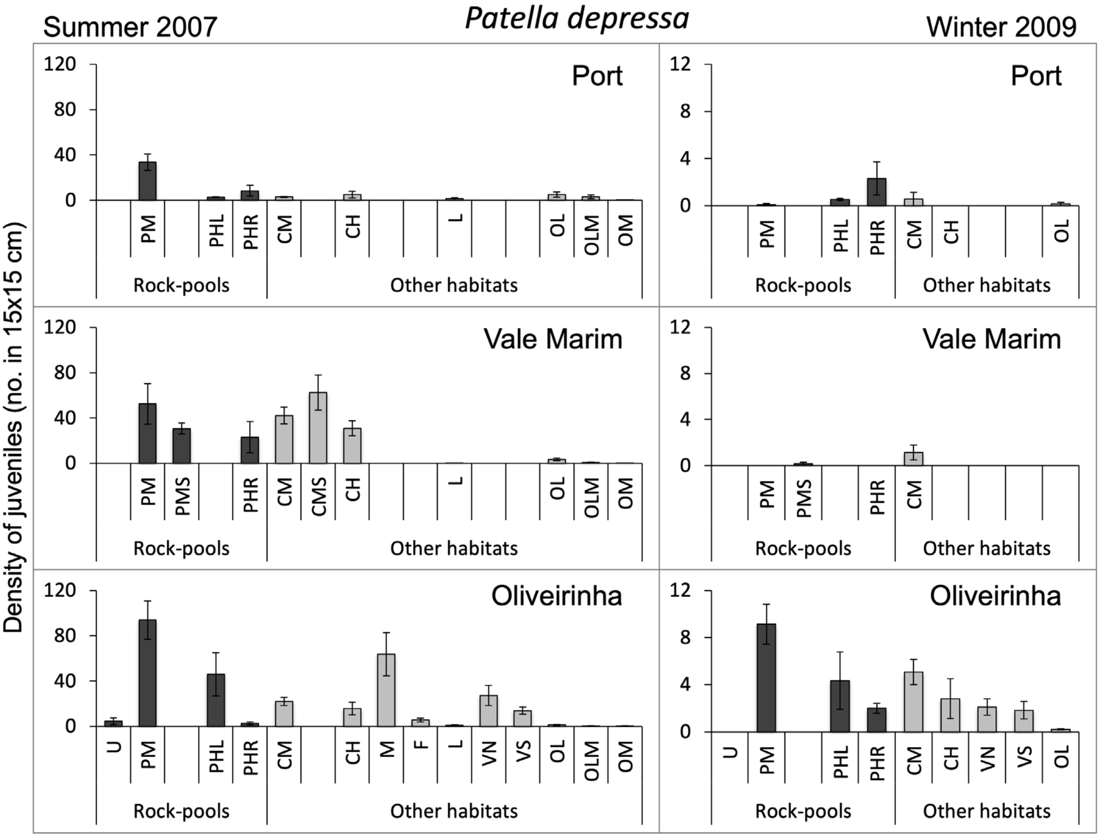

Figure 3.2 Abundance of juvenile *Patella depressa* (MSL ≤10 mm; mean ± SE, n = 4) in rock-pools (dark bars) and at other microhabitats (light bars), at three shores (Port, Vale Marim, Oliveirinha — inside the Port of Sines, 4 and 9 km away, respectively) on the SW coast of Portugal, during two surveys (Summer 2007 and Winter 2009). Note different scale of the *y*-axis between Summer 2007 and Winter 2009 graphs. Blank spaces displayed at the x-axes correspond to non-sampled microhabitats at a given shore/survey (should not be confounded with records of null/very low densities at sampled microhabitats, for which x-axes categories are labelled). microhabitat abbreviations in Table 3.1.

Table 3.3 Summary of results of analyses of variance to determine the distribution of juvenile *Patella depressa* and *Siphonaria pectinata* along a range of intertidal microhabitats, within three shores (Port, Vale Marim, Oliveirinha — inside the Port of Sines, 4 and 9 km away, respectively) in SW Portugal, in Summer 2007 and Winter 2009. microhabitat abbreviations listed in Table 3.1. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns $p > 0.05$.

Patella depressa juveniles (maximum shell length ≤ 10 mm)

Period	Shore	Data Transformation	df	MS	F	p value	SNK
Summer 2007	Port	sqrt(x+1)	8	7.47	9.79	***	PM > All others
	Vale Marim	sqrt(x+1)	9	27.56	12.83	***	(CMS,PM,CM,PMS,CH,PHR)>(OL=OML=L=OM); 2 groups defined but undefined pattern within the first group
	Oliveirinha	None	13	3196.91	9.60	***	PM > All others; 2 groups defined but undefined pattern within the second group
Winter 2009	Port	None (+)	5	3.03	1.98	0.1313, ns	
	Vale Marim	None (+)	3	1.18	2.65	0.0967, ns	
	Oliveirinha	sqrt(x+1)	8	1.78	5.73	***	undefined pattern but PM > All others except CM

Siphonaria pectinata juveniles (maximum shell length ≤ 5 mm)

Period	Shore	Data Transformation	df	MS	F	p value	SNK
Summer 2007	Port	None	8	1.65	3.37	**	undefined pattern but PM > (OM = OL)
	Vale Marim	None	9	72.74	4.29	**	undefined pattern but CMS > All others except CM, PM and PMS
	Oliveirinha	None	13	3.13	6.62	***	undefined pattern but (PHL=PM=PHR) > (OML=OM=OL=L=F=CH=U)
Winter 2009	Port	None	5	24.51	1.09	0.3988, ns	
	Vale Marim	None	3	751.33	1.49	0.2683, ns	
	Oliveirinha	None	8	39.88	15.92	***	(PM=PHL) > All others; 2 groups defined but undefined pattern within the second group

df, degrees of freedom; MS, mean sum of squares; F, F-statistic; SNK, Student–Newman–Keuls test
(+) no homogeneity of variances, even after data transformation

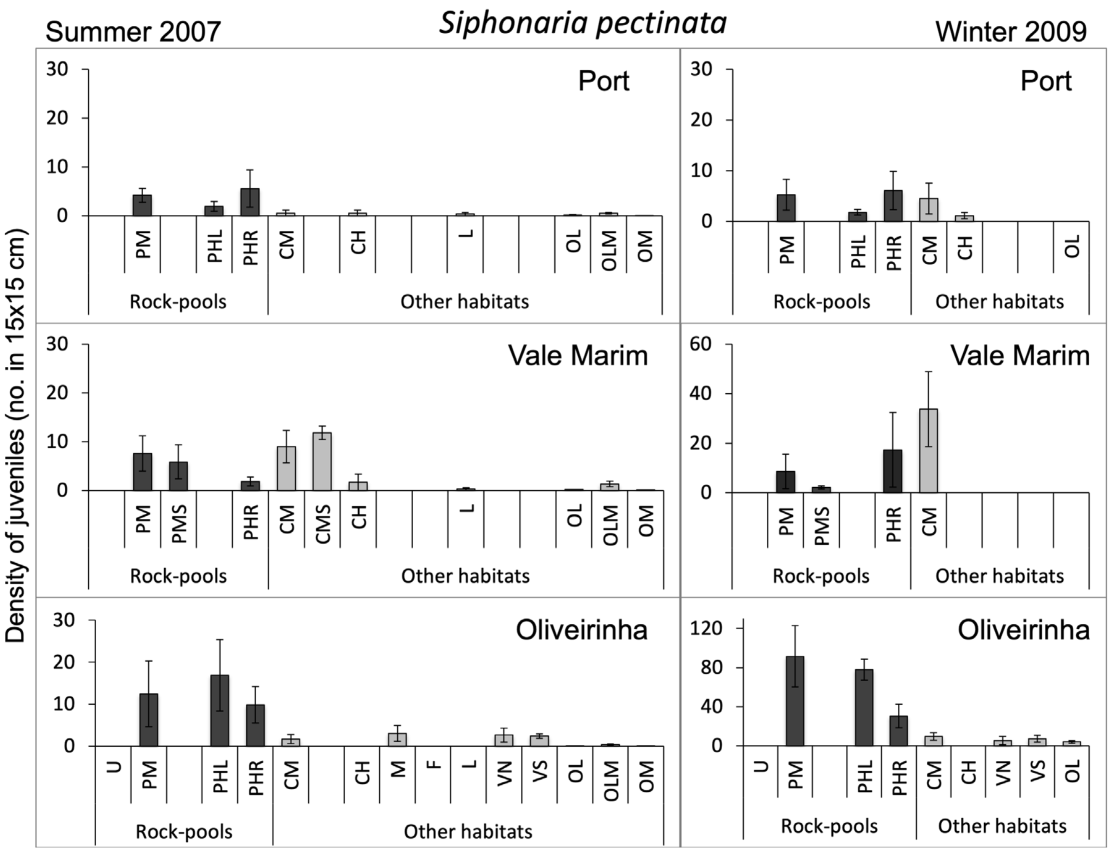

Figure 3.3 Abundance of juvenile *Siphonaria pectinata* (MSL ≤5 mm; mean ± SE, n = 4) in rock-pools (dark bars) and at other microhabitats (light bars), at three shores (Port, Vale Marim, Oliveirinha — inside the Port of Sines, 4 and 9 km away, respectively) on the SW coast of Portugal, during two surveys (Summer 2007 and Winter 2009). Note different scale of the y-axis among Winter 2009 graphs. Blank spaces displayed at the x-axes correspond to non-sampled microhabitats at a given shore/survey (should not be confounded with records of null/very low densities at sampled microhabitats, for which x-axes categories are labelled). microhabitat abbreviations in Table 3.1.

3.4.2 Temporal and spatial variation in limpet recruitment

3.4.2.1 Temporal recruitment patterns

Temporal patterns of recruitment into rock-pools censused quarterly for the three species showed seasonal and inter-annual variations (Figure 3.4). Considering mean recruit density of the three species on each shore and in each period, the maxima (number in 15×15 cm) recorded over the whole sampling period were the following: 64 *P. depressa* during the spring of 2008, 45 *P. ulyssiponensis* during the spring of 2007 and 134 *S. pectinata* during the summer of 2005, all in Oliveirinha. Despite variation among shores, the temporal pattern of recruit density over time for each species was mostly consistent across shores. Recruitment over three months of *P. depressa* and *P. ulyssiponensis* was consistently low during all summers of the whole study period and also during the autumn of 2007 (Figure 3.4). Considering mean densities of patellid recruits in all shore/periods, yearly recruitment peaks were recorded during winter, spring and autumn periods for both species. For *P. depressa* peaks were frequent across these three seasons, while for *P. ulyssiponensis* peaks were more frequent during winter and less during autumn (Figure 3. 4). Overall annual recruitment of both patellids was relatively steady, with the exception of *P. depressa* during 2006 (Figure 3.4), for which recruit density was two times lower on average than in the other years.

Density of *S. pectinata* recruits was highest during the summer of 2005 and this was consistent on all shores; overall annual recruitment was 4 to 10 times higher during 2005 than in the other years. During the following years, mean recruitment of *S. pectinata* was comparatively low (not above 35 individuals in 15×15 cm considering mean densities in all shore/periods), but usually higher during summer and autumn periods, and being almost absent during the spring of 2007 (Figure 3.4).

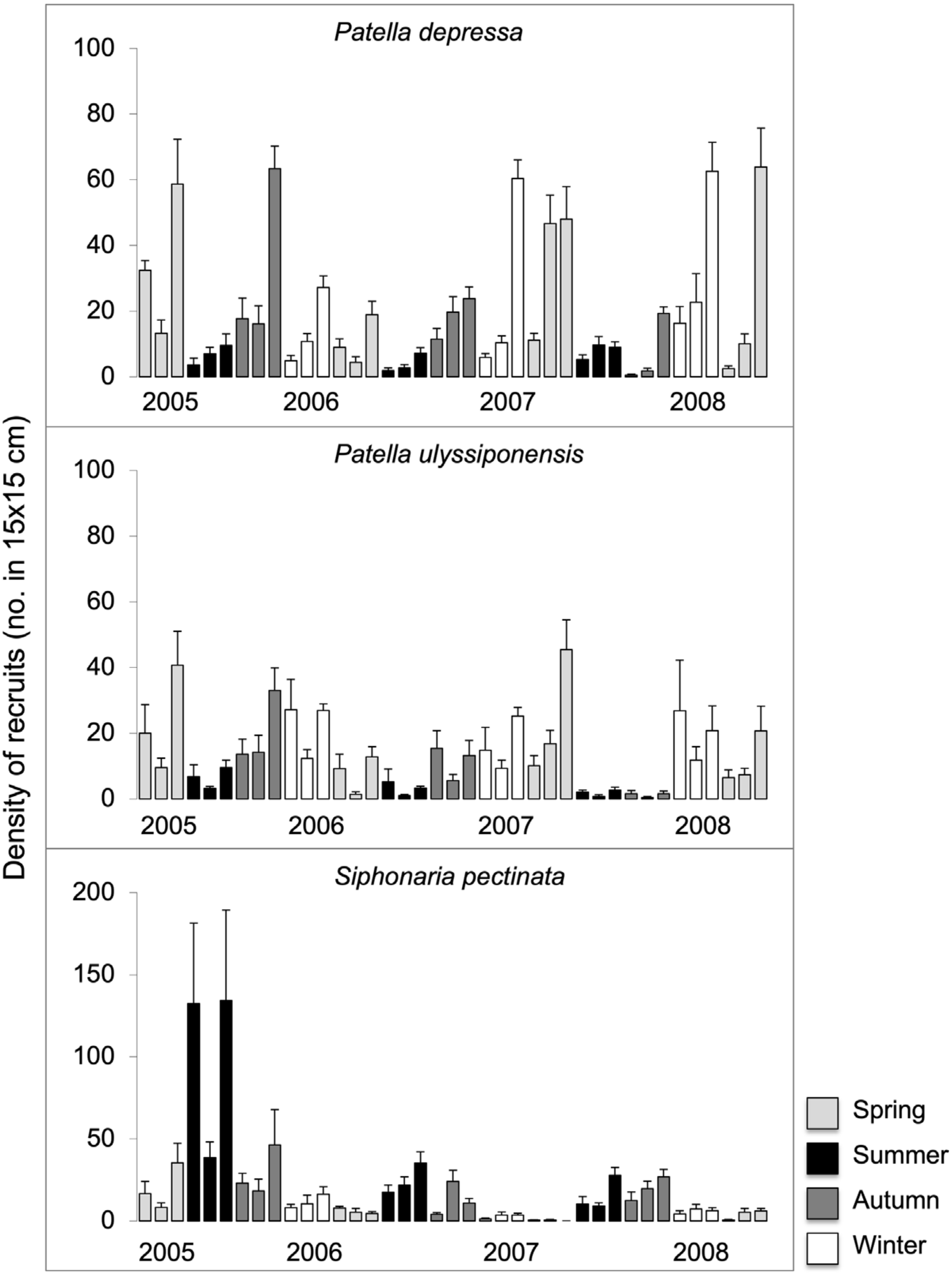

Figure 3.4 Abundance (mean density + SE) of three species (*Patella depressa*, *Patella ulyssiponensis* and *Siphonaria pectinata*) recruits (MSL < 5 mm) into rock-pools during three years (May 2005 to July 2008). See Table 3.2 for information on seasonal periods. The first, second and third bars of each quarterly period refer to recruitment in Port, Vale Marim and Oliveirinha, respectively. Note different scale of the y-axis between patellids and *S. pectinata* graphs.

3.4.2.2 Spatial recruitment patterns

For all the three species, there was among-shore variation in the intensity of recruitment into rock-pools monitored at a quarterly scale (Figure 3.4). Quarterly density of *P. depressa* recruits was significantly different among all the three shores (Pseudo-F = 19.10, p = 0.001, square-root transformed data), with mean densities being lowest in the Port, intermediate at Vale Marim (double than in Port) and highest at Oliveirinha (five times higher compared to Port and double than in Vale Marim) (Figure 3.5). Quarterly density of both *P. ulyssiponensis* and *S. pectinata* recruits differed significantly between the farthest shore from the Port of Sines (Oliveirinha) and the other two shores (Vale Marim and Port), being approximately twice as high in Oliveirinha, on average (*P. ulyssiponensis*, Pseudo-F = 2.75, p = 0.008 ; *S. pectinata*, Pseudo-F = 3.69 , p = 0.005) (Figure 3.5). Quarterly density of adult immigrants (all limpets with MSL > 10 mm) was also significantly different among shores (Pseudo-F: 2.59; P = 0.008, square-root transformed data), with mean adult migration into rock-pools being two to four times higher in Port (on average, four immigrants in 15×15 cm in Port) than on the two shores outside the Port.

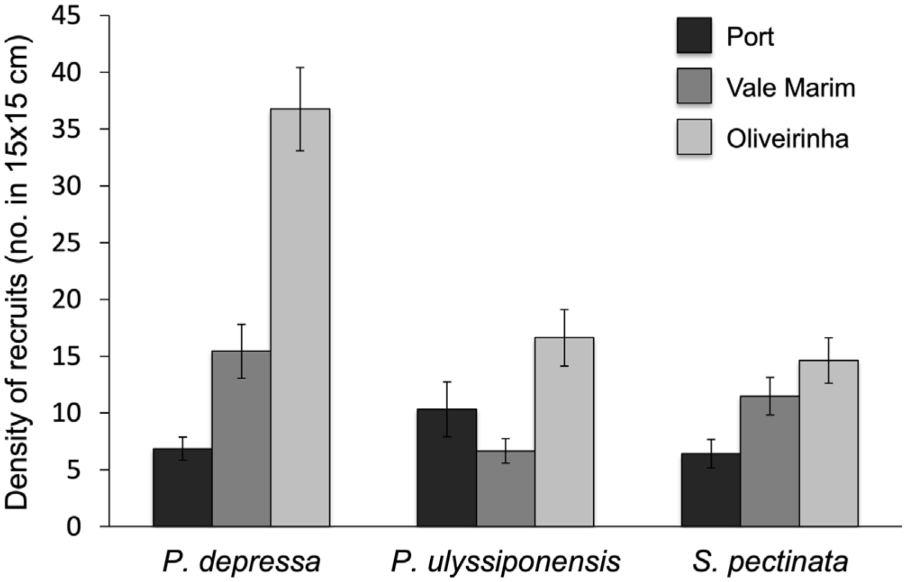

Figure 3.5 Abundance (mean density ± SE) of three species (*Patella depressa*, *Patella ulyssiponensis* and *Siphonaria pectinata*) recruits (MSL < 5 mm) into rock-pools at three shores: Port, Vale Marim and Oliveirinha (inside the Port of Sines, 4 and 9 km away, respectively).

3.6 Discussion

3.6.1 Primacy of rock-pools as nursery microhabitats for co-existing limpets

Intertidal rocky-shores of SW Portugal are the home of both patellid and siphonariid limpets, providing an intriguing comparison of the spatial and temporal recruitment patterns of three co-existing limpet species (*P. depressa*, *P. ulyssiponensis* and *S. pectinata*). We have presented field data, collected on three natural shores in the region of Sines, regarding: (i) the distribution of limpet juveniles across a wide range of intertidal microhabitats; (ii) a three-year time series of limpet recruitment into rock-pools. The combined information obtained from the two datasets provided substantial evidence for the role of rock-pools as nurseries for both patellogastropod and pulmonate limpets. To our knowledge, this is the first study to demonstrate the use of rock-pools by recruits/juveniles of a siphonariid species.

According to our hypothesis on the patterns of juvenile abundance of *P. depressa* and *S. pectinata* with respect to microhabitat, the distribution of juveniles would vary depending on the presence of rock-pools, predicting higher abundance in microhabitats of rock-pools for both species. Although our results generally confirmed this prediction, neither of the two species was found to be entirely dependent on rock-pools as a nursery area, since juveniles of both *P. depressa* and *S. pectinata* were present in the majority of sampled microhabitats. In fact, in comparison with the pool specialist *P. ulyssiponensis*, for which juvenile distribution was assessed in the same surveys (Seabra et al., 2019), these two species revealed more generalist patterns of juvenile microhabitat use. While the specificity of *P. ulyssiponensis* juveniles to a narrow microhabitat spectrum was due to an almost exclusive association with crustose coralline algae (CCA) presence (Seabra et al., 2019), the wider distribution of both *P. depressa* and *S. pectinata* juveniles indicated that shallow rock-pools located at mid and at high shore-levels, as well as other moist topographically complex microhabitats (mainly crevices but also mussel patches and vertical walls with overhangs or shaded surfaces), are alternative nursery grounds for these two species, possibly also providing protection from desiccation during their early shore-life. Remarkably, both *P. depressa* and *S. pectinata* juveniles were nearly absent in the microhabitat of rock-pools‘‘urchin-burrows’’ (‘‘U’’ in Figures 3.2 and 3.3 in Oliveirinha), where *P. ulyssiponensis* juveniles were commonly found (Seabra et al., 2019). This could be due to possible microhabitat unsuitability of these deeper rock-pools for *P. depressa* and *S. pectinata*, namely in the case of the pulmonate species which can breathe atmospheric air.

Large adult individuals of *P. depressa* (MSL > 20 mm) and *S. pectinata* (MSL> 10 mm) are common on open-rock at mid and lower-mid tidal levels (Section 3.3.2), despite juveniles of both species being rare on open-rock microhabitats (this study). Moreover, large *P. depressa* (MSL > 20 mm) inside mid-shore pools are frequently observed near pool edges in SW Portugal (Seabra et al. unpublished data), so they may live between the interface with the surrounding open-rock at this shore level, similarly to what has been described for *P. vulgata* in South Britain (Noel et al., 2009). These distribution patterns of adult limpets suggest that *P. depressa* and *S. pectinata* are possibly migratory species across mid-shore microhabitats, using rock-pools as nursery grounds with an unknown proportion of juveniles migrating at an unknown time/size after recruitment to open-rock and an unknown pro-portion of adults using pools temporarily for feeding/sheltering. A similar pattern has been suggested for *P. vulgata* in the British Isles, in contrast with *P. ulyssiponensis* for which recruits into pools may be permanent pool-dwellers across their whole life-time (Bowman, 1981; Delany et al., 1998; Firth and Crowe, 2008, 2010).

The similarities in the distribution and microhabitat associations of patellid juveniles revealed between our study and previous studies in the North-East Atlantic (Lewis and Bowman, 1975; McGrath, 1992; Delany et al., 2002; McGrath and Foley, 2005; Firth and Crowe, 2008) seem to indicate that there are region-wide common patterns in the nursery role of rock-pools and other damp intertidal microhabitats for the genus *Patella* with common processes probably driving recruitment in different regions.

In the case of *S. pectinata*, previous studies on population ecology (Ocaña, 2003; Rubal et al., 2013, 2015; Boukhicha et al., 2015) and reproductive biology (Dieuzeide, 1935 in Voss, 1959; Ocaña and Emson, 1999; Rubal et al., 2015) were exclusively made at open-rock microhabitats. In SW Portugal, however, higher monthly reproductive indices (number of egg ribbons per adult) of this species were found inside sheltered shallow rock-pools compared to adjacent emersed open-rock (Seabra and Cruz, unpublished data). Encapsulated embryonic development within egg masses of intertidal gastropods is often affected by exposure to several environmental stressors (variations in temperature, salinity, ultraviolet radiation and oxygen availability; reviewed by Przeslawski, 2004), with environmental carry-over effects (by which the parental influences embryonic viability and survival of offspring) being manifested across life stages and generations of siphonariids (Kessel and Phillips, 2018). Thus rock-pools where thermal stress is reduced may favour the recruitment of *S. pectinata* by providing effective protection against mortality of embryonic, larval and/or early-benthic stages.

Combined with results shown in Seabra et al. (2019), this study clarified the relative importance of rock-pools over most other sampled microhabitats as juvenile microhabitats for the three focal species. The primacy of shallow CCA-dominated rock-pools at mid-shore (designated as ‘’PM’’), as common nurseries for the three co-existing species was shown by significantly high densities of limpet juveniles almost invariably found within this microhabitat (present study; Seabra et al., 2019). Moreover, in this same microhabitat and consistently on all sampled shores, there was year-round presence of limpet recruits, with a maximum post-settlement age of three months. Thus we suggest that these particularly conspicuous microhabitats of rock-pools are likely to provide the most important nursery grounds for the three focal species in SW Portugal.

On the Portuguese coast, home-fidelity behaviour of intertidal blennies and gobies to rock-pools during early ontogeny (Roma et al., 2018) supported evidence on the functional importance of rock-pools for fish larvae/juveniles regarding sheltering (Dias et al., 2016) and feeding (Dias et al., 2014). Indeed, rock-pools in Europe are known to support high biodiversity and thereby enhance ecosystem functioning (Firth et al., 2014), sustaining complex food webs (Mendonça et al., 2018). Rock-pools within temperate regions may be seen as uniquely benign microhabitats in terms of physiology of intertidal fauna, due to water retention at low-tide (cf. Vinagre et al., 2018 for tropical regions). We thus suggest that rock-pools may also favour survival of limpet recruits as well as their food availability.

An alternative hypothesis of selection of rock-pools by limpet larvae based on CCA-enhanced settlement cues seems less likely, given results from recent experiments on larval settlement of *P. ulyssiponensis* (Seabra et al., 2019). However, it cannot be ruled out without settlement choice experiments with larvae of each species. The potential of rock-pools as predation refuges seems also unlikely for limpets in SW Portugal, given that juveniles of all species were found to occur mostly in pools with low structural complexity and low foliose algae abundance so protection from predators cannot be provided, and that *S. pectinata* is probably unpalatable for predators (references in Simone and Seabra, 2017).

Additional research is needed to test pre-and post-settlement processes that drive the observed patterns of limpet recruitment in different microhabitats, and to understand the factors that can explain the nursery value of rock-pools for each of the three focal species.

3.6.2 Temporal niche partitioning in recruitment of patellids and siphonariids into rock-pools

We found evidence of time displacement between patellids and siphonariids in the use of suitable microhabitats for recruitment. Considering the juvenile presence in the microhabitats that were sampled in common in the summer 2007 and winter 2009 surveys, density of *P. depressa* juveniles (MSL ≤10 mm) was 44 times higher on average in the summertime (probably corresponding to individuals that had mostly settled during the preceding were 13 times more abundant on average during the wintertime winter/spring), whereas juveniles of *S. pectinata* (MSL ≤5 mm) (probably corresponding to recruits of the preceding autumn). Opposite seasonal patterns were also found between both *Patella* species and *S. pectinata*, regarding the abundance of recruits (MSL < 5 mm) in pools that were quarterly cleared of all limpets. Considering mean recruit densities in all shore/periods, maximal yearly intensity of recruitment of *S. pectinata* during summer or autumn periods corresponded to yearly recruitment minima of one or both patellids. Seasonal patterns of recruitment of each studied species were generally in agreement with previous knowledge on their annual reproductive cycles in the study region (Section 3.3.2).

For both *Patella* species, similar temporal patterns in recruit density were detected over the whole period of study, with relatively low (only found for *P. depressa* in 2006) inter-annual variability in overall recruitment. The two species were largely synchronized in the periods of least recruitment during summer and in most yearly periods of highest recruitment during winter and spring. This similarity is likely due to the parallel phenology of reproduction of the two species. Multiple and temporally spread pulses of spawning of *P. ulyssiponensis* and *P. depressa* and a partial early summer pause (Ribeiro et al., 2009; Fernández et al., 2016) possibly drive several settlement events, explaining the continuous presence of spat of these species in pools and their mainly steady annual recruitment with a decline in intensity during summer. Settlement of *Patella* in Portugal had been previously described to be most successful if it occurred in late autumn to winter/early spring (Guerra and Gaudêncio, 1986). Synchrony in the arrival of recruits of the two patellids may also suggest the possible influence of similar oceanographic and meteorological processes driving larvae onshore. Recruitment of *P. vulgata* was found to be associated to rough seas and changes in temperature (Bowman and Lewis, 1977) and these conditions may also apply to the studied patellids.

For *S. pectinata*, there was a consistent pattern among the three years of high recruitment in the summer. This is likely due to a spawning peak during spring, described as the main pattern of the reproductive phenology of this species, for populations in Gibraltar (Ocaña and Emson, 1999) and across the northern and central Portuguese coast (Rubal et al., 2015). However, inter-annual variability was higher than seasonality in terms of absolute recruit density (highest in 2005). Ocaña and Emson (1999) found inter-annual variation in the length of the spring to summer/autumn period in which egg-ribbons were found in Gibraltar. The abundance of egg-ribbons was also found to vary between 2013 and 2014 in SW Portugal (Seabra and Cruz, unpublished data). Further research is needed on ecological factors affecting annual variation in reproduction and recruitment of this species.

Although the three species were found to share nursery microhabitats, the temporal mismatch on the recruitment of patellogastropod and pulmonate limpets might be important for success of their recruitment and ultimately for their coexistence. Additionally, we propose that microhabitat segregation after recruitment could be an important mechanism to allowing co-existence of the two patellid species in the mid-shore of SW Portugal, similarly to what has been demonstrated for *P. ulyssiponensis* and *P. vulgata* on the Irish coast (Firth and Crowe, 2010).

3.6.3 Variation of limpet recruitment inside and outside the Port of Sines

Recruitment of all the three species was significantly higher on the shore farthest from the Port of Sines. Several putative physical and biological factors can be responsible for this consistent among-shore pattern, by affecting recruitment at any pre or post settlement phases. We have shown that there was a higher abundance of adult immigrants in rock-pools located in the shore inside the Port, revealing a potentially negative relationship of recruitment and adult immigration intensities. On average, the percentage of the total of adult immigrants was 36% for *P. depressa*, 39% for *P. ulyssiponensis* and 25% for *S. pectinata*. So, the contribution of each species to any effect of adults on recruits arriving at the same times into sampled rock-pools would be similar. Espinosa et al. (2011) suggested the effect of bulldozing of limpet recruits by adult limpets when foraging. Competition between limpets of different size-classes was found to affect distribution and abundance of *P. depressa* on open-rock microhabitats on Portuguese shores (Silva et al., 2003; Boaventura et al., 2003). Evidence of intra-specific competition in rock-pools was also found for *P. ulyssiponensis* in Ireland (Firth and Crowe, 2010). We suggest that this process could affect the survival and growth of limpet recruits in pools inside the Port. The reason why adult immigration was higher in Port than in the shores outside the Port is unknown and should be investigated in the future.

Regarding other biological factors, the observed among-shore recruitment pattern could have been driven by a potentially lower fertility inside the Port, due to a lower number of reproductive individuals on the artificial structures adjacent to the sampled shore, which could determine a lower number of larvae/settlers. Low larval supply could be potentially responsible for the lower recruitment intensity within areas inside the Port of Sines. Future research is needed on processes affecting the larval pool and larval transport in this region at a variety of scales (see larger scale work by Gomes et al., 2016 on mussels).

Physical factors as differences in hydrodynamics and wave exposure among studied shores could also have potential effects on larval pool dynamics and larval transport processes, which could thus explain differential recruitment. The Port of Sines is a comparatively more sheltered environment than nearby rocky-shores outside the Port. However, the natural shore sampled inside the Port can be particularly exposed to the SW swell, so measurements are needed to understand water circulation patterns in this area as well as their potential influence on limpet recruitment. Other unstudied sources of variation among shores might also be responsible for explaining the detected among-shore pattern of limpet recruitment. In fact, although all sampled rock-pools were chosen so that their location, dimensions and biological assemblages were as similar as possible across shores, we cannot discard the possibility of any differences among shores in the physical or chemical environment of pools (not measured along the study) that could potentially influence the survival of limpet recruits across shores. Further research is needed regarding small-scale environmental variation of intertidal rock-pools in this region.

3.6.4 Conclusions

Assessing recruitment of marine species and the nursery value of a microhabitat are challenging issues. Recruitment is the net result of many factors operating during larval, settlement and post-settlement life stages (e.g. Pineda et al., 2009). Nursery value is the net result of a complex of interacting biotic, abiotic and landscape factors (e.g. Beck et al., 2001). Both vary at multiple temporal and spatial scales (e.g. Litvin et al., 2018). The present study describes, for the first time to our knowledge, limpet recruitment in SW Portugal. As our data were collected more than one decade ago, we suggest that new observations are needed to detect eventual recent changes, as coastal recruitment is dynamic over large temporal scales. We aimed to provide a better understanding of the microhabitats that serve as nurseries for patellogastropod and pulmonate limpets in SW Portugal and the factors that create site-specific variability in their nursery quality. Our results can be summarized in the following general patterns of limpet recruitment within the study region: (1) high juvenile abundance and year-round presence of recruits in microhabitats of rock-pools; (2) temporal displacement in the arrival of patellid and siphonariid recruits into rock-pools; (3) lower recruitment (and higher adult migration) into rock-pools located inside the Port of Sines than in the furthest shore outside the Port. These findings highlighted the importance of rock-pools for sustaining limpet populations in the region of Sines (SW Portugal), where natural rocky-shores have been gradually replaced by artificial defence structures with scarcity of these microhabitats (CIEMAR, 2018). This information should be taken into account regarding conservation and management strategies for this urbanized shoreline, namely in the light of the increasing coastal development and planned extension of the Port of Sines.

Finally, the system of rock-pools in any given intertidal area can be considered as a mosaic of interconnected nursery microhabitats, linked among each other through migration of adult limpets, which can in turn affect the success of juvenile recruitment. Rock-pools present in scarce natural areas remaining inside the Port of Sines are likely to be crucial nursery microhabitats that might ensure larval connectivity with adjacent artificial structures, the survival of recruits and the replenishment of adult limpet assemblages. Their protection in this Port is recommended, as well as the addition of rock-pools in artificial intertidal rocky substrata.

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CHAPTER 4: Variability and connectivity in populations of different limpet species across rockpool-generated mosaic landscapes

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4.1 Abstract

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 rock-pools and their surrounds. Our approach considered each rock-pool as a concentric system of three microhabitats where limpets were censused (species, counts, and size of individuals): inside rock-pool, 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 microhabitats) in Southwest Portugal. Additionally, open-rock surfaces far away (>25cm) from rock-pools 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 rock-pools on all shores, with rock-pools extending their vertical distribution upwards. *Patella vulgat*a was rare; *P. rustica* was absent from rock-pools and their edges. Intra-specific connectivity (correlations in density of the same species between adjacent microhabitats) 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 rock-pools 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 rock-pools and far from rock-pools. 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 rock-pools were positively associated with cover of coralline crusts 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 rock-pools 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, rock-pools/tidepools, emergent substrata, habitat connectivity, patellids, siphonariids, mosaic landscapes, co-existence

4.2 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 microhabitats: freely draining emersed surfaces (open-rock) and depressions retaining water during low tide (rock-pools 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 microhabitat variation also can determine distribution patterns of limpets living outside rock-pools, 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 microhabitats 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 rock-pools, 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 rock-pools as nurseries for coexisting limpets has been experimentally demonstrated across different regions (Delany et al., 1998; Seabra et al., 2020). Rock-pools 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 rock-pools 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 microhabitats happen (Delany et al., 2002; Noël et al., 2009). As limpet intra-specific competition occurs inside rock-pools (Firth et al., 2009; Firth and Crowe, 2010), coupled with the importance of these microhabitats as limpet nurseries (Seabra et al., 2020), rock-pools may act as sources of limpet emigration to surrounding open-rock areas. Conversely, as limpets with home scars outside rock-pools enter rock-pools when foraging at high tide (Noël et al., 2009), rock-pools 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 rock-pools (Delany et al., 1998). Rockpool edges are interfaces straddling neighboring microhabitats 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 microhabitats created by the presence of pools, likely to be important at emerging landscape scales.

Large variability at small spatial scales occurs among different rock-pools 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 rock-pools 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 microhabitat, 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 microhabitats) 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 rock-pools or patchy microhabitats 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 rock-pools and their surrounds. Here, we take an integrative view along the continuous gradient of shore height of the intertidal zone,focusing on microhabitats both inside and outside rock-pools 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 microhabitat and connectivity among them. Each species was quantified (number and size of individuals) in the following microhabitats: inside-rockpools (POOL), on the narrow edge-zone around the rim of rock-pools (EDGE), and on open-rock surrounding rock-pools (NEAR). Surveys were made over the whole intertidal gradient on six natural rocky shores in the southwest of continental Portugal (Figure 4.1), to assess diversity, distribution, abundance, and size structure of limpets throughout the distinct microhabitats and among shores. Additionally focusing only on the mid-shore, areas of open-rock at a minimum distance of 25 cm from any rock-pool (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 4.1). First, (1) we described the distribution of all limpet species present in each microhabitat 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 microhabitats (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 microhabitats 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 rock-pools (MidNEAR) and further away from rock-pools (FAR), and assessed whether the pattern of proximity to rock-pools (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 microhabitats, by measuring the relative occurrence of open-rock, rock-pools, 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 microhabitat 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 rock-pools, specifically testing the following hypothesis: inside rock-pools (POOL), each species occurs further upshore than its occurrence on the open-rock (NEAR). Third, (3) we described the small-scale (among rock-pools) variability in density and size-class structure of individual species (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*) within each microhabitat (POOL, EDGE, and NEAR), and we investigated which predictive variables explain most of these spatial patterns.

For every species and microhabitat, we assessed the influence of the following groups of predictors: i) metrics describing size, shape, topography, and position of rock-pools 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 microhabitat); and iii)“connectivity variables” describing inter-specific influences on connectivity (i.e., density of other limpet species in adjacent microhabitats).

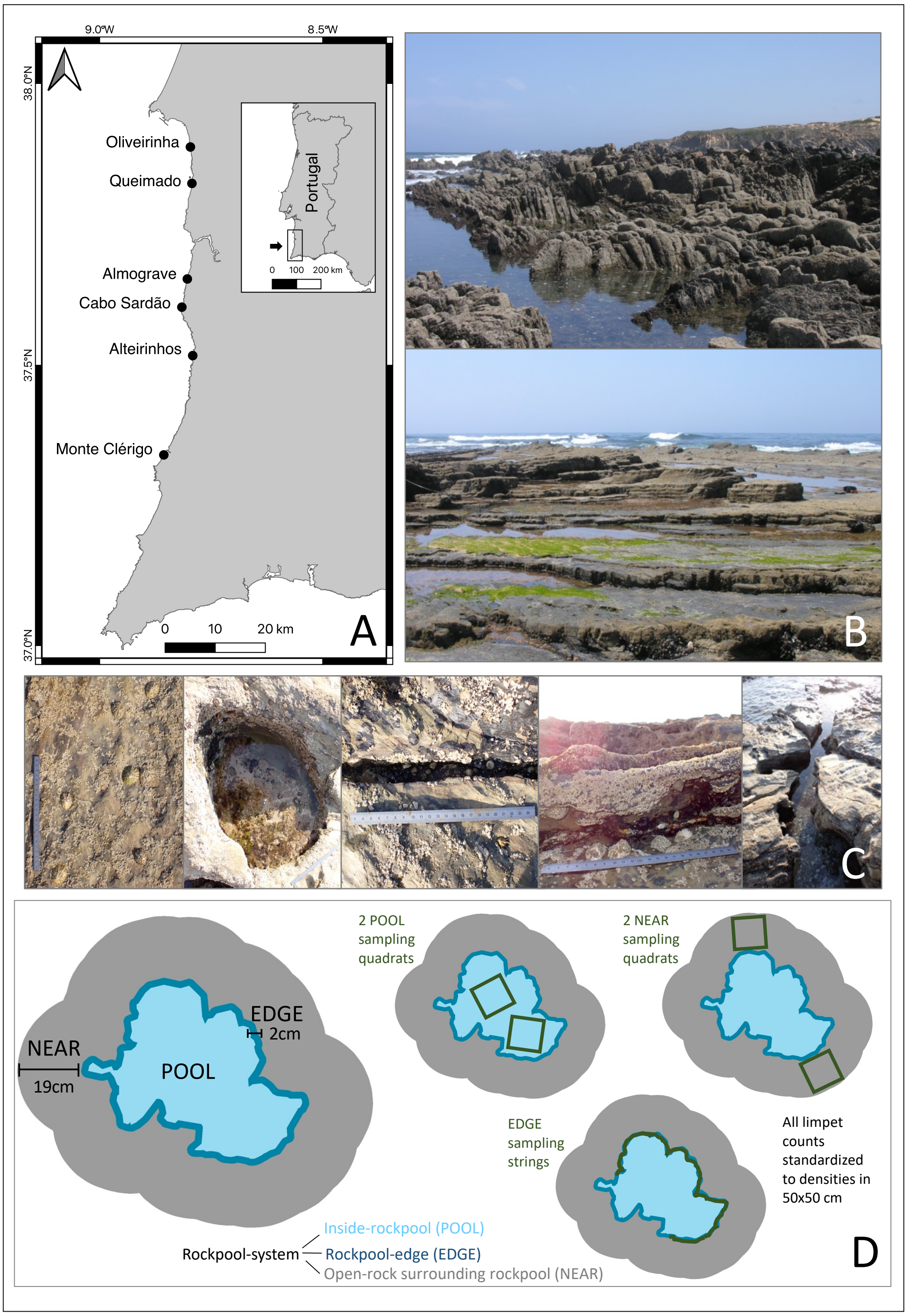

Figure 4.1. (A) Map of the study region and the six sampled shores; (B) shores varied in geomorphology, namely, in the cleavage orientation of shale strata, from flatter-layered platforms (Oliveiriinha, Queimado, and Monte Clérigo—example of Monte Clérigo on photograph below) to steeper bedrock (Almograve and Cabo Sardão; intermediate orientation in Alteirinhos—example of Almograve on photograph above); (C) five microhabitat categories considered for landscape variability assessment (open-rock, rock-pool, crevice, overhang, and channel); (D) diagram representing a rockpool system, with its three concentric microhabitats (POOL, EDGE, and NEAR) and respective sampling units (POOL and NEAR quadrats, EDGE strings).

Table 4.1 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 4.3.4.1 of Statistical analyses; Section 4.4.1 of Results)	1a) How do the abundance and size of each limpet species in three microhabitats (rock-pools, rockpool edges, open-rock surrounding rock-pools) vary on the six shores? (see Figure 4.1) 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 microhabitat?	9 response matrices analyzed separately (3 limpet species × 3 microhabitats); 3 limpet species: *Patella ulyssiponensis*, *P. depressa*, *Siphonaria pectinata*; 3 microhabitats: 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 microhabitat.	2 factors: Shore; Stretch within shore	Figures 4.2–4.4; Table 4.2 (nine two-way PERMANOVAs). Description for the least abundant species (*P. vulgata* and *P. rustica*): In text (Section 4.4.1) and in Supplementary Figures S4.1, S4.2).
	1b) Is the total density of the same species correlated between adjacent microhabitats (intra-specific connectivity)?	3 pairs of variables (3 groups of adjacent microhabitats—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 microhabitats		In text of Section 4.4.1 (three Spearman correlations per species).
	1c) Are there differences between open-rock surfaces surrounding rock-pools and further away from rock-pools 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 rock-pools 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 rock-pools; FAR—further away from rock-pools).	3 factors: Proximity to rock-pools, Shore, and Stretch within shore. Shores differed in landscape variability (relative availability of open-rock and topographic microhabitats, see Figure 4.1).	Figure 4.5; Table 4.3 (two three-way PERMANOVAs). Estimated values for landscape variability assessment: Supplementary Figure S4.3.
2 — Vertical distribution of limpet species inside and outside pools (Section 4.3.4.2 of Statistical analyses; Section 4.4.2 of Results)	2a) How does the abundance of each limpet species in three microhabitats (rock-pools, rockpool edges, open-rock surrounding rock-pools) vary with shore height on each shore?	15 response matrices (5 limpet species × 3 microhabitats; 5 limpet species: *Patella ulyssiponensis*, *P. depressa*, *P. vulgata*, *P. rustica*, *Siphonaria pectinata*; 3 microhabitats (POOL, EDGE, NEAR). Each response matrix: the total density of one species recorded within one microhabitat along the whole gradient of shore height of each shore.	Shore; shore height levels	Figure 4.6

	2b) We predict that inside rock-pools 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 rock-pools and on the open-rock.		Figure 4.7; Table 4.4 (four Binomial tests per species)
3 — Patterns among highly variable rockpool systems (Section 4.3.4.3 of Statistical analyses; Section 4.4.3 of Results)	What is the relationship between the spatial variability of individual limpet species within each microhabitat 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 microhabitat?	9 response matrices analyzed separately (3 limpet species × 3 microhabitats); 3 limpet species: *Patella ulyssiponensis*, *P. depressa*, *Siphonaria pectinata*; 3 microhabitats: 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 microhabitat.	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 4.2, 4.3.3, and 4.3.4.3; detailed definition and measurement in Supplementary Table S4.1; full list of predictors used to build each model in Supplementary Table S4.2).	Figures 4.8–4.10; Table 4.5 (nine DistLM models). Estimated values for habitat-composition and physical predictors: Supplementary Figures S4.4, S4.5 (percentage cover within POOL and within NEAR), Supplementary Figure S4.6 (counts of trochids and littorinids within POOL and within NEAR) and Supplementary Figure S4.7 (physical variables).

4.3 Materials and methods

4.3.1 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).

4.3.2 Study region and shores

Surveys were made in Southwest Portugal (Figure 4.1A), 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 rock-pools. 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 offrequency 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 4.1A): 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 4.1B), 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 microhabitats. The tidal regime is semidiurnal with 3.5 m maximum amplitude.

4.3.3 Field surveys

4.3.3.1 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 microhabitats 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, rock-pool, channel, crevice, or overhang (Figure 4.1C). The percentage frequency of occurrence was calculated for each category on each transect. The mean percentages of microhabitat 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 rock-pools intersected by transects, a minimum of 25 rock-pools per stretch were randomly selected and individually marked in the field for sampling (numbered removable epoxy tag on adjacent rock). We sampled 323 rock-pools (Oliveirinha, 52; Queimado, 51; Almograve, 57; Cabo Sardão, 56; Alteirinhos, 53; Monte Clérigo, 54).

Each rock-pool was treated as a system of three concentric microhabitats (Figure 4.1D): i) inside-rockpool (POOL); ii) edge of rock-pool (EDGE); and iii) open-rock surrounding the edge (NEAR). POOL was sampled using two equal-sized quadrats deployed on the bottom of each rock-pool, encompassing inside-pool surfaces located at least 2 cm 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 rock-pool, 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 rock-pool. 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 rock-pool. 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 microhabitat 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 rock-pool. Additionally, mid-shore surfaces of open-rock located at least 25 cm away from any rock-pool (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 rock-pools, 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 microhabitats (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, microhabitat, 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 microhabitat. 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 S4.1): 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.

4.3.3.2 Physical characteristics of rock-pools

A set of 19 physical variables was assessed for each of the 323 sampled rock-pools, in the field or through image analysis (details in Supplementary Table S4.1). 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 rock-pool, shore height, distance to low-water mark, and "barriers") of rock-pools 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 microhabitat (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 microhabitats; perimeter and surface area of a rock-pool are proportional to the ones of its surrounds).

4.3.4 Statistical analyses

Prior to multivariate analyses described in Sections 4.3.4.1 and 4.3.4.3, 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 microhabitat (hereafter "population size structure and density" or "size-class densities" of an individual species within a microhabitat)—was transformed (as a pre-treatment of the analysis for a given species-by-microhabitat combination): i) fourth-root transformation applied to response matrices inside rock-pools (POOL); ii) square-root transformation applied to response matrices of the other microhabitats (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).

4.3.4.1 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 microhabitats (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 rock-pools per stretch).

To assess intra-specific connectivity, correlations of the total density of the same species between pairs of microhabitats (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 rock-pools—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 microhabitat 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).

4.3.4.2 Vertical distribution of limpets inside and outside rock-pools

Binomial tests (Sokal and Rohlf, 1995) assessed if upward extension occurred in three species of limpets (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*) inside rock-pools (POOL), compared to outside rock-pools (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 microhabitats, 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 microhabitat (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 microhabitats was analyzed by individual binomial tests. In each binomial test, 12 POOL and 12 NEAR values were compared.

4.3.4.3 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 microhabitat) 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 microhabitat (full list of predictive variables used to build the model for each response matrix in Supplementary Table S4.2). 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 microhabitat or ii) connectivity variables if estimated in an adjacent microhabitat. 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 microhabitat code (Pool; Edge; Near). In the two cases where significant differences were detected among shores by the previously described two-way PERMANOVA tests (Section 4.4.1), 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 microhabitat, 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 microhabitat, 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.

4.4 Results

A total of 15,619 limpets were recorded. Within each microhabitat, 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 along 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 S4.1, S4.2).

4.4.1 Horizontal variation in the abundance and size structure of limpets

4.4.1.1 Horizontal patterns – 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 4.2). 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- habitats, the modal size class was PU 1–2 cm on most shores (Figure 4.2, 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 4.2, MidNEAR).

Population size structure and density within pools did not differ among shores or stretches within each shore (Table 4.2, PU within POOL). Within the surrounding microhabitats, differences were only found between stretches (Table 4.2, PU within EDGE and NEAR). Correlations of the total density of *P. ulyssiponensis* between adjacent microhabitats were all positively significant between POOL and EDGE (rs = 0.495, p<0.001), between EDGE and NEAR (rs = 0.423, p<0.001), and between POOL and NEAR (rs = 0.136, p=0.014).

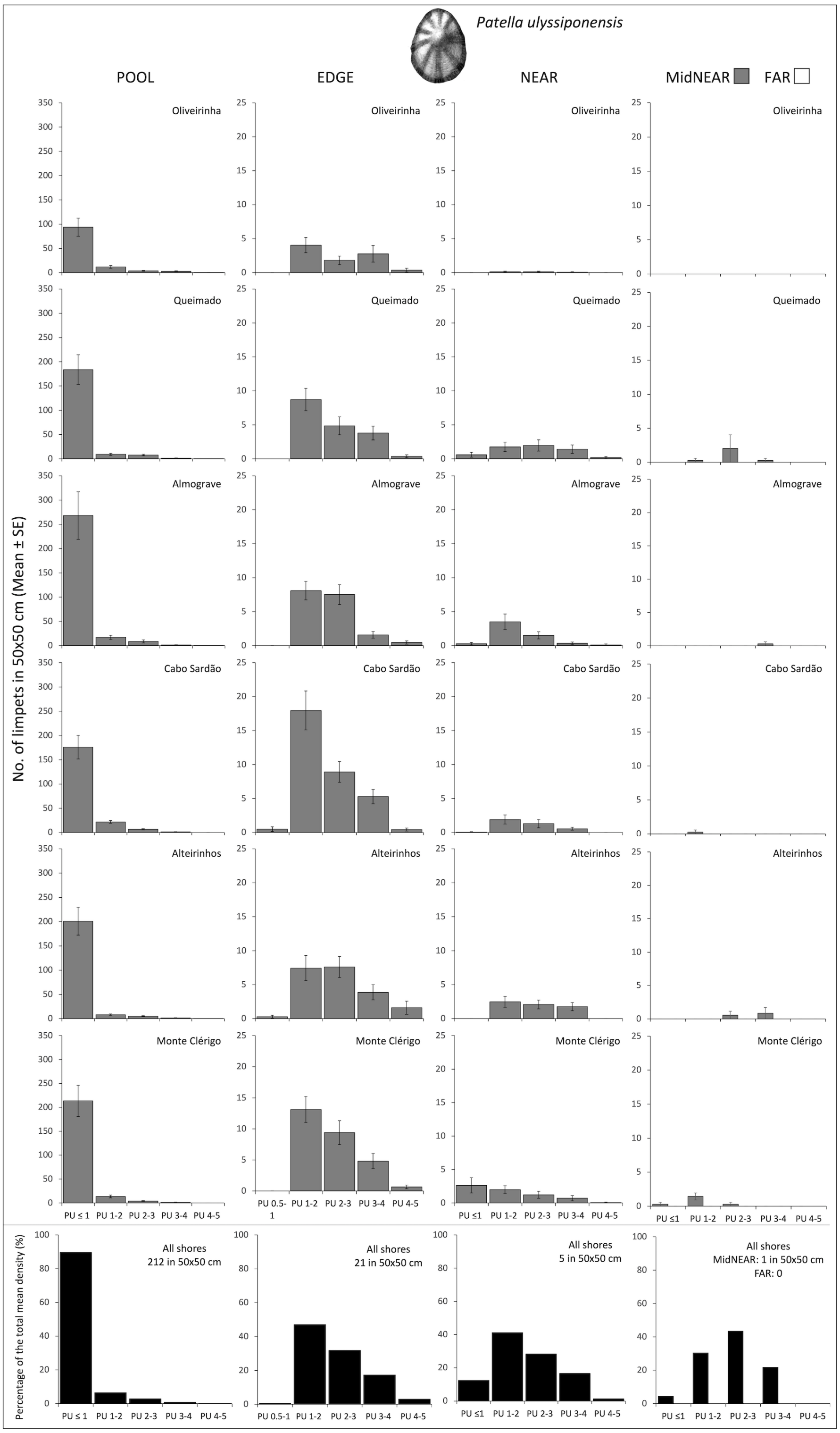

Figure 4.2 Densities and size-class structure of *Patella ulyssiponensis* on six shores within POOL (inside rock-pools), EDGE (edge of rock-pools), and NEAR (open- surrounding rock-pools) microhabitats (three left columns); and within areas of mid-shore open-rock at two proximity categories from rock-pools (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 microhabitat and across all shores. NB *P. ulyssiponensis* was absent in FAR.

Table 4.2 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 rock-pools), EDGE (in the edge of rock-pools) and NEAR (on the open-rock surrounding rock-pools) microhabitats.

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				
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 rock-pools 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.

4.4.1.2 Horizontal patterns – Patella depressa

On every shore, the abundance of this species was consistently higher inside rock-pools compared to the surrounding microhabitats: the total mean density of *P. depressa* in POOL was three times higher than in EDGE and five times higher than in NEAR (Figure 4.3). Inside pools and consistently on all shores, more than 90% of the total mean density corresponded to juveniles (PD ≤1cm) (Figure 4.3, 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 4.3, 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 4.3, NEAR, MidNEAR, and FAR).

Population size structure and density inside and along the edge of pools did not differ among shores but were significantly different among stretches within shores (Table 4.2, 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 4.2, 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 4.3). 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 4.3). Correlations of the total density of *P. depressa* between adjacent microhabitats 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 rock-pools, a significant interaction was found between factors Proximity and Shore, and significant differences were found among stretches at Oliveirinha and Alteirinhos (Table 4.3). Pair-wise tests on Proximity detected no differences between mid-shores surfaces close to and far from pools on any shore (Table 4.3, 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 4.3, MidNEAR); this was due to a higher density of PD 1–2 cm and PD ≤1 cm in Cabo Sardão (SIMPER, Figures 4.3, 4.5A); Cabo Sardão was the shore with the lowest relative proportion of open-rock (Figure 4.5B) (20% for the average of the two stretches, Supplementary Figure S4.3). 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 4.3, 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 4.3, 4.5C); these shores had more crevices and less open-rock (Figure 4.5D), 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 S4.3).

4.4.1.3 Horizontal patterns – Patella vulgata

This species (Supplementary Figure S4.1) 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 microhabitats 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 microhabitats (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.

4.4.1.4 Horizontal patterns – Patella rustica

This species (Supplementary Figure S4.2) 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 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).

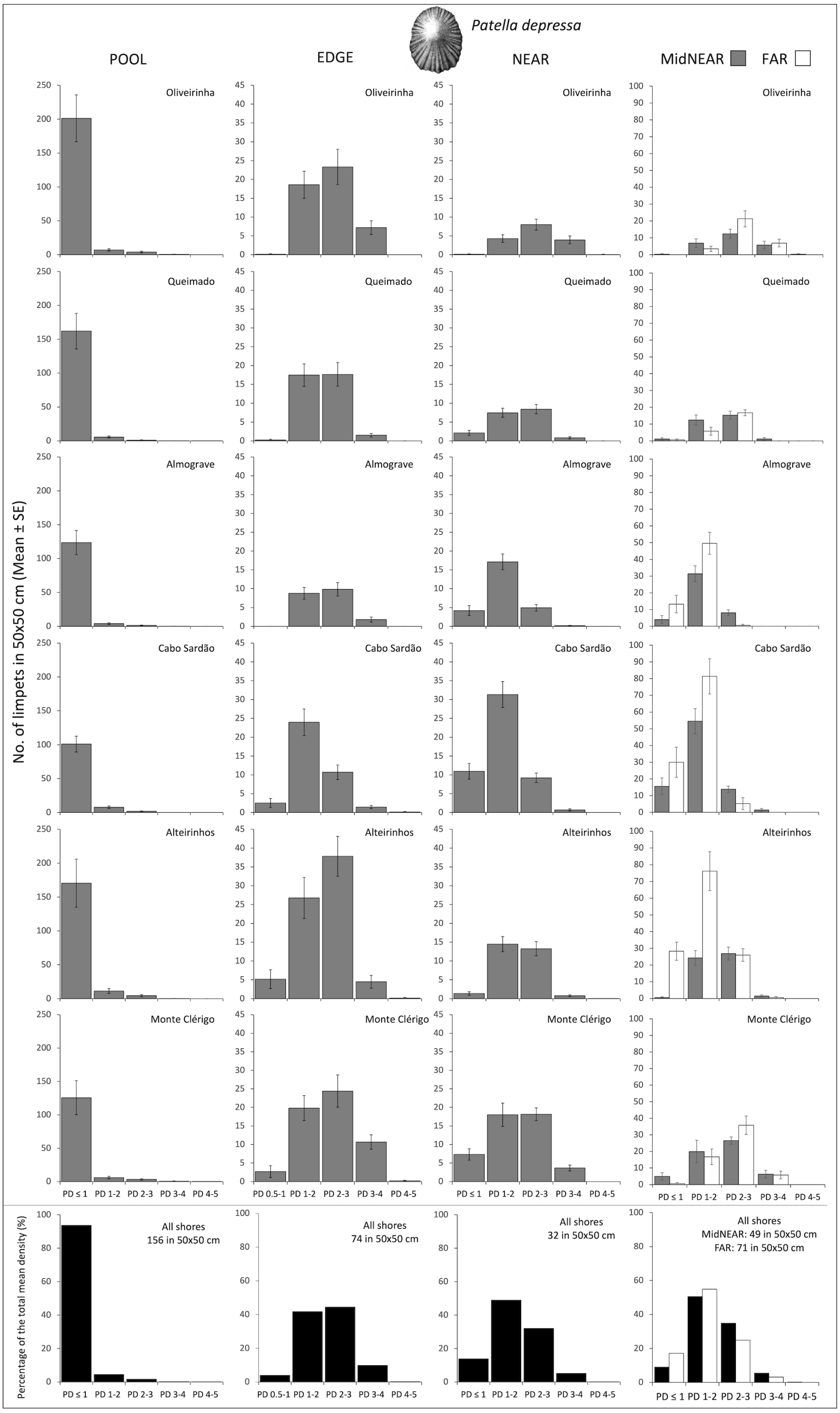

Figure 4.3 Densities and size-class structure of *Patella depressa* on six shores within POOL (inside rock-pools), EDGE (edge of rock-pools), and NEAR (open-rock surrounding rock-pools) microhabitats (three left columns); and within areas of mid-shore open-rock at two proximity categories from rock-pools (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 4.1. 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 microhabitat and across all shores.

4.4.1.5 Horizontal patterns – Siphonaria pectinata

On every shore, this species was consistently more abundant inside rock-pools compared to the surrounding microhabitats: 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 4.4). 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 4.4). Within pool 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 4.4, EDGE, NEAR, and MidNEAR). Away from pools, the modal size class was SP 0.5–1 cm on all shores (Figure 4.4, FAR). Monte Clérigo had the highest abundance of *S. pectinata* within all microhabitats (POOL, EDGE, and NEAR) and mid-shore categories (MidNEAR and FAR) (Figure 4.4). However, no significant differences were found among shores or stretches within each shore in population size structure and density within pools (Table 4.2, 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 4.2, 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 4.4). Moreover, the highest total mean density within EDGE (15 individuals per 50×50 cm) was at Monte Clérigo (Figure 4.4). On open-rock near pools, only differences among stretches were significant (Table 4.2, SP within NEAR). Correlations of the total density of *S. pectinata* between adjacent microhabitats 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 rock-pools, or among shores or stretches (Table 4.3).

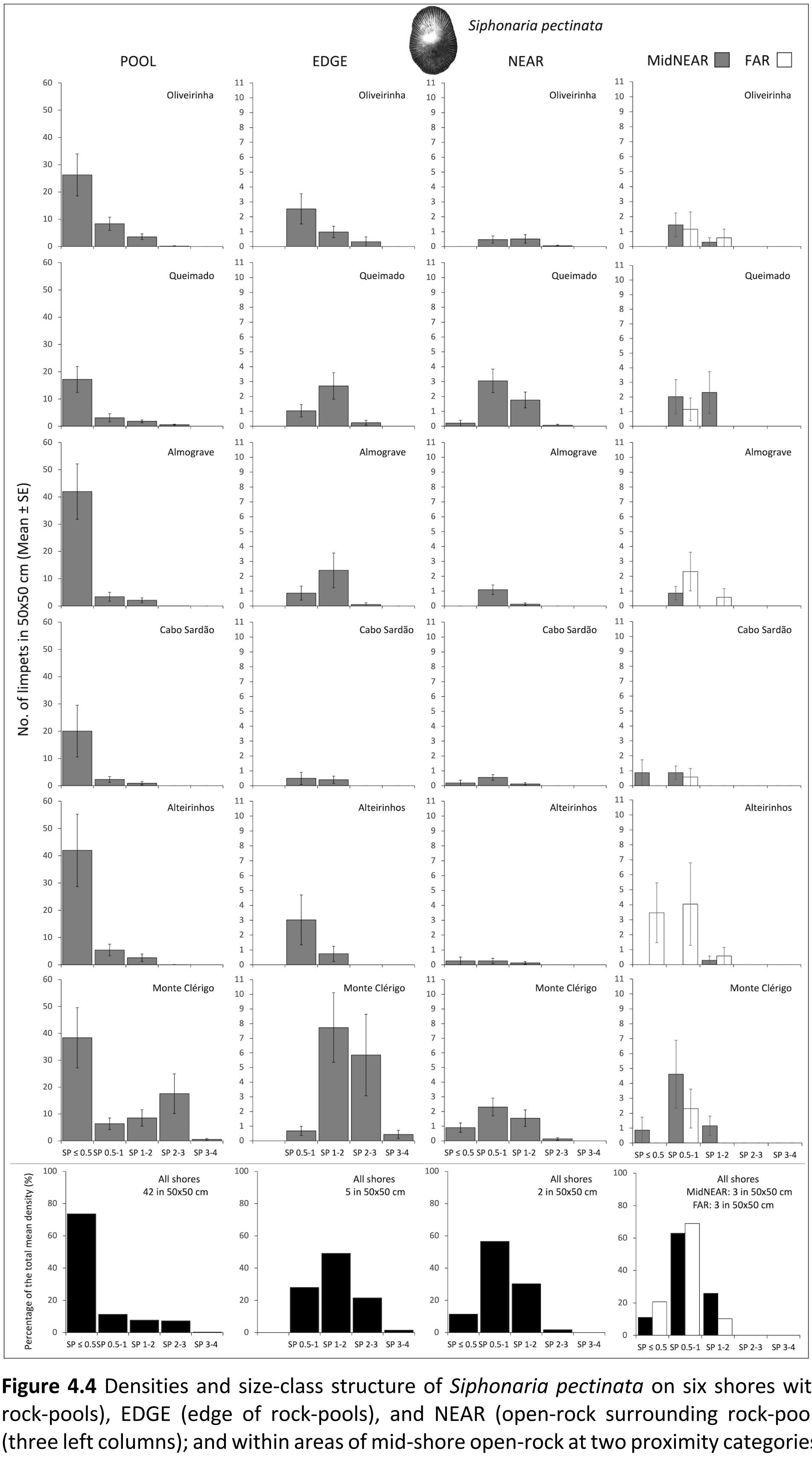

Figure 4.4 Densities and size-class structure of *Siphonaria pectinata* on six shores within POOL (inside rock-pools), EDGE (edge of rock-pools), and NEAR (open-rock surrounding rock-pools) microhabitats (three left columns); and within areas of mid-shore open-rock at two proximity categories from rock-pools (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 4.1. 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 microhabitat and across all shores.

Table 4.3 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 rock-pools, i.e., adjacent to rock-pools (MidNEAR) and located at least 25 cm away from any rock-pool (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		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.

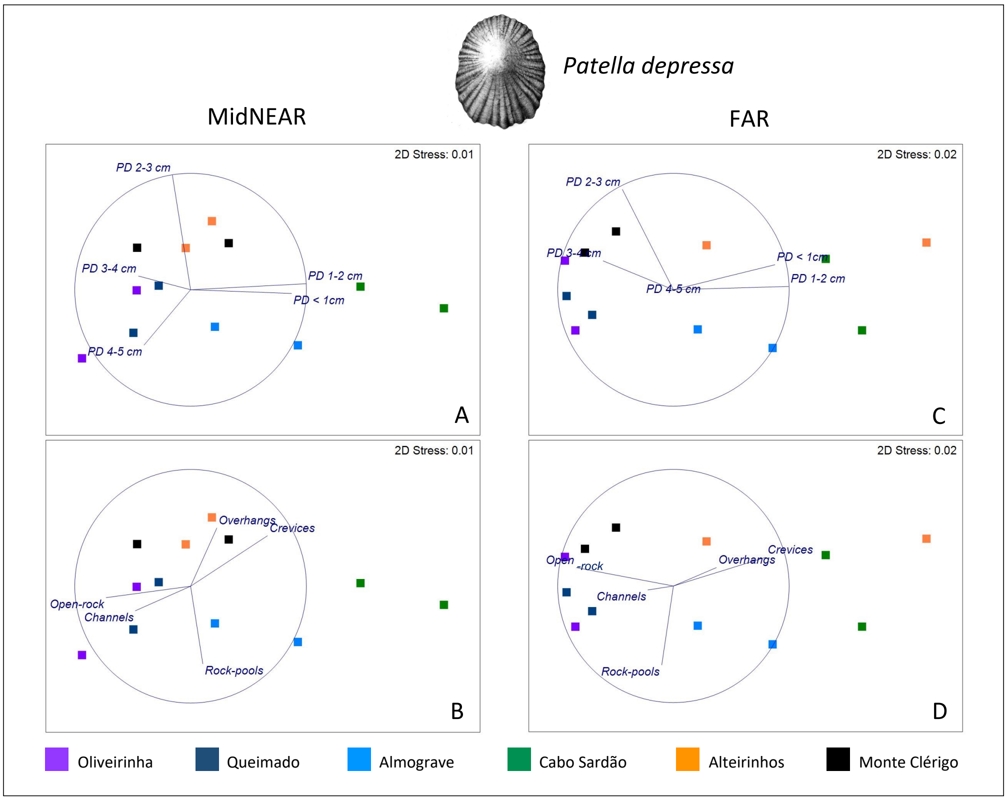

Figure 4.5 MDS plots for *Patella depressa* within areas of mid-shore open-rock at two proximity categories from rock-pools: MidNEAR (A, B) and FAR (C, D). Overlaid vectors of the response variables, i.e., densities of five size classes (A, C); overlaid vectors of predictors of landscape variability, i.e., proportions of open-rock and topographic microhabitats assessed on the mid-shore of two coastal stretches on every shore (B, D).

4.4.2 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 4.6). Moreover, the total number of rockpool systems sampled at the five shore levels differed among shores (N.B. sample sizes in Figure 4.6 caption).

4.4.2.1 Vertical patterns – Patella ulyssiponensis

The highest abundance inside rock-pools 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 higher abundance was found on the upper mid shore (2–2.5 m) (Figure 4.6, POOL). The highest abundance of this species in the open-rock surrounding rock-pools 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 4.6, NEAR). A similar pattern was found for rockpool edges, but with a less-abrupt decrease in abundance with increasing shore height (Figure 4.6, EDGE).

4.4.2.2 Vertical patterns – Patella depressa

Within every microhabitat, this species was generally most abundant at mid-shore levels; the exception was Monte Clérigo, where abundances of *P. depressa* within all the three microhabitats were similar across all three shore levels (Figure 4.6). 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 4.6, POOL and EDGE). Outside rock-pools, *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 4.6, NEAR).

4.4.2.3 Vertical patterns – 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 4.6).

4.4.2.4 Vertical patterns – Patella rustica

This species was most abundant at the highest shore levels, being usually found on the steeper shores (Almograve and Cabo Sardão) (Figure 4.6).

4.4.2.5 Vertical patterns – 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 4.6, 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 4.6, 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 4.6, 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 Sardão and Alteirinhos (Figure 4.6, 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 4.6).

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 4.7, Table 4.4). 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 4.4, Figure 4.7-A,C,E). 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 4.4). 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* (Figure 4.7-B,D,F).

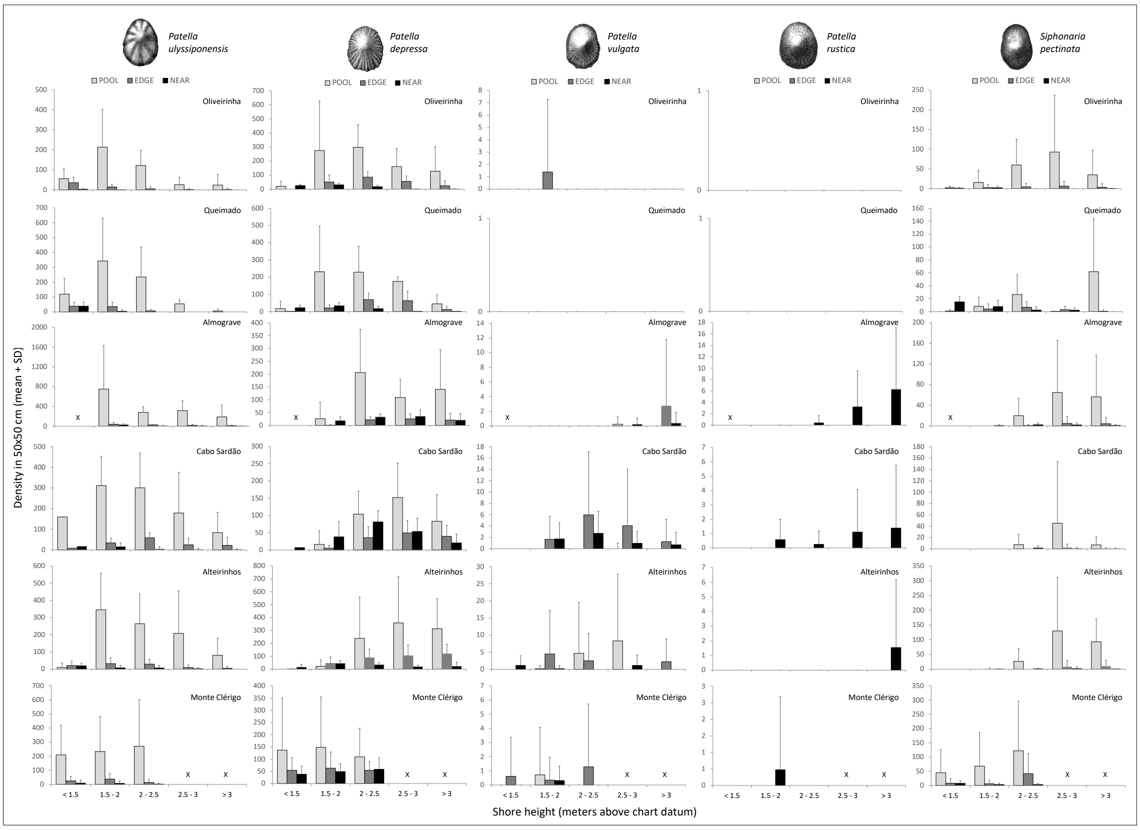

Figure 4.6 Total density of five limpet species along the vertical intertidal gradient of six shores within POOL (inside rock-pools, light grey), EDGE (edge of rock-pools, dark grey), and NEAR (open-rock surrounding rock-pools, black) microhabitats. Values are mean (± SD) number of limpets per 50×50 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.

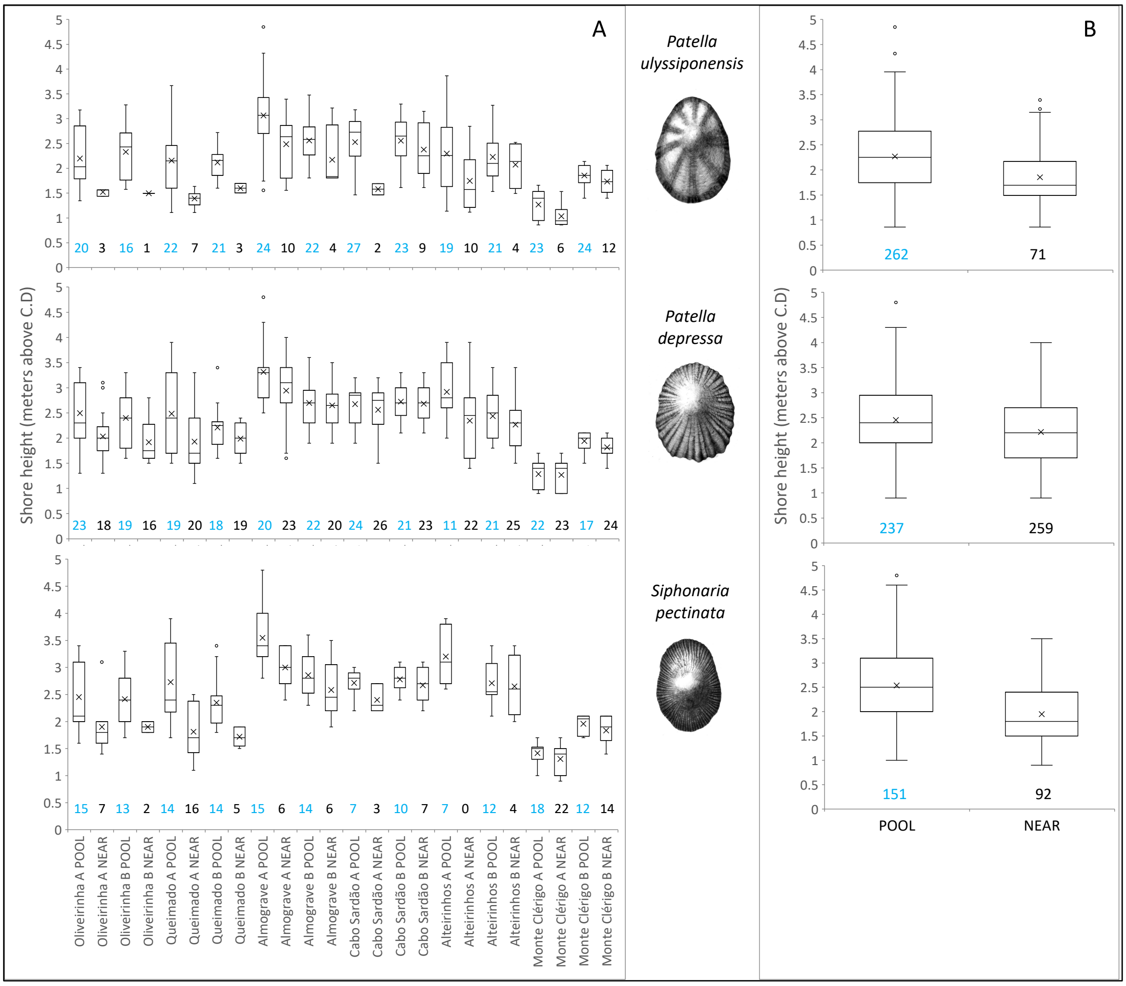

Figure 4.7 Vertical distribution of three species of limpets inside (POOL) and outside (NEAR) rock-pools. Values are shore-height records (meters above chart datum) of all rock-pools (POOL) or open-rock surfaces (NEAR) where each species was present (from a total of 323 sampled rock-pools and surrounding open-rock). (A) Data for each microhabitat on each of 12 coastal stretches (stretch A and B sampled within each of six shores). (B) Data for each microhabitat 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 rock-pools (blue, POOL) and open-rock surfaces (black, NEAR).

Table 4.4 Binomial tests comparing the vertical distribution of three species of intertidal limpets between microhabitats inside (POOL) and outside (NEAR) rock-pools.

	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 microhabitat; 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).

4.4.3 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 rock-pools present on all shores, encompassing cover of different substrata or sessile organisms (Supplementary Figures S4, S5), density of trochids and littorinids (Supplementary Figure S46), physical characteristics (Supplementary Figure S47), and total density of each co-occurring limpet species (Figures 4.2–4.4).

4.4.3.1 Models for *Patella ulyssiponensis*

The models retrieved by DistLM analyses for *P. ulyssiponensis* inside rock-pools (PU POOL) and on the open-rock surrounding rock-pools (PU NEAR) explained identical proportions of the variability among replicates (44%), higher than the model regarding this species in the edge of rock-pools (PU EDGE, 34%) (Table 4.5). 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 4.5). Two habitat-composition variables estimated as percentage cover were selected in common for these models (Table 4.5): articulated coralline algae (the first selected variable in both models, contributed alone to explain 13% and 30% of the variation inside and outside rock-pools, respectively) and CCA (explained 5% and 2% of the variation inside and outside rock-pools, respectively). Both were positively associated with overall abundance of this species within both microhabitats: articulated coralline algae was mostly related with density of *P. ulyssiponensis* juveniles (PU ≤1cm) inside rock-pools and with densities of *P. ulyssiponensis* adults (all size classes except PU ≤1 cm) outside rock-pools; whereas CCA was mostly related with *P. ulyssiponensis* adults inside and outside rock-pools (Figures 4.8A, 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 S4.2) 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 4.8A). Both the cover of sea anemones and barnacles were selected exclusively for the PU NEAR model (Table 4.5), both responsible for the segregation of a few samples (squares in the bottom part of the db-RDA plot, Figure 4.8C). Sea anemones were positively related with density of *P. ulyssiponensis* juveniles outside rock-pools, while barnacles were negatively related with density of *P. ulyssiponensis* adults (Figure 4.8C).

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 4.5). Shore height was selected as a “top predictor” for both models (Table 4.5), with a general negative association found between this predictor and *P. ulyssiponensis* abundance inside and outside rock-pools, particularly negatively related with density of juveniles inside rock-pools and with densities of adults outside rock-pools (Figures 8A, C). Outside rock-pools, distance to low-water mark was also negatively related with densities of *P. ulyssiponensis* adults (Figure 4.8C).

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 4.5). 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 4.8, middle). The other variables responsible for the spatial pattern of *P. ulyssiponensis* at rockpool edges were distance to low-water mark (lower overall abundance of *P. ulyssiponensis* further away from low-water mark), shore height and the abundance of the congener *P. depressa* (PD) (both negatively associated with the abundance of larger *P. ulyssiponensis*) (Table 4.5; Figure 4.8B).

Table 4.5 Distance based linear models (DistLM) for three species of limpets (*P. ulyssiponensis*, PU; *P. depressa*, PD; *S. pectinata*, SP) within three neighboring microhabitats (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 R2; AIC: 379.85		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: 4v + **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" 1stgroup: Habitat-composition (40%) 2ndgroup: Habitat-composition + Physical (44%)		
PU EDGE	7 variables: 36% in R2; AIC: 717.08	4 variables: 34% in R2; 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" 1stgroup: Connectivity (22%) 2ndgroup: Connectivity + Physical (31%) 3rdgroup: Connectivity + Physical + Habitat-composition (34%)		
PU NEAR	13 variables: 48% in R2; AIC: 287.5	6 variables: 44% in R2; 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 R2; AIC: 322.65	8 variables: 47% in R2; 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 rock-pool.
	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 R2; AIC: 954.78	7 variables: 26% in R2; 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" 1stgroup: Connectivity (12%) 2ndgroup: Connectivity + Physical (24%) 3rdgroup: Connectivity + Physical + Habitat-composition (26%)		
PD NEAR	14 variables: 52% in R2; AIC: 695.33	6 variables: 47% in R2; 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%) 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%)		
SP POOL	9 variables 28% in R2; AIC: 333.74	6 variables: 26% in R2; 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 R2; AIC: 442.59	5 variables: 22% in R2; 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 rock-pool.
	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 R2; AIC: 175.68	5 variables: 15% in R2; 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" 1stgroup: Habitat-composition (11%) 2ndgroup: 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 microhabitat. Physical variables: related with physical characteristics of rock-pools and surrounding rock. Connectivity variables: the total density of a co-occurring limpet species in an adjacent microhabitat; coded by the abbreviation of species name followed by "_adjacent_" and the microhabitat 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/microhabitat. Note that the habitat-composition group of EDGE models did not include cover and density of other species besides limpets.

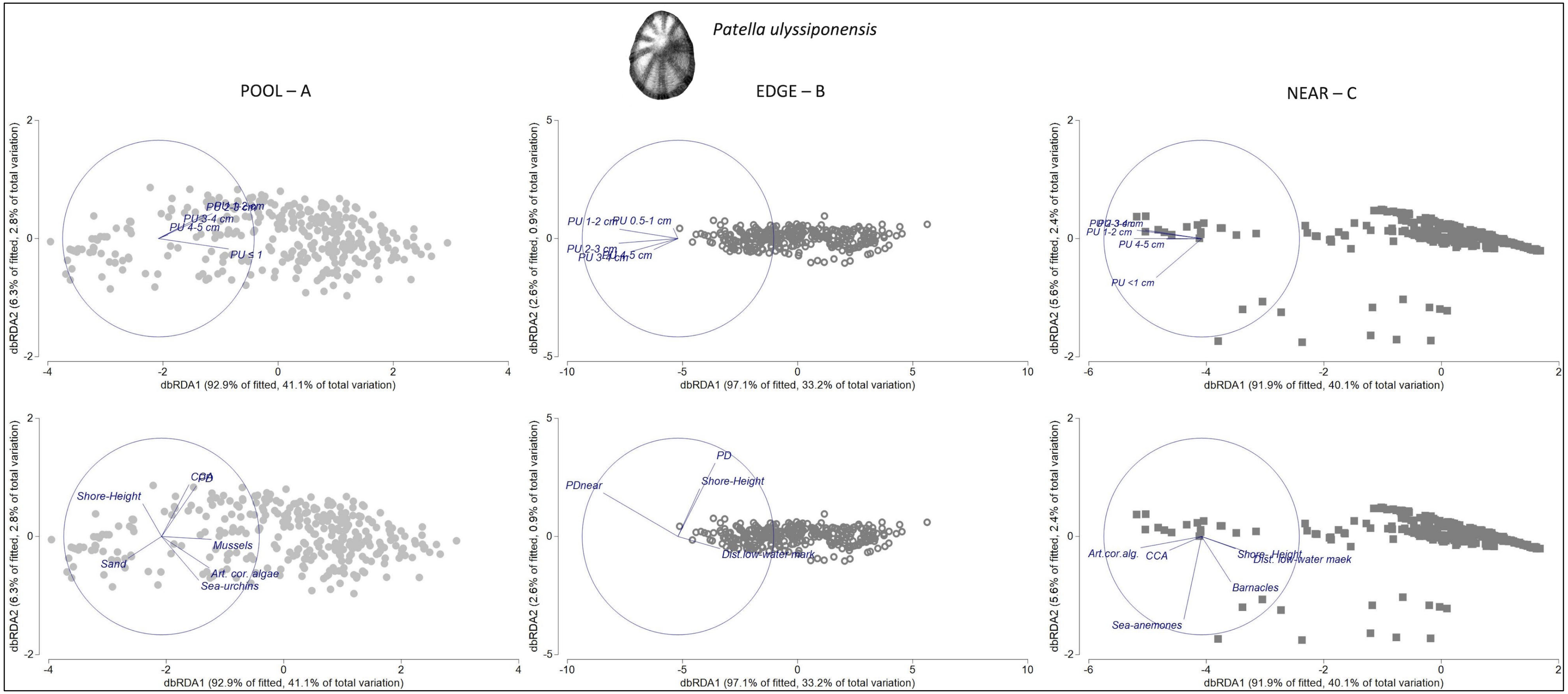

Figure 4.8 Distance-based redundancy analysis (db-RDA) plots for the spatial variability in abundance and size structure of *Patella ulyssiponensis* within each of three neighboring microhabitats: (A) POOL (rock-pools, gray circles); (B) EDGE (edge of rock-pools, open circles); (C) NEAR (open-rock adjacent to rock-pools, 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 4.5). 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

4.4.3.2 Patella depressa

Both DistLM models produced for *P. depressa* inside rock-pools (PD POOL) and on the open-rock surrounding rock-pools (PD NEAR) explained 47% of variation among rockpool systems, while the model regarding the EDGE explained 26% of the variability (Table 4.5).

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 rock-pools respectively (Table 4.5). These predictors greatly influenced spatial structuring within each microhabitat (samples along the horizontal axis db-RDA1), with CCA positively associated with overall abundance of *P. depressa* inside rock-pools and mussels positively associated with overall abundance of *P. depressa* outside rock-pools (Figures 9A, C). The other habitat-composition variables that also explained variation inside rock-pools were cover of sea urchins—negative relationship with juveniles (PD ≤1cm); total density of *P. ulyssiponensis* within the same microhabitat (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 microhabitat (SP)—positive relation with *P. depressa* adults; and cover of sand—negative relation with *P. depressa* adults (Table 4.5; Figure 4.9A). Outside rock-pools, besides the above-mentioned positive association with mussels, Verrucariaceae cover was negatively associated with density of small-sized *P. depressa* (PD ≤ 1 cm and 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 4.9C).

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 microhabitat (Table 4.5). Inside rock-pools, 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 4.9, top). Outside rock-pools, shore height was negatively associated with density of PD 3–4 cm (Figure 4.9C). An additional physical variable, the slope of the rockpool bottom, was negatively associated with PD ≤1cm inside rock-pools (Figure 4.9A).

While no connectivity variable was selected for the PD POOL model (Table 4.5), 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 rock-pools (Figure 4.9, bottom).

Furthermore, shore was included as a factor in the PD NEAR model (contributing to 12% of total explanation, Table 4.5), 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 4.9C).

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 4.5): 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 rock-pools (PU_adjacent_Pool) (Figure 4.9B). 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 associations with abundance of *P. depressa*) and confinement (Figure 4.9B). 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 4.9, middle). Finally, a habitat-composition variable was also selected for this PD EDGE model: abundance of *P. ulyssiponensis* within the same microhabitat (PU)— negatively related with large-sized *P. depressa* (Table 4.5; Figure 4.9B).

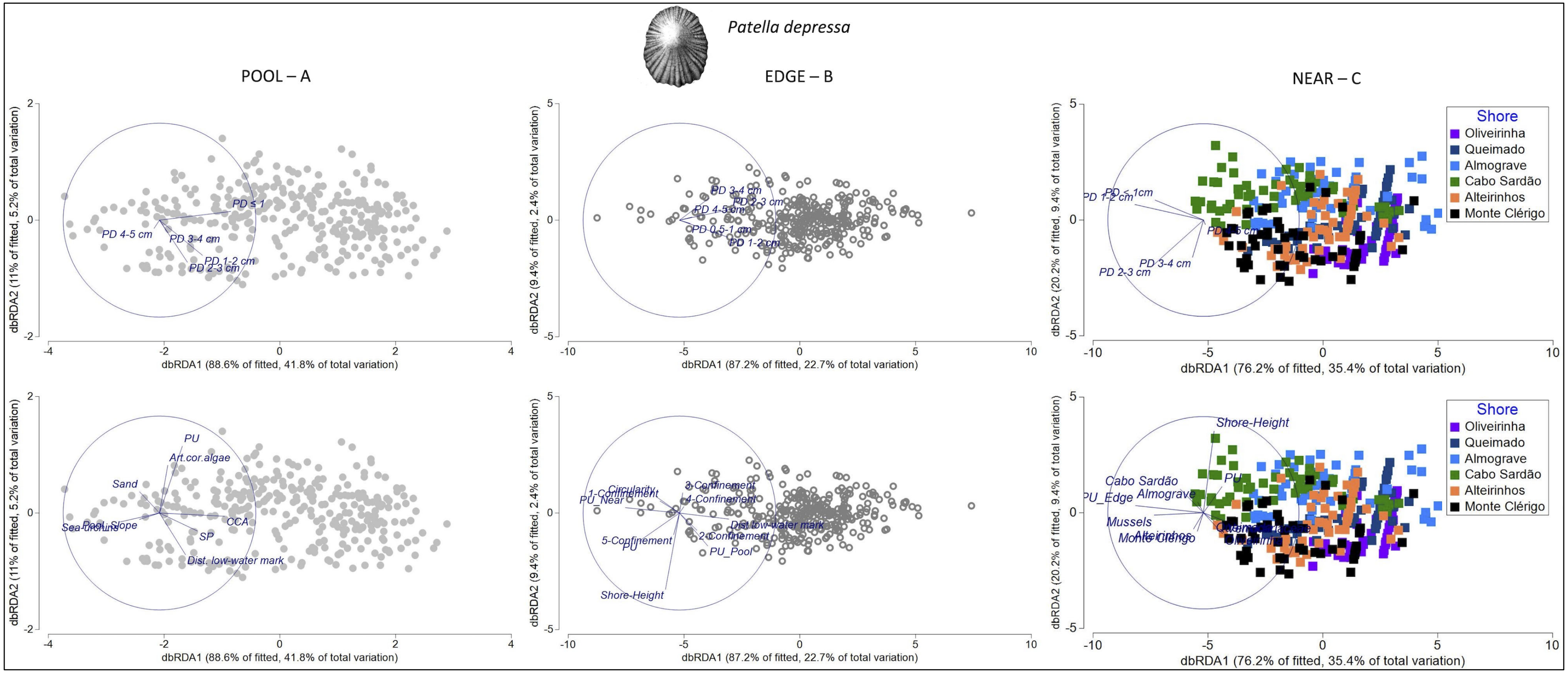

Figure 4.9 Distance-based redundancy analysis (db-RDA) plots for the spatial variability in abundance and size structure of *Patella depressa* within each of three neighboring microhabitats: (A) POOL (rock-pools, gray circles); (B) EDGE (edge of rock-pools, open circles); and (C) NEAR (open-rock adjacent to rock-pools, 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 4.5). 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).

4.4.3.3 Siphonaria pectinata

The DistLM models for *S. pectinata* inside rock-pools (SP POOL) and in the edge of rock-pools (SP EDGE) explained similar proportions of the variability (26% and 22%, respectively), higher than the model for this species on the open-rock surrounding rock-pools (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 4.5). The first selected predictor in the SP POOL model was the total density of *P. depressa* in the same microhabitat (PD) (Table 4.5), for which a positive relation with density of *S. pectinata* juveniles (SP ≤0.5cm) was suggested (Figure 4.10A). The other habitat-composition predictor included in the SP POOL model was the cover of articulated coralline algae (Table 4.5), for which a negative relationship was suggested with the density of the large and mid size-classes of *S. pectinata* (SP 2-3 cm, SP 3-4 cm and SP 1-2 cm) (Figure 4.10A). For the SP NEAR model, three different habitat-composition predictors were included: cover of crustose non-coralline algae was positively related with overall densities of *S. pectinata*; the variable "Other sessile invertebrates" was negatively associated with densities of larger-sized *S. pectinata* (SP 1–2 cm and SP 2–3 cm); and the total density of *P. depressa* in the same microhabitat (PD) was positively associated with densities of smaller-sized *S. pectinata* (SP ≤0.5 cm and SP 0.5–1 cm) (Table 4.5; Figure 4.10C).

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 rock-pools, namely, with juveniles within POOL (SP ≤0.5cm), and negatively associated with *S. pectinata* abundance outside rock-pools, namely, with mid- and large-sized *S. pectinata* within NEAR (SP 1–2 cm and SP 2–3 cm) (Table 4.5; Figures 10A, C). The other physical predictors selected in

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 4.5; Figure 4.10A). 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 4.5; Figure 4.10C).

In both the SP POOL and SP NEAR models, no connectivity variables were selected (Table 4.5). 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 rock-pools (PU_adjacent_Near)—negative association with mid-sized SP (SP 1–2 cm); *P. depressa* within open-rock surfaces surrounding rock-pools (PD_adjacent_Near)—negative association with with mid-sized *S. pectinata* (SP 1-2 cm); and *P. depressa* inside rock-pools (PD_adjacent_Pool)—positive association with small-sized *S. pectinata* (Table 4.5; Figure 4.10B). However, in the SP EDGE model, the variable that stood out was confinement, a physical predictor that explained 11% of the variation (Table 4.5), 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 4.10B). Besides the connectivity variables and confinement, the factor shore was selected in this model (Table 4.5), 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 4.10B). This shore had the highest percentage of very-low confinement (category 1): 24% of rock-pools (Supplementary Figure S4.7).

In summary, the cover of articulated coralline algae was a mutual habitat-composition “top predictor” in models of the three species inside rock-pools 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 4.5).

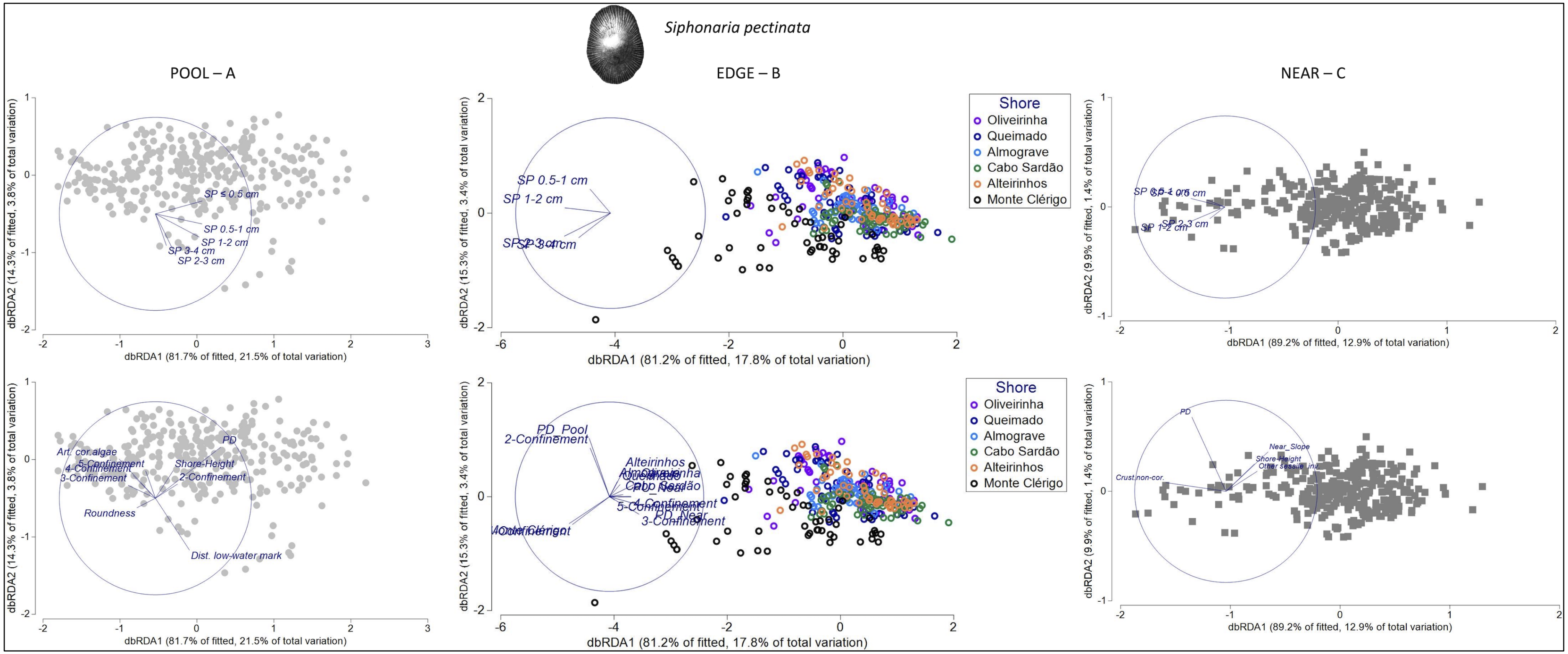

Figure 4.10 Distance-based redundancy analysis (db-RDA) ordination plots for the spatial variability in abundance and size structure of *Siphonaria pectinata* within each of three neighboring microhabitats: (A) POOL (rock-pools, gray circles), (B) EDGE (edge of rock-pools, colored circles), and (C) NEAR (open-rock adjacent to rock-pools, 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 4.5). 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.

4.5 Discussion

4.5.1 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 rock-pools, and along a large part of the vertical tidal gradient within rock-pools, 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 microhabitats 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 rock-pools and their edges, reflecting occupancy of open-rock and steeper shores (objective 1a); iv) a decreasing gradient of intra-specific connectivity from rock-pools 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 microhabitats), being steeper in *P. ulyssiponensis* than in *P. depressa* and *S. pectinata*, possibly due to *P. ulyssiponensis* being largely resident in rock-pools 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 rock-pools, which can be considered a single habitat for these species (objective 1c); vi) landscape variability within shores, and hence the microhabitat 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 rock-pools 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 microhabitat 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 microhabitats) (objective 3).

4.5.2 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 microhabitats; *P. vulgata* infrequently occurred mostly within EDGE and NEAR; and *P. rustica* was exclusively present on NEAR and FAR. Rock-pools 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 rock-pools 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 4.3.1) 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 S4.7) and a higher frequency of crevices were found (Supplementary Figure S4.3). 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 microhabitats surrounding rock-pools, 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.

4.5.3 Vertical distribution and among-shore patterns

When considering the microhabitat 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 rock-pools 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 rock-pools, 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 rock-pools and open-rock at low shore (Firth and Crowe, 2008). For *P. depressa* and *S. pectinata*, the lower distribution limit also varied between microhabitats. 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 rock-pools, Figure 4.7). This species can be particularly abundant upon open-rock and in rock-pools 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 rock-pools supporting more diverse assemblages compared to open-rock (Metaxas et al., 1994; Firth et al., 2014), and for the role of rock-pools 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 rock-pools 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 microhabitats 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 microhabitat 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 rock-pools, likely to warm quickly (the lowest values of maximum and mean depth, POOL topographic index and slope, and confinement, Supplementary Figure S4.7). 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 microhabitats, sporadic or frequent variation was found between stretches within each shore, which could be related to smaller-scale variation in physical and biological processes.

4.5.4 Horizontal distribution and intra-specific connectivity across microhabitats

On all shores, there were many more limpets of the three common species inside rock-pools 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 rock-pools 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 rock-pools (POOL versus NEAR, on average), but there were three times more *P. depressa* adults outside than inside rock-pools (NEAR versus POOL, on average). This suggests that rock-pools 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 microhabitats 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 rock-pools 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 rock-pools, possibly acting as a frontier for the largely pool-resident *P. ulyssiponensis* above the low shore and a potential 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 rock-pool system, the strength of correlations in the abundance of the same species between pairs of adjacent microhabitats 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 rock-pools to drier open-rock only suitable for older/larger limpets (e.g., Bowman, 1981; Bowman, 1985). However, the positive relationship between rock-pools 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 rock-pools 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 rock-pools 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 rock-pools 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 rock-pools (MidNEAR and FAR areas). Therefore, at this tidal level, the open-rock surrounding rock-pools can be considered as part of the open-rock habitat in general. Once emerged from mid-shore rock-pools 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 microhabitats for recently settled limpets) across the mid-shore landscape. Mid-shore crevices were identified as important juvenile microhabitats 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 microhabitats.

4.5.5 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 microhabitat. For all three species, the group of habitat-composition variables explained most of the variation in response matrices of the three species within both rock-pools 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 rock-pools (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 microhabitats, 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 microhabitats (connectivity variables selected only in the *P. depressa* NEAR model). On the contrary, abundances of other limpet species in the same microhabitat 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 rock-pools. 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 rock-pools. Abundances of both patellids inside rock-pools were positively related with cover of CCA, differentially related with articulated coralline algae (positively with *P. ulyssiponensis*, negatively with *P. depressa*), 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 rock-pools 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 both *S. pectinata* and *P. depressa* inside pools, a positive relationship was established with distance to low-water mark, and higher abundances were related to lower coverage of articulated coralline algae. Indeed, *S. pectinata* in SW Portugal is frequently found at high-shore rock-pools 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 microhabitat for the three species, possibly reflecting greater recruitment, survival, and growth lower down (Guerra and Gaudencio, 1986). The other highlighted relationships outside rock-pools 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 rock-pools in SW Portugal are very conspicuous within *Ralfsia verrucosa* and *Nemoderma tingitanum* patches at low and lower-mid shore open-rock (Castro, 2004; 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 rock-pools 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 of *P. ulyssiponensis* 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 differently related to the abundance of its congener at pool edges (negative relationship between *P. depressa* at pool edges and *P.ulyssiponensis* on adjacent open-rock; positive relationship between *P. ulyssiponensis* at pool edges and *P. depressa* on adjacent open-rock). A positive relationship was also established between the abundance of *S. pectinata* on pool-edges and the abundance of *P. depressa* within adjacent 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.

4.5.6 Concluding remarks

Limpets of the three most common species in SW Portugal co-occur in connected populations across a landscape of rock-pools, edges, and open-rock microhabitats. Rock-pools create a mosaic of microhabitats 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 microhabitats 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 rock-pools 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 rock-pool 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 rock-pool 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 rock-pool communities (as their role as keystone species has not been studied for rock-pool microhabitats in Portugal). The importance of rock-pools 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 rock-pools in sustaining limpet populations in the context of anthropogenic disturbance including harvesting, as part of biodiversity surveillance and conservation of temperate reefs.

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CHAPTER 5: Efficacy of eco-engineered rock-pools: long-term enhancement of limpet populations across intertidal artificial habitats.

Seabra, MI, Hawkin SJ, Espírito-Santo C, Celestino S, Sousa A, Mamede M, Costa A, Mamede N, Silva T, Castro JJ, Cruz T. Efficacy of eco-engineered rock-pools: long-term enhancement of limpet populations across intertidal artificial habitats. In preparation to be submitted to *Journal of Apllied Ecology*.

5.1 Abstract

1. Little is known about the efficacy of coastal eco-engineering on population responses over the long-term and beyond the intervention scale.

2. Here we report outcomes of a decadal experiment on eco-engineered rock-pools in sea defences of the Port of Sines (SW Portugal), focusing on limpet populations within and beyond these pools.

3. Drill-cored rock-pools were created on mid-tidal boulders of two rock-armour locations (General Cargo and Petrochemical terminals). On boulders with pools (Cored), we regularly estimated: percentage cover of macro-epibenthic taxa inside pools; density and size-structure of limpets within pools, on open-rock surrounding pools (Around) and on open-rock further away from pools (Away). Limpet populations on boulders without pools (Control) were also censused. We tested whether there were proximity (Around > Away) and landscape-unit (Cored > Control) effects of rockpool creation on the abundance of limpets over July 2010-July 2020.

4. A diverse assemblage colonized the pools, with contrasting composition between the two locations. Crustose coralline algae were significantly more abundant in pools of General Cargo.

5. Limpet recruits (shell length: ≤ 5 mm for patellids; ≤ 4 mm for siphonariids) were continuously found within pools at both locations – maxima of ten *Patella ulyssiponensis*, three *Patella depressa* and 15 *Siphonaria pectinata* (recruits in 126 cm2). Over time, recruits of *P. ulyssiponensis* and larger individuals (shell length > 5mm) of both patellids were significantly more abundant in pools at General Cargo; while egg masses of *S. pectinata* were significantly more abundant in pools at Petrochemical.

6. For *P. depressa*, a significant landscape-unit effect was found at General Cargo (four times more limpets on the open-rock of Cored than Control), whereas no proximity effect was detected. Proximity and landscape-unit effects were significant at both locations for *S. pectinata* (higher density Around than Away; nine times more limpets on the open-rock of Cored than Control).

7. Synthesis and applications Drill-cored rock-pools enhanced limpet abundance across distinct microhabitats, by providing nurseries and source-areas of ontogenic emigration to open-rock. Eco-engineering interventions are species-specific and locational context-dependent, and thereby should be informed by life-traits of target species and evaluated over extended spatial and temporal frames.

Keywords: greening of grey coastal infrastructure, intertidal artificial structures, long-term monitoring, beyond intervention effects, drill-cored rock-pools, patellid and siphonariid limpets, nurseries.

5.2 Introduction

Global coastlines are becoming intensively modified with proliferation of artificial structures associated with coastal protection, residential and industrial buildings, plus transport and maritime infrastructure (so called ocean sprawl – Duarte et al., 2012; Firth et al., 2016). This is a consequence of rapid population growth and economic development in the coastal zone leading to increasing land-claim (Firth et al., 2013a; Bugnot et al., 2021; Floerl et al., 2021), coupled with the inevitable need to defend people and property from rising and stormier seas driven by climate change (IPCC, 2023). Artificial habitats replace or modify natural habitats (Airoldi et al., 2005; Moschella et al., 2005; Bulleri and Chapman, 2010; Dugan et al., 2011; Firth et al., 2013a; Loke et al., 2019), pervasively changing the biodiversity and functioning of marine ecosystems (reviews by Bulleri and Chapman, 2010; Firth et al., 2016; Bishop et al., 2017; Heery et al., 2017).

Hard artificial structures used for coastal protection (sea defences, such as seawalls and breakwaters) are often built within highly modified contexts, such as industrial complexes and ports (e.g. Lai et al., 2015; Airoldi et al., 2016; Chapman et al., 2017; Mayer-Pinto et al., 2018), where changes to original ecosystem conditions are likely to be irreversible (Bulleri et al., 2020). Compared to natural shorelines, sea defences offer less diversity of microhabitats and spatial heterogeneity at local scales (Moschella et al., 2005; Chapman and Underwood, 2011; Firth et al., 2013b; Aguilera et al., 2014; Aguilera et al., 2022). An established consensus is that the smooth and relatively homogeneous surfaces of sea defences often act as poor surrogates of natural reefs, providing lower habitat suitability or narrower niche availability for colonization by marine organisms (Glasby, 1999; Bulleri, 2005; Bulleri et al., 2020; Bauer et al. 2024a,b).

Mobile species were found to be particularly negatively impacted, possibly reflecting reduction in reproductive output, decreased recruitment and/or increased mortality on sea defences (e.g. Chapman, 2003; Moreira et al., 2006; Espinosa et al., 2011). Various studies have found differences between populations of limpets living on artificial structures and on natural rocky-shores, such as genetic patterns (Fauvelot et al., 2009) or morphometric variation (Tablado and Gappa, 2001; Sempere-Valverde et al., 2024), trophic ecology (Burgos-Rubio et al., 2015; Sedano et al., 2020; Espinosa et al., 2021), physiological stress (Espinosa and Rivera-Ingraham, 2016), population structure and sex-ratios (Earp et al., 2023), distribution and abundance (Bulleri and Chapman, 2004; Cacabelos et al., 2016; Bauer et al., 2024a) or density and size-structure (Guerra-García et al., 2004; Rivera-Ingraham et al., 2011a,b). On the other hand, sea defences in ports have recently been proposed as Artificial Marine Micro Reserves: they can act as refuges from human collection, thereby promoting protection of endangered species in the

Mediterranean, such as the limpets *P. ferruginea* and *Cymbula nigra* (García-Gómez et al., 2011, 2015; Ostalé-Valriberas et al., 2022).

Design options, coastal management approaches and marine planning policies have been implemented to reduce adverse environmental impacts and enhance ecosystem services on sea defences (Dafforn et al., 2015b; Morris et al., 2019; Mamo et al., 2022). The ultimate aim is to promote their multifunctionality (Dafforn et al., 2015a; Evans et al., 2017; Mayer-Pinto et al., 2017; Sawyer et al., 2020), combining their primary engineering function along with conservation and other societal needs, consequently enabling more sustainable, environmentally-sensitive urbanised seascapes (Riisager -Simonsen et al., 2022; Suhui et al., 2025). A considerable effort to improve "value as habitat" of marine artificial structures (Chapman and Blockley, 2009; Borsje et al., 2011; Chapman and Underwood, 2011; Firth et al., 2016; Firth et al., 2014) has been pursued through ecologically informed engineering approaches ("eco-engineering" *sensus* Bergen et al., 2001; Mitsch and Jørgensen, 2003, also known as "greening of grey infrastructure" after Naylor et al., 2017b). These approaches can be applied in intertidal and subtidal artificial structures, without compromising their primary engineering function, to encourage colonization by native marine life and consequently enhance biodiversity, ecosystem functioning and service provision (recently reviewed by Strain et al., 2018; Morris et al., 2019; O'Shaughnessy et al., 2020; Airoldi et al., 2021; Evans et al., 2021; Firth et al., 2024a;).

Eco-engineering interventions on intertidal artificial structures include mimicking microhabitats that are missing from these structures but present on natural rocky shores. By resembling characteristics of natural habitats, the availability of refuges (from abiotic stress or biotic interactions) for marine organisms can be amplified at a range of spatial scales through changes in material (chemical composition) or shape (physical structure) of hard artificial substrata (Pioch et al., 2018; Evans et al., 2021). This is done by incorporation of topographical (e.g. Hall et al., 2018), biogenic (e.g. Ng et al., 2015) or both structural elements of habitat complexity (Bradford et al., 2020; Chee et al., 2021; Vozzo et al., 2021). These actions follow design guidelines (Burcharth et al., 2007; Firth et al., 2014; O'Shaughnessy et al., 2020), based on knowledge of natural history and experimental ecology of rocky shores as their closest natural analogues (reviewed by Hawkins et al., 2020). Recent comparisons among several design types of structural complexity have found that the provision of rock-pools was the most effective in bolstering biodiversity, consistently supporting the greatest abundances and species richness of algae and invertebrates, so their use has been especially advised for future eco-engineering in the intertidal zone (Bishop et al., 2022). Eco-engineered rock-pools (also called artificial tidepools) can be added as water-retaining features on intertidal levels of sea defences, during construction or retrospectively (e.g. O'Shaughnessy et al., 2020; Evans et al., 2021; Firth et al., 2014). Among the documented types of eco-engineered rock-pools, drill-cored rock-pools have been particularly recognized as a cost-effective means of habitat enhancement in both temperate (Evans et al., 2015) and tropical (Chee et al., 2020) regions.

Evidence-based practice for the use of eco-engineered rock-pools has been based on a limited suite of response variables and time-frames, resulting in little attention given to population dynamics or life-history traits of species using the pools. As usefully summarized by Evans et al. (2021), nearly all studies reporting effects of eco-engineered rock-pools have focused on the structure of macroalgal, invertebrate and/or fish communities, examining overall community composition and alpha diversity (richness of taxa or other diversity indices) to measure outcomes for biodiversity in general. Conversely, effects of rockpool creation at the population-level, namely on the abundance of target species or individual groups of species of functional significance, have been the subject of much fewer studies in the past decade (but see Chapman and Blockley, 2009; Browne and Chapman, 2014; Morris et al., 2017; Morris et al., 2018a). Moreover, the effects of rock-pools added to intertidal artificial structures have been mostly examined for short-term periods: often ≤ 2 years (e.g., Evans et al., 2015; Firth et al., 2016; Perkol-Finkel and Sella, 2016; Morris et al., 2018b; Ostalé-Valriberas et al., 2018; Waltham and Sheaves, 2018; Bone et al., 2024), rarely 3-5 years (Hall et al., 2019; Chee et al., 2020), with exceptional maximum of 7.5 years (Ostalé-Valriberas et al., 2023). This can be particularly limiting since assemblages on artificial structures take several years to develop (Hawkins et al., 1983), thus persistence of populations should be assessed after their stabilization in density and size (e.g. Firth et al., 2024b). Longer-term experiments or trials would determine consistency and generality of patterns through time, to thereby judge the ultimate usefulness of eco-engineering interventions in coastal artificial structures (Martins et al., 2016; Bishop et al., 2022).

Efficacy of eco-engineered rock-pools is usually evaluated through patch-scale comparisons with control habitats of intertidal artificial structures (open-rock surfaces) or analogue habitats at reference sites (nearby rocky-shores); but the connectivity among adjacent habitats has been mostly overlooked. Nevertheless, there is some evidence that the influence of eco-engineered pools can extend beyond their water-retaining limits to emersed areas of artificial structures. Ostalé-Valriberas et al. (2018, 2024) tested humidity buffering effects on benthic assemblages colonizing a 5-cm halo around rock-pools carved on rip-raps in Ceuta. They found a clear transition in the composition of these halo assemblages relative to the ones present inside the created rock-pools and on control open-rock, coupled with a differential distribution of several invertebrate species that mostly established within pools and surrounding halos. MacArthur et al. (2020) considered a “pool adjacent microhabitat” (~10-cm width surface immediately next to the edge of rock-pools created on rock-armour boulders), to assess enhancement effects on the presence of mobile species on coastal revetments in England. Furthermore, grazing halos (Bone et al., 2024) and adult limpet aggregations (Herbert et al., 2025) have been recorded on open-rock surfaces immediately surrounding artificial rock-pools bolted onto vertical seawalls in South England. Herbert et al. (2025) suggested that adults of the limpet *Patella vulgata* move into these pool-surroundings from areas further away from pools, to feed and/or find shelter from abiotic stress or predation. For intertidal species that naturally require the presence of protective microhabitats for recruitment (Bowman, 1981; Seabra et al., 2020), eco-engineered rock-pools might encourage early life stages usage and facilitate positive interactions among taxa within pools. If eco-engineered rock-pools can provide nurseries or temporary refuges for populations of mobile species, they could also act as source areas for emigration of individuals to the adjacent open-rock (Delany et al., 1998; Seabra et al., 2023). When selection by settling larvae or post-settlement survival is favoured by the newly created microhabitat and followed by cross-microhabitat migration, species abundance might be potentially enhanced across intertidal artificial habitats. An added biodiversity-value can thus spread from the spatial scale of the intervention (areas inside eco-engineered pools) to broader scales of artificial structures (outside-pool areas). This is especially important in the case of mobile grazers with key structuring roles for which artificial structures often fail to support viable populations (Moreira et al., 2006).

It has been well established that natural rock-pools are important nurseries for limpets of the genus *Patella* (e.g. Lewis and Bowman 1975; Delany et al., 1998), whose grazing controls algal vegetation on all coasts of the North-East Atlantic (Hawkins, 1981; Hawkins and Hartnoll, 1983; Jenkins et al., 2005; Coleman et al., 2006). Seabra et al. (2020) demonstrated the primacy of shallow mid-shore rock-pools dominated by crustose coralline algae (CCA) as nurseries for both patellid and siphonariid limpets co-occurring on natural rocky-shores near Sines (SW Portugal). They proposed a crucial value of these habitats for sustaining limpet populations on this coastline, which has undergone extensive changes since the 1970s due to setting and expansion of the Port of Sines. Inside this port, as in some other ports and harbours globally (e.g. Lai et al., 2015), nearly all of the intertidal shoreline is composed of featureless built structures that replaced original rocky and sedimentary habitats. Rock-pools are nearly absent on these hard coastal structures, where very low limpet abundance is found on the mid-shore (CIEMAR, 2018).

In contrast, adults of two limpet species (*Patella depressa* and *Siphonaria pectinata*) are very common on the mid-shore open-rock of nearby natural rocky shores, where rock-pools are conspicuously present (Seabra et al., 2023). Additionally, a massive settlement event of patellid post-larvae was previously recorded within plastic water-retaining collectors deployed on the mid-shore of both a rock-armour structure inside the Port and a natural shore outside the Port (Seabra et al., unpublished data; Appendix). This event supported that the scarcity of rock-pools, instead of lack of larval supply, is likely a main driver behind the paucity of limpets in sea defences of the Port of Sines.

Here we considered a long-term and landscape (cross-microhabitat) view of the efficacy of eco-engineering interventions for a key functional group of rocky-intertidal organisms (limpet grazers) in a highly human-modified port complex in Portugal. We followed colonization of pool biota and limpet population dynamics within drill-cored rock-pools over a ten-year period after their creation on the mid-shore of two rock-armour structures located in the Port of Sines. Most importantly we examined how this intervention added value across spatial scales beyond the cored-pool itself. Target species included patellid and siphonariid limpets occurring within mid-shore surfaces inside and outside the created rock-pools. Population responses were assessed through regular monitoring, by examining the recruitment of limpets onto the drill-cored pools as well as the variation in abundance and size of limpets on the surrounding emersed substrata. The general hypothesis of a positive effect of rockpool creation on the abundance of limpets on mid-tidal boulders of rock-armour structures of the Port of Sines was addressed.

Our first specific objective was to demonstrate that the core-drilled eco-engineered rock-pools can provide limpet nurseries or refuges, by describing their environmental temperature, colonization by epibenthic assemblages and abundance of limpets of different species and sizes over time (effects within pools – intervention-scale). Our second specific objective was to test whether eco-engineered rock-pools added to rock-armour boulders at mid-shore heights enhanced the abundance of limpets on the open-rock of such boulders (effects outside pools – beyond intervention-scale). This prediction was tested at two scales, considering the following specific hypotheses: (1) smaller-scale comparison (proximity effect) – within cored boulders (boulders where pools were added), the open-rock surfaces surrounding pools (“Around”) would support greater density of limpets than the ones further away from pools (“Away”); (2) larger-scale comparison (landscape-unit effect) – the open-rock surfaces of cored boulders would support greater density of limpets than the ones located on control boulders (unmanipulated boulders where pools were not added). We also discuss some implications for eco-engineering planning both in terms of site selection (context-dependency of efficacy) and desired outcomes, particularly how eco-engineered nursery grounds can be used to target valuable provisioning and regulatory ecosystem services provided by limpets on human-modified shorelines.

5.3 Materials and methods

5.3.1 Study area and sites

The Port of Sines is an oceanic deep-water port associated with a large industrial complex in SW Portugal. It is the main port of the Iberian-Atlantic coast in terms of vessel traffic and cargo handling, including crude oil and natural gas. Its international maritime importance, coupled with logistic and industrial infrastructure, has led to proliferation of multiple artificial structures on the foreshore and in nearshore environments. Apart from two natural rocky shores and an urban beach (Figure 5.1A), the coastline extending from the northern limit of the port (located at the leeward side of the very exposed headland of Cape of Sines – 37°57'27"N, 8°53'16"W) to its present southern limit (37°56'14"N, 8°50'6"W) has been completely modified by hard port infrastructure (more than 90 % of an approximately 12 km-stretch of coast). There are plans for the expansion of the existing port facilities and industrialized area to the South, which will increase the extent of artificial coastline.

Most of the rocky-intertidal shoreline inside the Port of Sines is composed of concrete units or rock-quarried boulders as part of coastal protection structures. At the outset of this study, abundance of the most common mid-shore limpet species was estimated on seven intertidal artificial structures inside the Port of Sines versus four nearby natural rocky shores outside this Port: *Patella depressa* was 70 times more common outside the Port (0.6 ± 0.1 vs. 43 ± 2.6); *Siphonaria pectinata* was almost three times more common outside the Port (3 ± 0.6 vs. 8 ± 0.9) – mean ± SE number of limpets per 50 x 50 cm (n = 224 inside the Port vs. 128 outside the Port; corresponding to four quadrats sampled in two coastal stretches of each location during two summer dates in 2001 and in 2005) (CIEMAR, unpublished data). In 2001 (no data available for 2005), the abundance of the two species estimated on a unique natural shore inside the Port (the one adjacent to General Cargo) was as follows: 35 ± 9.3 *P. depressa* and 2 ± 0.8 *S. pectinata* per 50 x 50 cm (n = 16 quadrats) (CIEMAR, unpublished data).

Two rock-armour structures in the Port of Sines, separated by ~2 km of shoreline, were considered in this study: General Cargo (37°56'42"N, 8°51'27"W) and Petrochemical (37°57'1"N, 8°52'40"W) Terminals, hereafter “General Cargo” and “Petrochemical” (Figure

5.1A). General Cargo is situated in the southern entrance to a yachting club and a tugboat quay. This location is publicly-accessible and adjacent to one of the two natural stretches of rocky coast remaining inside the Port (the natural shore named as "Port" described in Seabra et al., 2019, Figure 5.1A). Petrochemical is next to a jetty for docking of oil tankers (vessels up to 20.000 m^3 of cargo capacity) and an inland storage park for the handled crude-derived products (Figure 5.1B). Access to this location is limited by the port operator, nor are there any nearby natural shores (Figure 5.1A). The two rock-armour structures front reclaimed land and consist of coast-adherent seaward-facing revetments (rip-rap sloping seawalls) extending from the shallow subtidal to the high intertidal zones (Figure 5.1B). Both structures are composed of gabbrodiorite boulders with similar sizes (top surfaces with ~1 m^2) and the same origin (derived from a local quarry). The two structures have similar age: General Cargo was built in the 1980s, Petrochemical in 1977; both were subsequently reinforced during maintenance works in 1992/93. Their locations are characterized by well-mixed marine waters with considerable wave exposure in comparison with more sheltered locations within the Port. This region has a semi-diurnal tidal regime with a tidal range of approximately 3.5 m.

5.3.2 Experimental design and set-up

A manipulative experiment was set-up in both locations (the two rock-armour structures – General Cargo and Petrochemical), along a coastal stretch of ~80 m at mid-tidal height in each location. The top surfaces of mid-tidal boulders at both locations were dominantly covered by barnacles (70 % *Chthamalus montagui* and 30 % bare-rock, on average). Cylindrical rock-pools (8 cm diameter, ~3 cm depth) were created on horizontal surfaces of mid-tidal boulders (Figure 5.1 C-D), using a diamond-tipped drill corer. Two rock-pools were cored from the top surface of each boulder (Figure 5.1C). Dimensions of drill-cored rock-pools were within the ranges of those of natural rock-pools used as nurseries by limpets on nearby rocky-shores (Seabra et al., 2020). The base of each drill-cored rock-pool (a circular projected area of 50.24 cm^2) was marked by the shape of the drill bit during rockpool creation, defining three concentric zones: a central, a middle and a peripherical zone, respectively comprising 5 %, 60 % and 35 % cover of the bottom surface of each pool base (Figure 5.1D). These relative proportions were estimated at rockpool creation, through replicate weight measurements of aluminium sheets moulded onto the pool base substratum. The experiment was set-up at two start timings (two independent timings of rockpool creation): October 2009 (Start 1) and April 2010 (Start 2). At each start timing and location, three mid-tidal boulders were randomly selected to be cored with pools (Cored, Figure 5.1E) and three other mid-tidal boulders were randomly selected to remain without pools (Control, Figure 5.1E) (total per location: six cored boulders, six control boulders, and 12 pools).

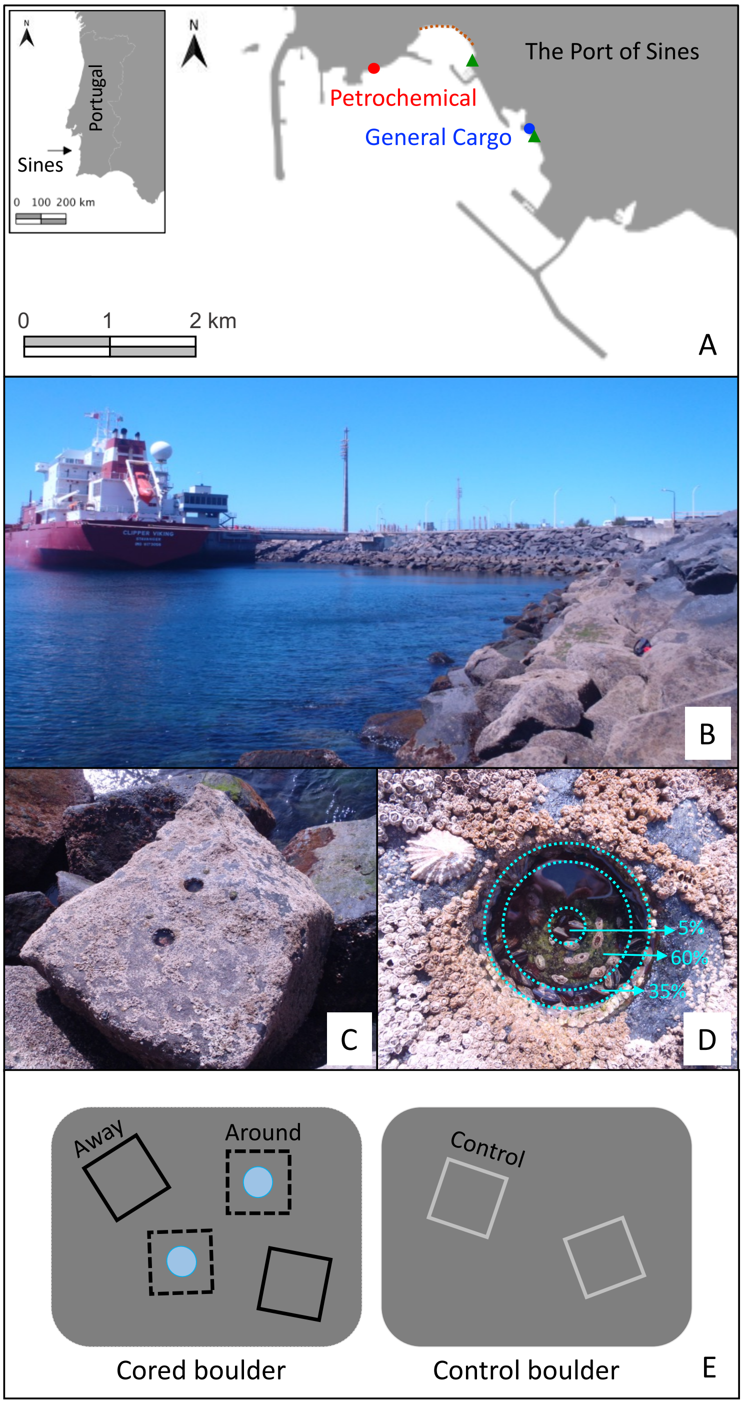


Figure 5.1 A: Study locations – General Cargo and Petrochemical, two rock-armour structures in the Port of Sines, SW Portugal. Map also shows the unique coastal stretches that were not replaced by hard artificial coastline within the Port – two natural rocky shores (green triangles) and an urban beach (brown dash curve). B: A rock-armour structure. C: A cored boulder (~1 m^2). D: A drill-cored rock-pool (8 cm diameter); dotted circles represent the borders of three concentric zones marked by the shape of the drill bit on the base of every cored pool with percentage values indicating their relative cover in proportion to the pool-base bottom surface. E: Cored (boulders with pools) vs. Control (boulders without pools); sampling areas included drill-cored rock-pools (blue circles; each pool – basal area of 50 cm^2 and total surface area of 126 cm^2) and open-rock surfaces categorized as "Cored Around" (two 25 x 25 cm areas centered on drill-cored rock-pools and excluding rockpool area; each dashed area after subtracting pool basal area – 575 cm^2), "Cored Away" (two 25 x 25 cm quadrats distancing more than 15 cm from pools; each black quadrat – 625 cm^2) and "Control" (two 25 x 25 cm quadrats on each control boulder; each grey quadrat – 625 cm^2). (Photographs B-D taken at Petrochemical).

5.3.3 Temperature measurement

Biomimetic loggers (DS1922L iButton, waterproofed by insertion into silicon-filled *Patella* spp. shells) were installed to describe differences in the thermal environment between two microhabitats (rock-pools vs. open-rock) on the two rock-armour structures (General Cargo and Petrochemical), and on a natural rocky-shore inside the Port (the natural shore adjacent to General Cargo, Figure 5.1). A single logger was deployed for each of six conditions: inside and outside a drill-cored rock-pool in both General Cargo and Petrochemical; plus inside and outside a shallow natural rock-pool (similar dimensions to drill-cored rock-pools) on the natural rocky-shore. Loggers outside pools were installed on open-rock surfaces ~15 cm away from the pool edge. At General Cargo and Petrochemical, loggers were installed on an additional cored boulder (a mid-tidal boulder cored with pools, but not used for other experimental purposes). All the six loggers were programmed to register temperatures with 0.5 °C resolution at 30 min intervals. Loggers were attached to substrata using epoxy (Z-Spar A-788 Splash Zone Compound) and interchanged approximately every three months to provide nearly continuous monitoring of temperature throughout the experiment.

5.3.4 Surveys

Following rockpool creation (time zero at Start 1 or Start 2), all experimental plots (drill-cored rock-pools and open-rock surfaces within cored and control boulders of both locations) were surveyed quarterly until November 2015, and biannually afterwards until July 2020 (total of 30 and 32 sampling times for plots initiated in Start 1 and in Start 2, respectively). All surveys were made during low-tide on daylight spring tides.

Colonization of drill-cored rock-pools was assessed on each sampling time and location as the percentage cover of free substrata (rock and sand/detritus) and biota (macro-epibenthic taxa, including algae and invertebrates) on the surface of each pool base (50 cm^2). During surveys within this sampling area, the three concentric zones (Figure 5.1D) were used as references with known percentage cover. The identity and percentage cover of each substratum type or macro-epibenthic taxon were recorded for each of the three zones; these records were then converted to give final values of percentage cover per pool base. Both primary (i.e. attached directly to the pool substrata) and secondary (i.e. growing onto the primary taxa) cover of taxa were assessed, and summed to give a total percentage cover that could exceed 100 %. Organisms inside pools were identified to species or to morphospecies in the field. Field records were subsequently lumped into the following 15 space-occupying categories: Rock (including bare-rock and biofilm); Sand/Detritus (including sediments and biodeposits, e.g. mussel shell remains); CCA (also called “Lithothamnia”); Non-coralline crustose algae (mostly *Nemoderma tingitanum*, *Ralfsia verruscosa* and *Hildenbrandia rubra*); Non-coralline turf (including cespitose algae and cyanobacteria, e.g. Sphacelariales, *Oscillatoria spp.*); Coralline turf (articulated coralline algae, mostly *Corallina/Elisolandia sp.*); Foliose green algae (mostly *Ulva* spp.); Foliose non-green algae (mostly *Caulacanthus ustulatus*); the mussel *Mytilus galloprovincialis*; *Chthamalus* spp. (mostly *Chthamalus montagui*); *Perforatus perforatus*; *Paracentrotus lividus*; Sea anemones (including Actiniidae, mostly *Actinia equina*, and Sagartiidae); Porifera (mostly *Hymeniacidon perlevis*); and Others (Serpulidae, Spirorbidae, Hydrozoa).

Limpets were censused (all individuals identified to species-level, allocated to size classes, and counted) on each sampling time and location, within four microhabitat categories: Pools (total bottom surface of drill-cored rock-pools, including the base and wall areas of each pool i.e. 126 cm^2), Cored Around (open-rock surfaces surrounding drill-cored rock-pools within cored boulders), Cored Away (open-rock surfaces located further away from pools within cored boulders), and Control (open-rock surfaces on unmanipulated boulders). On the open-rock of every cored boulder, Cored Around surfaces were comprised by two 25 x 25 cm quadrats centred on each drill-cored rock-pool (Figure 5.1E) and Cored Away surfaces were sampled by two 25 x 25 cm quadrats distancing more than 15 cm from any pool (Figure 5.1E). On every control boulder, two replicate 25 × 25 cm quadrats were sampled (Control, Figure 5.1E). Seven size classes (MSL, measured with calipers) were considered for *Patella* spp. (MSL in mm, ≤ 3, 3-5, 5-10, 10-20, 20-30, 30-40, > 40) and six for *S. pectinata* (MSL in mm, ≤ 2, 2-4, 4-10, 10-20, 20-30, 30-40). Additionally, egg masses of *S. pectinata* were counted inside pools.

Density of three species (*Patella ulyssiponensis*, *P. depressa* and *S. pectinata*) per pool were calculated separately for two size-groups: “Recruits” (MSL ≤ 5 mm for patellids; MSL ≤ 4 mm for siphonariids) and “Juveniles + adults” (all the other individuals, i.e. MSL > 5 mm for patellids; MSL >4 mm for siphonariids). For the other microhabitat categories, limpet counts per species and size-class were summed to give the density of each species per 625 cm^2. In the case of Cored Around, densities per quadrat were calculated after subtracting the projected area of the pool base (i.e. considering the open-rock sampling area: 575 cm^2) and then standardized to the quadrat area (625 cm^2).

5.3.5 Statistical analyses

Time-series of temperature registers during 12 periods of three-months were assembled. These were deployment periods over which data were successfully retrieved from the whole set of loggers (one logger per each of six contrasting environmental conditions). Minimum, mean and maximum values of daily averaged temperature profiles were calculated for each condition and period, and then averaged across the 12 periods.

The structure of colonizing assemblages on pool-base surfaces of drill-cored rock-pools was described over time for each set of pools created at the same start timing and location. Data consisted of the mean values of percentage cover of all space-occupying categories (15 response variables, including substrata, functional groups of algae, and key taxa of macro-epibenthic invertebrates) on every sampling time (replicates were all sampling times for each of the four combinations of start timing and location). Non-metric multidimensional scaling (MDS) was used to visualize the distance among replicates (labelled as 1 to 30 for Start 1, and 1 to 32 for Start 2) following the temporal sequence of the four successional trajectories, and the correlation vectors of response variables (space-occupying categories) with Pearson coefficient higher than 0.7. Hierarchical agglomerative clustering using group average linkage was applied on the same dataset to build up a dendrogram, followed by a SIMPER analysis (Clarke, 1993) to identify the taxa most responsible for the similarity/dissimilarity of replicates grouped within/between main clusters. Both the MDS plot and dendrogram were based on a resemblance matrix produced by the Bray-Curtis similarity coefficient of square-root transformed data.

For assessing effects of rockpool creation within drill-cored rock-pools over the period of July 2010 to July 2020, the following datasets were considered: the total amount of "Suitable substrata for limpet homes" (the sum of Rock, CCA and Non-coralline crustose algae), its individual parcels (Rock, CCA and Non-coralline crustose algae, separately), all expressed as percentage cover; "Recruits" and "Juveniles + adults" of *Patella ulyssiponensis*, *P. depressa* and *S. pectinata* (separately for the two size groups of the three limpet species) as well as egg ribbons of *S. pectinata*, all expressed as numbers per pool (126 cm^2). A matrix was organized for each of these datasets, in which the response variables were the 30 sampling times between July 2010 and July 2020. The replicates were each pair of pools located on the same boulder (to obtain a single estimate of percentage cover or density for each boulder, data were the averaged values of the two pools of each boulder for every sampling time). These multivariate datasets were individually analysed through two-way PERMANOVAs based on Euclidean distances, considering Start (Start 1 vs. Start 2) and Location (General Cargo vs. Petrochemical) as two fixed orthogonal factors with two levels, and n = 3.

For examining effects of rockpool creation on the abundance of limpets outside pools over the period of July 2010 to July 2020, the total density of each limpet species (*P. depressa* and *S. pectinata*) recorded within experimental plots of open-rock categories (Cored Around, Cored Away and Control) were averaged across the two replicate quadrats of the same boulder for every sampling time and taken as response variables (30 response variables). Data were assembled irrespectively of whether boulders were set-up in Start 1 or Start 2. This was because analyses including start timing as a factor did not reveal any significant effect of this factor within pools (see Tables 2 and 3) nor outside pools (preliminary three-way PERMANOVAs and three-way univariate ANOVAs for each individual sampling time, data not shown). Larger-scale (proximity) effects were tested separately for each species through two-way PERMANOVAs, considering Proximity (Around vs. Away) and Location (General Cargo vs. Petrochemical) as two fixed orthogonal factors with two levels, with three boulders as independent replicates for each level of Proximity (n = 3, randomly selected for Around and for Away from the six cored boulders of each location). Larger-scale (landscape-unit) effects were tested separately for each species through two-way PERMANOVAs, considering two fixed orthogonal factors with two levels, Landscape-unit (Cored vs. Control) and Location (General Cargo vs. Petrochemical), and n = 6 boulders (for the Cored treatment, the Cored Away estimates on all the six cored boulders of each location were used). All PERMANOVAs were based on Euclidean distances.

Data on the size-structure of each target species (*P. depressa* and *S. pectinata*) were described as the proportion (%) of every size-class relative to the total number of limpets sampled on every sampling time within all experimental plots of each microhabitat category (Pool, Cored Around, Cored Away and Control) and location. The size-structure of *P. ulyssiponensis* and the total number of *S. pectinata* egg masses, both inside pools of each location, were also plotted over time. Comparison of limpet size-structure among sampling times was not tested due to lack of independence over time (successively repeated monitoring of the same experimental plots).

All multivariate analyses were performed using PRIMER 7 (Clarke and Gorley, 2015) with the PERMANOVA + add-on (Anderson et al., 2008). PERMANOVA tests were employed using Type III sums of square and permutation of residuals under a reduced model, with P-values obtained using 999 random permutations. When significant differences were found between the two levels of a factor, the similarity percentages breakdown (SIMPER) procedure (Clarke, 1993) was applied to identify the pattern for the response variables that most contributed (cut off for low contributions: 70%) to the distance/dissimilarity between levels. Pair-wise tests were done when the interaction term was significant, to assess differences in the levels of a factor relatively to each level of the other factor. The PERMDISP routine (Anderson et al., 2008) was used to test for homogeneity of multivariate dispersions between levels of each factor (measured as deviations from centroids).

5.4 Results

5.4.1 Temperature

Thermal differences between microhabitats of rock-pool and open-rock within each location were as follows: i) mean temperatures were 1 °C lower inside than outside pools in the two rock-armour structures and in the natural rocky-shore; ii) minimum temperatures were generally similar across the two microhabitats (differences up to 0.5 °C) in the three locations; iii) maximum temperatures were 7 °C lower inside than outside drill-cored rock-pools at both rock-armour structures, and 6 °C lower inside than outside the natural rock-pool (averaged values of each variable across the 12 periods, Table 5.1).

Differences in mean averaged temperatures between all pairs of locations were consistently less than 0.5 °C for both microhabitats (Table 5.1). More extreme minimum temperatures were consistently found at Petrochemical than at General Cargo (1 °C lower on average, both inside and outside pools) (Table 5.1). Less extreme minimum temperatures (1 °C higher) were found in natural pools than in drill-cored pools of both structures. Minimum temperatures were relatively similar (0.3 °C higher on average) in natural open-rock vs. open-rock at General Cargo, and 1 °C higher on average in natural open-rock vs. open-rock at Petrochemical (Table 5.1). The maximum temperatures inside and outside rock-pools were, on average, consistently similar between General Cargo and Petrochemical, and consistently higher (3 °C) on both structures compared to the natural shore (Table 5.1). Daily averaged temperature maxima were reached during the summer of 2012 on both microhabitats: 32 °C and 40 °C, respectively in pools of General Cargo and open-rock of Petrochemical (Table 5.1).

The variation in extreme temperature (i.e. the averaged difference between maximum and minimum daily temperature over the 12 periods) for each condition was as follows: 9 °C in natural rock-pools (most stable); 12 °C in pools of General Cargo; 13 °C in pools of Petrochemical; 15°C on natural open-rock; 19 °C on open-rock of General Cargo; 20 °C on open-rock of Petrochemical (most variable) (Table 5.1).

Table 5.1 Minimum, mean and maximum values of daily averaged temperature profiles for each environmental condition and deployment period, and averaged across the 12 periods. One logger deployed on each of six contrasting environmental conditions: inside (Rock-pool) and outside (Open-rock) a pool within General Cargo (GC), Petrochemical (Pc) and a natural rocky-shore (Nat). Rock-pool refers to drill-cored rock-pools (8 cm diameter, ~3 cm depth) at General Cargo and Petrochemical, and a natural rock-pool (similar dimensions) in the natural rocky-shore. Open-rock refers to mid-shore rock adjacent to the rock-pool edge (~15 cm away) in the three locations. Over each period, time-series of temperature were registered with 0.5 °C accuracy and frequency of 30 minutes by biomimetic loggers (DS1922L iButton, waterproofed by insertion into silicon-filled *Patella* spp. shells). Note: results are rounded to whole numbers for clarity, but values in the last line are rounded to the precision of original registers.

	Mean daily temperature						Minimum daily temperature						Maximum daily temperature					
	Rock-pool			Open-rock			Rock-pool			Open-rock			Rock-pool			Open-rock		
Deployment period	GC	Pc	Nat	GC	Pc	Nat	GC	Pc	Nat	GC	Pc	Nat	GC	Pc	Nat	GC	Pc	Nat
May to August 2011	20	20	20	22	21	22	16	16	17	16	15	17	30	31	28	39	39	37
August to November 2011	20	19	19	21	20	20	16	16	17	16	15	16	27	27	24	33	31	29
May to August 2012	20	19	19	21	21	21	15	15	16	15	15	16	32	31	28	38	40	36
August to November 2013	20	20	19	20	20	20	17	15	17	17	16	16	26	26	24	32	32	29
November to February 2013	15	15	15	15	15	14	12	11	12	12	11	11	18	18	16	22	21	18
February to May 2014	16	16	16	17	17	17	13	12	13	13	12	12	25	25	23	31	31	28
May to August 2014	20	19	20	21	21	21	16	15	17	15	15	16	30	30	28	37	38	34
February to May 2015	17	17	16	17	18	16	12	11	13	11	12	12	26	26	22	32	33	28
May to August 2015	20	20	20	22	21	22	16	15	17	15	15	16	30	31	29	39	39	36
August to November 2015	19	19	19	20	20	19	16	15	17	16	16	16	26	26	24	33	31	28
May to August 2018	20	19	19	21	21	21	16	15	16	15	15	16	29	30	28	38	38	35
August to November2018	20	19	19	21	21	20	16	15	17	16	16	16	27	26	25	35	34	31
Average of all periods	18.9	18.5	18.4	19.9	19.6	19.5	15.1	14.2	15.7	14.8	14.2	15.2	27.3	27.3	24.7	34.1	33.9	30.6

5.4.2 Colonization of assemblages within drill-core rock-pools

Initially, drill-cored rock-pools at both locations were colonized by foliose green (mostly ephemeral) algae (39% in February 2010 for pools initiated in October 2009; 97% in July 2010 for pools initiated in April 2010 – mean cover averaged across locations for sampling times immediately after Start 1 and Start 2) (Figure 5.2 and 5.3A). Foliose green algae were almost only present initially during succession (never exceeding 9% after July 2011 on both start timings and locations) (Figure 5.2, 5.3A). Subsequently, a diverse assemblage of various functional groups of algae and key invertebrate taxa colonized the rock-pools of both start timings and locations (Figure 5.2), changing during succession over time (Figures 2 and 3A). Distinct epibenthic assemblages became established in pools at the two locations, with no discrimination between the two start timings after the initial period (see below) (Figures 5.2, 5.3A, 3B).

Discrimination of assemblages between locations was mostly explained by a higher cover of CCA in pools of General Cargo, and a higher cover of Foliose non-green algae in pools of Petrochemical (Figure 5.3A). The presence of CCA in pools of General Cargo was first recorded in February 2010 (Start 1) and January 2011 (Start 2), becoming consistently established (above 25% after November 2014) and reaching the highest cover (44%) in January 2020. In contrast, CCA rarely exceeded 1% cover at Petrochemical (mean cover values averaged across start timings; Figure 5.2). Foliose non-green (mostly brown) algae were recorded at all sampling times in pools at Petrochemical, where cover was almost invariably maintained above 20% after February 2015, reaching three similar peaks (35%) in November 2015, May 2016 and January 2020. In contrast, Foliose non-green algae at General Cargo were mostly below 3% cover, with the highest peaks registered in April 3013 (16%, Start 1) and in November 2015 (26%, Start 2) (mean cover values averaged across start timings; Figure 5.2).

Five clusters were formed according to dissimilarities among sampling times in the overall structure of colonizing assemblages of drill-cored rock-pools (Figure 5.3 B). The first cluster (a, Figure 5.3B) included early sampling times of pools created in the second start timing in the two locations, which were characterized solely by Foliose green algae (total contribution of 89% to average similarity of 77%; SIMPER). The second cluster (b, Figure 5.3B) included other early sampling times of pools of both start timings and locations and was characterized by Foliose green algae, Rock and Non-coralline turf (respectively, 38%, 30% and 11% of 79% total contribution to average similarity of 68%; SIMPER). The third cluster (c, Figure 5.3B) included a mixture of intermediate sampling times of pools of both start timings and locations, being characterized by uncolonised Rock, Mussels, and Non-coralline turf (respectively, 37%, 23% and

19% of 79% total contribution to average similarity of 75%; SIMPER). The fourth cluster (d, Figure 5.3B) included most sampling times of pools of General Cargo exclusively, being characterized by CCA, Rock and *M. galloprovincialis* (respectively 25%, 24% and 24% of 72% total contribution to average similarity of 75%; SIMPER). The fifth cluster (e, Figure 5.3B) included the majority of sampling times of pools of Petrochemical exclusively, being characterized by *M. galloprovincialis*, Rock, Foliose non-green algae, and Non-coralline crustose algae (respectively 26%, 26%, 18% and 13% of 82% total contribution to average similarity of 80%; SIMPER). Therefore, colonization of pools created at the two start timings showed convergence (i.e. a similar pattern in composition of assemblages was found in pools of Start 1 and Start 2 after a certain stage of the successional sequences). Convergence was earlier at General Cargo than at Petrochemical (Figures 5.2 and 5.3), being completed since April 2012 at General Cargo and since May 2014 at Petrochemical (i.e. by two and by four years after the second start timing, respectively) (Figure 5.3B).

The segregation of the clusters that were exclusive for each location (cluster d – General Cargo vs. cluster e – Petrochemical) were based on the following space-occupying categories: CCA, Foliose non-green algae, Non-coralline crustose algae, Non-coralline turf, *M. galloprovincialis* and Rock (respectively 24%, 17%, 10%, 7%, 7%, and 6% of 73% total contribution to average dissimilarity of 36%; SIMPER). As previously described, CCA was more abundant at General Cargo (cluster d). The other space-occupying categories were more abundant at Petrochemical (cluster e), as above described for Foliose non-green algae. Very low cover of Non-coralline crustose algae was consistently found at General Cargo (rarely above 5% and never exceeding 13%). In contrast, at Petrochemical, Non-coralline crustose algae become progressively established (above 10% after May 2017) after their first records in February 2010 (Start 1) and January 2011 (Start 2), with the highest cover (24%) reached in January 2020 (mean cover values averaged across start timings; Figure 5.2). Non-coralline turf was mostly found at initial successional stages of pools at both locations, with consistently higher cover at Petrochemical (maxima of 58% in February 2010 and 50% in October 2011, respectively for Start 1 and Start 2) than at General Cargo (maximum of 35% in October 2010 averaged across start timings) (Figure 5.2). The cover of the mussel *Mytilus galloprovincialis* fluctuated considerably, being generally higher in mid stages of the successional trajectories (Figure 5.2, 3A – General Cargo: above 50% in April 2013 (Start 1) and in February 2014, May 2014 and November 2015 (Start 2); Petrochemical: highest peaks above 40% in February 2014, May 2014 and May 2016 (on averaged across start timings)). A higher cover of mussels generally persisted at Petrochemical than at General Cargo during the final stages of succession (Figures 2, 3A – General Cargo:

consistently below 30% after October 2016; Petrochemical: above 30% after January 2019). Finally, the mean extent of Rock highly fluctuated over time at both locations (maxima of 65% in January 2011 at General Cargo and 61% in January 2012 at Petrochemical; the overall mean across the period of July 2010-July 2020 being 31% at General Cargo and 35% at Petrochemical).

5.4.3 Effects of rockpool creation within pools – Suitable substrata for limpet homes

There was no significant effect of Start timing nor significant interaction between Start timing and Location in any analysis of the effects of rockpool creation within pools (Table 5.2, Table 5.3).

The total cover of "Suitable substrata for limpet homes (bare-rock, CCA, non-coralline algal crusts)" within pools did not differ significantly between locations over time (Table 2). This meant that limpets had similar availability of suitable living space for home establishment in pools at both locations (overall mean cover of 52% across the study period). Similarly, the amount of uncolonized Rock in pools was not significantly different between the two locations over time (overall mean cover of 34% across the study period) (Table 2, Figure 2). The other two components of "Suitable substrata for limpet homes" differed significantly between the two locations, with contrasting patterns on all sampling times that contributed to around 70% of average distance (Table 2): CCA cover was higher at General Cargo compared to Petrochemical (by a factor of 49, on average across July 2010-July 2020); the cover of Non-coralline crustose algae (mostly *Ralfsia*) was higher at Petrochemical than at General Cargo (by a factor of three, on average across July 2010-July 2020) (Table 2, Figure 2). At the end of study (in July 2020): the mean cover of CCA was 40% at General Cargo but completely absent at Petrochemical; whereas the mean cover of Non-coralline crustose algae was 18% at Petrochemical and 1% at General Cargo (Figure 2).

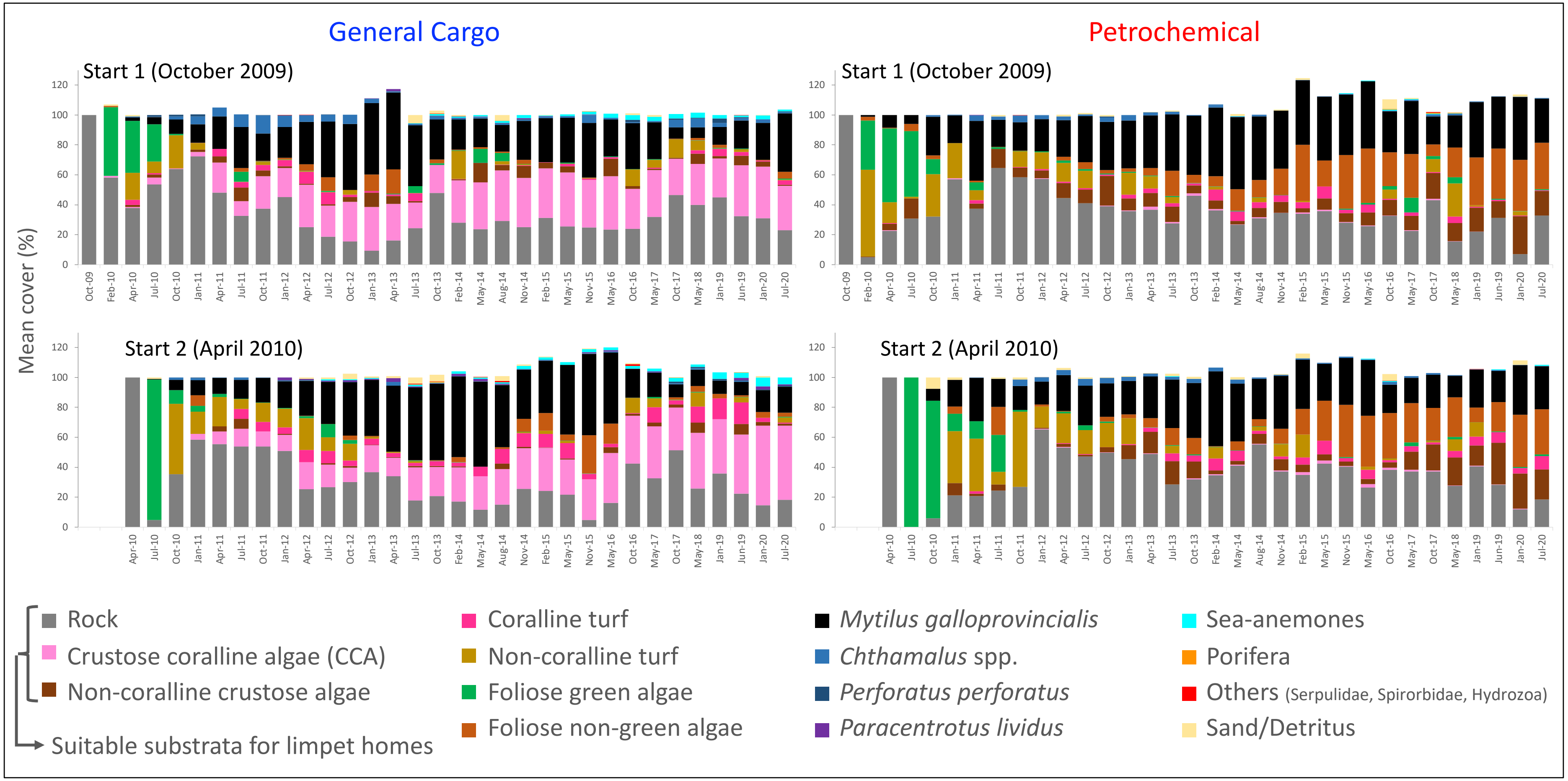
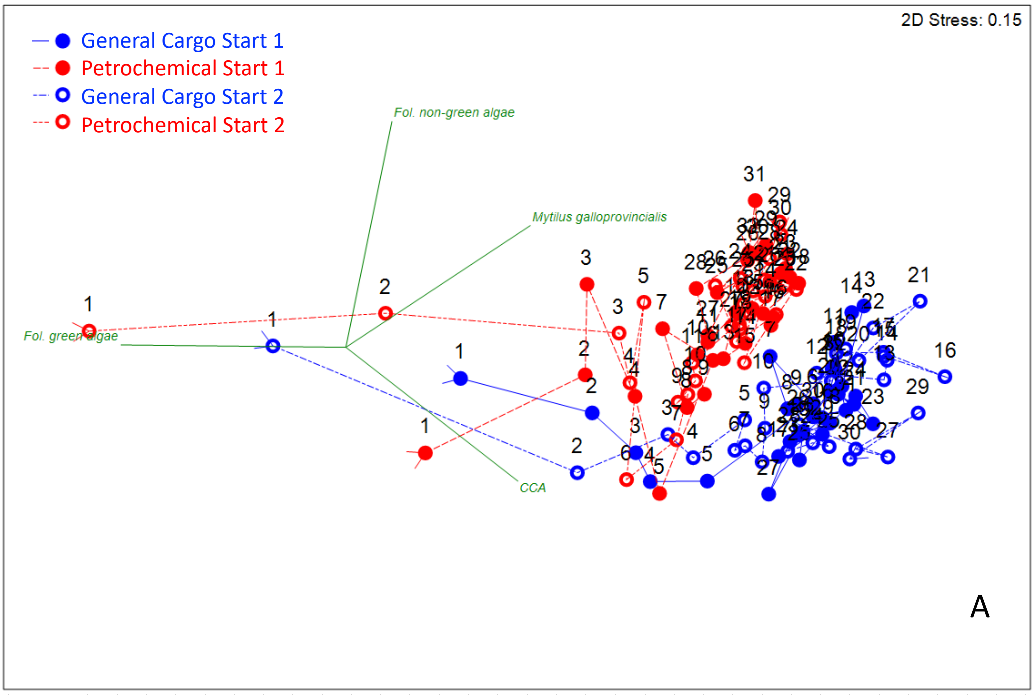
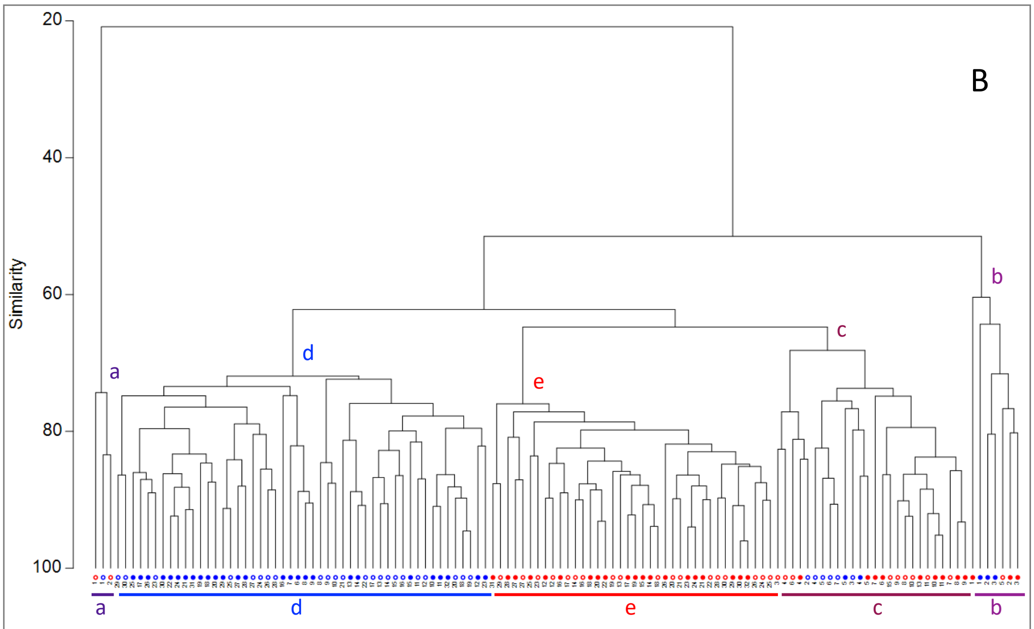


Figure 5.2 Relative abundance of 15 space-occupying categories (free substrata and macro-epibenthic taxa, including functional groups of algae and key taxa of invertebrates) during colonization of drill-cored rock-pools (base area of each pool: 50 cm^2) over time. Data for each sampling time consist of mean values of percent cover of each category, pooled across all the drill-cored rock-pools (n = 6) created at the same start timing (October 2009 – Start 1; April 2010 – Start 2) on each location (General Cargo and Petrochemical).



	Feb10	Apr10	Jul10	Oct10	Jan11	Apr11	Jul11	Oct11	Jan12	Apr12	Jul12	Oct12	Jan13	Apr13	July13	Oct13	Feb14	May14	Aug14	Nov14	Feb15	May15	Nov15	May16	Oct16	May17	Oct17	May18	Jan19	Jun19	Jan20	Jul20
Start 1 (Oct09)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Start 2 (Apr10)	-	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30



a: Jul10 of Start 2 in both locations; Oct10 of Start 2 in Petrochemical

b: Feb10 to Jul10 of Start 1 in both locations; Jul11 of Start 2 in Petrochemical

c: Oct10-Jan11 of Start1 in General Cargo; Oct10-Jul12 + Jan13 of Start1 in Petrochemical; Oct10-Jan12 of Start2 in General Cargo; Jan11 + Apr11 + Oct11-Jan13 + Feb14 of Start2 in Petrochemical

d: Apr11-Jul20 of Start 1; Apr12-Jul20 of Start 2 – General Cargo only

e: Oct12 plus Apr13-Jul20 of Start 1; Apr13-Oct13 + May14-Jul20 of Start 2 – Petrochemical only

Figure 5.3 Structure of colonizing assemblages of drill-cored rock-pools of both start timings and locations: response variables are space-occupying categories (free substrata and macro-epibenthic taxa); replicates are all sampling times (labelled as 1 to 30 for Start 1, and 1 to 32 for Start 2; corresponding dates are indicated in the middle). A: MDS plot; correlation vectors of response variables with Pearson coefficient higher than 0.7 (in green). B: Dendogram; five clusters (a, b, c, d, e) for which compositional differences were analyzed by SIMPER; replicates included in each cluster are indicated at the bottom. Both A and B based on a resemblance matrix produced by the Bray-Curtis similarity coefficient of square-root transformed data.

Table 5.2 Two-way PERMANOVAs analyses of the effects of Start timing (Start 1 vs. Start 2) and Location (General Cargo vs. Petrochemical) on the total abundance of "Suitable substrata for limpet homes" (the sum of Rock, CCA and Non-coralline crustose algae) within drill-cored rock-pools and of Rock, CCA and Non-coralline crustose algae, separately. Multivariate data consisted of values of percentage cover in every sampling time between July 2010-July 2020 (30 response variables) averaged across the two drill-cored rock-pools of the same boulder (n = 3 for each combination of Start and Location); transformation applied depending on the results of PERMDISP. Tests were based on Euclidian distances, considering two fixed and orthogonal factors with two levels.

Multivariate dataset (30 sampling times; 3 pairs of pools for each combination of Start and Location)	Suitable substrata for limpet homes		Rock		CCA		Non-coralline crustose algae	
	Square-root transformed		Untransformed		Fourth-root transformed		Square-root transformed	
	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)
Start	0.89	0.418	1.16	0.290	0.40	0.746	1.09	0.369
Location	1.72	0.168	1.62	0.171	11.483*	**0.012**	3.92	**0.008**
Start x Location	0.69	0.563	0.84	0.494	0.31	0.832	0.64	0.811
Pattern using SIMPER. The number of variables exhibiting the pattern in relation to the total number of variables explaining around 70% of the average distance between locations is shown in brackets.					Higher cover at General Cargo (18/18 contributing to 73.2% of average distance)		Higher cover at Petrochemical (14/14 contributing to 70% of average distance)	
PERMDISP p-values	0.029		0.572		0.041		0.147	

5.4.4 Effects of rockpool creation within pools – Limpets

Limpets belonging to the two size groups of the three species were first recorded in February 2010 at General Cargo, meaning that both larval settlement and immigration occurred into pools at this location during the three-months following the first Start (Figure 5.4). In contrast, in pools at Petrochemical, only patellids of the larger size group were found in February 2010 (recruits of both patellids and the larger size group of *S. pectinata* were first registered in July 2010, and *S. pectinata* recruits in October 2010) (Figure 5.4). Egg ribbons were first recorded in February 2010 at pools of both locations (Figure 5.4 - SP).

For each patellid species, there was some synchrony between pools at both locations in the trajectories of recruitment (peaks of Recruits at both General Cargo and Petrochemical – *P. ulyssiponensis*: April 2013, May 2015, and May 2016; *P. depressa*: April 2011, January 2012, April 2013, and May 2016) and in the abundance of larger individuals (peaks of Juvenile + adults at both locations – *P. ulyssiponensis*: April 2010, January 2011, May 2015, and October 2016; *P. depressa*: April 2011, April 2012, July 2013 and August 2014) (Figure 5.4 - PU, PD). Synchrony between the two patellid species was also seen within pools at each location (peaks of Recruits of both species: April 2013 and May 2016 at both locations; August 2014 and May 2017 at General Cargo; January 2012 at Petrochemical). Temporal patterns of recruitment were asynchronous between patellids and *S. pectinata* (Figure 5.4).

For *S. pectinata*, there was a clear synchrony between pools at both locations in the patterns of occurrence of egg ribbons (present at both General Cargo and Petrochemical in 15 out of the 18 sampling times in which they were recorded, with peaks in July 2011, July 2012, July 2013, August 2014, May 2017, May 2018, and July 2020) and recruits (peaks at both General Cargo and Petrochemical in April 2011, January 2013, October 2013, October 2016, January 2019, and January 2020) (Figure 5.4 - SP). Abundance of larger individuals of this species was asynchronous between pools of the two locations, being generally higher towards the end of the study period at General Cargo and in the middle of the study period at Petrochemical (Figure 5.4 - SP).

Considering the mean densities of limpets recorded over the study period within drill-cored rock-pools of each location, the maxima of each life-stage and species per pool-area were as follows (mean numbers in 126 cm^2, n = 6, Figure 4): i) Recruits – ten of *P. ulyssiponensis* (April 2013 and July 2013, General Cargo), three of *P. depressa* (April 2013, both shores) and 15 of *S. pectinata* (October 2012, Petrochemical); ii) Juveniles + adults – seven of *P. ulyssiponensis* (May 2018 and July 2020, General Cargo), six of *P. depressa* (July 2013, General Cargo) and 41 of *S.*

pectinata (July 2013, Petrochemical); iii) nine egg ribbons of *S. pectinata* (July 2012, Petrochemical).

From July 2010 to July 2020, the densities of each size group of *P. ulyssiponensis* within pools differed significantly between locations (Table 5.3). For both size groups, the pattern was explained by higher density of *P. ulyssiponensis* at General Cargo than at Petrochemical on almost all sampling times that contributed to around 70% of average distance (Table 5.3, Figure 5.4 - PU). There were not significant differences between pools of the two locations in the density of *P. depressa* recruits over the study period (Table 5.3). The density of larger individuals of *P. depressa* within pools was, however, significantly higher at General Cargo than at Petrochemical on almost all sampling times that contributed to around 70% of average distance (Table 5.3, Figure 5.4 - PD). On average across July 2010-July 2020, there were four times more Recruits of *P. ulyssiponensis*, three times more Juvenile + adults of *P. ulyssiponensis,* and three times more Juvenile + adults of *P. depressa* in pools of General Cargo compared to pools of Petrochemical. At final sampling (July 2020), there were twelve times more Recruits of *P. ulyssiponensis*, ten times more Juvenile + adults of *P. ulyssiponensis,* and three times more Juvenile + adults of *P. depressa* in pools of General Cargo compared to pools of Petrochemical (Figure 5.4 - PU, PD). Despite the highest densities of the two size groups of *S. pectinata* were registered within drill-cored pools of Petrochemical, their densities over time did not differ significantly between locations (Table 5.3, Figure 5.4 - SP). Egg masses of this species were significantly more abundant at Petrochemical than at General Cargo for all the eight sampling times that contributed to around 70% of average distance (Table 5.3, Figure 5.4 - SP) – there were two times more egg masses in pools of Petrochemical compared to pools of General Cargo on average across July 2010-July 2020; a magnitude of three times was found for the same pattern at the final sampling time (Figure 5.4 – SP).

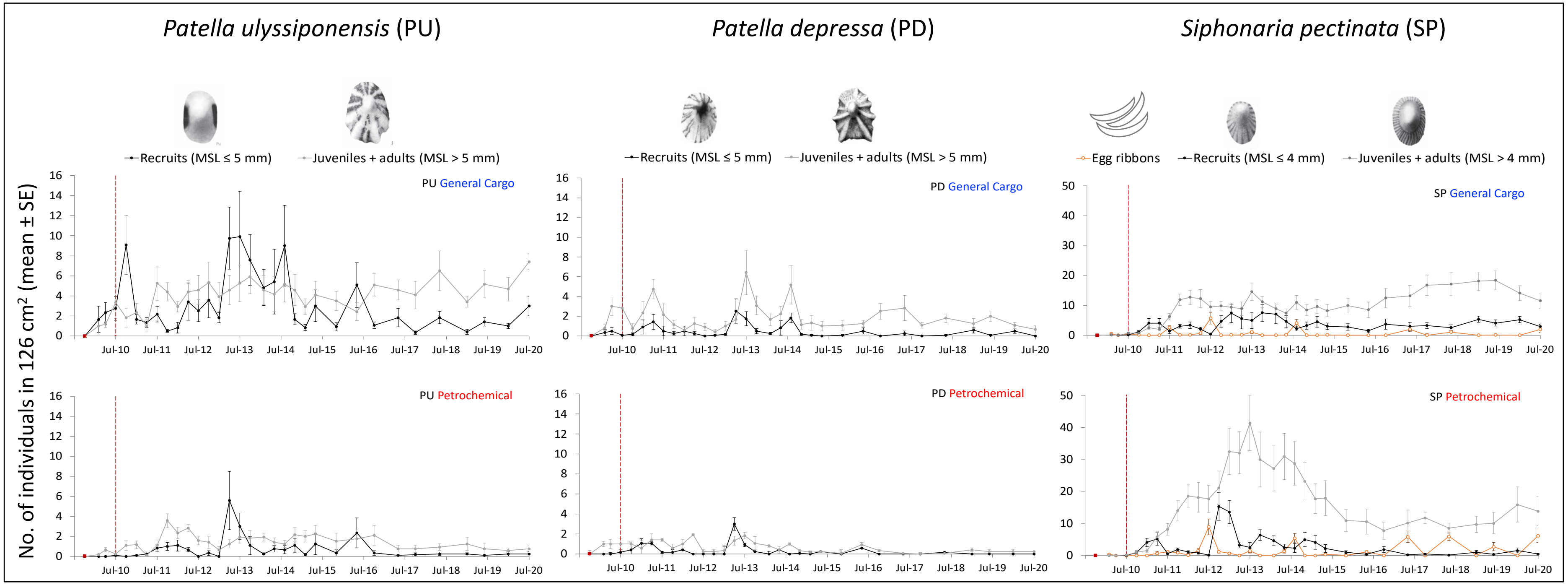


Figure 5.4 Abundance of three limpet species (*Patella ulyssiponensis*, *P. depressa* and *Siphonaria pectinata*) within drill-cored rock-pools of two locations (General Cargo and Petrochemical) over time. Number (mean ± SE) of individuals in 126 cm^2 (total surface area of each pool) plotted for: Recruits (MSL ≤ 5 mm for patellids and MSL ≤ 4 mm for siphonariids; black lines), Juveniles + adults (MSL > 5 mm for patellids and MSL > 4 mm for siphonariids; grey lines) and egg ribbons of *S. pectinata* (orange lines). Pools were created at two start timings (October 2009 and April 2010), and then sampled quarterly until November 2015 and semiannually afterwards. The vertical red line indicates the initial time of the period considered for data analyses (July 2010 to July 2020 – during which n = 6 pairs of drill-cored pools were monitored in each location). The red marker denotes initial null abundances (October 2009 – creation of the first 3 pairs of pools in each location). NB differences in y-axes between plots of patellids and siphonariids.

Table 5.3 Two-way PERMANOVAs of the effects of Start timing (Start 1 vs. Start 2) and Location (General Cargo vs. Petrochemical) on the abundance of Recruits (MSL ≤ 5 mm for patellids; MSL ≤ 4 mm for siphonariids) and Juveniles + adults (all the other individuals) of three limpet species (*Patella ulyssiponensis, P. depressa and Siphonaria pectinata*) and egg masses of *S. pectinata* within drill-cored rock-pools. Multivariate data consisted of the values of density in 126 cm^2 (total surface area of each pool) in every sampling time between July 2010-July 2020 (30 response variables) averaged across the two pools of the same boulder (n = 3 pairs of pools sampled for each combination of Start and Location); transformation applied depending on the results of PERMDISP. Tests were based on Euclidian distances, considering two fixed and orthogonal factors with two levels.

Multivariate dataset (30 sampling times; 3 pairs of pools for each combination of Start and Location)	*Patella ulyssiponensis*				*Patella depressa*				*Siphonaria pectinata*					
	Recruits		Juveniles + adults		Recruits		Juveniles + adults		Egg ribbons		Recruits		Juveniles + adults	
	Fourth-root transformed		Fourth-root transformed		Untransformed		Fourth-root transformed		Square-root transformed		Untransformed		Square-root transformed	
	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)
Start	0.74	0.652	1.45	0.19	1.25	0.300	1.19	0.30	0.89	0.472	0.88	0.409	0.28	0.868
Location	5.01	**0.008**	3.02	**0.04**	0.77	0.631	3.14	**0.01**	2.82	**0.035**	1.81	0.147	3.02	0.071
Start x Location	0.99	0.406	0.83	0.55	0.50	0.855	1.79	0.06	0.92	0.475	0.74	0.486	0.41	0.703
Pattern using SIMPER. The number of variables exhibiting the pattern in relation to the total number of variables explaining around 70% of the average distance between locations is shown in brackets.	Higher density at General Cargo (15/16 contributing to 70.7% of average distance)		Higher density at General Cargo (16/16 contributing to 72.4% of average distance)				Higher density at General Cargo (15/17 contributing to 71.5% of average distance)		Higher density at Petrochemical (8/8 contributing to 72.6% of average distance)					
PERMDISP p-values	0.196		0.807		0.209		0.229		0.053		0.58		0.211	

5.4.5 Effects of rockpool creation beyond pools – Proximity effect

No proximity effect was detected for *P. depressa* in any location: the density of this species over time did not differ significantly between open-rock areas surrounding pools and further away from pools (Cored Around = Cored Away – Table 5.4, Figure 5.5); the interaction between the factors proximity and location also was not significant (Table 5.4). The significant effect of location indicated that, independently of the distance to the pools, there was higher density of *P. depressa* on the open-rock of cored boulders at General Cargo than at Petrochemical for all the 17 sampling times that contributed to around 70% of average distance (Table 5.4, Figure 5.5). This spatial pattern (General Cargo > Petrochemical) was found to be greater by a factor of 14 on average across July 2010-July 2020, and by a factor of three at the final sampling time (July 2020) (Figure 5.5).

Main effects of proximity and location factors were both found for the small-scale comparison of *S. pectinata* within the open-rock of cored boulders (interaction term was not significant, Table 4). A significant proximity effect for *S. pectinata* was found at both locations: the density of this species over time was higher in areas surrounding pools (Around) than further away from pools (Away), on almost all sampling times contributing to around 70 % of dissimilarity (Table 4 – on average across July 2010-July 2020: Around > Away by a factor of eight at General Cargo and by a factor of 30 at Petrochemical (Figure 5)). Regarding the significant effect of location, densities of *S. pectinata* were higher at General Cargo on ten of the 18 sampling times contributing to around 70 % of average distance; whereas they were higher at Petrochemical on the other eight of these 18 sampling times (SIMPER; Table 4 – on average across July 2010-July 2020: General Cargo > Petrochemical by a factor of two in areas Around and by a factor of seven in areas Away (Figure 5)). At the final sampling time, mean densities of 3 and 1 individuals of *S. pectinata* in 625 cm^2 were respectively found in areas Around pools at General Cargo and Petrochemical, while being zero in areas Away from pools at both locations (Figure 5).

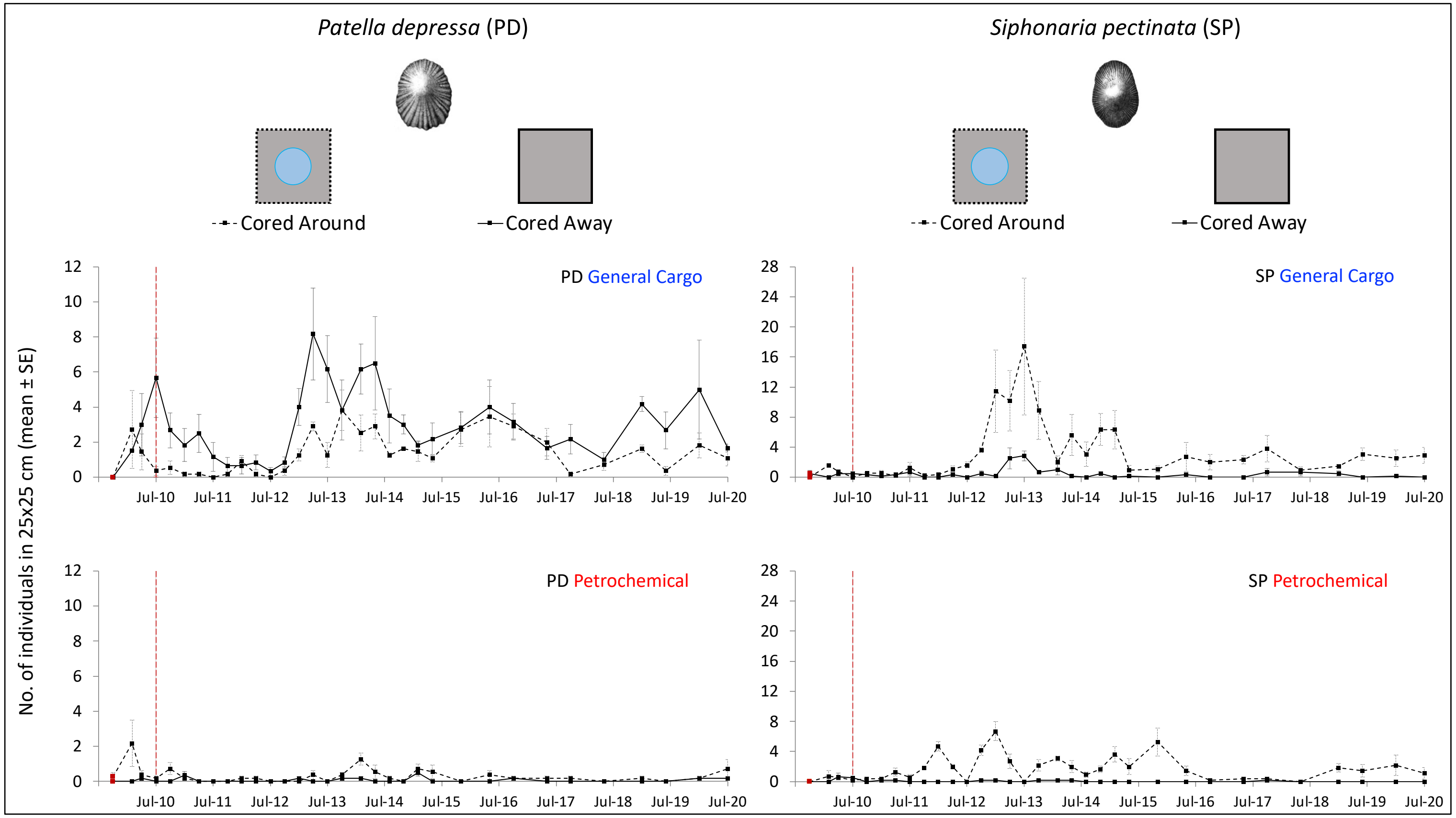


Figure 5.5 Proximity effect of drill-cored rock-pools on the abundance of two limpet species (*P. depressa* and *S. pectinata*) on the open-rock of two locations (General Cargo and Petrochemical) over time (small-scale comparison of effects of rockpool creation beyond rock-pools). Density (number of individuals per 625 cm^2, mean ± SE) within cored boulders at two proximity categories from drill-cored rock-pools: Cored Around – open-rock surfaces surrounding pools (dashed lines) and Cored Away – open-rock surfaces further away from pools (solid lines). Different boulders were randomly selected for each of the two proximity categories: n = 3 independent cored boulders for Cored Around and for Cored Around. Cored boulders were set-up in each location at two start timings (October 2009 and April 2010), their open-rock surfaces monitored quarterly until November 2015 and semiannually afterwards. The vertical red line indicates the initial time of the period considered for data analyses (July 2010 to July 2020). The red markers denote initial densities (October 2009 - first start timing). NB differences in y-axes between plots of patellids and siphonariids.

Table 5.4 Two-way PERMANOVAs of the effects of Proximity (Around vs. Away) and Location (General Cargo vs. Petrochemical) on the abundance of two limpet species (*P. depressa and S. pectinata*) on the open-rock of cored boulders. Multivariate data consisted of values of density standardized to 625 cm^2 in every sampling time between July 2010 - July 2020 (30 response variables) averaged across the two replicate quadrats of the same proximity category and boulder (n = 6 cored boulders in each location). Tests were based on Euclidian distances, considering two fixed and orthogonal factors with two levels.

Multivariate dataset (30 sampling times; N=3 randomly selected independent boulders for Around and Away in each location)	*Patella depressa*		*Siphonaria pectinata*	
	Fourth-root transformed data		Fourth-root transformed data	
	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)
Proximity	0.81	0.658	9.39	**0.002**
Location	10.83	**0.002**	2.5295*	**0.011**
Proximity x Location	1.76	0.105	1.26	0.210
Pattern using SIMPER. The number of variables exhibiting the pattern in relation to the total number of variables explaining around 70% of the average distance between locations is shown in brackets.	Higher density at General Cargo (17/17 contributing to 72.68% of average distance)		Proximity: Higher density Around than Away (16/17 contributing to 72.9% of average distance); Shore: Higher density at General Cargo (10/18) and higher density at Petrochemical (8/18).	
PERMDISP p-values	0.047		0.031	

5.4.6 Effects of rockpool creation beyond pools – Landscape-unit effect

Over July 2010 – July 2020, the maxima recorded for the mean density of target species in each combination of Cored/Control treatment and location were as follows (mean number of individuals in 625 cm^2, n=6, Figure 6): six *P. depressa* in Cored at General Cargo (April 2013); one *P. depressa* in Control at General Cargo (January 2013); one *P. depressa* in both Cored and Control at Petrochemical (both in February 2015); six *S. pectinata* in Cored at General Cargo (July 2013); one *S. pectinata* in Control at General Cargo (May 2017); two *S. pectinata* in Cored at Petrochemical (April 2013 and May 2017); and 0.3 *S. pectinata* in Control at Petrochemical (April 2013).

A significant landscape-unit effect (Cored > Control) in the density of *P. depressa* over time was found only at General Cargo, as significant differences were not found between the open-rock of cored boulders versus control boulders at Petrochemical (significant interaction term and Pair-wise tests to the interaction, Table 5.5). At General Cargo, the open-rock of cored boulders had four times more *P. depressa* than control boulders, on average across July2010 – July 2020; the same pattern was found by a factor of two in July 2020 (Figure 5.6). The abundance of *P. depressa* differed significantly between locations, being consistently higher at General Cargo than at Petrochemical for both Cored and Control (Table 5.5 – Pair-wise tests to the interaction) – on average across the study period: General Cargo > Petrochemical by 12 and 6 times respectively for the open-rock of cored boulders and control boulders; in July 2020: General Cargo > Petrochemical by 19 times for the open-rock of cored boulders (mean density of *P. depressa* was 1 individual in 625 cm^2 at General Cargo and null at Petrochemical on control boulders in July 2020) (Figure 5.6).

A significant landscape-unit effect was found for *S. pectinata* at both locations (no significant interaction term, Table 5.5), with higher density in Cored than Control on all the seven sampling times that contributed to around 70 % of average distance (Table 5.5). On average across July 2010 – July 2020, the open-rock of cored boulders had nine times more *S. pectinata* than control boulders consistently on both locations (same magnitude of effect at the two locations over time) (Figure 5.6). In July 2020, the density of *S. pectinata* was enhanced by three times at General Cargo (on the open-rock of cored versus control boulders) (Figure 5.6). At Petrochemical, the final mean density of *S. pectinata* was 0.4 individuals in 625 cm^2 in Cored and null in Control (Figure 5.6, July 2020). No differences were found between locations in the density of *S. pectinata* on rock-armour boulders over time (Table 5.5).

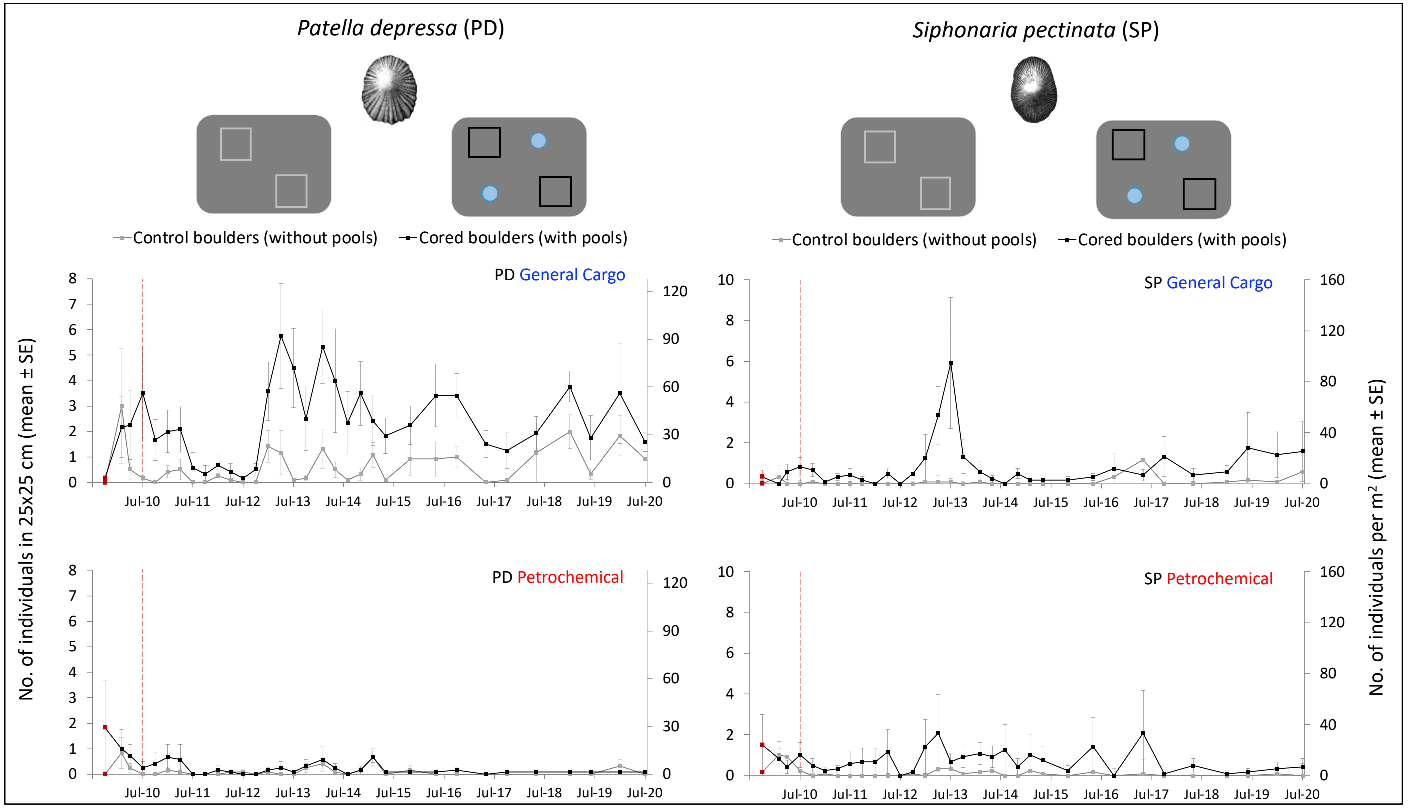


Figure 5.6 Landscape effect of drill-cored rock-pools on the abundance of intertidal limpets on open-rock surfaces of rock-armour structures (large-scale comparison). Density (number of individuals in 625 cm^2 and per m^2, mean ± SE) of two limpet species (*P. depressa* and *S. pectinata*) present on open-rock surfaces of Cored (black lines – boulders with two drill-cored rock-pools per boulder) and on Control (grey lines – boulders without pools, unmanipulated) at two locations (General Cargo and Petrochemical) over time – n = 6 boulders of each treatment sampled in each location). Boulders of both treatments were set-up in each location at two start timings (October 2009 and April 2010), their open-rock surfaces were monitored quarterly until November 2015 and semiannually afterwards. The vertical red line indicates the initial time of the period considered for data analyses (July 2010 to July 2020). The red markers denote initial densities (October 2009- first start timing). NB differences in y-axes between plots of patellids and siphonariids; second y-axis for extrapolation of densities per m^2. Sampling quadrats of cored boulders (with pools) corresponded to “Cored Away” surfaces (25 x 25 cm quadrats distancing more than 15 cm from both pools), but black lines in this figure were not the same as in Figure 5.5 due to different sample size.

Table 5.5 Two-way PERMANOVAs of the effects of Landscape-unit (Cored vs. Control boulders) and Location (General Cargo vs. Petrochemical) on the abundance of two limpet species (*P. depressa and Siphonaria pectinata*) on the open-rock of rock-armour boulders. Multivariate data consisted of values of density per 625 cm^2 in every sampling time between July 2010 - July 2020 (30 response variables) averaged across the two replicate quadrats of the same boulder (n = 6 cored boulders + 6 control boulders in each location). Tests were based on Euclidian distances, considering two fixed and orthogonal factors with two levels.

Multivariate dataset (30 sampling times; 6 Cored and 6 Control boulders for each location)	*Patella depressa*		*Siphonaria pectinata*	
	Fourth-root transformed data		Untransformed data	
	Pseudo-$F_{1,8}$	P(perm)	Pseudo-$F_{1,8}$	P(perm)
Landscape-unit	4.35	**0.003**	2.38	**0.023**
Location	11.07	**0.0001**	0.99	0.407
Landscape-unit x Location	2.61	**0.036**	1.13	0.313
Pair-wise tests to interaction	Cored > Control in General Cargo; Cored = Control in Petrochemical; General Cargo > Petrochemical for both Control and Cored.			
Pattern using SIMPER. The number of variables exhibiting the pattern in relation to the total number of variables explaining around 70% of the average distance between locations is shown in brackets.			Higher density in Cored than Control (7/7 contributing to 73.37% of average distance)	
PERMDISP p-values	0.123		0.24	

5.4.7 Size structure of limpet populations

Patella ulyssiponensis was very rarely found outside pools: this species was absent from all sampling areas of Control, Cored Away and Cored Around during respectively 28, 25 and 21 of the 30 sampling times of the study period; mean density never exceeded 0.5 individuals in 625 cm^2 (this maximum was registered in Cored Around at General Cargo both in October 2010 and January 2011). In pools of both locations, the size-structure of *P. ulyssiponensis* was clearly dominated by juveniles (MSL < 10 mm, red to yellow), but large adults (MSL > 20 mm, blues) were nearly continuously present over the study period (Figure 5.7). The proportion of large adults of *P. ulyssiponensis* increased considerably after October 2016 (by four times on average, compared with the period before October 2016), when larger-sized (MSL > 30 mm, dark blues) adults of this species started to be consistently present in pools at both locations (Figure 5.7). During this final 3.5 years (after October 2016), the proportions of recruits (MSL ≤ 5 mm, red and orange) of *P. ulyssiponensis* as well as of *P. depressa* were consistently reduced in pools at both locations (by half on average, compared with the period before October 2016), in parallel with an overall decrease in absolute abundances of both patellid species especially in pools of Petrochemical (Figure 5.7).

During most of the study period, the size-structure of *P. depressa* at General Cargo differed between drill-cored pools and the open-rock of cored boulders: dominated by older juveniles (5-10 mm, yellow) and recruits (3-5 mm, orange) in Pool *versus* dominated by adults with shell lengths between 10 and 30 mm (green and light blue) in both Around and Away (Figure 5.7). Larger size-classes of *P. depressa* (MSL > 30 mm, dark blues) were consistently found in Pool, Around and Away at General Cargo in the last 4.5 years of study (invariably present from November 2015 until the end of study) (Figure 5.7). In this final period, the size structure of *P. depressa* became more similar across these three microhabitats (Pool, Around and Away) at General Cargo (namely due to reduced proportions of the 5-10 mm size class inside pools in comparison with previously).

The absolute abundances of *P. depressa* on the open-rock of cored boulders (both Cored Around and Away areas) at Petrochemical remained very low across the whole study period (as no experimental effects were found for this species at this location) (Figure 5.7). This was also the case for the overall absolute abundances of each target species in Control areas of both locations (as these corresponded to unmanipulated boulders) (Figures 5.7 and 5.8). However, after November 2015, *P. depressa* became more frequently found in Control areas of General Cargo with a clear increase in the proportion of large adults (MSL > 20 mm) (Figure 5.7).

The size-structure of *S. pectinata* was generally similar across microhabitats at both locations over the study period, with dominance of the 4-10 mm and 10-20 mm size classes (yellow and green, Figure 5.8). Larger size-classes of *S. pectinata* (MSL > 20 mm, dark blues) were only occasionally present in the open-rock of cored boulders over most of the study period, but became consistently present inside pools in the last 4.5 to five years of study (from November 2015 at General Cargo and from May 2016 at Petrochemical) (Figure 5.8). This was coincident with a decrease in the proportion of recruits (MSL ≤ 4 mm, red and orange) and an overall decrease in absolute abundances of *S. pectinata* in areas Around and Away at both locations, as well as in pools of Petrochemical (Figure 5.8). Presence of egg masses were temporally followed by the subsequent presence of small recruits (MSL < 2 mm) inside pools of General Cargo; however, this was not consistently found inside pools of Petrochemical.

Figure 5.7 Absolute abundance and relative size-structure of patellid limpets (*P. ulyssiponensis* and *P. depressa*) in four microhabitat categories (Pool, Cored Around, Cored Away and Control), on two locations (General Cargo and Petrochemical) over time. *Patella ulyssiponensis* only plotted for the Pool environment (very rarely present in the other environments). NB differences in the y-axis scale between the two species in Pool. Data are the proportion of each size-class relative to the total number of individuals sampled within all sampling areas of each microhabitat category: Pool – inside all drill-cored rock-pools, each pool with 126 cm^2 of surface area), Cored Around (all open-rock areas of 575 cm^2 surrounding pools within cored boulders), Cored Away (all open-rock areas of 625 cm^2 located more than 15 cm away from pools within cored boulders), Control (all open-rock areas of 625 cm^2 within control boulders. Lack of columns corresponds to a sampling time when counts were nil for all sampled surfaces. The vertical red line indicates the initial time of the period from July 2010 to July 2020, when n = 12 pools per location in Pool, n = 24 open-rock areas in Cored Around, Cored Away and Control (sample size was half before that period).

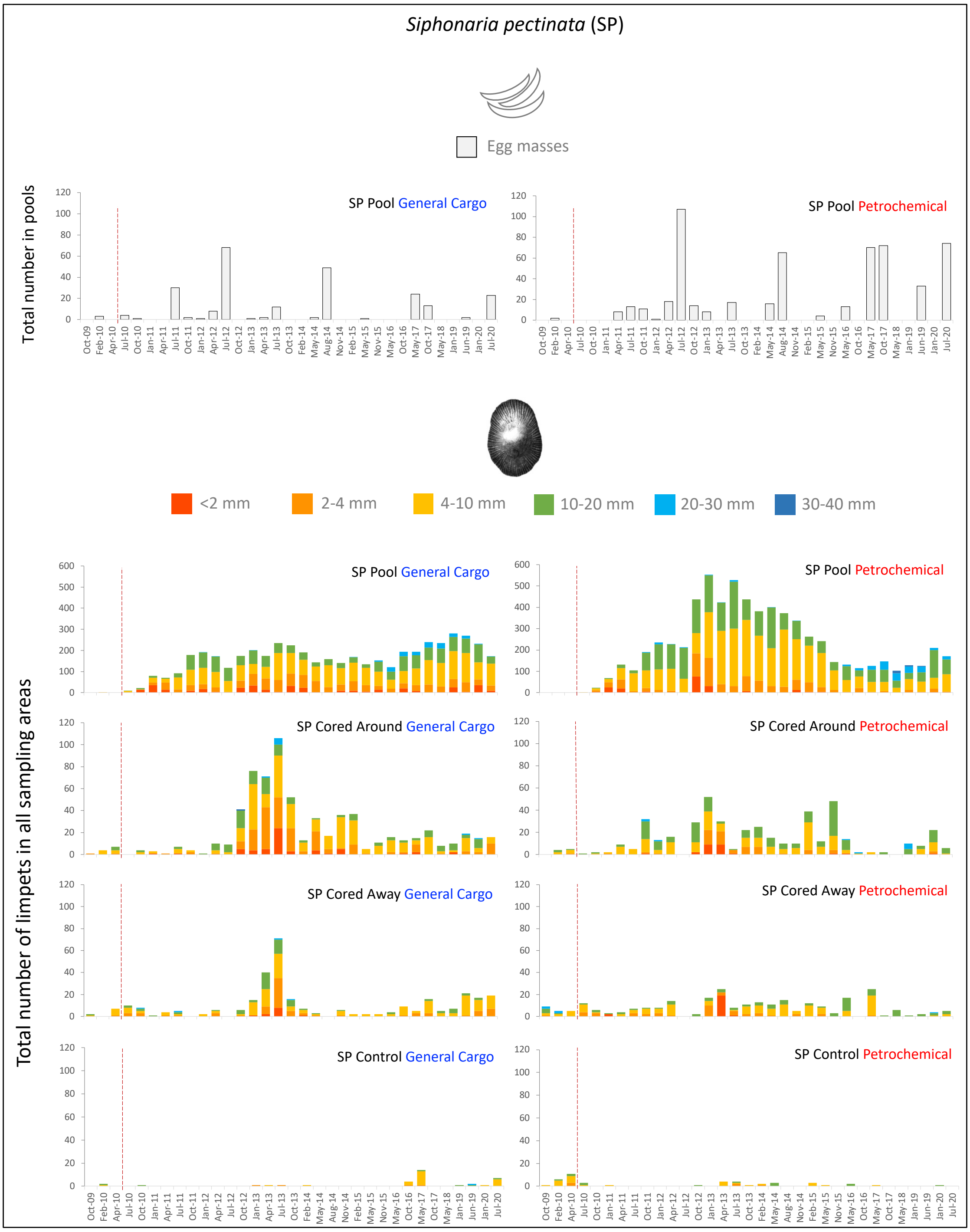


Figure 5.8 Absolute abundance and relative size-structure of *Siphonaria pectinata* in four microhabitat categories (Pool, Cored Around, Cored Away and Control), on two locations (General Cargo and Petrochemical) over time. The total abundance of *S. pectinata* egg masses recorded inside all drill-cored rock-pools of each location over time is also shown (non-stacked beige collumns). NB the following differences in the y-axis scale: Individuals in Pool vs. egg masses in Pool and individuals in the other three environments. Data are the proportion of each size-class relative to the total number of individuals sampled within all sampling areas of each microhabitat category (See legend of Figure 5.7 for information on sampling areas and sample size).

5.5 Discussion

Retrofitting of absent microhabitats, or their incorporation at construction phase, into sea defences or port installations, is increasingly being advocated as a nature-based solution to enhance native biodiversity and deliver ecosystem services (e.g. O'Shaughnessy et al., 2020; Naylor et al., 2023). Overall promising results have been reported, despite some criticisms and knowledge gaps (Firth et al., 2024a). To date, efficacy of coastal eco-engineering has been examined typically over 1-3 years (reviewed by Evans et al., 2021), maximal time-frames being 7 and 7.5 years on intertidal artificial structures (Martins et al., 2016; Ostalé-Valriberas et al., 2024; but see Bender et al., 2020 on subtidal artificial structures). Assessment is usually at the intervention itself (i.e. inside the created microhabitat features) and patch-scale controls (plain surfaces of artificial structures), with immediate surroundings only having been recently considered (5-cm halo: Ostalé-Valriberas et al., 2018, 2024; external surfaces of artificial rock-pools added to vertical seawalls: Bishop et al., 2022; Farrugia-Drakard et al., 2023b, 2024; Bone et al., 2024; Herbert et al., 2025). To the best of our knowledge, our study provided the first decade-long experimental test of the effects of eco-engineered microhabitats on the intertidal landscape. We considered both community succession and population-level responses of co-existing limpet species. Outcomes for limpet population dynamics were assessed within drill-cored rock-pools (126 cm^2) at two locations (Port of Sines, SW Portugal), with emergent outcomes on the abundance of limpets being assessed beyond pool edges at both proximity (surrounding open-rock, 575 cm^2) and landscape-unit (boulder, ~1 m^2) scales.

5.5.1 Summary of main findings

We found that the created water-retaining features: i) likely act as a refuge from local environmental conditions of high temperature and low humidity; ii) were colonized by diverse benthic assemblages; and iii) provided nursery grounds for the three most common limpet species in SW Portugal (*P. ulyssiponensis*, *P. depressa* and *S. pectinata*), whose nursery value spread from the scale of pools to broader scales of rock-armour structures for the two mid-shore species (*P. depressa* and *S. pectinata*). Our general hypothesis of a positive effect of eco-engineered rock-pools on the abundance of limpets in manipulated units of intertidal landscape (rock-armour boulders) was clearly demonstrated. Limpet recruits (sizes corresponding to a maximum post-settlement age of three months – Seabra et al., 2020) and egg ribbons of *S. pectinata* were found within drill-cored rock-pools at both locations throughout the study period, clearly demonstrating their long-term effectiveness as limpet nursery grounds. Besides early life stage usage by the three co-existing limpets, larger individuals (juveniles and adults) of the same species also established inside the created pools. As predicted, the abundance of each target species over time was significantly enhanced on the open-rock of cored boulders in comparison with control (unmanipulated) boulders at the same location. However, in the case of *P. depressa*, this larger-scale (landscape-unit) effect was only found at one location (General Cargo). The smaller-scale (proximity) effect was only found for *S. pectinata* (both locations). Moreover, the population structure of limpets exhibited considerable temporal and spatial variability across habitats. It was noteworthy that the colonization of pools by benthic assemblages contrasted greatly between the two locations, particularly regarding the taxa providing suitable substrata for limpet homes: over time, the cover of CCA was significantly higher in General Cargo than Petrochemical; while the cover of non-coralline crusts showed the opposite spatial pattern.

5.5.2 *Locational context dependency and species-specificity of tested effects*

Our findings are synthesized and interpreted diagrammatically in Figure 5.9. We focused on how differences in the colonization of eco-engineered rock-pools by epibenthic assemblages, namely their cover by CCA, shaped their nursery role for limpets and the consequences for abundance of limpet populations within and beyond rock-pools (see also Seabra et al., 2019, 2020, 2023). In turn, CCA was likely maintained by limpet grazing through a feedback mechanism (see Steneck, 1982).

The effects of pool creation within the pool itself revealed locational context dependency by following two different paths between locations of rock-armour structures (Figure 5.9A). Where pools were CCA-covered (General Cargo), the created microhabitats performed as functioning nursery grounds for the three limpet species and both target species could subsequently emerge to outside-pool areas. Where pools were not covered by CCA (Petrochemical), there was poor nursery functioning for patellids – densities of recruits of *P. ulyssiponensis* and larger individuals of both patellid species were significantly reduced. But there were significantly higher densities of egg masses of siphonariids. Consequently, *S. pectinata* become the dominant species within non-CCA-covered pools and the only one emerging to outside-pool areas.

The experimental effects beyond pools showed distinctive patterns for the two target species, revealing species-specificity as follows. In the case of *P. depressa*, the efficacy of pools was restricted to the location where pools were CCA-covered and acting as functioning nurseries (Cored > Control only in General Cargo) (Figure 5.9A,B). The subsequent distribution of *P. depressa* individuals at this location was even across the whole open-rock top surface of cored boulders (Around = Away, no proximity effect). Therefore, the quality of nurseries was conditional for the landscape-unit effect, which was possibly operated by a cross-habitat migration of older juveniles and young adults of *P. depressa* (from pools to the open-rock of cored boulders, independently of the distance to rock-pool edges) (Figure 5.9B). In the case of *S. pectinata*, efficacy of pools was general across locations (Cored > Control in both General Cargo and Petrochemical). There was also general small-scale variation in density within the open-rock of cored boulders (Around > Away, proximity effect in both locations). Therefore, the overall landscape-unit effect on *S. pectinata* implied that this species was less sensitive to environmental context than *P. depressa*, and the overall proximity effect on *S. pectinata* suggested that siphonariids might be commuting between pools and the surrounding open-rock after their ontogenic emergence from pools (Figure 8B). Species-specificity likely stemmed from differences in traits between the two target species.

There was a significant location effect on the density of juveniles and adults of *P. depressa* within drill-cored rock-pools, but not on the density of its recruits. Post-settlement survival of *P. depressa* was thus likely differential inside pools of the two rock-armour structures, thereby limiting their subsequent emigration to outside pool-areas. CCA cover was the space-occupying category that most contributed for the distinction of pool colonizing assemblages between the two locations in the present study. The cover of CCA inside pools was also previously found to be the most important predictor to explain the variation in size-class densities of *P. depressa* among rock-pools in natural rocky-shores of SW Portugal (Seabra et al., 2023). These authors found that CCA was positively associated with the overall abundance of this species inside rock-pools, which is consistent with results of the present study. CCA was present inside pools of this location since the first sampling time after set-up (February 2010), as well as recruits of *P. depressa* (MSL ≤ 5, benthic life ~three-months according to Seabra et al., 2020) and a small proportion of larger individuals of *P. depressa* (1-2 cm; adults according to Bowman, 1981). Therefore, besides settlement of CCA propagule and *P. depressa* larvae, some immigration of *P. depressa* (possibly derived from the vertical walls of boulders) probably occurred into these pools. Subsequent emergence from drill-cored pools likely occurred after the growth of recruits into adults of *P. depressa*, which then probably established long-term homes on the open-rock across cored boulders. Ontogenic migration from nursery grounds has been inferred for this species by data on the population structure in natural habitats or in settlement plates (e.g. Bowman, 1981; Seabra et al., 2023). Homing behaviour on the open-rock has been widely described for north-Atlantic patellids on natural rocky shores (e.g. Hartnoll and Wright, 1977).

Less locational context dependency was found for *S. pectinata,* both within and beyond pools. Within pools, differences between locations were only significant for the abundance of egg masses over time, but not reflected in differential intensity of recruitment between locations over time. Beyond pools, differences between locations were only significant for the smaller-scale comparison and showed a temporally inconsistent pattern over time. The generality of effects across locations is consistent with previous evidence of low sensitivity of *S. pectinata* to environmental context. High variability of living conditions, regarding abiotic and biotic variables and including human-modified contexts in harbours, has been described for this species in Tunisia (Slama et al., 2021). On the other hand, we found a localized distribution of *S. pectinata* on the open-rock of cored boulders: higher density within areas Around (the 25x25 cm sampling quadrat comprised maximum surrounding distances varying from 8.5 cm to 13.6 cm) than Away (distancing more than 15 cm from pools) on both locations over time. This resembles the spatial patterns of this species in nature, as small-scale variation at horizontal scales has been often found in the abundance of *S. pectinata* within the open-rock of natural shores (e.g. Rubal et al., 2013). However, Seabra et al. (2023) did not find differences in populations of *S. pectinata* between mid-shore open-rock surfaces sampled near (within a pool-surrounding radius of 20 cm) and far (at least 25 cm away) from natural rock-pools in SW Portugal. The difference between the two studies was perhaps due to the scale of the categories considered: larger for near/far of Seabra et al. (2023) in comparison with Around/Away in this study. The proximity effect found in the present study was probably underpinned by movements in and out of drill-cored rock-pools by highly vagrant *S. pectinata* adults, possibly with more flexible homing compared to patellids. Higher motility and lower tenacity of siphonariids compared to patellogastropods has been previously described (Hodgson, 1999). While individuals of *P. depressa* were usually censused while completely inactive, *S. pectinata* individuals (various shell sizes above 5 mm) were occasionally observed actively moving on the open-rock along pool edges during ebb low-tide and entering the pools as the tide receded. Ocaña and Emson (1999a,b) found that *S. pectinata* at Gibraltar forages when the relative humidity of the open-rock substratum exceeded 75 %, also moving away from the home scar to copulate and spawn. A possibly higher humidity of areas Around (surrounding pools) than Away (from pools) and the use of drill-cored rock-pools as preferential areas for depositing egg masses might have been the drivers behind the transitional movements of *S. pectinata* observed in the present study. Egg masses were recorded in pools where *S. pectinata* individuals were not present (e.g. February 2010 in Petrochemical - Figures 5.3 and 5.7). The preferential use of eco-engineered rock-pools as spawning areas for this species seems to be supported by Ostalé-Valriberas et al. (2018, 2024), who found a decreasing gradient in the percentage of *S. pectinata* egg masses inside pools, their 5-cm surrounding halos and control open-rock on rip-raps in Ceuta, both at one and 7.5 years after rock-pool creation. The experimental effects found for *S. pectinata* in the present study were consistent with the opportunistic nature previously proposed by Seabra et al. (2023) for the distribution of this species in SW Portugal.

5.5.3 Locational context – differences between the two locations

There were striking differences according to location in limpet population dynamics of target species within the created rock-pools. These patterns likely reflected contrasting composition of colonizing assemblages influencing the quality of the created rock-pools as limpet nursery grounds (Figure 5.9A). These were unanticipated differences between the two locations. They did not represent replicate sites as assumed at the start of study, despite their short distance apart and their overall similarities in horizontal and vertical extension, size and nature of rock-quarried boulders, age since construction, and composition of mid-shore communities. Within eco-engineered rock-pools deployed on vertical seawalls in Ireland, differences between sites were found in terms of seaweed colonization and productivity (Farrugia-Drakard et al., 2023b) as well as overall diversity and community composition (Farrugia-Drakard et al., 2024). Site-level variation was found despite general consistency of patterns among geographically and environmentally (estuarine/marine and urban/rural) broader contexts (Farrugia-Drakard et al., 2023b, 2024), emphasizing the influence of processes acting at local scales.

The two rock-armour structures of the present study might possibly differ in respect to: i) their baseline biodiversity and proximity to habitats influencing the local species pool; or ii) to key stressors such as wave exposure, aspect/orientation, flow dynamics; or iii) the more extreme minima temperatures registered at Petrochemical (1 °C colder on average compared to General Cargo and the natural shore) could be associated with more abiotically stressful conditions. One of the most plausible explanations for the differences between our study locations is the presence of a natural shore adjacent to General Cargo (Figure 5.1). This natural shore is likely to be a hotspot source for larval and propagule connectivity, given the scarcity of natural areas remaining inside the Port of Sines. On this natural shore, juvenile recruitment of the three limpets is successful in multiple habitats and adults of the two target species are common (Seabra et al., 2019, 2020). Furthermore, an exceptionally dense population of *P. ulyssiponensis* is present in the low-shore steep areas of this natural shore, which are dominated by CCA (Seabra et al., 2019). Comparatively less supply of limpet larvae and/or CCA propagule might arrive at Petrochemical than at General Cargo due to greater distance from breeding populations located at this natural shore. In fact, long-term survey data reveals that a higher abundance of *P. ulyssiponensis* and a more frequent CCA presence occur on the low-shore level at General Cargo compared to a contiguous area of Petrochemical (same rock-armour structure but where access is allowed) (CIEMAR, unpublished data). Distance to rocky shores was also identified as a key factor influencing the composition of both low- and mid-shore benthic assemblages of groynes in North Portugal (Kaffenberger et al., 2024). These authors found that *Patella* spp. greatly contributed to differences in assemblages between groynes located closer to versus further from natural shores, being significantly more abundant on groynes closer to rocky shores on both tidal levels.

Alternatively, Petrochemical might be slightly less wave-exposed than General Cargo (due to a more southwards shoreline orientation and embayment, considering that the dominant wave swell is from the NW). This variation in shoreline orientation might be related with slight differences in solar exposure and thermal environment at each of the two rock-armour structures. The conditions of 1 °C colder minima temperature in Petrochemical could be potentially inhibitory to the post-settlement survival of patellids, as *P. ulyssiponensis* and *P. depressa* are mostly winter breeders in SW Portugal (Castro, 2004) and recently settled patellids are known to be critically sensitive to temperature variations (Bowman, 1981; Bowman and Lewis, 1986). However, mortality events associated with recruitment failure of patellids due to cold stress were described to occur in the British Isles in severe winter conditions (air temperatures below 0 °C, with snow on the rock and ice frost in rock-pools, Bowman and Lewis, 1977) that do not occur in SW Portugal. Therefore, this explanation is unlikely given that the lowest value reported in the present study was a daily averaged temperature of 11 °C (during winter and early spring periods, Table 5.1). Colder temperatures could also have inhibited the establishment/growth of CCA, as this taxon were first detected in pools during winter sampling times. Further studies on the taxonomic identity, dispersal, and growth of CCA are needed in the study region.

Additionally, it is known that any eco-engineering efforts may be hampered by poor water quality (O'Shaughnessy et al., 2020) and the effects of chemical contamination can over-ride the effects of complexity at local scales when the survival of species is inhibited (Mayer-Pinto et al., 2016). Overall historical data on water quality assessments and levels of chemical contaminants in sediments and bioindicator organisms in the region of Sines suggests that pollution within the Port has been mostly confined to the commercial fishery harbour (CIEMAR, 2018). This harbour is closer to Petrochemical than to General Cargo. Therefore, the possibility of a higher exposure to contamination at Petrochemical that could have affected the survival of juvenile patellids in eco-engineered pools deserves further investigation.

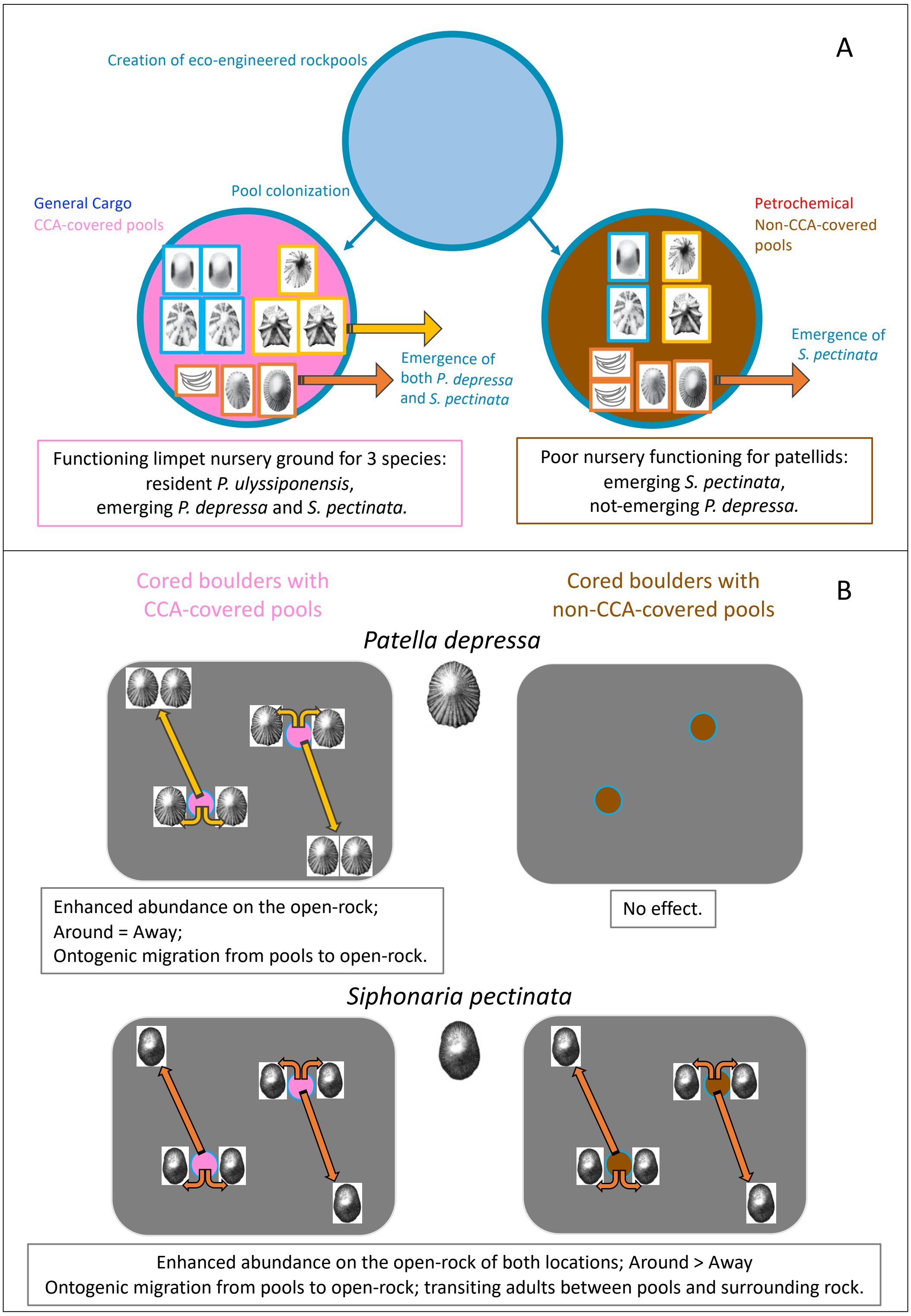


Figure 5.9 Efficacy of eco-engineered rock-pools for enhancing limpet abundance – context-dependency and species-specificity. A: Effects within pools – pools provide nurseries for recruits and juveniles of *P. ulyssiponensis* (blue), *P. depressa* (yellow) and *S. pectinata* (orange, including egg masses); B: Effects beyond pools (on the open-rock of cored boulders) – pools provide source-areas of limpet emigration to adjacent open-rock. Differences between locations in the colonization of pool assemblages (CCA cover) shape the nursery functioning of rock-pools, with consequences spreading across distinct microhabitats; differences in the patterns of the two target species related to their sensitivity to environmental context and life traits (see discussion).

5.5.4 No long-term influence of the timing of intervention

In contrast with the described location-specific effects, there was no-effect of start timing on the amount of living space for limpets nor on their population dynamics. Specifically, pools created in autumn (Start 1) and in spring (Start 2) at the same location developed similar cover of substrata for limpet establishment (bare-rock, CCA, non-coralline crusts) and similar density of limpets over time. This was not surprising considering that these experimental effects were tested over a long cumulative period. The two start timings tested in our study were planned based on the temporal displacement in recruitment of patellids (all year but lowest during summer) and siphonariids (mostly during summer) in SW Portugal (Seabra et al., 2020), predicting that patellids would be the first to settle in pools of Start 1 and siphonariids would be the first settlers in pools of Start 2. The expected seasonal pattern was found in density peaks of recruits of each species (corresponding to settlement/recruitment during the previous three-month period): April/May or July/August for *P. ulyssiponensis* (recruitment during winter and spring); April/May or January for *P. depressa* (recruitment during winter and autumn); October/November or January/February for *S. pectinata* (recruitment during summer and autumn). Despite seasonality, the density of limpets over time did not differ with the timing of rockpool creation. This finding supports the experimental evidence of no-impact of the timing of eco-engineering intervention on biodiversity outcomes, reported by Naylor et al. (2023). These authors found that eco-engineered tiles deployed at beginning versus end of the same season did not differ in their colonisation success after 18 months. We found that the successional sequences of pool assemblages initiated at the same location in the two Starts converged after two and four years in General Cargo and Petrochemical, respectively. The same temporal pattern of convergence has been generally found on artificial structures after disturbance (e.g. Foster et al., 2003; Farrugia-Drakard et al., 2023a). On Irish seawalls, Farrugia-Drakard et al. (2023a) found that the timing of disturbance (clearing times in winter and summer) influenced biofilm abundance up to 3 months but did not affect benthic community composition afterwards.

5.5.5 Functioning of the created pools and the time-course of efficacy

Our drill-cored rock-pools provided cooler microclimates compared to adjacent open-rock on both rock-armour structures, with differences in mean and maximum daily averaged temperature of 1 °C and 7 °C respectively. Chee et al. (2020) observed reductions in mean temperature (compared to open-rock surfaces on coastal revetments) of 1.8 °C and 2 °C driven by drill-cored rock-pools of respectively 5 or 12 cm depth on tropical artificial structures in

Penang, Malaysia. Bishop et al. (2022) found thermal maxima that were up to 10 °C cooler inside rock-pools incorporated in eco-engineered panels (not-specified pool dimensions) than on control surfaces of vertical seawalls in Sydney Harbour, Australia. In this study, maximum daily averaged temperatures reached 40 °C on the open-rock of rock-armour boulders over summer (Table 5.1). This value is close to the lethal temperature of *P. ulyssiponensis* (41.7 °C) and *P. depressa* (43.3 °C) (Evans, 1948), and above the critical thermal maximum (loss of foot attachment after heat exposure) of *P. depressa* (37.45 °C) and *S. pectinata* (32.96 °C) (Vinagre et al., 2019). As expected from comparisons of body temperatures and physiological performance of intertidal organisms between natural microhabitats (Helmuth and Hofmann, 2001), the temperature-buffering effect of drill-cored rock-pools of the present study likely translated in reduced thermal stress for colonizing organisms compared to horizontal open-rock of rock-armour boulders. This microhabitat amelioration was likely important for the recruitment of limpets into pools, and possibly facilitated their colonization by taxa that were otherwise very rare on the rock-armour structures (such as CCA, articulated corallines, sea anemones and urchins).

Maximum daily averaged temperatures were less extreme in the natural shore compared with both rock-armour structures (3 °C colder on average, both inside and outside pools). These differences in upper thermal conditions might explain why the densities of recruits of the three limpet species within drill-cored rock-pools of General Cargo were consistently lower than the ones registered by Seabra et al. (2020) within CCA-covered mid-shore rock-pools of this natural shore (by a factor of 1.3 for *P. ulyssiponensis*, seven for *P. depressa* and two for *S. pectinata*). This lower intensity of recruitment within drill-cored *versus* natural rock-pools coupled with harsher temperatures on the open-rock of rock-armour boulders *versus* natural open-rock likely explain why, the density of *P. depressa* on the open-rock of cored boulders at General Cargo in the end of study (25 ± 6 limpets per m^2, July 2020 - Figure 5.6) was still much lower than the density of this species on this adjacent natural shore (142 ± 6 limpets per m^2 – described in *Study area and sites*).

The landscape-unit effect yielded a similarly enhanced density of *S. pectinata* on both locations over time (July 2010-July 2020: 14 ± 1 limpets per m^2 in General Cargo and 11 ± 1 limpets per m^2 in Petrochemical, mean ± SE on the open-rock of cored boulders of each location) and of the same order of magnitude as the one given for the natural shore (9 ± 3 limpets per m^2 – described in *Study area and sites*). However, by the end of the study the density of *S. pectinata* was higher (four times on average) on the open-rock of cored boulders of General Cargo than of Petrochemical. This was possibly due to the drop in recruitment and absolute abundances of *S.*

pectinata within drill-cored rock-pools of Petrochemical in the last years of study (after November 2015), which was coincident with an increase in the cover of foliose non-green algae. These algae (mostly *C. ustulatus*, growing on top of mussels and the substratum) might have caused knock-on effects on later arrivals of *S. pectinata* larvae. Due to formation of dense patches, *C. ustulatus* was previously found to displace limpets and other macroinvertebrates and to benefit the growth of other intertidal seaweeds (Smith et al., 2014).

Interspecific relationships between limpets and space-occupying organisms during succession were evident within drill-cored rock-pools. CCA cover was likely particularly important for early-life stages and later survival of *P. ulyssiponensis* within pools, given the significant differences in densities of both size groups of this species between pools at the two locations. Residency of this limpet within drill-cored rock-pools was inferred by the larger size-classes present within pools in later sampling times and its rarity on the open-rock of mid-tidal boulders over the study period, in similarity to its microhabitat patterns in nature (Delany et al., 1998; Firth and Crowe, 2008; Seabra et al., 2023). However, as supported by Seabra et al. (2019) and Delany et al. (2002) the *P. ulyssiponensis*-CCA relationship was not obligatory, as recruits and adults of *P. ulyssiponensis* were present in pools at Petrochemical where CCA was absent in many sampling times (and the maximum cover was 2 %). As discussed above, our results on the association of *P. depressa* with CCA cover within drill-cored rock-pools emphasised its role on the survival of juveniles and adults. In the case of *S. pectinata*, we propose that non-coralline crusts might be important to provide an alternative substratum (in addition to rock and in the absence of CCA) for settlement and establishment of this species within rock-pools. Despite nearly absence of CCA within pools at Petrochemical, overall densities of the two size-groups of *S. pectinata* over time were similar compared to pools at General Cargo. The cover of non-coralline crusts (*Ralfsia verrucosa* and *Nemoderma tingitanum*) was the most important predictor of a model of population abundance and structure of *S. pectinata* on the open-rock of natural shores (Seabra et al., 2023).

The overall functioning of drill-cored rock-pools as limpet habitats was possibly promoted by a facilitating role of limpets, by gardening of CCA or non-coralline crusts, thus reinforcing limpet recruitment. Limpets are known to keep CCA surfaces free of overgrowing epiphytes through their grazing activity (Steneck, 1982). This has been described for *Patella ulyssiponensis* (Benedetti-Cecchi, 2000), which likely establishes a specialist mutualism with CCA (Gomes et al., 2021; Firth et al., 2023). Similar evidence of positive associations between siphonariids and *Ralfsia* (non-coralline crusts) have been found (Iwasaki, 1993 *in* Hodgson, 1999). This facilitation might have been possibly stronger in the first years after the intervention, as mid-term peaks in highest density were found for all limpet species within and beyond the created pools. A decrease in total abundance coincident with an increase in the proportion of large-sized individuals was apparent in later years. Therefore, a final inhibiting role by larger-sized limpets due to density-dependence mechanisms might have been present (through bulldozing of juveniles, therefore reducing limpet recruitment towards the end of the time series). The described pattern for the latter years of study could also reflect a limiting carrying capacity of drill-cored rock-pools and open-rock surfaces of rock-armour boulders.

Generally, our results support the findings of Seabra et al. (2023) on: i) the importance of biological predictors (benthic assemblages) in explaining among-rockpool variability in the occurrence of each limpet species; and ii) the importance of the presence of pools for the structure of limpet populations across mosaics of microhabitats connected at emergent landscape scales. Our interpretation of the enhanced limpet abundance on the open-rock of cored boulders was mostly based on the emigration of limpets from the created rock-pools to open-rock driven by the nursery role of pools, although we also reported immigration of adult limpets into the created pools. Other evidence of habitat enhancement on the abundance of limpets has been mostly interpreted as limpet immigration from areas outside the eco-engineered microhabitats to areas adjacent to those microhabitats driven by their humidity halos (Naylor et al., 2017a; MacArthur et al., 2020; Herbert et al., 2025). Martins et al. (2010), however, suggested the contribution of both immigration and increased recruitment. Compared to natural shores, the structural complexity of rock armour structures was found to be particularly deficient at large scales of 2–10 m and at fine 10 mm scales (Lawrence et al., 2021). Our intervention manipulated complexity at cm-scale with effects extended to the ~1-m scale of rock-armour boulders. Further extension of broader landscape-scale effects is possibly limited by the intervals between boulders, which possibly can hinder cross-boulder movement and thereby the connectivity of limpets across larger m-scales. However, a possible spill-over of a few large-sized *P. depressa* individuals from cored to control boulders in General Cargo during the last years of our study was suggested by the temporal variation on the total abundance and size-structure of this species in Cored areas.

5.5.6 Implications for management and lessons learnt

Cross-microhabitat connectivity of populations of mobile species should be considered when planning eco-engineering outcomes in areas beyond the scale of intervention. This approach can be used to enhance abundance of mobile species of commercial and/or conservation importance (see Martins et al., 2013). Enhanced populations of grazers may potentially clear microphytes (e.g. slippery filamentous cyanobacteria) (Skov et al., 2010) or minimize growth of nuisance algae (namely ephemeral greens) (Moschella et al., 2005; Jonsson et al., 2006), thus lessening safety hazards that compromise amenity or recreational value of artificial structures.

Our study highlighted the importance of performing experiments in different locations and the influence of site selection for the success of eco-engineering. It also highlighted the potential role of a natural shore inserted in the middle of a highly artificialized coastal area; we highly recommend the conservation of this natural area of the Port of Sines; its potential functioning as a hub for sources of larvae and propagule and its potential importance for regional biodiversity should be better investigated in the future. The importance of long-term monitoring was also highlighted, as ecological processes may lead to a different end-point that some mid-point during successional processes following the intervention. Species-specific responses to interventions highlighted the importance of identifying spatial-temporal variations in habitat use of target species, namely migration from nursery grounds. Increasing the abundance of CCA (the main habitat-forming species of limpet nurseries), by seeding it within the created rock-pools, could possibly serve to provide additional benefits to our approach. More research is needed to fully understand the biotic interactions described above and illustrated in the diagram of Figure 5.8. Extensive work has been recently published on the role of CCA as a settlement cue for patellids (e.g. Castejón et al., 2021, 2023, 2024). However, the processes conferring benefits of CCA for post-settlement survival of patellids *and S. pectinata* are still to be determined.

Non-destructive *in situ* sampling was employed in this study, as we were interested in the cumulative effects of eco-engineered rock-pools over time. Destructive sampling is usually required to fully document limpet recruitment (e.g. Seabra et al., 2019, 2020). Care was taken to thoroughly scan all sampling surfaces for cryptic small-sized limpets with minimal disturbance to developing habitats; censuses of limpets inside pools (where topographical complexity and limpet abundances are higher) were done by two experienced observers (MIS and CES). Nevertheless, it is possible that density of limpet recruits was underestimated, resulting in conservative estimates of the role of drill-cored rock-pools as limpet nurseries.

5.5.7 Concluding remarks

Based on the ecological role of natural rock-pools for limpets in the study region (Seabra et al., 2019, 2020, 2023), we predicted that the addition of drill-cored rock-pools to otherwise flat and featureless rock-armour units would positively affect the populations of limpets on the mid-shore of rock-armour structures in the Port of Sines. The creation of drill-cored rock-pools successfully enhanced the abundance of both target species, inside and outside the created pools, over a period of ten years. Importantly, the overall outcomes of the created microhabitats were context-dependent and species-specific, showing the influence of site selection and species life-traits on the magnitude and spatial scale of efficacy. We clearly demonstrated that eco-engineered rock-pools can enhance limpet populations over long time and across distinct microhabitats, by providing nurseries and source-areas of limpet emigration to adjacent open-rock. This can be important to target ecosystem services provided by mobile grazers on artificial structures, namely for increasing stocks of species with commercial or conservation importance or to lower cover of slippery algae. Effects of eco-engineering interventions should be assessed at different distances beyond their spatial scales and over longer time-frames to ultimately evaluate success or failure (Evans et al., 2021).

Authorship

MIS, TC, SJH and JJC conceived the ideas and designed methodology; MIS, CES, SC, AS, AC, MM, NM and TS collected the data (field surveys and temperature); MIS and TC analysed the data; MIS was responsible for data visualization and wrote the first draft of the manuscript; AS was involved in data curation and manuscript preparation; TC and SH participated in data interpretation and manuscript writing also providing supervision.

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CHAPTER 6: Overview and synthesis

In this final chapter, I give an overview of the outcomes of this thesis. A cohesive summary of evidence was synthesized in a set of diagrams (Figures 6.1 to 6.5) and gathered into six key findings on the role of rock-pools for limpets in SW Portugal (Section 6.1). This is presented by integrating across individual chapters, drawing together insights from the study of natural rock-pools (Chapters 2, 3, and 4) and the eco-engineered rock-pools explored in Chapter 5. Three cross-cutting themes are then discussed to place my work into the broader ecological context of temperate intertidal reefs (Section 6.2). Firstly, I discuss the link between processes that generate mosaic habitats and demographic connectivity of mobile fauna across the intertidal landscape. Then I discuss the consequences of mosaic microhabitats for the co-existence and niche-partitioning among different limpet species and different size-classes within a species. Lastly, I discuss the implications of this work for conservation and rehabilitation of coastal habitats. Limitations of the present work and needs for further studies are mentioned in each sub-section, with perspectives for future research being summarized before ending with brief concluding remarks.

6.1 Key findings

The biology and ecology of limpets in mainland Portugal have been extensively studied, including patellids (Guerra and Gaundêncio, 1986; Boaventura et al., 2002a, 2002b, 2003; Brazão et al., 2003, 2009; Cabral 2003, 2007; Silva et al., 2003, 2004, 2010; Castro, 2004; Jenkins et al., 2001; Coleman et al., 2006; Cabral and Simões, 2007; Lima et al., 2006, 2007, 2016; Sá-Pinto et al., 2007; Ribeiro et al., 2009; Xavier et al., 2010; Seabra et al., 2011, 2016; Sousa et al. 2012; Borges et al., 2015; Hines et al., 2017; Vasconcelos et al., 2019, 2021; Gomes et al., 2021; Pereira et al., 2022; Freitas et al. 2023; Magnússon et al., 2025) and *Siphonaria pectinata* (Rubal et al., 2013, 2015; Simone and Seabra, 2017). This wealth of knowledge was based on limpets inhabiting open-rock habitats (but see size-frequency data on *Patella ulyssiponensis in* Guerra and Gaundêncio, 1986; see also Hines et al., 2017; Gomes et al., 2021). Most studies focused on adult limpets, but some have also reported data on juveniles (Guerra and Gaudêncio, 1986; Lima et al., 2006; Silva et al., 2003, 2010; Sousa et al. 2012; Rubal et al., 2015; Gomes et al., 2021). My thesis added fundamental knowledge on the ecology of limpets occurring both inside and outside rock-pools in SW Portugal, including the importance of patchy microhabitats for recruitment of different species to various sizes (life stages).

6.1.1 Rock-pools are nurseries for three species of intertidal limpets (Chapters 2, 3, 4, 5)

The most general outcome of this thesis was to clearly demonstrate the importance of rock-pools for the early benthic life of the three most common limpet species in SW Portugal: the two patellids *Patella ulyssiponensis* and *P. depressa* and the siphonariid *S. pectinata*. This was found by studying limpet recruitment across different time periods and shore locations along with the spatial patterns of abundance and size of each limpet species.

Year-round arrival of individuals of the three species with a maximum post-settlement age of three months (recruits: maximum shell length not exceeding 5 mm) was consistently found into shallow CCA-dominated rock-pools on the mid intertidal level of natural shores (quarterly recruitment onto limpet-cleared rock-pools over three years, Chapter 3) (Figure 6.1). Over a ten-year period, an almost continuous presence of recruits of the three species was also found into shallow CCA-colonized rock-pools retrofitted to rock-armour boulders at mid-tidal levels (eco-engineered pools at General Cargo, Chapter 5) (Figure 6.1). Despite their shallow depth, both natural and eco-engineered pools had a lower mean and maximum temperature compared to the surrounding open-rock (Chapter 5). More stable thermal conditions were also found inside than outside these rock-pools (Chapter 5). Therefore, mid-shore rock-pools provide thermally buffered microhabitats that are likely crucial for limpet recruitment, namely to the first field-detectable sizes of the three species (1-2 mm shell sizes, Chapter 3) and possibly for their earlier field-undetectable sizes after-settlement (200-500 µm; Appendix). Benthic early-life stages of both patellids (Bowman, 1981, 1985; Bowman and Lewis, 1977, 1986) and siphonariids (Przeslawski, 2004; Kessel and Phillips, 2018; Rubal et al., 2015) are known to be highly sensitive to temperature variation. As shallow rock-pools lined by CCA are particularly ubiquitous on the mid intertidal zone of the study region, the primacy of these microhabitats as the main nursery grounds of the three species in SW Portugal (*P. ulyssiponensis*, *P. depressa* and *S. pectinata*) has been shown in my thesis.

High density of limpet juveniles (*P. ulyssiponensis* and *P. depressa*: MSL ≤ 10 mm; *S. pectinata*: MSL ≤ 5 mm) was also found in shallow to medium-depth rock-pools on low, mid, or high intertidal levels (Figure 6.1). This was shown by a summertime and a wintertime survey covering a wide range of intertidal microhabitats on three natural rocky shores located along ~9 km of coastline near the city of Sines (Chapters 2 and 3). The overall importance of rock-pools as juvenile habitats for the three most abundant focal species was reinforced when surveying across many (a total of 323) and diverse rockpool systems on six natural shores located along 60 km of coastline in the Southwest Portuguese coast (Chapter 4). Evidence for the same role of rock-pools was not found for the other two studied patellids: one species was restricted to open-rock (*P. rustica*) and the other was scattered across microhabitats inside and outside rock-pools (*Patella vulgata*), both being relatively rarer in the study region (Figure 6.2, Chapter 4). In turn, the following pattern was recognized in common for *P. ulyssiponensis* (PU), *P. depressa* (PD) and *S. pectinata* (SP): the total density of each species was consistently higher inside rock-pools than in the surrounding microhabitats (pool edges and adjacent open-rock), with the great majority of limpets found inside rock-pools being juveniles (~90 % of total PU, > 90 % of total PD, and 74 % of total SP, on average across all shores) (Figure 6.3, Chapter 4). The absolute ratios of mean density of juveniles inside rock-pools (POOL) to mean density of juveniles on surrounding open-rock surfaces (NEAR) were 318 for *P. ulyssiponensis*, 33 for *P. depressa* and 121 for *S. pectinata* (Chapter 4).

Collectively, the documented use of rock-pools by early-life stages of three limpet species in SW Portugal supports the nursery role of these microhabitats for North-east Atlantic limpets, as previously described for two abundant patellids in the British Isles (*P. ulyssiponensis* and *P. vulgata*: Bowman, 1981; Delany et al., 1998; Firth and Crowe, 2008). I found that the recruitment patterns within mid-shore rock-pools (both natural and eco-engineered pools) were, however, distinct for each studied species. The temporal variation in density of limpet recruits diverged mostly between patellids (*P. ulyssiponensis* and *P. depressa*) *versus* siphonariids (Chapters 3 and 5). Recruitment intensity into rock-pools was lowest for patellids and highest for siphonariids during summer periods (Figure 6.1); and more variable among different years for siphonariids than patellids (Chapter 3). These seasonal patterns can be explained by opposite breeding cycles in the study region: highest spawning of patellids in winter with a resting phase in summer (Castro, 2004); highest egg-laying of siphonariids in summer (Chapter 5). The causes for the high inter-annual variation in siphonariid recruitment onto rock-pools warrants further investigation, namely on the possible influence of seawater temperature. An on-going study focused on the temporal and spatial variation of seawater temperature in the coastline around Sines and its relationship with abundance patterns of egg-masses and potentially reproductive adults of *S. pectinata* will provide valuable insights on this subject (Seabra et al., unpublished data). During that on-going study, the hypothesis of rock-pools as microhabitats with higher egg-mass deposition compared to open-rock was supported by the observation of a higher number of egg masses per adult of this species in rock-pools (monthly estimates within both microhabitats on natural shores) (Seabra et al., unpublished data).

Associations between abundance of limpet juveniles inside rock-pools and a set of abiotic and biotic characteristics of rock-pools (habitat-composition and physical predictors) were distinctive for individual species (Figures 6.4 and 6.5, Chapter 4). Density of *P. ulyssiponensis* juveniles inside rock-pools was positively associated with the cover of articulated coralline algae, mussels, and sea urchins, being negatively associated with shore height (Figure 6.5). Juveniles of *P. depressa* inside rock-pools were predicted to be more abundant with higher CCA cover, lower cover of sea urchins and lower pool slope (Figure 6.5). High density of *S. pectinata* juveniles inside rock-pools was associated with higher total density of *P. depressa*, low pool confinement (low walls formed by surrounding rocks), higher shore height, and lower values of pool roundness (lower surface area to higher major axis of the pool) (Figure 6.5). These associations revealed species-specific patterns of distribution exhibited by juvenile limpets among different rock-pools. Lower-shore (and generally deeper) rock-pools colonized by habitat-forming (clumps of erect corallines and/or mussels) or habitat-modifying organisms (sea urchins, that generate burrows bordered by CCA-covered substratum) were mostly used by juveniles of *P. ulyssiponensis*. Conversely, a flatter CCA-covered substratum, characteristic of shallow mid-shore rock-pools without urchins, likely offered the highest habitat suitability as nursery grounds for *P. depressa*. The positive association between density of *S. pectinata* juveniles and total density of *P. depressa* (> 90 % juveniles) inside pools suggests a niche overlap in the distribution of juveniles of both species across microhabitats of rock-pools. Nevertheless, the lower-confined and less-rounded rock-pools on the highest intertidal levels (often coincident with very shallow pools lacking CCA) were particularly occupied by juveniles of *S. pectinata*. These spatial patterns likely reflect different habitat requirements of rock-pools as nurseries for each limpet species. However, the descriptive approach of Chapter 4 limits the interpretation of causal mechanisms underlying recruitment and habitat use of limpets among rock-pools with highly diverse microhabitat features. Manipulative studies are needed to understand the processes behind the distribution patterns identified for juveniles of each species and their associations with rockpool predictors, including direct and indirect effects (i.e., physical factors determining biological habitat context).

Additionally, more studies are needed to explain the presence of juveniles of *P. vulgata*: inside rock-pools on only the two most southern shores, and outside rock-pools mostly on the two steeper shores (Figure S4.1 of Chapter 4). The processes that determine the more general presence of *P. vulgata* (mostly adults) along pool edges (compared to inside rock-pools and surrounding open-rock) should also be investigated. Individuals of this species occupying pool edges in Southwest England have been found to use rock-pools to forage during high tide (Noël et al., 2009). Potentially parallel patterns in the use of rock-pools as foraging grounds in SW Portugal would be especially important in Cabo Sardão, where densities of *P. vulgata* were highest (namely of smaller sizes in microhabitats outside rock-pools) and where the relative availability of open-rock space was found to be least (Figure S4.3 of Chapter 4).

6.1.2 Positive patellid-CCA interactions (Chapters 2, 3, 4, 5)

Although the primacy of shallow CCA-dominated mid-shore rock-pools as limpet nurseries was consistent for the three most common species in the study region, the reliance of the association with CCA sequentially increased from *S. pectinata* (not important) to *P depressa* (important) and *P. ulyssiponensis* (most important) (Figure 6.1).

During surveys focusing on the search for small-sized limpets (MSL ≤ 10 mm defined for patellid juveniles), juveniles of *P. ulyssiponensis* were almost exclusively found in CCA-present microhabitats of natural shores: low-shore steep CCA-dominated open-rock, shallow CCA-dominated rock-pools on mid and high intertidal levels, urchin-inhabited burrows within medium-depth rock-pools on lower shore levels, and vertical north-facing walls (Chapter 2). During the same surveys, the abundance of *P. depressa* juveniles was almost invariably highest in CCA-dominated rock-pools on the mid-shore (Chapter 3). This pattern was found despite a much widespread distribution of juveniles of *P. depressa* on natural shores, including CCA-present and CCA-absent microhabitats: they were commonly found in a diversity of moist microhabitats, particularly mussel patches, crevices on both mid and high intertidal levels, high-shore rock-pools with both CCA-dominated or "bare-rock"-dominated substrata, both north- or south-oriented vertical walls, and beneath *Fucus* patches (Chapter 3).

The cover of CCA was positively associated with density of *P. ulyssiponensis* and *P. depressa* inside rock-pools, and with density of *P. ulyssiponensis* on the open-rock (models of size-class densities of each species within each microhabitat, Chapter 4; Figures 6.4 and 6.5). This was consistent with field-experimental responses of patellids to the creation of eco-engineered rock-pools (Chapter 5). Specifically, population-level effects over time differed between one location where CCA cover increased progressively within the created pools ("pink pools" at General Cargo) *versus* another location where CCA remained nearly absent within the created pools ("brown pools" at Petrochemical) as follows (Chapter 5):

a) Inside eco-engineered pools, recruits of *P. ulyssiponensis* (MSL ≤ 5 mm, sizes compatible to a maximum post-settlement age of three months according to Chapter 3) and larger individuals of both patellids (MSL > 5 mm) were more abundant at the location with CCA-colonized pools ("pink pools" > "brown pools");
b) Beyond eco-engineered pools (on barnacle-dominated open-rock), *Patella ulyssiponensis* was maintained as generally absent, and the abundance of *P. depressa* was enhanced

exclusively at General Cargo (i.e. only “pink pools” provided source areas of individuals to adjacent open-rock).

The described patterns suggested that both *P. ulyssiponensis* and *P. depressa* establish positive limpet-CCA interactions, with possibly different strength and time-course for the two limpets. The interaction of *P. ulyssiponensis* with CCA is possibly established early in ontogenic time (recruits in “pink pools” > “brown pools”, Chapter 5), greatly maintained during juvenile stages (Figure 6.1; Chapters 2) and can persist across the limpet life-span (positive associations established between *P. ulyssiponensis* adults and CCA cover within both rock-pools and open-rock; Figure 6.5; Chapter 4). The interaction of *P. depressa* with CCA is likely established later, mostly affecting the older juveniles and young adults inside pools (overall densities of *P. depressa* recruits were similar in eco-engineered rock-pools with contrasting CCA cover: “pink pools” = “brown pools”, Chapter 5).

Evidence of pre-settlement selection of CCA was not found in laboratory assays with *P. ulyssiponensis* larvae; notably, 51 % of settlers on CCA-encrusted substrata occupied tiny pits devoid of CCA (Chapter 2). Moreover, field evidence supported that CCA is not an obligatory substratum for early benthic life-stages of patellids in SW Portugal:

a) Early settlers of patellids non-identified to species-level (post-settlement age estimated to range from c. 2 to 12 days, M. A. Kendall, pers. comm.) were found in artificial water-retaining collectors lacking CCA (Appendix);

b) *Patella ulyssiponensis* individuals of the smallest size-class considered (MSL < 3 mm, sizes compatible to a maximum post-settlement age of one month according to Delany et al., 1998) were present within non-CCA-colonized “brown pools” (Petrochemical); this was recorded on several sampling dates, in synchrony with some of the recruitment peaks registered in CCA-colonized “pink pools” (General Cargo) (Chapter 5);

c) *Patella ulyssiponensis* juveniles were relatively abundant on mussel patches lacking CCA (Chapter 2). CCA-dominated microhabitats supported higher recruitment, but were not strictly essential for juvenile occurrence, which occurred in some CCA-absent areas (albeit minimally except for mussel patches) (Chapter 2).

Differential survivorship of both patellids with presence/absence or different magnitude in cover of CCA (i.e. a higher post-settlement survival in CCA-dominated microhabitats and/or a higher post-settlement mortality in CCA-absent microhabitats) is the most plausible process responsible for explaining the patterns described here. This process would operate shortly after settlement in the case of *P. ulyssiponensis* (assuming similarly-distributed settlers but higher number of recruits into CCA). The following alternative hypothesis could not be ruled out: settlement onto CCA-absent microhabitats being shortly followed by migration of settlers with field-undetectable sizes (before growing to the smallest field-detectable sizes) to CCA-present microhabitats. The hypothesis of a positive effect of CCA on patellid survival and growth, namely by providing a reliable food resource, needs to be assessed (both within rock-pools and on low-shore open-rock for *P. ulyssiponensis*, inside rock-pools for *P. depressa*).

The role of each of these two patellid species in maintaining, through their grazing, CCA surfaces free from being overgrown should also be tested by manipulative experiments. Both patellids have hard and strongly mineralized teeth typical of Patellogastropod radulae, with the strongest excavating capability in *P. ulyssiponensis* (Hawkins et al., 1989; Ornelas et al., in prep.). This species is possibly the most powerful ecosystem engineer positively influencing CCA cover inside pools (see Firth et al., 2023) and consequently limpet recruitment. Given the altered colour of the foot of *P. depressa* specimens inside pools (more orangish instead of olive green, personal observations), this species is likely to also consume CCA. Both patellids also probably feed on bacterial biofilms associated with CCA; CCA was found to host diverse bacterial communities that are highly variable at small spatial scales (Manea et al., 2025); these biofilms might differ among different rock-pools and contribute to the among-pool variability in the population structure of each limpet species.

Finally, results on larval settlement presented in Chapter 2 were considered as preliminary. More studies are needed to fully determine whether the two patellid-CCA interactions can be also caused by pre-settlement processes. Recent evidence has been gathered on CCA-inducing cues for settlement of patellids in Madeira (e.g. Castejón et al., 2021, 2024); this included *Patella aspera* that is considered a sister species of *P. ulyssiponensis* (Weber and Hawkins, 2005; Gomes et al., 2021), and *Patella ordinaria* that is phylogenetically related with *P. depressa* (Sá-Pinto et al., 2005). Post-larvae of these insular limpets were both responsive to the presence of CCA but showed specific requirements in relation to CCA surfaces (as an optimal settlement-inducing substratum) *versus* CCA-conditioned seawater (as a settlement enhancer for sub-optimal substrata): while settlement of *P. aspera* was higher on CCA surfaces, *P. ordinaria* settled similarly on CCA surfaces compared to other substrata exposed to CCA-conditioned seawater (Castejón et al., 2024). The eventual equivalence of these settlement patterns in continental species should be investigated. The response of *P. depressa* to water-soluble CCA-related cues (without requiring large cover of CCA substratum) could eventually explain the generalist distribution of juveniles of this species (Chapter 3), despite the role of CCA as an optimal substratum for juveniles of this species inside rock-pools (Chapters 3, 4 and 5). Reassessment of settlement patterns of *P. ulyssiponensis* using recent hatchery practices and choice assays would also be important, given the preliminary nature of results in Chapter 2, the specialist nature of the relationship between early benthic life stages of *P.* ulyssiponensis and CCA (Chapters 2 and 5) and the specific requirements of CCA surfaces for the sister species *P. aspera* during settlement (Castejón et al., 2024).

6.1.3 Siphonaria pectinata – opportunistic nature and association with non-coralline crusts (Chapters 3, 4, 5)

The opportunistic nature of *S. pectinata* can be inferred from the distribution of its individuals during early shore life (Chapters 3, 4, 5). The abundance of *S. pectinata* juveniles [defined as MSL ≤ 5 mm, based on the size at first sexual maturity of 5-6 mm assessed by Ocaña and Emson (1999) in Gilbraltar] in CCA-dominated rock-pools on both mid and high intertidal levels of natural shores was, in most cases (five of the six comparisons of three shores and two surveys), not significantly different from the ones recorded on two other microhabitats: mid-shore crevices or bare-rock-dominated high-shore rock-pools (Figure 6.1; Chapter 3). Moreover, similar densities of recruits (MSL ≤ 4 mm), as well as larger (MSL > 4 mm) siphonariids, were found in eco-engineered rock-pools with contrasting colonization by sessile assemblages (no difference in overall recruitment of *S. pectinata* between locations with “pink pools” vs. “brown pools”; Chapter 5). Importantly, in contrast with the experimental effect on the other migratory species (*P. depressa*), the abundance of siphonariids outside pools was consistently enhanced at both locations (i.e. both “pink pools” and “brown pools” provided functional nurseries). Neither CCA, nor any other biological predictor apart from the density of co-occurring *P. depressa*, was found to be associated with the density of *S. pectinata* juveniles in the model built for this species inside rock-pools (Figures 6.4 and 6.5; Chapter 4). All these results suggested that the importance of rock-pools for this species might be mostly due to water-retention rather than any biotic associations with sessile organisms.

Still, the role of non-coralline crustose algae was proposed as an alternative living substratum, in the absence of CCA and in addition to rock, for the establishment of *S. pectinata* within eco-engineered rock-pools (similar cover of “total suitable substrata for limpets” and uncolonized rock in pools of the two locations, but inverse patterns between General Cargo and Petrochemical in the cover of CCA and non-coralline crusts as two components of suitable substrata, Chapter 5). Moreover, a higher number of egg masses of *S. pectinata* over time was found into the “brown” eco-engineered pools at Petrochemical, where cover of non-coralline crustose algae was also significantly higher compared to General Cargo, despite the number of individuals being similar between locations (Chapter 5). An association with non-coralline crusts was not, however, recognized for rock-pools in nature, as the model of size-class densities of *S. pectinata* inside rock-pools did not include the cover of non-coralline crustose algae as a predictor (Figures 6.4 and 6.5; Chapter 4). This might be because natural mid-shore rock-pools with similar composition of assemblages to the eco-engineered rock-pools at Petrochemical (i.e. “brown pools” mostly covered by foliose algae, non-coralline crustose algae and mussels) are not common in the largely wave-exposed study region. Rock-pools with those characteristics can only be found in very sheltered settings in SW Portugal, where *S. pectinata* is usually the most abundant limpet species present and patellids are locally rare (Seabra et al., unpublished data). This is the case of a very wave- sheltered natural shore that lies between the urban beach and the Marina of Sines in an embayed area protected by a series of breakwaters, where shallow “brown” (high cover of *Ralfsia* crusts) natural rock-pools are found on the mid-shore (Seabra et al., unpublished data). The positive influence of wave-shelter generally agrees with the low tenacity of siphonariids (Hodgson, 1999) and has been related to horizontal variation in the abundance of individuals and egg-masses of *S. pectinata* on the open-rock (Ocaña and Emson, 1999; Slama et al., 2021), as well as in the size of individuals and egg-masses of *S. lessonii* inside rock-pools (Zabala et al., 2018). But the eventual link between wave-shelter and cover of non-coralline crusts plus population attributes of *S. pectinata* inside rock-pools was not yet investigated.

In the model built for *S. pectinata* occurrence on the open-rock, the cover of non-coralline crustose algae was the predictor with highest explanatory power, being positively related with overall density of siphonarrids (Figures 6.4 and 6.5; Chapter 4). This supports the existence of a possible interaction between *S. pectinata* and non-coralline crusts within open-rock patches of *Ralfsia verrucosa* and *Nemoderma tingitanum* that are commonly present at the low and lower-mid shore in the study region. A possibly positive *S. pectinata*-non-coralline-crusts interaction might be maintained through grazing in conditions of both rock-pools and open-rock. Given that the radula of *S. pectinata* is composed by small, uniform, non-mineralised, numerous teeth (Simone and Seabra, 2017), the areas covered by soft crust-forming algae might provide suitable feeding grounds for this limpet species. The radular morphology of *S. pectinata* and other siphonariids lacks the excavating properties of patellogastropod radulae, being unable to remove embedded microflora from layers below the rock surface and possibly hard crusts/CCA (Hodgson, 1999). Ocaña and Fa (2003) found that *S. pectinata* in Gilbraltar consumes microalgae, spores, and germlings/juvenile thalli of soft foliose algae that are rasped off the superficial substratum. The foraging ecology of this species was also previously associated with settlement and growth of *Ralfsia* spp. or *Calothrix* spp (non-coralline crustose algae), which were reported to colonize enclosures with the exclusive presence of *S. pectinata* (Ocaña and Fa, 2003 and references therein). Menge (1973) reported that the density and size of *Siphonaria normalis* in the Marshall Islands differed between pools covered by CCA and pools covered by a very slippery non-coralline crust: coralline pools had many small siphonariids and non-coralline pools contained a few large siphonariids; the main possible cause for this pattern was size-selective predation of siphonariids by *Thais* in coralline pools, but a "subtle effect of the substratum or food" was also suggested. Further studies are needed on the factors affecting survival and growth of possibly interacting *S. pectinata* and non-coralline crusts, namely on the grazing and reproductive patterns of this limpet species within rock-pools and open-rock of variable assemblage composition on natural shores.

Siphonaria pectinata is a generalist grazer, ingesting many components of the microbial film, feeding on a variety of items on the rock surface and extracting nutrients from the organic material ingested (Ocaña and Fa, 2003). This enables its "opportunistic" presence in very diverse conditions at different tidal heights, often lacking the presence of other macro-epibenthic organisms. Accordingly, models built for the occurrence of this species inside and outside rock-pools in SW Portugal predicted a higher abundance of *S. pectinata* adults respectively in high-shore rock-pools and on the low-shore open-rock, with conditions of low cover of major space-occupiers in both microhabitats (articulated coralline algae inside pools and other sessile invertebrates on the open-rock) (Figures 6.4 and 6.5; Chapter 4). While these intertidal sessile invertebrates (such as *Sabellaria alveolata* or Vermetidae) often form steep reefs that are thermally benign microhabitats for associated species (e.g. Muller et al., 2021), the subtropical *S. pectinata* is likely resistant to thermal stress in the study region (Rubal et al., 2013, 2015), being more abundant and larger in size in the shore with the flattest profile (i.e. Monte Clérigo, also the most southern shore and the one with the highest proportion of low-confined rock-pools) (Figures 6.4 and 6.5; Figure S4.7, Chapter 4).

Siphonaria pectinata remains as the most enigmatic of the focal species, given its highly variable zonation patterns across microhabitats and shores (Figure 6.3; Chapter 4). This is reflected in the low predictive power of models to explain the structure of its populations inside, along the edge of pools and on the adjacent open-rock (see lowest percentages values of explained variation in Figure 6.5). The ecological processes responsible for local conditions of habitat segregation of *S. pectinata* with patellids, namely sheltered sites or very-shallow/very-low-confined rock-pools on the splash-zone, as well as the occurrence of this siphonariid in environmentally extreme and periodically-disturbed microhabitats (such as sand burial or salinity variation) should be investigated in the future.

6.1.4 *Rock-pools provide habitat for adult limpets and extend the vertical distribution range of limpet species (Chapters 4 and 5)*

Rock-pools in SW Portugal host limpets (*P. ulyssiponensis, P. depressa*, and *S. pectinata*) of various sizes among diverse assemblages (Chapters 4 and 5), extending the vertical distribution of limpet species across the intertidal zone (Chapter 4).

Adult limpets (patellids with MSL > 1 cm and siphonariids with MSL > 0.5 cm) were consistently found inside both natural and eco-engineered rock-pools on all sampled natural shores and sea defences (Chapters 4 and 5). By taking into account their relative abundances across concentric microhabitats of rockpool systems (on average of all shores): i) rock-pools were found to be the main habitats for the adults of *P. ulyssiponensis* and *S. pectinata,* being respectively five and six times more abundant inside rock-pools than on the open-rock; ii) whereas rock-pools were primarily used as nurseries by *P. depressa*, since there were three times more adults of *P. depressa* on the open-rock than inside rock-pools (Figure 6.3; Chapter 4). Additionally, adults of *S. pectinata* might use rock-pools as preferential microhabitats for egg-mass deposition (in alternative, egg masses might have higher survival inside rock-pools) (Chapter 5; Seabra et al., unpublished data).

Processes controlling the differences in habitat-composition predictors are possibly the main drivers of patterns of occurrence of adults of the three species inside rock-pools (Figures 6.4 and 6.5; Chapter 4). Adults of the two patellids were more abundant in rock-pools with high CCA cover and reduced sand cover; siphonariid adults were more abundant in rock-pools with less cover of articulated corallines (Figures 6.4 and 6.5; Chapter 4). Additionally, adults of *P. ulyssiponensis* were more abundant in rock-pools with higher total density of *P. depressa*, whereas adults of *P. depressa* and juveniles of *S. pectinata* were mutually more abundant in pools with higher total density of each other (Figures 6.4 and 6.5; Chapter 4); this suggested mostly positive inter-specific associations of limpets living inside rock-pools. However, the abundance of the largest size-class of *P. depressa* was higher inside rock-pools with lower total density of *P. ulyssiponensis* and less cover of articulated corallines (Figures 6.4 and 6.5; Chapter 4). This suggested a competitive interaction between the two patellids inside rock-pools that is possibly conditional on the size of the largely migratory species (*P. depressa*), and possibly more important in rock-pools located further from the sea/higher on the shore, where adults of *P. depressa* are most abundant and migration of this species to the adjacent open-rock is possibly reduced (due to lack of habitat suitability of high-shore open-rock). The following relationships of adult limpets with physical predictors were found (Figures 6.4 and 6.5; Chapter 4): i) abundance of both *P. depressa* and *S. pectinata* adults inside rock-pools was directly related to the distance of rock-pools to the low water-mark; and ii) the categorical variable of pool confinement (height of surrounding walls) explained the abundance of *S. pectinata* of all shell sizes (associations with several size-classes) within rock-pools and their edges. More studies are needed to understand the possible effect of each predictor on the survival and growth of adult limpets and the role of interactions between different limpet species inside pools.

My approach unequivocally demonstrated that rock-pools extend the vertical distribution of *P. ulyssiponensis, P. depressa*, and *S. pectinata* upwards (Chapter 4). While zonation of each species in the study region was as previously known on the open-rock (*P. ulyssiponensis* on the low-shore, *P. depressa* mainly on the mid-shore, scattered *S. pectinata* with non-consistent vertical pattern across shores), their presence inside rock-pools was found along most of the intertidal gradient, with high abundances at many intervals of shore height on all shores (Chapter 4). The median shore height inside rockpools, in comparison to open-rock, extended upwards in 0.6 m for *P. ulyssiponensis*, 0.2 m for *P. depressa*, and 0.7 m for *S. pectinata* (Figure 6.3; Chapter 4). This extension included both the lower and upper limits of vertical distribution of each species, except for the lower limit of distribution of *P. ulyssiponensis* (Chapter 4), possibly due to equal habitat suitability between rock-pools and open-rock at low shore, as previously suggested by Firth and Crowe (2008). As rock-pools on the mid-shore in SW Portugal provide a more stable thermal environment compared to adjacent open-rock (Chapter 5), they likely act as temperature and desiccation refuges for limpets of all sizes. As microhabitats that ameliorate extreme temperatures and/or desiccation stressors could increase in importance with increasing tidal elevation (Bertness and Callaway, 1994), high-shore rock-pools possibly provide crucial habitat for adult limpets as they became progressively scarce on the high-shore open-rock. Nonetheless, variable salinity on very high-shore rock-pools (raised by evaporation during warm days, lowered by run-off of freshwater from terrestrial sources or precipitation) might act as ecological traps for limpets (see Vinagre et al., 2018). An episodic mortality of adult patellids after prolonged “mushrooming behaviour”, coinciding with lowered salinity and survival of siphonarrids, was reported during summer days in very high-shore “splash pools” in the study region (Seabra et al., unpublished data). Further studies are needed on the ecophysiology of patellids occupying rock-pools and open-rock at different tidal levels. More studies are also needed on the physical-chemical environmental conditions of rock-pools inhabited by limpets at different shore levels in SW Portugal.

6.1.5 *Connectivity of limpet populations occur across distinct microhabitats at horizontal scales (Chapters 3, 4, 5)*

Three limpet species in SW Portugal (*P. ulyssiponensis*, *P. depressa*, and *S. pectinata*) co-occur in connected populations across a landscape of rockpools, pool edges, and open-rock microhabitats; the interchange of individuals across the landscape was inferred from the studied spatial patterns as follows.

a) Immigration into rock-pools (Chapters 3 and 5)

Adult immigrants (all limpets with MSL > 10 mm; their total number was composed by 39 % of *P. ulyssiponensis*, 36 % of *P. depressa*, and 25 % of *S. pectinata*, on average) were found on mid-shore rock-pools that were quarterly limpet-cleared on three natural shores; given their dimension, these individuals could not have arrived into the rock-pools directly from the plankton; their densities were significantly higher on the shore located inside the Port of Sines, where the densities of recruits of the three limpet species were lower; it was thus suggested that adult immigration rates could negatively affect limpet recruitment, either through direct competition or bulldozing (Chapter 3). Limpet immigration was also present in eco-engineered rock-pools, as individuals of *P. ulyssiponensis* and *P. depressa* with MSL > 10 mm were found in pools of the two locations three months after the first starting timing of rockpool creation (Chapter 5). Immigration into the eco-engineered pools was likely derived from the vertical faces of the mid-tidal boulders that were cored with pools (as rock-pools and crevices were not available, the abundance of limpets on horizontal surfaces of mid-tidal boulders was very low, and the interval between adjacent boulders possibly hampers the movement of individual limpets). The origin of immigrants into natural rock-pools could be more diverse (surrounding open-rock, other rock-pools, other microhabitats available on natural shores, perhaps including other tidal levels).

b) Ontogenic emigration from rock-pools to open-rock (Chapters 4 and 5)

Work of Chapter 4 suggested strong intra-specific connectivity at the scale of the rockpool system for the three limpet species based on this common pattern (Figure 6.3): densities of each species consistently decreasing from inside to outside rock-pools, significant positive correlations in the total density of each species between pairs of adjacent microhabitats, and the strength of these correlations lessening with distance from the pool. Also in common for all three species, most individuals within rock-pools were juveniles and most individuals on the open-rock were adults (Chapter 4). These patterns were interpreted as ontogenic emergence of vagrant small-sized individuals of the three species from rock-pools to open-rock areas where large-sized limpets became established. *Patella ulyssiponensis* showed the sharpest gradient in densities and a significant correlation between POOL and NEAR, respectively suggesting large dependency on rock-pools for permanent residence above the low-shore, and a direct spill-over from pools to the surrounding open-rock on the low shore (Figure 6.3; Chapter 4). In contrast, *P. depressa* and *S. pectinata* exhibited a continuum of positive correlations between rock-pools and their edges, and between edges and their surrounding open-rock, denoting a gradual dispersal from pools to open-rock (gradual migration of young juveniles outward from rockpool-nursery areas) (Figure 6.3; Chapter 4). Movement of individuals across pool edges, as intermediate zones of migration from inside to outside rockpools, was thus likely present for the three species, but to a possibly lowest degree for *P. ulyssiponensis* (this was also the only species for which the abundance within EDGE was higher compared to NEAR on all shores, Chapter 4). The pool-edge interface possibly acts as: i) an insurmountable border for *P. ulyssiponensis* above the low shore as a likely non-migratory species at mid and high shore levels; and ii) a potential transition route for the other two species that are likely migratory to open-rock at least on mid shore levels (*P. depressa* and *S. pectinata*) (Chapters 4 and 5). The role of rock-pools as source areas of individuals of *P. depressa* and *S. pectinata* to the surrounding open-rock on mid tidal levels was experimentally supported by work on Chapter 5.

c) Inter-specific influence on connectivity (Figures 6.4 and 6.5; Chapter 4)

Connectivity predictors (density of other limpet species in adjacent microhabitats, named with "_adj_" in Figures 6.4 and 6.5) were included in models of each of the three species within rockpool edges, suggesting possible modulation of population patterns by both facilitative or competitive interactions established between different limpet species within this transition microhabitat. Specifically, interactions between the two patellids explained part of the variation of both *P. ulyssiponensis* and *P. depressa* within pool edges, and interactions between siphonariids and patellids explained part of the variation of *S. pectinata* within pool edges (Figures 6.4 and 6.5; Chapter 4). In fact, for response variables of both gradually migratory species (*P. depressa* and *S. pectinata*) within pool edges, negative relationships of overall/larger size-class densities with density of other limpet species on the open-rock ("_adj_Near"), as well as positive relationships of smaller size-class densities with density of other limpet species inside rock-pools ("_adj_Pool"), were recognized as a common pattern; whereas for largely-resident *P. ulyssiponensis*, only a positive relationship of smaller size-class densities within pool edges with density of *P. depressa* on the open-rock was found (Figures 6.4 and 6.5; Chapter 4). These associations might suggest that: i) the emigrants of the three species belong to smaller-size classes at the timing of migration outward from pools; ii) emigration of *P. depressa* and *S. pectinata* from pools to their edges is motivated by higher abundances of other limpet species inside rock-pools; ii) the larger-sized individuals of *P. depressa* and *S. pectinata* that stay at pool edges are negatively affected by other limpet species on the open-rock; and iii) *P. ulyssiponensis* do not migrate from pool edges to open-rock above the low-shore (where density of *P. depressa* is high). Much work is needed on the influence of the identified connectivity predictors (the possibly interacting limpet species pairs) on the occurrence of each species along pool edges, and for improving our understanding of limpet cross-microhabitat migration.

d) Populations living across the mid-shore open-rock

Population density and size-structure of *P. depressa*, the most abundant species present on the mid-shore open-rock in SW Portugal, did not differ with proximity to rock-pools (MidNEAR = FAR areas in Chapter 4; Cored Around = Cored Away areas in Chapter 5). Therefore, on both natural shores and rock-armour boulders, there was a unique population of this species within the open-rock at this tidal level.

The same spatial pattern was found for *S. pectinata* on natural shores (MidNEAR = FAR areas in Chapter 4), but not on rock-armour boulders of two sea defences where significant differences in the overall density of this species were found over a decade between open-rock surrounding eco-engineered rock-pools and open-rock located further away in the same boulders (Cored Around > Cored Away areas in Chapter 5). The pattern on rock-armour boulders was explained by possible transiting movements of siphonariid adults between rock-pools and open-rock on short-temporal scales, enhancing the density more closely to pools (Chapter 5). The difference in the described patterns of *S. pectinata* between natural and artificial open-rock might be due to the different spatial and temporal scales assessed in the two studies: proximity categories were smaller for Cored Around versus Cored Away than for MidNEAR versus FAR; natural shores sampled during a unique sampling period in Chapter 4 and over 33 sampling dates in Chapter 5. In alternative, this species might need to use rock-pools as temporary refuges on rock-armour boulders, but not on natural shores (as maximum temperatures on the open-rock of rock-armour boulders are much higher than on the natural open-rock, Chapter 5). Further work is needed on the movement patterns of siphonariids across microhabitats in different environmental contexts.

Finally, the nature of rock-armour structures possibly breaks connectivity at larger scales. Connectivity of *P. depressa* and *S. pectinata* populations occurred at landscape-unit scales of costal defences (i.e. within cored boulders, horizontal surface area of ~1 m^2), but the colonization of unmanipulated control boulders by limpets spreading out from cored boulders was either null (in the case of *S. pectinata*) or rare if possible (in the case of *P. depressa*) (Chapter 5).

6.1.6 The influence of geomorphology in creating landscape variability (Chapters 2, 3, 4, 5)

Sampling across several spatial scales and microhabitat types revealed nuances in habitat use patterns among limpet species and among their size-classes or life-stages that were not solely CCA or rockpool dependent, illustrating that a variety of microhabitats and landscape configurations might support essential nursery functions (all chapters).

Three natural shores with different lithology (vulcanic/basalt – "Port", metamorphic rock – Vale Marim, and sedimentary/shale – Oliveirinha) varied in the diversity of microhabitats available (Chapter 2 and 3). Six shores with similar lithology (i.e. all composed of shale) but varying geomorphology (i.e. different orientation of cleavage strata) varied in the availability of shore height levels and in the landscape variability present on the mid intertidal zone (measured as the relative proportion of open-rock surfaces and several patchy microhabitats) (Chapter 4). Differences in the density and size structure of limpets among these six shores were not found for *P. ulyssiponensis* within any of the three concentric microhabitats of rockpool systems; but were found for *S. pectinata* within pool-edges (higher density and larger shell sizes in Monte Clérigo, as described in sub-section 6.1.3) and for *P. depressa* within the surrounding open-rock (higher density and smaller shell sizes in Cabo Sardão; see below) (Figure 6.3; Chapter 4).

I showed that in comparison to the so-called "flatter shores" (characterized by a more platform-like profile and more horizontal cleavage of shale strata: Oliveirinha, Queimado and Monte Clérigo), the so-called "steeper shores" (more up-right cleavage of shale strata: Cabo Sardão and Almograve) are associated with a more broken-up bedrock, and therefore with a higher proportion of patchy and topographically-complex microhabitats and a lower proportion of open-rock on the mid-shore (Chapter 4). These differences in landscape variability were associated with differences in the structure of limpet populations on the mid-shore open-rock, namely with a pattern of higher density and lower shell sizes of *P. depressa* on steeper shores in SW Portugal (Chapter 4). This pattern can be discussed as this species response to:

a) the amount of area available, as shores with less open-rock have less space of the main microhabitat of the adults of *P. depressa* (the mid-shore open-rock), likely resulting in a concentration effect of more individuals per unit of area (and consequently less growth due to intra-specific competition, explaining the lower individual sizes);

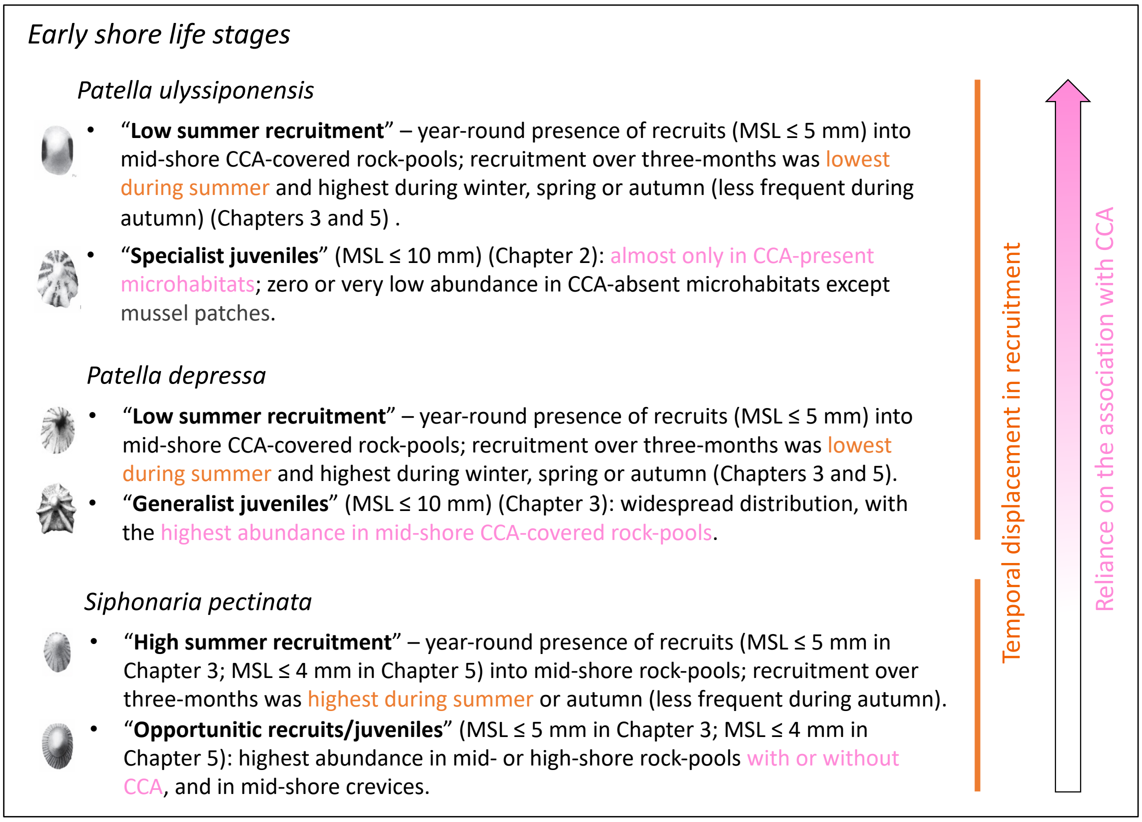
b) higher topographical complexity, promoting higher diversity of limpets (not only *P. depressa*, but also *P. rustica* and *P. vulgata* were more abundant in steeper than flatter shores);

c) higher availability of nurseries or refuges, promoting higher recruitment of limpet juveniles;

d) the importance of the heterogeneity of microhabitats for connectivity across microhabitats, namely the presence of rock-pools, that form mosaic networks resulting in more connected limpet populations across the wider intertidal matrix.

In agreement with this last point, positive effects of the experimental creation of water-retaining features, which provided nurseries and source areas of limpet emigration to the open-rock, were revealed in Chapter 5 by assessing areas within and beyond the created rock-pools in rock-armour structures of the Port of Sines.

Although the rocky coastline in SW Portugal is dominated by shale, localized sandstone stretches are also relatively widespread, generally as a few isolated blocks in the middle of shale rocky shores, but also present as extended platforms or islets (Pedra da Casca, Ilha do Pessegueiro/North of Queimado, Vila Nova de Milfontes, Castelejo). Shores with this lithology in SW Portugal are generally associated with a topographically complex substratum forming a “lunar-like landscape”, with mid-depth rock-pools separated by sharp rocky ridges. Pilot studies on shores with this geological nature showed that: i) both *P. depressa* and *P. rustica* can be very abundant and generally attaining small shell sizes on the open-rock; ii) large-sized *P. ulyssiponensis* are common in mid-shore rock-pools; and iii) large-sized *P. depressa* are common in higher-shore rock-pools (Seabra et al., unpublished data). This thesis did not include information on the spatial patterns of limpet populations living on intertidal shores composed of sandstone and further studies should fill this gap.



Early shore life stages

Patella ulyssiponensis

- “**Low summer recruitment**” – year-round presence of recruits (MSL ≤ 5 mm) into mid-shore CCA-covered rock-pools; recruitment over three-months was lowest during summer and highest during winter, spring or autumn (less frequent during autumn) (Chapters 3 and 5) .
- “**Specialist juveniles**” (MSL ≤ 10 mm) (Chapter 2): almost only in CCA-present microhabitats; zero or very low abundance in CCA-absent microhabitats except mussel patches.

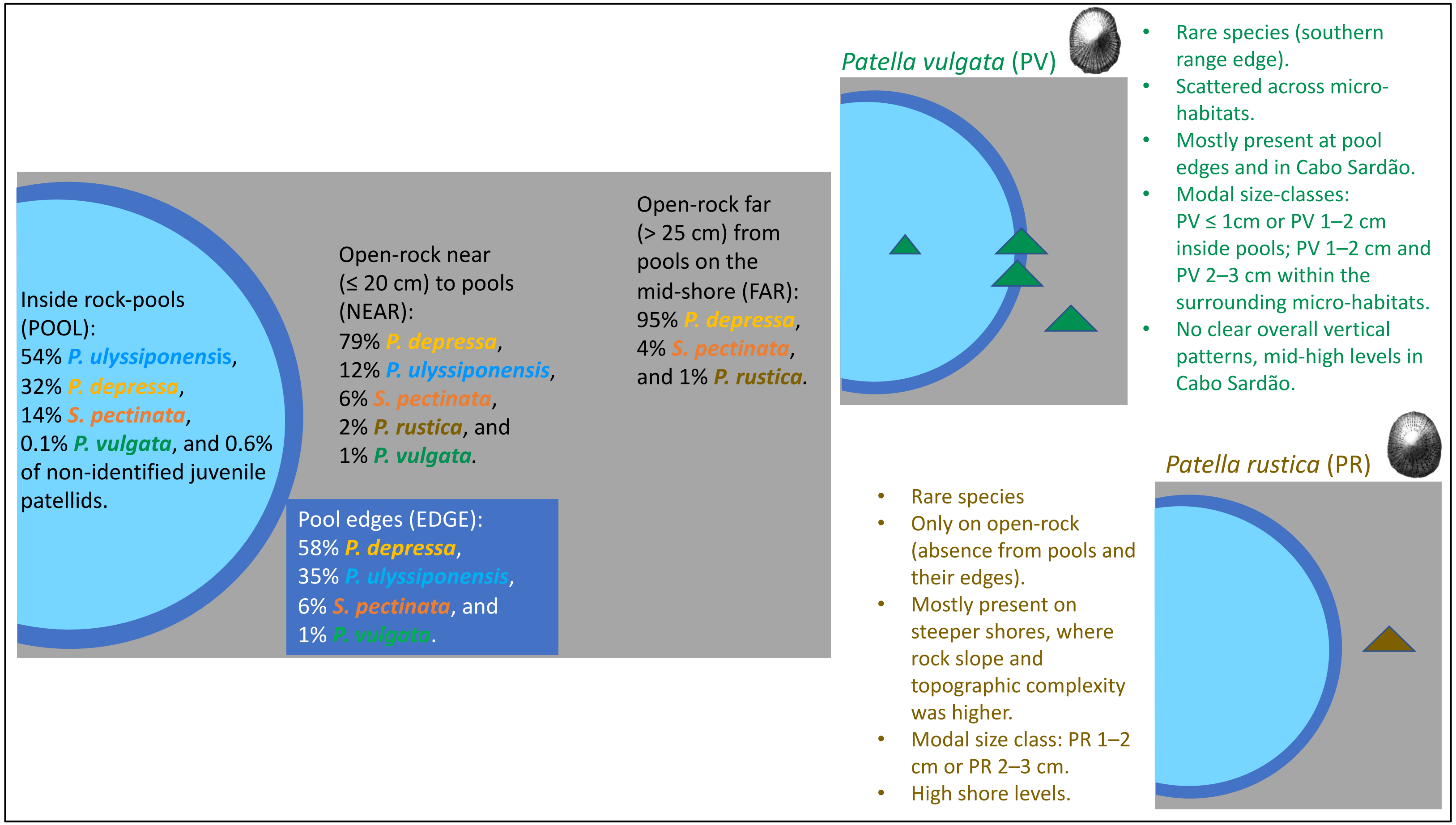
Patella depressa

- “**Low summer recruitment**” – year-round presence of recruits (MSL ≤ 5 mm) into mid-shore CCA-covered rock-pools; recruitment over three-months was lowest during summer and highest during winter, spring or autumn (Chapters 3 and 5).
- “**Generalist juveniles**” (MSL ≤ 10 mm) (Chapter 3): widespread distribution, with the highest abundance in mid-shore CCA-covered rock-pools.

Siphonaria pectinata

- “**High summer recruitment**” – year-round presence of recruits (MSL ≤ 5 mm in Chapter 3; MSL ≤ 4 mm in Chapter 5) into mid-shore rock-pools; recruitment over three-months was highest during summer or autumn (less frequent during autumn).
- “**Opportunitic recruits/juveniles**” (MSL ≤ 5 mm in Chapter 3; MSL ≤ 4 mm in Chapter 5): highest abundance in mid- or high-shore rock-pools with or without CCA, and in mid-shore crevices.

Figure 6.1 Summary of findings regarding recruitment patterns of the three most common limpet species in SW Portugal, including the two patellids *P. ulyssiponensis* and *P. depressa* and the siphonariid *Siphonaria pectinata* (Chapters 2, 3 and 5).



Inside rock-pools (POOL):
54% ***P. ulyssiponensis***,
32% ***P. depressa***,
14% ***S. pectinata***,
0.1% ***P. vulgata***, and 0.6% of non-identified juvenile patellids.

Open-rock near (≤ 20 cm) to pools (NEAR):
79% ***P. depressa***,
12% ***P. ulyssiponensis***,
6% ***S. pectinata***,
2% ***P. rustica***, and
1% ***P. vulgata***.

Open-rock far (> 25 cm) from pools on the mid-shore (FAR):
95% ***P. depressa***,
4% ***S. pectinata***,
and 1% ***P. rustica***.

Pool edges (EDGE):
58% ***P. depressa***,
35% ***P. ulyssiponensis***,
6% ***S. pectinata***, and
1% ***P. vulgata***.

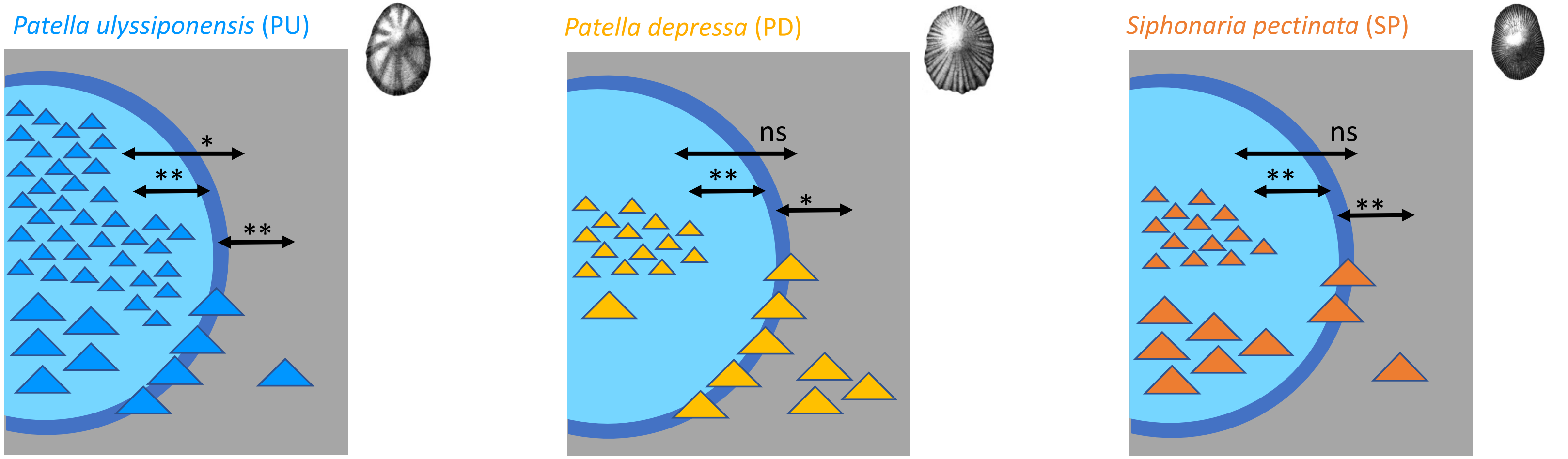
Patella vulgata (PV)

- Rare species (southern range edge).
- Scattered across micro-habitats.
- Mostly present at pool edges and in Cabo Sardão.
- Modal size-classes: PV ≤ 1cm or PV 1–2 cm inside pools; PV 1–2 cm and PV 2–3 cm within the surrounding micro-habitats.
- No clear overall vertical patterns, mid-high levels in Cabo Sardão.

Patella rustica (PR)

- Rare species
- Only on open-rock (absence from pools and their edges).
- Mostly present on steeper shores, where rock slope and topographic complexity was higher.
- Modal size class: PR 1–2 cm or PR 2–3 cm.
- High shore levels.

Figure 6.2 Summary of findings regarding diversity and relative species abundance of limpets within each studied microhabitat, plus major patterns of distribution and size-structure of rare species (Chapter 4). Apart from FAR (only assessed on the mid-shore), the other microhabitats were assessed across the whole intertidal gradient of six shores in SW Portugal (a total of 323 rockpool systems composed by POOL, EDGE and NEAR).



Patella ulyssiponensis (PU)

- Steep gradient in total mean density:
  44 times higher in POOL compared to NEAR,
  > 10 times higher in POOL than in EDGE,
  4 times higher in EDGE than in NEAR,
  null in FAR (mid-shore only).
- Largely resident in rock-pools: 5 times more adults inside than outside rock-pools (POOL > NEAR).
- Significant positive correlations between the 3 pairs of microhabitats, including direct link POOL vs. NEAR.
- Modal size-class: ~ 90% juveniles (PU ≤ 1 cm) in POOL; PU 1–2 cm or PU 2-3 cm in both EGDE and NEAR (PU 1–2 cm on most shores).
- No differences among shores in population density and size-structure within each microhabitat.
- Low-shore open-rock; upper distribution limit is higher inside rock-pools compared to open-rock (median shore height extended upwards in 0.6 m).

Patella depressa (PD)

- Total mean density in POOL: 3 times higher than in EDGE and 5 times higher than in NEAR.
- 3 times more adults outside pools (NEAR > POOL).
- Gradual migration outward from pool-nursery areas: not significant correlation POOL vs. NEAR.
- Modal size-class: > 90% juveniles (PD ≤ 1 cm) in POOL; PD 1–2 cm in NEAR on steeper shores; PD 2–3 cm in EDGE on most shores and in NEAR on flatter shores.
- Similar density and size-structure on mid-shore open-rock near to vs. far from rock-pools (MidNEAR = FAR).
- Relationship between population structure on the mid-shore open-rock and landscape variability: more abundant and smaller-sized limpets on shores with less open-rock and more crevices (steeper shores).
- Most abundant at mid shore levels within every microhabitat; vertical range (both upper and lower distribution limits) extended upwards inside rock-pools compared to open-rock (0.2 m in median shore height).

Siphonaria pectinata (SP)

- Total mean density in POOL: > 8 times higher than in EDGE and 19 times higher than in NEAR.
- 6 times more adults in POOL than in NEAR.
- Not significant correlation POOL vs. NEAR.
- Modal size-class: 74% juveniles (SP ≤ 0.5 cm) in POOL; SP 0.5–1 cm or SP 1–2 cm (or equal proportions of these two size classes) in both EDGE and NEAR; SP 0.5–1 cm in FAR.
- MidNEAR = FAR; and no among-shore differences in density and size-structure on the mid-shore open-rock.
- Higher density and larger-sized limpets around pool edges on the flattest shore with least-confined rock-pools (Monte Clérigo).
- Variable vertical distribution patterns among shores; vertical range largely extended upwards inside rock-pools compared to open-rock (0.7 m in median shore height).

Figure 6.3 Summary of findings regarding patterns of horizontal variation in population density and size-structure, intra-specific connectivity across microhabitats, and vertical distribution for the three most common limpet species in SW Portugal (Chapter 4). Limpets surveyed within microhabitats of rock-pools (POOL), pool edges (EDGE), open-rock near rock-pools (NEAR), and mid-shore open-rock far from rock-pools (FAR) on six shores (a total of 323 rockpool systems composed by POOL, EDGE and NEAR).

Patella ulyssiponensis (PU)

Positively associated with:

- **Articulated coralline algae** (PU juveniles inside pools, PU adults on open-rock);
- **CCA** (PU adults within pools and open-rock);
- **Mussels** and **Sea urchins** (PU juveniles inside pools);
- ***P. depressa*** (PU adults inside pools);
- ***PD_adj_Near*** (PU 0.5-2 cm on pool edges);
- **Sea anemones** (PU juveniles on open-rock).

Negatively associated with:

- **Sand** (PU adults inside pools);
- **Shore height** (PU juveniles inside pools, PU > 3 cm on pool edges; overall PU on open-rock).
- ***P. depressa*** (PU > 3 cm on pool edges);
- **Distance to low-water mark** (PU adults on both pool edges and open-rock);
- **Barnacles** (PU adults on open-rock).

Patella depressa (PD)

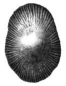
Positively associated with:

- **CCA** (overall PD inside pools);
- **Mussels** (overall PD on open-rock);
- ***S. pectinata*** (PD 1-4 cm adults inside pools);
- **Distance to low-water mark** (PD 2-4 cm inside pools, overall PD on pool edges);
- ***PU_adj_Pool*** (PD 0.5-2 cm on pool edges);
- ***PU_adj_Edge*** (PD 1-2 cm on open-rock).

Negatively associated with:

- **Sea urchins** and **pool Slope** (PD juveniles inside pools);
- ***P. ulyssiponensis*** (PD 4-5 cm inside pools, PD 3-4 cm on pool edges, PD 2-3 cm on open-rock);
- **Articulated coralline algae** (PD 4-5 cm inside pools);
- **Sand** (PD 1-4 cm adults inside pools);
- ***PU_adj_Near*** (overall PD on pool edges);
- **Shore height** (larger PD on both pool edges and open-rock, particularly PD 3-4 cm);
- **Very-low** (PD 0.5-2 cm) **and very-high** (PD 3-4 cm) **pool Confinement** (pool edges);
- **pool Circularity** (PD 0.5-2 cm on pool edges);
- **Verrucariacea** (PD juveniles and PD 1-2 cm on open-rock).

❖ **Shore** (open-rock): PD juveniles and PD 1-2 cm in steeper shores; PD > 2 cm in flatter or intermediate shores.



Siphonaria pectinata (SP)

Positively associated with:

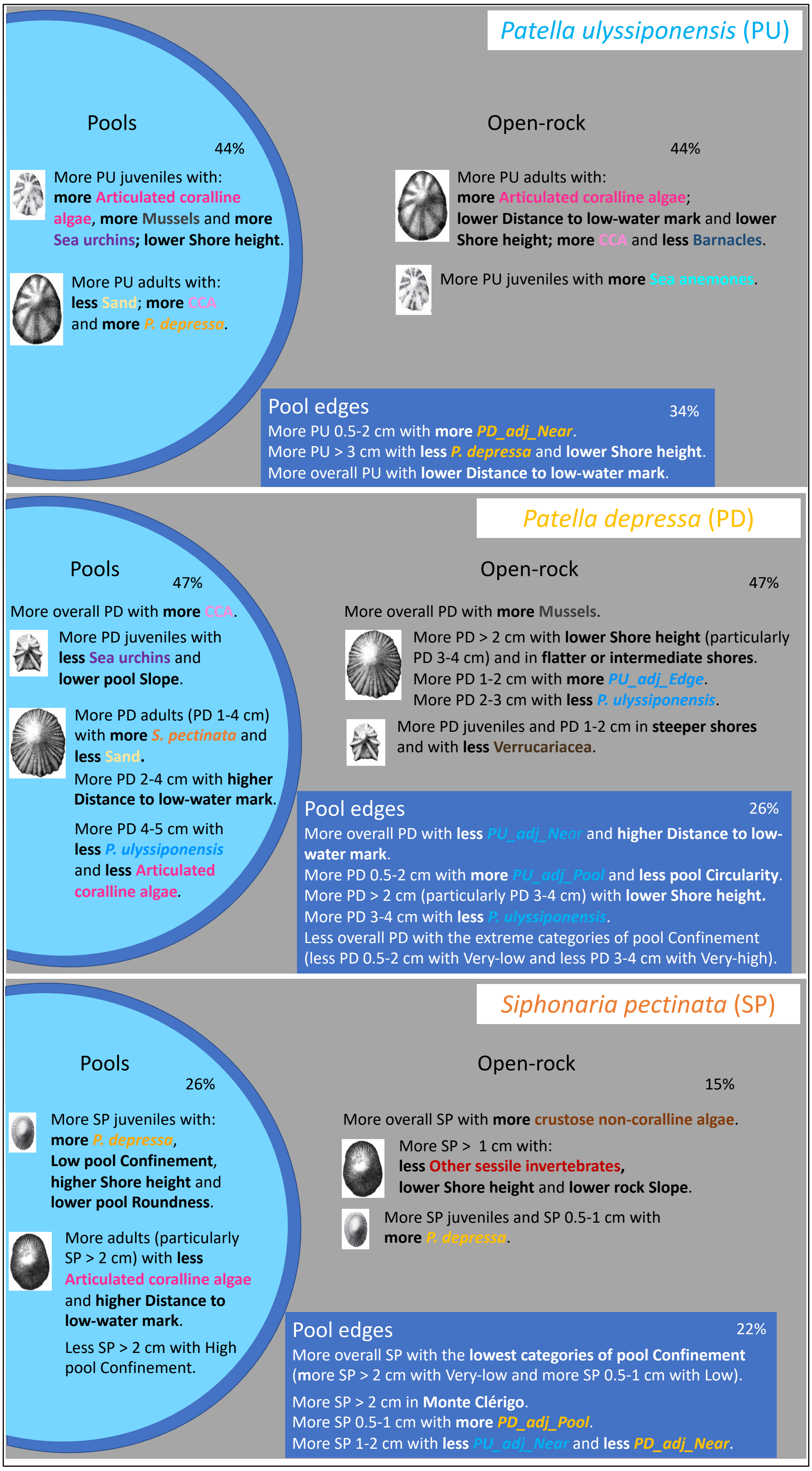
- **Crustose non-coralline algae** (overall SP on open-rock).
- ***P. depressa*** (SP juveniles inside pools, SP juveniles and SP 0.5-1 cm on open-rock);
- **Shore height** (SP juveniles inside pools);
- **Distance to low-water mark** (SP > 2 cm inside pools);
- **pool Confinement Very-low** (SP > 2 cm on edges) **and Low** (SP juveniles inside pools, SP 0.5-1 cm on pool edges);
- ***PD_adj_Pool*** (SP 0.5-1 cm on pool edges).

Negatively associated with:

- **Articulated coralline algae** and **High pool Confinement** (SP > 2 cm inside pools);
- ***PU_adj_Near*** and ***PD_adj_Near*** (SP 1-2 cm on pool edges)
- **Other sessile invertebrates**, **Shore height** and **rock Slope** (SP > 1 cm on open-rock);
- **pool Roundness** (SP juveniles inside pools).

❖ **Shore** (pool edges): SP > 2 cm in Monte Clérigo.

Figure 6.4 Summary of positive and negative associations of three limpet species with predictors (Chapter 4), including habitat-composition variables (cover of major substratum types and sessile taxa, plus density of other limpet species and other molluscan grazers in the same microhabitat), physical variables (metrics describing size, shape, topography, and position of rock-pools or surrounding rock), and connectivity variables (density of other limpet species in adjacent microhabitats; named with "_adj_"). Predictors were selected by distance-based linear models (DistLM) and associations derived from plots of distance-based redundancy analysis (db-RDA). Response variables of each model were size-class densities of an individual limpet species within an individual microhabitat. Limpets were surveyed and predictors were assessed across 323 rockpool systems composed by three concentric microhabitats (inside rock-pools, along the edge of rock-pools, adjacent open-rock).



Patella ulyssiponensis (PU)

Pools 44%

More PU juveniles with: **more** Articulated coralline algae, **more** Mussels and **more** Sea urchins; **lower Shore height**.

More PU adults with: **less** Sand; **more** CCA and **more** *P. depressa*.

Open-rock 44%

More PU adults with: **more** Articulated coralline algae; **lower Distance to low-water mark** and **lower Shore height**; **more** CCA and **less** Barnacles.

More PU juveniles with **more** Sea anemones.

Pool edges 34%

More PU 0.5-2 cm with **more** ***PD_adj_Near***.
More PU > 3 cm with **less** ***P. depressa*** and **lower Shore height**.
More overall PU with **lower Distance to low-water mark**.

Patella depressa (PD)

Pools 47%

More overall PD with **more** CCA.

More PD juveniles with **less** Sea urchins and **lower pool Slope**.

More PD adults (PD 1-4 cm) with **more** *S. pectinata* and **less** Sand.
More PD 2-4 cm with **higher Distance to low-water mark**.

More PD 4-5 cm with **less** *P. ulyssiponensis* and **less** Articulated coralline algae.

Open-rock 47%

More overall PD with **more** Mussels.

More PD > 2 cm with **lower Shore height** (particularly PD 3-4 cm) and in **flatter or intermediate shores**.
More PD 1-2 cm with **more** *PU_adj_Edge*.
More PD 2-3 cm with **less** *P. ulyssiponensis*.

More PD juveniles and PD 1-2 cm in **steeper shores** and with **less** Verrucariacea.

Pool edges 26%

More overall PD with **less** *PU_adj_Near* and **higher Distance to low-water mark**.
More PD 0.5-2 cm with **more** *PU_adj_Pool* and **less pool Circularity**.
More PD > 2 cm (particularly PD 3-4 cm) with **lower Shore height.**
More PD 3-4 cm with **less** *P. ulyssiponensis*.
Less overall PD with the extreme categories of pool Confinement (less PD 0.5-2 cm with Very-low and less PD 3-4 cm with Very-high).

Siphonaria pectinata (SP)

Pools 26%

More SP juveniles with: **more** *P. depressa*, **Low pool Confinement**, **higher Shore height** and **lower pool Roundness**.

More adults (particularly SP > 2 cm) with **less** Articulated coralline algae and **higher Distance to low-water mark**.

Less SP > 2 cm with High pool Confinement.

Open-rock 15%

More overall SP with **more** crustose non-coralline algae.

More SP > 1 cm with: **less** Other sessile invertebrates, **lower Shore height** and **lower rock Slope**.

More SP juveniles and SP 0.5-1 cm with **more** *P. depressa*.

Pool edges 22%

More overall SP with the **lowest categories of pool Confinement** (**more** SP > 2 cm with Very-low and **more** SP 0.5-1 cm with Low).
More SP > 2 cm in **Monte Clérigo**.
More SP 0.5-1 cm with **more** ***PD_adj_Pool***.
More SP 1-2 cm with **less** *PU_adj_Near* and **less** ***PD_adj_Near***.

Figure 6.5 Schematic representation of models built for each species inside rock-pools, along the edge of rock-pools, and on adjacent open-rock (Chapter 4). Percentage values correspond to the proportion of variation explained by each model. DistLM followed by db-RDA were applied (see legend of Figure 6.4).

6.2 Broader contributions to the knowledge of temperate intertidal reef ecology

Three cross-cutting themes were selected to briefly discuss the implications of this thesis in a broader ecological context. This section aimed to provide a more general understanding of the ecological roles of rock-pools in temperate intertidal reefs, emphasizing their importance in demographic connectivity and recruitment processes, with potential applications to conservation issues on coastal habitats and marine biodiversity and to implementation of nature-based solutions in highly modified coastlines.

6.2.1 Patchiness of mosaic habitats and connectivity of populations of mobile fauna

I showed the relationship between mosaic patterns of patchy habitats (namely rock-pools and surrounding habitats) and trait-mediated connectivity in populations of limpets across the intertidal landscape. This link sheds light on potential processes that can affect and maintain the interplay between a given species and the environment.

Geogenic features (lithology and geomorphology) primarily generate complexity on the rocky substratum, which influences spatial variation in abundance or movement of organisms (e.g. Underwood and Chapman, 1990, 1992; Firth et al., 2015; Fairchild et al., 2023). Topographic complexity creates landscape variability, via different proportions of microhabitats and their fragmentation within a given rocky-shore; topography-based niches can meet ecological needs regarding food, water, shelter, and space (Bauer et al., 2024); patchy microhabitats thus represent “potential nurseries” and/or “potential refuges”. Our findings showed that rocky-shores with a common lithology can drastically differ in this physical complexity, and thus on the frequency of occurrence of microhabitats that potentially promote demographic connectivity of mobile intertidal fauna. This is also likely to be important to promote beta-diversity at landscape scales (e.g. O'Shaughnessy et al., 2023), and ecosystem functioning (e.g. Giller et al., 2004; Hawkins, 2004; Crowe, 2005) in open ecosystems, especially considering the role of mosaic habitats as hotspots of biodiversity (e.g. Firth et al., 2013; Firth et al., 2014a,b) and the generalized lack of structural complexity of artificial shorelines (e.g. Lawrence et al., 2021).

Additionally, biogenic features secondarily generate complexity of intertidal substrata, influencing spatial distribution and dispersal of fauna (e.g. Crowe, 1996; Crowe and Underwood, 1999; Buršić et al., 2023). Biogenic complexity can be due to the role of habitat-forming organisms on habitat provisioning, by increasing the three-dimensional structure of the primary substratum (erect algae, sessile gregarious or colonial invertebrates), or by replacing the primary substratum by their two-dimensional growth (such as algal crusts). Habitat-forming organisms often dominate the composition of rockpool communities, their disturbance is frequent, and recovery is slow (Dethier, 1984, 1994; Dethier and Steneck, 2001). On the other hand, biogenic complexity can also be derived from the role of habitat-modifying organisms in changing the structure of assemblages in space and time through their biological activity, namely the roles of both specialist and generalist grazers (e.g. Dethier 1981, 1982; Dethier and Duggins, 1984). Therefore, biogenic complexity enhances landscape variability and has the potential to create "realized nurseries", i.e. effective and functioning microhabitats for early shore life stages and source areas allowing their recruitment to the adult population.

Both CCA and *P. ulyssiponensis* can be individually considered as ecosystem engineers that drive both ecosystem structure and functioning within rock-pools and surroundings. CCA have high productivity and greatly contribute to oceanic carbon cycle (Schubert et al., 2024); CCA increase rugosity of the rocky substratum (from millimetric-scale rugosity of "Lithothamnia" to attached or free-living bioconstruction nodules commonly known as rhodolits or maerl; see Teichert, 2024 for a review); CCA modify benthic assemblages by providing chemical cues for settlement and metamorphosis of a plethora of marine invertebrates and influencing algal spore settlement (references in Chapter 2; Twist et al., 2023); and CCA form diverse and life-long positive biotic interactions with associated taxa and epibiontic assemblages (Steneck, 1982; Piazzi et al., 2022; Bulleri et al., 2024). Our findings suggested the role of CCA in limpet recruitment, emphasizing survivorship rather than settlement cues. CCA might be likely critical for post-settlement survival of marine invertebrates, by providing a food source for grazers as well as structural complexity, reducing predation or environmental stress (see Bulleri et al., 2024 for a review). Both habitat-forming (through provision of associational refuges on their shells) and habitat-modifying (through grazing/bulldozing and bioerosion) roles of *P. ulyssiponensis* as a powerful ecosystem-engineer have been described on both open-rock and rock-pools (Firth et al., 2023 and references therein). The likely positive feedback mechanisms of *P. ulyssiponensis* on CCA and the likely negative density-dependent effects of this species on other limpets should be assessed experimentally within rock-pools and along its distribution range.

I argue that the habitat templates marked by the patchiness of rockpools influence the connectivity of limpet populations across distinct microhabitats in the intertidal landscape, thereby possibly influencing the structure of whole intertidal communities through trophic and facilitation cascades mediated by limpets. Thus, intertidal landscapes with higher geogenic and biogenic complexity are characterized by higher patchiness and likely enable higher demographic connectivity of mobile fauna and biodiversity benefits.

6.2.2 Co-existence

Here I focus on the consequences of the habitat template for the coexistence of different limpet species and sizes. Rock-pools in SW Portugal were used by both early life stages and adults of the three most common species (*P. ulyssiponensis, P. depressa*, and *S. pectinata*); their use by juveniles of *P. vulgata* was only found on a few shores, but adults of *P. vulgata* occurred relatively frequently along pool edges; neither rock-pools nor their edges were used by any size class of *P. rustica* (Figures 6.1 and 6.2). The three most common limpet species shared most of the rocky-intertidal space in the study region, given their co-occurrence along a large part of the vertical gradient of intertidal height inside rock-pools on all sampled shores. Across concentric rockpool systems: *P. ulyssiponensis* was the dominant species inside rock-pools; *P. depressa* was dominant within pool edges, open-rock surrounding rock-pools and mid-shore open-rock further away from rock-pools; *S. pectinata* was the third most abundant species within all of these microhabitats (Figures 6.2 and 6.3). Coexistence of these limpet species in the studied region is likely mainly driven by: i) microhabitat partitioning with spatial segregation and ii) temporal niche displacement.

Microhabitat partitioning of species and sizes was found when looking at spatial patterns of each limpet species, mostly relative to variability in the assemblage composition of rockpool systems or other microhabitats present on the intertidal landscape (sub-sections 6.1.2 and 6.1.3). Partial segregation among species (with some spatial niche overlap) was shown both during early benthic life and as adults (sub-sections 6.1.1 and 6.1.4), with differential occupancy of each species along small-scale horizontal gradients and edge transitions, while also following broader vertical and across-shore distribution patterns (Figure 6.3). Evidence of microhabitat partitioning between recruits and adults of each limpet species was found both inside and outside rock-pools (Figure 6.5). Age/size spatial segregation is likely driven by the patchy distribution of early benthic stages and nurseries areas, ontogenic differences in environmental tolerance or in biotic associations, ontogenic migration of older juveniles vs. home fidelity of adults, other processes driving intra-specific connectivity and individual movements, such as habitat suitability of surrounding microhabitats and possible inter-specific interactions between different limpet species within each microhabitat and between adjacent habitats (sub-sections 6.1.5 and 6.1.6). Similar patterns and their underlying processes causing differentiation between distribution of juveniles and adults (e.g. Branch 1975a,b) and among a much higher diversity of species (e.g. Branch, 1971; 1975c; 1976; Branch and Newell, 1978; Branch and Marsh, 1978) have been extensively studied for limpets on South-African shores.

According to models built in Chapter 4, adults of *P. ulyssiponensis* are generally more abundant inside rock-pools where the total density of *P. depressa* (composed by more than 90% of juveniles) is also higher (Figures 6.4 and 6.5). This might be due to a positive effect of the largely resident species (*P. ulyssiponensis*) on the overall densities of patellid juveniles by enhancing the cover of CCA through grazing. Their use of rock-pools at different time-frames (permanent lifetime for *P. ulyssiponensis* above the low intertidal level vs. mostly temporary for *P. depressa* that ontogenically shift to the open-rock at mid-shore) likely contributed to limit competition between these two congeneric species. However, the densities of large size classes of *P. depressa* within the three concentric microhabitats are consistently inversely related with the total density of *P. ulyssiponensis* in the same microhabitat; additionally, higher densities of largest shell sizes of *P. depressa* were predicted within pool-edges and open-rock located at lower shore heights, where the cooccurrence with adults of *P. ulyssiponensis* is more likely (Figures 6.4 and 6.5). Competition between the two most abundant patellids was recently revealed to set the upper limit of vertical distribution of *P. ulyssiponensis* on the open-rock in SW Portugal (Ornelas et al., in prep.). In that recent study, overall negative effects of the presence of naturally occurring adults of *P. depressa* on adults of *P. ulyssiponensis* transplanted above their natural zone of occurrence (from the low-shore open-rock to caged areas on the mid-shore open-rock) were found: the survival, growth rate and grazing intensity of *P. ulyssiponensis* were significantly reduced in the presence of *P. depressa* compared to controls. During the same experiment, independently from the described effects of interspecific competition, positive effects of habitat amelioration (artificially-enhanced moisture of the rocky substratum) were significantly found on growth and grazing intensity of *P. ulyssiponensis*. The findings of Ornelas et al. (in prep.) regarding interspecific competition might apply throughout the Atlantic rocky-shores of South-Western Europe, where *P. depressa* is the most abundant species on the mid-shore open-rock. The dominance of *P. depressa* in SW Portugal is progressively replaced by *P. vulgata* further north, with *P. depressa* being absent in Ireland (e.g. Coleman et al., 2006; Firth et al., 2009); interspecific competition between *P. depressa* and *P. vulgata* on the open-rock was previously found to be fairly symmetrical and weaker in comparison to intra-specific competition (Boaventura et al., 2002b). The reported effects of Ornelas et al. (in prep.) on the habitat amelioration by moisture enhancement on the open-rock substratum are also expected to be weaker in higher latitudes of the biogeographical distribution range of *P. ulyssiponensis*, where intertidal physical harshness and desiccation stress should be comparatively lower than in SW Portugal. The hypothesis of competition between *P. ulyssiponensis* and *P. depressa* inside rock-pools remains to be tested; we predict that effects of this process would be stronger in CCA-covered rock-pools located on higher intertidal levels (more distant to low-water mark) where the larger sizes of *P. depressa* are more abundant (Figure 6.5). Testing this hypothesis would be also relevant considering that: Firth et al. (2009) found a negative effect of *P. depressa* on the growth of *P. vulgata* only when these two species were caged in rock-pools together with *P. ulyssiponensis* (effects on *P. ulyssiponensis* were not studied); and that Firth and Crowe (2010) found no evidence of interspecific competition between *P. ulyssiponensis and P. vulgata* either in rock-pools or open-rock, consistently in both short-term dispersal (days) and long-term cage-enclosure (months) experiments.

Associations between siphonariids and patellids identified in the models of their occurrence within rock-pools and open-rock were all positive: juveniles of *S. pectinata* inside and outside rock-pools were directly associated with the total density of *P. depressa* in the same microhabitats, adults of *P. depressa* inside rock-pools were directly associated with the total density of *S. pectinata* in the same microhabitats (~70% composed by juveniles) (Figures 6.4 and 6.5, Chapter 4). Moreover, juveniles of both species cooccurred in humid microhabitats in the studied region (Chapter 3). However, negative associations were found in the model of *S. pectinata* within pool edges with the total density of the two patellids on the adjacent open-rock, and *P. depressa* is usually absent in microhabitats where adults of *S. pectinata* are particularly abundant (as mid-shore open-rock at sheltered places, very-shallow splash pools). The relative importance of differential habitat suitability and/or potential competition between adults of *S. pectinata* and patellid species has not been assessed. This is important in the context of climate change, given the recent spread of the geographic limits of *S. pectinata* to the north in the Atlantic and to the east in the Mediterranean coasts (Rubal et al., 2015 and references therein). Any potential competitive interactions between *S. pectinata* and patellids would most probably be for space and not for food, given their known differences in radular morphology (e.g. Simone and Seabra, 2017). Temporal displacement of recruitment between patellids (all year except during summer) and siphonariids (mostly during summer) in SW Portugal, caused by seasonality of reproductive cycles, likely reduced direct competition in rock-pools and small space-limiting microhabitats in this region. Seasonal partitioning in population dynamics, as well as different radular morphology and possibly differential homing behaviour of adults (more rigid in patellids *versus* more flexible in siphonariids) might thus be important to facilitate coexistence of *S. pectinata* with patellid species across its currently extending range.

6.2.3 Implications for conservation and management

Based on the work of this thesis, the conservation of scarce natural areas remaining inside the Port of Sines is highly recommended. This particularly applies to the natural shore (37°56'39.45"N, 8°51'24.43"W) designated as "Port" in Seabra et al. (2019) and Seabra et al. (2020) (Chapters 2 and 3, respectively), which is adjacent to the rock-armour structure designated by General Cargo in Chapter 5. According to recent plans for the Port's expansion, this natural shore will likely be replaced by artificial infrastructure in the coming years. Despite its small extent (nearly 50 meters of straight coastline) of this natural shore, I consider that there is a pressing need for its conservation, based on several findings that highlighted its ecological importance and uniqueness. This is one of the two natural shores remaining inside the Port of Sines. Its geological nature and geomorphology (formed by steeply sloping basaltic massifs separated by surge channels) contrasts with the shale-dominated shores of SW Portugal, located to the south from the Port. The intertidal communities present on this shore differ drastically from the other natural shore that remains inside the Port (which is extremely wave-sheltered) and from the coastline of the Cape of Sines located to the north from the Port (which is more extremely wave-exposed), despite the similar geological nature of these locations. Given its insertion in the middle of the highly artificialized coastal area of Sines, it is probably a hub for larval and propagule connectivity inside the Port of Sines. Distance to rocky shores is a key factor influencing the composition of benthic assemblages of groynes in North Portugal (Kaffenberger et al., 2024) and the colonization of isolated sea defences elsewhere (Dethier et al., 2010). This is relevant considering the low abundance of mid-shore limpets in artificial structures of this Port, and that patellid adults are abundant in this natural shore, where juvenile recruitment of the three most common limpet species in SW Portugal is also successful (Seabra et al., 2019, 2020).

Another argument to support the special status of this natural shore is that an exceptionally dense population of *P. ulyssiponensis* is present within its low-shore steep areas, which are dominated by CCA (Seabra et al., 2019). Similar densities of limpets were not found in any other sampled shore in the study region, where presence of CCA in low-shore areas is usually spatially confined to patches amid foliose or turf-forming algae or in gaps among beds of locally abundant sessile invertebrate species (stalked barnacles, mussels, reefs of honeycomb worms or Vermetidae). Additionally, in the low-shore areas of this natural shore adjacent to General Cargo, many shells of *P. ulyssiponensis* adults are completely overgrown by rugose forms of CCA and host many juvenile limpets; previous surveys have found that these epibionts are mostly conspecifics, forming conspicuous home scars (mother-of-pearl/silver marks) upon the shells of large-sized *P. ulyssiponensis*, in comparatively higher numbers (and with lower median sizes, suggesting higher recruitment rates) than in similarly-sized individuals of *P. ulyssiponensis* living in the low-shore open-rock of other nearby natural shores (Seabra et al., unpublished data).

I highly recommend the conservation of this natural area of the Port of Sines, based on its potential functioning as a hub of source populations of limpets and other species, and its potential importance for regional biodiversity.

The findings of Chapter 5 support a huge body of knowledge demonstrating that the incorporation of missing habitats to the homogenous surfaces of coastal defences has the potential to improve ecological outcomes. The implementation of such nature-based solutions should be encouraged by stakeholders, supportive policies, and integrative legislation (Firth et al., 2024 and references therein; Dodds et al., 2025). It also reinforced the premise that local ecological knowledge is fundamental to implement successful interventions. The evaluation of effects of eco-engineering interventions beyond the "feature" itself at relevant landscape-unit spatial scales, as well as the long-term monitoring were novel in this thesis. Drill-cored rock-pools enhanced limpet recruitment, and colonization by diverse assemblages within their water-retaining edges, confirming their role as effective nursery and biodiversity-enhancement hubs over a decade. Site-specific factors, including biotic interactions and possible differences in environmental conditions, namely proximity to natural shores, mediated the locational context-dependency of the success of the intervention. Species-specific responses of limpets highlighted the importance of tailoring eco-engineering designs to target species ecological requirements and taking in consideration ontogenic migration of mobile fauna. Importantly, the creation of rock-pools increased limpet densities on adjacent open-rock habitats, demonstrating broader cross-habitat benefits beyond the interventional areas.

Extra limpet density can convey important ecosystem services by their grazing on intertidal artificial structures (Jonsson et al., 2006): lower cover of slippery green algae makes access easier for recreational activities such as swimming, snorkelling, or fishing. The recreational and educational use of marine artificial structures might be very important in several world regions where local human populations do not have other nearby access to the sea. Although this is not the case of the study region (where there is an urban beach within the limits of the Port of Sines and natural coastlines located within short distances both to the north and the south from the Port), the conservation of the remaining natural coastline and the application of eco-engineering practices should be promoted on sea defences within this Port as coastal sustainability and rehabilitation measures in the highly modified seascape.

Given the inseparable relationship between humans and the sea in SW Portugal, the planning and implementation of nature-based solutions in marine infrastructure of the Port of Sines would be critical for increasing local environmental stewardship, decision-making on coastal management and marine resources, and ocean literacy. The possibility of extended ecological benefits of eco-engineered rock-pools beyond their boundaries will hopefully advance our knowledge on ways to promote biodiversity at whole landscape scales, particularly at the intertidal ecosystem-level of urbanized or industrialized coastal environments. Despite site-specific variability of effects reported in Chapter 5, the long-term stability of ecological benefits reinforced the value of drill-cored rock-pools as a scalable tool for biodiversity enhancement.

Rock-pools were found to play a crucial role in the spatial patterns and dynamics of patellid populations, and three species (*P. ulyssiponensis*, *P. depressa*, *P. vulgata*) are harvested for direct consumption and use as bait in SW Portugal. This might be relevant for regional monitoring assessments of harvested stocks in intertidal systems, namely those designed for evaluating the effectiveness of protection within particular special areas of the marine park, where fishing is totally or partially forbidden and other human activities are regulated (Castro et al., 2015; Castro et al., 2021). These special protection areas were established since 2011 in the context of the last review of the Management Plan of the marine park, and presently occupy less than 10% of the marine area of the park (Monteiro et al. 2020). If new areas are to be selected for protection in the future, they should have high heterogeneity of microhabitats (landscape variability) to contain high biodiversity, nursery grounds and the presence of habitat-forming species including CCA in addition to canopy algae and kelps. Of all studied limpet species, *P. ulyssiponensis* is the most intensively exploited (Castro, 2004) and is likely to be most sensitive to exploitation. It is more dependent on rock-pools, CCA-dominated and/or low-shore habitats (this thesis). It is also protandrous and at risk of larger females being over exploited as happened with its sister species *P.aspera* in the Azores (e.g. Hawkins et al., 2000). Harvesting likely causes a direct reduction on the abundance of the larger size-classes of patellids (minimum catch size is 2 cm of MSL in mainland Portugal), contributing to the small-scale variability detected for each limpet species and overall assemblages within each microhabitat.

Given the cascading effects promoted by *P. ulyssiponensis* as a powerful ecosystem engineer (e.g. Firth et al., 2023), this species should be the one where conservation efforts need to be primarily focused. It is known that harvesting of *P. ulyssiponensis* in this region can cause direct and indirect persistent impacts on limpet populations (abundance, size-structure, and size of grazed cleared areas) and biotic communities on the open-rock (Castro, 2004), but effects of limpet harvesting have not been studied for microhabitats inside rockpools and their edges in this coast. Studies on other coastal regions where limpets are exploited (e.g. the Azores; Martins et al., 2008, 2010a,b, 2011, 2016, 2017) can be informative for the sustainability of exploitation of these resources in SW Portugal, namely on the effectiveness of protection by specific regulations and for preventing the risks of overexploitation. Greater focus should be placed on the contribution of rock-pools to limpet populations, in the face of human disturbances like harvesting, as part of broader biodiversity monitoring and conservation efforts for temperate reefs.

6.3 Future work

Despite the contribution of this thesis to a better understanding of limpet populations in different intertidal microhabitats of temperate reefs, many relevant questions remain to be answered, several of them were generated by the work of this thesis and referred as limitations or knowledge gaps in sections 6.1 and 6.2.

Well-established protocols are currently developed for rearing patellids with high survival rates, from earlier developmental stages to millimetric specimens with well-developed teleoconch, both in the context of aquaculture (e.g. Castejón et al., 2022) or for controlled reproduction of endangered species for population restocking or reintroduction actions on natural shores (Ferranti et al., 2018, 2021). Recent studies on larval behaviour and identification of settlement cues for different limpet species, including the relative importance of CCA presence and the role of other habitat features (such as limpet mucus and presence of conspecifics), have been advancing our knowledge on the early benthic life stages of limpets. This will hopefully allow future studies to deepen our understanding of the importance of pre- and post-settlement processes for the focal species of this thesis: namely allowing the design of collectors for limpet larvae and field experiments with limpet recruits.

The nature of CCA-limpet interactions will probably be a primary issue for further investigation, namely their role for post-settlement survival and growth of juvenile and adult patellids. To improve our knowledge on these interactions in space and ontogenic time, future studies should look at feeding behaviour (namely gardening or territoriality) of individuals of different patellid species and sizes, on CCA and non-CCA substrata within rock-pools and on low-shore open-rock. The possibly similar interaction between *S. pectinata* and non-coralline crustose algae should also be considered. Much remains to be known on the species identity and life histories of intertidal CCA complexes and non-coralline crusts in the Northeast Atlantic, as well as on their specific responses to herbivory and the potential role of limpets on their cover and persistence.

Further work is crucial on the migration patterns of the studied limpet species between rock-pools and open-rock, and on the transitioning passage from vagrant to homing behaviour. The role of intra-specific class interactions (see Boaventura et al., 2003), and influence of inter-specific competition on ontogenic migration, as well as individual movements at short-temporal scales, should be investigated to identify the mechanisms of how connectivity of limpet populations operates.

Manipulative experiments and ecophysiological approaches are needed to clarify the influence of physical and biological processes on the described patterns and associations, particularly the use of rock-pools by adult limpets as possible thermal refuges, feeding and breeding grounds.

The two rarer species (*P. vulgata* and *P. rustica*) need further attention, namely on the occurrence of their early life stages and recruitment patterns (and surveying very-exposed shores and shores composed of sandstone in the case of *P. rustica*).

A better understanding of the specific environmental factors (e.g., hydrodynamics, water quality, physical-chemical microhabitat conditions) that drive recruitment patterns of limpets and other intertidal invertebrates in the study region, including areas inside and outside the Port of Sines, is essential for regional management. Long-term monitoring is needed to detect any potential changes in population dynamics due to ongoing coastal development and climate change.

Modern techniques (e.g. drone imagery and digital terrain models) should allow more precise metrics of landscape variability, enabling a more holistic approach on the diversity and spatial configuration of microhabitat patches plus the species they contain to be embraced. Scaling up in both time and space is essential to inform conservation and management including future eco-engineering approaches.

6.4 Concluding remarks

Taking a long-term (Chapter 5) and a landscape scale approach (Chapters 4 and 5) is essential in ecology. This holistic approach can be broken down into more reductionist elements as distribution patterns and settlement/recruitment processes (Chapters 2 and 3). Ultimately geomorphology and oceanography provide the topographic template and the physical-chemical settings in which biogenic complexity acts and population and community processes occur.

Rock-pools are both an aesthetical delightful and an essential ecological component of intertidal shore systems. Much remains to study on their ecological importance. Ironically, recent work on rock-pools has been stimulated by their absence on artificial structures. Eco-engineering trials creating pools for biodiversity enhancement and ecosystem service provision have thus greatly benefited fundamental knowledge on ecology.

This thesis underscores the ecological importance of rock-pools as nurseries and adult habitats for intertidal limpets, demonstrating their roles in sustaining limpet populations. The following issues were especially emphazised:

1. the possibly mutualistic nature of CCA-patellid interactions;
2. the primacy of shallow mid-shore rock-pools as nurseries for limpets in the study region;
3. the ecological importance of patchy microhabitats for demographic connectivity across the rocky intertidal landscape, and the consequences for the coexistence of different species and benthic life stages of limpets;
4. the potential of eco-engineered rock-pools to generate persistent and cross-microhabitat biodiversity benefits.

By integrating natural habitat studies with innovative eco-engineering approaches, it highlights practical pathways for conserving and enhancing intertidal biodiversity in the face of increasing human pressures.

Moving forward, a combination of long-term monitoring, experimental manipulative research, and interdisciplinary approaches will be essential to address emerging challenges and advance sustainable management of temperate intertidal reefs.

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SUPPLEMENTARY MATERIAL

Supplementary material of Chapter 1 – General Introduction

Supplementary Table S1.1 Limpet species that occur in rock-pools or their edges globally. Information was mostly gathered for the occurrence of limpets in natural rock-pools, but in a few cases this was complemented with the occurrence of limpet species in eco-engineered rock-pools. Studies in which size was not-specified likely refer to limpets across the size-range of each species or only the adults/larger limpets.

Species name according to WoRMS Editorial Board (2025)	**World region and/or country**	**Limpet size or life-stage**	**Reported occurrence in rock-pools or use of rock-pools**	**References**
***Cellana capensis* (Gmelin, 1791)**	East coast of South Africa	Not-specified	Occurs in the upper-shore "*Littorina* zone pools".	Huggett and Griffiths, 1986; De Pirro and Marshall, 2005.
***Cellana exarata* (Reeve, 1854)**	Hawai	Not-specified	Occurs in small rock-pools characterized by relatively low diversity and abundance of fauna.	O'Day, 2004.
***Cellana grata* (A. Gould, 1859)**	Hong Kong	Not-specified.	Pool edges as refuges from high temperatures and desiccation when inactive during summer.	Williams and Morritt, 1995.
Cellana karachiensis (Winckworth, 1930)	India	Not-specified	Occurs in intertidal holes, pools, puddles, and crevices.	Vakani and Kundu, 2020.
***Cellana radiata* (Born, 1778)**	India	Not-specified.	Density is high in pools and crevices.	Balaparameswara Rao and Ganapati, 1971.
***Cellana sandwicensis* (Pease, 1861)**	Hawai	Not-specified	Occurs in small rock-pools characterized by relatively low diversity and abundance of fauna.	O'Day, 2004.
***Cellana toreuma* (Reeve, 1854)**	Hong Kong; China.	Not-specified	Rock-pools as refuge from hot temperatures and large salinity during the summer monsoon. Refuge from winter cold-stress. Grazes in pools.	Wai and Williams, 2006; Firth and Williams, 2009; Sun et al. 2023.
***Cellana tramoserica* (Holten, 1802)**	Australia (Cape Banks)	Not-specified	Coexistence with the starfish *Parvulastra exigua* inside rock-pools. Abundance varied with the stratum of a pool (i.e. the position below the top of a pool) and the depth of pools.	Branch and Branch, 1980; Arrontes and Underwood, 1991; Astles, 1993.
***Cymbula miniata* (Born, 1778)**	South Africa	Not-specified	Association with CCA ("lithothamnion"). Restricted to rock-pools in the intertidal zone; also occurs in the subtidal habitat.	Branch, 1973.
***Cymbula oculus* (Born, 1778)**	South Africa	Juveniles	Rock-pools as refuge from desiccation. Mid-shore species, larger individuals migrate from pools onto bare rocks.	Branch, 1975a; Branch and Newell, 1978.
***Fissurella maxima* (G. B. Sowerby I, 1834)**	Central Chile	Adults.	Larger specimens occur mainly on boulder beaches, intertidal pools with permanent water exchange, and in the subtidal. Juveniles are frequently found in the mussel bed (on the open-rock). Human exploited species.	Oliva and Castilla 1986.
***Fissurella* Bruguière, 1789 (Fissurella sp.)**	Ceuta	Not-specified	Occurred inside eco-engineered rock-pools and surrounding rock (but much more abundant in pools).	Ostalé-Valriberas et al., 2024.
***Fissurella radiosa* (R. P. Lesson, 1831)**	Patagonia (Argentina)	Not-specified	Predated by the sea star *Anasterias minuta* within rock-pools.	Gil and Zaixso, 2008.

***Helcion concolor* (Krauss, 1848)**	South Africa	Adults	Occurs in high-shore rock-pools, possibly extended upper limits in pools. Juveniles on low-shore in moist crevices and damp sand.	Branch, 1975b.
Lottia digitalis (Rathke, 1833)	Northeastern Pacific (Oregon, USA)	Not-specified	Higher abundance in pools and boulders inacessible to oystercatchers.	Frank, 1982.
***Lottia limatula* (P. P. Carpenter, 1864)**	Northeast Pacific (California, USA)	Not-specified	Occurs in high-shore pools; Responsive behaviour to the sea-star *Pisaster ochraceus* in rock-pools.	Feder, 1963; Bracken et al., 2018, 2024.
***Lottia paradigitalis* (Fritchman, 1960)**	Northeastern Pacific Ocean (Vancouver Island, British Columbia, Canada)	Not-specified	Occurs in pools, possibly as means of spatial segregation with *Lottia digitalis* (that does not occur in pools in the same sites).	Breen, 1968.
***Lottia pelta* (Rathke, 1833)**	Northeastern Pacific (Vancouver Island, British Columbia, Canada; Washington, California, USA)	Not-specified	Occurs in pools, possibly as means of spatial segregation with Lottia digitalis (that does not occur in pools in the same sites). Mid to high pools occupied by *Ralfsia crusts*; Responsive behaviour to the sea-star *Pisaster ochraceus* in rock-pools.	Feder, 1963; Breen, 1968; Dethier, 1981, 1982, 1994.
***Lottia persona* (Rathke, 1833)**	Northeastern Pacific (Oregon, USA)	Not-specified	Higher abundance in pools and boulders inacessible to oystercatchers.	Frank, 1982.
***Lottia scabra* (A. Gould, 1846)**	Northeast Pacific (California, USA)	Not-specified	Occurs in high-shore pools; Responsive behaviour to the sea-star *Pisaster ochraceus* in rock-pools.	Feder, 1963; Bracken et al., 2018, 2024.
***Lottia scutum* (Rathke, 1833)**	Northeast Pacific (Washington, California, USA)	Not-specified	Eat crustose algae in pools. Responsive behaviour to the sea-star *Pisaster ochraceus* in rock-pools. Predated by oystercatchers in rock-pools.	Feder, 1963; Frank, 1982; Dethier, 1994.
***Lottia strigatella* (P. P. Carpenter, 1864)**	Northeast Pacific (southern California, USA)	Not-specified	Occurs in high-shore rock-pools, and in mid to high rock-pools occupied by *Ralfsia* crusts.	Dethier, 1981; Bracken et al., 2018, 2024.
***Montfortula rugosa* (Quoy and Gaimard, 1834)**	Australia	Juveniles and adults.	Abundant in rock-pools or damp areas on low-shore.	Creese, 1981; Chapman and Blockley, 2009.
***Nacella concinna* (Strebel, 1908)**	Antarctic Peninsula and adjacent islands	Adults	Temporary (seasonal) residence; migratory species from subtidal habitats during summer after ice retreat (another fraction of the population remains submerged). During the austral winter, rock-pools have a protective ice layer that protects the limpets from low air temperature and wave swell with crushing ice. During the summer, limpets in large rock-pools are less subjeted to reduced salinity due to meltwater compared to air-exposed limpets. Higher density and stronger grazing effect in rock-pools than open-rock. Two limpet morphotypes (the rock-pool *N. concinna polaris* and the subtidal *N. concinna concinna*), their phenotypic variation (smaller,	Shabica, 1976; Davenport, 1997, 2001; Abele et al., 1998; Chwedorzewska et al., 2010; Choy et al., 2011; Segovia-Rivera and Valdivia, 2016.

			lighter and flatter shells in rock-pools) is not due to genetic divergence.	
***Nacella magellanica* (Gmelin, 1791)**	Patagonia (Argentina)	Not-specified	Predated by the sea star *Anasterias minuta* in rock-pools.	Gil and Zaixso, 2008.
***Patella caerulea* (Linnaeus, 1758)**	Mediterranean (Italy; Ceuta)	Not-specified	Occurs inside rock-pools (within CCA patches or uncolonized rock). Inside eco-engineered rock-pools and surrounding rock (but more abundant on the open-rock).	Benedetti-Cecchi and Cinelli 1995, 1996; Ostalé-Valriberas et al., 2024
***Patella depressa* (Pennant, 1777)**	Northeast Atlantic (Bristish Isles, France, Portugal)	Juveniles and adults	Rock-pools as nursery-grounds. Cooccurence of juveniles and adults with *Patella ulyssiponensis* in CCA-covered pools. Adults may be almost confined to pools and cracks above "the C*hthamalus*-zone".	Eslick, 1940; Evans, 1947, 1948, 1957; Bowman, 1981; Guerra and Gaudêncio, 1986.
***Patella ferruginea* (Gmelin, 1791)**	Ceuta	Not-specified	Inside eco-engineered rock-pools and surrounding rock (but much more abundant on the open-rock).	Ostalé-Valriberas et al., 2024.
***Patella pellucida* (Linnaeus, 1758)**	Northeast Atlantic (Ireland)	Juveniles	Association with CCA ("Lithothamnia"). Recently-settled individuals found only on living "Lithothamnia" in rock-pools and on low-shore open-rock. Subsequent migration to macroalgal fronds.	Goss-Custard et al., 1979; McGrath, 1992
***Patella ulyssiponensis* (Gmelin, 1791)**	Northeast Atlantic (Bristish Isles, France, Portugal) and Mediterranean (Italy)	Juveniles and adults, confined to pools above low-shore.	Association with CCA ("Lithothamnia"); pool-dweller in CCA-covered "weed-free pools". Rock-pools as settlement site and nursery, refuge of early benthic stages from temperature fluctuations, and feeding grounds.	Evans, 1947, 1948, 1957; Bowman, 1978; Goss-Custard et al., 1979; Bowman, 1981; Bowman and Lewis, 1986; Guerra and Gaudêncio, 1986; Kooistra et al., 1989; Benedetti-Cecchi and Cinelli, 1995, 1996; Delany et al., 1998, 2002; Firth and Crowe, 2008, 2010; Firth et al., 2009; Nöel et al. 2009; Griffin et al., 2010.
***Patella vulgata* (Linnaeus, 1758)**	Northeast Atlantic (Bristish Isles, Portugal)	Juveniles and adults, but mostly juveniles.	Rock-pools as settlement site and nursery, refuge of early benthic stages from temperature fluctuations. Adults with home-scars in pool edges use pools for foraging during high-tide.	Eslick, 1940; Evans, 1947, 1948, 1957; Lewis and Bowman, 1975; Bowman and Lewis, 1977; Bowman, 1978; Goss-Custard et al., 1979; Bowman, 1981; Guerra and Gaudêncio, 1986; Delany et al., 1998, 2002; Firth and Crowe, 2008, 2010; Firth et al., 2009; Nöel et al., 2009.
***Patelloida alticostata* (Angas, 1865)**	Australia	Not-specified	Occurrence in natural rock-pools but not in eco-engineered rock-pools in close locations.	Creese, 1980; Chapman and Blockley, 2009.
***Patelloida latistrigata* (Angas, 1865)**	Australia (Cape Banks)	A few of the largest adults (12-15 cm).	Shallow pools are a occasional/marginal microhabitat (main microhabitat of juveniles and adults was among the barnacles *Tesseropora rosea*)	Creese, 1980, 1982.
***Patelloida mimula* (Iredale, 1924)**	Australia	Not-specified	Occurrence in natural rock-pools but not in eco-engineered rock-pools in close locations.	Chapman and Blockley, 2009.

Scurria araucana **(d'Orbigny, 1839)**	Southeastern Pacific (Chile)	Not-specified	Abundant in rock-pools, with mostly dispersive distribution pattern and with establishment of home scars.	Aguilera et al., 2013
Scutellastra argenvillei **(Krauss, 1848)**	West coast of South Africa	Adults.	Recovery of limpet density after exploitation by settlement and growth of recruits and by immigration of adults from inaccessible lower intertidal regions and rock pools.	Eekhout et al., 1992.
Scutellastra cochlear **(Born, 1778)**	South Africa	Not-specified	Association with CCA. Occasional occurrence in rock-pools, but more generally on low-shore open-rock, where it lives in CCA-dominated areas ("the cochlear zone").	Gusha et al., 2021.
Scutellastra granularis **(Linnaeus, 1758)**	South Africa	Not-specified	Use of pool edges as means of evaporative cooling.	Branch, 1975a; Gusha et al., 2021.
Scutellastra longicosta **(Lamarck, 1819)**	South Africa	Not-specified	Association with CCA. Can occur inside rock-pools in association with CCA ("lithothamnion"), in which it lives and feeds during the second year of life. Th first year of life is spent on shells (*Oxystele sinensis* or conspecifics, where they feed on epiphyte *Ralfsia* crusts), and before establishing their territorial gardens of *Ralfsia expansa*.	Branch, 1973.
Scutellastra peronii **(Blainville, 1825)**	Australia (Cape Banks)	Not-specified	Occurred inside eco-engineered rock-pools, with high among-pool variation in abundance.	Chapman and Underwood, 1999.
Siphonaria acmaeoides **(Pilsbry, 1894)**	Indo-West Pacific (Japan)	Not-specified	Occurs on exposed rocky shores in crevices and small shallow rock pools on upper littoral level, rarely on rock faces.	Jenkins and köhler, 2024.
Siphonaria alternata **(Say, 1826)**	Bermuda	Not-specified	Occurs as flatter limpets in rock-pools compared to ledges. Longer activity period (during low-tide) in pools than in ledges.	Cook, 1979
Siphonaria asghar (H. E. J. Biggs, 1958)	India	Not-specified	Occurred only in the rocky-muddy coast which has bigger pools and puddles on substratum.	Vakani and Kundu, 2020
Siphonaria australis **(Quoy and Gaimard, 1833)**	New Zealand (Wellington region)	Egg masses; Adults	High abundance of egg masses in rock-pools, despite being the microhabitat where egg masses suffered the greatest mortality (potential cost of pool immersion on benthic egg masses).	Russell and Phillips, 2009; Kessel and Phillips, 2018.
Siphonaria belcheri (Hanley, 1858)	India	Not-specified	Occurs on smooth rocky substratum with pools and puddles.	Vakani and Kundu, 2020
Siphonaria capensis **(Quoy and Gaimard, 1833)**	South Africa	Individuals with large range of sizes and egg masses	High-shore pools as primary microhabitats for this species and where its egg masses are deposited. More time of activity during diurnal low-tides and less tenacity in rock-pools than on open-rock. Homing reduces osmotic stress in pools (homing reduces desiccation and wave-dislodgement on open-rock).	Branch and Cherry, 1985; Bosman and Hockey, 1986; Chambers and McQuaid, 1994; Gusha et al. 2021.
Siphonaria compressa **(Allanson, 1958)**	South Africa (endemic to Langebaan Lagoon on the west coast, and Knysna Estuary	Not-specified	Occasional occurrence in permanent rock-pools above the eelgrass beds, suggested as possible refuge from air exposure (lives only on blades of *Zostera capensis*).	Angel et al., 2006.

	on the south coast)			
Siphonaria denticulata **(Quoy and Gaimard, 1833)**	Australia	Egg masses and adults.	Rock-pools as refuges from desiccation for egg masses (egg masses did not survive when exposed to air). Occurs inside natural and eco-engineered rock-pools.	Creese, 1980; Przeslawski, 2005; Przeslawski et al., 2005; Przeslawski and Benkendorff, 2005; Przeslawski and Davis, 2007; Chapman and Blocley, 2009; Morris et al, 2018.
Siphonaria exulum **(Hanley, 1858)**	Indo-West Pacific, Australia (Anson Bay)	Not-specified	Occurs in rock-pools.	Jenkins and köhler, 2024.
Siphonaria gigas **G. B. (Sowerby I, 1825)**	Pacific coast of Panama	Adults	Seldom occurs in rock-pools, being much more abundant in fissures and other protective microhabitats. May avoid small pools because they become very hot and may avoid cooler larger pools because of fish predators.	Lombardo et al., 2013.
Siphonaria kurracheensis (Reeve, 1856)	India	Not-specified	Occurs on smooth rocky substratum with rock-pools and puddles	Vakani and Kundu, 2020
Siphonaria lateralis **(A. Gould, 1846)**	Subantarctic (South Georgia)	Adults	Mostly occurs in shallow pools on mid-shore platforms protected from heavy wave action by rocks. Feeding grounds in rock-pools and pool edges.	Davenport and MacAlister, 1996; Davenport, 1997
Siphonaria lessonii **(Blainville, 1827)**	Argentina (Quequén harbour)	Egg masses, juveniles and small-sized individuals	Occurs at high and mid-intertidal levels on rocky shores, at wet areas such as crevices and rock-pools. Rock-pools as marginal microhabitat, but with enhanced growth in high-shore pools dominated by the green alga *Ulva lactuca*.	Tablado et al., 1994; Zabala et al., 2018
Siphonaria maura **(G. B. Sowerby I, 1835)**	Pacific coast of Panama	Not-specified	Occurs in crevices and rock-pools as the main microhabitats	Garrity, 1984
Siphonaria pectinata **(Linnaeus, 1758)**	Ceuta	Individuals (not-specified size) and egg masses	Occurrence of individuals and egg masses inside eco-engineered rock-pools and surrounding rock (egg masses were much more abundant in pools).	Ostalé-Valriberas et al., 2018, 2024.
Siphonaria plicata **(Quoy and Gaimard, 1833)**	Indo-West Pacific (Tonga)	Not-specified	Occurs in sheltered positions, including small pools, hollows on beach rock platforms and hollows of rocky cliff bases. Also occurs on exposed rocky shores or landside of lagoons at mid and lower littoral levels.	Jenkins and köhler, 2024.
Siphonaria stowae **(Verco, 1906)**	Indo-West Pacific, Southern coasts of Australia	Not-specified	Association with CCA. Often occurs in rock-pools and associated with white "Lithothamnion" algae, in sheltered rocky intertidal platforms and rocky areas, at mid littoral level.	Jenkins and köhler, 2024.
Siphonaria zelandica (**Quoy and Gaimard, 1833)**	Indo-West Pacific, Endemic to Australia	Not-specified	Common in sheltered positions, often within runoff areas and shallow platform pools, on exposed to moderately exposed rocky intertidal	Jenkins and köhler, 2024.

			marine shores across upper and mid littoral levels.	
Testudinalia testudinalis **(O. F. Müller, 1776)**	Subarctic western North Atlantic (Gulf of Maine, USA)	Recruits, juveniles and adults	Mutualist association with CCA (*Clathromorphum circumscriptum*). Rock-pools as recruitment sites and preferred food source for adults. Individuals in pools were smaller and less-desiccation tolerant than individuals in subtidal microhabitats and in high-shore vertical walls.	Willcox, 1905; Wallace, 1972; Steneck, 1982; Lyczkowski, 2008; Lord et al., 2011.

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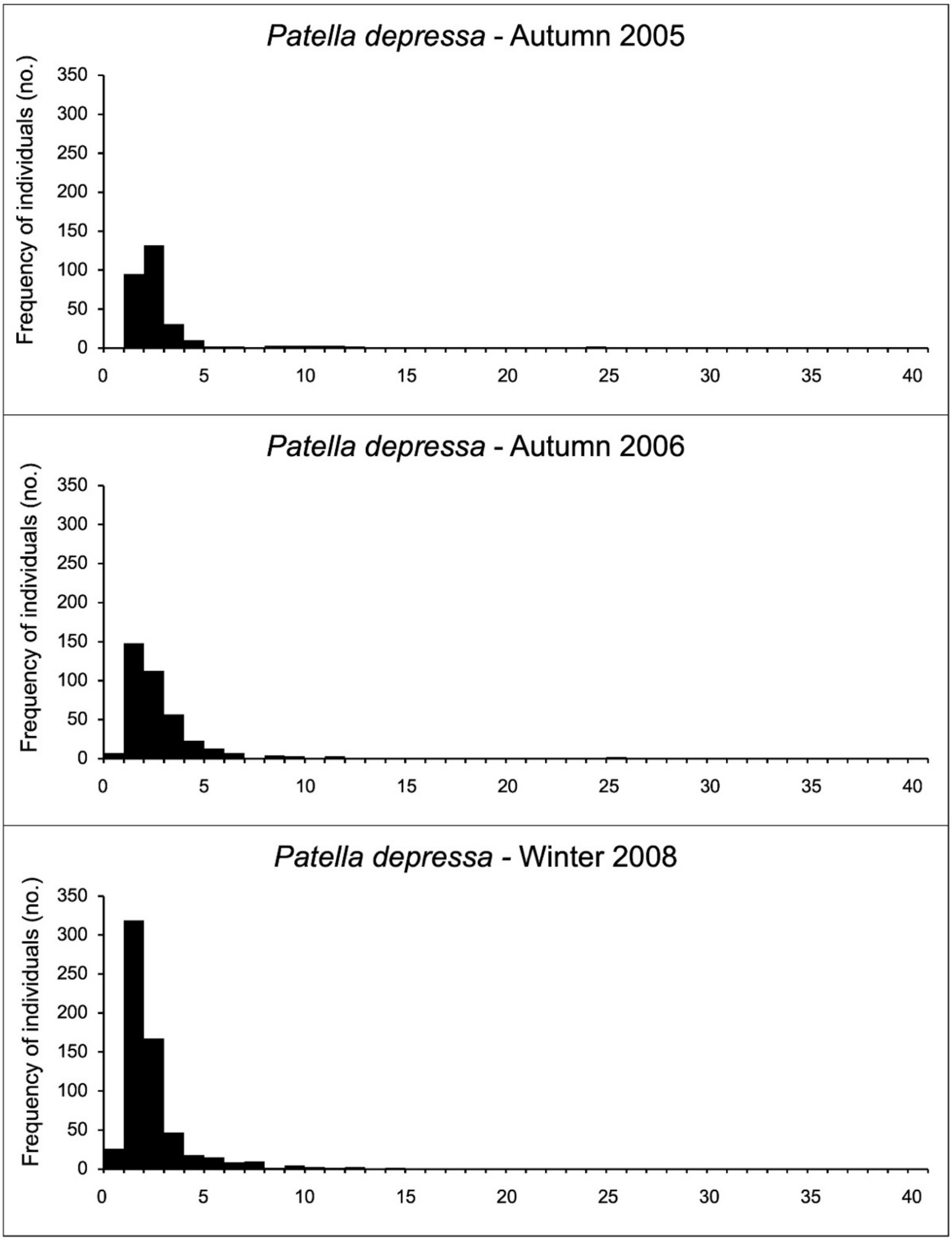
Supplementary material of Chapter 3 – Rock-pools as nurseries for co-existing limpets: Spatial and temporal patterns of limpet recruitment (Seabra et al., 2020)



Patella depressa - Autumn 2005

Patella depressa - Autumn 2006

Patella depressa - Winter 2008

Figure. S3.1 Size-frequency histograms (1mm size-class intervals are shown, labelled at 5mm intervals) of *Patella depressa* recorded into all sampled rock-pools for three selected periods (Autumn 2005, Autumn 2006 and Winter 2008 see Methods).

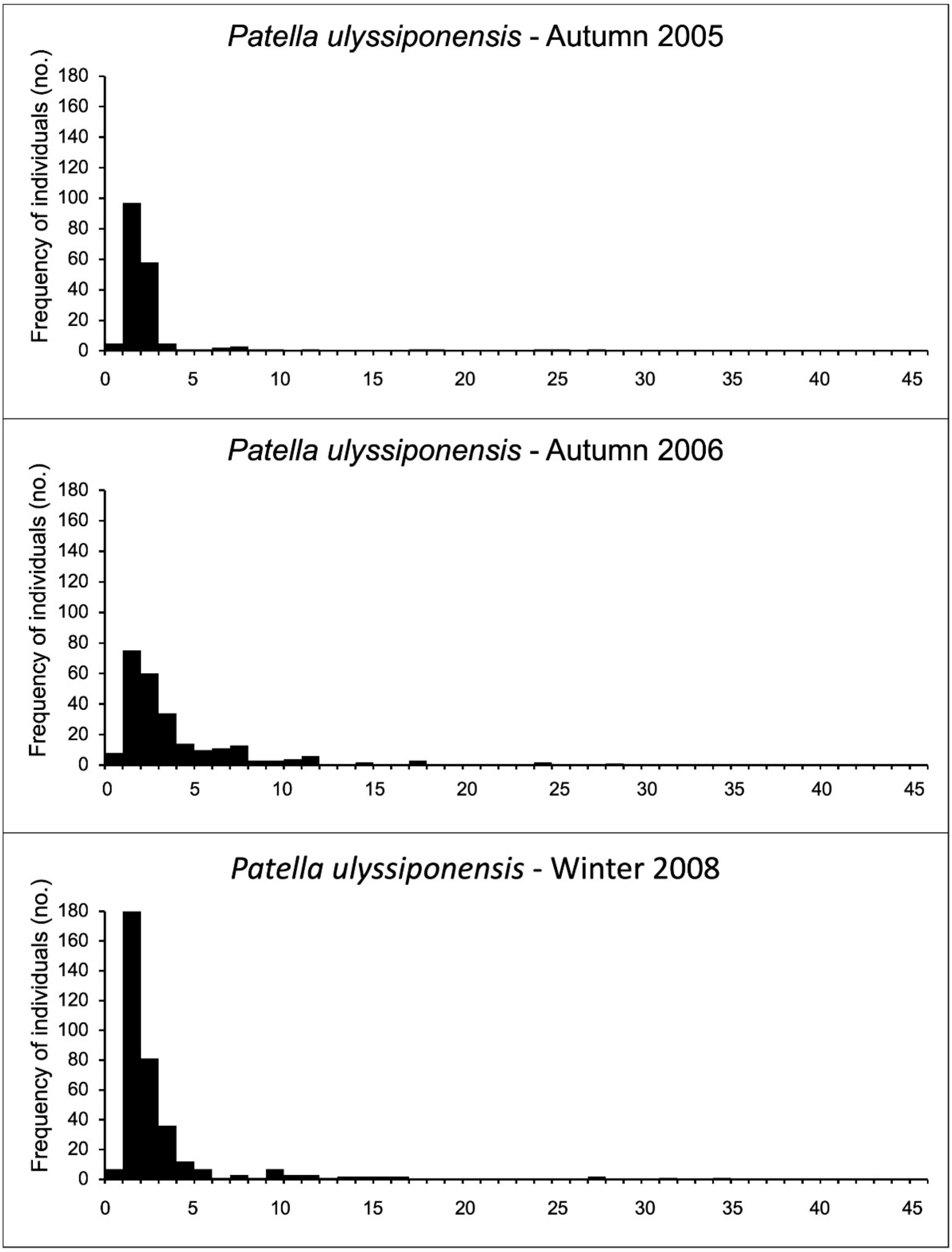


Figure S3.2 Size-frequency histograms (1mm size-class intervals are shown, labelled at 5mm intervals) of *Patella ulyssiponensis* recorded into all sampled rock-pools for three selected periods (Autumn 2005, Autumn 2006 and Winter 2008 see Methods).

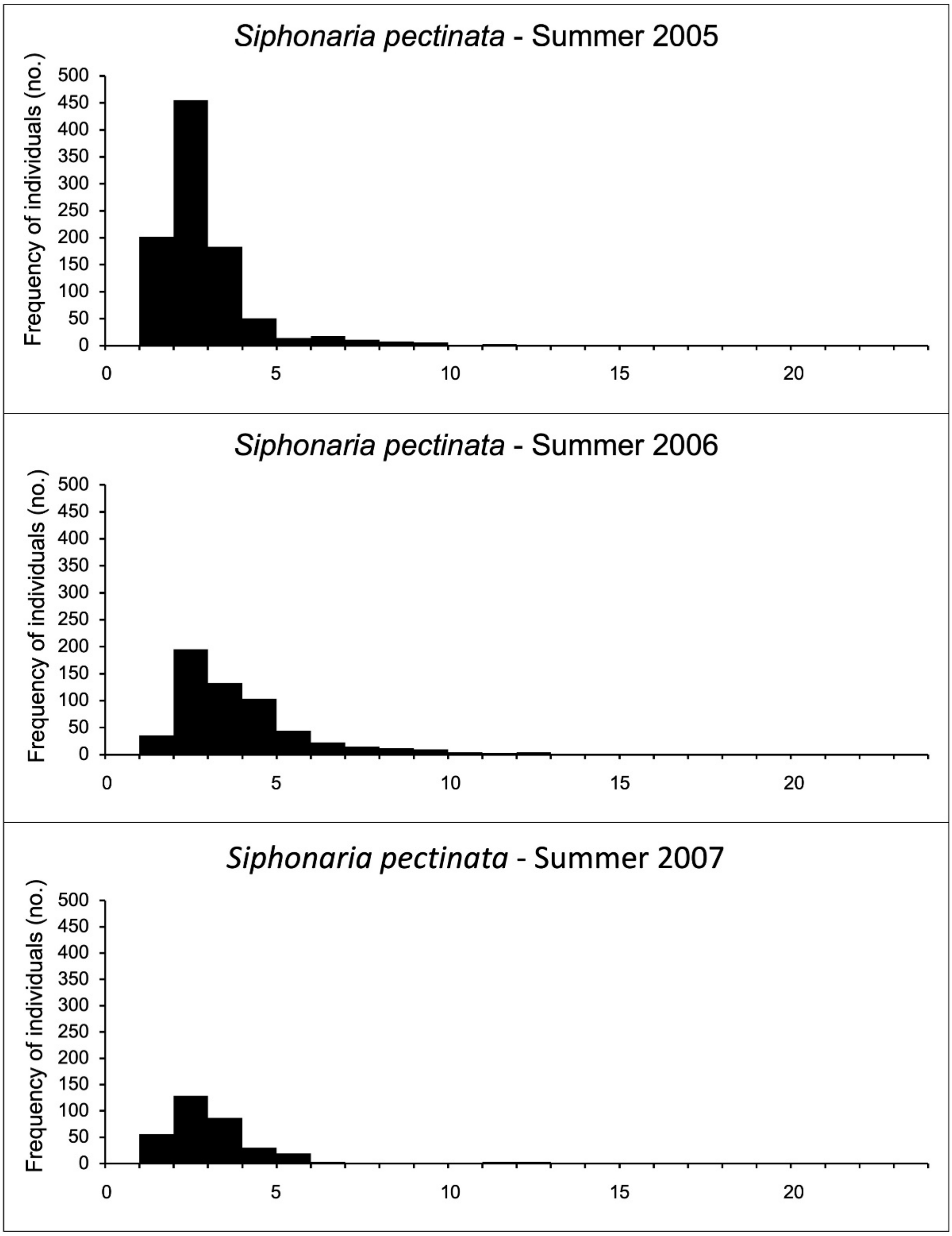


Figure S3.3 Size-frequency histograms (1mm size-class intervals are shown, labelled at 5mm intervals) of *Siphonaria pectinata* recorded into all sampled rock-pools for three selected periods (Summer 2005, Summer 2006 and Summer 2007 see Methods).

Table S3.1 Mean percentage of the densities of *Patella depressa* and *Patella ulyssiponensis* individuals measuring less than 3 and 5 mm in maximum shell length (MSL) relatively to the total density of each species. Values averaged across all sampled rock-pools for three selected periods (Autumn 2005, Autumn 2006 and Winter 2008 – see Methods).

Period	*Patella depressa*		*Patella ulyssiponensis*	
	Mean % MSL < 3 mm	Mean % MSL < 5 mm	Mean % MSL < 3 mm	Mean % MSL < 5 mm
Autumn 2005	74	85	87	90
Autumn 2006	66	87	61	80
Winter 2008	77	91	73	84

Table S3.2 Mean percentage of the densities of *Siphonaria pectinata* individuals measuring less than 2, 3, 4 and 5 mm in maximum shell length (MSL) relatively to the total density of this species. Values averaged across all sampled rock-pools for three selected periods (Summer 2005, Summer 2006 and Summer 2007 – see Methods).

Period	*Siphonaria pectinata*			
	Mean % MSL < 2 mm	Mean % MSL < 3 mm	Mean % MSL < 4 mm	Mean % MSL < 5 mm
Summer 2005	20	64	84	90
Summer 2006	6	36	65	81
Summer 2007	12	44	73	81

Supplementary Material of Chapter 4 – Variability and connectivity in populations of different limpet species across rockpool-generated mosaic landscapes (Seabra et al. 2023)

Supplementary Text

Methodological details of distance-based linear models (DistLM) to examine the relationship between the spatial structure of individual limpet species within each microhabitat and several predictors (physical, habitat-composition, and connectivity variables).

Preliminary procedures were done for predictive-variables: i) after data visualization on Draftsman' plots (pairwise scatter plots), the variable maximum-depth was eliminated due to collinearity with mean-depth; ii) categorical variables were transformed in presence/absence data for each individual category; iii) based on visualization of shade-plots, transformations (Supplementary Table S4.2) were applied for several quantitative predictors. For each response-matrix (i.e. size-class densities of a species within a microhabitat), the following sequence of DistLM were run with the following sub-sets of predictors (after preliminary procedures of elimination and transformation): one partial model with habitat-composition variables measuring percentage cover of space-occupying groups (13 for POOL, not applicable for EDGE analyses, 14 for NEAR, Supplementary Table 2-S), another partial model with all the physical variables (16 for POOL, 12 for EDGE and 14 for NEAR, Supplementary Table S4.2), and another partial model with habitat-composition and connectivity variables measuring density of all other molluscan grazers (8 for POOL, 10 for EDGE, 8 for NEAR, all square-root transformed, Supplementary Table S4.2). Then, a final model with all variables selected in the previous partial models was run.

Supplementary Table S4.1 Definition and measurement details of all predictive-variables of three groups (physical, habitat-composition and connectivity) assessed for every sampled rock-pool (n = 323). Under the column "Microhabitat", "only" are indicated for variables that refer exclusively to one microhabitat, whereas "All" identifies those variables that were considered as physical descriptors of each rockpool-system across POOL, EDGE, and NEAR microhabitats.

Variable	Group and Units	Microhabitat	Definition
Maximum-depth	Physical; cm	POOL only	Water-height at the deepest point of each rock-pool; field measurement with a ruler.
Mean-depth	Physical; cm	POOL only	Averaged water-height at several points along the two major pool-axes of each rock-pool; field measurement with a ruler.
POOL Topographic complexity	Physical; Dimensionless	POOL only	Ratio of the length of the major axis of each rock-pool along the contoured surface of substratum to its stretched-line length at the water-level; field measurements with a tape measure.
POOL Slope	Physical; Angle degrees	POOL only. One record taken per each POOL quadrat and the mean from both quadrats considered for each rock-pool.	Slope of the substratum surface inside each rock-pool; field measurements with an inclinometer directed to the center of the pool.
NEAR Topographic complexity	Physical; Dimensionless	NEAR only. One record taken per each NEAR quadrat and the mean of both quadrats considered for each rock-pool.	Ratio of the length of a NEAR quadrat taken along the contoured surface of substratum to its stretched-line length (i.e. the quadrat side: 19 cm); field measurements with a tape measure.
NEAR Slope	Physical; Angle degrees	NEAR only. One record taken per each NEAR quadrat and the mean from both quadrats considered for each rock-pool.	Slope of NEAR substratum surface surrounding each rock-pool; field measurements taken with an inclinometer directed to the center of the rock-pool.
Perimeter	Physical; cm	All	Length of the outline of each rock-pool; estimated by image analysis of a scaled digital photograph.
Surface-area	Physical; cm^2	All	Projected area of each rock-pool; estimated by image analysis of a scaled digital photograph.
Volume	Physical; cm^3	POOL only	Estimated volume of each rock-pool. Calculated based on the volume of a pyramid as $V = (1/3) Bh$, where "B" is the surface-area and "h" is the maximum-depth.

Circularity	Physical; Dimensionless	All	Shape descriptor corresponding to a function of the perimeter and the surface-area of the rock-pool; estimated by image analysis of a scaled digital photograph of each rock-pool. *Circularity = 4π surface-area / (perimeter)^2*
Aspect ratio	Physical; Dimensionless	All	Shape descriptor corresponding to a function of the largest diameter of the rock-pool (major axis) to the smallest diameter orthogonal to it (minor axis); estimated by image analysis of a scaled digital photograph of each rock-pool. *Aspect Ratio = major axis / minor axis*
Roundness	Physical; Dimensionless	All	Shape descriptor corresponding to a function of the surface-area and the largest diameter of the rock-pool (major axis); estimated by image analysis of a scaled digital photograph of each rock-pool. *Roundness = 4 surface-area / π (major axis)^2*.
Straight-line distance to the nearest rock-pool	Physical; cm	All	Minimum distance from the rockpool-edge to the edge of the nearest rock-pool, taken as the stretched linear lenght between the two points (field measurement with a tape measure).
Contoured distance to the nearest rock-pool	Physical; cm	All	Minimum distance from the rockpool-edge to the edge of the nearest rock-pool, taken as the length along the contoured surface of substratum between the two points (field measurement with a tape measure).
Shore-height	Physical; Metres above C.D.	All	The intertidal height of each rock-pool was calculated from field measurements taken during low-tide at the rock-pool edge and at the low-water mark, using a SOKKIA C3-30 auto level surveying equipment. Height measurements were later standardized by convertion to height above chart datum, based on the sea level values retrieved from http://neptuno.fis.ua.pt for the date and time of the low-water mark measurements.
Distance to low-water mark	Physical; m	All	The distance of each rock-pool to the low-water mark was calculated from field measurements taken during low-tide using a SOKKIA C3-30 auto level surveying equipment, regarding the positions of the rockpool-edge and the low-water mark.
Barriers	Physical; Categorical variable: two categories.	All	Classification of each rock-pool in the field. Category depends on whether there is absence (0) or presence (1) of a rocky outcrop located seaward at the maximum distance of 1m from the rockpool-edge.

Curved or Straight edges	Physical; Categorical variable: two categories.	All	Classification of each rock-pool in the field. Category depends on whether the edges of the rock-ppool are curved (1) or straight (0), regarding the total or great majority of their extent.
Confinement	Physical; Categorical variable: five categories.	All	Classification of each rock-pool in the field. Category based on the height of the rocks surrounding rockpool-edges. Total or majority of surrounding rocks forming: the same plane as rockpool-edge (category 1); low walls (less than 3cm, category 2); mid-height walls (up to 6cm, category 3); high walls (up to 10cm, category 4); very high walls often with obtuse angles in slope (greater than 10cm, category 5).
Rock	Habitat-composition; Percentage cover	Estimated within both POOL and NEAR of each rock-pool separately (for each microhabitat, two sampling quadrats were sampled and their mean was considered). On each POOL quadrat (inside-pools): i) the percentage cover of each sessile taxon or substratum type was assessed by counting the number of intersection points above it; ii) both primary and secondary (after moving away canopy algae) cover were sequentially assessed; iii) superimposed taxa occurring in a same intersection point (both epibiont and basibiont organisms) were equally scored; iv) consequently, the total percent cover could exceed 100%. On each NEAR quadrat (outside-pools), cover estimates were assessed using the following semi-quantitative index (and respective percent cover intervals): 0	Bare-rock and rock covered by biofilm.
Sand			Mobile substrata, mostly inorganic; mostly sand, but including detritus and cobbles in minor proportions; detritus include organic material such as shell-deposits intermixed with gravel.
Lichinaceae			*Lichina pygmaea* - exclusively present outside-rockpools.
Verrucariaceae			Mostly *Hydropunctaria maura.*
Cyanophytes			Genera *Calothrix* and *Oscillatoria,* and unidentified cyanobacteria.
Crustose non-coralline algae			Mostly *Nemoderma tingitanum*, *Ralfsia verrucosa*, *Codium adhaerens* and *Hildenbrandia rubra.*
CCA			Crustose coralline algae, mostly *Lithophyllum byssoides* and "Lithothamia" *sensu* Hawkins and Jones (1992) (including *Lithophyllum incrustans* and *Mesophyllum lichenoides*).
Articulated coralline algae			Genera *Corallina and Ellisolandia*, also *Jania rubens* and *Amphiroa rigida.*
Seaweed			Non-calcareous and non-crustose algal species belonging to phyla Chlorophyta, Ochrophyta and Rhodophyta, i.e. all "fleshy algae".
Porifera			*Hymeniacidon perlevis* and non-identified sponges.

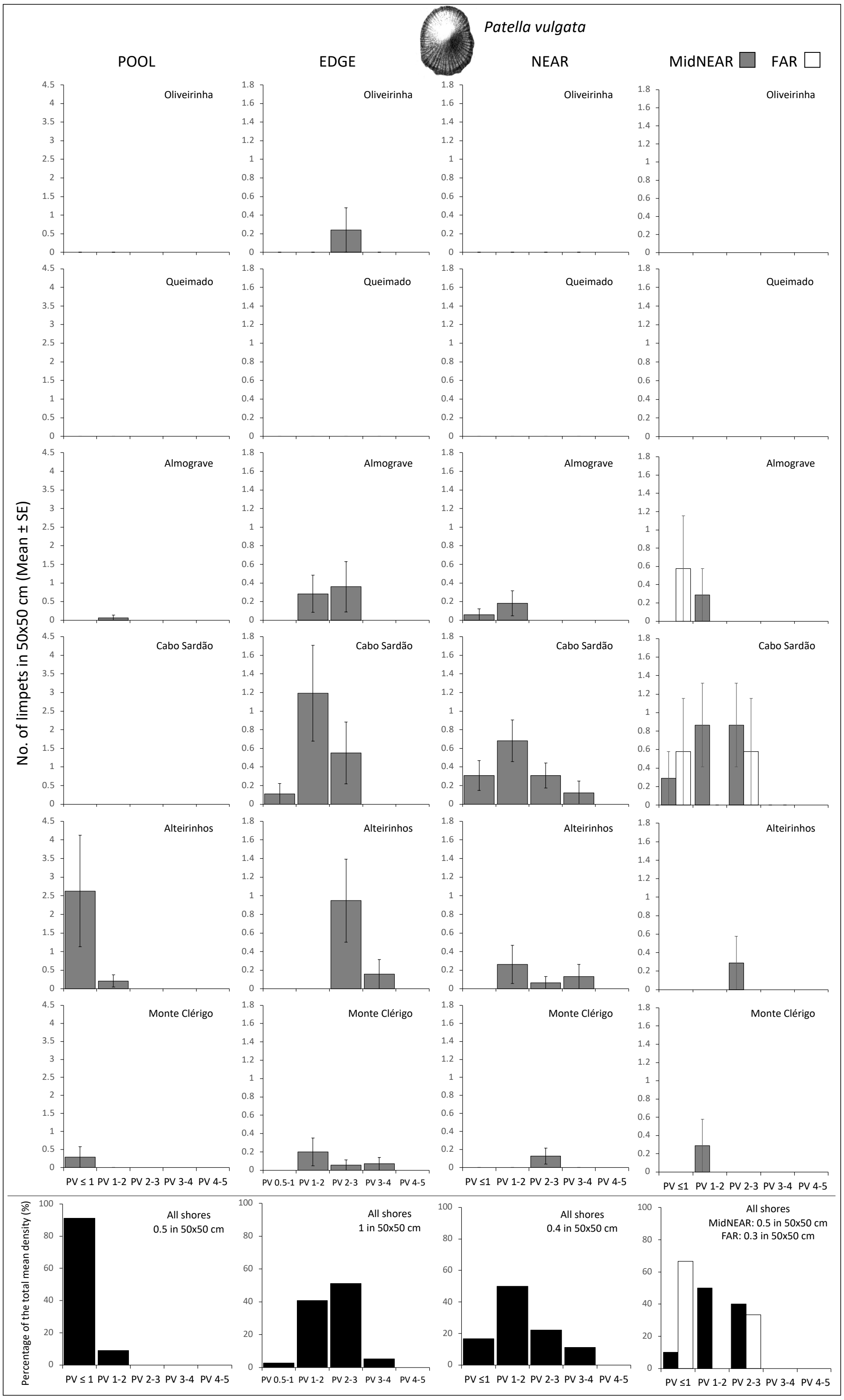
Sea-anemones		(not found); 1 (< 1%); 2 [1-5%[; 3 [5-10%[; 4 [10-25%[; 5 [25-50%[; 6 [50-75%[; 7 [75-90%[; 8 [90-95%[; 9 (≥ 95%).	Inside-rockpools including Actiniidae (*Anemonia viridis*, *Actinia equina*, *A. fragacea, Bunodactis verrucosa, Urticina felina*) and Sagartiidae (e.g. *Cereus pedunculatus*); outside-rockpools only represented by *Actinia equina*.
Barnacles		Each sampling quadrat had 36 intersection points and 25 equal sub-quadrat divisions (percentage cover of 2.8% for each point and 4% for each division).	*Acorn-barnacles* (*Chthamalus* spp., *Perforatus perforatus*) and stalked-barnacles (*Pollicipes pollicipes*).
Mussels			Mostly *Mytilus galloprovincialis* but occasionally including other bivalves (e.g. other mytilids or *Anomia spp.*).
Sea-urchins			*Paracentrotus lividus* - exclusively present inside rock-pools.
Other sessile invertebrates			*Sabellaria alveolata*, *Serpulidae*, *Spirorbidae*, *Vermetidae*, *Hydrozoa.*
Trochids	Habitat-composition; non-limpet grazers; counts standardized to densities in 50x50 cm.	Estimated within both POOL and NEAR of each rock-pool separately (two sampling quadrats were sampled for each microhabitat and their mean was considered).	The total number of trochids (including *Steromphala umbilicalis*, *S. pennanti*, *Phorcus sauciatus*, *P. lineatus*); recorded within the two sampling quadrats of each microhabitat (POOL and NEAR).
Littorinids			The number of individuals of the littorinid *Melarhaphe neritoides*; sub-sampled within each microhabitat (POOL and NEAR) using two quadrats of 40 cm^2.
PU- *Patella ulyssiponensis*	Habitat-composition; every other limpet species in the same microhabitat; counts standardized to densities in 50x50 cm.	Estimated within POOL, EDGE and NEAR of each rock-pool separately (sum of all size-class densities estimated for each of the other limpet species within the same microhabitat)	Total density of *P. depressa* coexisting with the target-species (*P. depressa* or *S. pectinata*) within the same microhabitat.
PD- *Patella depressa*			Total density of *P. ulyssiponensis* coexisting with the target-species (*P. depressa* or *S. pectinata*) within the same microhabitat.
PV- *Patella vulgata*			Total density of *P. vulgata* coexisting with the target-species (*P. ulyssiponensis*, *P. depressa* or *S. pectinata*) within the same microhabitat.
PR- *Patella rustica*			Total density of *P. rustica* coexisting with the target-species (*P. ulyssiponensis*, *P. depressa* or *S. pectinata*) within the same microhabitat (NEAR-only).
SP- *Siphonaria pectinata*			Total density of *S. pectinata* coexisting with the target-species (*P. ulyssiponensis* or *P. depressa*) within the same microhabitat.

PU_adjacent_Pool	Connectivity; other limpet species in an adjacent microhabitat; counts standardized to densities in 50x50 cm.	POOL only (sum of all size-class densities estimated for each of the other limpet species within POOL)	Total density of *P. ulyssiponensis* within POOL (adjacent to EDGE).
PD_adjacent_Pool			Total density of *P. depressa* within POOL (adjacent to EDGE).
PV_adjacent_Pool			Total density of *P. vulgata* within POOL (adjacent to EDGE).
SP_adjacent_Pool			Total density of *S. pectinata* within POOL (adjacent to EDGE).
PU_adjacent_Edge		EDGE only (sum of all size-class densities estimated for each of the other limpet species within EDGE)	Total density of *P. ulyssiponensis* within EDGE (adjacent to POOL or to NEAR).
PD_adjacent_Edge			Total density of *P. depressa* within EDGE (adjacent to POOL or to NEAR).
PV_adjacent_Edge			Total density of *P. vulgata* within EDGE (adjacent to POOL or to NEAR).
SP_adjacent_Edge			Total density of *S. pectinata* within EDGE (adjacent to POOL or to NEAR).
PU_adjacent_Near		NEAR only (sum of all size-class densities estimated for each of the other limpet species within NEAR)	Total density of *P. ulyssiponensis* within NEAR (adjacent to EDGE).
PD_adjacent_Near			Total density of *P. depressa* within NEAR (adjacent to EDGE).
PV_adjacent_Near			Total density of *P. vulgata* within NEAR (adjacent to EDGE).
PR_adjacent_Near			Total density of *P. rustica* within NEAR (adjacent to EDGE).
SP_adjacent_Near			Total density of *S. pectinata* within NEAR (adjacent to EDGE).

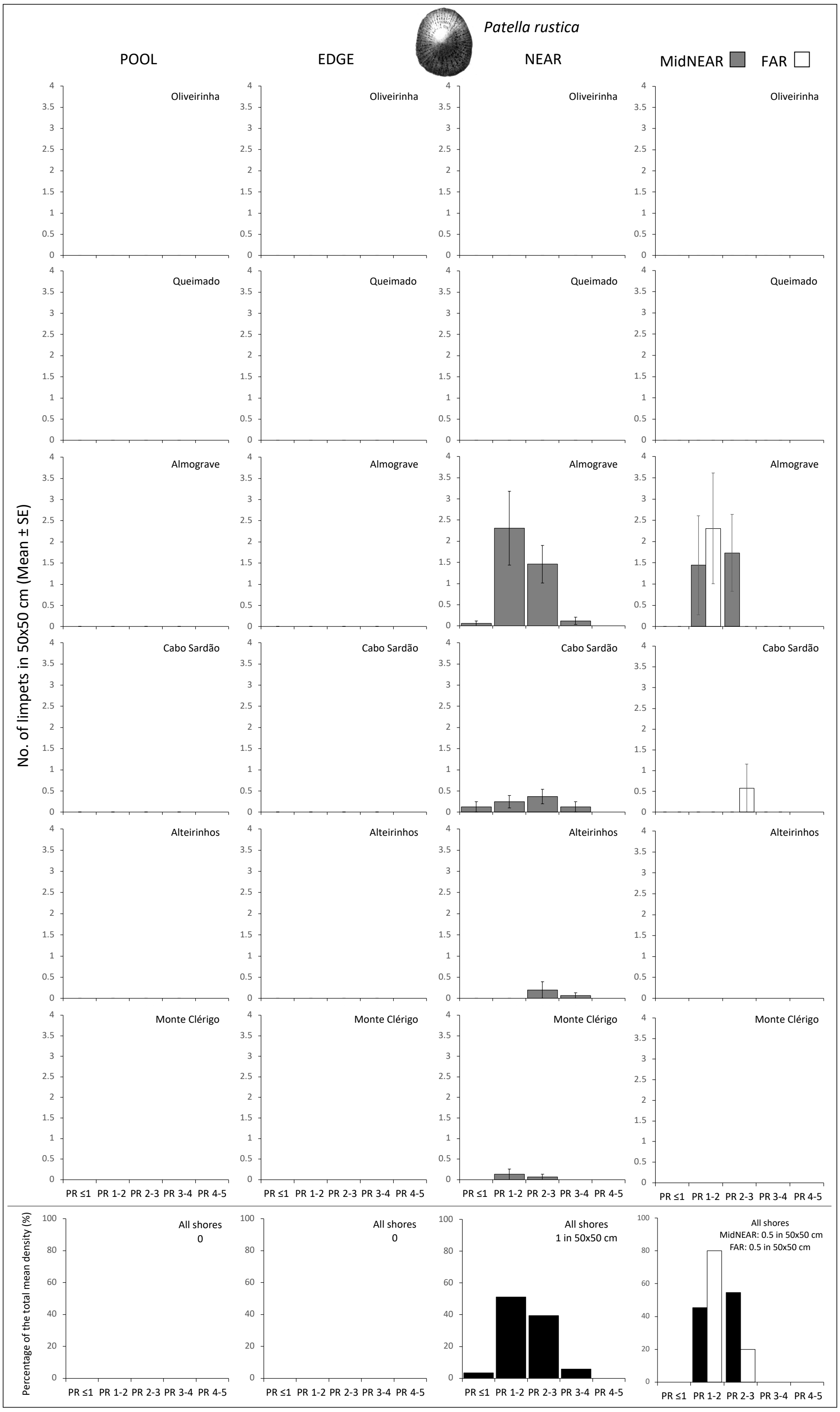
Supplementary Table S4.2 List of all predictive-variables used to build the distance-based linear model (DistLM) for each of the three species (*Patella ulyssiponensis* – PU; *P. depressa* – PD; *Siphonaria pectinata* – SP) within each of the three microhabitats (POOL; EDGE; NEAR). "Transf." refers to 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).

		9 Response-matrices **(3 limpet species x 3 microhabitats)** Each response-matrix: densities of 5 size-classes of a species within a microhabitat								
		POOL			EDGE			NEAR		
Predictor variables	Transf.	PU	PD	SP	PU	PD	SP	PU	PD	SP
Physical										
Maximum-depth *										
Mean-depth	sqrt	x	x	x						
POOL Topographic complexity	sqrt	x	x	x						
POOL Slope		x	x	x						
NEAR Topographic complexity	sqrt							x	x	x
NEAR Slope								x	x	x
Perimeter	sqrt	x	x	x	x	x	x	x	x	x
Surface area	sqrt	x	x	x	x	x	x	x	x	x
Volume	sqrt	x	x	x						
Circularity	sqrt	x	x	x	x	x	x	x	x	x
Aspect ratio	sqrt	x	x	x	x	x	x	x	x	x
Roundness		x	x	x	x	x	x	x	x	x
Straight distance to the nearest rock-pool	sqrt	x	x	x	x	x	x	x	x	x
Contoured distance to the nearest rock-pool	sqrt	x	x	x	x	x	x	x	x	x
Shore-height		x	x	x	x	x	x	x	x	x
Distance to low-water mark	sqrt	x	x	x	x	x	x	x	x	x
Barriers		x	x	x	x	x	x	x	x	x
Curved or Straight edges		x	x	x	x	x	x	x	x	x
Confinement		x	x	x	x	x	x	x	x	x
Habitat-composition										
Rock	sqrt	x	x	x				x	x	x
Sand	sqrt	x	x	x				x	x	x
Lichinaceae	sqrt							x	x	x
Verrucariaceae	sqrt	x	x	x				x	x	x
Crustose non-coralline algae	sqrt	x	x	x				x	x	x
CCA	sqrt	x	x	x				x	x	x
Articulated coralline algae	sqrt	x	x	x				x	x	x
Seaweed	sqrt	x	x	x				x	x	x
Porifera	sqrt	x	x	x				x	x	x
Sea-anemones	sqrt	x	x	x				x	x	x
Barnacles	sqrt	x	x	x				x	x	x
Cyanophytes	sqrt	x	x	x				x	x	x
Mussels	sqrt	x	x	x				x	x	x
Sea-urchins	sqrt	x	x	x						
Other sessile invertebrates	sqrt	x	x	x				x	x	x
Trochids		x	x	x				x	x	x
Littorinids	sqrt	x	x	x				x	x	x

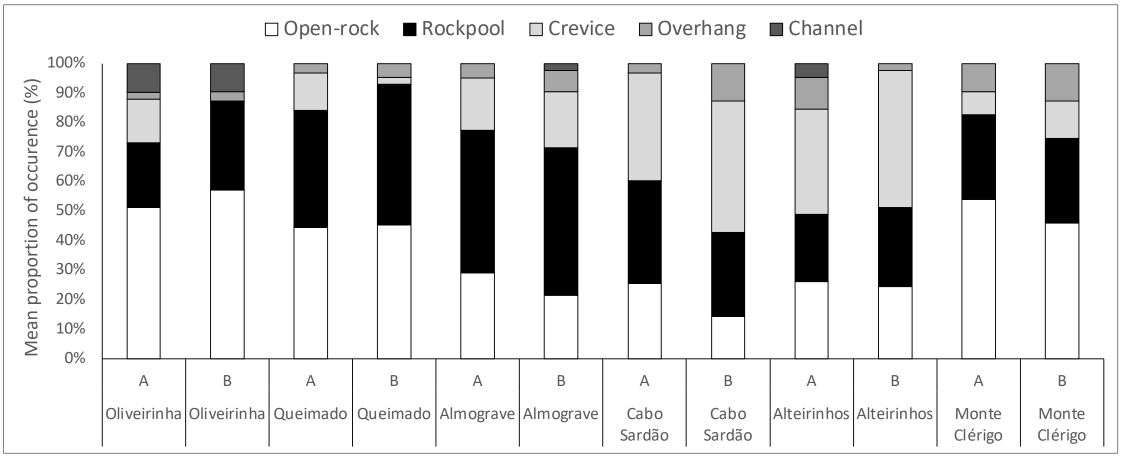
PU- *Patella ulyssiponensis*	sqrt		x	x		x	x		x	x
PD- *Patella depressa*	sqrt	x		x	x		x	x		x
PV- *Patella vulgata*	sqrt	x	x	x	x	x	x	x	x	x
PR - *Patella rustica*	sqrt							x	x	x
SP- *Siphonaria pectinata*	sqrt	x	x		x	x		x	x	
Connectivity										
PU_adjacent_Pool	sqrt				x	x	x			
PD_adjacent_Pool	sqrt									
PV_adjacent_Pool	sqrt				x	x	x			
SP-adjacent_Pool	sqrt				x	x	x			
PU_adjacent_Edge	sqrt							x	x	x
PD_adjacent_Edge	sqrt	x	x	x				x	x	x
PV_adjacent_Edge	sqrt	x	x	x				x	x	x
SP_adjacent_Edge	sqrt	x	x	x						
PU_adjacent_Near	sqrt				x	x	x			
PD_adjacent_Near	sqrt									
PV_adjacent_Near	sqrt				x	x	x			
PR_adjacent_Near	sqrt							x	x	x
SP_Adjacent_Near	sqrt				x	x	x			
Shore			x				x			



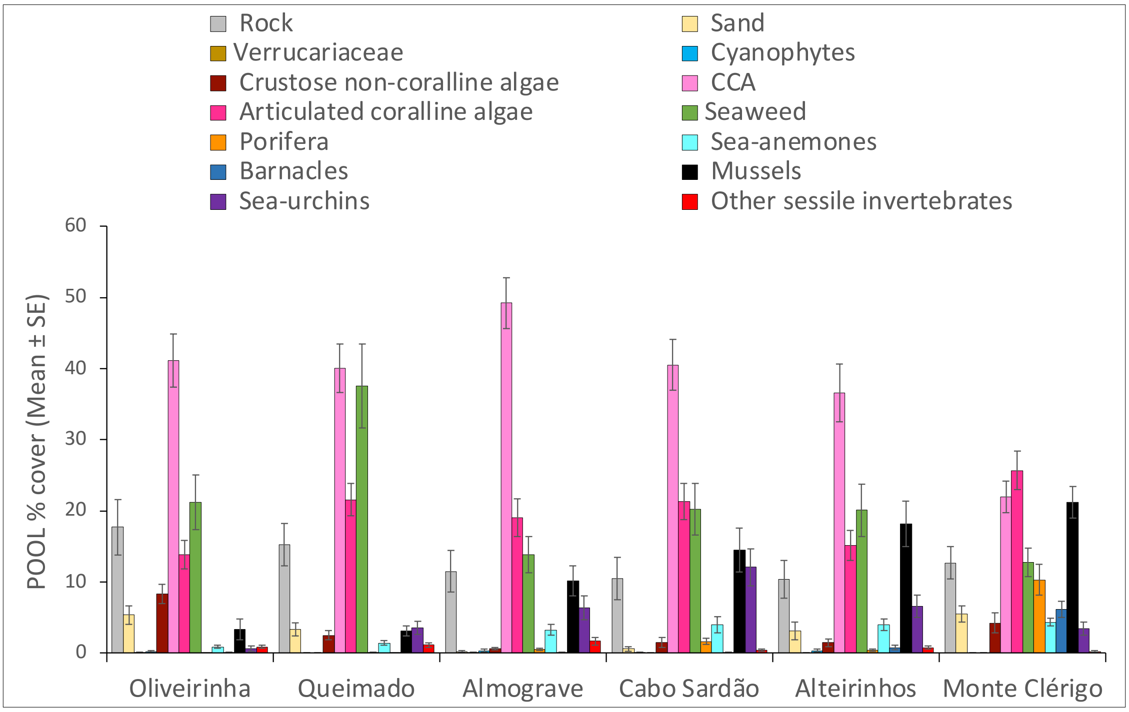
Supplementary Figure S4.1 Densities and size-class structure of *Patella vulgata* on six shores within POOL (inside rock-pools), EDGE (edge of rock-pools) and NEAR (open-rock adjacent to rock-pools) environments, and within areas of mid-shore open-rock at two proximity categories from rock-pools (MidNEAR and FAR). 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 4.2. Black and white bars (bottom row) are percentages of every size-class relatively to the total density of this species (value indicated on the top of each graph) within each environment and across all shores.



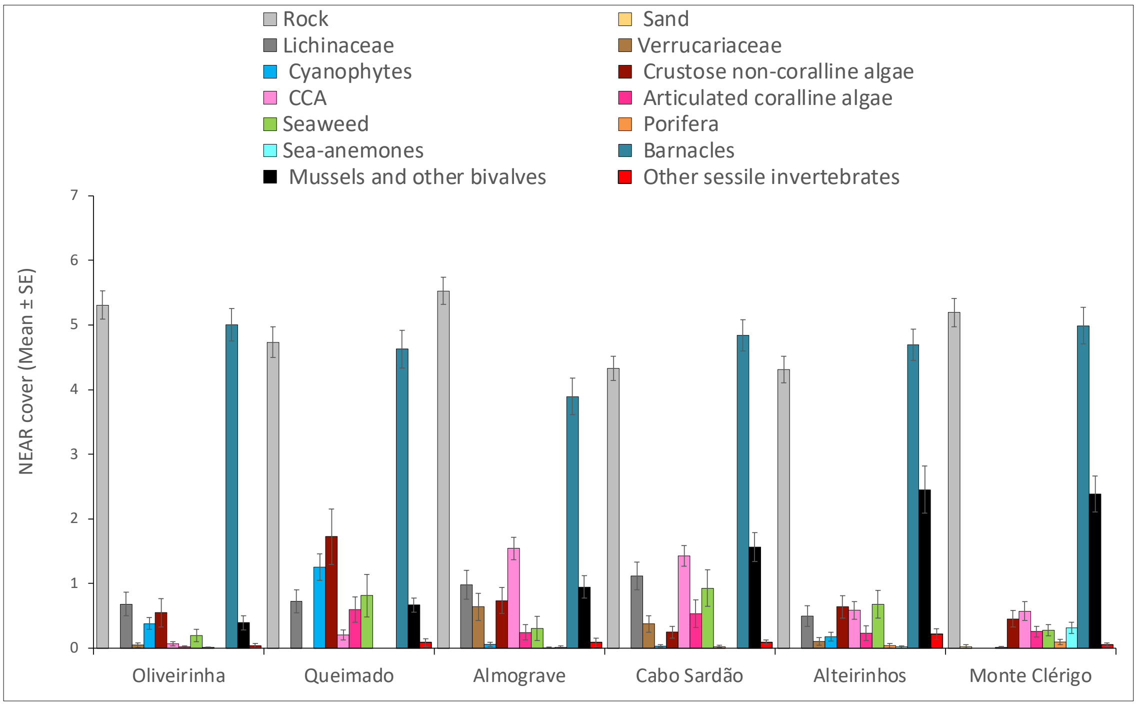
Supplementary Figure S4.2 Densities and size-class structure of *Patella rustica* on six shores within POOL (inside rock-pools), EDGE (edge of rock-pools) and NEAR (open-rock adjacent to rock-pools) environments, and within areas of mid-shore open-rock at two proximity categories from rock-pools (MidNEAR and FAR). Grey and white bars are mean (± SE) number of limpets of five size-classes per 50x50 cm; number of replicates indicated in caption of Fig 2. Black and white bars (bottom row) are percentages of every size-class relatively to the total density of this species (value indicated on the top of each graph) within each environment and across all shores.



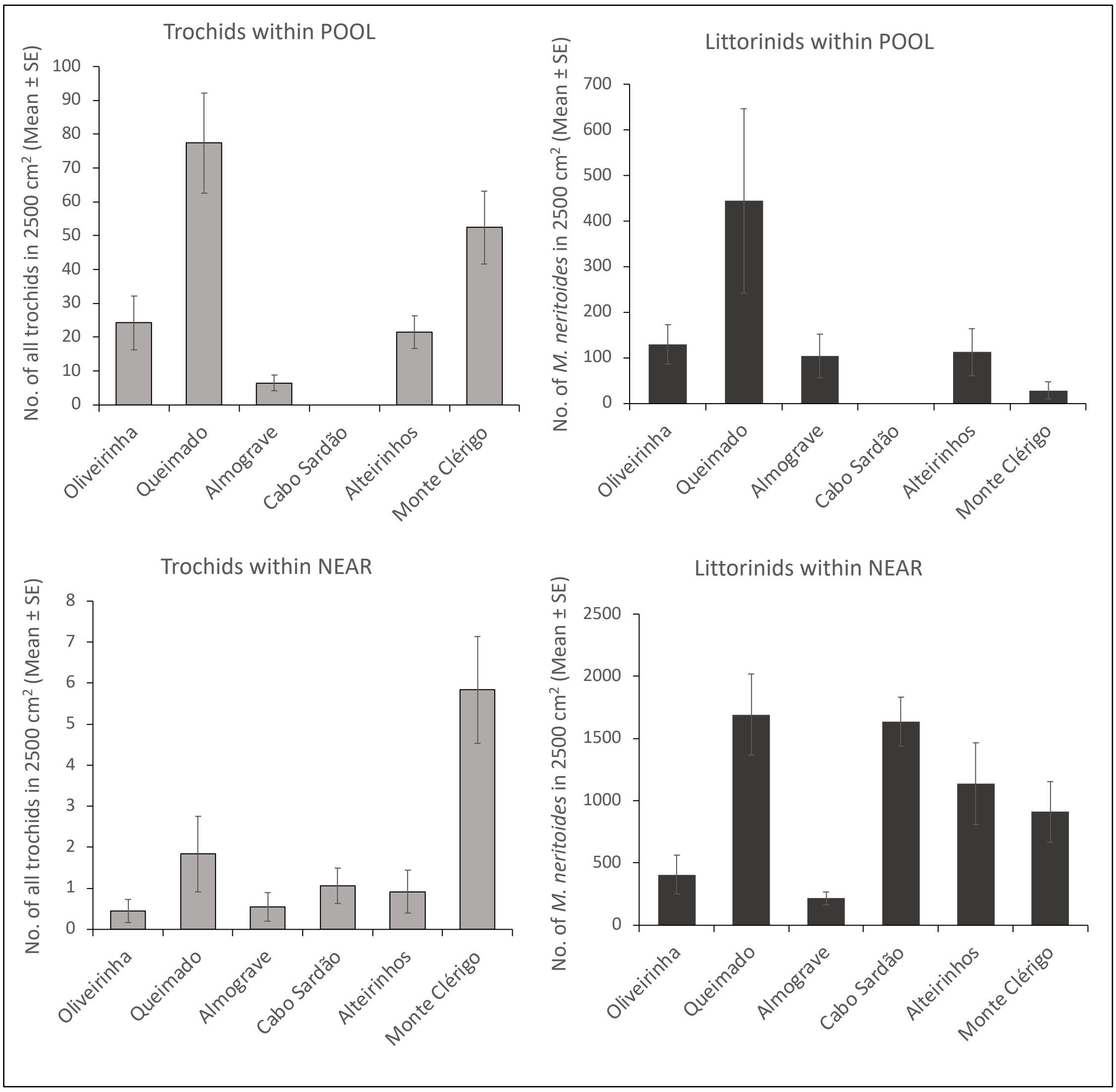
Supplementary Figure S4.3 Mean proportion (%) of occurrence of emersed open-rock, rock-pools, crevices, overhangs, and channels on the mid-shore of twelve coastal stretches (two random stretches, A and B, within each of six shores). Number of replicates within each stretch varied between 2 and 4 transects paralell to the sea.



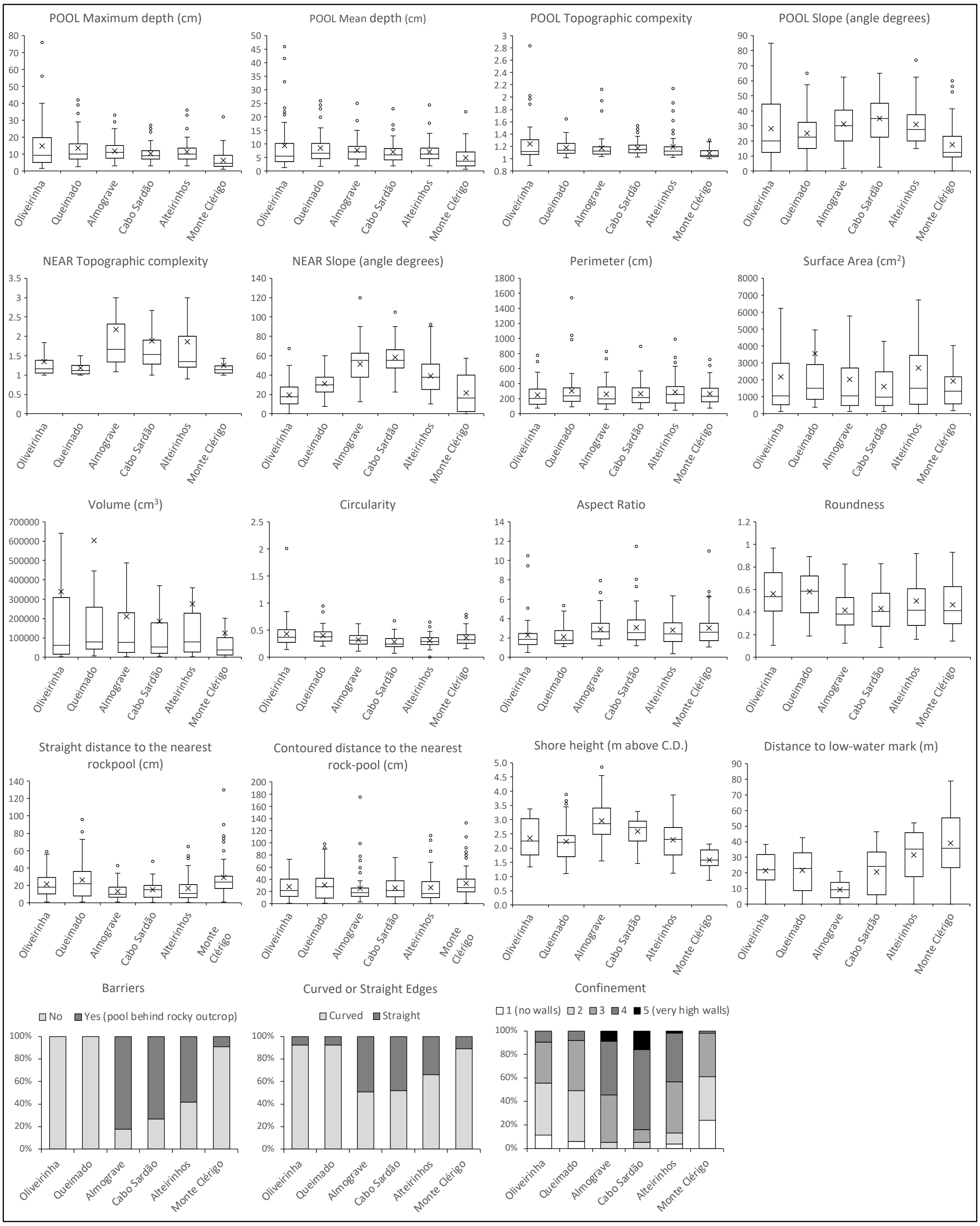
Supplementary Figure S4.4 Percentage cover of space-occupying categories within the POOL microhabitat. Values are mean % cover (± SE) of each substratum type or functional group of sessile organisms recorded inside rock-pools on six shores. The number of replicates in each shore was the following: 52 in Oliveirinha, 51 in Queimado, 57 in Almograve, 56 in Cabo Sardão, 53 in Alteirinhos and 54 in Monte Clérigo.



Supplementary Figure S4.5 Percentage cover of space-occupying categories (substratum types and functional groups of sessile organisms) within NEAR microhabitat. Values are mean (± SE) estimates assessed on open-rock surfaces of six shores using the following semi-quantitative index (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 S4.6 Densities (mean ± SE) of the total number of trochids (including the species *Steromphala umbilicalis*, *Steromphala pennanti*, *Phorcus sauciatus*, *Phorcus lineatus*) and the number of littorinids *Melarhaphe neritoides* within POOL and NEAR microhabitats. 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 S4.7 Physical variables of rockpool-systems on six shores of SW Portugal (see Table S4.1 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 microhabitats of rockpool-systems: inside-rockpools (POOL), rockpool-edges (EDGES), and surrounding emersed open-rock (NEAR).

APPENDIX

Seabra M.I., Cruz T., Jacinto D., Fernandes J., Espirito-Santo C., Castro J.J., Hawkins S.J. 2008. A Massive Settlement Event of Patellid Limpets in Artificial Tidepools (Sines, SW Portugal). 8th Larval Biological Symposium. Lisboa (Portugal), 6-11 Julho (Poster)

A MASSIVE SETTLEMENT EVENT OF PATELLID LIMPETS IN ARTIFICIAL TIDEPOOLS (SINES, SW PORTUGAL)

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Patellid limpets have a life history characterized by planktonic dispersal of larvae and a sedentary adult stage. Successful larval settlement and the emergence of limpet recruits on the shore depends on the conditions of early shore life [1]. Immediately post-settlement phases are non-detectable by naked-eye and the observation of Patellid settlers in nature have been rarely described in the literature (exceptions are [1,2,3,5]).

Here we report a massive event of settlement of limpets (*Patella* sp.) in the SW coast of continental Portugal. This was observed on a single episodic (so-far unrepeatable) occasion, in the region of the Port of Sines, a deep oceanic port with international importance.

Observations - Outside the Port of Sines, limpets are very common occupants on rocky shores; most abundant species are *Patella ulyssiponensis*, *P. depressa* and *Siphonaria pectinata* but *P. rustica* and *P. vulgata* can also occur. In these natural shores, juvenile limpets are found mostly in tidepools and damp/shaded microhabitats. In contrast, inside the port, almost all rocky shores consist of breakwaters composed by artificial blocks or by big boulders where tidepools and small crevices are rare and limpets (both adults and juveniles) are much less abundant.

Methods - We tested the hypothesis of different patterns of settlement of limpets in two locations: a natural shore outside the port (Oliveirinha) and an artificial shore inside the port (TGC - General Cargo Terminal); see Figure 1. Settlement of limpets was measured on both locations using artificial tidepools (AT), i.e. small collectors designed to retain seawater at low-tide. From February to April 2005, AT of various shapes, sizes and materials were deployed at mid-shore level as a pilot study to select the most suitable types of AT (see Fig. 2). In Oliveirinha, AT were set up both on open rock and in natural tidepools.

Figure 1 – Setting of sampling locations.

Figure 2 – Examples of artificial tidepools deployed during experiments outside (on the left: Oliveirinha) and inside (on the right: TGC) the Port.

Results and discussion

At the end of the experiment, recently-settled spat of Patellid limpets (early benthic-stage postlarval individuals with protoconch still attached; Fig. 3) were found in all AT present on both locations (mean density ± S.E.: 4.6 ± 0.32 ml^{-1}, N = 15 and N = 2 AT, outside and inside the port, respectively). Intensity of settlement did not differ significantly between the two locations (PERMANOVA: Pseudo-F = 1.352, p = 0.16, 136 unique permutations), neither between open rock vs. natural tidepool microhabitats in Oliveirinha (PERMANOVA: Pseudo-F = 2.4287, p = 0.11, 455 unique permutations). Among the types of AT tested, the highest densities of settlers were recorded in triple-grooved PVC panels and their maximum density was up to 20 ml^{-1}.

Regarding the size of limpet settlers (200-500 μm in maximum length of developing adult shell, Fig. 3), their post-settlement age was estimated to range from c. 2 to 12 days (M. A. Kendall, personal communication).

Settlement timing of these newly-arrived limpets overlapped annual active reproductive periods of *Patella ulyssiponensis*, *P. depressa* and *P. rustica* in Portugal [4]. Patelliform development was evident, with oriented displacement of the larval shell-cap relatively to the long axis of the adult shell [6]. Species identification was not accessible due to the absence of a diagnostic description of specific morphological characters in postlarval benthic stages. Genetic typing is being considered.

Similar experiments, with more replication in space and time, were carried out in 2005, 2006 and 2007, but no other event like this was detected. During these experiments, limpet settlement in AT was nil, although emerged recruits with calcified shell (≥ 1 mm) were observed in tri-monthly monitored natural tidepools, both inside and outside the port. Asynchronous breeding, with frequent small-intensity spawnings and continuous gonad redevelopment can influence settlement predictability and success and might have accounted for our difficulties in settlement detection.

Figure 4 – Size gradient of Patellid post-larvae settled in artificial tidepools. Note the presence of the protoconch, which becames progressively shed with increasing size of the adult shell.

Conclusion - Our results suggest that the differences in the abundance of limpets found inside and outside the Port of Sines are not caused by constrains on larval supply inside the port but most likely by the scarcity of suitable microhabitat for limpet settlement and/or by putative higher post-settlement limpet mortality inside this port.

References – [1] 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 P. G. Moore & R. Seed (eds.), The Ecology of Rocky Coasts; [2] Corpuz G.D., 1981. Settlement and emergence in a supratidal Hawaiian limpet, Cellana exarata Reeve (Prosobranchia: Patellidae). Pacific Science, 35: 265-266; [3] Gardner J.P.A. 1986. A method for the investigation of the shell structure of newly settled limpets. Journal of Molluscan Studies, 52: 35-37; [4] Guerra, M. T. & M. J. Gaudêncio, 1987. Aspects of the ecology of Patella spp. on the Portuguese coast. Hydrobiologia 142: 57- 69.; [5] McGrath D. 1992. Recruitment and growth of the blue-rayed limpet, Helcion pellucidum (l.), in South East Ireland. Journal of Molluscan Studies, 58: 425-431.; [6] Wanninger, A., Ruthensteiner, B., Dictus, W.J.A.G., Haszprunar G. 1999. The development of the musculature in the limpet Patella with implications on its role in the process of ontogenic torsion. Invertebrate Reproduction and Development, 36: 1-3

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