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Key Exploited Species as Surrogates for Coastal Conservation in an Oceanic Archipelago:
Insights from topshells and limpets from Madeira (NE Atlantic Ocean)

DOCTORAL THESIS

Ricardo Jorge Silva Sousa

DOCTORATE IN BIOLOGICAL SCIENCES

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Note:

The present thesis presents the results of work already published (chapters 2 to 11), in accordance with article 3 (2) and article 6 (2) of the Specific Regulation of the Third Cycle Course in Biological Sciences of the University of Madeira.

Resumo

As lapas e os caramujos estão entre os herbívoros mais bem adaptados ao intertidal do Atlântico Nordeste. Estas espécies-chave fornecem serviços ecossistémicos valiosos, desempenhando um papel fundamental no equilíbrio ecológico do intertidal e têm um elevado valor económico, estando sujeitas a altos níveis de exploração e representando uma das atividades económicas mais rentáveis na pesca de pequena escala no arquipélago da Madeira. Esta dissertação visa preencher as lacunas existentes na história de vida e dinâmica populacional destas espécies, e aferir os efeitos da regulamentação da apanha nos mananciais explorados. A abordagem conservacionista implícita ao longo desta tese pretende promover: (i) a regulamentação adequada da apanha de caramujos (*Phorcus sauciatus*) e (ii) a avaliação dos efeitos da regulamentação da apanha de lapas nas populações exploradas (*Patella aspera*, *Patella candei*). Atualmente, os mananciais de lapas e caramujos são explorados perto do rendimento máximo sustentável, e a monitorização e fiscalização são fundamentais para evitar a futura sobre-exploração. A regulamentação da apanha de lapas produziu um efeito positivo nas espécies de lapas exploradas, com um aumento no tamanho, na proporção de indivíduos reprodutores, no tamanho de maturação e num maior equilíbrio na proporção de sexos. A apanha de caramujos não está regulamentada e com o atual nível de exploração ocorrem alterações na estrutura de tamanhos, abundância e potencial reprodutivo das populações exploradas, pelo que urge implementar a regulamentação da apanha desta espécie, por forma a mitigar os efeitos negativos desta atividade. O efeito da proximidade das populações humanas e acessibilidade costeira na estrutura de tamanhos e abundância de gastrópodes explorados mostrou que a proporção de reprodutores e a abundância eram geralmente menores em áreas mais próximas das populações humanas e em áreas mais acessíveis. Os efeitos das Áreas Marinhas Protegidas na proteção das populações de lapas resultaram num aumento diferencial do tamanho, da maturidade sexual e da captura por unidade de esforço de acordo com o grau de proteção. O esclarecimento e envolvimento das comunidades locais, reguladores, decisores políticos e partes interessadas, baseados em informação e educação, são cruciais para uma gestão eficaz e sustentável destes gastrópodes marinhos e ecossistemas a médio e longo prazo.

Palavras-chave

Caramujos, *Phorcus sauciatus*, lapas, *Patella aspera*, *Patella candei*, *Patella piperata*, parâmetros biológicos, estrutura populacional, regulamentação, Áreas Marinhas Protegidas, exploração sustentável, intertidal, Arquipélago da Madeira, Atlântico Nordeste.

Abstract

Limpets and topshells are among the most successful intertidal grazers in the North-eastern Atlantic. These keystone species play a pivotal role in structuring rocky shores communities, and provoding valuable ecosystem services. Than have an important economic value, being subject to high levels of exploitation and representing one of the most profitable economic activities in small-scale fisheries in the archipelago of Madeira. This thesis aims to fill the gaps on the life-traits and population dynamics of these species, and assess the effects of harvesting regulations on the exploited stocks. A focus on conservation is implicit throughout this thesis since it addresses the promotion of: (i) proper regulation of the unregulated harvesting of topshells (*Phorcus sauciatus*) and (ii) provide additional information on the effects of harvesting regulations on limpets (*Patella aspera*, *Patella candei*). Currently, limpets and topshells stocks are being exploited near the maximum sustainable yield and monitoring and enforcement must be accomplished to avoid future overexploitation. Conservation measures prompted a positive effect on both exploited limpet species with an increase in length, reproductive individuals, size-at-first maturity and a more balanced sex-ratio after harvesting regulations. The harvesting of topshells is not regulated and with the current level of exploitation there are changes in the size structure, abundance and reproductive potential of the exploited populations, so it is imperative to implement the harvesting regulations for this species, in order to mitigate the negative effects of harvesting. The effect of proximity to human settlements and coastal accessibility on the size-structure and abundance of exploited gastropods showed that the mean-size, proportion of reproductive individuals and abundance were generally smaller in areas closer to human settlements and in more accessible areas. The effects of protection from the Marine Protected Areas on limpet populations resulted in a differential increase on size, size-at-first maturity and catch-per-unit-effort according to the degree of protection. The understanding and commitment of local communities, regulators, policymakers and stakeholders, based on information and education are crucial to the effective management and to ensure the sustainability of these marine gastropods and ecosystems at medium and long term.

Keywords

Topshells, *Phorcus sauciatus*, limpets, *Patella aspera*, *Patella candei*, *Patella piperata*, life-traits, population structure, harvesting regulations, Marine Protected Areas, sustainable exploitation, intertidal, Madeira archipelago, North-eastern Atlantic.

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List of abbreviations

AChE – Acetylcholinesterase

ANCOVA – Analysis of covariance

ANOVA – Analysis of variance

ARDITI - Regional Agency for the Development of Research, Technology and Innovation

A – Atresia

A_{50} – Age at median maturity

$A_{0.95}$ – Potential longevity

Ca. – Approximately

CPUE – Capture per unit effort

CPUEn – Capture per unit effort in number

CPUEw – Capture per unit effort in weight

db-RDA – Distance-based redundancy analysis

DRP – Regional Fisheries Department

DRP-RAM – Regional Fisheries Department of Autonomous Region of Madeira

DSI – Research Service Directorate

DST – Desertas

d180 – Oxygen isotope ratio

E – Exploitation rate

e.g. – For example

ELEFAN – Electronic Length Frequency Analysis

F – Fishing mortality

F= – Brown-Forsythe F test

FEDER – Regional Development Fund

FCT – Foundation for Science and Technology

$F_{current}$ – Current fishing mortality

F_{MAX} – Maximum production

F_{MSY} – Fishing mortality at maximum sustainable yield

FISAT – Fish Stock Assessment Tools FAO-ICLARM

g – Gram

g min^{-1} – Grams per minute

GRJ – Garajau

GSI - Gonadosomatic index

GST – Glutathione-S-transferase

H_0 – Null hypothesis

H_1 – Alternative hypothesis

i.e. – That is

IFCN IP-RAM – Institute of Forests and Nature Conservation

JV – Joana Vasconcelos

K – Growth coefficient

Kg – Kilograms

KW – Kilowatts

KW= – Kruskall-Wallis statistics

L – Shell length

L_{50} – Size at sex change

Lc_{25} – Lengths at which 25% of the specimens are captured

Lc_{50} – Lengths at which 50% of the specimens are captured

Lc_{75} – Lengths at which 75% of the specimens are captured

Lm_{25} – Lengths at which 25% of the specimens are mature

Lm_{50} – Size at first maturity at which 50% of the specimens are mature

Lm_{75} – Lengths at which 75% of the specimens are mature

L_t – Mean shell length at age t

L_∞ – Asymptotic shell length

M – Natural mortality

Ma – Million years

M€ – Millions of euros

MACAROFOOD – Valorisation of marine products from Macaronesia: tourism, gastronomy and professional training

MARE – Center for Marine and Environmental Sciences

MARISCOMAC – Development of technical and scientific conditions, training, transfer of technology and knowledge, aiming to promote the sustainable exploitation and marketing of shellfish in Macaronesia

MDS – Multidimensional scaling

mm – Millimeter

MPA – Marine Protected Area

MPAs – Marine Protected Areas

N.° – Number

NE – North eastearn

NEOLI – No-take, Enforced, Old, Large, and Isolated

nMDS – Non-metric multidimensional scaling

NORMSEP – Method for decompose composite length-frequency distributions

OOM – Oceanic Observatory of Madeira

P – Logistic equation

PAHs – Polycyclic aromatic hydrocarbons

PCBs – Polychlorinated biphenyls

Pers. comm. – Personal communication

Pers. obs. – Personal observation

Pers. observ. – Personal observation

POF – Post-ovulatory follicles

$P<0.05$ – 5% significance level

$P<0.01$ – 1% significance level

r^2 – Coefficient of determination

RN – Rocha do Navio

RR – Rodrigo Riera

RS – Ricardo Sousa

SI – Separation index

S_L – Logistic curve

SLV – Selvagens

Specimens/min – Specimens per minute

Spp. – Species

SST – Sea surface temperature

ST – Seminiferous tubules

T – Annual mean environmental temperature

t – Age

TAC – Total allowable catches

Tons – Tonnes

t= – Student’s t test

t_0 – Hypothetical age at which $L_t = 0$

T_c – Age at first capture

T_r – Age at recruitment

TW – Total weight

UE – European Union

UK – United Kingdom

U= – Mann-Whitney *U* Test

VO – Vitellogenic oocytes

vs. – Versus

W – Total weight

W= – Levene’s statistics

WoRMS - World Register of Marine Species

W/L – Weight length relationship

w_{∞} – Asymptotic weight

$\bar{x}$ – Median

Z – Total mortality

Z= – Kolmogorov Smirnov two-sample test

y^{-1} – Years

Y/R – Yield per recruit

ϕ' – Growth performance index

χ^2 – Chi-square

& – And

°C – Centigrade degrees

PART I

GENERAL INTRODUCTION

Chapter 1

General introduction

Introduction

Rocky shores are extremely productive ecosystems supporting a high diverse range of assemblages of algae and animals (Raffaelli & Hawkins, 1999; Gamfeldt & Bracken, 2009). Its easy accessibility has made them susceptible to a variety of human-induced impacts such as harvesting (Thompson et al., 2002; Nakin & McQuaid, 2014; Riera et al., 2016), pollution (Walsh et al., 1995) and habitat modification (Cole et al., 2012).

Human exploitation of organisms on the rocky shores is a significant cause of disturbance of intertidal communities' occuring since prehistoric times (Bustamante & Castilla, 1990; Boer & Prins, 2002; Martins et al., 2008). Anthropogenic activities frequently lead to changes in species richness and biomass resulting in shifts in community composition of the exploited ecosystems around the world (Moreno et al., 1984).

Molluscs are some of the most exploited intertidal organisms worldwide (Roy et al., 2003; Sagarin et al., 2007), being extensively harvested for recreational, subsistence and commercial purposes (Moreno et al., 1984; Siegfried, 1994) in several geographic regions (Moreno et al., 1984; Keough et al., 1993). The effects of harvesting are not limited to alterations in targeted species, but they extend through cascading trophic effects to the whole ecosystem (Scheffer et al., 2005; Martins et al., 2017). The exploitation of these resources is greatly influenced by human demography, tradition, and economy (Rius & Cabral, 2004).

Intertidal grazers such as topshells and limpets are considered keystone species because of their pivotal role in the ecological balance of the rocky shores with no other species fulfilling the same role (Hawkins & Hartnoll, 1983; Bowman & Lewis, 1986; Jenkins et al., 2005; Coleman et al., 2006; Donald et al., 2012; Templado & Rolán, 2012). They have often been used as biological indicators to evaluate the consequences of anthropogenic impacts on the rocky shore ecosystem (Lima et al., 2007; Sousa et al., 2019).

Topshells, limpets and winkles are essential in the structuring and regulating the ecological balance of intertidal communities, directly through the key process of grazing on algae that determines macroalgal abundance, and indirectly by influencing the establishment of other organisms (Hawkins & Hartnoll, 1983; Jenkins et al., 2005; Coleman et al., 2006). These intertidal grazers are extremely vulnerable because of their particular life-traits and having a restricted habitat easily accessible by humans (Nakin & McQuaid, 2014). In extreme cases, harvesting has led to the fragmentation of limpets assemblages (Riera et al., 2016) and even to the disappearance of populations from some areas, as occurred for *Patella ferruginea* Gmelin, 1791 in the Mediterranean Sea (Espinosa, 2009), and *Cellana* *exarata* (Reeve, 1854), *Cellana sandwicensis* (Pease, 1861) and *Cellana talcosa* (Gould, 1864) in Hawaii (Valledor, 2000).

The most important molluscs exploited in the archipelago of Madeira are topshells of the genus *Phorcus* Risso, 1826 and limpets of the genus *Patella* Linnaeus, 1758, representing one of the most profitable economic activities of small-scale fisheries (Henriques et al., 2012). The easy accessibility to the intertidal prompted the exploitation of marine shellfish along the coast of this archipelago. The harvest activity began in the fifteenth century when the archipelago was discovered and colonized (Silva & Meneses, 1921) and became progressively more intensive because of the demographic increase of coastal human settlements and the technological advances that promoted easier accessibility to the coast at previously inaccessible areas (Sousa et al., 2018).

The genus *Phorcus* comprises nine living species of herbivorous topshells occurring in the intertidal zones from the Mediterranean Sea to the Northeastern Atlantic Ocean including the NE Atlantic archipelagos of Madeira, Azores, Canaries and Cape Verde (Crothers, 2001). The evolution of this important intertidal group was clarified by a complete molecular phylogenetic study performed by Donald et al. (2012) that provide strong evidences for nine distinct species including species previously under the genus *Monodonta* Lamarck, 1799, or *Osilinus* Philippi, 1847. The most abundant topshell present in the archipelago of Madeira is *Phorcus sauciatus* (Koch, 1845), occurring in the islands of Madeira, Porto Santo and Desertas, as well as in the Selvagens Islands, while *Phorcus atratus* (Wood, 1828), is restricted to the Selvagens islands as the endemic subspecies *Phorcus atratus selvagensis* (Donald et al., 2012; Sousa et al., 2018).

The species of the genus *Phorcus* are considered useful tools in assessing coastal habitats quality, being frequently used as bioindicators in the establishment of conservation measures concerning these environments (Donald et al., 2012). These keystone species are a useful biomonitoring system of pollutants and good indicators of the effects of climate change due to their reduced mobility, widespread distribution, ability to accumulate high metal concentrations and abundance all year-round (Crothers, 1994; Walsh et al., 1995; Wang & Ke, 2002; Mieszkowska et al., 2007). In fact, *Phorcus lineatus* (da Costa, 1778) has been used as an indicator of climate changes around the coasts of Western Europe due to its wide biogeographic distribution (Crothers, 1994; Mieszkowska et al., 2007). Also, the expansion of *P. sauciatus* distribution into the north of mainland Portugal and south of Galicia has been linked to the warming of sea surface temperature in the Northeastern Atlantic due to global warming (Lima & Wethey, 2012; Rubal et al., 2014). Another example is the recent colonization of Santa Maria Island in the archipelago of Azores by *P. sauciatus,* probably after 2009, coinciding with sea water temperature increase (Ávila et al., 2015).

Several works have focused on the biology, reproduction, population dynamics, ecology, age and growth of topshells (Bode et al., 1986; Gaudêncio & Guerra, 1986; Foale & Day, 1997; Boucetta et al., 2010). However, most of the studies focused on *P. lineatus* and scarce information is available on *P. sauciatus*. Nevertheless, regional information on the size-distribution is available for *P. sauciatus* from the north-west Iberian Peninsula (Rubal et al., 2014) and the Canary Islands (Ramírez et al., 2005, 2009; Alfonso et al., 2015). An integrated study on *P. sauciatus* including the geographic and environmental particularities of the archipelago of Madeira, and how they affect this species is required for the sustainable exploitation in the medium and long term.

Patellid limpets are another group of successful marine gastropods inhabiting the intertidal ecosystem (Branch, 1981), occurring from the supratidal to the subtidal zones. Together with topshells, these organisms are subject to one of the most variable and unpredictable environments in nature (Harley et al., 2009), caused by gradients of wave action, temperature variation and desiccation that can prompt significant changes on the overall morpho-physiological characteristics of patellid limpets (Davies, 1969). For instance, shifts on temperature and storminess (Branch, 1974; Bowman & Lewis, 1986), latitude and temperature (Lewis, 1986), climate change (Moore et al., 2011), parasitism (Firth et al., 2017) and anthropogenic pressures (Boukhicha et al., 2013; Henriques et al., 2017) can deeply influence the reproductive biology and phenology of limpets.

The genus *Patella* currently comprises of 14 living species with a geographical distribution restricted to the North-Eastern Atlantic and the Mediterranean Sea (Henriques et al., 2017), but only four species are reported for the Macaronesian archipelagos sensu lato. Three limpet species are present in the archipelagos of Madeira and Canaries namely, *Patella aspera* Röding, 1798, *Patella candei* d' Orbigny, 1840 and *Patella piperata* Gould, 1846, while two species are present in the archipelago of Azores (*P. aspera* and *P. candei*) and in the archipelago of Cape Verde (*Patella lugubris* Gmelin, 1791 and *P. piperata*) (WoRMS, 2019).

A plethora of studies on the genus *Patella* has been performed concerning their morphology, ecology, behaviour, abundance, habitat distribution, life history, reproduction, taxonomy and genetics (Orton et al., 1956; Christiaens, 1973; Côrte-Real et al., 1996; Ridgway et al., 1998; Koufopanou et al., 1999; Boaventura et al., 2002; Arrontes et al., 2004; Weber & Hawkins, 2005; Henriques et al., 2012; Faria et al., 2015; Faria et al., 2016), but most of the available scientific information is related to *Patella vulgata* Linnaeus, 1758, *Patella ulyssiponensis* Gmelin, 1791 and *Patella depressa* Pennant, 1777.

Although several genetic studies aiming to clarify the taxonomy of the genus *Patella* have been undertaken in recent decades (Côrte-Real et al., 1996; Weber & Hawkins, 2002; Sá-Pinto et al., 2005), the taxonomic status of Macaronesian limpets remains unclear. More recently, Faria et al. (2015, 2016) using novel microsatellite markers and next generation sequencing for *P. candei* and *P. aspera* suggested that *P. candei* from each archipelago are geographically and/or ecologically isolated populations and recommended the use of the denomination of *P. candei* in Selvagens, *Patella gomesii* in the Azores, *Patella ordinaria* in Madeira, and *Patella crenata* in the Canaries (Faria et al., 2017). These molecular tools can be useful to further establish the genetic structure and patterns of connectivity of patellid limpets in the archipelago of Madeira, pivotal tools in the definition of stocks and establishment of specific conservation strategies aiming at their sustainable exploitation.The biological life history of limpets varies inter- and intraspecifically because of genetic differences and environmental influences (Bowman & Lewis, 1986). Differences in limpet populations from distinct geographic areas are probably caused by specific environmental and anthropogenic conditions, essentially food availability, seawater temperature, and harvesting pressure (Henriques et al., 2017) and as such, regional studies are crucial to implement regulations considering the behaviour of the species in their particular ecosystem. One of the consequences of the life history of limpets is the ability to change sex in low population densities, since under such conditions hermaphroditism is supposed to increase the species reproductive efficiency (Charnov, 1979; Munday et al., 2006).

Hermaphroditism constitutes an advantageous strategy in the evolution of limpets, since it apparently increases the likelihood of successful fertilization for semi sessile broadcast spawning, especially when occurring in low population densities (Branch, 1981; Guallart et al., 2013). The sex change is triggered by specific environmental cues and/or is determined genetically, occurring mainly after the first reproductive season (Wright, 1989; Fretter et al., 1998). Knowledge on these aspects is important and needs to be taken into consideration in the conservation and management of heavily exploited populations (Espinosa et al., 2006), since size-selective harvesting negatively affects the reproductive output of threatened species (Fenberg & Roy, 2008).

For instance, exploited populations of limpets are known to have reduced reproductive potential due to the decrease in abundance and mean size (Oliva & Castilla, 1986), which contributes to the reduction of reproductive output in these broadcast spawners. Moreover, in protandrous hermaphrodite species the removal of larger females may result in skewed sex-ratios and sex change occurring at a smaller size compromising the reproductive output of these populations as such, continuous monitoring and assessesment of the status of the exploited populations are crucial to promote a sustainable exploitation over the years (Martins et al., 2017).

Phorcus sauciatus is commercially exploited in the archipelago of Madeira without the existence of regulations or of a management strategy and as such, no information concerning the harvesting yield and economical value is available. On the other hand, the commercial harvest of *P. aspera* and *P.*

candei in a mixed exploitation is known to reach annual landings of up to 150 tons yielding an overall first value of *ca*. 0.7 M€ (Sousa et al., 2017). These limpet species are endemic to the Macaronesian archipelagos and occur in the Madeira archipelago together with *P. piperata*, traditionally exploited by local inhabitants for their own consumption. In the last three decades, a shift in the nature of limpet harvest has occurred from a traditional (upper intertidal) to a commercial exploitation (subtidal harvest), due to the reduction in limpet availability in the upper zones of the intertidal (Delgado et al., 2005).

Both topshell and limpet consumption in the archipelago of Madeira is part of the gastronomic cultural heritage of the region, appreciated by locals and tourists alike, and has a high socioeconomic importance. Therefore, harvesting pressure on these resources is one of the greatest concerns to their conservation, since this long-term exploitation promoted changes in the population dynamics of these exploited marine gastropods over the years. These disturbances essentially focus on the abundance, size structure, and alterations of the reproductive output and replenishment of the exploited populations as result of the size-selective nature of harvest (Lindberg et al., 1998; Kido & Murray, 2003; Riera et al., 2016; Martins et al., 2017; Sousa et al., 2019). The slow growth and long lifespan of these marine gastropods contributes to their extreme vulnerability to exploitation and as such life history and the impacts of exploitation, need to be assessed to provide the proper management tools required to ensure their sustainable exploitation. Suitable regulation, considering the biological and ecological specificities of the exploited species in their particular habitat, should be applied to promote their preservation as well as their habitats at medium and long term.

The anthropogenic pressures to wich topshells and limpets are exposed to, such as harvesting (Martins et al., 2008; Ramírez et al., 2009; Turrero et al., 2014; Riera et al., 2016) and habitat modification and loss (Fischer-Piétte, 1963; Cole et al., 2012; Rubal et al., 2014), prompt a decrease of populations (Kido & Murray, 2003; Martins et al., 2008), a reduction in abundance and shifts in size composition throughout the last decades worldwide (Núñez et al., 2003; Ramírez et al., 2009; Rubal et al., 2014; Riera et al., 2016). Several works on the effects of anthropogenic impacts on limpets populations have been carried out in South Africa (Branch & Odendaal, 2003), Mediterranean (Espinosa et al., 2009), Canary Islands (Riera et al., 2016) and the Azores (Hawkins et al., 2000; Martins et al., 2017), but for the archipelago of Madeira no studies exist on this subject although regulation of limpet harvest has been in effect since 2006. Knowledge of the impact of harvesting pressure on topshell populations is even more scarce, nonetheless Ramírez et al. (2009) assessed the harvesting pressure over the abundance and size of *P. sauciatus*, *P. atratus*, *P. aspera*, *P. candei* and *P. piperata* in the Canary Islands and the authors concluded that human activities turned out to be the major driver for the observed differences among islands, calling into question the effectiveness of the current shellfish regulations in this archipelago. As such it is essential to assess the effect of harvesting regulations on the exploited limpets' populations in the archipelago of Madeira to evaluate the effectiveness of the regulations.

The conservation of exploited topshell and limpet populations is of concern especially in coastal isolated areas, where no adjacent populations are present to supply larvae for settlement and recruitment (Cowen et al., 2000). The management measures should consider the biological parameters estimated in their specific habitat, their ecological role in the ecosystem and accurate characterizations of population dynamics and stock status. Several regulation measures have been introduced for limpet harvest in the archipelagos of Madeira and Azores (*Patella* spp.) comprising closed seasons and areas, minimum catch size, capture limits per day, obligation of harvester licenses and declaration of catches on logbooks (Ferraz et al., 2001; Delgado et al., 2005). In the archipelago of Madeira, regulators established a maximum allowable commercial catch of 15 kg/person/day or 200 kg/vessel/day, being exempted of any license the traditional harvest that does not exceed 3 kg/day per person, a minimum catch size of 40 mm, logbook provision, the landings and first auction sale and a closed season lasting from December to March to protect spawning, larvae development, and settlement, and to increase the reproductive success (Legislative Orders N.˚ 11/2006/M, 18 April 2006 and N.˚ 40/2016, 17 February 2016). Despite the existence of limpets harvesting regulations, an accurate assessment of the stocks, current biological studies and comparative studies before and after the implementation of regulation are pivotal to assess the effectiveness of management measures and to promote a sustainable exploitation at medium and long term.

Several studies have shown that limpet populations exploited worldwide respond positively to harvesting regulations, as long as management measures are adequately enforced by the responsible authorities and with the involvement of local communities (Fenberg et al., 2012; Coppa et al., 2016). For instance, Núñez et al. (2003) verified that *P. candei* populations were better preserved in terms of abundance and size composition in areas protected by regulations in the island of Fuerteventura. The imposition of total allowable catches (TAC) has also been suggested and the involvement of authorities and environmental agencies in a global conservation plan has been proposed, establishing small MPAs where dense populations settle, being useful as a focus for resettlement of depleted areas (Eekhout et al., 1992; Espinosa et al., 2009).

The implementation of MPAs is considered a key tool for the conservation of marine biodiversity in coastal areas (Ballantine, 1991; Zann, 1996; Edgar et al., 2014) due to its ecosystem-level approach for exploited species (Henriques et al., 2017). MPAs defined as no take zones, are an alternative to traditional management measures of marine resources (Halpern & Warner, 2002), since the exploited organisms, usually attain higher density, biomass, and size in these zones (Hockey & Bosman, 1986;

Keough et al., 1993; Halpern, 2003). The effect of harvesting prohibition of limpets such as *Patella* spp. and trochids such as *Phorcus* spp. in MPAs promoted an increase on their abundances (Ceccherelli et al., 2006; Bertocci et al., 2012; Shears et al., 2012). MPAs re-establish and protect marine resources within their boundaries, mainly the reproductive component, and may also act as a source of larvae that could eventually contribute for settlement and recruitment outside of the reserves (Rakitin & Kramer 1996; Pelc et al., 2009), due to larval connectivity between MPAs and unprotected areas (Christie et al., 2010).

Considering the knowledge gap on biological and ecological information of the exploited species of intertidal grazers in the archipelago of Madeira, as well as the lack of information regarding the impact of harvest and of effectiveness of conservation measures, the present work aims to provide the framework necessary to develop the management tools required to ensure the long-term sustainable productivity of the stocks of both harvested limpets (*P. aspera* and *P. candei*) and the topshell *P. sauciatus*, in order to avoid commercial extinction as has previously occurred in other geographic regions.

Aims

The main goals of the present dissertation were two-fold, firstly investigate the autecology, i.e. biological and ecological particularities, of the exploited limpets (*P. aspera* and *P. candei*) and of the topshell *P. sauciatus* in a highly harvested scenario, i.e. the archipelago of Madeira. This is the first step to develop management measures for the conservation of the topshell commercially harvested. Secondly, the evaluation of harvesting impact on these intertidal resources to get an accurate depiction of the current situation of commercial stocks of limpets and topshells in Madeira, which is necessary to provide guidelines for future conservation efforts and guarantee their sustainable exploitation.

To achieve the above-mentioned aims, the present dissertation has been structured in order to reach the following specific objectives:

(i) Compilation and review of the biology and ecology of *Phorcus* species. Evaluation and discussion of the consequences of anthropogenic impacts on these keystone species and their potential as bioindicators on coastal environments.

(ii) Determination of the life history and the exploitation rate of *P. sauciatus* in Madeira and evaluation of the effect of size at first capture on exploited stocks. Development of a proposal of management measures for the regulation of topshell harvest in this region.

(iii) Review and discussion of biology and ecology of patellid limpets, discussion of the anthropogenic impacts and identification of the effects of MPAs in the stock's recovery.

(iv) Determination of the life history parameters of *P. aspera*, such as growth, age structure, reproduction, sexual maturity, recruitment, and mortality rates and evaluate the effect of size at first capture on exploited stocks.

(v) Identification of histological evidences of hermaphroditism in *P. piperata*.

(vi) Characterization of limpet and topshell harvesting in the archipelago of Madeira, considering and discussing the evolution of landings, landed values and describing the harvesting, the composition of fishing fleets, the exploited species and the yields for a period of 27 years (1990-2017). Description of the established management measures regulating the harvest of marine gastropods and discussion of their impact on exploited stocks.

(vii) Assessment of the effect of limpet harvesting regulation on the reproductive parameters of *P. aspera* and *P. candei*.

(viii) Analysis of the effects of anthropogenic pressure on the size structure and abundance of populations of *P. aspera* and *P. candei* in the archipelago of Madeira.

(ix) Determination of the effects of harvesting pressure on the size-structure and abundance of historically highly exploited populations of *P. sauciatus* in Madeira considering the proximity to human settlements and the accessibility to topshells populations.

(x) Determination of the effects of MPAs on the population dynamics of *P. aspera* and *P. candei* through a comparative analysis of size composition, reproduction (Lm_{50}) and abundance in the archipelago of Madeira. Assessment of the impact of NEOLI features on population dynamics, reproduction, and abundances of both limpet species.

Thesis outline and structure

The thesis presented herein is focused on the most important exploited molluscs in the archipelago of Madeira (limpets and topshells). These species are ecologically fundamental grazers responsible for the balance of the intertidal, bioindicators of pollutants and good indicators of climate changes that require prioritizing conservation policies. This thesis consists of four parts comprising twelve chapters.

The first part includes the objectives and an integrative description of all the chapters (chapter 1). The second part consists of five chapters on the autecology of the limpets *P. aspera*, *P. candei* and the topshell *P. sauciatus*, dealing with different ecological and biological aspects of these species. The chapters 2 and 4 focus on the biology and ecology of the studied species and the anthropogenic pressure on these resources. These chapters deal with the anatomy, growth, reproduction, mortality, behaviour and the ecological role of these keystone species and their interactions in the rocky shores as well as the effects of harvesting, habitat loss and pollution to which they are subject. The determination of life history parameters such as growth, age, reproduction, recruitment and mortality of *P. sauciatus* and *P. aspera* and the assessment of the exploited stock status were addressed in chapters 3 and 5. Additionnaly, a proposal of harvesting regulation for *P. sauciatus* in the archipelago of Madeira is given in chapter 3. This second section is concluded with an example of hermaphroditism in *P. piperata* (chapter 6), elucidating the importance of sex change on the management and conservation of patellids exhibiting this advantageous reproductive strategy.

The third part contains five chapters dealing with the conservation biology of the above mentioned exploited limpets and topshells. The characterization of the small-scale harvesting of gastropods in the archipelago of Madeira was performed in chapter 7. In this chapter the evolution of the landings, landed values and description of the harvesting activity, the fishing fleets, the exploited species and the yields are also discussed. The assessment of the effect of harvesting regulation on the reproductive parameters, on the size structure and abundance and the effectiveness of the MPAs on population dynamics, size structure and abundance of *P. aspera* and *P. candei* are presented in chapters 8, 9 and 11. This part also includes the determination of the harvesting pressure on *P. sauciatus* (chapter 10), to disentangle the exploitation status of this species and provide insights for its conservation, by assessing the effect of proximity to human settlements and coastal accessibility on the size-structure, proportion of reproductive individuals and abundance of topshell populations.

The fourth part includes the final discussion integrating the main findings of all chapters and some perspectives on species conservation and management contributing to the sustainable exploitation of limpets and topshells in the archipelago of Madeira in the medium and long term.

Ten of the chapters were published in peer reviewed journals and books (seven as papers and three as book chapters).

Candidate´s list of publications

Published book chapters

1. **Sousa R**, Delgado J, González JA, Freitas M, Henriques P (2018) Marine snails of the genus *Phorcus*: Biology and ecology of sentinel species for human impacts on the rocky shores. In *Biological Resources*

of Water. Ray S (Ed). IntechOpen, Rijeka. pp. 141-167. https://doi.org/10.5772/intechopen.71614 (Chapter 2);

2. Henriques P, Delgado J, **Sousa R** (2017) Patellid limpets: An overview of the biology and conservation of keystone species of the rocky shores. In *Organismal and Molecular Malacology*. Ray S (Ed). IntechOpen, Rijeka. pp. 71-95. https://doi.org/10.5772/67862 (Chapter 4);

3. **Sousa R**, Riera R, Vasconcelos J, Gouveia L, Pinto AR, Delgado J, Alves A, González JA, Freitas M, Henriques P (2019). Artisanal harvest of benthic shellfish in the north-eastern Atlantic: the example of limpet and topshell fisheries in the archipelago of Madeira. In *Invertebrates - Ecophysiology and Management*. Ray S, Diarte-Plata G, Escamilla-Montes R (Eds). IntechOpen, Rijeka. pp. 1-18. https://doi.org/10.5772/intechopen.85728. (Chapter 7).

Published papers

1. **Sousa R**, Vasconcelos J, Delgado J, Riera R, González JA, Freitas M, Henriques P (2018) Filling biological information gaps of the marine topshell *Phorcus sauciatus* (Koch, 1845) (Gastropoda: Trochidae) to ensure its sustainable exploitation. *Journal of the Marine Biological Association of the United Kingdom* 99(4): 841-849. https://doi.org/10.1017/S0025315418001054 (Chapter 3);

2. **Sousa R**, Delgado J, Pinto AR, Henriques P (2017) Growth and reproduction of the North-Eastern Atlantic keystone species *Patella aspera* (Mollusca: Patellogastropoda). *Helgoland Marine Research* 71(8): 1-13. https://doi.org/10.1186/s10152-017-0488-9 (Chapter 5);

3. **Sousa R**, Henriques P, Vasconcelos J, Faria G, Riera R, Pinto AR, Delgado J, Hawkins SJ (2019) First observations of hermaphroditism in the patellid limpet *Patella piperata* Gould, 1846. *Journal of the Marine Biological Association of the United Kingdom* 99(7): 1615-1620. https://doi.org/10.1017/S0025315419000559 (Chapter 6);

4. **Sousa R**, Vasconcelos J, Riera R, Pinto AR, Delgado J, Henriques P (2019) Potential impact of harvesting management measures on the reproductive parameters of the limpets *Patella aspera* and *Patella candei* from Madeira Island. *Estuarine Coastal and Shelf Science* 226: 106264. https://doi.org/10.1016/j.ecss.2019.106264 (Chapter 8);

5. **Sousa R**, Vasconcelos J, Henriques P, Pinto AR, Delgado J, Riera R (2019) Long-term population status of two harvested intertidal grazers (*Patella aspera* and *Patella candei*), before (1996-2006) and after (2007-2017) the implementation of management measures. *Journal of Sea Research* 144: 33-38. https://doi.org/10.1016/j.seares.2018.11.002 (Chapter 9);

6. **Sousa R**, Vasconcelos J, Riera R, Delgado J, González JA, Freitas M, Henriques P (2019) Disentangling exploitation of the intertidal grazer *Phorcus sauciatus* (Gastropoda: Trochidae) in an oceanic archipelago: Implications for conservation. *Marine Ecology* 40(2): 1-8. https://doi.org/10.1111/maec.12540 (Chapter 10);

7. **Sousa R**, Henriques P, Vasconcelos J, Pinto AR, Delgado J, Riera R (2020) The protection effects of marine protected areas on exploited molluscs from an oceanic archipelago. *Aquatic Conservation: Marine and Freshwater Ecosystems* 1-13. https://doi.org/10.1002/aqc.3285 (Chapter 11).

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PART II

AUTECOLOGY OF INTERTIDAL GRAZERS IN THE HISTORICALLY HARVESTED ARCHIPELAGO OF MADEIRA

Chapter 2

Marine snails of the genus *Phorcus*: Biology and ecology of sentinel species for human impacts on the rocky shores

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Abstract

In this review article, the authors explore a broad spectrum of subjects associated to marine snails of the genus *Phorcus* Risso, 1826. Namely, distribution, habitat, behaviour and life history traits, and the consequences of anthropological impacts such as, fisheries, pollution, and climate changes on these species. This work focuses on discussing the ecological importance of these sentinel species and their interactions in the rocky shores as well as the anthropogenic impacts to which they are subjected. One of the main anthropogenic stresses that affect *Phorcus* species is fisheries. Topshell harvesting is recognized as occurring since pre-historic times, and has evolved through time from a subsistence to commercial exploitation level. However, there is a gap in information concerning these species that hinders stock assessment and management required for sustainable exploitation. Additionally, these keystone species are useful tools in assessing coastal habitats quality, due to their eco-biological features. Contamination of these species with heavy metals carries serious risk for animal and human health due to their potential of biomagnification in the food chain. Thus, the use of these species as bioindicators is warranted to the establishment of conservation measures targeting marine coastal environments. Climate change increases the level of environmental stress to which intertidal organisms are subjected to, affecting the functioning of biological systems at different levels of organization. *Phorcus* species have been widely used as indicators of the effect of climate change on local disturbances of intertidal ecosystems and geographic distribution shifts of these organisms. Further studies concerning biological parameters of *Phorcus* species and how they react to exploitation, pollution and climate change will consolidate these species as indicators of large scale ecological impacts of anthropogenic activities.

Keywords

Phorcus, topshells, life history traits, fisheries, pollution, climate change.

1. Introduction

Topshells are marine gastropods that inhabit rocky shores. These marine snails together with limpets and winkles are the most successful algal grazers present in the intertidal of the North-eastern Atlantic and Mediterranean Sea [1]. Topshells occupy the rocky-sea shores from the supratidal to the subtidal, one of the most extreme, heterogeneous, and dynamic environments in nature, that expose these organisms to different levels of thermal and desiccation [2, 3]. These unpredictable environmental conditions are therefore responsible for many of their peculiar morphological and biological characteristics that can be perceived as adaptations to the intertidal environment [4]. The marine snails of the genus *Phorcus* are ecologically important algal grazers that play a major role in regulating the ecological balance of their habitat and have often been used as biological indicators in evaluating the consequences of anthropogenic impact on this ecosystem [4, 5].

The diversity and ecological importance of the genus *Phorcus* prompted intensive research over the past years. Recently, this genus was redefined by Donald et al. [5] to include species previously under the genus *Monodonta* Lamarck, 1799, or *Osilinus* Philippi, 1847, allowing to trace the biogeographic history of this genus' origin to 40-20 Ma, prior to the closure of the Tethyan Seaway.

Intertidal invertebrates' life history traits vary inter- and intraspecifically because of genetic differences and environmental effects. Growth, reproductive strategy, and mortality depend on a complex combination of selective forces and are fundamental to understand the distribution and abundance of these species along the intertidal zone [6, 7]. As such, knowledge of life history traits of *Phorcus* populations provides important information required to understand how these species adapt to an ever-changing environment, whether because of human activities, such as fisheries, habitat disturbance, pollution and climate change, or natural causes.

One of the main causes of disturbance in the intertidal ecosystem is the harvest of gastropods in the rocky shores, which has occurred since prehistorical times, resulting in shifts in abundance and/or size structure of these species [4]. Another cause of disturbance is the contamination of coastal waters, by the presence of unnatural chemicals, as result of industrial spillage and sewage discharges among others. Gastropod molluscs are frequently used as bioindicators to assess the health status of the coast and determine the effect of marine pollution [8]. Walsh et al. [9] recorded that these sentinel species have the potential to act as a useful biomonitoring system of pollutants in the marine environment. As such, they act as pollution indicators by tracing metals, providing information required for the establishment of protective measures of the ecosystem.

Phorcus species are recognized as good bioindicators of water quality due to their reduced mobility, easy sampling, adequate size for tissue analysis, widespread distribution, abundance all year-round, and ability to accumulate high metal concentration in their shell and tissues, reflecting heavy metal availability in coastal waters [10, 11].

Global climate change also causes disturbance in the intertidal ecosystem that results in changes in the geographical distribution of marine gastropods. Intertidal invertebrates are known to respond to climate change through alterations in biogeographic distributions following a latitudinal gradient, from warmer towards cooler regions. *Phorcus* species are bioindicators and changes in their distribution have been successfully linked to hypothesis of climate change on North-eastern Atlantic shores, particularly in species presently at their northern limits which may be expected to move further north as the coastal waters continue to warm, as has happened in the last decades [36].

The aim of this work is to compile and review a wide array of subjects related to *Phorcus* species biology and ecology, comprising anatomy, growth, reproduction, mortality, behaviour and ecological role and also to evaluate and discuss the consequences of anthropogenic impacts such as fisheries, pollution, and climate changes on these keystone species and their potential as bioindicators of the effect of human activities on coastal marine environments.

2. Biology and ecology of topshells

2.1. Anatomy

Gastropods are comprised essentially of two main parts: the shell and the body. These asymmetrical molluscs have a twisted, spirally coiled shell around its body, which protects them from biotic and abiotic factors present in their environment, and a corneous or calcareous operculum, a flat plate that rests on the upper dorsal side of the foot that acts as a supporting pad for the shell. When the snail actively moves or blocks the aperture, the body withdraws, protecting the animal from predators and preventing water leakage in exposed rocky shores [12, 13].

In topshells, the shell is complete and usually pyramidal, moderately large, conical to globose in shape, with rounded to angular body whorls and often with a flattened base and an interior consisting of mother-of-pearl. This structure is formed in the embryonic stage, with the secretion of protein fibres from the outer skin of the visceral mass and from the mantle, while they are free-swimming larvae and they are followed by the secretion of calcium carbonate from the same cells. Posterior to the embryonic phase, the shell continues to grow through the addition of a protein mesh and calcium carbonate mostly on its margins but also on its interior. Shell growth is not continuous and it frequently leaves different growth lines since maturity and adverse environmental conditions may cease growth. The shell offers refuge both from predators and from desiccation being impervious to gasses and liquids and resistant to crushing [12-14]. Colour patterns of the shell are usually highly variable in topshells and are mostly related to diet rather than to genetic control (Figure 1) [12].

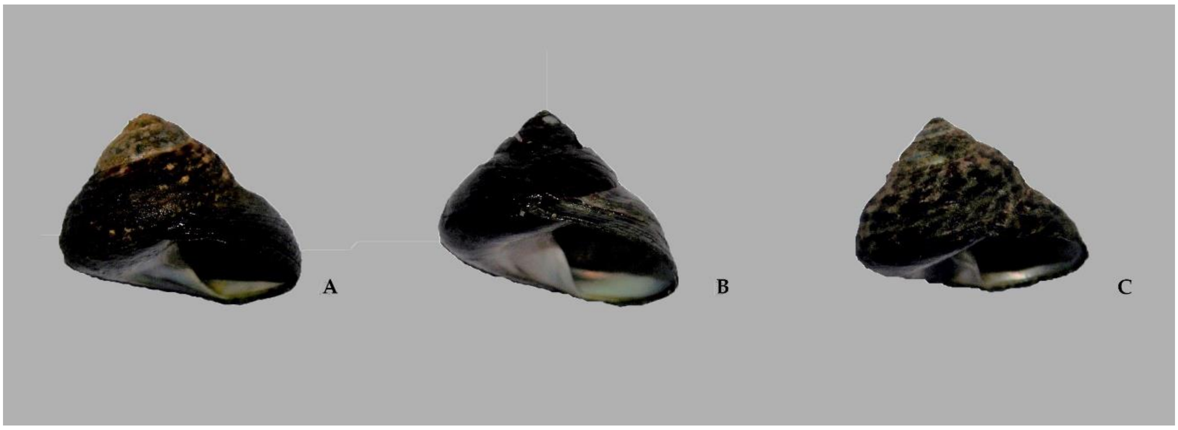

Figure 1 - Shell phenotypic variability of *Phorcus sauciatus*. A – Portugal mainland, B – Madeira Island, C – Gran Canaria Island.

The soft body consists of two compartments connected by a waist and present a dark ash colour with a greenish tint [15]. The lower compartment encompasses the muscular foot and the head. The foot is used for locomotion over the substrate, swimming, jumping, and returning the animal to an upright position when overturned. Also, it helps to detect food. The upper compartment is used for respiration, digestion, excretion, gamete production and shell secretion. The body of these organisms comprises a head with a short snout, a pair of conical and papillate tentacles, cup-shaped open eyes on distinct stalks, a foot, a muscular ventral organ with a flattened base used for locomotion, and a visceral mass, which fills dorsally the spire of the shell and contain most organ systems and the mantle, a collar-like tegument, which lines and secretes the shell, and forms a mantle cavity normally provided with respiratory gills for breathing in water and a well-vascularised mantle cavity, which allows the animals to breathe in air [13, 14].

2.2. Taxonomy and geographic distribution

Phorcus Risso, 1826 are herbivorous marine snails (Gastropoda: Prosobranchia) belonging to the family Trochidae Rafinesque, 1815, that inhabit rocky shores from the Mediterranean Sea through the North-eastern Atlantic Ocean including the Macaronesian Archipelagos of Madeira, Canaries, Azores, and Cape Verde [14].

This genus of gastropod grazers is currently represented by nine recognized living species [5-6] and is comprised of *Phorcus articulatus* (Lamarck, 1822), *Phorcus atratus* (Wood, 1828), *Phorcus lineatus* (da Costa, 1778), *Phorcus mariae* Templado & Rolán, 2012, *Phorcus mutabilis* (Philippi, 1851), *Phorcus punctulatus* (Lamarck, 1822), *Phorcus richardi* (Payraudeau, 1826), *Phorcus sauciatus* (Koch, 1845), and *Phorcus turbinatus* (Born, 1778) [1, 5].

There is a clear separation between the species of *Phorcus* that occur in the Atlantic and the Mediterranean. This split distribution is thought to result of the barrier imposed by the Strait of Gibraltar, since there is no species overlap in the adjacent area, and the nearby Alboran front that act as biogeographic breaks for animals with short larval stages, such as *P. lineatus*, whose lecithotrophic veliger larvae remain in the water column for, at the most, 6 to 7 days [5, 14, 16]. As such, four species of this genus are restricted to the Mediterranean Sea, specifically *P. turbinatus*, *P. mutabilis*, *P. articulatus* and *P. richardi* and the remaining five species occur in the North-eastern Atlantic Ocean, namely *P. lineatus*, *P. sauciatus*, *P. atratus*, *P. punctulatus,* and *P. mariae* (Figure 2) [5].

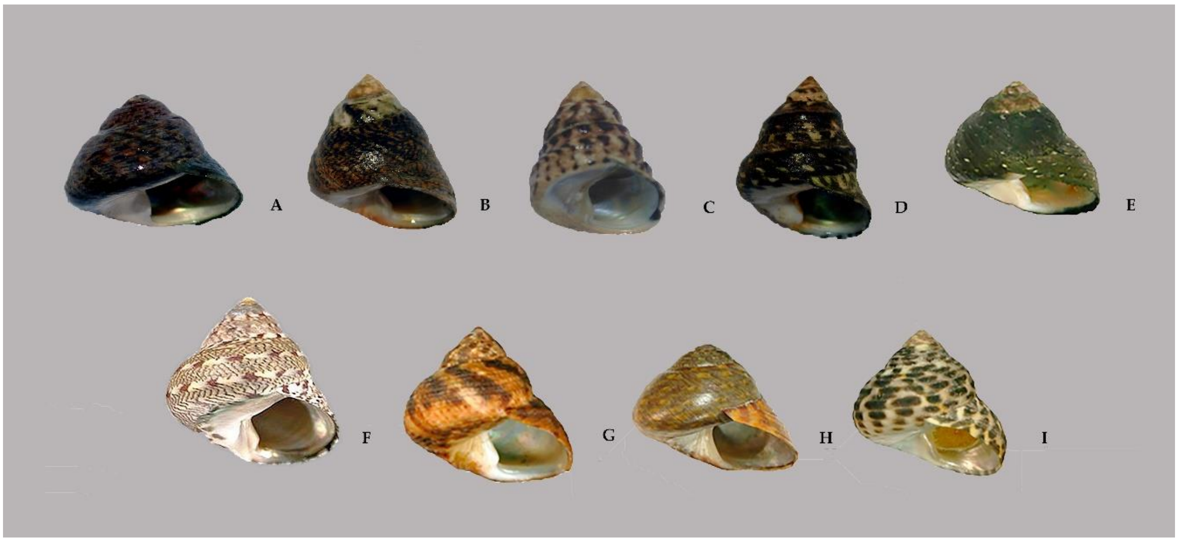

Figure 2 - Shells of the nine species of the genus *Phorcus*. A – *Phorcus sauciatus* from Madeira archipelago, B – *Phorcus lineatus* from mainland Portugal, C – *Phorcus atratus* from Selvagens Islands, D – *Phorcus mariae* from Cape Verde archipelago, E – *Phorcus punctulatus* from Senegal (NMR 36429) [17], F – *Phorcus articulatus* from Spain (NMR 36447) [17], G – *Phorcus mutabilis* from Greece (NMR 36658) [17], H – *Phorcus richardi* from Greece (NMR 36669) [17], I – *Phorcus turbinatus* from Greece (NMR 36606) [17]. Images E, F, G, H, I by Joop Trausel and Frans Slieker and available online at NMR – Natural History Museum Rotterdam [17].

In the North Atlantic Ocean, *P. lineatus* is the species that reaches the northernmost geographic limits of the genus *Phorcus* in North Wales and Ireland and *P. punctulatus* the southernmost limits in Senegal. *P. mariae* is restricted to Cape Verde archipelago, *P. atratus* to the Canaries archipelago and Selvagens Islands, and *P. punctulatus* to Senegal [1, 5, 14]. *P. lineatus* has a wide distribution ranging from North Wales and Ireland to Morocco and *P. sauciatus* includes the Macaronesian archipelagos of Madeira, Canary, and Azores with its northern boundary in the Iberian Peninsula and its southern limit in the African mainland, with negligible genetic differentiation between them, suggesting either recent or continuing dispersal among these areas [5, 18, 19].

Concerning the geographic distribution of the genus *Phorcus* in the Mediterranean Sea, *P. turbinatus* occurs from Spain to Cyprus, *P. articulatus* from Spain to Tunisia, *P. richardi* from Spain to Croatia and *P. mutabilis* from Italy to Turkey [5].

Topshells and limpets are subject to an array of environmental stresses due to their extended vertical distribution, which ranges from the upper to the lower shore levels. Thus, these organisms can exhibit varying degrees of structural adaptations since their position relative to the shore influences their exposure to desiccation, hydrodynamic action of the waves, temperature variation and tidal width [20-23] resulting in a wide array of intraspecific phenotypic variability.

2.3. Respiratory system

Marine snails of the genus *Phorcus* have a gill for water breathing and a well vascularised mantel cavity which allows the animal to breathe in the air [14]. The mantle cavity placed between the body and its overhanging mantle skirt, is constituted by a single gill in the front part of the mantle cavity and thin-walled organs that absorb oxygen from the sea water [12].

The marine snails' blood, the haemolymph, contains haemocyanin, a copper-containing protein that can fix and transport two to three times more oxygen, from the gills to the heart, than organisms without this protein. The heart pulsations push the oxygen-rich blood over a closed system of arteries that lead the blood to a system of open arteries, without epithelial walls, that surround the viscera and the muscles covering all organs with oxygen-rich blood. The body organs receive the oxygen from the haemolymph and release carbon dioxide into it, which then returns to the gills, via a system of veins, where it releases the carbon dioxide and again receives oxygen [12].

2.4. Feeding habits, behaviour, and ecological importance

Molluscan grazers are known to have an important influence on the overall structure of benthic marine communities, because of the influence and control they exert on algae [24-25]. Removal of grazers often leads to an imbalance on the population dynamics of the species involved on the rocky shores ecosystem, due to a dramatic development of seaweed beds [25].

Topshells, winkles and limpets form a guild of microphagous herbivores that feed on microbial biofilms, by grazing the rocky substrate with the radula, a specialized rasping organ unique to molluscs, on which successive rows upon rows of backwards-pointing teeth are placed. The teeth crack, break, and wear away during use, by the food or the hard substrate from which the sea snail scrapes [12]. Marine snails, can all be found together, grazing on the open shore and it is probable that these various snail species do not feed in exactly the same place, at the same time, in the same manner or on exactly the same food [14] in order to avoid interspecific competition. The feeding adaptations between these species can be behavioural through spatial differentiation or anatomical through adaptations in the radula. Among these species, radulae show different hardness and patterns, being multi-fine-toothed rhipidoglossan in topshells, less complex taenioglossan in winkles and simple docoglossan in pattelid limpets; therefore, it is easy to conclude they feed in different ways [14].

In several species of sea snails, the digestive fluids contain the cellulase enzyme that breaks down cellulose. This is one of the very few cases throughout the animal kingdom of an animal producing an enzyme capable of breaking down cellulose [12]. Feeding behaviour in topshells is assumed to occur at night or during high tide as stated by Crothers [14] for *P. lineatus*. Food particles are gathered by the radula, squashed by the jaws, and then transported inward into the mouth where the digestive track begins, in the front of the body, and then transported back along the body through the oesophagus to the stomach where most of digestion occurs, and finally, digested food loops and descends forwards to the intestine where faeces are formed and expelled by the anus, which drains into the mantle cavity, at the front of the body [12].

Common topshells and edible winkles, swing their head from side to side while crawling and may leave grazing tracks on the rock surface and visible slime trails. Usually, the more active species secrete a thicker layer on which to crawl and this may show up as a pale band over the rock surface. Trail-following, namely the crawling over existing mucus trails, will reduce the expense of producing a mucus trail. These trails might also be used in locomotion back home, to find mates, and assist in feeding, by trapping food particles from the water column [12]. Marine snails crawl by squeezing the front end of the foot against the substrate and by means of a ripple of muscle contraction, pass that point of contact forcing the mass of the snail forwards. In topshells, the two halves of the foot work independently of each other, out of phase, producing a characteristic slime trail [26].

Contrary to limpets, topshells are active at low tide and respond very rapidly to changes in weather conditions, moving out into the open when the sun shines and hiding from rain or cold winds in crevices or under boulders [26]. These species are limited in their vertical zonation by their tolerance to temperature variation; as such, they undertake vertical migrations up and down the shore over the seasons [27].

Wave action also acts as a limiting factor on suspension feeders and on semissessile and sessile organisms that are favoured on exposed conditions, since the water movement allows the flow of food, propagules, nutrients, and preys to these organisms. However, in these habitats, the increase of exposure to wave action involves an increase on the risk of dislodgement and physical damage, limiting the range of susceptible and physically fragile species [2]. In order to overcome the adverse conditions of the exposed areas, intertidal gastropods inhabiting these areas have a thin and smooth shell with large aperture due to the large foot required to cope with the higher risk of wave displacement and to be able to maintain a firm hold on rocky surfaces [28, 29]. In dangerous circumstances, a snail withdraws into its shell and adheres firmly to the substrate, so as to not be detached by waves or predators [12]. In the North-eastern Atlantic, *P. lineatus* is usually used as an indicator of sheltered rocky shores [30] contrary to *P. sauciatus* that seems to be more tolerant to wave action being found lower on the shore but also able to establish on sheltered zones [18]. The anatomic features of these two species corroborate this hypothesis since *P. sauciatus* thinner shell, larger foot, and consequently large aperture, implies that this species is more tolerant to wave action than *P. lineatus* with thicker shell and smaller aperture. On the other hand, these anatomical differences result in *P. sauciatus* being less tolerant to desiccation than *P. lineatus*.

2.5. Growth

Growth is a key variable in determining the survivability of any given animal, and it is important to understand the factors that drive it [31]. Biological parameters such as growth rate, asymptotic length, longevity, and age structure reflect the overall state of health of a population and are commonly used as stock assessment tools of exploited marine organisms [4]. In gastropods, growth rates have been determined through several features such as growth lines and rings in shells [32, 33], opercula [34], and statoliths [35]. Size and age of topshells are positively related, thus allowing to investigate population structure [36].

Size and growth rates in the species of the genus *Phorcus* are influenced by fluctuations in food supply [26, 37], competition [38], and wave action [39], while population density is mainly controlled by the successful settlement of larvae and predation [26, 38]. The oceanographic current systems are known to be largely responsible for the water temperature and nutrients of the coastal ecosystems, which marks the distribution and behaviour of organisms throughout the coastlines [2]. As such, temperature also influences growth in the species of the genus *Phorcus*. For instance, Crothers [14] and Mannino et al. [40] observed that a decrease in water temperature promotes a metabolic deceleration, resulting in the interruption of growth during the winter in *P. lineatus*. However, after this season growth continues rapidly through the year, slowing only in the next winter. In general, in the first year, the growth rate of this species is high and decreases thereafter [14] as a possible result of achieving sexual maturity. In the first six months post settlement, specimens can grow up to 8 mm diameter, reaching 11 to 15 mm by the end of the year [41]. Although the growth rates slow down dramatically after the achievement of the sexual maturity, since energy is mostly directed towards reproduction, growth continues throughout the life cycle of this species. In habitats with low abundance, *P. lineatus* grows rapidly to a large size and reaches maturity early but has lower longevity. While individuals that live in habitats where they are more abundant, grow slowly, do not achieve great size and may live to an older age. These differences in growth are likely related to different levels of food availability depending on population density, which in turn is related to settlement success and predation evasion [26]. The specimens of this species have been known to reach a size of 34 mm in shell height and a longevity of 15 years of age in southern Britain [36]. *P. sauciatus* have approximately the same size range of *P. lineatus*. For instance, in the Madeira archipelago, *P. sauciatus* size ranges from 2 to 28 mm (pers. obs.); in the Canary Islands, this species size ranges from 5 to 26 mm [42]; and in the Portuguese mainland coast its size ranges from 7 to 24 mm (pers. obs.). There is, however, a great gap in knowledge concerning life history parameters of *Phorcus* species. Most studies focused on *P. lineatus* due to their wide geographical distribution spanning from Morocco to North Wales/Ireland. Life history parameters such as growth rates, asymptotical length, size at first maturity, recruitment patterns, and mortality of *Phorcus* species are likely to differ inter- and intraspecifically as a result of different biotic and abiotic factors. Further studies on the biology and population dynamics of *Phorcus* are therefore required in order to guarantee the implementation of successful conservation strategies and a sustainable exploitation based on effective management measures.

2.6. Reproduction

The reproductive system of topshells is usually strikingly simple, with a genital duct opening into the mantle cavity through the right kidney. Sea snails commonly have separate sexes, but these species are not externally sexually dimorphic and sex determination is only possible through macroscopic observation of the gonads. Internally, the most reliable character for sorting them is the appearance of the urogenital aperture. In males, the lips of this organ are unpigmented and smaller, while in females, the lips are yellow and swollen. Nevertheless, in the ripe state, males have cream testis and females greyish-green ovary covering the digestive gland and viscera [43, 44], being therefore easily differentiated in the breeding state. The lobes of the gonad, whether ovary or testis, lie near the apex of the visceral hump, among the lobes of the digestive tube, and they drain into the pericardium [12].

Prior to the breeding season, adults migrate up shore to the high eulittoral zone. It seems that this migration brings the animals into a region of higher temperature required for spawning. An increase in temperature may stimulate spawning as suggested by Desai [44], who observed that adults that have migrated furthest up shore were the first to spawn.

In fact, spawning in intertidal organisms seems to be promoted by environmental triggers such as, temperature, high wind speed, and wave action. Biological factors as an increase in phytoplankton concentration may also stimulate spawning as occurs in limpets [38, 45]. As such, breeding stages of a given species may differ according to their geographical position. In fact, in the northernmost range limit, breeding seasons are shorter with a single spawning period while in southern regions the breeding season is longer with multiple spawning events [46, 47]. For instance, in *P. lineatus* from Asturias, Spain, the gonadal development occurs from November to June and the breeding stages from June to September and may last until November in some specimens [46]. Spawning occurs between May and August [48]. Further north in Wales, the same species is reported to have a shorter spawning season, lasting from July to August [14]. On the other hand, *P. turbinatus* that occurs in the Mediterranean Sea appears to have a longer breeding period with two spawning events in spring and autumn [49].

Fertilisation is external, with both sexes releasing their gametes into the sea and the whole process occurs directly in the water. During the reproductive season, males and females approach each other and then females send out chemical signals, leading to sperm being discharged in the water by males, which in turn stimulates females to release the oocytes [12]. According to Desai [44], males discharge clouds of spermatozoa that become very active 2 or 3 minutes after being released, and females liberate oocytes separately, a few at each spasm. This process of external fertilisation, regarded as a primitive trait in snails, becomes a high-risk strategy and improbable to succeed unless the species is locally common [14]. The fertilised egg develops within approximately a day and becomes a trochophore larvae which are capable of independent locomotion. The veliger larvae enclosed in a tiny shell develops in one or two days. At metamorphosis, the veliger turns upside down with the foot becoming ventral and the shell dorsal. Posterior to the snail's development, the back dorsal rotates in 180º anticlockwise in relation to the head and foot. Veliger larvae remain in the water column for at most 6 to 7 days [5, 14, 16] and at settlement, the shell measures a little over 1 mm across [14]. According to Heller [12], the trochophores of the genus *Phorcus* hatch down shore, within approximately one day and the veliger settles 4-5 days with about 1 mm. For *P. lineatus* in the United Kingdom, the recruits achieve 5 to 6 mm shell length by the first autumn and are detected on the bare rock between September and November and recognized, with 6 to 14 mm, through their first year [33].

The gap in size at settlement and size at first capture reported for topshells may be understood as a potential argument for the existence of nursery areas, underneath boulders or fissures, in which small juveniles are much commoner, but there appears to be no uniform pattern [14]. For instance, in Madeira archipelago, the juveniles of *P. sauciatus* are commonly found under boulders, with the smallest individuals having 2 mm in diameter (pers. observ.). These boulders may function as a nursery for topshell juveniles as they provide protection against abiotic factors, such as wave action and desiccation, and biotic factors, such as predation and substrate competition.

3. Anthropogenic impacts on the genus *Phorcus*

3.1. Harvesting

Intertidal and shallow-water grazers are extremely vulnerable organisms because of their limited habitat and its accessibility to human activity [50]. Hunter-gatherers have exploited intertidal grazers, since prehistoric times, and there are evidences that the densities and the maximum sizes of several species were reduced by the exploitation [51, 52]. Studies performed in Northern Spain, showed that topshells and limpets were collected, at subsistence exploitation levels, from intertidal areas of exposed shores, leading to the formation of huge shell middens [53]. In fact, intertidal resources have always been collected by humans as food supplement or used as a bargaining chip with other products worldwide [54, 55].

Several studies were carried out aiming to investigate the temporal patterns of topshells exploitation worldwide. A proven approach to study these temporal patterns of prehistoric shellfish exploitation is the analysis of the oxygen isotopic ratio (d18O) of the latest growth increment of mollusc shells [56, 57]. Variations in oxygen isotope ratios from shell carbonates are mostly dependent on sea surface temperature (SST), which enables the estimation of temperatures during periods of shell growth and helps to determine the season of the year when the mollusc died [58]. Colonese et al. [59] applied this approach to the topshell *P. turbinatus* from archaeological sites in Italy and concluded that Mesolithic *P. turbinatus* exploitation was carried out almost exclusively during the colder and intermediary seasons, with very sporadic harvesting during the warmer seasons.

The same approach has been followed by Gutiérrez-Zugasti et al. [58] that confirmed the potential of oxygen isotope analysis on *P. lineatus* for paleoclimate reconstruction and also showed that the aragonite of those shells grew under conditions of isotopic equilibrium, opening new avenues for future research. This species is commonly found in Holocene archaeological deposits of Atlantic Europe and is one of the most abundant subsistence resources utilized during the Mesolithic in northern Spain.

Continued exploitation of these species is likely to incur in shifts on size and shape over time. Colonese et al. [59] observed a significant change in shell shape of *P. turbinatus*, with slender Mesolithic shells being replaced by squatter forms in the Meso-Neolithic. These differences were explained with collection shifting from sheltered shores in the Mesolithic to exposed rocky shores in the Meso-Neolithic, thus confirming the potential effect of human collection on size and shape of this exploited species.

In recent times, however, the pattern of exploitation has changed both quantitatively and qualitatively, due to the expansion of human population, to the commercial value of several species and to the industrial development that facilitated shipping and flying products around the world [25]. Limpets, abalones, chitons, winkles, and topshells are common gastropods of intertidal rocky shores; however, some species are in serious decline mainly as a consequence of overexploitation [60]. The exploitation of these resources has plentiful direct and indirect effects on the trophic chains of marine ecosystems, with potential complex cascading effects [61].

The direct effects of exploitation are the decline of the exploited species´ abundance and a shift in size composition of their populations, that results from the size-selective nature of harvest. Ramírez et al. [42] assessed the effects of human impacts over the abundance and size patterns of topshells (*P. atratus* and *P. sauciatus*) and limpets (*Patella aspera* Röding, 1798, *Patella candei* d'Orbigny, 1840, and *Patella rustica* Linnaeus, 1758), usually collected in the Canaries archipelago. The author's observed significant differences in size structure of these species among islands, according to the level of human influence and verified that not only all large-sized individuals disappeared from the most populated island, but also that there was a decrease in numbers for the majority of size ranges, concluding that the observed differences among islands were mainly a consequence of the human activities. In fact, in exploited populations of broadcast spawners such as topshells and limpets, the decrease of larger individuals will reduce the reproductive success leading to a decrease in population abundance and, in extremes cases, conduct to the disappearance of the species [62, 63].

Also, differences on spatial distribution of the abundance and biomass of *P. articulatus* were observed by Cheour et al. [64] along the coast of Tunisia. The authors concluded that these differences were related to several anthropogenic and environmental factors.

Even though species of the genus *Phorcus* have been exploited by humans since prehistoric times, information regarding the status of exploited stocks is scarce and exploitation is generally unregulated. Recently, some efforts have been undertaken in the Canaries archipelago, aiming to contribute to the recovery of the stock of *P. sauciatus* and *P. atratus* in a short and medium term, namely by implementing a minimum capture size of 15 mm of shell length for both species [65].

Overexploitation of marine organisms prompts the implementation of management policies in order to protect the exploited populations and mitigate human impacts. Currently, protection of *Phorcus* species is mostly guaranteed by the implemented Marine Protected Areas (MPAs); however, further measures should be equated especially in regions where exploitation of these species is more intensive. Management measures and regulation aiming at a sustainable exploitation of these species are therefore warranted, as is the improvement in enforcement of existing legislation and involvement of all interested stakeholders; otherwise, protection of topshells will remain ineffective.

3.1.1. Harvest of *Phorcus sauciatus* in the Madeira archipelago: an historical perspective

P. sauciatus is the more abundant of the two species of the genus *Phorcus* described for the Madeira archipelago and has a wide geographical distribution, occurring in all islands including Madeira, Porto Santo, Desertas, and Selvagens. *P. atratus* is also present in this archipelago as the endemic subspecies *Phorcus atratus selvagensis* restricted to the Selvagens Islands. However, according to Donald et al. [5], the classification of this subspecies needs additional clarification.

P. sauciatus is harvested in the Madeira archipelago since early colonization times, remounting back to the beginning of the fifteenth century. According to Silva and Meneses [66], *P. sauciatus*, formerly identified as *Trochus colubrinus* Gould, 1849, occurred in the intertidal zone of all the islands and was consumed salted or pickled, being imported from the Selvagens Islands in a relatively large quantity. This species was also used as bait for fisheries.

Nowadays, *P. sauciatus* continues to be caught in Madeira and Porto Santo, except in marine protected areas, without harvest regulation or auction obligation. The harvest of this species in the Madeira archipelago became more intensive due to the development of their commercial exploitation supported by technological advances in methods of collection, processing, storage, and transportation, but also due to the increase in human population density and the accessibility to the coastal zones. As such, shifts in abundance and/or size structure of this species occurred mainly in the south coast of Madeira Island, resulting in a reduction in abundance levels and sizes of the caught specimens, due to the existence of more favourable environmental conditions, higher population density, and easier accessibility.

Given the current scenario, it has become vital to know the biological and ecological traits of *P. sauciatus* in Madeira archipelago and its population dynamics. As such, the Fisheries Research Service from the Regional Directorate of Fisheries of the Autonomous Region of Madeira, presently developed a full study on this species. Its aim to establish proper conservation strategies, in order to preserve this important keystone resource of the intertidal zone, that would contribute towards the reduction of the risks of overexploitation and promotion of a sustainable harvest of *P. sauciatus* in the Madeira archipelago, through the implementation of suitable regulation and management measures considering the biological and ecological specificities of this species in this region. At a first glance, the implementation of regulation concerning harvest techniques, maximum allowable catch weight and minimum catch size should be considered and also mandatory landings. Depending on the results obtained in the study, other measures might have to be pursued in order to provide an adequate management for a sustainable exploitation of this resource such as the establishment of a closed season to ensure optimal reproductive success.

3.2. Pollution: topshells as bioindicators of habitat health

The ecological effects of increasing levels of heavy metals concentrations in the environment are of great concern due to their high bioaccumulative nature, persistent behaviour, and high toxicity [67].

The increase of human population and anthropogenic activities, such as the development of industry on the coastline, are the major responsible factors for pollution hot spots that occur predominantly close to major ports, industrial areas, and cities [68]. Maritime traffic also acts as a source of pollution due to the antifouling paints of boats [69]. Marine and especially coastal ecosystems are increasingly endangered by the large amounts of metal pollutants, arriving to this environment mainly by superficial runoff of rain, by direct atmospheric deposition, and by discharges from sewage effluents, spillage, and industrial establishments [70, 71]. Biological and physiological alterations in benthic communities may occur due to the toxic effects of metals and to the sedentary lifestyle of these species [72]. Aquatic organisms can accumulate petrogenic and anthropogenic compounds such as n-alkanes, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) from the environment into their lipid tissues, some of which can be either carcinogenic and/or highly toxic for living organisms [71]. Most of the comparative studies between taxonomic groups indicate that bioaccumulation of pollutants in molluscs is, in general, much superior than in fish [73]. Molluscs shell and tissues reflect the higher degree of environmental pollution by heavy metals and are the most useful bioindicator tools. The metals body burden in molluscs may reflect the concentrations and availability of heavy metals in the surrounding water and sediment and may thus be an indication of quality of the surrounding environment [74]. These organisms accumulate comparatively higher concentrations of metals, both from water and sediment, because of their sedentary nature [67].

The worldwide increase of pollution levels on coastal zones, has led to the awareness of the need to perform ecotoxicological research and to define sensitive bioindicators that allow the evaluation of contamination degrees, aiming to recommend the appropriate measures to conserve the ecology of the coastal areas. The species of the genus *Phorcus* act as sentinel species due to their particular eco-biological characteristics, as abundance and wide geographical distribution, long life span, suitable dimensions, easy identification, and collection, becoming a useful biomonitoring system of pollutants in the marine environment and making these organisms suitable to measure for hazard and risk assessment. These molluscs are also sturdy enough to survive in laboratory and field studies and tolerant to environmental alterations and various contaminants [67].

P. turbinatus is generally considered as a bioindicator of metal pollution in coastal areas [69, 75], because of their ability to tolerate temperature and salinity fluctuations and survive even in hypoxia [75]. Boulajfene et al. [77] evaluated the degree of metal contamination in *P. turbinatus* and monitored the impact of metals on metallothioneins functioning in the North-eastern and the Eastern coasts of Tunisia and found that sedimentological metallic contents of copper, zinc, and cadmium vary according to the area where these species live. It seems that this species has an ability to accumulate these metals and that the metal effect on protein induction may be linked to physical factors such as temperature, oxygen, and copper contents in sediment. Boucetta et al. [78] assessed the health status of *P.*

turbinatus on the Algerian East coast through the analyses of the concentrations of trace metals in this species tissue, and of biomarkers such as the activity of acetylcholinesterase (AChE) and Glutathione-S-transferase (GST) and verified that the alteration of the activity of AChE with the induction of GST was mainly due to the presence of high concentrations of trace metals, and abiotic factors including salinity and pH.

In fact, several environmental factors such as water current, water flow, renewal of water, pH, and salinity affect the distribution of heavy metals in molluscs as reported by Grupta and Singh [67]. Survival is significantly affected by salt concentration and by temperature, as well as by the interaction between them, so that the toxicities of salts are generally enhanced at higher temperatures.

Other studies support the efficacy of topshells as bioindicators, such as Bordbar et al. [79] who investigated the impact of a ferronickel smelting plant on the coastal zone of Northern Greece, through the study of metal bioconcentration in *Patella caerulea* and *P. turbinatus*, concluding that the ferronickel smelting plant had heavily impacted the coastal zone. Another study on the southeast coast of Tenerife reports the use of *P. atratus* in the evaluation of the concentrations of n-Alkanes and PAHs in the visceral mass and demonstrated that this species is contaminated with a chronic background of aliphatic and hydrocarbons strongly retained in his lipid tissues and suggested this species as a bioindicator of petrogenic contamination [71]. Cabral-Oliveira et al. [80] presented further evidence by comparing the accumulation of trace elements in edible molluscs *Mytilus galloprovincialis* Lamarck, 1819, *Patella ulyssiponensis* Gmelin, 1791, and *P. lineatus*, between one sewage-impacted area and two reference areas in central western coast of Portugal and suggested that the concentrations of trace elements in the soft tissues of these molluscs can be affected by the presence of sewage discharges.

Measurement bioaccumulation of pollutants in molluscs, in general, is much superior to that in fish due to their sedentary nature. Thus, their shell and tissues reflect the levels of environmental pollution and are the most useful bioindicator regarding the quality of the surrounding environment. As such, there is a growing interest in the use of these marine gastropods as bioindicators, due to their ecobiological features, both in a scientific and ecosystem management perspective. This approach will contribute to the establishment of conservation measures targeting marine coastal environments. Also, all species of the genus *Phorcus* are a food source for other species; therefore, if these species are contaminated there is a potential for biomagnification in the food chain that can carry serious risks both to wildlife and human health.

3.3. Climate change effects on intertidal communities: impacts on topshells of the genus Phorcus

The history of earth is riddled with events that have shaped different ages, each with specific conditions that characterized them. One of these characteristics is global temperature that has oscillated numerous times over the course of earths' long history and thus shaped biodiversity throughout the ages. For instance, the change in mean temperature between the late Pleistocene (colder conditions) and the early Halocene (warmer conditions) lead to a *taxa* alteration between these two periods. The more abundant species adapted to cold water, such as the periwinkle *Littorina littorea* (Linnaeus, 1758) and the limpet *Patella vulgata* Linnaeus, 1758 in the late Pleistocene, were replaced by species better suited to warmer conditions such as *P. lineatus*, *Patella depressa* Pennant, 1777, and *P. ulyssiponensis* in the Holocene [81, 82]. A similar pattern is visible today in the Cantabrian coast, with a predominance of warmer species such as *P. lineatus* and the absence of *L. littorea* [53].

Nowadays, however, global climate change is recognized as a reality, driven mostly as a direct consequence of human activity [83, 84], namely, through the cumulative post-industrial carbon emissions to the Earth's atmosphere [85]. Known consequences of climatic change in the marine environment are the increasing global temperature, perturbed regional weather patterns with increasing wind velocity and storm frequency, rising sea levels, ocean acidification, changed nutrient loads and altered ocean circulation [86]. These and other physical consequences are affecting marine biological processes from genes to ecosystems, over scales from rock pools to ocean basins, impacting ecosystem services and threatening human food security [85]. The rates of physical change are unprecedented in some cases and biological changes are also likely to occur at a quick rate, although the resilience of organisms and ecosystems is highly variable. Biological changes founded in physiological response manifest as species range changes, invasions and extinctions, and ecosystem regime shifts [85].

Coastal ecosystems are among the most vulnerable to climate change, especially the intertidal areas, which have shown faster biogeographic changes [88, 89] than those found in terrestrial environments [89]. Long-term monitoring studies have shown that the distribution limits of the intertidal biota of hard substrates have progressed towards the poles at a rate of over 50 km per decade [88, 90, 91].

Invertebrates and seaweeds, inhabiting the intertidal zone, may be particularly vulnerable to fluctuating temperatures, since individuals must adapt to the extreme temperatures of both the terrestrial and marine environments [92]. Even in small spatial scales in the intertidal zone, a broad range of thermal conditions are found that may exceed the range of large latitudinal bands. Therefore, intertidal organisms are believed to be at the limit of their physiological tolerance since these organisms are sorted by zonation in which the upper limit of one species is set by physiological stress, and species replace one another moving up the shore [88, 93]. The species most tolerant to heat and desiccation live at the top of these zones [94]. Since these organisms are thought to live at the utmost extremes of their physiological tolerance limits, any changes in abiotic parameters such as temperature and air exposure time could lead to death or local extinction [95, 96].

On the other hand, these changes can also lead to the expansion of the range and distribution area of some species. Thus, intertidal ecosystems are thought to be amongst the first to show responses to increases in global temperatures [95, 97] and are potential environments to assess the effects of climate change [98].

Rising temperatures can result in increased thermal stress and desiccation at low tide and in latitudinal changes in species abundance and distribution. However, not only changes in temperature affect the rocky intertidal; for instance, rising sea levels can result in altered zonation of intertidal biota and compression on vertical engineered defences. Also, increased storm frequency can result effectively in higher levels of wave exposure, resulting in shifts in community structure, due to a replacement of grazers by filter feeders, and shifts in direction of trophic control [85].

Intertidal organisms are subject to other factors that can lead to significant physiological stress and mortality such as shifts in salinity, increased levels of siltation, and prolonged oxygen or nutrient deprivation [99-102]. These factors play an important role in reproduction and survival of these organisms and are predicted to change in the coming decades as a result of global climate change. In fact, some of these changes are believed to have already occurred as ecological impacts on coastal ecosystems [103].

A species geographic limit reflects the interactions of organisms and their environment and is likely one of the first signals of the effect of climate change on the biota of the planet [89]. Geographical range limits impose environmental stresses, such as temperature, to populations that restrict adult survival or juvenile recruitment [88, 93]. This is related to the organisms' physiological tolerance to temperature. Exceeding these tolerance limits results in the organism's death and can lead to the local extinction of a population if temperatures are extreme enough [89]. Changing climatic conditions results in shifts of geographical limits in which populations can survive and reproduce thus acting as indicators of the processes of long-term climate change [88, 89, 93].

Species of the genus *Phorcus* like other intertidal organisms are considered good indicators of the effects of climate change in marine ecosystems. For instance, *P. lineatus* has been identified as an indicator species for monitoring climate changes around the coasts of Western Europe [104] due to its extensive biogeographic distribution, ranging from North Wales and Ireland to Morocco [5, 14]. Crothers in 1994 [33] showed evidence that the geographical range edge of this species has extended in the Bristol Channel eastwards along the Somerset coast for a least 20 kilometres in the past 50 years and suggests that it may be still advancing. In fact, a decade after Mieszkowska et al. [105] reported that *P. lineatus* and *Gibbula umbilicalis* (da Costa, 1778) have undergone North and North-eastern range extensions in Britain, with the increased in abundance of the populations and a decrease in adult size. According to Mieszkowska et al. [104], the range limits of *P. lineatus* in the British Isles have extended by up to 55 km, between the 1980s and the 2000s, even though the extremely cold winter of 1963 in the west and south of Britain [106] prompted a cold induced mortality [107]. The recovery of these populations occurred in subsequent warmer years with breeding populations being found up to and beyond their limits before the cold spell [105]. These shifts have been synchronous throughout this geographic region, strongly suggesting that a large-scale factor such as climate is responsible for the observed changes.

The extension of northernmost geographic limits of *P. lineatus* in the North-eastern Atlantic is one among several evidences of range shifts that have been reported in recent years and is in accordance with Helmuth et al. [88] that reported that intertidal species range limits may be shifting by up to fifty kilometres per decade.

Another possible example of geographic range extension due to climate change could be the colonization of Santa Maria Island in the Azores archipelago, by *P. sauciatus* that occurred very recently, probably after 2009 [19]. The founder population has been able to recruit itself and is currently mostly constituted by specimens under 2 years of age. Presently, this species is restricted to the most occidental island of the Azores archipelago, the nearest island to the Portuguese mainland, and to the archipelagos of Madeira and Canaries where this species is well established since colonization times. According to the same authors, a successful colonization of the remaining islands of the Azores is predicted. Even though the driving forces that lead to the recent establishment of a population of this species in this island are unknown, there is a strong possibility that it is related to the increase of sea surface temperature (SST) in the North-eastern Atlantic (Figure 3) [108-110]. One of the determinants of successful reproduction and recruitment of *P. sauciatus*, a sub-tropical species, is sea surface temperature. According to Hutchins [110] sub-tropical species require warmers summers in order to guarantee reproductive success; as such, an increase in SST in Azorean waters could have played an important role in the successful establishment of *P. sauciatus* in Santa Maria Island.

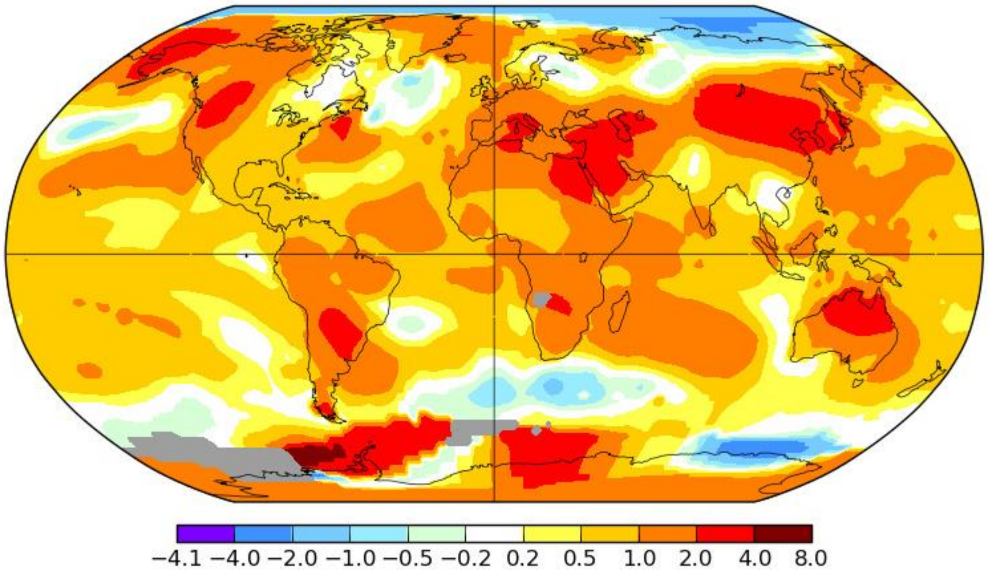

Figure 3 - Map representing the spatial variability in surface warming. The temperature anomaly (°C) is colour coded for July 2017 compared to the mean surface temperature for the period of 1951 to 1980. Grey pixels indicate missing data [109, 110].

Changes on the abundance and distribution of *P. sauciatus* could be directly or indirectly related to climate and oceanographic events that result in an increase of SST [18]. Historically, the geographic range of this species on the Northwest coast of the Iberian Peninsula is characterized by the existence of a distribution gap between southern Galicia and northern Portugal possibly related to upwelling events in the region. The first records of *P. sauciatus* having colonized, in the early years of the twenty-first century, at least partially its distribution gap were presented by Rubal et al. [18]. These authors suggested that colonization occurred from the east and north in westward and southward direction from South Galicia to North Portugal. The beginning of the expansion in the distribution of this species in South Galicia coincided with a warming in SST in the North-eastern Atlantic due to global warming [111, 112] by the end of the 1980s and early 1990s that was responsible by similar range expansion of warm water species in the English Channel [103, 113]. Rubal et al. [18] suggest that the weakening of the upwelling since the 1940's lead to an increase in SST that could have been responsible for the recent colonization of these regions by *P. sauciatus*.

These changes in oceanographic conditions could result in shifts on the distribution and abundance of *P. sauciatus* along its northern boundary; such patterns have already been reported for other gastropods in this area [114] and other *Phorcus* species at northern latitudes [104, 105].

Climate change increases the level of environmental stress to which intertidal organisms are usually subjected to and these may severely affect the functioning of biological systems at different levels of organization. The reviewed works of several authors provide strong evidence of the suitability of *Phorcus* species as indicators of global climate change. This is particularly true for populations in the geographic boundaries of these species that can expand up to fifty km per decade, affecting ecological interactions and community structure of the intertidal ecosystems. Alteration of vertical zonation of these assemblages is another consequence of climate change that can be inferred using *Phorcus* as indicators, since these species occur at their physiological tolerance limits. Changes in temperature, climatic patterns, and oceanographic features directly affect biological processes, which can scale up to the assemblage level, thus affecting different levels of biological organization. For instance, reduction in body size and changes in reproductive cycles are recognized as universal responses of intertidal organisms to global warming. Further studies are required, in order to provide information concerning biological parameters of *Phorcus* species and how they are affected by climate change, consolidating these species as indicators of large scale ecological impacts of climate change.

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Chapter 3

Filling biological information gaps of the marine topshell *Phorcus sauciatus* (Gastropoda: Trochidae) to ensure its sustainable exploitation

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Abstract

Topshells play a pivotal role in intertidal rocky ecosystems and are adapted to harsh thermal and desiccation. *Phorcus sauciatus*, a common grazer in the Macaronesian region (Madeira and the Canaries), has rarely been studied due to its restricted geographic distribution compared to *Phorcus lineatus*. Monthly samples were taken throughout 2017 to analyse biological parameters and evaluate the harvesting effect on the stocks of this species in Madeira. Individuals of the first age classes (<4 years) were dominant (approximately 89%), while immature individuals were more abundant during the summer season. The spawning season occurs between March and August, especially from March to June. The size at first maturity was 12.95 mm long (1.68 years), with a continuous recruitment pattern throughout the year. Yield-per-recruit analysis (*Y*/*R*) showed that maximum production is achieved at a fishing mortality of 1.7 y^{-1}, corresponding to a *Y*/*R* of 0.023 g. Currently, *Phorcus sauciatus* seems to be moderately exploited in Madeira, but urgent conservation measures, such as a landing obligation, the establishment of a minimum catch size of 15 mm length, and a closed season (February-May), are warranted to preserve stocks of this species in the medium to long term.

Keywords

Life history, north-eastern Atlantic, *Phorcus sauciatus*, sustainable exploitation, yield-per-recruit.

1. Introduction

Topshells, limpets and winkles are among the most successful intertidal algal grazers in the North-eastern Atlantic Ocean and the Mediterranean Sea (Templado & Rolán, 2012). These marine gastropod molluscs are subject to one of the most extreme and dynamic natural environments, occurring from the supratidal to the subtidal zones in rocky shores. Topshells are exposed to different levels of thermal and desiccation that result in specific morphological and biological characteristics related to adaptations to the harsh environmental conditions on rocky shores (Ramírez et al., 2005; Donald et al., 2012; Henriques et al., 2017). They play a pivotal role in the intertidal ecological balance and are frequently used as biological indicators of anthropogenic impacts (Sousa et al., 2018).

The ecological importance of the genus *Phorcus* Risso, 1826 on the overall structure of intertidal communities, particularly their influence and control on algae, prompted intensive research over the past decades, highlighting that the removal of these grazers leads to imbalances on rocky shore communities (Creese, 1988; Branch & Moreno, 1994). However, most of the studies concerning this genus focused on *Phorcus lineatus* (da Costa, 1778), and little information is available on *Phorcus sauciatus* (Koch, 1841). Nonetheless, local information on the population structure and distribution of *P. sauciatus* is available from the north-west Iberian Peninsula (Rubal et al., 2014) and the Canary Islands (Ramírez et al., 2005, 2009; Alfonso et al., 2015).

Phorcus sauciatus, a common temperate-subtropical grazer that inhabits extensive and gently sloping rocky shore platforms in the eastern Atlantic, including the Macaronesian archipelagos of Madeira, the Canaries, and the Azores, reaches its northern boundary in the Iberian Peninsula (Rubal et al., 2014; Ávila et al., 2015). The life history traits of this species vary intraspecifically due to genetic differences and environmental effects. The size and age of these gastropods are positively related, thus allowing studies on population structure, reproductive strategy, growth rates, mortality, and longevity (Crothers, 1998). These parameters depend on a complex combination of selective forces and are required to understand the distribution, abundance, and adaptations of this species to an ever-changing environment. Additionally, the knowledge on the life history traits of these populations will play a pivotal role in providing proper background information for effective management of this important resource.

Exploitation of *P. sauciatus* in Madeira dates back to the fifteenth century when the archipelago was colonized by the Portuguese and has become more intensive due to the demographic increase of human settlement of the islands' coast and the technological advances that facilitate access to the whole coast (Silva & Menezes, 1921; Sousa et al., 2018).

The harvesting of these molluscs is not regulated in the Madeira archipelago except for coastal natural reserves where harvest is forbidden. The absence of proper harvest regulation results in the landings of this species not passing through auction, which prohibits knowing how much effort is exerted on the harvested populations. Knowledge gaps regarding the life traits and population dynamics of this species are one of the key factors contributing to the lack of harvest regulation (Sousa et al., 2018). These gaps prompted an intensive collection of biological data in the scope of a comprehensive biological and ecological study that aimed to provide the scientific information required for the implementation of conservation measures and the proper regulation of harvesting activities.

Herein, we aim to (i) provide information on the biological parameters and exploitation rate of *P. sauciatus* in Madeira (NE Atlantic Ocean) and (ii) evaluate the effect of size at first capture on the exploited stock by applying a yield-per-recruit model. These objectives are pivotal for developing a series of conservation measures to preserve stocks of this mollusc, such as the establishment of a minimum catch size, the introduction of a closed season and the implementation of mandatory landings and first sale at auction of this commercially harvested species.

2. Material and methods

2.1. Data collection

Monthly samples of *P. sauciatus* were collected from three locations (São Vicente, Santa Cruz and Ribeira Brava) throughout the coast of Madeira (32.00°-33.05°N; 15.05°-18.00°W), located in the north-eastern Atlantic, between January and December 2017. Specimens were randomly collected from the mid-to-lower intertidal zone of the rocky shores during low tide, without selecting for size, for a period of 15 minutes. Shell length (L) was measured using a Vernier calliper to the nearest 0.01 mm, and total weight (W) to the nearest 0.01 g was measured using an electronic scale. Specimens were removed from the shell and dissected for sexing purposes by macro- and microscopic observation of the gonads (Desai, 1966). Macro- and microscopic inspection of the gonads allowed for the assignment of each specimen to one of the five gonad maturation stages based on an adaptation of Desai's (1966) maturation scale. In stage I, female and male gonads have pink-brown pigmentation. The germinal epithelium in both sexes are ill-defined and oocytes are approximately 25 µ in diameter; in stage II, females have greenish gonads, and males also have irregularly greenish gonads. Females show a well-defined germinal epithelium with numerous oocytes, measuring up to 45 µ in diameter, in each trabecula divided by connective tissue. Males possess a germinal epithelium with rounded spermatogonia; in stage III, both female and male gonads are intumescent and have uniformly greenish pigmentation. In stage IV, females have a well-defined honeycomb structure of connective tissue and oocytes up to 50 µ, and males have spermatocytes and spermatids; both sexes have fully developed gonads. Female gonads exhibit intense green pigmentation, and males have pink and yellowish gonads. Females have numerous large oocytes that are freely present in the connective tissue. Oocytes up to 165 µ seem to remain intact when released into seawater. In stage V, males produce spermatozoa; females have green gonads, and males pink and yellowish gonads. Females ovaries are filled almost exclusively with mature oocytes, and male gonads are filled with active spermatozoa. After macroscopic observation, the gonads were removed, damp-dried, weighed to 0.001 g accuracy and prepared for histological confirmation of the maturation stages. All measurements were performed on fresh samples.

The data were analysed for deviations to the parametric assumptions of analysis of variance (ANOVA). The data distribution was tested for normality using the Kolmogorov-Smirnov two-sample test. Homogeneity of variance was determined using Levene's statistics. ANOVA was used to test for differences in the shell length and total weight between the sexes (Sokal & Rohlf, 1995).

2.2. Growth and age

The shell weight-length relationship (W/L) was estimated by least-squares linear regression after the logarithmic transformation of both variables ($\log W = \log a + b \log L$) using the potential relationship $W = aL^b$ (Bagenal & Tesch, 1978), where W is the total weight (g), L is the shell length (mm), a is the intercept (condition factor) and b is the slope (relative growth rate). The coefficient of determination, r^2, was used as an indicator of the quality of the regression. A Student's t-test (King, 1995) was used to test the hypothesis of an isometric relationship (H_0: $b = 3$; H_1: $b \neq 3$, at the 5% significance level).

Monthly length-frequency distributions were inferred through apparent shifts of the modes in the time series of the length-frequency samples by means of modal progression analysis (MPA) and used to estimate absolute growth using Bhattacharya's method, which is included in the package FISAT II (Fish Stock Assessment Tools FAO-ICLARM), VER 1.2.0 (Gayanilo et al., 2005). This method implies the identification of mean values through the decomposition of composite distributions into their components followed by the determination and linking of means perceived to belong to the same cohort, and finally, the estimation of growth parameters is determined using the growth increments and size-at-age resulting from the linking of the means. All of the identified size-age groups resulted from at least three consecutive points, and the selection of the best results was based on the separation index (SI) values (>2) for the different age groups and the number of individuals per age group. Only size-age groups with an SI greater than 2 were considered, since values below 2 are unreliable (Sparre & Venema, 1997). NORMSEP by Hasselblad & Tomlinson (1971) was applied to decompose the mixtures of normal distributions based on Hasselblad's maximum likelihood method (Hasselblad, 1966).

The von Bertalanffy growth parameters were estimated by means of the Gulland and Holt method (Gulland & Holt, 1959) for non-linear parameter estimation in the routine ELEFAN I available in FISAT II (Gayanilo & Pauly, 1997) using the equation $L_t = L_\infty \{1 - \exp[-k\ (t-t_0)]\}$ (Gulland & Holt, 1959), where L_t is the mean shell length at age t (mm), L_∞ is the asymptotic shell length (mm), K is the growth coefficient (year^{-1}), t is the age of *P. sauciatus* (years), and t_0 is the hypothetical age at which $L_t = 0$ (years).

The growth performance index (ϕ') is generally considered a better tool for comparing growth dynamics between species, phylogenetically related groups, and the same species in different areas. The estimated parameters L_∞ and K were used to determine ϕ' through the application of the equation $\phi' = \log_{10}(K) + 2 \log_{10}(L_\infty)$ (Pauly, 1997).

The inverse von Bertalanffy growth equation, $t = t_0\ (1/K) \ln (1-L_t/L_\infty)$, was used to determine the age at length of *P. sauciatus* (King, 1995), and the potential longevity ($A_{0.95}$) was estimated from the equation $A_{0.95} = t_0 + 2.996/K$ (Taylor, 1960).

2.3. Reproduction and recruitment

The sex ratio of *P. sauciatus* was determined, and the existence of differences in the proportion of sexes was tested using the chi-square goodness-of-fit statistic. A Pearson's χ^2 test was applied to test for the presence of differences in sexual proportions between months. The gonadosomatic index (GSI) was estimated according to GSI = (wet gonad weight / total body wet weight) x 100. Differences in the mean GSI values between sexes among months were tested through ANOVA, considering a significance level of 0.05. To estimate the spawning season, the proportion of immature/mature specimens per month was plotted; all developing individuals between stages 1 and 3 and all ripe individuals in stages 4 or 5 of gonadal development were considered (Desai, 1966). The existence of a correlation between monthly sea surface temperature (SST) and the GSI was determined using the Pearson correlation.

The size at first maturity (i.e., the size at which 50% of all specimens in a stock are mature, Lm_{50}) was estimated from the correlation between the proportion of mature individuals and length according to the logistic equation: $P = 1/(1+\exp(^{(-(a+bL))})$ (Sparre & Venema, 1997), where P is the balanced probability, and a and b are the equation parameters estimated by the linear least square method using a logarithmic transformation. The mean size at maturity was defined as the size at which 50% of the population is mature, when $P = 0.5$, then $Lm_{50} = (-a)/b$ (King, 1995). The lengths at which 25% (Lm_{25}) and 75% (Lm_{75}) of the topshells were mature was also determined.

The recruitment pattern was determined through the projection of the length-frequency data backwards on the time axis using the estimated growth parameters (Moreau & Cuende, 1991), and a normal distribution of this pattern was obtained by the NORMSEP routine (Pauly & Caddy, 1985) in FISAT.

2.4. Mortality, exploitation rate, probability of capture and yield-per-recruit

Total mortality (Z) was estimated using the length-converted catch curve procedure, where the percentage of samples in the length groups are pooled to simulate a steady-state population. The natural mortality rate (M) was determined by Pauly's empirical model: $\log_{10}M = -0.0066-0.279\ (\log_{10}L_\infty) + 0.6543\ (\log_{10}K) + 0.4634\ (\log_{10}T)$ (Pauly, 1980), where L_∞ is the asymptotic shell length (mm), K is the growth coefficient (year^{-1}), and T is the annual mean environmental temperature, which was 20.5°C for the habitat of *P. sauciatus* in the study area. Harvesting mortality (F) was calculated as the difference between Z and M, and the exploitation rate (E) was estimated from $E = F/Z$ (Gulland, 1971).

The probability of capture for *P. sauciatus* was calculated by using a logistic transformation of the probabilities obtained from the small topshells using the ascending left arm of the length-converted catch curve by plotting the cumulative probability of capture against the middle point of the length class intervals. The length at first capture (i.e., the cumulative probability of 50%, Lc_{50}) was obtained from the resulting curve according to the equation: $S_L = 1/[1 + \exp(S1\text{-}S2 \times L)]$ (Sparre & Venema, 1997), where S_L is the logistic curve, $S1$ and $S2$ are constants in the equation for the length-based logistic curve, and L is the topshell length. The lengths that correspond to the cumulative probabilities of 25% (Lc_{25}) and 75% (Lc_{75}) were also estimated.

Estimates of the relative yield-per-recruit (Y/R) and the harvesting mortality corresponding to the maximum production (F_{MAX}) were estimated according to Beverton & Holt's (1957) length-based method:

$$\frac{Y}{R} = Fe^{(-M(T_c - T_r))} W_\infty [\frac{1}{Z} - \frac{3S}{Z+K} + \frac{3S^2}{Z+2K} - \frac{S^3}{Z+3K}]$$

where Y/R is the catch in weight, w_∞, K and t_0 are growth parameters, T_c is the age at first capture, T_r the age at recruitment, F the harvesting mortality, M the natural mortality, Z the total mortality, and $S = e^{(-K(T_c - T_0))}$.

To assess the effect of harvesting smaller and larger specimens on harvesting mortality and Y/R, the sizes of capture estimated previously (Lc_{25}, Lc_{50} and Lc_{75}) were applied to the Y/R model to simulate the effect of size.

3. Results

A total of 5480 specimens, 56.48% females and 43.52% males, were sampled throughout the study (Figure 1). Individual size varied between 6.09 and 29.25 mm L ($\bar{x}$ = 14.64 ± 3.55 mm *L*) in females and between 6.73 and 28.78 mm L ($\bar{x}$ = 14.74 ± 3.50 mm *L*) in males. The total weight ranged from 0.11 to 6.08 g ($\bar{x}$ = 1.06 ± 0.79 g) in females and from 0.13 to 5.68 g ($\bar{x}$ = 1.11 ± 0.84 g) in males. The smallest sample specimen was collected in July at 2.34 mm *L*, and the largest specimen was collected in October at 29.25 mm *L*.

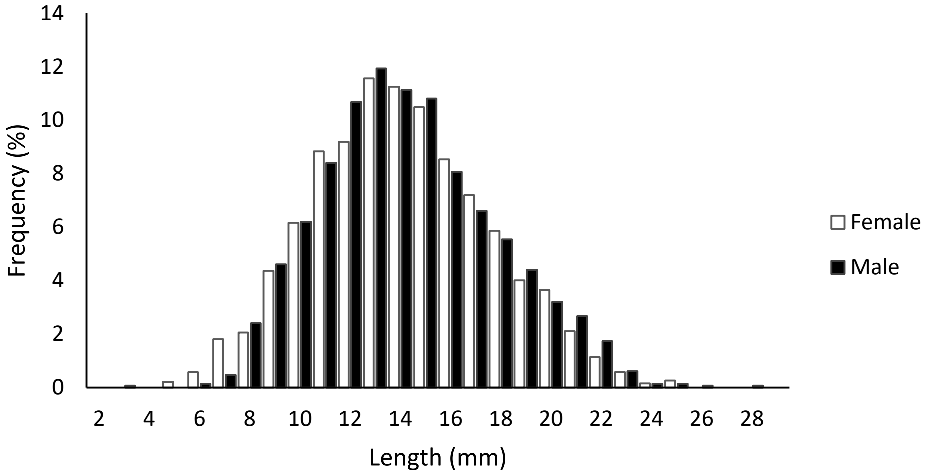

Figure 1 - Length-frequency distributions for females and males, pooled from all collections made of *Phorcus sauciatus*, collected from January to December 2017.

3.1. Growth and age

Phorcus sauciatus size-frequency of all samples pooled over a year, showed a normal distribution (Z = 1.861, $P < 0.05$) and a homogenous variance between the sexes (W = 0.145, $P > 0.05$); the same pattern was observed regarding weight, with a normal distribution (Z = 7.946, $P < 0.05$) and a homogenous variance between the sexes ($W = 3.659$, $P > 0.05$). No significant differences were found in the mean length ($F = 0.749$, $P > 0.05$) or in the mean weight ($F = 2.668$, $P > 0.05$) between females and males; thus, monthly length and weight frequency estimates were combined.

The correlation between shell length and total weight showed a negative relationship, and the parameters of the regression were estimated as $W = -3.325 + L^{2.815}$, $r^2 = 0.95$, $P < 0.05$. The *b* coefficient returned values less than 3, and statistical differences were highly significant ($t = 21.762$, $P < 0.05$).

The monthly length-frequency distributions of *P. sauciatus* are shown in Figure 2. The von Bertalanffy growth parameters were obtained for the best fit with $L_\infty = 31.90$ mm *L* and $K = 0.31$ year^{-1}. The growth performance index (ϕ’) was calculated as 2.50. *P. sauciatus* showed a predominance of individuals in the first age classes, with 88.94% of all specimens from the studied population being < 4 years. The most representative age class was 2 years, representing 54.71% of the studied specimens. Potential longevity, assuming $t_0 = 0$, was determined to be 9.66 years.

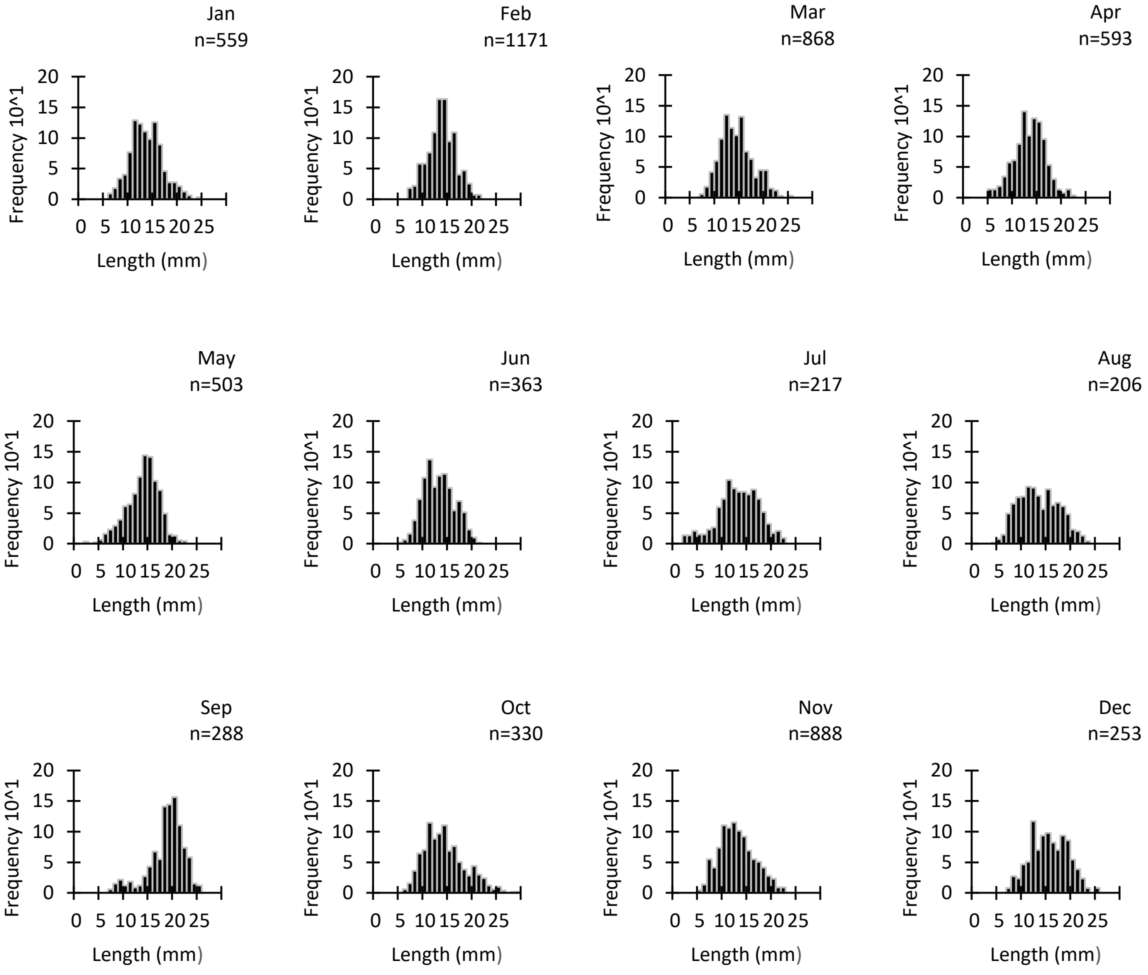

Figure 2 - Monthly length-frequency for *Phorcus sauciatus*, collected from January to December 2017.

3.2. Reproduction and recruitment

Phorcus sauciatus does not exhibit observable external sexual dimorphism. The overall sex ratio favoured females (1:1.30). The Chi-square goodness-of-fit test showed that the observed differences were significant (χ^2 = 57.791, $P < 0.05$). Monthly sex ratio analysis showed that females were predominant all year, except in February, when males were predominant (χ^2 = 64.605, $P < 0.05$).

Immature and mature specimens were found all year (Figure 3). Immature specimens were more abundant in summer, and mature individuals were more abundant in the remaining seasons. The highest proportion of immature specimens occurred in August (54.92%), and the lowest proportion occurred in February (24.12%). Mature individuals were predominant (approximately 61%) from September to May, with the highest proportion found in February (75.88%) and the lowest in August (45.08%).

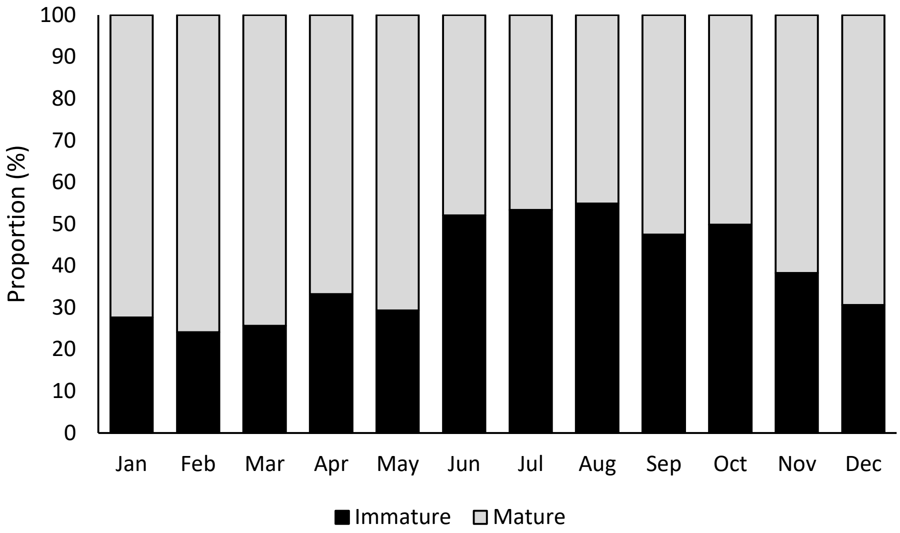

Figure 3 - Monthly distribution of immature (stages 1 to 3) and mature individuals (stages 4 to 5) of *Phorcus sauciatus* from Madeira archipelago, collected from January to December 2017.

GSI values only showed significant differences between sexes in March (F = 5.763, $P < 0.05$) and April (F = 6.764, $P < 0.05$) and monthly differences within females (F = 18.291, $P < 0.05$) and males (F = 22.053, $P < 0.05$) (Figure 4). Monthly proportions revealed an increase from October to March for both sexes. The highest GSI values occurred in March for both sexes, with 6.78% for females and 7.42% for males. After the observed peak in March, there was a decrease until August for both sexes, suggesting that the spawning season of *P. sauciatus* occurs between March and August with higher values from March to June. The observed monthly variations in the GSI were in accordance with the observed proportions of immature and mature individuals, increasing when the proportion of mature specimens was higher but decreasing when the proportion of immature specimens increased. The Pearson correlation factor ($r^2 = 0.86$) showed that the mean GSI of *P. sauciatus* was negatively correlated with SST ($F = 298.433$, $P < 0.05$).

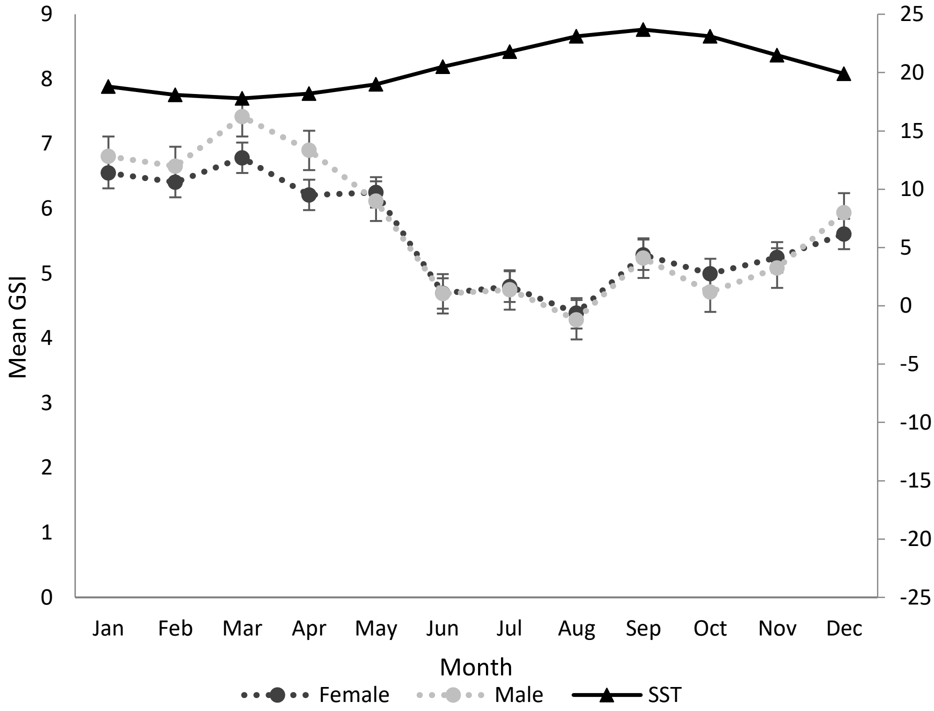

Figure 4 - Monthly variation in sea surface temperature and seasonal changes in gonadosomatic index (GSI) for females and males of *Phorcus sauciatus* from Madeira archipelago collected from January to December 2017.

The median size at first maturity (Lm_{50}) for combined sexes was 12.95 mm *L*, corresponding to 1.68 years. The size at which 25% and 75% of the study population reached sexual maturity was estimated at 12.17 and 13.73 mm *L*, respectively. The recruitment pattern was continuous throughout the year, reaching higher values between April (10.51%) and August (16.18%) and peaking in June (16.73%). Individuals with < 5.00 mm shell length were only collected from April to August.

3.3. Mortality, exploitation rate, probability of capture and yield-per-recruit

Total (*Z*) and natural mortality (*M*) were estimated at 1.61 and 0.71 per year, respectively. Harvesting mortality (*F*) was 0.90 per year, and the exploitation rate (*E*) was estimated as 0.56. The length at first capture for the combined sexes (Lc_{50}) was estimated as 13.19 mm *L*, corresponding to 1.72 years. The analysis of yield-per-recruit (*Y/R*) showed that, at the current exploitation rate, maximum production is achieved at F_{MAX} of 1.7 per year, corresponding to a *Y/R* of 0.023 g. The simulation of *Y/R* varying the length-at-capture resulted in a decrease in the harvesting effort allowed to maintain a sustainable yield, with an F_{MAX} of 1.1 per year. The simulation also showed a decrease in maximum production that can be achieved, with a *Y/R* of 0.021 g for Lc_{25}; for Lc_{75}, even though the harvesting effort can be maximized to an F_{MAX} of 2.0 per year, the gains in maximum production are negligible, with a *Y/R* of 0.024 g (Figure 5).

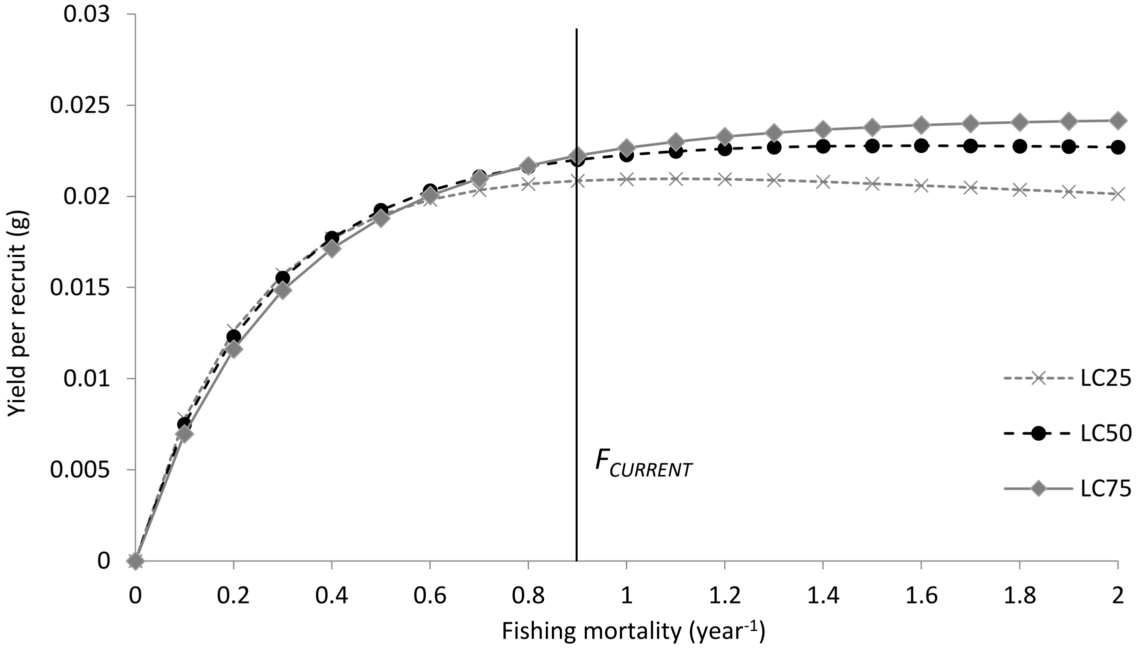

Figure 5 - Beverton-Holt yield-per-recruit curves on *F* for *Phorcus sauciatus*, considering the size at first capture (Lc_{50}), and the lengths at which 25% (Lc_{25}) and 75% (Lc_{75}) of the topshells were captured.

4. Discussion

Information on the life history traits of *P. sauciatus* provides significant knowledge, which is required to explain and understand the adaptations of this species to an ever-changing environment and to perceive how human activities such as fisheries, habitat disturbance or natural causes affect their abundance and population dynamics (Sousa et al., 2018).

The size distribution estimated for *P. sauciatus* from the Madeira archipelago is in accordance with what was previously reported for this species in the Canary Islands (Ramírez et al., 2009). The relative growth coefficient obtained for the combined sexes revealed a negative allometric growth for this gastropod, which implies a disproportional increase in total weight in relation to the increase in individual shell growth. Thus, this species allocates more energy to growth than to reproduction in this geographic area. The value of the *b* parameter obtained for this species in the Madeira archipelago is well within the range of values usually obtained in other geographic areas (2.5 - 3.5) (Bagenal & Tesch, 1977; Froese, 2006), indicating normal growth dimensions and/or the well-being of the studied population (Carlander, 1969; Bagenal & Tesch, 1977; King, 1995).

Intertidal gastropods from different geographic areas in temperate seas frequently show intraspecific variation in shell size and shape (Reimchen, 1982) as a result of adaptations to predation and wave action (Preston & Roberts, 2007) or to the conditions of their preferred habitats (Boulding et al., 1999). The negative growth pattern obtained for *P. sauciatus* might be explained by the harvesting pressure on this resource, which leads to lower population densities. The reduction in population density results in an increase in food and substrate availability, promoting an increase in the population density of other intertidal gastropods, such as *Patella aspera* Röding, 1798 and *Patelloida alticostata* (Angas, 1865) (Black, 1977; Sousa et al., 2017). This pattern may also result from the instability of the intertidal conditions, since *P. sauciatus* is highly vulnerable to sea temperature, wave action and desiccation like *Patella ulyssiponensis* as observed by Thompson (1979).

Growth in molluscs is influenced by several biotic factors (e.g. predation, competition and population density) (Haven, 1973) and abiotic factors (e.g. temperature, insolation, photoperiod and food availability) (Crothers, 2001, 2012). Moreover, it seems that molluscs have a strategy of diverting energy to reproduction or to growth according to the organisms’ requirements, and this life history strategy will influence the growth rates of these organisms (Haven, 1973; Branch, 1974; Underwood, 1979; Clarke et al., 2004). Growth in molluscs is generally conditioned by the allocation of the majority of their energy to shell production; once they reach sexual maturity, molluscs divert most of their energy to reproduction (Crothers, 1994).

The estimated asymptotic length of 31.90 mm *L* for *P. sauciatus* from Madeira is consistent with the length of the largest sampled specimen (29.25 mm *L*). This species had a moderately low growth rate of 0.31 year^{-1}, which may be partially explained by the oligotrophic nature of the coastal waters of Madeira (Caldeira et al., 2002). Similar growth rates were observed in other intertidal gastropods, such as *Patella candei* d'Orbigny, 1840 (Henriques et al., 2012) and *P. aspera* (Sousa et al., 2017), in the same geographic area.

Phorcus sauciatus in Madeira have a median lifespan of 9.66 years, meaning the population is moderately long-lived, which is in contrast to its congeneric species, *P. lineatus*, which may reach an age of 15 years at its northern/eastern limits in Great Britain (Crothers, 1998). The shorter lifespan observed for *P. sauciatus* in Madeira is in accordance with Clarke et al. (2004), who stated that molluscs in colder regions grow more slowly and reach a larger maximum size, thus achieving a longer lifespan than molluscs inhabiting warmer regions.

Phorcus sauciatus does not exhibit observable external sexual dimorphism in the studied populations, similarly to other topshells, such as *P. lineatus*, *Steromphala umbilicalis* (da Costa, 1778) and *Steromphala cineraria* (Linnaeus, 1758) (Desai, 1966; Underwood, 1972; Crothers, 2001), and other British trochids (Fretter & Graham, 1962); the sexes of *P. sauciatus* are only distinguishable by gonadal observation.

The gonadal cycle of *P. sauciatus* was established based on the monthly variation of the GSI, which reflects the periods of accumulation before the ripening and release of gonadal material during spawning as well as the use of stored energy reserves (Toro et al., 2002). *Phorcus sauciatus* seems to be reproductively active all year in Madeira, with mature and partially spawned individuals present throughout the year. The obtained results also showed a synchronous gametogenesis cycle between the sexes of *P. sauciatus*, with females and males exhibiting a similar pattern all year, with minor differences in the proportion of mature specimens. The reproductive cycle of this species seems to involve two key periods, namely, development and spawning. The gonadal development phase lasts from September to February when an increase in the GSI values and in the number of mature individuals can be observed. The main spawning pulse begins in March, when the highest GSI values for both sexes were found, indicating maximum development of the gonads, which consistently decreased until August, when the lowest GSI values for both sexes were observed; this finding agrees with the reduction in the proportion of ripe individuals. During this phase, gametes are released into the sea where fertilization occurs (Crothers, 2001). The occurrence of a noticeable spawning pulse between March and June indicates that, for this species, the majority of spawning occurs in the spring and extends more subtly throughout the summer. The observed reproductive cycle did not exhibit a resting phase, agreeing with Bode et al. (1986) for *P. lineatus* in northern Spain. These results are contrary to those observed from the northernmost populations in England, which exhibit a resting phase between the spawning phase and the gonadal development phase, which is typically shorter (Desai, 1966; Underwood, 1972). This is probably related to differences in sea surface temperature; it is known that reproduction cycles in marine gastropods are influenced by temperature, with populations inhabiting colder habitats developing a more pronounced spawning period and a shorter development phase, while in warmer conditions, the phases of the reproductive cycle tend to be less pronounced (Crothers, 2001).

Underwood (1972) observed that *P. lineatus* spawns throughout the summer and early autumn in England. Bode et al. (1986) verified that this period could be extended up to November for some individuals of the same species in Spain. In Madeira, the majority of *P. sauciatus* spawning seems to occur earlier in spring, suggesting that variations in reproductive seasons and in the duration of these periods are likely related to the geographic region, mainly due to the influence of temperature.

In Madeira, the spawning pulse of *P. sauciatus* is synchronised with the increase in seawater temperature, which is in concordance with Crothers (2001), who stated that effective spawning appears to require an environmental trigger leading to gamete release at the same time with cascading effects but with sharp differences in the extent of the spawning seasons, with the northern populations characterized by short, mid-summer breeding periods and the southernmost populations with lengthened breeding periods that extend in some cases throughout much of the year but with little or no activity during mid-summer (Lewis, 1986).

Within the same geographic region, other environmental factors, such as high wind speed allied to stimulation by wave action and the increase in phytoplankton concentrations, seem to act as triggers on the intertidal limpet species *P. aspera* (Sousa et al., 2017) and *P. candei* (Henriques et al., 2012), which are winter breeders, suggesting that the trigger that stimulates reproduction is different between limpets and the topshells in Madeira.

The estimated length at first maturity for *P. sauciatus* in Madeira was 12.95 mm *L*, corresponding to 1.68 years. More than 60% of the sampled population was sexually mature. In the Canaries, the size at first maturity was estimated to be 9.50 mm *L* (González et al., 2012). The differences in abiotic conditions between the two archipelagos may explain the larger size at first maturity estimated for the population of Madeira. It is well known that a decrease in growth rate can be related to a decrease in temperature, resulting in delayed sexual maturation at larger sizes (Berrigan & Charnov, 1994). Another explanation may be due to the exploitation level of this species in the Canaries, where it is considered overexploited (González et al., 2012). Additionally, since *Phorcus* harvest is size-dependent, as larger individuals are more visible and are thus more prone to being harvested, it is likely that the difference in the size at maturity between archipelagos is influenced by the different exploitation pressures. In overexploited populations, size at first maturity may suffer shifts as a result of changes in the population size structure, resulting in smaller sizes at first maturity (Fenberg & Roy, 2008).

The recruitment pattern of *P. sauciatus* was continuous throughout the year, as indicated by the presence of developing individuals (stages 1 to 3) all year, but with higher levels of new recruits occurring during the main recruitment season from April to August. In the present study, recruits (2-5 mm *L*) were detected on rocky shores from April to August. Crothers (2001) stated that settlement occurs when individuals reach lengths > 1 mm *L* and verified that most settlement for *P. lineatus* in Great Britain occurs in early September, and by the end of that month, recruits could grow to 3 mm and even 4 mm. The recruitment trend obtained for *P. sauciatus* in Madeira is most likely a consequence of warmer temperatures, which result in continuous reproductive activity throughout the year with a period of intense reproduction. Therefore, the entrance of new recruits in the adult population follows the same trend, with a more prominent recruitment period occurring within a few months following the reproductive peak.

The analysis of the mortality rates estimated for *P. sauciatus* from Madeira suggests that harvesting mortality applies a high pressure on this resource compared to natural mortality. A combination of traditional and commercial fishing methods put harvesting pressure on the stocks of this topshell in the study area, which also occurs for the limpet *P. candei* (Henriques et al., 2012) and for *P. sauciatus* in the Canary Islands (Alfonso et al., 2015). The relatively high total mortality together with the moderate slow growth rate estimated suggest that its biomass is maximized at an early age (King, 1995), which is supported by the fact that approximately 89% of the sampled specimens were < 4 years old. The low frequency of specimens in the age classes between 4 and 9 years (approximately 11%) seems to be a consequence of intensive size-selective exploitation of the larger topshells, resulting in a decrease in the reproductive output. The reproduction of this species is size gamete density-dependent, as occurs in other gastropods, such as the patellids *Patella candei crenata* and *P. aspera* (Riera et al., 2016; Sousa et al., 2017).

The catch selectivity analysis resulted in a shell length of 13.19 mm for the length at first capture, corresponding to an individual of 1.61 years old and indicating that most individuals are harvested after *P. sauciatus* achieves sexual maturity. The yield-per-recruit analysis suggests that this species' stock is being moderately exploited in Madeira, considering the current levels of mortality are < 1.7 year^{-1}, which returns a yield of 0.023 g. The data showed that even if the harvesting effort is doubled, there will only be a slight increase of 0.001 g in the yield, and since the relationship between mortality and yield is essentially asymptotical, the harvesting effort required to take approximate mortality to F_{MAX} would most likely be excessive for a profitable yield.

The simulation of the effect of size at first capture on the yield-per-recruit showed that the F_{MAX} is 1.1 year^{-1} at Lc_{25}, indicating that this fishery is vulnerable to the harvest of smaller specimens that will result in a decrease in yield, which is most likely due to lower levels of recruitment because of the lower reproductive output of smaller individuals. Increasing the size at first capture to Lc_{75} (F_{MAX} increases to 2.0 year^{-1}) would produce negligible benefits in terms of yield.

Knowledge of the life history parameters on stocks of exploited marine species is one of the major contributors to the identification and implementation of harvesting management policies for sustainable exploitation. The present study showed that the urgent implementation of management measures is required to preserve the commercial stock of *P. sauciatus* in the medium and long term. As such, the data reported here will contribute to the establishment of a properly regulated harvest that is both profitable and sustainable. The recommended conservation measures are the following: (i) the definition of different harvesting typologies (e.g. non-commercial harvesters with catches for personal use and professional harvesters for commercialization) with the establishment of maximum catches per day (we suggest no more than 2 kg per day for non-commercial use and 20 kg per day for professionals); (ii) the implementation of landing obligations and first sale at auction of *P. sauciatus* in Madeira to gain exact knowledge about the status of the commercial catches and to monitor the harvesting effort by local authorities; (iii) the establishment of a minimum catch size of 15 mm shell length to ensure that a sufficient proportion of reproductively active individuals contributes to the reproductive effort of the exploited population, and (iv) the establishment of a closed season between February and May to prevent all types of harvesting during the main spawning pulse.

Finally, the data provided in the present study come from a single time period, and some yearly variations may occur because of the particularities of abiotic and biotic conditions, e.g. temperature and turbidity. As such, the continuation of the monitoring of exploited populations and further studies focused on the reproduction of *P. sauciatus* are warranted to verify whether the observed patterns are consistent throughout time.

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Chapter 4

Patellid limpets: An overview of the biology and conservation of keystone species of the rocky shores

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Abstract

This work reviews a broad spectrum of subjects associated to hit the biology of Patellid limpets' biology such as growth, reproduction, and recruitment, also the consequences of commercial exploitation on the stocks and the effects of marine protected areas (MPAs) in the biology and population dynamics of these intertidal grazers. Knowledge of biological traits of limpets plays an important role in providing proper background for their effective management. This chapter focuses on determining the effect of biotic and abiotic factors that influence these biological characteristics and associated geographical patterns. Human exploitation of limpets is one of the main causes of disturbance in the intertidal ecosystem and has occurred since prehistorical times resulting in direct and indirect alterations in the abundance and size structure of the target populations. The implementation of MPAs has been shown to result in greater biomass, abundance, and size of limpets and to counter other negative anthropogenic effects. However, inefficient planning and lack of surveillance hinder the accomplishment of the conservation purpose of MPAs. Inclusive conservation approaches involving all the stakeholders could guarantee future success of conservation strategies and sustainable exploitation. This review also aims to establish how beneficial MPAs are in enhancing recruitment and yield of adjacent exploited populations.

Keywords

Patellidae, limpets, fisheries, MPAs, conservation.

1. Introduction

The Patellidae are one of the most successful families of gastropods that inhabit the rocky shores from the supratidal to the subtidal, a marine habitat subject to some of the most variable and unpredictable environmental conditions. Therefore, many of their peculiar morphological and biological characteristics can be understood as adaptations to this environment. The biological traits of limpets vary inter- and intraspecifically as a result of genetic differences and environmental influences [1]. Parameters such as growth, reproduction, and mortality are dependent on a complex array of selective forces and are important in understanding the distribution and abundance of a species [2, 3]. Differences in limpet populations from distinct geographic areas are most probably explained by specific environmental and anthropogenic conditions, essentially oligotrophy, sea water temperature, and fishing pressure. Thus, for some of the biological traits, it is expected to find patterns, like temperature which changes somewhat consistently with latitude and has a profound effect on the growth of limpet species, with species inhabiting higher latitudes growing more slowly and achieving larger maximum sizes, therefore having a longer lifespan than limpets from lower latitudes.

Patellid limpets are also subjected to anthropogenic impacts on the coastal ecosystems such as, pollution, habitat removal, and harvest which in some cases has led to the reduction of abundance or even the disappearance of limpets from large areas. The decline of these species, which may have been further accelerated by the progressive deterioration of the coastline, continues at an alarming rate and many of their stocks are on the verge of disappearance. To avert this situation, regulators have established several measures including the implementation of closed seasons and areas where limpet harvest is interdicted, minimum size of capture, and catch limits. Limpet populations seem to respond, in general, in a positive way to these measures; however, the response is closely linked to the ability of the regulators to enforce said measures.

Another popular strategy adopted in the protection of the rocky shores and limpets is the implementation of marine protected areas (MPAs). The effectiveness of MPAs in protecting exploited populations of limpets and underlying their overall success in increasing density and abundance as well as promoting healthy size composition with impact on the reproductive output of these species is well known. Nonetheless, several limitations are recognized that can negatively affect the protective role of MPAs such as, naturally occurring variations of the species biology and ecology as well as limitations regarding the management of MPAs, for instance, the lack of surveillance and enforcement of protection regulations.

The aim of this work is to review a broad spectrum of subjects associated with the biology of patellid limpets such as growth and reproduction, also the consequences of commercial exploitation on the stocks of these species and the effects of marine protected areas on the biology and population dynamics of these intertidal grazers. The focus is on determining the effect of identified biotic and abiotic factors that influence these biological characteristics and geographical patterns recognized to be closely connected to growth and reproduction, such as latitude. Regarding conservation of Patellidae, the authors aim to elucidate how beneficial MPAs are in their role of protection of exploited populations and in enhancing recruitment and yield of adjacent exploited populations.

2. Biology and ecology of patellid limpets

2.1. Taxonomy and distribution

Patellid limpets are marine gastropod grazers belonging to the family Patellidae Rafinesque, 1815 that comprise the genera *Patella* Linnaeus, 1758, *Cymbula*, H. Adams & A. Adams, 1854, *Helcion* Montfort, 1810, and *Scutellastra* H. Adams & A. Adams, 1854. The worldwide distribution of species of Patellidae anti-tropical with half of the known species restricted to southern Africa and the North-Eastern Atlantic where a high diversity of species is found, while relatively few species are present in the Indian and Pacific Oceans [4, 5]. The Patellidae family is currently represented by, at least, 49 recognized species [6]. The genus *Patella* is comprised of 14 recognized species with a geographical distribution restricted to the North-Eastern Atlantic and the Mediterranean Sea; the genus *Cymbula* includes 10 species found in Southern Africa, South-Eastern Atlantic, and Mediterranean; the genus *Helcion* is represented by four species restricted to Southern Africa, while *Scutellastra* encompasses 21 species with a wide distribution ranging from Southern Africa to the Indo-West and Eastern Pacific [4, 7–10]. Limpets are subject to an array of environmental stresses as a result of their extended vertical distribution, which ranges from the upper to the lower shore levels. Thus, limpets can exhibit varying degrees of structural adaptations since their position relative to the shore influences their exposure to desiccation, hydrodynamic action of the waves, temperature variation, and tidal width [11–14]. This impressive phenotypic plasticity allied to the relatively simple shell geometry, convergent shell shape, and sculpturing results in an unclear taxonomy of patellid limpets, in such a way that the initial generic names, with broad geographical range, had to be re-evaluated based on superficial similarities [15].

2.2. Feeding habits and ecological importance

Limpets are grazing herbivores that feed, by scraping the rocky substrate with the radula, on microbial biofilms which are primarily composed of cyanobacteria and microalgae, including diatoms, spores, and other propagules of macroalgae and invertebrates [16, 17]. The feeding habits of limpets are essential in structuring intertidal communities [16–19] since limpet grazing is a key process in rocky shores involved in determining macroalgal abundance and in modifying ecosystem stability, indirectly enhancing or inhibiting the establishment of other organisms [17].

The decline of population density of limpets might result in an abnormal development of algae diversity as reported by Boaventura et al. [20] or in the occupation of their ecological niche by competing organisms such as barnacles or sea urchins [21–23]. However, the effect of these grazers is not limited to the removal of algae, and very often they can affect other animal species through competitive interactions [24] and by providing secondary habitats for other invertebrates that settle either on top of, or beneath, their shells [19, 25, 26]. Grazers may also affect the rate of succession [27] or cause different assemblages to develop [28]. Thus, limpets are rightfully considered to be keystone species in intertidal communities [29].

2.3. Movement and homing

Patellid limpets are considered to some extent semi-sessile organisms; nonetheless, they perform small movements in the area surrounding their usual fixation site. This behaviour is designated as homing and can often be observed through the scar that remains in the rocky substrate where the limpet settles. Limpet movement patterns and homing behaviour have been extensively studied for *Patella vulgata* Linnaeus, 1758 [30], *Patella depressa* Pennant, 1777 [31], *Patella rustica* Linnaeus, 1758 [32], *Patella ferruginea* Gmelin, 1791 [33], *Scutellastra flexuosa* (Quoy & Gaimard, 1834), and *Scutellastra argenvillei* (Krauss, 1848) [34]. This homing behaviour has different functions in different species such as avoiding desiccation [35, 36], reducing predation and intraspecific competition [37–40], responding to wave action [41, 42] and defending territory or asserting dominance [43, 44]. The mechanism that is most widely accepted as being responsible for the homing behaviour reports limpets following chemical trails, laid down on the outward trip, on their way back to the fixation site [31, 45, 46].

2.4. Growth

Biological parameters such as growth rate, asymptotic length, and age structure reflect the overall state of health of a population and are commonly used as stock assessment tools of exploited marine organisms. Growth, reproductive strategy, and mortality are dependent on a complex array of environmental factors [2] and are important in understanding the distribution and abundance of a species [3]. To determine these parameters, most studies usually resort to the capture-recapture method [22, 47–50] or length-frequency distribution analysis [51–53].

Over the past decades, intensive research has focused on the biology of limpets, due to their diversity and ecological significance; however, there remain gaps in the knowledge concerning these species' age structure and growth patterns.

Patellid limpets, like many marine gastropods, exhibit both intra- and interspecific seasonal variation in growth rates [54]. Although some intraspecific variation may be genetically controlled [55], external factors such as changes in food availability [56, 57], wave action [58–60], and vertical distribution on the shore [61] are thought to influence growth rates. Other factors such as population density, available grazing area, predation, and competition are indicated as influencing growth rates of molluscs supporting the idea that the strategy of diverting energy to reproduction and vice versa, according to the organisms' needs, influences growth rates [24, 49, 62, 63]. It has been suggested that limpets with greater growth rates have smaller lifespan while limpets with slow growth are generally long-lived [46]. As such, rapidly growing limpets are usually associated with early maturation, high mortality, and a short lifespan [46, 64].

Clarke et al. [49] observed a latitudinal cline in annual shell growth of the polar limpet *Nacella concinna* (Strebel, 1908). This latitudinal pattern could nevertheless be masked by inter-annual variability. The authors suggest that the observed variation could be the result of a simultaneous change in both growth rate and the duration of the growth period. This change would result from the shorter duration of the seasonal blooming of epiphytic microalgal and microbial biomass at higher latitudes. Another factor influencing growth rates in *N. concinna* is seawater temperature, with warmer temperatures that last longer producing higher growth rates.

Scutellastra and *Cymbula* species that occur at similar latitudes present variations in terms of growth, namely in maximum size and growth rates. When compared to tropical limpets belonging to the genus *Cellana* H. Adams, 1869, limpets from temperate regions are generally larger, with wider lifespan and slower growth rates. Additionally, limpets inhabiting the artic regions such as *N. conccina* achieve larger sizes, even wider lifespans, and slower growth rates. This latitudinal pattern has been usually associated with the latitudinal variation of temperature, photoperiod, and insolation [49]. Even though it is generally agreed that species from lower latitudes grow more rapidly than species from higher latitudes [49, 52], it is not yet clarified whether physiological constraints, a reduced or prolonged growing season, or combination of both might be the cause of dissimilar growth rates at differing latitudes [49].

Nevertheless, due to anti-tropical distribution of patellids, growth patterns are difficult to observe, particularly when considering latitude. Within this family, variations in growth are mostly derived from prevalent local environmental factors. Nonetheless, when comparing to other Patellogastropoda, a latitudinal pattern becomes apparent, in which at lower latitudes limpets grow at faster rates and achieve smaller sizes, while at higher latitudes, they grow at slower rates and achieve larger sizes. For instance, for the polar limpet *N. concinna* reported growth rates range between 0.059 and 0.323 year^{-1}, while the highest growth rate is exceptionally high for a limpet inhabiting the Polar Regions, probably due to specific characteristics of the habitat in Signy Island [49]. The overall growth rates are inferior to those reported for limpets of the genus *Cellana* that inhabit lower latitudes in temperate and tropical regions with growth rates ranging from 0.400 to 1.661 year^{-1}. Patellid limpets exhibit intermediate growth rates ranging from 0.117 year^{-1} in *Scutellastra cochlear* (Born, 1778) and 1.020 year^{-1} in *Cymbula oculus* (Born, 1778) reflecting their anti-tropical distribution.

However, the nonlinearity of growth of marine organisms renders the direct comparison of growth parameters impossible [65]. As such, determination and comparison of the overall growth performance of different marine species is achieved using the growth performance index (GPI) of Pauly and Munro [66], which relates the asymptotic length and growth rate [66].

Nonetheless, the growth performance index in Patellogastropod limpets exhibits the same pattern as growth rates with decreasing GPI as latitude increases and ranging from 1.942 in *N. concinna* to 3.653 in *Cymbula granatina* (Linnaeus, 1758), suggesting that growth performance of limpets varies with latitude. Within the Patellidae family the variation of GPI is reduced with values ranging from 2.42 for *S. cochlear* to 3.65 for *C. granatina* from South Africa [62], which is in agreement with Sparre et al. [67] who claim that the growth performance index remains relatively constant at similar rates between related taxa. The variability results therefore due to abiotic and biotic factors that different species are subject to, such as greater or lesser extent of hydrodynamics, desiccation, predation, competition, and temperature.

2.5. Reproduction

Patellid limpets have a simple reproductive system, consisting of a simple gonad occuring in the visceral mass and a reduced gonoduct leading to the right nephridium [68, 69]. These species are not externally sexually dimorphic, and sex determination is only possible through macroscopic observation of the gonads. Spawning results in the release of oocytes and sperm directly in the ocean where fertalization occurs. According to Orton et al. [68], spawning is stimulated by environmental triggers, such as high wind speed and wave action. An increase in phytoplankton concentration may also stimulate spawning as suggested by Underwood [24] who observed that gastropod species with planktotrophic larvae spawn when phytoplankton concentration is high.

Most limpet species have a reproductive cycle with a gonadal development stage culminating in a spawning period followed by a resting phase. The spawning period varies inter- and intraspecifically; it may also vary from year to year and is supposed to be triggered by temperature variations, increased wave action, and onshore winds [70]. In regions with higher temperatures, spawning occurs in a short period contrary to what happens in regions with colder waters, where the development of the gonads requires a longer time period [71]. *P. vulgata* is believed to be a winter breeder, with spawning occurring from October to March; however, in colder localities, sexual maturation occurs earlier [68]. On the other hand, in south-west England, *P. depressa* is considered a summer breeder [72] with spawning occurring between late July and early September and without a resting phase unlike *P. vulgata*. The same authors suggested that an increase in temperature associated with wave action stimulates spawning in this species. *Patella ulyssiponensis* Gmelin, 1791 has a spawning period that lasts from October to December, being also considered a winter breeder in south-west England [59, 68, 73]. Orton et al. [68] and Orton and Southward [72] suggested that although the development of the gonad in *P. vulgata* and *P. depressa*, respectively, is well related with temperature, the act of spawning is triggered by violent onshore storms. Thompson [59] also found *P. ulyssiponensis* spawning during the autumn storms. Hence, it seems likely that spawning cannot take place until a population is sufficiently mature, but after that stage is reached, the first strong windstorm will trigger spawning [59]. Another factor that potentially affects the timing of spawning in limpets is food availability; Underwood [24] reported that species with planktotrophic larval stage time spawning with periods when phytoplankton concentrations are high. One such case is that of the closely related species of *P. ulyssiponensis* from the Portuguese mainland and *Patella aspera* Röding, 1798 from Madeira Island. *P. ulyssiponensis* is reported to be a summer breeder while *P. aspera* was reported to be a winter breeder with spawning occurring when the phytoplankton concentration is higher (P. Henriques, pers. comm.). Similarly, it has been reported that in limpets with restricted geographic distribution, the reproductive cycle is influenced by geographic locality, namely in the timing of gametogenesis and spawning [62, 74]. For limpets with broader geographic distribution, it is possible that the reproductive cycle is adjusted to regional environmental conditions [74].

Limpets, like many sessile or sedentary marine invertebrates, have life cycles that include a prolonged pelagic larval phase that can last up to 2 weeks as reported by Hawkins et al. [75] for *Patella* species. Veliger larvae remain in the water column as plankton until eventually fixating in the rocky substrate on the inferior level of the coast. As the juveniles grow, they begin a slow vertical migration, colonizing different levels of the rocky shores [76], leading to variability in patterns of recruitment [77]. Moreover, larvae in the water column are subject to processes of physical transport that can disperse them from the site of reproduction [78]. Thus, the number of recruits on a specific location may be independent of the local larvae production [16, 79] and influenced by current regimes. Nonetheless, limpet populations cannot be considered fully open or fully closed, since some local larval retention is likely to occur despite larval dispersal [80, 81]. Orton [82] suggested the existence of the phenomenon of protandrous hermaphroditism in limpets of the genus *Patella* based on sexual dimorphism in size-frequency of *P. vulgata*; subsequently Thompson [59], Branch [46], and Le Quesne and Hawkins [83] observed that some individuals reach maturity as males and become females in the more advanced stages of their life cycle.

This phenomenon of sequential hermaphroditism is also suggested to occur in species of the genera *Cymbula* [46], *Helcion* [74], and *Scutellastra* [62, 84, 85]. Not all male limpets change sex, since a considerable proportion of males can be found in the larger size-groups, these individuals might eventually change sex or remain as males if the signals that lead to sex change are not present [86]. Also, some limpet species are sequential hermaphrodites in which the sex change can be reverted as reported for *P. ferruginea* by Guallart et al. [87] and for *P. vulgata* by Le Quesne and Hawkins [83].

Sex change in limpet species is thought to be genetically controlled. However, high variability in the timing or on the limpet size at which the change occurs suggests that environmental factors may influence the process. Species such as *C. oculus* have a relatively fixed timing of sex change [88], while in other species, the sex change occurs at sizes that are highly variable. These differences in size and age at which the sex change occurs are often mediated by environmental factors [46, 89–92]. For instance, sex change in molluscs can be delayed in populations where large females are present [89, 90]. Additionally, in populations subjected to higher mortality rates or slower growth rates, sex change seems to occur earlier [93]. Also, it has been reported that social control of sex change occurs in Patellogastropod limpets [91, 92]. In this case, several possible cues for sex change have been suggested such as, contact frequency between individuals, available movement area, food availability, growth rate, pheromonal information, and communication by mucus traces left by individuals during foraging excursions [91].

Hermaphroditism is an evolutionarily advantageous strategy for species with low population densities or low mobility such as limpets, since under such conditions, hermaphroditism is supposed to increase the likelihood of successful fertilization [87]. Reproductive success in broadcast spawners, such as limpets, is correlated to the quantity of gametes released into the water column. It is believed that larger limpets produce more gametes than smaller individuals and also due to the fact that the production of sperm is cheap and an animal could be a functional male when small and the same individual a female when large and not growing so much.

Additionally, sex change in protandrous hermaphrodite species results in an increase of female individuals in the larger size classes. Thus, the sex distribution through sizes in protandrous hermaphrodite limpets makes these species extremely vulnerable to harvest [33], since the depletion of larger and more fecund individuals and females in a higher percentage may potentially alter the sex ratio and reduce the reproductive output of populations [86].

3. Anthropogenic impact on Patellid limpets

Patellid limpets are common gastropods of intertidal rocky shores; however, some species are in serious decline mainly as a consequence of overexploitation [94]. These intertidal and shallow-water grazers are highly vulnerable because of their restricted habitat and its accessibility to human activity [26]. Worldwide, shellfish exploitation has often been shown to lead to decreased biomass and species richness and cause shifts in community composition [95–98]. These effects are driven by the increase of human population density along the coast, the replacement of subsistence by commercial exploitation, and technological advances in methods of collection, processing, storage, and transport [99, 100]. As a result, the effects of human exploitation add to those of natural processes that influence population size of exploited limpets and are a concern in conservation biology [101]. Limpets have been exploited by human populations since the Palaeolithic period [102] at a subsistence level and used as food and bait in several parts of the world, including Mexico, the United States of America [101], Hawaii [103], Australia [104], South Africa [105], Chile [106], and Macaronesia [53, 107, 108]. More recently, this subsistence activity has been replaced, in many parts of the world, by heavy and highly profitable commercial exploitation, increasing the pressure on these species' stocks. Limpet harvest results in reductions in density and shifts toward smaller individuals and can decrease reproductive output since individual fecundity is greater in larger individuals [44, 109, 110]. Thus, harvesting has both direct and indirect effects on these species. There are also effects on the overall community composition as removal of grazing limpets facilitates the growth of algae [20, 111, 112], leading to further changes within the rocky shore communities [16, 17].

The direct effects of limpet exploitation are the decline of the exploited species' abundance and a shift in size composition of their populations that results from the size-selective nature of limpet harvest [100]. This is a result of larger individuals being more visible, thus more prone to be caught, and due to their greater commercial value [22, 113, 114]. The loss of older and larger individuals results in cascading effects on the biology of these species and the affected populations, including changes in life-history parameters, demographics, reproductive success, and ecological interactions [98].

For instance, the decline of larger individuals in an exploited population of limpets might lead to the complete disappearance of the population's viable size as a consequence of a seriously diminished reproductive success, affecting different species in a differentiated manner, as observed by Martins et al. [115] in the Azores. Protandrous hermaphrodite species are particularly susceptible to changes in their population size composition that promote a decline of frequency of larger individuals, since it directly affects the sex ratio of the population resulting in a decrease in female specimens that in natural conditions occur with higher frequency in the larger size classes. Also, larger individuals represent a greater contribution to the reproductive effort in limpets [104], thus the harvest of larger individuals contributes to a decrease in the reproductive success of marine invertebrates such as reported for *P. ferruginea* [33] and may eventually result in the collapse of exploited populations [86, 116, 117].

Reduction of sizes and abundance of larger individuals in exploited populations of limpets have been reported for *Patella candei* d'Orbigny, 1840 [116] and *Patella candei crenata* [114] in the Canaries*, P. cande*i and *P. aspera* in the Azores [115], *Helcion concolor* (Krauss, 1848) [44], and *P. ferruginea* in Algeria [118] and Spain [86], as well as for the species *C. oculus* in South Africa [88]. The overexploitation of limpets has prompted the implementation of management strategies in order to protect the exploited populations and mitigate human impacts in several parts of the world [26, 53]. The establishment of species-specific total allowable catch, minimum size of capture, closed seasons, and closed areas has been the most common measures ensued with this objective. These strategies are thought to maintain sex ratios, preserve age structure, prevent sperm limitation, enhance yield, and restrict evolutionary changes in response to fishing, such as shifts to early maturation [119–122]. When considering limpets, due to the phenomenon of protandrous hermaphroditism, in addition to minimum size limits used to prevent recruitment overfishing, management policies should also consider minimum and maximum size limits [122].

For instance, in Madeira archipelago the harvest of *Patella candei* sensu lato and *P. aspera* is regulated since 2006, enforcing the maximum allowable commercial catch of 15 kg/person/day or 200 kg/boat/day and a minimum capture size of 40 mm. Additionally, the competent authorities became responsible for issuing harvest licenses, limiting the number of active fishermen involved in limpet harvest. A closed season was also implemented between November and February in order to prevent limpet harvest during the reproductive season. More recently, the closed season was modified in order to more effectively provide protection to these heavily exploited species, now lasting from December to March. In the Azores, the overexploitation of limpets resulted in a drastic decline in population density and abundance of limpet populations, and in order to prevent a complete collapse of the stocks, regulation was implemented through the establishment of limpet protected zones that comprise stretches of coast of a few kilometres where the collection of limpets is strictly prohibited throughout the year, seasonal fishing closures, and minimum legal catch sizes [123].

Martins et al. [123] studied the effect of regulation on the recovery of the exploited populations of limpets in the Azores and concluded that the legislation and current levels of enforcement were insufficient to protect the exploited populations and greater levels of enforcement, such as the establishment of physical barriers and other protective strategies should be considered to protect limpet populations. The authors further elaborate that in the absence of adequate enforcement, a complementary approach that has had positive results is co-management [124], due to increasing awareness of the need to increase ownership of conservation areas and to involve all interested parties in the development of management schemes [125, 126].

4. Marine protected areas and their protective role in exploited limpet populations

Marine protected areas are frequently considered as a key tool in the conservation of marine biodiversity in coastal regions [127, 128] due to its ecosystem-level approach for exploited species. Reserves are supposed to restore and protect exploited marine organisms within their boundaries and have been shown to harbour denser populations, larger individuals, and higher biomass of exploited species [129].

MPAs potentially offer a way to conserve marine biodiversity by prohibiting harvest and at the same time sustaining fisheries by re-establishing natural conditions for reproduction [129–131]. Thus, protected populations would have higher densities and larger individuals leading to greater production of larvae that would eventually settle outside of the protected area [88, 132–134]. However, increase in recruitment outside reserves can be difficult to verify in the field [135, 136], and there is debate about whether marine reserves can benefit fisheries, as well as act as a conservation tool [137–139].

Human harvesting of limpets is usually size-selective with a strong preference for larger individuals [98] that may potentially alter the sex ratio and reduce the reproductive output of populations in successive hermaphrodite species [75, 140]. A reduction in the abundance of large limpet species, induced by high harvesting pressure, has been observed worldwide with several documented cases of drastic declines such as in the case of the endemic limpets *P. aspera* and *P. candei* in the Macaronesian Archipelagos [115, 141, 142], *P. ferruginea* considered one of the most endangered marine invertebrates on western Mediterranean rocky shores [118, 143] and *C. oculus* in South Africa [88]. In a more extreme case, the overexploitation as a food source and adornments [144], since pre-Columbian times [145] of *Scutellastra mexicana* (Broderip & G. B. Sowerby I, 1829), resulted in this species being thought extinct [146]. However, some populations of this species were reported to have survived and now the species is considered endangered [147, 148].

MPAs are zones where the harvest of marine organisms is interdicted and are considered a popular alternative to traditional marine resource management measures [149]. Exploited marine organisms in general achieve higher abundance, biomass, and size in MPAs [104, 150]. Halpern [129] reported that abundance and species diversity of marine invertebrates were significantly higher in MPAs regardless of their size.

Halpern and Warner [149] reported that establishing MPAs results in significant increases in the average level of density and biomass in a period of 3 years and that these values are persistent over time. Even though it is considerably difficult to predict the amount of time needed for a community to respond to MPA protection, evidence collected by some authors suggests that the response occurs within 2 years [151, 152]. The speed of response to MPA protection depends on the degree of exploitation to which the species is subjected. If exploitation levels are high, the species are more probable to respond rapidly to the MPA protection, when recruitment occurs at the required levels, as a consequence of the removal of the fishing activity that limits population size, demographics of the species [153–155], and the trophic level occupied by the species, since recruitment is associated to the species' life-history parameters. In general, for marine invertebrates with a long lifespan and slow growth, it is assumed that the response to protection from MPAs occurs at a slower rate [149]. Some limpet species such as *P. candei* sensu lato and *P. aspera* are considered to have slow growth and relatively long lifespan, thus they are extremely vulnerable to size-selective harvest and would have a slower response to MPA protection [53] (P. Henriques, pers. comm.).

Another possible effect of MPAs is the enhancement of recruitment on adjacent exploited populations, since the higher densities and larger individuals in reserves are expected to lead to greater production of larvae than in nearby exploited areas [88]. Therefore, MPAs are expected to enhance adjacent fisheries through the export of larvae [132, 133]. However, it is still unclear how and to what extent reserves influence exploited populations regarding the renewal of recruitment on these populations, due to the export of larvae originated in MPAs [137, 138, 156]. For instance, Hockey and Branch [157] found that limpet populations closer to protected areas benefit from an increase in juvenile individuals, suggesting a spillover of recruitment from MPAs. Nevertheless, the correlation between larvae production in MPAs and recruitment on exploited populations is difficult to predict, due to the difficulties in determining patterns of physical transport, especially at small scales [78, 79, 158].

According to Halpern [129], the average values of several biological variables are 20 to 30% higher in populations of MPAs when compared to exploited populations, independent of MPA size, indicating that small MPAs can also produce high values. Several studies have reported a pattern of better preserved populations of limpets in MPAs regarding abundance and biometric structure, for example *P. candei* in Fuerteventura [116], *P. candei crenata*, *P. aspera* and *P. rustica* in the Canaries archipelago [114], *P. ferruginea* in the Mediterranean [159], *C. oculus* in South Africa [88] as well as *H. concolor*, *Scutellastra longicosta* (Lamarck, 1819) and *Scutellastra granularis* (Linnaeus, 1758) in South Africa [26].

Núñez et al. [116] studied the abundance and size composition of eight populations of the heavily exploited *P. candei* in the island of Fuerteventura, two of which were included in two protected areas, and reported that these two populations were the best preserved in terms of abundance and size composition, while the areas closer to human settlement, thus more accessible, exhibited less abundance and smaller size individuals. Another study in the Canaries archipelago by Ramírez et al. [114] showed that the populations of limpets exposed to anthropogenic effects return lower levels of abundance and smaller size composition compared to more isolated populations; even when the populations are encompassed in an MPA, the non-enforcement of the imposed regulations and lack of surveillance may compromise their effectiveness. Coppa et al. [159] also reported that the impact of MPAs in the protection of the endangered limpet *P. ferruginea* in terms of population density, spatial distribution, and morphometric characteristics is inversely correlated to accessibility.

The effect of MPAs in population density, size structure, and biomass of the exploited limpet *C. oculus* in South Africa was assessed by Branch and Odendaal [88], resulting in important increases of the studied parameters in MPAs when compared to exploited populations. Also, survivability, sex ratio, and reproductive output were significantly higher in MPAs. Other examined parameters such as growth rate and age at maturity were apparently unaffected by the protection of MPAs. Conversely, recruitment was higher in exploited populations than in protected areas. These results clearly show the necessity for MPAs among the tools used for coastal management.

Nakin and McQuaid [26] reported the effect of MPAs in the populations of heavily exploited limpets *S. longicosta* and *H. concolor* and the less exploited *S. granularis*. The authors evidenced a subtle enhancement of population density and size structure, more evident in heavily exploited species. However, the effects of spatial and temporal variation allied to the existence of poaching activities appear to dilute the effect of marine reserves.

Even though these studies put in evidence the overall benefits of establishing MPAs in protecting the intertidal habitat and the species that inhabit it, they also raise important questions regarding their effectiveness. If on one side, MPAs allow exploited limpet populations to recover in regard to certain biological parameters, on the other hand their effectiveness is in some cases hindered by the lack of surveillance and poor enforcement of protection regulations. In fact, these two factors seem the most important in determining the effectiveness of MPAs. Nonetheless, the implementation of MPAs even when unable to fully stop illegal harvest of limpet species, results in direct improvements for the protected populations in terms of abundance, size structure, and population density and indirect effects regarding reproductive output of these broadcast spawners. For this reason, the implementation of MPAs has become one of the most widely advocated tools for the management and conservation of coastal marine ecosystems in the recent decades [160, 161].

Several factors affect the response of protected populations, thus comparison between different MPAs is somewhat difficult. In fact, the recovery indicators reported for protected populations may be a consequence not only of MPA protection but also of changes in environmental conditions, biological characteristics of the species and, level of exploitation to which they are subjected [162–164]. The degree of exposure to wave action, as well as the vertical distribution of the species is thought to play an important role in the recovery of limpet populations; limpets more exposed to wave action as well as species exposed for longer periods to desiccation have a less pronounced response to the protection given by MPAs as shown by Branch and Odendaal [88] for *C. oculus* in South Africa.

Unsatisfactory results generally occur in those MPAs that are affected by inappropriate planning, ineffective surveillance, poor acceptance by local communities, and the lack of political will to reinforce the importance of environmental protection [140, 165–167]. For instance, Coppa et al. [159] concluded that although the designation of MPAs as a tool to preserve the remaining populations of the heavily exploited *P. ferruginea* is of extreme importance, for these MPAs to fulfil their goal, additional measures must be considered. In 2015, Coppa et al. [163] suggested that without a joint effort toward the protection of intertidal habitats by enforcement bodies, regulators, researchers, and sea users, the MPAs will not be able to achieve their conservation objectives.

The effectiveness of MPAs in the conservation of limpet populations could be enhanced through the implementation of several additional measures that encompass a broader view of these exploited populations and the biological and ecological factors that influence their capacity to recover. For instance, it is necessary to determine which actions are required to ensure the reproductive success of individuals, essential to maintain the genetic biodiversity of overexploited species, particularly in species with absent gene flow between populations, since inbreeding increases the extinction probability of wild populations [168]. Also, the reintroduction or reinforcement of recruitment of depleted populations with allochthonous specimens produced by artificial fertilization procedures could be considered as a strategy to further fulfil the MPAs’ conservation objective [169].

The establishment of MPAs as a conservation tool of marine coastal habitats and species has returned valuable contributions over the years, particularly in terms of density, abundance, and size structure of exploited species. However, to overcome limitations a possible route to improve the success of conservation strategies could be the establishment of networks of MPAs based on solid scientific information that identifies the type of measures that need to be implemented.

Planning should consider the number and size of MPAs, which should be large enough to ensure the recovery of protected populations but sufficiently spaced in order to allow the spillover of recruits and adults to the exploited populations. MPA planning should ultimately target the ecosystem and not a specific exploited species, since the success of a reserve depends not only on the recovery of a single species but on the recovery of the ecosystem to which the species belongs. Additionally, due to geographic specificities, the prevalent abiotic factors and how they influence the target ecosystem should be considered when planning MPAs. Also, continuous monitoring of the effects of MPAs on the exploited populations would allow for a more adequate management of MPAs, allowing for the adjustment of the protective measures as needed.

Besides adequate planning of MPAs, new conservation strategies are required to implement measures that raise public awareness and the political will of decision makers that would allow for innovative approaches involving not only decision makers but also the end users of these marine resources in the conservation effort of exploited species, particularly to avoid illegal poaching, which is one if not the greatest factors hindering MPA success.

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Chapter 5

Growth and reproduction of the north-eastern Atlantic keystone species *Patella aspera* (Mollusca: Patellogastropoda)

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Abstract

The growth and reproductive biology of the limpet *Patella aspera* were studied in the north-eastern Atlantic, Madeira archipelago, to enhance the knowledge concerning biological parameters and population dynamics of this species. This study comprised the estimation of growth rates, sexual maturity, reproduction, recruitment patterns, mortality coefficients and the exploitation rate, and yield-per-recruit (*Y*/*R*) based on monthly shell-length frequency data. A total of 16,941 specimens were sampled from January to December 2015. The relative growth pattern exhibited a negative allometric nature of growth for both sexes. The estimated von Bertalanffy growth parameters showed an asymptotic length of 84.15 mm for females and 80.51 mm for males with a growth coefficient of 0.36 and 0.32 year^{-1} respectively. *P. aspera* in this geographical area is a moderately long-lived limpet with a predominance of specimens younger than 3 years old. This species is a winter breeder with a reproductive cycle encompassing three main periods namely development, spawning and resting with a synchronous gametogenesis for both sexes. Gonadal development lasts from October to December, spawning likely occurs from January until April and resting occurs from May to September. The mean size at sexual maturity was determined as 41.78 mm for females and 38.29 mm for males and the length at first capture as 42.62 mm. The recruitment pattern was continuous throughout the year with a major peak identified in March. The natural, fishing and total mortalities were similar between sexes, with fishing mortality exerting greatest pressure on this resource. However, yield-per-recruit analysis showed that the stock of *P. aspera*, in the study area, is exploited at levels below the fishing mortality that returns maximum sustainable yield. This study revealed that currently the stock of *P. aspera* is under-exploited, nonetheless due to its slow growth and long life, continuous monitoring and the enforcement of the existing harvest regulations must be accomplished if future overexploitation is to be avoided. Further genetic studies are necessary to establish connectivity of the populations and improve present conservation strategies.

Keywords

Patella aspera, growth, reproduction, recruitment, mortality.

1. Background

Limpets are marine gastropods that inhabit rocky shores. These species play an important role in regulating and structuring communities in their habitat and have often been used as biological indicators in evaluating the consequences of anthropogenic impact on this ecosystem [1, 2].

Patella aspera Röding, 1798 is one of the fourteen worldwide recognized species of the genus *Patella* Linnaeus, 1758. This species is endemic to the Macaronesian archipelagos, where it is highly exploited for human consumption [3], and occurs in Madeira archipelago together with *Patella candei* d' Orbigny, 1840 and *Patella piperata* Gould, 1846. In the Madeira archipelago, it is caught mostly by scuba diving in a mixed exploitation with *P. candei* and has considerable commercial importance, both species combined reaching annual catches of up to 100 tons [4] with a peak of 150 tonnes in 2015 that yielded a total first sale value of *ca.* 0.7 M€. *P. aspera* is a protandric species [5] reported to occur in the intertidal and subtidal [6]. It has a subpentagonal flattened shell with numerous regular furrows more or less similar and imbricated with an apex situated above. Internally, the shell is white to blue delimited by a brown band [7]. The foot has orange or yellow colour and the mantle is surrounded by translucent tentacles [8].

The life history parameters of intertidal invertebrates vary inter- and intraspecifically as a result of genetic differences and environmental influences [9]. Parameters such as growth, reproductive strategy and mortality are dependent on a complex combination of environmental factors [10, 11] and are important in understanding the distribution and abundance of a species [12].

Limpet's diversity and ecological significance prompted intensive research over the past decades. However, the knowledge concerning life-history, age structure and growth patterns for these species is still limited [13]. Most information available on the biology of *Patella* species is focused on *Patella vulgata* Linnaeus, 1758. Even so, some aspects of limpet biology such as reproduction have been studied for other species such as *Patella ulyssiponensis* Gmelin, 1791, *Patella depressa* Pennant, 1777, *Patella rustica* Linnaeus, 1758 and *Patella candei* [6, 9, 14–17].

This work aims to provide information on growth, age structure, reproduction, sexual maturity, recruitment, and mortality rates of *P. aspera* contributing with additional information and filling the gaps on the knowledge on the life traits of this species and also to evaluate the effect of size at first capture on the exploited stock applying a yield-per-recruit model. Therefore, contributing to the sustainable exploitation of stocks by providing proper background for effective management of these resources.

2. Methods

2.1. Collection of data

From January to December 2015 monthly fresh samples of *Patella aspera* were collected at Madeira archipelago, north-eastern Atlantic (32°00′–33°30′N; 15°30′–18°00W) encompassed in a Fisheries Research Project of the Fisheries Research Service (DSI) from the Regional Directorate of Fisheries of the Autonomous Region of Madeira. All samples were collected randomly from the inter- and subtidal by snorkelers executing several dives during 30 min, without selecting species or size of specimens. All dives were performed by DSI technicians.

Shell length (*L*) was measured using a Vernier calliper (+0.1 mm) and total weight (*W*) using an electronic scale with 0.01 g accuracy. Specimens were sexed according to gonad pigmentation. Yellow gonads correspond to males and reddish brown to females. Dissection of specimens and macroscopic examination of the gonads allowed assigning each specimen to one of the six gonad maturation stages based on an adaptation of Orton et al. [18] maturation scale in which each stage is determined according to the progression of the gonads volume in the hemocele. Stage I—start of development, detectable sex, slight increase in the volume of the gonads; stage II—gonads developed up to 1/3 of the total volume; stage III—gonads in size between 1/3 and 2/3 of the total volume; stage IV—gonads with 2/3 of the total volume; stage V—gonads fully developed occupying the entire hemocele; neuter stage—specimens in pre-reproductive phase and adults in the resting phase between breeding cycles, after full release of gametes, in which the gonads are not macroscopically observable. Gonads were removed, damp-dried and weighed to 0.01 g accuracy. All measurements were taken from fresh samples.

The data were analysed for deviations to the parametric assumptions of ANOVA. Normality of the distribution of the sample was determined by the Kolmogorov–Smirnov two-sample test and the homogeneity of variance was tested using Levene’s statistics. The existence of differences in shell length and total weight between sexes was determined using an analysis of variance (ANOVA).

2.2. Relative growth

The relationship between weight (*W*) and shell length (*L*) of *P. aspera* was determined adjusting the data to a potential relationship as $W = aL^b$ [19], where *W* is the total weight (g), *L* is the shell length (mm), *a* is the intercept (condition factor) and *b* is the slope (relative growth rate).

The parameters *a* and *b* were calculated by linear regression analysis fitted by the least-squares method over log-transformed data ($\log W = \log a + b \log L$). The coefficient of determination r^2 was used as an indicator of the quality of the linear regression and the nature of growth was tested using the Student's t test in order to determine if the *b* coefficient was different from 3 with a significance level of 0.05 [11].

2.3. Absolute growth and age

Growth was estimated from the modal class progression analysis in the monthly length-frequency distributions, using the Bhattacharya method included in the package FISAT II (Fish Stock Assessment Tools FAO-ICLARM), VER 1.2.0 [20]. All the identified size-age groups were derived from at least three consecutive points and selection of the best results was based on the values of the separation index (>2) for the different age groups and the number of individuals per age group [21]. NORMSEP by Hasselblad and Tomlinson [22] was used to decompose the mixtures of normal distributions based on Hasselblad's maximum likelihood method [23].

The parameters of the von Bertalanffy growth function $L_t = L_\infty \{1 - \exp[-k(t - t_0)]\}$ [24], were estimated by nonlinear regression using the FiSAT II software package, where, Lt is the predicted length at time t (mm), L_∞ is the asymptotic length (mm), *k* is the growth constant (year^{-1}), *t* is the age (year) of the *P. aspera*, and t_0 is the age at which $L_t = 0$ [25].

The estimated parameters L_∞ and *K* were used to calculate the growth performance index (ϕ') using the equation: $\phi' = \log_{10}(K) + 2\log_{10}(L_\infty)$ [26].

The age at length was determined using the inverse von Bertalanffy growth equation and the potential longevity ($A_{0.95}$) was estimated from: $A_{0.95} = t_0 + 2.996\,K$ [27, 28].

2.4. Reproduction and recruitment

The sex ratio of *P. aspera* was estimated and tested for the existence of differences in the proportion of sexes in the studied population using a χ^2 goodness-of-fit test and between months using Pearson's Chi square (χ^2) considering a significance level of 0.05. The gonadosomatic index (GSI) was calculated according to the equation: GSI = (wet gonad weight / total body wet weight) x 100. Differences in the mean GSI values between sexes and among months were assessed using an analysis of variance (ANOVA) considering a significance level of 0.05.

The proportion of specimens in each maturation stages was plotted per month in order to estimate the spawning season considering all the individuals with gonad maturation stage equal or superior to III mature following Orton et al. [18].

Size at sexual maturity (Lm_{50}) corresponds to the size at which 50% of all individuals in a stock are mature and was estimated from the relationship between the proportion of mature individuals and length, described by the logistic equation: $P = 1/(1+\exp^{(-(a+bL))})$ [29], where P is the balanced probability, a and b are the equation parameters estimated by the linear least square method using the logarithmic transformation. The mean size at maturity was defined as the size at which 50% of the population is mature, when $P = 0.5$ then $Lm_{50} = -a/b$ [11]. The values of Lm_{25} and Lm_{75} (length at which 25 and 75% of limpets are mature) were also estimated.

The recruitment pattern was estimated by projecting the length-frequency data backwards on the time axis using the growth parameters [30] and the normal distribution of this pattern was obtained using the NORMSEP [31] routine in FISAT.

2.5. Mortality, exploitation rate, probability of capture and yield-per-recruit

Total mortality (Z) was estimated using the length converted catch curve method. Natural mortality rate (M) was estimated using Pauly's empirical model [32]: $\log_{10}M = -0.0066 - 0.279(\log_{10}L_{\infty}) + 0.6543(\log_{10}K) + 0.4634(\log_{10}T)$, where L_{∞} the asymptotic shell length (mm), K the growth coefficient (year^{-1}) and T the annual mean habitat temperature (°C) which was 20.1°C in the habitat of the species in the study area. Fishing mortality (F) was obtained by subtracting M from Z and the exploitation rate (E) was obtained from $E = F/Z$ [33].

The probability of capture was estimated by means of the logistic transformation of the probabilities obtained from the lower-sized limpets, using the left hand-side of the length-converted catch curve, by plotting the cumulative probability of capture against middle length of class intervals. The length at first capture (Lc_{50}) was assessed from the resultant curve and represents the length which corresponds to the cumulative probability of 50%, according to Sparre and Venema [29]:

$SL = 1/[1 + \exp(S1 - S2 \times L)]$ where SL is the logistic curve, $S1$ and $S2$ are constants in the formula for length-based logistic curve and L is the limpet length. Additionally, the lengths that correspond to the cumulative probabilities of 25 and 75% (Lc_{25} and Lc_{75}) were also estimated.

Relative yield-per-recruit (Y/R) and the fishing mortality that returns the maximum sustainable yield (F_{MSY}) were obtained according to Beverton and Holt [34] length based method:

$$\frac{Y}{R} = Fe^{(-M(T_c - T_r))}W_\infty[\frac{1}{Z} - \frac{3S}{Z+K} + \frac{3S^2}{Z+2K} - \frac{S^3}{Z+3K}]$$

where Y/R is the catch in weight, per recruit, W_∞, K and t_0 are growth parameters, T_c is the age at first capture, T_r is the age at recruitment, F is the fishing mortality, M the natural mortality, Z the total mortality and $S = e^{(-K(T_c - T_0))}$.

The variation of yield-per-recruit resulting from different size of capture was simulated by applying the previously estimated Lc values of 25, 50 and 75% to the model, in order to evaluate the effect of harvest of smaller and larger individuals in the fishing mortality and Y/R.

3. Results

A total of 16,941 specimens were sampled including 5,074 (29.95%) females, 6,239 (36.83%) males and 5,628 (33.22%) neuters (Fig. 1). The size-frequency showed that the sampled data had a normal distribution (Z = 2.946, P < 0.001). However, size did not have homogenous variance between sexes (W = 171.142, p < 0.001). In regards to weight the sampled data was normally distributed (Z = 13.862, P < 0.001) and not homogeneous (W = 597.136, P < 0.001). As such, all ANOVA's were performed considering the Brown–Forsythe *F* test.

The size in females varied from 15.42 to 82.96 mm ($\bar{x}$ = 45.66 ± 8.02), in males from 17.30 to 79.83 ($\bar{x}$= 44.31 ± 7.60) and in neuters from 11.00 to 67.00 ($\bar{x}$ = 41.51 ± 6.18), the observed differences in mean length between groups was significant (F = 351.202, P < 0.05). The total weight varied from 1.07 to 67.19 g ($\bar{x}$ = 9.99 ± 6.16) in females, 1.08 to 54.07 g ($\bar{x}$ = 8.62 ± 4.80) in males and 0.37 to 42.62 g ($\bar{x}$ = 6.12 ± 2.87) in neuters, the observed differences in mean weight were significant (F = 906.667, P < 0.05).

The largest sampled specimen was collected in July with 82.96 mm and the smallest in March with 11.00 mm. Specimens with more than 80.00 mm were only collected in July.

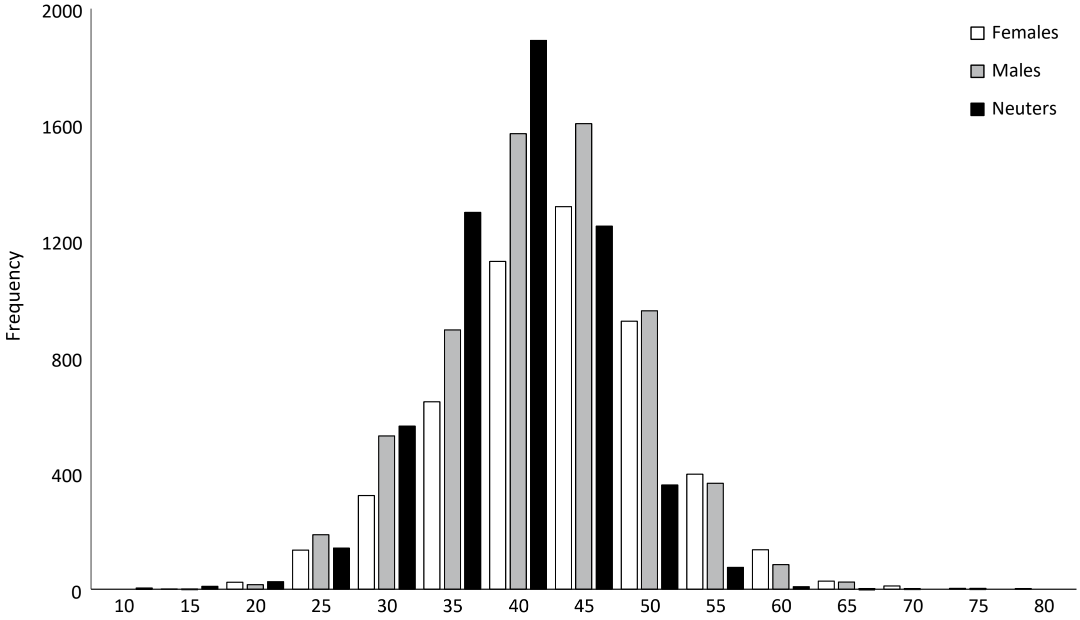

Figure 1 - *Patella aspera* length-frequency distributions of females, males and neuters, collected from January to December 2015.

3.1. Relative growth

The regression between shell length and total weight showed a negative relationship and the parameters of the regression were estimated as: $W = -3.797 + L^{2.859}$, $r^2 = 0.82$, $P < 0.05$ and $W = -3.729 + L^{2.805}$, $r^2 = 0.85$, $P < 0.05$ for females and males respectively. The *b* coefficient returned values inferior to three for both sexes and the difference was highly significant ($P < 0.001$), indicating negative allometry.

3.2. Absolute growth and age

The *P. aspera* monthly length-frequency distributions are shown in Fig. 2. The estimated von Bertalanffy growth parameters for females were $L_\infty = 84.15$ mm and $K = 0.36$ year^{-1} whereas for males they were $L_\infty = 80.51$ mm and $K = 0.32$ year^{-1}. The growth performance index (ϕ′) was calculated as 3.41 for females and 3.32 for males based on the collected data.

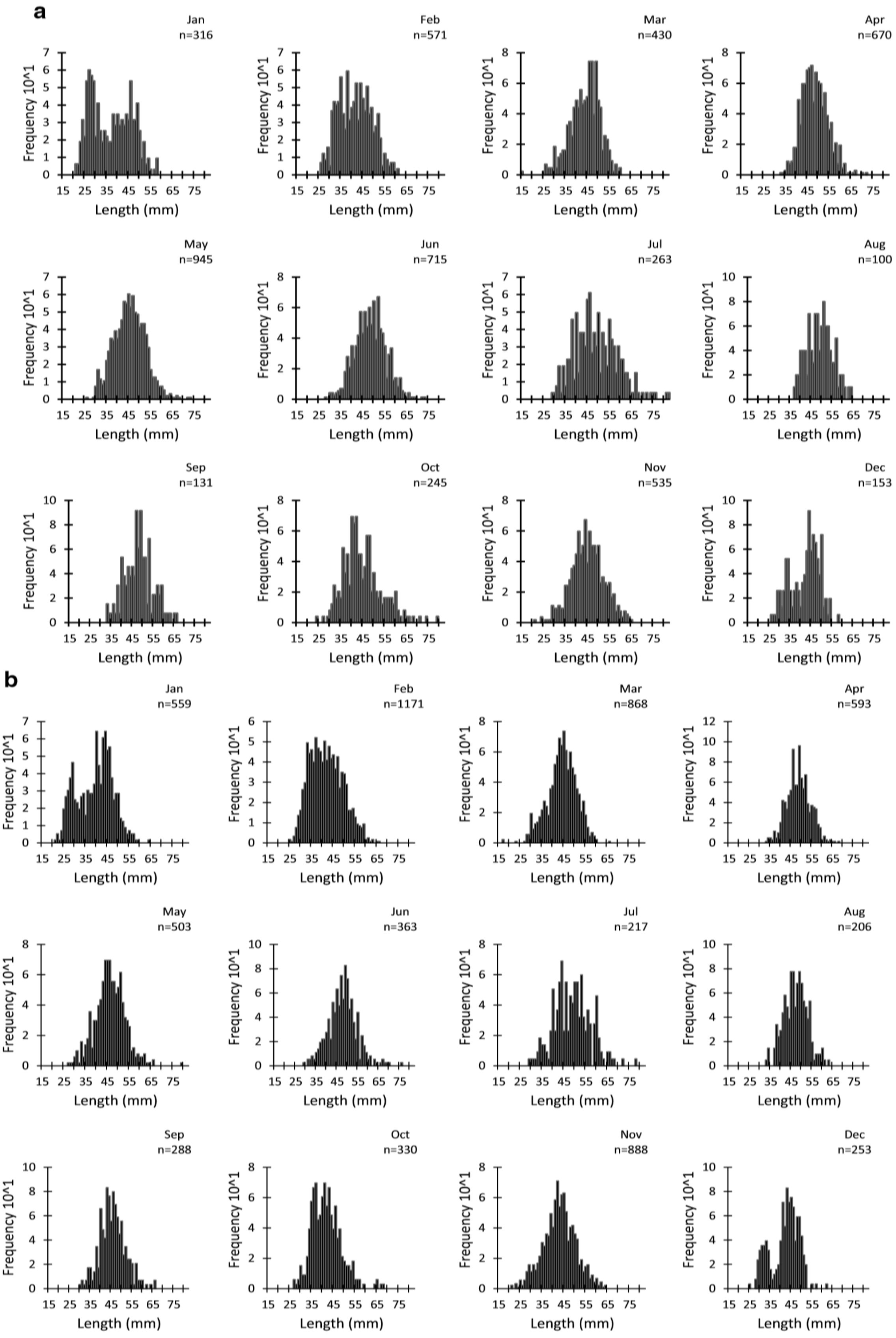

Figure 2 - Monthly length-frequency distribution for females (a) and males (b) of *Patella aspera*, collected from January 2015 to December 2015.

The species *P. aspera* showed a predominance of specimens in the first age-classes for both sexes. More than 80% of all individuals from the studied population were younger than 3 years, 93.81 of the females and 84.12% of the males. Potential longevity, assuming t_0= 0 was estimated as 8.32 years for females and 9.36 for males.

3.3. Reproduction and recruitment

Patella aspera does not exhibit observable external sexual dimorphism. The overall ratio between females and males was 1:1.23 (female: male), slightly favouring the males. The Chi^2 goodness-of-fit test showed that the observed differences were significant (χ^2 = 124.765, $P < 0.05$). The analysis of the sex ratio per months showed that males were predominant between August and March and females from April to July (χ^2 = 784.460, $P < 0.05$). Immature, mature and neuter individuals were present all year round (Fig. 3).

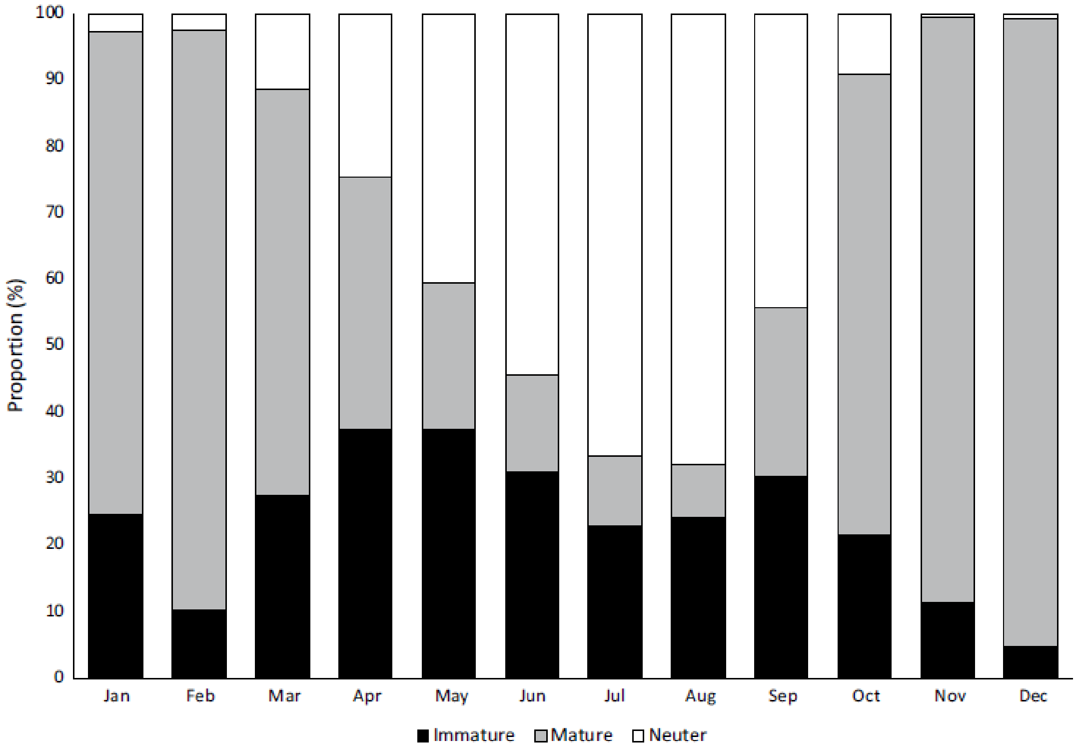

Figure 3 - Monthly distribution of immature, mature and neuter individuals of *Patella aspera* from Madeira archipelago, collected from January to December 2015.

Immatures were more abundant in spring and summer, matures predominated in autumn and winter wile neuters dominated in late spring and summer. The highest proportion of immature individuals occurred in April with 37.58% and the lowest in December (4.88%). Immatures females and males (stage I and II) were more abundant in April (38.83%) and March (27.50%) respectively (Fig. 4).

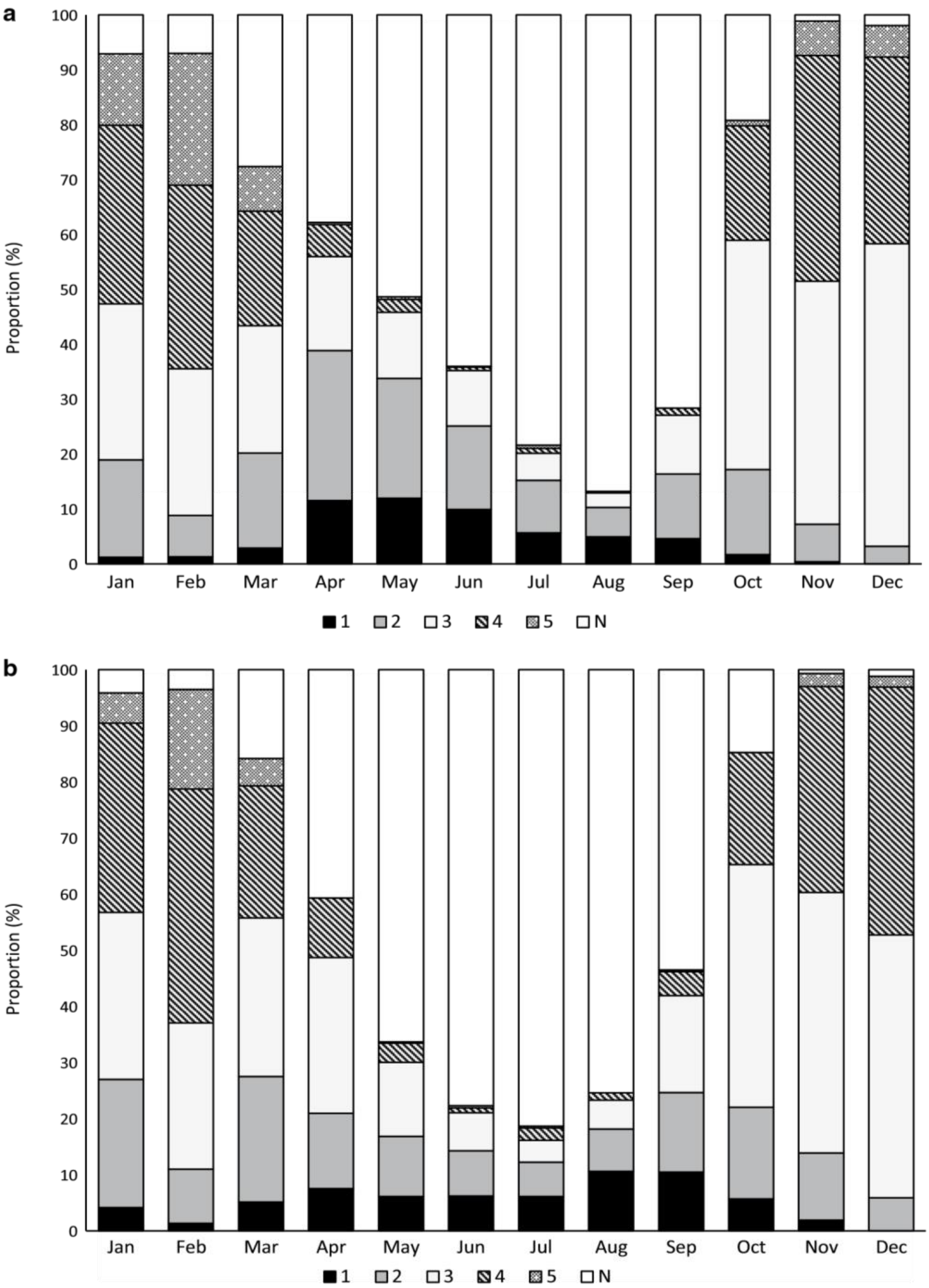

Figure 4 - Annual gametogenesis cycle of *Patella aspera* from Madeira archipelago collected from January to December 2015. a - Females, b - Males.

Mature specimens were predominant from October to March with more than 61.00% of specimens per month. The greatest proportion of mature individuals was found in December (94.38%) and the lowest in August (7.93%). The pulse of mature females and males (stages III, IV and V) occurred in

December with 94.87 and 92.97% respectively. Neuters were present all year and predominated from May to September with a peak in August (67.74%). The smallest and largest mature specimen were both females with a shell length of 21.07 and 82.96 mm respectively.

The GSI values for *P. aspera* did not significantly differ between sexes (F = 1.638, $P > 0.05$), however significant differences between months within females (F = 12.292, $P < 0.05$) and males (F = 13.530, $P < 0.05$) were observed. Variations in the GSI for females and males are shown in Fig. 5. Monthly values revealed an increase from November to January. The highest GSI values were found in January for both sexes with 7.44% for females and 7.30% for males. After that peak it consistently decreases until April and between May and November GSI fluctuated between 0.79 and 2.68% for both sexes, suggesting that the spawning season for this species occurs from January to April. The monthly variation of GSI values is in accordance with the observed proportions of immature, mature and neuter limpets, increasing when the proportion of mature individuals is higher and decreasing when the proportion of neuters and immature limpets is higher.

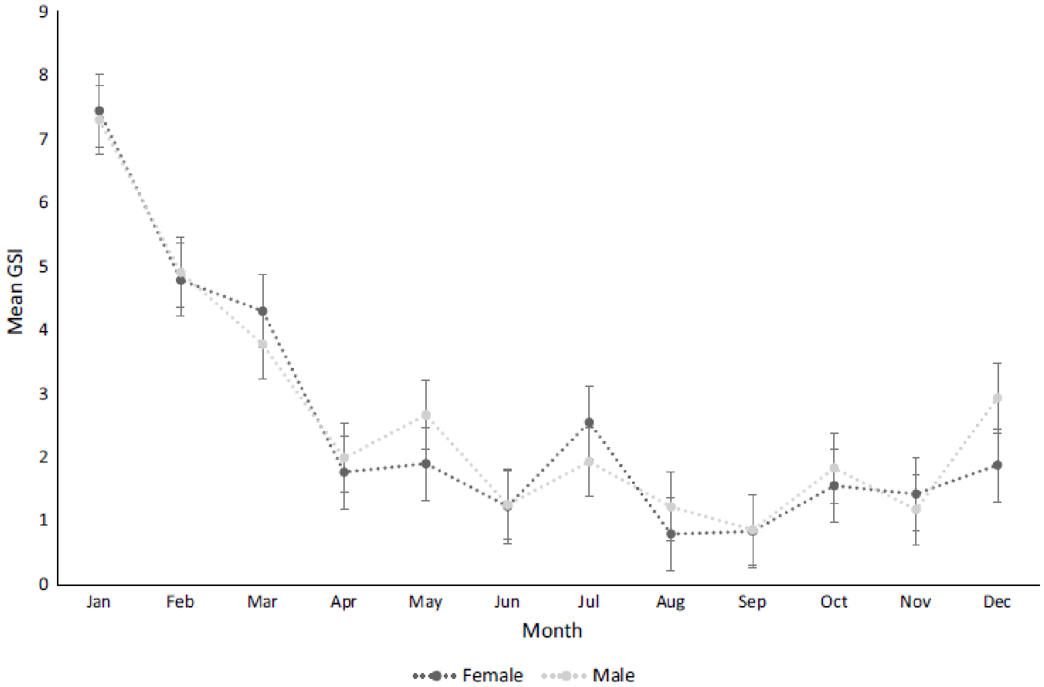

Figure 5 - Seasonal changes in gonadosomatic index (GSI) for females and males of *Patella aspera* from Madeira archipelago collected from January to December 2015.

The mean size at sexual maturity was determined (Lm_{50}) as 41.78 mm for females and 38.29 for males corresponding to 1.91 and 2.01 years of age respectively. The size at which 25 and 75% of the population reach sexual maturity was estimated at 31.61 and 51.97 mm for females, and 24.96 and 51.61 mm for males.

The recruitment pattern was continuous throughout the year, with a major peak being identified in March with 18.76% and a minor peak in October with 7.76%. The smallest specimen was collected in March.

3.4. Mortality, exploitation rate, probability of capture and yield-per-recruit

Total mortality (*Z*) and natural mortality (*M*) for females were estimated at 1.38 and 0.59 per year respectively. Fishing mortality (*F*) was 0.79 per year and the exploitation rate (*E*) was estimated at 0.57. For males *Z* was 1.33 year^{-1}; *M* was 0.55 year^{-1}; *F* was 0.78 year^{-1} and *E* was 0.58.

The probability of capture returned an estimate of length-at-first capture for combined sexes (Lc_{50}), of 42.62 mm during the study period corresponding to 1.99 years. The values of Lc_{25} and Lc_{75} were estimated at 38.10 and 47.14 mm, and corresponded to 1.70 and 2.32 years of age respectively.

The analysis of yield-per-recruit showed that at the current exploitation level the maximum production is achieved at F_{MSY} of 1.0 per year corresponding to a *Y/R* of 2.923 g. The simulation of *Y/R* varying length-at-capture resulted in an F_{MSY} of 0.8 per year and *Y/R* of 2.674 g for Lc_{25}, while for Lc_{75} the F_{MSY} obtained was 1.4 per year with a *Y/R* of 3.161 g (Fig. 6).

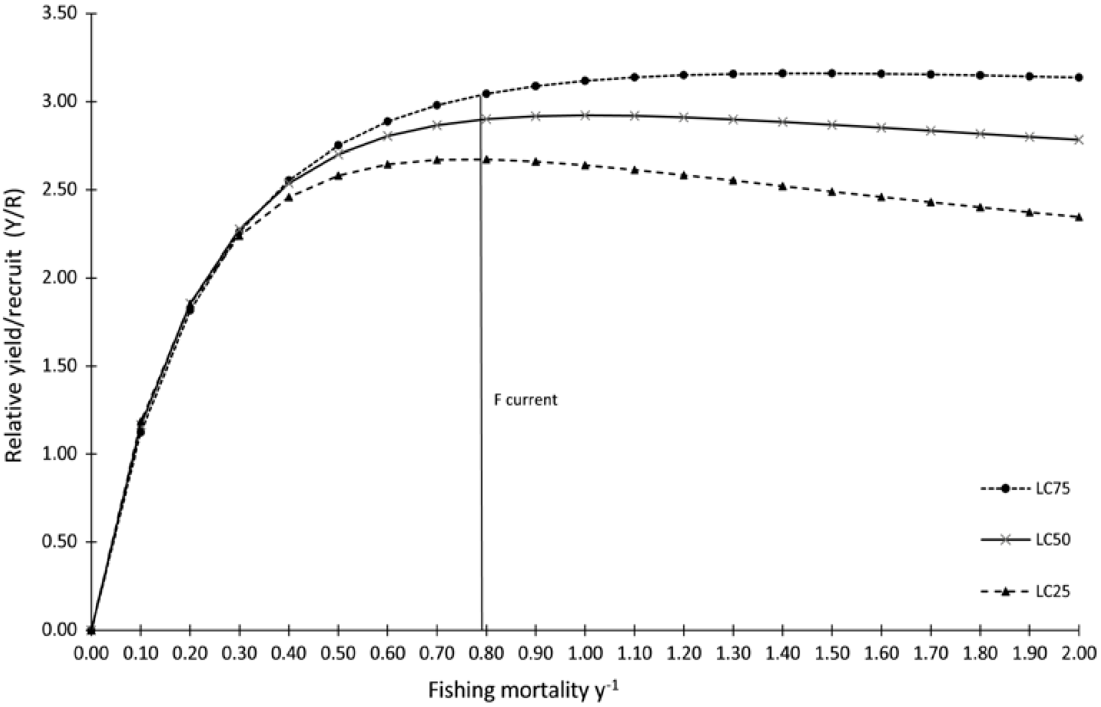

Figure 6 - Beverton-Holt yield-per-recruit curves on *F* for *Patella aspera*, considering considering the size at first capture (Lc_{50}), and the lengths at which 25% (Lc_{25}) and 75% (Lc_{75}) of individuals of *Patella aspera* were captured.

4. Discussion

Intertidal gastropods are especially useful in population differentiation studies generally because they inhabit heterogeneous environments and exhibit conspicuous variation in behaviour, morphology and life-history [20, 35–37].

The present study on the exploited limpet *P. aspera* fills the gap and enhances knowledge on life history traits of this keystone species of the intertidal and subtidal lower zones in the north-eastern Atlantic, Madeira archipelago.

4.1. Relative growth

The relationship between length and weight plays a major role in fisheries research since it is often associated with providing population parameters necessary for proper fisheries management and sustainable yield of the stocks [38].

The relative growth coefficient obtained for *P. aspera* was significantly lower than 3 for both sexes suggesting that this species growth has a negative allometric nature supported by high values of correlation indicating an elevated predictability between shell length and weight for this species. The *b* values obtained for females and males of *P. aspera* in the Madeira archipelago were in accordance with the range of values usually encountered for this parameter in marine species, which lies between 2.5 and 3.5 [19, 39]. This growth constant notably demonstrates normal growth dimensions and/or well-being of the studied population [19, 40, and 41]. The exhibited negative allometric growth pattern implies that the rate of increase in shell length was higher than the rate of increase in weight. This relates to how *P. aspera* allocates energy use. The obtained nature of relative growth indicates that this species in Madeira archipelago invests more energy in growth than in reproduction.

The growth pattern obtained may be explained by the existence of lower population densities in the preferred habitat of occurrence of *P. aspera*, possibly as a result of the traditional exploitation of this resource [42] whereas the decrease in population density is translated in an increase of food and substratum availability promoting the increase in growth as reported for *P. alticostata* by Black [43]. The negative allometric pattern observed might also be related to the instability of the environmental conditions in the intertidal zone, since this species is vulnerable to many factors such as desiccation, insolation, air temperature fluctuation and wave action which was shown by Thompson [44] to influence growth in *P. vulgata* and *P. ulyssiponensis* and to biotic factors like age and sex [11].

4.2. Absolute growth and age

In general, the growth parameters obtained for *P. aspera* in the archipelago of Madeira were similar to those found for other limpet species. The estimated asymptotic lengths for *P. aspera* at 84.15 mm for females and 80.51 for males are consistent with the lengths of the largest sampled female (82.96 mm) and male (79.83 mm) and identical to that determined by Henriques et al. [17] for the congener species *P. candei* in the same geographical area. Factors such as population density, predation, competition and limited food supply are indicated as influencing growth rates on molluscs supporting the idea that the strategy of diverting the energy to reproduction and vice versa according to the organisms needs influences growth rates [42, 45–48].

Growth rates in limpets are most likely influenced by the environmental factors to which they are exposed, such as temperature, insolation and/or photoperiod that changes with latitude [48]. The estimated growth rates of 0.36 year^{-1} for females and 0.32 year^{-1} for males, are relatively low and probably explained by the oligotrophic nature of the water in the Madeira archipelago [49] and the warmer sea water temperature [50]. Identical growth rates were estimated for *P. candei* (0.32 year^{-1}) from Madeira archipelago [17], *P. rustica* (0.30 year^{-1}) from the Adriatic Sea [13], *Scutellastra cochlear* (Born, 1778) (0.33 year^{-1}) and *Scutellastra longicosta* (Lamarck, 1819) (0.30 year^{-1}) from South Africa [51].

The nonlinearity of growth of marine organisms prevents direct comparison of the growth parameters [52], as such determination and comparison of the overall performance of different marine species growth is performed using the growth performance index of Pauly and Munro [26] which relates the asymptotic length and growth rate. This index is considered to be species-specific and to represent the physiological capacity of the organism with genetically predetermined factors [13]. For *P. aspera* the calculated growth performance index was 3.40 similar to *P. candei* (3.32) from the Madeira archipelago [17] and *Patella ferruginea* Gmelin, 1791 (3.30) from North Africa [53]. Nevertheless, the variation of this index among species of the Patellidae family is reduced with values ranging from 2.42 for *S. cochlear* to 3.65 and *Cymbula granatina* (Linnaeus, 1758) from South Africa [47], which it is in agreement with Sparre et al. [54] who claim that the growth performance index remains relatively constant at similar rates between related taxa. The variability is likely due to abiotic and biotic factors such as hydrodynamics, desiccation, predation, competition, and temperature to which the different species are subject.

The median lifespan of 8.32 years for females and 9.36 for males depicted by *P. aspera* probably influences their growth in a way that makes them reach the asymptotic length at a moderated rate.

The potential longevity of *P. aspera* in this work describes the population as a moderately long-lived species like other temperate limpets such as *Lottia gigantea* Gray in G. B. Sowerby I, 1834, *P. candei* and *P. ferruginea* [5, 17, 53]. On the contrary tropical limpets have shorter lifespan as reported by Khow [55] for *Cellana testudinaria* (Linnaeus, 1758). This pattern is in agreement with Clarke et al. [48] who states that limpets in temperate regions grow more slowly and reach larger maximum size and therefore have a longer lifespan than tropical limpets.

4.3. Reproduction and recruitment

The sex ratio of the studied population of *P. aspera* was slightly skewed in favour of males. Imbalances in the sex ratio of this species might be related to the possibility of *P. aspera* being a protandric hermaphrodite. Also, size selective harvest of this species might induce phenotypic plasticity in protandric hermaphrodite limpets as recently reported for *Patella vulgata* by Borges et al. [56]. In heavily exploited populations of protandric hermaphrodite limpets, sex-change has been reported to occur at smaller sizes as a consequence of the size selective nature of limpet harvest, skewing sex-ratios and altering the reproductive output of those populations. Thus, further studies are required to determine if *P. aspera* is indeed a protandric hermaphrodite and if so to infer the impact of long term continuous exploitation of this species in the archipelago of Madeira.

The gonadal cycle of *P. aspera* was described using the monthly variation of the GSI. This method is commonly used in species with gonads that are easily dissected from the rest of the soma, since it is a good indicator of gonadal changes throughout the year [57, 58]. Seasonal changes in GSI reflect periods of accumulation and release of gonad material as well as utilization of stored energy reserves [59].

Patella aspera is reproductively active from January to April. Neverteless, the presence of mature and partially spawned specimens in the sampled population throughout the year, suggested that some individuals could spawn all year round. Results showed that *P. aspera* had a synchronous gametogenesis for both sexes, with females and males showing the same pattern all year round, with small monthly differences in the proportion of mature individuals. The reproductive cycle encompasses three main periods namely development, spawning and resting. The phase of gonadal development seems to be moderately short, lasting from October to December as shown by the increase in GSI and proportion of gonadal maturity stages III and IV. Spawning likely occurs from January until April, in accordance with the decrease in the proportion of specimens with ripe gonads and the higher GSI values that consistently decrease during this period when the gonads of *P. aspera* were in maximum development for both sexes. Spawning is followed by a longer resting phase that lasts 5 months from May to September, when neuters are prevalent in the population and minimum GSI values occur. The moderately short gonadal development period might be explained by the negative allometric nature of growth of *P. aspera* in the archipelago of Madeira which indicates a stronger investment of energy in growth than in reproduction.

The occurrence of a spawning pulse from January to April indicates that *P. aspera* is a winter breeder like *P. candei* in the Madeira archipelago [17], *P. vulgata* in the British Isles [18, 60] and unlike *Patella ulyssiponensis* and *Patella depressa*, in the central Portuguese coast, described as summer breeders [6, 9, 15, 16].

Patella aspera as most other patellids exhibits a restricted reproduction season generally with a main spawning event per year, followed by a long resting state [18, 47] with the duration of these periods varying according to the region of occurrence mostly as a consequence of the influence of temperature. In regions with higher temperatures spawning occurs in a shorter period, contrary to what happens in regions with colder waters, where the development of the gonads requires a longer time period [18]. Several other factors such as high wind speed allied to stimulation by wave action and increase in phytoplankton concentration might also induce spawning, acting as environmental triggers. In Madeira archipelago, the spawning of *P. aspera* is concurrent with higher phytoplankton concentration which is in agreement with Underwood [46] that states that gastropod species with planktotrophic larvae spawn when phytoplankton concentration is higher. In general, the reproductive patterns obtained in the present study generally conform to the latitudinal trend previously described for other species of the genus *Patella* and other intertidal species, consisting in progressively longer reproductive seasons and spawning occurring later in the year towards the south [9].

The observed pattern in the reproductive cycle of *P. aspera* in Madeira archipelago is consistent with previous findings reported by Delgado et al. [42] for a time series encompassing 5 years. This indicates that the obtained pattern is consistent throughout the years and does not result from specific particularities of the studied period. Even though small variations are expected to occur between years, as a result of different abiotic conditions, the observed pattern is likely to be maintained.

The estimated shell length at first maturity of *P. aspera* in the archipelago of Madeira was 41.78 mm for females and 38.29 mm for males corresponding to 1.91 and 2.01 years and most of the sampled population was distributed between one and three years of age (>95%) meaning that most individuals in the population are sexually mature.

The recruitment of juveniles to the studied populations of *P. aspera* was continuous throughout the year, with a major peak in March and a less prominent peak in October. According to Henriques et al.

[17] *P. candei* also exhibits a continuous recruitment pattern throughout the year, with the major recruitment event occurring in January. The differences in recruitment pulses between these two species could be explained by specific physical factors like temperature, habitat, topography of the bottom and humidity of surface and biological factors such as the presence of competitor species [61].

The specimens with shell length inferior to 10 mm were not sampled in this study. Likewise, Henriques et al. [17] stated that the smallest *P. candei* sampled was around 15 mm. This could suggest the existence of a specific nursery habitat for these limpet species in the Madeira archipelago since the behaviour of these two species is similar. In this case juveniles, would only migrate to the adult population after reaching a specific shell length/age as previously reported for *Patella pellucida* [62] and other mollusc species [63].

4.4. Mortality, exploitation rate, probability of capture and yield-per-recruit

The natural, fishing and total mortalities were almost similar between sexes for *P. aspera* in the Madeira archipelago. Fishing mortality exerts more pressure on the resource in the study area contrary to natural mortality that was inferior and similar to the exploitation rate, indicating that the combination of traditional and commercial fishery of this resource exerts considerable pressure on the stock of *P. aspera*. Nonetheless, the results suggest that the stock of this species in the Madeira archipelago is moderately under-exploited. Similar results were found for the congener species *P. candei* in the same geographical area [17].

Analysis of catch selectivity returned a value of 42.62 mm for the length at first capture corresponding to 1.96 years of age for females (41.78 mm) and 2.35 years of age for males (38.29 mm), suggesting that the bulk of capture occurs shortly after *P. aspera* reaches sexual maturity for both sexes which seems to ensure reproduction of the species, as long as the minimum capture size allowed (40 mm) and the closed season (from December to March) are respected. However, the results also indicate that 99.5% of captured limpets are younger than 4 years old. The low frequency of older individuals in the natural populations of *P. aspera* could probably be a consequence of intensive size selective exploitation of larger limpets which could result in a decrease in reproductive output, since reproduction of this species is gamete density dependent.

The analysis of Y/R showed that *P. aspera* is exploited in the archipelago of Madeira at levels of fishing mortality that are below the F_{MSY} of 1.0 year^{-1}, returning a yield of 2.901 g. These results indicate that the stock of *P. aspera* in the region is not overexploited, however an increase in fishing mortality to the level of F_{MSY} would result in only a slight increase in yield from 2.901 to 2.923 g and since the relationship between yield and fishing mortality is fundamentally asymptotical the fishing effort required to approximate fishing mortality to F_{MSY} would most likely be too great to be fruitful. Moreover, the simulation of the effect of size at first capture on the Y/R showed that at Lc_{25} the F_{MSY} is of 0.8 year^{-1} corresponding to a yield of 2.647 g. This decrease in F_{MSY} when considering smaller size at first capture indicates that this fishery is vulnerable to the harvest of smaller individuals. This is related to the size at first maturity being greater than the size at first capture, in which case the removal of smaller individuals compromises the reproductive output of the exploited populations. Thus, this simulation corroborates the importance of limiting fishing mortality of younger limpets by ensuring that the current minimum size of capture of 40.00 mm is enforced.

An increase in the size at first capture on the other hand would allow for higher fishing mortality and yield, even though initially it would result in a decrease of yield due to capturing less individuals, on the long term it would allow for the capture of larger and heavier individuals, thus making up for the loss of number of individuals captured in total weight. In fact, the close values obtained for length at first capture and length of maturation suggest that an increase in the minimum size of capture should be considered in order to maintain the reproductive output of the stock of *P. aspera*.

5. Conclusions

The life history of *P. aspera* in the archipelago of Madeira exhibited similar characteristics and behaviour to its congener *P. candei* in the same geographical area. The observed differences between *P. aspera* and other patellids from distinct geographic areas are probably explained by specific environmental and anthropogenic conditions such as oligotrophy, sea water temperature and fishing pressure.

Patella aspera in this geographical area is a moderately long-lived limpet with a predominance of specimens younger than 3 years old. This species is a winter breeder with a reproductive cycle encompassing three main periods namely development, spawning and resting with a synchronous gametogenesis for both sexes. Even though fishing mortality exerts more pressure on the populations of *P. aspera*, in the study area, than natural mortality, the Y/R results indicate that the stock of this species is under-exploited. However, it also indicates that the stock is susceptible to changes in minimum size of capture.

Continuous monitoring of the stock is advised in order to prevent future over-exploitation as a consequence of the slow growth and long life of this species. Also, further genetic studies are necessary to discern between the existence of a single meta-population with considerable gene flow between the south and north coasts of Madeira and the islands of Porto Santo and Desertas or, in contrast, if populations are isolated. This is, in our view, a very important issue to establish proper conservation strategies to preserve the resource taking in consideration its connectivity.

Abbreviations

L: shell length; W: weight; Fisat II: Fish Stock Assessment Tools; GSI: Gonadosomatic Index; Lm_{25}, Lm_{50}, Lm_{75}: length at which 25, 50 and 75% of the population is mature; Z: total mortality; M: natural mortality; F: fishing mortality; F_{MSY}: fishing mortality at Maximum Sustainable Yield; E: exploitation rate; Lc_{25}, Lc_{50}, Lc_{75}: length at which the cumulative proportion of capture is 25, 50 and 75%; Y/R: relative yield-per-recruit.

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Availability of data and materials

The data that support the findings of this study are available from the Regional Directorate of Fisheries of Madeira but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the Regional Directorate of Fisheries of Madeira.

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Chapter 6

First observations of hermaphroditism in the patellid limpet *Patella piperata* Gould, 1846

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Abstract

Hermaphroditism is thought to be an advantageous strategy common in marine molluscs that exhibit simultaneous, sequential or alternating hermaphroditism. Several species of patellid limpets have previously been shown to be protandrous hermaphrodites. The present study aimed to confirm whether this phenomenon occurs in *Patella piperata*. Transitional forms of simultaneous protandrous hermaphroditism were found in intermediate size classes of *P. piperata,* in Madeira (north-eastern Atlantic). Sequential hermaphroditism was confirmed after histological analysis. The overall sex-ratio was biased towards females but approached similar proportions in the larger size classes. Analysis of size at sex change showed that at a shell length of 36 mm 50% of the population probably have changed sex. The results reported confirm the occurrence of sequential hermaphroditism. These findings are of utmost importance to the understanding of the reproductive biology of this species with direct effect on management and conservation of this traditionally harvested limpet.

Keywords

Mollusca, Patellogastropoda, intertidal, sex change, Madeira, Atlantic Ocean.

1. Introduction

Patellid limpets are among the most successful marine gastropods inhabiting rocky shores (Branch, 1981) from the supratidal to the subtidal zones and as such, are subject to the most variable and unpredictable environmental conditions in nature (Harley *et al.*, 2009). The sharp environmental gradients of wave action, temperature variation and desiccation can prompt significant changes to the overall morpho-physiological characteristics of patellid limpets (Davies, 1969). For instance, the reproductive biology and phenology of limpets are deeply influenced by factors such as temperature and storminess (Branch, 1974; Bowman & Lewis, 1986), latitude and temperature (Lewis, 1986), climate change (Moore *et al.*, 2011), parasitism (Firth *et al.*, 2017) and anthropogenic pressures such as harvesting, pollution and habitat loss (Boukhicha *et al.*, 2013; Henriques *et al.*, 2017).

Hermaphroditism is regarded as an evolutionarily advantageous strategy supposed to increase the likelihood of successful fertilization for sedentary broadcast spawning species such as limpets, especially when occurring in low population densities (Branch, 1981; Guallart *et al.*, 2013). Hermaphroditism may be simultaneous, with one individual releasing both types of gametes during the same season, or sequential, with one individual functioning first as one sex and then changing to another later in life (Orton, 1928; Branch, 1974; Ghiselin, 1987). Sequential hermaphroditism is designated as protandry when an individual is first a male and then changes to female (Branch, 1981). Protogyny is when an individual first matures as female and then changes to male. In some limpet species the sex change can be reversed (Le Quesne & Hawkins, 2006; Rivera-Ingraham *et al.*, 2011), whereby males that became females can become males again. This could be linked to their reproductive fitness, since small females produce fewer oocytes and they would have a greater reproductive fitness as males (Guallart *et al.*, 2013). When sex change occurs repeatedly it is known as alternating sexuality (Hoagland, 1984). Sex can also be determined by environmental circumstances where one individual can influence the sex of another (Heller, 1993). Sex change in limpets has been shown to be density dependent by experimental manipulation in *Lottia gigantea* Gray in G. B. Sowerby I, 1834 (Fenberg & Roy, 2008) and in *Patella vulgata* Linnaeus, 1758 (Borges *et al*., 2015). Earlier onset of switching to females has been shown in heavily exploited populations of *Patella aspera* Röding, 1798 in the Macaronesian Islands (Martins *et al.*, 2017). In some species, sex change is triggered by specific environmental cues, in other species, sex change seems to be determined genetically, occurring mainly after the first reproductive season (Fretter *et al.*, 1998; Wright, 1989).

Hermaphroditism has been described in several families of limpets, for example Nacellidae (Mau *et al.*, 2017), Siphonariidae (Pal & Hodgson, 2005) and Patellidae (Orton, 1928). This phenomenon is prevalent in some genera of the Patellidae as *Cymbula* (Branch, 1981), *Scutellastra* (Branch, 1974; Lindberg, 2007), *Helcion* (Henninger & Hodgson, 2001) and, especially in the genus *Patella*. *Patella vulgata* (Orton *et al.*, 1956; Le Quesne & Hawkins, 2006), *Patella ulyssiponensis* Gmelin, 1791 (Thompson, 1979), *Patella caerulea* Linnaeus, 1758 (Montalenti, 1958), *Patella aspera* (Martins *et al.*, 2017), *Patella ferruginea* Gmelin, 1791 (Espinosa *at al.*, 2009) and *Patella rustica* Linnaeus, 1758 (Prusina *et al*., 2014) are known to be protandrous hermaphrodites (Dodd, 1956; Le Quesne & Hawkins, 2006; Rivera-Ingraham *et al*., 2011, Martins *et al.*, 2017). Simultaneous hermaphroditism has been reported for the Azorean endemic limpet *Patella candei gomesii* (Cunha *et al.*, 2007). Protandry does not seem to occur in populations of *Patella depressa* Pennant, 1777 in southern England (Orton & Southward, 1961; Guerra & Gaudencio, 1986) however, more recently some indications of protandry in populations from Portugal have been reported (Borges *et al.*, 2015).

Hermaphroditism in patellid limpets is an important trait that needs to be taken into consideration in the conservation and management of heavily exploited populations (Espinosa *et al.*, 2006). It is known that size-selective harvesting negatively impacts the reproductive output threatening species survival (Fenberg & Roy, 2008). In fact, larger specimens are more visible and valuable thus more prone to being harvested and at the same time contribute the most to the reproductive effort, since in these species the reproductive output is directly related to size, with larger individuals with larger gonads producing more gametes (Creese, 1980). Removal of these individuals will therefore diminish the reproductive success of exploited populations (Espinosa *et al.*, 2009; Henriques *et al.*, 2017). In hermaphroditic species, removal of larger individuals might also cause alterations in population dynamics, since the absence of the predominant sex at larger size is considered one of the cues that triggers sex change to occur earlier at smaller sizes (Rivera-Ingraham *et al*., 2011).

Patella piperata Gould, 1846 is one of the fifteen worldwide recognized species of the genus *Patella* Linnaeus, 1758 (WoRMS, 2018). This species is endemic to the Macaronesian archipelagos of Madeira, Canaries and Cape Verde (Christiaens, 1973) and occurs together with *P. aspera* and *Patella candei* d' Orbigny, 1840 in the Madeira archipelago (Sousa *et al*., 2017). It is closely related to *P. rustica,* a sister species occurring on continental Atlantic coasts and throughout the Mediterranean (Côrte-Real *et al.*, 1996; Sá-Pinto *et al.*, 2005). This important microphagous grazer controls algal biomass of the intertidal rocky shores (Bergasa *et al.*, 2007) and is collected for human consumption by locals throughout the Canaries (Moro & Herrera, 2000; Bergasa *et al.*, 2007) and occasionally in Madeira. A potentially hermaphroditic individual of *P. piperata* was macroscopically identified, while studying this species' reproductive biology. This find prompted the need to confirm, whether hermaphroditism occurs in this species similarly to its protandrous sister clade *P. rustica* (Prusina *et al.*, 2014). Our specific objectives were: (i) to report the histological validation of sex change in *P. piperata* and (ii) to determine the size at which sex change occurs.

2. Materials and methods

Monthly samples of *P. piperata* were collected from the coast of Madeira north-eastern Atlantic (32°00'-33°30'N; 15°30'-18°00'W), at two different sites (São Vicente and Santa Cruz), between 2017 and 2018, as part of a study focussing on growth and reproduction of this species. Specimens were randomly collected from the supra-tidal rocky shore, removing all the specimens found without considering their size, for a period of 30 minutes. Shell length (L) was measured using a Vernier calliper to the nearest 0.01 mm, and total wet weight (W) to the nearest 0.01 g was measured using an electronic scale balance. Specimens were dissected for macroscopic observation of the gonads and sexed according to Orton *et al.* (1956) considering gonad pigmentation, with males exhibiting pale white to pink gonads and females brown to red gonads.

Gonads were removed, damp-dried, weighed to 0.01 g accuracy and preserved in a 10% buffered formaldehyde solution. All measurements were taken from fresh samples.

Histological analysis was performed on dissected gonads in five stages: (I) fixation; (II) dehydration (alcohol with different concentrations: 70%, 90% and 95%); (III) embedding in resin; (IV) preparation of trimming blocks and (V) sectioning. A portion of about 1 cm^3 from the middle of each gonad was embedded in Technovit 7100 resin, following standard protocols. Histological sections (3–4 μm thickness) were cut and stained with methylene blue solution. The slides of each specimen were sealed with a drop of neo-mount resin, and image acquisition using the visual image analysis system composed of the Leica DMLB microscope coupled to the Leica EC 3 camera and the software LAS 4.5. (Vasconcelos, 2017).

An analysis of variance (ANOVA) was employed to compare limpet size among males, females and hermaphrodites. The size cumulative frequency was analysed using the non-parametric Kolmogorov-Smirnov test and a Pearson Chi-square was employed to compare the sexual proportion by size classes between areas. All statistical analyses were performed using SPSS v.24.0 (IBM Corp., Armonk, NY). For all tests, statistical significance was accepted when $P < 0.05$.

The size at sex-change (L_{50}) corresponds to the size at which 50% of mature individuals are the second sex (male for protogynous, female for protandrous) and was determined using a logistic regression (Allsop & West, 2003).

3. Results

A total of 1164 specimens of *P. piperata* were sampled between 2017 and 2018. Among the sampled individuals, 53 were macroscopically identified as potential hermaphrodites (Figure 1), representing 5% of the total sample. 65% were females and 30% were males. Hermaphrodites were only found from March to June.

Figure 1 - Hermaphrodite gonad of *Patella piperata*, where the ovary is represented by the central dark area and the testis is represented by the lighter areas.

The shell length of the hermaphrodites varied between 21.72 and 37.81 mm ($\bar{x} = 31.54$ ± 4.98 mm), female shell length varied between 11.17 and 37.34 mm ($\bar{x} = 25.11$ ± 4.56 mm), and male shell length varied between 11.65 and 37.52 mm ($\bar{x} = 22.99$ ± 5.34 mm). Differences in mean shell length among sexes were statistically significant ($F = 94.067$, $P < 0.001$).

The overall sex ratio was 2.16:1 (female: male) favouring females. The distribution of the cumulative frequency of males, females and hermaphrodites per size is represented in figure 2. The observed differences were statistically significant ($Z = -4.037$, $P < 0.001$).

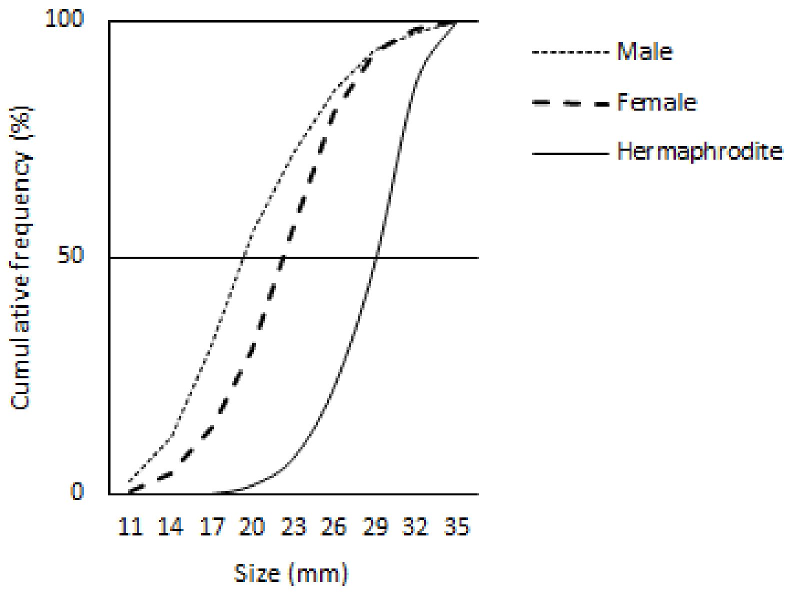

Figure 2 - Cumulative size frequency of males, females and hermaphrodites of *Patella piperata*.

The Pearson's Chi-square showed that sexual proportion between size classes did not differ between sampling sites ($\chi^2 = 2.006$, $P = 0.157$) and that females were predominant between 17.00 and 35.00 mm ($\chi^2 = 33.999$, $P = 0.005$), indicating that the size structure of the populations was similar between localities and that males were more abundant between 11.00 and 16.00 mm and females between 17.00 and 37.00 mm. The size at sex change (L_{50}) was estimated at a shell length of 36.01 mm, and at 29.22 mm 25% of limpets had started to change sex.

The histological validation of the potential hermaphrodites showed that defined sections of ovary and testis occurred simultaneously in specimens of *P. piperata* between March and June, suggesting that these individuals represent a transitional stage of sequential sex-change, probably occurring in the late stages of the spawning season (Figure 3). Testicular tissue, with some distinguishable seminiferous tubules but without sperm was observed, implying that male structures during transition are not functional. Ovarian tissue with vitellogenic oocytes and some atresia was also observed, suggesting post spawning degeneration of female structures (Figure 4).

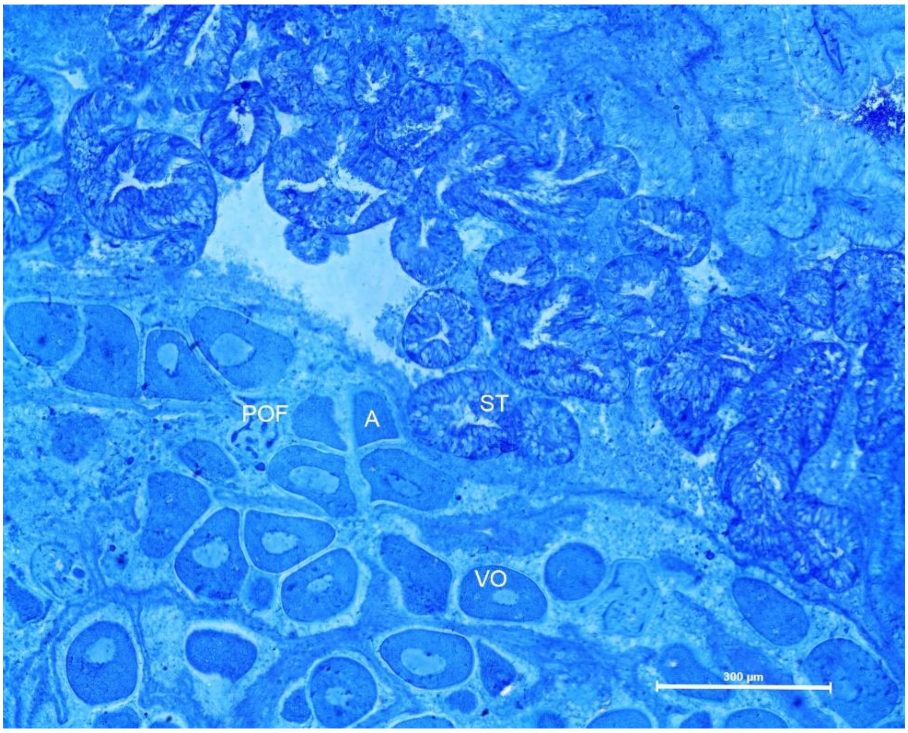

Figure 3 - Histological section of a hermaphrodite specimen of *Patella piperata* gonads. General view highlighting the coexistence of spermatogenic and oogenic stages. ST-seminiferous tubules; VO-vitellogenic oocytes; POF-post-ovulatory follicles; A - atresia

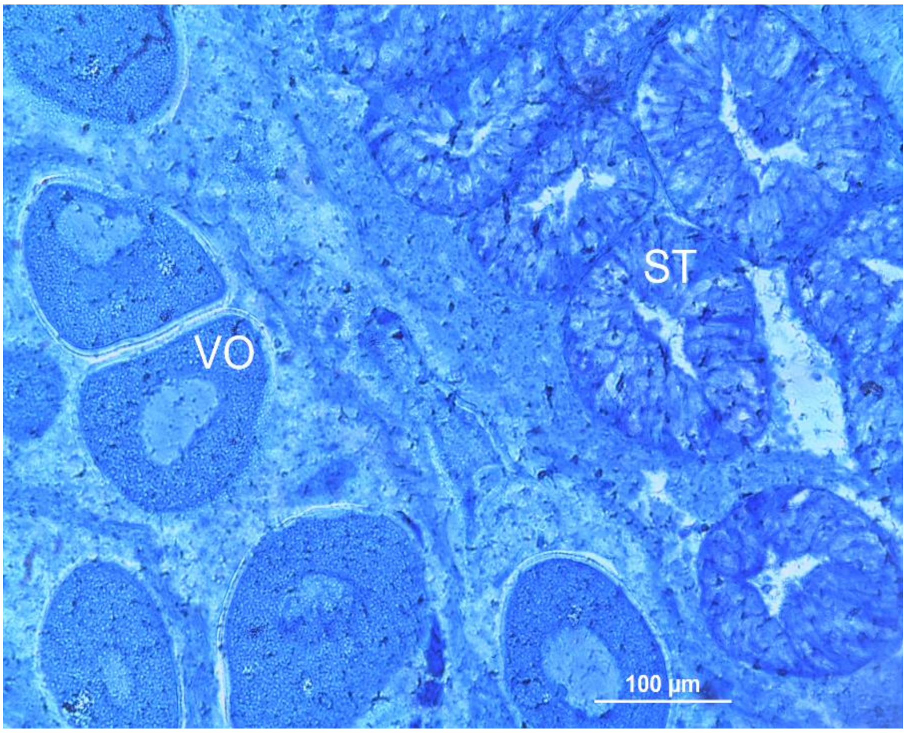

Figure 4 - Histological section of a hermaphrodite specimen of *Patella piperata* gonads. View detailing the spermatogenic and oogenic stages. ST-seminiferous tubules; VO-vitellogenic oocytes.

4. Discussion

Hermaphroditism is thought to represent an advantageous strategy in increasing the reproductive success of organisms, based on the assumption that an individual changing sex will increases its reproductive efficiency (Charnov, 1979; Munday *et al.*, 2006). When male and female reproductive values are closely related to size or age, natural selection will favour genes coding for sex change (Warner, 1988). The direction of sex change is thought to be determined by the relative fitness returns for the two sexes over the course of the limpet's lifetime and not by size (Allsop, 2003; Guallart *et al.*, 2013). If males have lower reproductive success than females in smaller sizes and greater in larger sizes, sex change will probably be protogynous to maximize the reproductive success of males later on life when their fitness accelerates above that of the females (Guallart *et al.*, 2013). The inverse holds for protandrous sex change (Cunha *et al*., 2007). This sex changing allocation mechanism ensures that individuals have the optimal sex for the predominant circumstances, thus maximizing the organism's genetic contribution to the next generation (Warner *et al*., 1996).

Sequential hermaphroditism assumes the existence of sexual dimorphism in size-classes (Orton, 1919; Branch, 1974; Thompson, 1979; Creese *et al.*, 1990), with the occurrence of a small number of simultaneous hermaphrodites in intermediate size classes (Bacci, 1947; Branch, 1974; Creese *et al*., 1990). In fact, direct observations have confirmed this pattern for the protandrous hermaphrodite *P. vulgata* (Le Quesne & Hawkins, 2006). Two forms of simultaneous hermaphroditism can occur, the very rare 'mosaic' form, in which patches of male and female gametes appear interspersed, usually considered aberrations since they mostly occur in gonochoristic species (Dodd, 1956) and the 'transitional' form, in which defined sections of male and female gamete have been observed to occur simultaneously and is thought to represent a gonad in a transitional state of sex change (Branch, 1974). Both these forms have been observed in the order Patellogastropoda (Orton, 1928; Branch, 1974), the mosaic form in *P. vulgata* and the transitional form in *P. caerulea* and *Cymbula oculus* (Born, 1778) (Dodd, 1956; Branch, 1974).

The results reported herein are the first observations of a transitional form of sequential hermaphroditism in *P. piperata*. This species size distribution ranges from 3 mm to 46 mm shell length (R. Sousa, personal communication) and the hermaphroditic individuals occurred in the size classes ranging from 20 to 38 mm shell length representing approximately 5% of the examined individuals. The size at which sex change is estimated to occur was 36.08 mm shell length. This corresponds to the size class at which 50% of the individuals in this population are of the second sex. Nonetheless, sex change in *P. piperata* starts to occur earlier, with 25% of limpets having changed sex at 29.22 mm shell length and the smallest hermaphrodite found had 21.72 mm shell length. The sex ratio was biased towards females in most of the sampled size range, with males being predominant in the smaller size classes and females in the larger size classes, corresponding to the size at which hermaphrodites become more common. The skewed sex ratio to females is uncommon in protandrous species where small males are often numerically dominant (Allsop & West, 2004). This could be a result of incomplete sampling with smaller recently matured males occurring lower on the shore or in cryptic habitats.

In gastropods, sex change is also thought to be determined genetically and to occur mainly after the first reproductive period (Fretter *et al*., 1998). In some species, sex change is triggered by specific environmental signals and this may be the case of *P. piperata*. However, the environmental cues that trigger sex change in this species are yet to be determined.

Hoagland (1978) stated that in protandrous gastropods the presence of females or other larger conspecifics delays or inhibits sex change in males, with abundance acting as a trigger for sex change. As such, sex change in *P. piperata* in Madeira may be density dependent, where a greater overall abundance of one sex may prompt a sex change to balance the sex ratio of these populations as reported for *P. aspera* (Martins *et al*., 2017), *P. vulgata* (Borges *et al*., 2015), and *P. ferruginea* (Espinosa *et al*., 2009; Rivera-Ingraham *et al*., 2011).

The occurrence of 5% of simultaneous hermaphroditic individuals in the sampled populations in the size classes between 20 to 38 mm, warrants further studies to better understand the impact of this phenomenon on the reproductive biology and population dynamics of *P. piperata* in Madeira namely, whether the mature gametes produced in hermaphrodites are functional or not and provide evidence of the direction of sex change. Another aspect that requires additional research is the environmental cues that control this phenomenon in this species.

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PART III

CONSERVATION OF INTERTIDAL GRAZERS IN THE HISTORICALLY HARVESTED ARCHIPELAGO OF MADEIRA

Chapter 7

Artisanal harvest of shellfish in the north-eastern Atlantic: the example of limpet and topshell fisheries in the archipelago of Madeira

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Abstract

The harvesting of littoral benthic shellfish in the archipelago of Madeira dates back to the fifteenth century when the Portuguese discovered and colonized the archipelago. The consumption of littoral shellfish is part of the gastronomic cultural heritage of this region, appreciated by the local population and tourists, and has a high social and economic importance. Therefore, harvesting pressure on these resources is one of the greatest concerns, and as such, a sustainable exploitation based on proper regulation, considering the biological and ecological specificities of these species in their particular habitat, is crucial to promote the preservation of species and habitats at medium and long term. This study presents the current harvesting management regime for gastropods in the archipelago of Madeira and characterizes the artisanal harvest through a period of 27 years (1990-2017) providing new insights for future research in these topics. This artisanal harvesting operates mostly by small vessels (<10 m), with low tonnage and capacity, in nearby areas preferentially in the North coast of Madeira and around Desertas Islands. During the studied period, management actions resulted in the reduction of 50% of the vessels operating in the harvesting of limpets and in slightly recovering of the stocks of limpets. The economic impact of limpets gradually increased over the years, representing in 2017 96% of the economic value landed for molluscs and 2% of the total landings in this region. The present characterization provides a comprehensive outlook of the evolution of the marine gastropod harvest in the archipelago of Madeira and allows future comparisons with other regions where gastropods are commercially exploited.

Keywords

Harvesting, management, sustainable exploitation, limpets, topshells, archipelago of Madeira.

1. Introduction

The artisanal fisheries of marine invertebrates in the archipelago of Madeira (NE Atlantic) target mainly gastropods and cephalopods. The main gastropod species harvested are the limpets *Patella aspera* Röding, 1798 and *Patella candei* d'Orbigny, 1840, the topshell *Phorcus sauciatus* (Koch, 1845), and the whelk or redmouthed rocksnail *Stramonita haemastoma* (Linnaeus, 1767). Regarding cephalopods, the common octopus *Octopus vulgaris* Cuvier, 1797; the squid European *Loligo vulgaris* Lamarck, 1798; and the orangeback squid *Sthenoteuthis pteropus* (Steenstrup, 1855) are the main target species of this fishery. Additionally, the cuttlefish *Sepia officinalis* Linnaeus, 1758, and the European flying squid *Todarodes sagittatus* (Lamarck, 1798) are sporadically captured.

The artisanal harvest of gastropods in the archipelago of Madeira is a low-cost activity, usually carried out by the owner of the vessel accompanied by professional snorkelers. This activity is one of the most important small-scale fisheries in this region, due to the economic and social benefits it provides directly to the coastal communities and indirectly to the whole community. This fishery dates back to the fifteenth century when the Portuguese colonized the archipelago. The good accessibility to the rocky shores prompted the exploitation of marine shellfish resources along the coast. The harvest activity becomes progressively more intensive with the demographic increase of human settlement around the islands' coasts and with the technological progresses that simplify the access to the coast at previously inaccessible areas [1, 2]. This long-term exploitation has changed the population dynamics, due to shifts on the abundance and/or size structure and density of the exploited marine gastropods over the years [3].

In the past, four species of the genus *Patella* were reported for the archipelago of Madeira, namely *Patella aspera* as the most abundant species, *Patella caerulea*, *Patella lusitanica,* and *Patella vulgata* (known locally as "concharéu" due to its large size, sharp edges, and helmet shape). The distribution of *P. candei* (formerly identified as *P. vulgata*), which once occurred in all the islands of the archipelago, became restricted, in the beginning of the twentieth century, to the Selvagens Islands [1]. Presently, *P. aspera* and *P. piperata* (formerly identified as *P. lusitanica*) are common species in all islands from the archipelago of Madeira, *P. candei* (formerly identified as *P. caerulea*) is present in Madeira Island, Porto Santo, and Desertas, while the real *P. candei* is restricted to the Selvagens Islands. Recently new molecular tools developed with novel microsatellite markers using next-generation sequencing suggest the use of *P. candei* for the species from the Selvagens and *Patella ordinaria* for the species of Madeira, Desertas, and Porto Santo islands [4].

Concerning topshell exploitation, two species of the genus *Phorcus* have been harvested in Madeira archipelago since early colonization times. *Phorcus sauciatus* (formerly identified as *Trochus colubrinus* Gould, 1849), the most common species and with a wider geographical distribution, occurring in all islands of the Madeira archipelago, and *Phorcus atratus* are restricted to the Selvagens Islands as the endemic subspecies *Phorcus atratus selvagensis* [5]. Nowadays, *P. sauciatus* continues to be exploited in Madeira, Porto Santo, and Desertas, except in the Marine Protected Areas (MPAs) and *P. atratus selvagensis* is not commercially exploited since its distribution is restricted to the MPA of the Selvagens where harvesting is not allowed.

Both consumption of limpets and topshells in Madeira archipelago are part of the gastronomic cultural heritage of this region, appreciated by the local population and tourists alike, and has a high socioeconomic importance. Therefore, harvesting pressure on these resources is one of the greatest concerns. As such, a sustainable exploitation, based on suitable regulation considering the biological and ecological specificities of these species in their particular habitat, is crucial to promote the preservation of species and habitats at medium and long term.

The aim of present work is to compile and characterize the harvest of limpets and topshells in the archipelago of Madeira, considering and discussing the evolution of the landings and economic values and describing the activity, fishing fleets, exploited species, and yields for a period of 27 years (1990-2017). Additionally, the impact of this activity on selected aspects of limpet and topshell population dynamics is analyzed and discussed.

The implemented management measures regulating the harvest of marine molluscs in the archipelago of Madeira are characterized in detail and their impact on the exploited stocks critically discussed through a comparative analysis of any relevant available data on these species from this region. Finally, the economic and social contextualization of this fishery is made in the overall fisheries sector in the region.

2. Study area

The archipelago of Madeira is located in the north-eastern Atlantic Ocean and is included in the Macaronesian biogeographical region together with the Azores, Canary, and Cape Verde (**Figure 1**). The islands of these archipelagos are of volcanic origin, resulting from the activity of several geological hotspots and sharing the oceanic nature, the geographic location and the climatic regime. However, with specific characteristics according to the proximity of the islands to the mainland regions [6].

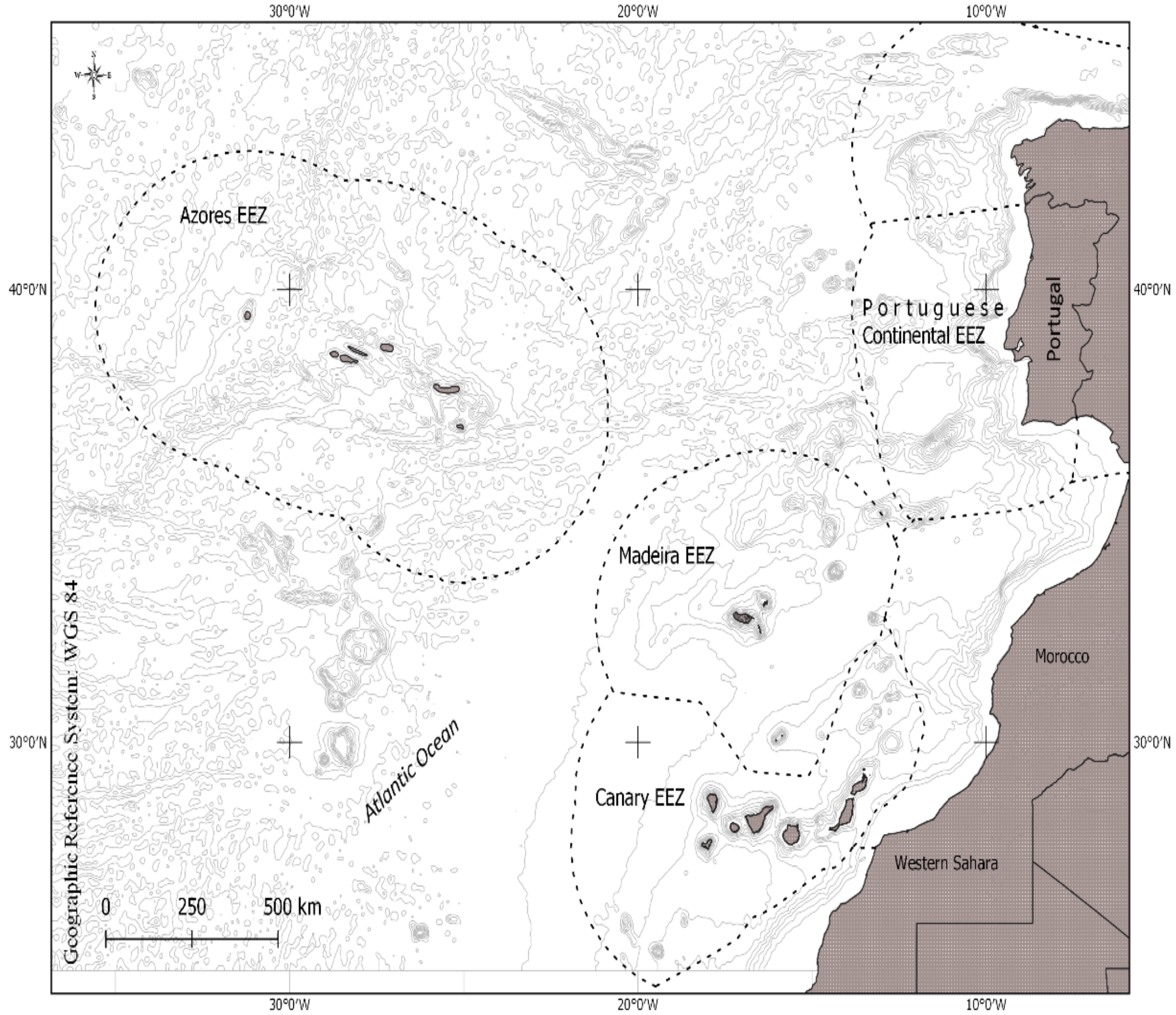

Figure 1 - Representation of the southern part of the north-eastern Atlantic showing the study area, the archipelago of Madeira, included in the Macaronesian biogeographical region.

The archipelago of Madeira comprises the islands of Madeira (741 km^2 of area), Porto Santo (42 km^2), Desertas (14 km^2), and the Selvages islands (3 km^2). The island of Madeira is located approximately at 635 km from Morocco and at 900 km from the Portuguese mainland. The Selvagens islands are the southernmost territory of Portugal at 239 km from the island of Madeira and at 375 km from the coast of Morocco. This subtropical archipelago is influenced by the Azores anticyclone, the Gulf and the Canary currents, the continental anticyclonic centre of Northwest Africa and Western Europe, and the frontal systems associated with the lower pressure centre of the polar front [7].

The islands of Madeira and Desertas represent the most recent islands of the archipelago of Madeira with 4.6 and 3.6 million years, respectively. Porto Santo has an estimated age of 14.3 million years and the Selvages islands an estimated age between 24 and 29 million years [8-9].

The population living in the archipelago of Madeira in 2017 was *ca.* 254 thousand inhabitants, and the fishing activity employed 618 registered fishermen (DRP-RAM). The annual landings increased 38.6% in relation to 2016 with 6.739 tonnes of fish and molluscs corresponding to 21,636 thousand €.

2.1. Data collection

Data on the landings (i.e., species, day, weight, and economic value) and on the artisanal fleet (i.e., length of the fishing vessel, tonnage, capacity, metier, fishery license) were obtained from the Regional Fisheries Department of Autonomous Region of Madeira (DRP-RAM) for both limpets and topshells.

Logbooks data analyses were only available to characterize the limpets harvesting activity (i.e., harvesting area, typology of bottom, depth of harvesting, number of divers, number of snorkelers per vessel, and landing place and time), since it is mandatory to fill the logbooks according to the regulation of the limpet harvest in the archipelago of Madeira. For topshells these data are not available due to this activity not being regulated.

Landings data were compiled and analyzed covering the period from 1990 to 2017 and the logbook data from 2008 to 2017, concerning the period after the regulation of the harvesting of limpets that required the filling of all the harvesting information in logbooks.

3. Harvesting regulation in the archipelago of Madeira: a driver to sustainable exploitation

The fisheries sector should protect fish resources and environment through an effective legal regulation and an appropriate compliance and enforcement to ensure the sustainable resource exploitation [10]. Based on this principle, the regulation of limpet harvesting in the archipelago of Madeira sets the basis for a sustainable and responsible exploitation of these resources.

The rules of governing the harvesting of limpets in Madeira were initially set by the necessity to establish proper measures to the regional specificities, with regard to the practice of underwater hunting. These measures were implemented through the article 6 of the Regional Government under the Regional Legislative Decree N^{o} 11/1995/M on 21 June 1995 and setting a maximum allowable catch of 3 kg/day per person for limpets. This was the first management action regarding the harvesting of gastropods in the archipelago of Madeira.

The legal regulation of the limpets' harvesting method was decreed by the Legislative order N^{o} 1102-B/2000, 22 November 2000, which established the tools and instruments to be used in limpets harvesting.

The current limpets' management in Madeira was based in technical measures implemented by the Regional Government under the Regional Legislative Decree N^{o} 11/2006/M, 18 April 2006, which establishes the legal regime for the harvesting of these gastropods in this region [3]. The management measures were implemented in 2006 based on the knowledge obtained from studies on the biology, population dynamics, and assessment of the stocks and intended to advise a precautionary approach capable of harmonizing the need to protect stocks with the preservation of the economic activities associated with their capture and gastronomic use [11]. For this purpose, regulators established several management measures for the traditional and commercial exploitation of the species *P. aspera* and *P. candei*. For the commercial exploitation, a minimum catch size of 40 mm, the obligation of harvesting licenses, logbook provision, the landings and first auction sale, and catches limits were implemented, enforcing the maximum allowable commercial catch of 15kg/person/day or 200kg/boat/day, being exempted of any license the traditional harvest that does not exceed 3 kg/day per person.

Additionally, the Legislative Orders N^{o} 80/2006 and N^{o} 81/2006, 17 June 2006, set the establishment of a closed season between November 1 and January 31 to avoid limpet harvest during the reproductive season, and the rules concerning the harvesting card.

In 2009, based on continuous limpets' population monitoring, the closed season was changed to become effective between the December 1 and February 28 (Legislative Order N^{o} 5/2009, 4 July 2009). In 2016, the closed season was extend through the Legislative Order N^{o} 40/2016, 17 February 2016, in result of the data obtained from the continuous monitoring of the stocks, in order to more efficiently provide protection to these heavily exploited species, now lasting from December 1 to March 31. The extension of the closed season is intended to result in biological benefits, allowing greater protection of spawning, larvae development, and settlement and to increase the success of annual recruitment and subsequent development of limpets. Additionally, the competent authorities implemented a reduction in the number of harvesting licenses aiming to diminishing the pressure on the resource [3, 12].

Since the implementation of limpet harvest regulation, the non-compliance of the imposed management measures is, punishable with administrative offenses and penalties. The sanctions includes monetary fines (between 49.88 € and 44,891.81 €), harvesting prohibition, and suspension of licenses. Nevertheless, poaching continues to occur during the closed season, without abiding the minimum catch size of 40 mm of shell length [3].

The regulation of limpets in the archipelago of Madeira allowed safeguarding the peak of reproduction, the immature specimens and the catches of both *P. aspera* and *P. candei*. Although the management measures on these resources have been implemented for several years only a slightly recover on the limpets populations has been observed, maybe due the peculiar life traits characteristics of this species, as slow growth and longevity. Hence, it is of paramount importance to keep up with the enforcement of the implemented measures in order to achieve higher recovery rates of the exploited limpet stocks.

Currently, topshell harvest in the archipelago of Madeira is not regulated, with the exception of harvest ban in MPAs [2]. As such, the effort exerted and the shifts on the populations' dynamics of the harvested populations, exploited for more than 500 years, are unknown. However, management measures based on recent studies [2], are in progress and shall enter into force during the year 2019, aiming to promote both profitable and sustainable harvest. The establishment of a maximum catch of 2 kg per day for non-commercial use, and 20 kg per day for commercial use, the implementation of landing obligations and first sale at auction, the establishment of a minimum catch size of 15 mm shell length and the establishment of a closed season between February and May are recommended [2].

Further European legislation through the European Community Council Regulation N^{o} 199/2008, 25 February 2008, prompted an increase in the knowledge regarding the fisheries sector in the European Union, including smaller sections such as limpet harvest, meeting the demands generated by the necessity to evolve towards a sustainable fisheries sector, with its management based on the fleet and fishing areas, rather than based on fish stocks. As such, the collection of data and their availability by region will provide the basis for the better scientific advice.

4. Harvesting

Limpets and topshells are collected by hand, in the intertidal zone by the local population (traditional harvesting) and in the subtidal zone by snorkelers executing several dives per day (commercial harvesting), from 1 to 6 m deep ($\bar{x}$ = 1.74 ± 1.31). The harvesting fleet operates preferentially on the northern coast of Madeira and Desertas Islands (**Figure 2**), the least accessible zones while traditional harvesters collect limpets from all around the island preferably in areas with easy access and milder sea conditions.

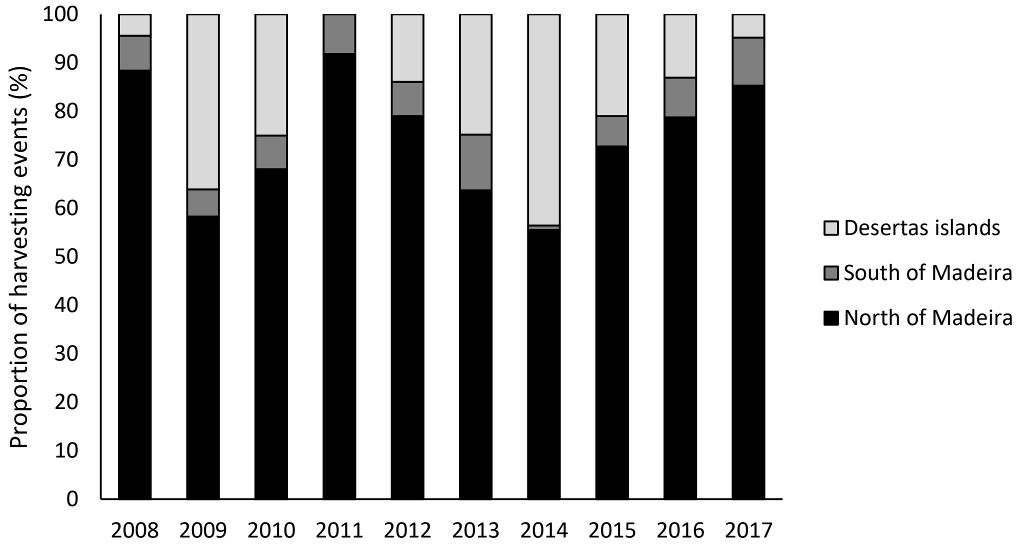

Figure 2 - Proportion of harvesting limpets' events per zone based in data from logbooks.

The harvest of limpets in the archipelago of Madeira is operated from April to November for limpets and all year round for topshells mostly by small vessels (< 10 m). Between 1990 and 2017, the number of vessels operating on the harvesting of gastropods was reduced from 17 (1990) to 9 (2017) (**Figure 3**). The observed reduction in the number of vessels and licenses results from the implementation of regulation, which among other management measures included the gradual reduction in the number of fishing licenses and vessels in order to reduce the harvesting pressure on limpet stocks.

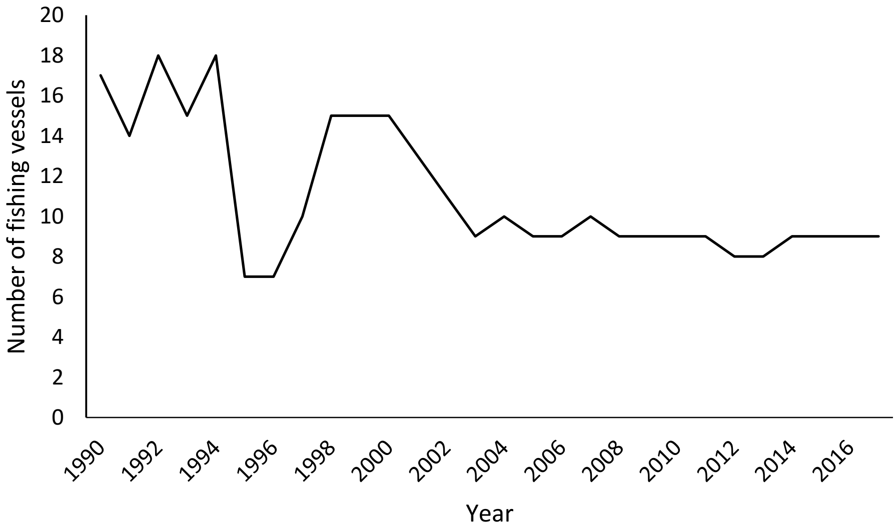

Figure 3 - Number of fishing vessels operating in the harvesting of limpets from 1990 to 2017 in the archipelago of Madeira.

The majority of the fishing vessels have less than 10 m length (78%), and the remaining ranges between 10-12 m (11%) and between 12-18 m (11%). This artisanal fleet includes vessels of low tonnage (0.74 to 17.28 gross tonnage) and capacity between 11 to 136 KW, usually operating in nearby areas reachable in a short time.

Limpet harvest is deeply rooted in the local community and particularly on coastal fishing communities representing an additional revenue source for many families, thus contributing to the local economy. The number of snorkelers per fishing vessel, between 2008 and 2017, varied between 2 and 8 depending on the vessel size ($\bar{x}$ = 4.9 ± 1.29). The number of professional harvesters registered per vessel and nonprofessional harvesters (15kg/person/day) decreased over the study period following the management measures implemented (**Figure 4**).

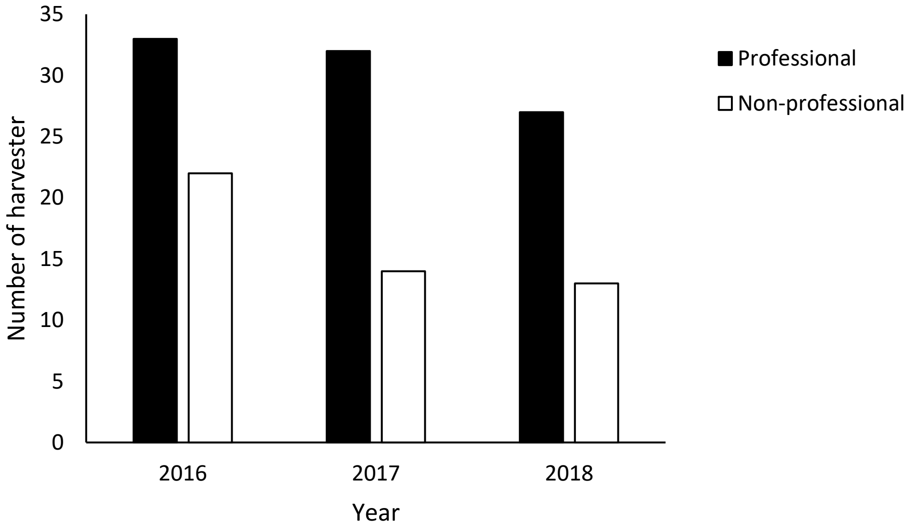

Figure 4 - Number of professional and non-professional harvesters registered in the archipelago of Madeira between 2016 and 2018.

A remarkable proportion of the Madeiran fishing fleet focuses solely on the harvesting of gastropods; nevertheless due to the implementation of a closed season, some fishing fleets operate with two or three metiers. In 2017, 56% of the fishing fleet operated exclusively in the harvesting of gastropods; 33% operated with 3 metiers and 11% with 2 metiers (**Figure 5**). The complementary metiers were essentially directed to the capture of tuna fish and demersal fish.

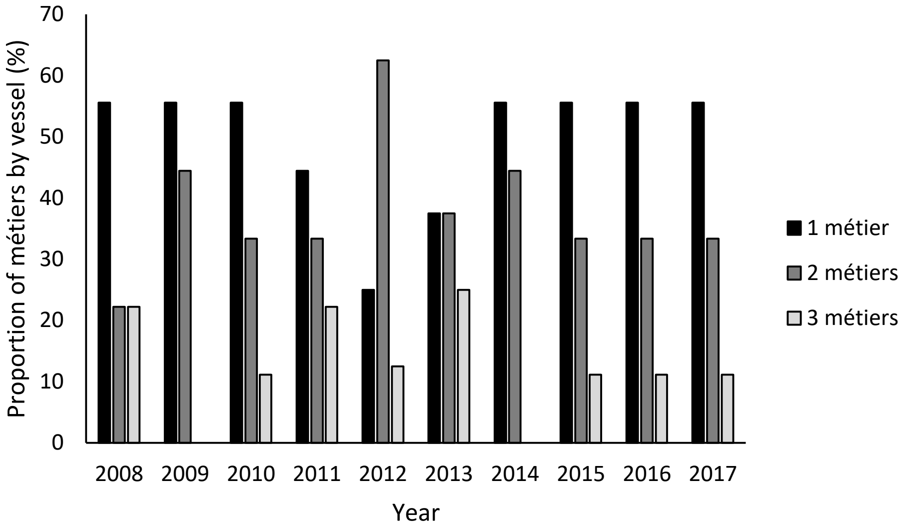

Figure 5 - Proportion of metiers operated by the fishing vessels involved in the harvesting of gastropods in the archipelago of Madeira.

4.1. Species and yields

In the archipelago of Madeira, limpets are mostly harvested by snorkelling in a mixed exploitation of *P. aspera* and *P. candei* with relevant commercial importance [13-14] for the involved population. This activity represented approximately 1.5% of the total of the fisheries and 2% of the total of the economic value (€) in 2017, reaching an average value of 4 € per Kg [3]. In 2011, this activity represented *ca.* 5% of the total economic value (€) of the fisheries which landed in the archipelago of Madeira.

The commercial landings in weight of limpets harvest varied from *ca.* 5 tonnes in 1990 to *ca.* 111 tonnes in 2017 (**Figure 6**). The maximum value landed was 150 tonnes in 2015 yielding a first auction sale value of 0.7 M€. The decrease in landings in 2016 and 2017 is related to the increase of the closed season from 3 to 4 months, reducing the harvesting activity in one month which was reflected in the annual landings values. Data from 1990 to 2006, needs to be analysed carefully since limpet harvesting was not regulated and obligatory reporting of landings were not mandatory.

The limpets landed in Madeira are for internal consumption and, to a minor extent, for exportation, mainly to the archipelago of Azores. However, data on the exportations of limpets are not available and as such is not possible to determine accurately.

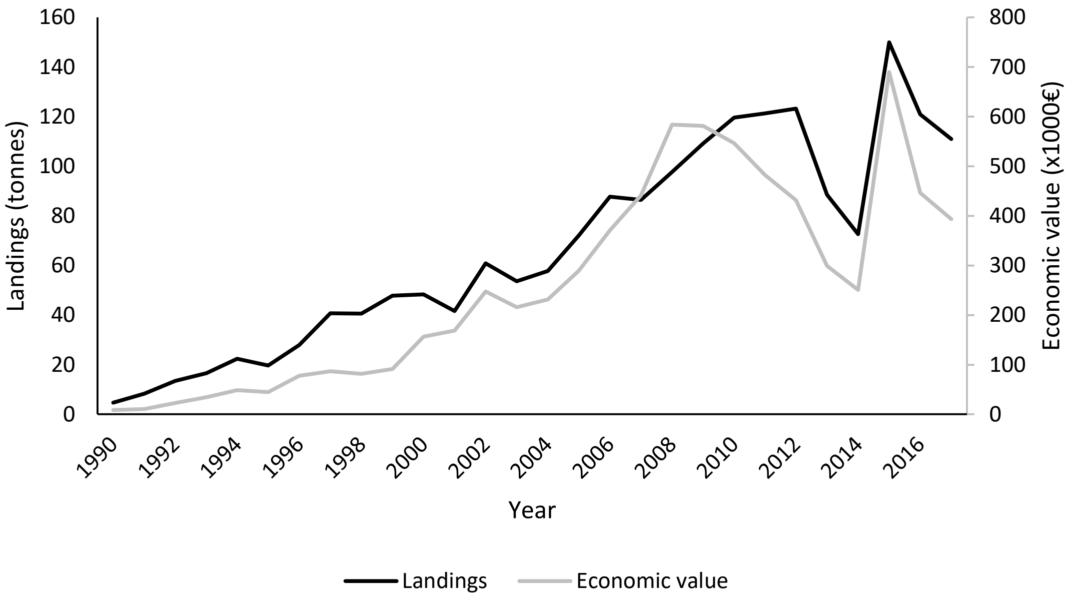

Figure 6 - Representation of landings (tonnes) and economic value (thousand euros) of limpets from 1990 to 2017 in the archipelago of Madeira.

The average annual value of limpets per kg landed in the archipelago of Madeira fluctuated considerably, from 1990 to 2017, increasing from 1.80 € in 1990 to 3.65 € in 2017 ($\bar{x}$ = 3.33 ± 1.25 €). For this period the average annual price varied from 1.21 € in 1990 to 5.90 € in 2008 (**Figure 7**). Since 2008, the value per kg decreased due to the increase in landings and the demand for this resource. Even so, the value per kg is still very attractive for the professionals involved in this activity.

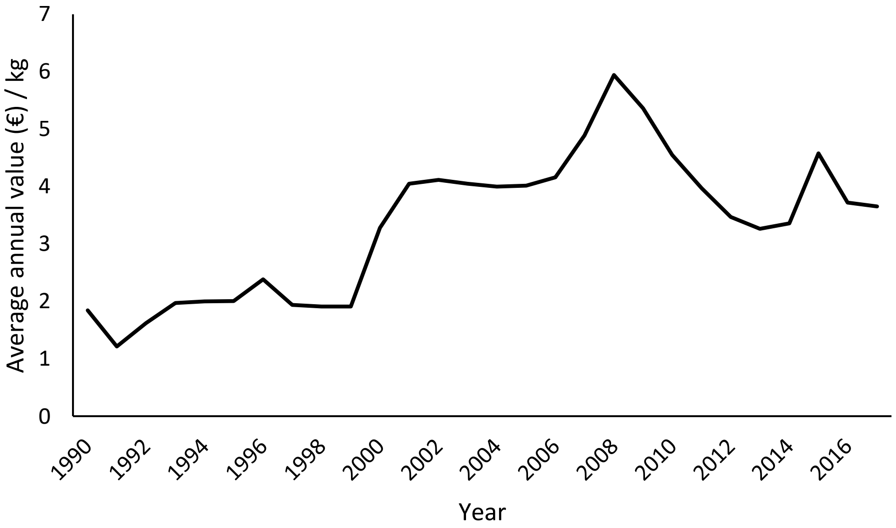

Figure 7 - Average annual price of the limpets landed in the archipelago of Madeira from 1990 to 2017.

The harvesting effort, represented by the number of days employed in the harvest of limpets, decreased from 1448 days in 2008 to 655 days in 2017. However, in general the reduction in fishing effort contrary to what would be expected led to an increase in the landings (*ca.* 98 tonnes in 2008 to *ca.* 111 tonnes in 2017). From 2013 to 2017, the oscillations on the landings were related to different sea conditions among years, e.g. in 2015 the milder sea conditions resulted in 361 more days at the sea. The increase verified in the landings seems to result from the management measures implemented in 2006 (**Figure 8**). In fact, if the management measures implemented were successful then it is expected that the exploited stocks have greater biomass, not only in density but also larger individuals are supposedly more abundant which in return means that lower effort returns similar or superior yields.

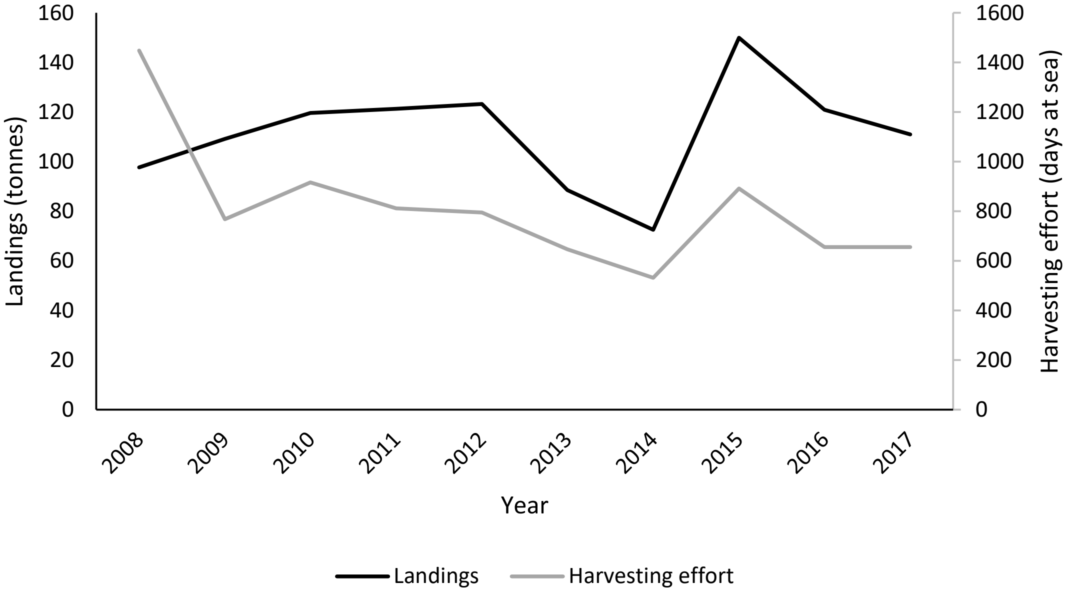

Figure 8 - Landings and harvesting effort from 2008 to 2017 in the harvesting of limpets in the archipelago of Madeira.

The highest proportion of landings of limpets, for the considered time period, occurred from May to August, corresponding to the months with better sea conditions in the archipelago of Madeira (**Figure 9**).

The comparison between monthly landings before and after the implementation of the closed season showed that the highest proportion of landings remained identical between the two periods, except for the months of the closed season, when limpet harvesting is not allowed.

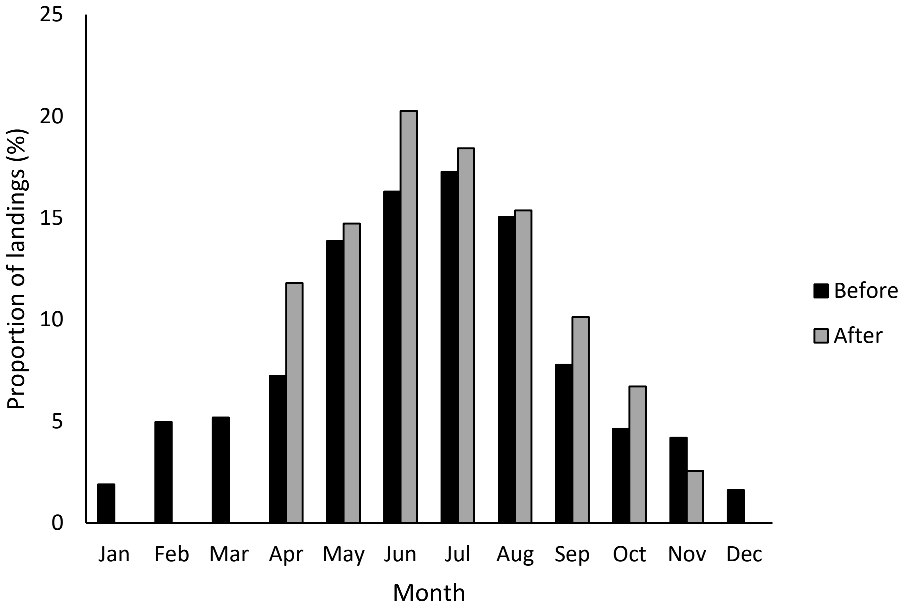

Figure 9 - Proportion of landings of limpets per month before (1990 to 2007) and after (2008-2017) after the implementation of management measures in the archipelago of Madeira.

In the archipelago of Madeira, landings of limpet commercially harvested, between 1990 and 2017, occurred in ten ports namely, Funchal, Câmara de Lobos, Calheta, Madalena do Mar, Paúl do Mar, Porto Moniz, Santa Cruz, Machico, Caniçal, and Porto Santo.

The total landings per port in tonnes are represented in **Figure 10**. During this period, Porto Moniz, Funchal, Caniçal, and Paúl do Mar, were the most important ports representing approximately 96% of the limpets landed in 1990 and 100% of the limpets landed in 2017. Porto Moniz showed the highest annual limpet landings with 1.9 tonnes in 1990 and 76 tonnes in 2017, representing approximately 69% of the total of the landings of limpets in 2017 (**Figure 11**).

Six fishing vessels landed *ca.* 96 tonnes representing 86% of the total of limpets landed in 2017. Since 2014, landings of commercially harvested limpets occur only in four ports (Porto Moniz, Caniçal, Paúl do Mar, and Funchal), mainly due to the proximity of the ports to the harvesting zones.

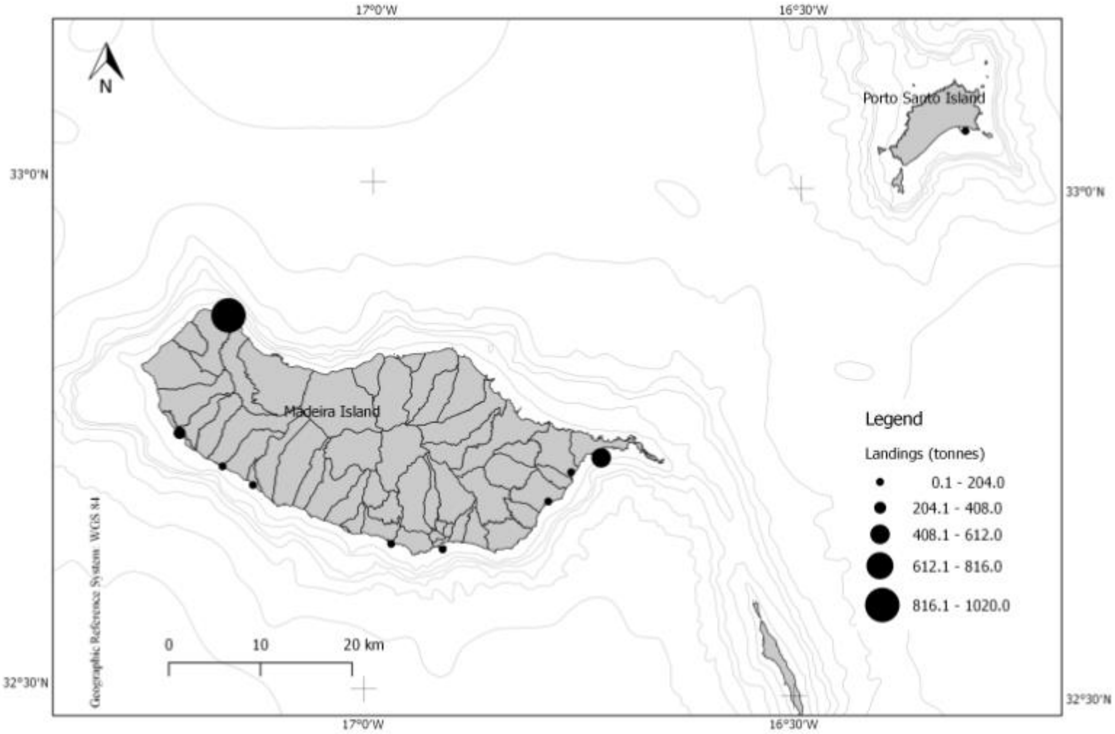

Figure 10 - Total landings of limpets per port between 1990 and 2017.

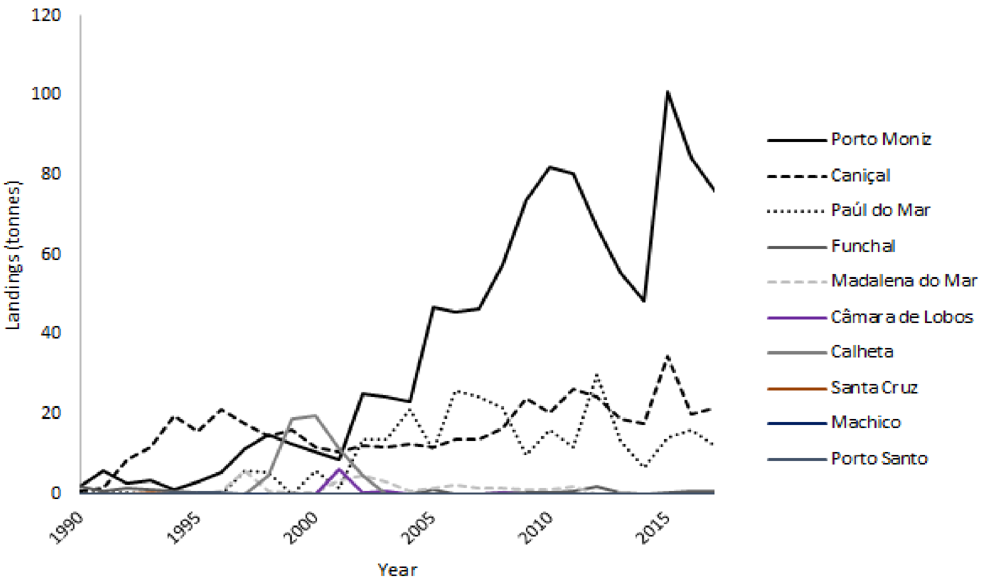

Figure 11 - Landings of limpets per year considering the landing port.

The commercial landings of limpets (in weight) in the archipelago of Madeira increased from 12% in 1990 to 96% of the total of molluscs landed in this region (**Figure 12**). Since 1998, limpets represent >90% of the landed molluscs in weight, increasing in importance over the years.

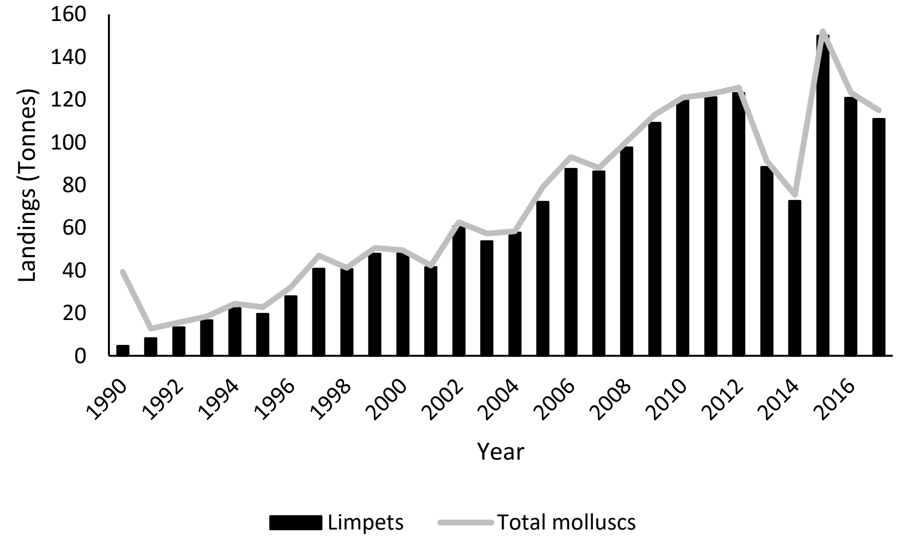

Figure 12 - Landings of limpets and the landings of the total molluscs per year, between 1990 and 2017 in the archipelago of Madeira.

The economic importance of limpets in the fisheries sector of the archipelago of Madeira gradually increased over the years. In 1990, limpets represented approximately 26% of the total economic value landed for molluscs and in 2017 approximately 96%. Since 1991, limpets represent the majority of the landed value for molluscs. From 1998 to 2017 this resource represents over 90% of the landings of molluscs (**Figure 13**).

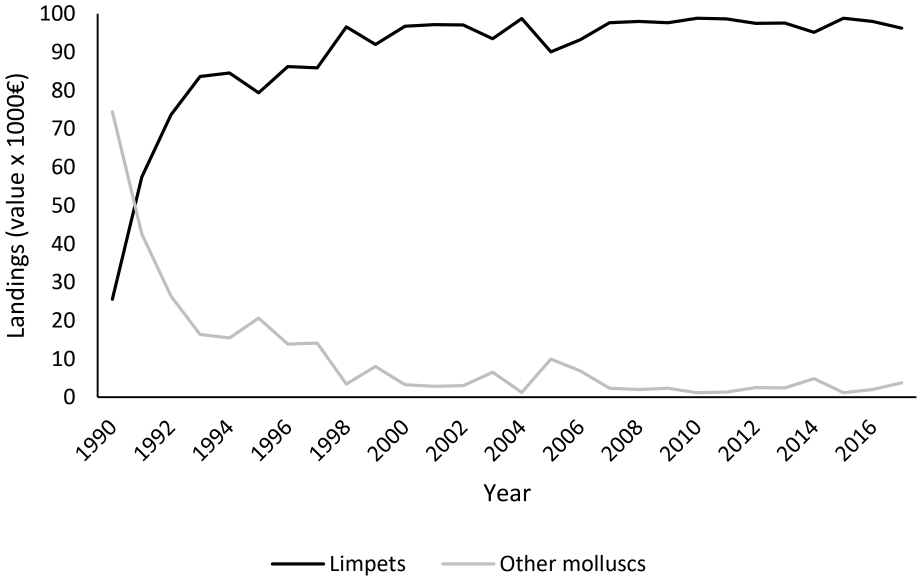

Figure 13 - Economic value of the landings of limpets and the economic value of the landings of the other molluscs per year, between 1990 and 2017 in the archipelago of Madeira.

The traditional harvesting of limpets carried out by the local populations, is not represented in the landings since current harvesting regulation does not require first auction sale for this activity (< 3 kg/day/person), thus making it difficult to quantify the real impact of the traditional harvest on the exploited populations. Also, illegal harvest is not considered on the total of landings. Nonetheless, it is known that commercial harvest occurs preferentially on certain locations (north coast of Madeira Island and around the Desertas) and, for these regions at least there is a better understanding of the harvesting effort on the exploited stocks.

The topshell (*P. sauciatus)* harvest in the archipelago of Madeira is carried out unregulated and without auction obligation, and as such, the landings of this species are residual, being impossible to estimate the real harvesting effort exerted on this species [2]. The landings varied from *ca.* 3 kg (2015) to *ca.* 230 kg (1993) between 1991 and 2017, yielding annual landed values between 14 and 520 € (**Figure 14**). Usually this species is sold directly to the markets at very high prices, reaching prices, i.e. between 15 and 20 € per kg.

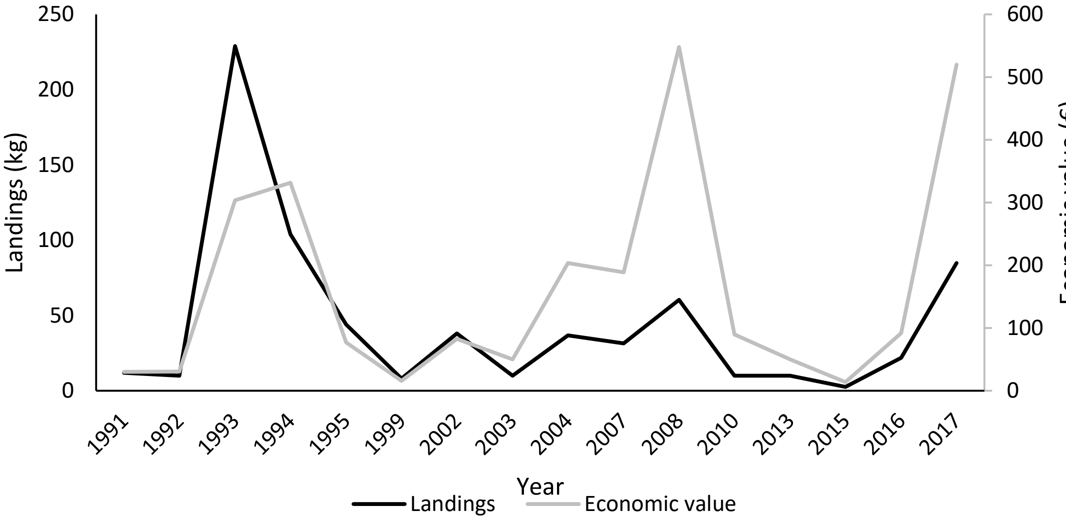

Figure 14 - Landings and economic value of topshells from 1990 to 2017 in the archipelago of Madeira.

4.2. Stocks status

Specific stock assessments for limpets in the archipelago of Madeira begun in 1994 and in the mid-2000s and were the basis for regulation of shellfish harvest in the region [11]. Nevertheless, due to the increasing interest in these molluscs, preliminary monitoring of limpet populations began in 1996. More recently, studies on the biology and stock assessment of *P. candei* and *P. aspera* were accomplished by Henriques et al. [13] and Sousa et al. [14].

The stocks of *P. candei* and *P. aspera* were found to be under-exploited, but with exploitation occurring near their maximum sustainable yield in the archipelago of Madeira. However, limpets' specific life traits, like slow growth rates and long life-span, make these molluscs extremely vulnerable to over-exploitation, and as such continuous monitoring of the stocks and the enforcement of the existing harvest regulations must be accomplished if future overexploitation is to be avoided [13-14]. Surveillance and enforcement of the closed season are also particularly important to avoid poaching and safeguard the reproduction of these species. Specifically, for *P. aspera* since this species is a protandrous hermaphrodite, and thus especially vulnerable to size selective harvest, since after reaching sexual maturation a percentage of males shifts to females, thus the removal of larger individuals will target primarily females leading to a decreased reproductive success.

Presently, some concerns persist regarding the management of the commercial exploitation of these two species in the region, namely, the lack of knowledge on the genetic connectivity between populations. In this sense, studies in this field are ongoing to establish proper conservation strategies considering connectivity or isolation of the populations. Another important concern relates to illegal poaching in the closed season and in MPAs and new conservation policies raising public awareness using innovative approaches involving not only decision makers but also the local communities which will be pivotal for the conservation of these species [12].

The first approach to assess the status of the stock of the topshell *P. sauciatus* in the archipelago of Madeira was held in 2018 although this species has been exploited since the fifteenth century. The stock of *P. sauciatus* seems to be moderately exploited in the region, but vulnerable to the harvest of small specimens. Conservation measures on harvesting regulation, considering the biological and ecological specificities of this species in this region, are required to preserve the stock in the medium and long term and efforts in implementing it are currently underway.

5. Effects of management measures in limpets populations

The implementation of limpet harvest management measures in the archipelago of Madeira was of paramount importance due to the life history traits of these species allied to their economic importance that results in a high harvesting pressure on these resources.

The continuous monitoring of the limpets populations over time allowed the regulators to readjust harvesting regulation in Madeira, namely the closed season period that initially lasted from November to January and presently ranges from December to March, to protect more effectively the breeding period of limpet species. This continuous monitoring clearly maximizes the returns that can be achieved through the regulation of this activity; since the responses of exploited stocks to harvest are continually changing, regulation has to adapt to these changes in order to promote its sustainability. The reduction of the number of harvesting licenses, the prohibition to capture immature individuals (< 40 mm shell length), and the catch limits have also contributed to the increase of the mean size of the specimens, to the recovery of the size-composition and to the increase of the abundance of the limpets' populations, in the archipelago of Madeira [3].

Conservation measures prompted an overall improvement of the exploited stocks on both exploited limpet species in Madeira. Comparative studies on the effectiveness of the implemented management measures demonstrated that the stocks of *P. aspera* and *P. candei* are slightly recovering since regulatory measures entered into force. The harvesting regulation was conducted to an increase in limpets mean shell length and to a more balanced size structure of the populations and a dominance of reproductive individuals. This effect was more noticeable in *P. aspera* populations due to the greater exploitation pressure exerted on this species [3].

The implemented management measures and the current levels of enforcement in the archipelago of Madeira showed positive results contrarily to those in the archipelago of Azores and the Canaries where management and enforcement were insufficient to protect the exploited limpet populations [15-16].

The management measures led to an increase of 14% in proportion of reproductive individuals and to an increase in the size and age of first maturity for both species. A shift from a biased sex ratio before harvesting regulation to a balanced sex ratio after regulation occurred for *P. aspera* (R. S., pers. Obs.).

Marine protected areas also contributed to the protection of limpets populations in the archipelago of Madeira. The increase of size-at-first maturity, shell size, balanced size composition, and capture per unit effort (CPUE) is evident in MPAs when compared to exploited populations. Also in the oldest and well-enforced MPAs, a high representation of large adults and more balanced populations for both limpet species was found (R.S., per. Obs.). In fact, MPAs play a pivotal role in the recovery of the exploited stocks of limpets in the archipelago of Madeira, considering their potential to promote replenishment and recruitment in nearby coastal areas where limpets are or have been heavily exploited. However, further studies are required to confirm this possible positive effect of MPAs on limpet stocks.

6. Conclusions

The harvesting of limpets in the archipelago of Madeira is protected by legislation that provides the basis for a sustainable exploitation. However, it is still very common for violations to the current management policies and regulations which are punishable with the application of penalties when detected by the local authorities to occur. The two major weaknesses in this regard are the lack of compliance by the fishermen, which is mostly due to the lack of knowledge about the importance of the implemented management measures, and to the poor enforcement by the regional authorities. A greater effort in the enforcement of regulations is required to ensure compliance of the fishing communities and an increase of the surveillance by the authorities to discourage illegal harvesting of these molluscs. Concerning topshells it is crucial to establish management measures on the harvesting of this resource to promote a sustainable exploitation in a medium and long term.

The ecological role that these key resources play in the coastal ecosystem and the importance of their survival not only from a conservational perspective but also economical needs to clearly transmitted to the fishermen and the general public, stakeholders, and authorities. This can be achieved through a proximity approach promoting training and involvement of all interested parties in the management and protection of these species. Promoting the awareness and providing informed advice to consumers to avoid purchasing illegally caught limpets to reduce illegal harvesting. Also, by instilling entrepreneurial spirit in fishermen by complementing their fishing activity with tourism through vessel trips and dives, adding value to these resources and increasing the fishermen income.

The implementation of obligatory species-specific landings for limpets is urgent to more accurately quantify the landings of each species and monitoring the exploited stocks, since they are landed together as a mixed exploitation.

The encouragement of aquaculture investments and post-harvest facilities will reduce fishing pressure on exploited gastropods in the archipelago of Madeira and opening the possibility of stock replenishment by reintroduction of cultured individuals to their natural habitat. This would also positively contribute to the socioeconomic development of the region, not only through production, but also through job creation.

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Chapter 8

Potential impact of harvesting management measures on the reproductive parameters of the limpets *Patella aspera* and *Patella candei* from Madeira Island

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Abstract

Intertidal and shallow subtidal molluscs are highly sensitive to overharvesting because of their restricted habitat, limited mobility and easy access to humans. Several parameters have been previously used to determine their exploitation status, e.g. sizes, abundances and reproductive stages. Herein the reproductive parameters of two exploited limpets, *Patella candei* and the protandrous hermaphrodite *Patella aspera* were examined. Limpets were collected “before” (1996-2006) and “after” (2007-2017) the implementation of management measures aiming to preserve limpet populations in Madeira Island (NE Atlantic Ocean). The proportion of reproductive individuals increased approximately 14% after the implementation of management measures for both species. An increase in the size and age at first maturity of both species (from 34.6 to 37.5 mm in *P. aspera* and from 33.4 to 37.4 mm in *P. candei*) also occurred after such implementation. “Before” samples of *P. aspera* showed male-biased populations (1.7:1) shifting towards a balanced sex ratio (1:1) after the regulations. No “before” nor “after” sex ratio variations were observed in *P. candei* populations. Reproductive parameters revealed feasible to determine the efficiency of the implementation of management measures and to detect a slight improvement in reproductive potential of both limpet species.

Keywords

Limpets, size-structure, sex ratio, harvesting, management measures, North-eastern Atlantic.

1. Introduction

Limpets are pivotal for structuring and regulating the ecological balance of intertidal communities, directly through the key process of grazing that determines macroalgal abundance, and indirectly by enhancing or inhibiting the establishment of other organisms (Jenkins et al., 2005; Coleman et al., 2006).

Limpets are common grazing marine gastropods on rocky shores and are highly vulnerable because of their restricted habitat and easy access to humans (Nakin and McQuaid, 2014). These molluscs have been used as a food resource worldwide and exploited by human since the Palaeolithic (Turrero et al., 2014). The exploitation of limpets is known to induce decreased species richness and biomass and to promote shifts on the communities' structure in rocky shores (Durán and Castilla, 1989; Lasiak, 1998; Sagarin et al., 2007; Fenberg and Roy, 2008). Exploited limpet populations show a reduction in density and shifts in population structure toward smaller sizes which can lead to a lower reproductive output since individual fecundity is size-dependent (Branch, 1975; Levitan, 1991; Tegner et al., 1996). These effects are related to the size-selective nature of limpet harvesting, with the removal of older and larger individuals (more visible and with greater commercial value) resulting in changes in demographics, life-history parameters and reproductive success of the exploited populations (Griffiths and Branch, 1997; Lindberg et al., 1998; Kido and Murray, 2003; Fenberg and Roy, 2008; Ramírez et al., 2009).

The impact of limpet harvesting has been observed for *Patella candei* d'Orbigny and *Patella aspera* Rödin, 1798 in Madeira Island (Sousa et al., 2019), for *P. candei* and *Patella candei crenata* in the Canaries Islands (Ramírez et al., 2009; Núñez et al., 2003), *P. candei* and *P. aspera* in the Azores Islands (Martins et al., 2008) and *Patella ferruginea* Gmelin, 1791 in Algeria and Spain (Espinosa, 2009; Espinosa et al., 2009). This situation has led to the implementation of management measures including the establishment of closed seasons, species-specific daily allowable catches, and minimum size of capture. These measures aim to preserve size/age structure, maintain yields within sustainable limits, prevent significant changes in sex ratios and sperm limitation, and avoid shifts to early maturation. Additionally, the establishment of closed areas where harvest is prohibited, such as Marine Protected Areas (MPAs) have proven to be an effective key tool in marine biodiversity conservation in coastal regions (Ballantine, 1991; Zann, 1995; Halper and Warner, 2002; Edgar et al., 2014) due to its ecosystem-level approach for exploited species (Henriques et al., 2017). MPAs promote the recovery and protect exploited marine organisms within their boundaries while sustaining fisheries at the same time. This is accomplished by re-establishing natural conditions for reproduction (Halpern, 2003; Lubchenco et al., 2003), increasing density and size leading to greater production of larvae, and enhancing adjacent fisheries through the export and settlement of larvae outside the protected zones (Branch and Odendaal, 2003; Gell and Roberts, 2003; Pelc et al., 2009).

Harvesting regulation is of paramount importance because limpets are broadcast spawners that do not exhibit external sexual dimorphism, whose reproductive success is size- and density-dependent. Therefore, changes in population structure towards a greater frequency of smaller individuals may lead to a decline in gamete production resulting in decreased reproductive fitness, as reported by Kido and Murray (2003) for *Lottia gigantea* (Sowerby, 1834).

Also, several species of limpets, such as *Patella vulgata* Linnaeus, 1758, *P. ferruginea* and *P. aspera* are known protandrous hermaphrodites (Le Quesne and Hawkins, 2006; Rivera-Ingraham et al., 2011; Martins et al., 2017). In these species the size-selective harvesting is of particular concern, since after reaching sexual maturation a fraction of males changes to females, thus the removal of larger individuals will target primarily females (Martins et al., 2017). There are also evidences that harvesting leads to decreases in both shell size and size at sex change, suggesting a shift to earlier sex change in exploited populations (Fernberg and Roy, 2012). Even though sex change is thought to be determined genetically, occurring mainly after the first maturation period (Fretter et al., 1998), external cues are involved in the timing of sex change of many protandrous hermaphrodite gastropods (Guallart et al., 2013). Among those cues, greater abundance of large females delays or inhibits sex change in males (Hoagland, 1978), while conversely size-selective harvesting targets the larger females which may promote sex change in protandrous hermaphrodites. In fact, in highly exploited populations of protandrous hermaphrodite limpets, such as *P. aspera* and *P. vulgata*, sex change tends to occur at an earlier size/age (Martins et al., 2017; Borges et al., 2016).

Another effect of heavy exploitation of hermaphrodite species is the occurrence of disruptions in the population sex ratio, often skewing towards the sex that matures at smaller size/age. Therefore, the sex ratio of populations subject to size-selective exploitation will be biased, resulting in reproductive failure in harvested populations (Alonzo and Mangel, 2004; Hamilton et al., 2007).

In the Madeira archipelago, limpets are harvested since the early years of colonization, dating back to the early 15th century (Silva and Menezes, 1921). Currently, limpets are landed in a mixed exploitation of *P. aspera* and *P. candei*, representing approximately 1.5% of the total of the fisheries of the region and reaching an average value of 4 € per Kg (Sousa et al., 2019). In 2017, the commercial landings in weight reached annual catches of up to 111 tonnes yielding a first value of *ca.* 0.4 M€ (Fig. 1). This long-term continuous exploitation is likely to have changed the reproductive dynamics of *P. aspera* and *P. candei* in the Madeira archipelago. In the present study, the status of exploited populations is represented by data gathered before the implementation of harvesting management measures in 2006. Subsequently, continuous monitoring of the exploited stocks provided data for a comparative study to analyse the effects of harvesting regulations on the species targeted by this locally important socioeconomic activity.

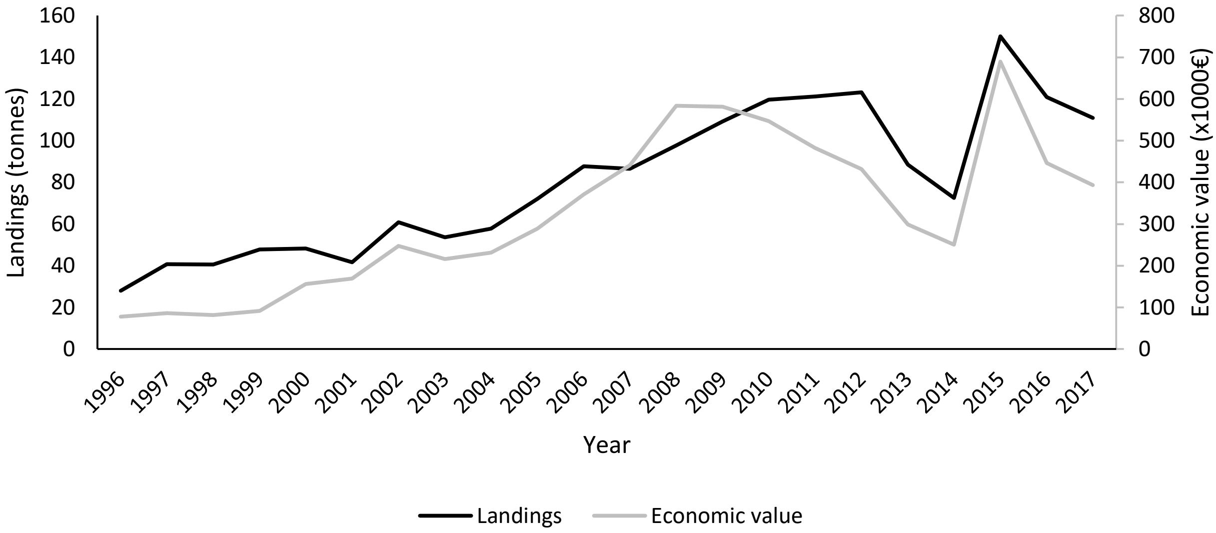

Figure 1 - Representation of landings (tonnes) and economic value (thousand euros) of limpets from 1996 to 2017 in the archipelago of Madeira.

The present study assessed whether the implementation of those management measures improved the reproductive parameters in exploited populations of *P. aspera* and *P. candei*, namely in terms of: i) the proportion of reproductive individuals in the populations, which is hypothesised to increase in both species after the implementation of management measures; ii) the size at first maturity, which is expected to increase after the introduction of harvesting regulations and iii) the sex ratio of the populations, particularly in *P. aspera* (a protandrous hermaphrodite), since the establishment of the minimum catch size is expected to positively impact the proportion of females in the larger size classes.

2. Material and methods

2.1. Sampling surveys and data collection

From 1996 to 2017 specimens *of P. aspera* and *P. candei* were randomly collected from inter- and subtidal rocky shores in Madeira Island, north-eastern Atlantic (Fig. 2) by snorkelling during 30 minutes without selecting species or specimen size. All dives were performed by the technicians of the Fisheries

Service Directorate of the Regional Fisheries Department. A total of nine sampling areas throughout Madeira Island, six in the south coast (Calheta, Ponta do Sol, Ribeira Brava, Funchal, Santa Cruz and Machico) and three in the north coast (Porto Moniz, São Vicente and Ponta de São Lourenço) of Madeira were sampled all year round, including during the legally established closed season from December to March.

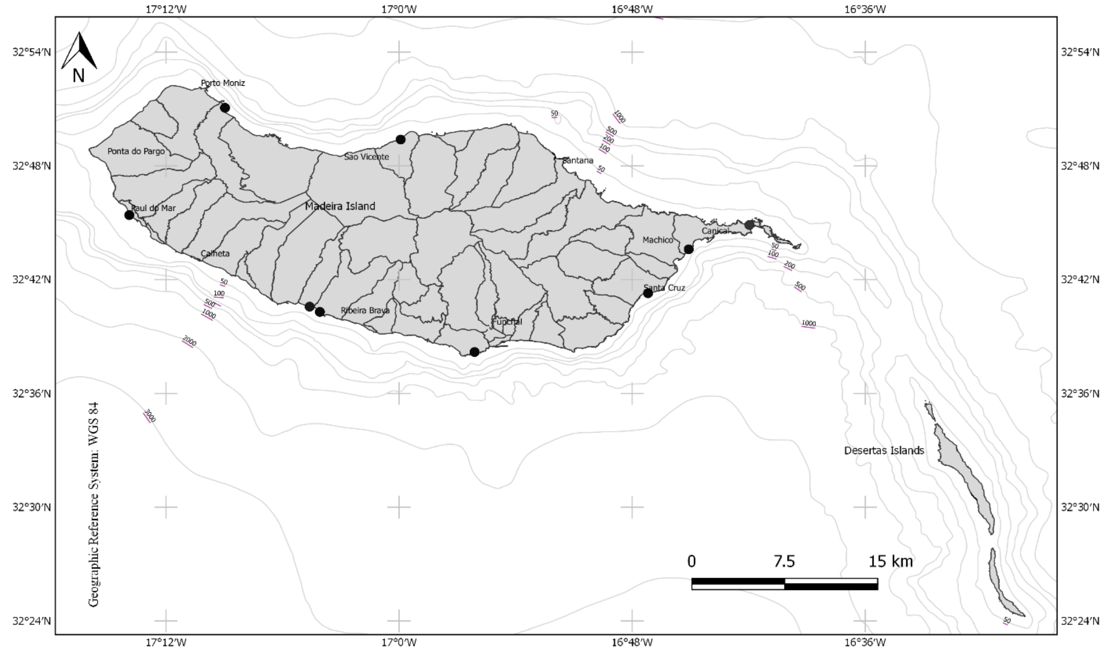

Figure 2 - Representation of sampling locations of *Patella aspera* and *Patella candei* in the Madeira Island.

All specimens were measured (total shell length, L) using a Vernier calliper (0.1 mm) and weighed (total weight, W) on a digital balance (0.01 g). Individuals were sexed according to gonad pigmentation, pale white or pink in males and brown to red in females. The dissection of individuals and macroscopic examination of the gonads allowed assigning each specimen to a maturation stage, determined according to the progression of the gonadal volume in the haemocoel, based on an adaptation of Orton et al. (1956) as described by Sousa et al. (2017).

2.2. Effect of harvesting regulations on the proportion of reproductive individuals

The proportion of reproductive individuals in the populations of *P. aspera* and *P. candei* was analysed before and after the implementation of harvesting management measures. For that purpose, specimens were classified as non-reproductive, reproductive, and resting based on the maturation scale and the estimated size at first maturity of both species.

2.3. Effect of management measures on the size at sexual maturity

The size at first sexual maturity of *P. aspera* and *P. candei* before and after the implementation of harvesting regulations were compared to determine the effectiveness of those management measures. For that purpose, the size at first maturity (Lm_{50}), was estimated from the relationship between the proportion of mature specimens and shell length, according to the balanced logistic equation:

$$P = {}^{1}\!/_{(1 + exp^{(-(a+bL))})} \text{ (Sparre and Venema, 1997)}$$

where P is the balanced probability, a and b are the equation parameters determined by the linear least square method using the logarithmic transformation. The mean size at maturity was defined as the size at which 50% of the population is mature, when P = 0.5 then $Lm_{50} = {}^{(-a)}\!/_{b}$ (King, 1995).

Age at median maturity (A_{50}) was calculated by the inverse von Bertalanffy growth function (von Bertalanffy, 1938):

$$A_{50} = t_0 - ({}^{1}\!/_{K})\ln(1 - {}^{Lm_{50}}\!/_{L_\infty}) \text{ (Jennings et al., 2001)}$$

where, L_∞ is the asymptotic shell length, K is the growth coefficient, t_0 the theoretical age at zero shell length and Lm_{50} is the size at first maturity.

2.4. Effect of management measures in the sex ratio

To assess the impact of the implementation of harvesting management measures on the reproduction of *P. aspera* and *P. candei*, the sex ratio (male: female) of both species was investigated for the two time-series (before and after those regulations).

2.5. Statistical analysis

The effect of harvesting regulations on the proportion of reproductive individuals was analysed by comparing the proportion of reproductive individuals between time-series and among size-classes within each time-series using the Pearson's chi-square test.

The existence of differences in limpet size-at-maturity between time-series was identified by comparing the slopes of the maturity curves using an analysis of covariance (ANCOVA). This test allows comparing the size-at-maturity before and after implementation of management measures, while statistically controlling for discrepancies in size-at-maturity caused by variation in size classes.

Additionally, the existence of differences in the proportion of sexes between time-series and among sizes-classes within each time-series was tested using the Pearson's chi-square test.

Statistical analyses were performed using SPSS v.24.0 (IBM Corp., 2016) with significance level considered for $p < 0.05$.

3. Results

A total of 51,380 limpets from both *P. aspera* and *P. candei* were analysed from the rocky shores of Madeira from 1996 to 2017. The shell length of the 26,273 sampled specimens of *P. aspera* ranged from 3.1 to 82.9 mm ($\bar{x}$ = 43.2 ± 7.8 mm) while for the 25,107 specimens of *P. candei* shell length varied from 6.0 to 81.8 mm ($\bar{x}$ = 46.7 ± 7.9 mm).

3.1. Proportion of reproductive individuals before and after harvesting regulations

Before the implementation of management measures in 2006, of the total *P. aspera* analysed, 3.9% were non-reproductive, 56.4% reproductive and 39.7% resting. After the implementation of those measures (2007-2017), an increase in the proportion of non-reproductive (12.8%) and reproductive (70.5%) and a decrease in the resting individuals (16.7%) was observed. In *P. candei* sampled between the specimens sampled between 1996 and 2006, 1.2% were non-reproductive, 69.5% reproductive and 29.3% in resting stage. Similarly to *P. aspera*, following the implementation of management measures *P. candei* displayed an increase in the proportion of non-reproductive (4.4%) and reproductive (83.5%) and a decrease in resting specimens (12.1%).

Regarding the size distribution, non-reproductive individuals of *P. aspera* were dominant (>50%) between 10 and 35 mm L, reproductive specimens predominated in the larger size-classes (40-65 mm L) and resting individuals prevailed between 35 and 40 mm before the implementation of management measures (Fig. 3A). The time-series after the management measures was characterized by a broader range of size-classes, with the predominance of non-reproductive specimens in the smaller classes (0-35 mm L) and the prevalence of reproductive individuals in the remaining classes (35-85 mm L) with more than 80% in the size-classes from 40 to 85 mm L (Fig. 3B). The differences in the proportions within each time-series were statistically significant before (χ^2=4141.763, $p < 0.05$) and after (χ^2=12106.199, $p < 0.05$) the implementation of management measures. The differences between the two sampling periods (1996-2006 *vs.* 2007-2017) were also statistically significant (χ^2=1554.921, $p < 0.05$).

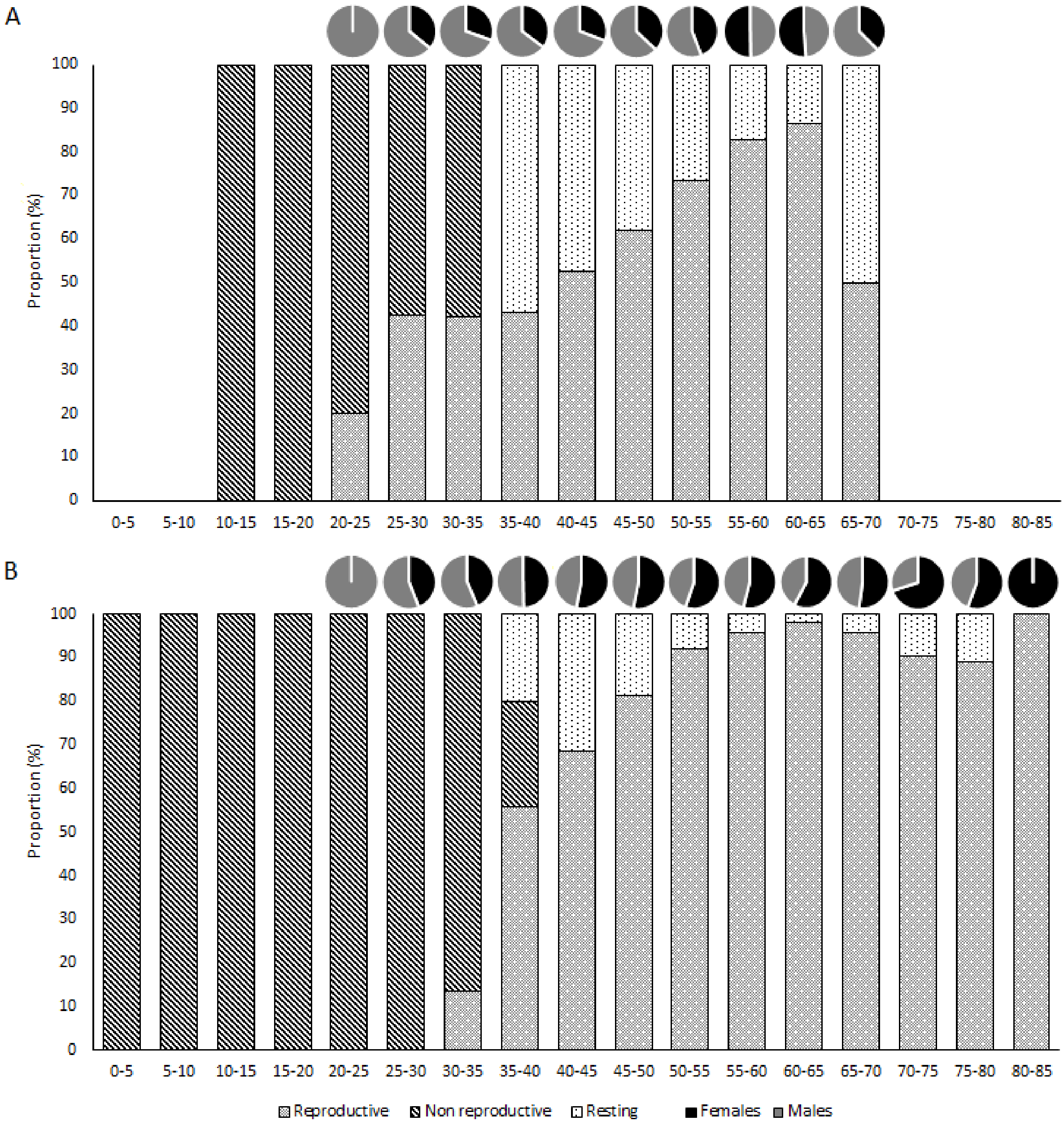

Figure 3 - Relative proportions of non-reproductive, reproductive, and resting individuals by size-class (bars) and sexual proportion (pie) of *Patella aspera*: A) before (1996-2006) and B) after (2007-2017) the implementation of harvesting management measures in Madeira Island.

Similarly, non-reproductive individuals of *P. candei* were predominant in the smaller size-classes (15-30 mm L), reproductive individuals in the larger classes (35-80 mm L) and resting specimens more abundant between 35 and 40 mm L (45%) during the period before management measures (Fig. 4A).

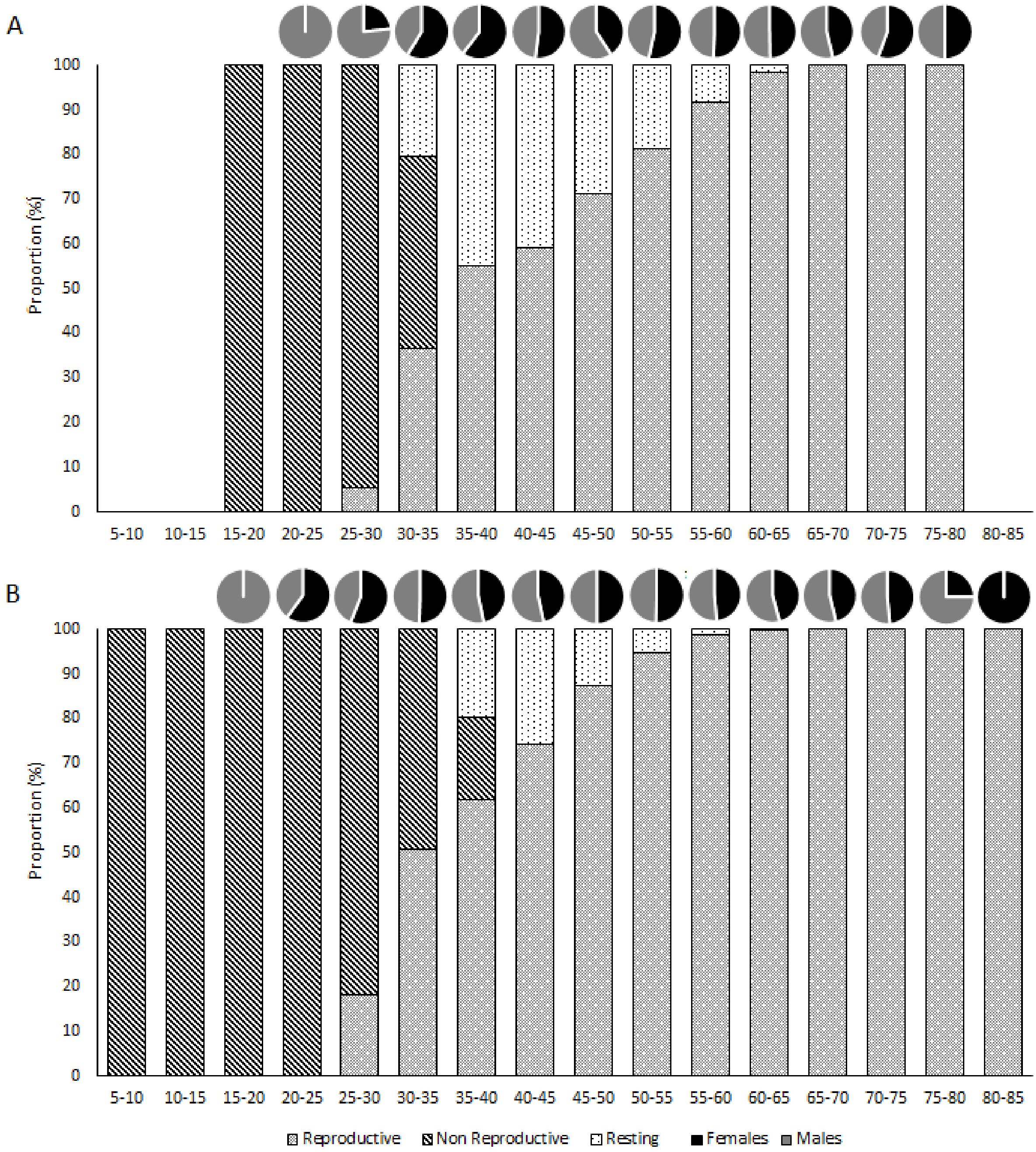

Figure 4 - Relative proportions of non-reproductive, reproductive, and resting individuals by size-class (bars) and sexual proportion (pie) of *Patella candei*: A) before (1996-2006) and B) after (2007-2017) the implementation of harvesting management measures in Madeira Island.

These differences in the proportion of non-reproductive, reproductive, and resting individuals were statistically significant (χ^2 = 5206.493, $p < 0.05$). Concerning the period after implementation of harvesting regulations, *P. candei* presented a wider range of size-classes, with non-reproductive specimens more abundant from 25 to 35 mm L and completely dominant in the smaller classes (0-25 mm). Reproductive specimens prevailed between 40 to 85 mm, with more than 75% from 45 to 85 mm L (Fig. 4B). The differences among size classes were statistically significant (χ^2 = 6440.242, $p < 0.05$), as well as those between the two sampling periods (χ^2 = 1155.485, $p < 0.05$).

3.2. Impact of management measures in size at first sexual maturity

The mean size at first maturity (Lm_{50}) of both *P. aspera* and *P. candei* increased with the implementation of management measures. The Lm_{50} in *P. aspera* increased from 34.6 mm during the period 1996-2006 to 37.5 mm during the period 2007-2017. The estimated mean A_{50} of *P. aspera* increased gradually from 1.8 to 2.0 years between 1996-2006 and 2007-2017. Concerning to *P. candei*, Lm_{50} increased from 33.4 mm L (1996-2006) to 37.4 mm L (2007-2017), corresponding to an increase in A_{50} from 1.7 years before to 2.0 years after the implementation of harvesting regulations. These slight differences in Lm_{50} between time-series were not statistically significant neither for *P. aspera* (F = 0.020, p = 0.889) nor for *P. candei* (F = 0.116, p = 0.735).

3.3. Effect of management measures in the sex ratio

From the sexed individuals of *P. aspera*, in the period before the implementation of the harvesting regulations (1996-2006), 63.5% were males and 36.5% females, whereas in *P. candei* 53.2% were males and 46.8% females. During the period after the implementation of regulations (2007-2017) 47.6% of the sexed specimens of *P. aspera* were males and 52.4% females, whereas *P. candei* presented 48.9% males and 51.1% females.

The sex ratio (male: female = 1.7:1) was male-biased for *P. aspera* during 1996-2006 and displayed an inverse trend during 2007-2017 (male: female = 1:1). The differences in sex ratio between time-series were statistically significant (χ^2=296.127, $p < 0.05$). Before the implementation of the management measures the sexual proportion among size-classes was in favour of males in all size-classes (20-70 mm L), with a totality of males (100%) in the 20-25 mm L size-class (Fig. 3A). These differences in the sexual proportion among size-classes were statistically significant during 1996-2006 (χ^2=52.941, $p < 0.05$).

After the implementation of the management measures the populations showed an increase in the size-range of the individuals and a shift in the sex ratio. In this period, females predominated in the larger size-classes (40-85 mm L) and males in the smaller classes (20-35 mm L) (Fig. 3B). The differences in the sex ratio among size-classes during this period (2007-2017) were also statistically significant ($\chi^2 = 59.821$, $p < 0.05$).

The overall sex ratio of *P. candei* was slightly male-biased (1.1:1) before the implementation of management measures (1996-2006). During this period, *P. candei* populations were characterized by the predominance of males on the first size-classes (20-30 mm L), on the remaining size-classes males and females were balanced, except for the 70-75 mm size-class where the females prevailed (63%) (Fig. 4A). In the period following the harvesting regulations, the sex ratio (male: female =1.05:1) was similar to the previous time-series with an increase in the size range towards smaller size-classes (15-20 mm L) with only males, an inversion of the proportion of females (> 55 %) in the size-classes from 20-30 mm L and higher homogeneity in the sexual proportion between 30 and 75 mm (Fig. 4B). The differences in the proportion of sexes between the two time-series were significant ($\chi^2 = 6.990$, $p < 0.05$), but not among size-classes for 1996-2006 ($\chi^2 = 16.946$, $p = 0.109$) nor 2007-2017 ($\chi^2 = 21.155$, $p = 0.098$).

4. Discussion

The establishment of an effective stock management strategy of marine gastropods requires a combined approach considering the traditional tools of stock assessment, such as abundance and size structure, together with the reproductive potential of the exploited populations. In that sense, reproductive parameters like proportion of reproductively active individuals, sex ratio and size at first maturity should be taken into consideration when establishing management measures.

In the present case, the protective effects of the implemented management measures on limpet stocks was evident through the increased proportion of reproductive individuals, more balanced sex ratio and larger size at first maturity.

The reproductive potential of an exploited population of limpets is affected by the reduction in abundance and mean size (Oliva and Castilla, 1986), resulting in a decreased reproductive output of these broadcast spawners. This is due to the fact that reproductive success in limpets is dependent of the quantity of gametes released into the water column and that larger limpets produce more gametes than smaller individuals. Moreover, in protandrous hermaphrodite species the removal of larger females may induce skewed sex ratios.

Several management measures were implemented in 2006 in order to prevent harvesting pressure from causing a decline of limpet populations in Madeira Island, namely the establishment of the maximum allowable commercial catch of 15kg/person/day or 200 kg/boat/day, aimed at reducing the overall harvesting effort, and the minimum catch size of 40 mm, to allow adults to contribute to the reproductive fitness, as well as the establishment of a closed season from December to March to avoid disturbance during the reproductive season (Henriques et al., 2017).

Highly exploited populations of limpets are characterized by smaller size ranges, reduced abundances, individuals maturing at smaller sizes and younger ages, reduced proportion of reproductive individuals, reduced maximum life-span and, in the case of protandrous hermaphrodites, male-biased populations and earlier sex change as seen in fish (Hamilton et al., 2007). Prior the implementation of management measures, the reproductive parameters of *P. aspera* and *P. candei* populations from Madeira Island were consistent with these features.

The overall results show that *P. aspera* larger than 70 mm were absent in the populations sampled prior to 2007, while for *P. candei* the full-size spectrum was found. This might result from different levels of exploitation of these species, with the preferential target species (*P. aspera*) suffering a greater reduction in mean size than *P. candei*, which is similar to what occurs in most exploited stocks (Oliva and Castilla, 1986; Pombo and Escofet, 1996; Fenberg and Roy, 2012). Additionally, the fact that *P. aspera* is a protandrous hermaphrodite makes it more vulnerable to overexploitation, since it reaches sexual maturity as males and changes to females later in life, consequently the size-selective nature of limpet harvest will target mostly females leading to male-biased populations (Martins et al., 2017).

The sex ratio by size-class in *P. aspera* prior to the implementation of management measures was male biased, which is frequent in protandrous patellids (Branch, 1974; Creese et al., 1990). After the implementation of harvesting regulations, the species sex ratio shifted towards a ratio 1:1, although males were still more abundant than females in the smaller size classes and females became more abundant in the larger size classes.

One expected impact of limpet harvesting in protandrous hermaphrodite species is the decrease of the size at which the sex change occurs as a compensation for the removal of the larger females due to size-selective harvesting, as observed for *P. vulgata* in UK (experimental harvesting farm exploited populations) (Borges et al., 2016) and *P. aspera* in the Azores Islands and Canary islands (Martins et al., 2017). Prior to the establishment of management measures in Madeira Island, *P. aspera* reached the size of sex-change earlier than after those regulations, suggesting a possible recovery of the exploited stock, since this species potentially changed sex at larger sizes during the period 2007-2017. Even though the regulations apparently had a positive effect on the exploited populations, these stocks could still benefit from the enforcement of a maximum size of capture, allow a more effective protection of larger females, safeguarding the renewal of the exploited populations.

Many studies worldwide have reported changes in maturation dynamics of heavily exploited stocks, namely smaller size and younger age at which individuals start reproducing and the decreasing spawning biomass (Trippel, 1995; Law, 2000; Sharp and Hendry, 2009). Size at first maturity and age at median sexual maturity are important reference points in fishery management (Hilborn and Walters, 1992), whose shifts are mainly attributed to overfishing that leads to a decrease in stock density and to changes in environmental factors (Trippel, 1995; Gerritsen et al., 2003; Domínguez-Petit et al., 2008).

The present trend of increasing size and age at first maturity after the implementation of management measures for both limpet species in Madeira Island should be analysed with some caution since, poaching still represents a serious threat that can undermine the success of the implemented management measures. Nevertheless, this slight increase likely results from an improved reproductive potential of the exploited populations, due to measures that ensure higher reproductive output, such as the closed season during the main spawning season and the prohibition of harvesting specimens smaller than 40 mm. The increase in the size at first maturity corroborates the increase in the asymptotic length (L_∞) estimated at 71.80 mm for *P. aspera* and 77.98 mm for *P. candei* before the management measures (Delgado et al., 2005) and at 84.2 mm for female and 80.5 mm for male *P. aspera* after regulations (Sousa et al., 2017) and 80.81 mm for *P. candei* (Henriques et al., 2012).

One consequence of size-selective harvesting is the removal of larger, reproductively active individuals from the target populations. In fact, for the 1996-2006 time-series both limpet species in Madeira Island exhibited smaller proportions of reproductive individuals, which was more evident in *P. aspera* (*ca.* 56%) than in *P. candei* (*ca.* 70%). After the implementation of management measures an improvement of approximately 14% in the proportion of reproductive individuals was observed for both species. These similar results are likely related to the close size at first maturity of both species and to the fact that management measures are equal for all harvested limpets. In *P. aspera* not only the proportion of reproductive individuals increased but also the size range, with specimens larger than 70 mm being only sampled in the time-series 2007-2017. Additionally, in both species increase the proportion of non-reproductive individuals, coupled with smaller specimens being sampled in the period post implementation of management measures, suggesting an improvement of the reproductive potential of the harvested populations.

The opposite trend was reported for *Patella candei crenata* and *P. aspera* in the Canaries where populations were characterized by highly fragmented assemblages dominated by non-reproductive specimens (Riera et al., 2016). This scenario was observed even after implementation of management measures, due to ineffective surveillance and increasing human population in coastal areas. The poor recovery of these species resulted from intense harvesting and showed the low viability of limpet populations at medium and long-term in this region (Riera et al., 2016). The same has been observed for *P. candei* in the Azores Islands by Martins et al. (2011), who stated that the efficiency of the established measures was insufficient to ensure the recovery of exploited populations, mostly due to illegal harvesting linked to lack of enforcement of regulations.

The present parameters on the reproductive potential of exploited populations of *P. aspera* and *P. candei* are valid indicators of the efficiency of the management measures implemented, when these parameters are considered in the establishment of harvesting regulations, as occurred for these species in Madeira. In fact, the present results indicate a slight improvement in the reproductive potential of these heavily exploited limpets, although the implementation of management measures could have resulted in greater improvements because their efficiency directly depends on a thorough enforcement of the regulations. Occasional poaching is still a threat to these species, together with ecosystem disturbances related to other anthropogenic activities, such as pollution and habitat removal (reviewed in Henriques et al., 2017). To enhance the positive impacts of the implemented legislation, continuous monitoring of the exploited stocks is paramount and the establishment of a maximum size of capture should be considered aiming to ensure higher reproductive outputs. Overall the improvements in reproductive potential of *P. aspera* and *P. candei* result from a successful management strategy, although the enforcement of regulations needs to be reinforced to protect these species from illegal harvesting, while the involvement of the local population in the conservation effort is of pivotal importance to achieve this goal.

Several genetic studies including both *P. aspera* and *P. candei* were carried out in the past decades (Ridgway et al., 1998; Weber et al., 1998; Koufopanou et al., 1999; Weber and Hawkins, 2005). Recently, molecular tools using microsatellites were developed for conservation genetics of both species (Faria et al., 2015; 2016). Local applications of those tools are recommended in order to clarify whether these limpet populations represent a single stock or a network of smaller local stocks, with consequences on their future management. Also, studies regarding the impact of the various marine protected areas existent in Madeira, would allow a better understanding of their effect on the recovery of exploited stocks.

Overall, the present results suggest a slight improvement on the exploited limpet stocks in Madeira Island, however further development requires continuous monitoring of harvesting activities, effective vigilance of no-take areas and during the closed season to prevent poaching, further awareness and engagement of local communities on promoting a sustainable fishery, and if needed, more specific regulations, such as a maximum size of capture for *P. aspera*, to counter the negative effects of size-selective harvesting in this protandrous hermaphrodite species.

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Chapter 9

Long-term population status of two harvested intertidal grazers (*Patella aspera* and *Patella candei*), before (1996-2006) and after (2007-2017) the implementation of management measures

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Abstract

Intertidal limpets are subject to harvesting pressure in regions, e.g. oceanic islands, where marine organisms are a more accessible source of protein. These molluscs are very sensitive to human exploitation which often results on a decrease of their densities and an over-representation of immature individuals, because of the loss of large-sized reproductive specimens. Two species of exploited limpets (*Patella aspera* and *Patella candei*) were assessed throughout 21 years, before (1996-2006) and after (2007-2017) the implementation of conservation measures for their sustainable management in Madeira (North-eastern Atlantic Ocean). Different levels of anthropogenic pressure were also taken in account in this comparative study: (i) proximity to coastal settlements ("Near" *vs.* "Far") and (ii) accessibility to the coast (North *vs*. South), that may be considered surrogates of harvesting pressure on the intertidal of Madeira. The present results showed that the stocks of *P. aspera* and *P. candei* are slightly recovered after regulatory measures entered into force, with an increase of mean shell length and dominance of reproductive individuals (> 40 mm). *P. aspera* populations showed a clearer effect mainly due to the higher exploitation rate relative to *P. candei*. Conservation measures prompted a positive effect on both exploited limpet species, but further assessment studies are necessary to address the evolution of stocks over time.

Keywords

Limpets, size-structure, harvesting, management measures, North-eastern Atlantic.

1. Introduction

Limpets play a key role in regulating the community structure of littoral ecosystems and are of significant economic importance, being used worldwide as food since prehistoric times (Bowman and Lewis, 1986; Stearns, 1992; Gutiérrez-Zugasti, 2011). These intertidal grazers are extremely vulnerable because of their particular life-traits, restricted habitat and its accessibility to human activity (Nakin and McQuaid, 2014).

Patellid limpets are exposed to anthropogenic impacts on the littoral ecosystems such as, harvest (Martins et al., 2008; Riera et al., 2016) and habitat modification (Cole et al., 2012). The expansion of coastal settlements resulting in the increase of human population density along the coast, has prompted a consistent decrease of limpet populations throughout the last decades worldwide (Kido and Murray, 2003; Martins et al., 2008). In several cases, this phenomenon has led to the reduction in abundance and/or shifts in size composition of their populations that result from the size-selective nature of limpet harvest, with larger specimens, with higher commercial value, being subject to higher harvesting pressure. Size reduction and abundance decreases in exploited populations of limpets have been reported for several species such as, *Patella candei crenata* (Ramirez et al., 2009) and *P. candei* d'Orbigny, 1840 (Núñez et al., 2003) in the Canaries, *P. candei* and *P. aspera* Röding, 1798 in the Azores (Martins et al., 2008), *Helcion concolor* (Krauss, 1848) (Branch, 1985), and *Patella ferruginea* Gmelin, 1791 in Algeria (Espinosa, 2009) and Spain (Espinosa et al., 2009), as well as for the species *Cymbula oculus* (Born, 1778) in Southern Africa (Branch and Odendaal, 2003).

The loss of large individuals produces cascading effects on the biology of limpets in exploited populations, including changes in life-history parameters, demographics, reproductive success, genetics, as well as changes in ecological interactions and limpet behaviour (Fenberg and Roy, 2008; Espinosa et al., 2009; López et al., 2012; Henriques et al., 2017). In the most extreme cases, harvesting pressure is recognized to have led to the high fragmentation of limpet assemblages as occurred *for P. candei crenata* and *P. aspera* in the Canaries (Riera et al., 2016) and even to the disappearance of populations of *P. ferruginea,* an endemic and endangered species from the Mediterranean Sea (Espinosa, 2009), and of the endemic species *Cellana sandwicensis* (Pease, 1861), *Cellana exarata* (Reeve, 1854) and *Cellana talcosa* (Gould, 1846) in Hawaii (Valledor, 2000). Local extinction events are particularly worrying in oceanic islands due to the low connectivity existing between insular limpet populations (Bird et al., 2007; Goldstien et al., 2009).

In Madeira, the harvesting targeted limpets are *P. aspera* and *P. candei* representing one of the most profitable commercial activities on small-scale fisheries, reaching annual catches of up 150 tonnes in

2015 yielding a total first sale value of *ca.* 0.7 M€ (Henriques, 2010; Sousa et al., 2017). Therefore, harvesting pressure is one of the greatest concerns for limpet conservation in Madeira since their high economic value, reaching in average *ca.* 4 € per Kg in 2017, together with their biological characteristics could lead to the decline of populations and conduct to the overexploitation of the stocks. To prevent this situation, regulators established several management measures enforcing the maximum allowable commercial catch of 15 kg/person/ day or 200 kg/boat/day, a minimum catch size of 40 mm and the obligation of harvesting licenses (Regional Legislative Decree N.˚ 11/2006/M, 18 April 2006). A closed season was also implemented between December and March to avoid limpet harvest during the reproductive season (Henriques et al., 2017). Several studies have shown that limpet populations respond positively to implemented management measures, as long as the enforcement of those measures is adequately accomplished by the responsible authorities and more particularly when there is an active participation of the local communities (Fenberg et al., 2012; Coppa et al., 2016; Henriques et al., 2017).

Herein the effects of anthropogenic pressure on the size structure and abundance of populations of *P. aspera* (white-footed limpet) and *P. candei* (black-footed limpet) in Madeira (North-eastern Atlantic Ocean) are analysed. We hypothesized that the proximity to human settlements will result in a decrease in mean limpet size and lower abundance in “near” stations (< 1 km from human settlements) compared to “far” stations (> 3 km from human settlements). Additionally, we hypothesized that accessibility to limpet populations also affects negatively the size structure and abundance of the more accessible populations (South coast) compared to least accessible populations (North coast). Moreover, a comparative study was carried out considering two time-series, “before” (1996-2006) and “after” (2007-2017) the implementation of management measures in order to verify their effectiveness along the Madeira coast.

2. Materials and methods

Fresh samples of *P. candei* and *P. aspera* were collected in the rocky shores of Madeira, NE Atlantic, in the framework of the European Fisheries Research Projects ‘Programa Nacional de Recolha de Dados da Pesca’ and MARISCOMAC- MAC/2.3d/097.

A total of 9 coastal settlements throughout the South (6) (Calheta, Ponta do Sol, Ribeira Brava, Funchal, Santa Cruz and Machico), and North coasts (3) (Porto Moniz, São Vicente and Ponta de São Lourenço) of Madeira were sampled all year round between 1996 and 2017. Three natural reserves, where limpet commercial harvest is not allowed, were also sampled as control areas (Garajau, Rocha do Navio and

Desertas) (Fig. 1). At each locality, a minimum of 2 sites were selected according to the classification of Riera et al. (2016) as “near”, <1 km from the nearest human settlement, and “far”, >3km from the nearest human settlement. Accessibility to the coast was grouped into “North”, the least accessible due to rough seas, and “South”, as more accessible due to milder conditions. Sampling was randomly performed at the subtidal by snorkelers in several dives over a period of 30 minutes without selecting for size or species. The dives were performed by the technicians of the Fisheries Service Directorate of the Regional Fisheries Department. All individuals were measured (total shell length, *TL*, mm) using a Vernier calliper (0.1 mm) and weighted (total weight, *TW*, g) on an electronic scale with 0.01 g accuracy.

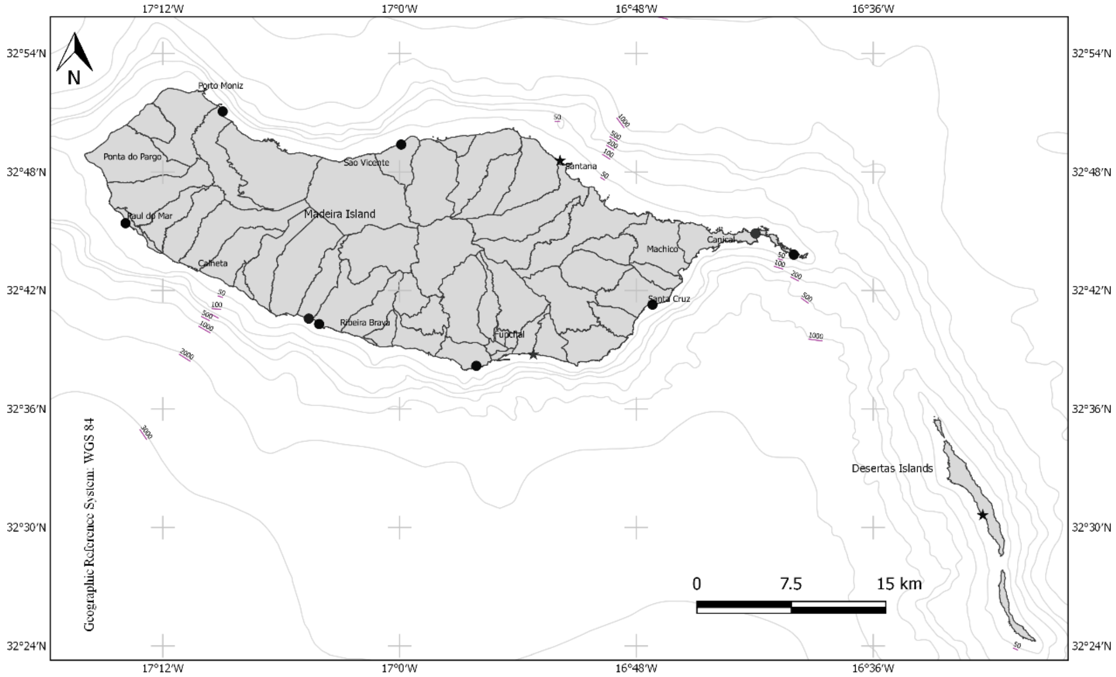

Figure 1 - Representation of sampling locations of *Patella aspera* and *Patella candei* in the Madeira archipelago (• exploited zones, ⋆ natural reserves).

Data were tested for normality of distribution of samples and for the homogeneity of variance using the Kolmogorov-Smirnov test (two samples) and the Levene’s statistics respectively. All analysis of variance were performed considering the Brown-Forsythe *F* test, when the variance of the data was not homogeneous. Proximity to human settlements and accessibility were considered with the data to determine the influence of harvesting on limpet populations.

A univariate comparison of the size of *P. aspera* and *P. candei* was performed using an analysis of variance (ANOVA) to verify the effect of each categorical variable (proximity and accessibility) on the shell length, and a two-way analysis of variance (ANOVA) was performed to examine the influence of the interaction of the two categorical independent variables (proximity and accessibility) on the continuous dependent variable (shell length).

A comparative study was conducted considering two time-series, before (1996-2006) and after (2007-2017) the implementation of the management measures. Specimens were separated in non-reproductive and reproductive considering the size at first maturity of both species, 36.70 mm for *P. candei* (Henriques et al., 2012) and 38.29 mm for *P. aspera* (Sousa et al., 2017). The comparison of limpet size from both time series was carried out using an analysis of variance (ANOVA). The size structure of the exploited populations was analysed for both periods (before and after management measures implementation) by the non-parametric Mann-Whitney test (two samples) in order to determine any differences observed in limpet's size between the two time-series when the data was not normally distributed.

All statistical analyses were performed using SPSS v.20.0 (IBM Corp., Armonk, NY). For all tests, statistical significance was accepted when $P < 0.05$.

3. Results

3.1. Effect of proximity and accessibility

A total of 33112 limpets from 9 selected locations and 3 control locations of the rocky shores of Madeira were analysed. The mean shell length of the 16345 sampled specimens of *P. aspera* was 44.63±7.27 mm while for the 16767 specimens of *P. candei* shell length showed a mean of 47.03±6.78 mm. The size-frequency showed that the sampled data had a normal distribution for *P. aspera* (Z = 3.095, $P < 0.001$) and *P. candei* (Z = 3.788, $P < 0.001$). However, size did not exhibit homogeneous variance in neither *P. aspera* (W = 12.196, $P < 0.001$) nor *P. candei* (W = 17.492, $P < 0.001$).

The size distribution of both species showed that larger sizes (>40 mm length) were dominant with 75% of *P. aspera* and 86% of *P. candei* specimens. In both cases the observed size range indicates that most specimens are reproductive individuals, having reached the size at first maturity. *P. aspera* and *P. candei* assemblages were dominated by individuals ranging from 40 to 50 mm (50.53 and 53.33% of the overall abundance, respectively). However, the modal class for *P. aspera* (40-45 mm) was slightly smaller than for *P. candei* (45-50 mm).

For *P. aspera*, 10490 specimens were caught from near sites, 3944 from far sites and 1911 from the control sites. Differences in mean shell length were found between the three sites, with smaller individuals in near sites (42.69 ± 6.56 mm) than in far sites (47.26 ± 6.99 mm). Control zones, where no commercial harvest is allowed showed the highest mean shell length (49.82 ± 7.31mm). The same pattern was observed for the 12404, 3653 and 710 sampled specimens of *P. candei* from near (46.27± 6.47 mm), far (48.68 ± 6.98 mm) and control (51.99 ± 7.30 mm) sites, respectively. The observed differences in mean shell length between sites were significant for both *P. aspera* (*F* = 1185.716, *P* < 0.05) and *P. candei* (*F* = 345.701, *P* < 0.05) (Fig. 2).

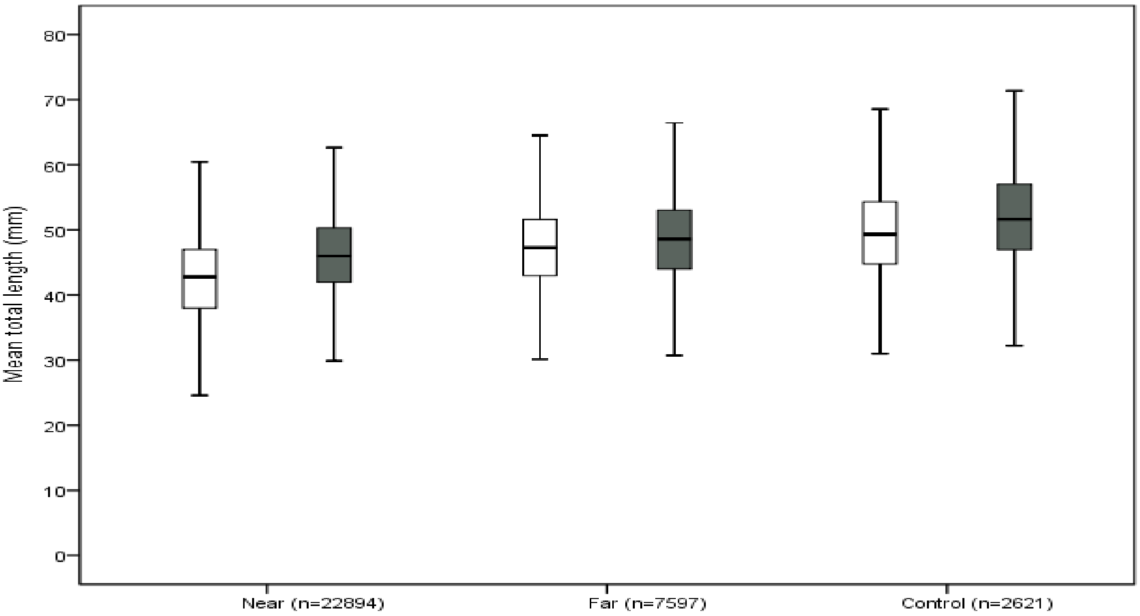

Figure 2 - Mean total length according to proximity to human settlements for white and black footed limpets. White bars represent *Patella aspera* and dark grey bars represent *Patella candei*. Box-plot showing median (black line) and upper and lower quartiles of the data.

The effect of accessibility was observed for *P. aspera* with larger individuals on the north coast (44.09 ± 6.53 mm) than those from the southern coast (43.07 ± 7.55 mm). However, this trend was not observed for *P. candei* that exhibited a smaller mean shell length in the north coast (46.69 ± 6.39 mm) than in the south coast (46.94 ± 7.23 mm). Differences in mean shell length between the north and south coasts were significant for *P. aspera* (*F* = 58.807, *P* < 0.05) but not for *P. candei* (*F* = 3.763, *P* > 0.05) (Fig. 3).

The effect of the interaction of proximity and accessibility was observed for both species with larger specimens occurring on far sites on the north coast. Differences in mean shell length between the north and south coast, considering proximity to human settlements were statistically significant for *P. aspera* (*F* = 10.790, *P* < 0.05) and *P. candei* (*F* = 11.814, *P* < 0.05). This analysis highlights differences in mean shell length for *P. candei* between the north and south coast which are not evident when considering only the accessibility.

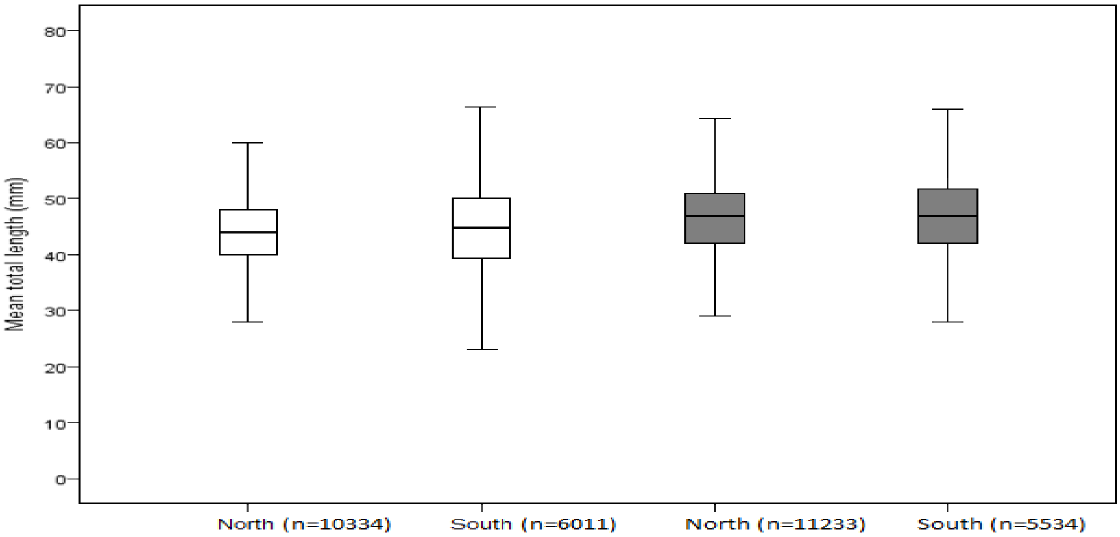

Figure 3 - Mean total length according to accessibility of white and black footed limpets populations. White bars represent *Patella aspera* and dark grey bars represent *Patella candei*. Box-plot showing median (black line) and upper and lower quartiles of the data.

3.2. Effects of management measures on the stock of P. aspera and P. candei

The assemblages of *P. aspera* and *P. candei*, before the implementation of management measures (1996-2006), were characterized by a wide range of sizes, from 11.00 to 68.00 mm and 16.00 to 76.00 mm respectively. In the period following the regulation of limpet harvesting (2007-2017) an increase in size range was observed for *P. aspera*, ranging from 3.10 to 82.86 mm. For *P. candei* this effect was less pronounced with sizes ranging from 25.46 to 78.31 mm. The mean size of *P. aspera* increased from 43.53 ± 6.23 mm before regulation to 45.38 ± 7.82 mm after, these differences between time-series were statistically significant ($F = 281.295$, $P < 0.05$). An increase in size was also observed for *P. candei* from a mean shell length of 46.26 ± 6.34 mm before regulation to 47.89 ± 7.14 mm after, this difference in mean size was also significant ($F = 240.469$, $P < 0.05$).

The proportion of size classes was slightly right-skewed after implementation of management measures for *P. aspera*, with the highest percentages in classes 40 to 45 mm before and 45 to 50 mm after. Limpets smaller than 10 mm and larger than 70 mm were only observed following regulation. Specimens of *P. aspera* with mean shell length between 25 and 35 mm and from 50 to 85 mm were predominant after regulation (Fig. 4). However, the observed differences in size classes between the two time-series were not significant ($U = 121.000$, $P > 0.05$). The proportion of reproductive individuals

(>38.29 mm) remained unaltered for *P. aspera* before and after implementation of management measures.

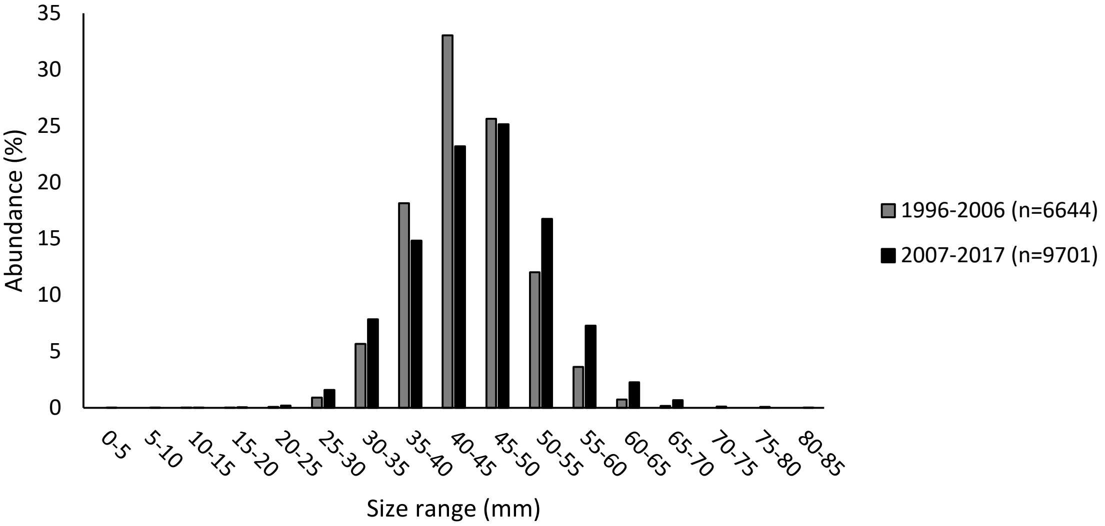

Figure 4 - Variation of size ranges of *Patella aspera* before (1996-2006) and after (2007-2017) the implementation of management measures in Madeira archipelago.

For *P. candei*, the proportion of size classes remained mostly similar before and after implementation of management measures, the size class with the highest percentages was the same for both time-series (45-50 mm). Specimens of *P. candei* with mean shell length between 50 to 85 mm were slightly more abundant after regulation (Fig. 5).

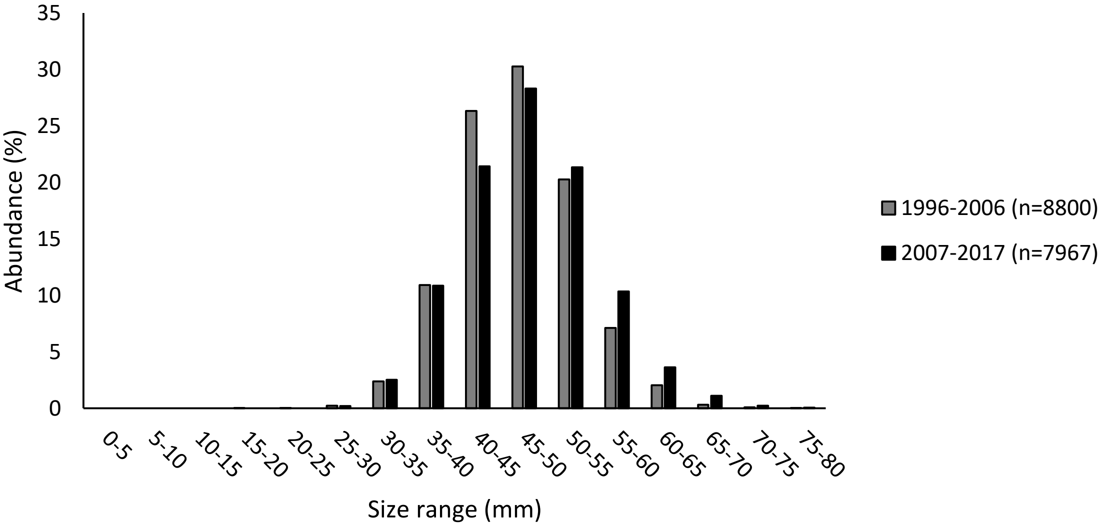

Figure 5 - Variation of size ranges of *Patella candei* before (1996-2006) and after (2007-2017) the implementation of management measures in Madeira archipelago.

Differences in size classes between the two time-series were not significant ($U = 141.000$, $P > 0.05$). As occurred for *P. aspera* no changes in the proportion of reproductive individuals for *P. candei* (>36.70 mm) were observed.

4. Discussion

Anthropogenic activities negatively affect populations of marine gastropods of commercial interest, such as limpets, namely by altering population structure of the affected species, resulting in decreased abundance, and altered size structure (Tuya et al., 2006). Management measures are thought to preserve age structure, maintain sex-ratios, prevent sperm limitation, enhance yield, and restrict evolutionary changes in response to fishing or harvesting, such as shifts to early maturation (Alonzo and Mangel, 2004; Baskett et al., 2005; Heppell et al., 2006; Hamilton et al., 2007). Limpet harvesting is a traditional activity in Madeira archipelago, dating back to the early years of colonization (Silva and Meneses, 1921). Since 2006, this activity has been regulated, following the guidelines obtained from the first stock assessment performed on the stocks of both species (Delgado et al., 2005). The implementation of management measures is of utmost importance considering the harvesting pressure resulting from the importance of limpets in the local gastronomy, both for locals and tourists' consumption.

The overall results of this study indicate that the size composition of the populations of *P. aspera* and *P. candei* in Madeira, are going through a slight recovering phase as a result of the implementation and enforcement of management measures on limpet harvesting. The populations are currently characterized by the increase in mean shell length and by the dominance of individuals with larger sizes in both species. Most of the specimens are adults (>40 mm) with high reproductive potential and as such, main contributors to the fitness of the exploited populations. This pattern is more evident in *P. aspera* where the consolidation on the recruitment is also evident by the current increase of specimens in the smaller size classes when comparing the "before" (1996-2006) and "after" (2007-2017) time-series. The small differentiation observed in the recovery of the exploited populations is more evident in *P. aspera* due to the fact that this species is thought to be more heavily exploited than *P. candei* and as such the effect of the management measures is more noticeable for this species.

Consistent differences were found in limpet size structure when considering the effect of proximity to human settlements, with larger individuals being more abundant in distant sites. This trend was observed for both limpets (*P. aspera* and *P. candei*) with smaller individuals being more common in sites closer to human settlements than in more distant sites. When considering the control sites, where no harvesting has been allowed for over 20 years it is evident that these populations are in balance by the highest mean shell length and abundances obtained at the three considered sites.

The observed recovery pattern in the exploited populations of both limpets agreed with Martins (2009) and Martins et al. (2017) that observed the same correlation between limpets' abundances and the proximity to coastal settlements in Azores (NE Atlantic). Also, when considering the control sites, these results are concurrent with Lopez et al. (2012) that observed that sites where harvesting is not allowed, e.g. marine protected areas (MPAs), assemblages had larger individuals and higher abundances, and also an increase of their populations was observed. In general, the implementation of MPAs results in direct improvements for the protected populations in terms of size structure, abundance, and population density and indirect effects regarding reproductive output of these broadcast spawners (Carr, 2000; Claudet et al., 2011). Therefore, these areas should be used as complementary tools for the recovery of depleted ecosystems as well as for the management and protection of the exploited resources (Jenkins and Hartnoll, 2001).

The accessibility to *P. aspera* populations was shown to also affect negatively the size structure and abundance of these populations on the south coast which are more accessible than the populations in the north coast. Nevertheless, this pattern was not observed for *P. candei* since there were no significantly differences in the size structure and abundance when considering the factor accessibility. The differences in how accessibility effects these two species are likely related to the greater harvesting pressure that *P. aspera* is subject to, or to specific characteristics of this species that confer greater vulnerability and/or a more pronounced effect to its size structure, such as the fact that this species is a protandrous hermaphrodite (Martins et al., 2017). In fact, the size selective nature of harvest would more than likely contribute to a decrease in the percentage of females (more common in larger size classes), hindering the reproductive success of this species making it more vulnerable to harvesting. Therefore, the implementation of the minimum size of catch (40 mm) was pivotal to ensure a sufficient percentage of the population reaches sexual maturity, thus increasing the reproductive output of these populations. Despite the effect of accessibility being less pronounced in *P. candei*, when considering the interaction of accessibility and proximity the differences in mean shell length of *P. candei* were also significant, even though less marked than in *P. aspera*. This might imply that the effect of accessibility on *P. candei* is related also to the proximity to human settlements with smaller sizes occurring in near sites in the south coast (more accessible) where harvesting pressure, habitat loss and pollution are higher.

The effects of regulation on the recovery of the exploited populations of limpets in Madeira were characterized by an increase in the range of sizes and in the mean shell length. This pattern was more pronounced in *P. aspera,* with individuals smaller than 10 mm and larger than 70 mm were only observed following the introduction of harvest regulation. Concerning *P. candei*, this effect was mainly observed by the increase in the percentages of larger specimens (>50 mm) after the regulation. The implementation of harvesting regulation for *P. aspera* in Madeira seems to have been effective in not only allowing individuals to reach larger sizes, but also to guarantee that these specimens reproduce and contribute to recruitment of new individuals into the exploited populations, thus increasing the proportion of smaller individuals. For *P. candei*, these measures have allowed the exploited populations to recover mainly in terms of the abundance of larger specimens, but no effect is expected to have occurred in terms of its reproductive success since the proportion of reproductively active specimens is similar before and after the implementation of the management measures. Even though the implementation of harvesting regulations has resulted in a slight recovery of the exploited populations, a greater effect was expected to be evident after 11 years since its introduction. This could be explained by poaching which is known to occur during the closed season, without abiding the minimum catch size and the maximum allowable catch weight. This could be mitigated by raising awareness of the fishery and restauration communities to the need of conservation of these species and by actively involving them in the conservation effort, namely by not commercialising limpets during the closed season. Also, the increase of population density along the coast, the technological advances introduced in methods of processing and storage of limpets as well as a booming tourism activity is likely to add pressure to the exploited populations.

Martins et al. (2011) showed the legislation and current levels of enforcement were insufficient to protect the exploited limpet populations in Azores. Thus, they proposed that greater levels of enforcement should be considered, through the establishment of physical barriers and other protective strategies. Co-management has been observed to have positive results for conservation of exploited stocks (Costello et al., 2008), taking into consideration the need to enhance ownership of conservation areas and to involve all interested parties in the development of management schemes (Baxter, 2001; Thompson et al., 2002). Riera et al. (2016) showed that limpet harvesting has led to a sharp decrease in the mean size of both *P. aspera* (7 mm) and *P. candei crenata* (5 mm) in Tenerife (Canary Is.), together with a low representation of reproductive individuals (>35 mm). Even though limpet harvesting is controlled by regulations, the obtained results highlighted the low viability of limpet populations at medium and long-term in Tenerife as a consequence of ineffective surveillance due to a lack of means, coupled with a high human population density in coastal areas (Riera et al., 2016).

The scenario in Madeira seems to be more favourable, with the implemented measures having contributed to a slow but steady recovery of the exploited limpet populations. However, continuous monitoring of both species is required to address the evolution of the stocks over time, and to ensure the sustainable exploitation of these coastal resources. In the future, monitoring surveys should be accomplished using non-destructive methods in order to minimize the pressure exerted on these species. For that end, survey samplings should be performed in field, and after measuring, weighing and sexing, specimens should be returned to their habitat. The most adequate method for sexing specimens without killing them would be taking a biopsy of the gonad with a hypodermic needle (Baxter, 1982; Wright and Lindberg, 1979; LeQuesne and Hawkins, 2006).

One major difficulty in the management of these species in Madeira is to accurately quantify the landings of each species since they are landed together, as such it would be fruitful to implement obligatory species-specific landings, to allow for a more accurate monitoring of the exploited stocks. Other anthropogenic pressures have also to be considered, proximity to and accessibility of limpet populations not only increases their vulnerability to harvest but also to habitat loss and pollution among other factors that negatively impact these still fragile populations. The overall improvement of the exploited populations will have greater benefits in the medium and long term if management measures are fully fulfilled and involve the local community, thus assuring the sustainability of these species. Additionally, genetic studies should complement the continuous monitoring of these species, particularly in defining whether these limpets represent a metapopulation or segregated populations, allowing to adapt the management measures to the particularities of the exploited populations.

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Chapter 10

Disentangling exploitation of the intertidal grazer *Phorcus sauciatus* (Gastropoda: Trochidae) in an oceanic archipelago: Implications for conservation

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Abstract

Harvesting of intertidal grazers such as topshells is known to affect negatively the exploited populations by altering population structure and decreasing abundance. *Phorcus sauciatus* has a wide geographic distribution in the North-eastern Atlantic Ocean and is subject to increasing levels of harvesting pressure due to the expansion of human population on coastal areas. The effect of proximity to human settlements and coastal accessibility on the size-structure and abundance of *P. sauciatus* populations was examined in Madeira archipelago. Mean size, proportion of reproductive individuals and abundance of this species were generally smaller in areas closer to human settlements and in more accessible coastal areas. Marine protected areas returned the highest mean sizes evidencing their effectiveness in preserving the size-structure of this species. The results highlight the necessity to regulate the harvest of *P. sauciatus* in Madeira archipelago, as well as the implementation of management measures aiming at the sustainable exploitation and conservation of this species, exploited in this region since the early 15th century.

Keywords

Human-induced pressure, harvesting, intertidal, molluscs, *Phorcus sauciatus*, Atlantic Ocean.

1. Introduction

Intertidal reefs are highly productive ecosystems supporting an extremely diverse range of assemblages of algae and animals (Gamfeldt & Branken, 2009). Their accessibility has made them susceptible to a variety of human-induced impacts such as harvesting (Thompson, Crowe, & Hawkins, 2002). Highly exploited populations are characterized by low abundances and a decrease of mean size of the targeted species (Castilla & Durán, 1985; Riera et al., 2016). However, the effects of harvesting are not limited to changes in targeted species, but they extend through cascading trophic effects to the whole habitat (Scheffer, Carpenter, & Young, 2005). Molluscs are one of the most exploited intertidal organisms all over the globe (Roy, Collins, Becker, Begovic, & Engle, 2003; Sagarin et al., 2007) due to the easy accessibility to their habitat having been extensively harvested in several geographic regions since prehistoric times (Martins, Jenkins, Hawkins, Neto, & Thompson, 2008; Siegfried, 1994; Turrero, Múñoz-Colmenero, Pola, Arbizu, & García-Vásquez, 2012).

The harvesting of intertidal organisms is steadily increasing throughout the last decades due to the expansion of human population to coastal areas (Neumann, Vafeidis, Zimmermann, & Nicholls, 2015). Several species are considered highly threatened such as the limpet *Patella ferruginea* Gmelin, 1791, a Mediterranean species that is currently concentrated in a limited number of coastal locations (Espinosa et al., 2014), and the Hawaiian endemic limpet species *Cellana sandwicensis* (Pease, 1861), *Cellana exarata* (Reeve, 1854) and *Cellana talcosa* (Gould, 1846), that have been disappearing in extensive intertidal areas of islands with high population density (Valledor, 2000). However, the situation concerning other intertidal mollusc species, e.g. sea snails, still remains overlooked for most of their geographic regions though having recently received some attention in Atlantic oceanic islands (Navarro et al., 2005; Ramírez et al., 2005; Sousa et al., 2018).

The lack of management measures for the conservation of sea snails is alarming, especially in coastal isolated places where no adjacent populations are present to supply larvae for settlement and recruitment, though it has been observed that a high proportion of larvae settle < 100 km from their source population (Cowen, Lwiza, Sponaugle, Paris, & Olson, 2000), and large genetic connectivity among populations has been shown in Atlantic and Mediterranean populations of *Osilinus* and *Phorcus* (Donald et al., 2012).

The Macaronesian region, comprising the oceanic archipelagos of Azores, Madeira, Selvagens and Canaries, is characterized by high harvesting pressure on intertidal ecosystems (Martins, Thompson, Neto, Hawkins, & Jenkins, 2010; Núñez, Brito, Riera, Docoito, & Monterroso, 2003). This situation has been demonstrated through a sharp decrease of exploited molluscs populations in Azores (Martins, 2009; Martins et al., 2010), Madeira (Sousa et al., 2018) and the Canaries (Ramírez, Tuya, & Haroun, 2009), however, food culture greatly varies among these archipelagos. For example, Madeira has an old tradition of harvesting sea snails, namely the species *Phorcus sauciatus* (Koch, 1845) (Sousa et al., 2018), while the consumption of this species is not extensive in the Canaries and varies among islands, being more intense in western islands (Moro & Herrera, 2000; Tuya et al., 2006; Ramírez et al., 2009). Thus, the harvesting pressure of *P. sauciatus* show variations throughout the Macaronesian region and adjacent areas, and conservation measures need to be context-dependent and focused on local harvesting pressures to ensure the viability of this sea snail species in the region.

The topshells of the genus *Phorcus* are ecologically important algae grazers that play a key role in regulating the ecological balance of their habitat (Ramirez et al., 2005). These species occupy the rocky shores from the supratidal to the subtidal and are subject to an array of environmental pressures due to their extended vertical distribution, resulting in structural adaptations since their position relative to the shore influences their exposure to wave action, temperature variation, desiccation and tidal width (Ramirez et al., 2005; Sousa et al., 2018). They are essentially preyed by prawns, crabs and fishes (Crothers, 2001).

Phorcus sauciatus has a wide geographic distribution in the North-eastern Atlantic, including the Macaronesian archipelagos of Azores, Madeira, and Canaries with its northern boundary in the Iberian Peninsula and its southern limit in the African mainland, with small genetic differentiation between them, suggesting either recent or continuing dispersal among these areas (Ávila et al., 2015; Donald et al., 2012; Rubal, Veiga, Moreira, & Sousa-Pinto, 2014).

Herein, the effects of harvesting pressure on the size-structure and abundance of populations of *P. sauciatus* in Madeira (NE Atlantic Ocean), where this species has been historically highly-exploited, were investigated considering: (i) that proximity to human settlements would result in a decrease in topshell mean size and lower abundance in “near” stations (< 1 km from human settlements) compared to “far” stations (> 3 km from human settlements); (ii) and that accessibility to topshell populations affects negatively the size-structure and abundance of the more accessible populations (South sectors) compared to the least accessible populations (North sectors).

2. Methods

The study was conducted on specimens randomly collected from the upper to the lower intertidal zones of the rocky shores of the Madeira archipelago (Figure 1), during low tide. Each harvesting set was performed by the same experienced harvester during a standardized period of 15 minutes.

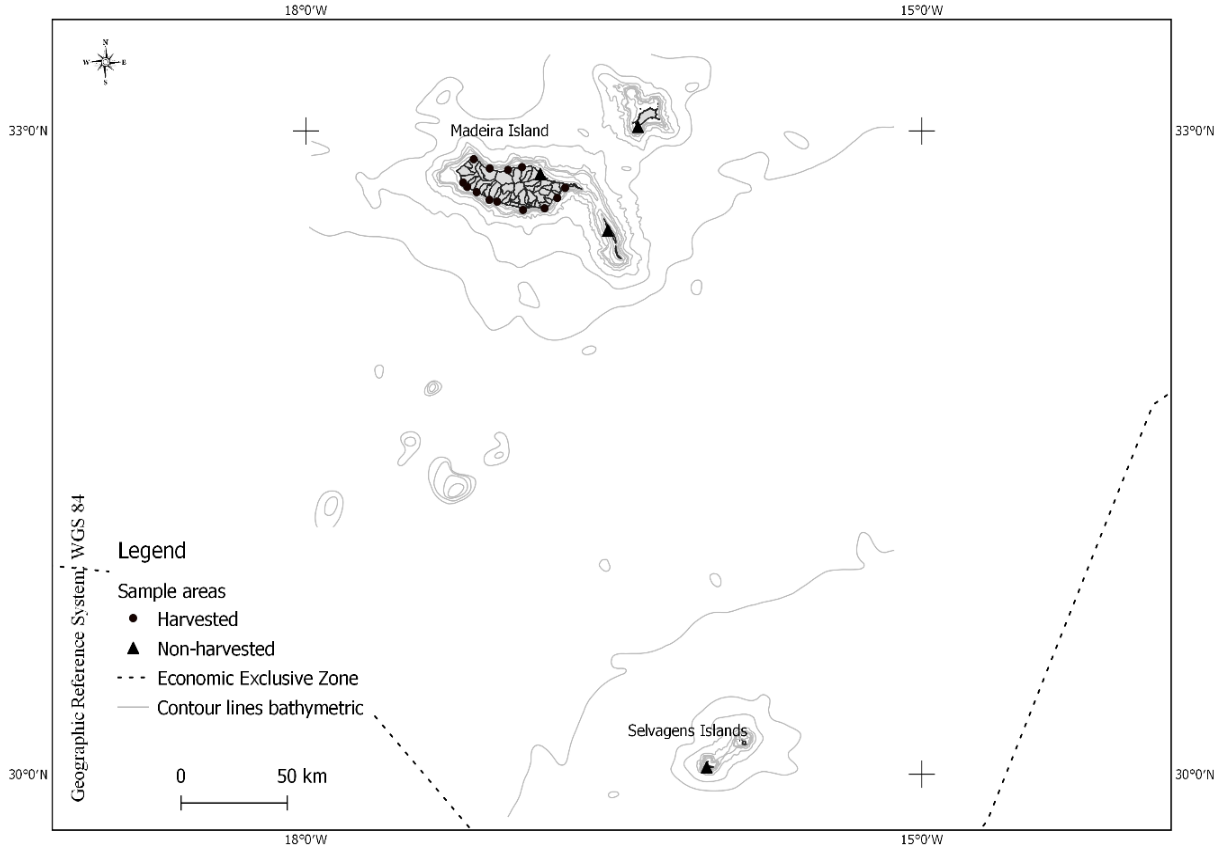

Figure 1 - Map of the sampling locations of *Phorcus sauciatus* in the archipelago of Madeira.

A total of 17 coastal settlements (locations) throughout the rocky shores were sampled between January 2017 and May 2018 (13 in harvested and 4 in not harvested zones). The locations were as similar as possible to each other and were selected considering the coastal settlements with analogous conditions (e.g. type of substrate, slope of the coast, rugosity).

All specimens were measured (total shell length, *TL*, mm) using a Vernier calliper to the nearest 0.01 mm.

Data were analysed for deviations to the parametric assumptions of analysis of variance (ANOVA). Three factors were considered, (i) Locations (random factor); (ii) Proximity (fixed factor, with three levels "Near" and "Far" from coastal settlements and MPAs); (iii) Accessibility (fixed factor, with three levels "North", "South" and MPAs). Normality of the distribution of the data was verified through the Kolmogorov-Smirnov two-sample test and homogeneity of variance was determined using Levene's statistics. Analysis of variance was performed considering the Brown-Forsythe *F* test, when the variance of the data was not homogeneous. All statistical analyses were performed using SPSS v.24.0 (IBM Corp., 2016). For all tests, statistical significance was accepted when $P < 0.001$ to reduce the chance of false positives.

2.1. Effect of proximity and accessibility on size-structure and CPUEn

The effect of proximity was analysed for each coastal settlement of Madeira archipelago, by selecting a minimum of two sites according to proximity to human settlement as described by Riera et al. (2016). Sites at <1 km from the nearest human settlement were classified as “near”, and sites >3 km from the nearest human settlement were considered as “far”. Sites were also grouped according to accessibility to the coast, samples from the northern coast, the least accessible due to rough seas were classified as “North”, and samples from the more accessible southern coast, due to milder conditions, were classified as “South”. Four Marine Protected Areas (MPAs), where topshell harvest is not allowed, were also sampled as areas with no harvesting pressure throughout the year, because of their inaccessibility and protection measures, namely Porto Santo island (NE Madeira); Rocha do Navio (N Madeira); Desertas Islands (SE Madeira) and Selvagens Islands (between Madeira and the Canaries).

A univariate comparison of the size of *P. sauciatus* was performed using an ANOVA. For the comparison of the proportion of reproductive individuals, considering the size at first maturity as 12.95 mm *TL* (Sousa et al., 2018), a statistical Pearson Chi-square test was applied. Proximity to human settlements and accessibility were considered within the data to determine the influence of harvesting on *P. sauciatus* size-structure throughout Madeira archipelago.

Relative abundance was estimated using the catch per unit of effort in number (CPUEn), corresponding to the ratio between the total number of captured specimens at each harvesting set and time. The effect of proximity and of accessibility on the abundance of *P. sauciatus* was evaluated using a Pearson Chi-square and a Mann-Whitney *U* Test respectively.

3. Results

A total of 6,329 specimens of *P. sauciatus* from 13 selected locations of rocky shores and four MPAs of Madeira archipelago were analysed. The mean size of total topshells sampled in Madeira was 14.39 ± 3.44 mm TL. The size-frequency distribution showed that the sampled data had a normal distribution ($Z = 1.883$, $P < 0.001$). Size did not exhibit homogeneous variance for proximity ($W = 55.125$, $P < 0.001$); however, it was homogenous for accessibility ($W = 1.613$, $P = 0.204$).

3.1. Effect of proximity and accessibility on size-structure and CPUEn

The study populations were characterized by a wide range of sizes, from 2.40 to 23.49 mm in near areas, from 2.91 to 25.07 mm in far areas and from 7.35 to 25.79 mm in MPAs. The size distribution showed that reproductive specimens (>12.95 mm TL) were dominant, representing 51% of the total specimens in "near" areas, 65% in "far" areas, and 94% in MPAs (Figure 2). This suggests that most specimens are reproductive individuals, having reached the size at first maturity. The differences in the proportion of reproductive individuals according to proximity was statistically significant (χ^2 = 682.946, P < 0.001). Pairwise comparisons showed significant differences in the proportion of reproductive specimens between near and far sites (χ^2 = 67.203, P < 0.001), near sites and MPAs (χ^2 = 223.514, P < 0.001) and far sites and MPAs (χ^2 = 607.858, P < 0.001). Statistical differences were also observed between the proportions of reproductive specimens according to the level of proximity on each of the 13 studied localities (*Pearson Qui-square statistical test*, P < 0.001).

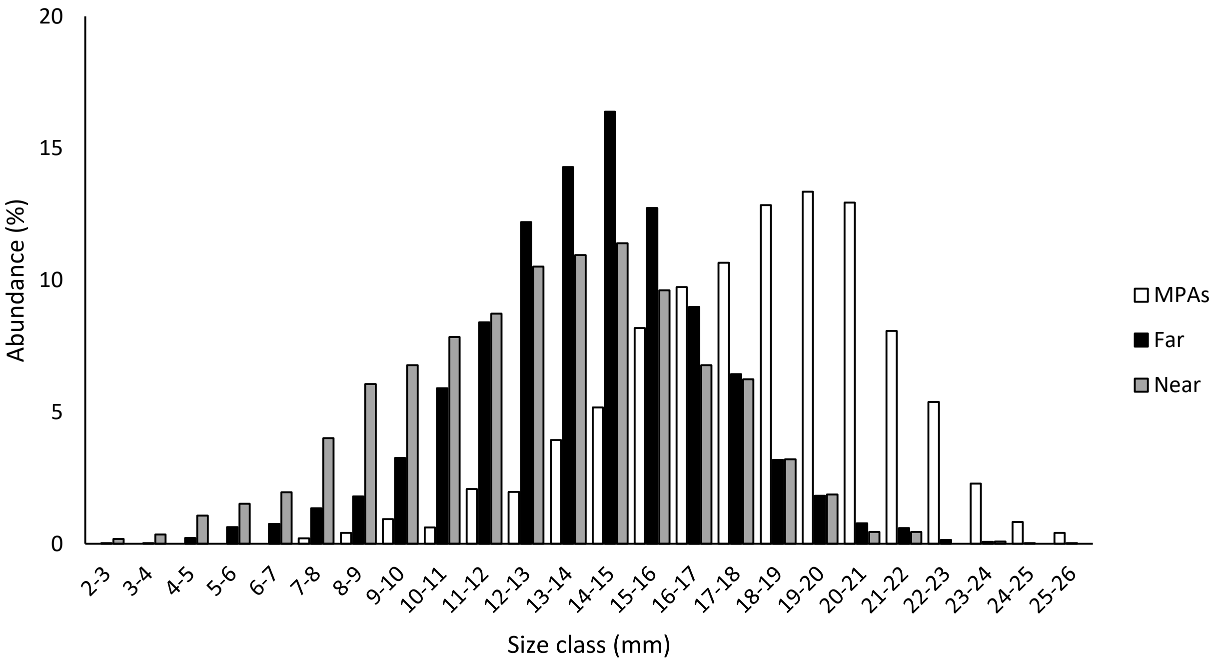

Figure 2 - Abundance of *Phorcus sauciatus* per size class according to proximity to human settlement in Madeira archipelago.

Mean size of the specimens caught in "near" areas was 12.87 ± 3.52 mm *TL*, 13.94 ± 2.86 mm *TL* in "far" areas, and 18.13 ± 3.10 mm *TL* in MPAs (Figure 3). Statistical differences in mean size among levels of proximity were significant (F = 806.524, P < 0.001) (Table 1). Post-hoc analysis confirms significant differences between near and far sites (*ANOVA Tamhane's T2 statistical test*, P < 0.001), near sites and MPAs (*ANOVA Tamhane's T2 statistical test*, P < 0.001) and far sites and MPAs (*ANOVA*

Tamhane's T2 statistical test, $P < 0.001$). Statistical differences were also observed between levels of proximity on each of the 13 studied localities (*ANOVA*, $P < 0.001$).

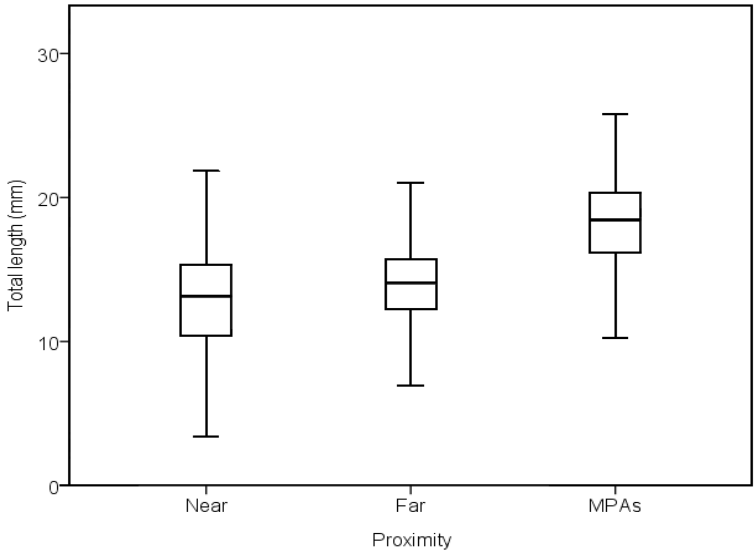

Figure 3 - Total length of *Phorcus sauciatus* considering proximity to human settlements in Madeira archipelago. Box-plot showing median (black line) and upper and lower quartiles of the data.

Table 1 - Results of analysis of variance on the size-structure of *Phorcus sauciatus*, in Madeira archipelago, according to locality, proximity and accessibility to the coastal settlements

Factor	Sum of Squares	df	Mean Square	*F*	*p*-Value
Locality	1,833.904	12	15.825	17.763	0.000
Proximity	16,959.324	2	8,479.662	806.524	0.000
Accessibility	4,285.856	2	4,285.856	422.210	0.000

Regarding accessibility, specimens ranged from 2.40 to 23.39 mm in the more accessible areas and from 2.97 to 25.07 mm in the least accessible areas. The size distribution showed that reproductive individuals were more dominant in the least accessible areas (70%) than in the more accessible areas (57%). The differences in the proportion of reproductive individuals among levels of accessibility were statistically significant ($\chi^2 = 1052.511$, $P < 0.001$) (Figure 4). Pairwise comparisons confirmed significant differences in the proportion of reproductive specimens between north and south ($\chi^2 = 56.057$, $P < 0.001$), north and MPAs ($\chi^2 = 585.149$, $P < 0.001$) and south and MPAs ($\chi^2 = 791.710$, $P < 0.001$).

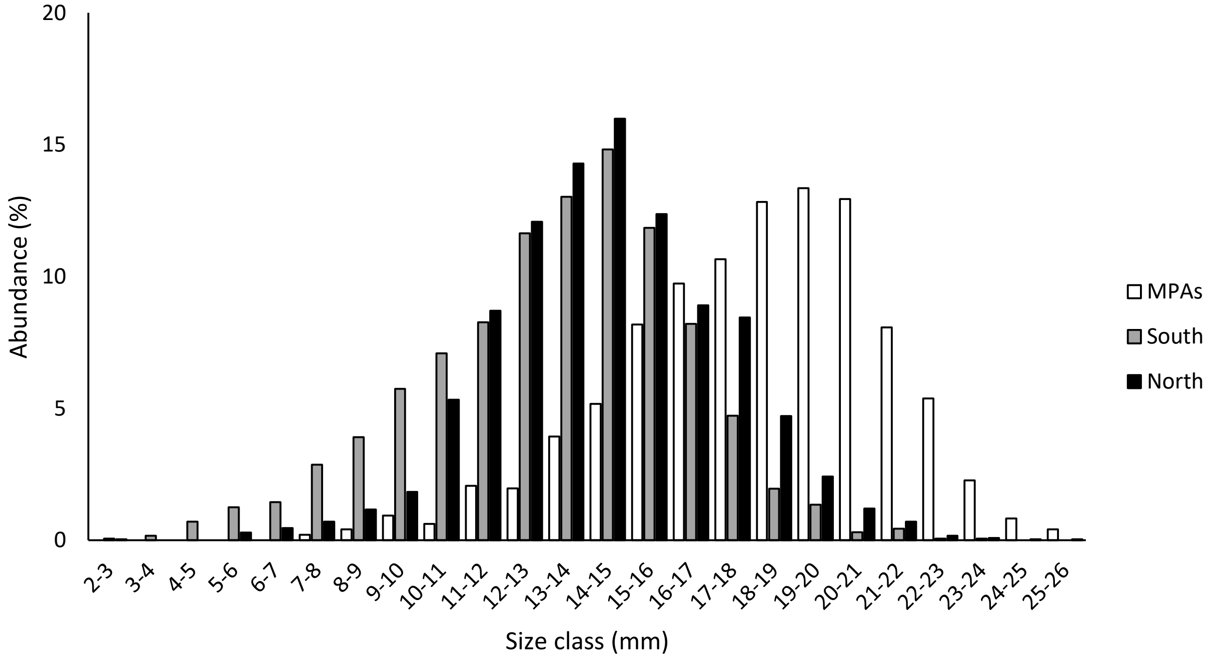

Figure 4 - Abundance of *Phorcus sauciatus* considering accessibility of sampling sites in Madeira archipelago. Box-plot showing median (black line) and upper and lower quartiles of the data.

The analysis of specimens mean size showed larger individuals on less accessible areas (north coast), with a mean size of 14.92 ± 3.24 mm *TL*, than on more accessible areas (south coast) with a mean size of 13.18 ± 3.14 mm *TL* (Figure 5).

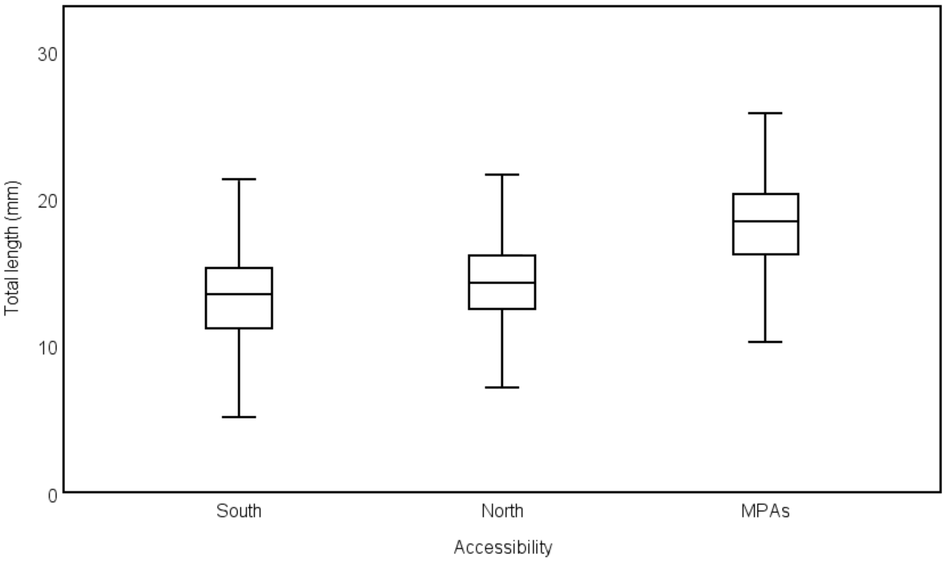

Figure 5 - Total length of *Phorcus sauciatus* considering accessibility of sampling sites in Madeira archipelago. Box-plot showing median (black line) and upper and lower quartiles of the data.

The differences observed in mean size of topshell according to accessibility were statistically significant (*F* = 422.210, *P* < 0.001) (Table 1). Post hoc analysis also showed significant differences within the north and south coasts (*ANOVA Tamhane's T2 statistical test*, *P* < 0.001), north and MPAs (*ANOVA Tamhane's T2 statistical test*, *P* < 0.001) and south and MPAs (*ANOVA Tamhane's T2 statistical test*, *P* < 0.001).

Concerning the CPUEn, abundances ranged from 0.23 to 22.73 specimens per minute with a mean abundance of 8.30 ± 6.20 specimens/min. The highest mean abundances were recorded for MPAs ($\bar{x}$ = 17.30 ± 2.93 specimens/min) and "far" areas ($\bar{x}$ = 13.38 ± 5.89 specimens/min), and the lowest for "near" areas ($\bar{x}$ = 3.40 ± 2.13 specimens/min) (Figure 6). Differences found in mean abundances between areas were statistically significant (*KW* = 8.160, *P* < 0.001). Pairwise comparisons showed significant differences within the near and far sites (*U* = 3.000, *P* < 0.001), near sites and MPAs (*U* = 0.000, *P* < 0.001). Concerning far sites and MPAs the observed differences were not statistically significant (*U* = 11.000*, P* = 0.240).

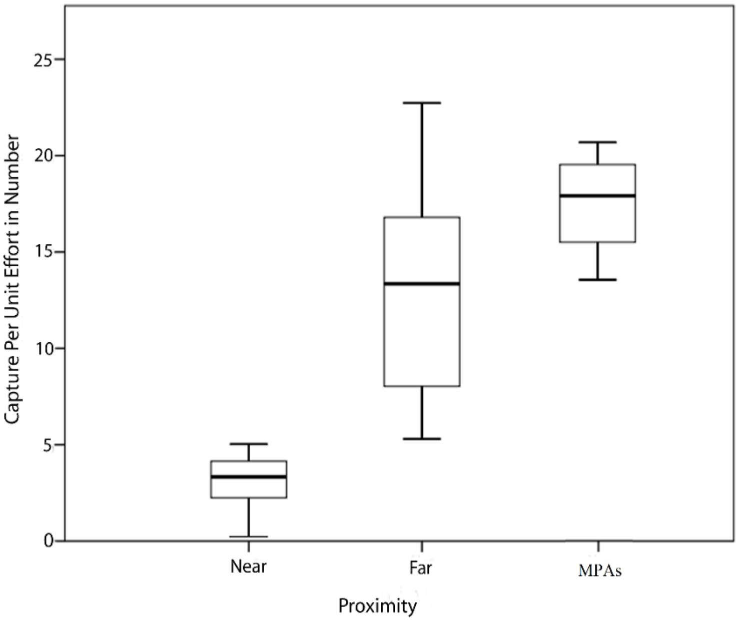

Figure 6 - Capture per unit effort in number of *Phorcus sauciatus* considering proximity to human settlements in Madeira archipelago. Box-plot showing median (black line) and upper and lower quartiles of the data.

Regarding to accessibility, the highest abundance occurred at the least accessible coast ($\bar{x}$ = 12.08 ± 7.26 specimens/min) and the lowest at the more accessible coast ($\bar{x}$ = 6.58 ± 5.89 specimens/min) (Figure 7). Statistical differences found in mean abundance according to accessibility were statistically significant (*KW* = 8.160, *P* = 0.017). The pairwise comparisons showed significant differences between the south and MPAs (*U* = 5.000, *P* < 0.001) contrarily to the observed differences between south and north coasts (*U* = 22.000, *P* = 0.080) and between north and MPAs (*U* = 6.000, *P* = 0.201) which were not statistically significant.

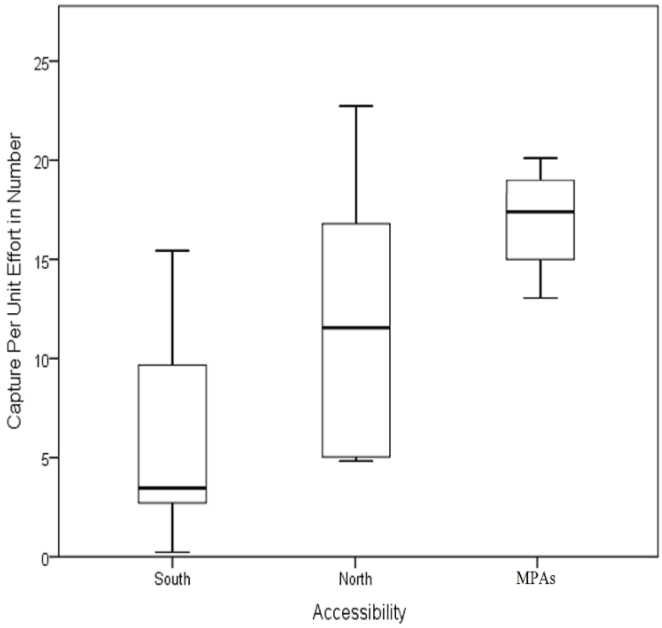

Figure 7 - Capture per unit effort in number of *Phorcus sauciatus* considering accessibility of sampling sites in Madeira archipelago. Box-plot showing median (black line) and upper and lower quartiles of the data.

4. Discussion

The limited habitat and its easy accessibility to human activity make intertidal and shallow-water grazers extremely vulnerable to anthropogenic pressures (Nakin & McQuaid, 2014). Harvesting of intertidal grazers such as topshells and limpets is known to affect negatively the exploited populations by altering their population structure, resulting in a decrease of abundance, and altered size-structure (Riera et al., 2016; Tuya et al., 2006). Additionally, the removal of grazers can often lead to an imbalance on the population dynamics of the species inhabiting rocky shores due to both direct and indirect effects on the trophic chains, with potential cascading effects (Branch & Moreno, 1994; Crowder & Norse, 2008).

The overall present results indicated that the populations of *P. sauciatus* in Madeira archipelago are dominated by reproductive specimens (>12.95 mm *TL*). However, in areas closer (<1 km) to human settlements the mean size and the proportion of these specimens are smaller than in distant areas (>3 km) as a result of size selective harvesting. The effect of proximity is more pronounced on the south coast due to a greater density of human population allied to an easier accessibility to this coast. This effect is even more pronounced when comparing with MPAs where harvesting is not allowed, and

>90% of individuals are mature. Even though, reproductive individuals are overly more abundant, it is known that in exploited populations of broadcast spawners such as, topshells and limpets the decrease of larger individuals, results in the reduction of their reproductive success and can lead to their decline over time (Guerra-García, Corzo, Espinosa, & García-Gómez, 2004; Núñez et al., 2003). In fact, present results suggest that, for *P. sauciatus* in Madeira, harvesting has led to a decrease in reproductive individuals according to a gradient of proximity to human settlements, varying from 90% in no take zones to 51% in areas closest to human settlements. This trend currently exists although there are many MPAs in and around Madeira Island from where larval dispersal and settlement may occur and provide recruits to nearby harvested populations.

The effect of human influence has also been demonstrated for *Phorcus atratus* (Wood, 1828) and *P. sauciatus* in the Canaries (Ramírez et al., 2009), where significant differences in size-structure of these species among islands have been observed. Ramírez et al. (2009) also verified that proximity to human settlements resulted in the disappearance of larger individuals and a decrease in number for the majority of size ranges of these species in the Canaries. The reduction in size and the increase in mortality rates are the primary effects of size-selective harvest, however harvest also prompts shifts on the reproductive potential of these species through changes in reproductive investment and relative fecundity. Additionally, it promotes ecological effects like changes in community structure and interspecific competition (Fenberg & Roy, 2008; Fenberg et al., 2012).

The size-structure of *P. sauciatus* populations is also influenced by accessibility, with larger individuals being found in the least accessible coast (North) and it is clear that on the south coast there are more significant differences in average sizes between localities. This pattern may be due to the fact that populations on the north coast are more protected, having less harvesting pressure and therefore being more balanced in terms of population structure. When considering MPAs, where harvesting is not allowed, it is evident that these populations have a more balanced size-distribution, with higher mean shell length and greater abundance of larger size class specimens.

The effect of accessibility on the abundance of *P. sauciatus*, however, did not return significant differences between northern and southern populations. Regardless, least accessible populations still showed higher abundance than more accessible populations due to the existence of rougher environmental conditions and lower human density in the North coast. These impacts on the spatial distribution of *P. sauciatus* abundance in Madeira archipelago, as a result of proximity and accessibility to human settlements, have also been reported for *Phorcus articulatus* (Lamarck, 1822) along the coast of Tunisia by Cheour, Cherif, Messaoud, Aloui-Bejaoui, & Ali (2014) and in the Canaries by Ramírez et al. (2009).

As expected, harvested populations always showed smaller mean size and lower proportion of reproductive individuals than non-harvested populations, suggesting that harvesting is impacting the population dynamics of *P. sauciatus*. However, differences in environmental conditions such as, sea surface temperature, tidal width, degree of exposure to wave action and productivity, between locations may also impact the population dynamics of these populations. These factors were not considered in the current analysis however, most of these factors are not expected to vary greatly between the studied locations, for instance sea surface temperature and productivity. On the other hand, the degree of exposure to wave action is likely to affect population dynamics of *P. sauciatus* as shown by our data, locations subject to harsher wave action have larger sized and more reproductively active individuals, as an indirect consequence to lower levels of exploitation due to these populations being less accessible to humans.

The present results highlight the necessity to regulate the harvest of *P. sauciatus* in Madeira archipelago, as well as the implementation of management measures aiming at the sustainable exploitation of this species. The effect of regulating topshell harvest is evidenced by the smaller size in harvested compared to non-harvested areas in Madeira. These management measures should be considered together with no take zones, as it has been shown that they contribute to the preservation of the size structure of *P. sauciatus* and complemented with continuous monitoring effort of the exploited local (Madeira) and regional (Macaronesia) populations.

Further studies with a wide range of regions and considering the harvesting pressure together with the biotic (interspecific and intraspecific competition) and abiotic factors (temperature, productivity, salinity) of the regions are warranted to evaluate the role of these factors in the size structure and abundance of *P. sauciatus* in the north-eastern Atlantic.

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Chapter 11

Are NEOLI features also valid to preserve exploited molluscs assemblages? Insights on the protection effects of MPAs from an oceanic archipelago

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Abstract

1. Limpets are one of the most successful intertidal algal grazers in the North-eastern Atlantic. They provide valuable ecosystem services, playing a pivotal role in the rocky shores ecological balance and have an important economic value, being subject to high levels of exploitation and representing one of the most profitable economic activities on small-scale fisheries in the Madeira archipelago. However, these species are extremely vulnerable to anthropogenic impacts such as overharvesting, habitat fragmentation and pollution.

2. The protection effects and the effectiveness of MPAs on the population dynamics of two historically highly exploited limpet species, *Patella aspera* and *Patella candei* were analysed through a comparative study of size, reproduction and abundance in Madeira archipelago, and the impact of the NEOLI features (regulation, enforcement, MPA age, MPA size and isolation) assessed.

3. The effects of protection from the MPAs on limpet populations resulted in a differential increase on size-at-first maturity, shell size and capture per unit effort according to the degree of protection.

4. MPAs with four NEOLI features as Selvagens and Desertas, were manifestly in greater balance than MPAs with no NEOLI features (Rocha do Navio) and consequently than exploited zones. NEOLI features of MPAs were pivotal to explain the observed variability among MPAs. Age and Enforcement were overwhelmingly the most relevant drivers to explain the observed variability among MPAs.

5. A thorough and multidisciplinary study is necessary to carry out in the near future to get a reliable picture of commercial stocks of the two targeted species, specifically genetic analysis and detailed studies on food source in order to shed light to future scenarios that are crucial for management conservation strategies.

Keywords

Intertidal, conservation evaluation, invertebrates, fishing.

1. Introduction

Human exploitation of organisms on the rocky shores is an important cause of disturbance of intertidal communities' occurring since prehistoric times (Bustamante & Castilla, 1990; Boer & Prins, 2002; Martins, Jenkins, Hawkins, Neto, & Thompson, 2008). Human activities often lead to the reduction of densities and maximum sizes of several species around the world (Moreno, Sutherland, & Jara, 1984). Among these species, molluscs are one of the most exploited intertidal organisms worldwide (Roy, Collins, Becker, Begovic, & Engle, 2003; Sagarin et al., 2007), being extensively harvested in several geographic regions (Moreno, Sutherland, & Jara, 1984; Keough, Quinn, & King, 1993). Currently, the exploitation of these organisms comprises recreational, subsistence and commercial activities (Moreno, Sutherland, & Jara, 1984; Siegfried, 1994) and depends on factors such as human demography, tradition, and economy (Rius & Cabral, 2004).

The removal of organisms for feeding, bait, aquariums, and shell collection for decorative purposes is one of the human activities that most affects the mollusc populations in coastal zones (Ramírez, Tuya, & Haroun, 2009), resulting in direct and indirect disturbance of intertidal populations (Kingsford, Underwood, & Kennelly, 1991; Addessi, 1994; Lindberg, Estes, & Warheit, 1998). These disturbances essentially focus on the abundance, size structure, and alterations on the reproductive output and replenishment of the exploited populations as result of the size-selective nature of harvest (Lindberg, Estes, & Warheit, 1998; Kido & Murray, 2003; Martins et al., 2017; Riera et al., 2016; Sousa et al., 2019). The larger specimens are the more attractive, visible, and prone to be caught adding to its higher commercial value (Lindberg, Estes, & Warheit, 1998; Kido & Murray, 2003; Ramírez, Tuya, & Haroun, 2009). This preferential removal of the larger and older specimens leads to a decrease in the reproductive success of the exploited population. Also, a decrease in the effective population size which might culminate in the disappearance of the species, from extensive intertidal areas, as occurred for the limpets *Cellana sandwicensis* (Pease, 1861), *Cellana exarata* (Reeve, 1854) and *Cellana talcosa* (Gould, 1846) in Hawaii (Valledor, 2000). Eventually becoming highly threatened as *Patella ferruginea* Gmelin, 1791, currently restricted to a few Mediterranean coastal locations (Espinosa, 2009) and in extreme cases lead to extinction (Núñez, Brito, Riera, Docoito, & Monterroso, 2003; Guerra-García, Corso, Espinosa, & García-Gómez, 2004). Nevertheless, the effects of harvesting pressure are not restricted to changes in targeted species extending to the whole ecosystem through cascading trophic effects (Scheffer, Carpenter, & Young, 2005).

The exploitation of rocky shores molluscs in the archipelago of Madeira dates to the 15th century and focuses mainly on limpets (*Patella candei* d'Orbigny, 1840 and *Patella aspera* Röding, 1798) and topshells (*Phorcus sauciatus* (Koch, 1845)) (Silva & Menezes, 1921; Sousa et al., 2018). This activity has been traditionally carried out by the coastal populations in the intertidal zone and commercially in the subtidal zone by snorkelers. The decrease in limpet availability in the intertidal has led to an increase of the impact of commercial diving on the exploited populations in Madeira archipelago (Delgado et al., 2005). Even though, the stocks of *P. aspera* and *P. candei* are not over-exploited they are being exploited near their maximum sustainable yield in this archipelago. The slow growth and long life contribute to these species' extreme vulnerability to exploitation and as such the enforcement of the existing harvest regulations must be accomplished to prevent future over-exploitation of these keystone resources (Henriques et al., 2012; Sousa, Delgado, Pinto, & Henriques, 2017). The first management efforts of this commercial activity implemented in 2006 were determinant to avoid the progress to a state of overexploitation of this resource (Sousa et al., 2019).

The conservation of exploited limpet populations is of concern, especially in coastal isolated areas, where no adjacent populations are present to supply larvae for settlement and recruitment (Cowen, Lwiza, Sponaugle, Paris, & Olson, 2000). The implementation of Marine Protected Areas (MPAs) is considered a key tool for the conservation of marine biodiversity in coastal areas (Ballantine, 1991; Zann, 1995) due to its ecosystem-level approach for exploited species (Henriques, Delgado, & Sousa, 2017).

Marine reserves defined as no take zones, are a popular alternative to traditional management measures of marine resources (Halpern & Warner, 2002). The exploited marine organisms, as a rule, reach higher density, biomass, and size in MPAs (Hockey & Bosman, 1986; Keough, Quinn, & King, 1993, Halpern, 2003). Reserves may also re-establish and protect marine resources within their boundaries, mainly the reproductive component, and act as a source of larvae that could eventually contribute for settlement and recruitment outside of the reserves (Rakitin & Kramer 1996; Pelc, Baskett, Tanci, Gaines, & Warner, 2009), as evidenced by Christie et al. (2010) that showed larval connectivity between marine reserves and unprotected areas.

The effectiveness of marine reserves depends on their size, suggesting that the impact of marine reserves increases directly with its size, as such, larger reserves may be required to achieve the proposed objectives (Halpern, 2003). Additionally, Christie et al. (2010) further highlighted the importance of joining oceanographic, genetic, and ecological data in order to predict, validate and quantify larval connectivity among marine populations. More recently, Edgar et al. (2014) showed that the conservation value in fish communities within MPAs, is affected by the cumulative effects of five key features, i.e. regulation, enforcement, MPA age, MPA size and isolation (hereafter summarized as "NEOLI"). These authors concluded that the conservation benefits increase exponentially in no-take, well enforced, old, large and isolated MPAs. However, these features were not previously tested in the

MPAs from the Madeira archipelago, and we propose herein to explore its feasibility on two intertidal harvested limpet species.

Herein, the protection effects of MPAs on the population dynamics of *P. aspera* and *P. candei* was analysed through a comparative analysis of size composition, reproduction (Lm_{50}) and abundance in Madeira (NE Atlantic Ocean), where these species have been historically highly-exploited. To achieve this objective: (i) it was hypothesized that populations in MPAs will have an increase in limpet mean size compared to exploited areas, (ii) it was assumed that limpet populations in protected areas will have higher size-at-first maturity compared to exploited populations, and (iii) it was also hypothesized that the removal of harvesting pressure in limpet populations in MPAs increases the abundance of individuals comparatively to the exploited populations.

Additionally, the effectiveness of existing MPAs on the preservation of limpet populations was investigated by assessing the impact of NEOLI features through a comparative analysis in which it was hypothesized that no-take, well enforced, older, larger and isolated MPAs produce a more pronounced effect on population dynamics, reproduction, and abundances of both limpet species (*P. aspera* and *P. candei*).

2. Methods

The study was conducted on fresh specimens of *P. aspera* and *P. candei*, collected from the subtidal zone of the rocky shores of the Madeira archipelago, NE Atlantic, during low tide, without selecting species or size of specimens. Each harvesting set was performed for a standardized period of 30 minutes by the same experienced harvesters. All dives were performed by the technicians of the Fisheries Service Directorate of the Regional Fisheries Department. The locations were selected considering areas with similar abiotic and biotic conditions among each other (e.g. type of substrate, slope of the coast, rugosity, hydrodynamic conditions, and type of community).

Sampling was performed on four MPAs (Garajau, Rocha do Navio, Desertas and Selvagens), and twelve exploited coastal zones throughout the South (7) (Paúl do Mar, Madalena do Mar, Ponta do Sol, Cabo Girão, Caniço, Santa Cruz and Ponta de São Lourenço south), and North coasts (5) (Ponta de São Lourenço north, Porto Moniz, Ribeira da Janela, Ponta Delgada and Ponta do Pargo) of Madeira, between 2017 and 2018 (Figure 1). For the MPA of Selvagens Islands only *P. aspera* was considered since taxonomy status of *P. candei* in this region is not fully solved. According to Faria *et al.* (2017) it is very probable that *P. candei* from each Macaronesian archipelago are geographically and/or ecologically isolated populations and as such for this comparative study only were considered specimens of *P. candei* from Madeira and Desertas islands.

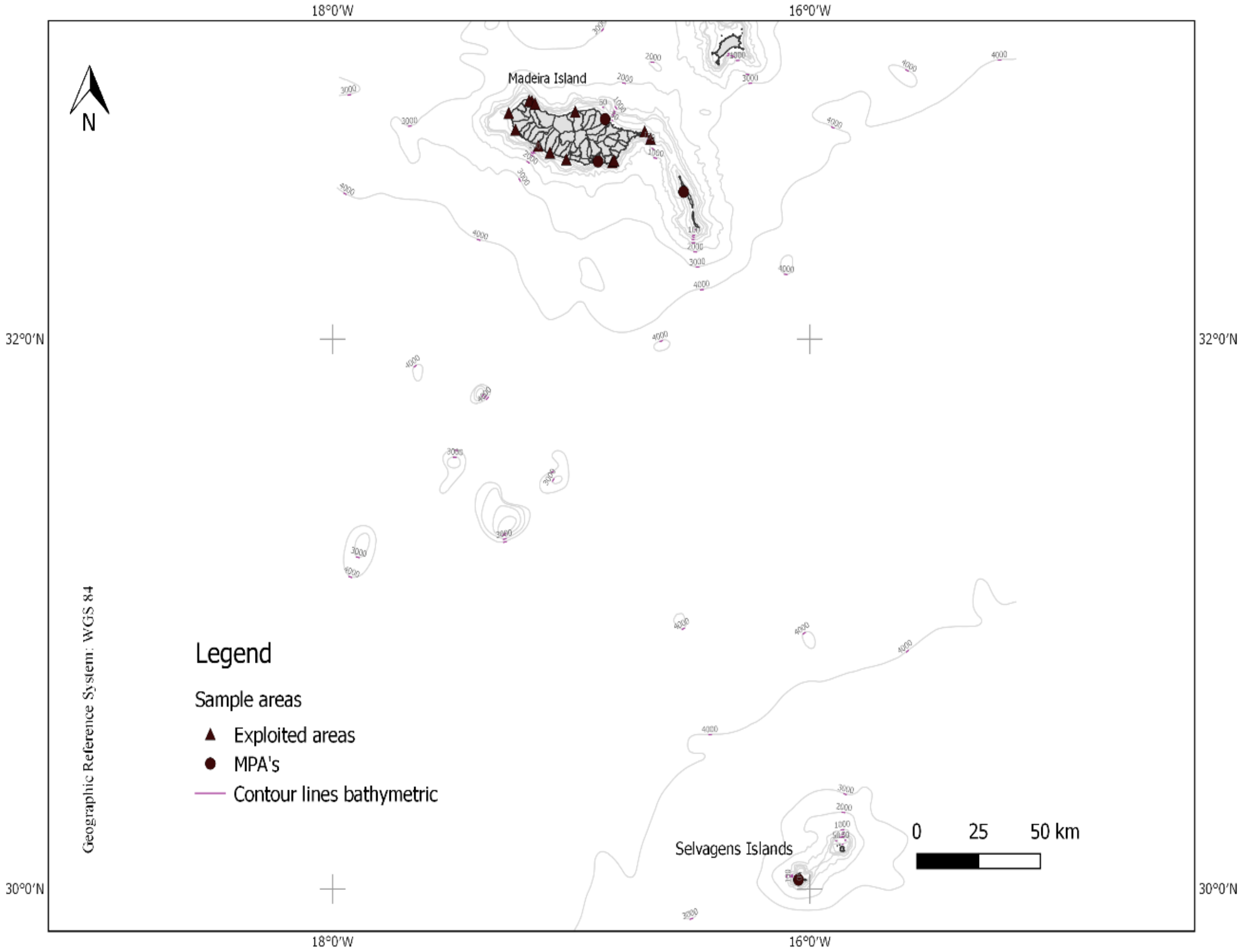

Figure 1 - Representation of sampling areas of *Patella aspera* and *Patella candei* in north-eastern Atlantic, Madeira archipelago.

All specimens were sorted by species, counted, measured (total shell length, *TL*, mm) using a Vernier calliper to the nearest 0.01 mm and weighted (total weight, *TW*, g) on an electronic scale with 0.01 g accuracy. Individuals were dissected to inspect the gonads and then sexed according to gonad pigmentation, in males the gonads are pale white or pink and brown to red in females (Orton, Southward, & Dodd, 1956). Individuals without visible sex characteristics were considered neuter (Martins, Santos, & Hawkins, 1987; Vale, 2016).

Data were analysed for deviations to the parametric assumptions of analysis of variance (ANOVA). Normality of the distribution of the data was verified through the Kolmogorov-Smirnov two-sample test and homogeneity of variance was determined using Levene's statistics. Analysis of variance was performed considering the Brown-Forsythe *F* test, when the variance of the data was not homogeneous. All statistical analyses were performed using SPSS v.24.0 (IBM Corp., 2016). For all tests, statistical significance was accepted when $P < 0.05$.

2.1. Impact of MPAs on size-structure of P. aspera and P. candei populations

A comparative study to verify the effects of MPAs in populations' length size-frequency of *P. aspera* and *P. candei* was conducted throughout the rocky shores of Madeira archipelago, considering individuals from MPAs (4) and exploited areas (12).

The comparison of the impact of MPAs on limpet size was carried out using an analysis of variance (ANOVA) and the comparison of the proportion of individuals per size-class between both not exploited and exploited zones was performed using a Pearson's Chi-square analysis to determine any differences observed in limpets size distribution between the two areas.

2.2. Impact of MPAs on the reproduction and CPUE of P. aspera and P. candei populations

The impact of MPAs in sexual maturation was evaluated trough the analysis of size- and age-at-first-maturity for each species, considering populations from MPAs and exploited areas. For this purpose, the size-at-first maturity (Lm_{50}), was estimated from the relationship between the proportion of mature individuals and size (5 mm size classes), applying the balanced logistic function: $P = 1/(1+\exp^{(-(a+bL))})$ (Sparre & Venema, 1997), where P is the balanced probability, a and b were the equation parameters determined by the linear least square method, after logarithmic transformation. The mean size-at-maturity was defined as the size in which 50% of the individuals from a population are mature, when $P = 0.5$ then $Lm_{50} = (-a)/b$ (King, 1995).

Age-at-maturity (A_{50}) was estimated applying the inverse von Bertalanffy growth function (von Bertalanffy, 1938), $A_{50} = t_0 - (1/k)\ln(1-(Lm_{50}/L_\infty))$ (Jennings, Kaiser, & Reynolds, 2011), where, L_∞ is the asymptotic shell length, K the growth coefficient, t_0 the theoretical age at zero length of the limpet and the Lm_{50} the size-at-first maturity. The existence of differences between exploited and not exploited population of limpets was determined by testing the slopes of the maturity curves using an analysis of covariance (ANCOVA).

Relative abundance was estimated using the catch per unit of effort in weight (CPUE), corresponding to the ratio between the total weights of captured specimens by species at each harvesting set and time.

The effect of MPAs was estimated through the comparison of the abundance of *P. aspera* and *P. candei* in MPAs and exploited areas applying the Pearson Chi-square statistics.

2.3. Impact of NEOLI features on the effectiveness of MPAs in the conservation of P. aspera and P. candei populations

The analysis of the impact of planning and management features on MPAs effectiveness on the conservation of limpet populations was accomplished following an adaptation of NEOLI features (regulations, enforcement, age, size and isolation) proposed by Edgar et al. (2014), to the specificities of the studied species and the protected areas.

The influence of all investigated features on the both limpet species within the surveyed MPAs, was categorized at three levels: low (L), medium (M) and high (H). The categories of each feature are given below (Table 1):

Table 1 - Category of NEOLI feature by Marine Protected Area in the Madeira archipelago (L - low, M - medium and H - high).

	Regulations	Enforcement	Age	Size	Isolation
Rocha do Navio	M	M	L	M	M
Garajau	H	M	M	L	L
Desertas	H	H	L	H	H
Selvagens	H	H	H	M	H

(i) Regulations concerns to the restriction of fishing at survey area (L – area with no additional fishing restrictions to those applied in the region; M – area inside the MPA but with some fishing methods authorized and H – no-take area within the surveyed MPA);

(ii) Enforcement concerns to the extent of compliance to the law that prohibits fishing, through policing and community support (L – small attempt at control; M – moderate control with policing attempted but with apparent infractions and H – well enforced but poaching may occur);

(iii) Age relates to the time elapsed between the fishing restriction and the undertaking of the surveys (L – MPA with ages comprised between 20 and 30 years; M – encompassed reserves with ages between 31 and 40 years and H - MPAs with over 40 years old);

(iv) Size of MPAs as described in the management plan (L – MPA area < 5 km^2; M – MPA area between 5 and 100 km^2 and H – MPA area > 100 km^2);

(v) Isolation refers to the degree of human pressure in the surveyed MPAs as a correlate of population density (L – MPA near a human settlement with population density > 100 inhabitants/km^2; M – MPA near a human settlement with a population density between 10 and 100 inhabitants/Km^2 and H – MPA near a human settlement with a population density < 10 inhabitants/Km^2).

The influence of the abovementioned features on the size-structure, reproduction and abundance was evaluated within each MPA. The effect of these features on population size-structure was assessed through an analysis of variance (ANOVA), on the size-at-maturity using an Analysis of covariance (ANCOVA), and on abundance applying a Pearson's Chi-Square statistic for both species according to each category.

2.3.1. Patterns of limpet size structure according to NEOLI features

To examine patterns of limpet size structure among the studied MPAs, an n-MDS procedure was conducted based on Bray-Curtis similarity on square-rooted transformed data; the MDS was used to visualize data spatially. Only centroids (i.e. means) for each coastal MPA were plotted to facilitate visualization of multivariate patterns in the ordination space. A distance-based redundancy analysis (db-RDA) (Legendre & Anderson, 1999) tested whether variation in any of the NEOLI features of MPAs, i.e. Age, Size, Enforcement, Isolation and Regulation (Edgar et al., 2014), contributed to explain variations in limpet size structure in the studied MPAs. Multivariate multiple regression, using the DISTLM routine (Anderson, 2001), then tested the significance of these relationships by fitting a linear model based on Bray-Curtis dissimilarities from square-rooted transformed abundance data. To retain variables with good explanatory power, as a result of collinearity among variables, the AIC routine was used as a selection criterion (Legendre & Anderson, 1999). All multivariate procedures were carried out via the PRIMER v6 (Clarke & Gorley, 2006) and PERMANOVA+ (Anderson, Gorley, & Clarke, 2008) statistical package.

3. Results

A total of 7,090 specimens from the 16 selected locations, 12 from exploited zones and 4 MPAs locations, of the rocky shores of the Madeira archipelago were investigated. The mean shell length of the 4,226 sampled individuals of *P. aspera* was 45.10 ± 9.97 mm, while for the 2,864 specimens of *P. candei* shell length showed a mean of 48.56 ± 8.22 mm. The analysis of size-frequency showed a normal distribution of data for both *P. aspera* (Z = 3.966, P <0 .01) and *P. candei* (Z = 1.709, P < 0.01).

Nevertheless, size did not show homogenous variance for *P. aspera* ($W = 27.728$, $P < 0.01$) contrarily to *P. candei* that exhibit a homogenous variance ($W = 0.937$, $P > 0.01$).

3.1. Impact of MPAs on size-structure of P. aspera and P. candei populations

For *P. aspera*, 1,019 specimens were collected from MPAs and 3,207 from exploited zones. The study populations were characterized by a wide range of sizes, from 3.07 to 75.22 mm in exploited areas and from 12.56 to 84.22 mm in MPAs. Differences in mean shell length of *P. aspera* were observed between locations, with smaller specimens in exploited locations (41.82 ±7.65 mm) than in MPAs (55.40 ± 9.37 mm). The same pattern was observed for the 2,864 specimens of *P. candei* caught (883 from MPAs and 1,981 from exploited locations), with smaller individuals in exploited locations (45.65 ± 6.61 mm) than in MPAs (55.64 ± 7.21 mm) (Figure 2A).

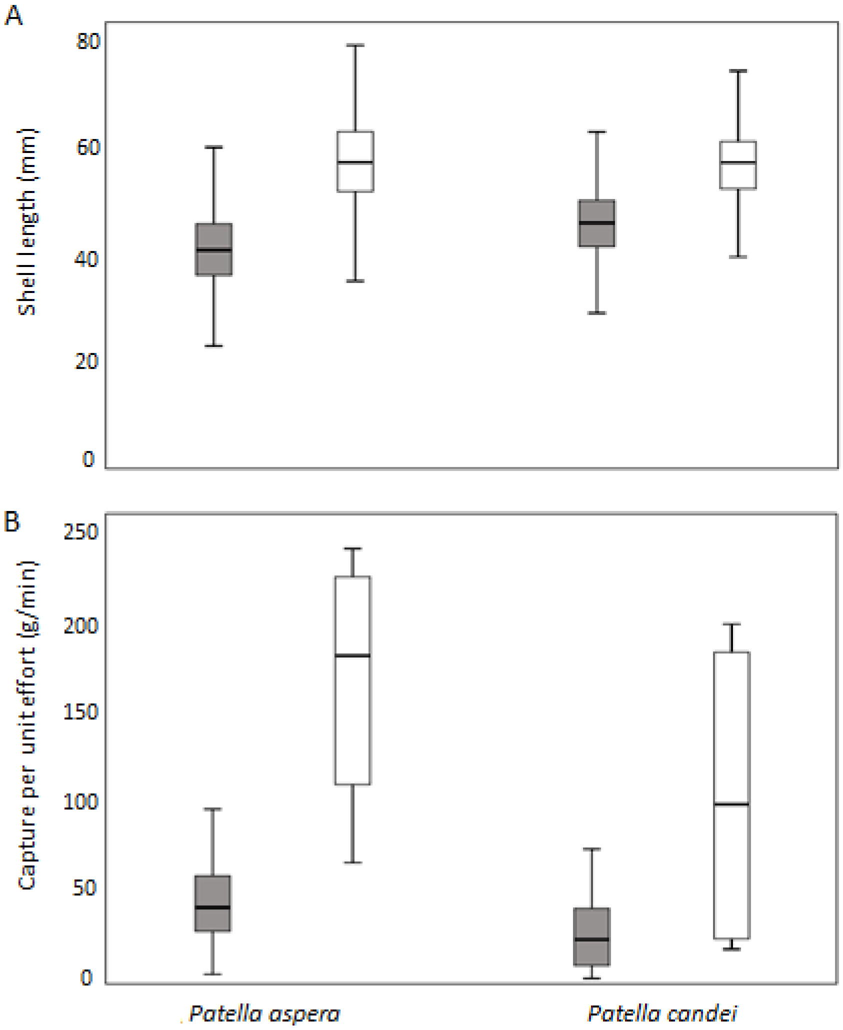

Figure 2 - Shell length (A) and capture per unit effort (B) for *Patella aspera* and *Patella candei*. White bars represent MPAs and dark grey bars represent exploited areas. Box-plot showing median (black line) and upper and lower quartiles of the data.

This species also showed a wide range of sizes, from 25.25 to 68.04 mm in exploited areas and from 14.22 to 79.63 mm in MPAs. The observed differences in mean shell length between exploited areas and MPAs were statistically significant for both *P. aspera* ($F = 1214.03$, $P < 0.01$) and *P. candei* ($F = 345.701$, $P < 0.05$).

The proportion of specimens of *P. aspera* per size-class was dominated by larger individuals in MPAs, with the highest percentages of specimens in classes 55 to 60 mm (*ca.* 26% of the total of the specimens) and 45 to 50 mm (*ca.* 30%) in exploited areas. The same pattern was observed for *P. candei*, with the highest percentages of specimens in classes 55 to 60 mm (*ca.* 22%) and 45 to 50 mm (*ca.* 26%) in exploited areas (Figure 3).

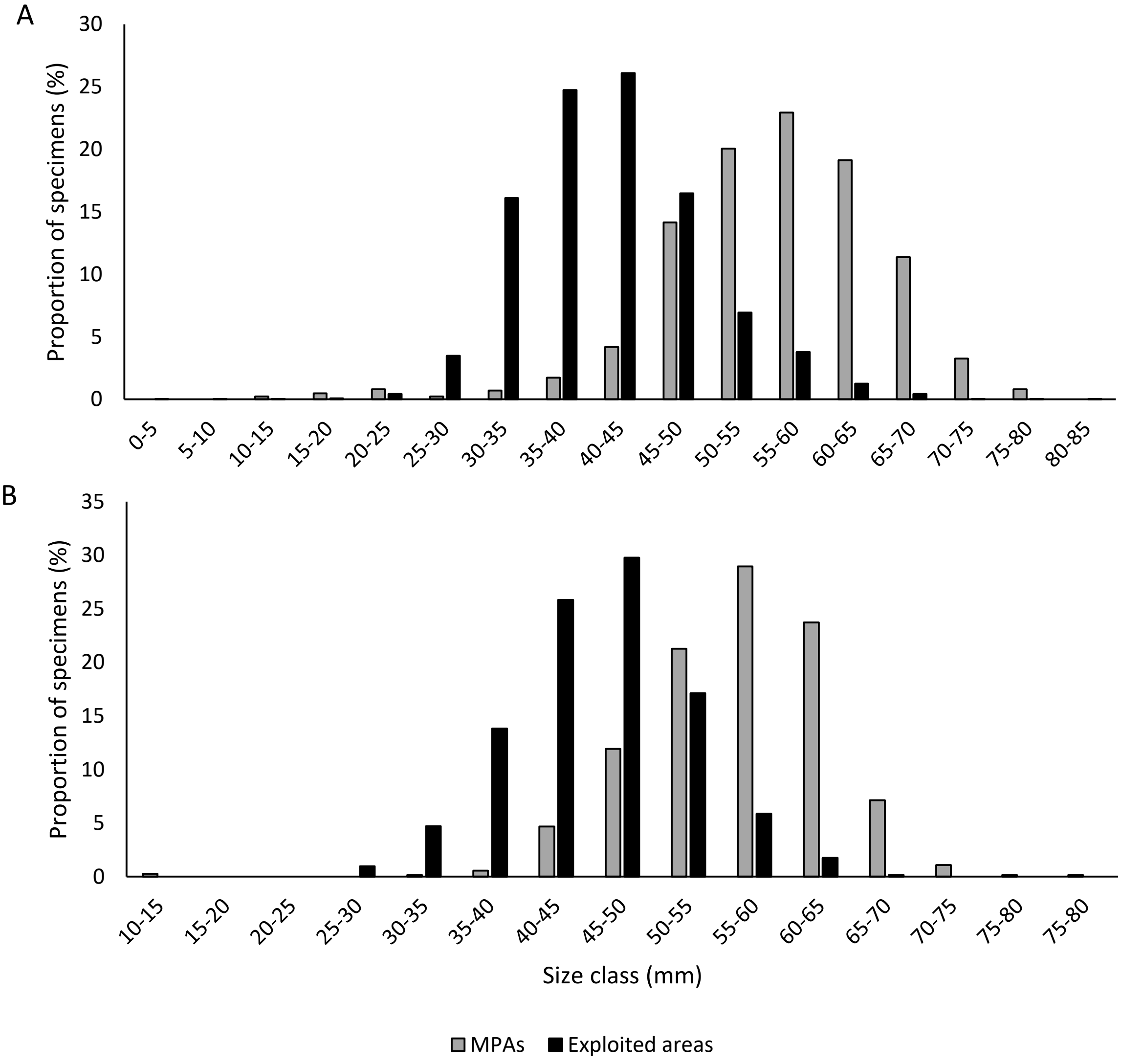

Figure 3 - Proportion of specimens of *Patella aspera* (A) and *Patella candei* (B) per size-class in exploited and protected areas.

The observed differences in the proportion of specimens per size classes between the two areas (MPAs and exploited areas) were statistically significant for *P. aspera* ($\chi^2 = 1689.301$, $P < 0.05$) and for *P. candei* ($\chi^2 = 930.859$, $P < 0.05$).

3.2. Impact of MPAs on the reproduction and CPUE of P. aspera and P. candei populations

The determined mean size-at-first maturity exhibited higher values in MPAs for both *P. aspera* and *P. candei*. The Lm_{50} increased from 37.02 mm in exploited areas to 40.47 mm in MPAs for *P. aspera*. Consequently, an increase in the estimated mean A_{50} for this species was also observed from 1.9 (exploited areas) to 2.2 years (MPAs). Concerning *P. candei*, the Lm_{50} increased from 37.14 mm (exploited areas) to 42.82 mm (MPAs), corresponding to an increase in A_{50} from 1.9 years in the exploited areas to 2.4 years in MPAs. The observed differences in Lm_{50} between exploited areas and MPAs were statistically significant for *P. aspera* ($F = 69.417$, $P < 0.001$) and for *P. candei* ($F = 64.116$, $P < 0.001$).

The abundance showed higher rates in MPAs than in exploited areas for both limpet species. Concerning *P. aspera*, abundance ranged from 66.5 to 238.96 g min^{-1} (166.47 ± 37.81 g min^{-1}) in MPAs and from 5.28 to 95.96 g min^{-1} (45.89 ± 25.89 g min^{-1}) in exploited areas. Regarding *P. candei*, the abundance varied from 19.04 to 197.52 g min^{-1} with a mean abundance of 103.42 ± 91.84 g min^{-1} in MPAs and from 2.95 to 74.07 g min^{-1} (28.08 ± 21.47 g min^{-1}) in exploited areas (Figure 2B).

The differences in abundances between MPAs and exploited areas were statistically significant for *P. aspera* ($\chi^2 = 14.792$, $P < 0.05$) and for *P. candei* ($\chi^2 = 8.571$, $P < 0.05$).

3.3. Impact of NEOLI features on the effectiveness of MPAs in the conservation of P. aspera and P. candei populations

The effect of planning and management measures, on the mean shell length of the populations of *P. aspera* among MPAs was observed, with larger specimens occurring in more effectively protected MPAs (4 NEOLI features), as Selvagens (57.34 ± 9.22 mm) comparatively with the less effectively protected MPA Rocha do Navio (0 NEOLI features) that showed smaller individuals (51.74 ± 10.00 mm). The same pattern was observed for the populations of *P. candei* that showed larger individuals in MPAs with 4 NEOLI features as Desertas (58.03 ± 7.73 mm) relatively to the MPAs with no NEOLI features as Rocha do Navio (53.78 ± 6.59 mm). Significant differences in mean shell length between MPAs were found for *P. aspera* ($F = 18.368$, $P < 0.05$) and for *P. candei* ($F = 28.318$, $P < 0.05$). However, post hoc analysis performed applying Tamhane's test showed that no differences in mean size of the specimens of *P. aspera* were observed between MPAs without NEOLI features and with one NEOLI feature (Rocha do Navio and Garajau) (*P* = 0.112) and between MPAs with 4 NEOLI features (Desertas and Selvagens) (*P* = 1.000).

P. aspera showed higher proportion of specimens in the size-class 50 to 55 mm in MPAs without (Rocha do Navio) or with one NEOLI feature (Garajau) (approximately 26% and 27%, respectively), and in the size-classes 60-65 mm in MPAs with 4 NEOLI features (Desertas) (approximately 22%) (Figure 4).

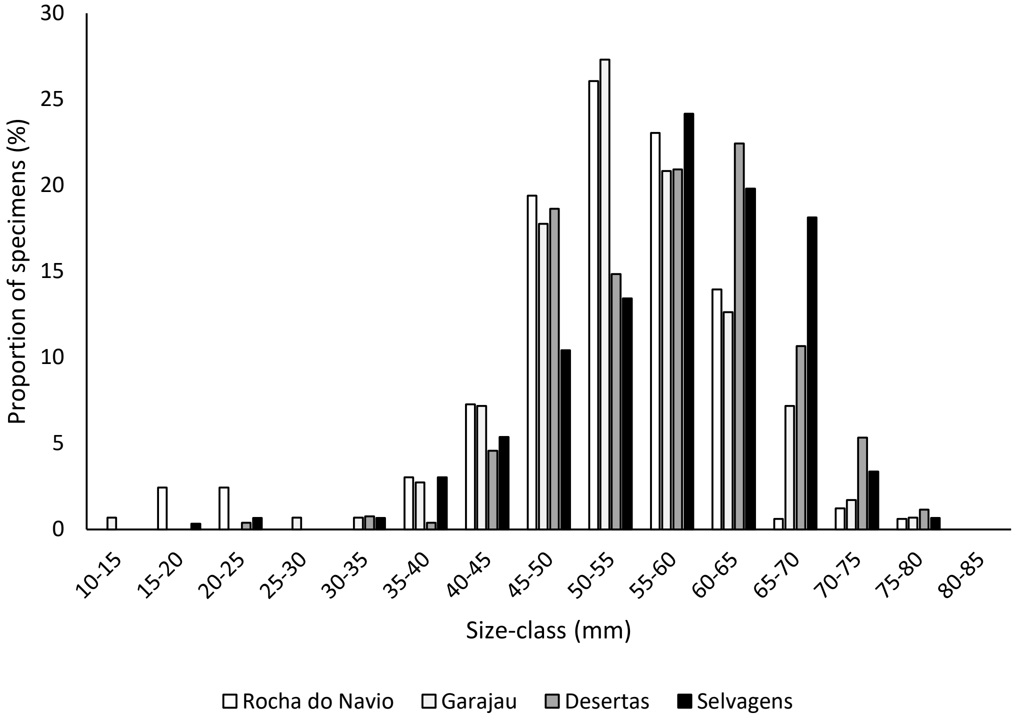

Figure 4 - Proportion of specimens of *Patella aspera* per size-class according to MPA.

Concerning *P. candei*, a similar pattern was observed with a higher proportion of specimens occurring in the size-class 55-60 mm for Rocha do Navio (0 NEOLI features) (approximately 29%) and in the size-class 60-65 mm for Desertas (4 NEOLI features) (approximately 31%) (Figure 5). Differences in the proportion of specimens per size classes between MPAs were statistically significant for *P. aspera* (χ^2 = 132.489, *P* < 0.001) and for *P. candei* (χ^2 = 98.834, *P* < 0.001).

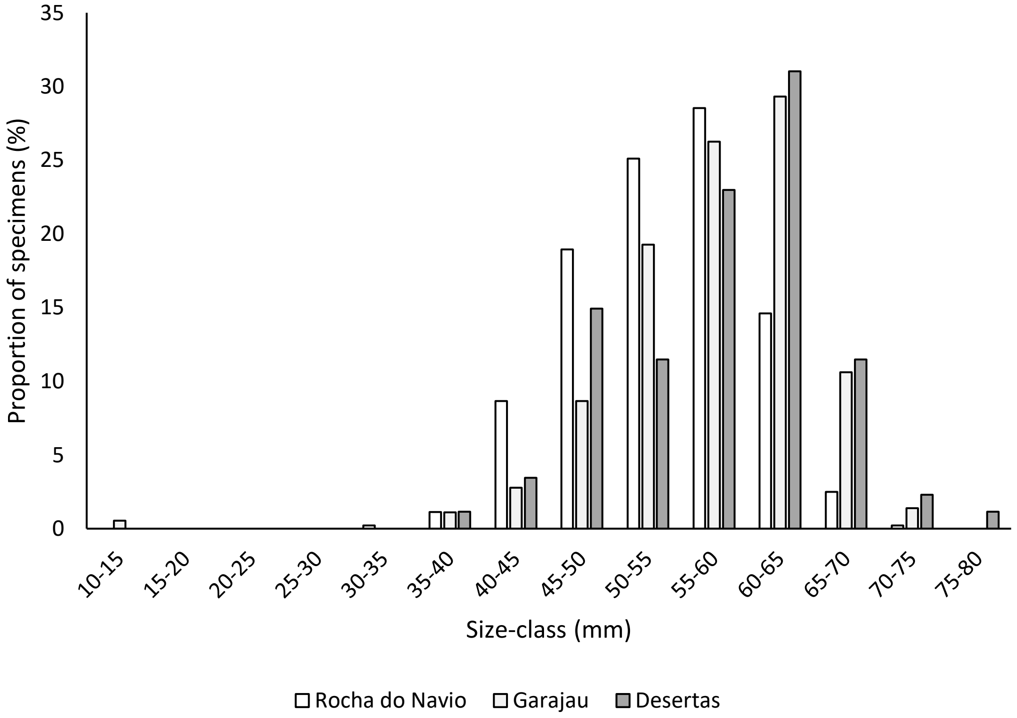

Figure 5 - Proportion of specimens of *Patella candei* per size-class according to MPA.

The estimated values of the mean size-at-first maturity for *P. aspera*, showed a progressive increase from the MPA without NEOLI features (Rocha do Navio) (38.27 mm) to the MPA with 4 NEOLI features (Selvagens) (44.52 mm). Regarding to *P. candei*, the same pattern was observed with an increase from 38.15 mm (Rocha do Navio with 0 NEOLI features) to 44.84 mm (Desertas with 4 NEOLI features). The observed differences in Lm_{50} between MPAs according to the protection measures were statistically significant for *P. aspera* ($F = 151.174$, $P < 0.001$) and for *P. candei* ($F = 95.144$, $P < 0.001$).

The abundance rates of *P. aspera* increased progressively from the less effectively protected MPA Rocha do Navio (66.55 g min^{-1}) to the more effectively protected MPA Selvagens (238.96 g min^{-1}). Regarding *P. candei*, the abundance was higher in Garajau with 197.52 g min^{-1}. The differences in abundances between MPAs were statistically significant for *P. aspera* ($\chi^2 = 863.00$, $P < 0.001$) and for *P. candei* ($\chi^2 = 728.00$, $P < 0.001$).

3.3.1. MPA regulations

The effects of the NEOLI feature "regulations" on the populations of *P. aspera* was evident, with larger specimens in no-take MPAs (56.11 ± 9.09 mm) (Desertas, Selvagens and Garajau) comparatively to MPAs where traditional fishing methods are authorized (51.74 ± 10.00 mm) (Rocha do Navio). The same pattern was observed for the populations of *P. candei* that showed larger individuals in no-take MPAs (57.47 ± 7.33 mm) relative to MPAs where traditional fisheries is allowed (53.78 ± 6.59 mm). Significant differences in mean shell length between MPAs according to regulations were found for *P. aspera* ($F = 26.981$, $P < 0.05$) and for *P. candei* ($F = 61.655$, $P < 0.05$). The differences in the proportion of specimens per size classes according regulations of MPAs were also statistically significant for *P. aspera* ($\chi^2 = 53.203$, $P < 0.001$) and for *P. candei* ($\chi^2 = 84.863$, $P < 0.001$).

The estimated values of Lm_{50} showed a progressive increase from unregulated MPAs to the no-take MPAs for both limpet species. Subsequently, it was also observed an increase in the estimated mean A_{50} for both limpet species. The observed differences in Lm_{50} between MPAs according to this NEOLI feature were statistically significant for *P. aspera* ($F = 105.683$, $P < 0.001$) and for *P. candei* ($F = 108.206$, $P < 0.001$).

The abundance rates followed the same pattern as size and Lm_{50}, with higher values in no-take MPAs (Selvagens) for *P. aspera* and *P. candei* (Garajau). The differences in abundances between MPAs according to regulations of the protected area were statistically significant for *P. aspera* ($\chi^2 = 187.065$, $p < 0.001$) and for *P. candei* ($\chi^2 = 44.755$, $P < 0.001$).

3.3.2. MPA enforcement

The impact of the MPAs enforcement on the shell size was verified for both *P. aspera* and *P. candei*, with larger individuals occurring on well-enforced MPAs and smaller specimens in MPAs with moderate control. For *P. aspera* significant differences were found in the mean shell length ($F = 46.691$, $P < 0.05$) and in the proportion of specimens per size-class ($\chi^2 = 75.404$, $P < 0.001$), according to enforcement of the MPA. *P. candei* populations followed the same trend with differences in the mean size ($F = 46.691$, $P < 0.05$) and in the proportion of specimens per size-class ($\chi^2 = 23.711$, $P < 0.05$).

The values of the mean size- and age-at-first maturity increased from the MPAs with moderate control to well enforced MPAs for both *P. aspera* and *P. candei*. The observed differences in Lm_{50} between MPAs according to enforcement applied to the protected areas were statistically significant for *P. aspera* ($F = 35.255$, $P < 0.001$) and for *P. candei* ($F = 100.226$, $P < 0.001$).

P. aspera showed an increase in the abundance in MPAs with moderate control compared to well enforced MPAs. Concerning *P. candei* the higher abundances rates occurred in Garajau. The differences in abundances between MPAs according to the degree of enforcement on the protected area were statistically significant for *P. aspera* ($\chi^2 = 863.00$, $P < 0.001$) and for *P. candei* ($\chi^2 = 728.00$, $P < 0.001$).

3.3.3. MPA age

The impact of the age of the MPA on the shell size was evident for *P. aspera*, with larger individuals occurring on the older MPAS and smaller specimens in younger MPAs. This trend was also observed for *P. candei* that showed a higher mean shell length in reserves with more than 30 years old than in the younger MPAs. Differences in mean shell length between MPAs according to age were significant for *P. aspera* ($F = 10.613$, $P < 0.05$) and for *P. candei* ($F = 33.886$, $P < 0.05$). The proportion of specimens per size class also showed statistically significant differences for *P. aspera* ($\chi^2 = 82.675$, $P < 0.001$) and for *P. candei* ($\chi^2 = 58.347$, $P < 0.001$).

The estimated Lm_{50} exhibited a progressive increase from the younger to the older MPAs for both limpet species. Subsequently, it was also observed an increase in the estimated mean A_{50}. The observed differences in Lm_{50} between MPAs according to the size of the protected areas were statistically significant for *P. aspera* ($F = 119.067$, $P < 0.001$) and for *P. candei* ($F = 103.542$, $P < 0.001$).

The abundance rates increased progressively from the younger to the older MPAs for both limpet species. The differences in abundances between MPAs according to the age of the protected area were statistically significant for *P. aspera* ($\chi^2 = 1726.00$, $P < 0.001$) and for *P. candei* ($\chi^2 = 863.00$, $P < 0.001$).

3.3.4. MPA size

The populations of both species were characterized by a wide range of sizes in the three MPAs size categories analysed. Both species showed a pattern of increase in the mean shell length from the smaller to the larger MPAs. Differences in mean shell length were observed between MPAs for *P. aspera* ($F = 8.396$, $P < 0.05$) and *P. candei* ($F = 28.318$, $P < 0.05$) according to the size of the MPA. The differences in the proportion of specimens per size classes according to the size of the MPAs were statistically significant for *P. aspera* ($\chi^2 = 65.530$, $P < 0.05$) and for *P. candei* ($\chi^2 = 98.834$, $P < 0.05$).

Mean size- and age-at-first maturity presented an increase from the smaller to the larger MPAs for both *P. aspera* and *P. candei*. The observed differences in Lm_{50} between MPAs according to the size of the protected areas were statistically significant for *P. aspera* ($F = 118.779$, $P < 0.001$) and for *P. candei* ($F = 114.995$, $P < 0.001$).

The abundance rates increased from the smaller to the larger MPAs for *P. aspera*. Concerning *P. candei* the abundance was higher in the medium-sized MPAs. The differences in abundances between MPAs considering the reserve size were statistically significant for *P. aspera* ($\chi^2 = 863.00$, $P < 0.05$) and for *P. candei* ($\chi^2 = 728.00$, $P < 0.05$).

3.3.5. MPA isolation

The effect of isolation in relation to the shell size showed larger individuals occurring on more isolated MPAs and smaller specimens in MPAs near to the human settlements for both species. For *P. aspera,* significant differences in the mean shell length ($F = 26.661$, $P < 0.05$) and in the proportion of specimens per size-class ($\chi^2 = 108.105$, $P < 0.001$) were found. *P. candei* populations followed the same trend with differences in the mean size ($F = 28.318$, $P < 0.05$) and in the proportion of specimens per size-class ($\chi^2 = 98.834$, $P < 0.05$).

The values of the mean size- and age-at-first maturity increased from the MPAs near to human settlements to isolated MPAs for both *P. aspera* and *P. candei*. The observed differences in Lm_{50} between MPAs according to isolation of the protected areas were statistically significant for *P. aspera* ($F = 124.830$, $P < 0.001$) and for *P. candei* ($F = 100.226$, $P < 0.001$).

The abundance rates progressively increased from MPAs near to the human settlements in relation to more isolated MPAs for the species *P. aspera*. Concerning *P. candei* the abundance increased from the less isolate MPA to the medium isolate MPA. The differences in abundances between MPAs considering the degree of isolation were statistically significant for *P. aspera* ($\chi^2 = 863.00$, $P < 0.05$) and for *P. candei* ($\chi^2 = 728.00$, $P < 0.05$).

3.4. Patterns of limpet size structure according to NEOLI features

Limpet size structure varied among the studied MPAs, the Selvagens clearly being separated from the remaining ones due to the high representation of larger-size classes (>60 mm long). Also, a high heterogeneity within Desertas was observed, relative to the high similarity among within-replicates from the other MPAs (Rocha do Navio and Garajau).

The first two axes of the db-RDA explained approximately 68% of overall variation in *P. aspera* size structure (Figure 6A). Two (Enforcement and Age) out of the five NEOLI features contributed significantly to explain the observed variability on *P. aspera* size classes. Age was the most important variable, followed by Enforcement explaining 48% and 18% of populations' variability, respectively (Table 2). For *P. candei*, the MPA from Selvagens was not included due to taxonomic discrepancies concerning the identification of this species in this small archipelago. For this species, the first two db-RDA axes explained 48% of total variability of the size structure of this species (Figure 6B).

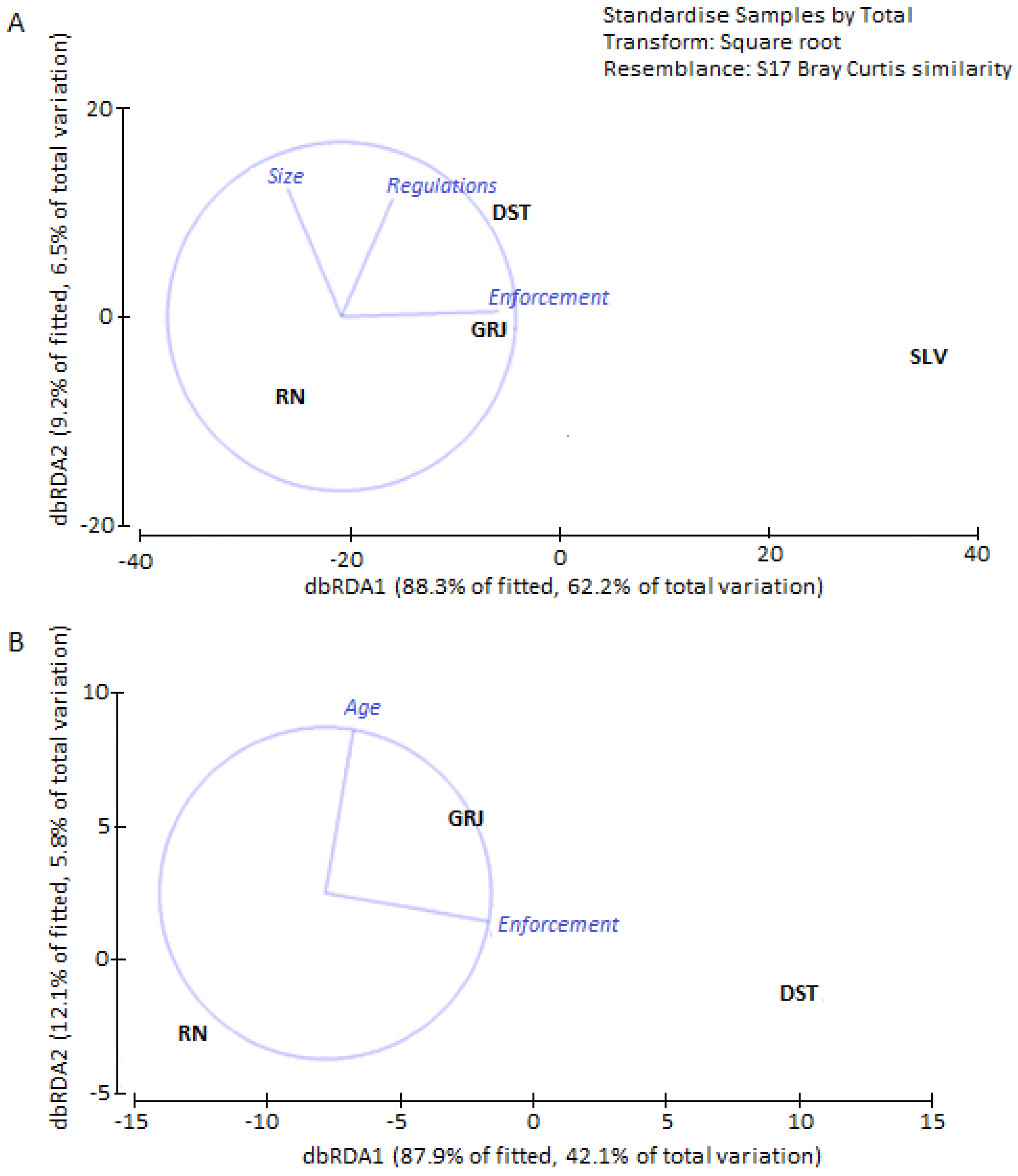

Figure 6 - Distance-based redundancy analysis (db-RDA) biplot of first and second axes relating environmental variables that affected significantly (see Table 2) the size structure of targeted limpet species for *Patella aspera* (A) and *Patella candei* (B). Centroids for each distance ar plotted. RN – Rocha do Navio, GRJ – Garajau, DST – Desertas and SLV – Selvagens.

Table 2 - Results of multivariate multiple regression testing the relationship between the measured set of NEOLI features (Table 1) and limpet size structure of both species. To retain variables with explanatory power, the adjusted r^2 procedure was chosen as model selection criterion using sequential tests. *No *Patella candei* specimens in Selvagens MPA.

Species	Variable	SS (trace)	Pseudo-F	P	% of explained variation
Patella aspera	Age	4,713.40	10.336	0.0012	0.484
	Enforcement	1,819.10	5.69	0.002	0.187
	Size	325.12	1.019	0.432	0.033
*Patella candei**	Enforcement	731.19	4.385	0.009	0.354
	Age	257.15	1.67	0.188	0.124

Enforcement was the most important variable explaining the observed variability of *P. candei* among sites, being responsible for 35%. Age was also responsible for the observed variability (12%), but not significantly (Table 2). Hence, these two variables were relevant drivers explaining patterns in limpet size structure, and the differences of age and enforcement regimes explaining differences in size composition of the two studied limpets among the studied MPAs.

4. Discussion

The effects of protection from the studied MPAs on limpet stocks are not only restricted to an increase of biomass inside non-harvested sites. An increase of size-at-first maturity and shell size was also observed in the four surveyed MPAs but at different rates. NEOLI features of MPAs were pivotal to explain the observed variability among MPAs, specifically Age and Enforcement. In all analysed MPAs was observed an improvement of the marine ecosystem and population health for both limpet species. However, it was evident that those MPAs with four NEOLI features (Selvagens and Desertas) were distinctly in greater balance, than the remaining MPAs and consequently than the exploited zones. It was also notable that MPAs with the NEOLI feature present better recovery of the limpets' populations, than those without that key feature. The present results showed that old and well-enforced MPAs harbour a high representation of large adults and more balanced populations for both *P. aspera* and *P. candei*.

Unfortunately, the implementation of MPAs is steadily increasing only recently worldwide (Lubchenco & Grorud-Colvert, 2015) instead of several decades ago. This is probably to the conventional conception of oceans as an endless source of resources and the perception that anthropogenic activity is not a cause of extinction of marine organisms is slowly changing (Roberts & Hawkins, 1999), being increasingly evident that fishing activity affects the target species as well as the entire ecosystem through the trophic cascade effect (Castilla, 1999). The impact of anthropogenic activities is complex in biological systems (Lindberg, Estes, & Warheit, 1998), and the effect of exploitation on the upper trophic levels is difficult to predict. The islands have marine biological systems less diverse than those observed in the corresponding continental habitats (Paulay, 1994). Hence, over-exploitation on the islands could lead to irreversible impacts such as local extinctions, substantial decreases or even a total elimination of the ecosystem function provided by the exploited species through the alteration of the food web (Martins, Jenkins, Hawkins, Neto, & Tompson, 2008).

In Madeira archipelago, *P. candei* and *P. aspera* are subject to high levels of exploitation, representing one of the most profitable economic activities on small-scale fisheries, that reached an annual catch of up 111 tonnes in 2017, yielding a total first sale value of *ca.* 0.38 M€ (DRP). The direct effects of intensive harvesting pressure on the exploited resources are the reduction of abundances and shifts in limpet size structure, because of the preferential harvesting of larger specimens (Griffiths & Branch, 1997). The loss of larger and consequently older specimens due to selective harvesting influences multiple cascading effects on the biology of the target species and populations, including changes in life history, demographics, breeding success, and ecological interactions (Fenberg & Roy, 2008).

The obtained results showed that the impact of commercial harvesting of limpets in Madeira archipelago negatively affects the abundance, size and reproduction of *P. candei* and *P. aspera*, which are significantly less abundant, have lower sizes and early maturation in exploited areas. Similar patterns of size decrease and abundance reduction, specifically the depletion of large adults were reported for *P. candei* (Núñez, Brito, Riera, Docoito, & Monterroso, 2003) and *P. candei crenata* (Ramírez, Tuya, & Haroun, 2009) in the Canary Islands, and *P. candei* and *P. aspera* in the Azores archipelago (Martins, Jenkins, Hawkins, Neto, & Tompson, 2008; Martins et al., 2017). This pattern has been also observed in South Africa for *Patella concolor* Krauss, 1848 (Branch, 1975) and in the Mediterranean coast of Spain for *P. ferruginea* (Espinosa, Guerra-García, Fa, & García-Gomez, 2006; Espinosa, 2009). Even in other limpet genera such as, *Cymbula oculus* Born, 1778 in South Africa (Branch & Odendaal, 2003), *Lottia gigantea* Gray in G.B. Sowerby I, 1834 in Mexico (Pombo & Escofet, 1996) and southern California (Sagarin et al., 2007) and other species of harvested molluscs, e.g. *Fissurella* spp. in Chile (Moreno, Sutherland, & Jara, 1984), South Africa (Hockey & Bosman, 1986), Australia (Keough, Quinn, & King, 1993) and Tanzania (Newton, Parkes, & Thompson, 1993).

Limpets have relatively high longevity and slow growth rates (e.g. Núñez, Brito, Riera, & Docoito, 2004), thus extremely vulnerable to the selective harvesting of larger specimens (Lindberg, Estes, & Warheit, 1998; Kido & Murray, 2003; Ramírez, Tuya, & Haroun, 2009; Henriques et al., 2012; Sousa, Delgado, Pinto, & Henriques, 2017). Additionally, the illegal harvesting of limpets in Madeira archipelago, which are marketed directly to the consumer, also contributes to changes in size-structure of the populations. The decrease in the abundance of specimens in the higher size-classes leads to the illegal collection of specimens with smaller sizes than the minimum catch size (< 40 mm) established by regulators and since these catches are not declared, the real impact of the exploitation is underestimated, as also observed in the Azores (Martins, Jenkins, Hawkins, Neto, & Tompson, 2008).

The scarcity of larger specimens may lead to a decrease in the effective population size as consequence of the decrease in reproductive success, differentially affecting *P. candei* and *P. aspera* as previously observed in the Azores (Martins, Jenkins, Hawkins, Neto, & Tompson, 2008). *P. aspera* is a protandrous hermaphrodite (Hawkins, Côrte-Real, Pannacciulli, Weber, & Bishop, 2000; Delgado et al., 2005), being particularly susceptible to changes in its population structure that favour the decrease of the frequency of larger individuals. This situation leads to the reduction of females, which under natural conditions are more abundant in the larger size classes, with a reversal of the sex ratio in the larger size classes for this species in exploited areas in Madeira archipelago (unpublished data), as also verified for *L. gigantea* in southern California (Kido & Murray, 2003). Hence, management measures promoting the protection of larger adults are pivotal for the replenishment of exploited populations, and concomitantly an increase of effective population size of *P. aspera*, these measures are urgently needed in Madeira archipelago. The fact that larger specimens present a greater contribution to the population reproductive effort (Keough, Quinn, & King, 1993) implies that the harvesting impact on larger specimens in *P. candei* will not be overlooked. This biased harvesting will negatively affect the breeding success, as occurs in *P. ferruginea* (Espinosa, Guerra-García, Fa, & García-Gomez, 2006) and may culminate in the population collapse (Núñez, Brito, Riera, Docoito, & Monterroso, 2003; Guerra-García, Corso, Espinosa, & García-Gómez, 2004). For example, the Canarian populations of *P. candei* showed a marked decline due to over-exploitation (Navarro et al., 2005), suggesting that even this species could be susceptible to the population decline resulting from intensive exploitation. Both *P. candei* and *P. aspera* are Macaronesian endemisms and, only local populations contribute to the total recruitment, thus becoming more vulnerable and reducing the reproductive success of local populations (Martins, Jenkins, Hawkins, Neto, & Tompson, 2008).

MPAs are one of the most important tools in the conservation of coastal marine biodiversity (Ballantine, 1991; Zann, 1995; Edgar et al., 2014). In fact, the establishment of marine reserves with continued fishery closure and protection (Micheli et al., 2008), results in significant increases in density and biomass over a period of three years that persist over time (Halpern & Warner, 2002). MPAs also play an important role in the natural replenishment of stocks (Manríquez & Castilla, 2001). The effectiveness of response of species to the protection provided by MPAs greatly depends on the degree of exploitation to which the species are subject. Species subject to high levels of exploitation are more likely to respond rapidly when recruitment occurs at sufficient levels, because of the removal of fishing activity that limited the population size and demographics of the species (Polacheck, 1990; Carr, & Reed, 1993; Rowley, 1994; Lotze et al., 2011). The position on the trophic chain also plays an important role on the species recovery, since it is correlated with its life history parameters. For marine invertebrates, with high longevity and slow growth, temporal responses to reserve protection are assumed to be slower (Halpern & Warner, 2002). *P. candei* and *P. aspera* are moderate long-lived organisms with slow growth rates (Núñez, Brito, Riera, & Docoito, 2004; Henriques et al., 2012; Sousa, Delgado, Pinto, & Henriques, 2017). They seem to be extremely vulnerable to the selective harvesting of larger specimens and have a slower temporal response to the protection in reserves.

According to Halpern (2003) the mean values of different biological variables are 20 to 30% higher in protected zones compared to explored areas and regardless of MPA size, indicating that small reserves can produce high values. The increase observed in the shell length of *P. candei* and *P. aspera* from protected areas suggests a positive effect of the MPAs in the populations of both species. The fact that the reserves ages ranged from 21 (Rocha do Navio) to 47 years (Selvagens), showed a marked differentiation in the composition of sizes in both *Patella* species between exploited and protected zones. An increase in approximately 14 mm in mean shell size for *P. aspera*, and 10 mm in mean shell size for *P. candei* on the MPAs, shows the key role of protected zones on the recovery of the populations of these important intertidal grazers in Madeira archipelago. This trend becomes more pronounced when considering well-established and old MPAs with an increase of approximately 16 mm and 13 mm in mean shell length for *P. aspera* and *P. candei*, respectively. The evidenced pattern results mainly from the removal of the greater anthropogenic impact, the harvesting activity in protected areas that results in the recovery of the balance in the size structure of the populations of *P. candei* and *P. aspera* in Madeira archipelago, which agrees with the observed for *P. ferruginea* in the Alboran Sea (Guerra-García, Corso, & García-Gómez, 2003).

The progressive increase in mean size of the specimens in protected areas may result mostly from the biological characteristics of these species, i.e. slow growth, late maturity, and high longevity, combined with the occurrence of some illegal harvesting, especially on Garajau and Rocha do Navio. Additionally, the regulatory measures entered into force in 2006 have contributed to a slight recovery of the exploited populations stocks in this region and contributed to reduce the differences in shell length between exploited and protected zones (Sousa et al., 2019).

In the Canaries, a similar pattern was obtained for *P. candei* in Fuerteventura (Núñez, Brito, Riera, Docoito, & Monterroso, 2003; Núñez, Brito, Riera, & Docoito, 2004), and also for the remaining limpet species (*P. candei crenata*, *P. aspera* and *P. rustica*) in the Canaries (Ramírez, Tuya, & Haroun, 2009) with better preserved populations, in terms of abundance and size structure, in areas with reduced anthropogenic impact. The effectiveness of protected areas has been reported for the black abalone (*Haliotis picta* Röding, 1798) in Tasmania (Edgar & Barrett, 1999) showing declines of small-sized individuals and consistent increases of large-sized adults within one MPA relative to full-access sites. Micheli et al. (2008) showed recovery of exploited abalone populations (*Haliotis rufescens* Swainson, 1822 and *Haliotis cracherodii* Leach, 1814) in California coasts. However, high natural mortality and illegal harvesting may be responsible of no signs of recovery comparable to preceding levels of stock collapse. A plausible explanation of size increases within MPAs may be related to the role of protected areas on natural replenishment of targeted populations, as observed for the abalone *Concholepas concholepas* (Bruguière, 1789) in SE Pacific coast (Manríquez & Castilla, 2001).

We herein explore the protection effects of MPAs using several variables, i.e. body (size), reproduction (Lm_{50}) and size structure, of the most important targeted coastal invertebrates by harvesters. NEOLI features of MPAs demonstrated to be reliable to protect these resources, especially age and enforcement measures in MPAs since old and well-enforced protected sites, e.g. Selvagens, showed well-represented limpet size structures. These assemblages were dominated by adults, with a good representation of large-sized specimens, i.e. > 60 mm long. It was clear that the increase in the number of NEOLI features enable a better understanding on how MPAs distinct features may play together to increase the conservation benefits and achieve their desired conservation value. In addition, the understanding and commitment of local communities, management regulators, policymakers and stakeholders, based on information and education are pivotal to the effective management of MPAs and to ensure the sustainability of the species and ecosystems at medium and long term.

The present study constitutes a first step to characterize the current condition of exploited limpet assemblages in the study area. A thorough and multidisciplinary study needs to be carried out in the near future to get a reliable picture of commercial stocks of the two targeted species (*P. aspera* and *P. candei*), specifically genetic analysis to detect speciation process in isolated assemblages, and detailed studies on food source (taxonomic identification, energy content, interaction with other species, etc.). These studies will shed light to future scenarios that are crucial for management conservation strategies.

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PART IV

GENERAL DISCUSSION

Chapter 12

General discussion

Discussion

The islands have marine biological systems less diverse than those observed in the corresponding continental habitats (Paulay, 1994; Hawkins et al., 2000), and as such they are more susceptible to over-exploitation (Martins et al., 2008). Harvesting activities can in fact, lead to irreversible impacts by affecting not only the target species but also the entire ecosystem through a trophic cascading effect (Castilla, 1999). This is more evident when small islands or archipelagos like Madeira are involved in.

The harvest of marine gastropods in the archipelago of Madeira dates back to the fifteenth century and is one of the most important small-scale fisheries in this region. The exploitation of these oceanic, insular resources for almost 600 years resulted in changes in the life history, demographics, reproductive success, and ecological interactions of the exploited species, as well as in alterations in the affected ecosystems. As such, the focal reasoning behind this thesis lies in the need to identify and assess the impacts of harvesting on littoral gastropods to better preserve the exploited species and ecosystems concerned.

The present thesis fills the information gaps on the life traits and population dynamics of limpets and topshells in the archipelago of Madeira and assesses the effects of harvesting on the exploited stocks. The impact of harvesting regulations on limpets' populations is evaluated herein and recommendations to improve the existing management measures of limpets are advised. Additionally, a proposal of harvesting regulations for the topshell *Phorcus sauciatus* (Koch, 1845) is suggested.

Autecology of intertidal grazers in the historically harvested archipelago of Madeira

Limpets and topshells are among the most important keystone species on the overall structure of rocky shores communities, because they provide valuable ecosystem services (Bowman & Lewis, 1986), particularly in the ecological balance of this ecosystem by playing an important role in the regulation of algae and also because they are suitable bioindicators of anthropogenic impacts and have a high economic value (Jenkins et al., 2005; Coleman et al., 2006; Mieszkowska et al., 2007).

Marine gastropods are subject to environmental and anthropogenic stresses that affect the functioning of biological systems, ecological interactions and community structure (Hawkins et al., 2003; Southward et al., 2005). The sharp environmental gradients of wave action, temperature variation and desiccation can prompt significant changes on the overall morpho-physiological characteristics of patellid limpets (Davies, 1969). For instance, the reproductive biology and phenology of marine gastropods are deeply influenced by factors such as temperature and morphodynamics

(Bowman & Lewis, 1986), latitude and temperature (Lewis, 1986), climate change (Moore et al., 2011) and parasitism (Firth et al., 2017), which vary accordingly to the geographic region.

Anthropogenic impacts like over-harvesting, habitat loss and pollution also prompt significant shifts in size composition and population dynamics of the affected species. As such, knowing and understanding the life history traits and the ecological role of limpets and topshells in the species-specific intertidal ecosystem of the archipelago of Madeira, considering the impacts to which they are subject are crucial to assess the exploitation status of these resources and then promote a sustainable exploitation in the near future.

To the knowledge of the author the present work includes the first full biological study on *P. sauciatus*, despite its wide geographic distribution including the Macaronesian archipelagos of Canaries, Madeira and Azores with its northern boundary in the Iberian Peninsula and its southern limit in the northeastern Africa mainland (Donald et al., 2012). The main results showed that populations of this species are dominated by individuals of the first age classes (<4 years). The spawning season occurs between March and August and the size at first maturity was estimated at 12.95 mm shell length. The yield-per-recruit analysis showed that *P. sauciatus* is being exploited near their maximum sustainable yield and urgent conservation measures are warranted to preserve stocks in the medium to long term. This analysis also showed that this fishery is susceptible to the size selective nature of harvest, resulting in a decrease of the yield mostly due to the lower reproductive output of small specimens. The increase of the size of capture to Lc_{75} would produce negligible benefits in yield at short term, but will increase at medium and long term due to the increase of the spawning biomass.

A proposal for the regulation of *P. sauciatus* harvest in the archipelago of Madeira is suggested to preserve this topshell species, exploited since the beginning of the fifteen century and still unregulated. As such, to ensure this species conservation the following five conservation measures are recommended (Sousa et al., 2018):

(i) Definition of different harvesting typologies and the establishment of maximum catches per day, e.g. non-commercial harvesters with catches for personal use (2 kg/day/person) and professional harvesters for commercialization (20 kg/day/person).

(ii) Implementation of landing obligations and first sale at auction to provide exact knowledge about the status of the commercial catches and to monitor the harvesting effort by local authorities.

(iii) Obligation of harvester licenses and declaration of catches on logbooks.

(iv) Establishment of a minimum catch size of 15 mm shell length to ensure that a sufficient proportion of mature individuals would contribute to the reproductive effort of the exploited populations.

(v) Establishment of a closed season between February and May to prevent all types of harvesting during the main spawning pulse and to safeguard the breeding season.

The implementation of harvesting regulation for *P. sauciatus* in the archipelago of Madeira will provide protection to this heavily exploited species, resulting in improvements in terms of size composition, abundance and reproductive output of the exploited populations. For instance, the establishment of a closed season during the spawning season would protect the reproductively active individuals, thus safeguarding spawning, larvae development and settlement contributing to the increase of the annual recruitment. Additionally, the establishment of a minimum size of capture (15 mm) would protect the immature specimens and consequently allow the contribution of more than 75% of the individuals to the reproductive output of the populations.

The present study on the commercially exploited species *Patella aspera* Röding, 1798 fills the existing gap and enhances knowledge on the life-history of this species in the archipelago of Madeira. The obtained results showed that *P. aspera* is a moderately long-lived limpet with a predominance of young specimens (<3 years old). This species is a winter breeder with gonadal development lasting from October to December, and spawning likely occurs from January until April while resting occurs from May to September.

The size at first maturity is now determined at a shell length of 41.78 mm for females and 38.29 mm for males. *P. aspera* is being exploited near the maximum sustainable yield nonetheless, due to its slow growth and long life, continuous monitoring and the enforcement of the existing harvest regulations have to be accomplished to avoid future overexploitation. Since this species is susceptible to changes in the minimum size of capture, a proposal for increasing the size at first capture for 45 mm is here suggested to improve the reproductive output, allowing the contribution of more specimens to the fitness of the exploited populations, and consequently to promote an increase of the spawning biomass at medium and long term. In the future, the increase of the length at first capture will result in an increase in the yield through the harvesting of larger and heavier specimens, thus making up for the initial losses for not harvesting smaller specimens (< 45 mm).

The growth parameters now determined for *P. aspera* are similar to those estimated for *P. candei* in the same geographic region (Henriques et al., 2012). *P. candei* also showed continuous recruitment throughout the year with slight differences in the recruitment peak explained by the different levels of harvesting pressure exerted on each species and by environmental factors such as temperature, habitat, insolation and topography (Black, 1977; Thompson, 1979; Underwood, 1979; Clarke et al., 2004). Predation, competition, limited food supply and population density are also indicated as influencing growth rates. The similarity of the obtained life history parameters between both limpet species indicates that the undifferentiated regulation by species was the right management measure to implement.

The sex ratio of the exploited populations was slightly skewed in favour of males. This imbalance might be related to the fact that *P. aspera* is a suspected protandric hermaphrodite at the archipelago of Madeira. Sex change is induced by the size selective nature of the harvest that conducts to this change in smaller sizes, resulting in skewed sex ratios and changing the reproductive output of those heavily exploited populations. In fact, hermaphroditism is an evolutionary advantageous strategy to increase the likelihood of successful fertilization for semi-sessile broadcast spawners, particularly when low population densities occur (Branch, 1981; Guallart et al., 2013).

This work confirms worldwide for the first time sequential hermaphroditism for *Patella piperata* Gould, 1846. This species is endemic to the archipelagos of Madeira, Canaries and Cape Verde (Christiaens, 1973) and occurs in the supratidal zone, the most accessible zone and as such is subject to harvesting pressure by the local population.

The results reported herein showed that this phenomenon occurred in approximately 5% of the examined specimens. As sex change represents an advantageous strategy in increasing the reproductive success of organisms through an increase of its reproductive efficiency, further studies are warranted to better understand the impact of this phenomenon on the reproductive biology and populations' dynamics, and to determine the biotic or abiotic cues controlling hermaphroditism in this species and how this phenomenon correlates to exploitation pressure.

Histological validation of hermaphroditism in *P. candei* and *P. aspera* is also important, since both species are suspected to be protandrous hermaphrodites in the archipelago of Madeira, as reported for the archipelago of Azores (Cunha et al., 2007; Martins et al., 2017). This information is of utmost importance to better understand the reproductive biology of these species and may warrant changes on existing harvest regulations. The histological validation of protandrous hermaphroditism in both species, in the archipelago of Madeira, will substantiate the implementation of specific regulation to counter the negative effects of the size-selective harvest, such as the specification of a maximum allowable size of capture ensuring the protection of larger specimens. The protection of larger specimens is fundamental for the replenishment of exploited populations since these specimens provide a greater contribution to the population reproductive effort (Keough et al., 1993).

Conservation of intertidal grazers in the historically harvested archipelago of Madeira

The artisanal harvesting of gastropods in the archipelago of Madeira is operated mostly by small vessels in nearby areas preferentially on the north coast of Madeira and Desertas Islands. The economic impact of limpets gradually increased over the years, representing in 2017 96% of the economic value landed for molluscs and 2% of the total landings in this region. Limpets harvest is protected by legislation that aims to provide the basis for a sustainable exploitation. However, it is still very common to occur violations to the current management policies and regulations, and as such a greater effort in the enforcement of regulations is required to ensure compliance of the fishing communities and an increase of the surveillance by the authorities to discourage illegal harvesting of these molluscs. Concerning topshells, the establishment of management measures on the harvesting of this resource is crucial to promote a sustainable exploitation in a medium and long term, since several areas already seem to be depleted in this region.

The direct effects of severe harvesting pressure on the exploited populations are the reduction of abundances and changes size structure, because of the preferential harvesting of larger specimens (Griffiths & Branch, 1997). Harvesting regulations are supposed to preserve age structure, enhance yield, preserve balanced sex ratios, prevent sperm limitation, and restrict evolutionary as shifts to early maturation (Alonzo & Mangel, 2004; Baskett et al., 2005; Heppell et al., 2006; Hamilton et al., 2007).

The current limpet harvesting regulations in the archipelago of Madeira are based in technical measures established by the Regional Government under the Regional Legislative Decree Nº11/2006/M, 18 April 2006 (see chapter 7). The harvesting regulations were based in preliminary biological studies on *P. aspera* and *P. candei* and intended to protect stocks and preserve the economic activity. Since 2006 the closed season was extended, now lasting from December 1st to March 31st and a reduction in the number of harvesting licenses was promoted. Topshell harvest is presently unregulated.

Despite the existence of limpets harvesting regulations, comparative studies had never been carried out to verify the effectiveness of the established management measures, and it was urgent to assess the status of the stocks, since management measures were implemented more than a decade ago. As such the effectiveness of the harvesting regulations and of the marine protected areas (MPAs) on the size composition, abundance and reproductive parameters were herein assessed for the first time for the archipelago of Madeira.

The long term analysis of exploited limpet populations before (1996-2006) and after (2007-2017) the implementation of harvesting regulations indicates that the size composition of these populations is going through a slight recovery phase characterized by: i) a dominance of the proportion of the reproductive individuals; ii) an increase of the size at first maturity; iii) an increase of mean shell length, and iv) a more balanced sex ratio after the implementation of management measures for both limpet species. Even though conservation measures prompted a positive effect on both exploited limpet species, continuous monitoring is required and further assessment studies are necessary to address the evolution of stocks over time to ensure the sustainable exploitation of limpets. The recovery of the exploited populations was more evident for *P. aspera* due to the fact that this species is under higher harvesting pressure and hence, the effect of harvesting regulations was more noticeable (see chapter 9).

Overall, limpet harvesting regulations in the archipelago of Madeira prompted positive results, contrarily to what observed for the archipelago of Azores (Martins et al., 2017) and for the Canary Islands (Riera et al., 2016) where legislation and current levels of enforcement were insufficient to protect and promote the recovery of the populations. Despite of the observed recovery of exploited limpet populations, a more pronounced effect of the implemented harvesting regulations was expected. This less pronounced recovery of the exploited populations is most likely explained by poaching which is known to occur during the closed season, without abiding the minimum permissible catch size and the maximum allowable catch in weight established by regulations. Also, the estimated slow growth rates and long life-span of *P. aspera* and *P. candei* in the archipelago of Madeira are thought to contribute to the slow recovery of the exploited populations. In fact, for marine invertebrates with high longevity and slow growth, temporal responses to marine protection are assumed to be slower than in other marine organisms such as fishes (Halpern & Warner, 2002).

The overall improvement of the populations could have been greater if an effective enforcement of the implemented management measures was fully assured. However, poaching is still possibly the greatest threat to these species, together with other anthropogenic impacts such as habitat removal and pollution. Reinforcing surveillance, creating further awareness and instigating a more committed engagement of local communities are pivotal to achieve better results on promoting the conservation of these species while maintaining their sustainable and economically viable exploitation.

Further evidence showing the negative effects of these anthropogenic impacts is provided by the analysis of the effect of proximity to human settlements and accessibility to the coast. In fact, the size-structure and abundance of topshells and limpets showed consistent differences in regard to mean size, proportion of reproductive individuals and abundance which were generally smaller in areas closer to human settlements and in more accessible coastal areas. The same pattern was observed for Martins et al. (2008) in Azores for limpets' abundances. Concerning accessibility, it was showed to affect negatively the size structure and abundance of *P. sauciatus* and *P. aspera*. However, this trend was not observed for *P. candei*, possibly due to greater harvesting pressure being exerted on *P. sauciatus* and *P. aspera* or to species specific characteristics that confer greater vulnerability to these species (Sousa et al., 2019). In fact, proximity and accessibility to limpet and topshell populations not only rises their vulnerability to harvest but also to habitat loss and pollution that negatively impact the exploited populations and as such, it is suggested to consider these two factors in future harvesting regulations in order to better preserve populations near the human settlements and from more accessible areas (see chapters 9 and 10).

In areas where the impact of anthropogenic activities is reduced to a minimum such as MPAs, where harvesting has been prohibited for over 20 years, topshell and limpet populations showed the highest abundances and size composition. These results confirm the mainstream opinion that MPAs are one of the most important tools in the conservation of coastal resources (Micheli et al., 2008; Pérez-Ruzafa et al., 2008; Edgar et al., 2014), contributing to increasing density and biomass and to the natural replenishment of stocks (Manríquez & Castilla, 2001; Halpern & Warner, 2002).

The comparative study between exploited and protected populations showed that commercial harvesting of limpets in Madeira archipelago negatively affects the abundance, size and reproduction of *P. aspera* and *P. candei*, which are considerably less abundant, have lower sizes and early maturation in exploited areas. Size decrease and abundance reduction in exploited populations were also reported for exploited limpets in South Africa (Branch, 1975), Chile (Moreno et al., 1984), Australia (Keough et al., 1993), Tanzania (Newton et al., 1993), Mexico (Pombo & Escofet, 1996), Canary Islands (Núñez et al., 2003; Ramírez et al., 2009), Mediterranean (Espinosa et al., 2006) and Azores (Martins et al., 2017).

The effects of protection from MPAS on limpet populations resulted in a differential increase on size-at-first maturity, shell size and capture per unit effort according to the degree of protection. The conservation value within MPAs is affected by the cumulative effects of five key features: regulation, enforcement, age, size and isolation (NEOLI features) (Edgar et al., 2014). MPAs with four NEOLI features as Selvagens and Desertas Islands were manifestly in greater balance than reserves with no NEOLI features (Rocha do Navio) and consequently than exploited zones. It was notorious that old and well-enforced MPAs harbour a high representation of large specimens and more balanced populations with an increase of 16 mm in shell length for *P. aspera* and 13 mm for *P. candei*. NEOLI features of MPAs were applied for the first time in marine gastropods and demonstrated to be reliable to protect these resources, particularly the features age and enforcement. The results obtained herein should be used to corroborate the positive effects of MPAs in the protection of the exploited resources; especially in oceanic islands were species are more susceptible to over-exploitation.

Overall, the implementation in 2006 of limpet harvesting regulation in the archipelago of Madeira has yielded positive returns contributing to a slight recovery of the exploited populations and to the reduction of size differences in both limpet species between exploited zones and MPAs (Sousa et al., 2019). These positive returns are more evident for *P. aspera,* allowing specimens to achieve larger sizes and guaranteeing reproduction and recruitment, while for *P. candei* regulations contributed mainly in terms of the abundance of larger specimens. The harvesting regulation in topshells would benefit the exploited population as occurred for limpets since they share some similar life history traits and are subject to analogous environmental and anthropogenic pressures and as such, it would be expected that the implementation of management measures would yield similar results to those verified for limpets.

In short, to improve the recovery of the exploited populations, to counter the negative effects of size selective harvesting, to ensure the effective management of marine gastropods and the sustainability of the studied species and ecosystems at medium and long term it is crucial to promote further awareness, engagement and commitment of local communities, management regulators, policymakers and stakeholders, based on information and education of the local populations.

Conclusions and recommendations

1.- The establishment of limpet harvesting regulations in the archipelago of Madeira in 2006 proved to be effective in the recovery of the exploited populations. However, the slight recovery observed is justified by limpets in this region, having low growth rates allied to their long life which leads to a prolonged time of recovery. Another factor that also influenced the low recovery of the populations was the ineffective surveillance during the closed season to prevent poaching. As such, further awareness of all actors involved in this activity is crucial to ensure a more effective recovery of the exploited populations. Furthermore, genetic studies should complement the continuous monitoring of limpet populations, mainly in defining the stock in this region, allowing adapting the established regulations to the particularities of the harvested populations.

2.- MPAs demonstrated to be an effective tool in protecting the populations of limpets in the archipelago of Madeira, resulting in a differential increase of size at first maturity, shell size and capture per unit of effort. Age and enforcement were the most relevant drivers to explain variability among protected areas.

3.- Topshell harvesting regulations should be implemented as soon as possible to prevent the possibility of overexploitation. The recovery of harvested topshell populations is expected to be analogous to that observed for limpets because of the similar conditions of life history parameters and habitat of the studied species.

More specifically, to increase the effectiveness of the established harvesting regulations the following series of 10 recommendations is presented as a complement to the management measures in effect.

Recommendations to insure sustainable exploitation of limpets and topshells

i) Implementation of the management measures developed herein is of paramount importance to establish the proper conservation strategies of topshells in the archipelago of Madeira at medium and long term.

ii) Feasibility of topshells and limpets as bioindicators of anthropogenic pressures, based on how they respond to pollution, exploitation and climate change to provide information required for the establishment of management measures of the rocky shore's ecosystem.

iii) Continuous monitoring studies of the exploited populations of topshells and limpets are warranted to verify the variation of the biological parameters and population dynamics of these species throughout time, allowing the adjustment of harvesting regulations as needed.

iv) Establishment of obligatory species-specific landings for limpets is crucial to accurately quantify the landings by species and to better assess the status of the exploited stocks.

v) Implementation of a maximum size of capture for both species, to counter the negative effects of the size-selective harvest in these species.

vi) Application of new molecular tools and next generation sequencing to verify whether the populations represent one single stock or a network of smaller stocks, and to determine connectivity among populations and if they are geographically and ecologically isolated, contributing to the establishment of specific conservation strategies if necessary.

vii) Encouragement of aquaculture investments and stock replenishment by reintroduction of cultured specimens to reduce harvesting pressure on the exploited populations.

viii) Instilling entrepreneurial spirit on harvesters by complementing their activity through vessel trips and dives increasing ecosystem value of these resources.

ix) Reinforcement of the implemented management measures to achieve a better stock by raising public awareness, political will and engagement of local communities through further sensitization and more effective vigilance by the local authorities to prevent poaching.

x) Information and advice to the consumers to avoid purchasing illegally harvested limpets to reduce illegal harvesting.

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