Universidade de Évora - Instituto de Investigação e Formação Avançada

Programa de Doutoramento em Biologia

Tese de Doutoramento

# The interplay between ecology and management of the stalked barnacle *Pollicipes pollicipes* (Gmelin, 1791 [in Gmelin, 1788–1792]) fishery

Alina de Sousa Marcelino

Orientadores | Teresa Paula Cruz
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Évora 2025

*…*

*See the line where the sky meets the sea? it calls me*

*And no one knows how far it goes*

*If the wind in my sail on the sea stays behind me*

*One day I'll know*

*If I go, there's just no telling how far I'll go…*

*How far I'll go*

*(Moana, Disney).*

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# Abstract

This thesis contributes to the knowledge of the ecology of the stalked barnacle *Pollicipes pollicipes* that can directly or indirectly be used to a better or more sustainable management of the fisheries and conservation of this important resource. All studies were developed on locations along the European distribution of this species, at different spatial scales.

A large-scale assessment of the abundance and size of *Pollicipes pollicipes*, using a standardised methodology, showed a strong north-south pattern in the Iberian Peninsula, consisting of a lower density of *P. pollicipes* in Asturias and Galicia (Spain), comprising larger animals, and a higher density of barnacles on the SW coast of Portugal, consisting of smaller individuals.

*P. pollicipes* growth rate was assessed for the first time at a large-scale. A higher growth rate was registered in Galicia comparing to the growth rate of this species in Brittany, Asturias and SW Portugal regions, namely in the juveniles size class, which can indicate that this species can reach sexual maturity faster in Galicia than in the other regions.

The study of the phenotypic variability that affects *P. pollicipes* quality and commercial value detected significant differences between the morphometry of the two extreme phenotypes (more elongated barnacles are associated with bad quality and have lower commercial value), but no evidence of genetic (using the amplified fragment length polymorphism – AFLP method) or epigenetic (using the methylation sensitive amplification polymorphism – MSAP method) variation were found. The main causes mentioned by fishers for this variation were related to the characteristics of the rock and the hydrodynamics. Potential drivers explaining this variation were tested in the field through a manipulative experiment. Density of *P. pollicipes* at the clump scale and microhabitat conditions can affect *P. pollicipes* morphology.

The timing of exploitation (summer, autumn or spring) had no effect on the abundance and recovery of *P. pollicipes* after harvesting, but regional differences were found between two experimental sites

(Berlenga and Sines) where a field manipulative experiment was carried out. A slower recovery potential after harvesting was detected in Berlenga.

The local ecological knowledge of the fishers of Berlengas was used to classify the state of *P. pollicipes* and the state of this fishery. An acceptable state of this fishery management and an increasing acceptance of the possibility of implementation of a co-management system was revealed.

Overall, the present thesis highlights the importance of assessing ecological patterns at a regional-scale, and of integrating the local ecological knowledge of the fishers, for a more sustainable management of this resource and of its fisheries.

**Keywords:** stalked barnacle, regional variability, ecological knowledge, commercial quality, epigenetic variation

# Resumo

**A interação da ecologia com a gestão da apanha do percebe *Pollicipes pollicipes***

A presente tese contribui com conhecimento ecológico sobre o percebe *Pollicipes pollicipes* que pode ser direta ou indiretamente aplicado a uma melhor e mais sustentável gestão e conservação deste importante recurso. Todos os estudos aqui apresentados foram desenvolvidos em locais ao longo da distribuição Europeia desta espécie, a diferentes escalas espaciais.

A variação da abundância e tamanho de *P. pollicipes* a uma larga escala espacial foi realizado utilizando uma metodologia standardizada, tendo revelado um padrão espacial norte-sul na Península Ibérica, em que uma menor densidade foi observada nas Asturias e na Galiza (Espanha), maioritariamente composta por indivíduos grandes, e uma maior densidade foi observada na costa SW de Portugal, essencialmente composta por indivíduos pequenos.

O crescimento de *P. pollicipes* foi estudado pela primeira vez a uma larga escala espacial. Uma maior taxa de crescimento foi registada na Galiza quando comparada com a taxa de crescimento obtida nas regiões da Bretanha, Astúrias e SW Portugal, nomeadamente na classe dimensional dos juvenis, o que poderá indicar que esta espécie na Galiza atinge a maturidade sexual mais rapidamente do que nas restantes regiões amostradas.

O estudo da variabilidade fenotípica que afeta a qualidade e o valor comercial de *P. pollicipes* detetou diferenças significativas entre as morfometrias dos dois fenótipos extremos (percebes mais longos estão associados a uma menor qualidade e menor valor comercial), mas não foi detetada variação genética (usando o método 'AFLP') ou epigenética (usando o método 'MSAP') entre estes dois fenótipos. As possíveis causas desta variação fenotípica referidas pelos apanhadores estão relacionadas com características das rochas e com o

hidrodinamismo. Foram testados potenciais fatores responsáveis por esta variação fenotípica de uma experiência manipulativa no terreno. A densidade dos grupos de percebes, bem como as condições do microhabitat podem afetar a morfologia de *P. pollicipes*.

O momento em que se efetuou a exploração de *P. pollicipes* (verão, outono e primavera) não teve efeito na abundância e recuperação de grupos de percebes explorados de forma experimental. No entanto, foram detetadas diferenças regionais na abundância e recuperação de grupos explorados entre os dois locais onde foi instalada esta experiência manipulativa (Berlenga e Sines), tendo os grupos explorados na Berlenga apresentado um potencial de recuperação mais lento.

O conhecimento ecológico dos pescadores das Berlengas foi usado para classificar o estado do percebe e o estado desta pescaria. O estado da gestão desta pescaria foi considerado aceitável e os pescadores revelaram uma maior aceitação em relação à implementação de um sistema de cogestão na Reserva Natural das Berlengas.

De um modo geral, a presente tese realça a importância do estudo de padrões ecológicos a uma escala espacial regional, e da integração do conhecimento ecológico dos apanhadores de percebe na gestão desta pescaria, de forma a promover a sustentabilidade deste recurso e das suas pescarias.

**Palavras-chave:** Percebe, variabilidade regional, conhecimento ecológico, qualidade comercial, variação epigenética

# Contents

# **Chapter 1.** General introduction

Integrating ecological knowledge into the management of a fishery is fundamental for ensuring sustainable fishing. Understanding and even being able to predict the dynamics of a population can have a major impact in the conservation of marine biodiversity and the management of fisheries (Gebremedhin et al. 2021). The present thesis is focused on the interplay of ecology and management in the context of an exploited marine resource, the stalked barnacle *Pollicipes pollicipes*, contributing to the goals of SDG 14 (Life Below Water) to conserve and sustainably use the oceans.

Within the crustacean cirripedes of the genus *Pollicipes*, four species can be identified (Cruz et al. 2022): *Pollicipes polymerus* distributes along the north-eastern Pacific Ocean; *Pollicipes elegans* occurs in the tropical eastern Pacific Ocean; *Pollicipes pollicipes* inhabits the north-eastern Atlantic Ocean; and *Pollicipes caboverdensis* is an endemic species from the Cape Verde islands (Cruz et al. 2022). All *Pollicipes* species are exploited, and several fisheries were identified for each species, being *Pollicipes pollicipes* (**Fig. 1.1**) considered to be the most intensively exploited species, namely in Spain and Portugal (Cruz et al. 2022).

**Fig. 1.1** – The stalked barnacle *Pollicipes pollicipes* at “Reserva Natural das Berlengas”. Photo by David Mateus.

In order to provide an overview of the information on the ecology of *Pollicipes pollicipes* and its exploitation and management, the introduction to this thesis first presents a literature review and then a section where the structure of this thesis is presented. The literature review is divided in two main sub-sections. The first sub-section (1.1) reviews the information on the ecology of the stalked barnacle *Pollicipes pollicipes*, and the second sub-section (1.2) reviews the information on *Pollicipes pollicipes* fisheries and management strategies.

This literature review is mainly based on the review article of Cruz et al. (2022) on the "Pedunculate cirripedes of the genus *Pollicipes*: 25 years after Margaret Barnes' review", in which I was actively involved as a co-author. Sub-section 1.1 on the ecology of *P. pollicipes* summarises the information presented in Cruz et al. (2022), while section 1.2 on the fisheries and management of *P. pollicipes* is completely taken from the "Fisheries, management and conservation" section of Cruz et al. (2022), as it was the section in which I was most heavily involved. Whenever more recent information that was not included in Cruz et al. (2022) is available, it has been included in the present literature review.

## 1.1 The ecology of stalked barnacle *Pollicipes pollicipes*

### 1.1.1 Geographical distribution

Based on the most recent review of the genus *Pollicipes* of Cruz et al. (2022), the stalked barnacle *Pollicipes pollicipes* is mainly distributed along the north-eastern Atlantic Ocean, from Land's End peninsula, UK (50°4'N; Cruz et al. 2022) to Dakar, Senegal (14°38'N; Stubbings 1967, Fernandes et al. 2010) (**Fig. 1.2**).

On the European Atlantic coast, exploitable populations of this species are found in Brittany, France, on the north and north-west coast of Spain and on the west coast of Portugal (Cruz et al. 2022). Along the African Atlantic coast, this species occurs in Morocco, through Western Sahara, Mauritania, until Senegal (Cruz et al. 2022). *P. pollicipes* is also present in the Canary Islands, at

Tenerife (Marín & Luengo, 1998) and Fuerteventura (González et al. 2012). In the Mediterranean North African Coast, there are records in Algeria (Bachetarzi et al. 2016) and in the Western Mediterranean Sea at Isla the Alborán in the Alboran Sea (Mas et al. 1996).

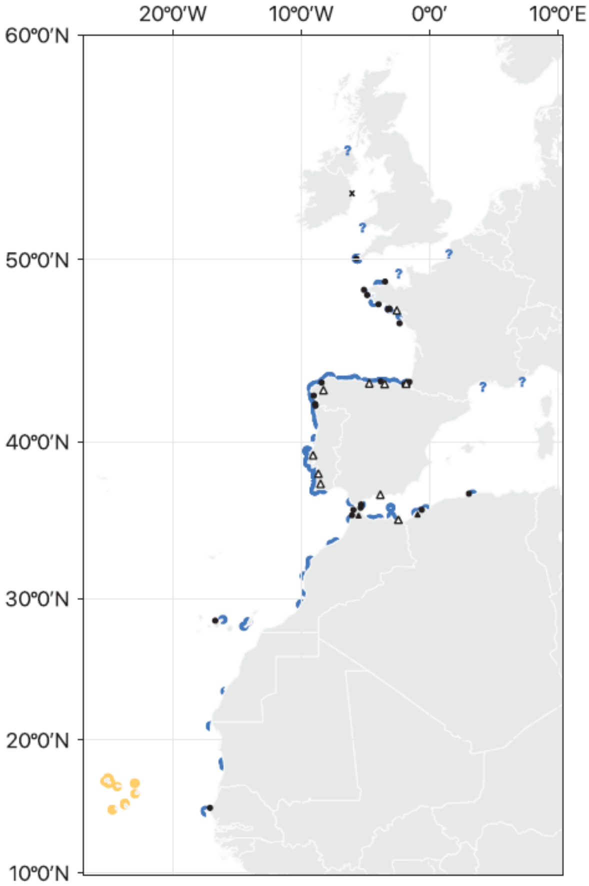


**Fig. 1.2**. Figure taken from Cruz et al. (2022) on the geographic distribution of *Pollicipes pollicipes*. Map with georeferenced sites where *P. pollicipes* was detected or sampled, based on published information after Barnes (1996) or not referred to in Barnes (1996), in personal observations and communications and on websites with geographical information (for more specific information see Cruz et al. 2022). Symbols: • *P. pollicipes*; • *P. caboverdensis*; • sites mentioned in Barnes (1996); × sites mentioned in Barnes (1996) that were considered an error; "?" dubious records of *Pollicipes*; ▲ Pre-historic shell middens where *Pollicipes* were found and cited by Barnes (1996); **Δ** Pre-historic shell middens where *Pollicipes* were found after Barnes (1996). Projected coordinate system used: WGS 84/Pseudo-Mercator (EPSG: 3857).

### 1.1.2 Abundance, size, and habitat

As all the other *Pollicipes* species, *Pollicipes pollicipes* is distributed on wave-exposed rocky coasts (Sousa et al. 2013, Cruz et al. 2022), mainly on vertical or steep-sided rocks that face a strong wave action (Macho 2006, Boukaici et al. 2015), but also in less wave-exposed areas with a constant water turbulence (Barnes 1996). This species is also found on crevices, rock fissures and caves (Cruz 2000, Fernandes et al. 2010). The only record of *Pollicipes pollicipes* in other substrata than rock is from a population on a ship stranded on a sandy beach in Nouakchott, Mauritania (Joana Fernandes personal communication).

*Pollicipes pollicipes* is an intertidal species that distributes mainly in the mid-shore (Cruz 2000, Macho 2006, Fernandes et al. 2010) being also present in low-shore (e.g. Cruz 2000, Macho 2006, Sousa et al. 2013), with their occurrence extended to the shallow subtidal zone (Cruz 2000, Borja et al. 2006a, b). The maximum upper limit recorded for this species is 4-5m above chart datum (SW Portugal, Cruz 2000) and the lowest record at 100 m of depth in channels (Barnes 1996). This species presents a gregarious distribution, forming clumps of varying sizes.

The assessments of quantitative abundance in *P. pollicipes* populations have been done locally, being accessed either by measuring percentage cover (Spain – Borja et al. 2006a, Parada et al. 2012, Bidegain et al. 2017; Portugal – Sousa et al. 2013, Jacinto & Cruz 2016, Neves 2021) or by quantifying their number and/or weight of individuals per unit area (Spain – Borja et al 2006a, b, Bidegain et al. 2017; Portugal – e.g. Sousa et al. 2013, Cruz et al. 2015b, Neves 2021; Morocco – Boukaici et al. 2012, Bourassi et al. 2019). As an example, the estimated *P. pollicipes* biomass ranged between 1.3 and 7.7 $Kg/m^2$ on mid-shore and from 0.5 to 2.4 $Kg/m^2$ on low-shore populations within three regions in Portugal (Sousa et al. 2013).

The studies that report this type of data were made to serve multiple objectives and used different methodologies. Some studies focus on *P. pollicipes* stock assessment surveys and on protocols for monitoring this species fisheries (e.g. Sousa et al. 2013, Parada et al. 2012, Boukaici et al. 2012), providing abundance data (e.g. Borja et al. 2006a) that can support the evaluation of the state of the resource (e.g. Cruz et al. 2015b) and the effects of management strategies (e.g. Borja et al. 2006a). As *Pollicipes* present a role as a habitat-forming species, the information on abundance has been also an important factor for the conservation of priority areas (Rubidge et al. 2020, Neves 2021).

The methodology that was used to estimate biomass/density in a more standardized way was based on estimates of relative abundance/biomass taken from destructive samples and corrected by the total area of *P. pollicipes* coverage, allowing the assessment of the total population and also its harvestable part (Sousa et al. 2013).

Recently a newer approach, using unmanned aerial vehicles (drones), was used to estimate the intertidal areas occupied by *P. pollicipes*, with very promising results to access *P. pollicipes* populations at central and SW coast of Portugal (Neves 2021). This new approach could be a very useful tool to obtain multiple abundance estimates at relevant scales, that can be used information for the management of this resource (Cruz et al. 2022).

Several post-settlement processes can influence *P. pollicipes* abundance and distribution, such as physical factors (e.g. air/water temperature, wave action), intra and/or inter-specific competition, and predation (Cruz et. al. 2022). As an exploited species, with important fisheries along its range (see section 1.2 of the present General introduction), these processes can also affect the recovery of this species after being exploited. A first approach on the study of *P. pollicipes* recovery after exploitation, in Asturias, indicates that this recovery is highly variable and slow (Geijer et al. 2024). The recovery of the gaps produced by *P. pollicipes* exploitation was easier when adult conspecifics were present in the margin of a gap (Gomez-del Campo, et al. in press).

### 1.1.3 Morphology

Externally, the morphology of *Pollicipes pollicipes* consists, as in all pedunculated cirripedes of a flexible peduncle that supports the capitulum. The capitulum is formed by a series of calcite plates, such as the scutum (S; paired), the tergum (T; paired), the rostro (R) and the carina (C) and several smaller plates that can be paired or unpaired (Newman 1987) and positioned between or below the mentioned plates. The peduncle consists of an elastic organic matrix, covered by calcareous scales or spicules, that is generally longer and narrower than the capitulum (Chaffe & Lewis, 1988).

Briefly, the internal morphology of *P. pollicipes,* based on Anderson (1994), Molares (1994) and Barnes (1996), consists of a body and cirri that are enclosed by the capitulum. The capitulum is formed by a bivalved carapace, and the aperture of the capitulum consists of the opening of the capitular valves. The cavity that is enclosed by the capitular plates is called mantle cavity and is where the body and cirri are located. The body is composed by a prosoma, thorax and a vestigial abdomen.

The most obvious phenotypic variability in *Pollicipes pollicipes* is the variation in the length of the peduncle and water content (Cruz et al. 2022). This phenotypic variation is associated to food quality by the harvesters and consumers (Parada et al. 2013). Two extreme forms are recognized in the Iberian Peninsula. One of the forms is described by several authors using terms/characteristics as: standard form and barnacles with a smooth peduncle (Galicia, Spain, Parada et al. 2012); barnacles with the peduncle with a greater amount of muscle (Asturias, Spain, Rivera et al. 2014); and barnacles with a large and short morphology (Portugal, Cruz et al. 2016b, Sousa et al. 2021). The other extreme form is described using terms/characteristics as: elongated form, barnacles with a thin and long morphology (Portugal, Cruz et al. 2016b, Sousa et al. 2021); or barnacles with a wrinkled peduncle (Galicia, Spain, Parada et al. 2012).

The barnacles with an elongated morphology are considered by the fishers and by the market as having low quality and a lower commercial value (Parada et al. 2012).

Several morphometric relations were used to describe this variation in the commercial quality of *P. pollicipes*, such as the relationship between the length, width and weight of the barnacle (Molares et al. 1987) and the ratio of the capitular base diameter to total height (Parada et al. 2012) or the ratio between maximal rostral-carinal length and total height (Cruz et al. 2016b). These morphometric relations present lower values for low-quality barnacles. The factors that can cause this variation in the peduncle length are not clear and more research is needed (Cruz et al. 2022).

### 1.1.4 Reproduction and recruitment

The study of the patterns of reproduction and recruitment of a species are extremely important to consider in its management, as they might directly influence the resource that can be available to be exploited.

The life cycle of *P. pollicipes* consists of two phases, comprising a planktonic larval phase (Molares et al. 1994, Kugele & Yule 1996) and benthic adults. Like all other *Pollicipes* species, *P. pollicipes* is a simultaneous hermaphrodite. Pseudo-copulation is the mating process of cross-fertilization that has been observed in *P. pollicipes* (Cruz et al. 2022), which consists in the release of sperm into the mantle cavity of a functional female. There is no evidence of self-fertilization for this species (Cruz & Hawkins 1998). The embryos are brooded inside the mantle cavity of the adults until hatching as naupliar larvae (Cruz et al. 2010). Then, the naupliar larvae are released into the sea. The planktonic stage consists of six naupliar stages and a final cyprid stage. The cyprid larvae settle on a substrate and after a final metamorphose become a sessile juvenile.  It is recognized that this species settles heavily on conspecifics (Barnes 1996), which promotes the gregarious distribution observed in this species.

The main breeding season of *P. pollicipes* considering all the studies developed along the European range of this species (Brittany, in France, Asturias and Galicia in Spain and in the SW coast of Portugal) consists of the summer months, with variations in extension and intensity along the geographic range (Aguión et al. 2022b, Cruz et al. 2022). The main recruitment season of *P. pollicipes* occurs from mid-summer until mid-autumn, also with variations observed along its range (Aguión et al. 2022a, Cruz et al. 2022). Regional differences of the interval between the beginning of the main reproduction season and the beginning of the recruitment season have been found along *P. pollicipes* European range, with a lower interval identified near the northern distribution and increasing through the south (Aguión et al. 2022a).

In the Iberian Peninsula, several factors have been identified that can influence the reproduction and recruitment patterns of *P. pollicipes*, such as air and sea water temperature, chlorophyll a concentration and upwelling events and their intensity (Cardoso & Yule 1995, Cruz & Hawkins 1998, Cruz 2000, Macho 2006, Fernandes et al. 2021, Aguión et al. 2022a, Nolasco et al. 2022).

### 1.1.5 Growth

Unlike most of the crustaceans, *Pollicipes pollicipes* does not grow by completely moulting of the exoskeleton, it grows by accretion of the plates in the capitulum and by increasing the length of the peduncle continuously throughout life (Anderson 1994).

The growth of *P. pollicipes* has been estimated by:  field measurements of *in situ* marked individuals (Cruz 21993); population size structure analysis (Cardoso 1998, Cruz 2000, Sestelo & Roca-Pardiñas 2007, Cruz et al. 2010, Sestelo & Roca-Pardiñas 2011, Boukaici et al. 2012); monitoring of size increment of individuals recruited on clear surfaces (natural or artificial; Cruz 2000, Cruz et al. 2010, Mateus 2015, Cruz et al. 2016a, b, Mateus 2017, Belela 2018, Santos 2019, Cruz et al. 2020); and estimates of the growth rates of chemically  marked individuals (Cruz et al. 2016a, b, Figueira 2015, Jacinto et al. 2015, Neves 2021).

The techniques used to individually mark *P. pollicipes* include mapping of the individuals in relation to marks made in the adjacent substrate or using individual marks (such as insect tags) glued to the capitular plates (e.g. Phillips 2005, Cruz et al. 2010). Those individual marking techniques often present some constrains to implement in the field, and the number of observations might be low. The development of a chemical marking technique (using calcein as a chemical marker), that allows mass marking of individual barnacles of different cohorts within a short period (e.g. Jacinto et al. 2015, Cruz et al. 2010, Neves 2021) was an important asset in the study of the growth rate of this species.

The growth of *P. pollicipes*, as of the other species of this genus, was considered highly variable at both temporal and spacial scales. The maximal length between the plates rostro and carina (RC) in the capitulum is often used as the best descriptor to access *P. pollicipes* size (Cruz 2000) and consequently to measure growth rate. The mean growth rates of juveniles (RC<15 mm) were considered higher (0.18 – 5.20 mm/month) and more variable, while of larger individuals (RC> 15 mm) is lower (0.08 – 0.48 mm/month) (data from Cruz et al. 2022). The estimates of growth rates available for *P. pollicipes* suggest a higher growth rate during the first year (with individuals reaching 11-17 mm RC), with most individuals reaching maturity within the first year (Cruz 2000, Cruz et al. 2010, Boukaici et al. 2012, Parada et al. 2012, 2013).

Several biotic, such as intertidal height or density, and abiotic factors, such as wave period, wind velocity and direction, are described in the literature as factors that can affect *P. pollicipes* growth rate, although the relative importance of each of those factors is difficulty to measure, as they can covary and interact (Cruz et al. 2022).

Being *P. pollicipes* an exploited species with a high economic and ecological importance, the study of *P. pollicipes* growth is of extreme importance, since this is a very relevant information to be incorporated in the fisheries management (Cruz et al. 2022).

## 1.2 The Stalked barnacle *Pollicipes pollicipes* fisheries and management

This section was completely retrieved from the "fisheries, management and conservation" section of Cruz et al. (2022), adding new information in the sub-section of Portugal, regarding recent information on the "Reserva Natural das Berlengas" fishery.

*Pollicipes pollicipes*

*Pollicipes pollicipes* is the only *Pollicipes* species that is heavily harvested throughout its range, wherever significant populations are present (i.e. France, Spain, Portugal and Morocco). The species has long been considered a seafood delicacy in Spain and Portugal, where it is the most important fishery in the rocky intertidal (Cruz et al. 2010, Aguión et al. 2022b). The main fishery is located in Galicia (Spain) (average of 333 t and 8.9 million € per year between 2015 and 2019), which is larger in terms of volume harvested and market value than all of the other *Pollicipes pollicipes* fisheries combined (Aguión et al. 2022b). In Brittany (France) and Morocco, this species is also extensively harvested, but, since it is rarely sold locally, most catches are exported to the

Iberian countries, where it costs much less than the locally fished species. In Western Sahara, Mauritania and Senegal, harvesting appears to be residual.

*Brittany, France*

The *Pollicipes pollicipes* fishery in Brittany is the third largest in the world, after Galicia and Portugal, in terms of landed weight (around 55 t in recent years, but with peaks over 100 t in the early 2000s), although its socio-economic relevance is much smaller than in the Spanish and Portuguese fisheries (Aguión et al. 2022b). Around 90 % of the landings in Brittany come from the department of Morbihan, with the rest from Finistère (Dominique Davoult, pers. comm.). In Morbihan, a co-management system has been implemented with around 50 harvesters involved. Fishers can harvest large amounts of barnacles per day (120 kg), the highest in any *Pollicipes* fishery, that nevertheless fetch a very low market value (5–8 €/kg) (**Table 1.1**) due to the lack of a local market, with almost everything being exported to Spain and Portugal. A similar system in terms of governance and management measures is found in Finistère, although at a much smaller scale (Table 1 and Dominique Davoult, pers. comm.). Despite the strong tradition in French cuisine for seafood (e.g. bivalves, gastropods and decapods), *Pollicipes pollicipes* has never been locally appreciated, which has prevented the development of a more locally significant fishery. In the 1970s, Spanish middlemen went to France, attracted by the amount of unharvested stock and the low prices, and today, the fishery is driven by Spanish demand. Consequently, this strong link with Spanish markets has created a trans-national poaching system, from France to Spain, due to the large respective differences in governance, control and surveillance, in the social structure of the fishery, and in the demand and market prices (Geiger et al. 2022).

*Spain*

In Spain, only commercial harvesting of *Pollicipes pollicipes* is allowed, while recreational fishing is forbidden, with the exception of a residual recreational fishery in the Basque Country. The species is mainly harvested in Galicia, but also in Asturias, Cantabria and residually in the Basque Country, while in the Canary Islands, its small fishery has been closed by the regional government since 2011, due to overfishing (‘*Order 2 Mayo, 2011, Gobierno de Canarias*’). *Pollicipes pollicipes* is a highly appreciated seafood in Spain, with an average first-sale price of 17–32 €/kg, which is much higher in premium areas (e.g. 65 €/kg in Cangas, Galicia), with record prices at Christmas up to 250–350 €/kg (Pescadegalicia.gal 2021).

Historically, this species has been commercially harvested in the NW of Spain since at least the 1930s (Dirección General de la Marina Civil y Pesca, 1935), without much regulation until its collapse in the 1970s and 1980s (Molares & Freire 2003), despite initial measures being introduced, such as a summer reproductive closure in Galicia (Goldberg 1984). Since the 1970s, and while the local stocks were becoming depleted, the large Spanish market demand was partly met through importation from France, Portugal, Morocco, and even Canada and Peru (Molares & Freire 2003). Imports to Spain from Canada and Peru continued until the 2000s, when they stopped for a combination of reasons: the difficulty of importing fresh product from so far away, the collapse of the Peruvian stocks and the recovery of the Spanish stocks. Nevertheless, importation from France, Portugal and Morocco was consolidated during the last two decades and continues until the present.

Barnes’ (1996) review concluded with the depletion of the Spanish populations of *Pollicipes*, and she noted the recent implementation of “strict conservation measures”. A profound change has occurred since that time, not merely through the implementation of new management measures, but mainly due to a totally new governance approach. This required the strengthening and empowerment of the fisher’s associations, who were granted exclusive access to the fishing beds under a comanagement approach (Molares & Freire 2003, Macho et al. 2013, Rivera et al. 2014, 2016a, Aguión et al. 2022b). The most prominent examples are Galicia and Asturias, with steps in this direction also taking place in other regions (Cantabria and Basque Country).

In Spain, several professional *Pollicipes pollicipes* fisheries currently operate in place in Galicia, Asturias, Cantabria and the Basque Country. The main management measures for each fishery are summarized in **Table 1.1** and include a maximum number of harvesting licences (limiting access to the fishery), minimum sizes, temporal and spatial closures (including no-take zones), and even individual daily quotas, fishing bed rotation and self-enforcement in the most developed fisheries in Galicia and Asturias, based on exclusive access to fishing grounds (*i.e.* Territorial User Rights for Fishing – TURF), which are granted to the fishers' organizations locally known as *cofradias* (Aguión et al. 2022b).

Galicia supports the main and oldest regulated *Pollicipes pollicipes* Spanish fishery. Historically, despite the secular tradition of the *cofradias* since the Middle Ages, shellfishing was mostly a *de facto* open access system until the 1990s (Macho et al. 2013). In 1992, the first co-management system in Spanish fisheries started in Galicia using TURF, where the responsibility for the exploitation was shared between the cofradías (fishers' guilds supervised by the regional government) and the fishery authorities (Molares & Freire 2003). This change opened new opportunities for innovation and improvement in the management system, following an adaptive process necessary to design and implement fishery management plans that have become mandatory since 1992 (Molares & Freire 2003). The management plans specify annually (triennially for the future 2022–2024 period) the different components of the management system: authorized fishers, fishing grounds, general objectives, state of the fishery and stock assessment analyses, harvesting and trade plans, actions for stock enhancement, and a financial plan (Macho et al. 2013). The *cofradias* have to design the management plan and seek approval from the regional fishery administration, who evaluates them. The introduction of management plans was a key step in the management of this fishery, and their numbers quickly grew, from 12 plans in 1992, to 29 in 2001 and to 37 in 2021 (Molares & Freire 2003, Aguión et al. 2022b), now covering all the fishing beds. The performance of the fisheries managed by the *cofradias* using the plans was generally positive, and the production (both in biomass and in economic value) showed an increasing trend, despite some isolated cases of overexploitation (Molares & Freire 2003). One key aspect when developing these plans, and in general for the management of the fishery, is the role of the biologist, directly working for the *cofradias* with government funding. This role matches the 'barefoot ecologist' concept (Prince 2003, 2010), who gives management advice and facilitates communication between stakeholders. Formally known as technical assistants, these biologists enable the provision of good-quality and organized fisheries data, to facilitate and support decision-making processes. They also build robust social capital, by acting as knowledge collectors and translators between fishers, managers and scientists (Macho et al. 2013). In 2018, there were 41 technical assistants in Galician cofradías, overseeing almost all *Pollicipes pollicipes* fishery management plans (our unpublished data). Another key aspect is that most of the *cofradias* have their own surveillance service, co-paid by the fishers. This effectively enforces the management measures internally, and externally promotes collaboration with the government fishery inspection service to avoid poaching by illegal fishers (Molares & Freire 2003). Stalked barnacle harvesters also participate in the enforcement activities personally, in coordination with the surveillance service (Aguión et al. 2022b). Furthermore, the *cofradias* also have the capacity to commercialize the catch as they generally manage the first-sale markets (Molares & Freire 2003), giving them strong economic status.

The stalked barnacle fishery is one of the most important artisanal fisheries in Galicia, from a socio-economic point of view (~1300 harvesters, 333 t and ~9 millions of € per year), although still far from the clam fishery, the largest artisanal fishery in Spain (~7100 fishers, ~7.900 t and ~74 millions of € per year) (Domínguez et al. 2021). Around 80 % of the harvesters access the intertidal fishing grounds by boat, and the rest by land (*i.e.* by car, on foot). The latter specialize in harvesting stalked barnacles, but the boat fishers also use other gear during the year, mainly octopus traps, depending on the market. Harvesters actively participate in all aspects of the management and share responsibilities with the administration in decision-making. The key decisions deal with the rotation scheme between fishing beds and the daily individual quotas allowed for each harvester, although the system is very flexible and adaptive to accommodate changes regarding new and unforeseen circumstances. The Galician stalked barnacle fishery has a very strong governance framework, focused on promoting participation by harvesters, which has rendered a very high number of sustainability attributes in a recent European stalked barnacle fisheries review (Aguión et al. 2022b).

In Asturias, there are two very different stalked barnacle fisheries, a co-management system in the west and a top-down system in the east. The former, as in Galicia, is a highly participatory system, based on adaptive management plans and exclusive access rights to the fishing beds (TURF) granted to the *cofradias*, who share responsibilities with the administration in the decision-making (Rivera et al. 2014). In Asturias-West, the eight management plans are subdivided into 250 zones, according to resource quality, and catch monitoring is done at this micro-/patch scale (from single rocks 3 m long up to 3.3 km extents of coastline) (Rivera et al. 2014). Such a detailed spatial scale is only possible due to the close collaboration between harvesters and managers (Rivera et al. 2014). Another key attribute of this fishery is the strong monitoring and control system (MCS) at various scales: (1) the official control and surveillance system from the regional government, (2) the presence of one enforcement officer on each of the cofradías with TURF, who are mainly focused on this fishery and (3) the direct involvement of the stalked barnacle harvesters in the surveillance and control activities (Rivera et al. 2014). Before the early 1990s, stalked barnacles in Asturias were only harvested sporadically, but in 1994, and led by the fisheries administration, a pilot TURF programme started in the *cofradia* of Ortiguera, which was expanded to seven *cofradias* by 2001 (Rivera et al. 2014). The system has received public approval, where 73 % of the stakeholders indicated that the only way to maintain a sustainable stalked barnacle fishery in Asturias is through the current management regime (Rivera et al. 2016a). Recently, the TURF system in Asturias has also been found to achieve high sustainability scores (Aguión et al. 2022b), where social factors (e.g. conflict resolution mechanisms and strong leadership) are the key drivers for the sustainability of this bottom-up management system (Rivera et al. 2019).

The other *Pollicipes pollicipes* fishery of Asturias, on the east coast, is a top-down limited-entry system. It has similar management measures (size limit and daily individual quotas), except that the open harvesting period is set from May to September, but with much less involvement of the harvesters in the decision-making, a much broader spatial scale of management and a much weaker MCS (Aguión et al. 2022b). This fishery has much less socio-economic significance (**Table 1.1**).

In Cantabria, a small top-down limited-entry system is in place in the stalked barnacle fishery. It is not clear how many fishers are involved, since it is not mandatory for the harvesters to be associated with any *cofradia*, but a regional census was established in 2018 (‘*Orden MED/25/2018, Gobierno* *de Cantabria*'). Since 2016, a daily reporting system requires all catches to go through official landing points so that catch statistics are available (annual landings of 4.6 t and average price of 22 €/kg) (Gorka Bidegain, pers. comm.). The fishery is managed based on three measures: a minimum size, a temporal closure and a spatial harvesting system with areas permanently open, seasonally closed and permanently closed (Bidegain et al. 2015, '*Orden MED/7/2021, Gobierno de Cantabria*'). In 2017, the regional government of Cantabria promoted a pilot co-management plan with some harvesters, but the lack of a united harvesters' association led to failure (Gorka Bidegain, pers. comm.).

Finally, in the Basque Country, a residual stalked barnacle fishery takes place. There are two management plans in Orio and Bakio under a co-management approach, with fewer than 10 harvesters involved and annual catches of only 0.1 t (Aguión et al. 2022b). In the rest of the region, a top-down open access system is in place for the small fishing beds available. The Basque Country is also the only region in Spain where recreational harvesting of stalked barnacles is allowed, although not in the areas of Bakio, Orio and the MPA Biotopo Protegido de San Juan de Gaztelugatxe (Borja et al. 2006b).

*Portugal*

In Portugal, there is a long tradition of exploiting *Pollicipes pollicipes* by professional and recreational fishers. The fishery is regulated differentially along the Portuguese mainland coast. The first Portuguese legislation relating specifically to this fishery was in 1989, when the '*Reserva Natural da Berlenga*' (called '*Reserva Natural das Berlengas*' after 1998, RNB, an archipelago in the central coast of Portugal) was created. At that time, the *Pollicipes pollicipes* fishery was totally banned in the RNB area. In 2000, the first specific regulation for this fishery in a marine protected area (RNB) was published, together with the first Portuguese general fisheries legislation with specific reference to *Pollicipes pollicipes* (Sousa et al. 2013). After 2000, several changes were made to these professional fishing regulations (RNB, modified in 2011; general legislation modified in 2006 and 2011). Specific regulation for this professional fishery was also created in another marine protected area, the '*Parque Natural do Sudoeste Alentejano e Costa Vicentina*' (PNSACV) in 2006, and modified in 2008 and 2011 (Sousa et al. 2013). Additionally, recreational harvesting with specific reference to *Pollicipes pollicipes* has been regulated since 2006 and changed in 2009, 2011 and 2014 (Cruz et al. 2015b). Consequently, three main *Pollicipes pollicipes* fisheries can be identified in Portugal: RNB and PNSACV, corresponding to two marine protected areas, and the rest of the mainland coast outside the marine protected areas (referred to as Portugal General, Aguión et al. 2022b). A large marine protected area where *Pollicipes pollicipes* harvesting is prohibited is the '*Parque Marinho Professor Luiz Saldanha*', a 38 km area of coast near to Cape Espichel, central Portugal (Sousa et al. 2013). The current management measures for each fishery are summarized in **Table 1.1**. They include temporal and spatial closures, individual quotas, minimum size (maximum distance between the carinal and rostral plates in relation to a given catch volume), a ceiling of harvesting licences (in all fisheries) and catch reporting in logbooks (in RNB and PNSACV) (Sousa et al. 2013, Aguión et al. 2022b). In Portugal, and in contrast to Spain, *Pollicipes pollicipes* is harvested both professionally and recreationally, with the exception of RNB and a few small areas in PNSACV, where recreational harvesting is prohibited (**Table 1.1**).

Official statistical data from the exploitation of *Pollicipes pollicipes* in Portugal are centralized and managed by the '*Direcao Geral de Recursos Naturais, Seguranca e Servicos Maritimos*' (DGRM). Most of the catches of this species are sold directly to intermediaries or final consumers, and not in official auctions. Until 2006, the official data did not include catches sold outside of auctions, which meant that the official statistics could not be considered representative of the amounts caught. Since 2006, professional fishers have also been required to report what they sell outside of auctions. Thus, the most recent official estimates are more representative of the professional fishing effort. However, it is known that there are many unreported catches, and there are also no records of the recreational fishing effort. The most recent statistical data available on this fishery in Portugal (2015–2019, unpublished data from DGRM) report an average of 456 licensed professional fishers and 136 t of annual catches (maximum of 146 t in 2016). This corresponds to a mean annual value of €1,622,131 and reveals a slight positive trend in the price of €10.1 $kg^{-1}$ in 2015 to €11.3 $kg^{-1}$ in 2019.

Nevertheless, the average first-sale prices charged by fishers, based on surveys conducted in 2013 (and 2018 only for RNB), was higher than these official data, being higher in the RNB than in other fisheries. The variation reported is as follows: RNB, €23.3 kg−1 (2013), €28.8 kg−1 (2018) (maximum of €173 kg−1 in 2013 and €100 kg−1 in 2018) (*n* = 32 in 2013, *n* = 39 in 2018); central coast, €14.4 kg−1 (maximum of €70 kg−1) (*n* = 26); PNSACV, €13.1 kg−1 (maximum of €168 kg−1) (*n* = 49) (Cruz et al. 2016; unpublished observations).

In a study of European *Pollicipes pollicipes* fisheries, of the three main Portuguese fisheries identified (RNB, PNSACV and Portugal General), the RNB fishery showed the highest levels of governance and sustainability attributes (based on Gutiérrez et al. 2011) (Aguión et al. 2022b). RNB was considered a bottom-up harvester-governed fishery at an intermediate sustainability level, while PNSACV and Portugal General scored low in sustainability, despite PNSACV being subjected to bottom-up governance. The rest of Portugal (Portugal General) has governance that was considered to be top-down. The classification of bottom-up versus top-down governance was based on a governance score obtained by summing the levels of four governance elements: spatial scale of management, co-management, access structure and participation of fishers (Aguión et al. 2022b). Several factors contribute to RNB having the highest sustainability classification among Portuguese fisheries: no recreational harvesting, being a marine reserve and being the first area in Portugal with a managed *Pollicipes pollicipes* fishery (Sousa et al. 2013), low accessibility (i.e. it is a group of islands), long-term professional licences granted in this fishery and a constant number of licences through time (Jacinto et al. 2011). Furthermore, several scientific projects and studies, which monitor the state of the resource and the state of management, have the participation of fishers (e.g. Sousa et al. 2013, Cruz et al. 2015b, Sousa et al. 2020, Neves 2021). In the RNB, a higher biomass of stalked barnacles (mid-shore, 7.7 kg/m2) and a higher proportion of adults with commercial value were observed when compared to other Portuguese fisheries (PNSACV and the central coast, data from 2011, Sousa et al. 2013). Recently, Portuguese commercial fisheries legislation has changed and now includes the possibility of implementing co-management (‘*Decreto-Lei n.o 73/2020*’). Consequently, a formal co-management system for the *Pollicipes pollicipes* fishery in RNB was implemented in 2021 (‘*Portaria n.o 309/2021*’). This is the first case of co-management of a fishery in Portugal. The first co-management plan for this fishery was approved in 2023 (‘*Portaria n.º 16/2023*’). Co-management of *P. pollicipes* fishery at RNB has become more cemented as all the necessary changes to the regulation to accommodate co-management in *P. pollicipes* fishery in RNB were recently finalized and approved by the ‘*Comité de cogestão para a apanha de percebe na Reserva Natural das Berlengas*’ (2024). This committee is the responsible for the management decisions in this fishery. The next step is the approval by the government of the proposed changes, Consequently, we have classified the management level of this fishery in Cruz et al. (2022) as ‘Co-management Mid-Level’, but with all the recent improvements we consider that it has progressed to ‘Co-management High-Level’.

An assessment of the state of the fishery and the management of *Pollicipes pollicipes* in RNB, PNSACV and the coastal area from Cape Carvoeiro to Cape Raso (in the central coast of Portugal, regulated by Portugal General legislation) was made in 2013 using different approaches (independent observations, enquiries to the fishers and logbook information) (Cruz et al. 2015b). This assessment has not been repeated in the PNSACV or the central coast, but there have been recent monitoring studies in RNB (Sousa et al. 2020, Neves 2021). An overall decline in the state of the fishery and conservation of this resource was observed in all regions in 2013, with the exception of a stable tendency detected in the PNSACV when using the enquiries approach. The worst situation was observed in the central coastal area. Reasons for this include the following: not being part of a marine protected area; less management measures in practice; no specific licences for exploiting barnacles in this area (Cruz et al. 2015b); and the fact that the maximum number of licences available for this coast has not yet been reached (‘*Direcao Geral de Recursos Naturais, Seguranca e Servicos Maritimos – DGRM*’ information, 2021). This diagnosis was also identified by Aguión et al. (2022b), where the Portugal General fishery, which includes the central Portuguese coast, was classified as low in sustainability. In the PNSACV, although also scoring low on sustainability (Aguión et al. 2022b), the *Pollicipes pollicipes* fishery is more regulated and prospects are more promising, as there is bottom-up involvement through consultative participation of the fishers in the management of the fishery (Aguión et al. 2022b). Consequently, the management level of the PNSACV was considered as 'incipient co-management' (**Table 1.1**). Furthermore, there are several characteristics of this fishery that might favour improvement in the current management and promote the sustainability of this activity, such as the existence of specific professional licences, a constant number of licences over time, and several associations that represent the fishers of this area. Studies conducted in the PNSACV recommend greater involvement of fishers and the local community in the management of *Pollicipes pollicipes* (Castro & Cruz 2009, Stewart et al. 2014, Cruz et al. 2015b, Carvalho et al. 2017). Based on public debates, surveys and information from professional fishers, the main problems of the RNB fishery are poaching and poor surveillance (Sousa et al. 2020, Geiger et al. 2022), while in the PNSACV (Stewart et al. 2014, Cruz et al. 2015a, b, Carvalho et al. 2017) and the central coast (Cruz et al. 2015b, 2016b), there is excessive exploitation, poaching, unorganized harvesting, lack of association and union among fishers, and insufficient surveillance.

*Morocco*

Of the fisheries that exist in Africa, a regulated fishery of *Pollicipes pollicipes* exists only in Morocco. According to Hakima Zidane from the laboratory '*Prospections des Ressources Littorales*', *Institut National de Recherche Halieutique* (INRH), Morocco (June 2021), the exploitation of this species is not a traditional activity in this country and local consumption of these barnacles is very limited. Boukaici (2015) described this fishery in the Mirleft region, southern Morocco. Hakima Zidane (pers. comm.) added that *Pollicipes pollicipes* is harvested all along the Atlantic coast, namely in Mansouria, Sidi Abed and Souiria Kdima, and that there are no fisheries on the Mediterranean coast. This fishery is regulated by several ministerial decrees (Bourassi et al. 2019), which include the establishment of the following management measures: seasonal closure (exploitation is allowed from 1st November to 31st May and prohibited from 1st June to 31st October), size limit (RC of 2.5 cm, since 2015) and licences for professional fishmongers (Hakima Zidane pers. comm.). Hakima Zidane (pers. comm.) stated that these professionals mainly sell barnacles for export to Spain and

Portugal and to a few five-star hotels in the Casablanca region. These professionals hire the services of an intermediary, who in turn sub-contracts the services of several fishers who collect the barnacles (Hakima Zidane pers. comm.). According to Hakima Zidane (pers. comm.), this fishery has increased in the last 10 years. The price charged by fishers at first sale is around €3 to €7 $kg^{-1}$, depending on the quality and the size of the barnacles, while the price charged by professional fishmongers is, on average, 60–80 DH/kg (~€6–€8 $kg^{-1}$) and can reach 120 DH per kg (~€12 $kg^{-1}$). Boukaici (2015) presented photographs of large quantities of barnacles stored in burlap sacks in the intertidal zone of the Bay of Agadir, illustrating the intermediate step of the sales circuit which precedes their export, carried out by professional fishmongers. According to Boukaici (2015) and Hakima Zidane (pers. comm.), poaching is the biggest threat to the fishery of *Pollicipes pollicipes* in Morocco.

*Western Sahara and Mauritania*

In the Western Sahara and Mauritania, there is indication of disturbance by *Pollicipes pollicipes* fishers at the Cape Blanco Monk Seal Colony (Fernández de Larrinoa & Cedenilla 2003). These fishers descend from the clifftops to harvest the barnacles in the intertidal zone, and although they do not interact negatively with the seals, they do cause disturbance in the locations occupied by these animals. Fernández de Larrinoa & Cedenilla (2003) determined through interviews with these fishers that this activity originated at a time when the territory was still a Spanish colony. At present, Pablo Fernández de Larrinoa (pers. comm.) considers that this unregulated fishery is not important in the Cape Blanco peninsula and that these barnacles are not consumed locally, being sold abroad. According to this researcher, it is currently forbidden to harvest *Pollicipes pollicipes* in the seal reserve.

*Senegal*

Although Senegal corresponds to the southern limit of distribution of *Pollicipes pollicipes* (see section 'Geographical distribution'), this species is considered to be an exploited species in this country

(‘*Direction des peches maritimes*’, Senegal, 2002). Although this fishery is not regulated, there are records of the sale of these barnacles in Senegal to foreigners (informal online information in 2021 of the sale at 3000 West African CFA franc per kg (~4.6 euros)).

**Table 1.1** - Table adapted from Cruz et al. (2022) on *Pollicipes pollicipes* fisheries identified by country, main management measures (for recreational and professional harvesting), management level, access type, number of fishers and official annual landings. References in this table include references used for building this table and other references found related to each fishery. DBC – diameter of the *capitulum* base, IQ-day – Individual quota per day, IQ-month – Individual quota per month, MLS – Minimum legal size, MPAs – Marine protected areas, NA – Not applicable, ND – No data, PNSACV – '*Parque Natural do Sudoeste Alentejano e Costa Vicentina*', SE – self-enforcement, RC – maximum distance between the *carina* and *rostrum* plates, Rotation – rotation of the harvesting areas, RNB – '*Reserva Natural das Berlengas*', TAC – Total allowable catch per year, TC – Temporal closure, TL – total length, TURF – Territorial use rights for fisheries, ? – We have doubts on the existence of a type of harvesting or we have a recent reference (personal communication, online information) that the species is exploited or sold, but no further data were obtained. *- Based on personal communications. In bold – alteration to the original table presented in Cruz et al. (2022), following the more recent information available for RNB.

| Species/Country/Fishery | *Recreational harvesting?* Main management measures | *Professional harvesting?* Main management measures | Management level | Access type | Number of fishers | Official annual landings (tonnes) | References |
|---|---|---|---|---|---|---|---|
| *France* | | | | | | | |
| Finistère | *Yes*<br>NTZs (areas with total and partial protections: Cap Sizun Special Protection Area), IQ-day (3 kg) | *Yes*<br>TC (Jul-Aug), NTZs (areas with total and partial protections: Cap Sizun Special Protection Area), IQ-day (90 kg) | Co-management<br>Mid-level | Limited entry | 18<br>(In 2020) | 5,6*<br>(Annual average estimated) | Aguión et al. (2022b), *'Comite Regional des Peches Maritimes et des Elevages Marins de Bretagne* (161-2020)', Dominique Davoult pers. comm. |
| Morbihan | *Yes*<br>NTZs (areas with total and partial protections), IQ-day (3 kg) | *Yes*<br>TC (Jul-Aug), NTZs (2 no-take areas in Groix Island), IQ-day (120 kg) | Co-management<br>Mid-level | Limited entry | 30<br>(On average from 2013 to 2016) | 50*<br>(On average from 2013 to 2016) | Aguión et al. (2022b), '*Comite Regional des Peches Maritimes et des Elevages Marins de Bretagne (181-2020)'* |
| *Spain* | | | | | | | |
| Orio and Bakio | *No*<br>NA | *Yes*<br>MLS (TL>40 mm = RC>17 mm), TAC (4 t) | Co-management<br>Mid-level | Limited entry | <10<br>(On average from 2013 to 2016) | 0,1<br>(On average from 2013 to 2016) | Aguión et al. (2022b) |
| Basque Country General | *Yes*<br>MLS (40 mm TL = 17 mm RC), TC (closed May-September), NTZs (Gaztelugatxe Marine Reserves), IQ-day (0,5 kg) | *Yes*<br>MLS (TL>40 mm = RC>17 mm), NTZs (Gaztelugatxe Marine Reserve) | Top-down | Open access | ND | ND | Bald et al. (2006), Borja et al. (2006a, b), Aguión et al. (2022b) |

**Table 1.1** - Continued

| Species/Country/Fishery | *Recreational harvesting?* Main management measures | *Professional harvesting?* Main management measures | Management level | Access type | Number of fishers | Official annual landings (tonnes) | References |
|---|---|---|---|---|---|---|---|
| Cantabria | *No*<br>NA | *Yes*<br>MLS (RC>18 mm), TC (May-Sep), NTZs (three types of protection regimes; permanently open, seasonally closed, and permanently closed -Sonabia-) | Top-down | Limited entry | ND | 4,6<br>(On average from 2019 to 2020 – before 2019 landings were very low due to misreporting) | Gutiérrez-Cobo & Bidegain. (2012), Bidegain et al. (2015, 2017), '*Orden MED/15/2020, de 20 de julio*' |
| Asturias East | *No*<br>NA | *Yes*<br>MLS (RC>18 mm), TC (Oct-Apr), IQ-day (5-8 kg) | Top-down | Limited entry | 234<br>(On average from 2013 to 2016) | 11<br>(On average from 2013 to 2016) | Rivera et al. (2013, 2014), Rivera (2015), Rivera et al. (2016a, b, c, 2017, 2019), Aguión et al. (2022b) |
| Asturias West | *No*<br>NA | *Yes*<br>MLS (RC>18 mmC), TC (May-Sep), Rotation, IQ-day (5-8 kg), SE | Co-management<br>High-level | TURF | 204<br>(On average from 2013 to 2016) | 44<br>(On average from 2013 to 2016) | Aguión et al. (2022b) |
| Galicia | *No*<br>NA | *Yes*<br>MLS (DBC > 15 mm = RC > 18.3 mm), rotation, IQ-day (3–10 kg), SE | Co-management<br>High level | TURF | 1308<br>(On average from 2013 to 2016) | 333<br>(On average from 2015 to 2019) | Freire & Garcia-Allut (2000), Molares & Freire (2003), Molares et al. (2008), Navarrete (2009), García-Negro et al. (2009), Parada et al. (2012, 2013), Macho et al. (2013), Vázquez-Rowe et al. (2013), Pita et al. (2019), Aguión et al. (2022b) |
| Canary Islands | *No*<br>NA | *Yes*<br>But closed since 2011 | Top-down | ND | ND | ND | Marín & Luengo (1998), '*Orden 2 de mayo de 2011*' |

**Table 1.1** - Continued

| Species/Country/Fishery | *Recreational harvesting?* Main management measures | *Professional harvesting?* Main management measures | Management level | Access type | Number of fishers | Official annual landings (tonnes) | References |
|---|---|---|---|---|---|---|---|
| *Portugal* | | | | | | | |
| RNB | *No*<br><br>NA | *Yes*<br><br>MLS (RC≥23 mm– at least in 50% of the volume), TC (Jan-Mar & Aug-Sep), NTZs, IQ-day (20 kg) | Co-management<br><br>**High-level** | Limited entry | 40<br><br>(Maximum allowed) | 16<br><br>(On average from 2015 to 2019) | Aguión et al. (2022b), Albuquerque (2014), Albuquerque et al. (2016), Cruz et al. (2015c), Jacinto et al. (2010, 2011), Neves (2021), Sousa et al. (2013, 2020) |
| PNSACV | *Yes*<br><br>MLS (RC≥20 mm – at least in 75% of the volume), TC (15 Sep – 15 Dec), NTZs, IQ-day (2 kg) | *Yes*<br><br>MLS (RC≥20 mm – at least in 75% of the volume), TC (15 Sep – 15 Dec), NTZs, IQ-day (10-15 kg) | Incipient Co-management | Limited entry | 80<br><br>(Maximum allowed) | 16,4<br><br>(On average from 2007 to 2012) | Aguión et al. (2021), Carvalho et al. (2017), Castro (2004), Castro & Cruz (2009), Costa (2012), Cruz (2000), Cruz et al. (2015c), Diogo et al. (2020), Jacinto (2016), Jacinto & Cruz (2016), Sousa et al. (2013), Jesus (2004), Penteado (2011), Stewart (2010) |
| Portugal General | *Yes*<br><br>MLS (RC≥20 mm – at least in 75% of the volume), TC (15 Sep – 15 Oct), IQ-day (2 kg),<br><br>NTZs (Marine Park – Parque Marinho Luíz Saldanha) | *Yes*<br><br>MLS (RC≥20 mm – at least in 75% of the volume), TC (15 Sep - 15 Oct), IQ-day (20 kg), NTZs (Marine Park – Parque Marinho Luíz Saldanha) | Top-down | Limited entry | 456 | 136<br><br>(On average from 2015 to 2019 - Including RNB & PNSACV) | Sousa et al. (2013), Cruz et al. (2015b), Aguión et al. (2022b) |
| *Morocco* | *?*<br><br>ND | *Yes*<br><br>MLS (RC>25mm), TC (Jun-Oct) | Top-down | Limited entry | ND | ND | Boukaici et al. (2012, 2015), Boukaici (2015), Bourrassi et al. (2019), Hakima Zidane (pers. comm.) |

**Table 1.1** - Continued

| Species/Country/Fishery | *Recreational harvesting?* Main management measures | *Professional harvesting?* Main management measures | Management level | Access type | Number of fishers | Official annual landings (tonnes) | References |
|---|---|---|---|---|---|---|---|
| *Western Sahara & Mauritania* | *?*<br>NA | *Yes*<br>Mainly unregulated | Mainly unregulated and unreported | ND | ND | ND | Fernández de Larrinoa & Cedenilla (2003), Pablo Fernández de Larrinoa (pers. comm.) |
| *Senegal* | *?*<br>NA | *Yes*<br>Mainly unregulated | Mainly unregulated and unreported | ND | ND | ND | '*Direction des pêches maritimes − Rapport statistique 2002 − La mer: richesse et avenir du Sénégal*'; online information of selling barnacles |

## 1.3 Aims and structure of the thesis

The main aim of this thesis is to contribute with fundamental knowledge about the ecology of the stalked barnacle *Pollicipes pollicipes* that can be used to a better and more sustainable management of the fisheries of this species.

In addition to the present chapter 1, which includes the literature review, this thesis is organised into five more chapters (2 to 6) where experimental work was carried out and a final chapter of general conclusions and final remarks (chapter 7). Some of these studies have already been published (the paper in chapter 4.1 and a paper that corresponds to chapter 6), are in the process of revision (the paper in chapter 4.2) or are in preparation (manuscripts that correspond to chapters 2, 3 and 5). The manuscripts "in preparation" presented in this thesis were not reviewed by all the co-authors.

In chapters 2 and 3, we studied how the abundance, biomass and size (chapter 2), as well as the growth rate (chapter 3) of *P. pollicipes* varied on a European scale, including sampling locations in France, Spain and Portugal. This geographical distribution area comprises the most relevant *P. pollicipes* fisheries, that present several differences on fishing efforts and respective management strategies (see sub-section 1.2 The stalked barnacle *Pollicipes pollicipes* fisheries and management). Here, we have used for the first time a standardised methodology to estimate the abundance, biomass, size and growth rate of *P. pollicipes* across several fisheries within the *P. pollicipes* European range. This ecological knowledge can be directly or indirectly used to understand the exploitation patterns of these fisheries, as well as applied to their management strategies. Both manuscripts of Chapter 2 and 3 are "in preparation", chapter 2 is entitled 'Abundance and body size of the exploited stalked barnacle *Pollicipes pollicipes* along its European distribution: an inverse pattern of density and size along Iberia' and chapter 3 'Assessing growth rates of the stalked barnacle *Pollicipes pollicipes* across its European distribution range: higher growth of juveniles in Galicia'. These studies were developed as part of a European project ("PERCEBES-Tools for the transition to spatial management of coastal resources: the stalked barnacle fishery in SW Europe", funded by BiodivERsA) that facilitated the interaction among scientists from various countries. These two studies were not included in the original proposal of the project.

Chapter 4 comprises two studies on the topic of *Pollicipes pollicipes* phenotypic variability. Two extreme phenotypes can be identified. More elongated barnacles are associated with bad quality and have lower commercial value. So, describing this pattern of morphological variation and understanding its nature is of fundamental importance and could have a direct influence in the management of these fisheries. We began by investigating how Portuguese and Galician fishers described these two morphotypes and how they explained this variation, then whether the morphological patterns corresponded to genetic or epigenetic patterns, based on barnacles sampled in Asturias and Galicia (Spain) and in Portugal (published paper 'Phenotypical variability affecting the commercial value of the stalked barnacle *Pollicipes pollicipes*: no evidence for epigenetic variation' Sousa et al. (2024)). Within this problematic, we also investigated whether the fishers perception of this variation was the same as that of the scientists and whether there was a variation in biochemical composition between the two extreme morphotypes, and finally we investigated the influence of two potential drivers (density and microhabitat) of the *P. pollicipes* morphology variation through a manipulative experiment (paper in review 'Morphological and commercial quality variation of the stalked barnacle *Pollicipes pollicipes* (Gmelin, 1791 [in Gmelin, 1788–1792]): patterns and drivers').

Chapter 5 tests the hypothesis that the timing of exploitation may have an effect on the abundance of barnacles after harvesting. Harvesting was experimentally manipulated in clumps of barnacles. Control areas with groups of unmanipulated clumps of barnacles were also considered. This hypothesis was tested through a manipulative field experiment at two sites (Cape of Sines and Berlengas Nature Reserve) with different types of governance. This experiment was carried out on two vertical levels of the intertidal zone and was monitored up to two years after the experimental harvesting. The results from this study are fundamental ecological knowledge that can be applied on fisheries management, namely on the pertinency of the timing of temporal closures. As the different exploitation timings are related to different phases of the biological cycle of *P. pollicipes* (spring-reproduction and no recruitment; summer- reproduction and recruitment; autumn- no reproduction and recruitment), if there is, for example, a slower recovery of the groups exploited in a given period, we will have arguments to recommend a closure during that period. A manuscript of this chapter is being prepared and is entitled “No effect of timing of exploitation on the abundance of stalked barnacles (*Pollicipes pollicipes*) after harvesting: a small-scale approach”

Chapter 6 emphasises the importance of integrating the local ecological knowledge (LEK) of the fishers regarding the state of a *P. pollicipes* fishery and also assesses their perception about its management and the possibility of implementation of a co-management system in the future (published paper, ‘Temporal variation of the fishers’ perception about the stalked barnacle (*Pollicipes pollicipes*) fishery at the Berlengas Nature Reserve’ Sousa et al. (2020)). This study was carried out in the fishery of ‘*Reserva Natural das Berlengas*’ (RNB), that is the fishery within Portugal that showed the highest scores for governance and number of sustainability attributes (Aguión et al. 2022b). This study was developed as part of the project (‘Co-Pesca 2 – Implementação do comité de cogestão para a apanha de percebe na Reserva Natural das Berlengas’, funded by MAR2020). This study was not in the original proposal of this project.

Finally, chapter 7 consists of the “Conclusions and final remarks”, Here, we summarize and discuss the main information and whenever appropriate we provide management/conservation strategies, as well as suggestions for future research.

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# **Chapter 2.** Abundance and size of the exploited stalked barnacle *Pollicipes pollicipes* along its European distribution: an inverse pattern of density and size along Iberia

Sousa, A.; Macho, G.; Acuña, J.L.; Aguión, A.; Arrontes, J.; Broudin, C.; Castro, J.J.; Davoult, D.; Feis M. E.; Fernandes, J.N.; Fernandez, C.; Geiger, K.; Jacinto, D.; Leandro, S.M.; Mateus, D.; Muniz, C.; Neves, F.; Román, S.; Silva, T.; Thiébaut, E.; Vázquez, E.; Cruz, T. *in preparation.* Abundance and size of the exploited stalked barnacle *Pollicipes pollicipes* along its European distribution: an inverse pattern of density and size along Iberia.

## 2.1 Abstract

The stalked barnacle *Pollicipes pollicipes* is an important economic resource that is exploited throughout its geographical range (from Brittany, France to Senegal). The aim of the present study was pursuing a large-scale standardized comparison of the percentage cover, density, biomass and size structure of this species on the mid shore along its European distribution. Within the European range of this species six relevant fisheries/regions have been sampled: Brittany, France; Asturias and Galicia in Spain; Berlengas Nature Reserve in Portugal and the Central cost and the SW coast of Portugal. Considering the "abundant-centre-theory", we would expect a greater abundance and larger size of *P. pollicipes* in the SW coast of Portugal, a region that is close to the centre of the geographical distribution of this species (Morocco) and smaller abundance and size in the northern limit of its distribution (Brittany). Results do not support this theory. The strongest pattern observed was a north-south pattern on the Iberian Peninsula, consisting of a lower density in Asturias and Galicia (Spain), comprising larger animals, and a higher density of *P. pollicipes* in the SW coast of Portugal, made up of smaller individuals. The remaining three regions (Brittany, Berlengas Nature Reserve and the Central coast of Portugal) can be considered intermediate as, depending on the variables studied, they may be more similar to the Spanish regions or to the SW coast of Portugal.

In contrast to the variation of density of *P. pollicipes*, no differences were found in the biomass of this species among regions. However, the size structure of the relative biomass varied among regions. In average an area of 100 cm2 completely covered with *P. pollicipes* corresponds to a biomass of 192 g of barnacles. However, the relative average contribution of the size class corresponding to barnacles with moderate and high commercial value (> 20 mm RC) to this biomass is much greater in Spain (71.5 % in Asturias and 54 % in Galicia) than in SW Portugal (5.6 %). In SW Portugal, around 90 % of this biomass is made up of juvenile barnacles (< 12.5 mm RC). These findings have important implications for fisheries management. Harvesting and managing a fishery composed mainly of small individuals (e.g. centre and SW coast of Portugal) differs from one where there are fewer but larger specimens (e.g. Asturias and Galicia).

## 2.2 Introduction

The abundance and body size (hereafter referred as size) of a sedentary marine species can vary across its geographical distribution due to variation in natural processes like reproduction (e.g. Gilman 2006a, b, Lester et al. 2007), recruitment (Hidas et al. 2010, Lathlean et al. 2010), growth (Gilman 2006a, b, Lathlean et al. 2013) and mortality (DeRivera et al. 2005, Lathlean et al. 2010, Lathlean et al. 2013), as well as variable fishing mortality if the species is exploited. Size-selective harvesting can negatively impact the abundance and size of a species, potentially affecting other processes such as reproduction and recruitment (Fenberg & Roy 2008).

Patterns of abundance and size of a species along its geographic distribution have long attracted the attention of scientists (e.g.Brown 1984, Blackburn et al. 1999). One of the most studied theories is the "abundant-centre theory", which predicts that a species reaches its highest abundance and size at the centre of its range, decreasing towards the edges of the distribution range (Hengeveld & Haeck 1982, Brown 1984, Virgós et al. 2011, Pironon et al. 2017). The basic assumptions of this theory rely on environmental variables being spatially correlated throughout a species´ range (Brown 1984, Enquist et al. 1995, Brown et al. 1996, Sagarin et al. 2006). Rocky intertidal sedentary species are well suited for studying these biogeographical patterns (e.g. along the Pacific coast of North America, Sagarin & Gaines 2002; in south-eastern Pacific, Rivadeneira et al. 2002), as many species are conspicuous, abundant and easily observed and sampled. Regarding the geographical variation in abundance (see examples in Sagarin & Gaines 2002, Fenberg & Rivadeneira 2011) and size (see examples in Tam & Scorsati 2011) some species, such as the keyhole limpet *Fissurella volcano* and the turban snail *Tegula funebralis*, have distributions that support the "abundant-centre theory", others do not, such as the barnacle *Tetraclita rubescens* or the mussel *Mytilus edulis*. However, as many species occur along large stretches of coastline, sampling their abundance and size can be difficult from a logistical point of view. Furthermore, some of these species are exploited throughout their geographical range (e.g. the keyhole limpet *Fissurella* spp. and the muricid gastropod *Concholepas concholepas* in Chile, see Castilla 1999; the stalked barnacles of the genus *Pollicipes*, Cruz et al. 2022) with variable fishing effort and under different governance models or in an unregulated manner. Monitoring studies of the abundance and size of exploited rocky intertidal sedentary species over a wide geographical area and using the same methodology are scarce.

The stalked barnacle *Pollicipes pollicipes* is a cirripede crustacean that mainly inhabits the intertidal zone of very exposed rocky shores along the north-eastern Atlantic Ocean, from Land's End peninsula, UK (50°4'N) to Dakar, Senegal (14°38'N). It is harvested whenever significant populations are present along its range (Cruz et al. 2022). Several fisheries of *P. pollicipes* have been identified in Europe and Africa, including those near its northern (Morbihan, Brittany, France, ~50 t/year) and southern limits (Mauritania and Senegal) (Aguión et al. 2022b; Cruz et al. 2022). The most important fishery is located in Galicia (333 t/year and ~9 million € per year) (see revision of Cruz et al. 2022). Throughout its European geographical distribution, there are other important fisheries of *P. pollicipes* in Spain (Asturias-West, 44 t/year) and in Portugal (*Reserva Natural das Berlengas* - RNB, *Parque Natural do Sudoeste Alentejano e Costa Vicentina* – PNSACV, as well as general *Pollicipes* fisheries in Portugal) (Aguión et al. 2022b). However, the data on official annual landings for Portugal (average value of 136 t/year) are, with the exception of RNB (16 t/year, value included in the average value for Portugal), generally described as being underestimated (Cruz et al. 2022). An additional problem that was identified in several of these European fisheries was poaching, which is the illegal harvesting of this species (Geiger et al. 2022). There are no estimates of the amounts of *P. pollicipes* that are illegally caught, but Geiger et al. (2022) considered that poaching has the potential to affect the sustainability of this resource. Discards of small individuals (including juveniles and adults) is also an issue in these fisheries since mixed sizes are unavoidably harvested together in clumps due to heavy recruitment taking place on the stalks of adult conspecifics (Cruz et al. 2022). In Galicia it was estimated that up to 42 % of the *P. pollicipes* catches are discarded (Macho et al. 2013). The sustainability level of these fisheries was assessed, presenting Galicia and Asturias-west the highest sustainability scores, followed by RNB (Aguión et al. 2022b). These three fisheries are co-managed (bottom-up governance approach) with higher levels of participation by fishers in management and exclusivity of the access structure, while the other fisheries have governance models with less participation by fishers or top-down approaches (Aguión et al. 2022b, Cruz et al. 2022). All these issues hinder an assessment of the state of this resource on a European scale.

Besides, there is no study addressing the patterns of distribution, abundance and size of *P. pollicipes* throughout its geographical range. Abundance of *P. pollicipes* has only been locally or nationally assessed, either by measuring percentage cover (Spain – Borja et al. 2006a, Parada et al. 2012, Bidegain et al. 2017; Portugal – Sousa et al. 2013, Jacinto & Cruz 2016) or by quantifying density or biomass (Spain – Borja et al 2006a, b, Bidegain et al. 2017; Portugal – Sousa et al. 2013, Cruz et al. 2015; Morocco – Boukaici et al. 2012, Bourassi et al. 2019).  The largest study was done by Sousa et al. (2013) including a standard methodology to measure the relative density and biomass of various size classes of *P. pollicipes* in three regions of Portugal (RNB, Central coast of Portugal and SW coast of Portugal). These size classes were established on the basis of the life cycle of *P. pollicipes* (e.g. juveniles and adults) and on commercial importance criteria (e.g. low commercial value, high commercial value). By using standardised methodologies, comparisons between regions or countries are facilitated.

Here, we present, for the first time, results on a European survey to describe the patterns of percentage cover, density, biomass and size structure of *P. pollicipes* in the mid shore, along its entire distribution range in Europe (Brittany in France, Asturias and Galicia in Spain, and Berlengas Nature Reserve, the Central coast and the SW coast in Portugal), using a standardized methodology. Taking into account the "abundant-centre theory" and the geographical distribution of *P. pollicipes* (~14ºN to 50ºN), the centre of its distribution corresponds to Morocco (~27ºN to 35ºN), and the southernmost European region with exploited barnacles is the SW coast of Portugal (~37ºN). It is therefore expected that the abundance and size of *P. pollicipes* will increase from north to south in Europe and will reach the highest values in SW Portugal.

## 2.3 Materials and methods

*Study area*

The present study was carried out along the European distribution of *P. pollicipes*, from Brittany in France to the southwest coast of Portugal. Within the European distribution of *P. pollicipes* six regions were defined. The northern region was coincident with the northern limit of distribution of *P. pollicipes*, Brittany (BR), in France. In Spain two regions were considered: Asturias (AS) in the north coast of Spain; and Galicia (GL) located on the west coast. On the coast of Portugal three regions were defined: Berlengas Nature Reserve (RNB-PT - a group of islands located approximately 10 miles west from Cape Carvoeiro), the region of the central coast of Portugal (C-PT - defined as the coastal area between Cape Carvoeiro and Cape Raso), and the region of the southwest coast of Portugal (SW-PT - defined as the coastal area between Cape of Sines and Cape São Vicente). Within each region, three random sites were considered (see **Fig.2.1** and **Table 2.S1** in supplementary material). In all sampled sites, *P. pollicipes* is harvested by fishers, but these sites differ in their fishing management strategies and respective levels of governance (see details in Aguión et al. 2022b)

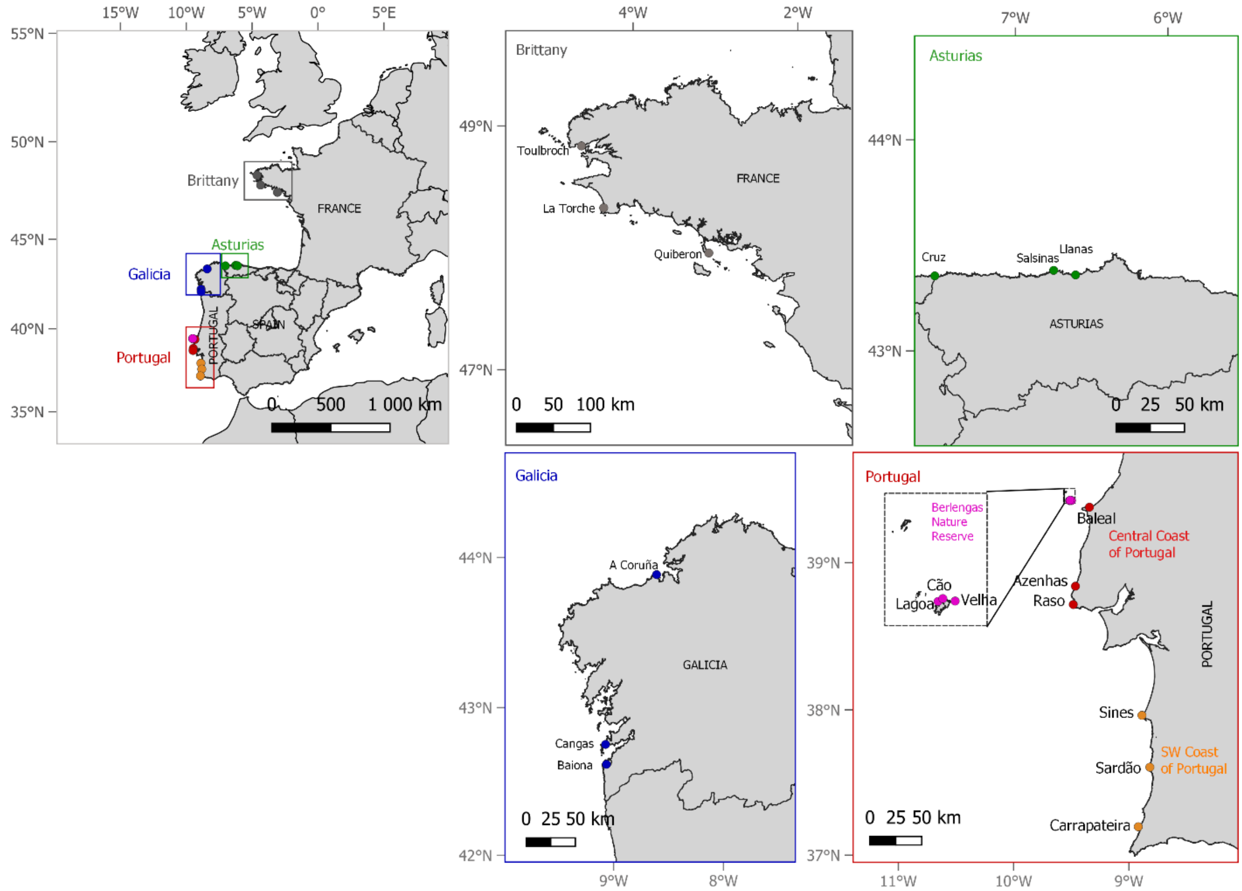


**Fig.2.1** - Studied regions: Brittany (BR – France, in dark grey), Asturias (in green) and Galicia (in blue) (AS and GL, respectively, Spain), Berlengas Nature Reserve (RNB-PT - Portugal, in pink), Central Coast of Portugal (C-PT - coastal area between Cape Carvoeiro, Peniche and Cape Raso, Cascais, in red), and the SW Coast of Portugal (SW-PT - coastal area between Cape of Sines and Cape São Vicente, Sagres, in orange).

## <u>Distribution, abundance and size structure of *P. pollicipes*</u>

### *Data collection*

Sampling was carried out from May to July of 2019. At each site in each region (**Fig. 2.2**), we estimated the percentage cover, biomass, density, and size structure of *P. pollicipes*.

In all sites, *P. pollicipes* data were collected in the mid shore tidal level, which corresponds to the middle/upper intertidal distribution of *P. pollicipes* (~1.5 m to 3 m above MLWS).

The sampling methods for estimating the percentage cover, density, biomass and size structure of *P. pollicipes* were based on Sousa et al. (2013). The percentage cover in each site was estimated from digital photographs of 50 x 50 cm quadrats (n = 6). A grid of equidistant rows (2.5 cm) was superimposed to the area of the quadrat and counted the number of intersection points over *P.*

*pollicipes* (out of the 361 intersection points). All the image analyses were performed using QGIS software (www.qgis.org).

Density, biomass and size structure of *P. pollicipes* were estimated by sampling 15 x 15 cm quadrats (n = 3 to 6) in each site. The quadrats were placed over *P. pollicipes* clumps, avoiding rocky fissures, photographed and completely scraped. All scraped organisms were frozen at -20 ºC until posterior analysis. The percentage cover of *P. pollicipes* in each quadrat was estimated by image analysis (25 intersection points) in a similar way to that described above. The percentage cover of each quadrat was used to calculate the area of the clump as described below.

$$Area\ of\ the\ clump\ (cm^2)\ =\frac{\%\ cover\ of\ the\ 15\ x\ 15\ quadrat}{100}\times 225$$

The barnacles from each replicate were then thawed, individualised, measured (maximum distance between the rostrum and carina plates, RC) with callipers (precision 0.1 mm), grouped in 6 size classes and counted. The range of each size (RC) class was related to *P. pollicipes* life cycle stages and its socio-economic importance, as defined in Sousa et al. (2013): RC ≤ 5.0 mm considered recruits; [range of 5.0 mm - 12.5 mm] juveniles with no commercial value; [12.5 - 17.5 mm] small adults, mostly with < 1 year old, with no commercial value; [17.5 - 20.0 mm] adults with low commercial value; [20.0 - 22.5 mm] adults with moderate commercial value; and RC ≥ 22.5mm representing adults with high commercial value. The groups of barnacles in each size class were immersed in tap water for 30 min and dried on absorbent paper for 20 minutes, before being weighted using a digital scale (precision 0.1 g). The total number of individuals and total biomass of each quadrat corresponded respectively to the sum of the number of individuals and biomass of each size class of that replicate.

As the clumps of barnacles occupied different areas in each quadrat, the total density and the total biomass were standardised for a clump of barnacles occupying 100 cm$^2$ (an approximate average value of the area occupied by a clump of barnacles). The formulas used to estimate the standardised density and standardised biomass per 100 cm$^2$ of barnacles are described below:

$$Standardised\ density\ per\ 100\ cm^2\ (ind/100\ cm^2)\ =\ \frac{total\ number\ of\ individuals\ (15x15cm\ quadrat)}{area\ of\ the\ clump} \times 100$$

$$Standardised\ biomass\ per\ 100\ cm^2\ \ (g/100\ cm^2) = \frac{total\ biomass\ of\ P.pollicipes\ (15x15cm\ quadrat)}{area\ of\ the\ clump} \times 100$$

The relative density and biomass of each size class of *P. pollicipes* in each site was calculated after pooling the data of all quadrats of that site (n between 3 and 6), in order to increase the number of barnacles in each size class. In each site, the size structure of *P. pollicipes* relative density and relative biomass was calculated using the formulas below, being $x$ one of each of the 6 size classes defined above.

$$Relative\ density\ of\ x\ size\ class = \frac{number\ of\ individuals\ of\ x\ size\ class}{Total\ number\ of\ individuals\ (all\ size\ classes)}$$

$$Relative\ biomass\ of\ x\ size\ class = \frac{biomass\ of\ P.pollicipes\ of\ x\ size\ class}{Total\ biomass\ (all\ size\ classes)}$$

Finally, to estimate the density and biomass of *P. pollicipes* per $m^2$ in each site, the data of total number of individuals and total biomass were corrected with the mean value of percentage cover of each site (obtained from the 50 x 50 cm quadrats). This correction was necessary as *P. pollicipes* form dense aggregates of individuals and the sampling of 15 x 15 cm quadrats was performed over clumps of barnacles instead of randomly along the sampled site. The formulas used to estimate density per $m^2$ and biomass per $m^2$ in each site are described below:

$$Density\ per\ \ m^2 (individuals\ per\ m^2) = standardised\ density\ per\ 100\ cm^2 \times mean\ \%\ cover\ of\ the\ site$$

$$Biomass\ per\ m^2\ (\ Kg\ of\ wet\ weight\ per\ m^2) =\ standardised\ biomass\ per\ 100\ cm^2 \times mean\ \%\ cover\ of\ the\ site$$

*Data Analysis*

The hypothesis of different percentage cover of *P. pollicipes* along the sampled regions was tested by analysis of variance (ANOVA). Two factors were considered: region (fixed factor with six levels: BR, AS, GL, RNB-PT, C-PT, SW-PT), and site (random factor with three levels, nested in factor region). Sample size was 6 (the 6 photo quadrats of 50 x 50 cm). Homogeneity of variance was tested using Cochran's C test and SNK tests were used when appropriate (Underwood 1997). The analysis of variance was performed according to Underwood (1997), using the software GMAV5 for windows (Institute of Marine Ecology, University of Sydney).

The hypothesis of different spatial distribution of density (standardized density per 100 $cm^2$ of barnacles and density per $m^2$) and biomass (standardized biomass per 100$cm^2$ of barnacles and biomass per $m^2$) of *P. pollicipes* along its European geographic distribution were analyzed by permutational multivariate analysis of variance, PERMANOVA (Anderson 2001), including two factors: region (fixed factor with six levels: BR, AS, GL, RNB-PT, C-PT, SW-PT), and site (random factor with three levels) nested in region. Sample size was variable (3 to 6 quadrats). For the biomass analyses the region of Brittany was not considered due to methodological problems that were detected.

Analyses of density and biomass were based on Euclidian distance of fourth root transformed data. Permutation of residuals under a reduced model and type III sums of squares were applied (Anderson et al. 2008). PERMANOVA was used to analyze univariate data due to an unbalanced design resultant of the different number of replicates collected in each site.

The size structure of the relative density and of the relative biomass were analyzed by PERMANOVA with one fixed factor, Region, with six levels (BR, AS, GL, RNB-PT, C-PT, SW-PT) (density data) and five levels (AS, GL, RNB-PT, C-PT, SW-PT) (biomass data). These analyses were based on the Bray Curtis similarity coefficient, calculated from a multivariate data matrix of the relative density and the relative biomass of *P. pollicipes* in each size class per replicate. SIMPER procedure (Clarke 1993) was used to identify which were the variables (size classes) that most contribute for the dissimilarity between regions. The relative biomass size structure data from Brittany were excluded from the analysis due to methodological problems.

Principal coordinate analysis, PCO, was performed on the two matrices (density and biomass) of Bray Curtis similarity, calculating raw Spearman correlations of the variables (size classes) with the PCO axes. Correlations of the variables with PCO axes were also overlaid on the plots in order to visualize the possible relationships between those variables and the PCO axes.

In all PERMANOVA analyses, the homogeneity of dispersion based on Euclidean distance (standardized density per 100 $cm^2$ of barnacles, density per $m^2$, standardized biomass per $100cm^2$ of barnacles, biomass per $m^2$) or Bray Curtis similarity (relative size structure of density and of biomass) was tested using the PERMDISP routine (Anderson, 2006). When appropriate pair-wise a posteriori comparisons were conducted. The software PRIMER 6 & PERMANOVA+ (www.primer.com; Anderson et al. 2008) was used to perform all the density and biomass statistical analyses.

## 2.4 Results

The percentage cover of *P. pollicipes* in the mid shore along the stalked barnacle European distribution (**Fig. 2.2**) ranged between 0.05 % (at Baleal, Central coast of Portugal) and 69.0 % (at Cão, Berlengas Nature Reserve, Portugal). Significant differences were detected among regions (ANOVA analysis, **Table 2.1**). However, no clear pattern was detected among regions, the percentage cover was higher in the region of Brittany, France (49.7 % mean barnacle coverage), and lower in the region of the central coast of Portugal (5.8 %) (**Fig. 2.2**, **Table 2.1**). Significant variation among sites was also detected (**Table 2.1**).

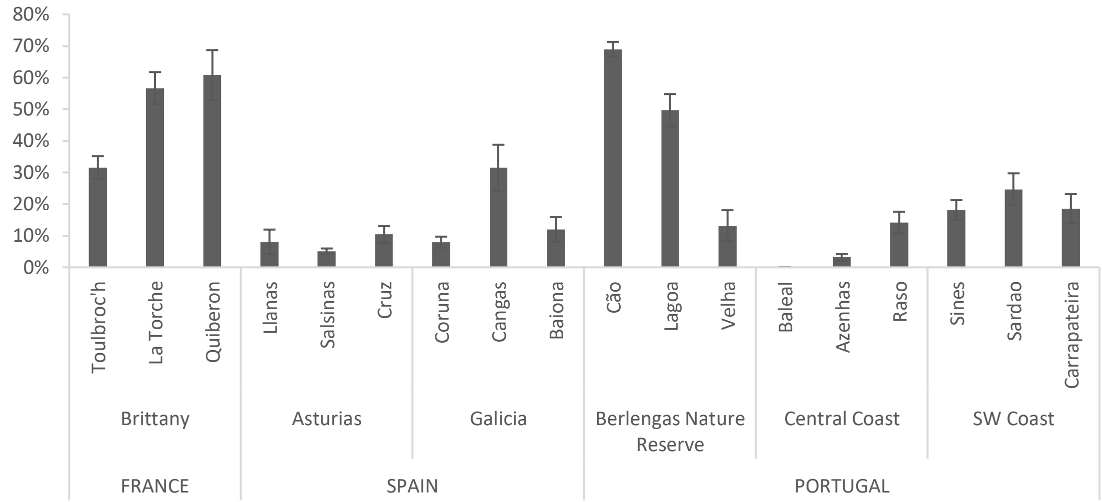


**Fig. 2.2** – Percentage cover (mean ± SE; n=6) of *Pollicipes pollicipes* in each site of the six sampled regions.

**Table 2.1** – ANOVA analysis on *Pollicipes pollicipes* percentage cover in relation to "Region" and "Site". n=6 replicate quadrats. Non-transformed data. Cochran's test: C=0.1978 (p>0.05). Significant effects are indicated in bold (p<0.05). SNK tests for the main factor "Region".

| Source | df | MS | *F* | *p* |
|---|---|---|---|---|
| Region | 5 | 6144.9325 | 4.77 | **0.0124** |
| Site (Region) | 12 | 1288.1459 | 10.20 | **0.0000** |
| Residual | 90 | 126.3425 | | |
| SNK | Region: Brittany > Central Coast of Portugal; no clear pattern for other regions | | | |

The standardised density and biomass of *P. pollicipes* in an area of 100 $cm^2$ (area occupied by an average clump of *P. pollicipes*) of barnacles were calculated for all regions (**Fig. 2.3**). The variation in the area of the clumps of barnacles that were sampled in each site within each region is shown in **Fig. 2.S1** in Supplementary material. The standardised density of *P. pollicipes* per 100 $cm^2$ of barnacles ranged between 82 ind/100 $cm^2$ (at La Cruz, Asturias, Spain) and 846 ind/100 $cm^2$ (at Sardão, SW Coast of Portugal; **Fig. 2.3A**). The PERMANOVA analysis to this variable revealed significant differences among regions (**Table 2.2**), however, there was no clear pattern of variation among regions, with the group of regions of Asturias, Galicia and Berlengas Nature Reserve showing the lowest density values and the Central coast and the Southwest coast of Portugal the highest

(**Fig. 2.3A**, **Table 2.2**). The region of Brittany was considered similar (several pair wise tests, $P>0.05$) to the other regions. Significant variation among sites was also detected (**Table 2.2**).

The standardised biomass of *P. pollicipes* per 100 $cm^2$ of barnacles ranged between 105 g/100 $cm^2$ (at Velha, Berlengas Nature Reserve, Portugal) and 350 g/100 $cm^2$ (at Cangas, Galicia, Spain; **Fig. 2.3B**), with an average of 192 g/100 $cm^2$. The PERMANOVA analysis revealed no significant differences of this variable among regions (**Table 2.2**), but significant variation among sites was detected (**Table 2.2**).

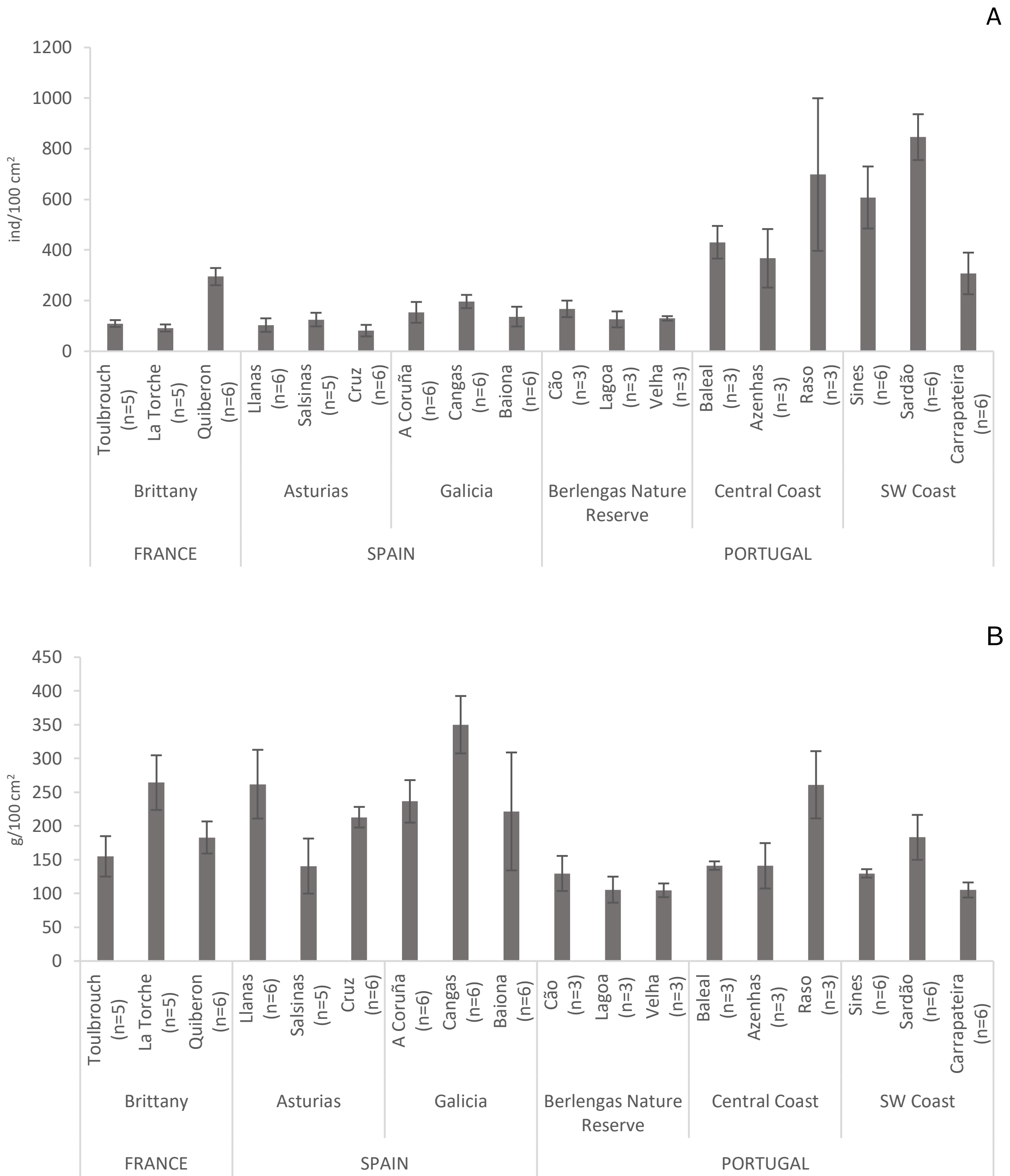


**Fig. 2.3** - *Pollicipes pollicipes* (**A**) standardised density per 100 $cm^2$ of barnacles (mean individuals per 100 $cm^2$ ± SE; n= 3 to 6), (**B**) standardised biomass per 100 $cm^2$ of barnacles (mean g per 100 $cm^2$ ± SE; n= 3 to 6); of the six sampled regions. n = sample size in each site .

**Table 2.2** – PERMANOVA analysis on *Pollicipes pollicipes* density per 100cm$^2$ of barnacles and biomass per 100 cm$^2$ of barnacles in relation to factors "Region" and "Site". Analyses were based on Euclidean distances of fourth root (for density data) and square root (for biomass data) transformed data. P-values were obtained using 9999 random permutations. n= 3 to 6 replicate quadrats. PERMDISP tests: F = 1.915 (density; p > 0.05) and F = 2.666 (biomass; p > 0.05). Significant effects are indicated in bold (p<0.05). Pair-wise tests:" >" or "<" (p < 0.05). BR – Brittany, France; AS – Asturias, Spain; GL – Galicia, Spain; C-PT – Central Coast of Portugal; RNB-PT – Berlengas Nature Reserve, Portugal; SW_PT – Southwest Coast of Portugal.

| | *Standardised density (ind/100 cm$^2$)* | | | | *Standardised biomass (g/100 cm$^2$)* | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | MS | *Pseudo-F* | *p* | df | MS | *Pseudo-F* | *p* |
| Region | 5 | 7.208 | 8.18 | **0.004** | 5 | 20.753 | 2.97 | 0.067 |
| Site (Region) | 12 | 0.891 | 3.38 | **0.001** | 12 | 17.248 | 2.14 | **0.025** |
| Residual | 70 | 0.263 | | | 70 | 8.062 | | |
| Pair-wise | Region:<br>No general pattern defined<br>AS=GL=RNB-PT < C-PT=SW-PT<br>BR = all regions | | | | | | | |

The size structure based on the relative density of *P. pollicipes* (**Fig. 2.4A** and **2.4B**) revealed significant differences among the six regions (**Table 2.3**), but no general pattern of variation among regions was detected by the pair-wise tests (**Table 2.3**). The most different region was SW-PT, which was considered to be different from all regions except the Portuguese regions (RNB-PT and C-PT) (**Table 2.3**). This pattern seems to be due to the greater relative abundance of the smaller size classes, namely the class comprising recruits (barnacles with RC < 5.0 mm), and the lower relative abundance of adults (RC > 12.5 mm) (**Fig. 2.4A** and **2.4B**). In fact, the proportion of juvenile classes (RC < 12.5mm) in Portugal is higher than in other regions, varying between 58 % and 94 %, while in the Spanish regions and in Brittany (France), adult classes are relatively more abundant, varying between 28 % and 75 % (**Fig. 2.4A**). The Asturias region is the most different from the others, namely because it exhibits a higher proportion of the largest size classes (RC > 20.0 mm) (**Fig. 2.4A** and **2.4B**). It is the only region where, in relative terms, the abundance of adults of moderate and high commercial interest (RC > 20.0 mm) can exceed 30%. In addition, the differences between Asturias and Galicia also seem to be due to a different proportion of the two smallest classes, with a higher proportion of recruits (RC < 5.0 mm) and a lower proportion of juveniles (5.0 - 12.5 mm, RC) in Asturias (**Fig. 2.4A**).

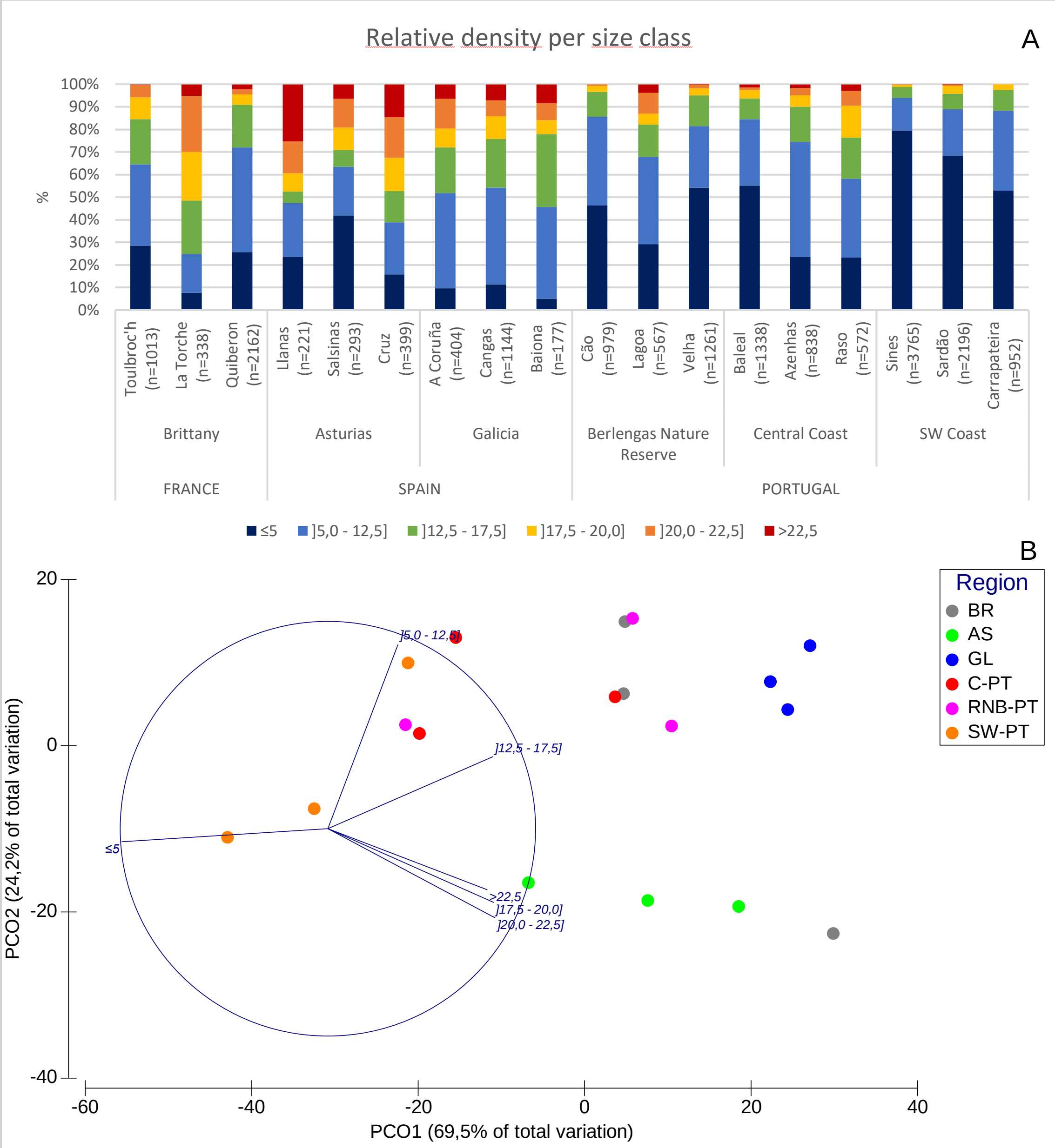


**Fig. 2.4** – *Pollicipes pollicipes* (**A**) relative density per size class (six size classes, see methods) and (**C**) Principal coodinate analysis (PCO) performed on a matrix of Bray Curtis similarity for *P. pollicipes* size structure of relative density, considering 6 size classes as variables and the sites for each of the six regions as samples.Vector overlays represents the raw Spearman correlations of variables (size classes) with the PCO axes. Region codes as in **Table 2.2** caption.

The size structure based on the relative biomass of *P. pollicipes* (**Fig. 2.5A** and **2.5B**) revealed significant differences among the five regions (**Table 2.3**). It was not possible to statistically analyse the Brittany region because there was only data from one site (**Fig. 2.5A** and **2.5B**). The two most different regions were once again Asturias and the southernmost region sampled, the SW coast of Portugal (**Table 2.3**). In fact, Asturias was considered to be different from all the Portuguese regions (**Table 2.3**). This pattern was mostly driven by a higher relative biomass of adults with moderate and high commercial value (RC > 20 mm) in Asturias, and a higher relative biomass of juveniles and small adults (RC < 17.5 mm) in the three Portuguese regions (**Fig. 2.5A** and **2.5B**). The SW coast of Portugal, showed significant differences with all the other regions, except for Central coast of Portugal (**Table 2.3**). Considering the two largest size classes (RC > 20.0 mm, adults with moderate or high commercial value), the proportion of biomass of these large barnacles varied between 60 and 85 % in Asturias, between 50 and 58 % in Galicia, and in SW Portugal between 0 and 16 %. In the SW coast of Portugal, juveniles and adults with no commercial interest (RC < 17.5 mm) accounted for the majority of the biomass (54 – 81 %). Brittany is similar to the regions considered intermediate (GL, RNB and C_PT).

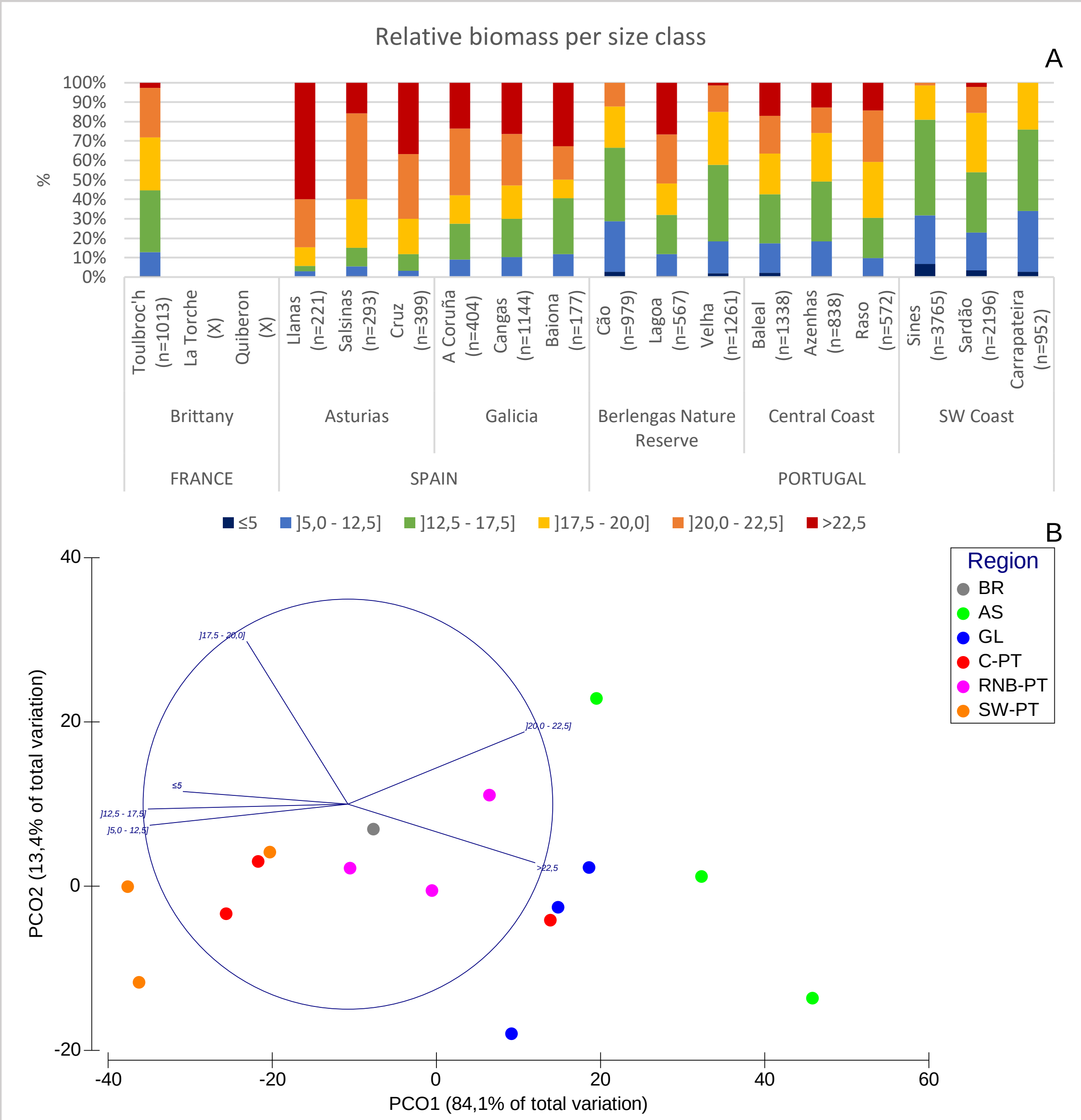


**Fig. 2.5** – *Pollicipes pollicipes* (**A**) relative biomass per size class (six size classes, see methods) and (**C**) Principal coordinate analysis (PCO) performed on a matrix of Bray Curtis similarity for *P. pollicipes* size structure of relative biomass, considering 6 size classes as variables and sites for each of the six regions as samples. Vector overlays represents the raw Spearman correlations of variables (size classes) with the PCO axes. Region codes as in **Table 2.2** caption.

**Table 2.3** – PERMANOVA analysis on *Pollicipes pollicipes* size structure of relative density and of relative biomass in relation to the factor "Region". Analyses were based on Bray Curtis similarity of untransformed data. P-values were obtained using 9999 random permutations. n= 3 replicate sites (pooled data from the 3 – 6 quadrats per site). PERMDISP tests: F = 2.580 (density; p>0.05) and F = 0.935 (biomass; p>0.05). Significant effects are indicated in bold (p<0.05). Pair-wise tests: "≠" (p <0.05). Region codes as in Table 2 caption. BR was not included in the analysis of relative biomass.

| | *Size structure of relative density* | | | | *Size structure of relative biomass* | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | MS | *Pseudo-F* | *p* | df | MS | *Pseudo-F* | *p* |
| Region | 5 | 1556.9 | 5.390 | **0.001** | 4 | 1841.7 | 6.150 | **0.003** |
| Residual | 12 | 288.9 | | | 10 | 299.5 | | |
| Pair-wise | Region:<br>No clear pattern defined<br>BR: = to all regions with exception of SW-PT<br>AS: = BR and RNB-PT; ≠ GL, C-PT and SW-PT<br>GL: = BR and RNB-PT; ≠ AS, C-PT and SW-PT<br>RNB-PT = to all regions<br>C-PT: = BR, RNB-PT, SW-PT; ≠ AS and GL<br>SW-PT: = RNB-PT and C-PT; ≠ BR, AS and GL | | | | Region:<br>No clear pattern defined<br>AS: = GL ≠ all Portuguese regions<br>GL: = AS, RNB-PT and C-PT; ≠ SW-PT<br>RNB-PT = GL and C-PT; ≠ AS and SW-PT<br>C-PT: = all regions except ≠ AS<br>SW-PT: = C-PT; ≠ all other regions | | | |

Finally, the density and biomass of *P. pollicipes* per m$^2$ (**Fig. 2.6A** and **2.6B**) were estimated for each region. In both analyses, significant variation among sites was detected (**Table 2.4**). The density of *P. pollicipes* per m$^2$ ranged between 22 ind/m$^2$ (at Baleal, in the Central Coast of Portugal) and 17,927 ind/m$^2$ (at Quiberon, Brittany in France; **Fig. 2.6A**). The region that was once again the most different was Asturias, as it had lower density values than three other regions (Brittany, Berlengas Natures Reserve and the SW Coast of Portugal) (**Table 2.4**, **Fig. 2.6A)**. With the exception of Asturias, there was no significant differences among the other regions (**Table 2.4**). Contrarily to density per m$^2$, no significant differences were found between regions in the case of biomass per m$^2$. The biomass of *P. pollicipes* per m$^2$ ranged between 0.01 Kg/m$^2$ (at Baleal, in the Central Coast of Portugal) and 15.0 Kg/m$^2$ (at La Torche, Brittany in France; **Fig. 2.6B**), with an average of 4.5 Kg/m$^2$.

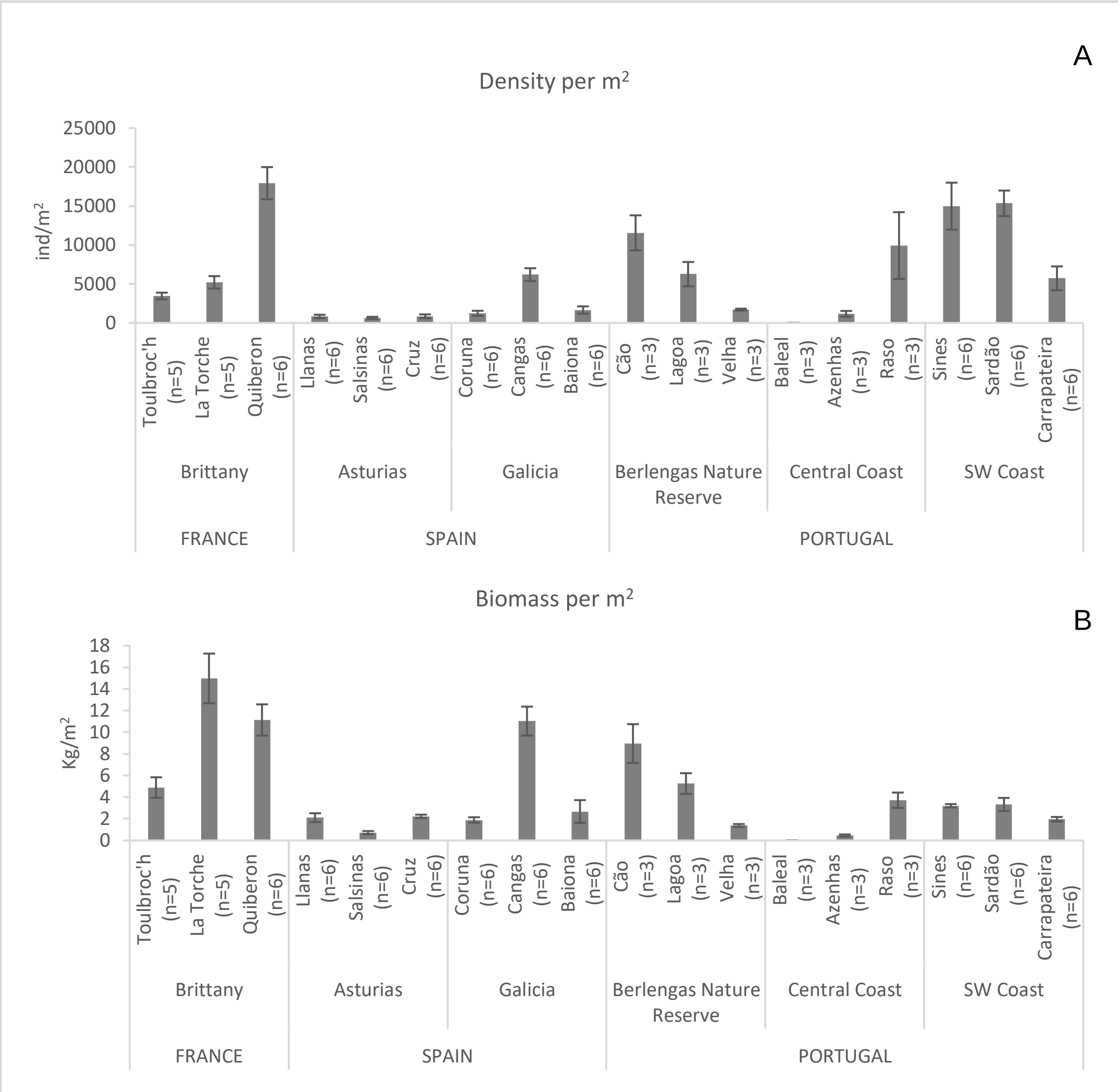


**Fig. 2.6 –** *Pollicipes pollicipes* (A) Density per m² (mean ind/m² ± SE; n= 3 to 6) and (B) Biomass per m² (mean Kg/m² ± SE; n= 3 to 6), for each sampling sites of the six regions.

**Table 2.4** – PERMANOVA analysis on *Pollicipes pollicipes* density per $cm^2$ and biomass per $m^2$ in relation to "Region" (Re) and "Site" (Si). Analyses were based on Euclidean distances of fourth root transformed data. P-values were obtained using 9999 random permutations. n= 3 to 6 replicate quadrats. PERMDISP tests: F = 5.960 (density; p<0.05) and F = 11.525 (biomass; p<0.05). Significant effects are indicated in bold (p<0.05). Pair-wise tests: ">" or "<" (p <0.05). Region codes as in **Table 2.2** caption.

| | *Density per $m^2$ (ind/ $m^2$)* | | | | *Biomass per $m^2$ (Kg/$m^2$)* | | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | MS | *Pseudo-F* | *p* | df | MS | *Pseudo-F* | *p* |
| Region | 5 | 62.158 | 3.566 | **0.037** | 4 | 0.649 | 1.529 | 0.272 |
| Site (Region) | 12 | 17.699 | 17.523 | **0.000** | 10 | 0.434 | 26.762 | **0.000** |
| Residual | 70 | 1.01 | | | 56 | | | |
| Pair-wise | Re:<br>AS < BR, SW-PT, RNB-PT;<br>no clear pattern for GL and C-PT (similar (P>0.05) to all regions) | | | | | | | |

## 2.5 Discussion

Contrary to what was expected by the 'abundant-centre theory', a clear pattern of higher density and larger size of *P. pollicipes* was not observed in SW Portugal (closer to the centre of the geographical distribution of this species, which is Morocco) than in the extreme north of its distribution (Brittany, France). The fact that *P. pollicipes* is an exploited species and that there is variation in the intensity of exploitation throughout its geographical range, could interact with natural latitudinal patterns. Nevertheless, the high percentage of cover of this species in the northern limit of its distribution (Brittany), where it is also exploited (Aguión et al. 2022b), supports the contradiction with this theory.

The strongest pattern observed was a north-south pattern on the Iberian Peninsula, consisting of a higher density of *P. pollicipes* in the SW coast of Portugal, made up of smaller individuals, and a lower density in Asturias and Galicia (Spain), comprising larger animals. Considering all the variables measured (percentage cover, standardized density per 100 $cm^2$ of barnacles, density per $m^2$, relative frequency of the density of various size-classes, standardized biomass per 100 $cm^2$ of barnacles, biomass per $m^2$, relative frequency of the biomass of various size-classes), in general terms, the other regions studied (Brittany, Central coast of Portugal and Berlengas Nature Reserve) did not display such clear patterns of regional variation, as the variation between Asturias and Galicia in relation to SW Portugal. This pattern was generally detected in all analyses, with the exception of the analysis of percentage of cover.

In fact, the percentage of cover of *P. pollicipes* does not seem to be a good proxy for the density and size of this species, as there was no pattern of variation in the percentage cover similar to that observed for standardised density and for the size structure of relative density or relative biomass (pattern Asturias and Galicia versus SW Portugal). However, the percentage cover of *P. pollicipes* could be a good indicator in relation to the degree of exploitation, as the greatest differences were found between Brittany, where exploitation has been considered lower than in Galicia and Portugal (Cruz et al. 2022) and the central coast of Portugal which was considered the most exploited and problematic fishery in Portugal in the study by Sousa et al. (2013) (where the Central coast of Portugal was compared to Berlengas Nature Reserve and SW Portugal). In addition, the Central coast of Portugal fishery was scored low in sustainability in relation to other European fisheries of this species (Central coast of Portugal included in Portugal General, see Aguión et al. 2022b). In the future, the relationship between the percentage of cover and the degree of exploitation of this species could be further investigated, as the percentage cover is a variable that can be easily and quickly measured by analysing in situ or drone images (e.g. Neves 2021).

With regard to the standardised density in an area of 100 $cm^2$ covered by *P. pollicipes* (similar to the average area of a group of stalked barnacles), there is a clear pattern of variation in the Iberian Peninsula, with lower density in the northernmost regions (Asturias, Galicia and Berlengas) and higher density in the two southernmost regions (Central and SW coast of Portugal). Regional differences were also found in the case of the size-structure of relative density, with SW Portugal being the most different region, particularly from the Spanish regions. While in SW Portugal, barnacles of moderate or high commercial value (> 20.0 mm) represent less than 1 % of individuals, in Asturias they represent around 30 % and in Galicia around 20 %. In contrast, in SW Portugal, around 90 % of the barnacles are juveniles (< 12.5 mm, RC), while in Spain juveniles account for around 50 %. When the density per $m^2$ was estimated by correcting the standardised values found by the percentage cover estimated for each shore, the differences were not so clear, although the lowest density persisting in Asturias compared to SW Portugal. This Iberian pattern of higher density and smaller size in SW Portugal compared to the northern regions of Spain could be related to biological processes and/or to exploitation pressure and governance: higher recruitment in SW Portugal (partially supported by a past study where recruitment in SW Portugal was much higher than in Galicia, but similar to Asturias, see Aguión et al. 2022a); greater harvesting pressure in SW Portugal with a consequent reduction in larger individuals (partially supported by this fishery being scored as low in sustainability compared to Asturias-West and Galicia, scored as high in sustainability in the study of Aguión et al. 2022b); higher growth rate in Asturias and Galicia compared to SW Portugal (partially supported by observation of higher growth rates in Galicia compared to Asturias and SW Portugal (unpublished observations, chapter 3).

Concerning the two biomass variables measured (standardized biomass per 100 $cm^2$ of *P. pollicipes* and biomass per $m^2$), no differences were found among regions, but differences were found in the size-structure of the relative biomass, i.e. regional differences in the biomass contribution of each size class. Thus, in the regions studied, the average biomass of a group of *P. pollicipes* occupying an area of 100 $cm^2$ was 192 g and the average biomass of *P. pollicipes* per $m^2$ was 4.5 kg. If we consider the average contribution of the size classes corresponding to animals with moderate and high commercial value (> 20 mm, RC) to the standardised biomass (71.6 % in Asturias, 51.5 % in Galicia and 5.6 % in SW Portugal), we can estimate the biomass per $m^2$ of *P. pollicipes* with moderate and high commercial value in each of these more contrasting regions: 3.2 kg in Asturias, 2.3 kg in Galicia; and 252 g in SW Portugal. If we now consider a capture of 5 kg of these barnacles of greatest commercial value, we can estimate that in Asturias it is necessary to exploit 1.6 $m^2$, in Galicia 2.2 $m^2$, while in SW Portugal it is necessary to exploit 19.8 $m^2$. However, it should be remembered that the fishery of *P. pollicipes* is not completely size-selective, as clumps are usually a mix of sizes from recruits and juveniles to big adults, due to heavy recruitment on the stalk of conspecific adults (Barnes 1996). As a result, there are always discards of commercially valueless adults and juveniles (Macho et al. 2013, Cruz et al. 2022). Consequently, we can expect a more negative perception of the state of the resource by fishers in SW Portugal, where a fisher will have to cover more space than in Asturias or Galicia to be able to exploit the same amount of barnacles with moderate and high commercial value.

In terms of temporal variation, the data in this study for the Portuguese regions (fieldwork carried out in 2019) can be compared with that obtained by Sousa et al.(2013) (fieldwork carried out in 2011) which used the same methodology: Berlengas Nature Reserve – percentage cover of 42.7 % (2011) and of 43.9 % (2019), density per $m^2$ of 5680 individuals (2011) and 6560 individuals (2019), biomass per $m^2$ of 7.7 kg (2011) and 5.2 kg (2019); central coast of Portugal – percentage cover of 27.4 % (2011) and of 5.8 % (2019), density per $m^2$ of 6870 individuals (2011) and 4040 individuals (2019), biomass per $m^2$ of 2.4 kg (2011) and 1.4 kg (2019); SW coast of Portugal – percentage cover of 35.2 % (2011) and of 20.5 % (2019), density per $m^2$ of 12,000 individuals (2011) and 14,000 individuals (2019), biomass per $m^2$ of 3.3 kg (2011) and 3.0 kg (2019). There was no apparent consistent pattern of variation in the percentage cover, density and biomass per $m^2$ observed within 8 years in the Berlengas Nature Reserve and in the SW coast of Portugal. However, the decrease in percentage cover, density and biomass per $m^2$ observed at the central coast of Portugal, combined with the lower sustainability level of this fishery (Aguión et al. 2022b), raise a concerning for the sustainability of this *P. pollicipes* fishery and must be carefully considered in the management strategy of this region.

It should be emphasised that it is in the co-managed fisheries at the time of the field observations (Asturias-West and Galicia) that the state of the resource seems to be better, if this is defined as the number of large animals per unit area. However, in terms of the state of the species, if this is defined as the total number per unit area, it is in the south of its European distribution that we find the highest values.

However, a caveat of the present study is that it was carried out on the mid shore, while exploitation might be more intense in the low shore, namely in Portugal (Cruz et al. 2015). Therefore, estimates of density, biomass and size of *P. pollicipes* should also be obtained for the low shore in the future, as variation in the abundance and size of *P. pollicipes* between vertical levels (mid shore versus low shore) has already been detected (see Sousa et al. 2013).

Finally, variation at the site scale was observed in all the variables where variation among shores was measured. This pattern indicates that there are other processes that could explain this variation, such as differences in recruitment as observed in other studies (Cruz et al. 2010; Aguión et al. 2022b), in the degree of exploitation (e.g. related to variation in shore accessibility and poaching) or differences in the degree of wave exposure that could indirectly affect the mortality of this species (e.g. by variation in predation, Cruz et al. 2022).

Overall, this study enabled us to compare, for the first time, the abundance and size of *P. pollicipes* in various European fisheries using a standardised methodology. The inverse north-south pattern of density and size observed in the Iberian Peninsula is an important pattern to take into account when managing these fisheries. Managing a fishery where there are many small barnacles and few of high commercial value (SW Portugal) may be different from managing a fishery where there are fewer barnacles but relatively larger ones (Asturias and Galicia).

### 2.6 Acknowledgments

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## 2.8 Supplementary material

**Table 2.S1** – Geographic coordinates of all sampling sites.

| Country | Region | Site | Geographical coordinates (Datum wgs84) |
|---|---|---|---|
| France | Brittany | Toulbroc'h | 48°20'21"N 4°37'33"W |
| | | La Torche | 47°49'82"N 4°20'87"W |
| | | Quiberon | 47°49'82"N 4°20'87"W |
| Spain | Asturias | Llanas | 43°33'58"N 6°06'08"W |
| | | Salsinas | 43°37'77"N 6°14'02"W |
| | | Cruz | 43°33'18"N 7°01'69"W |
| | Galicia | A Coruña | 43°23'14"N 8°24'33"W |
| | | Cangas | 42°15'09"N 8°52'25"W |
| | | Baiona | 42°07'06"N 8°51'57"W |
| Portugal | Berlengas Nature Reserve | Velha | 39°25'11"N 9°29'52"W |
| | | Lagoa | 39°25'08"N 9°30'50"W |
| | | Cão | 39°25'09"N 9°30'32"W |
| | Central coast of Portugal | Baleal | 39°22'27"N 9°20'30"W |
| | | Azenhas do Mar | 38°50'30"N 9°27'45"W |
| | | Raso | 38°43'04"N 9°28'55"W |
| | Southwest coast of Portugal | Sines | 37°57'47"N 8°53'10"W |
| | | Sardão | 37°36'07"N 8°49'08"W |
| | | Carrapateira | 37°11'08"N 8°54'46"W |

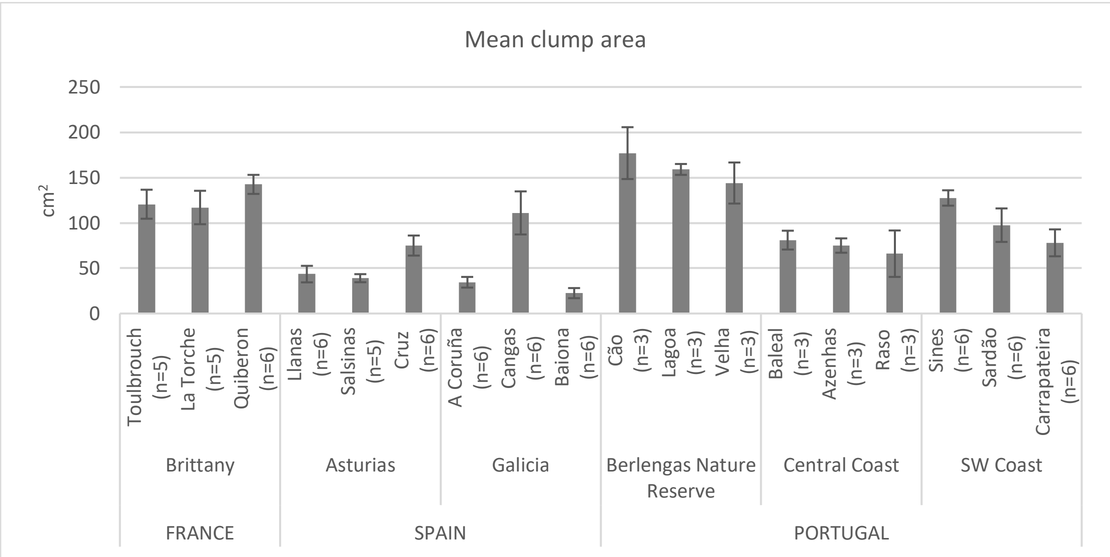


**Fig. 2.S1** – Mean area of the clumps (cm2) of barnacles that were sampled in each site within each region.

# **Chapter 3.** Assessing growth rates of the stalked barnacle *Pollicipes pollicipes* across its European distribution range: higher growth of juveniles in Galicia

Sousa, A. Macho G., Acuña, J.L., Aguión, A., Arrontes, J., Broudin, C., Castro, J.J., Davoult, D., Feis, M.E., Fernandes, J.N., Fernandez, C., Geiger, K., Jacinto, D., Leandro, S.M., Mateus, D., Neves, F., Silva, T., Thiébaut, E., Cruz, T. *in preparation*. Assessing growth rates of the stalked barnacle *Pollicipes pollicipes* across its European distribution range: higher growth of juveniles in Galicia.

## 3.1 Abstract

The aim of the present study was to evaluate the growth rate of the stalked barnacle *Pollicipes pollicipes* along its European range using a same methodology and during a same time period, enabling direct comparisons of growth rates among regions. *P. pollicipes* is an important economic resource, with several fisheries identified throughout its geographic distribution.

Observations were done during the summer of 2018 in four regions within the European range of *P. pollicipes*, namely Brittany (France), Asturias and Galicia (Spain) and the SW coast of Portugal. Growth rates were estimated using capture-mark-recapture methods with calcein as a chemical marker, allowing for mass marking of individuals with relatively low field effort.

The most relevant pattern observed was the higher average growth rates of juveniles (RC= ]5.0 mm – 12.5 mm]; mean growth rate 1.39 mm/month) in Galicia compared to other regions. Significative differences among regions were also detected for the size classes of recruits, adults with low and moderate commercial value, with Galicia presenting the higher mean growth rate among the regions. In Galicia, we estimated that *P. pollicipes* can reach sexual maturity in approximately 8 months, whereas in other regions, this process takes at least 1 year.

As in previous studies, higher and more variable growth rates were obtained for the smaller size classes (recruits and juveniles), with growth rates consistently decreasing as size increased.

## 3.2 Introduction

Investigating the processes that may influence the distribution, abundance and body size patterns of a species along its biogeographic range is paramount to better understanding its demography. Most of the research on these processes is related to reproduction (e.g. Jones & Simons 1983, Lester et al. 2007, Aguión et al. 2022a), growth (e.g. Dehnel 1955, Barnes & Arnold 2001, Moss et al. 2016, Reed et al. 2021) and lifespan (e.g. Moss et al. 2016) of a species.

There are not many studies in the literature on the growth rate of marine invertebrates over a wide spatial range of a species´ distribution. Several studies use historical growth data from previous local studies or the maximum size of individuals of museum collections to study growth along a larger range of a species distribution (e.g. López-Gappa & Tablado 1997, Moss et al. 2016, Reed et al. 2021). In addition, few studies use a standardized methodology to assess growth rates along a wide range of a species distribution (Barnes & Arnold 2001, Phillips 2005, Linse et al. 2006). While some studies revealed that growth rate decreases with latitude (bivalves, Moss et al. 2016; bryozoans, Linse et al. 2006) or that maximum size increases with latitude (chitons, López-Gappa & Tablado 1997), another study revealed a non-linear relationship of growth with latitude (bivalves, Reed et al. 2021).

The stalked barnacles of the genus *Pollicipes* are marine crustaceans that inhabit very exposed rocky shores (Cruz et al. 2022). All four species of this genus (*P. polymerus* – in the north Pacific; *P. elegans* – in the central and south Pacific; *P. caboverdensis* – in the Cape Verde islands and *P. pollicipes* – in the north Atlantic) are exploited, but *P. pollicipes* is the only one that is heavily harvested along its geographic range, wherever exploitable populations are present (France, Spain, Portugal and Morocco) (Cruz et al. 2022). A recent study on the latitudinal variation in the abundance and size of *P. pollicipes* throughout its European distribution has identified a pattern of greater density and smaller size of this species in SW Portugal compared to the regions of Galicia and Asturias, Spain (unpublished observations, chapter 2). Based on this study, no clear patterns of density or size variation were found in the northern range of this species distribution, Brittany, France, compared to other regions of the Iberian Peninsula. One of the processes that could explain these patterns is a regional variation in the growth of this species.

The growth of pedunculated barnacles occurs in a narrow zone between the capitulum and the peduncle (Chaffee & Lewis 1988) and by calcareous accretion of the plates in the capitulum (Anderson 1994). Research on the growth rate of *Pollicipes* has been carried out by analyzing population size structure data, by monitoring the individual increment in size of individuals that have recruited in cleared substrates and by estimating growth rates of marked individuals (see review in Cruz et al. 2022). Mean estimates of growth rates obtained to date are highly variable, being usually higher and more variable in juveniles, and gradually decreasing with barnacle size (e. g. Cruz 1993, Phillips 2005, Jacinto et al. 2015, Cruz et al. 2022). Variations in *Pollicipes* growth patterns have been associated with different ecological and/or oceanographic conditions, such as intraspecific competition for space and food (*P. polymerus*, Helms 2004), intertidal height (*P. pollicipes*, Cruz 2000, Cruz et al. 2010), wave period, wind velocity and direction (*P. pollicipes*, Pavón 2003) and water temperature (*P. polymerus*, Phillips 2005).

A major advance in the study of *Pollicipes* growth rates was made by using chemical marking with calcein (Helms 2004, Jacinto et al. 2015). Calcein is incorporated into the calcium carbonate present in the calcified plates, leaving a fluorescent mark on these calcified structures (Moran 2000, Helms 2004). The chemical marking technique enables the rapid marking of a large number of barnacles of various sizes (Jacinto et al. 2015), facilitating the investigation of growth patterns across the entire size range of a population (Cruz et al. 2022).

The focus of the present study was to investigate, for the first time, the patterns of growth rate of *P. pollicipes* populations in Europe using a standardized methodology (chemical marking with calcein). Four regions within the European distribution of *P. pollicipes* were considered: 1) Brittany, France; 2) Asturias-West, Spain; 3) Galicia, Spain; and 4) SW coast of Portugal. The hypothesis is that there is regional variation in the growth rate. In addition, seawater temperature was also measured in situ to investigate whether the hypothetical regional growth rate pattern was related to variation in seawater temperature.

## 3.3 Materials and methods

Study sites

The growth rate of *P. pollicipes* was assessed in four regions along the European distribution of this species (**Fig. 3.1**). Brittany (BR), in France, was the northern region defined and was coincident with the northern limit of the exploitable populations of *P. pollicipes*. In Spain, two regions were considered: Asturias (AS), in the north coast; and Galicia (GL), on the west coast. On the Portuguese coast the growth rate of *P. pollicipes* was studied on the SW coast (between the Cape of Sines and Cape São Vicente, Portugal). Within each region 2 to 3 sites were randomly selected.

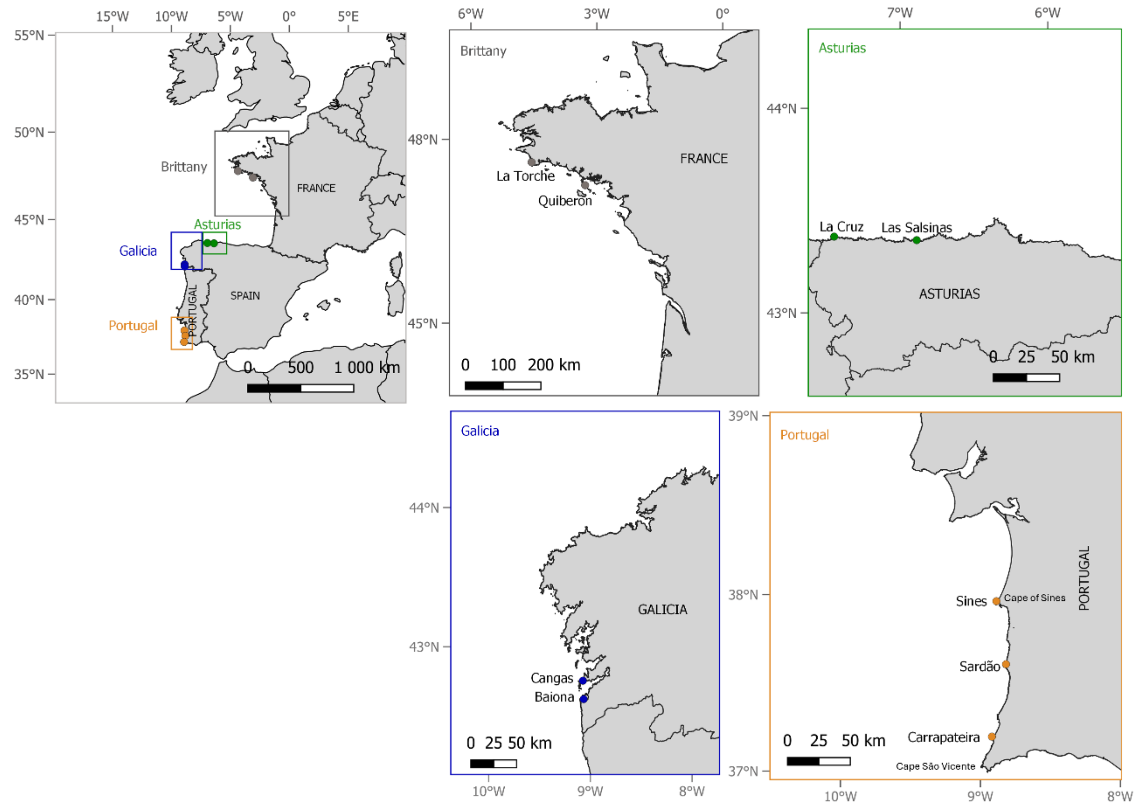


**Fig. 3.1** - Study regions: Brittany (France, in grey), Asturias and Galicia (Spain, in green and blue, respectively), and the SW Coast of Portugal (in orange).

*Growth rate (field and laboratory protocol adapted from Jacinto et al. (2015))*

During the summer of 2018 (June in Brittany, July in the other regions), several chips of rock (6 to 11) with *P. pollicipes* clumps attached (including barnacles with a wide range of sizes), were detached (using a hammer and chisel) from the middle intertidal level of *P. pollicipes* distribution (~1.5 m to 3 m above MLWS) of all sampled sites and transported to the laboratory. The chips of rock with barnacle clumps were immersed overnight (~20 h – marking period) in a solution of filtered seawater at 125 mg calcein $l^{-1}$ and kept in a closed circuit, continuously aerated with diffusive air stones, at room temperature. Barnacles were kept without being fed during the marking period. After the marking period, the chips of rock with barnacles attached were transported back to the same site (where they were collected) and randomly fixed across the same vertical level, using Z-Spar Splash Zone Epoxy (Kop-Coat Inc., Pittsburgh, PA). After a growth period of 59-89 days (depending on the site) in the field, the chips of rock with barnacles attached were collected and frozen (-20 ºC) until further analysis.

The laboratory analysis of the rock chips with attached barnacles consisted of thawing the samples, detaching the barnacles from the rock and individualizing all barnacles (RC > 1mm). All barnacles were initially measured (maximum rostro-carinal length, RC) with calipers (precision = 0.1 mm) and immersed in commercial bleach (sodium hypochlorite 3.5 %) from 1 minute up to 24h, depending on the size of the barnacle (see Jacinto et al. 2015). After the immersion in bleach, the scuta were detached from the capitulum and observed at a dissecting microscope (Leica M165FC) equipped with a UV light source and a GFP3 filter and photographed using a camera (Leica DF 295) coupled to the dissecting microscope and connected to a computer, for a initial visual inspection. If the calcein marking was successful, a fluorescent mark would appear on the calcareous plates. This mark allows the identification of the original scutum at the time of marking. Whenever calcein marks were identified, the maximal length of the scutum (sc_f), and the maximal length of the original scutum at the time of marking (edges detectable by the fluorescent calcein mark; sc_i), were measured on the right scutum with digital image analysis software (**Fig. 3.2**). Measurements were obtained with a digital image analysis software (Leica Application Suite v4.12).

A total of 514 individuals with visible marks on the right scutum (from a total of 1196 analyzed individuals, 307 in Brittany, 87 in Asturias, 284 in Galicia, 518 in Portugal) were used to estimate *P. pollicipes* growth rate in the 4 sampled regions (103 individuals from Brittany, 63 from Asturias, 133 from Galicia and 215 from the SW coast of Portugal).

The initial and final maximal rostro-carinal length were estimated as described by Jacinto et al. (2015), where a model based on the linear relationship between RC and the maximal scutum length was used to convert the measurements made in the right scutum (sc_i and sc_f) into RC units (RC_i and RC_f, respectively), using the formula bellow:

$$RC = 1.66 \times sc$$

Monthly growth rate (dRC30) was calculated based on the RC_i and RC_f, using the formula:

$$dRC30 = \frac{RC\_f - RC\_i}{number\ of\ days\ of\ the\ marked\ clump\ in\ the\ field} \times 30$$

Data were then organised into size classes according to the estimated RC_i of each marked barnacle: recruits – RC ≤ 5 mm; juveniles RC= ]5.0 mm – 12.5 mm]; small adults RC= ]12.5 mm – 17.5 mm]; adults with low commercial value RC= ]17.5 mm – 20.0 mm]; adults with moderate commercial value RC= ]20.0 mm – 22.5 mm]; and adults with high commercial value RC > 22.5 mm. As the number of barnacles that survived the marking period and had their scutum marked varied greatly from site to site, the variation between sites was not analysed. Consequently, the analysis of regional variation was done by pooling all the marked barnacles from each region. The hypothesis of a differential monthly growth rate (dRC30) along the European distribution of *P. pollicipes* was tested for each size class, using permutational multivariate analysis of variance PERMANOVA (Anderson 2001), considering one factor: region (fixed factor with four levels, BR, AS, GL and SW-PT). Sample size was variable and correspond to the number of barnacles analyzed in each region and size class (ranged between 2 and 89).

Analysis of the monthly growth rate was based on Euclidian distances. When appropriate, data were transformed with square root or fourth root. Unrestricted permutation of raw data and type III sums of squares were applied (Anderson et al. 2008). PERMANOVA was used to analyze univariate data due to an unbalanced design resultant of the different number of individuals of each size class analyzed in each region. In all analyses, the homogeneity of dispersion based on the Euclidean distances was tested using the PERMDISP routine (Anderson 2006). When appropriate pair-wise a posteriori comparisons were conducted. Software PRIMER 6 & PERMANOVA+ (www.primer.com; Anderson et al. 2008) was used to perform all statistical analyses.

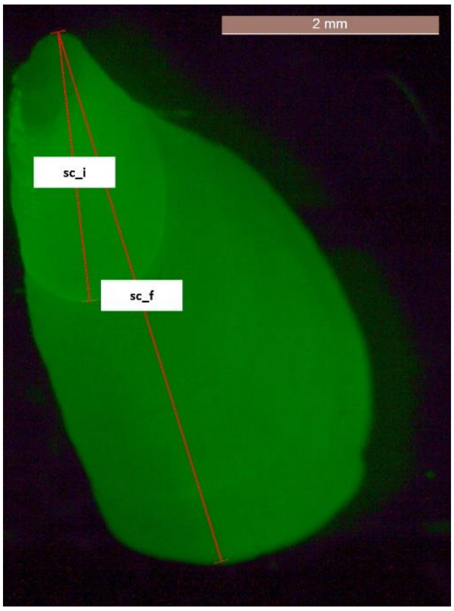


**Fig. 3.2** – Right scutum plate from a marked juvenile barnacle with the calcein mark visible. Red lines indicate the measurements of sc_i – initial scutum length and sc_f – final scutum length.

*Sea water temperature*

Seawater temperature was registered in the field, from 16th of July 2018 until 16th of September 2018 (with the exception of the SW Portugal temperature was registered in the field from 8th of August until 16th of September 2018), by the deployment of stand-alone temperature loggers (ibutton Thermochron® 1922L) in the midshore of all sample sites of the four regions, that registered temperature with 1h frequency.

A mean daily seawater temperature value per region was calculated by selecting and averaging the two daily values that coincided with the predicted times of peak high tide at each site and by calculating the mean daily value (2-3 sites) for the region.

## 3.4 Results

The percentage of *Pollicipes pollicipes* that presented visible calcein marks on the right scutum was 72 % in Asturias, 47 % in Galicia, 42 % in the SW coast of Portugal and 34 % in Brittany.

The mean RC measured with calipers was 11.4 ± 5.91 mm (mean ± SD) and the mean of RC_f estimated from the scutum measurement was 11.7 ± 5.73 (mean ± SD).

The monthly growth rate (dRC30, mm) was highly variable in all size classes, namely in recruits and juveniles (**Fig. 3.3**). Considering all size classes, the average growth rate was higher in Galicia than in the other regions (**Fig. 3.4**), with a more expressive result in the juveniles size class. However, regional variation was not always detected by the PERMANOVA analysis, but when this variation was significant (recruits, juveniles, adults with low commercial value, adults with moderate commercial value), the growth rate of *P. pollicipes* was always higher in Galicia (**Table 3.1**).

Considering all size classes, there was a decrease in the growth rate as the size of the animals increased (**Fig. 3.3** and **Fig. 3.4**). For instances, in Galicia, the mean growth rate ranged from 0.08 mm, RC (adults with moderate commercial value), to 1.39 mm, RC (juveniles) (**Fig. 3.3**). The average growth rate in the other regions was always highest in the size class of recruits (from 0.66mm, RC, in the SW coast of Portugal, to 0.98mm, RC, in Brittany) and lowest for adults (always less than 0.2mm, RC). (**Fig. 3.4**)

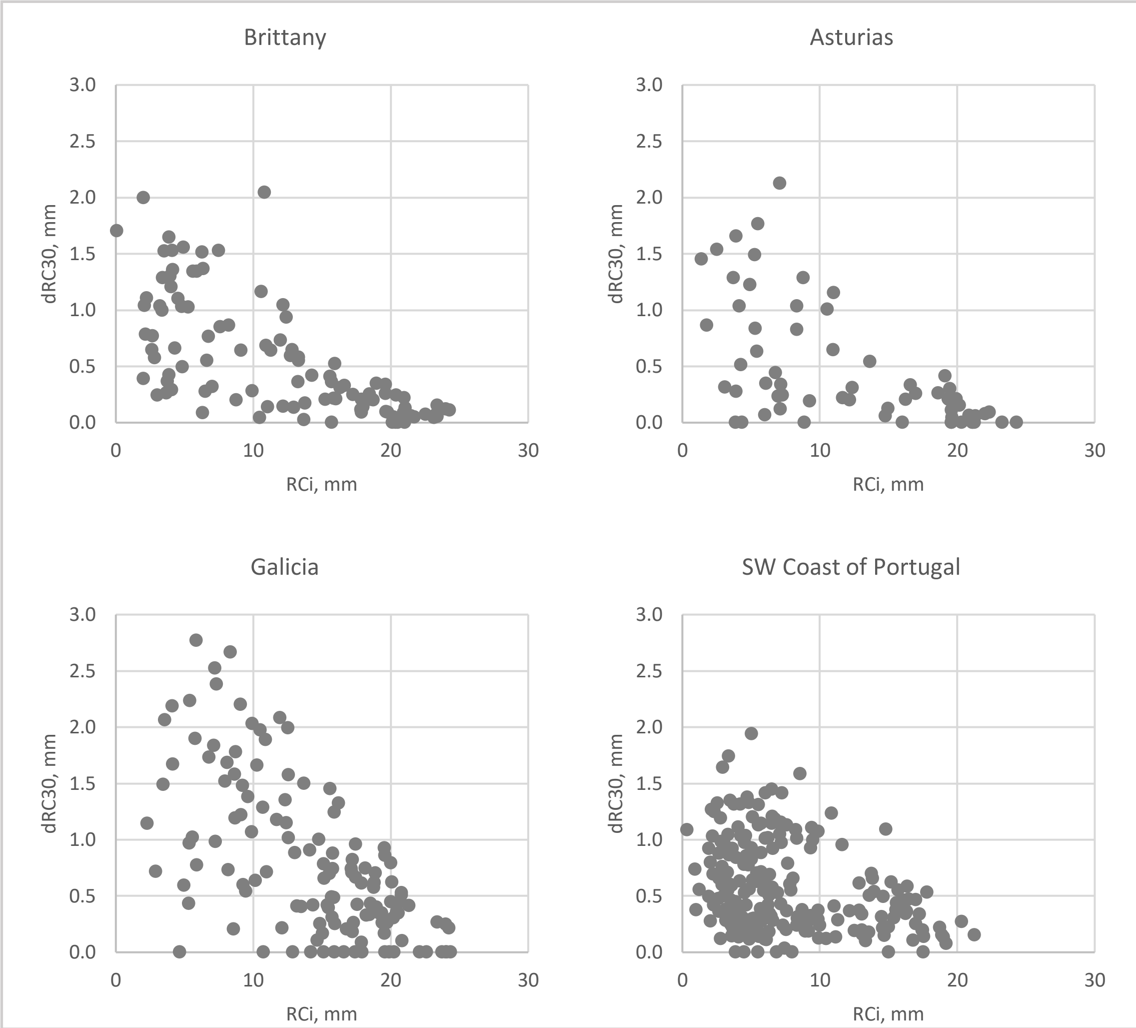


**Fig. 3.3** – Monthly growth (dRC30) of *Pollicipes pollicipes* in relation with the estimated initial maximal rostro-carinal length (RCi) in each of the sampled regions (Brittany in France, Asturias and Galicia in Spain, and the SW Coast of Portugal).

Regarding the variation in seawater temperature during the common deployment period of temperature loggers, the lowest mean value was registered in Galicia (15.4 ºC) (**Fig. 3.5**). In the other three regions the mean seawater temperature was approximately 1ºC higher (16.6 ºC in Brittany, 16.6 ºC in Asturias, and 17.4 ºC in the SW coast of Portugal; **Fig. 3.5**).

**Table 3.1** - PERMANOVA analysis on *Pollicipes pollicipes* monthly growth (dRC30) for each size class (size classes were based on the estimated initial maximal rostro-carinal length RCi, recruits - RC≤5 mm, juveniles RC= ]5.0 mm – 12.5 mm], small adults RC= ]12.5 mm – 17.5 mm], adults with low commercial value RC= ]17.5 mm – 20.0 mm], adults with moderate commercial value RC= ]20.0 mm – 22.5 mm] and adults with high commercial value RC>22.5 mm) in relation to factor "Region" (Re). Analyses were based on Euclidean distances of untransformed data (for recruits, adults with moderate and high commercial value data) and square root (for juveniles, adults with low commercial value data), fourth root transformed data (small adults data). P-values were obtained using 9999 random permutations. n= 2 to 40 replicate individuals. PERMDISP tests: F = 4.956 (recruits; $p < 0.05$), F = 0.491 (juveniles; $p > 0.05$), F = 4.208 (small adults; $p > 0.05$), F = 1.767 (adults with low commercial value; $p > 0.05$), F = 4.310 (adults with moderate commercial value; $p < 0.05$) and F = 24.484 (adults with high commercial value; $p < 0.05$). Significant p-values in bold. Pair-wise test for the significant factor in the previous analysis for each variable. > or < ($p < 0.05$); BR – Brittany, AS – Asturias/Spain, GL – Galicia/Spain, SW_Pt – Southwest Coast of Portugal.

| | Recruits | | Juveniles | | Small adults | | Adults with low commercial value | | Adults with moderate commercial value | | Adults with high commercial value | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | pseudo-F | p | pseudo-F | p | pseudo-F | p | pseudo-F | p | pseudo-F | p | pseudo-F | p |
| Re | 5.99 | **0.00** | 14.43 | **0.00** | 0.90 | 0.45 | 2.99 | **0.04** | 10.11 | **0.00** | 0.83 | 0.47 |
| Pair-wise | GL > SW-Pt, BR > SW-Pt, SW-Pt = AS, BR = AS, BR = GL, AS = GL | | GL > all other regions | | | | GL > AS GL = BR, GL = SW-Pt, AS = BR, AS =SW-PT BR= SW-Pt | | GL > AS, GL > BR, GL = SW-Pt, AS = BR, AS =SW-PT BR= SW-Pt | | | |

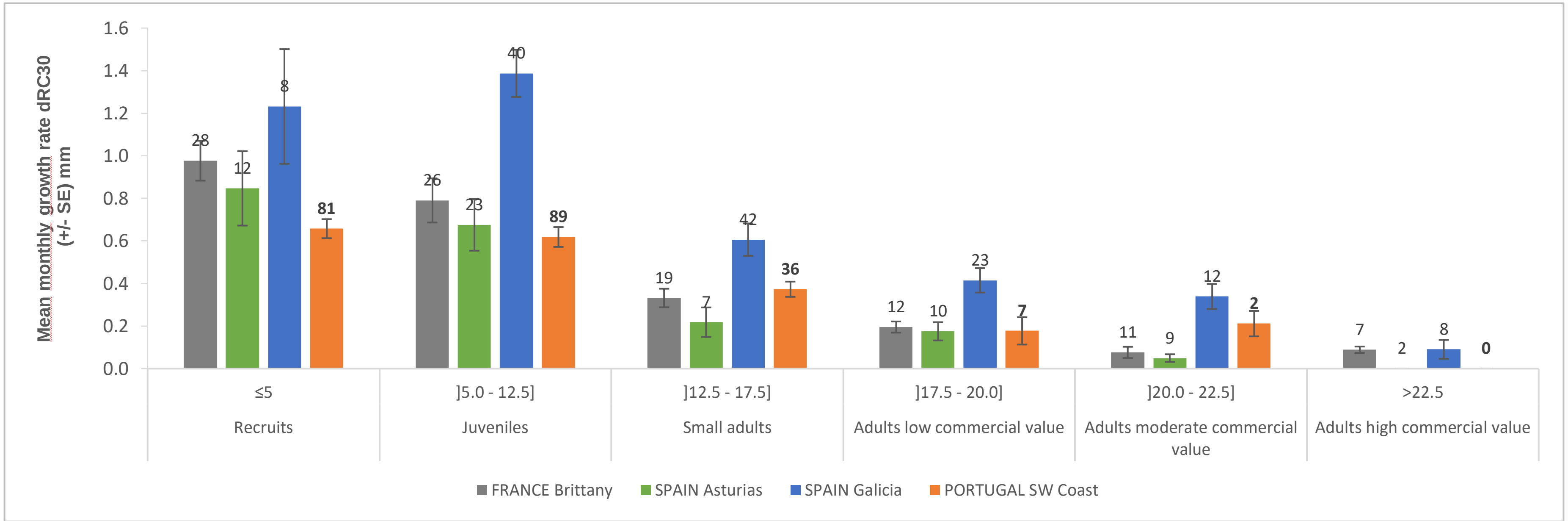


**Fig. 3.4 –** Mean monthly growth rate (+/- SE) (dRC30, mm) of the stalked barnacle *Pollicipes pollicipes* for each size class (size classes based on the estimated initial maximal rostro-carinal length RCi, recruits - RC≤5 mm, juveniles RC= ]5.0 mm – 12.5 mm], small adults RC= ]12.5 mm - 17.5 mm], adults with low commercial value RC= ]17.5 mm - 20.0 mm], adults with moderate commercial value RC= ]20.0 mm - 22.5 mm] and adults with high commercial value RC>22.5 mm), in each region (Brittany, France; Asturias and Galicia, Spain and the SW coast of Portugal). The number above each bar represents the sampling size.

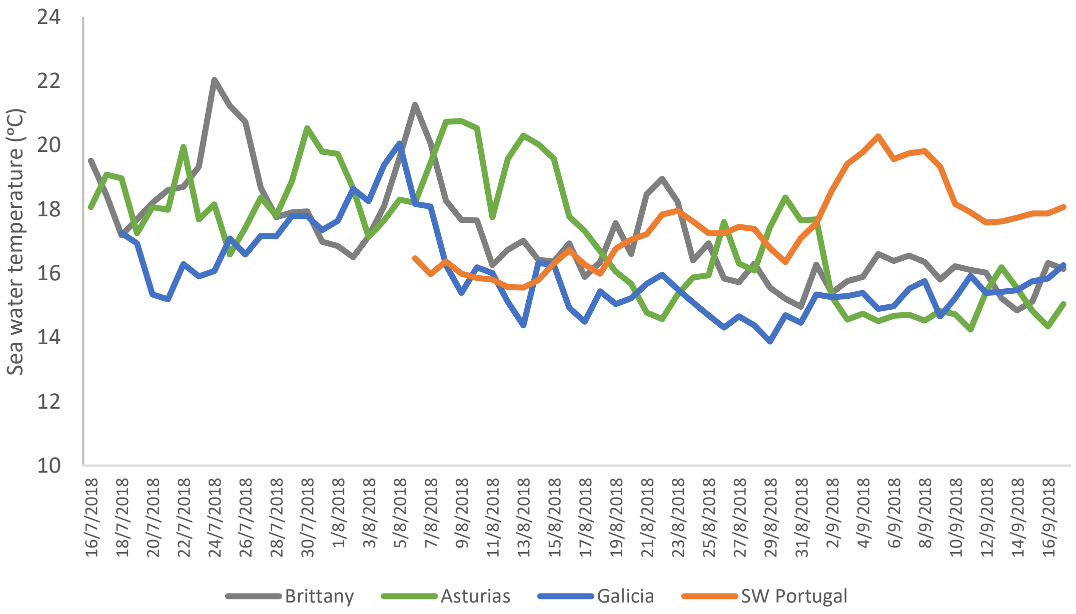


**Fig. 3.5 –**Average daily seawater temperature (ºC) in each region from 16th of July 2018 until 16th of September 2018.

### 3.5 Discussion

We present, for the first time, a comparison of the growth rate of *Pollicipes pollicipes* in various European regions by using a standardised methodology, the chemical marking with calcein. The most relevant result was that in all size classes the mean growth rate of *P. pollicipes* in Galicia was higher than in the other regions (Brittany, Asturias and SW Portugal). In the size class of juveniles, this result was expressive, about twice that of other regions. Assuming that this growth rate is maintained throughout the year (this work was done in the summer), we estimate that *P. pollicipes* can reach sexual maturity in 8 months in Galicia, while in other regions it takes at least 1 year. These estimates are consistent with what has been observed in other studies of the genus *Pollicipes* (see review of Cruz et al. 2022), in which it is stated that most individuals reach maturity within 1 year.

One possible explanation for the higher growth rate in Galicia might be indirectly related to seawater temperature. In fact, the average seawater temperature measured in situ during the present study was around 1ºC lower in Galicia than in the other regions. Also, based on Aguión et al. (2022b) in which SST was measured using satellite images for the same period of time (summer 2018), a lower average seawater temperature was observed in Galicia than in the other regions. Furthermore, based on Aguión et al. (2022b), we can identify that in the summer of 2018: the average chlorophyll a values in Galicia and SW Portugal were higher than in Asturias and Brittany; and the average upwelling index values in Galicia and SW Portugal are higher than in Asturias (there is no upwelling index data for Brittany). Therefore, we can consider that the lower temperature in Galicia in summer was associated with upwelling events and a higher primary productivity (measured by chlorophyll a) and probably to a greater availability of food that could be related to the higher growth rate of *P. pollicipes* that was observed in this region. This apparent relationship between lower seawater temperature and higher growth rate that we detected in Galicia is contrary to what has been observed in other studies, where an inverse relationship has been observed (e.g. in *P. polymerus*, Phillips 2005; in other crustaceans, Sanford & Menge 2001, Inatsuchi et al. 2010, Nishizaki & Carrington 2015).

Contrarily to Galicia, the pattern of lower growth rates of *P. pollicipes* during Summer in the SW coast of Portugal does not seem to be related to upwelling and the availability of food, which seems favourable, based on the upwelling and chlorophyll a data presented in Aguión et al. (2022b). One explanation could be that the growth rate in SW Portugal is being regulated by the high density of groups of barnacles in this region (unpublished data, chapter 2), associated with a possible greater intraspecific competition. In a study with *P. polymerus* in Oregon, USA, Helms (2004) also suggested that there may be a negative effect of density on the growth of juveniles of this species.

Another relevant pattern that had already been found in previous studies with *P. pollicipes* (Cruz 1993, 2000, Jacinto et al. 2015), with *P. polymerus* (Barnes & Reese 1960, Helms 2004, Phillips 2005) and with *P.elegans* (Pinilla 1996, Samamé & Quevedo 2001) is the higher and more variable growth rate in juveniles compared to adults. Further investigation is needed to better understand the great variability of the growth rate, namely of juveniles.

We present an updated version of the table revising the growth rates of the species of the genus *Pollicipes* presented in Cruz et al. (2022), including the estimates from the present study (**Table 3.2**). Thus, data from new locations has been added to the original table (Brittany and Asturias) and information on growth rates in Galicia and SW Portugal has been added. Overall, the estimates obtained on *P. pollicipes* growth rates from the present study were within what was presented in past studies for all the regions except the region of Galicia. In Galicia, past estimates based on size structure analysis of natural populations (Sestelo & Roca-Pardiñas, 2007, 2011) indicated a lower growth rate of large barnacles (RC > 15 mm) than those estimated in the present study using the calcein marking method (**Table 3.2**).

Compared to another study carried out in SW Portugal using the same method of marking with calcein (Jacinto et al. 2015), we obtained a much lower success rate in marking the barnacles (94 % success in Jacinto et al. 2015; 34 % to 72 % in the present study). This difference could be explained by the different concentration of calcein used in Jacinto et al. (2015) (220 mg calcein $l^{-1}$) and in the present study (125 mg calcein $l^{-1}$). In future studies, we propose using a calcein solution with the concentration used in Jacinto et al. (2015) study.

Studies such as this one, in which the variation of a fundamental biological process is analysed, in this case the growth rate, over a considerable area of the geographical distribution of a commercial species, are essential for improving fisheries management.

**Table 3.2** – Growth rates of *Pollicipes pollicipes* – original table from Cruz et al. (2022), updated with the data from the present study. Two size classes were considered: Small – RC < 15 mm; Large – RC > 15 mm.

| Country | Methods and location | Growth rates (mm RC/month) | | References |
|---|---|---|---|---|
| | | Small | Large | |
| France | Size increments of marked individuals (calcein tags). Brittany | 0.03 – 2.05 | 0 – 0.52 | Present work |
| Spain | Size increments of marked individuals (calcein tags). Asturias. | 0 – 2.13 | 0 – 0.41 | Present work |
| | Size structure analysis of natural population. Galicia. | - | 0.34 | Sestelo & Roca-Pardiñas (2007, 2011) |
| | Size increments of marked individuals (calcein tags). Galicia. | 0 – 2.77 | 0 - 1.45 | Present work |
| Portugal | Size increments of marked individuals (physical and calcein tags) on natural or transplanted clumps and artificial substrata; size structure analysis of recruits on cleared surfaces and artificial substrata. SW Portugal; RNB Portugal | 0.17 – 0.66 | 0.08 – 0.48 | Cruz (1993) |
| | | 0.18 – 5.20 | 0.11 – 0.47 | Cruz (2000), Cruz et al. (2010), Figueira (2015), Jacinto et al. (2015), Mateus (2015), Cruz et al. (2016a,b), Darras (2017), Mateus (2017), Belela (2018), Fernandes (2018), Santos (2019), Cruz et al. (2020), Neves (2021) |
| | Size increments of marked individuals (calcein tags). SW Portugal | 0 – 1.94 | 0 – 0.62 | Present work |
| Marocco | Size structure analysis of natural population; SW Morocco | 1.03 | 0.20–0.45 | Boukaici et al. (2012) |

## 3.7 Acknowledgments

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# **Chapter 4.** Morphological variation

## *4.1* Phenotypical variability affecting the commercial value of the stalked barnacle *Pollicipes pollicipes*: no evidence for epigenetic variation

Sousa A., Morán P., Acuña J.L., Vázquez E., Cruz T., & Macho G. 2024. Phenotypical variability affecting the commercial value of the stalked barnacle *Pollicipes pollicipes*: No evidence for epigenetic variation. Estuarine, Coastal and Shelf Science 303 108807. doi:10.1016/j.ecss.2024.108807

### 4.1.1 Abstract

The stalked barnacle *Pollicipes pollicipes* is an important economic resource in Portugal and Spain. Two extreme phenotypes can be identified, based on their morphology. More elongated barnacles are associated with bad quality and have a lower commercial value.

The fishers perception about the existence, definition and causes for this phenotypical/quality variation was evaluated through a survey performed in Portugal and Galicia, Spain. The existence of two extreme commercial qualities was validated. Good quality barnacles were mainly defined as thick and short in both countries. In Spain (Galicia), the definition of bad quality animals corresponded mainly to the terms long and thin, while in Portugal, fishers used a wider variety of terms including watery, thin and soft. The characteristics of the rock and the hydrodynamics were the causes most referred by the fishers for this variation.

The morphological variation of *P. pollicipes* was described by the ratio between maximal rostro-carinal length (RC) and total height (TH): RC/TH values >0.4 indicate good quality barnacles; and values <0.4 indicate poor quality barnacles.

Although morphological variation between the two extreme qualities/phenotypes was found, no genetic (amplified fragment length polymorphism - AFLP) or epigenetic (methylation sensitive amplification polymorphism - MSAP) differences were detected.


### 4.1.2 Introduction

The economic value of marine exploited species is influenced by several factors such as the size (Santojanni et al. 2005), geographical location (Lahbib et al. 2010, Ramírez-Valdez et al. 2021), market factors (Natividad 2016) and/or quality attributes (Reynolds and Wilen 2000, Rocha et al. 2019). Quality attributes includes, for example, sex and variation in gonad colour in sea urchins (Rocha et al. 2019) and in mud crabs (Waiho et al. 2020), freshness and storage methods of several species of fishes such as sardines, cod and swordfish (e.g. Ababouch et al. 1996, Erkan and Özden 2008, Ishimura and Bailey, 2013, Lee 2014), and morphology in fishes (e.g. Lee 2014, Sjöberg

2015), mud crabs (Fazhan et al. 2020) and stalked barnacles (e.g. Lessard et al. 2003, Parada et al. 2012).

The most evident morphological variability in stalked barnacles of the genus *Pollicipes* is the variation in the peduncle. Two extreme phenotypes were described in the literature for *Pollicipes polymerus* Sowerby, 1833 and for *Pollicipes pollicipes* Gmelin, 1791 [in Gmelin, 1788–1792]. One phenotype was characterized as: stouter individuals (Chaffee and Lewis 1988) with strong peduncles, relatively short and that attach to the substrate with a considerable basal area (Barnes and Reese 1960) (*P. polymerus*); and barnacles with a smooth peduncle (Parada et al. 2012) containing a large amount of muscle (Rivera et al. 2014, Seoane-Miraz 2015) (*P. pollicipes*). The other extreme phenotype was described as: slender individuals (Chaffee and Lewis 1988) with a greatly elongated peduncle and a smaller attachment area of the peduncle to the substrate (Barnes and Reese 1960) (*P. polymerus*); and barnacles with a wrinkled peduncle (Parada et al. 2012) that is thin, long and has a high water content (Seoane-Miraz 2015) (*P. pollicipes*).

In *P. polymerus* this phenotypical variation was mainly related to wave exposure (Barnes and Reese 1960) and to the relative position of the barnacles on the clump (Chaffee and Lewis 1988). The barnacles with strong and short peduncles were associated with very wave-exposed locations, while those with elongated peduncle were associated with more sheltered places (Barnes and Reese 1960). Also, slender individuals were generally located in the centre of the barnacles clumps of this species (Chaffee and Lewis 1988). In *P. pollicipes*, this phenotypical variation was associated with differences in individual density, with locations with higher densities having thinner and longer animals (Cruz 2000). However, this factor might be confounded with other factors such as hydrodynamics or predation (Cruz 2000). Seoane-Miraz (2015) related the barnacles with the long peduncles as inhabiting shaded locations, as opposed to individuals in the sun which had more robust peduncles. However, these causes have not been experimentally tested.

Stalked barnacles of the genus *Pollicipes* are exploited all over the world. The most intensively exploited species is *P. pollicipes*, mainly in Portugal and Spain, but also in France (Molares and Freire 2003, Cruz et al. 2022). The annual economic value of the *P. pollicipes* fishery in Europe is of EUR 10 million, representing 500t of landings and involving 2,100 professional fishers (Aguión et al.

2022). In Spain (Galicia and Asturias) and also in the SW coast of Portugal, the economic value of this species was considered to be influenced by its quality/morphology, with elongated barnacles being associated with bad quality and having a lower commercial value (Parada et al. 2012, Rivera et al. 2016). In fact, following the fishers Local Ecological Knowledge (LEK), sections of the rocky coast have been classified according to their perceived quality/morphology of *P. pollicipes*, both in Galicia (Parada et al. 2012) and west Asturias (Rivera et al. 2014, 2016). Also, in the SW coast of Portugal, fishers have classified sections of the coast in relation to their perception on the quantity and quality of *P. pollicipes* (Carvalho et al. 2017). However, despite of the importance of the variation in quality/morphology for the fishery, we are not aware of any study that investigated whether fishers, in general, consider that there are individuals of *P. pollicipes* of different qualities, what is the definition of good and bad quality barnacles, and what is their perception on the causes of this quality variation. Furthermore, the fishers from Galicia have been concerned about the barnacles with the elongated morphology, since they have the perception that this morphology had become more abundant (Quinteiro et al. 2006). As a consequence, the fishers' guilds ("cofradías") had contacted the scientific community in order to obtain more information about this phenotype, namely if it was a different species.

Phenotypical variability can be related to genetic differences but can also be a result of environmental factors (Mokady et al. 2000). In a preliminary study carried out in Galicia, no evidence of genetic differences between the two *P. pollicipes* phenotypes was found (Quinteiro et al. 2006). However, by studying the expression of 5 genes in the peduncular muscle, a differential genetic expression for the two extreme *P. pollicipes* phenotypes was observed, namely an overexpression of 4 of the 5 studied genes in the peduncular muscle of the phenotype of barnacles with short and robust peduncles (Seoane-Miraz 2015). This author suggested that the overexpression of these genes, mainly related to the muscular and cuticular integrity of the peduncle (guanine nucleotide-binding protein, chitin based cuticle attachment to epithelium, cuticular protein 11B and cuticular protein 47Ee genes), provides these barnacles the enough strength to maintain themselves attached to the rocky substrate. On the other hand, the phenotype of barnacles with long and thin peduncles showed a reduced expression of these genes (Seoane-Miraz 2015).

Epigenetics is the study of hereditary alterations in the expression and genetic functions that cannot be explained by alterations in the DNA sequence (Richards 2006, Bird 2007, Bossdorf et al. 2008). DNA methylation is one of the main epigenetic mechanisms for the regulation of gene expression in eukaryotes. DNA methylation was described in mammals and other species as a mark repressing transcription, where the presence of DNA methylation at CpG-rich gene promoters, called CpG islands, would block transcription factor binding leading to gene silencing (Bird 2002, Kaluscha et al. 2022). In vertebrates, gene bodies with substantially enriched DNA methylation are positively correlated with the level of gene transcription (gene expression), suggesting that methylation at these regions has a positive role in gene regulation (Keller et al. 2016).  while there is evidence in some invertebrates that this correlation does not exist (Dixon and Matz. 2022). Among invertebrate species, methylation has been extensively studied in *Daphnia* spp. and in the Pacific oyster (*Crassostrea gigas*) where the role of methylation in gene regulation has been widely demonstrated (see Song et al. 2017, Kvist et al. 2018 references there). The role of methylation in gene expression has also been studied in other crustacean species such as the mud crab *Scylla paramamosain* (Jiang et al. 2020) and the Kuruma shrimp, *Marsupenaeus japonicus* (Wang et al. 2020).

The aims of the present study were: (1) to evaluate the perception of the fishers from Spain and Portugal about the existence of different commercial qualities of P. *pollicipes*, in order to describe the definition of two extreme qualities of  barnacles  (good and bad), and about the potential causes that may be determining this variation; (2) to characterize the morphometry of both extreme qualities/phenotypes of P. *pollicipes* from Spain (Galicia and Asturias) and Portugal (Alentejo) and (3) to determine the genetic and DNA methylation patterns of both extreme qualities/phenotypes of P. *pollicipes* from Spain (Galicia and Asturias) and Portugal (Alentejo).

### 4.1.3 Material and Methods

The present study includes three parts: (1) a survey to professional fishers of *P. pollicipes*; (2) a morphological analysis; and (3) genetic/epigenetic analyses of this species.

*Survey to fishers*

The survey to the fishers (in Supplementary Material **S4.1**) was performed in Portugal and in Galicia (Spain). In Portugal, fishers from two marine protected areas, “Reserva Natural das Berlengas - RNB” and “Parque Natural do Sudoeste Alentejano e Costa Vicentina - PNSACV” (see **Fig. 4.1.1**), were interviewed. In Galicia, surveyed fishers belonged to 11 “cofradías”(**Fig. 4.1.1**).


**Fig. 4.1.1.** Map with the sampled locations used for the morphological, genetic and epigenetic study (indicated in bold). Survey to professional fishers was run in several “cofradías” in Galicia, Spain – black dots (from North to South: Vicedo, A Coruña, Malpica, Laxe, Camelle, Camariñas, Muxia, O Pindo, Lira, Cangas and Baiona); and at RNB (“Reserva Natural das Berlengas”) and PNSACV (“Parque Natural do Sudoeste Alentejano e Costa Vicentina”) in Portugal.

Fishers were interviewed by telephone during the spring/summer of 2020. All interviews were conducted in their native language (Portuguese or Spanish). In Portugal, 52 professional fishers participated in the survey, 25 from RNB (which represents 63 % of the professional fishing licences for *P. pollicipes* in RNB) and 27 from PNSACV (which represents 34 % of the professional fishing licences for *P. pollicipes* in PNSACV). In Galicia, a total of 45 professional fishers from several “cofradías” participated in the survey: Vicedo (n=4), A Coruña (5), Malpica (5), Laxe (2), Camelle (3), Camariñas (5), Muxia (1), O Pindo (5), Lira (5), Cangas (5), and Baiona (5). Based on official data from 2022, these 45 fishers represent 8 % of the total number of *P. pollicipes* licences in Galicia, and approximately 17 % of the active fishers (not all fishers with licence are active fishers, it has been estimated that only around half of the licences are actually in use, based on data from the cofradía of Bueu – unpublish data, Gonzalo Macho).

The fishers were questioned about (1) their opinion on the existence of stalked barnacles with different qualities, (2) their definition of both extreme qualities of stalked barnacles (good and bad) and (3) their perception on what is causing this variation. Questions 2 and 3 were open-ended questions and to analyse the respective answers, similar responses (in the original language) to each question were grouped into a single English term/expression representing the responses (Tables **S4.1.1**, **S4.1.2** and **S4.1.3**).

All interviews performed were confidential and anonymous and all the fishers gave their consent to answer to the survey and knew about the objective of the study.

*Morphological analysis*

*Pollicipes pollicipes* of the two extreme phenotypes (good quality – short and thick barnacles and bad quality – thin and long barnacles) were sampled by professional fishers or scientists in three locations: Cudillero (43°33'43.7"N 6°06'21.0"W), Asturias (hereafter called Cudillero) in July 2017; Baiona (42°07'06.5"N 8°52'00.2"W), Galicia (hereafter called Baiona) in October 2016; and Cape of Sines (37°57'46.49''N 8°53'10.04''W), Alentejo, Portugal (hereafter called Sines) in September 2017 (see **Fig. 4.1.1**). All samples were preserved in 99% alcohol and kept at -4○C until further analysis.

In the laboratory, 11/12 (from Baiona) and 19/20 individuals (from Sines and Cudillero) with a maximal rostro-carinal length (RC) of more than 16 mm were randomly selected from different clumps of stalked barnacles of each quality. The minimum size of 16 mm was defined to ensure that the analysed individuals were adults (minimum sexual maturity size (RC) is 12.5 mm, Cruz and Araújo 1999) and have some commercial value.

All individuals were measured with a calliper (precision 0.1 mm) to register RC and the total height (TH) in order to calculate the individual RC/TH ratio. The RC/TH ratio variability was analysed by permutational multivariate analysis of variance, PERMANOVA (Anderson 2001) including two factors: 1) Quality (fixed factor with two levels: good quality and bad quality barnacles), 2) Location (random factor with three levels: Cudillero, Baiona and Sines). Analyses were based on Euclidean distances of untransformed data. Unrestricted permutation of raw data and Type III sums of squares were applied (Anderson et al. 2008). PERMANOVA was used to analyse univariate data due to the unbalanced design (different sample size among locations) and PERMDISP (Anderson 2006) to test homogeneity of univariate dispersion. When appropriate, pair-wise a posteriori comparisons were conducted. The software PRIMER 6 & PERMANOVA+ (Anderson et al. 2008) was used to perform the morphological statistical analysis.

*Genetics and epigenetics analyses*

The genetic and epigenetic analyses of stalked barnacles were run considering the same samples that were used in the morphological analysis.

Genomic DNA was isolated from muscle tissue of the two qualities of barnacles of the three sampled populations (Cudillero, Baiona and Sines) using the E.Z.N.A®Mollusc DNA Kit (Omega Bio-Tek), following the manufacturers instructions. Subsequently DNA quality and concentration were checked with a Nanodrop-1000 spectrophotometer. DNA extractions were adjusted to a final concentration of 50 ng/µL and frozen until use.

Amplified fragment length polymorphism (AFLP) methodology was based on a modified version of Vos et al. (1995). For each individual, 50 ng of DNA were digested and ligated using 5 U of EcoRI and 3 U of MseI (New England Biolabs). The obtained DNA fragments were ligated with specific adapters and subjected to two consecutive amplification rounds. A first pre-selective PCR, using EcoRI-A and MseI-C preselective primers was followed by a second selective PCR with 6-FAM labelled EcoRI-ACT and MseI-CAC selective primers.

A methylation sensitive amplification polymorphism (MSAP) protocol was adapted from Reyna-López et al. (1997). Briefly, each DNA sample was digested in parallel reactions with either EcoRI/HpaII or EcoRI/MspI endonucleases. The obtained DNA fragments were ligated with specific adapters and subjected to two consecutive amplification rounds. A first pre-selective PCR, using an HpaII/MspI+T and EcoRI+A primer pair was followed by a second selective PCR with 6-FAM labelled

HpaII/MspI+TAG and HpaII/MspI+TCC primers. A detailed protocol of the entire procedure is given in Morán and Pérez-Figueroa (2011).

PCR products, (AFLPs and MSAP) were loaded simultaneously with a GeneScan 500 ROX size standard (Thermofisher) into an ABI Prism 310 Genetic Analyzer Fragment analysis and AFLP scoring was performed using GeneMapper v.3.7 software (Thermofisher).

AFLP markers were scored as dominant binary markers 1 and 0, for fragment presence and absence, respectively and analysed with the R package MSAP (Pérez-Figueroa 2013) using the option meth = FALSE. MSAP profiles were assessed from the resulting absence/presence matrix with the R package MSAP. Loci were categorized as non-methylated (NMT) on specimens amplifying bands for both HpaII and MspI digestions, internal cytosine methylated (ICM) or hemimethylated (HMM), if bands were respectively present only on either MspI or HpaII, or hypermethylated (HPM) whenever both bands were not present for a given specimen. The absence of genetic differentiation between phenotypes was checked. The option no.bands = "h" was used for the analysis assuming that HPA-/MSP- (no band for both isoschizomers) pattern represents full methylation of cytosine6.3s in the target (hypermethylation), ignoring the chance of genetic change in the target. Loci below a 5 % error rate threshold and showing less than two occurrences of each state were systematically excluded from the analysis. Differences among phenotypes for each population were assessed with a multivariate Principal Coordinate Analysis (PCoA) and Analysis of Molecular Variance (AMOVA), using the R package MSAP (Pérez-Figueroa 2013). To further assess whether locus-specific methylation is dependent on phenotype, Fisher exact tests were used to detect candidate loci among the Methylation-Susceptible Loci (MSL). After statistical adjustment of the resulting *P-values* according to Benjamini and Hochberg false discovery rate (FDR), only loci showing $P < 0.05$ were selected (Benjamini & Hochberg 1995).

### 4.1.4 Results

*Survey to fishers*

Most fishers from Galicia and Portugal, considered the existence of stalked barnacles with different qualities (96 % and 100 %, respectively).

When asked about a definition for good quality barnacles, 24 % of the Galician fishers (11 of the 45 surveys performed) and 23 % of the Portuguese fishers (12 of the 52 surveys performed), were not able to define them, consequently only answers of fishers that mentioned a definition were considered on the following analysis. The two terms that were more mentioned to define good quality barnacles were related to the morphometric characteristics "thick" (91 % in Galicia and 63 % in Portugal) and "short" (56 % in Galicia and 50 % in Portugal) (**Fig. 4.1.2**). Most of the Galician fishers also stated the term "red coloured" (53 %). Both Galician and Portuguese fishers also mentioned the term "hard" as relevant (44 % in Galicia and 38 % in Portugal). Other expressions less used (≤ 15 %) to define good quality barnacles were "dark colored", "yellow capitulum", "heavier", "tastier", "less water", "preserve longer time" and "sweeter" (**Fig. 4.1.2**).

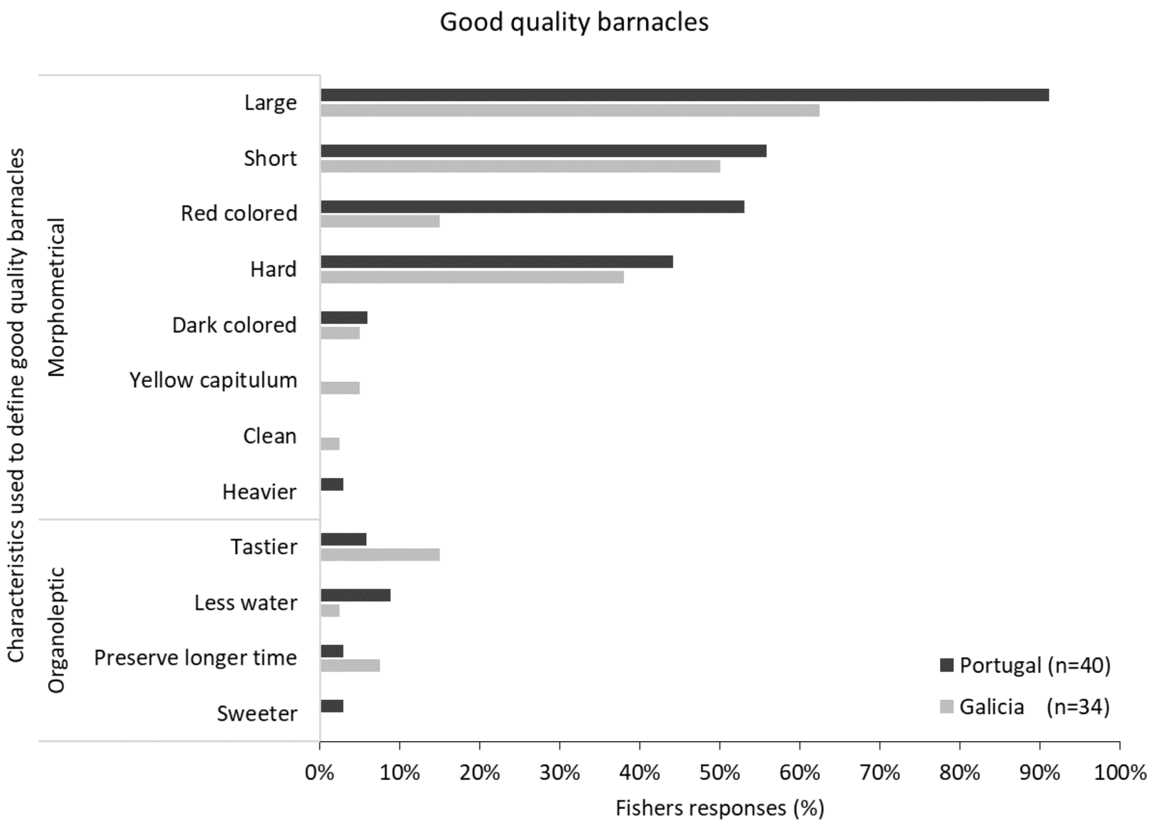


**Fig. 4.1.2**. Characteristics mentioned by Galician and Portuguese fisher to define good quality barnacles. The terms and expressions were grouped in one English term that better described the characteristic. The terms and expressions were mentioned in the native languages presented in supplementary material **Table S4.1.1**. Only the answers of fishers that mentioned a definition were considered (34 fishers in Galicia and 40 in Portugal).

Regarding the definition of bad quality barnacles, 22 % of the Galician fishers (10 of the 45 surveys performed) and 31 % of the Portuguese fishers (16 of the 52 surveys performed), were not able to define them, consequently only the answers of fishers that mentioned a definition were considered on the following analysis. The terms that were most mentioned by the fishers of Galicia to define bad quality barnacles were related to the morphometric characteristics “long” (86 %) and “thin” (51 %) (**Fig. 4.1.3**). The Portuguese fishers used a wider variety of terms to define bad quality barnacles, including the morphological characteristics “thin” (36 %), “soft” (31 %) and “long” (25 %), but also the organoleptic term “watery” (39 %), which was the one most frequently mentioned by these fishers (**Fig. 4.1.3**). Galician fishers also used the morphological term “soft” (40 %) and the organoleptic term “watery” (23 %). Both Galician and Portuguese fishers also mentioned the terms (<30 %): “dark”, “green capitulum”, “small”, “yellow capitulum” “empty”, “preserve less” and “flavourless” to define bad quality barnacles (**Fig. 4.1.3**).

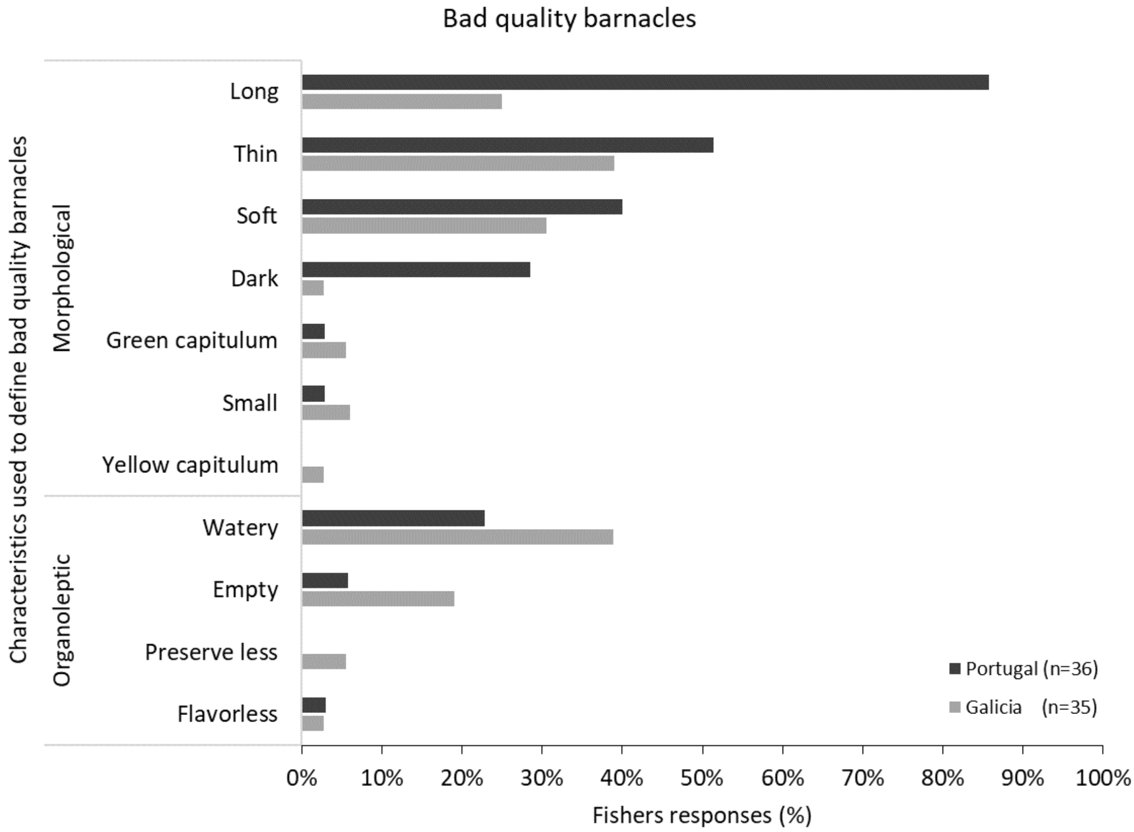


**Fig. 4.1.3**. Characteristics mentioned by Galician and Portuguese fishers to define bad quality barnacles. The terms and expressions were grouped in one English term that better described the characteristic. Terms and expressions mentioned in the native languages presented in supplementary material **Table S4.1.2**. Only the answers of fishers that mentioned a definition were considered (35 fishers in Galicia and 36 in Portugal).

When fishers were asked about their opinion on the causes of the variation in quality of the stalked barnacles, 31 % of the Galician fishers (14 of the 45 surveys performed) and 8 % of the Portuguese fishers (4 of the 52 surveys performed) were not able to explain them. Those who answered pointed out several causes, that differed slightly between the fishers of Portugal and Galicia. Almost the totality of the fishers from Portugal considered that the variation in the quality of stalked barnacles was related to some characteristics of the rock (96 %, **Fig. 4.1.4**), being the hydrodynamics the second more referred cause (31 %, **Fig. 4.1.4**). However, in Galicia there were no causes invoked by the large majority of the fishers, although the two most referred causes were the same: hydrodynamics (45 %) and characteristics of the rock (46 %) (**Fig. 4.1.4**). In addition to the above referred causes, the fishers from Portugal and Galicia also referred as causes for this quality variation (< 20 %): tidal level, general local characteristics, density of barnacles, food, solar exposure, and water/air temperature (**Fig. 4.1.4**). However, another regional difference was the greater number of causes cited by fishers in Galicia compared to Portugal (**Fig. 4.1.4**). The fishers from Galicia added the environmental conditions, the water quality, and genetic differences to the list of possible causes of this quality variation (**Fig. 4.1.4**).

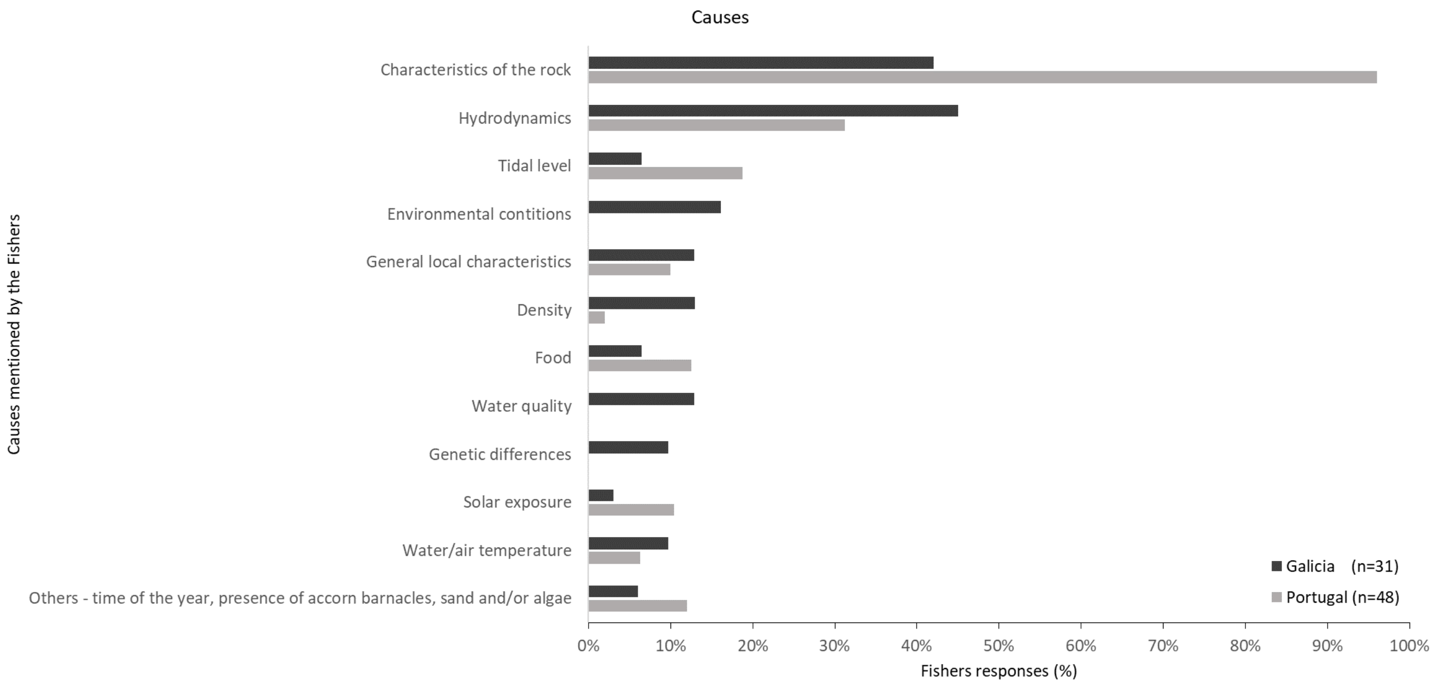


**Fig. 4.1.4** Causes mentioned by Galician and Portuguese fishers to explain the morphological variation between good and bad quality barnacles. The identified causes were grouped in one English term/expression that better described the cause. causes mentioned in the native languages presented in supplementary material **Table S4.1.3**. Only the answers of fishers that mentioned a cause were considered (31 fishers in Galicia and 48 in Portugal).

*Morphological analysis*

The average RC/TH ratio of good quality barnacles and bad quality barnacles ranged between 0.48 (Sines, n=20) and 0.55 (Cudillero, n=19) (**Fig. 4.1.5**), and between 0.33 (Baiona, n=11) and 0.41 (Cudillero, n=20) (**Fig. 4.1.5**), respectively.

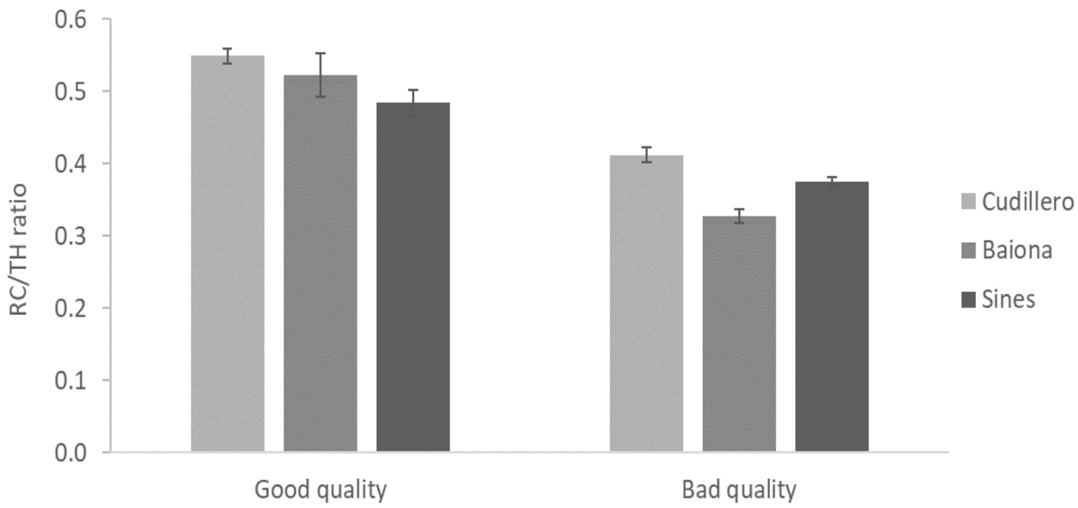


**Fig. 4.1.5.** *Pollicipes pollicipes* RC/TH ratio (mean± SE; n=19/20 for Cudillero and Sines and n=12/11 for Baiona) for the good and bad quality barnacles from the three sampled locations (Cudillero, Baiona and Sines).

The morphological analysis on the RC/TH ratio variability revealed a significant interaction of the factor quality with the factor location, but the RC/TH ratio was significantly higher for good quality barnacles when compared with low quality barnacles in all locations (**Table 4.1.1**). Homogenized dispersions were not observed among qualities (PERMDISP<0.05). A lower RC/TH ratio indicates longer barnacles.

**Table 4.1.1.** PERMANOVA main test and pair-wise test on the RC/TH ratio in relation to "Quality" (Qu) and "Location" (Lo). Analyses were based on Euclidian distance of untransformed data. p-Values were obtained using 9999 random permutations. n=12/11 for Baiona and n=20/19 for Cudillero and Sines. Significant effects are indicated in bold (p<0.05). PERMDISP test: F=6.76 (Quality; p<0.05).

| Source of variation | d.f. | MS | Pseudo-F | *p* |
|---|---|---|---|---|
| Qu | 1 | 0.515 | 35.40 | **0.039** |
| Lo | 2 | 0.034 | 9.52 | **0.000** |
| Qu x Lo | 2 | 0.013 | 3.81 | **0.029** |
| Res | 95 | 0.035 | | |
| Pair-wise tests | | | | |
| Qu x Lo | *Qu*<br>Good quality > Bad quality (*p*<0.01 in Cudillero, Baiona and Sines) | | | |

*Genetic and epigenetic analysis*

A total of 72 individuals were analysed for AFLP, 31 from Cudillero (13 good and 18 bad quality), 26 from Baiona (13 good and 13 bad quality) and 15 from Sines (7 good and 8 bad quality), resulting in

229 loci. 99 % of the loci were polymorphic. First, bad and good quality barnacles for each population were tested for differences. As no differences were found between phenotypes for each population, the samples were grouped for a comparison among populations. AMOVA showed significant differentiation among populations ($\Phi ST = 0.046$, $p < 0.001$). The principal coordinates analysis (PCoA, **Fig. 4.1.6**) clearly shows the among population differentiation. The first two principal coordinates (C1 and C2), account for 6.8 % and 6.2 %, respectively, of the total variance.

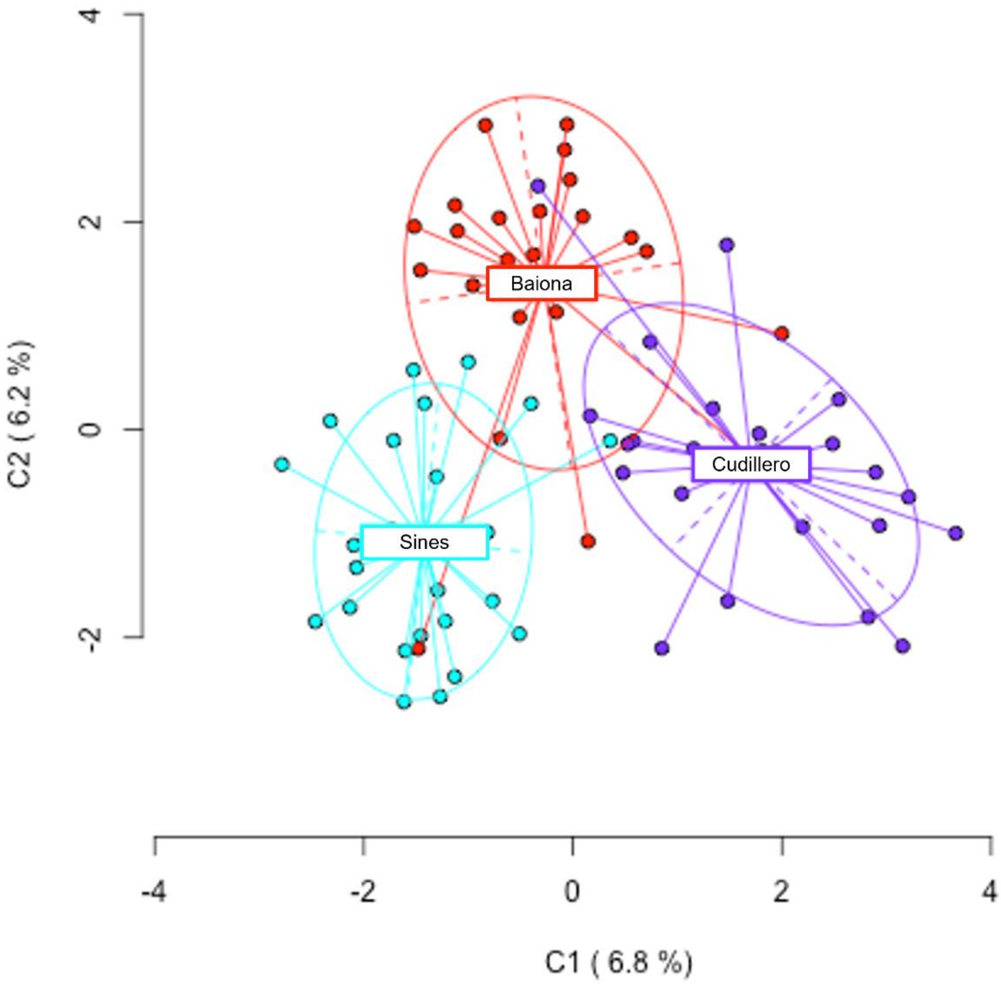


**Fig. 4.1.6.** Results of the Principal Coordinates Analysis (PCoA) with respect to the genetic differences detected among populations. The first two coordinates (C1 and C2) are displayed indicating the explained variance percentages in brackets. Scores represent individual samples. Labels indicate the centroids of each population. Ellipses represent the dispersion associated with each value with the long axis showing the direction of the maximum dispersion, while the short axis depicts the direction of minimum dispersion. In purple, barnacles from Cudillero (Asturias, Spain); in red, barnacles from Baiona (Galicia, Spain); in blue, barnacles from Sines (Alentejo, Portugal).

MSAP analysis detected a total of 222 loci. Since there are remarkable genetic differences among the three analysed populations, the differences in methylation between phenotypes were evaluated for each population although the results of presence and absence of bands were obtained globally, and were run simultaneously in GeneMapper. The number of Methylation-Susceptible Loci (MSL) in each population ranges from 43 % to 61 %. The relative frequency of the different states of methylation for the two phenotypes in each population is given in **Table 4.1.2**.

**Table 4.1.2.** Frequency of polymorphic Methylation-Susceptible Loci (MSL). AMOVA results between phenotypes for each population. Frequency (%) of the different states of methylation for the sampled populations. Good=good quality morphotype, Bad=bad quality morphotype.
[a] Methylation-susceptible loci

| State of Methylation | Cudillero | | Baiona | | Sines | |
|---|---|---|---|---|---|---|
| | Good | Bad | Good | Bad | Good | Bad |
| % polymorphic MSL[a] | 48 % | | 61 % | | 43 % | |
| AMOVA | $F_{ST}$ = 0.001 ($p$ = 0.4158) | | $F_{ST}$ = 0.020 ($p$ = 0.1202) | | $F_{ST}$ = -7.744e$^{-05}$ ($p$ = 0.4577) | |
| HPA/MSP+ (unmethylated) | 14.0 % | 13.3 % | 14.8 % | 17.3 % | 14.9 % | 13.5 % |
| HPA/MSP- (hemimethylated) | 11.5 % | 10.6 % | 11.8 % | 12.3 % | 11.3 % | 9.8 % |
| HPA/MSP+ (internal cytosine methylated) | 12.1 % | 11.0 % | 18.0 % | 16.0 % | 10.7 % | 11.4 % |
| HPA-/MSP- (full methylation) | 62.3 % | 64.9 % | 55.1 % | 54.2 % | 62.9 % | 65.2 % |

It can be observed that the frequency of unmethylated, hemimethylated, methylated states in the internal cytosine and full methylation are quite similar between phenotypes and populations. The absence of epigenetic variation between phenotypes was confirmed by both PCoA and the AMOVA. Genomewide methylation patterns were not statistically significant between phenotypes for each population (AMOVA, **Table 4.1.2**). The PCoA of the three populations with regard to the phenotypes is depicted in **Fig. 4.1.7**. The first two principal coordinates (C1 and C2), account for 15.2 to 12.2 % and 12.2 to 8.4 % respectively of the total variance. However, the clusters of each phenotype are overlapping and show no difference between phenotypes for any of the populations.

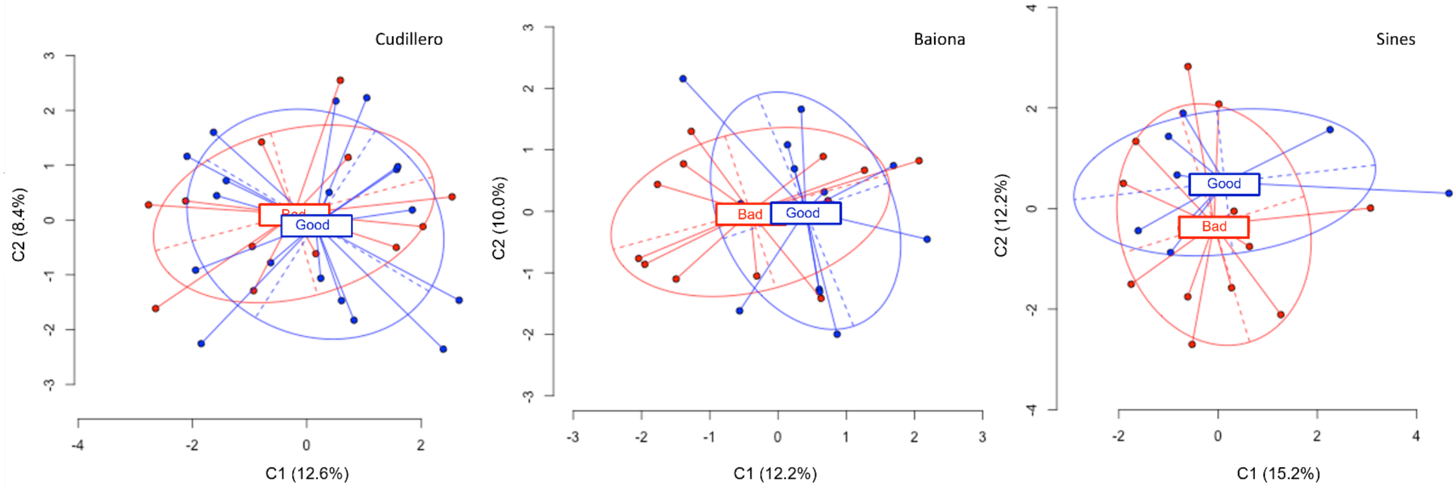


**Fig. 4.1.7.** Results of the Principal Coordinates Analysis (PCoA) with respect to the epigenetic differences detected between phenotypes in populations from Cudillero (Asturias, Spain), Baiona (Galicia, Spain) and Sines (Alentejo, Portugal). The first two coordinates (C1 and C2) are displayed indicating the explained variance percentages in brackets. Scores represent individual samples. Labels indicate the centroids of each morphotype. Ellipses represent the dispersion associated with each value with the long axis showing the direction of the maximum dispersion, while the short axis depicts the direction of minimum dispersion. In blue good quality barnacles and in red bad quality barnacles.

The single-locus analysis by means of Fisher exact test revealed up to 1 loci, 0 loci and 3 loci displaying significant ($P < 0.05$) methylation differences between phenotypes in Asturias, Galicia and Portugal populations respectively. However, none of these loci remained significant ($P < 0.05$) after FDR adjustment. Therefore, by means of the MSAP technique, no direct evidence of genome-wide methylation changes was detected between phenotypes.

### 4.1.5 Discussion

The survey on the professional fishers' perception about the existence of different qualities of *Pollicipes pollicipes* revealed that the large majority of the fishers considered that there are differences in the quality of the harvested stalked barnacles. Most of the fishers from Galicia and Portugal had the same perception about the quality of the barnacles and generally use similar terms to define them, although there are slight regional differences. In general, both Galician and Portuguese fishers defined quality of barnacles privileging morphological terms instead of organoleptic characteristics such as taste.

The terms most referred in the present study by the fishers to define good quality barnacles in Galicia and Portugal were thick and short, but also hard in both regions and red coloured in Galicia. The same or similar terms were also associated with good quality of barnacles in other studies of *Pollicipes* (Barnes and Reese 1960, Chaffee and Lewis 1988, Lessard et al. 2003, Parada et al. 2012 Rivera et al. 2014), which indicates a consensus in this description.

Regarding the definition of bad quality barnacles, the terms that were more mentioned by the professional fishers of the present study were slightly different between regions. Most of the fishers in Galicia mentioned the terms long and thin, while there was no term referred in Portugal by the majority of fishers. In Portugal, the terms more used to define bad quality barnacles were “watery” and “thin” by 39 % of the fishers, while “long” was mentioned by 25 % of the fishers. The fact that “watery” was a relevant term to define bad quality barnacles in Portugal might be related to the fact that in Portugal the fishers call bad quality barnacles as “*percebe mijão*” (“pissing” barnacles), due to the fact that these barnacles have a high content in water and can squirt water when caught or eaten. In Galicia, bad quality barnacles are called “*picholón*” ("big dick”) related to the most common term used, “long”. Overall, the terms most referred by the fishers of Galicia and Portugal for bad quality barnacles or similar terms were also referenced in the literature regarding *Pollicipes* spp. quality (Barnes and Reese 1960, Chaffee and Lewis 1988, Parada et al. 2012).

In relation to what is causing the variation in quality in stalked barnacles, the fishers from Galicia and Portugal referred slightly different causes. Almost the totality of the fishers from Portugal agreed that the cause responsible for the quality variation was related to some characteristic of the rock, while the second most referred cause was the hydrodynamics. The answers given by the fishers of Galicia were more diverse than those from Portuguese fishers. The two most commonly identified causes were also hydrodynamics and characteristics of the rock, but there was a greater dispersion of answers across a higher number of potential causes. This pattern might indicate that the fishers of Galicia have been thinking more about what is causing this variation of quality. In fact, fishers from Galicia have considered that the morphology of *P. pollicipes* with an elongated stalk was becoming more abundant on the coast (Quinteiro et al. 2006), which might be a major concern for these fishers due to its lower price in the market (Parada et al. 2012). On the other hand, in Portugal, based on surveys conducted with fishers in "*Reserva Natural das Berlengas*" (RNB), the majority of fishers reported that there had been no change in the quality of *P. pollicipes* in the last 5 years (in relation to 2013 and 2018) (Sousa et al. 2020). Assuming that the size of the barnacles can also be an indication of quality, also in "*Parque Natural do Sudoeste Alentejano e Costa Vicentina*" PNSACV, based on surveys carried out in 2013 (Cruz et al. 2015) and in 2016 (Carvalho et al. 2017), the situation seems similar, as the majority of fishers in both studies indicate that there have been no changes in the size of *P. pollicipes* in the last 5 years.

Several actions were taken in Galicia in the 2000s to deal with the issue of the elongated barnacles. Initially the biologists of the "*cofradías*" (see Macho et al. 2013) did several trials removing patches of elongated barnacles in the rocks to check if they were again recolonized by the same elongated barnacles or not. Results from these experiences were not conclusive (Alberto Garazo, Biologist from the cofradia of Bueu, personal communication). Due to the pressure of the "*cofradías*", the Fisheries administration in Galicia granted two projects, one to do a genetic study comparing the two morphologies and another one to look for processed products using elongated barnacles. As stated above, the first project found that both morphotypes were genetically the same species (Quinteiro et al. 2006). The second project aimed to produce processed products based on the bad quality barnacles and led to the creation of a company *Mar de Silleiro* formed by fishers from the "*cofradías*" of Baiona, A Guarda and Bueu, which was operational for 10 years. Nowadays, the elongated morphology is being exploited and commercialized, despite its lower commercial value (Miguel Verea, stalked barnacle harvester from Baiona, personal communication).

The identification that variation in hydrodynamics and in characteristics of the rock may be related to morphological/quality changes had also been mentioned in previous studies with *P. polymerus*: relatively short individuals, with strong peduncles, that attach with a considerable basal area, associated to locations with a strong wave exposure, while more elongated barnacles with a relatively small area of attach were related to more sheltered locations, and also with the presence of sand (Barnes and Reese 1960); local conditions such as wave exposure to explain *P. polymerus* variation in body shape and size (Lessard et al. , 2003). In contrary, in the Gaztelugatxe marine reserve, north of Spain, no significant relation was found between the total length of *P. pollicipes* and the degree of wave exposure (Borja et al. 2006), which can indicate that the occurrence of both phenotypes of *P. pollicipes* are not related exclusively with the degree of wave exposure. In Asturias, the quality of *P. pollicipes* was associated not only with wave exposure, but also with the configuration of the shore and the distance from the coast, with good quality barnacles associated with more exposed and convex areas that distance from the coast approximately 700m or more (Rivera et al. 2016). Other potential causes invoked to explain the morphological variation in these two species of the genus *Pollicipes* were: the position of *P. polymerus* in the clump (Chaffee and Lewis 1988); the density of *P. pollicipes* of the clump (Cruz 2000); and the sun exposure (Seoane-Miraz 2015). The overall conclusion is that the causes of this phenotypical variation in *Pollicipes* are still hard to understand, and more investigation is needed.

The RC/TH ratio expresses the relation between the height and width of a stalked barnacle. If the barnacle is thicker and shorter the RC/TH ratio is higher, and if the barnacle is longer and thinner the RC/TH ratio obtained is lower. As expected, the morphometric analysis on the RC/TH ratio variability on *P. pollicipes* of the two extreme qualities of the three sampled locations (Cudillero, Baiona and Sines) revealed that RC/TH ratio was significantly higher for good quality barnacle when compared with low quality barnacles, although both morphotypes differ in dispersion of RC/TH values. In the past, the commercial quality of *P. pollicipes* was defined by the relationship between capitulum height, capitulum width and the weight of the barnacle (Molares et al. 1987). Based on ecological fishers' knowledge, Lessard et al. (2003) considered that the configuration of the stalk is the most important attribute to classify *P. polymerus* quality, even more important than its weight. More recent studies used the ratio between the diameter of the capitulum base and the total height of the individuals to determine *P. pollicipes* quality (Parada et al. 2012). Based in the present study, we propose that the RC/TH ratio can be used to describe the morphological variation between the two extreme phenotypes of *P. pollicipes*: RC/TH ratio > 0.4 – good quality barnacles, RC/TH ratio < 0.4 – bad quality barnacles.

Regarding the genetic analysis, AFLPs were analysed, and although no differences were detected between both phenotypes, differences among the three analysed populations (Sines, Baiona and Cudillero) were detected. This is the first time that AFLPs were used to estimate the differences among populations of the genus *Pollicipes*. These results are partially consistent with data obtained with other genetic markers such as microsatellites where genetic differentiation among populations of juveniles of the same three geographical areas was detected (Parrondo et al. 2022). Contrarily, in the same study no spatial genetic differentiation of adults was found. Concordant or contradictory results based on the use of these two types of molecular markers have already been found in previous studies (e.g. Mariette et al. 2001; Gaudeul et al. 2004; Alacs et al. 2011), although comparisons of AFLPs and microsatellites are more common in studies to detect diversity in plants (Sønstebo et al. 2007).

The results on the MSAP analysis revealed that, although some methylation differences were found, no methylation differences were associated with the two extreme phenotypes studied in the three populations. In fact, although some significant single locus methylation differences were detected in two of the populations (Asturias and Portugal), these differences were not detected after multiple test correction. These results contradict the preliminary results obtained by Seoane-Miraz (2015). In recent studies, that used a similar methodology to the present study, with the Brown mussel *Perna perna* (Watson el al. 2018) and two species of reef corals (*Acropora cervicornis* and *A. palmata*) (Hackerott et al. 2023), it was found a relationship between phenotypic plasticity and variation in epigenetic DNA methylation. As the MSAP method is general, we cannot reject that other more specific DNA methylation approaches (see extensive review for marine organisms by Eirin-Lopez and Putnam 2019) could detect differential gene expression mediated by methylation.

Considering the lower commercial value that seems to be associated with the bad quality barnacles (Parada et al. 2012 in Galicia; Cruz 2000 and unpublished observations in Portugal), and the perception of Galician fishers that this quality of barnacles seems to be increasing in relation to the better quality ones, we recommend that the assessment of the quality/morphology of barnacles should be included as part of the monitoring of the state of this resource. On the other hand, in a previous study, although it was only carried out at one site (a big crevice in Cape Sardão, Alentejo, Portugal), it was observed that there was less sexual activity and less intense recruitment associated with more elongated barnacles (Cruz and Araújo 1999, Cruz 2000). Thus, in addition to the potential socio-economic impacts associated with a variation in quality, there may also be a potential variation in fundamental biological processes that should be better investigated in relation to the quality/morphology of the barnacles.

### 4.1.6 Conclusion

Professional fishers both from Portugal and Spain consider that there are *P. pollicipes* with different qualities, defining good quality barnacles as thick and short, and bad quality barnacles as long, thin, watery and soft. The fishers' answers validated the definitions and terms that were used in previous studies to define extreme phenotypes in *Pollicipes* species.

Regarding the causes of this morphological/quality variation of *P. pollicipes*, the large majority of the professional fishers considered that this variation can be caused by characteristics of the rock and also by hydrodynamics.

Based on the results of the present study, we propose the use of RC/TH ratio to distinguish the two extreme phenotypes of *P. pollicipes*, in the Iberia Peninsula, where values > 0.4 indicates good quality barnacles and values < 0.4 indicates bad quality barnacles.

Differences between both phenotypes were detected when using the RC/TH ratio, but no epigenetic differences were found, using the MSAP methodology. Further studies are needed using other DNA methylation approaches and to investigate the causes of this variation.

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### 4.1.9 Supplementary material

**S.4.1.** Survey to fishers:

1. In your opinion, are there barnacles with different qualities (good quality and bad quality)?

____Yes _____No

2. Please define:

2.1. Good quality barnacles ______________________________________________________

______________________________________________________________________________

2.2. Bad quality barnacles ________________________________________________________

______________________________________________________________________________

3. In your opinion, what is causing these differences?

**Table S4.1.1** – Characteristics (in English) and respective terms and expressions used by the fishers from Galicia (in Spanish) and Portugal (in Portuguese) to define good quality barnacles.

| **Characteristic (in English)** | **Terms and expressions mentioned by the fishers of Galicia (*in Spanish*)** | **Terms and expressions mentioned by the fishers of Portugal (*in Portuguese*)** |
|---|---|---|
| Thick | *grueso, gordito, gordo, regorditod, anchos* | *gordo, grosso, maior diâmetro, gande encorpado* |
| Short | *corto, pequeñito, pequeño* | *curto, pequeno* |
| Red colored | *color rojo, rojo en el pie donde se sujeta, pie de um color rojizo, el culo rojo* | *Vermelho, rosado, vermelhinho, pedúnculo laranja, bico vermelho* |
| Hard | *duro, firmeza, denso, macizo* | *rijo, consistente, carnudo, inteiro, cheio, tudo é carne, com mais carne* |
| Dark colored | *más escuro, arriba negro* | *negro, mais escuro* |
| Yellow capitulum | | *unha amarela* |
| Clean | | *limpo* |
| Heavier | *pesa más* | |
| Tastier | *major sabor* | *mais saboroso* |
| Less water | *no lleva mucha agua, com poco líquido* | *menos água* |
| With a longer shelf life | *aguanta más dias en la câmara fria* | *dura mais tempo depois de apanhado* |
| Sweeter | *más dulce* | |

**Table S4.1.2** - Characteristics (in English) and respective terms and expressions used by the fishers from Galicia (in Spanish) and Portugal (in Portuguese) to define bad quality barnacles.

| **Characteristic (in English)** | **Terms and expressions mentioned by the fishers of Galicia (*in Spanish*)** | **Terms and expressions mentioned by the fishers of Portugal (*in Portuguese*)** |
|---|---|---|
| Long | *largo, alongado, alargado* | *longo comprido* |
| Thin | *delgado, fino* | *fino, magro, esguio, delgado, mirrado* |
| Soft | *blando, flojo, flaco* | *mole, fraco, não se aguenta, macio* |
| Dark | *oscuros, negro* | *negro* |
| Green capitulum | *verde en la uña* | *unha verde, com muito limo* |
| Small | *pequeño* | *pequeno* |
| Yellow capitulum | *uña amarela* | *unha amarela* |
| Watery | *es todo agua, mucha agua, mixóns, aguoso* | *aguado, mijão* |
| Empty | *poca carne* | *vazio, sem miolo, leve* |
| Preserve less | | *perde qualidade depressa* |
| Flavourless | *no tiene el mismo sabor* | *sem sabor* |

**Table S3** - Causes (in English) and respective terms and expressions used by the fishers from Galicia (in Spanish) and Portugal (in Portuguese) to explain the causes that explain the morphologic/quality variation in the stalked barnacle *P. pollicipes*.

| **Cause (in English)** | **Terms and expressions mentioned by the fishers of Galicia (*in Spanish*)** | **Terms and expressions mentioned by the fishers of Portugal (*in Portuguese*)** |
|---|---|---|
| Characteristics of the rock | *piedra, tipo de roca, forma de la roca* | *pedra, calcário na pedra, qualidade da pedra* |
| Hydrodynamics | *oleaje, corrientes* | *exposição ao mar, oxigenação, hidrodinamismo, posição virada ao mar, movimento do mar, correntes* |
| Tidal level | *altura a que se crian, tiempo somergido* | *profundidade, marés, se está debaixo de água* |
| Environmental conditions | *clima* | |
| General local characteristics | *la zona, el sítio* | *zona* |
| Density | *exces de individuos formando piña, se juntam muchos* | *quantidade* |
| Food | *la alimentación* | *alimento, disponibilidade de plankton, acesso a nutrientes* |
| Water quality | *calidad de agua, contaminación* | |
| Genetic differences | *componente genético* | |
| Solar exposure | *la luz que recibe* | *exposição solar, sol, sol/sombra* |
| Water/air temperature | *temperature del agua, temperature, calor* | *calor, temperatura* |
| Others – time of the year, presence of acorn barnacles, sand, algae | *época do año, cerca de arena* | *altura do ano, craca, areia, limo* |

## *4.2* Morphological and commercial quality variation of the stalked barnacle *Pollicipes pollicipes* (Gmelin, 1791 [in Gmelin, 1788–1792]): patterns and drivers

Sousa A., Jacinto D., Penteado N., Pereira D., Silva T., Castro J.J., Cruz T. *in review*. Morphological and commercial quality variation of the stalked barnacle *Pollicipes pollicipes* (Gmelin, 1791 [in Gmelin, 1788–1792]): patterns and drivers. Regional Studies in Marine Science.

### 4.2.1 Abstract

Fishers and consumers recognise that there is important variation in the morphology and quality of the stalked barnacle *Pollicipes pollicipes*. Two extreme phenotypes can be identified: thick/short barnacles associated with a high commercial quality; and thin/long barnacles associated with low commercial quality.

The perception of the variation in quality and morphology of *P. pollicipes* by scientists and fishers was considered similar. The ratio of the width of the capitulum base of *P. pollicipes* to its total length (CB/TL) was used and validated as a good proxy variable for evaluating quality. Barnacles with a relatively longer peduncle/lower quality shown lower values of CB/TL. There were no significant differences in the individual biomass of high and low quality barnacles.

The biochemical composition of the edible part of the peduncle of *P. pollicipes* of high and low commercial quality was evaluated for the first time in three Portuguese regions. High commercial quality barnacles presented a higher protein content, energetic value and fat content, and low commercial quality barnacles presented a higher water content. Regional differences were also detected in the biochemical composition of *P. pollicipes.* On the SW coast of Portugal (SW), barnacles presented a higher ash, protein and water content, and a lower glucose and energetic value, than in the other two regions located on the centre coast (RNB and CENTRE).

The influence of two potential drivers (density and microhabitat) of variation in morphology/quality of *P. pollicipes* was tested through a manipulative experiment. The results of this experiment suggest that the elongation of *P. pollicipes* is related to the greater density of the barnacles on a group scale. On the other hand, the shortening of *P. pollicipes* may be related to the lower density of the barnacles at the group scale and to specific microhabitat environmental conditions, such as hydrodynamics.

### 4.2.2 Introduction

Phenotypic plasticity is the term used to describe all types of environmental-induced phenotypical variation within individuals of the same species (Stearns, 1989). Phenotypic plasticity has been reported in several species of marine invertebrates, such as bryozoans (e.g. Okamura & Partridge, 1999), bivalves (e. g. Hamdoun et al. 2003; Freeman & Byers, 2006; Wang et al. 2021), gastropods (e.g. Trussell, 1996; Melatunan et al. 2013; Bourdeau et al. 2015; Broitman et al. 2018; Vasconcelos et al. 2021), and barnacles (e.g. Bertness, 1989; Mokady et al. 2000; Arsenault et al. 2001; López et al. 2007). The phenotypic variation that is most frequently described in marine intertidal species is related to variation in thickness of calcified parts (Trussell, 1996; Leonard et al. 1999; Melatunan et al. 2013; Lardies et al. 2021), variation in the feeding apparatus (Arsenault et al. 2001; Marchinko & Palmer, 2003; López et al. 2007, 2010), physiological changes (Hamdoun et al. 2003; Melatunan et al. 2013; Broitman et al. 2018, 2021), and changes in the shape/size of the body (Barnes & Powell, 1950; Lively, 1986; Mokady et al. 2000; Melatunan et al. 2013; Broitman et al. 2018; Lardies et al. 2021; Pardal et al. 2021; Vasconcelos et al. 2021).

Several environmental causes have been suggested as potential sources of the phenotypical plasticity observed in marine invertebrate species that inhabit intertidal rocky shores, such as water temperature (Hamdoun et al. 2003), ocean acidification (Melatunan et al. 2013; Broitman et al. 2018; Lardies et al. 2021), presence of predators (Lively, 1986; Trussell, 1996; Mokady et al. 2000; Freeman & Byers, 2006), hydrodynamics (Kaandorp, 1999; López et al. 2010; Marchinko & Palmer, 2003; Marzouk et al. 2016; Watson et al. 2018; Pardal et al. 2021; Vasconcelos et al. 2021; Peñas-Torramilans et al. 2024), diet (Marzouk et al. 2016), and density (Barnes & Powell, 1950; Bertness, 1989; López et al. 2007). Those environmental forces can act combined or isolated, and sometimes it is hard to identify the source(s) responsible for the detected phenotypical variation.

Phenotypic plasticity has been studied in several intertidal barnacle species, namely morphological variation of the cirri and of shell shape. A shell dimorphism was detected in the acorn barnacle, *Chthamalus anisopoma*, that presented a typical form when a carnivore gastropod was absent and an atypical, bent-over form when the predator was present (Lively, 1986). Also, at high densities, the acorn barnacles *Semibalanus balanoides* (Barnes & Powell, 1950; Bertness, 1989; Bertness et al. 1998) and *Balanus crenatus* (Barnes & Powell, 1950) form groups (hummocks) of tall, densely packed individuals, as a response of intraspecific competition for primary space to settle. Regarding the morphological variation of the cirri, several studies have been carried out in acorn barnacles (e.g. Marchinko & Palmer, 2003; Marchinko et al. 2004; Chan & Hung, 2005; Miller, 2007; López et al. 2010) and in the stalked barnacle *Pollicipes polymerus* (Marchinko & Palmer, 2003; Marchinko et al. 2004). For example, in several species (e.g. *Balanus glandula, Chthamalus dalli, Pollicipes polymerus*), significantly longer cirri have been observed in sites that are relatively less wave-exposed (Marchinko & Palmer, 2003).

Regarding the edible stalked intertidal barnacles of the genus *Pollicipes*, the most obvious phenotypic variability is probably the peduncle shape and the corresponding variation in morphology and commercial quality (see review of Cruz et al. 2022). In general, stalked barnacles with a longer peduncle have been associated with a lower commercial quality and economic value, compared to barnacles with a thicker and shorter peduncle. Of the four *Pollicipes* species (*P. polymerus* – north-eastern Pacific Ocean, *P. elegans* – tropical eastern Pacific Ocean, *P. caboverdensis* – Cape Verde islands, and *P. pollicipes* – north-eastern Atlantic Ocean), *Pollicipes pollicipes* is the most intensively exploited species, namely in Spain and Portugal (Cruz et al. 2022). In a recent study, professional harvesters defined high quality barnacles of this species as thick and short, and low quality barnacles as long, thin, soft and watery (Sousa et al. 2024). This study also investigated whether this morphological variation was associated with genetic or epigenetic variation, but no significant differences were found between the two morphotypes of *P. pollicipes*. A different quality of this species might also be associated with a different biochemical composition, although this has never been investigated.

The causes of this phenotypic variation in *Pollicipes* have been associated with: wave exposure (*P. polymerus* with short peduncles in very wave-exposed locations, Barnes and Reese, 1960; *P. pollicipes* with stubby, wide peduncles in more wave-exposed locations, Peñas-Torramilans et al. 2024); relative position within the clump of barnacles (*P. polymerus* with slender peduncles in the center of the clumps, Chaffee and Lewis, 1988); and density of the clump of barnacles (*P. pollicipes* with thinner and longer peduncles when in high densities, Cruz, 2000). In a recent study (Sousa et al. 2024), the causes of this variation were also investigated through the perception of professional harvesters of *P. pollicipes* from Portugal and Galicia (Spain). This study revealed that the local characteristics of the rocks, including hydrodynamics, were the most likely causes identified to explain the differences in the quality of the barnacles.

The first objective of the present study is to compare the classification of the quality of *P. pollicipes* made by professional harvesters and by scientists. To fulfill this objective, a variable relating the width of the capitulum base of the barnacle to its total length (CB/TL) was used, with animals with a relatively longer peduncle/lower quality showing lower values of this variable. The accomplishment of this objective means that the measurement of this variable can be interpreted as a variation in the quality of *P. pollicipes.* Secondly, three hypotheses were tested: 1) that CB/TL would increase with a reduction in the density of barnacles with long peduncles; 2) that CB/TL would increase when barnacles with long peduncles were transplanted to a microhabitat with barnacles with relatively shorter peduncles; and 3) that CB/TL would decrease when barnacles with short peduncles were transplanted to a microhabitat with barnacles with longer peduncles. Additionally, it was also investigated whether there was any variation in the biochemical composition of the edible part of the peduncle of *P. pollicipes* with the two morphologies/qualities.

### 4.2.3 Materials and methods

The present study includes three parts: (1) classification and variation in the morphology/quality and biomass of *P. pollicipes* according to professional harvesters and scientists; (2) a manipulative experiment involving the transplantation of groups of barnacles and the reduction of barnacles density; and (3) an analysis of the biochemical composition of the edible part of the peduncle.

The classification of the morphology/quality of *P. pollicipes* according to professional harvesters and scientists and the analysis of the biochemical composition of the edible part of the peduncle were done considering three regions in the coast of mainland Portugal: the marine protected area of “Reserva Natural das Berlengas” (RNB), the coastal area between Cape Carvoeiro and Cape Raso (CENTER) and the SW coast of Portugal, from Cape of Sines to Cape São Vicente, that includes the west coast of “Parque Natural do Sudoeste Alentejano e Costa Vicentina” (SW, **Fig. 4.2.1**).

The manipulative experiment was performed at Cape Sardão, located within the “Parque Natural do Sudoeste Alentejano e Costa Vicentina” (37°36′25.78″N 8°49′02.50″W, **Fig. 4.2.1**).

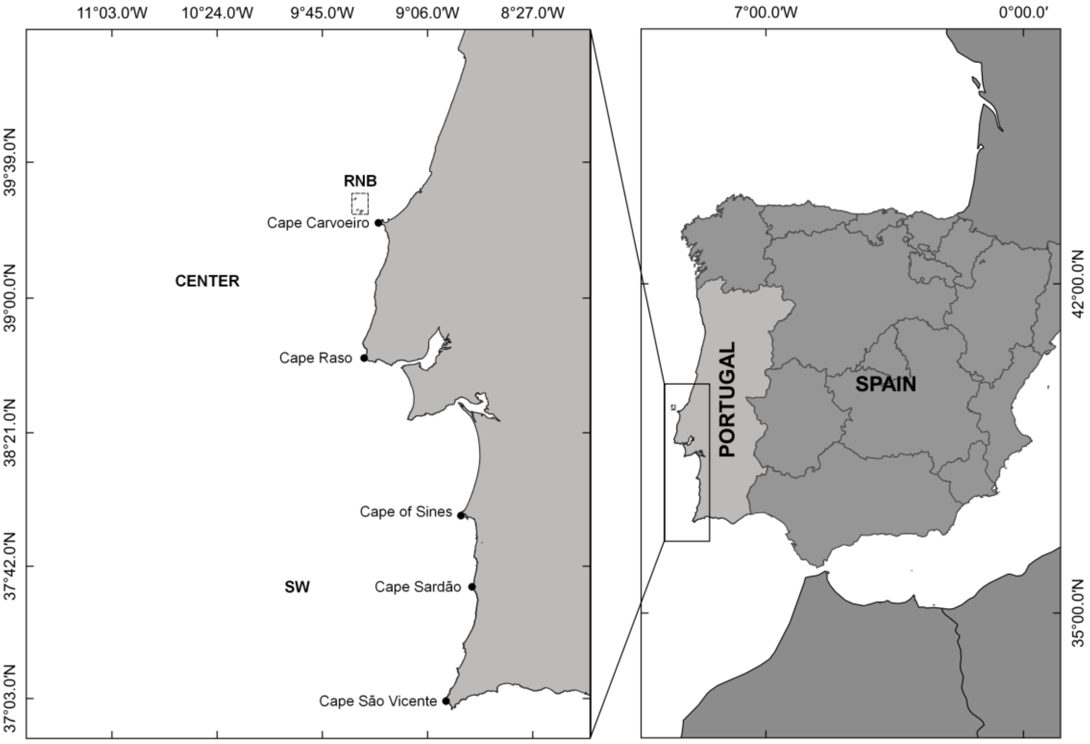



**Fig. 4.2.1 –** Map of mainland Portugal with the three coastal regions considered: "Reserva Natural das Berlengas" (RNB); the coastal area between Cape Carvoeiro and Cape Raso (CENTER); and the SW coast of Portugal, that includes the west coast of "Parque Natural do Sudoeste Alentejano e Costa Vicentina" and the Cape of Sines (SW). The location of the manipulative experiment (Cape Sardão) is marked on the map.

*Classification and variation in the morphology/quality and biomass of P. pollicipes according to professional harvesters and scientists*

With the aim of classifying the morphology/quality of *P. pollicipes* according to the perception of professional harvesters and scientists, professional harvesters from three regions (RNB, CENTER and SW) were interviewed individually and directly in 2013. The number of interviews conducted, and the total number of licenses of professional harvesters issued in each region, are shown in **Table 4.2.1**.

**Table 4.2.1** – Total number of inquiries to professional harvesters and total number of professional harvesters licenses for each region. NA – in the period of this study (2013), there were no individual specific licenses for professional harvesting of *Pollicipes pollicipes* at CENTER.

| **Region** | **Number of inquiries** | **Total number of specific licenses for professional harvesting of *Pollicipes pollicipes*** |
|---|---|---|
| RNB | 32 | 40 (since 2011) |
| CENTER | 49 | NA |
| SW | 35 | 80 (since 2006) |

The professional harvesters were interviewed about *P. pollicipes* quality, with the objective to validate if their perception about quality was similar to the scientists that co-authored the present study. Professional harvesters were asked to classify a collection of 10 stalked barnacles (ethyl alcohol preserved) according to their perception about the quality of each barnacle. Four barnacle collections with similar characteristics were prepared, to ensure simultaneous interviews. In each enquiry, one collection was used. All the barnacles from each collection had a similar maximum distance between the *rostrum* and the *carina* plate (RC ~ 21 mm, that corresponds to an average of the maximum distance between the base of *subcarina* and the base of the *subrostrum* (CB) of ~ 14 mm), but their ratio between CB and total length (TL) was different. The CB/TL ratio of all the barnacles of each collection was calculated (see example of two barnacles with contrasting CB/TL ratios in **Fig. 4.2.2**) and the respective classification given by scientists is shown in **Fig. 4.2.4A**. The barnacles B, E and G had a lower total length (short peduncle) and consequently a higher value of CB/TL (mean ratio of 0.43, ranging between 0.41 and 0.44) and were classified by the scientists as having "high quality". The barnacles A, F and I had a higher total length (elongated morphology), and consequently a lower value of CB/TL (mean ratio of 0.17, ranging between 0.15 and 0.18) and were classified by the scientists as having "low quality". Finally, the barnacles C, D, H and J had a mean value of CB/TL ratio of 0.29 (ranging between 0.27 and 0.31) and were classified by the scientists as having an "intermediate quality" between the two extreme categories defined above.

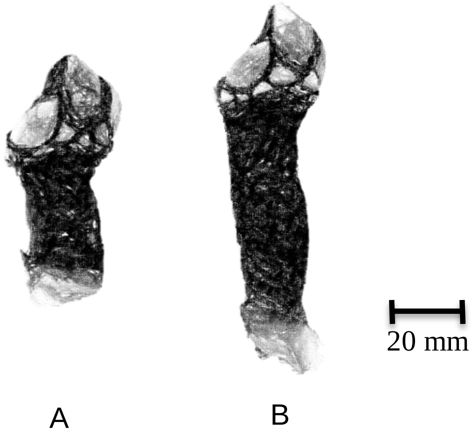



**Fig. 4.2.2** – A – Representation of *Pollicipes pollicipes* with a relatively shorter peduncle (higher CB/TL value), B – Representation of *Pollicipes pollicipes* with a relatively longer peduncle (lower CB/TL value).

Additionally, with the aim of analyse if the variation in the morphology and individual biomass of high and low quality *P. pollicipes* collected by professional harvesters and scientists was similar, barnacles of both qualities (approximately 1.5 kg each) were independently sampled by professional harvesters and by scientists, at two random sites within each of the three regions (RNB, CENTER and SW), between June and November of 2013.

In the case of the analysis of morphological variation, we used a sub-sample of 60 adult barnacles (RC > 12.5 mm) collected at each site by professional harvesters and by scientists. Barnacles were individualized and we measured (precision of 0.1mm) their RC, CB and TL and calculated the respective CB/TL ratio.

Morphological variability data (CB/TL ratio) was analysed with ANOVA including four factors: Harvester (HR – fixed factor with two levels, "professional harvester – H" and "scientists – S"), Quality of *P. pollicipes* (QL – fixed factor with two levels, "high quality" and "low quality"), Region (RG – fixed factor with three levels, "RNB", "CENTER" and "SW") and Site (Si – random factor with two levels). The first three factors are orthogonal, and the factor Site is nested in Harvester, Region and Quality. Sample size is 60.

The analysis of variance was performed according to Underwood (1997), using R environment (R Core Team, 2018) combined with GAD (Sandrini-Neto and Camargo, 2018). Homogeneity of variance was assessed using Cochran's C test and SNK tests were used when appropriate (Underwood, 1997).

In the study of individual biomass variation, we only used the barnacles from each sub-sample of 60 barnacles that had RC values ranging from 22.5 mm to 25 mm (large adults). Each of these barnacles was individually weighted (fresh weight, precision of 0.01 g).

The biomass variability of large adults of *P. pollicipes* (RC ranging between 22.5 and 25.0 mm) was analysed with PERMANOVA (Anderson 2001), using the same design described above. Sample size varied between 2 and 46. We used PERMANOVA instead of analysis of variance due to the unbalanced design of the biomass analysis. This analysis was based on Euclidean distances of untransformed data, and on permutation of residuals under a reduced model, type III sums of squares and 9999 permutations (see Anderson et al. 2008). Homogeneity of dispersions based on Euclidean distances was tested using PERMDISP routine (Anderson, 2006). The software PRIMER 6 & PERMANOVA+ (Anderson et al. 2008) was used to perform these statistical analyses.

*Biochemical composition of the edible part of the peduncle*

The barnacles with high and low quality that were collected for the morphological and individual biomass analyses were used to perform an analysis of the biochemical composition of the edible part of the peduncle. In this analysis, each replicate consisted of approximately 200 g of the inner part of *P. pollicipes* peduncle (the edible part), taking into account the large adults used in the biomass study, as well as smaller adults that had been collected at each site by harvesters or scientists. The edible part of the stalk was scraped with the help of a metallic spatula. This procedure was done in the first six hours after collection. All replicates were frozen (~-20º C) until the biochemical laboratory analyses were performed. The biochemical variables analysed are the content of fat, total protein, carbohydrates, glucose, energetic value, water content and ash content. These analyses were performed at *Instituto Nacional de Saúde Doutor Ricardo Jorge* (Lisbon, Portugal), that has accredited laboratory procedures for the biochemical analysis of food products. All the variables analysed, and respective methods are listed in **Table 4.2.2**.

**Table 4.2.2** – Biochemical variables and the respective accredited method of analysis.

| Variable | Method |
|---|---|
| Water content (g/100 g) | DAN URQ-PE32_01 L (Gravimetric method) |
| Ash content (g/100 g) | DAN URQ-PE23_01 L (525º C incineration method) |
| Fat content (g/100 g) | DAN URQ-PE25_01 L (Acid hydrolysis with extraction) |
| Total protein (g/100 g) | DAN URQ-PE40_02 L (Kjeldahl method) |
| Carbohydrates (g/100 g) | DAN URQ-PE46_01 L (Calculation) |
| Glucose (g/100 g) * | Enzymatic method |
| Energetic value (kcal/100 g, kJ/100 g) | DAN URQ-PE47_01 L (Calculation) |

* Non-accredited method

The biochemical multivariate structure of *P. pollicipes* with different qualities was analysed with PERMANOVA (Anderson, 2001) using two orthogonal factors: Region (RG – fixed factor with three levels, "RNB", "CENTER" and "SW"), and Quality of *P. pollicipes* (QL – fixed factor with two levels, "high quality" and "low quality"). Sample size is 4. This analysis was based on Euclidean distance of normalized and untransformed data, and on permutation of residuals under a reduced model, type III sums of squares and 9999 permutations (see Anderson et al. 2008). Homogeneity of dispersions based on Euclidean distance matrix was tested using PERMDISP routine (Anderson, 2006). In addition, a principal components analysis (PCA) was used as an ordination technique to map the replicates based on their biochemical composition and identify which were the variables most associated with the variation between regions and qualities of *P. pollicipes*. The software PRIMER 6 & PERMANOVA+ (Anderson et al. 2008) was used to perform these statistical analyses.

*Manipulative experiment*

To test the influence of density/microhabitat on *P. pollicipes* morphology/quality, a manipulative experiment was performed at Cabo Sardão, Portugal (37º36’25.78’’N 8º49’02.50’’W), between July 2012 and July 2013.

Two microhabitats were identified: a high quality *P. pollicipes* microhabitat, placed at low shore, which corresponds to the lower intertidal level of this species distribution, where stalked barnacles have a short peduncle that corresponds to a thick/short morphology and occur in dispersed clumps of lower density; and a low quality *P. pollicipes* microhabitat, placed at a mid shore large crevice, where barnacles occur in higher densities and have an elongated peduncle corresponding to a thin/long morphology.

In this experiment, low quality barnacles were transplanted from their original microhabitat to the high quality microhabitat and vice versa. The density of *P. pollicipes*, another identified putative factor that can affect the morphology of barnacles, was manipulated in the low quality microhabitat in two ways: by reducing the density of barnacles within the clump by cutting the *capitulum* of about 75% of the large barnacles; and by reducing the density around the clump of *P. pollicipes*, so that there is a margin of approximately 5 cm without barnacles. These treatments and the respective controls are described in **Fig. 4.2.3** and **Table 4.2.3**. For each treatment, we considered three experimental areas corresponding to clumps of *P. pollicipes* measuring around 5x5 cm. In transplanted treatments, transplantation consisted of removing a piece of rock with barnacles attached (an area of approximately 5x5 cm) from the original microhabitat and gluing the lower surface of this piece of rock with Z-Spar Splash Zone Epoxy (Kop-Coat Inc., Pittsburgh, PA) on the rock of the destination microhabitat (**Table 4.2.3**).

Treatments T3, T5, T6, T8, and T10 had a cage over *P. pollicipes* for approximately 6 months . The purpose of this cage (plastic-coated metal mesh, mesh size of 1 cm) was to protect the animals from the more severe predation that could occur at the start of the experiment due to the greater vulnerability of the animals to predation caused by transplantation or density reduction. Comparisons made at the end of the experiment between treatments that had a cage at the start of the experiment and corresponding treatments without a cage (T9 versus

T10, T2 versus T3) revealed no significant differences and consequently there was no cage effect (results not shown).

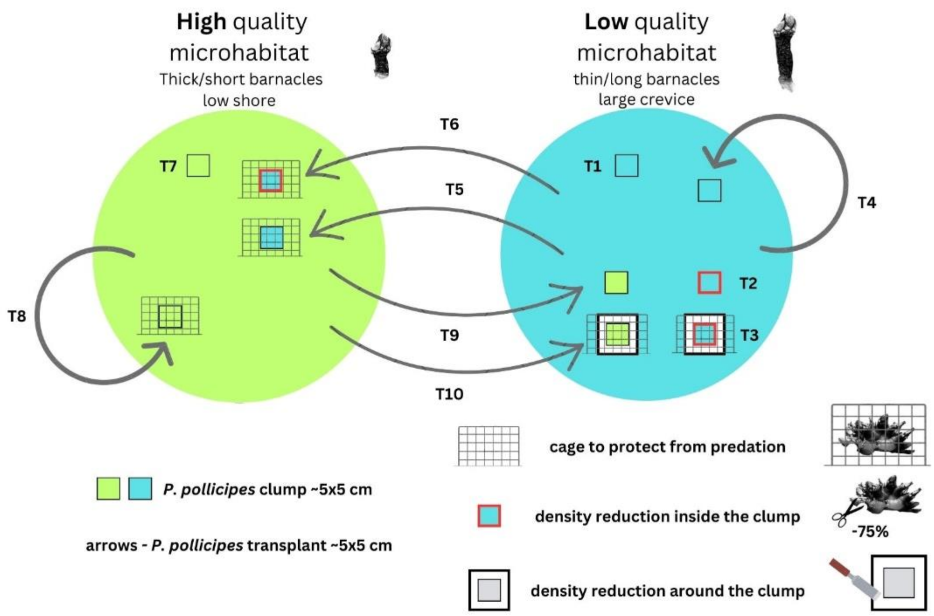



**Fig. 4.2.3** – Schematic representation of the manipulative experiment that tested the effect of density/microhabitat on *P. pollicipes* quality with 10 treatments (T). Three experimental clumps were considered in each treatment. Replicates were the large barnacles (RC>12 mm) from these clumps that survived until the end of the experiment (July 2012 to July 2013). Treatments T3, T5, T6, T8, and T10 had a cage over *P. pollicipes* for approximately 6 months.

**Table 4.2.3** – Description of the original microhabitat and type of manipulation performed in the 10 treatments (T) of the manipulative experiment presented in **Fig. 4.2.3**. ↓ **–** reduction of the density of *P. pollicipes*. Low – habitat with low commercial quality barnacles. High – habitat with high commercial quality barnacles.

| Name of treatment | Original habitat | Type of manipulation | | | |
|---|---|---|---|---|---|
| | | Reduction of the density of barnacles within a group by 75% | Removal of macro-organisms around the group of barnacles | Cage during the first months | Transplant and destination microhabitat |
| T1 - Low control | Low | No manipulation | | | |
| T2 - Low, ↓ density within | Low | Yes | No | No | No |
| T3 - Low, ↓ density within and ↓ around clump | Low | Yes | Yes | Yes | No |
| T4 - Low to Low | Low | No | No | No | Yes. Low to Low |
| T5 - Low to High, Cage | Low | No | No | Yes | Yes, Low to High |
| T6 - Low to High, ↓ density within | Low | Yes | No | Yes | Yes, Low to High |
| T7 - High control | High | No manipulation | | | |
| T8 - High to High, Cage | High | No | No | Yes | Yes, High to High |
| T9 - High to Low | High | No | No | No | Yes, High to Low |
| T10 - High to Low, ↓ around clump | High | No | Yes | Yes | Yes, High to Low |

The response variable that was measured is the CB/TL ratio of *P. pollicipes* with a RC larger than 12 mm. We used this dimensional threshold because we only wanted to consider barnacles that were already present in each treatment at the start of the experiment and not barnacles that settled *a posteriori*. This assumption was based on an average growth of 1 mm RC each month (see Cruz et al. 2022). The sample size in each treatment was variable and depended on the number of barnacles with RC > 12 mm that survived in the three experimental clumps that were considered in each treatment (n from 14 to 200). The hypotheses tested are described in **Table 4.2.4**.

**Table 4.2.4** – Description of the hypotheses that were tested in the manipulative experiment presented in **Fig. 4.23**. Response variable is CB/TL. Barnacles with a relatively longer peduncle/lower quality shown lower values in this variable. Low – habitat with low commercial quality barnacles. High – habitat with high commercial quality barnacles.

| | |
|---|---|
| **A** – Relation between control treatments in each microhabitat<br>We expected the barnacles in the low quality microhabitat to be longer than those in the high quality microhabitat. | T1 (control low)<br><<br>T7 (control high) |
| **B** – Density effect<br>If a higher density is associated to a lower quality of barnacles (more elongated barnacles), we expected that when we reduced the density of the barnacles (within or around a group of barnacles) in the low quality microhabitat (treatments T2 and T3), these barnacles will shorten. | T1 (control low)<br><<br>T2, T3 |
| **C** – Transplantation effect<br>In order to distinguish that the transplant to a different microhabitat is not due to the transplant manipulation itself, we expected that there will be no differences between the transplant carried out at the same microhabitat (T4 and T8) and the respective control for that microhabitat (T1 and T7). | T1 (control low) = T4<br><br>T7 (control high) = T8 |
| **D** – Microhabitat effect<br>If a particular microhabitat is associated with a better quality of barnacles, we expected that by transplanting the barnacles from the low to the high quality microhabitat, regardless of the density of the group (T5 and T6), they will shorten compared to control treatment (T1) and to reduction of density treatments that remained in the low quality microhabitat (T2 and T3), and became more similar to the control barnacles of the high quality microhabitat (T7). | T1 (control low), T2, T3<br><<br>T5, T6, T7 (control high) |
| **E** – Microhabitat effect<br>If a particular microhabitat is associated with a worse quality of barnacles, we expected that by transplanting the barnacles from the high to the low quality microhabitat (T9 and T10), they will elongate compared to the control treatment (T7) and became more similar to the control barnacles of the low quality microhabitat (T1). | T9, T10, T1 (control low)<br><<br>T7 (control high) |

Hypothesis testing regarding changes of the CB/TL ratio among the treatments were analysed with PERMANOVA (Anderson, 2001) considering the factor treatment (T – fixed factor). This analysis was based on Euclidean distances, on unrestricted permutation of raw data, type III sums of squares and 9999 permutations (see Anderson et al. 2008). Data were transformed when appropriate. Homogeneity of dispersions based on Euclidean distances was tested using PERMDISP routine (Anderson, 2006) The sample size is variable due to a variable number of barnacles that have survived in each treatment (n = 14 to 200). The software PRIMER 6 & PERMANOVA+ (Anderson et al. 2008) was used to perform these statistical analyses.

### 4.2.4 Results

*Classification of the morphology/quality of P. pollicipes according to professional harvesters and scientists*

The classification of the barnacles by harvesters was similar to the classification previously made by scientists. More than 78 % of the harvesters agreed that the three barnacles with thick and short morphology, and consequent higher CB/TL ratio (B, E and G), had high quality (**Fig. 4.2.4**). Again, as the scientists, more than 91 % of the harvesters classified the three barnacles with a thin and long morphology, and a lower CB/TL ratio (A, F and I) as having low quality (**Fig. 4.2.4**).

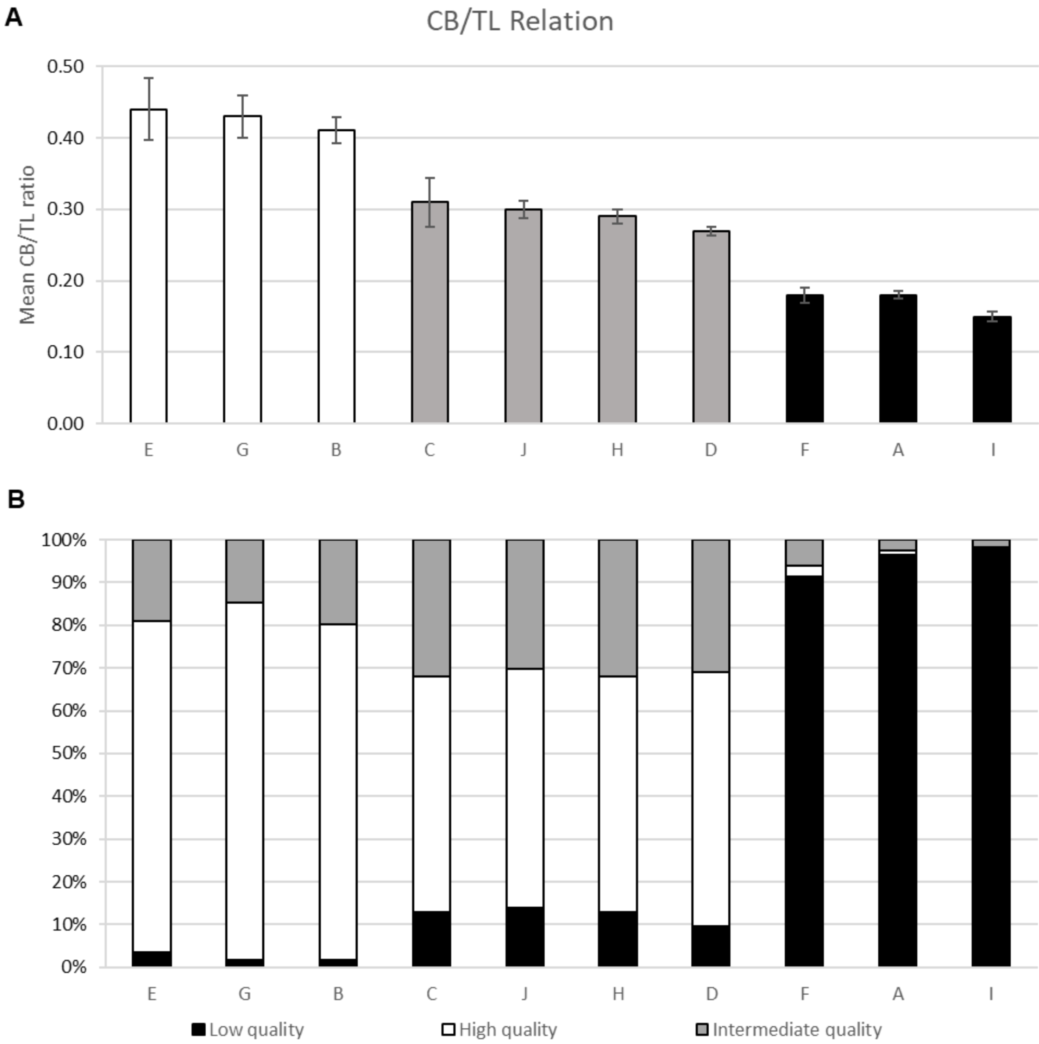



**Fig. 4.2.4** – **A** – mean CB/TL ratio (± standard deviation) of each barnacle considered in each collection of *P. pollicipes* with different qualities that was presented to the professional harvesters. The barnacles were presented in a random way (considering their CB/TL ratio), from A to J. Barnacles B, E and G had a mean CB/TL ratio of 0.43, and were classified by the scientists as high quality barnacles, C, D, H and J had a mean CB/TL ratio of 0.29, and were classified by the scientists as intermediate quality barnacles, and A, F and I had a mean CB/TL ratio of 0.17, and were classified by the scientists as low quality barnacles. **B** – Classification of the 10 barnacles seen by professional harvesters (n = 116) in the interviews. All barnacles had a similar RC value of approximately 21 mm.

Regarding the morphometry of high quality and low quality barnacles collected independently by professional harvesters and scientists, there were no significant differences on the CB/TL ratio between them, but significant effects ($P < 0.05$) of factors Quality and Region were found (**Table 4.2.5**). High quality barnacles presented a higher CB/TL ratio (mean value = 0.35) than low quality animals (mean value = 0.23, **Fig. 4.2.5A, Table 4.2.5**). Concerning the region factor, RNB and SW barnacles presented a significantly higher CB/TL ratio than CENTER barnacles (**Fig. 4.2.5A, Table 4.2.5**). PERMANOVA to individual biomass revealed a significant effect of factor Region, but no effect of the other fixed factors (Harvester and Quality) was found. The individual biomass of barnacles collected at SW (mean individual biomass = 5.17 g) was lower than the observed in barnacles from RNB (mean individual biomass = 6.58 g) (**Fig. 4.2.5B, Table 4.2.5**). The biomass of *P. pollicipes* in the region CENTER was considered similar to RNB and SW . In both analyses (CB/TL and individual biomass), small-scale variation (between sites) was detected (**Table 4.2.5**).

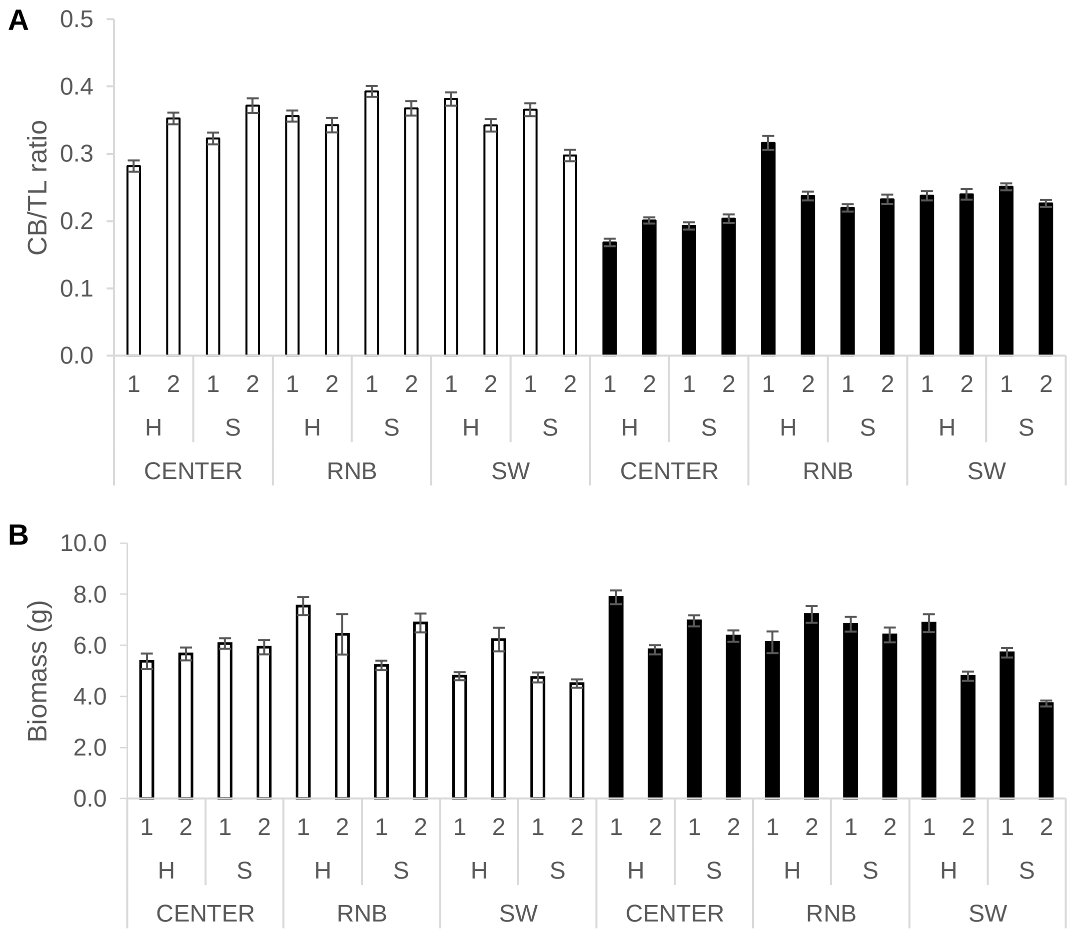



**Fig. 4.2.5** – **A** – Mean values (± standard error) of CB/TL ratio (n = 60) of stalked barnacles (*Pollicipes pollicipes*) with different morphologies/qualities ("high quality" – white bars, "low quality" – black bars) harvested by professional harvesters (H) and by scientists (S) in two sites (1 and 2) of each region (CENTER, RNB and SW). **B** – Mean values (± standard error) of the individual biomass (n= 2 to 46) of *P. pollicipes* within the size class of 22.5 mm – 25.0 mm of RC, with different morphologies/qualities ("high quality" – White bars, "low quality" – black bars), harvested by professional harvesters (H) and by scientists (S) in two sites (1 and 2) of each region (CENTER, RNB and SW).

**Table 4.2.5** – Results of ANOVA to CB/TL ratio and of PERMANOVA to individual biomass in relation to factors "Harvester" (HR), "Quality" (QL), Region (RG) and "Site" (Si). Significant effects are indicated in bold ($p < 0.05$). *ns* – non significant; df – degrees of freedom; MS – mean square.

| | **ANOVA** | | | | **PERMANOVA** | | | |
|---|---|---|---|---|---|---|---|---|
| **Source** | **CB/TL Ratio** | | | | **Biomass** | | | |
| | Cochran's test: *ns* | | | | PERMDISP to Region: *ns* | | | |
| | Transformation: Ln(x) | | | | Data not transformed | | | |
| | df | MS | F | *p* | df | MS | Pseudo-F | *p* |
| HR | 1 | 0.0 | 0.00 | 0.997 | 1 | 18.0 | 1.42 | 0.250 |
| QL | 1 | 67.4 | 64.50 | **0.000** | 1 | 17.6 | 1.39 | 0.266 |
| RG | 2 | 4.7 | 4.54 | **0.034** | 2 | 67.8 | 4.72 | **0.0347** |
| HRxQL | 1 | 0.5 | 0.44 | 0.521 | 1 | $2.7 \times 10^{-4}$ | $2.20 \times 10^{-5}$ | 0.997 |
| HRxRG | 2 | 0.7 | 0.70 | 0.515 | 2 | 15.4 | 1.07 | 0.371 |
| QLxRG | 2 | 1.7 | 1.58 | 0.245 | 2 | 9.9 | 0.69 | 0.511 |
| HRxQLxRG | 2 | 1.4 | 1.31 | 0.306 | 2 | 4.3 | 0.30 | 0.728 |
| Si(HRxQLxRG) | 12 | 1.1 | 31.90 | **0.000** | 12 | 20.3 | 9.40 | **0.000** |
| Residual | 1416 | 0.0 | | | 642 | 2.2 | | |
| | SNK tests: | | | | Pair-wise tests: | | | |
| | **QL** | | | | **RG** | | | |
| | High quality > Low quality | | | | RNB > SW (no defined pattern for CENTER) | | | |
| | **RG** | | | | | | | |
| | (SW=RNB) > CENTER | | | | | | | |

*Biochemical composition of the edible part of the peduncle*

The analysis of the biochemical composition of *P. pollicipes* (per 100 g of the edible part of the peduncle, **Table 4.2.6**) revealed that the variable with the highest values is water content (mean content of high quality barnacles = 76.53 g/100 g, mean content of low quality barnacles = 78.54 g/100 g), followed by the protein content (mean content of high quality barnacles = 18.99 g/100 g, mean content of low quality barnacles = 17.52 g/100 g). Ash content, fat content, carbohydrates and glucose were variable compounds and presented lower values. Finally, the energetic value, calculated based on the protein, fat and carbohydrates content, was higher in the high quality *P. pollicipes* (mean = 93.25 kcal/100 g) than in low quality barnacles (mean = 82.67 kcal/100 g).

The PERMANOVA analysis detected a significant effect of factors Region and Quality (**Fig. 4.2.6**, **Table 4.2.7**) in the biochemical composition of *P. pollicipes*. No interaction between these two factors were detected. The PCA revealed that the differences between *P.*

*pollicipes* qualities could be associated to a higher protein content and energetic value of the high quality barnacles and a higher water content of the low quality barnacles (**Fig. 4.2.6** and **Tables 4.2.6, 4.2.7**). The Pair-wise tests for factor Region showed that SW differed significantly from the other two regions (CENTER and RNB), probably due to a higher ash content and a lower content of glucose and energetic value (PCA, see **Fig. 4.2.6** and **Tables 4.2.6, 4.2.7**).

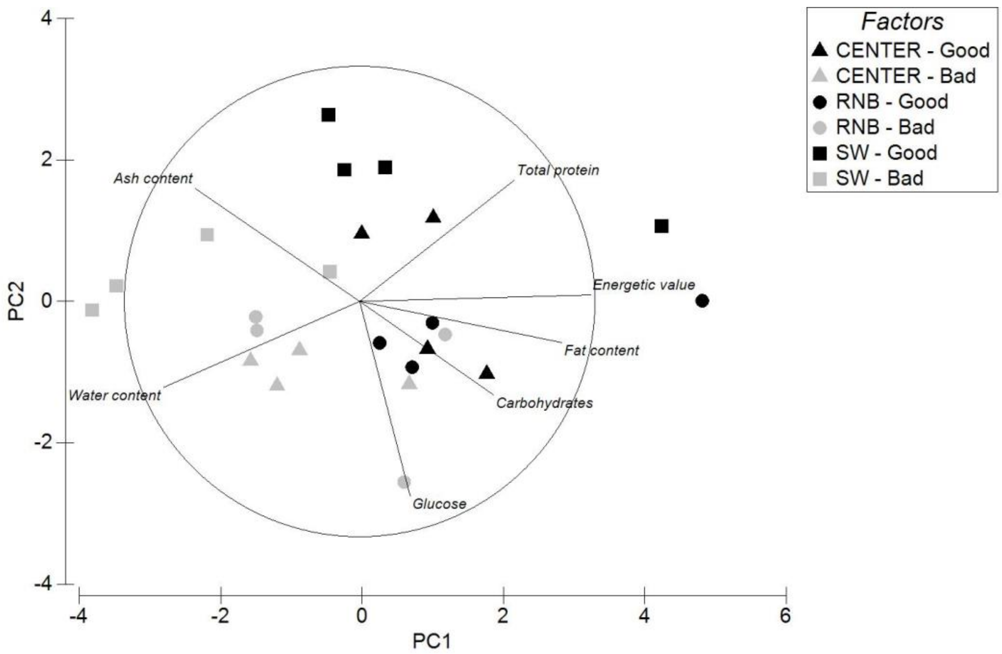



**Fig. 4.2.6 –** PCA results for the biochemical composition of the stalked barnacle *Pollicipes pollicipes* (edible part of the peduncle) in relation to factors Region (CENTER, RNB and SW) and quality (Good – high commercial quality barnacles; Bad – low commercial quality barnacles).

**Table 4.2.6** – Biochemical composition of the stalked barnacle *Pollicipes pollicipes* (edible part of the peduncle) of high and low quality, in the three sampled regions (RNB, CENTER and SW).

| Variables | High quality | | | | Low quality | | | |
|---|---|---|---|---|---|---|---|---|
| | RNB | CENTER | SW | **Mean** | RNB | CENTER | SW | **Mean** |
| Water content (g/100 g) | 76.58 | 76.90 | 76.10 | **76.53** | 78.08 | 78.50 | 79.05 | **78.54** |
| Protein content (g/100 g) | 18.70 | 18.93 | 19.35 | **18.99** | 17.95 | 17.33 | 17.28 | **17.52** |
| Ash content (g/100 g) | 2.16 | 2.17 | 2.53 | **2.29** | 2.17 | 2.20 | 2.52 | **2.29** |
| Fat content (g/100 g) | 1.88 | 1.60 | 1.58 | **1.65** | 1.33 | 1.30 | 0.95 | **1.19** |
| Carbohydrates (g/100 g) | 0.70 | 0.43 | 0.49 | **0.54** | 0.50 | 0.68 | 0.23 | **0.47** |
| Glucose (g/100 g) | 0.28 | 0.23 | 0.05 | **0.18** | 0.28 | 0.18 | 0.06 | **0.17** |
| Energetic Value (kcal/100 g) | 94.50 | 91.75 | 93.50 | **93.25** | 85.75 | 83.75 | 78.50 | **82.67** |

**Table 4.2.7** – PERMANOVA to the biochemical composition of *P. pollicipes* (edible part of the peduncle) in relation to factors Region (RG) and Quality (QL). PERMDISP to quality and to region: non significant. Normalized data. Significant effects are indicated in bold ($p < 0.05$).

| Source | df | MS | Pseudo-F | *p* |
|---|---|---|---|---|
| RG | 2 | 13.07 | 2.78 | **0.023** |
| QL | 1 | 42.356 | 8.99 | **0.000** |
| RGxQL | 2 | 3.87 | 0.82 | 0.543 |
| Residual | 18 | 4.71 | | |

Pair-wise tests:
**RG**
SW ≠ (CENTER = RNB)
**QL**
High quality ≠ Low quality

*Manipulative experiment*

The influence of the density and/or microhabitat on *P. pollicipes* quality was assessed by a manipulative experiment that was set at Cabo Sardão (**Fig. 4.2.2**). The variation of the CB/TL ratio, considering the five main hypotheses specified in **Table 4.2.4** are described in **Table 4.2.9**.

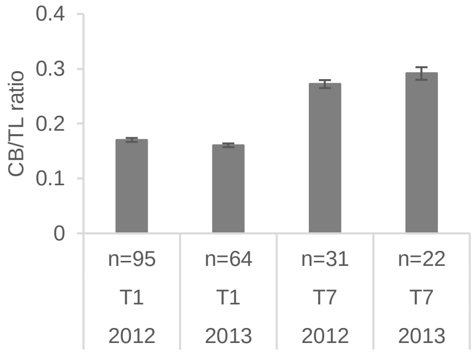
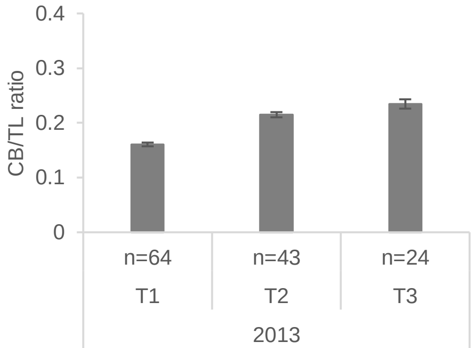
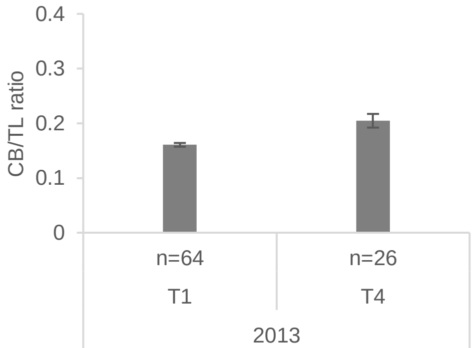
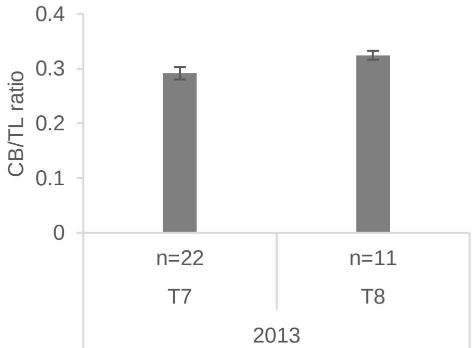
As expected, the animals in the control low quality microhabitat treatment (T1) were longer than those in the control high quality microhabitat treatment (T7), and for each microhabitat there were no significant differences between the two years (2012 and 2013) in which these treatments were applied (**Table 4.2.9A**).

It was supported that density had an effect on the morphology of *P. pollicipes*, because when the density was reduced (through a reduction in density within the clump (T2) or around the clump (T3)), the animals became shorter than in the control low quality microhabitat treatment (T1), since the barnacles in T3 showed significantly higher CB/TL values than in T2 (**Table 4.2.9B**).

In order to distinguish that the transplant to a different location/microhabitat from the original was not due to the transplant manipulation itself, a comparison was made in each microhabitat between the control treatment and a transplant treatment to the same microhabitat (low quality microhabitat – T1 versus T4; high quality microhabitat – T7 versus T8). Whilst there was no effect of transplantation in the high quality microhabitat, this was not the case in the low quality microhabitat, where the barnacles that were transplanted into this microhabitat (T4) became shorter than in the control treatment (T1) (**Table 4.2.9C**).

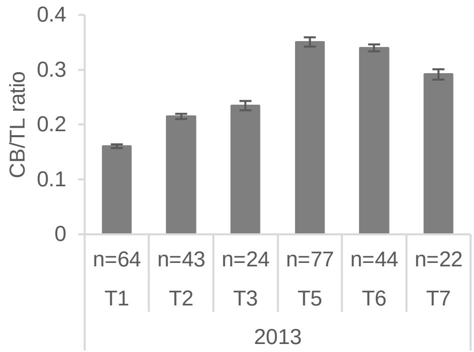
The study of the hypotheses to test the possible effects of the microhabitat on *P. pollicipes* morphology was investigated separately for the two microhabitats considered (**Table 4.2.9D** and **4.2.9E**). A microhabitat effect was detected on *P. pollicipes* transplanted from the low quality microhabitat to the high quality microhabitat, which seems to have been added to the effect of density reduction, as the barnacles that were transplanted with density reduction from the low quality microhabitat to the high quality microhabitat (T5 and T6) not only became shorter than those that remained in the low quality microhabitat (control low – T1 and reduced density treatments – T2 and T3), but even shorter than the control barnacles in the high quality microhabitat (T7) (**Table 4.2.9D).** On the contrary, no microhabitat effect was detected on *P. pollicipes* transplanted from the high quality microhabitat to the low quality microhabitat, as these transplanted barnacles (T9 and T10) did not elongate and remained similar to the control animals that remained in the high quality microhabitat (T7) (**Table 4.2.9E).**

**Table 4.2.9** – Mean CB/TL ratio (± standard error) of *Pollicipes pollicipes* from different treatments and PERMANOVA results for the tests of hypotheses described in **Table 4.2.3** (A to E). The nomenclature of the treatments is described in **Table 4.2.4**. Low – habitat with low commercial quality barnacles. High – habitat with high commercial quality barnacles.

| Hypothesis | Results | PERMANOVA |
|---|---|---|
| (**A**) Relation between control treatments in each microhabitat:<br><br>T1 (control low)<br><<br>T7 (control high) |  | Permdisp: $p > 0.05$<br>Data transformed – square root<br><br>*Pseudo-F*$_{3,208}$ = 139.27<br>p-value = 0.0001<br><br>pair-wise tests:<br>T1(2012) = T1(2013)<br><<br>T7 (2012) = T7 (2013) |
| (**B**) Density effect:<br><br>T1 (control low)<br><<br>T2, T3 |  | Permdisp: $p > 0.05$<br>Data transformed – square root<br><br>PERMANOVA:<br>*Pseudo-F*$_{2,128}$ = 65.47<br>p-value = 0.0001<br><br>pair-wise tests:<br>T1 < T2 < T3 |
| (**C**) Transplantation effect:<br><br>T1 (control low) = T4<br><br>T7 (control high) = T8 |  | Permdisp: $p > 0.05$<br>Data transformed – square root<br><br>PERMANOVA:<br>*Pseudo-F*$_{1,88}$ = 44.66<br>p-value = 0.0001<br><br>pair-wise test:<br>T1<T4 |
| |  | Permdisp: $p > 0.05$<br><br>PERMANOVA:<br>*Pseudo-F*$_{1,31}$ = 3.61<br>*p-value* = 0.0696<br><br>T7=T8 |

(**D**) Microhabitat effect:

T1 (control low), T2, T3
<
T5, T6, T7 (control high)



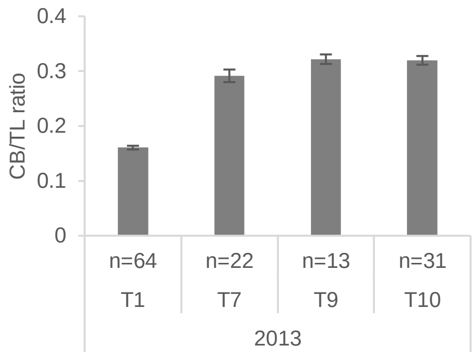

Permdisp: $p > 0.05$
Data transformed – square root

PERMANOVA:
$Pseudo\text{-}F_{5,268} = 177.15$
p-value = 0.0001

pair-wise test:
T1 < T2 < T3 < T7 < (T5 = T6)

(**E**) Microhabitat effect:

T1 (control low), T9, T10
<
T7 (control high)




Permdisp: $p > 0.05$
Data transformed – square root

PERMANOVA:
$Pseudo\text{-}F_{3,126} = 187.11$
p-value = 0.001

pair-wise test:
T1
<
T7, T9, T10
No clear pattern among T7, T9 and T10.

### 4.2.5 Discussion

The recognition that there is a better and a worse commercial quality of *Pollicipes pollicipes* had already been described in previous studies (Parada et al. 2012; Rivera et al. 2014, 2016; Sousa et al. 2024). Differences in quality perceived by fishers and consumers have been associated with morphological differences that can be typified into two extreme morphotypes (Parada et al. 2012; Sousa et al. 2024). Thinner and more elongated barnacles have been considered to be of lower quality, while barnacles with thicker and short peduncles are considered to be of high quality (Parada et al. 2012; Rivera, 2015; Sousa et al. 2024). In the present study, the novelty is to investigate whether the scientists and the professional harvesters assess the quality of the individuals of *P. pollicipes* similarly. We used a proxy variable for quality measured as the ratio of the length of the capitulum base of the barnacle to its total length (CB/TL). Barnacles with a relatively longer peduncle/lower quality show lower values in this variable.

Previous studies have validated the classification of the commercial quality of *P. pollicipes* that professional harvesters have given in various geographical areas through the morphological analysis of the barnacles (Parada et al. 2012; Peñas-Torramilans et al. 2024). In the present study, the validation of the definition of quality by scientists and fishers was carried out at the level of the individual, using two methods: classification by fishers of the quality of barnacles previously categorized by scientists; and comparison of CB/TL of barnacles harvested by scientists and fishers as being of high or low quality. Harvesters had a similar perception of quality as scientists: 78 % of the harvesters identified barnacles with a higher CB/TL ratio (mean CB/TL ratio = 0.43) as having high quality; 90 % of the harvesters identified barnacles with a lower CB/TL ratio (mean CB/TL ratio = 0.17) as having low quality; significant differences in CB/TL were found between the barnacles harvested as having high (mean CB/TL ratio of 0.34) or low quality (mean CB/TL ratio of 0.23) by scientists and fishers, and no differences were found between CB/TL of barnacles harvested by fishers and scientists. Consequently, the CB/TL ratio was validated as a good proxy to evaluate the two extreme qualities (high and low quality) of *P. pollicipes*. In previous studies, other variables were used to typify the two extreme qualities of *P. pollicipes* in Spain and Portugal, but in all the studies a variable associated with the width of the barnacles (capitulum base diameter in Parada et al. 2012 and Peñas-Torramilans et al. 2024; and maximal rostro-carinal length (RC) in Sousa et al. 2024) was related to total length of the barnacles (width versus total length or the inverse).

Morphological differences were not the only ones found between *P. pollicipes* with better or worse commercial quality. The present study investigated for the first time whether the biochemical composition of the edible part of the peduncle varied with the quality of this species and significant differences were found. High quality barnacles presented a higher protein content, energetic value and fat content, and low quality barnacles presented a higher water content. A higher protein content in high quality barnacles and a higher water content in low quality barnacles might be related to the characteristics utilized by harvesters to define high (“thick/hard”) and low (“watery”) quality barnacles in Portugal and Galicia (Sousa et al. 2024). In Galicia, a previous study described the high quality barnacles as having a greater amount of muscle in the peduncle (Rivera et al. 2014), which can be indicative of a higher protein content. In Portugal, the fishers call the low quality barnacles “percebe mijão” (“pissing” barnacles), as these barnacles can squirt water when caught or eaten (Cruz et al. 2022), which can be associated with its higher water content. Nevertheless, the water content that can easily and abundantly be found inside low quality fresh or cooked barnacles, when manipulated and opened for human consumption was not analysed in the present study.

However, the individual biomass of *P. pollicipes* was not considered a good proxy to evaluate this species commercial quality, since no differences were detected in this variable between the two barnacle qualities sampled in the present study. Regarding the individual biomass, regional differences were detected, being lower in SW than in RNB. Regional differences were also detected in the biochemical composition of *P. pollicipes* (edible part of the peduncle), being the SW region the one that differed from the other two studied regions. *P. pollicipes* in SW presented a higher ash, protein and water content, and a lower glucose and energetic value. Although temporal replication in sampling is advisable (not carried out in this study), as the samples were all taken at the same time of the year (summer), we can speculate that the regional differences may be more related to regional variation in the quantity and quality of the food or to other factors than to temporal variation of sampling. In past studies, seasonal and regional differences in biochemical composition of molluscs, such as mussels and other bivalves, were considered to be influenced by the reproductive cycle and/or by environmental conditions, such as water temperature, salinity and quantity and quality of available food (Ruiz et al. 1992; Orban et al. 2002; Ren et al. 2003; van der Meeren et al. 2008; Celik et al. 2012, 2014; Cheng et al. 2021). Previous work investigating the temporal and spatial variability of near-surface phytoplankton pigment concentrations along the western Iberia coast indicated higher concentrations of phytoplankton in the regions north of Cape of Roca (Peliz & Fiúza, 1999). This result suggests that the food supply may be higher in the RNB and CENTER regions (located north of Cape of Roca) than in the SW region, which could explain the variability in the biomass and biochemical composition of *P. pollicipes* in these regions. Further studies are needed to better understand these spatial patterns.

The general analysis of the biochemical composition of *P. pollicipes* revealed that this species can be classified has having a high protein content, as ≥ 20% of its energetic value consists of proteins (Regulation (EC) No 1924/2006 of the European Parliament and of the Council). It is also low in fat and sugar, since the fat content is < 3 g/100 g and the sugar content is <5 g/100 g (Regulation (EC) No 1924/2006 of the European Parliament and of the Council). This classification was also found in some bivalve mollusc species, such as mussels, oysters and clams (Celik et al. 2014), and in crustaceans, as lobsters (Barrento et al. 2009) and crabs (Pires and Batista, 2023). The published reference values for the biochemical composition of *P. pollicipes* in Portugal (INSA, 2023) and Spain (Anta et al. 2021) generally present a higher water content (> 80 g/100 g) and a lower content of proteins (< 16 g/100 g), lipids (< 0.5 g) and carbohydrates (< 0.1 g) than the ones obtained in the present study. This discrepancy must be further investigated in the future, evaluating the seasonal variability of the biochemical composition of this species and sampling more regions and more sites in each region of the Portuguese coast.

With the aim to investigate the main drivers that can influence the morphological variation/quality of *P. pollicipes,* we performed a manipulative experiment. This experiment was conducted at Cape Sardão (a location within the SW region), where there are two microhabitats associated to *P. pollicipes* quality: high quality microhabitat located at low shore (lower intertidal level of *P. pollicipes* distribution), where the barnacles apparently have a short peduncle (thick/short morphology) and are distributed in small clumps of low density; and a low quality microhabitat, located inside a mid shore large crevice, where barnacles present an elongated peduncle (thin/long morphology) and occur in clumps of high density. This experiment involved transplanting the barnacles between microhabitats and artificially reducing the density of the clumps in the low quality microhabitat.

As expected, the control barnacles from the low quality microhabitat were longer (lower CB/TL values – 0.17 and 0.16 in 2012 and 2013, respectively) than those from the high quality microhabitat (higher CB/TL values – 0.27 and 0.29 in 2012 and 2013, respectively). The elongated morphology and corresponding lower quality of *P. pollicipes* was associated with the higher density of the clumps in the low quality microhabitat, as when the density was artificially reduced in this microhabitat, the barnacles became shorter. The pattern of barnacles with long peduncles in very dense clumps may be related to a greater intraspecific competition for space in these groups, since the combined reduction in density around and within the clump seems to have had a more intense effect on altering morphology than the reduction in density just within the clump.

A similar pattern was identified in the acorn barnacles *Semibalanus balanoides* (Barnes & Powell, 1950; Bertness, 1989; Bertness et al. 1998) and *Balanus crenatus* (Barnes & Powell, 1950), that form hummocks of tall densely packed individuals at high densities. Hummocked *S. balanoides* tend to invest less energy in the structural support than solitary individuals, as in the hummocks, the individuals share the structural support with the others around them (Bertness et al. 1998). A comparable relationship may also occur in *Pollicipes*, as in very dense groups, the support provided by the group may be more important than in smaller groups, where individual support should be relatively higher. According to Walker and Anderson (1990), the highest haemolymph pressures in barnacles are recorded in the peduncles of barnacles that occur in the intertidal zone of very wave-exposed shores, namely *P. pollicipes* and *P. polymerus*. Burnett (1987) states that these high pressures are necessary for a stalked barnacle to maintain the turgidity of the peduncle and support the *capitulum* in an elevated position. These high pressures in *Pollicipes* spp. are probably maintained by muscle contraction, and the haemolymph spaces are small compared to other species of pedunculate cirripedes like *Lepas anatifera* that live adrift in the ocean (Walker and Anderson, 1990). We hypothesise that *P. pollicipes* in the low quality microhabitat has greater amounts of haemolymph (higher water content) and lower amounts of muscular tissue (lower protein content), and consequently lower turgidity pressures, because, as the clumps are very dense, the turgidity of the peduncle may not be as important as the muscle to withstand the action of factors such as wave exposure and impact. On the other hand, in less dense groups and in very wave-exposed conditions, we hypothesise that these turgidity pressures are higher as a result of a greater investment in muscle tissue in the peduncle (higher values of protein content).

In *S. balanoides*, hummocking was suggested to be a response to high recruitment densities and growth rates, which intensifies competition for primary substrate space (Bertness et al. 1998). However, in a previous study where recruitment of *P. pollicipes* was measured in the same low and high quality microhabitats of the present study, an inverse pattern was observed, with lower recruitment intensity in the low quality microhabitat (Cruz, 2000). In this study, it was suggested that the high density of barnacles in the low quality microhabitat was associated with a greater survival in this microhabitat. The reduction in density was not the only driver of the shortening of *P. pollicipes*. In fact, stalked barnacles from the low quality microhabitat also became shorter when transplanted to the high quality microhabitat, which also suggests that the characteristics of this microhabitat (e.g. higher wave-exposure) might also be important in altering morphology. This association between wave-exposure and morphology was also reported in a past study with *P. polymerus*: strong peduncles, that are relatively short and attached over a considerable basal area in wave exposed situations; and more elongated peduncles that are attached to a smaller basal area in less exposed situations and among mussel beds (Barnes & Reese, 1960). The characteristics of the rock (such as type of rock, shape and composition of the rock) and hydrodynamics (including wave direction and currents) were the causes most cited by fishers from Spain and Portugal to justify the variation in quality in *P. pollicipes* (Sousa et al. 2024). In a recent study, a linear relation was found between shifts in hydrodynamics (winter wave induced orbital currents) and the quality/morphometry of *P. pollicipes*, with lower wave forcing leading to a decrease in quality (Peñas-Torramilans et al. 2024).

Contrary to the shortening hypotheses (B and E in **Table 4.2.4**) that were supported in the present study, the elongation hypothesis (D in **Table 4.2.4**) was not supported. When individuals of *P. pollicipes* were transplanted from the high quality microhabitat to the low quality microhabitat, they did not lengthen. This result may be related to the difficulty of placing the transplanted barnacles in similar conditions of high density that characterise the clumps of barnacles in the low quality microhabitat. Nevertheless, this result suggest that the characteristics of the low quality microhabitat (e.g. wave-exposure) are not the proximate cause of the elongation of the barnacles, as the barnacles did not elongate one year after the transplantation. As suggested above, this elongation of the peduncle might be a result of the higher density and consequent greater intraspecific competition.

Further investigation is essential to fully comprehend the results outlined in this study. One constrain of the manipulative experiment is that it was only performed in one location. However, conducting manipulative experiments in very exposed locations such as those where *P. pollicipes* occurs is challenging. Indeed, experimental manipulative studies in the extremely exposed rocky shores inhabited by *P. pollicipes* are scarce. In the future, it can be relevant to investigate if the phenotypic variability described in the present study and recognized by harvesters, consumers and scientists, corresponds to differences in the epigenetics of *P. pollicipes*. Epigenetic factors may play an important role in the physiological adaptability of species to the environmental conditions associated with microhabitats (Clark et al. 2018). However, in a recent study, where epigenetics differences between *P. pollicipes* of the two morphotypes/qualities were investigated using the methylation sensitive amplification polymorphism (MSAP) method, no significant differences were detected (Sousa et al. 2024).

### 4.2.6 Conclusions

Professional harvesters and scientists revealed a similar perception about *P. pollicipes* commercial quality. Although the individual biomass of the barnacles was not considered a good proxy to evaluate *P. pollicipes* quality, the CB/TL ratio was validated as a good proxy. Barnacles with higher quality presented higher CB/TL ratio values.

The biochemical composition of the peduncle of *P. pollicipes* revealed that high quality barnacles presented a higher protein content, energetic value and fat content, and low quality barnacles presented a higher water content. Regional differences were also detected in the biochemical composition, as in SW region, *P. pollicipes* presented a higher ash, protein and water content, and a lower glucose and energetic value.

Results from a manipulative experiment support that morphological variation of *P. pollicipes* can be related to the density of barnacles and/or to some local effects. Putative drivers may vary at a small spatial scale. Further investigation is needed to better understand the mechanisms that can influence *P. pollicipes* morphology, quality and commercial value.

#### 4.2.7 Acknowledgements

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# **Chapter 5.** No effect of timing of exploitation on the abundance of stalked barnacles (*Pollicipes pollicipes*) after harvesting: a small-scale approach

Sousa, A., Mateus, D., Neves, F., Penteado, N., Maia, S., Fernandes, J.N., Seabra, M.I, Silva, T., Castro, J.J., Jacinto, D., Leandro, S.M., Cruz, T. *in preparation.* No effect of timing of exploitation on the abundance of stalked barnacles (*Pollicipes pollicipes*) after harvesting: a small-scale approach

## 5.1 Abstract

The stalked barnacle *Pollicipes pollicipes* is a highly valuable intertidal resource of European rocky shores. Here we followed clumps of *P. pollicipes* for a maximum of 2 years with the aim to evaluate the impact of *P. pollicipes* exploitation on the abundance and recovery of *P. pollicipes* at a small spatial scale. We have performed a field experiment in two sites of the Portuguese coast (Berlenga and Sines). We considered small areas (15 x 15 cm) where the exploitation of this species was manipulated (exploited areas) and areas that were not manipulated (controls). We measured three response variables in several monitoring dates: the percentage of *P. pollicipes* cover in a 15 x 15cm area; the area of the clump; the state of the clump - recovery (exploited areas) or growth (controls). We have considered a timing of the exploitation / set-up factor (summer, autumn and spring) and an intertidal level factor (low and mid shore).

The hypothesis that the recovery of the exploited areas or clumps would be faster if the exploitation had been carried out in summer or autumn (recruitment season) and slower in spring (non-recruitment season) was not supported. As in previous studies, the recovery of *P. pollicipes* was considered to be variable and slow, as two years after the initial exploitation most of the clumps only had partially recovered the area they had before exploitation.

The main pattern we found was that the results showed regional differences. Recovery of *P. pollicipes* in Berlenga was slower than in Sines. Regional ecological studies are fundamental for a regional sustainable governance.

## 5.2 Introduction

Rocky intertidal landscapes can be described as a mixture of patches of sessile organisms (e.g. barnacles, mussels, algae) and patches of space for attachment also designated as gaps. Assemblages of sessile species on intertidal rocky shores are affected by disturbance (sensu Dayton 1971) which can be physical (e.g. wave impact, log battering, sand inundation, e.g. Dayton 1971, Paine & Levin, 1981, Sousa 1985, Underwood 1999) or biological (e.g. natural predation, e.g. Dayton 1971) that might produce gaps. If those species are harvested, such as mussels (Dye 1992), ascidians (Monteiro et al. 2002) and stalked barnacles (Cruz et al. 2022, Geiger et al. 2024), there is an extra source of disturbance - human disturbance – that creates gaps.

Most research has been carried out into the recovery of these gaps (e.g. within mussels, Paine & Levin 1981, Sousa 1984; within algae, Benedetti-Cecchi & Cinelli 1994, Kim & DeWreede 1986) and on gap attributes that might influence this recovery (e.g. size, shape, location and time of creation, see Sousa 1985 and revision in Kim & DeWreede 1996). If the gap has been created by harvesting of a group of sessile animals, it is also worth monitoring the abundance of these animals in the exploited area, as the group may have been partially or fully exploited.

Stalked barnacles of the genus *Pollicipes* are sessile gregarious species that are distributed in clumps of varying size mainly in the intertidal zone of very exposed shores (Barnes 1996, Cruz et al. 2022). All *Pollicipes* species are exploited for human consumption, though *P. pollicipes* (exploitable populations in the Atlantic from Brittany, France to Senegal) is considered to be the most exploited species, namely in Portugal and Spain (Cruz et al. 2022). Most of this harvesting is done at low tide with a scraper allowing the larger barnacles to be extracted within the clumps (Cruz et al. 2022). However, as recruitment is high on conspecifics (e.g. Fernandes et al. 2021), smaller animals are always discarded during exploitation, as groups of barnacles are made up of animals of varying size (Sousa et al. 2013). The impact of exploitation on *P. pollicipes* has not been much studied, but recent studies indicate a highly variable and slow recovery of *P. pollicipes* (Geiger et al. 2024) facilitated by the presence of adult conspecifics in the margins of the gaps (Gomez-del Campo et al. in press).

Considering that the main recruitment period for *P. pollicipes* occurs in summer and autumn (Cruz et al. 2022), we can expect that the impact of exploitation will vary with season. We can expect that the recovery of the resource will be facilitated during summer and autumn and more difficult in spring, as this season may correspond to the recruitment season of other species that cohabit the intertidal zone and that may occupy the exploited area. In fact, the assumption that the recovery of *P. pollicipes* after exploitation is facilitated during summer and autumn has in the past justified the decision to establish a temporal closure in Portugal in summer and/or autumn (August and September in the Berlengas Nature Reserve, between 15th September and 15th December on the SW coast of Portugal and between 15th September and 15th October on the rest of the Portuguese coast).

The aim of this study was to analyse the variation in the abundance of *P. pollicipes* (percentage cover in 15 x 15 cm and area of a selected clump) between control and exploited (manipulation of exploitation) quadrats/clumps. Experimental harvesting on a clump was always partial, with a part of the clump of varying size remaining unexplored. In addition, the state of the clump (control or exploited) was classified into different categories ranging from total loss to growth of the clump.  The monitoring of the quadrats/clumps was made in two sites of the Portuguese coast up to a maximum of 24 months, considering two tidal levels were *P. pollicipes* occurs (mid and low shore) and different timings of experimental exploitation (summer, autumn, and spring). The hypothesis is that the recovery of the exploited clump is faster when the exploitation was carried out in summer or autumn and slower in spring.

## 5.3 Materials and methods

*Study sites*

The field experiment was carried out in two sites along the Portuguese coast: in the Berlenga island (39°24'54''N 9°30'58''W) and in Cape of Sines (37°57'47"N 8°53'10"W) (**Fig. 5.1**).  The Berlenga island site was located within the *P. pollicipes* no-take zone of

“Berlengas Nature Reserve” (for more information see Sousa et al. 2013). The Cape of Sines is located in a zone adjacent to a marine protected area (“Parque Natural do Sudoeste Alentejano e Costa Vicentina” (PNSACV)), where *P. pollicipes* harvesting is allowed following the PNSACV regulations (for more information see Sousa et al. 2013).

Three timings of exploitation were considered (**Table 5.1**): at the beginning of summer of 2020 (corresponds to the beginning of recruitment season); at autumn of 2020 (corresponds to the end of recruitment season); and in spring of 2021 (corresponds to the non-recruitment season). In each site, two vertical levels were defined (mid and low shore). Mid shore was considered the middle/upper *P. pollicipes* intertidal distribution (~1.5 m to 3 m above MLWS) and Low shore was defined as the lower intertidal level of *P. pollicipes* distribution (~MLWS to +1.5 m). In each site and vertical level, 15 x 15 cm experimental quadrats were randomly selected among areas with *P. pollicipes* clumps and permanently marked on the shore. The permanent mark on the shore consisted of three inox screws, that allow the identification of those same quadrats over time. The number of experimental quadrats marked in each tidal level varied, being higher at mid that at low shore, due to the lower accessibility of this last intertidal level. In each tidal level, two types of quadrats were marked: quadrats where *P. pollicipes* was experimentally exploited (exploited quadrats), and control quadrats where *P. pollicipes* remained untouched. Sample size was 7-10 at mid shore and 3-6 at low shore.

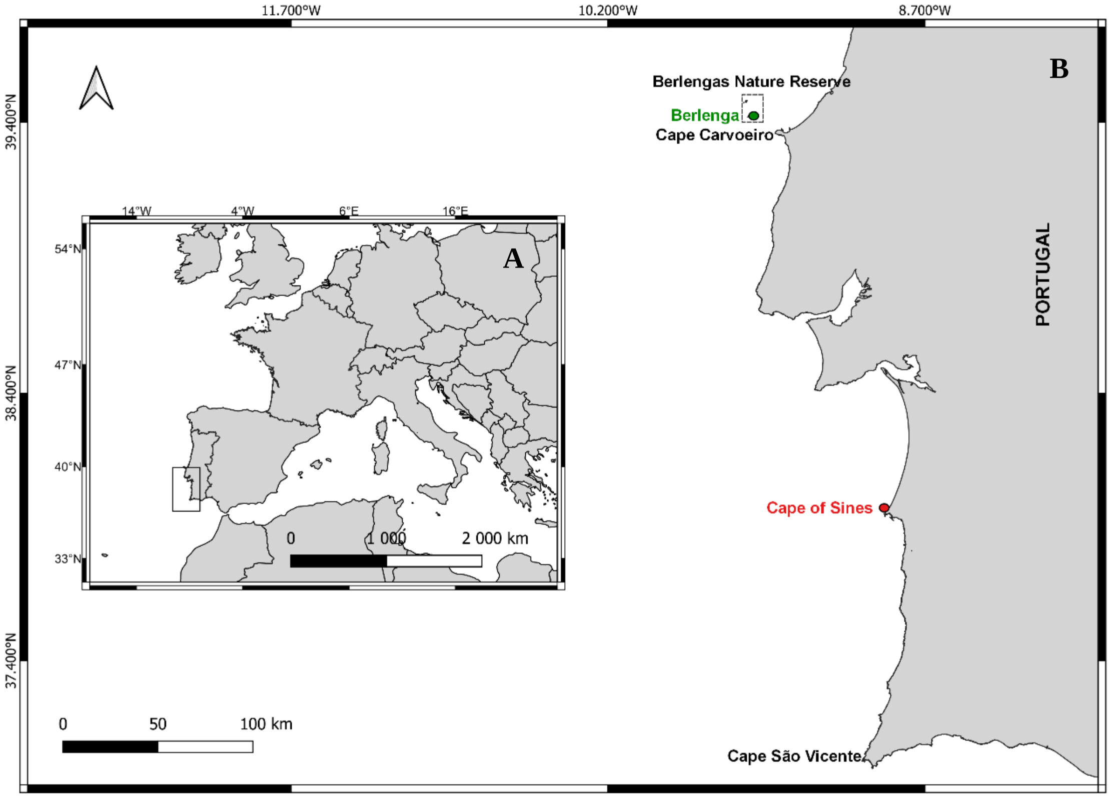


**Fig. 5.1** – (**A**) Map of Europe with the location of the zone where experimental sites were located, in Portugal, (**B**) Location of the two experimental sites: Berlenga (in green) and Cape of Sines (in red).

At the beginning of the experiment the quadrats of both types were photographed (T0-set-up time). The quadrats where experimental exploitation occurred were also photographed after the experimental exploitation (T1-set-up, post exploitation time). The experimental exploitation of *P. pollicipes* clumps was carried out by the scientists of the research team. In each manipulated quadrat a portion of a clump was harvested (ranging from 2 to 91% of exploited clump area, average area of 39%). The manipulated exploitation was directed towards the bigger individuals (RC > 20 mm) of the clump, with the aim of proceeding as similarly as possible to professional harvesters. To fulfill this approach, different portions of the clump might have been harvested in each exploited clump.

The variables that were measured in this study were: (1) the percentage cover of *P. pollicipes* in the 15x15cm area; (2) the area of a *P. pollicipes* clump ($cm^2$); and (3) the state of a selected clump, a categorical variable that classified the state of each control or exploited clump (see definition of levels of this variable for control and exploited quadrats in **Table 5.2**). All these variables were measured approximately every 6 months, for a maximum of 2 years (T6, T12, T24) after each experimental set-up. The summer set-up was monitored for a period of 24 months, while the autumn and spring timings were monitored for a period of 12 months (**Fig. 5.2**, **Table 5.1**). The monitoring consisted of photographing the same quadrats in each monitoring date (T6, T12, T24).

**Table 5.1** – Chronogram of the field experiment carried out in two experimental sites (Berlenga and Cape of Sines) with indication of the dates of the set-up of the experiment (T0 and T1) and monitoring dates (T6, T12 and T24). Seasons: Su – Summer, Au – Autumn, Wi – Winter, Sp – Spring. T0 – set-up time before exploitation, T1 – set-up time after exploitation, T6 - 6 months after exploitation, T12 - 12 months after exploitation and T24 –24 months after exploitation

| | | Su 2020 | Au 2020 | Wi 2020 | Sp 2021 | Su 2021 | Au 2021 | Wi 2021 | Sp 2022 | Su 2022 |
|---|---|---|---|---|---|---|---|---|---|---|
| Berlenga | Su Set-up | T0, T1 | | - | | T12 | | | | T24 |
| | Au Set-up | | T0, T1 | | T6 | | T12 | | | |
| | Sp Set-up | | | | T0, T1 | | T6 | | T12 | |
| Sines | Su Set-up | T0, T1 | | T6 | | T12 | | | | T24 |
| | Au Set-up | | T0, T1 | | T6 | | T12 | | | |
| | Sp Set-up | | | | T0, T1 | | T6 | | T12 | |

*Image analysis*

In each photograph of a control quadrat, a clump of *P. pollicipes* was randomly selected to follow over time. In the exploited quadrats, the clump that was selected was the one that was exploited. The area of the selected clump was measured (in $cm^2$) in the set-up photos (T0 and T1, when appropriate) and in each monitoring time (T6, T12 and T24). The error of measuring the clumps was assessed by measuring the same clump (for the same monitoring time) 10 times in different occasions, using 10 different clumps. The average assessed error was 1 $cm^2$.

In addition, in each quadrat of both types, the percentage cover of *P. pollicipes* inside the 15 x 15 cm quadrat was estimated, following a methodology similar to the described by Sousa et al. (2013). Briefly, a grid of equidistant rows (1 cm) was superimposed on the photo of the quadrat, and the number of intersection points of the grid that were over barnacles were counted (out of 196 intersection points).

Finally, the state of each selected clump (control or exploited) was classified in all monitoring times, considering the categories defined in **Table 5.2**. For each site, timing of exploitation, tidal level, and in each monitoring time, the percentage of clumps classified in each category was calculated.

QGIS software (www.qgis.org) was used for all the image analyses.

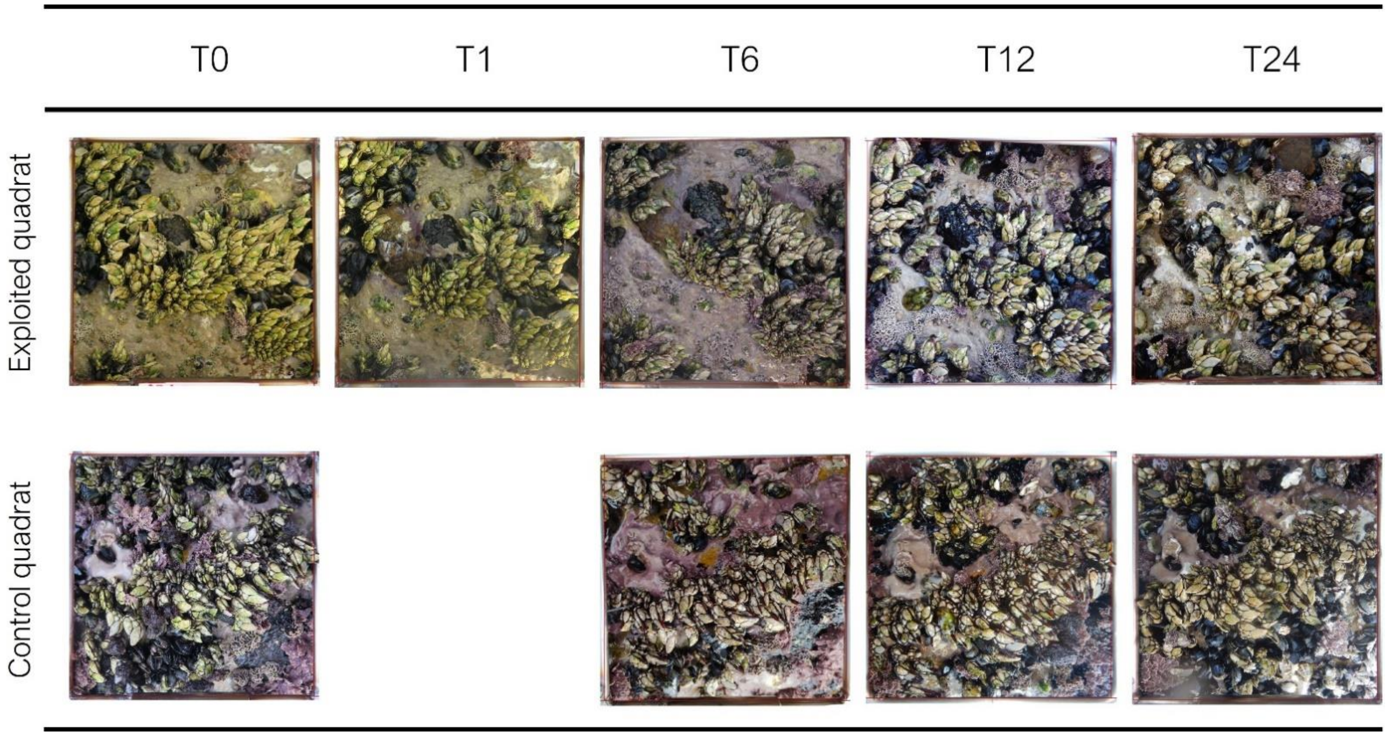


**Fig. 5.2** – Representative example of an exploited and a control quadrat followed through time. T0 – set-up time before exploitation, T1 – set-up post-exploitation, T6 – 6 months after exploitation, T12 – 12 months after exploitation and T24 –24 months after exploitation.

*Data analyses*

The hypotheses of variation of percentage cover of *P. pollicipes* and of clump area ($cm^2$) between exploited and non-exploited (control) quadrats and clumps were analyzed for each site (Berlenga or Sines) and set-up time (T0 – set-up time before exploitation, T1 – set-up post-exploitation) and monitoring time, (T6 – 6 months after exploitation, T12 – 12 months after exploitation and T24 –24 months after exploitation) by permutational multivariate analysis of variance, PERMANOVA (Anderson 2001), including three factors: timing of exploitation (fixed factor with three levels - Summer, Autumn and Spring), intertidal level (fixed factor with two levels - mid shore and low shore) and exploitation level (fixed factor with two treatments: - control and exploited). Sample size was variable (7 - 10 at mid shore and 3 - 6 at low shore). The analyses were run separately for Berlenga and Sines, since previous differences regarding the percentage cover of *P. pollicipes* between the two sites were observed (Sousa et al. 2013).

The hypothesis of different state of the clumps of *P. pollicipes* were analyzed separately for control and exploited clumps in each monitoring time (T6 – 6 months after exploitation, T12 –12 months after exploitation and T24 – 24 months after exploitation) by permutational multivariate analysis of variance, PERMANOVA (Anderson 2001) with three factors: site (fixed factor with two levels - Berlenga and Sines), timing of exploitation (fixed factor with three levels - Summer, Autumn and Spring), and intertidal level (fixed factor with two levels - mid shore and low shore). Sample size was variable (7 - 10 at mid shore and 3 - 6 at low shore).

All PERMANOVA analyses, were based on Euclidian distance with data transformed when appropriate. Permutation of residuals under a reduced model and type III sums of squares were applied (Anderson et al. 2008). PERMANOVA was used to analyze univariate data due to an unbalanced design resultant of the different sampling size. The homogeneity of dispersion based on Euclidean distance was tested using the PERMDISP routine (Anderson 2006). When appropriate pair-wise a posteriori comparisons were conducted. The software PRIMER 6 & PERMANOVA+ (www.primer.com; Anderson et al. 2008) was used to perform all statistical analyses.

**Table 5.2** – Description of the classifications of the state of the clumps over time.

| Classification | Description of state of the clump | Classification |
|---|---|---|
| | **Exploited clumps** | |
| Total loss | Clump totally disappeared | 1 |
| Partial loss | The area of the clump at the monitoring time was smaller the area of the clump post-exploitation (T1) ± 1cm$^2$ | 2 |
| No change | The area of the clump at the monitoring time was similar to the area of the clump post-exploitation (T1) ± 1cm$^2$ | 3 |
| Partial recovery | The area of the clump at the monitoring time was higher than the area of the clump post-exploitation (T1) ± 1cm$^2$, but did not reach the area of the clump prior to exploitation (T0) ± 1cm$^2$ | 4 |
| Total recovery | The area of the clump at the monitoring time was similar to the area of the clump prior to exploitation (T0) ± 1cm$^2$ | 5 |
| Growth | The area of the clump at the monitoring time was higher than the area of the clump prior to exploitation (T0) ± 1cm$^2$ | 6 |
| Growth by joining to a nearby clump | The area of the clump at the monitoring time was higher than the area of the clump prior to exploitation (T0) ± 1cm$^2$ due to joining to a nearby clump | 7 |
| | **Control clumps** | |
| Total loss | Clump totally disappeared | 1 |
| Partial loss | The area of the clump at the monitoring time was smaller than the area of the clump at the beginning of the experiment (T0) ± 1cm$^2$ | 2 |
| No change | The area of the clump at the monitoring time was similar to the area of the clump at the beginning of the experiment (T0) ± 1cm$^2$ | 3 |
| Growth | The area of the clump at the monitoring time was higher than the area of the clump at the beginning of the experiment (T0) ± 1cm$^2$ | 4 |
| Growth by joining to a nearby clump | The area of the clump at the monitoring time was higher than the area of the clump at the beginning of the experiment (T0) ± 1cm$^2$ due to joining to a nearby clump | 5 |

## 5.4 Results

*Percentage cover of Pollicipes pollicipes*

The analysis of the variation in the percentage cover of *P. pollicipes* in 15 x 15 cm over time (T0, T1, T6, T12 and T24) in Berlenga did not show the same results as those obtained in Sines (see **Table 5.3** and **Fig. 5.3**).

In Berlenga, in the post-exploitation time (T1), not all combinations of timing of exploitation and intertidal level revealed that the percentage of barnacle cover was lower in the exploited quadrats than in the controls. On the contrary, in Sines, there was a clear pattern of a lower percentage of cover of *P. pollicipes* in the exploited quadrats than in the controls at T1. However, 6 months after exploitation (T6), the pattern of lower percentage cover in exploited quadrats was significant in both Berlenga and Sines, and remained significant in the Berlenga low shore after 12 months and 24 months post exploitation (T12 and T24). This was not the case in the Berlenga midshore and in Sines (both intertidal levels) where the percentage cover in exploited quadrats and controls was similar in T12 and T24.

With regard to factor timing of exploitation (= set-up time), in Sines, a lower general percentage cover of *P. pollicipes* was observed when the experiment starts in Autumn (T0 and T1) or when monitoring was carried out in Autumn (spring set-up after 6 months, autumn set-up after 12 months). In Berlenga, a pattern of generalised variation in the percentage cover of barnacles depending on the set-up time was only observed after 12 months. At this site and time (T12), the percentage cover of *P. pollicipes* was higher when monitoring was carried out in summer and autumn than in spring.

At both sites, there was no consistent pattern of variation of percentage cover of *P. pollicipes* between vertical levels.

*Area of Pollicipes pollicipes clumps*

Similarly to the analysis of the percentage of cover, the analysis of the variation in clump area ($cm^2$) of *P. pollicipes* in Berlenga did not show exactly the same results as those obtained in Sines (see **Table 5.4** and **Fig. 5.4**).

In Berlenga, after exploitation (T1), not all combinations of timing of exploitation and intertidal level revealed that the clump area was smaller in the exploited quadrats than in the controls. On the contrary, in Sines, a clear pattern of lower clump area was observed in the exploited quadrats than in the controls at T1. However, 6 months after exploitation (T6), the pattern of lower clump area in exploited quadrats was significant in both Berlenga and Sines, and remained significant in Berlenga after 12 months (T12) and in the Berlenga lowshore after 24 months (T24). This was not the case in Sines where after 12 and 24 months post-exploitation, the percentage cover of exploited quadrats and controls was similar.

With regard to factor timing of exploitation, no clear patterns of variation of clump area of *P. pollicipes w*ere found in Berlenga, and in Sines, this factor was never significant. In relation to variation between intertidal levels, in Berlenga no clear pattern of variation was observed, while in Sines this factor was never significant.

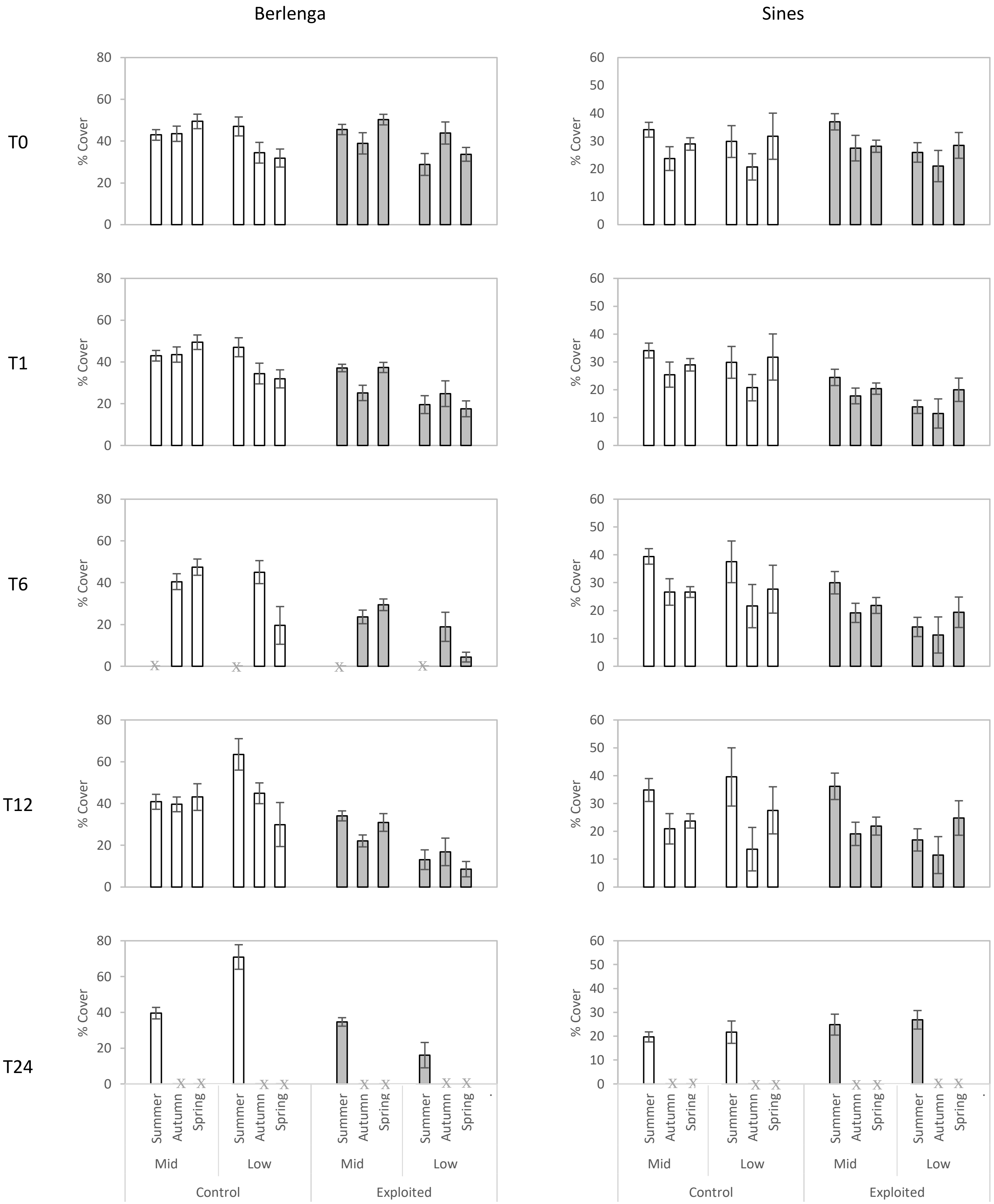


**Fig. 5.3** – *Pollicipes pollicipes* percentage cover (mean ± SE; n= 7 - 10 at mid shore and 3 - 6 at low shore) in 15 x15 cm, assessed in each site (Berlenga and Sines), considering two types of quadrats (control and exploited), two intertidal levels (mid shore and low shore), and three set-up seasons (Summer, Autumn and Spring), for each monitoring time (T0 – initial time before exploitation, T1 – initial time after exploitation, T6 - 6 months after exploitation, T12 - 12 months after exploitation and T24 –24 months after exploitation).

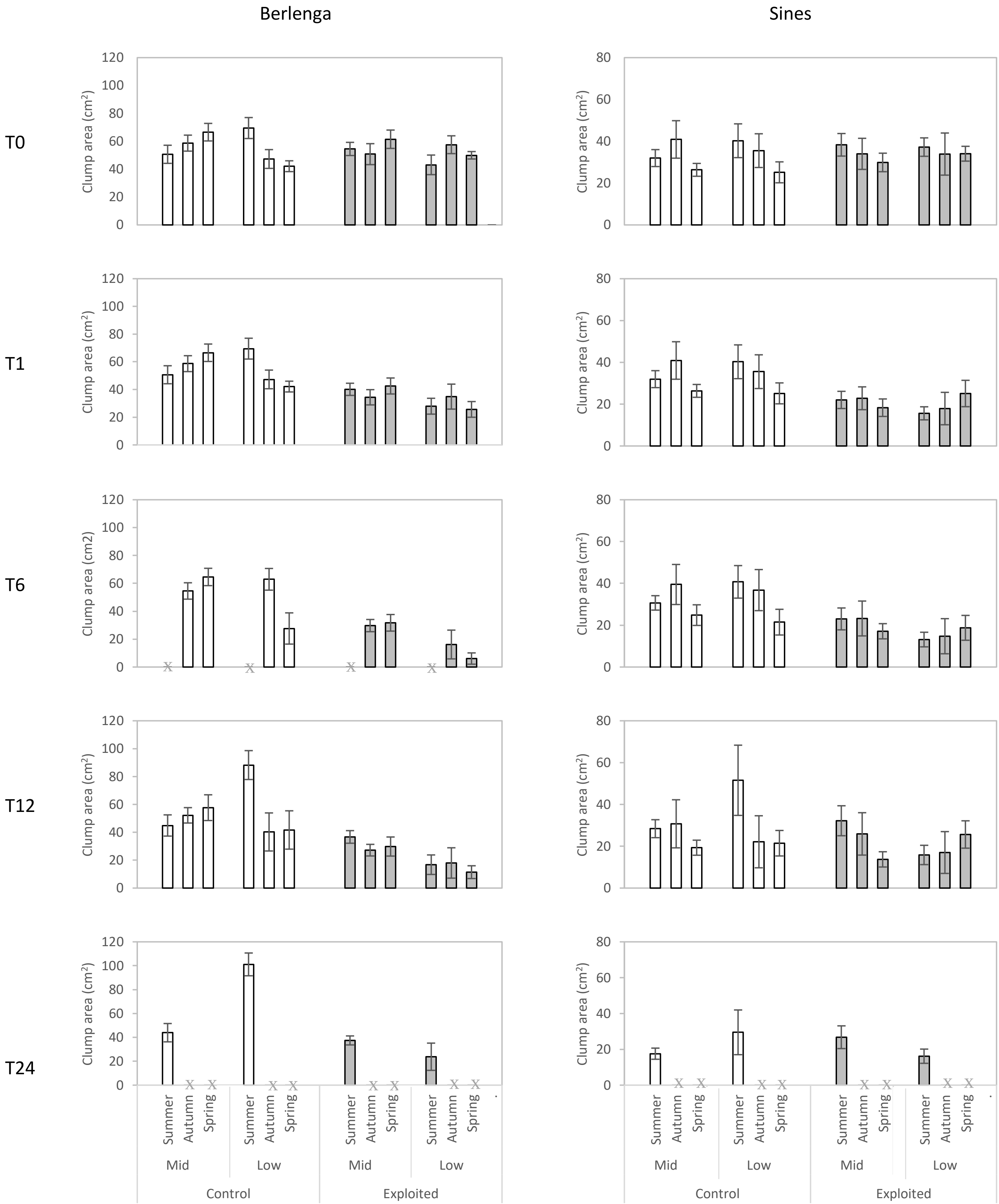


**Fig. 5.4** – *Pollicipes pollicipes* clump area (in cm$^2$; mean ± SE; n= 7 - 10 at mid shore and 3 - 6 at low shore) assessed in each site (Berlenga and Sines), considering two types of quadrats (control and exploited), two intertidal levels (mid shore and low shore), and three set-up seasons (Summer, Autumn and Spring), for each monitoring time (T0 – initial time before exploitation, T1 – initial time after exploitation, T6 - 6 months after exploitation, T12 - 12 months after exploitation and T24 –24 months after exploitation).

**Table 5.3** – *P*-values from the PERMANOVA analyses on *Pollicipes pollicipes* percentage cover in 15x15cm for each time of monitoring (T0 – initial time before exploitation, T1 – initial time after exploitation, T6 - 6 months after exploitation, T12 - 12 months after exploitation and T24 –24 months after exploitation) in relation to factor timing of exploitation/season (Se, fixed factor with three levels), factor Intertidal level (Le, fixed factor with two levels) and factor type of quadrats/exploitation (Ex, fixed factor with two levels). Analyses were based on Euclidean distances of untransformed data except for Berlenga T12 where data were Fourth root transformed. *P-values* were obtained using 9999 random permutations. n= 7 - 10 at mid shore and 3 - 6 at low shore. PERMDISP tests for Berlenga: F = 0.1297 (T0; p>0.05), F = 0.0000 (T1; p>0.05), F = 0.5881 (T6; p>0.05), F = 0.2397 (T12; p>0.05) and F = 5.9584 (T24; p<0.05); PERMDISP tests for Sines: F = 0.0043 (T0; p>0.05), F = 0.7523 (T1; p>0.05), F = 0.7892 (T6; p>0.05), F = 4.6739 (T12; p>0.05) and F = 2.0346 (T24; p>0.05). Significant *p-values* that were interpreted are in bold. Pair-wise tests for significant factors or interactions: > or < (p <0.05); Timing of exploitation/Seasons: Su – Summer, Au – Autumn, Sp – Spring; Intertidal level: Mid and Low shore; Type of quadrats/Exploitation level – Ex – exploited, Co – control.

| | Berlenga | | | | | Sines | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | T0 | T1 | T6 | T12 | T24 | T0 | T1 | T6 | T12 | T24 |
| Se | 0.9145 | 0.2111 | 0.0479 | **0.0005** | - | **0.0316** | **0.0441** | **0.0087** | **0.0019** | - |
| Le | 0.0007 | 0.0001 | 0.0005 | 0.0105 | 0.3433 | 0.1109 | 0.0897 | 0.0541 | 0.2551 | 0.6104 |
| Ex | 0.5676 | 0.0001 | **0.0001** | 0.0001 | 0.0070 | 0.8495 | **0.0001** | **0.0002** | 0.1392 | 0.1963 |
| Se x Le | 0.0329 | 0.0215 | **0.0005** | 0.8917 | - | 0.2412 | 0.2374 | 0.4115 | 0.3065 | - |
| Se x Ex | 0.1502 | 0.7936 | 0.4680 | 0.8786 | - | 0.8743 | 0.7206 | 0.2580 | 0.4350 | - |
| Le x Ex | 0.6750 | 0.2558 | 0.6305 | **0.0026** | **0.0070** | 0.4520 | 0.4149 | 0.2250 | 0.2089 | 0.9963 |
| Se x Le x Ex | **0.0105** | **0.0191** | 0.3843 | 0.9436 | - | 0.8897 | 0.9067 | 0.6176 | 0.2170 | - |
| Pair- Wise | **Se x Le x Ex**<br>**Se:**<br>MidEx, MidCo,<br>LowEx –<br>Su=Au=Sp<br>LowCo – n.p.d.<br>(Su > Sp)<br><br>**Le:**<br>SuEx, SpEx,<br>SpCo –<br>Mid > Low<br>SuCo, AuEx,<br>AuCo –<br>Mid = Low<br><br>**Ex:**<br>SuMid, AuMid,<br>AuLow, SpMid,<br>SpLow – Co =<br>Ex<br>SuLow –<br>Co > Ex | **Se x Le x Ex**<br>**Se:**<br>MidCo, LowEx<br>– Su=Au=Sp<br>MidEx<br>– Su = Sp >Au<br>LowCo – n.p.d.<br>(Su>Sp)<br><br>**Le:**<br>SuEx, SpEx,<br>SpCo –<br>Mid > Low<br>SuCo, AuEx,<br>AuCo –<br>Mid = Low<br><br>**Ex:**<br>SuMid, AuLow<br>– Co = Ex<br>SuLow, AuMid,<br>SpMid, SpLow<br>– Co > Ex | **Ex**<br>Co > Ex<br><br>**Se x Le**<br>Se:<br>Mid – Sp > Au<br>Low – Au > Sp<br><br>Le:<br>Au –<br>Mid = Low<br>Sp – Mid > Low | **Se**<br>Su = Au > Sp<br><br>**Le x Ex**<br>Le:<br>Ex – Mid > Low<br>Co – Mid = Low<br><br>Ex:<br>Mid – Co = Ex<br>Low –<br>Co > Ex | **Le x Ex**<br>Le:<br>Ex – Mid = Low<br>Co – Low > Mid<br><br>Ex:<br>Mid – Ex = Co<br>Low – Co > Ex | **Se**<br>Su = Sp > Au | **Se**<br>Su = Sp > Au<br><br>**Ex**<br>Co > Ex | **Se**<br>Su > Au = Sp<br><br>**Ex**<br>Co > Ex | **Se**<br>Su = Sp > Au | |

**Table 5.4** – *P*-values from the PERMANOVA analyses on *Pollicipes pollicipes* clump area (cm$^2$) for each time of monitoring T0 – initial time before exploitation, T1 – initial time after exploitation, T6 - 6 months after exploitation, T12 - 12 months after exploitation and T24 –24 months after exploitation) in relation to factor timing of exploitation/season (Se, fixed factor with three levels), factor intertidal level (Le, fixed factor with two levels) and factor type of quadrats/exploitation (fixed factor with two levels). Analyses were based on Euclidean distances of untransformed data except for Berlenga T12 where data were square root transformed, Berlenga T24 data were Fourth root transformed and Sines T12 data were Log (x + 1) transformed. *P-values* were obtained using 9999 random permutations. n= 7 - 10 at mid shore and 3 - 6 at low shore. PERMDISP tests for Berlenga: F = 0.4142 (T0; $p>0.05$), F = 2.0013 (T1; $p>0.05$), F = 0.3305 (T6; $p>0.05$), F = 0.0005 (T12; $p>0.05$) and F = 0.0000 (T24; $p<0.05$); PERMDISP tests for Sines: F = 0.1013 (T0; $p>0.05$), F = 1.8168 (T1; $p>0.05$), F = 0.7652 (T6; $p>0.05$), F = 0.1975 (T12; $p>0.05$) and F = 0.34841 (T24; $p>0.05$). Significant *p-values* that were interpreted are in bold. Pair-wise tests for significant factors or interactions: > or < ($p <0.05$); Timing of exploitation/Seasons: Su – Summer, Au – Autumn, Sp – Spring; Intertidal level: Mid and Low shore; Type of quadrats/Exploitation level – Ex – exploited, Co – control.

| | Berlenga | | | | | Sines | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | T0 | T1 | T6 | T12 | T24 | T0 | T1 | T6 | T12 | T24 |
| Se | 0.9584 | 0.7385 | 0.1116 | 0.9813 | - | 0.1468 | 0.4302 | 0.2115 | 0.2332 | - |
| Le | 0.1727 | 0.0532 | 0.0020 | **0.0002** | 0.4746 | 0.8354 | 0.8980 | 0.5742 | 0.4255 | 0.9217 |
| Ex | 0.4718 | 0.0001 | **0.0001** | **0.0003** | 0.0003 | 0.7456 | **0.0004** | **0.0008** | 0.2463 | 0.7644 |
| Se x Le | 0.0944 | 0.0438 | **0.0062** | 0.9506 | - | 0.7964 | 0.6591 | 0.8237 | 0.1803 | - |
| Se x Ex | 0.3329 | 0.6759 | 0.4100 | 0.8276 | - | 0.5591 | 0.1771 | 0.2800 | 0.7546 | - |
| Le x Ex | 0.9738 | 0.6079 | 0.6029 | 0.5337 | **0.0003** | 0.9582 | 0.7471 | 0.3686 | 0.6915 | 0.1117 |
| Se x Le x Ex | **0.0265** | **0.0372** | 0.1092 | 0.9117 | - | 0.6222 | 0.3384 | 0.3700 | 0.6130 | - |
| Pair- Wise | **Se x Le x Ex**<br>**Se**<br>MidEx, MidCo, LowEx – Su=Au=Sp<br>LowCo – n.p.d. (Su > Sp)<br><br>**Le**<br>SpCo – Mid > Low<br>SuEx, SpEx, SuCo, AuEx, AuCo – Mid = Low<br><br>**Ex**<br>SuMid, AuMid, AuLow, SpMid, SpLow – Co = Ex<br>SuLow – Co > Ex | **Se x Le x Ex**<br>**Se**<br>MidEx, MidCo, LowEx – Su=Au=Sp<br>LowCo – n.p.d. (Su>Sp)<br><br>**Le**<br>SpCo – Mid > Low<br>SuEx, SuCo, SpEx, AuEx, AuCo – Mid = Low<br><br>**Ex**<br>SuMid, AuLow – Co = Ex<br>SuLow, AuMid, SpMid, SpLow – Co > Ex | **Ex**<br>Co > Ex<br><br>**Se x Le**<br>Se:<br>Low – Au > Sp<br>Mid – Au = Sp<br><br>Le:<br>Au – Mid = Low<br>Sp – Mid > Low | **Le**<br>Mid > Low<br><br>**Ex**<br>Co > Ex | **Le x Ex**<br>Le:<br>Ex – Mid = Low<br>Co – Low > Mid<br><br>Ex:<br>Mid – Ex = Co<br>Low – Co > Ex | | **Ex**<br>Co > Ex | **Ex**<br>Co > Ex | | |

*State of the clumps of Pollicipes pollicipes*

The variation of the state of the clumps was analysed separately for the control and exploited clumps (**Fig. 5.5**). The analysis of variation of the state of the clumps in relation to the sampling site (Berlenga or Sines), timing of exploitation (Summer, Autumn or Spring) and intertidal level (low or mid shore) in the control clumps is shown in **Table 5.5**, and in the exploited clumps in **Table 5.6**.

The state of the control clumps on the two sites scored differently in the first 6 months after set-up (T6). In Berlenga, the control clumps grew more from Autumn to Spring (autumn set-up) than from Spring to Autumn (spring set-up). In Sines, there were no differences in growth over 6 months in relation to set-up time, i.e. clump growth was independent of the period of the year. With regard to the annual or two-year growth of the control clumps (T12, T24), this growth seems to be independent of the initial date to which the growth period was reported (set-up time), both in Berlenga and Sines. When comparing the two sites (in T12 and T24), growth in the control clumps on low shore over 12 months and 24 months was higher in Berlenga than in Sines. This pattern was not observed in the mid-shore. Regarding the intertidal level variation, in Berlenga, the growth of control clumps was higher in the low shore than in the mid shore, while in Sines no significant differences were found between intertidal levels.

In the case of the exploited clumps, in the first 6 months after exploitation (T6), their recovery and growth did not show any clear variation in relation to the timing of exploitation, either in Berlenga or in Sines. When comparing the two sites (T12 and T24), recovery and growth in the exploited clumps in 12 months was higher in Sines than in Berlenga. Although not tested, this pattern is also apparent in T6 (top graph at right in **Fig. 5.5**). However, this pattern ceased after 24 months. Relatively to the intertidal level variation of the recovery and growth of exploited clumps in Berlenga and Sines, no significant differences or no consistent pattern of variation between intertidal levels were found.

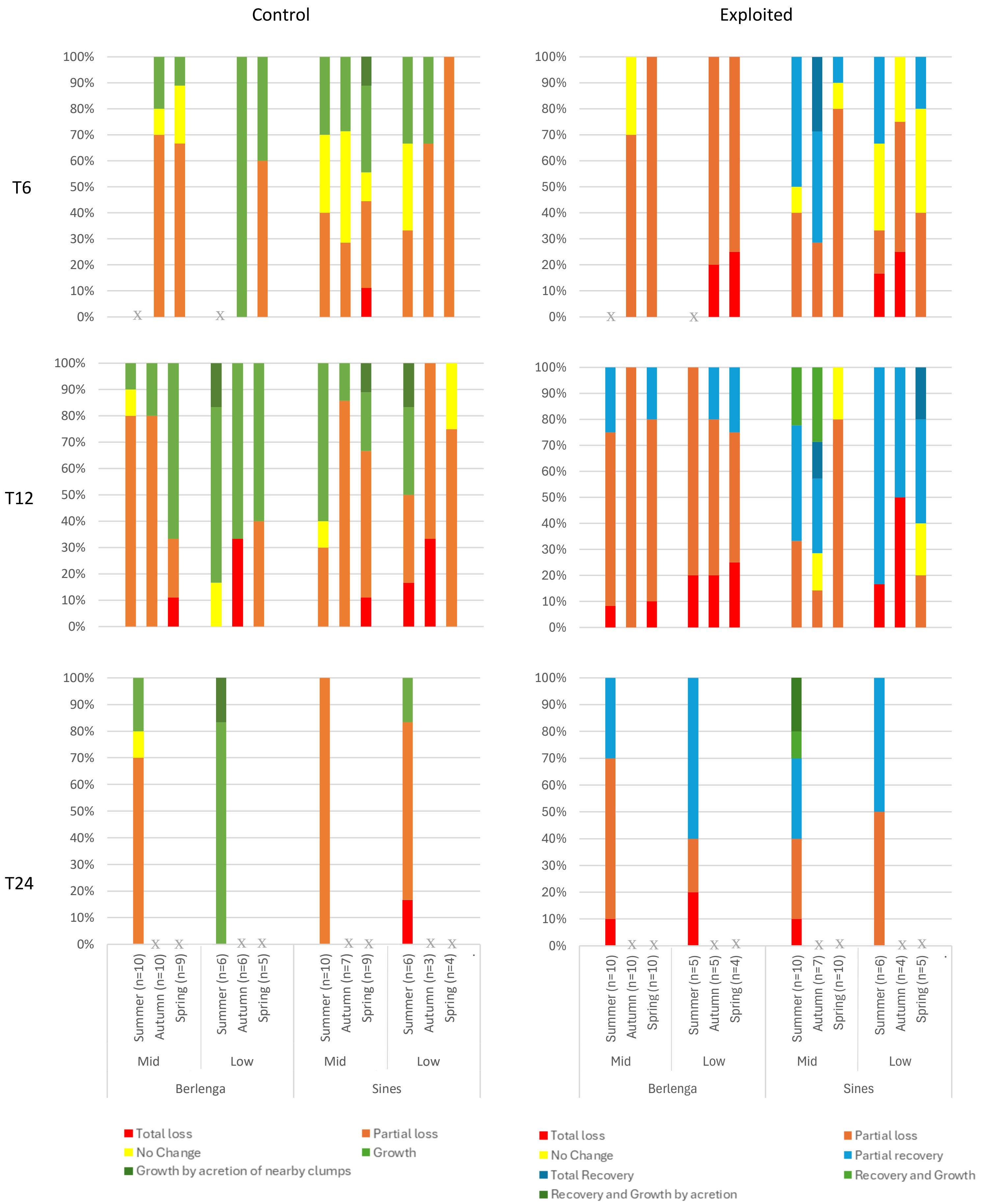


**Fig. 5.5** – State of the control clumps (left column of graphs) and exploited clumps (right column of graphs) of *Pollicipes pollicipes* (see **table 5.2** for description of categories of the state of the clumps in the case of control or exploited clumps) in each site (Berlenga and Sines), and in two intertidal levels (mid shore and low shore), and in three set-up/exploitation times (Summer, Autumn and Spring); n= 7 - 10 at mid shore and 3 - 6 at low shore. Monitoring time: T6 - 6 months after set-up/exploitation, T12 - 12 months after set-up/exploitation and T24 –24 months after set-up/exploitation.

**Table 5.5** – *P*-values from the PERMANOVA analyses on the state of control clumps of *Pollicipes pollicipes* for each time of monitoring (T6 - 6 months after set-up, T12 - 12 months after after set-up and T24 –24 months after after set-up) in relation to factor site (Si, fixed factor with two levels), factor timing of set-up/season (Se, fixed factor with three levels) and factor Intertidal level (Le, fixed factor with two levels). Analyses were based on Euclidean distances of untransformed data. *P-values* were obtained using 9999 random permutations. n= 7 - 10 at mid shore and 3 - 6 at low shore. PERMDISP tests: F = 8.144 (T6, Berlenga; p>0.05), F = 0.907 (T6, Sines; p>0.05), F = 1.798 (T12; p>0.05) and F = 23.806 (T24; p<0.05). Significant *p-values* that were interpreted are in bold. Pair-wise tests for significant factors or interactions: > or < (p <0.05); Seasons: Su – Summer, Au – Autumn, Sp – Spring; Intertidal level: Mid and Low shore; Exploitation level – Ex – exploited, Co – control.

| | T6 | | T12 | T24 |
|---|---|---|---|---|
| Source | Berlenga | Sines | | |
| Si | - | - | 0.0213 | 0.0002 |
| Se | **0.0406** | 0.3901 | **0.0259** | - |
| Le | **0.0040** | 0.2609 | 0.5188 | 0.0012 |
| Si x Se | - | - | 0.3251 | - |
| Si x Le | - | - | **0.0088** | **0.0082** |
| Se x Le | 0.0637 | 0.4254 | 0.3082 | - |
| Si x Se x Le | - | - | 0.2258 | - |
| Pair- Wise | **Se**<br>Au > Sp<br><br>**Le**<br>Low > Mid | | **Se**<br>n.p.d. (Su > Au)<br><br>**Si x Le**<br>Si:<br>Mid –<br>Berlenga = Sines<br>Low – Berlenga > Sines<br><br>Le:<br>Sines – Mid = Low<br>Berlenga – Low > Mid | **Si x Le**<br>Si:<br>Mid – Ber = Sin<br>Low – Ber > Sin<br><br>Le:<br>Ber – Low > Mid<br>Sin – Mid = Low |

**Table 5.6** – *P*-values from the PERMANOVA analyses on the state of exploited clumps of *Pollicipes pollicipes* for each time of monitoring (T6 - 6 months after exploitation, T12 - 12 months after exploitation and T24 –24 months after exploitation) in relation to factor factor site (Si, fixed factor with two levels), factor timing of exploitation/season (Se, fixed factor with three levels) and factor intertidal level (Le, fixed factor with two levels). Analyses were based on Euclidean distances of untransformed data. P-values were obtained using 9999 random permutations. n= 7 - 10 at mid shore and 3 - 6 at low shore. PERMDISP tests: F = 3.427 (T6, Berlenga; p<0.05), F = 4.434 (T6, Sines; p<0.05), F = 25.445 (T12; p>0.05) and F = 1.813 (T24; p>0.05). Significant p-values are in bold. Pair-wise tests for significant factors or interactions: > or < (p <0.05); Seasons: Su – Summer, Au – Autumn, Sp – Spring; Intertidal level: Mid and Low shore; Exploitation level – Ex – exploited, Co – control.

| | T6 | | T12 | T24 |
|---|---|---|---|---|
| Source | Berlenga | Sines | | |
| Si | - | - | **0.0001** | 0.3231 |
| Se | 0.2683 | 0.5211 | 0.4000 | - |
| Le | **0.0244** | 0.1262 | 0.4863 | 0.3154 |
| Si x Se | - | - | 0.2546 | - |
| Si x Le | - | - | 0.9331 | 0.6595 |
| Se x Le | 0.4272 | **0.0344** | 0.0701 | - |
| Si x Se x Le | - | - | 0.0971 | - |
| Pair- Wise | **Le**<br>Mid > Low | **Se x Le**<br>Se:<br>Mid – n.p.d. (Au > Sp)<br>Low – Su = Au = Sp<br><br>Le:<br>Su, Au, Sp – Mid = Low | **Si**<br>Sines > Berlenga | |

## 5.5 Discussion

In this study, the impact of *P. pollicipes* exploitation on the abundance and recovery of *P. pollicipes* was analysed at a small spatial scale by performing a field experiment, considering small areas (15 x 15 cm) where the exploitation of this species was manipulated (exploited areas) and areas that were not manipulated (controls). As this species is naturally distributed in clumps made up of barnacles of various sizes, many of which are attached to other individuals (see Cruz et al. 2022), this resource is traditionally exploited by extracting some or all of the barnacles from the clump (especially the larger animals) and leaving a gap. Thus, the experimental exploitation of clumps of barnacles was simulated in a similar way to traditional harvesting, with a mean percentage of exploited area in a clump of 39%. Our approach involved measuring three response variables up to a maximum of 24 months after set-up: the percentage of *P. pollicipes* cover in a 15 x 15 cm area that could include several clumps (in the case of exploited quadrats, it included an exploited clump); the area of the exploited clump or of an unmanipulated clump (control); and the state of the clump in relation to the set-up time of the experiment, i.e. whether it had recovered in the case of exploited clumps or whether it had grown in the case of control clumps. This experiment analysed the variation in relation to the exploitation factor (exploited and control areas), the timing of the exploitation (in the case of the exploited areas, equivalent to the set-up time in the control areas) and to two intertidal levels, having been installed on two sites along the Portuguese coast.

The hypothesis that the recovery of the exploited areas or groups would be faster if the exploitation had been carried out in summer or autumn and slower in spring was not supported in the two locations where this experiment was carried out (Berlenga and Sines). It was expected that there would be a significant interaction between the exploitation factor (control areas and exploited areas) and the timing of the exploitation/set-up factor (start of the experiment in summer, autumn or spring), with the exploited areas recording a higher percentage of cover/clump area/recovery level of *P. pollicipes* when the experiment started in summer or autumn, which corresponds to the recruitment season for *P. pollicipes* on this coast (Cruz et al. 2010; Fernandes et al. 2021). However, this was not observed.

This result is an important contribution of ecological knowledge that can be applied to the management of this fishery, particularly in Portugal. In fact, the closed season that exists on the Portuguese coast (August and September in the Berlengas Nature Reserve, between 15 th September and 15 th December on the SW coast of Portugal and between 15 th September and 15 th October on the rest of the Portuguese coast, see Sousa et al. 2013) was defined in the past based on the assumption that it would be important to protect the recruitment period (personal knowledge), as it would facilitate the recovery of the resource at that time, particularly after a potentially more intense exploitation season (summer, better sea conditions). What we observed in this study is that recovery is not greater if the exploitation was carried out during the recruitment season (summer and autumn), i.e. we observed that the recovery of the resource is independent of the timing of the exploitation. However, the existence of temporal closures can be important in reducing the fishing effort at a given time.

The importance of the date of birth of a gap in the middle of a matrix of sessile organisms (e.g. algae) has already been investigated in other studies in which the timing of cleaning was manipulated (e.g. Benedetti-Cecchi & Cinelli 1994, Kim & DeWreede 1996). The results of these studies suggest that the occurrence of this effect depends on the species.

The recovery of exploited clumps of *P. pollicipes* presented a slow and highly variable pattern, as two years after the exploitation most of the clumps have only partially recovered the area they had before being exploited. This result was not in line with the perception of professional fishers about *P. pollicipes* clump recovery in Berlengas islands (Neves 2021). These fishers considered that, if a clump was only partially harvested, the clump will have completely recovered or even outgrowth the initial area of the clump after 6/12 months (Neves 2021). Nevertheless, a similar pattern of highly variable and slow recovery was found in previous studies on *Pollicipes: P. polymerus* on Oregon, USA, Bingham 2016; *P. polymerus* on Vancouver Island, British Colombia, Edwards 2020; *P. pollicipes* in Asturias, Spain, Geiger et al. 2024; *P. pollicipes* from several locations in Europe, Gomez-del Campo et al. in press). One of the limitations of most of these studies estimating *Pollicipes* recovery is that they are based on estimates of the percentage of cover, without estimating the biomass or size of these animals, which are fundamental aspects to consider when evaluating the recovery of the resource.

Other factors can explain the variation in the recovery of a gap in the middle of a group of sessile organisms (e.g. location and size of the gap, see Sousa 1984, Benedetti-Cecchi & Cinelli 1994). In this study, we also investigated the variation related to the intertidal level, but the patterns we found were not consistent for the response variables analysed. In future studies, predictive variables related to the physical or biological attributes of the gap or of its surroundings should be analysed in order to explain the high variability of the states of clumps that were observed. Recently, Gomez-del Campo et al. (in press) analysed the recovery of gaps in stands of *P. pollicipes* and estimated that the presence of adult conspecifics in the margins of the gaps increased by at least four times the probability of initiation of their recovery. It should also be noted that some of the clumps that were followed in the present study and in which total loss was observed may have been exploited.

The main pattern we found was that the results showed differences related to the location of the experiment: Berlenga, on the centre coast of Portugal and within a nature reserve; and Sines, located on the SW coast of Portugal. The results of the three response variables analysed indicate that recovery of *P. pollicipes* in Berlenga is slower than in Sines. This regional difference might be related to the high recruitment intensity observed in SW Portugal, including Sines (Aguión et al. 2022b) and to an apparent lower intensity of recruitment intensity in Berlenga (Neves 2021). Heavy recruitment might facilitate the recovery process.

The main conclusion of this study is that regional variation is more important than temporal variation of the timing of exploitation on the abundance and recovery of *P. pollicipes* after exploitation. Regional ecological studies are essential for sustainable regional governance based on the best ecological knowledge. The governance of *P. pollicipes* in Europe (Aguión et al. 2022a) is already a governance that can be considered regional, as different fisheries have been identified.

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# **Chapter 6.** Temporal variation of the fishers' perception about the stalked barnacle (*Pollicipes pollicipes*) fishery at the Berlengas Nature Reserve

Sousa, A., Jacinto, D., Penteado, N., Pereira, D., Silva, T., Castro, J.J., Leandro, S.M. & Cruz, T. 2020.Temporal variation of the fishers' perception about the stalked barnacle (*Pollicipes pollicipes*) fishery at the Berlengas Nature Reserve (Portugal). Regional Studies in Marine Science 38, 101378, doi:10.1016/j.rsma.2020.101378.

## 6.1 Abstract

The engagement of the fishers and other stakeholders in the management of the resources is considered the key ingredient for a good fishery governance.

The stalked barnacle *Pollicipes pollicipes* can be considered the most important economic resource on rocky shores of northern Spain and continental Portugal. This species is highly prized as food and it is heavily exploited.

The main objective of the present study was to evaluate the temporal variation in the perception of *P. pollicipes* fishers at Berlengas Nature Reserve (RNB, the first area in Portugal to be managed for this fishery, since 2000) regarding the state of the fishery and the state of the management, and the implementation of a co-management system applied to the harvesting of this resource at RNB.

The fishers' perception was evaluated by individual and direct interviews to professional *P. pollicipes* fishers with licence to harvest at RNB, performed in 2005, 2013 (past studies) and 2018 (present study).

The perception of the fishers about the state of *P. pollicipes* revealed an overall negative tendency of this resource at RNB, as they considered that the amount and size of the barnacles had decreased over time, and the quality remained the same. Most fishers also considered that *P. pollicipes* are being overexploited at RNB.

The state of the management was defined as acceptable, as a large majority of the fishers agreed with most of the management measures, except for the spatial closures. However, their sense of nonfulfillment was high regarding most management measures in practice.

Finally, an increasing percentage of the fishers agreed with the implementation of a co-management system applied to barnacle harvesting at RNB. Co-management might bring benefits perceived by fishers as a better state of the resource, a more sustainable fishery management and an increase of the economic value of the resource.

## 6.2 Introduction

The key ingredient for a good fishery governance is public participation (Coffey, 2005). The aim of public participation is the public engagement, conflict resolution and the improvement of the quality of the decisions (Coffey, 2005).

One of the causes of the current crisis in world fisheries is often related with the historical failure to include the major stakeholders in meaningful decision-making (Pita *et al.* 2010). This lack of cooperation is also pointed out as a weakness of the fisheries management process (Jentoft, 1989; Cochrane, 1999), namely the lack of involvement of the fishers.

The way in which fishers may participate in the fishery management range from more passive (e.g. getting their perception of the state of a resource and their opinion in how management is in practice) to more active ways (e.g. co-management systems). The traditional knowledge that can be retrieved from local fishers is considered a useful complement to the available information on the evolution of fisheries and to track changes in marine resources (Coll *et al.* 2014).

Two main models are being used in Europe to manage the stalked barnacle (*Pollicipes pollicipes*) fishery: 1) bottom-up approaches as the co-management systems in practice in Galicia (Molares and Freire, 2003) and in Asturias (Rivera *et al.* 2014), Spain; and 2) top-down regional or national systems, as in the Basque Country, Spain (Borja *et al.* 2006) and in Portugal (Sousa *et al.* 2013). In the co-management systems of Galicia and Asturias, the regional authorities and the fishers participate in the management and data gathering and there are official and representative data about the fishery (Molares and Freire, 2003; Macho *et al.* 2013; Rivera *et al.* 2014). In the top-down system that manages barnacle harvesting in Portugal, the National Administration centralizes the management and fishers' participation is residual. Additionally, in the past, fishery data have been considered as not estimating the real pressure upon this resource (Cruz *et al.* 2015).

*P. pollicipes* can be considered the most important economic resource on rocky shores of northern Spain and continental Portugal (Molares and Freire, 2003; Sousa *et al.* 2013; Rivera *et al.* 2014). It is highly prized as food and it is heavily exploited (Cruz *et al.* 2010, 2015). Its commercial value can range from 20 to 200 euros per kg in restaurants of Portugal and Spain (Cruz *et al.* 2015).

In Portugal, the specific regulation to manage barnacle harvesting is recent (since 2000) and different along the Portuguese coast and has been frequently changed (see revision in Sousa *et al.* 2013).

The first management plan for barnacle harvesting in Portugal was implemented at Berlengas Nature Reserve (RNB) in 2000 (modified in 2011), and it includes spatial and temporal closures, size and bag limits and a limited number of professional licences (no recreational harvesting is allowed in RNB) (Jacinto *et al.* 2010; Sousa *et al.* 2013) (**Table 6.1**).

The state of the fishery and conservation of *P. pollicipes* in RNB was recently studied using a combination of different methodologies (biomass and size independent data, logbook data, and inquiries to professional fishers) and was considered as having a negative tendency, while the state of the management was considered acceptable based on fishers' responses to inquiries (Cruz *et al.* 2015). The inquiries used to classify the state of the fishery and the management at RNB were performed in 2005 and 2013, and included questions on: (1) the fishers' perception on the evolution of the resource (in terms of size, amount and quality of the barnacles); (2) the concordance of the fishers about the management measures in practice; and (3) their sense of nonfulfillment.

In order to continue to monitor the state of this resource and of its management at RNB, there is a need to perform similar inquiries as the ones carried out in 2005 and 2013 to explore the temporal variation of the professional barnacle fishers' perception about: (1) the state of the fishery and the management of *P. pollicipes*; and (2) the implementation of a co-management system applied to this fishery at RNB. This evaluation is the main goal of the present study and was based in interviews to professional barnacle fishers performed in the present study (2018) and in two previous studies (Cruz *et al.* 2008, 2015).

### 6.3 Material and methods

*Study area*

The Berlengas archipelago is situated ~5,7 miles off Cape Carvoeiro (Peniche, Portugal, **Fig. 6.1**) and is constituted by three sets of islands: Berlengas, Estelas and Farilhões.

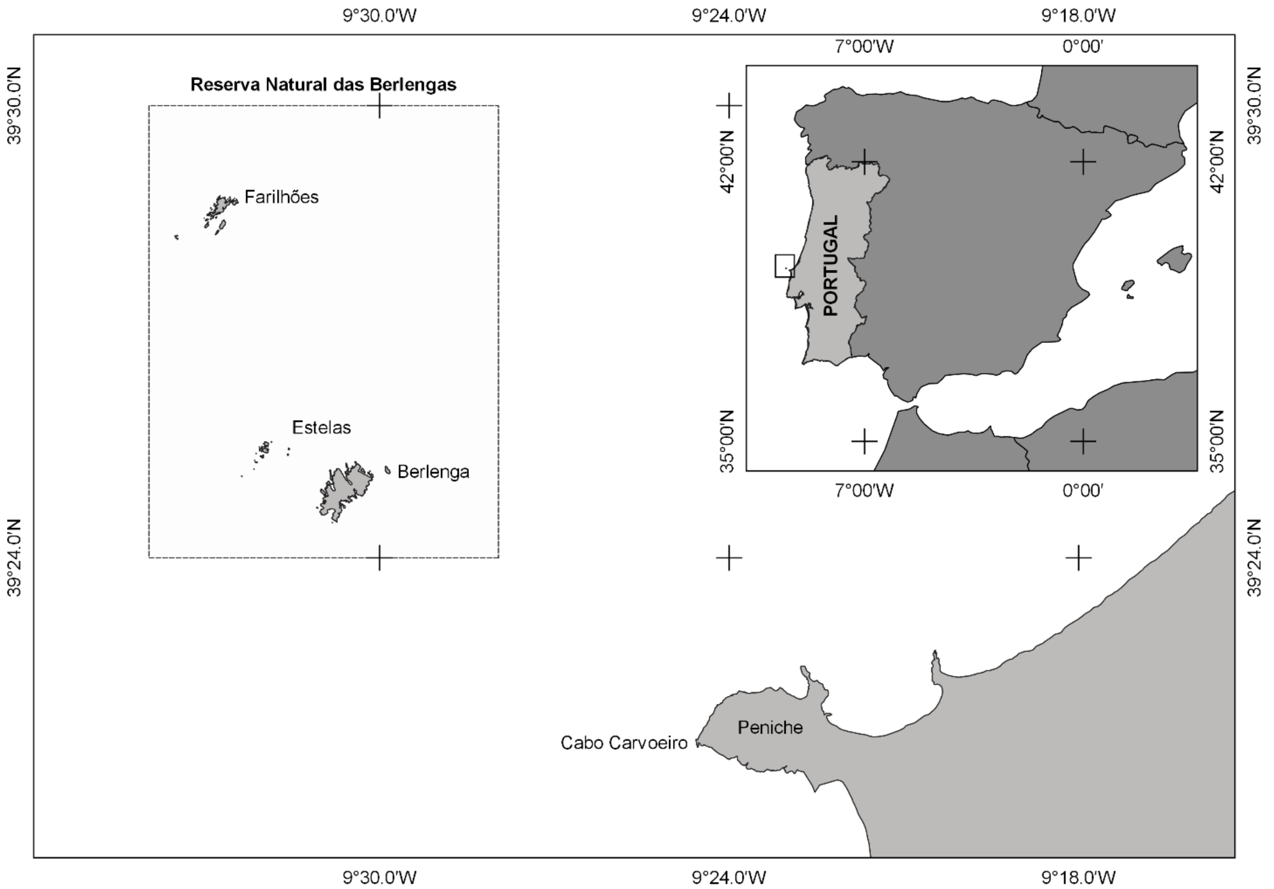


**Fig. 6.1**. – Location of the Berlengas Nature Reserve (RNB) relative to Cabo Carvoeiro and the mainland Portugal (inset). The reserve limits (since 1998) are shown as dotted lines.

The Berlenga Nature Reserve (RNB) was designated in 1981, aiming to preserve a rich natural heritage and to ensure sustainable development of human activities in the area. Initially, it included the Berlenga and Estelas islands and a marine protected area defined by the 30 m bathymetric line around these two islands. In 1989, barnacle harvesting was forbidden in the area of the RNB (**Table 6.1**). In 1998, the reserve was reclassified as an area limited by meridians and parallels (39º24' to 39º30' N and 09º28' to 09º34' W) that included the area defined in 1989, the Farilhões islands and a larger marine protected area. Between 1989 and 2000, barnacle harvesting was forbidden in the area of the reserve, but barnacles were exploited and harvesting control was considered to be ineffective (Cruz, 2000).

The first management plan was implemented at RNB in 2000 and its management measures are described in **Table 6.1**. Modifications of the original plan were made in 2011 on temporal closures, rotational harvest, size limit and number of harvesting licences (**Table 6.1**).

In 2011, the Berlengas archipelago was designated as a UNESCO Biosphere Reserve by the International Coordination Council of Man and Biosphere Programme (MAB), in order to promote solutions reconciling the conservation of biodiversity with its sustainable use.

**Table 6.1** – Summary of the evolution of the management measures for *Pollicipes pollicipes* harvesting at Berlengas Nature Reserve (RNB). RC – maximal rostro-carinal distance.

| Since | Management measures |
|---|---|
| **1989** | *Decree law no. 293/1989* |
| | Barnacle harvesting not allowed |
| **2000** | *Decree order no. 378/2000* |
| Temporal closure | August and September<br>Monday, Friday, weekends and holidays<br>Night time |
| Spatial closure | Permanent no-take zones |
| Rotational harvest | Annual rotation between two harvested zones |
| Bag size | 20 $kg.day^{-1}$ per fisher (without sorting) |
| Size limit | 25 mm (RC) – at least 50% of the harvested volume |
| Harvesting licences | Maximum number of licences issued yearly |
| Catch report | Individual semestral logbook |
| **2011** | *Decree order no. 232/2011* |
| Temporal closure | Added a temporal closure from January to March |
| Spatial closure | No changes |
| Rotational harvest | End of the annual rotation of harvested zone |
| Bag size | No changes |
| Size limit | 23 mm (RC) – at least 50% of the harvested volume |
| Harvesting licences | Maximum of 40 licences |
| Catch report | No changes |

### *Individual Interviews*

The state of the fishery and management of *P. pollicipes* at RNB was defined based on interviews to professional fishers with a valid licence to harvest barnacles at RNB.

Professional fishers of RNB were individually and directly interviewed during December 2018. All interviews performed were confidential and anonymous and all fishers gave their consent to answer to the inquiry and knew about the objective of the study (see first paragraph of the inquiry in **A.1**). The total number of inquiries performed was 39 (total number of licences was 41).

The state of the fishery was evaluated by asking the fishers about their perception of the evolution of the amount, size and the quality of *P. pollicipes* during the previous 5 years (see **A.1**). Similar questions were performed in previous studies at RNB (2005, n=38, Cruz *et al.* 2008; and 2013, n=32, Cruz *et al.* 2015). Also, the fishers were inquired about their perception of *P. pollicipes* being excessively exploited at RNB (see **A.1**). A similar question was performed in 2013 (Cruz *et al.* 2015).

The state of the fishery in 2018 was defined as showing a negative, positive or stable tendency if the perception of the fishers of the temporal evolution of *P. pollicipes* was negative, positive or stable, respectively, and these results were compared with 2005 and 2013 data from previous studies (Cruz *et al.* 2015) to assess if the tendencies identified in the past were maintained or changed.

The assessment of the state of the management of *P. pollicipes* harvesting at RNB was made using a methodology similar to the one used by Cruz *et al*. (2015) in which a set of closed questions (see **A.1**) was used to gather the fishers opinion regarding the management rules in practice, namely their agreement and their sense of nonfulfillment about temporal closures, spatial closure, bag and size limits, catch report and maximum number of licences, and their opinion about the occurrence of illegal harvesting practices (e.g. harvesting at night, harvesting with scuba and harvesting without a licence).

The state of the management was classified as weak, acceptable or good, adapting the methodology used by Cruz *et al*. (2015). A good management state was defined if the majority of the fishers agreed with all the rules and the perception of the compliance with all the rules by the majority of the fishers was positive. An acceptable management state was defined if half of these conditions was satisfied, while a weak management state was inferred if most of these conditions was not satisfied. These results were compared with 2005 and 2013 data from previous studies (Cruz *et al.* 2015) to assess if the classifications identified in the past were maintained or changed.

The fishers' opinion about the enforcement of the law was evaluated by asking their perception of the surveillance that takes place at the RNB (see **A.1**).

Finally, the fishers' opinion about the implementation of a co-management system applied to barnacle harvesting at RNB (see **A.1**) was also assessed and compared with results to this same question from a previous study in 2013 (Cruz *et al.* 2015).

*Data analysis*

The temporal variability on the fishers' perception of the evolution of the amount, size and quality of *P. pollicipes* at RNB considering the previous 5 years was individually analysed by PERMANOVA (Anderson, 2001) to factor year (fixed factor), considering the inquiries as the independent replicates. In each inquiry, qualitative answers to each question were transformed as following: 1 – decreased; 2 – remained; and 3 – increased. A similar analysis was done to analyse the temporal variability on the fishers' perception of *P. pollicipes* being excessively exploited at RNB after transforming the qualitative answers as following: 1 – no and 2 – yes. In each of the four analyses, we have not considered the inquiries where fishers have considered the option "don't know/don´t want to answer". Sample size ranged from 31 (2013 – size data) to 39 (2018 – amount, quality and excessive exploitation data). In the case of the analyses to the amount and size of *P. pollicipes* at RNB, factor year had three levels (2005, 2013 and 2018), while in the case of the analyses to the quality of the barnacles and to the excessive exploitation at RNB, factor year had two levels (2013 and 2018) (no data for these variables regarding 2005).

The temporal variability of the fishers' concordance and of the sense of nonfulfillment/problems regarding the management plan in practice at RNB was analysed by two independent PERMANOVA (Anderson, 2001) to factor year (fixed factor with two levels, 2013 and 2018), and considering the inquiries as the independent replicates. We did not use the year of 2005 in these analyses as the number of variables/questions that were common to the three analysed years was low.

The multivariate data matrixes of the concordance and of the non-fulfilment/problems were transformed as following: for the concordance matrix (1 – totally disagree, 2 – disagree, 3 – don't know/don't want to answer, 4 – agree, 5 – totally agree); and for the non-fulfilment/problems matrix (1- absent, 2 – rare, 3 - don't know/don't want to answer, 4 – sometimes, 5 – a lot of times). The "don't know/don't want to answer" option was transformed in a mean score in order to not influence the outcome of the analyses and in order to not exclude the inquiries when a fisher choose this option in one of the variables. Sample size was 39.

All univariate and multivariate analyses were based on Bray-Curtis similarity of untransformed data, and on unrestricted permutation of raw data, Type III sums of squares and 999 permutations (see Anderson *et al.* 2008). Pair-wise tests were used when significant differences among years were detected (see Anderson *et al.* 2008). Homogeneity of univariate and multivariate dispersions based on Bray-Curtis similarity was tested for each analysis using PERMDISP routine (Anderson, 2006).

In the case of the multivariate analyses, SIMPER procedure (Clarke 1993) was used to identify which were the variables (management rules/problems) that most contribute for the dissimilarity between years.

The software PRIMER 7 & PERMANOVA+ (www.primer-e.com; Anderson *et al.* 2008) was used to perform all statistical analyses.

## 6.4 **Results**

*The state of the fishery*

The majority of the fishers considered that the amount of barnacles at RNB has decreased during the previous 5 years (50 % in 2005, 66 % in 2013 and 59 % in 2018), suggesting a consistent negative tendency regarding the amount of barnacles at RNB (**Fig. 6.2A**). No significant differences on the perception of the fishers regarding the amount of barnacles at RNB among years were identified (PERMDISP, $F_{2,103}$=5,66, $p$=0,064; PERMANOVA, $Pseudo\text{-}F_{2,103}$=1,23, $p$=0,303).

In relation to the evolution of the size of the barnacles, in 2013 and 2018 most (63 % and 56 %, respectively) fishers considered that it decreased in the previous 5 years (**Fig. 6.2B**). No significant differences among years for size were identified (PERMDISP $F_{2,102}$=1,17, $p$=0,145; PERMANOVA, $Pseudo\text{-}F_{2,102}$=2,81, $p$=0,061), but there was a trend for a more negative perception in 2013 and 2018, as in 2005, most fishers (61 %) have considered that size did not change (**Fig. 6.2B**).

The fishers' perception considering the quality of the barnacles over the previous 5 years was only evaluated in 2013 and 2018 and revealed a maintenance tendency in both years, since the majority of the fishers considered that the quality did not change (59 % in 2013 and 69 % in 2018, **Fig. 6.2C**). No significant differences on the perception of the fishers regarding the quality of barnacles at RNB between years were identified (PERMDISP, $F_{1,69}$=1,94, $p$=0,432; PERMANOVA, $Pseudo\text{-}F_{1,69}$=0,22, $p$=0,714).

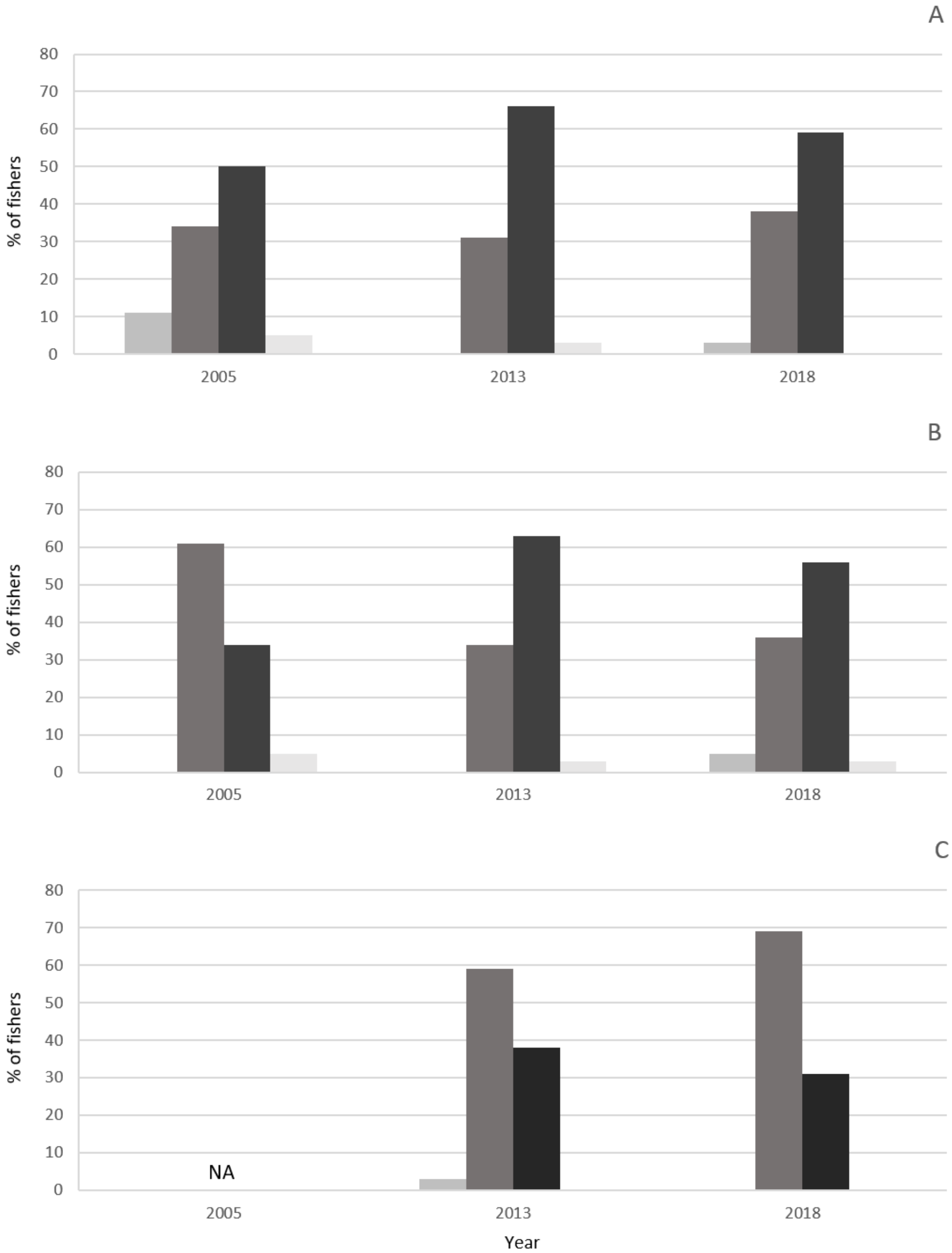


**Fig. 6.2.** Percentage of professional fishermen from RNB that answered that the amount (A) size (B) and quality (C) of *P. pollicipes* in this region has increased ( ), did not change ( ) or has decreased ( ) in the previous 5 years, when interviewed in 2005, 2013 and 2018 (n=38, 32 and 39, respectively). ( ) did not answer. NA – not assessed.

The fishers' perception regarding *P. pollicipes* being excessively exploited at RNB was worst in 2018 than in 2013, as 78 % of the fishers considered that there was excessive exploitation in 2018, and this value was 66 % in 2013. However, no significant differences on the perception of the fishers regarding *P. pollicipes* being excessively exploited at RNB between the years were identified (PERMDISP, $F_{1,68}$=0,40 $p$=0,703; PERMANOVA, $Pseudo\text{-}F_{1,68}$=0,10; $p$=0,807).

An overall negative tendency of the state of the fishery was identified in 2018, as it was in the past (Cruz *et al.* 2015), since most of the fishers considered that the amount and the size of the barnacles had decreased in the last 5 years, and the quality did not change.

*The state of the management*

A large majority of the fishers totally agreed or agreed with most of the management measures in practice at RNB, namely: the temporal closures, bag size, size limit, the catch report and the maximum of 40 licences (**Table 6.2**). In most management measures, their acceptance has been increasing over the years (**Table 6.2**). The only exception is the existence of no-take zones, which revealed to be the least accepted measure by the fishers in 2018 (28 %), while in 2013 it was accepted by most of them (56 %; **Table 6.2**). The analysis on the temporal variability of the fishers' concordance regarding the management plan at RNB revealed significant differences between 2013 and 2018 (PERMDISP, $F_{1,69}$=24,31, $p$=0,001; PERMANOVA, $Pseudo\text{-}F_{1,69}$=7,54, $p$=0.001). The SIMPER analysis (**Table 6.4A**) revealed that the dissimilarity between years is explained by an increasing concordance of all management measures, except for the "No-take zones" measure for which concordance is lower in 2018.

The sense of nonfulfillment of RNB barnacle fishers to the different management measures was evaluated in 2005, 2013 and 2018, with the exception of temporal closures, no-take zones and size limit measures, only assessed in 2013 and 2018, since the regulation for barnacle harvesting at RNB changed in 2011 (*Decree order no. 232/2011*) (**Table 6.1 and 6.3**). In 2018, the three management measures that fishers considered to be less fulfilled were the monthly and weekly closures and the no-take zones. The sense of nonfulfillment about these three measures has been increasing (<60 %

in 2005 and 2013; > 75 % in 2018). The major reason presented in 2018 to justify this sense of nonfulfillment is the illegal harvesting (harvesting without a licence) that has been identified by the fishers as the major problem in this fishery, as 85 % of them considered it to happen a lot of times or sometimes. The measures of bag and size limit revealed a sense of nonfulfillment of 59 % and 47 % in 2018, respectively, which is lower than the values obtained in 2013 (>67 %). Harvesting at night and using scuba was not a frequent problem identified by the fishers in the three sampling years (<27 %).

The analysis of the temporal variability of the fishers' sense of nonfulfillment/problems regarding the management plan at RNB revealed significant differences between 2013 and 2018 (PERMDISP, $F_{1,69}$=8,48, $p$=0,018; PERMANOVA, $Pseudo\text{-}F_{1,69}$=10,42, $p$=0.001). The SIMPER analysis (**Table 6.4B**) revealed that the dissimilarity between years is explained by a higher sense of nonfulfillment and a higher perception of problems in 2018, except for the bag and size limit measures in which the sense of the nonfulfillment was higher in 2013. The SIMPER analysis (**Table 6.4B**) also revealed that the variable contributing more for the dissimilarity between years was the harvesting without a license (with a contribution of 14,1 % for the total dissimilarity).

Most fishers considered that the enforcement of the law was low, since 95 % of them considered the surveillance that took place in the RNB area to be insufficient or inexistent.

The state of the management was defined, according to the method of Cruz *et al.* (2015), as acceptable, as a large majority of the fishers agreed with most of the management measures in practice since 2013 (except for the spatial closures). However, their sense of nonfulfillment was high regarding most management measures.

Finally, an increasing percentage of fishers (not assessed in 2005, 81 % in 2013 and 97 % in 2018) agreed with the implementation of a co-management system applied to barnacle harvesting at RNB. In 2018, 97 % of the fishers that were interviewed also considered that the implementation of a co-management system at RNB would bring advantages for their activity. The advantages that most of the fishers referred are the improvement of the state of the resource, a better fishery management (more sustainable) and the ability of the fishers to work less and earn more.

**Table 6.2** – Professional fishers' opinion on the management rules regarding the fishery of the stalked barnacle *Pollicipes pollicipes* at RNB in 2005 (n=38), 2013 (n=32) and 2018 (n=39). In bold all values higher than 50 %. Not assessed (-).

| | Totally agree or agree | | |
|---|---|---|---|
| ***Management measures*** | **2005** | **2013** | **2018** |
| Monthly closure – August and September | **55%** | **66%** | **80%** |
| Monthly closure – January to March | - | **81%** | **93%** |
| Weekly closure – Monday, Friday, weekends and holidays | **53%** | **78%** | **85%** |
| Daily closure - night time | **100%** | **97%** | **100%** |
| No-take zones | - | **56%** | 28% |
| Bag size – 20 kg | **81%** | **88%** | **94%** |
| Size limit – 23 mm (RC) – at least 50% of the harvested volume | - | **79%** | **95%** |
| Maximum of 40 licences | - | **88%** | **95%** |
| Mandatory catch report in logbooks | - | **94%** | **82%** |

**Table 6.3** - Professional fishers' opinion on the sense of nonfulfillment about the management measures and on the occurrence of problems regarding the fishery of the stalked barnacle *Pollicipes pollicipes* in RNB in 2005 (n=38), 2013 (n=32) and 2018 (n=39). All values higher than 50 % are in bold. Not assessed (-).

| | A lot of times or sometimes | | |
|---|---|---|---|
| ***Management measures*** | **2005** | **2013** | **2018** |
| Monthly closures – January to March, August and September | - | **50%** | **95%** |
| Weekly closure – Mondays, Fridays, weekends and holidays | **51%** | 40% | **79%** |
| No-take zones | - | **59%** | **92%** |
| Bag size – 20 kg | **55%** | **72%** | **59%** |
| Size limit – 50% of the volume of harvested barnacles need to have RC>23 mm | - | **69%** | 47% |
| ***Problems*** | | | |
| Harvesting at night | 26% | 12% | 21% |
| Harvesting using scuba | 21% | 12% | 24% |
| Harvesting without a licence | **63%** | 47% | **85%** |

**Table 6.4** - Average abundance of fishers' answers on the concordance about the management plan (**A**) and sense of nonfulfillment/problems (**B**) in each year (2013 and 2018), and individual and cumulative percentage contributing for Bray-Curtis dissimilarity between years for each variable (untransformed data).

| Variable | Average abundance | | Contribution % | Cumulative % |
|---|---|---|---|---|
| | 2013 | 2018 | | |
| **(A) *Concordance*** | | | | |
| No-take zones | 3,3 | 2,7 | 15,9 | 15,9 |
| Monthly closures – August and September | 3,7 | 4,1 | 15,0 | 30,8 |
| Weekly closure – Mondays, Fridays, weekends and holidays | 3,9 | 4,3 | 13,4 | 44,2 |
| Size limit – 50% of the volume of harvested barnacles need to have RC>23 mm | 3,9 | 4,7 | 12,7 | 56,8 |
| Monthly closures – January to March | 4,2 | 4,7 | 10,2 | 60,0 |
| Maximum of 40 licences | 4,0 | 4,5 | 10,1 | 77,1 |
| Bag size – 20 kg | 4,3 | 4,7 | 9,9 | 87,0 |
| Mandatory catch report in logbooks | 4,3 | 4,5 | 9,1 | 96,0 |
| Daily closure - night time | 4,7 | 5,0 | 4,0 | 100,0 |
| **(B) *Sense of nonfulfillment and problems*** | | | | |
| Harvesting without a licence | 3,0 | 4,1 | 14,1 | 14,1 |
| Size limit – 50% of the volume of harvested barnacles need to have RC>23 mm | 3,6 | 3,1 | 13,9 | 28,0 |
| Monthly closures – January to March, August and September | 3,0 | 4,5 | 13,8 | 41,8 |
| Weekly closure –Mondays, Fridays, weekends and holidays | 2,9 | 4,1 | 13,4 | 55,2 |
| Bag size – 20 kg | 3,8 | 3,5 | 13,1 | 68,3 |
| No-take zones | 3,5 | 4,4 | 11,5 | 79,9 |
| Harvesting using scuba | 2,2 | 2,4 | 10,4 | 90,3 |
| Harvesting at night | 2,3 | 2,3 | 9,7 | 100,0 |

## 6.5 Discussion

The state of the fishery and of the management of the stalked barnacle *P. pollicipes* at RNB were assessed for the third time in 14 years (2005, 2013, 2018) using individual interviews. This source of information is very important as it reflects the fishers' perception of the management of this fishery and of the conservation of this resource at the RNB fishery. Besides, by applying the same methodology through time, it is also possible to monitor the evolution of the perception of the fishers, as recommended by Coll *et al.* (2014).

The team of interviewers that worked in these studies have an in-depth interaction with the RNB community of fishers, and Moore *et al.* (2010) and Lozano *et al.* (2011) consider that the relation between the fishers and the interviewers has an influence on the accuracy and reliability of the obtained data.

An overall negative tendency of the state of the fishery of *P. pollicipes* was observed at RNB in 2018, as it was previously observed (Cruz *et al.* 2015). The interviews revealed that most of the fishers considered that the amount and the size of the barnacles had decreased in the last 5 years. Cruz *et al*. (2015) also described a similar negative tendency, when using three different sources of information: individual interviews; individual observations on the biomass and size of the barnacles and catch report data compiled from logbooks. A relevant observation regarding the evolution of the state of the fishery at RNB is that an increasing percentage of the fishers considered that *P. pollicipes* is being excessively exploited in this area (66 % in 2013 and 78 % in 2018), although no significative interannual differences were observed.

However, a more positive situation was observed in relation to the quality of the barnacles, as the 2013 and 2018 surveys have indicated a maintenance tendency, since most fishers considered that the quality did not change in the previous 5 years.

In monitoring programs to evaluate the state of this fishery it is crucial to include all sources of information used in the past, apart from the individual interviews data as presented here, as the independent observations on biomass and size of these animals, and logbook data. All sources are extremely important to correctly assess the state of the fishery and must be considered in the future.

In relation to the state of the management of *P. pollicipes* at RNB, individual interviews to the fishers were the unique source of information, as used in previous studies (Cruz *et al.* 2015), to perform this classification and the opinion of the fishers was obtained in relation to the acceptance and sense of nonfulfillment regarding the management rules in practice, the surveillance and the possibility of a change to a co-management system.

Regarding the management, an increasing acceptance of the management plan in practice at RNB was inferred, except for the no-take zones measure. In fact, with the exception of this measure, the degree of acceptance was higher in 2018, as it has increased from 2013 to 2018 for all management measures. In contrast, the degree of acceptance of the no-take zones measure has substantially decreased, and this result should be investigated in the future in order to understand the reasons for this disagreement and to specifically discuss this management measure and its impacts.

However, the sense of nonfulfillment about most of the management measures was very high, as the perception of the fishers regarding the sense of nonfulfillment/problems has significantly changed between 2013 and 2018, being more negative in 2018 than in 2013 for most of the management measures, except for the bag and size limit measures. Illegal harvesting (harvesting without a license) was identified as being the most important variable to explain the dissimilarity between years, being a stronger problem in 2018. Pouching was also identified in the past as being a factor willing to compromise the sustainability of this fishery at RNB (Jacinto *et al.* 2010). In contrast, the slight positive change in the fishers' perception regarding the sense of nonfulfillment of the bag and size limit measures should be investigated in order to understand this change.

In addition, the high sense of nonfulfillment regarding most of the regulations' measures might be related with a low enforcement of the law, namely the poor surveillance that is perceived by the fishers to happen in RNB area, as 95% of the fishers considered that the surveillance that takes place in the RNB was insufficient or inexistent. In 2005, both fishers and the RNB staff agreed that the surveillance at RNB was scarce and a major problem for *P. pollicipes* fishery (Jacinto *et al.* 2010, 2011).

Stewart *et al.* (2014) found a similar result for the *P. pollicipes* fishery in the Natural Park located in the SW coast of Portugal (Parque Natural do Sudoeste Alentejano e Costa Vicentina - PNSACV), where only 24 % of the inquired fishers considered that the law is enforced. Stewart *et al.* (2014) suggested that the non-enforcement of the law might be leading to a non-compliance of the regulation by the fishers and local population, since the chances of being penalised are low. Professional barnacle fishers from other regions of mainland Portugal also perceived surveillance as insufficient (Cruz *et al.* 2015).

As previously reported (Cruz *et al.* 2015), the evaluation of the state of the management of barnacle harvesting at RNB in 2018 was considered to be acceptable, as most of the fishers agreed with the management measures, but their sense of nonfulfillment was high.

A very important result from this study on the perception of the fishers about the management of *P. pollicipes* in RNB is the increasing percentage of fishers (81 % in 2013 and 97 % in 2018) that agreed with the implementation of a co-management system applied to this fishery. This management shift might bring benefits perceived by the fishers as a better state of the resource, a more sustainable fishery management and an increase of the economic value of the resource.

Oldekop *et al.* (2016) found that a greater benefit to the local communities is met when protected areas are co-managed by the local communities and the conservation bodies. D'Armengol *et al.* (2018) suggested that co-management can be the conduit to the sustainability of small-scale fisheries, and in some specific cases it can make targeted species more abundant and improve their habitat.

Cruz *et al.* (2015) suggested that RNB could be a pilot region to test a co-management approach in Portugal, since many fishers of this region are aware of the concept and agree with its implementation, and suggested that the implementation of a co-management system besides the expected improvement in the commercial value of the species, could also bring a better surveillance driven by a putative involvement of the fishers.

In the past, the implementation of a co-management system applied to barnacle harvesting inverted the overexploitation of *P. pollicipes* that took place in the Galician coast before the 1990s (Molares and Freire, 2003).

The implementation for the first time in Portugal, of a co-management system applied to *P. pollicipes* harvesting at RNB is being sought since 2018 within the framework of an on-going research project

("Co-Pesca 2") and will be a very important opportunity to test a paradigm shift from a centralized management to a co-management system for this fishery. The involvement of the fishers in this project and process is crucial and getting their opinion from individual interviews as done in the past and in the present study is of major relevance, namely if such information is used to discuss and ameliorate the management system in practice.

### 6.6 Conclusions

The assessment of the state of the fishery, conservation and management of the stalked barnacle *P. pollicipes* at RNB based on individual interviews to professional fishers revealed an overall negative tendency of the state of the fishery over the years and an acceptable state of the management.

Regarding the state of management, an increasing acceptance of the management plan in practice at RNB was inferred, except for the spatial closure. However, the sense of nonfulfillment about the management measures has increased, except for the bag and size limit measures. The problem of illegal harvesting perceived by the fishers is being increasing and the large majority of the fishers considered that the surveillance addressed to this resource at RNB is insufficient or inexistent.

The implementation of a co-management system applied to barnacle harvesting at RNB have reunited along the years an increasing acceptance by the fishers of this region.

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### 6.9 Supplementary material

**A.1 –** Inquiry performed to Berlengas Nature Reserve (RNB) professional barnacle fishers in 2018. The original inquiry was in Portuguese and this is an adapted English version of the original.

Project **"CO-PESCA2 – Implementation of a co-management committee to the stalked barnacle harvesting at Berlengas Nature Reserve**
(MAR-01.03.02-FEAMP-0018)**"**

Interview to professional barnacle harvesters with a valid licence to harvest at
**Berlengas Nature Reserve**

This interview is part of the investigation of "Co-PEsca2" project. It is directed to professional stalked barnacle harvesters that work and have a valid licence to harvest at the Berlengas Nature Reserve (RNB) and it is intended to better know the barnacle harvesting activity, as the harvester's opinion about the state of the resource and the management of this fishery at RNB. The interview is confidential and anonymous.

**1.** In your opinion, in the last 5 years, the **amount** of barnacles at RNB?
___ Increased ___Remained ___Decreased

**2.** In your opinion, in the last 5 years, the **size** of the barnacles at RNB?
___ Increased ___Remained ___Decreased

**3.** In your opinion, in the last 5 years, the **quality** of the barnacles at RNB?
___ Increased ___Remained ___Decreased

**4.** Do you consider that the stalked barnacles are being **excessively exploited** at RNB?
___ Yes ___ No

**5.** Do you **agree** with the following management measures:

| ***Management measures*** | **Totally agree** | **Agree** | **Disagree** | **Totally Disagree** | **Don´t know/ Don’t want to answer** |
|---|---|---|---|---|---|
| Monthly closure – August and September | | | | | |
| Monthly closure – January to March | | | | | |
| Weekly closure – Monday, Friday, weekends and holidays | | | | | |
| Daily closure - night time | | | | | |
| No-take zones | | | | | |
| Bag size – 20 kg | | | | | |
| Size limit – 23 mm (RC) – at least 50% of the harvested volume | | | | | |
| Maximum of 40 licences | | | | | |
| Mandatory catch report in logbooks | | | | | |

**6.** How do you classify your **sense of nonfulfillment** regarding the following management measures and occurrence of the following problems:

| ***Management measures*** | **A lot of times** | **Sometimes** | **Rare** | **Absent** | **Don´t know/ Don't want to answer** |
|---|---|---|---|---|---|
| Monthly closures – January to March, August and September | | | | | |
| Weekly closure – Mondays, Fridays, weekends and holidays | | | | | |
| No-take zones | | | | | |
| Bag size – 20 kg | | | | | |
| Size limit – 50% of the volume of harvested barnacles need to have RC>23 mm | | | | | |
| ***Problems*** | | | | | |
| Harvesting at night | | | | | |
| Harvesting using scuba | | | | | |
| Harvesting without a licence | | | | | |

**7.** Who do you classify the **surveillance** regarding the stalked barnacle fishery at RNB?

____ Inexistent ___ Insufficient ___ Sufficient ___ Good

Why? _________

_______________________________________________

**8.** Do you agree with the implementation of a **co-management** system applied to barnacle harvesting at RNB?

___ Yes ___ No

Why? _______________________________________________

**9.** Do you agree that the implementation of a **co-management** system applied to barnacle harvesting at RNB would bring advantages for your activity

___ Yes ___ No

Why? _______________________________________________

# **Chapter 7.** Conclusions and final remarks

This thesis provides ecological knowledge on the stalked barnacle *Pollicipes pollicipes* that can be used for a more sustainable management of the fisheries of this species.

Understanding the dynamics of a population and even being able to predict those dynamics has a major impact on the management of fisheries (Gebremedhin et al. 2021). In this thesis, a wide assessment of the patterns of *P. pollicipes* abundance, size and growth within this species European distribution is presented; two studies focused on the phenotypic variability affecting *P. pollicipes* quality and economic value; a study on the effects of the timing of exploitation on the abundance of *P. pollicipes* and recovery after harvesting is analysed; and finally a study on the fishers local ecological knowledge regarding the state of a *P. pollicipes* fishery and its management is described.

Chapter 2 and 3 pursued a large-scale standardized comparison of the percentage cover, density, biomass, and size structure (chapter 2) as well as growth rate (chapter 3) of the stalked barnacle *Pollicipes pollicipes* at a European scale, including sampling locations within regions of France, Spain and Portugal. This geographical distribution area comprises the most important fisheries of *P. pollicipes* (see sub-section 1.2) with several differences on fishing efforts and respective management strategies (Aguión et al. 2022b, Cruz et al. 2022). Regarding the abundance and size of *P. pollicipes* along its European range the most significant result was a north-south pattern observed on the Iberian Peninsula, consisting of a lower density of *P. pollicipes* in Asturias and Galicia (Spain), comprising larger animals, and a higher density in the SW coast of Portugal, made up of smaller individuals. Considering the detected regional pattern of density and size, it was expected a higher growth rate in the regions of Asturias and Galicia (Spain) and lower in SW Portugal. Nevertheless, a higher growth rate was only detected in the region of Galicia, namely in the size class of juveniles.

From the results of the study on the abundance and size (chapter 2) it was possible to estimate the biomass per of $m^2$ of *P. pollicipes* with moderate and high commercial value within the three contrasting regions (Asturias, Galiza and SW Portugal): 3.2 $kg/m^2$ in Asturias, 2.3 $kg/m^2$ in Galicia; and 252 $g/m^2$ in SW Portugal. Considering a capture of 5 kg of these barnacles with greatest commercial value, and these estimated biomass per $m^2$, the effort (measured in $m^2$ that a harvester needs to exploit) within each of these regions corresponds to: 1.6 $m^2$ in Asturias, 2.2 $m^2$ in Galicia, while in SW Portugal it is necessary to exploit 19.8 $m^2$. Also, from the results of the growth rate assessment (chapter 3) based on the greatest growth rate detected on the juvenile size class from Galicia it was possible to estimate that in this region, *P. pollicipes* can reach sexual maturity within 8 months, while in the other regions (Brittany, Asturias and SW Portugal) it will be necessary at least 1 year. The recruitment that was evaluated at the same spatial scale using a standardized methodology (Aguión et al 2022a) detected a higher recruitment in the SW Portugal and Asturias than in Galicia. Based on the regional ecological differences detected in both studies (chapter 2 and 3) and also in Aguión et al. (2022a), we can predict that a similar exploitation pressure on *P. pollicipes* in these three regions (Galicia, Asturias and SW Portugal) can potentially have different effects on their populations. The regional differences detected when studying the ecology of *P. pollicipes* along a wide range of this species distribution, highlights the need for regional-specific ecological data to ensure a better management of these fisheries.

A caveat of both studies presented in Chapters 2 and 3 is that *P. pollicipes* fishers exploit more barnacles in the low shore (Cruz et al. 2015) and only the mid shore tidal level was assessed. Also, further investigation is needed to fully understand what is causing the regional differences detected in the abundance, size and growth rate of this species.

The phenotypic variability that affects *P. pollicipes* quality was the focus of two studies presented in chapter 4 (chapters 4.1 and 4.2). Based on this species morphology, two extreme phenotypes can be identified. It is considered that more elongated barnacles have lower quality and lower commercial value (Parada et al. 2012). Both studies presented an evaluation based on the fisher's ecological knowledge to describe this pattern of morphological variation and understand its nature. Fishers from Galicia and Portugal defined good quality barnacles as thick and short, while the definition of bad quality barnacles presented regional variability being defined as long and thin, in Spain, and with a wider variety of terms including watery, thin and soft, in Portugal (chapter 4.1). Based on the perception of the fishers, the main causes of the phenotypic variation are related to the characteristics of the rock and the hydrodynamics (chapter 4.1).

Chapter 4.1 also assessed morphological, genetic and epigenetic differences between the two extreme qualities/phenotypes from samples collected in Asturias and Galicia (Spain) and in Portugal. Although, morphological variation was detected, no genetic (using the amplified fragment length polymorphism – AFLP method) or epigenetic (using the methylation sensitive amplification polymorphism – MSAP method) differences were detected. In chapter 4.2 the biochemical composition of the edible part of the peduncle of *P. pollicipes* of the two extreme phenotypes, collected in three Portuguese regions, was investigated for the first time. The higher commercial quality barnacles presented a higher protein and fat content and energetic value, while low commercial quality barnacles presented a higher water content. Also, regional differences were detected on the biochemical composition of *P. pollicipes* sampled in the SW coast of Portugal that presented a higher ash, protein and water content, and a lower glucose and energetic value, than in the other two sampling regions located on the central coast of Portugal ('*Reserva Natural das Berlengas'* and central coast of Portugal, between Cape Carvoeiro and Cape Raso). Finally, in chapter 4.2, a manipulative experiment was carried out to test the influence of two potential drivers (density and microhabitat) on the morphology of this species. The results suggest that the elongation of the stalk of *P. pollicipes* is related to a greater density of barnacles on a clump scale, while the shortening can be related to the lower density of the barnacles at the clump scale and to specific microhabitat environmental conditions, such as hydrodynamics. Further investigation is needed to fully understand this morphological variability of *P. pollicipes* and the processes that can cause it. However, given the importance of the question of the quality of *P. pollicipes* in terms of its fishing and management, we recommend that more studies are needed to continue this research.

The hypothesis that the timing of exploitation can influence the abundance and recovery of *P. pollicipes* after harvesting was investigated in chapter 5. To test this, a manipulative experiment was conducted at two sites (Cape of Sines and Berlengas Nature reserve), considering two tidal levels (mid and low shore). Three timings of exploitation were considered in relation to the different phases of *P. pollicipes* biological cycle: summer and autumn (both correspond to the recruitment season) and spring (non-recruitment season). At each site and tidal level, experimentally harvested clumps of *P. pollicipes* and control clumps of unmanipulated barnacles were monitored (abundance and recovery/growth) during a maximum of 24 months. Results revealed a highly variable and slow growth process, with most of the experimentally exploited clumps not being able to reach in two years the abundance they had before exploitation. Regional differences in the abundance and recovery/growth of exploited/control clumps were detected over time (higher recovery potential in Sines than in Berlenga). The hypothesis that the recovery of the exploited areas or clumps would be faster if the exploitation had been carried out in summer or autumn (recruitment season) and slower in spring (non-recruitment season) was not supported. This ecological result has a direct impact on the management of these fisheries, as it releases the timing of the temporal closure rule from biological justifications, allowing it to be defined on the basis of other criteria, such as the need to reduce fishing effort.

Further investigation is needed to better understand the factors driving regional variation on *P. pollicipes* abundance and recovery after the physical disturbance of harvesting.

Chapter 6 highlighted the importance of integrating local ecological knowledge of the fishers in fishery management. The main objective of the study presented on chapter 6 was to evaluate the temporal variation in the perception of *P. pollicipes* fishers regarding the state of the resource and the state of its management, and to ask them about the possibility of implementing a co-management system applied to the harvesting of this resource. The fishery that was assessed was the '*Reserva Natural das Berlengas*' (RNB), in Portugal (see chapter 1.2). The assessment was based on interviews made to the professional fishers of this region, over time (in 2005, 2013 and 2018). The state of *P. pollicipes* revealed an overall negative tendency at RNB, and the state of the management of this fishery was defined as acceptable.

In addition, between 2013 and 2018, the percentage of fishers that revealed an acceptance with the implementation of a co-management system applied to barnacle harvesting at RNB increased. The fishers considered that a management shift to a co-management system could bring benefits such as a better state of the resource, a more sustainable fishery management and an increase of the economic value of the resource. This fishery is the fishery that showed the higher scores for governance and number of sustainability attributes in Portugal (Aguión et al. 2022b). Since the study presented in chapter 6 (in 2020), several changes have occurred in the management of this fishery., Barnacle harvesting at RNB is now the first Portuguese fishery with a formal co-management system implemented since 2021 ('*Portaria n.º 309/2021*'). This co-management system is being cemented and the first co-management plan for this fishery is now in practice ('Portaria *n.º 15/2023*). Transferring ecological knowledge from fishers and scientists and promoting the sharing of this knowledge improves the management of this fishery and promotes its sustainability.

Overall, the present thesis highlights the importance of obtaining ecological knowledge on *P. pollicipes* at a regional scale, since regional variability was detected in several studies. The regional ecological knowledge is crucial for a more sustainable management of the fishery of each region. There are management decisions of these fisheries that can be justified on ecological terms (e.g. minimum sizes, seasonal closures, spatial rotation). Based on this study, we consider that a better management of these fisheries will be achieved if it is based on regional ecological knowledge. Other highlight of the present thesis is the importance of integrating the local ecological knowledge of *P. pollicipes* fishers on the management of these fisheries.

Further research is needed to better understand the processes responsible for the regional variability detected in abundance, size and growth of *P. pollicipes*, at a regional scale, but also at the clump scale. It is also important to further investigate the causes of this species phenotypical variability, as it has a strong influence on the market price of this species and is a concern for fishers. Finally, it would be important to be able to continue investigating and monitoring aspects related to the biology and ecology of *P. pollicipes* on a European scale, including the regional scale, using standardised methodologies. The privilege we had of being able to apply standardised methodologies from France to Portugal enriched this work and allowed us to compare ecological data in an unconfounded way.

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